

Center for Neuroscience and Cell Biology

Associate Laboratory

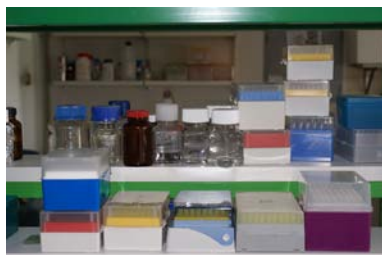
Annual Report | 2011

Experimental Biology and Biomedicine | A new culture through Scientific Research

Research Programmes | Biology | Neuroscience | Health and Disease | Biotechnology



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Introduction | General Objectives



CNC is an Institute committed to excellence in research in Neuroscience and Biomedicine with a main focus on “fundamental and translational research, advanced training and capacity to provide specialized services to the community”. Research is organized in 6 thematic areas, each coordinated by a senior scientist: Neuroscience and Disease, Molecular Biotechnology and Health, Cell and Molecular Toxicology, Microbiology, Biophysics and Biomedical NMR, Cell and Development Biology. The programme for each area is implemented by small groups headed by a research leader.

The scientific productivity of CNC in 2011 is demonstrated by 212 publications (more 80 in press), an effort supported by 115 grant projects (93 FCT Projects, 6 national projects, 16 international projects).

In 2011 CNC supported the training of 204 PhD Students, (31 PhD Thesis concluded) and 63 MSc Students (23 MSc Thesis concluded).

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From 2006 to 2011 980 papers were published, with a total number of citations of 12360, and an average number of citations of 21.9 per paper, placing CNC as a leading research institute in the biomedical and neurosciences field in the country.

The current aims at CNC are: 1) Fundamental and translational research in Neuroscience, Cell Biology and Molecular Biotechnology, 2) Advanced training; 3) Technology transfer and specialized services to the community; 4) outreach Programme (science and society).

The core scientific activity of CNC is the study of the molecular basis of neurodegenerative processes common to aging, neurodegenerative disorders, cerebral ischemia and epilepsy. In parallel, the research groups explore mechanisms of neuroprotection and regeneration, which may be future

candidates for the development of potential therapeutic strategies to manage these disorders. This core activity is complemented by supporting areas which also develop their own research activity, opening the scope of intervention of CNC in the biomedical field, while providing novel lines of research applicable to Neuroscience, namely: A) molecular biotechnology, with expertise in genetic screening of diseases, structure-function relation of proteins with biomedical or biotechnological interest, development of new vectors for delivery of drugs and genetic material and biomaterials for stem cell- based therapeutics; B) molecular and cellular toxicology, focused on the study of drug and disease-induced cell dysfunction, aiming to understand the molecular basis for clinical drug toxicity, with particular expertise in processes involving mitochondrial dysfunction and free radicals; C) biomedical NMR and metabolomics with a focus on development of inorganic compounds for medical diagnosis , intermediate metabolism and diabetes; D) cellular and developmental biology, whose programs focused on human infertility, disruption of human cell function in cancer, contact dermatitis, osteoarthritis, auto-immune disease, obesity and pathogens biology, involve close partnerships with clinicians at Coimbra University Hospital Center (CHUC) and Coimbra Portuguese Institute of Oncology (IPO); E) microbiology with emphasis on the strategies for adaptation of microorganisms to extreme environments, the screening and development of new anti-mycobacterial drugs and the susceptibility to legionella and fungal infection.

Translational research, organized as an Inter-institutional research programme, involves Hospitals, Pharmaceutical Companies and the Biotechnology Association Biocant.

Development of new technologies based on solid



fundamental research, and stimulated by the growing interest in translational research, led to reorganization of the services sector and to the creation of a technology transfer unit at Biocant, the UC/CNC/BIOTECH Institute. The promotion of technology transfer and the creation of novel biomedical and biotechnology enterprises is one of the aims of CNC at Biocant.

Graduated training includes a Doctoral Programme in Experimental Biology and Biomedicine and the participation in the MIT-Portugal Doctoral Program thus providing Master and PhD students with a multi-faceted education in molecular life sciences related to disease, contributing to national and international scientific networking. Since 2009 CNC is a partner of the European Neuroscience Campus Network, which offers an uniform PhD training in Neuroscience in Europe. CNC integrates



international networks such as the European Excellence Neuroscience Institutes Network (ENI-NET), the MIT-Portugal and Harvard Medical School-Portugal Programs, and is a founder of Health Cluster Portugal (HCP).

The Outreach Programme at CNC is devoted to promotion of science outside the scientific community, in a close collaboration with “Ciência Viva” Programme and “Instituto para a Educação e Cidadania” initiatives.

In the next future CNC will pursue its major mission, fostering fundamental and translational research and training in biomedical science with a particular focus on neurosciences.

Facts & Figures | 2011

RESEARCH STAFF

Members holding Ph.D.	175
Ph.D.Students	204
MSc Students	63
Grant Technicians	37
Service Staff	14
Technical Staff	18
Administrative Staff	12

Scientific papers published 212

Scientific papers published *In Press* 80

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THESIS CONCLUDED

Ph.D. thesis 31

MSc thesis 23

Organization

The Center for Neuroscience and Cell Biology (CNC) is a non-profit biomedical research center of public utility at the University of Coimbra. CNC brings together scientists from the Faculties of Science and Technology, Medicine and Pharmacy and from the University Hospital Center. CNC is a "Laboratório Associado".

Associate Members of CNC are: Universidade de Coimbra (principal associate – 50%), Centro Hospitalar da Universidade de Coimbra, Fundação para a Ciência e Tecnologia, AIBILI, Fundação Bissaya Barreto and two commercial firms – Reagente 5 and ILC.

1- Governing Body

President: Catarina Resende de Oliveira

Vice Presidents: Euclides Pires
Carlos Faro
João Ramalho Santos

Honorary President: Arsélio Pato de Carvalho

Executive Council Directors of the Departments

Research Council CNC members holding PhD

"Conselho Fiscal" I.L.C., Reagente 5, Leal e Carreira

"Revisor Oficial de Contas" Leal e Carreira, Sociedade Revisora de Contas

External Advisory Committee Enrique Cadenas (USA); Roberta Brinton (USA); George Perry (USA); Helmut Sies (Germany); Stephen Zinder (USA).

2- Scientific Areas and Research Groups

At present, research programmes and projects are organized in 6 scientific areas, each coordinated by a senior scientist. The programme for each area is implemented by small research groups each headed by a research leader in a specific field of study. In 2011, the research groups for each area can be identified, according to the following organization:

Neuroscience and Disease | Catarina Oliveira

Neuromodulation Group (Head: Rodrigo Cunha)

Glutamatergic Synapses Group (Head: Ana Luísa Carvalho)

Neuroprotection and Neurogenesis in Brain Repair Group (Head: João Malva)

Neuronal Cell Death and Neuroprotection Group (Head: Carlos B. Duarte)

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group (Head: A. Cristina Rego)

Molecular Mechanisms of Disease Group (Head: Sandra Cardoso)

Neuroendocrinology and Neurogenesis Group (Head: Cláudia Cavadas)

Emerging Group

Chronic Inflammation Group (Head: Margarida Carneiro)

Molecular Biotechnology and Health | Euclides Pires

Molecular Biotechnology Group (Head: Carlos Faro)

Molecular Systems Biology Group (Head: Armindo Salvador)

Structural and Computational Biology Group (Head: Rui Brito)

Vectors and Gene Therapy Group (Head: M. Conceição Pedroso Lima)

Biomaterials and Stem Cell-Based Therapeutics Group (Head: Lino Ferreira)

Pharmacometrics Group (Head: Amílcar Falcão)

Bioorganic and Medicinal Chemistry Group (Head: M^a Luisa Sá e Melo)

Cell and Molecular Toxicology | Rui A. Carvalho

Mitochondrial Toxicology and Disease Group (Head: Paulo Oliveira)

Redox Biology in Health and Disease Group (Head: João Laranjinha)

Microbiology | Milton Costa

Microbiology of Extreme Environments Group (Head: Milton Costa)

Medical Mycology - Yeast Research Group (Head: Teresa Gonçalves)

Biophysics and Biomedical NMR | Carlos Geraldes

Inorganic Biochemistry and Molecular Imaging Group (Head: Carlos Geraldes)

Intermediate Metabolism Group (Head: John Griffith Jones)

Cell and Development Biology | João Ramalho Santos

Cellular Immunology and Oncobiology Group (Head: Celeste Lopes)

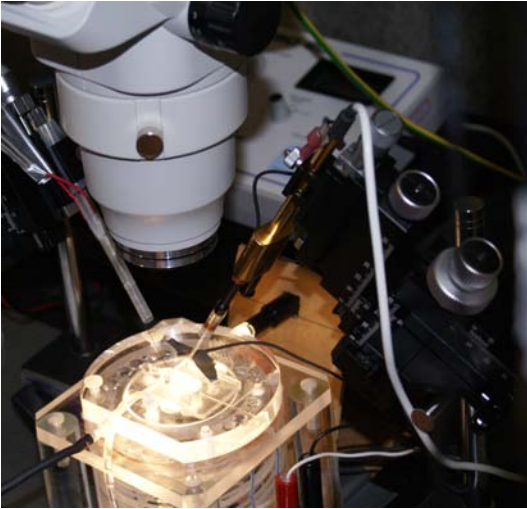
Biology of Reproduction and Human Fertility Group (Head: João Ramalho Santos)

Infection, Phagocytosis and Pathogens Group (Head: Otilia Vieira)

Insulin, Resistance and Adipocyte Group (Head: Eugénia Carvalho)

Neuroscience and Disease Area

Coordinator: Catarina Resende Oliveira



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Research activity is centered on: 1. synaptogenesis and synaptic function modulation; 2. cellular and molecular mechanisms leading to selective neurodegeneration; 3. development of neuroprotective and brain repair strategies. The aims of the 7 groups in this area are: *Neuromodulation*, studying purines and endocannabinoids as modulators of synaptic activity, neuro-inflammation and brain metabolism; *Glutamatergic Synapses*, analysing synapses formation and synaptic activity; *Neuroprotection and Neurogenesis*, crosslinking neuro-inflammation and neural stem cells; *Neuronal Cell Death and Neuroprotection*, studying excitotoxic neuronal damage and neurotrophic support; *Mitochondrial Dysfunction and Signaling in Neurodegeneration*, evaluating mitochondrial dysfunction, energy metabolism and glutamate receptors; *Molecular Mechanisms of Disease*, studying mitochondrial preconditioning, regulation of intracellular trafficking and ER-mitochondria crosstalk; *Neuroendocrinology and Neurogenesis*, focusing hypothalamic, hippocampal and adipose tissue factors effect on neuronal function. A *Neuroimmunology line* is emerging.

The groups in this Area achieved important research results as indicated in their individual reports, which can be summarized as follows:

Glutamatergic synapses plasticity and synaptic clustering of glutamate receptors were shown to be controlled by adenosine A_{2A} and P₂ (ATP) receptors and the cell adhesion molecule Caspr1, respectively. Using a microfluidic culture system axonal differentiation was induced and presynaptogenesis was shown to require mRNA axonal translation. Oxygen-glucose deprivation induced an alteration of genes transcription related to the activation of necroptosis, coding for synaptic scaffolds and proteins involved in synaptic translation. Under excitotoxic

conditions the vesicular GABA transporter was cleaved by calpains giving rise to a stable cleavage product that is not targeted to the synapse, thus affecting GABAergic neurotransmission.

Mitochondria dysfunction and cellular bioenergetics impairment were shown to be a common feature in neurodegenerative disorders, such as polyglutamine diseases, Alzheimer's (AD) and Parkinson's (PD) diseases, causing microtubule depolymerization and protein aggregation via the autophagic-lysosomal pathway impairment. GluN2B-containing NMDA extrasynaptic glutamate receptors were shown to play a key role in A β toxicity. NMDAR subunits are differentially implicated in A β induced ER stress which is a prominent feature of endothelial cell dysfunction in AD. Mitochondrial preconditioning induces a protective effect of endothelial and neuronal cells which is associated with ROS generation and HIF1 signaling.

Endogenous peptides such as NPY were shown to be involved in the regulation of caloric restriction – induced autophagy in hypothalamic neurons and together with adenosine are neuroprotective agents. Hypothalamic neurogenesis and up-regulation of NPY levels were shown to be promoted by fluoxetine, an effect mediated by BDNF.

The pro-neurogenic action of endogenous peptides and BDNF was identified and pharmacologically dissected.

Neuromodulation Group

Rodrigo A. Cunha PhD – *Head of group*

Ângelo José R. Tomé	PhD
Attila Köfalvi	PhD
Carla Sofia G. Silva	PhD
Geanne M. Andrade	PhD
Henrique B. Silva	PhD
Lisiane O. Porciúncula	PhD
Paula G. Agostinho	PhD
Catarina A. Gomes	Post-Doc Fellow
Daniela Pochmann	Post-Doc Fellow
Manuella P. Kaster	Post-Doc Fellow
Rui Prediger	Post-Doc Fellow
Adalberto Alves Castro	PhD Student
Ana Cristina F. Lemos	PhD Student
Ana Patrícia Simões	PhD Student
Daniel Rial	PhD Student
Elisabete O. Augusto	PhD Student
Francisco Queiroz	PhD Student
Jessié Gutierres	PhD Student
Jimmy George	PhD Student
Marco António P. Matos	PhD Student
Marta Regina S. Carmo	PhD Student
Nuno Miguel Machado	PhD Student
Pedro Manuel Garção	PhD Student
Samira C. Ferreira	PhD Student
Sílvia Viana da Silva	PhD Student
Stefania Zappettini	PhD Student
Tiago Manuel P. Alfaro	PhD Student
Anna Pliássova	MSc Student
Tiago Emanuel Silva	MSc Student
Filipe Marques Teixeira	Grant Technician
Caroline Veloso	Grant Technician

Glutamatergic Synapses Group

Ana Luísa Carvalho PhD – *Head of group*

João Peça-Silvestre	PhD (Collaborator)
Ramiro Almeida	PhD
Rui Costa	PhD
Carlos Matos	PhD Student
Joana Ferreira	PhD Student
Joana Pedro	PhD Student
Luís Ribeiro	PhD Student
Maria Inês Coelho	PhD Student
Maria Joana Pinto	PhD Student
Sandra Sofia Rebelo	PhD Student

Susana Louros	PhD Student
Swarna Pandian	PhD Student
Tatiana Catarino	PhD Student
Dominique Fernandes	MSc Student
Luís Pedro Leitão	MSc Student
Pedro Daniel Rio	MSc Student
Tânia Marisa Perestrelo	MSc Student

Neuroprotection and Neurogenesis in Brain Repair Group

João O. Malva PhD – *Head of group*

Ana Paula P. Silva	PhD (collaborator)
Armando Cristóvão	PhD
Catarina Sofia Pimentel	PhD
Fabienne Agasse	PhD
Raquel Ferreira	PhD
Jorge Gomez-Lobo	Post-Doc Fellow
Liliana Bernardino	Post-Doc Fellow
Sara Xapelli	Post-Doc Fellow
Alexandra Rosa	Post-Doc Fellow
Clarissa Schitine	PhD Student
Joana T. Gonçalves	PhD Student
Maria Francisca Eiriz	PhD Student
Sofia Grade	PhD Student
Tiago Alexandre Santos	PhD Student
Ismael Fonseca Neiva	MSc Student

Neuronal Cell Death and Neuroprotection Group

Carlos B. Duarte PhD – *Head of group*

Armanda E. Santos	PhD
Emília P. Duarte	PhD
Margarida V. Caldeira	PhD
Ana Rita A. Santos	Post-Doc Fellow
Graciano Leal	PhD Student
Joana F. C. Fernandes	PhD Student
João T. Costa	PhD Student
Marta Dias M. Vieira	PhD Student
Miranda Mele	PhD Student
Pedro João Afonso	PhD Student
Ivan Lalanda Salazar	MSc Student
Patrícia Rebelo	Grant Technician

Mitochondrial Dysfunction and Signaling in Neurodegeneration GroupAna Cristina Rego PhD – *Head of group*

Ildete Luisa Ferreira	PhD
M ^a Teresa C. Oliveira	Post-Doc Fellow
Tatiana R. Rosenstock	Post-Doc Fellow
Ana Cristina Silva	PhD Student
Carla Maria Lopes	PhD Student
Luana Carvalho Naia	PhD Student
Márcio Ribeiro	PhD Student
Mário Laço	PhD Student
Rita Perfeito	PhD Student
Sandra Mota	PhD Student
Paulo Santos	PhD Student
Ana Margarida Oliveira	MSc Student
Carolina Noronha	MSc Student
Gladys Tarcilia Caldeira	MSc Student
Joana C. Rodrigues	MSc Student
Sofia Isabel Sousa	Grant Technician Collab.

Molecular Mechanisms of Disease GroupSandra Morais Cardoso PhD – *Head of group*

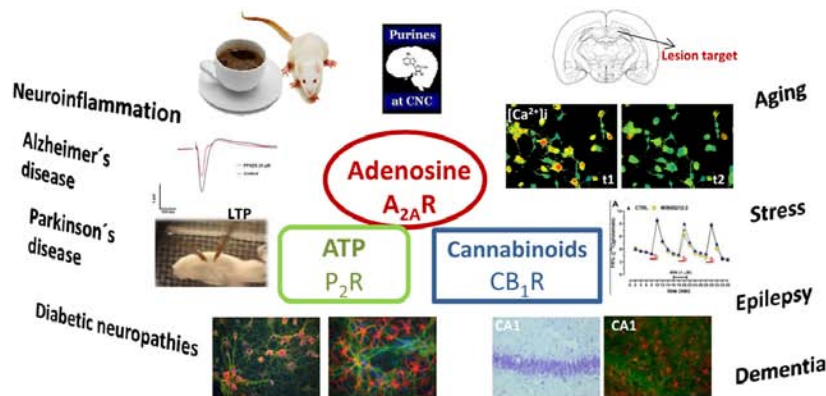
Catarina R. Oliveira MD, PhD	
M ^a Isabel J. Santana	MD, PhD
Cláudia M ^a F. Pereira	PhD
Paula Isabel Moreira	PhD
Ana Isabel Duarte	Post-Doc Fellow
Ana Raquel Esteves	Post-Doc Fellow
Elisabete B. Ferreiro	Post-Doc Fellow
Marina Marques Pinto	Post-Doc Fellow
Rosa M. Matos Resende	Post-Doc Fellow
Ana Catarina Fonseca	PhD Student
Cristina Carvalho	PhD Student
Daniel Santos	PhD Student
Daniela M. Arduíno	PhD Student
Diana FF Silva	PhD Student
Renato Xavier Santos	PhD Student
Sónia Correia	PhD Student
Sueli Cristina Marques	PhD Student
Susana Cardoso	PhD Student
Ana Isabel Fernandes	MSc Student
Diana Raposo	MSc Student
Diogo Martins Branco	MSc Student
Isaura Vanessa Martins	MSc Student
Sílvia Catarina F. Gomes	MSc Student
Emanuel Candeias	Grant Technician

Neuroendocrinology and Neurogenesis GroupCláudia Cavadas PhD – *Head of group*

Ana Rita Álvaro	PhD
António F. Ambrósio	PhD Collaborator
Bruno Carreira	PhD
Caetana Carvalho	PhD
Célia Avelaira	PhD
Inês Araújo	PhD Collaborator
Paulo F. Santos	PhD
Joana Salgado	Post-Doc Fellow
Ana S. Carvalho	PhD Student
Gabriel Costa	PhD Student
Joana Vindeirinho	PhD Student
Magda Santana	PhD Student
Maria Inês Morte	PhD Student
Mariana Botelho Rocha	PhD Student
João Felipe Martins	PhD Student
Ana Isabel Santos	MSc Student
Ana Sofia Lourenço	MSc Student
Fábia Sofia Vicente	MSc Student
Jorge Pascoal	MSc Student
Marta Maria Estrada	MSc Student
Vanessa Machado	MSc Student
Vera Raquel Cortez	Grant Technician

Emerging Group**Chronic Inflammation Group**M^a Margarida Carneiro PhD – *Head of group*

Helena M ^a Carvalheiro	PhD Student
Mónica Teresa P. Abreu	MSc Student
Tiago R. Sousa	MSc Student



The mechanisms of brain dysfunction are at present unclear but accumulating evidence indicates that both metabolic dysfunction and synaptic dysfunction may be early events in neurodegenerative diseases. More than understanding the mechanisms of brain diseases, there is an urgent need to devise novel strategies to manage these diseases. Our group focuses on the study of modulators of synaptic activity that can also affect brain metabolism, namely purines (adenosine, ATP) and cannabinoids. We began exploring the basic properties and function of the neuromodulation systems operated by adenosine and ATP in the nervous system: adenosine and ATP receptors (expression, binding characteristics, coupling to transducing systems, desensitisation), formation and inactivation of ATP and adenosine, physiological roles (control of neurotransmitter release, of ion channels and of synaptic transmission and plasticity) and we are now fostering understanding the role of these systems in physio-pathology using animal models of aging, hypoxia, epilepsy, diabetic neuropathies, stress, Alzheimer's and Parkinson's diseases and neuro-inflammation. Changes in endocannabinoid and adenosine levels in the brain are also associated with metabolic alterations and disturbances in energy homeostasis, which are often present in brain diseases; this makes metabolic control by neuromodulators an emerging and promising intervention strategy. Given the dual exploration of purines and on the other hand cannabinoids and

brain metabolism, the area was split into two groups: 'Purines at CNC' (lead by RA Cunha) and, on the other hand, 'Neuromodulation and Metabolism' (lead by A Köfalvi).

The major achievement was the identification of key mechanisms associated with the ability of adenosine A_{2A} receptors to neurodegeneration: the control of abnormal plasticity in glutamatergic synapses and the control neuro-inflammation, a key process in the evolution of neurodegenerative disorders. This paves the way for a better planning of the use of A_{2A} receptor antagonists as novel neuroprotective drugs.

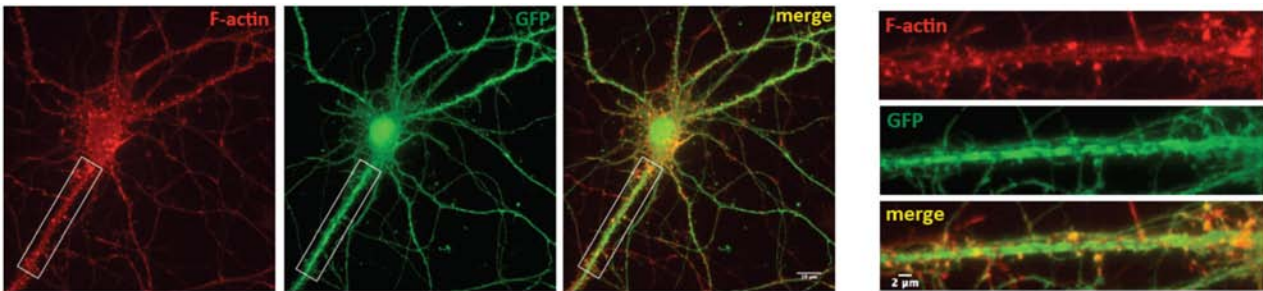
Another line of research exploited the role of ATP as a danger signal in brain diseases. We concluded that the extracellular levels of ATP are increased by different noxious brain insults, that glutamate excitotoxicity is abrogated by removing extracellular ATP, and that antagonists of P2 (ATP) receptors, namely of P2Y1 receptors, afford a robust neuroprotection against glutamate excitotoxicity and dysfunction/damage in animal models of epilepsy, ischemia and Alzheimer's disease. Overall, this provides a solid proof of concept for ATP as a danger signal in brain diseases.

We also described in a detailed manner for the first time that CB1 cannabinoid receptors - possibly situated in mitochondria - control the TCA cycle, i.e. the main "energy plant" of the cells. Therefore, it is not

surprising that the CB1R knockout mice exhibit lower glucose uptake and lower dissipative glucose metabolism rate. This can be a novel manner whereby the endocannabinoid system may control the synaptic availability of major neurotransmitters. However, we also found that in human Alzheimer's patients, the endocannabinoid signalling machinery is overactive contributing to synaptic dysfunction in the classical way

Glutamatergic Synapses Group

Head: Ana Luísa Carvalho



F-actin clustering in dendrites, detected in cultured rat hippocampal neurons (15 DIV) using rhodamine-coupled phalloidin, localizes to dendritic spines.

Our research group is interested in studying the formation of synaptic contacts between neurons, and their regulation throughout development and in response to neuronal activity. Alterations in synaptic strength are thought to be at the basis of learning and memory processes, and neurologic diseases such as schizophrenia, autism, Alzheimer's disease, polyQ diseases, are the result of perturbed synaptic function. The aim of the group is to understand aspects of synaptic function, using a combination of primary neuronal cultures, molecular cell biology and biochemistry tools. Some of the ongoing projects in the laboratory are described below.

Regulation of glutamatergic neurotransmission; PI: Ana Luísa Carvalho

Glutamate receptors of the AMPA type mediate fast excitatory neurotransmission in the CNS, and play key roles in synaptic plasticity. A proteomic screening performed in our laboratory identified novel binding partners for AMPA receptors (Santos et al. J. Proteome Res. 2010), whose function we are addressing. One of the proteins that caught our interest is Contactin associated protein1 (Caspr1), a protein of the neurexin family, which we have found to localize to glutamatergic synapses and to regulate the cell surface and synaptic expression of AMPA receptors (Santos et al., J. Biol. Chem. 2012). We have novel evidence suggesting that this protein may be

involved in post-transcriptional mechanisms of regulation of the transcripts of AMPA receptor subunits. Therefore, we are currently addressing the role of this protein in mechanisms of homeostatic plasticity, whereby neurons calibrate synaptic activity by adjusting their overall synaptic strength up or down to compensate for excessive excitation or inhibition.

Additionally, we are interested in stargazin, a member of the transmembrane AMPA receptor regulatory protein (TARP) family. Stargazin-mediated synaptic targeting of AMPA receptors requires the interaction of stargazin with PSD95, and phosphorylation of stargazin. Based on preliminary data from our laboratory, and in collaboration with Dr. Chinfei Chen at Harvard Medical School, we are testing the hypothesis that stargazin and its phosphorylation may play a role in synaptic scaling, a form of homeostatic synaptic plasticity.

NMDA receptors act as coincidence detectors in the induction of synaptic plasticity. There is controversy in the field regarding the differential role of NMDA receptors composed of different subunits. In collaboration with Dr. Ann Marie Craig, University of British Columbia, we are addressing this question. After studying the role of GluN1 splice variants in the synaptic traffic of NMDA receptors (Ferreira et al., J. Biol. Chem. 2011), we are now using neuronal cultures from knock-out mice for the GluN2 NMDA receptor subunits, to understand the role of

the NMDA receptors with different GluN2 subunit composition in the regulation of glutamatergic synapses. Proteomic quantitative analysis (iTRAQ, in collaboration with Dr. Ka Wan Li, VU University Amsterdam) of the postsynaptic densities of cortical neurons from GluN2B(-/-) mice has provided novel insights about the specific function of the GluN2B subunit of NMDA receptors, which we are currently exploring.

Appetite-regulating hormones target the hypothalamus, but have recently been described to affect hippocampal-dependent behavior. We are interested in how ghrelin, an appetite-stimulating hormone which was shown to enhance memory processes and synaptic plasticity in the hippocampus, regulates glutamatergic transmission. We found that the ghrelin receptor is expressed in glutamatergic synapses, and that its prolonged activation increases the synaptic expression of AMPA receptors. In collaboration with Dr. José Esteban at the Centro de Biología Molecular Severo Ochoa in Madrid, we are addressing the signalling pathways triggered by the activation of the ghrelin receptor which may impact glutamatergic transmission.

To establish the "hot spots" of axonal mRNA translation; PI: Ramiro Almeida

It has been known for many years that axons are capable of "locally responding" to guidance cues but only now are the mechanisms responsible for these phenomena starting to be

understood. Recent data has shown that local translation is required for other neurodevelopmental mechanisms like neuronal survival and axonal pathfinding. Also, the observation that distal axons have a diverse mRNA composition leads us to ask if local mRNA translation may play an important role in other neurodevelopmental processes like presynaptic differentiation.

The first goal of our research was to establish the “hot spots” of axonal mRNA translation. For that purpose our objectives were to determine if local mRNA translation is required for presynaptogenesis and if local protein synthesis occurs at the sites of nascent synapses.

We are interested in how AMPA receptor function is regulated in the brain, since the mechanisms for changing synaptic strength depend on the regulation of AMPA receptor function and traffic, and synaptic plasticity is at the basis of higher brain functions such as learning and memory (Santos et al. Neuroscience 2009). We performed a proteomic screening for interactors of long-form AMPA receptor subunits (Santos et al. J Proteome Res 2010), and identified novel proteins that bind to AMPA receptors. We have focused our attention on the cell adhesion molecule Caspr1, which interacts with AMPA receptor subunits. This protein localizes to excitatory synapses, and its overexpression in hippocampal neurons promotes the synaptic localization of GluA1, whereas its downregulation using shRNA decreases the synaptic clustering of GluA1-containing AMPA receptors. Additionally, Caspr1 increases the amplitude of glutamate-evoked currents. These data support a role for Caspr1 as a novel regulator of AMPA receptor function (Santos et al. J. Biol. Chem. 2012).

Another goal of the research in the group is to understand the mechanisms of synaptic accumulation of NMDA receptors. NMDA receptors in hippocampal neurons are tetramers comprised of two GluN1 subunits combined with two GluN2A and/or GluN2B subunits. GluN1 is essential for channel function and for trafficking GluN2 to synapses, and is

alternatively spliced. We have addressed the role of GluN1 splice variants in synaptic targeting in a physiological context, in collaboration with Ann Marie Craig at the University of British Columbia, and found that whereas the presence of the C2' cassette in the C-terminus of the GluN1 subunit drives the synaptic accumulation of NMDA receptors, NMDA receptors containing any of the GluN1 splice variants show homeostatic synaptic accumulation and PKC dispersal (Ferreira et al. J. Biol. Chem. 2011).

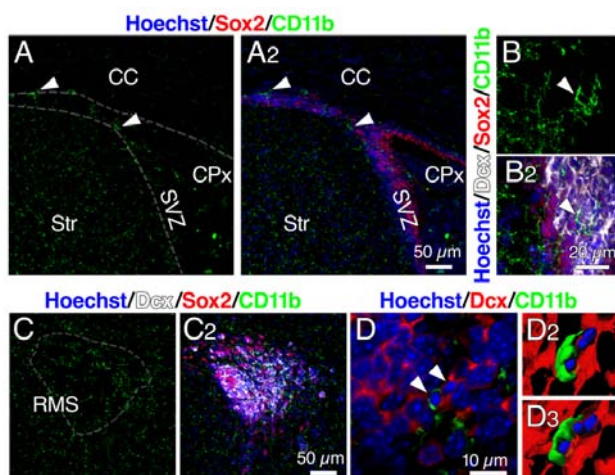
As mentioned in the previous section, the goal of the research work coordinated by Ramiro Almeida is to detect if local mRNA translation is required upon induction of presynaptogenesis. We have successfully established a microfluidic culture system and using this new platform we were able to specifically induced axonal differentiation. We observed that presynaptic assembly requires axonal translation, indicating that local protein translation can regulate the formation of new synapses. All nuclear transcribed RNAs have a 5' methylated GTP cap that binds to the translation factor eIF4E. In the unphosphorylated state 4EBP1 is bound to eIF4E, which sequesters this initiating factor and prevents translation. Specific stimuli lead to phosphorylation of the mammalian target of rapamycin (mTOR) which, in turn, phosphorylates and inactivates 4EBP1, releasing eIF4E and enhancing translation. Therefore, 4-pEBP1 staining is commonly used as a model for local translation. Our results show that 2h after FGF22 stimulation there is a significant increase in the phosphorylation of 4EBP1, suggesting that FGF22 induces presynaptogenesis through activation of local translation pathway.

Another research interests in the group relates to the polyQ disease Machado-Joseph disease (MJD). Several groups at the CNC (Cristina Rego, Luis P Almeida, e.g.) have a long experience in studying this disease, and we have become interested in the biologic function of ataxin-3, the protein whose polyQ expansion causes MJD (Matos et al., Prog. Neurobiol. 2011). In

collaboration with Sandra Macedo-Ribeiro at IBMC (University of Porto) we have characterized novel phosphorylation sites in ataxin-3, which we have found to modulate the catalytic activity of the protein. In collaboration with Patrícia Maciel (U Minho) and with Luis P Almeida (CNC-U. Coimbra) we are currently addressing the role of ataxin-3 phosphorylation in the pathogenesis of MJD.

Neuroprotection and Neurogenesis in Brain Repair Group

Head: João O. Malva



The long-term our objective is to contribute with fundamental knowledge, new tools and innovative strategies to treat brain diseases and to promote brain repair. Our scientific niche resides on coordinated investigation crosslinking neuroprotection, neuroinflammation and neural stem cells.

In 2011, the main specific objectives of our group included the following:

1-To reveal a role for neuropeptide Y as an important mediator of neuron-microglia crosstalk.

2-To identify novel factors (contact factors and intercellular communication through gap junctions) responsible for endothelial cell-neural stem cells communication in the neural stem cell niche.

3-To identify proneurogenic properties of peptides, such as galanin and somatostatin, in SVZ neural stem cells.

4-To identify novel mechanisms of action for AMPAkinases (focus on CX546) as proneurogenic compounds in SVZ neural stem cell cultures.

5-To develop new biocompatible nanoparticles to release retinoic acid and characterize the proneurogenic action of these nanoparticles in the SVZ stem cell niche.

6- To identify a role for endocannabinoids and hemoglobin-derived peptides in neurogenesis and oligodendrogenesis in SVZ neural stem cell cultures.

7-To identify a proneurogenic effect of histamine and to develop innovative

biocompatible histamine-releasing microparticles to modify the neural stem cell fate in the SVZ stem cell niche.

8-To reveal a role for endothelial BDNF signaling in neuroblast migration in the developing mice cerebellum.

9-To reveal a role for endothelial BDNF in rerouting neuroblast from the rostral migratory stream into the ischemic striatum of the mice.

We took advantage of an excellent network of national (in house and extramural) and international collaborators to develop new interdisciplinary projects. These collaborative and innovative strategies were also a pillar for internationalization of our group.

A key objective of the research group has been the training of new scientists at the master, PhD and postdoctoral level. Consistently with our commitment with science awareness in society we also contributed to Brain Awareness Week and other activities approaching the laboratory with schools.

In the year 2011, we successfully developed the main research projects conducted in our laboratory. In agreement with our defined strategic scientific pillars, new projects dedicated to neuroinflammation and neural stem cells will emerge in 2012.

1- We revealed a role for neuropeptide Y as an important mediator of neuron-microglia

crosstalk. The identification of NPY Y1 receptor-mediated inhibition of microglial cell migration and phagocytotic capacity was particularly relevant. Leading scientist: João O. Malva.

2- Novel factors (contact factors and intercellular communication through gap junctions) responsible for endothelial cell-neural stem cells communication in the neural stem cell niche were identified. A particular role for laminin-integrin signaling in the neural stem cell niche was dissected. Leading scientists: Fabienne Agasse; international collaboration with Florence Hofman (Los Angeles).

3-We identified, and pharmacologically dissected, a role for peptides, such as galanin and somatostatin, in SVZ neural stem cells differentiation of new neurons. Leading scientists: Fabienne Agasse; international collaboration with David Woldbye (Copenhagen).

4- We revealed proneurogenic properties of CX546 AMPAkinase in SVZ neural stem cell cultures. We observed that exposure of SVZ cells to CX546 increased proliferation and functional differentiation of new neurons. Leading scientist: João O. Malva; international collaboration with Fernando Mello (Rio de Janeiro).

5- We could develop (in close collaboration with Lino Ferreira, at CNC/Biocant) new biocompatible nanoparticles able to release retinoic acid in the stem cell niche. We showed that these particles are highly efficient in driving SVZ cell to neuronal fate. Leading scientists: Liliana

Bernardino and Lino Ferreira.

6- We could identify a novel role for endocannabinoids and hemoglobin-derived peptides in neurogenesis and oligodendrogenesis in SVZ neural stem cell cultures. A key action of CB1 receptors in nestin-positive cells was explored. Leading scientists: Sara Xapelli and João O. Malva

7- We could identify a new proneurogenic action of histamine (via H1 receptors) in SVZ stem cells and to develop innovative biocompatible histamine-releasing microparticles. These microparticles were highly efficient in promoting differentiation of neurons from SVZ cell cultures and to promote differentiation of new neuroblasts in SVZ cell grafted into hippocampal organotypic slice cultures. Leading

scientists: Liliana Bernardino, Lino Ferreira and João O. Malva

8-We dissected a role for endothelial BDNF signaling in neuroblast migration in the developing mice cerebellum. Leading scientist: João O. Malva; international collaboration with Armen Sagatelyan (Québec City).

9-We revealed a role for endothelial BDNF in re-routing neuroblast from the rostral migratory stream into the mice ischemic striatum. Leading scientist: João O. Malva; international collaboration with Armen Sagatelyan (Québec City).

Additionally, we contributed to the successful training of young and high-quality scientists at the master, PhD and postdoctoral level. In 2011,

Alexandra Rosa and Raquel Ferreira were awarded with a PhD degree. Jorge Valero and Catarina Pimentel were admitted as postdoctoral fellows/investigators at our research group. Fabienne Agasse, Liliana Bernardino and Sara Xapelli concluded their postdoctoral training/CNC contract and were admitted in extra-mural institutions, keeping a productive collaboration with CNC members at our research group.

The members of our group were highly active, together with other members of the CNC and the Portuguese Society for Neuroscience (SPN), organizing a variety of activities included in Brain Awareness week and European Night of Scientists.

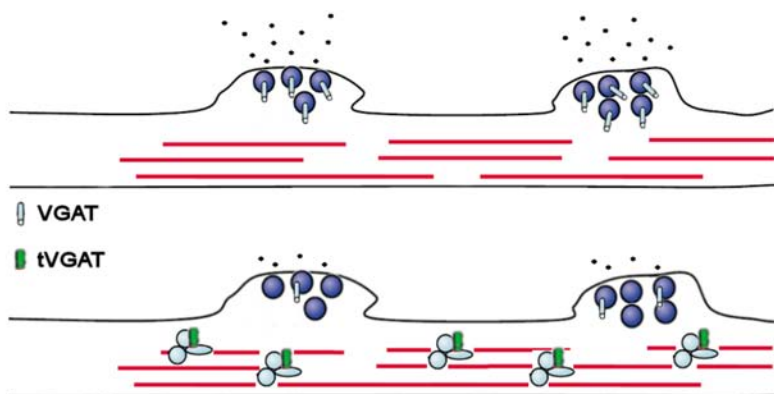


Center for Neuroscience and Cell Biology
A new Culture through Scientific Research



Neuronal Cell Death and Neuroprotection Group

Head: Carlos B. Duarte



Effect of VGAT cleavage on the distribution of the protein along axons. VGAT cleavage at the N-terminal region by calpain may eliminate trafficking signals that determine the subcellular distribution of the protein (adapted from J Neurosci 31, 4622-4635 [2011]).

Numerous disorders of the CNS are characterized by neuronal cell death, which may arise from the deregulation of the activity of neurotransmitter systems or insufficient neurotrophic support. In brain ischemia and in several neurological disorders there is an excessive accumulation of the neurotransmitter glutamate, and the resulting overactivation of glutamate receptors causes neuronal death (excitotoxicity). This group studies molecular mechanisms contributing to excitotoxic neuronal damage, particularly in the hippocampus, a brain region highly vulnerable to glutamate toxicity.

The $[Ca^{2+}]_i$ overload resulting from overactivation of Ca^{2+} permeable glutamate receptors activates Ca^{2+} -dependent signalling pathways that are coupled to neuronal death through regulation of transcriptional activity. This group is particularly interested in the contribution of the JNK and p38 signalling pathways in excitotoxic cell death, focusing on the signalling complex organization and on transcription regulation. Moreover, we are interested in excitotoxicity-induced mechanisms of Ca^{2+} -permeable AMPA receptors trafficking to the cell surface.

In addition to the activation of toxic signalling pathways, the $[Ca^{2+}]_i$ overload under excitotoxic conditions also upregulates the activity of calpains, which cleave several neuronal proteins. Many of these proteins are not degraded after cleavage, but their subcellular distribution and/or activity may be

affected. This group investigates changes in proteolysis under excitotoxic conditions and the impact on the activity of i) GABAergic and glutamatergic neurons, and ii) on the activity of neurotrophic factors. Changes in the glutamatergic and GABAergic systems in brain ischemia should affect the balance between excitatory and inhibitory activity, thereby modulating the demise process. Changes in the abundance of receptors for neurotrophic factors also affect the demise process.

Cerebral ischemia induces a transcriptional response that has an important role both in neuronal survival and in neuronal death. By means of a whole genome DNA microarray, we are investigating the transcriptional profile of rat hippocampal neurons challenged by an oxygen-glucose deprivation (OGD) stimulus in order to possibly identify new genes involved in neuronal death or survival. We performed an ontological analysis of the DNA microarray results, and observed that the genes related to metabolism, signalling pathways, transcriptional regulation, and receptor activity, were those whose transcription was most altered 7 and 24 h after an OGD stimulus. Some of the most promising genes obtained with this approach are related to the activation of the necroptosis cell death program, that can target the JNK pathway, as well as genes coding for synaptic scaffolds and proteins involved in synaptic translation. To confirm the DNA

microarray results, we are currently looking at the mRNA levels of selected genes by real time PCR while the level of corresponding proteins is being evaluated by Western Blotting. We expect that our approach will allow identifying a new therapeutic target in cerebral ischemia.

Under excitotoxic conditions there is also deregulation of proteolytic systems, and abnormal cleavage of key proteins. We found that the vesicular GABA transporter (VGAT) is cleaved by calpains in the N-terminal region, under excitotoxic conditions, giving rise to a stable cleavage product that is not targeted to the synapse. VGAT cleavage was also observed after transient middle cerebral artery occlusion in mice, a cerebral ischemia model, and following intrahippocampal injection of kainate, but no effect was observed in transgenic mice overexpressing calpastatin, a calpain inhibitor. Incubation of isolated cerebrocortical synaptic vesicles with recombinant calpain also induced the cleavage of VGAT and formation of stable truncated form of VGAT. The cleavage of VGAT is likely to affect GABAergic neurotransmission and may influence cell death during ischemia. (Gomes et al., J Neurosci 31, 4622-4635 [2011]). Excitotoxic and ischemic insults also induce the cleavage of VGLUT2 in the C-terminus (and VGLUT1 to a lower extent), and the cleaved transporter was found to a great extent in non-synaptic regions along neurites, when compared with the full-length VGLUT2 (Lobo et al., Neurobiol Dis 44,

292-303 [2011]). This is likely to affect glutamatergic transmission and cell death under excitotoxic conditions, especially in the neonatal period when the transporter is expressed at higher levels.

The TrkB.FL receptors for the neurotrophin BDNF are also down-regulated under excitotoxic conditions and in transient focal ischemia, and this is accompanied by an upregulation of a truncated form of the receptor (TrkB.T) lacking tyrosine kinase activity. Downregulation of TrkB.FL was mediated by calpains whereas the increase in TrkB.T protein levels required transcription and translation

activities. Down-regulation of TrkB.FL receptors under excitotoxic conditions was correlated with a decrease in BDNF-induced activation of the Ras/ERK and PLC γ pathways. However, calpain inhibition, which prevents TrkB.FL degradation, did not preclude the decrease in signaling activity of these receptors. On the other hand, incubation with anisomycin, to prevent the upregulation of TrkB.T, protected to a large extent the TrkB.FL signaling activity, suggesting that truncated receptors may act as dominant negatives to downregulate the signaling activity of the full-length receptors. The upregulation of TrkB.T

under excitotoxic conditions was correlated with an increase in BDNF-induced inhibition of RhoA, a mediator of excitotoxic neuronal death. These results indicate that BDNF protects hippocampal neurons by two distinct mechanisms: through the neurotrophic effects of TrkB.FL receptors and by activation of TrkB.T receptors coupled to inhibition of the excitotoxic signaling.

The Center for Neuroscience and Cell Biology (CNC) is an Associated Laboratory with research organized in six thematic areas:

- Biophysics and Biomedical NMR
- Cell and Developmental Biology
- Cell and Molecular Toxicology
- Microbiology
- Molecular Biotechnology and Health
- Neuroscience and Disease

The CNC gathers researchers from the Faculties of Medicine, Pharmacy and Science and Technology at the University of Coimbra, as well as from the University Hospitals (HUC). It has also its own group of hired researchers.

In order to translate basic knowledge into clinical applications a strong and valuable interaction has been developed between the CNC and the Clinical Faculty. From this interaction emerged the Biomedical Inter-Institutional Research Program involving CNC, HUC, CHC (Hospital Center of Coimbra) and IPO (Portuguese Institute of Oncology). Together with AIBILI - Association for Innovation and Biomedical Research on Light and Image, CNC offers specialized services to the community, namely to hospitals and pharmaceutical industry.

As a founding partner of Biocant - Biotechnology Innovation Center, the CNC has shown its commitment to foster technology transfer. The CNC is also a founding member of the Health Cluster Portugal (HCP).

The CNC integrates the Network of European Neuroscience Institutes (ENI-net), and is a partner of the MIT|Portugal Program in the focus area of Bio-Engineering Systems, and of the Harvard Medical School-Portugal Program.



The CNC hosts a PhD program in Experimental Biology and Biomedicine as part of its graduate training activity (<http://beb.cnc.pt/>). It also takes part in the European Neuroscience Campus Network Joint Doctorate Program (<http://www.enc-network.eu/>) awarded with an Erasmus Mundus grant.

Research

Committed to excel in Biomedical Research, particularly on aging and neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's, epilepsy, and retinal dysfunction), diabetes, regenerative medicine, stem cells, and disease and drug-associated toxicology.

Education

Offers graduate students a unique multidisciplinary environment.

Outreach

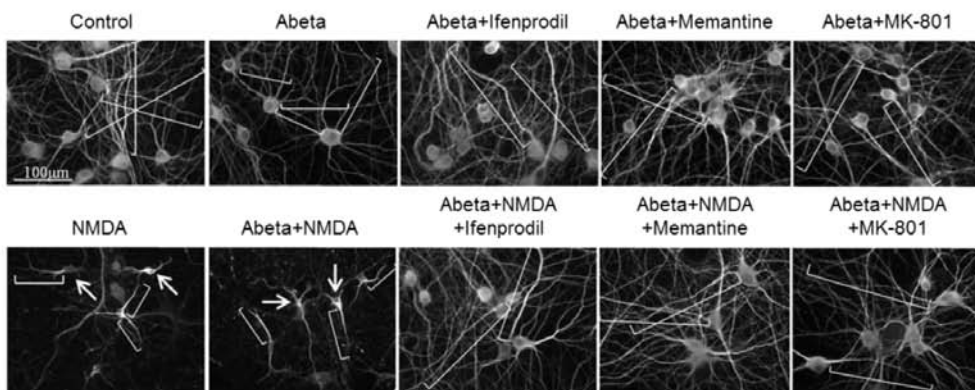
Strong commitment to science education programs intended for to the non-scientific community and to inspire the next generation of scientists.



Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Head: Ana Cristina Rego

Effect of A β ₁₋₄₂ and NMDA receptor activation on neurite length



Mota et al., *Curr. Alzheimer Res.* (in press)

In 2011 our group followed research activity with the objective to define the mechanisms of neurodegeneration in three neurodegenerative diseases, namely Huntington's disease (HD), Machado-Joseph disease (MJD), two polyglutamine expansion disorders, and Alzheimer's disease (AD). In this perspective we determined the alterations in mitochondrial function, energy metabolism and the involvement of glutamate receptors, namely the *N*-methyl-D-aspartate (NMDA) receptors (NMDAR), linked to the excitotoxic process, and further evaluated the protective effects of FK-506, insulin-like growth factor-1 (IGF-1) and NMDAR antagonists.

* Research in polyglutamine expansion disorders, HD and MJD:

Following the line of research in HD cybrid lines, for which the contribution of mitochondrial defects from patients peripheral cells is isolated, as described in the last report (Ferreira et al., 2010, *Exp. Neurol.* **222**, 243-255), in 2011 we investigated the mitochondrial-associated metabolic pathways in HD versus control (CTR) cybrids.

Moreover, we examined the striatal protective effects of FK506, an inhibitor of calcineurin, since this compound has shown neuroprotective effects in cellular and animal models of HD, including in a previous study performed by our research group (Almeida et al., 2004, *Neurobiol. Dis.* **17**, 435-444). The effects of FK506 were analysed in two striatal HD models, primary rat striatal

neurons and immortalized striatal *STHdh* cells derived from HD knock-in mice expressing full-length mutant huntingtin with 111 glutamines (*STHdh*111/111) or normal huntingtin (*STHdh*7/7).

Since central and peripheral metabolic abnormalities and altered IGF-1 levels have been described in HD, we hypothesized that restoration of IGF-1-mediated signaling pathways could rescue HD transgenic R6/2 mice from metabolic stress and behavioral changes induced by polyglutamine expansion. In this context, we analyzed the *in vivo* effect of continuous peripheral IGF-1 administration on diabetic parameters, body weight and motor behavior in the hemizygous R6/2 mouse model of HD subjected to continuously infused recombinant IGF-1 or vehicle, for 14 days.

Taking into account that mitochondrial dysfunction has been proposed as a mechanism of neurodegeneration in polyglutamine disorders, including HD, we used different cell models and transgenic mice of MJD to assess the importance of mitochondria on cytotoxicity observed in MJD.

* Research in AD:

Recent evidence demonstrates that glutamate receptors are dysregulated by amyloid beta peptide (A β) oligomers, one of the proteins affected in AD, resulting in disruption of glutamatergic synaptic transmission which parallels early cognitive deficits. Although it is well accepted that

neuronal death in AD is related to disturbed intracellular Ca²⁺ (Ca²⁺(i)) homeostasis, little is known about the contribution of NMDARs containing GluN2A or GluN2B subunits on A β -induced Ca²⁺(i) rise and neuronal dysfunction. Thus, the main goal of our work was to evaluate the role of NMDAR subunits in dysregulation of Ca²⁺(i) homeostasis induced by exposure to A β preparation containing both oligomers (in higher percentage) and monomers in rat cerebral cortical neurons. We further evaluated the role of NMDARs on A β -evoked neuronal dysfunction and cell death through changes in microtubule polymerization in mature hippocampal cultures. The involvement of NMDARs was evaluated by pharmacological inhibition with MK-801 or the selective GluN2A and GLUN2B subunit antagonists NVP-AAM077 and ifenprodil, respectively.

Main achievements:

A) Research in HD:

In the paper by Ferreira et al. (2011, *Exp. Neurol.*) we showed that HD cybrids exhibited an increase in ATP levels, when compared to CTR cybrids. Concomitantly, we observed increased glycolytic rate in HD cybrids, as revealed by increased lactate/pyruvate ratio. Nevertheless, pyruvate supplementation could not recover HD cybrids' ATP or phosphocreatine levels, suggesting a dysfunction in mitochondrial use of that substrate. Nevertheless, mitochondrial NADH/NADt levels

were decreased in HD cybrids, which was correlated with a decrease in pyruvate dehydrogenase activity and protein expression. Results obtained in this study showed that inherent dysfunction of mitochondria from HD patients affects cellular bioenergetics in a functional nuclear background (Ferreira et al., 2011).

Using rat primary striatal neurons (Rosenstock et al., 2011, *Neurochem. Int.*) we showed that FK506 abolished 3-nitropropionic acid (3-NP)-induced increase in caspase-3 activation, DNA fragmentation and necrosis. Treatment of *STHdh*(111/111) cells exposed to 10 nM staurosporine (STS) with FK506 effectively prevented cell death by apoptosis and moderate necrosis. Data evidenced that FK506 may be neuroprotective in HD against apoptosis and necrosis under mild cell death stimulus (Rosenstock et al., 2011).

By verifying the effects of peripheral IGF-1 administration in the R6/2 mice (Duarte et al., 2011, *Exp. Neurol.*), we showed that IGF-1 treatment prevented the age-related decrease in body weight in HD mice. Moreover, IGF-1 ameliorated poor glycemic control, which seemed to be associated with a decrease in blood insulin levels in R6/2 mice. Also, IGF-1 treatment highly improved paw clasping scores. These results showed that IGF-1 has a protective role against HD-associated impaired glucose tolerance, by enhancing blood insulin levels.

B) Research in MJD:

In the context of MJD (Laço et al., 2011, *BBA – Molecular Basis of Disease*), expression of expanded ataxin-3 increased the susceptibility to 3-NP in HEK cells, PC6-3 cells and cerebellar granule cells derived from MJD transgenic mice. Mutant PC6-3 cells differentiated into a neuronal-like phenotype with nerve growth factor (NGF) exhibited a decrease in mitochondrial complex II activity. Also, mitochondria from MJD transgenic mice and lymphoblast cell lines derived from MJD patients showed a trend toward reduced complex II activity. Our results suggested that mitochondrial complex

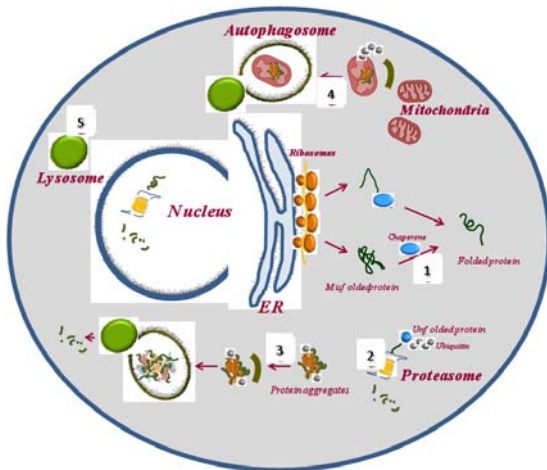
II activity is moderately compromised in MJD, possibly designating a common feature in polyglutamine toxicity.

C) Research in AD:

In primary cortical neurons (Ferreira et al., 2011, *Cell Calcium*), we showed that A β , similarly to NMDA, increased Ca²⁺(i) levels through activation of NMDARs containing GluN2B subunits. Moreover, A β modulated NMDA-induced responses and *vice versa*. Simultaneous addition of A β and NMDA potentiated Ca²⁺(i) levels, being this effect regulated by GluN2A and GluN2B subunits in opposite manner. Using mature rat hippocampal cultures (Mota et al., *in press, Curr. Alzheimer Res.*), we demonstrated that exposure to A β caused a decrease in total and polymerized beta-III tubulin and polymerized alpha-tubulin, suggesting microtubule disassembly. Moreover, the effects of A β on beta-III tubulin polymerization were correlated with reduced neurite length and neuronal DNA fragmentation. Interestingly, these effects were prevented by MK-801 and memantine, suggesting a role for extrasynaptic NMDARs in A β toxicity, and by ifenprodil, indicating the involvement of GluN2B-containing NMDARs. Data showed that A β -induced hippocampal neuronal dysfunction occurs through NMDAR-dependent microtubule disassembly associated to neurite retraction and DNA fragmentation in mature hippocampal cells (Mota et al., *in press*). These studies contributed to the understanding of the molecular basis of early AD pathogenesis, by exploring the role of NMDAR subunits in the mechanisms of A β toxicity in AD.

Molecular Mechanisms of Disease Group

Head: Sandra M. Cardoso



Major routes of intracellular clearance. Newly synthesized proteins may be incorrectly folded due to incorrect synthesis or to mutations, and as a result need to be removed from the cellular milieu. The first attempt by the cell is to correct the folding defect with the help of molecular chaperones (1). The second attempt is through the ubiquitin proteasome system (2) or via autophagy (3). The lysosome may also degrade dysfunctional organelles (4) or specific proteins with a target motif by a process called chaperone mediated autophagy. The lysosomal membrane may also invaginate to trap cytosolic regions by a process called microautophagy (5).

Mitochondria are involved in Alzheimer's and Parkinson's diseases (AD and PD) etiopathogenesis. We aimed to study the role of mitochondrial metabolism in the regulation of intracellular trafficking in PD and AD. We also evaluate mitochondrial signalling pathway and ER-mitochondria crosstalk in AD and in the disclosure of new therapeutic strategies.

Moreover, and since type 1 diabetic patients under insulin therapy experience daily fluctuations in blood glucose levels, ranging from low (hypoglycemia) to high (hyperglycemia) blood glucose levels, we investigate how brain cortical and hippocampal mitochondria are affected by STZ-induced diabetes, a model of type 1 diabetes, and insulin-induced acute hypoglycemia.

Finally, we evaluate the efficacy of mitochondrial preconditioning (a phenomenon in which small doses of noxious stimulus are required to afford robust protective responses against future injury) against glucotoxicity.

We provided evidence that mitochondrial impairment causes the loss of microtubule function, culminating in microtubule depolymerization that enhances α -synuclein aggregation, a pathological hallmark of PD, via autophagolysosomal pathway alteration.

The deleterious ER-mitochondria crosstalk in AD was supported by data obtained in mitochondrial DNA (mitDNA)-depleted cells and cybrids

(that recapitulate mitochondrial deficits of AD patients). In addition, we provided evidence that N-methyl-D-aspartate receptor (NMDAR) subunits are differentially implicated in $A\beta$ -induced neuronal ER stress and that ER stress is also a prominent feature of endothelial cell dysfunction in AD.

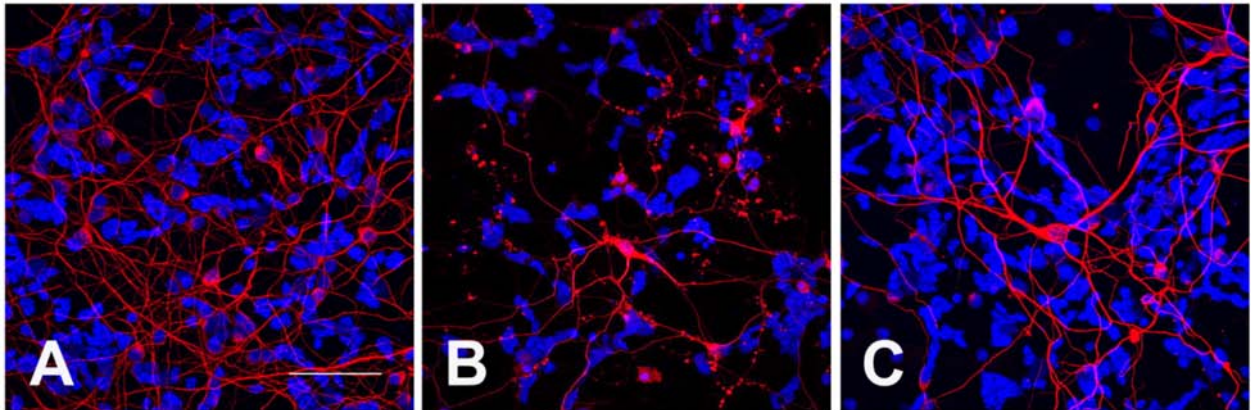
Although both hyperglycemia and insulin-induced hypoglycemia lead to an increase in ROS levels associated with a decrease in antioxidant defenses in brain cortical and hippocampal mitochondria, we observed that both brain areas behave differently in response to a metabolic insult. Furthermore, we showed that an acute episode of hypoglycemia induced by insulin potentiates the effects of STZ-induced diabetes having detrimental effects in cortical mitochondria bioenergetics and making the antioxidant defenses unable of overcome the increased oxidative stress. Therefore, the poor glycemic control that occurs in diabetic patients undergoing insulin therapy may have a negative impact in brain mitochondria, namely those of the cortex, predisposing the brain to degenerative events and cognitive impairment.

We also demonstrated that mitochondrial preconditioning induced by cyanide is effective in protecting both brain endothelial and NT2 neuron-like cells against high glucose-induced damage. Additionally, the cytoprotective effects of cyanide preconditioning are reliant on functional mitochondria, mitochondrial ROS generation and

induction of HIF-1 α signaling pathway. Elucidation of the role of the mitochondrial ROS and HIF-1 α in the protective mechanisms triggered by preconditioning may offer new avenues for the treatment of diabetes-associated neuronal and endothelial dysfunction.

Neuroendocrinology and Neurogenesis Group

Head: Cláudia Cavadas



NPY protects against neuronal cell death induced by glutamate.

Neurons (TUJ 1 – positive cells, red) integrity were assessed by immunocytochemistry in rat retinal neural cells. A - Control (no drug); B – Cells were incubated with glutamate 500 μ M for 24h; C – Cells were pre-incubated with NPY 100 nM, 1 hour before 500 μ M Glutamate. Cell nuclei were stained with Hoechst 33342 (blue). Scale bar 50 μ M

Our group continues studying the contribution and manipulation of hypothalamus, hippocampus, retina and adipose tissue to achieve a healthy lifespan.

1. Caloric restriction (CR) is a robust anti-aging intervention known to extend lifespan. Increase evidence shows that autophagy is an essential mechanism on the anti-aging effect of CR. In addition, CR increases neuropeptide Y (NPY) in the hypothalamic arcuate nucleus. NPY is a potent neuroprotective agent in several areas of the central nervous system; however its role in autophagy and consequently, lifespan extension, remains unknown. The aim of our group in this field is to investigate the role of NPY and the NPY receptors involved, on the regulation of autophagy in rat hypothalamic neurons. In addition, the involvement of NPY in CR-induced autophagy and the mechanisms underlying this process are also under investigation.

2. The understanding of pathophysiological and exogenous conditions that regulate proliferation and differentiation of endogenous neural progenitor cells is strategy to achieve neuronal repair by using neural stem cells. In this context our group is studying the role and mechanisms of inflammation in regulating rat neural stem cells proliferation and differentiation. In more detail, we are interested on

investigating the role of nitric oxide (NO) and calpains, and signaling pathways involved, on proliferation and migration of neural stem cells. Moreover, we will also investigate the potential of hypothalamic neurogenesis as a new approach on rescue hypothalamus that undergoes dysfunction and cell death in obesity status.

3. Chronic stress and depression have been associated to a state of "accelerated aging," In this context we are investigating the role of adenosinergic system in the adrenal gland as a protective system against certain changes induced by chronic stress.

4. Since retina is highly susceptible to eye diseases, somehow related with aging, we are interested on the identification of new strategies and targets to promote neuronal retinal protection and repair. The we are continuing to investigate the effect of diabetes or hyperglycemia on neuronal dysfunction and retina microglia changes, and specially the changes induced on adenosinergic system. The potential of neuropeptide Y (NPY) system and adenosinergic systems as a neuroprotective strategy in the retina will be also investigated. Moreover we are using inducible pluripotent stem (iPS) cells as promising cell therapy strategy.

5. Our group aims to investigate the conditions that negatively regulate neuronal protection and healthy lifespan, namely high food intake/obesity. Therefore we are interested on the understanding of adipose tissue regulation upon two conditions: hypoxia and anti-diabetic drugs (gliptins).

Main Achievements:

1- Our studies on proliferation of endogenous neural progenitor cells, as a strategy to promote neuronal repair, show that nitric oxide (NO) stimulates the proliferation of subventricular zone (SVZ) cells via the ERK-MAPK pathway. Furthermore, we found that in mixed cultures of SVZ cells and microglia, upon an inflammatory stimuli, cell proliferation is impaired and NO removal prevents this effect, suggesting that NO is responsible for the antiproliferative effect of inflammation. Moreover, mice lacking calpastatin have impaired proliferation and migration in the subgranular and granular zone of the dentate gyrus, as compared to their wild-type littermates.

Neurogenesis also occurs in the hypothalamus of adult rodents and the new neurons contribute to the maintenance of energy balance. Moreover, hypothalamic neurogenesis is pointed as a possible mechanism to remodel "faulty" feeding circuits in obesity and hypothalamic dysfunctions. We observed that

fluoxetine promotes the proliferation of hypothalamic neuroprogenitor cells and up-regulates the levels of orexigenic NPY; these effects are mediated by the neurotrophic factor BDNF. Possible therapeutic applications of fluoxetine as remodeling agent of feeding circuits should be further investigated. These studies open new perspectives to study hypothalamic neurogenesis in energy balance regulation and feeding dysfunctions.

2- Our results show that retinal adenosinergic system is affected by diabetes/hyperglycemia and may play a potential role in cell protection against the hyperglycemic environment. Targeting neuropeptide Y (NPY) system as a neuroprotective strategy in the retina, we observed that NPY inhibited glutamate-induced cell death mediated by the activation of Y_2 , Y_4 and Y_5 receptors through the activation of PKA; therefore, these results suggest that NPY system is a potential neuroprotective target in retinal degenerative diseases, such as glaucoma.

Inducible pluripotent stem cells (iPS cells) obtained from retinas and fibroblasts of reprogrammable mice produce iPS colonies that express the pluripotency markers. The *in vitro* differentiation of retina-derived iPS cells resulted on photoreceptor's shaped cells with photoreceptor markers. iPS cells obtained from the reprogrammable mice retina are pluripotent (with pluripotency markers) and were differentiated into photoreceptors with almost 100% efficiency. The use of iPS cells to obtain retinal cells, such as photoreceptors, could be a promising cell therapy strategy in retinal degenerative diseases.

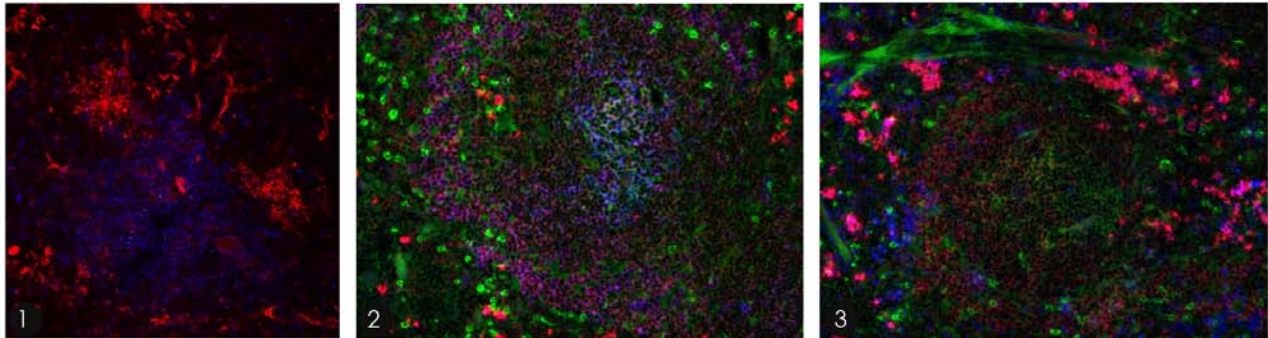
3 - Our results showed that, in mice, A_{2A} receptor antagonists modulate catecholamine release upon chronic stress, supporting the potential of A_{2A} receptor antagonists to manage modifications induced by chronic stress.

4- We investigated the role of the anti-diabetic drugs – gliptins (DPPIV inhibitors) - on adipose tissue regulation. The murine pre-adipocyte cell line, 3T3-L1, was used as a cell model. We observed that vildagliptin, sitagliptin and saxagliptin reduce intracellular lipid accumulation with inhibition of transcription factor expression, the PPAR γ 2 - crucial for adipocyte differentiation. It was also observed that gliptins inhibited adipogenesis, through PKA pathway, but do not induce lipolysis. These results suggest that gliptins may be used as new putative pharmacological strategies to prevent adipose tissue increase without the risk of dyslipidemia.

We also investigated the effect hypoxia on adipose tissue formation, namely on adipocytes differentiation from pre-adipocytes. Hypoxia induced lipid accumulation without PPAR γ 2 and perilipin changes, but increased miR27-a and miR27-b expression. Moreover, the lipid accumulation was accompanied with mitochondria dysfunction and with an increase of ROS. In conclusion, hypoxia induces lipid accumulation through a non-classical adipogenesis pathway.

5- Interestingly, NPY and caloric restriction (nutrient deprivation) induced the activation of autophagy in hypothalamic neurons (cell line N42) and in primary cell cultures of rat hypothalamic neurons. Moreover, mice overexpressing NPY in hypothalamus showed an increase of autophagy markers in the hypothalamus. NPY Y_1 , Y_2 and Y_5 receptor antagonists decreased autophagy marker induced by nutrient deprivation in hypothalamic neurons. Overall, these results show that autophagy activation under nutrient deprivation is, at least in part, mediated by NPY, in hypothalamic neurons. This observations support the hypothesis that NPY may act as a caloric restriction mimetic to extend lifespan.

Chronic Inflammation Group Head: M^a Margarida S. Carneiro



1- Follicular T cells (blue) near germinal center (red)
2- Follicular dendritic cells network (blue) in a germinal center; and follicular B cells (red)
3- Macrophages (blue) surrounding lymphoid follicle with B cells (red) and germinal center (green)

The Immunology Group studies the roles of B cells, CD8⁺ T cells and NK cells in the context of autoimmunity and Neuroimmunology .

Neuroimmunology Research Line:

Two projects have been recently approved on the role of Immune system in Parkinson and Alzheimer Disease.

Project 1:

LRRK2 role on auto-antibody production by human B cells
LRRK2 expression has been demonstrated in the brain and several organs, including the thymus and spleen, which are major immune system organs for B and T lymphocyte maturation and selection. Furthermore, LRRK2 expression has been described in different cells of the immune system, including B lymphocytes. Additionally, the presence of autoantibodies is a common finding in Parkinson's Disease (PD) patients, suggesting that a deregulation of the immune response – in particular B lymphocyte differentiation and function- might be associated with PD. This project aims to: 1) determine if LRRK2 mutations influence the expression of LRRK2 in peripheral blood B lymphocytes of PD patients, and how this expression relates to the presence of autoantibodies; 2) understand the role of LRRK2 expression during the cell differentiation and antibody production processes of human B lymphocytes.

Project 2:

Change in Alzheimer's Disease of NK and CD8⁺ T cells, a go-between innate and adaptive immune systems

Alterations in the peripheral immune system have been associated with early stages of dementia as well as with Alzheimer's disease (AD). Both the innate and the adaptive immune systems have been associated with memory performance in AD, to such an extent that vaccination therapies have been developed to manage AD; however, this has met variable success rates which appear to be dependent of the presence of particular HLA-DR alleles. Nevertheless, a unified model on how changes in the immune system might impact or specifically relate to AD pathogenesis is still lacking. Oddly, little attention has been devoted to the contradictory findings on the impact of AD on the function and phenotype of particular cell types known to play key roles in the communication between the innate and adaptive immune systems, such as Natural Killer (NK) and CD8⁺ T cells.

Overall, this project aims at clarifying to which extent the systemic changes in AD lead to altered NK and CD8⁺ T cell functionality in different AD model systems, and how such changes might contribute to and/or exacerbate neurological deterioration. Furthermore, this project will explore if NK and CD8⁺ T cell function can be considered potential biomarkers for AD progression, and/or potential targets

for novel therapeutic strategies aimed at modulating the immune system in AD.

Basic / translational Immunology

Chronic Inflammation Research Line:

Project 1: CD8⁺ T cell subsets in rheumatoid arthritis, and their potential in the initiation and maintenance of the disease (see inter-Institutional Programme)

Project 2: Immune response in Ncf1-deficient mice with DSS induced colitis

Chronic granulomatous disease (CGD) is a genetically heterogeneous immunodeficiency disorder caused by deficiency in oxidative burst, essential for the clearance of phagocytized micro-organisms causing increased susceptibility to severe bacterial and fungal infections. CGD is caused by mutations in the phagocyte NADPH oxidase complex, the enzyme that generates microbicidal oxygen radicals. One of the more common clinical complications in CGD is chronic intestinal inflammation. Ncf1 mutation leads to lack of reactive oxygen species in mice which are described as susceptible to autoimmune diseases and hypersensitivity to some pathogens. We used these mutant mice to study and comprehend how this lack of ROS influences a DSS colitis immune response, the recovery and a second DSS induction.

Project 3: B cells against *Candida albicans*: the role of reactive oxygen species in response to infection

Classically, patients with CGD will suffer from recurrent infections due to the decreased capability of their immune system to fight off disease-causing organisms, for the deficit of these reactive products; the organisms mainly involved in these infections are catalase-positive, such as bacteria (e.g. *Staphylococcus aureus*) and fungi (e.g. *Aspergillus fumigatus* and *Candida albicans*). In this study, the potential involvement of B cells in the immune response against the *C. albicans* infection was investigated, *in vitro*, in CGD and in control mice by: migration assay, cytometric analysis of lymphocyte and apoptotic phenotype upon fungal stimulation, quantification of ROS production by fluorimetric analysis and analysis of the mRNA expression of five genes involved in the B cells response.

Main Achievements:

Neuroimmunology Research Line:

LRRK2 role on auto-antibody production by human B cells :

Our preliminary data show that parkinsons disease patients have less B cells and more T cells in the blood than healthy controls.

Change in Alzheimer's Disease of NK and CD8+ T cells, a go-between innate and adaptive immune

Our preliminary data show that Alzheimer patients have a reduction of the total circulating B cells in particular the memory subset. There is a reduction of the T cell pool which show a higher production of pro-inflammatory cytokines. Additionally there is an accumulation of cytotoxic NK cells.

Chronic Inflammation Resarch Line:

Project 1: CD8⁺ T cell subsets in rheumatoid arthritis, and their

potential in the initiation and maintenance of the disease

In this project we aim at identifying the CD8⁺ T cell subsets present in the peripheral blood and synovial fluid or RA patients, and infer their contribution to the disease according to the patients' clinical diagnosis.

Our results demonstrate that CD8⁺ T cells are highly activated in rheumatoid arthritis patients when compared to healthy controls, and that activated CD8⁺ T cells seem to accumulate in the remission state of the disease when compared to the active state. Moreover, the correlation analysis of CD8⁺ T cell subsets present in the peripheral blood and synovial fluid indicate that some subsets have a high positive correlation. Thus, this study lead us to the conclusion that CD8⁺ T cells may have an important role in the pathogenesis of RA, as they appear to be potential initiators of the disease and responsible for its relapse.

Project 2: Imune response in Ncf1-deficient mice with DSS induced colitis

In this study we observed that the Ncf1 mice present worst clinical scores during an acute colitis induction and indications of more enhanced and aberrant immune Th1/Th17 response, with a weaker recovery during the rest period and signs of a chronic evolution when subject to a subsequent DSS induction. Thus, we suggest that ROS deficiency in these mice leads to abnormalities in the immune response at the intestinal mucosa, leading to aberrant immune responses with sever clinical outcome and a poor resolution evolving into a chronic inflammation.

Project 3: B cells against *Candida albicans*: the role of reactive oxygen species in response to infection

We observed, that the B cells are *chemoattracted* to the fungal antigens which was accompanied by an increased expression of CD69 (an early-activation cell surface maker on leukocytes), and a time-dependent increase of CD205 (an endocytic type I C-type lectin responsible for the

fungal PAMPS recognition). Moreover, programmed cell death and the release of ROS were the two ways, used by the B cells to overcome the infection. In fact the apoptosis was observed not only in the B cells, probably to elude the *C. albicans* attack, but , more interesting, was induced also in the fungal cells; proving in this way an antifungal response by the B cells. After reculture of *C. albicans* the cells have shown a decrease of viability. Additionally, we could show that, despite both WT and Ncf1 B cells increase ROS production, the Ncf1 B cells produced less ROS than the WT counterparts. Moreover, after the first hours of fungal contact we could see that the expression of four important genes linked with B cell maturation, proliferation and differentiation, was significantly reduced in the Ncf1-deficient B cells when compared to the wild-type ones. Thus, suggesting that the weaker anti-fungal response in Ncf1-deficient B cells is partially due to an impaired activation and differentiation of B cells as consequence of ROS deficiency. Overall, we have proved a hitherto unknown role for B cells in anti-fungal responses that should be explored in designing new therapeutic strategies. Moreover, by proving that a deficient production of ROS impairs the anti-fungal B cell response, we propose that the recurrent fungal infections observed in CGD patients, are not solely due to a deficient phagocytic capacity by macrophages and neutrophils, but also due to insufficient B cell activation and differentiation. Thus, future studies should concentrate on defining strategies to improve B cell differentiation and activation mechanisms and overcome deficient ROS production on B cells.

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Molecular Biotechnology and Health Area

Coordinator: Euclides Pires



The research activity performed by the groups of this scientific Area aims at the elucidation of the function of macromolecules either as single entities or as a part of complex systems. This research involves genomic, proteomic, metabolic and structural biology approaches; purification and characterization of new proteins, production of recombinant proteins and the use of system biology strategies to predict and integrate metabolic pathways. Relevant information obtained is then incorporated into translational projects aiming at new biotechnological or biomedical applications, in particular, development and pharmacokinetic characterization of new drugs, development of new drugs carriers and development of new biomaterials.

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Each group had developed a deep know-how and expertise in a defined set of approaches and techniques, mentioned above, mainly the techniques they use extensively in their own specific projects. Currently the groups make available and share their expertise with other CNC researchers interested in using those techniques.

Fundamental and Technological know-how of this research line is being widened by incorporation of two coming groups: 1) The Pharmacometrics group, with strong expertise in quantitative Pharmacology; 2) The Bioorganic and Medical Chemistry group, with expertise in chemo-enzymatic syntheses, an emerging group whose members were previously in the Vectors and Gene Therapy group.

The main achievements of 2011 are:

Pollens from 10 common trees were shown to contain serine or amino peptidases that increase transepithelial permeability through disruption of transmembrane proteins and degrade airways bioactive peptides that can disturb the balance between the anti- and pro-inflammatory effects in the lung.

Synthetic niches to potentiate in vivo stem cell engraftment and therapeutic effect were developed.

Silence of tumour-specific protein surviving was shown to sensitize tumour cells to cytotoxic action of conventional chemotherapeutic agents.

Characterization of the molecular mechanisms of amyloid formation by transthyretin, in particular the refolding kinetics of TTR and the kinetics of the early stages of oligomerization were carried out.

A method for profiling mitotic-cycle dependent metabolism without having to synchronize cells was devised.

A method for simultaneous determination of norfloxacin, lomefloxacin and ciprofloxacin by fast isocratic liquid chromatography was developed and validated.

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Biomaterials and Stem Cell-Based Therapeutics

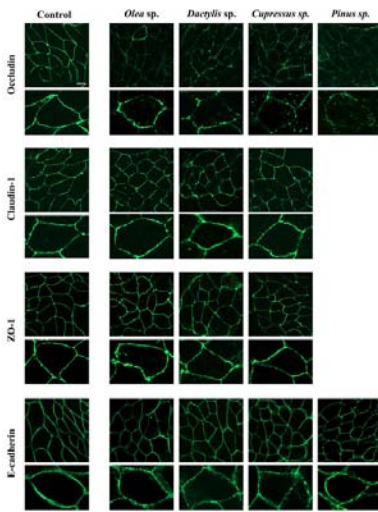
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The initial research interest of the group was on plant aspartic proteases and their biotechnological applications. Studies of the milk-clotting enzymes from the Asteraceae family and on the atypical aspartic proteases from Arabidopsis known to participate in defence mechanisms against biotic and abiotic stresses constitute a specific research line oriented towards the economic relevance of these subsets of enzymes. More recently the group became interested in studying proteases involved in rickettsioses and allergy where they are known to play critical roles in infection and invasion respectively. Research has been focused on the structure-function relationship of pollen aminopeptidases and rickettsia aspartic proteases aiming at developing novel therapeutic strategies to fight those diseases.

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Finally and taking advantage of the mass spectrometry facility, wide and specific proteome approaches were undertaken to elucidate the molecular mechanisms underlying the involvement of proteases in disease through the study of their degradomes. Similar approaches have also been pursued in collaboration with other CNC groups to search for potential biomarkers of some neurological diseases.

During the past year the aspartic proteinase (cirsin) responsible for the milk-clotting activity of *Cirsium vulgare* flower extracts was cloned,

expressed, purified and subsequently characterized. Recombinant procirsin displayed all typical proteolytic features of aspartic proteinases and showed high specificity towards κ -casein and milk-clotting activity, suggesting it might be an effective vegetable rennet. We have also worked on the identification of the first typical aspartic proteinase from *Chlamydomonas reinhardtii*. This enzyme, named chlapsin, accumulates in chloroplasts and shows a pattern of complexity in what concerns sequence features, localization and biochemical properties. This is the first time an aspartic protease is shown to occur in green algae.

To continue our work on plant aspartic protease, selected representative members of the atypical subgroup, known to be involved in defensive mechanisms against biotic and abiotic stresses, were subcloned and prepared for expression in both *E. coli* and *K. lactis*. Expression optimization procedures were carried out and some of these enzymes are currently being expressed for further characterization. We are currently focused on the atypical proteinase NP_181826 which showed interesting biochemical properties such as a strict redox dependence of the activity and alternative chloroplast targeting mechanism.

Rickettsiae are gram-negative strict

intracellular bacteria and many of them are pathogenic to humans causing severe infections like Rocky Mountain spotted fever and Mediterranean spotted fever. As a result of a bioinformatics analysis we have identified a single-lobed aspartic proteinase gene highly conserved in Rickettsial genomes (Gram-negative bacteria). The soluble domain of APRick was produced as a GST-fusion protein and shown to be enzymatically active and capable of undergoing multi-step processing over activation time as described to other retroviral-like APs. The similarities between APRick and retroviral-like APs and the demand for a strong inhibitor for APRick, prompted us to investigate possible interactions of HIV protease inhibitors with the activated form of APRick. We have tested eight therapeutic HIV protease inhibitors and our results indicate that indinavir, nelfinavir, amprenavir and atazanavir significantly inhibit the activity of APRick (ca. 50-70%). Taken together, these results provide evidences that this new AP from Rickettsia is indeed an active enzyme and that it shares some of the features previously described for single-lobed APs of retroviral origin.

Pollen allergy has a remarkable clinical impact all over Europe. Proteolytic activity in 10 pollen diffusates with distinct allergenicity: *Olea sp*, *Dactylis sp*, *Cupressus sp*,

Pinus sp, *Quercus sp*, *Betula sp*, *Chenopodium sp.*, *Plantago sp.*, *Platanus sp.*, *Eucalyptus sp* was evaluated through several enzymatic assays (1D, 2D Zymography, fluorogenic assays). All pollen diffusates were shown to have high molecular weight proteases with low pI, and predominant serine and/or aminopeptidase activity. These proteases increased transepithelial permeability through disruption of transmembrane proteins and degrade airways bioactive peptides that can disturb the balance between the anti- and pro-inflammatory effects in the lung. Pollen proteases are likely to be involved in the sensitization to a range of airborne allergens by facilitating allergen delivery across the epithelium, and also contribute

directly to the inflammation characteristic of allergic diseases. Our results suggest that even less allergenic pollens are likely to be involved in the allergic sensitization and in chronic respiratory inflammation. The presence of these enzymes may explain the extent of respiratory symptoms in subjects with non-IgE mediated rhinitis or asthma that takes place at the peak of pollen season.

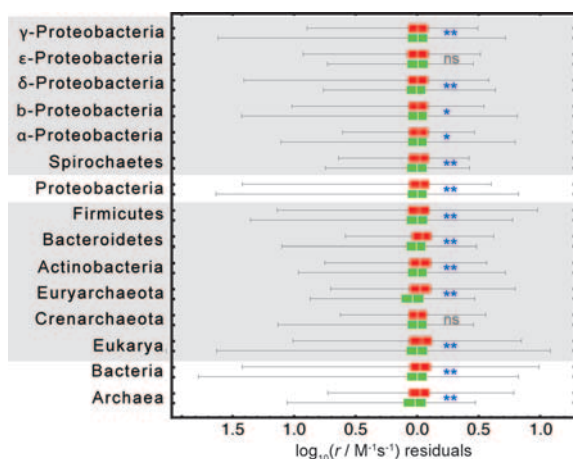
Finally, the degradomics platform started to be implemented using pollen proteases and specific targets, aiming to identify protease cleavage sites. This approach resulted in a high ranked publication in the field and will be further extended to non-targeted

analysis. Proteomics approaches to elucidate changes in protein levels have been successfully used in the past in the identification of potential biomarkers for multiple sclerosis disease. During the reported period, we collaborated in an Alzheimer's' disease biomarkers project for the validation of new and also known biomarker. Moreover, a collaboration in a large scale proteomics analysis of plant tissues resulted in a recently accepted publication in a highly ranked journal in the field.



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Comparison of HO• reactivity per aminoacid (r) between proteins of O₂-tolerant unicellulars (green) and those of O₂-intolerant unicellulars (red) after statistically controlling for the effect of protein size. Lineages in the shadowed set evolve independently. Whiskers, full range; bars, interquartiles; white lines, medians. Significances by Mann-Whitney U statistic after Bonferroni correction: **, $p < 10^{-9}$; *, $p < 5 \times 10^{-4}$; ns, $p > 5 \times 10^{-4}$.

Objectives:

1. Understanding the genesis, fate and evolutionary adaptations to biologically relevant reactive chemical species (RS). We seek to understand the following questions: (1) How are some of the main RS that damage cellular components generated? (2) At what point in the evolution of living organisms did oxidative stress arise and when did the main antioxidant defenses evolve? (3) Does (and how?) the monomer composition and the structure of proteins undergo evolutionary adaptation towards minimizing reactivity with RS? (4) How does the naturally evolved design of antioxidant defense systems relate to the function of these systems, and what design features are key for their effectiveness?

2. Developing a method for profiling mitotic-cycle-dependent metabolism without having to synchronize cells.

3. Developing an Internet-based platform for distributed collaboration in kinetic modeling of biochemical processes. Current solutions for archival and communication of kinetic models just store “frozen” versions of the models and do not promote discussion and further development. This is a major

limitation because model development should be viewed as a dynamic process reflecting the evolving knowledge about biochemical processes. We seek to develop a platform — WikiModels — for developing models as a community activity through constant open peer-review of modeling decisions, recording successive states of a model and tracking credit for contributions.

Main Achievements:

Did oxidative stress originate only after O₂ in the atmosphere accumulated to >1% of present values, ~2 Ga ago? Seeking to address this question, we developed mathematical models of the generation of reactive chemical species (RS) in the Archean ocean (3.8 Ga ago, origin of life) and in modern oceans. Our modeling indicates that water photolysis by the intense ultraviolet C irradiation of the photic zone of the Archean ocean (then not shielded by an atmospheric ozone layer) maintained much higher concentrations of free radicals than those in the modern oceans, resulting in much higher oxidative damage rates [Pais, Salvador, Ferreira, Antunes (in preparation)]. Corroborating the suggestion that oxidative stress was an early evolutionary factor, a phylogenetic analysis indicates that antioxidant defenses such as glutathione

synthetase, glutaredoxin reductase, CuZnSOD, originated before 3.2 Ma ago. [Pais, Salvador, Ferreira, Antunes (in preparation)].

Could organism species adapt to O₂-rich environments also by making proteins that are less susceptible to oxidative damage? We analyzed indices of reactivity with the hydroxyl radical (HO•) for the proteome sequences of 735 species of unicellular organisms to clarify this question. We found that compared to proteins from O₂-intolerant organisms those from O₂-tolerant organisms are on average (a) made of less HO•-reactive aminoacids, and (b) less HO•-reactive in a per-aminoacid basis even after controlling for protein-size differences. These trends are consistent across 10 independently evolving clades spanning the three domains of Life (Archaea, Bacteria and Eukarya), indicating that they are the result of evolutionary adaptation to the presence of O₂ rather than of phylogenetic inertia [Salvador, Moura, Alves (2011) In Barbosa, Caires (Eds.). “Proceedings of InForum 2011”, pp. 66-71].

Seeking to clarify the pathways to reactive scission products from the autoxidation of polyunsaturated fatty acyl chains (PUFA) we are using a computational approach to generate and analyze the corresponding reaction networks. In a proof-of-principle our approach managed to

explain the formation of all products from linoleic acid autoxidation hitherto detected in experiments *in vitro* under mild conditions, except for pyranosic and furanosic products, which we are currently addressing. Applying graph theoretical algorithms we could then trace minimal pathways to each product. Some of these pathways are more plausible than those proposed in the literature. Finally, we selected potential markers for the occurrence of specific scission mechanisms *in vivo* [Saravanan, Piñeiro & Salvador, manuscript in preparation].

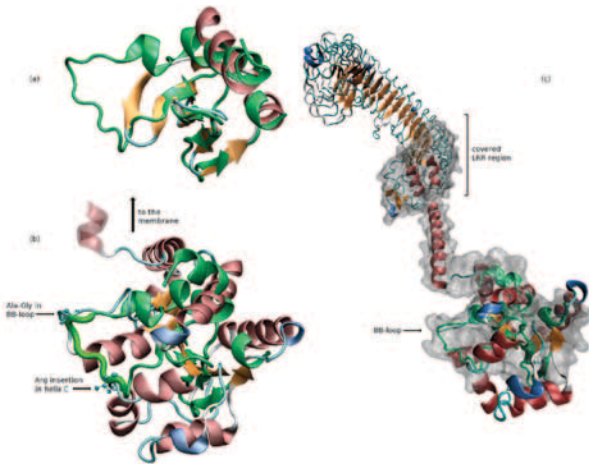
Why do human erythrocytes use both catalase (Cat) and peroxiredoxin (Prx2) to defend against H_2O_2 ? Our

kinetic modeling [Benfeitas, Antunes, Salvador, in preparation] indicates that Cat and Prx2 complement each other in the defense against H_2O_2 as follows. Under low/mild oxidative stress Prx2 is the main H_2O_2 scavenger. However, the flux capacity of the Prx2 system is limited to $\sim 0.23 \mu M H_2O_2/s$. For higher H_2O_2 influx Cat gradually becomes the main H_2O_2 scavenger. Thus, Cat is necessary as a backup defense at high oxidative loads. But why don't erythrocytes rely solely on Cat for H_2O_2 elimination, thus sparing reducing equivalents? Fully replacing Prx2 such that the same $[H_2O_2]$ as in normal erythrocytes would be retained would require a Cat amount corresponding to $\sim 32\%$ of the hemoglobin contents. In turn, Prx2, with its higher specific second-order

rate constant, accomplishes the same at an amount corresponding to just 1.7 % of hemoglobin contents. Thus, reliance on both Cat and Prx2 resolves a trade-off between space and operation costs. Namely, by combining both defenses erythrocytes achieve effective protection over the whole physiological range of H_2O_2 loads at both modest protein allocation and limited NADPH expenditure.

Structural and Computational Biology

Head: Rui M. M. Brito



Structural model of the ASFV-pI329L protein, an inhibitor of the TLR3 signalling pathway.

Its intracellular domain (green cartoon representation) results similar to the SARS-coV domain-C (superposed in (a)), and overlaps key residues for TLR3-TIR dimerization (as depicted in (b)). (c) The gray transparent surface delimits the TLR3 regions for which the entire pI329L could have mimetic (antagonist) action.

The Structural and Computational Biology group combines the reach of experimental and computational methodologies to pursue the following objectives:

I. Characterization of the molecular mechanisms of amyloid formation by the protein transthyretin (TTR)

Ia. Refolding kinetics of TTR

The small differences observed in the crystal structures of different TTR variants, as well as the thermodynamics and kinetics of tetramer dissociation, do not seem to completely justify the amyloidogenic potential of different variants. With this in mind, we set out to study the refolding kinetics of WT-TTR and its amyloidogenic variant V30M-TTR.

Ib. Kinetics of the Early Stages of TTR Oligomerization

Conversion of native TTR to amyloid fibrils is a multi-step process initiated by the dissociation of the native protein to non-native monomers which are prone to self-assemble into small soluble oligomers and eventually into amyloid fibrils.

We proposed to characterize the kinetics of TTR oligomerization using circular dichroism, intrinsic tryptophan fluorescence, and multi-angle-laser-light-scattering (MALLS) to follow protein conformational changes accompanying amyloid formation, and to identify the oligomeric nature of the

intermediates in the aggregation pathway.

II. Rational design of inhibitors of amyloid formation

IIa. Characterization of Structural and Energetic Properties of known TTR binders.

Isothermal titration calorimetry (ITC) and Saturation Transfer Difference NMR (STD-NMR) experiments coupled with computational mapping of the protein molecular interaction fields (MIFs) and electrostatics calculations were used to characterize in detail the structural and energetic determinants of known TTR binders.

IIb. Receptor-based and ligand-based virtual screening campaigns to search for potential TTR amyloid inhibitors among virtual libraries of millions of compounds.

As a result of the virtual screening campaigns, 50 of the most promising virtual compounds were purchased from chemical vendors and assayed *in vitro* for inhibitory activity against TTR amyloid formation. Several of these were in fact active, and are presently being chemically optimized.

IIc. Relational Learning Approaches to Structure-Activity Relationships in Drug Design Toxicity Studies

The goal was to develop a tool to allow the use of Relational Learners in the task of identifying molecules or molecular fragments with potential to produce toxic effects, and thus help in stream-lining drug design *in silico*.

III. Structural modelling of viral proteins and rational design of new anti-viral agents

IIIa. Modeling of the Toll-like receptor 3 and a putative Toll-like receptor 3 antagonist encoded by the African swine fever virus (ASFV).

ASFV viral protein pI329L was found to inhibit the Toll-like receptor 3 (TLR3) signaling pathway. To explore the idea that pI329L might act as a TLR3 decoy, we used comparative modeling and other structure prediction protocols to present (a) a model for the TLR3-Toll-IL1 receptor homodimer and (b) a structural model for pI329L.

IIIb. Identification and characterization of conserved features among different strains/serotypes of selected influenza A proteins.

We used bioinformatic tools for comparative sequence and structure analysis, for a selected set of proteins within serotypes H1-H3 and H5, and N1 and N2, with the goal of highlighting conserved regions and critical positions of frequent

mutations in several protein domains.

IV. Ibercivis - A volunteer computing platform for the Iberian Peninsula

Throughout 2011 efforts were made in increasing the general public awareness about the project and the integration of new Portuguese research teams.

Main achievements:

The main results achieved by the Structural and Computational Biology group are presented below:

I. Characterization of the molecular mechanisms of amyloid formation by the protein transthyretin (TTR)

Ia. Refolding kinetics of TTR

Our results demonstrate that the *in vitro* refolding mechanisms of WT- and V30M-TTR are similar, involving a dimeric intermediate. However, there are large differences in the refolding rate constants for the two variants, specially at nearly native conditions. Interestingly, tetramer formation occurs at a much slower rate in the amyloidogenic variant V30M-TTR than in WT-TTR, resulting in higher susceptibility for aggregation and amyloid formation instead of spontaneous refolding.

Ib. Kinetics of the Early Stages of TTR Oligomerization

The initial steps of TTR oligomerization can be described by a three-state process. Our results suggest that prior to fibril formation, there is the accumulation of an oligomeric intermediate state. After the initial conformational changes, TTR aggregation proceeds via a nucleation-and-growth mechanism.

II. Rational design of inhibitors of amyloid formation

IIa. Characterization of Structural and Energetic Properties of known TTR binders.

From ITC experiments it was possible to characterize the binding properties (association constants and cooperativity effects) and the thermodynamic profiles of the binding between WT-TTR and four different carefully selected ligands. The average total interaction energy computed at structural level follows the same trend of the ΔG values determined by ITC. The computational study also suggests that the trend in ΔG values could be primarily determined by shape complementarity (translated into vdW interactions).

IIb. Relational Learning Approach to Structure-Activity Relationships in Drug Design Toxicity Studies.

iLogCHEM, an interactive tool for chemo-informatics based on ILP, was developed. This system is designed to allow search and manipulation of drug patterns. The input is given as a standard formatted description of the molecules. The output is provided visually, or as text files in standard chemical file formats.

III. Structural modelling of viral proteins and rational design of new anti-viral agents

IIIa. Modeling of the Toll-like receptor 3 and a putative Toll-like receptor 3 antagonist encoded by the African swine fever virus (ASFV).

Using homology modeling and other structure prediction simulation protocols, we proposed (a) a model for the so far experimentally unsolved TLR3-TIR structure, assembled within the context of the overall TLR3-dsRNA recognition complex, and (b) a structural model for the intracellular extension of the p1329L viral protein that reinforces the idea that the viral protein is a TLR3 antagonist. The proposed computational models are consistent with the available experimental data.

IIIb. Identification and characterization of conserved features among different strains/serotypes of selected influenza A proteins.

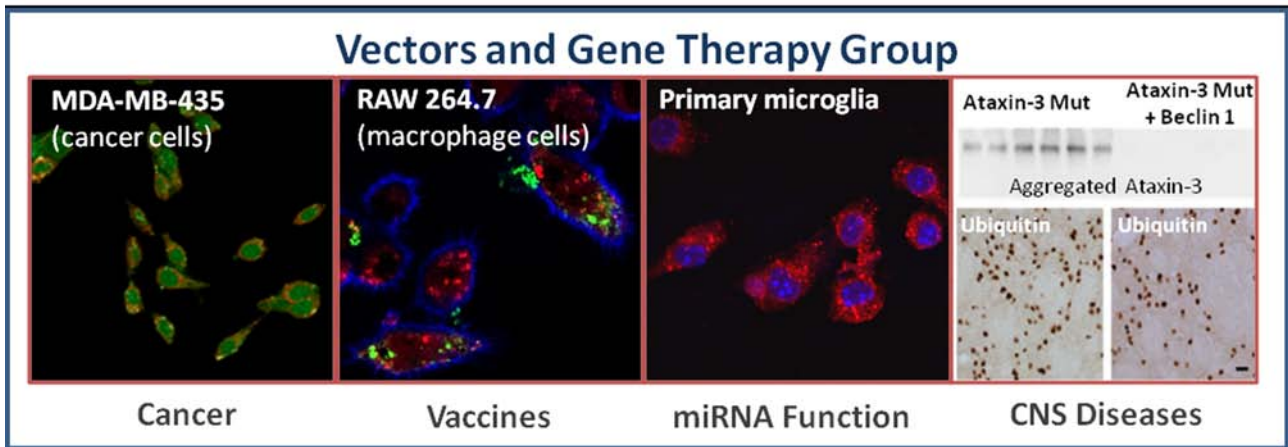
ClustalW2 was employed to analyse the sequence alignments of sets of selected viral proteins. The set of clusters obtained identifies conserved sequence regions. These results are currently being interpreted in conjunction with additional relevant epidemiologic information on the disease.

IV. Ibercivis - A volunteer computing platform for the Iberian Peninsula

In July 2011, a new Portuguese project, named Soluvel, joined Ibercivis and started benefiting from the computational power made available by thousands of volunteer citizens all over the world.

Vectors and Gene Therapy Group

Head: M. Conceição P. Lima



The research in the Group of Vectors and Gene Therapy has been devoted to the design and development of carriers, including viral and non-viral vectors, for nucleic acid and drug delivery aiming at their application as technological platforms for 1) establishment of disease models, 2) study of disease mechanisms and 3) development of new molecular therapeutic and prophylactic strategies.

Our studies on non-viral vectors have been mainly focused on the evaluation of the potential of novel lipid-based nanosystems and polymeric nanoparticles in gene therapy strategies for the treatment of both cancer and neurodegenerative disorders, and for the development of vaccines for infectious diseases.

Non-viral vectors, such as cationic liposomes, stable nucleic acid lipid particles and cell-penetrating peptides have been explored as carrier systems to deliver nucleic acids, including plasmid DNA encoding therapeutic proteins, as well as antisense oligonucleotides, siRNAs and anti-miRNA locked nucleic acids, aiming at promoting silencing of known oncogene proteins and both cancer-related and pro-inflammatory miRNAs. The group is interested in investigating the anti-tumoral effect of gene therapy strategies, either *per se* or in combination with chemotherapeutic agents, both *in vitro* and in animal models for different types of cancer. In addition, non-viral vectors are currently being developed to study the role of miRNAs in neuroinflammation, aiming at promoting neuronal survival

by targeting the inflammatory pathways associated with neurodegenerative diseases.

Fundamental research work addressing the development and physicochemical characterization of new nucleic acid delivery systems has also deserved the attention of our group. Research efforts have been developed to define, through a biophysical approach, the architecture parameters that endow vectors with the ability to transpose membranes and efficiently deliver their cargo into the cell. In this context, some significant data have already been collected from the analysis of the physicochemical properties of Gemini surfactant/lipid-based gene carriers with different transfection competence, from which we expect soon to be able to establish structure-activity relationships.

Viral vectors, particularly lentiviral and adeno-associated viruses are powerful technological platforms for gene delivery to the CNS, which we have been using for investigating the pathogenesis and modeling of neurodegenerative diseases, with a focus on Machado-Joseph disease (MJD). This knowledge is expected to allow the generation of disease-modifying approaches for MJD therapy. Encouraging results have already been obtained with the strategies of gene silencing, proteolysis inhibition, autophagy activation, adenosine receptor blockage and modulation of the interaction of ataxin-3 with other

polyglutamine proteins. It is expected that these studies will contribute to the finding of new therapies for this fatal disorder for which no effective therapy is available.

Mucosal vaccination (oral and nasal) with the antigen encapsulated in polymeric nanovectors to target the lymphoid structures of the mucosal immune system is also addressed by our group. Related with this theme two projects started in 2010: "Development of a mucosal anthrax vaccine: designing a prototypic multi-antigen polymeric delivery system" and "Development of chitosan-based nanoparticles for nasal immunization against hepatitis B". Regarding these two projects, the main objectives of the first year were the development and optimization of the processes for preparing the delivery systems. New chitosan-based delivery systems able to simultaneously encapsulate antigens and a second adjuvant were generated. An important objective for the next year will be to evaluate possible synergistic effects of chitosan and the second adjuvant (mast cell activator c48/80 and aluminum compounds).

Regarding the development of non-viral gene delivery and gene silencing approaches for the treatment of cancer, several important achievements were made in 2011. Concerning the development of new therapeutic strategies for cancer, we observed that the silencing of the tumor-specific protein survivin

sensitizes tumor cells to the cytotoxic action of conventional chemotherapeutic agents, which may represent an advance toward a targeted gene therapy to cancer with reduced side effects. Another strategy involved silencing of the oncomir miR-21, overexpressed in glioblastoma multiforme, through combination of tumor-targeted stabilized nucleic-acid lipid particles with anti-miRNA LNA oligonucleotides, which resulted in significant reduction in miR-21 expression levels and consequently decrease in tumor cell viability.

In a different approach, a proprietary targeted lipid-based nanocarrier for siRNA delivery towards cancer cells and the tumor microenvironment was developed, which exhibited high encapsulation efficiency of nucleic acid, ability to protect the encapsulated siRNA, small size and a surface charge close to neutrality. Moreover, the attachment of the F3 peptide onto the liposomal surface resulted in a high extent of internalization by both cancer and endothelial cells from angiogenic blood vessels, but not by non-transformed cells. Sequence-specific downregulation of enhanced green fluorescent protein (eGFP) in *eGFP*-overexpressing human cancer cell lines, both at the protein and mRNA levels, was further observed upon delivery of anti-*eGFP* siRNA by F3-targeted liposomes. The present work represents an important contribution towards a nanoparticle with multi-targeting capabilities, both at the cellular and molecular levels.

Lipid-based nanoparticles have also been applied in gene therapy strategies targeting pro-inflammatory miRNAs. We have investigated the role of miR-155 in microglia cells and observed that the downregulation of this miRNA inhibits the release of pro-inflammatory factors, contributing to neuronal survival.

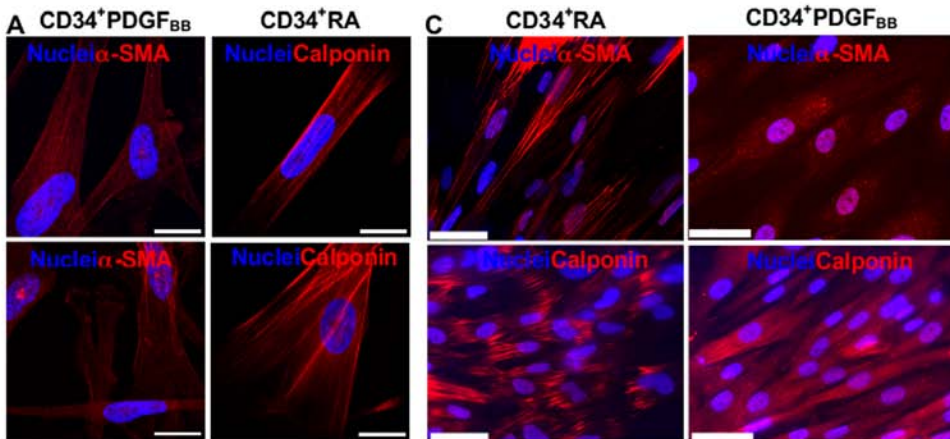
Biophysical studies on membrane interactions of Gemini surfactant and CPP-based vector formulations have provided interesting insights into the mechanisms through which vectors modulate membrane physical properties and these regulate vector efficiency in the delivery process.

Regarding the use of viral-mediated gene delivery, we investigated the implication of autophagy in the accumulation of mutant ataxin-3 aggregates and neurodegeneration found in Machado-Joseph disease and assessed whether specific stimulation of this pathway by beclin-1 overexpression could mitigate the disease. Using tissue from Machado-Joseph disease patients, transgenic mice and a lentiviral-based rat model, we found an abnormal expression of endogenous autophagic markers, accumulation of autophagosomes and decreased levels of beclin-1, a crucial protein in autophagy. Lentiviral vectors-mediated overexpression of beclin-1 led to stimulation of autophagic flux, mutant ataxin-3 clearance and overall neuroprotective effects in neuronal cultures and in a lentiviral-based rat model of Machado-Joseph disease. These data demonstrate that autophagy is a key degradation pathway, with beclin-1 playing a significant role in alleviating Machado-Joseph disease pathogenesis.

Regarding mucosal vaccine projects, several polymeric delivery systems were developed. One objective was to encapsulate not only antigens but also immunopotentiators. In this regard, we were able to produce aluminum chitosan-based particles that were used to prepare complexes with a plasmid encoding the hepatitis B antigen. These complexes were able to transport and protect DNA in the presence of DNases and were able to transfect A549 cultured cells. In the second project, mast cell activator c48/80 was adsorbed on the surface of chitosan/alginate particles and entrapped in chitosan particles. A decrease of the cytotoxicity of the C48/80 compound was observed in this system.

Biomaterials and Stem Cell-Based Therapeutics

Head: Lino Ferreira



Currently, the Biomaterials and Stem Cell-Based Therapeutic research group has three main avenues of research: (i) development of 3D biomaterials to create synthetic (stem) cell niches in order to maximize the therapeutic potential of stem cells and to understand their biology, (ii) development of nanomaterials to manipulate stem cells and control their differentiation, and (iii) the development of biomaterials with antimicrobial properties.

1- 3D biomaterials as synthetic (stem) cell niches

Cells do not only connect to each other, but also to a support structure called the extracellular matrix (ECM). The ECM has multiple functions in cell growth, differentiation, and cell maintenance, as well as in tissue morphogenesis. It functions via a number of cell-surface receptors, including integrins, which are involved in anchoring the cells to the ECM, and in transmitting and controlling information across cell membranes, which regulates processes such as migration and differentiation among others. To mimic the 3D architecture and biological role of the ECM, researchers have developed biomaterials capable of modulating the biological activity of stem cells. The 3D scaffolds are important to overcome some of the limitations of 2D culture systems. First, 2D cell culture systems promote unnatural interaction with soluble factors. In 2D culture systems only a part of the cell (the basal region) interacts with the ECM and the neighboring cells, while the apical

region of the cell is exposed to culture media. Therefore, it is conceivable that the distribution of integrins at the cell surface and the organization of the intracellular machinery is affected by this unnatural polarization, and thus changing the cellular response. Second, the efficiency of the differentiation process varies, depending in the final cellular lineage. Some experimental data indicates that 2D cell culture may not adequately reproduce features important for the differentiation of stem cells into specific cell lineages. Third, 2D culture systems are not exact models of *in vivo* embryonic development, and therefore, many aspects of human development might be underrepresented. 3D culture systems might be important to study the regulatory mechanisms in morphogenesis- the development of form in embryo.

One of the main objectives of the Biomaterials and Stem Cell-Based Therapeutic research group is to develop biomaterials for the efficient differentiation and transplantation of the stem cells and their progenies at the injured site. The group is focused in developing 3D scaffolds capable of retaining the cells at the desired location, while serving as a template for 3D cell assembly, survival and engraftment.

2- Development of nanomaterials to manipulate stem cells and control their differentiation

The development of a wide spectrum of nanotechnologies (referred as Nanomedicine by National Institutes of Health for applications in the biomedical area) during the last years are very promising for the study of stem cell biology and for the development of new approaches for their expansion, differentiation and transplantation. The second goal of our research group is to develop nanotechnologies able to manipulate the differentiation program of the stem cells.

3- Development of biomaterials with antimicrobial properties

A major problem associated with the implantation of biomedical devices in the human body is the inherent risk of microbial infections. A separate goal of our research group is the design of biomaterials with antimicrobial properties. We are developing effective strategies to control antimicrobial infections by developing coating technologies to immobilize antimicrobial agents.

Main achievements:

Three major achievements have arisen from our recent work: first, the development of a new set of nanomaterials to control the differentiation of stem cells (Maia *et al.*, ACS Nano 2011) by the efficient

spatio-temporal delivery of biomolecules; second, the development of synthetic niches to potentiate *in vivo* stem cell engraftment and therapeutic effect (Pedroso *et al.*, PLoS One 2011; Kraehenbuehl *et al.*, Biomaterials 2011; Vazão *et al.*, PLoS One 2011); and third, the development of new antimicrobial nanomaterials and coatings (Paulo *et al.*, Biomacromolecules 2010).

Our recent work shows that the 3D culture of human embryonic stem cell-derived smooth muscle precursor cells (hESC-derived SMPCs) modulates gene expression towards the expression observed on complete differentiated smooth muscle cells (Vazão *et al.*, PLoS One 2011). SMPCs were encapsulated in fibrin gels for 3 days after which the cells were characterized at protein and gene levels. Gene expression of SMPCs was compared to human vascular smooth muscle cells (hVSMCs) under the same culture conditions. The culture of SMPCs in 3D gels modulated the expression of smooth muscle cell (SMC) genes (α -SMA, SM-MHC or SMA-22) towards the one observed for hVSMCs cultured in 3D gels. We complemented these studies by evaluating the expression of extracellular matrix and adhesion molecules by a quantitative real-time PCR array. This array evaluated the expression of 84 genes involved in cell-cell and cell-matrix interactions. Again, the 3D culture of SMPCs modulated extracellular matrix and adhesion molecule genes towards the expression observed in hVSMCs. The number of genes with similar expression increased from 9 to 53 when SMPCs were cultured in 2D or 3D, respectively.

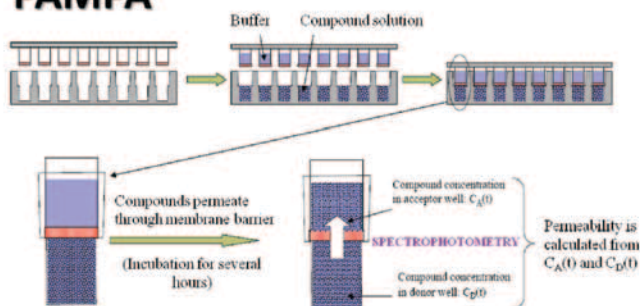
Our results show that 3D scaffolds may induce further the differentiation of SMPCs into SMCs. Several factors might account for the differences found between 2D and 3D culture systems including (i) ECM stiffness and (ii) ECM 3D environment. We are conducting further work to evaluate the effect of both factors in the

modulation of geno- and phenotype of the differentiated cells over the time and study the underlining mechanism.

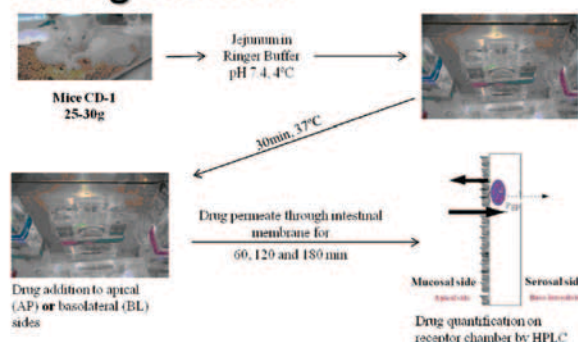
In collaboration with João Malva research group, we have developed nanomaterials to manipulate stem cells and control their differentiation. Recently, we demonstrated the ability of nanomaterials to induce neurogenesis exclusively after being internalized by SVZ stem cells (Maia *et al.*, ACS Nano 2011). The nanoparticles are not cytotoxic for concentrations equal or below to 10 mg/mL. The internalization process is rapid and nanoparticles escape endosomal fate in few hours. Retinoic acid-loaded nanoparticles increase the number of neuronal nuclear protein (NeuN)-positive neurons and functional neurons responding to depolarization with KCl and expressing NMDA receptor subunit type 1 (NR1). These nanoparticles offer an opportunity for *in vivo* delivery of proneurogenic factors and neurodegenerative diseases treatment.

Recently, we have reported a novel methodology to immobilize covalently an antifungal agent, amphotericin B (AmB), into nanomaterials. Silica nanoparticles (SNP) were chosen due to their non-cytotoxicity, low price, high stability and durability and ease of modification by organosilane chemistry, allowing the incorporation of an array of different functional groups. We demonstrated that these NPs have high antifungal activity against several strains of *Candida* and can be reused without losing their antifungal activity. Importantly, the antifungal activity was not due to the leaching of AmB from the surface of the NP, since media that have been in contact with the antifungal NPs had no significant antifungal activity. We further showed that these NPs can be immobilized into flat surfaces forming an antifungal coating.

PAMPA



Ussing Chamber



Pharmacometrics aims to assess quantitatively the pharmacokinetics and pharmacodynamics of drugs, using data from various phases of drug development which are then linked together and quantitatively related to each other.

Given the relevance of pharmacokinetics, more specifically the intestinal absorption and distribution, on drugs pharmacological potential, we intended to develop mathematical and statistical models that could be applied in initial phases of drug discovery to predict these characteristics in humans. To this end, data are obtained from *in silico*, *in vitro* and *in vivo* investigations and then correlated to each other. Firstly, new *in vitro* methodologies must be developed in the scope of Pharmacometric group.

Data obtained in the aforementioned paragraph is also correlated with physicochemical properties of drug candidates in order to define the characteristics that compounds should have to be considered good candidates of future drugs, contributing to a rational drug design.

Throughout the year of 2011, we developed and validated a new *in vitro* PAMPA technique to predict the intestinal transcellular absorption and plasma protein binding of compounds. Furthermore an *in vitro* Ussing chamber technique was also developed and validated in order to evaluate the permeability of drug candidates across mouse small intestine and estimate their intestinal

human fraction absorbed. This technique also demonstrated to be able to adequately identify compounds that are substrates of P-glycoprotein (P-gp). This is an efflux transporter expressed in intestinal membrane, liver, kidneys, brain tissue and blood-brain barrier and it may, hence, compromise the bioavailability and biodistribution of drugs.

These techniques were applied to antiepileptic drugs and derivatives [carbamazepine (CBZ), oxcarbazepine (OXC), eslicarbazepine acetate (ESL), *S*-eslicarbazepine (*S*-Lic), *R*-licarbazepine (*R*-Lic), carbamazepine-10,11-epoxide (CBZ-E), 10,11-*trans*-dihydroxy-10,11-dihydro-carbamazepine (*trans*-diol), BIA 2-024, BIA 2-059 and BIA 2-265] for which relevant observations were found (*please see references*).

However, it is intended to expand their application to other pharmacological groups (new inhibitors of catechol-*O*-methyltransferase for Parkinson's disease, fluoroquinolones and cardiovascular drugs).

Data from *in vitro* pharmacokinetic screens of CBZ, OXC, ESL, *S*-Lic, *R*-Lic, CBZ-E, *trans*-diol, BIA 2-024, BIA 2-059 and BIA 2-265 were combined with that obtained *in vivo* after the administration of each compound to male CD-1 mice by oral gavage. Plasma and brain (biophase of antiepileptic drugs) concentrations obtained for each compound and main metabolites were determined up to 12 h. The time to reach peak concentration, bioavailability (given

by area under the curve) and metabolic stability of the derivatives seemed to be possible responsible for the distinct activity previously reported *in vivo* for the compounds in study. Moreover correlations between plasma and brain concentrations were found, and linear equations defined to predict pharmacokinetics in biophase considering plasma concentrations.

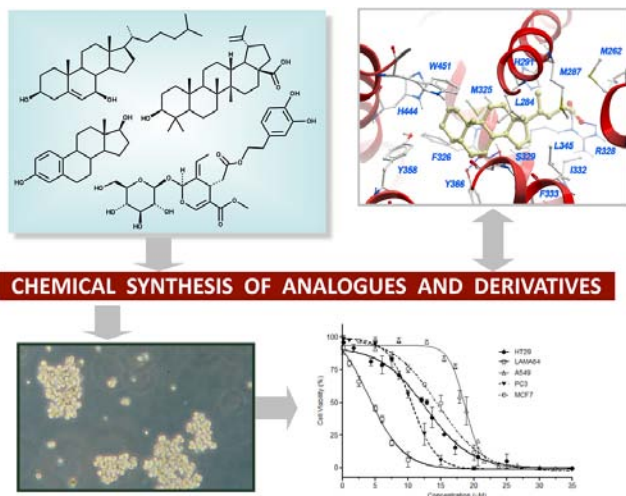
On the other hand, the potential for pharmacological interactions between herbal extracts and conventional drugs with narrow therapeutic index has been investigated.

At the moment we have developed a methodology to quantify amiodarone and its main metabolite in human and rat plasma, as well as in several tissues of rat. For amiodarone various studies were already performed in Wistar rats in order to evaluate the interaction between *Fucus vesiculosus* extract (a slimming herbal extract) and amiodarone, using for that purpose the previously developed method. After the administration of the herbal extract, in single dose or during a pre-treatment period of 14 consecutive days, the pharmacokinetics of a single dose of amiodarone was assessed. The data obtained from these pharmacokinetic studies supported the existence of interaction between *Fucus vesiculosus* extract and amiodarone in Wistar rats.

Studies aiming to evaluate the potential of other herbal extracts for significant herb-drug interactions are ongoing.

Bioorganic and Medicinal Chemistry Group

Head: Maria Luísa Sá e Melo



Drug discovery and development is the core research of the Bioorganic and Medicinal Chemistry Group. At present, the main focus is on drug discovery in oncology.

Oxysterols are oxygenated derivatives of cholesterol endogenously found and formed through enzymatic and non-enzymatic processes. Oxysterols exert profound biological effects in cholesterol and fatty-acid metabolism, immune regulation, neurodegenerative mechanisms and cell differentiation and proliferation. Our group has been interested in the study of the structural requirements of oxysterols to display cytotoxicity (*J. Med. Chem.* 2009, 52, 4007-4127; *J. Med. Chem.*, 2010, 53, 7632-7638). Aiming to push forward potency and selectivity, a large number of chemically diverse sterols, heavily oxygenated in rings A and B, has been recently prepared and evaluated for cytotoxicity against human cancer and non-cancer cells. The SAR analysis of the oxysterols synthesized to define the sterol structural determinants for a selective activity will complement our aim.

Pentacyclic triterpenoids are a class of pharmacologically active and structurally rich natural products with privileged motifs for further modifications and structure-activity relationship (SAR) analyses. The naturally occurring lupane-type triterpenoids betulin and betulinic acid have been thoroughly investigated during the past years for their

promising chemopreventive and antitumor activities. We focused our attention on the synthesis of lupane-type imidazole carbamates and *N*-acylimidazole bearing derivatives (*Bioorg. & Med. Chem.*, 2009, 17, 6241-6250). The promising results prompted us to extend our study to 2'-methylimidazole and triazole derivatives, in order to establish meaningful SAR (*Bioorg. & Med. Chem.*, 2010, 18, 4385-4396). The compounds with better cytotoxicity, were tested for their ability to induce apoptosis and cell cycle arrest in HepG2, Hela and Jurkat cells.

The rapid growth in the number of high quality X-ray crystal structures bound to multiple ligands, and the recent large online publicly available chemical databases with annotated activity for thousands of known modulators creates an opportunity to develop accurate computational models for fast in silico prediction of ligand binding affinity. We are currently interested in deriving high quality benchmarking test sets for docking and scoring, as well as developing and validating new software algorithms that account for the flexible nature of protein-ligand interactions.

The research activities of the group are supported by the following expertise:

- Computational approaches in drug discovery: 4D (pocket ensemble) molecular docking; pharmacophore- and structure-based drug design; virtual screening; focused library design based on hit and target.
- Synthesis in drug discovery: asymmetric synthesis for chiral drugs; biocatalysis; chemo-enzymatic methods; clean processes.
- Analysis of structure-activity relationships (SAR) to predict potency and improve "hits" to "lead candidates" by optimizing their selectivity against the target and pharmacokinetics.

The most cytotoxic endogenous oxysterols, the 4 β -OHChol, 7 β -OHChol and 3 β , 5 α , 6 β -triOHChol, were used as scaffolds and different oxygen functionalities, as hydroxy, oxo, oxime, acetamide, acetate and alkoxy groups were introduced on rings A and B of the steroid. The 33 oxygenated compounds synthesized were evaluated in vitro for cytotoxicity in a panel of human cancer and noncancer cells, using the Alamar Blue assay. This panel encompassed HT-29 (from colorectal adenocarcinoma), HepG2 (from hepatocellular carcinoma), A549 (from lung adenocarcinoma epithelium), PC3 (from prostate metastasis), MCF-7 (from breast adenocarcinoma) and SH-SY5Y (from neuroblastoma bone marrow). ARPE-

19 (from retinal pigment epithelium) and BJ (from skin fibroblast cells) were used as models of human noncancer cells to gain insights on the preferential cytotoxicity against cancer cells. These studies revealed a broad antiproliferative activity for the oxysterols in a low micromolar range with increased activities on LAMA-84, HT-29, HepG2 and MCF-7 cells. From the selectivity indexes and SAR analysis, a set of compounds were identified with very high selectivity. The neuroblastoma cells showed resistance to the majority of the oxysterols, although some derivatives presented increased cytotoxicity. This work demonstrated how structural modifications in natural oxysterols have an impact on their selective cytotoxicity (*J. Med. Chem.* 2011, 54, 6375-6393). The synthetic work, including ours, in the use of enzymes in biocatalytic transformations of steroids, under chemo-, regio- and stereoselective control, has been compiled in a review article (*Curr. Org. Chem.*, 2011, 15(6), 928-941).

49 Recently, we focused our attention on the synthesis of lupane-type imidazole carbamates, *N*-acylimidazole bearing derivatives and 2'-methylimidazole and triazole derivatives, in order to establish meaningful SAR and study their ability to induce apoptosis and cell cycle arrest in HepG2, Hela and Jurkat cells. The overall findings suggest that some of the new lupane-type derivatives are strong regulators of tumor cells proliferation, inducing cell cycle arrest and apoptosis through a caspase-based mechanism. (*Biochimie*, 2011, 93, 1065-1075). The synthesis of new related triterpenoid compounds is currently underway (*Adv. Synth. Catal.*, 2011, 353, 2637-2642).

To demonstrate the usefulness of virtual ligand screening in structure-based drug discovery, flexible docking and scoring was benchmarked for ligand binding mode prediction against high quality co-crystal structures available in the Protein Data Bank. The Internal Coordinate

Mechanics method for virtual ligand screening was tested against the 40 DUD target benchmarks and 11-target WOMBAT sets. The self-docking accuracy was evaluated for the top 1 and top 3 scoring poses at each ligand binding site with near native conformations below 2 Å RMSD found in 91% and 95% of the predictions, respectively. The virtual ligand screening using single rigid pocket conformations provided the median area under the ROC curves equal to 69.4 with 22.0% true positives recovered at 2% false positive rate. Significant improvements up to ROC AUC= 82.2 and ROC (2%) = 45.2 were achieved following our best practices for flexible pocket refinement. Our results confirm the usefulness of conformation ensembles to improve protein-ligand recognition using the structure-based approach. The benchmarking results, current developments in the docking-based drug design and future prospects were presented during a symposium at the 241st ACS National Meeting held in March 2011 in Anaheim, CA, and were accepted for publication in the *Journal of Computer-Aided Drug Design*.

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Cell and Molecular Toxicology Area

Coordinator: Rui Carvalho



Mitochondrial Toxicology and Disease Group

The main general objective of this research group is to provide an insight into the role of mitochondria as a primary intracellular target in the initiation of drug- and disease-induced cell dysfunction, as well as the modulation of mitochondrial bioenergetics and cell metabolism during carcinogenesis. One particular objective is to understand how mitochondria are involved in the pathophysiology of diseases such as diabetes, cholestasis, cancer and how mitochondrial function can be altered by chemotherapy, not only to decrease the side effects of agents commonly used in oncology, but also to specifically identify new mitochondrial targets in tumor cells.

Redox Biology in Health and Disease

The main general objective of this research group is to determine both the dynamics of production and steady-state levels of reactive oxygen/nitrogen species, known to be critically involved in the redox regulation of cell functions but also connected to selective responses, including the extensive oxidative damage to biomolecules, leading to cell death, either by turning off vital processes or by upregulating toxic cascades.

Main Achievements

Mitochondrial Toxicology and Disease Group

Significant achievements were made in several distinct but complementary lines of research: **a)** anticancer drugs and mitochondrial toxicology; **b)** survival and death pathways during cancer and anti-cancer therapy (e.g., inhibition of B-RAF/ERK survival signaling facilitates the cell death response triggered by berberine; **c)** mitochondrial alterations induced by xenobiotics: resveratrol disturbs mitochondrial hydroperoxide production through the

modulation of superoxide dismutase activity; **d)** mitochondrial signaling and metabolic dysfunction (MitoLab): SIRT1 and AMPK activation are able to counteract metabolic dysfunction by stimulating mitochondria and stimulation of autophagy by dibenzofuran induce alterations on the cellular energetic status; **e)** mitochondria, carcinogenesis and chromium toxicity: an higher cellular malignant phenotype is related with a more glycolytic behaviour than the precursor cell lines; **f)** metabolic profiling and toxicology: the evaluation of metabolic pathways and their regulation was performed in cell cultures (stem cell vs differentiated), isolated perfused organs (heart and liver) and whole organisms by means of methodologies based in ^2H and ^{13}C NMR isotopomer analysis.

Redox Biology in Health and Disease

For the first time was provided a quantitative and temporal basis for the understanding of the biological actions of the ubiquitous intercellular messenger, nitric oxide (NO), that conveys information in a novel and radical way in the brain, associated with its concentration dynamics. Along this notion we have: **a)** established in vivo the concentration dynamics of nitric oxide along the trisynaptic loop in connection with the expression of the neuronal isoform of nitric oxide synthase (nNOS), highlighting that the regulation of nNOS is a critical determinant of NO signaling in the brain; **b)** identified the major mechanisms for NO decay in the brain; i.e., the critical determinants that shape NO signals in the brain. Specifically, erythrocytes represent the major NO inactivation pathway in the brain; **c)** determined NO life-time in hippocampus in vivo; **d)** assessed NO diffusion in brain tissue, evidencing, contrary to the current paradigm, an heterogenous diffusion pattern and the occurrence of facilitated paths.

Mitochondrial Toxicology and Disease Group

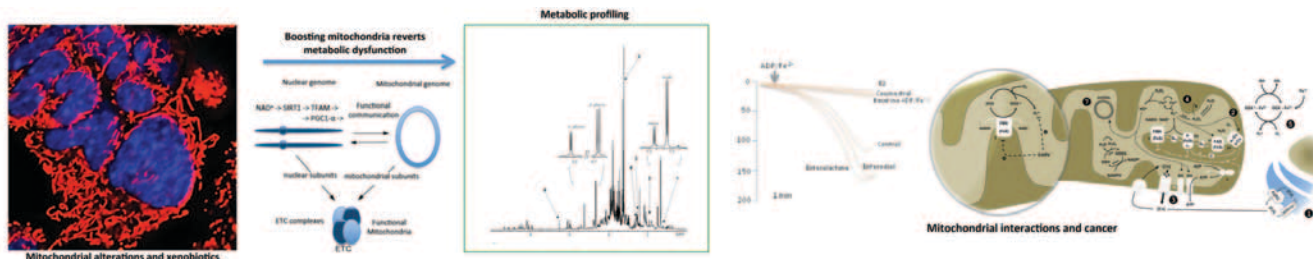
Paulo J. Gouveia Oliveira	PhD – <i>Head of group</i>
Anabela Pinto Rolo	PhD
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Ignacio Vega-Naredo	PhD
José Antunes Custódio	PhD
M ^a Carmen Alpoim	PhD
M ^a Sancha J. Santos	PhD
Rui Carvalho	PhD
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Ana Carolina Moreira	PhD Student
Ana Filipe R. Branco	PhD Student
Ana Francisca Soares	PhD Student
Ana Patrícia Gomes	PhD Student
Ana Teresa F. Varela	PhD Student
Claudia Sofia A. Pereira	PhD Student
Carlos F. Rodrigues	PhD Student
Cátia M. Vieira Diogo	PhD Student
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Kátia Mesquita	PhD Student
Ludgero C. Tavares	PhD Student
Mariana Ponte Ribeiro	PhD Student
Nuno Gabriel Machado	PhD Student
Paulo G. Guerreiro	PhD Student
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João Fonseca	MSc Student
Mariana Val	MSc Student
Susana Sampaio	MSc Student
Ana Cristina Lemos	Grant Technician
Rute Marisa Loureiro	Grant Technician
Telma Bernardo	Grant Technician

Redox Biology in Health and Disease Group

João Laranjinha	PhD – <i>Head of group</i>
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Leonor Almeida	PhD
Teresa Dinis Silva	PhD
Rui Barbosa	PhD
Carla Nunes	Post-Doc Fellow
Bárbara Rocha	PhD Student
Cassilda Pereira	PhD Student
Cátia Marques	PhD Student
Diana Serra	PhD Student
Joana Paixão	PhD Student
Nuno Ricardo Ferreira	PhD Student
Ricardo Santos	PhD Student
Ana Rita Fonseca	MSc Student
Elisabete P. Ferreira	MSc Student
Joana Cristina Viegas	MSc Student
Marcelo Rodrigues	Grant Technician
Sara Monteiro Lopes	Grant Technician
Sónia Neto Pereira	Grant Technician

Mitochondrial Toxicology and Disease Group

Head: Paulo J. Gouveia Oliveira



Mitochondria are critical organs in cell metabolism during health and disease. Also, mitochondrial regulation of cell death pathways is now a widely studied aspect of cell biology, as well as the mitochondrial control of calcium homeostasis, redox regulation and intermediate metabolism. Mitochondrial alterations have been implicated in cancer and in cardiovascular and hepatic diseases. Also, drug-mediated mitochondrial liability is nowadays a critical hinder factor in the safety of many pharmaceuticals. The main and general objective of the “Mitochondrial Toxicology and Disease Group” is to provide an insight into the role of mitochondria as a primary intracellular target in the initiation of drug- and disease-induced cell dysfunction, as well as the modulation of mitochondrial bioenergetics and cell metabolism during carcinogenesis. One particular objective is to understand how mitochondria are involved in the pathophysiology of several diseases, including diabetes, cholestasis, cancer and how mitochondrial function can be altered by chemotherapy, not only to decrease the side effects of agents commonly used in oncology, but also to specifically identify new mitochondrial targets in tumor cells. We use different in vitro (isolated mitochondrial fractions, cultured cell lines) and in vivo models (animal models of drug or disease-induced mitochondrial alterations) in order to analyze mitochondrial function, toxicology and metabolism. From polarographic, spectrophotometric and fluorimetric techniques to NMR spectroscopy, the objective of our group is to explore the interplay between mitochondria, metabolism, disease, and human pathology.

Our group has produced significant scientific achievements in several distinct lines of research including the following topics:

Anticancer drugs and mitochondrial toxicology:

The combination of retinoids with antiestrogenic drugs exhibits additive antiproliferative effects and it is a promising anticancer therapy. Moreover, antiestrogenic metabolites prevent mitochondrial disruption effects induced by retinoic acids.

Survival and death pathways during cancer and anti-cancer therapy

Inhibition of B-RAF/ERK survival signalling facilitates the cell death response triggered by the phytochemical berberine. Also, new caffeic and ferulic acid lipophilic derivatives show increased cytotoxicity toward human breast cancer cell lines.

Mitochondrial alterations induced by xenobiotics:

The toxicity of selected phytoestrogens (coumestrol, enterolactone, enterodiol and resveratrol) was evaluated on isolated mitochondria from brain, heart and liver from male and female Wistar-Han rats. Although having a protective effect against lipid peroxidation, several PEs caused alterations of mitochondrial function in different target tissues. Resveratrol disturbs mitochondrial hydroperoxide production apparently not due to direct effects on Complex I, but probably through the modulation of superoxide dismutase activity.

The effects of endoxifen on mitochondrial bioenergetics were evaluated, as well as its protective effect on mitochondrial permeability transition pore induced by retinoic drugs.

Mitochondrial signalling and metabolic dysfunction (MitoLab): Mitochondria are a main driver of hyperglycemic memory, transforming a transient insult in

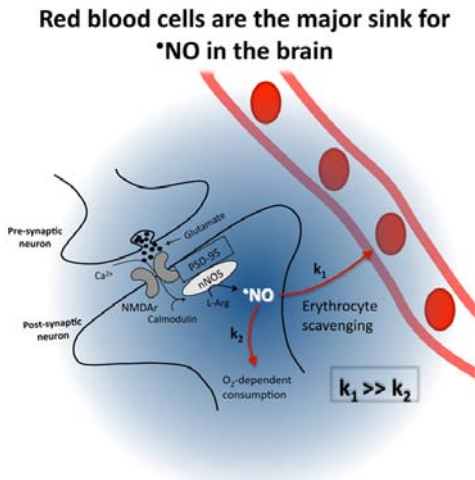
permanent cellular damage. SIRT1 and AMPK activation are able to counteract metabolic dysfunction by stimulating mitochondria. Activation of farnesoid X receptor by bile acids and stimulation of autophagy by dibenzofuran induce alterations on the cellular energetic status.

Mitochondria, carcinogenesis and chromium toxicity: Cell lines with increasing malignant phenotype and different migration capacities were established. Specific biomarkers were determined. A higher cellular malignant phenotype is related with a more glycolytic behaviour than the precursor cell lines.

Metabolic profiling and toxicology: The evaluation of metabolic pathways and their regulation was performed in cell cultures, isolated perfused organs and whole organisms by means of methodologies based in ^2H and ^{13}C isotopomer analysis: characterization of substrate selection by the heart under ischemia/reperfusion; evaluation of hepatic fat and glucose oxidation in rats with lipid-induced hepatic insulin resistance; determination of metabolic fluxes in tumor cell lines.

Drug- and disease-induced cardiovascular injury:

Two cardioplegic solutions, Celsior (Cs) and Histidine Buffer Solution (HBS) are not particularly effective in preventing the trigger of apoptosis by ischemia/reperfusion (I/R). Also, the addition of NaHS to HBS enhances glycolysis during ischemia and decreases mitochondrial dysfunction and apoptosis during I/R. The differentiation state of H9c2 myoblasts alter isoproterenol (ISO) toxicity, which may involve calcineurin, p38-MAPK, and Bax/Bcl-2 alterations.



The production of reactive oxygen/nitrogen species and the occurrence of antioxidants are critically involved in the redox regulation of cell functions but their steady-state levels and dynamics may be connected to selective responses, including the extensive oxidative damage to biomolecules (oxidative and nitrosative stresses), leading to cell death, either by turning off vital processes or by upregulating toxic cascades.

We are interested in: (a) the study of the molecular mechanisms inherent in neuromodulation, and aging that critically involve nitric oxide, connecting the dynamic profiles of nitric oxide in the brain with its role as a neuromodulator and as the mediator of neurovascular and neurometabolic coupling; (b) the analysis of the mechanisms of action of plant-derived dietary phenolic compounds, particularly those present in wine, in terms of protection against vascular endothelial dysfunction, anti-inflammatory properties, as well as their impact on nitrite-driven regulatory processes, encompassing the non-enzymatic production of nitric oxide from dietary nitrite in the gastric compartment.

We have provided for the first time a quantitative and temporal basis for the understanding of the biological actions of the ubiquitous intercellular messenger, nitric oxide (NO), that conveys information in a novel and

radical way in the brain, associated with its concentration dynamics. Along this notion we have:

Established *in vivo* the concentration dynamics of nitric oxide along the trisynaptic loop in connection with the expression of the neuronal isoform of nitric oxide synthase (nNOS), highlighting that the regulation of nNOS is a critical determinant of NO signaling in the brain;

Identified the major mechanisms for NO decay in the brain; i.e., the critical determinants that shape NO signals in the brain. Specifically, erythrocytes represent the major NO inactivation pathway in the brain;

Determined NO life-time in hippocampus *in vivo*;

Assessed NO diffusion in brain tissue, evidencing, contrary to the current paradigm, an heterogenous diffusion pattern and the occurrence of facilitated paths.

We have provided the biochemical foundations for a hypothesis proposing that post-translational protein modifications in the gastric compartment are part of dietary nitrate metabolism and can influence physiological and pathological responses.

We have expanded our knowledge on the molecular mechanisms involved in the vascular cytoprotection

afforded by anthocyanins, in particular, malvidin-3-glucoside, against endothelial cells injury in terms of improved NO bioavailability and inhibition of pro-inflammatory mediators. These effects are potentiated by association with aminosalicylic acid, thus opening new windows for therapeutic approaches against inflammatory processes.

We have elucidated a free radical-induced pathway leading to cell death involving the concerted action of DOPAC and NO and the critical role of glutathione in maintaining a functional mitochondria.

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Microbiology Area

Coordinator: Milton Costa



The Extreme Environment Group studies the microbiological diversity in extreme environments to isolate and characterize novel organisms for basic studies and for biotechnological applications. The microbial diversity and population dynamics in a low saline alkaline groundwater will be determined.

We will identify new compatible solutes, carbohydrate hydrolyzing enzymes; elucidate biosynthetic pathways and role in stress tolerance. We will also assess the contribution of natural environmental *Legionella pneumophila* strains into the molecular evolution of crucial genes in host infection.

We will focus on the characterization of the biosynthetic pathway for methylglucose lipopolysaccharides exclusively found in mycobacteria.

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The Medical Mycology - Yeast Research Group focused its studies in unravelling how *Alternaria infectoria* modulates the cell wall components and the genes coding for FKS, CHS and melanin synthesis in stressful situations

The role A2A adenosine receptors in the interaction of *Candida albicans* with phagocytic and non-phagocytic cells is one of the major points of interest of MMYRG. In the period considered we aimed to map the distribution of A2A receptor in the host cells infected by *C. albicans*, the modulation of ATP release and of *C. albicans* ectonucleotidase

The Extreme Environments Group has participated in the first Portuguese exploration of the Atlantic sea-floor and in the international expedition Middle and Mamba 09 to the deep brine basins of the Mediterranean Sea, retrieving a large number of samples from those unexplored environments. We have isolated strains from several

environments leading to the description of 3 new Genera and 5 new Species of bacteria. The performed analyses of alkaline groundwater showed the presence of a diverse and very stable microbial community.

We discovered of a unique MpgS gene from the spikemoss *Selaginella moellendorffii*, and identified the gene for glycosyl hydrolases, crucial for MG and GG hydrolysis in organisms that accumulate these solutes as response to stress conditions. We also identified the first MGGP hydrolase, catalysing the final step in the synthesis of MGG in *Rhodospirillum rubrum*. This work has been completed and the manuscripts submitted for publication.

The evolution of *Legionella pneumophila* type II-related genes suggested an ancient divergence attributed to the substrate playing a specific role in a subset of niches, and not related with more virulence-related phenotypes within mammalian cells.

We have purified a highly specific phosphatase crucial for the second step in the biosynthetic pathway for MGLPs, allowing us to isolate the gene and recombinantly produce the enzyme in soluble bioactive form.

The Medical Mycology - Yeast Research group has characterised the cell beta-glucan and chitin modulation by treatment with the antifungals caspofungin (inhibitor of Fks) and Nikkomicin Z (inhibitor of Chs) proving that this fungus does not exerts a response similar to other fungi, such as *Aspergillus fumigatus*. More the signalling pathways governing the synthesis of these cell wall components are not similar either to *A. fumigatus* or to *C. albicans*. More, the most resistant *A. infectoria* strain is characterised by a higher basal content in chitin. The co-culture of *A. infectoria* with macrophages resulted in a massive death of the fungal conidia, with an exacerbated response when compared to other fungi.

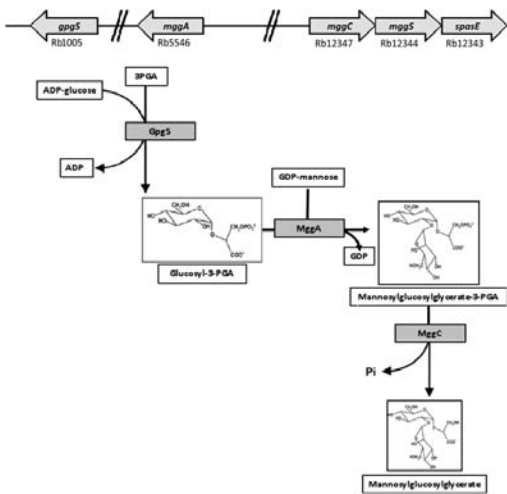
When *Candida albicans* is internalised by macrophages the A2A receptors co-localize with the phagosome containing yeast cells. In keratinocytes, the endocytosis of *C. albicans* cells does not trigger the expression of Adora gene, that codes for A2A receptors, opposed to what happens when exposed to LPS. The activity of ectonucleotidase in a group of 50 *C. albicans* strains was quantified leading to the conclusion that a higher activity of ectonucleotidase is found in *C. albicans* isolated from more severe infection situations.

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Proposed pathway for the synthesis of MGG in *R. baltica* and genomic organization of the genes involved. *GpgS*, glucosyl-3-phosphoglycerate synthase; *MggA*, mannosylglucosyl-3-phosphoglycerate synthase; *MggC*, mannosylglucosyl-3-phosphoglycerate phosphatase; *MggS*, putative mannosylglucosyl-glycerate synthase; *Spase*, putative sucrose phosphorylase. The MGG structural representation has been adapted from reference (Jorge et al 2007).

The objectives for 2011 were:

To isolate and characterize novel organisms from extreme environments for basic studies and for their biotechnological potential.

To continue our studies on the mechanisms involved in stress adaptation of thermophilic, halophilic and desiccation-resistant bacteria and also in members of the *Planctomycetes*, an unusual deep-rooted lineage of bacteria.

To identify new compatible solutes and elucidate their biosynthetic pathways and their role in stress tolerance.

To elucidate the biosynthetic pathway for methylglucose lipopolysaccharides (MGLPs) exclusively found in mycobacteria.

To determine the contribution of natural environmental *Legionella pneumophila* strains into the molecular evolution of genes crucial for infection under distinct environmental conditions.

To identify horizontally transferred genes between protozoa and *L. pneumophila*.

To determine the structural microbial diversity in a low saline alkaline groundwater environment by pyrosequencing, and to assess the population dynamics using DGGE analyses in the same environment.

During 2011:

We have expressed a unique mannosylglycerate synthase (MgS) gene from the spikemoss *Selaginella moellendorffii*, and found that the corresponding enzyme has unique catalytic features, as it efficiently synthesizes both mannosylglycerate (MG) and glucosylglycerate (GG). We have also identified the gene for glycosyl hydrolases crucial for MG and GG hydrolysis in the organisms that accumulate these solutes as response to stress conditions.

We have expressed three genes for the synthesis of MGG in *Rhodopirellula baltica*, a member of *Planctomycetes*. Those comprise the first mesophilic mannosylglucosylphosphoglycerate (MGPG) synthase and we also have identified the first MGPG hydrolase, catalysing the final step in the synthesis of MGG.

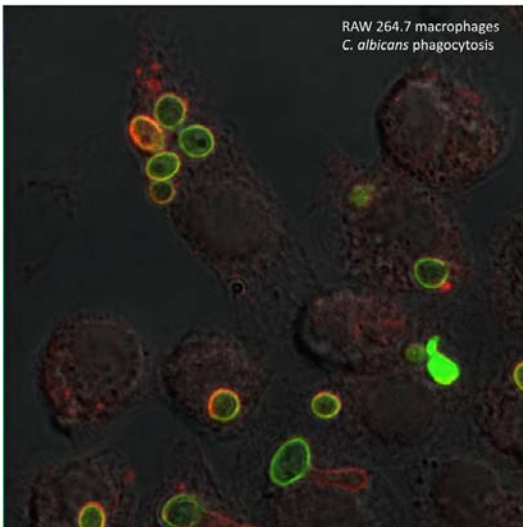
We have purified, from mycobacterial extracts, a highly specific phosphatase crucial for the second step in the biosynthetic pathway for MGLPs. This allowed us to isolate the gene and recombinantly produce the enzyme in soluble bioactive form.

We have determined the allelic diversity of five *Legionella pneumophila* type II Lsp-related genes, in strains isolated from natural and man-made environments and disease-related, suggesting an ancient divergence for each group, indicating

that the operating selective pressures have equally affected the evolution of the five Lsp-related genes. The observed allelic diversity could be attributed to the substrate playing a specific role in a subset of niches, and not related with more virulence-related phenotypes within mammalian cells.

Pyrosequencing analyses of alkaline groundwater showed the presence of a diverse community. The majority of bacterial populations affiliated with chemolithoautotrophic taxonomic lineages, namely with hydrogen-oxidizing bacteria. Archaeal populations were less diverse and were mainly related with to Phylum "Euryarchaeota". DGGE analyses evidenced a very stable microbial community.

Description of the first haloarchaea from the deep brines of the Mediterranean at 3050 m. Description of several extremophiles from hot springs.



Projects and objectives

1. “*Alternaria infectoria* FKS, CHS and melanin synthesis genes: the combination to opportunism”

1. Modulation of CHS and FKS gene expression by Caspofungin and Nikkomycin
2. Identification of the pathways involved in the regulation of chitin and glucan synthesis
3. Construction of a melaninless mutant
4. Macrophage in vitro infection by *A. infectoria* spores – effect of caspofungin treatment

2. “Role of adenosine and adenosine receptors in the resistance of *Candida albicans* to macrophage attack”

1. Role of adenosine A2A receptor in *C. albicans* infection
2. Adora gene expressio
3. *C. albicans* infection of A2A knockout mice peritonea macrophages

3. “Survey on virulent traits and resistance to caspofungin of a collection of clinical *C. albicans* isolates”

1. Ectophosphatase activity
2. Caspofungin susceptibility and genomic modifications of the FKS1 gene

4 “HIV-Vpr1 variants and AIDS progression”

1. Analysis of Vpr sequences of 31 perinatal HIV-infected children. Retrospective clinical evaluation of the children by several clinical markers such as haematological parameters, viral load, development delay, opportunistic infections, other

pathophysiological conditions.

2. Completion of a yeast model of expression
3. Initiation of this same study in the adult population

Main Achievements

1. “*Alternaria infectoria* FKS, CHS and melanin synthesis genes: the combination to opportunism”

Inter-strain variability on the susceptibility to caspofungin and nikkomycin

Two strains were studied in what regards the susceptibility to caspofungin and to nikkomycin and we found that discret differences in the MEC value results in different modulations of gene expression and synthesis of the main components of the cell wall: chitin and beta-glucan. More, it was demonstrated that the regulatory pathways of chitin synthesis in *Alternaria infectoria* work differently from *C. albicans* or *Aspergillus fumigattus*. The cell changes upon caspofungin and nikkomycin treatment are being studied by electronic microscopy in collaboration with Professor Neil Gow of the Institute of Medical Sciences of Aberdeen, UK.

2. “Role of adenosine and adenosine receptors in the resistance of *Candida albicans* to macrophage attack”

a) By immunocytochemistry it was demonstrated that the infection RAW 264.7 macrophages by *C. albicans* results in the increased number of adenosine A2A receptors in the phagosome membrane inclosing the yeast cells. Confocal microscopy clearly showed that

the receptor proteins are localized in the phagosome cell membrane whenever the phagosome contains *C. albicans* cells. The plasma membrane seems to be devoid of A2A protein. When the macrophages are stimulated by LPS the A2A receptors are clearly in the plasma membrane. Nevertheless, the relative Adora gene (that codes for A2A adenosine receptor) increases 11 times in the LPS-treated macrophages while it remains unchanged in the macrophages infected with candida cells.

b) In order to compare whether this A2A differential regulation is also found in non-phagocytic cells we studied the impact of keratinocytes exposure to *C. albicans* cells. The results showed that no Adora gene expression increase was observed, while in LPS-treated keratinocytes the increased ranges the 50 times. By fluorescence microscopy we could observe that keratinocytes internalise yeast cells and that compared to control yeast cells the co-culture cells have a higher rate of yeast-to-hypha transition.

3. “HIV-Vpr1 variants and AIDS progression”

1. Among the children studied 11 carried the mutation R77Q. At the time of first medical appointment the children infected with the Vpr variant carrying the mutation showed lower viral load than children with no mutation. During the period considered (2005-2010) these children remained with no clinical signs of disease and with no need of aggressive therapy.

2. A total of 30 adult patients were included in this study and the vpr gene is being sequenced .

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Biophysics and Biomedical NMR Area

Coordinator: Carlos Geraldes



General Objectives

1. Develop integrated measurements of hepatic glycogen and lipid metabolism in spontaneously feeding animals using D_2O .
2. Quantify hepatic transaldolase exchange activity and its effects on estimates of gluconeogenesis in subjects with normal and impaired glucose tolerance.
3. Develop and study new diagnostic imaging tools - metal based nanoparticles and chelates as multimodal targeted agents *in vitro* and in animal models.
4. Use paramagnetic chelates as covalent and non-covalent tags for NMR studies of protein structure, dynamics and protein-protein interactions.
5. Study inorganic-based drugs for therapy –Vanadium complexes as oral insulin-mimetic agents– mechanisms of action in adipocytes and animal models.
6. NMR and DFT studies of ion-polymer complexes, of complexes of transition metal ions with hydroxyacids and their phosphorylated derivatives and of metal ion interactions with polyelectrolytes.
7. Study physiologic role of the large-conductance Ca^{2+} -sensitive K^+ (BK) channel in pancreatic islets.

Main Achievements

1. Development of labeled meal tolerance test for assessing glucose kinetics and hepatic and peripheral insulin sensitivity.
2. Noninvasive sampling of hepatic acetyl-CoA enrichment from stable-isotope tracers in mice with *p*-amino benzoic acid.
3. New MRI contrast agents (CA) based on Gd(III) complexes were developed: strong supramolecular adducts of negatively charged Gd(III) chelates with positive derivatized cyclodextrins could be an alternative strategy as efficient contrast agents in preclinical MRI studies.
4. A new methodology useful for the design of target-specific MRI contrast agents was developed and studied with target proteins (RCA₁₂₀ lectin and HSA) using STD NMR and molecular modeling.
5. Several new nanoparticulate molecular imaging agents were developed as relaxometric responsive CAs to enzymatic protease activity and evaluated *in vitro* and in cell systems by MRI; Yeast cell wall particles are very efficient microcarriers for multimodal (MRI and Optical) imaging; the effect of the silica coating on core-shell $Fe_2O_3@SiO_2$ nanoparticles was studied quantitatively by magnetometry and NMRD relaxation techniques, showing that the silica layer is partially porous to water; new silica nanoparticles with the surface grafted with different complexes of Ln(III) ions were developed as cell imaging agents for Bimodal MRI-Optical modalities .
6. Several new radiolabeled compounds for nuclear imaging were developed and studied *in vitro* and in Wistar rats: ^{67}Ga -DOTA derivatives for SPECT, bone targeting ^{68}Ga complexes of phosphonate macrocycles, and a new Ga(III) tripodal tris hydroxypridinone complex.
7. NMR studies of proteins and membranes using covalent probes of protein-protein interactions in solution were developed providing a new method to refine existing x-ray crystal structures.
8. $V^{IV}(dmpp)_2$ significantly increases glucose uptake and inhibits free fatty acid release in primary adipocytes via phosphorylation of Akt1.
9. Potent actions of charybdotoxin on beta-cell electrical activity.

Inorganic Biochemistry and Molecular Imaging Group

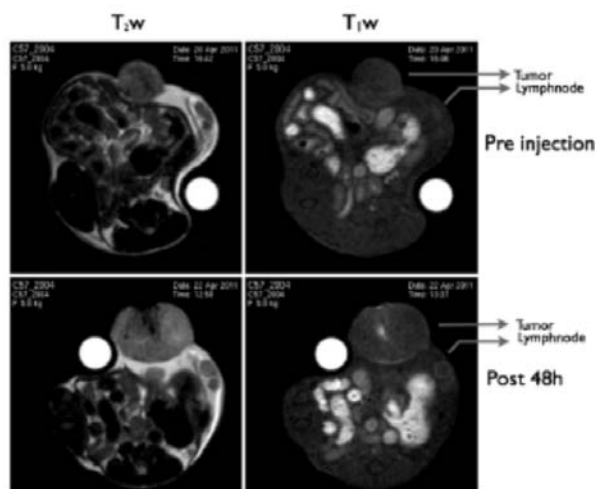
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T2-weighted and fat-suppressed T1-weighted multislice multislice MR images of C57BL/6 mice grafted subcutaneously with B16 melanoma cells. Images were obtained before and after the injection of Gd-labeled VCWPs (post 48H).

Our general objectives are the study of inorganic compounds for medical diagnostic imaging (in particular MRI contrast agents), inorganic drugs for medical therapy, and biological applications of inorganic and polymeric compounds.

The design and development of metal based agents for multimodal targeted molecular imaging agents is followed by *in vitro* cell studies and animal model evaluation using MRI and nuclear imaging techniques. These agents include Ln^{3+} -based paramagnetic complexes of Gd^{3+} and paramagnetic nanoparticles and liposomes with interesting photoluminescent properties for optical imaging (OI), and/or high r_1/r_2 relaxivities, especially at high fields, yielding positive or negative contrast in T1/T2-weighted MRI imaging and as bimodal molecular imaging agents for preclinical animal studies. The increase of r_1 and r_2 relaxivities of these agents as efficient reporters for Molecular Imaging has been pursued. A series of ^{67}Ga and ^{68}Ga -labeled with targeting capacities will also be analysed *in vitro* and in animal models as potential nuclear imaging (gamma imaging and PET) agents.

Paramagnetic chelates will also be used as covalent and non-covalent tags to study by high resolution NMR protein structure and dynamics and protein-protein interactions.

Several types of new inorganic vanadium(IV/V) complexes are being synthesised, chemically characterized

in aqueous solution and their potential use as efficient oral insulin-mimetic agents to treat diabetes has been investigated. Thus parameters indicative of insulin mimetism (increase of glucose uptake, decrease of free fatty acid release) have been evaluated *in vitro* using different cell systems (in particular primary adipocytes) and *in vivo* (plasma glucose and insulin levels, glucose tolerant test) with animal models of type 2 diabetes and obesity (Zucker rats). Toxicity tests have accompanied all these studies to evaluate the toxicity of the effective dose. The interaction of V(IV, V) species with serum components (human serum albumin and transferrin, citrate, glucose, oxalate) has been studied using different techniques, in particular the interaction with albumin has been investigated by STD-1H NMR. The mechanism of action of the vanadium complexes at the molecular level will be further investigated, in particular the effect on target proteins of the insulin signaling cascade, using western blot analysis and antibodies for key proteins of this signaling pathway. *In vivo* MRI and ^1H MRS will be used in animal models of type II diabetes. to measure hepatic triglycerides content and metabolic studies will continue to be carried out with cell and tissue extracts by high resolution ^1H and ^{13}C NMR, the latter using of ^{13}C -labeled substrates, and animal biopsies using HR-MAS NMR techniques. This methodology has been used particularly with a vanadium

compound – the bis(1,2-dimethyl-3-hydroxy-4-pyridinone)oxovanadium(IV)- which has shown promising anti-diabetic and anti-obesity properties.

Inorganic Chemistry projects include a) NMR structural and computational theoretical studies of conjugated oligomers and polymers for molecular electronic device applications, such as of poly(9,9-dialkylfluorene)s; b) synthesis and structural studies of metal complexes with relevant molecules using NMR spectroscopy, computational methods and luminescence studies, with reference to their potential ability to chelate metals *in vivo* and their use as sensors of metals in surface waters and biological fluids

c) Study on the structure and interaction with metal ions of polyelectrolytes with particular reference to their applications in biosensors of nucleic acids, sugars, proteins and in optoelectronic devices.

d) Bursting electrical activity is a distinctive trait of pancreatic beta-cells in intact islets, related to oscillatory Ca^{2+} influx and pulsatile insulin release. The possible physiological role of the large-conductance Ca^{2+} -sensitive K^+ (BK) channel as a 'burster channel' or modulator of action potential firing was investigated mainly by electrophysiological methods and pharmacological interventions.

Main Achievements

A) New Gd(III) containing formulations as MRI contrast agents (CA):

1) Supramolecular Adducts of negatively charged Ln(III) Chelates and positive derivatized cyclodextrins showing that they could be used as an alternative strategy as efficient contrast agents in preclinical MRI studies.

2) The interactions at the molecular level of several Ln³⁺ Complexes of with the RCA120 lectin and human serum albumin were studied using STD NMR and molecular modeling, providing a methodology useful for the design of target-specific MRI contrast agents

B) Nanoparticulate molecular imaging agents:

3) Supramolecular protamine/Gd-loaded liposomes were developed as relaxometric responsive contrast agents to enzymatic protease activity and evaluated in vitro and in cell systems by MRI.

4) Yeast cell wall particles were studied in vitro as a promising new class of nature-inspired microcarriers for multimodal (MRI and Optical) imaging. The r_1 relaxivity of the obtained Gd(III) loaded particles obtained is presently the highest reported and their efficient uptake by antigen-presenting cells gave very good contrast in MRI studies of loaded cells, also giving indication of promising applications in cell labeling and tracking in vivo.

5) Quantitative analysis of effect of the thickness of the coated silica layer of g-Fe₂O₃@SiO₂ core-shell NPs on the r_1 , r_2 , and r_2^* relaxivities by magnetometry and NMRD relaxation techniques. The silica layer is partially porous to water and its thickness can be tuned to allow for a high response as CA, and adequate grafting of targeted biomolecules.

6) Silica NPs with the surface grafted with different complexes of Gd³⁺ and Eu³⁺/Tb³⁺ were evaluated in vitro by luminescence and NMR spectroscopy and by MRI and fluorescence

microscopy of labeled cells as imaging agents for Bimodal MRI-Optical Imaging.

C) Radiolabeled compounds for nuclear imaging:

7) Several amphiphilic DOTA-type chelates of Ga(III) were characterized chemically and radiolabeled with SPECT active g-emitting ⁶⁷Ga, their stability in human serum, biodistribution and scintigraphic imaging in Wistar rats were studied, showing hepatic and renal uptake compatible with their lipophilicities and total elimination at 24 h.

8) A Ga(III) complex with a new tripodal tris-hydroxypyridinone was characterized chemically and by NMR as a potential nuclear diagnostic agent. This study was complemented by in vivo rat studies of the ⁶⁷Ga-labeled species.

9) Several phosphorus-containing macrocyclic ligands labeled with ⁶⁸Ga were evaluated in vitro and in vivo in rats using PET-imaging, providing information about their bone-targeting properties and information on bone metabolism.

D) NMR studies of proteins and membranes

10) Positively and negative Gd(III) chelates were analysed as NMR probes of protein-protein interactions in solution. The NMR study with the rubredoxin / Cytochrome c3 pair gave new information on the molecular details of the interaction, providing a new methodology for further studies on other biological systems.

11) The structure and dynamics of the catalytic domain of MMP-1 was studied by paramagnetic NMR through tagged macrocyclic chelates containing paramagnetic lanthanides, using a combination of pseudo-contact shifts and residual dipolar couplings. A method was proposed of refining existing x-ray crystal structures.

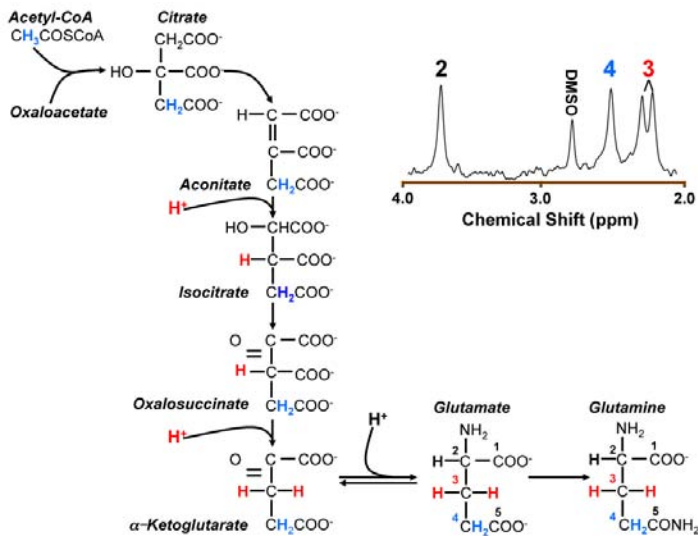
12) Other NMR studies carried out in collaboration – ³¹P NMR studies with membrane models.

E) Vanadium-based insulin-mimetic agents:

13) Non-toxic concentrations of V^{IV}(dmp)₂ significantly increase glucose uptake by primary adipocytes in the absence of insulin and inhibit free fatty acid release. This compound promotes the phosphorylation of Akt1 in the insulin signaling cascade, being a promising candidate as antidiabetic drug.

F) Electrophysiology of beta-cells:

14) Superficial intracellular recording revealed potent actions of charybdotoxin on beta-cell electrical activity. BK channels contribute to shaping action potential firing by controlling its amplitude and frequency, being essential to sustain burst plateau and regularity.



The overall objective of our group is to develop and apply new technologies for the study of hepatic intermediary metabolism and to apply these methods to better understand how liver metabolism is altered in diseases such as Diabetes and under different nutritional states and diets. We are developing methods based on nonradioactive stable isotope tracers and the use of common pharmacological agents such as paracetamol, phenylbutyric acid and *p*-amino benzoic acid to noninvasively sample hepatic metabolites in the urine. Among other things, these approaches allow dietary and endogenous sources of hepatic glycogen or glucose to be identified and their contributions to glucose and glycogen synthesis resolved. Also, they provide insights into hepatic lipid synthesis and oxidation rates under normal and pathophysiological states. In addition to our longstanding interests in human and rodent metabolism, we are also applying our methods to study glucose and amino acid metabolism in fish with the aim of optimizing growth and feed utilization for farmed fish including the seabass and seabream, (Robalo and Dourada) –key species in Portuguese aquaculture.

Our deuterated water method for analysis of glucose metabolism in humans is now well established and we are considered as a Reference Lab for this analysis by Leading Diabetes Laboratories in both USA

and Europe. This has resulted in productive collaborations and has secured ~90000 euros in International funding. To expand this modest funding stream we need to develop faster and more robust sample analyses and throughput and to this end we are investing heavily in the development of LC-MS/MS methods in collaboration with the LC-MS facility at Biocant. This methodology is particularly important for developing dynamic tracer measurements of glucose metabolism in both humans and mouse models, since these require frequent sampling of small blood samples.

Since 2011, the Group P.I. is also one of the founder members of the APDP-RC, a newly-established Clinical Research Center at the Portuguese Diabetes Association in Lisbon - one of the oldest Diabetes Associations in the World and one of the largest Diabetes Outpatient Clinics in Europe. This will provide unique opportunities for further development of our tracer metabolic studies in both Type 1 and Type 2 diabetes patients and integration with other leading Diabetes Research Laboratories in Portugal and beyond.

Main Achievements

1. Development of labeled meal tolerance test for assessing glucose

kinetics and hepatic and peripheral insulin sensitivity:

Labeled meal tolerance tests have been developed where absorbed endogenous contributions to plasma glucose levels are calculated by modelling the appearance of infused and meal-borne glucose tracers in the plasma glucose pool. This approach relies on frequent sampling of plasma for insulin levels and glucose enrichments from the meal and baseline-infused tracers. For the tracer analysis, the sampling method has to be sufficiently sensitive for analysis of small blood volumes and the sampling throughput needs to be fast in order to accommodate the large number of samples. The LC-MS method that we developed permitted precise analysis of tracer enrichments with coefficients of variation of ~5% for each time point (determined by 4 replicate measurements) and fast throughput (~15 minutes per measurement). With this approach, the endogenous insulin secretion profile can be correlated with glucose excursions. The appearance of the meal-borne [6,6-²H₂]glucose tracer in plasma glucose allows the contribution of absorbed glucose to be determined relative to total while enrichment of plasma glucose from the constantly infused [U-¹³C]glucose tracer provides a means of following glucose R_a over the course of the meal. For upcoming proposed studies, these data will be applied to a circulatory model of glucose kinetics that will resolve endogenous and meal-derived glucose rates of

appearance. Insulin and c-peptide excursions are then integrated with the glucose kinetic data to describe insulin secretion and sensitivity.

2. Noninvasive sampling of hepatic acetyl-CoA enrichment from stable-isotope tracers in mice with *p*-amino benzoic acid:

Acetyl-CoA is a key hepatic metabolite but is difficult to analyze for enrichment from metabolic tracers. We developed a method that allows acetyl-CoA enrichment from ^{13}C - and ^2H -enriched precursors to be quantified. *p*-Amino benzoic acid (PABA) is acetylated via hepatic cytosolic acetyl-CoA to form *N*-acetyl PABA. This is rapidly cleared into urine where it is analyzed. To demonstrate this sampling method, acetyl-CoA enrichment from

deuterated water ($^2\text{H}_2\text{O}$) was quantified by ^2H NMR of *N*-acetyl-PABA in order to determine the true ^2H -precursor enrichment for *de novo* lipogenesis. Second, the contribution of an $[\text{U-}^{13}\text{C}]$ glucose load to hepatic acetyl-CoA was determined by ^{13}C NMR analysis. As proof-of-concept, 4 mice were injected with $^2\text{H}_2\text{O}$ in saline (3g/kg body water) one hour into the light phase, and their drinking water was also enriched with 3% $^2\text{H}_2\text{O}$. 24 hours later, they received an injection of PABA (15 mg/kg) dissolved in saline containing 3% $^2\text{H}_2\text{O}$. Urine was collected for 6 hours following PABA injection. In a second protocol, 4 mice were fasted overnight, then injected with $[\text{U-}^{13}\text{C}]$ glucose (2 g/kg) and PABA (15mg/kg) in saline. Urine was collected over the following 6 hours. Urinary *N*-acetyl PABA was purified by solid phase extraction and the acetyl

carbon and hydrogen enrichments from $^2\text{H}_2\text{O}$ and $[\text{U-}^{13}\text{C}]$ glucose were successfully analyzed by NMR. The analyses revealed that a surprisingly small fraction of hepatic acetyl-CoA (< 2%) was derived from an intraperitoneal glucose load given to 24-hour fasted mice. This suggests that the liver was not committed to glycolytic and lipogenic utilization of the administered glucose under these conditions. Following $^2\text{H}_2\text{O}$ administration, hepatic acetyl-CoA and body water hydrogens are enriched to the same levels hence body water enrichment can be used as a surrogate for the true acetyl-CoA precursor.

The Center for Neuroscience and Cell Biology, University of Coimbra, is a partner of



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Cell and Development Biology Area

Coordinator: João Ramalho-Santos



The key identifying feature of the “Cell and Development Biology” area is CNC Researchers whose programs involve close partnerships with clinicians at FMUC/HUC, both in terms of basic research with human samples, setting up novel clinically-relevant services and trials, and furthering translational research. Partnerships already in place include: Immunology, Oncobiology, Genetics, Neurology, Dermatology, Reproduction, Endocrinology (Obesity, Diabetes), and likely others.

One of the major strengths of the groups, included in the “Cell and Development Biology” area, is the strong collaboration with clinical departments, allowing the collection of human tissues and samples for the development of translational investigation in several distinct, yet interconnected research lines. In line with this, the major goal in past years has been the consolidation of the research projects being carried out, which was achieved as the publication record for the various groups in this area demonstrates.

The main purpose for this area was to continue the consolidation of the research carried out. The quality of publications has clearly increased, and this promises to continue in 2012. Funding does not seem to be a critical issue for this area, and the past two years have been particularly successful.

The Reproduction group has now established solid grounds in the field of stem cell biology, namely in the metabolic regulation of pluripotency. This work has been extended to induced pluripotent cells, and papers on this topic were published in 2011 with more in preparation.

The Cellular Immunology and Oncobiology group was able to continue the strengthening of national and international collaborations, apparent by the publication of joint manuscripts. Also underway is a reformatting of this group, to better highlight the different lines of independent research carried out within. The current report was already prepared with this strategy in mind.

The Phagocytosis and Pathogens group reached a significant dimension in line with the process of new recruitments, and has now achieved good financing status with a good publication record in the several areas of intervention it pursues.

The Metabolism, Insulin Resistance and Complications group is now more firmly established within CNC, especially due to collaborations with HUC services and CNC's groups, and has continued to publish well and attract funding in the area of diabetes, obesity and wound healing.

Cellular Immunology and Oncobiology Group

Celeste Lopes	PhD – <i>Head of group</i>
Alexandrina Mendes	PhD
Ana Bela Sarmiento	MD, PhD
Ana Luisa Vital	PhD
Anália do Carmo	PhD
Bruno Miguel Neves	PhD
Fernando Judas	MD, PhD
Maria Teresa C. Rosete	PhD
Marta Viegas da Silva	PhD
Teresa Maria C. Martins	PhD
Silvia Neves	PhD
Ana Cristina Gonçalves	PhD Student
Ana Inês Crespo	PhD Student
Ana Teresa Rufino	PhD Student
Carlos Manuel Melo	PhD Student
Diana Diniz Azenha	PhD Student
Diana M. Carvalho	PhD Student
Humberto Ferreira	PhD Student
Joana Balsa Silva	PhD Student
Joana Liberal	PhD Student
João Martins	PhD Student
Mariana Freitas	PhD Student
Patrícia Domingues	PhD Student
Raquel Alves	PhD Student
Sara Tavares Melo Lima	PhD Student
Vera Lúcia G. Francisco	PhD Student
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Diana Matias	MSc Student
Isabel Ferreira	MSc Student
Patrícia Guarino	MSc Student
Vera Gonçalves	Grant Technician
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Hermínio Tão E. Santo	MD
M ^a Olinda Rebelo	MD

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Ana Sofia Rodrigues	PhD Student
Ângela Crespo	PhD Student
Beatriz L. de Sousa	PhD Student
Carla Patrícia R. Paiva	PhD Student
Marília H. Cordeiro	PhD Student
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Renata Tavares	PhD Student
Rodrigo Santos	PhD Student
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Bárbara Lourenço	MSc Student
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Patricia Morais	MSc Student

Infection, Phagocytosis and Pathogens Group

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Luís Estronca	Post-Doc Fellow
Luísa Jordão	Post-Doc Fellow Collaborator
Ângela Inácio	PhD Student
Michelle Stump	PhD Student
João Silva	MSc Student
Elda Bonifácio	Grant Technician

Insulin Resistance and Adipocyte Group

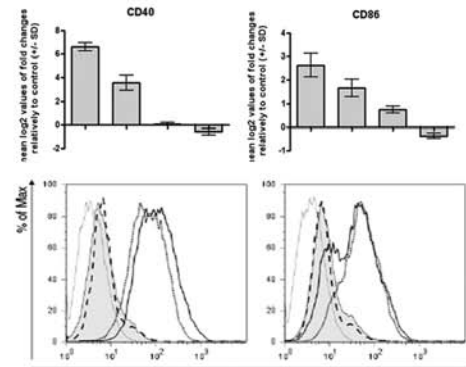
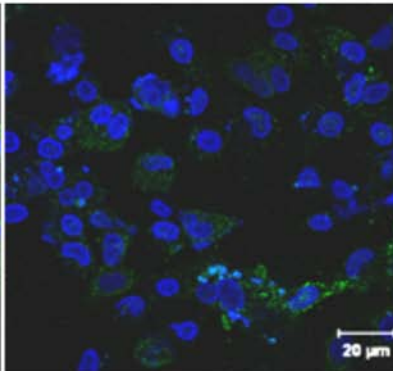
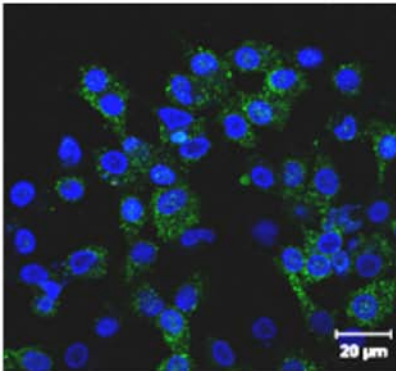
Eugenia Carvalho	PhD – <i>Head of group</i>
Manuel Aureliano Alves	PhD Collaborator
Ermelindo Leal	Post-Doc Fellow
Ana Tellechea	PhD student
Maria Joao Pereira	PhD student
Liane Moura	PhD student
Patrícia Lopes	PhD student
Daniel Espinoza	Grant Technician

Biology of Reproduction, Stem Cells and Human Fertility Group

João Ramalho Santos	PhD – <i>Head of group</i>
Ana Paula Sousa	PhD
M ^a Alexandra Amaral	Post-Doc Fellow
Sandra Amaral	Post-Doc Fellow

Cellular Immunology and Oncobiology Group

Head: Celeste Lopes



The researchers of the Cellular Immunology and Oncobiology group share common interests in identifying the cellular mechanisms that regulate the function of normal human cells and in understanding how disruption of these processes leads to disease, namely to allergic contact dermatitis, osteoarthritis, autoimmunity and cancer.

One of the strengths of this group is the variety of approaches, ranging from *in vitro* studies in human primary cell cultures and established cell lines, to *in vivo* experiments with animal models and analysis of clinical samples made in close collaboration with hospital clinical units, namely with the: i) Dermatology Department of the University Hospital of Coimbra (HUC); ii) Orthopaedic and Bone Bank Departments of HUC; iii) Clinical Hematology Department of HUC; iv) Portuguese Oncology Institute of Coimbra; v) Neuropathology Laboratory and Neurosurgery Service of HUC and vi) Center for Cancer Research of the Salamanca University, Spain.

1a) Research on Cellular Immunology focused in:

Immunobiology of antigen presenting cells:

- to study modifications on the signalling profiles, cytokines and chemokines expression in dendritic cells induced by skin and respiratory chemicals to establish *in vitro* tests that can predict the sensitizing potential of chemicals.

- screening of lead molecules with anti-inflammatory properties obtained from medicinal plants.

Chondrocyte biology and osteoarthritis:

- characterizing the subunit composition of ATP-dependent K⁺ channels expressed in normal, aged and OA human chondrocytes and the role of high glucose in modulating their composition and function.

- identifying new compounds in plant volatile extracts with potential anti-osteoarthritic activity, as well as with potential activity against other diseases with a chronic inflammatory component, namely inflammatory bowel disease.

CD38 in immune function:

- to study the role of CD38 in immune regulation, namely during mycobacterial infections and development of systemic autoimmunity.

1b) Research on Oncobiology focused in:

Cell signalling pathways involved in cancer and chemoresistance:

- to evaluate the role of oxidative stress and mitochondrial dysfunction and the deregulation of apoptotic and survival pathways that can allow the identification of new molecular therapeutic targets.

Pathways involved in thyroid and breast cancer:

- to investigate new players in thyroid and breast carcinogenesis, based on previously obtained clinical data which highlighted the possible role of LRP1B, a modulator of tumour microenvironment, and Casp1n, a protein involved in checkpoint responses and DNA replication, as tumour suppressors in these two types of cancer, respectively.

Signaling pathways and genetic abnormalities in brain tumors:

- to evaluate chromosomal and genetic abnormalities involved in human gliomas and to identify new genes (and cell signaling pathways) potentially relevant for glioblastoma development and progression.

Main Achievements

2a) Research on Cellular Immunology Immunobiology of antigen presenting cells:

We developed a dendritic cell-derived *in vitro* test to detect skin sensitizers. This test was protected through a patent and is currently ongoing validation by the European Centre for the Validation of Alternative Methods (ECVAM).

We found that *Cymbopogon citratus* has anti-inflammatory properties by

inhibiting TNF- α and CCL5 production in macrophages through NF- κ B, p38 MAPK and JNK pathways modulation. The suppression of NF- κ B pathway by *Cymbopogon citratus* is mediated through inhibition of proteasome activity.

Chondrocyte biology and osteoarthritis:

In human chondrocytes, ATP-dependent potassium channels are composed of Kir6.1 and Kir6.2 pore forming subunits and SUR1 and SUR2B regulatory subunits, being involved in the regulation of the availability of glucose transporters.

One essential oil was found to inhibit catabolic and inflammatory responses in human chondrocytes, which is strongly predictive of potential anti-osteoarthritic activity. Although with lower potency, the same essential oil also inhibited inflammatory signalling pathways in a human intestinal epithelial cell line, suggesting potential activity in inflammatory bowel disease.

CD38 in immune function:

Using CD38KO mice, we found that CD38 is required for effective macrophage activation by T cells, NO production, chemotaxis and chemokine secretion during immune responses against mycobacteria; and for the control of systemic autoimmunity.

resistance to conventional chemotherapy and new targeted therapies is a problem and contribute to disease relapse.

Pathways involved in thyroid and breast cancer:

We unravelled a new pathway involved in non-medullary thyroid cancer involving LRP1B and the modulation of the extracellular microenvironment; we investigated the transforming potential of new RET mutations; and identified changes in Claspin associated with increased susceptibility to breast cancer and analysed the functional implications of these mutations in cell cycle regulation, namely in Chk1 activation.

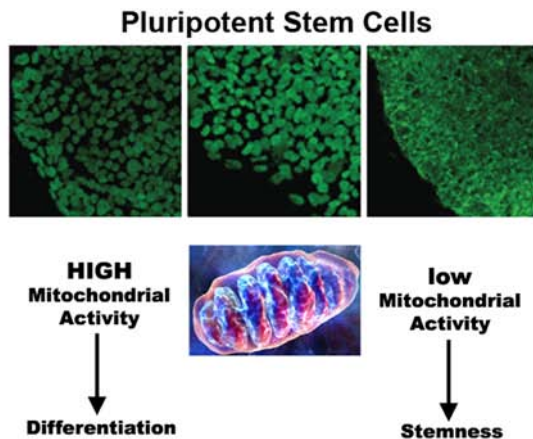
Signaling pathways and genetic abnormalities in brain tumors:

The study of gliomas by iFISH revealed a complex cytogenetic heterogeneity and distinct clonal pathways of glioma evolution. In addition, the analysis of gene expression profile (GEP) demonstrated clear association between the GEP of gliomas and tumor histopathology and, among grade IV astrocytoma, GEP are significantly associated with the cytogenetic profile of the ancestral tumor cell clone. Regarding the cell signalling pathways, our results indicate that the activation of PI3K/Akt, MAP Kinase and CXCR4 signaling pathways may contribute to the chemoresistance that characterizes glioma cells.

2b) Research on Oncobiology

Cell signalling pathways involved in cancer and chemoresistance:

we found the involvement of oxidative stress and mitochondrial dysfunction in neoplastic development, as well as the levels of apoptotic modulators that can be related with the resistance to cell death. Our results also demonstrate that the farnesyltransferase inhibitor, α -HFFPA, is effective independently of Ras mutations and that epigenetic modulators show a synergistic effect dependent on schedule of administration. Besides that,



The main goals involve determining the metabolic cues that govern gonad homeostasis, proper mammalian gamete function, and pluripotent stem cell status, with the goal of increasing the success rates of Assisted Reproduction in humans, model and endangered species, as well as to develop efficient methods to improve stem cell propagation and differentiation into specific fates.

Current projects include continuing research to characterize the most viable human gametes, both in terms of basic science and for application in Assisted Reproduction. In this regard more functional sub-populations of sperm from a heterogeneous ejaculate are being isolated and characterized (by classical methods or flow cytometry), and long-term sperm *in vitro* culture systems perfected in order to prolong the time window in which male gametes can be used following collection. Projects involving the evaluation of oocyte quality using novel simple non-invasive assays, as well as the proper cryopreservation of ovarian tissue for the preservation of fertility of patients undergoing chemotherapy are also underway.

Other projects involve both the preservation of the male germline by testicular xenotransplantation using the cat as a model, and further characterization of testis bioenergetics, with an emphasis on mitochondrial function and how it can be affected by xenobiotics, such as dioxins or pesticides. Given that testicular mitochondria seem to be completely different from other mitochondria normally used for *in vitro*

assays (namely liver mitochondria), we postulate that they may more accurately serve as models for toxicology studies involving substances thought to impair reproductive function. Parallel studies are being carried out with mature sperm, and also include other candidate substances that may modulate sperm function.

Finally the group is pursuing the modulation of stem cell pluripotency and differentiation using metabolic cues, and this work is being expanded to also include the generation, propagation and differentiation of induced pluripotent cells (iPS cells).

Recently concluded research includes:

1. Characterization of testicular mitochondrial bioenergetics and the finding that they are very distinct from that of mitochondria from other tissues, both in terms of basic function and how it is modulated by different substances.
2. Establishing the basic conditions for a realistic use of xenografting of testicular tissue from endangered felids under field conditions using the domestic cat as a model, thus providing novel tools in preserving the germline of rare individuals.
3. Development of novel assays to improve the analysis of human sperm function and the diagnosis of male infertility, given that the methodology currently employed is unreliable. Namely, a simple assay to monitor human sperm DNA status, an important parameter that is not

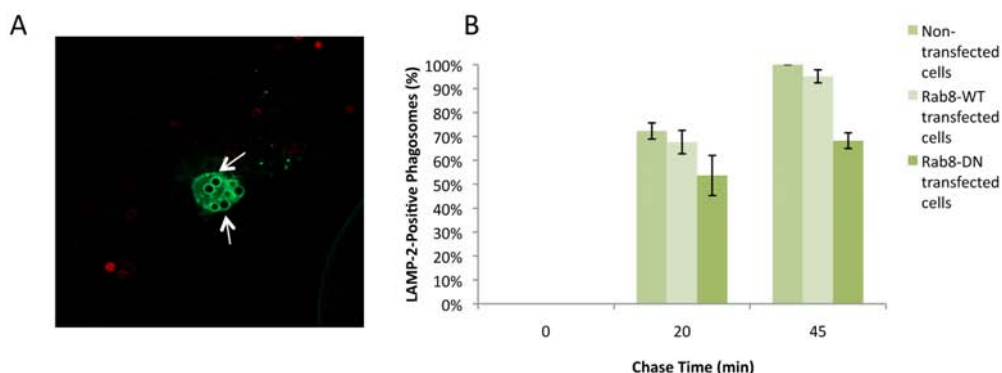
usually quantified in routine semen analysis, was introduced.

4. Using fluorescence assisted cell sorting (FACS) to determine that mitochondrial activity in human sperm is directly related to fertilization potential, and that mitochondrial probes can be used to separate a subpopulation of sperm with higher functionality from a heterogeneous ejaculate.

5. Characterization of a simple system that allows for the maintenance of viable and motile human sperm in culture for about two weeks, a week longer than previous research had indicate, and thus creating a tool for the long-term study of human sperm function.

6. Discovery of a role for mitochondria in maintaining human embryonic stem cell pluripotency and in inhibiting stem cell differentiation into specific fates via a ROS-dependent mechanism, suggesting that metabolic modulation may play an important role in stem cell biology.

7. Comparative characterization of the metabolism in human embryonic stem cells (hESC) and human induced pluripotent stem cells (iPSC). It was determined that, although slightly different, both cell types share a preference for aerobic glycolysis and show many characteristics that are also common with cancer cells. This has implications for the study and use of pluripotent stem cells and will be the subject of future research.



Rab8 associates transiently with phagosomes and is required for phagolysosome formation. RAW cells were transfected with wild-type (WT) and inactive (DN) Rab8. A) Shows Rab8 distribution during phagocytosis. B) Shows the effect of the Inactive Rab8 in LAMP-2 acquisition by phagosomes. RAW cells were pulsed with IgG-opsonized latex beads for 15 min (0 min chase). The external beads were stained and the cells chased for the times indicated in the graph. Results are means (\pm SD) of three to four independent experiments.

We have three main objectives in our group:

Establish etiological hierarchies among the chemical constituents of Oxidized-LDL with regard to foam cell formation;

Identify the molecular machinery involved in phagosomal maturation of opsonized inert particles and *Mycobacterium tuberculosis*

In vitro study of surfactant structure-toxicity relationships: implications for surfactant use in sexually transmitted infection prophylaxis

Our main achievements were:

For the first Project:

Cholesteryl-hemiesters (compounds formed during LDL oxidation) are responsible for:

- 1) Formation of foam cells with lipid accumulation in the endolysosomal compartment;
- 2) Incapacity of affected cells to metabolize (hydrolyze and re-esterify) internalized cholesteryl esters;
- 3) Irreversible and uncontrolled lipid accumulation in the endolysosomal compartment; and 4) Apoptotic cell death.

For the second Project:

- 1) Rab8 a and b are required for phagocytosis of both *Mycobacterium tuberculosis* and IgG-opsonized beads;
- 2) Rab8 a and b are also required for phagosomal maturation and
- 3) The overexpression of Rab8a is sufficient to change the fate of *Mycobacterium*-containing phagosomes.

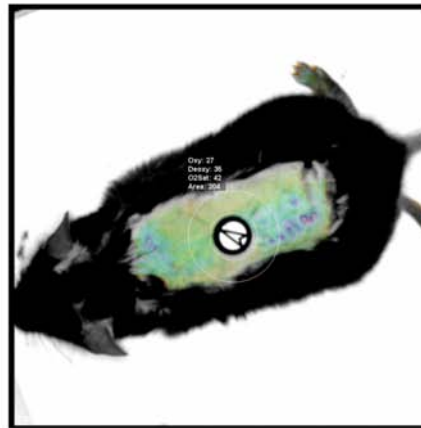
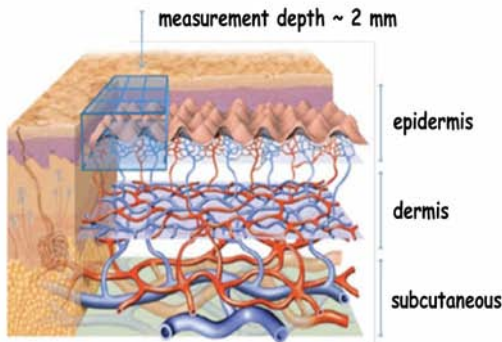
For the third Project:

- 1) All cationic surfactants were toxic at concentrations far below their Critical Micellar Concentration (CMC) and showed significant differences in their toxicity toward polarized as compared with non-polarized cells;
- 2) Their toxicity was also dependent on the chemical nature of the polar head group and
- 3) Our results suggest an intracellular locus of action for cationic surfactants and show that their structure-activity relationships could be profitably exploited for Sexually Transmitted Infections prophylaxis in vaginal gel formulations

Insulin Resistance and Adipocyte Group

Head: Eugenia Carvalho

• Wound healing in Diabetes



Objectives

Immunosuppressive agents (IA), such as cyclosporine (CsA), tacrolimus (FK) and rapamycin (Rap) can cause dyslipidemia as well as new-onset diabetes (NODAT) in solid organ-transplantation patients. The aim of this study was to investigate whether adipose tissue plays a role in the perturbations of glucose and lipid metabolism caused by IAs. This was evaluated in abdominal subcutaneous adipose tissue obtained from healthy volunteers.

To understand the molecular events associated with NODAT, we investigated the effect of IA on glucose metabolism, insulin action, heart rate and blood pressure *in vivo* in Wistar rats.

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Diabetes mellitus is one of the most widespread diseases in the world. It may cause chronic and non-healing diabetic foot ulcers (DFU), which decrease the welfare of patients. Peripheral neuropathy impairs wound healing in diabetes. We have evaluated if neurotensin (NT) is promoting wound healing via iNOS by using the iNOS knockout (iNOSKO) mice or treating wounds with an iNOS inhibitor, 1400w.

The G protein-coupled metabotropic cannabinoid receptor type-1 (CB1R) is a major regulator of metabolism,

growth and inflammation, and is present in most tissues of the body. For the first time, we aimed to test the expression of CB1R and other factors of inflammation and regeneration in the skin of diabetic and in CB1R knockout mice (CB1R KO).

Recent studies suggest that neuropeptides and mast cells participate in wound healing but the mechanisms of their action are not clear. Our main hypothesis is that skin mast cells are dysfunctional in diabetes due to neuropeptide deficiency, contributing to impaired wound healing. We assessed wound healing in both streptozotocin-induced diabetic (STZ-DM) and non-diabetic (non-DM) mast cell deficient mice (KitW/KitW-v) and their wild type (WT) littermates.

Natural biopolymers like chitosan, collagen and their derivatives, are presently receiving greatest attention as wound dressing materials for wound healing applications. Employing these chitosan derivatives simultaneously as dressings and as platforms for the delivery of a neuropeptide, neurotensin (NT) has not yet been evaluated and it is being addressed in our work.

In the last decades some reports reveal the neuropeptide neurotensin (NT) as an immune mediator in the Central Nervous System and in the gastrointestinal tract, however its

effects on skin immunity were not identified. Our present studies investigated the effect of NT on signal transduction and on pro/anti-inflammatory function of skin dendritic cells, fibroblasts and macrophages. Furthermore, we investigated how neurotensin can modulate the inflammatory responses triggered by LPS in skin dendritic cells, fibroblasts and macrophages.

Main Achievements

1. In collaboration with Prof. Jan Eriksson, Gothenburg University, Sweden, and with Dr Flavio Reis from the Institute of Pharmacology & Experimental Therapy at the Medicine Faculty of the University of Coimbra we investigated the role of glucocorticoids (GCs) and immunosuppressive agents (IA) as important players in the impairment of glucose and lipid metabolism in the metabolic syndrome in isolated rat and human adipocytes. Immunosuppressive agents, such as cyclosporine (CsA), tacrolimus (FK) and rapamycin (Rap) can cause new-onset diabetes (NODAT) as well as dyslipidemia in solid organ-transplantation patients. The studies of the effects of IA in human adipocytes are few, and it is believed that it could be a good model to understand the effects of IA in humans. The aim of this study was to investigate whether IA could cause alterations on glucose and lipid

metabolism as well as on gene expression in human subcutaneous adipose tissue. We have demonstrated for the first time that in human adipocytes CsA and FK at therapeutic concentrations inhibits both basal and insulin-stimulated glucose uptake, but did not change the initial steps of the insulin signaling cascade or GLUT4. Moreover, Rap may impair insulin action on glucose uptake by reducing IRS2 protein levels and AKT Ser473 phosphorylation in the human subcutaneous adipose tissue. All three IAs increased lipolysis but only Rap was found to alter gene expression of important lipolysis regulators and adipokines that may induce insulin resistance. These effects of IA in the adipose tissue could potentially contribute to the development of new-onset diabetes and dyslipidemia in solid organ-transplant patients. To follow up on these studies we are carrying out in vivo studies where we will treat animals with IA for a period of time of 3, 6 and 9 weeks, we will study the effects of IA on glucose and lipid metabolism in fat, muscle and liver.

In collaboration with Dr Aristides Veves at Harvard Medical School we have been trying to understand the role of neuropeptides and mast cells (MC) in wound healing. We have found that substance P (SP) is a neuropeptide of major importance in wound healing in diabetes. The results indicate that impaired wound healing in diabetic animals is related with abnormal expression of angiogenic markers and neuropeptides. In addition, we hypothesized that skin MC are dysfunctional in diabetes due to neuropeptide deficiency, contributing to impaired wound healing. We assessed wound healing in both streptozotocin-induced diabetic (STZ-DM) and non-diabetic (non-DM) MC deficient mice (KitW/KitW-v) and their wild type (WT) littermates. Wound healing was delayed in MC deficient mice compared to WT mice. SP treatment accelerated wound closure in both non-DM ($p < 0.05$) and STZ-DM WT mice ($p < 0.05$), but had no effect in the KitW/KitW-v mice. WT STZ-DM mice showed higher MC degranulation

levels in the skin, compared to WT non-DM mice ($p < 0.05$). Wounds from MC deficient mice showed more inflammatory cell infiltrate, than wounds from WT mice ($p < 0.05$). At day 10, skin gene expression of VEGFR2 and KC was increased in KitW/KitW-v mice ($p < 0.05$). We conclude that MC deficiency severely impairs wound healing in both DM and non-DM settings and leads to skin hypoxia and aberrant expression of growth factor and cytokines. STZ-DM affects skin MC function. SP exerts its beneficial effect on wound healing, at least partly, through MC function.

Furthermore, at the CNC, we have evaluated if neurotensin (NT) is promoting wound healing via iNOS by using the iNOS knockout (iNOSKO) mice or treating wounds with an iNOS inhibitor, 1400w. We studied wound healing in wild-type (WT) C57BL6/J, WT diabetic, iNOSKO, and diabetic iNOSKO mice. NT improves wound healing in WT, WT diabetic and iNOSKO mice ($p < 0.05$), but not in diabetic iNOSKO mice. Our results show that NT has a pro-inflammatory effect by increasing iNOS, IL6 and KC expression and an anti-inflammatory effect by decreasing TNF alpha expression. Moreover, NT has an angiogenic effect increasing the expression of VEGF, VEGFR2 in normal and diabetic mice but not in iNOSKO mice. These results suggest that the effects of NT in wound healing can be via iNOS signaling.

4. In collaboration with Dr Attila Köfalvi, at the CNC, we have for the first time, aimed at evaluating the expression of critical factors of inflammation and regeneration in the skin of diabetic rats, wild-type (WT) and CB₁R knockout mice (CB₁R KO). Our main hypothesis is that CB₁R plays a role in skin homeostasis, especially in diabetes, and this study will suggest novel therapeutic role for existing cannabinoid system-targeting medicines as e.g. topical treatment in diabetic skin complications. We found that CB₁R expression is decreased in the skin of diabetic mice, indicating the possible impairment of the

expression of these CB₁R-dependent homeostatic markers.

5. In Collaboration with Dr Teresa Cruz, at the CNC, we have also, evaluated the effects of neurotensin on different skin cells. We have shown that neurotensin downregulates the pro-inflammatory properties of skin-dendritic cells and increases its epidermal growth factor (EGF) expression. In addition, neurotensin induces human skin fibroblast-mediated expression of EGF and down regulates the immunomodulatory chemokines IL-8 and IL-6 under inflammatory conditions. Moreover, we have also shown that there is a decrease in the inflammatory response of macrophages induced by NT under hyperglycemic conditions, probably due to a decrease in the expression of NT receptors.

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Biomedical Inter-Institutional Research programme



The interaction of CNC researchers with clinicians at CHUC, Paediatric Hospital and IPO led to the development of a Biomedical Inter-institutional Research Programme. The main ongoing joint research projects include: 1. Psychiatry Research: Molecular genetics studies of complex disorders; 2. Neurology Research: Biochemical and genetic studies of neurodegenerative disorders; 3. Paediatric Research: Biochemical and genetic research of metabolic disorders including mitochondrial cytopathies; 4. Pharmacogenomics Research; 5. Dermatology Research: Contact Dermatitis; 6. Arthritis Research: Rheumatoid Arthritis and Inflammation; 7. Research in Brain Cancer: Genetic heterogeneity of gliomas; 8. Analysis of Human sperm function in the diagnosis of male infertility.

1. Psychiatry Research

Carlos Pato, Michele Pato (University of Southern, Califórnia), António Ferreira de Macedo, Ana Telma Pereira (FMUC, CNC)

1.1 Molecular Genetics Studies of Complex Disorders

Our team has over 20 years experience in population studies of schizophrenia (Sz) and Bipolar Disorder (BP) focusing on the identification of susceptibility genes for these disorders through the use of linkage and the more recent state-of-the art association analysis with genome wide association studies (GWAS) and whole genome and exome sequencing. For this purpose several populations have been analyzed: a relatively homogenous population from Azores, augmented by a similarly homogenous subsample from Madeira, and a mainland Portuguese population. To date we have collected over 3000 DNA samples, including 700 schizophrenic patients, 500 bipolar patients, and 1400 unaffected family members. Additionally, 350 unaffected (i.e. no history of psychiatric disorder) subjects of Azorean descent have been collected as a control group. The schizophrenic sample includes 100 multiplex (2 or more affected members) families, and the bipolar sample includes 120 multiplex families. This sample is being expanded by Dr Pato at The University of Southern California (USC-Center for Genomic Psychiatry), with a project integrating a US- wide network of academic medical centers that have created the Genomic Psychiatry Cohort (GPC). The aims of this project are to assemble a cohort of 10,000 patients with schizophrenia and 10,000 controls without schizophrenia or a family history of schizophrenia, from 8 sites and in the future, assemble a similar sample of bipolar patients. The cohort from the USA and Portugal has reached 30,000 individuals.

In the GPC as well as in the International Schizophrenia Consortium (ISC) that we have also formed we intend to use whole genome approaches to define the genomics of schizophrenia and bipolar disorder. Of the total 30,000, 9,000 are drawn from long-term studies of specific populations, and over 21,000 have joined as partner participants. These participants have all contributed DNA, and cells, that are sharable through the NIMH repository. All have agreed to prospective follow-up. Further, over 80% have agreed to be contacted for future studies. The Genomic Cohort includes 4,000 African-American, close to 6,000 Latino, and over 20,000 Euro-Caucasian participants. We have just begun a very large genotyping effort as a partnership between USC and the BROAD. It includes over 20,000 subjects. Over 4,000 African Americans will make up wave 1. Immediately followed with over 5,200 Latino subjects that will make up wave 2. We are also planning wave 3 focused on Caucasian subjects that may include over 12,000 subjects. We are performing a genome-wide analysis of common SNPs, common haplotypes, and CNVs using the Illumina Omni Express Platform. We will also do a genome-wide analysis of low-frequency variation in the genome's protein-coding sequences using the newly designed Exome Array. This is a unique opportunity to study populations that trace ancestry to continents other

than Europe. We believe this has the potential to lead us to novel risk factors and to alleles for which discovery power is different in different populations. As well as, increase our understanding of the genetics of human populations and population admixture. Further we are actively doing whole genome sequencing on over 3,000 cohort members with the ability to impute newly discovered variants into the cohort in general.

Our studies have utilized the more recent DNA and RNA microarray technology to identify chromosomal regions of linkage to each disorder, genetic association information, as well as areas of differential gene expression in the presence of illness. This convergent genetic-genomic approach has led to the identification of several areas in the human genome that may harbour susceptibility genes for Sz or BP. In Sz, our group identified a region on 5q31–5q35 with a NPL score of 3.28 which was replicated in the BP sample with psychosis. Further study of this region showed positive SNP associations with several GABA receptor subunit genes in patients with SZ. In BP, the identification of a region on 6q22 (NPL-Z=4.2), was also an important finding. In our case-control studies a number of significant associations were reported for several genes: syntaxin 1A; NRG1, GABA receptor subunit genes; Neurogranin; CHRNA7, and DRD2. More recently, as published in *Nature*, our studies with copy number variants (CNVs) led to the identification of 22q11.2, 15q13.2 and 1q21.1 as regions with excess CNVs in Sz.

An exploratory WGA study in the Portuguese Sz probands was carried out on the Affymetrix GeneChip® Mapping 500K Assay. We identified a total of 55 SNPs that showed nominally significant associations with schizophrenia at a threshold of $P < 1 \times 10^{-4}$. Two of these SNPs survived FDR correction (rs6638512 on chromosome X, and rs4907606 on chromosome 13). However, in this study, when considering the region of maximal linkage on Chromosome 5q31-35, only one of the 22 candidate genes, glutamate receptor, ionotropic, AMPA 1 (GRIA1) was found to have multiple SNPs showing significant association at $p < 10^{-4}$ (Middleton et al, submitted).

However, the problem of the phenotypic heterogeneity in the area of psychosis still remains to be solved and we have to face the possibility that it could even be increased in samples of the magnitude used in GWAS. It is necessary, in parallel with these large GWAS, to implement nested studies, using clinical covariates that shows high familiarity and are potentially under the control of a smaller set of genes, defining more homogeneous sub-samples. One of the areas of expertise of our team is phenotypic definition, and in this context, we intend to use phenotypic measures potentially more adequate to dissect the underlying pathologic mechanisms.

Some of the phenotypes that have received greatest attention to date are those relating to psychosis because both population-based studies and molecular genetic studies, either linkage or association studies, show evidence that SZ and BP partly share a common genetic cause. Thus, based on the assumption that we can expect substantial overlaps of genetic susceptibility across diagnostic categories and substantial heterogeneity within diagnostic categories we are now also interested in investigating some key phenotypic measures/symptom dimensions selected for their heritabilities in order to better characterize the genetic architecture of psychosis.

In the last trimester of 2011 we have obtained limited funding from the “Programa de Estímulo à Investigação” (Program to Incentive Research) from Faculty of Medicine-University of Coimbra, to develop a research project entitled “*Phenotypic Dimensions in Psychosis*” (PHEDIP/PEI-FMUC, 2011). Our aims include: 1. To contribute with 300 SZ/BD/SzA probands (from multiplex families and unrelated cases) to the GPC, which implies to assess these 300 cases (diagnostic classification and lifetime-ever occurrence of symptoms using all available clinical information) and deposit the 300 Blood/DNA samples in the FMUC repository for future studies and in the NIMH repository as part of GWA, replication, and epigenetic studies. 2. To contribute to phenotypic refinement and formulation of alternative phenotypes: symptom dimensions and subphenotypes. The project has begun last January and at the moment we are collecting phenotypic data and blood samples in the Coimbra University Hospital and we are establishing collaborations to expand the data collection to other hospitals, not only in the area of Coimbra, but also in other cities/hospitals, such as Oporto and Aveiro.

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1.2 Phenotypic Studies of Complex Disorders

In parallel with the genetic studies of schizophrenia and bipolar disorder, we have developed a range of clinical investigations in areas in which a more clear understanding of the phenotypic definitions and boundaries were needed. These studies have focused in the area of personality, namely studying the perfectionism and the relationship between this trait and psychopathology. Our correlational studies have established an association between the maladaptive aspects of perfectionism and a broad range of psychopathological conditions and health problems (e.g. sleep problems). However, the cognitive mechanisms that mediate this association are not fully understood, and the main cognitive processes and cognitions underlying perfectionist behavior and its negative emotional consequences wait for further clarification. We are now launching a new project to investigate the role of multilevel cognitive processes in the relationship between psychological distress (PD) and perfectionism in a non-clinical sample of undergraduate students and a clinical sample of depressive and anxiety disorders.

Another important area of interest in which we have developed a line of research is the study of affective disorders in the perinatal period, a topic which have been relatively neglected.

Our team have also acquired an extensive expertise in the field of psychometrics and diagnostic methodologies, developing and adapting diagnostic tools, and several scales which have been validated to be used in the above mentioned studies.

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2. Neurology Research: studies on neurodegenerative disorders

Luis Cunha, Isabel Santana (FMUC, CHUC); M^a Rosário Almeida (CNC); Inês Baldeiras, Catarina Oliveira (FMUC, CNC)

Early diagnosis of Alzheimer's Disease (AD) is one of our main areas of interest. In this context, new research criteria have been developed (Dubois et al, 2007), that include the evidence of significant episodic memory impairment, assessed by the Free and Cued Selective Reminding Test (FCSRT), associated with at least one biological footprint of the disease. Recently, we have studied the performance of Cerebrospinal Fluid (CSF) markers - amyloid β (1-42) protein (A β 42), total tau (t-tau) and phosphorylated tau protein (p-tau) - in Mild Cognitive Impairment (MCI) cases, and found that a combination of these markers could help stratify MCI patients into those with very low or high risk for future development of AD. Additionally, we have evaluated the relation between the CSF markers and the FCSRT scores in MCI and AD patients and found that A β 42 levels were significantly correlated with Total Immediate Recall scores of the FCSRT (FCSRT-IR), while the correlation for both t-tau and p-tau was inverse. The results of the FCSRT allowed us to divide the initial MCI group into stable MCI and prodromal AD (cut-off scores $\leq 2SD$, based on a control population). CSF t-tau and p-tau were similar in the prodromal AD and AD group and significantly higher than in the stable MCI group. CSF A β 42 levels were lower in the prodromal AD and AD group than in the stable MCI group. The results thus show a relation between biological markers of AD pathology and memory impairment and support early AD diagnose based on the Free and Cued Selective Reminding Test and CSF biomarkers. Although CSF biomarkers have proven to be valuable for dementia diagnosis, its collection is an invasive procedure and therefore sampling is limited. In contrast, blood is easily collected, and as approximately 500 mL of CSF are absorbed every day, plasma may be a rich source of biomarkers for screening of neurodegenerative disease. Therefore, the search for potential peripheral markers in dementia has been another of our research interests. We have an ongoing collaboration with the Cell Biology Unit of

Biocant, to study the plasma proteome of patients with AD and MCI and of healthy control individuals. Two-dimensional gel electrophoresis (2D-PAGE) coupled with mass spectrometry was used to identify differences in protein expression and protein posttranslational modifications resulting from disease. The results were analyzed using univariate and multivariate statistical and numerical techniques, and allowed the identification of a set of protein spots that are differentially expressed in plasma samples from patients with AD, MCI and controls. Validation of the results obtained, using a different experimental strategy, is currently underway and may contribute to the development of useful clinical tools in the early diagnosis of AD.

In this line of research, Transthyretin (TTR) has recently received large attention and has been suggested to bind to A β , hence avoiding its accumulation, aggregation and toxicity, being a potential marker for AD. We have, in collaboration with the Molecular Neurobiology Unit at IBMC, investigated the levels of TTR in plasma from patients with MCI, AD and non-demented controls, by radial immunodiffusion, and found that TTR is significantly decreased in the MCI and AD groups, particularly in women. Thyroxine binding to TTR in plasma was also assessed, and TTR from MCI and AD patients had a reduced capacity to carry the hormone. Finally, plasma estradiol levels in women from the MCI and AD groups was also reduced when compared to women in the control group. Thus, this study shows that plasmatic TTR decrease is a gender-dependent early event in AD and opens new perspectives on the mechanisms involved in TTR disease-modulation.

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Carvalho, C., Cardoso, S., Correia, S., Santos, R., Santos, M.S., Baldeiras, I., Oliveira, C.R., and Moreira, P.I. Metabolic Alterations Induced by Sucrose Intake and Alzheimer's Disease Promote Similar Brain Mitochondrial Abnormalities. *Diabetes*. (in press)

Ribeiro, C.A., Santana, I., Oliveira, C., Baldeiras, I., Moreira, J., Saraiva, M.J., Cardoso, I. Transthyretin decrease in plasma of MCI and AD patients: investigation of mechanisms for disease modulation. *Curr. Alz. Res.* (in press)

3. Translational Bi-Genomics and Pharmacogenomics

Head: Manuela Grazina

3.1. Biochemical genetics study in Metabolic and proliferation disorders

Manuela Grazina (FMUC, CNC), Luisa Diogo (CHUC, CNC), Catarina R. Oliveira (FMUC, CNC)

Collaborators:

Carmo Macário, Paula Garcia, Guiomar Oliveira, Paulo Moura (CHUC); Lina Carvalho (FMUC, CHUC), Filipe Silva (IBILI)

Mitochondrial respiratory chain diseases (MRCD) are a diverse group of disorders with a broad spectrum of clinical manifestations, characterised by defects in mitochondrial energetic function. The precise pathogenic mechanisms by which these biochemical abnormalities induce tissue dysfunction are not clearly understood and diagnosis of these disorders is complex, requiring specialised techniques and correlation between clinical and biochemical/ genetic data. The genetic causes of these complex disorders are located either in mtDNA or nuclear DNA, affecting the subunits of MRC system and all factors involved in mitochondrial biogenesis or mtDNA replication, transcription or stability.

The implementation of mtDNA copy number/mutation quantification by real time PCR was an important step for patients' diagnostic workup, but also for translational research projects, and represents a major advance for our centre in this area. We have gathered the results of the first 18 months of studies and compared copy number with mtDNA pathogenic mutations findings in the same sample. We have found that depletion is 4-5 fold more frequent in children than point mutations, suggesting that the screening in paediatric samples should start by copy number investigation. Furthermore, we have found that about 40% of the depletion patients have mutations in the nuclear encoded gene DGUOK, which has an important role in mtDNA replication. Additionally, depletion in heart has not been characterized in detail. Given the high number (~30) of myocardium samples in LBG from patients remaining without definitive diagnosis, we have investigated it for depletion and we have found 3 cases

with depletion in heart. These results are being gathered for publication.

A collaborative project is in progress with Dr. Fernando Scaglia and Prof. Lee-Jun Wong (Baylor College of Medicine, Houston, Texas, USA) for the study of MRCD and autism patients, for the study of complete mtDNA sequence and several nuclear genes affecting mtDNA biogenesis and maintenance. The results are being gathered for publication.

We have continued the set up of the evaluation of coenzyme Q10, Pyruvate dehydrogenase and Krebs cycle enzyme activities for diagnostic and research purposes.

A research project to evaluate the prenatal history of the cases with mtDNA mutations identified in LBG has been accomplished, representing a valuable contribution for the investigation of prenatal manifestations of MRCD. The results are being gathered for publication.

We have also accomplished a project to evaluate the role of mtDNA content as a possible biomarker in lung cancer. We have compared the results in blood and both tumour and normal tissue of the same patient. Values in blood cannot be used as a biomarker, but the mtDNA content is highly increased in tumour tissue. Additionally, normal lung tissue of active smokers' present mtDNA levels identical to tumour tissue. The results are being gathered for publication.

PUBLICATIONS

Santos, M.J., Marques, M., Pratas, J., Simões, M., Mendes, C., Diogo, L., Garcia, P., Scaglia, F., Oliveira, C.R., Wong, L.J., Grazina, M. (2011) mtDNA depletion investigation in myocardium samples. *Journal of Inherited Metabolic Disease*, 34 (S3): 236-P.

Domingues, J., Diogo, L., Grazina, M., Macario, M.C. (2011) A mitochondrial tRNA mutation lysine causing isolated severe mitochondrial myopathy. *Journal of Inherited Metabolic Disease*, 34 (S3): 544-P.

Isidoro, L., Letra, L., Matias, F., Bento, M.C., Grazina, M., Rebelo, O., Macario, M.C. (2011) MELAS syndrome in adult Neurology Department. *Journal of Inherited Metabolic Disease*, 34 (S3): 545-P.

Massano, A., Teotónio, R., Silva, F., Isidoro, L., Domingues, J., Garcia, P., Diogo, L., Grazina, M., Macario, M.C. (2011) Leigh's adult syndrome and its phenotypical variability in the adult metabolic population. *Journal of Inherited Metabolic Disease*, 34 (S3): 546-P.

Henriques, M., Diogo, L., Garcia, P., Pratas, J., Simões, M., Grazina, M. (2011) Mitochondrial DNA 8993T>G mutation in a child with OTC deficiency and Leigh syndrome- an unexpected association. *J. Child. Neurol.* (in press)

Grazina, M. Mitochondrial Respiratory Chain: Biochemical Analysis and Criterion for Deficiency in Diagnosis. In Mitochondrial disorders: Methods and Protocols. Edited by Lee-Jun C. Wong. Chap 6. *Humana Press. New York. Methods Mol. Biol.* (in press)

3.2. Bigenomic investigation in Neurodegenerative disorders

Manuela Grazina (FMUC, CNC), Isabel Santana (FMUC, CHUC, CNC), Catarina R. Oliveira FMUC, CNC)

Collaborators: Beatriz Santiago, Diana Duro (CHUC), Filipe Silva (IBILI)

Neurodegenerative disorders are complex and the mechanisms underlying the phenotypic expression of this group of diseases are not clearly understood. Finding genetic risk factors, either from nuclear or mitochondrial genome origin, will contribute to identify new tools for early diagnosis. Our aim is to search for genetic risk factors in our population and identify disease risk groups.

We have finished, in collaboration with Neurology Department of University Hospitals, a Research Project for Medical Students, concerning the evaluation of mtDNA *ND1* sequence variations in a larger sample of FTD patients, following the evidences of the involvement of MRC complex I in FTD, reported in 2004 (Grazina M, Silva F, Santana I, Santiago B, Oliveira M, Cunha L, Oliveira C. Frontotemporal dementia and mitochondrial DNA transitions. *Neurobiol. Dis.* 2004; 15-2: 306-311). Our results point to the involvement of mtDNA and MRC in FTD. The role of mtDNA needs further examination, but our results support mitochondrial cascade hypothesis in FTD etiopathogeny.

One of the most complex neurodegenerative diseases is Multiple Sclerosis, and we aimed to investigate the role of mitochondrial respiratory chain (MRC) and mtDNA genetic variations, including haplogroups, in this disease and we have found that 48% of patients have MRC deficiency correlating with haplogroup J and with the presence of mtDNA sequence variations (3 fold higher).

Additionally we have continued the genetic characterization of dementias related to 5HTR2A. Accordingly, the project of the PhD student Daniela Luís entitled "Genetic Regulation of 5HT2A receptor in Frontotemporal Dementia", assigned by FCT in 2008 (SFRH/BD/45387/2008), aiming to analyse the coding exons and the flanking intronic regions of 5HTR2A gene, in 92 samples from FTD patients was concluded. We have found 174 sequence variations, 3 of which are novel, 2 in the coding region (no aminoacid alteration) and 1 intronic (does not affect splicing), undergoing *in silico* characterization, to evaluating possible pathogenicity and selection for further functional studies.

Additionally, collaboration within CNC/UC has been started with the group of Sandra Cardoso for the analysis of mtDNA in Parkinson cybrids. The samples were extracted and sequencing of the 7 mtDNA-encoded *ND* genes has been initiated.

We have continued the genetic studies in eye disorders, namely Kjer type optic atrophy in collaboration with IBILI - FMUC and "Serviço de Oftalmologia" - CHUC.

PUBLICATIONS

Luís, D., Silva, F., Santana, I., Oliveira, C.R., Grazina, M. Investigation of 5HTR2A gene sequence in Frontotemporal Dementia. (in preparation)

3.3. Pharmacogenomics

Manuela Grazina (FMUC, CNC), Carolina Ribeiro (CHUC)

Collaborators: Ana Valentim, Ana Eufrásio, Teresa Lapa, Luís Rodrigues (CHUC), Filipe Silva (IBILI), Isabel Santana (FMUC, CHUC, CNC), Ana Raposo (FMUC), Adrián Llerena, Eva Peñas-Lledó (Univ. Extremadura)

Since 2007, we have developed several projects aiming to identify genetic variants that will contribute for either identification of susceptibility factors or to support the development of more rationale therapies, including a pharmacogenetic approach.

We have concluded a pharmacogenomic project in Alzheimer's disease, studying CYP2D6, which is involved in the oxidative metabolism of many different classes of commonly used drugs including donepezil.

The aim of this study was to investigate the association between four CYP2D6 alleles: *2, *3, *4 and *10 in a group of 96 patients with probable diagnosis of Alzheimer's disease and their clinical characteristics. Our results reveal a positive association with the age, age of onset and

depression features with alleles *4 and *10, suggesting that genetic variations previously associated to decreased CYP2D6 activity may be a protective factor on the manifestation and progression of Alzheimer's disease.

We have performed the evaluation of 40 DNA samples from women undergoing epidural after labouring, on the scope of a MSc study, for genetic analysis of CYP2D6 alleles *2, *3, *4 and *10. We have found that profiles of poor metabolizers are more associated to higher pain scores. The results are being gathered for publication.

Other projects applying pharmacogenomics approaches in pain are in progress.

PUBLICATIONS

Grazina, M., Ribeiro, C., Santana, I., Martins, P., Silva, F., Santiago, B., Peñas-Lledó, E., Llerena, A. Pharmacogenomics approach in Alzheimer's disease: genetic variation of CYP2D6 associated with susceptibility to disease onset. (*in preparation*).

Raposo, A., Valentim, A., Ribeiro, C., Silva, F., Grazina, M. CYP2D6 genetic variation in labor analgesia: pharmacogenomics approach. (*in preparation*).

4. Dermatology research

Margarida Gonçalo (CHUC), Américo Figueiredo (FMUC, CHUC), Teresa Cruz (FFUC, CNC), Bruno Neves (UA), Celeste Lopes (FFUC, CNC)

4.1. Contact dermatitis

Allergic contact dermatitis (ACD) and delayed drug eruptions are T-cell mediated skin reactions with different degrees of dermo-epidermal T cell infiltration and keratinocyte apoptosis, but pathomechanism are not fully understood, namely in which considers the sensitizing phase. As shown in previous work by our group, contact allergens directly activate intracellular signalling pathways in dendritic cells (DC) in vitro and modify the expression of adhesion and activation molecules, cytokine and chemokine receptors and the release of cytokines and chemokines that promote DC maturation and migration to induce T cell sensitization.

Epicutaneous skin tests, as those used for the diagnosis of ACD, are often positive in drug eruptions, suggesting that skin DC can also be involved in antigen presentation of these systemic drugs. It is our objective to evaluate if cultured DC suffer the same activation and maturation process in the presence of systemic drugs as they do in the presence of contact allergens. Our studies using a DC-like cell line, have shown direct DC activation by carbamazepine. It is our intention to characterize further phenotypic and functional DC modifications induced by different drugs (amoxicillin, allopurinol and oxypurinol), namely in which concerns the patterns of expression of adhesion and activation molecules, cytokine and chemokines receptors and cytokine/chemokine production.

PUBLICATIONS

Gonçalo, M., Bruynzeel, D. (2011) Patch testing in drug eruptions. *In: Contact Dermatitis. Johanssen J-D, Frosch P, Lepoittevin J-P (Eds), 5th Ed., Springer-Verlag Berlin/Heidelberg, Cap. 26., pp 475-492.*

Andrade, P., Brinca, A., Gonçalo, M. (2011) Patch testing in fixed drug eruptions. A 20 year review. *Contact Dermatitis*, 65: 195-201.

Pereira, N., Canelas, M.M., Santiago, F., Brites, M.M., Gonçalo, M. (2011) Value of patch tests in clindamycin-related drug eruptions. *Contact Dermatitis*, 65: 202-207.

Serra, D., Gonçalo, M., Mariano, A., Figueiredo, A. (2011) Pustular psoriasis and drug-induced pustulosis. *G. Ital. Dermatol. Venereol.*, 146:155-8.

Andrade, P., Gonçalves, M. (2011) Fixed Drug Eruption caused by etoricoxib – 2 cases confirmed by patch testing. *Contact Dermatitis*, 64: 118-120.

Neves, B., Gonçalves, M., Figueiredo, A., Duarte, C.B., Lopes, M.C., Cruz, M.T. (2011) Signal transduction profile of chemical sensitizers in dendritic cells: An endpoint to be included in a cell-based in vitro alternative approach to hazard identification? *Toxicol. Appl. Pharmacol.*, 250: 87-95.

5. Arthritis research

Fernando Judas (HUC, FMUC), Alexandrina Mendes (FFUC, CNC), Carlos Cavaleiro (FFUC/CEF), Ali Mobasheri (U.Nottingham,U.K.), Celeste Lopes (FFUC, CNC)

5.1. Inflammation and osteoarthritis

In collaboration with the Orthopedic and Bone Bank Departments of HUC, we are using normal and osteoarthritic (OA) human articular cartilage and chondrocytes to identify 1) cellular and molecular mechanisms relevant in OA pathogenesis that can be translated into new therapeutic strategies, and 2) compounds in essential oils with potential anti-

osteoarthritic activity. Two essential oils were found to simultaneously inhibit catabolic and inflammatory responses in human chondrocytes, which is strongly predictive of potential anti-osteoarthritic properties. Fractionation of one of those oils identified one active fraction. Further fractionation and isolation of pure compounds are underway along with pharmacological characterization to identify the active compound(s).

PUBLICATIONS

Rosa, S.C., Rufino, A.T., Judas, F., Tenreiro, C., Lopes, M.C., Mendes, A.F. (2011) Role of glucose as a modulator of anabolic and catabolic gene expression in normal and osteoarthritic human chondrocytes. *J. Cell. Biochem.*, 112: 2813-2824.

Rosa, S.C., Rufino, A.T., Judas, F., Tenreiro, C., Lopes, M.C., Mendes, A.F. (2011) Expression and function of the insulin receptor in normal and osteoarthritic human chondrocytes: modulation of anabolic gene expression, glucose transport and GLUT-1 content by insulin. *Osteoarthritis Cartilage*, 19:719-727.

Judas, F., Saavedra, M.J., Mendes, A.F., Dias, R. (2011) Cortical strut allografting in reconstructive surgery in orthopaedic surgery. *Acta Reumatol. Port.*, 36: 24-28.

5.2. Studies on rheumatoid arthritis

Margarida Souto-Carneiro (CNC), José António Pereira da Silva (CHUC, FMUC)

94 CD8⁺ T cells play a major role in destroying cells infected by virus or cytosolic bacteria. However, they comprise up to 40% of the T cells infiltrating the synovial membrane in rheumatoid arthritis (RA), and make 50% of the T cells present in the rheumatoid synovial fluid. Thus, in this project we aim at identifying the CD8⁺ T cell subsets present in the peripheral blood and synovial fluid of RA patients, and infer their

contribution to the disease according to the patients' clinical diagnosis. Peripheral blood from RA patients and healthy controls were stained for CD8⁺ T cell markers and intracellular cytokines and analyzed by flow cytometry. Whenever possible, paired collection of peripheral blood and synovial fluid was performed, the subset differences between both sample types were determined and a correlation analysis was performed.

PUBLICATIONS

Carvalho, H., Antunes, D., Duarte, C., Silva-Cardoso, S., Rodrigues-Sousa, T., Pereira da Silva, J.A., Souto-Carneiro, M.M. (2011) Characterization of CD8⁺ T cell subsets in the Synovial fluid and Peripheral blood of rheumatoid Arthritis patients. *Ann. Rheum. Dis.*, 70(Suppl 2): A46.

Abreu, M., Carvalho, H., Duarte, C., Pereira da Silva, J.A., Souto-Carneiro, M., Rodrigues-Santos, P. (2011) Expression of CD62L, CD27 AND CD11B define a marked Cytotoxic effector role for NK cells in Rheumatoid Arthritis. *Ann. Rheum. Dis.* 70(Suppl 2): A55.

Moura, R.A., Cascão, R., Perpétuo, I., Canhão, H., Vieira-Sousa, E., Mourão, A.F., Rodrigues, A.M, Polido-Pereira, J., Pereira da Silva, J.A., Rosário, H.S., Souto-Carneiro, M.M., Graça, L., Fonseca, J.E. (2011) Spondyloarthritis and Rheumatoid Arthritis: Different clinical manifestations, similar cytokine network. *Ann. Rheum. Dis.* 70(Suppl 2): A82.

6. Research in brain cancer

Alberto Orfão (CSIC, University Salamanca), Maria Dolores Taberero (University Hospital, Salamanca), Hermínio Tão (CHUC), Olinda Rebelo (CHUC), Marcos Barbosa (FMUC, CHUC), Celeste Lopes (FFUC, CNC)

6.1. Genetic heterogeneity of gliomas

Gliomas are tumors derived from glial cells of brain and they account for more than 70% of all neoplasms of the central nervous system and vary considerably in morphology, localization, genetic alterations and response to therapy.

The project entitled “Genetic Heterogeneity in Gliomas: correlation with clinical and biological features of the disease” is being developed in collaboration with Neuropathology Laboratory and Neurosurgery Service of the University Hospital of Coimbra and with Center for Cancer Research of Salamanca. In this project, we first analysed the incidence of numerical/structural abnormalities of chromosomes in a group of 90 human gliomas by using interphase fluorescence *in situ* hybridization (iFISH). Overall, iFISH analysis revealed complex and heterogeneous cytogenetic profiles in this type of tumors with distinct pathways of clonal evolution being detected, which were associated with both the histopathological subtype and the grade of the tumor.

In a second step, the gene expression profiles (GEP) of

tumor cells were analysed in a subset of 40 tumors using cDNA oligonucleotide microarrays, in order to assess the potential impact of individual chromosomal changes and cytogenetic profiles in the tumors-associated patterns of gene expression. The results of this study demonstrated a clear association between the GEP of gliomas and tumor histopathology, and the most discriminating genes between low- and high-grade being genes involved in the regulation of cell proliferation, apoptosis, DNA repair and signal transduction. Regarding the cell signalling transduction pathways, our results performed in glioma cell lines indicate that the activation of PI3K/Akt and MAP kinase signaling pathways contribute to the chemoresistance that characterizes glioma cells.

Presently, high-density (500K) single-nucleotide polymorphism array is being performed to investigate genome-wide copy number (CN) alterations in glioblastoma multiforme (GBM) samples. We have shown that combining both genomic and transcriptional data to differentiate genes with concordant CN alterations and expression patterns is crucial to disclose which of those genes may have functional relevance in GBM pathogenesis.

PUBLICATIONS

Crespo, I., Vital, A.L., Nieto, A.B., Rebelo, O., Tão, H., Lopes, M.C., Oliveira, C.R., French, P.J., Orfao, A., Taberero, M.D. (2011) Detailed characterization of alterations of chromosomes 7, 9 and 10 in glioblastomas as assessed by single-nucleotide polymorphism arrays. *J. Mol. Diagn.*, 13: 634-647.

Carmo, A., Carvalheiro, H., Crespo, I., Nunes, I., Lopes, M.C. (2011) Effect of Temozolomide on the U-118 glioma cell. *Oncol. Lett.*, 2:1165-1170.

Carmo, A., Crespo, I., Lopes, M.C. (2011) Genetics and Biology of Glioblastoma Multiforme. In: *Molecular Targets of CNS Tumors*, cap 13, pp. 289-314.

Matarraz, S., González-González, M., Jara, M., Orfao, A., Fuentes, M. (2011) New technologies in cancer. Protein microarrays for biomarker discovery. *Clin. Transl. Oncol.*, 13(3):156-61.

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7. Yeast nosocomial infections

M. Santos-Rosa (FMUC), Ana Luísa Costa (FMUC, Dentistry Department), Alice Mirante (CHUC), João Maló de Abreu (FMUC, Dentistry Department) Teresa Gonçalves (FMUC, CNC)

7.1. Oral yeast carriage in type I diabetic children

This collaboration, under the leadership of Faculty of Medicine (FMUC), aimed to characterize the yeast species of normal and type I diabetic children, together with the yeast load in each individual. In this study, undertaken from 2009 until 2011, the Medical Mycology Yeast Research Group was responsible for the identification of the yeast isolates obtained from saliva and mucosal specimens from 200 patients. In these specimens it was quantified the yeast load, using a CFU based methodology.

The identification procedures used were molecular biology based.

Yeasts were recovered from stimulated saliva and mucosal surface swabs of 133 diabetic children and 72 control subjects. Diabetic children were grouped according to HbA1c.

The main conclusions of this study was that T1D children had higher levels of CD4+T-cells in their saliva than control subjects. The higher level of CD4+ cells in the saliva of these patients was correlated with a lower passive colonization by yeast cells.

PUBLICATIONS

Costa, A.L., Silva, B., Soares, R., Mota, D., Alves, V., Mirante, A., Ramos, J.C., Maló de Abreu, J., Santos Rosa, M., Gonçalves, T. (2011) Impact of salivary CD4+ T-cells in the oral yeast colonization of T1D children. *Submitted*

7.2. HIV-1 Vpr variants and disease progression. Using a yeast model to predict AIDS progression

Graça Rocha (CHUC, FMUC), A. Meliço-Silvestre (CHUC, FMUC), António Vieira (CHUC), Andrea Spiegel (CHUC), Cindy Rodrigues (CNC), Rui Soares (CNC), Teresa Gonçalves (FMUC, CNC)

The biological functions of HIV-1 Vpr have been involved in the replication and pathogenesis of the virus. Infants with perinatal acquired HIV-1 infection have widely variable courses, the long-term non-progressors and fast progressors. Part of this collaboration is an ongoing work aimed to study the correlation, in a population of perinatal infected children, between the Vpr variant present and disease progression. In this population

The analysis of Vpr sequences in 31 patients showed that 11 carried the mutation R77Q. At the time of first medical appointment the children infected with the Vpr variant carrying the mutation showed lower viral load than children with no mutation. During the period considered (2005-2011) these children remained with no clinical signs of disease and with no need of aggressive therapy.

During 2011 a protocol of collaboration was signed between CHC and FMUC/CNC in order to make a similar study in an adult population.

We believe that with this study it will be possible to identify Vpr not only as a bio-marker of disease progression, but also as therapeutic efficiency flag and a potential novel therapeutic target. It is expected to construct a model to predict HIV virulence based on the effect of Vpr variants on mitochondrial dysfunction.

8. Novel Techniques for the Diagnosis and treatment of Human Infertility

Teresa Almeida Santos (CHUC, FMUC), Ana Paula Sousa (CHUC, CNC), Alexandra Amaral (CNC), Renata Tavares (CNC), Marta Baptista (CNC), Raquel Brito (CHUC), J. F. Velez de la Calle (Clinique Pasteur, Brest, France), Helena Figueiredo (Gaia Hospital, Portugal), Vasco Almeida (University of Oporto, Portugal), João Ramalho-Santos (CNC, FCTUC)

Infertility is a growing problem, affection about 15% of couples worldwide. A partnership has been established between CNC and the Assisted Reproduction Laboratory of the University Hospitals of Coimbra (HUC) to develop novel assays to monitor human sperm and oocyte quality with the ultimate goal of improving Assisted Reproduction.

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For sperm analysis the focus has been on complementing traditional analysis by including new parameters with a higher predictive value in terms of defining proper sperm function. These parameters include sperm viability, sperm mitochondrial activity, and sperm chromatin status, monitored using simple, easy and quick assays that can be implemented clinically with minimal effort. The collaboration has recently been extended to two other Portuguese labs (University of Oporto and Gaia Hospital)

and one in France (Clinique Pasteur, Brest) for a multi-center evaluation and validation of procedures. Papers describing a novel methodology to assess sperm chromatin routinely, and how to correctly determine sperm mitochondrial function and use it to select the best sperm have been published (below).

In terms of oocyte evaluation novel non-invasive techniques are being pioneered to select the best oocytes (and, ultimately, the best embryos) to be used in Assisted Reproduction.

In addition, the collaboration also involves improving the cryo-banking and subsequent use of ovarian tissue for patients undergoing chemotherapy, as this type of treatment often leads to female infertility.

PUBLICATIONS

Sousa AP, Amaral A, Baptista M, Tavares RS, Caballero-Campo P, Caballero-Peregrín P, Freitas A, Paiva A, Almeida-Santos T, Ramalho-Santos J. (2011) Not all sperm are equal: Functional mitochondria characterize a subpopulation of human sperm with better fertilization potential. *PLoS ONE*, 6(3):e18112.

9. Research in Neurodegenerative Diseases: Prevalence of the proven pathogenic *LRRK2* mutations in Parkinson disease patients

Maria do Rosário Almeida (CNC), Ana Morgadinho, Fradique Moreira, Cristina Januário(CHUC, FMUC)

Parkinson disease (PD) is the second most common neurodegenerative disorder affecting approximately 3% of the population over age 65. In most instances, PD manifest in sporadic form, but mutations in 6 genes (*SNCA*, *LRRK2*, *PRKN*, *DJ1*, *PINK1*, and *ATP13A2*) have conclusively been shown to cause familial parkinsonism and were found in about 10% of cases diagnosed as PD. Mutations in *LRRK2*, the gene that encodes leucine-rich repeat kinase 2 (*LRRK2*), are associated with autosomal dominant and sporadic forms of PD and are the most common genetic causes of PD. There is currently convincing evidence to suggest that 6 mutations are disease causing (R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T). Of all the mutations in *LRRK2*, Gly2019Ser has the highest frequency, which varies among ethnic groups and consequently should influence

diagnostic genetic testing. This mutation occurs in 6.1% of PD Portuguese patients, 15% to 20% of Ashkenazi Jewish patients, and approximately 40% of North African Arabs with PD. The aim of this study was to determine the contribution of these six known pathogenic mutations in a cohort of PD patients seen at the Movement Disorders Clinics of the Neurology Department of University Hospital of Coimbra with a positive family history (one or more relatives with the disease) and/or age at onset above 50 years. Exon 31 (Arg1441Cys, Arg1441Gly, Arg1441His), exon 35 (Y1699C) and exon 41 (Gly2019Ser, Ile2020Thr) were PCR amplified and subsequently direct sequenced to the mutation frequency evaluation.

9.1. Research in Vision-related Disorders: Evaluation of *Optineurin* gene mutations in unrelated microphthalmia, anophthalmia and coloboma (MAC) spectrum cases

Maria do Rosário Almeida (CNC), Isabel Carreira (FMUC, CNC), Joana Barbosa (FMUC), Eduardo Silva (CHUC,FMUC)

Microphthalmia, anophthalmia and coloboma (MAC) are major structural eye malformations and are responsible for around 25% of severe visual impairment in childhood, with microphthalmia reported in up to 11% of blind children. Anophthalmia is characterized by the complete absence of the eye, microphthalmia is characterized by a small eye while the coloboma results from a failure in the closure of the optic fissure. MAC is observed in 1–3 per 10,000 births and is often associated with systemic anomalies such as: developmental delay, kidney or heart defects and cleft lip and palate. MAC may occur isolated or as part of a syndrome. Mutations in several different transcription factors have been implicated in MAC, *CHX10*, *GDF6*, *RAX*, *SOX2* and *OTX2*. They have been recognized in dominant or

recessive forms of the disease and the two most commonly mutated genes in these monogenic forms are the *SOX2* and *OTX2*. Therefore, the aim of this project was to identify the genetic contribution of *OTX2* gene in MAC patients to allow their classification into distinct subtypes based on the specific ocular and/or other systemic features associated with specific genetic causes. Genotype–phenotype correlations, if identified, can be used to target genetic testing and also to guide the diagnosis and management of patients with MAC. The coding exons 1-3 of *OTX2* (GenBank accession number NM_172337.1) were amplified by PCR using primers located in flanking intronic sequences and subsequently direct sequenced.

Internationalization



Internationalization has been a permanent concern of the CNC strategy. To attain this goal the researchers have been encouraged to establish collaborations and joint projects with laboratories abroad, and to collaborate in the organization of international scientific meetings. A third action line of the Internationalization strategy is the Graduate Studies Programme which is described in the next section of this report.

Projects jointly with laboratories abroad

Neurosciences and Disease

A function for MeCP2 in synapse remodelling. Chinfei Chen (Harvard Medical School, Boston, MA, USA), Ana Luisa Carvalho (CNC, Portugal).

Alteration of hippocampal synaptic function and plasticity in models of Alzheimer's disease. Christophe Mulle (CNRS, Univ. Bordeaux2, France), Rodrigo Cunha (CNC, Portugal).

Caloric restriction increases lifespan: role of neuropeptide Y on autophagy regulation. Tamas Horvath (Section of Comparative Medicine, Yale School of Medicine PO Box 208016, New Haven, USA), Claudia Cavadas (CNC, Portugal).

Changes in the ubiquitin-proteasome system in brain ischemia. Lorella M.T. Canzoniero (University of Sannio, Italy), Carlos Duarte (CNC, Portugal).

Characterization of adenosine neuromodulation in the development of hippocampal circuits. Christophe Bernard (INSERM U 751CNRS; Univ. Méditerranée, France), Rodrigo Cunha (CNC, Portugal).

Control by ATP P2X receptors of NMDA receptor. Juan Lerma, Neuroscience Inst., Alicante, Spain), Rodrigo Cunha (CNC, Portugal).

Effect of the Contactin/Caspr complex on AMPA receptor-mediated excitatory postsynaptic currents in hippocampal neurons in culture. Christophe Mulle (University of Bordeaux, Bordeaux, France), Ana Luisa Carvalho (CNC, Portugal).

Hypoxic preconditioning as a trigger of neurovascular protection in Alzheimer's disease and diabetes: role of HIF signalling pathway and mitochondria. Joseph LaManna and Gemma Casadesus (Institute of Pathology, Case Western Reserve University, USA), Paula Moreira (CNC, Portugal).

Identification of new ubiquitinated proteins at the synapse. Manuel S. Rodríguez (CIC-BioGUNE. Parque Tecnológico de Vizcaya, Derio, Spain), Ka Wan Li (Center for Neurogenomics and Cognitive Research, University Amsterdam, The Netherlands), Carlos Duarte (CNC, Portugal).

Impact of aging and gender in diabetic brain metabolism, oxidative stress and inflammation: Is there a (protective) role for insulin therapy? Patrik Brundin, Jia-Yi Li, Maria Björkqvist (Wallenberg Neuroscience Center, Section for Neuronal Survival Department of Physiological Sciences, University of Lund, Swede); Ana Cristina Rego (CNC, Portugal).

Interaction between A_{2A} and BDNF. Patrizia Popoli (Institut Sanità, Rome, Italy), Rodrigo Cunha (CNC, Portugal).

Interaction between cannabinoid CB1 receptor and A_{2A} receptors in the control of striatal glutamatergic transmission. Laurent Venance (Collège de France), Rodrigo Cunha (CNC, Portugal).

Interactions between the purinergic and the endocannabinoid system in pain sensation. László Köles (Semmelweis University, Budapest, Hungary), Rodrigo Cunha (CNC, Portugal).

Joint project in epileptogenesis (University of Coimbra, CNPq). Esper Cavalheiro (Univ. São Paulo), João Malva (CNC, Portugal).

Localization and function of dopamine D4 receptors – interaction with adenosine A_{2A} receptors. Sergi Ferré (NIDA, NIH, Bethesda, USA), Rodrigo Cunha (CNC, Portugal).

Localization and role of adenosine receptors in amygdalar circuits. Ki Ann Goosens (MIT, Boston, USA), Rodrigo Cunha (CNC, Portugal).

Mapping the metabolic and neuromodulator role of insulin in the hippocampus. Tibor Harkany (Univ. Aberdeen, Scotland), Rodrigo Cunha (CNC, Portugal).

Mechanisms involved in the ability of caffeine to prevent memory impairment. Diogo Souza (UFRGS, Brazil), Rodrigo Cunha (CNC, Portugal).

Mechanism of adenosine A_{2A} receptors in the control of neurodegeneration. Jiang Fan Chen (Boston Univ., USA), Rodrigo Cunha (CNC, Portugal).

Mitochondrial axonal transport deficits in a transgenic mice model of Alzheimer's disease. Jorge Busciglio (School of Biological Sciences, University of California, Irvine, USA), Claudia Pereira (CNC, Portugal).

Modulation of the glutamatergic synapses by BDNF. Clive Bramham (University of Bergen, Norway), Carlos Duarte (CNC, Portugal).

Neuroinflammation in the neurodegeneration. Marina Lynch (Trinity College Dublin, Ireland), Rodrigo Cunha (CNC, Portugal).

Neurophysiological role of the dopamine-adenosine systems in attention-deficit hyperactivity disorder: a new therapeutic target for caffeine. Francisco Ciruela (Univ. Barcelona, Spain), Rodrigo Cunha (CNC, Portugal).

Novel agonists of leptin's receptor as therapeutical approaches for Alzheimer's disease. Laszlo Otvos (Department of Biology, Temple University, Philadelphia, USA), Claudia Pereira (CNC, Portugal).

P2 receptor-mediated control of neurodegeneration. Geanne M. Andrade (Fac. Medicine, Federal Univ. Ceará, Brazil), Rodrigo Cunha (CNC, Portugal).

PGC-1 α role in mitochondrial function and its contribution to AD neurodegeneration. Russel H Swerdlow (Kansas University, USA), Sandra Morais Cardoso (CNC, Portugal).

Proneurogenic effect of AMPAkinases in SVZ neural stem cell cultures. Fernando Mello and Ricardo Reis (Univ. Federal do Rio de Janeiro, Brasil), João Malva (CNC, Portugal).

Protein cleavage in the ischemic rat brain. Takaomi C. Saido (Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, Wako, Saitama), Tadeusz Wieloch (Wallenberg Neuroscience Center, Lund Sweden), Carlos Duarte (CNC, Portugal).

100 *Quantitative proteomic analysis of postsynaptic densities from GluN2B knockout neurons.* Ka Wan Li (Center for Neurogenomics and Cognitive Research, Faculty of Earth and Life Sciences, VU University Amsterdam, The Netherlands), Ana Luisa Carvalho (CNC, Portugal).

Regulation of glutamatergic transmission by ghrelin in the hippocampus. José Esteban (Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid/CSIC, Madrid, Spain), Ana Luisa Carvalho (CNC, Portugal).

Role of adenosine in depression. Jean-Marie Vaugeois (Fac. Pharmacy, Univ. Lyon 1, France), Rodrigo Cunha (CNC, Portugal).

Role of adenosine receptors in the control of mood disorders. Detlev Boison (Legacy Foundation, Portland, USA), Rodrigo Cunha (CNC, Portugal).

Role of astrocytic adenosine A_{2A} receptors in the control of neurodegeneration in animal models of Parkinson's disease. Michael Schwarzschild (MGH, Harvard Univ., Boston, USA), Rodrigo Cunha (CNC, Portugal).

Role of calpains in excitotoxic neuronal damage. Ben A. Bahr (University of Connecticut, Storrs, USA), Carlos Duarte (CNC, Portugal).

Role of calpains in neural stem cell migration. Alan F. Horwitz (University of Virginia, Charlottesville, VA, USA), Patrik Brundin (Lund University, Lund, Sweden), Claudia Cavadas (CNC, Portugal).

Role of diffusible and contact factors from endothelial cells in stemness and neurogenesis. Florence Hofman (Univ. of South California, Los Angeles), João Malva (CNC, Portugal).

Role of endothelial BDNF in migration of neuroblasts into the cerebellum and ischemic striatum. Armen Sagatelyan (Univ. Laval, Québec City, Canada), João Malva (CNC, Portugal).

Role of the JNK/c-Jun pathway on excitotoxic cell death. Michael Courtney (Molecular Signalling Laboratory, Department of Neurobiology, A. I. Virtanen Institute, University of Kuopio, Finland), Armanda Santos (CNC, Portugal).

Role of N-Methyl-D-Aspartate receptor subunits on endoplasmic reticulum stress induced by amyloid beta oligomers. William L. Klein (Cognitive Neurology and Alzheimer's Disease Center, Northwestern University Institute for Neuroscience, Northwestern University, Evanston, IL, USA), Claudia Pereira (CNC, Portugal).

Role of nitric oxide in adult neurogenesis. Patrik Brundin (Lund University, Lund, Sweden), Claudia Cavadas (CNC, Portugal).

Role of stargazin in homeostatic synaptic plasticity. Chinfai Chen (Harvard Medical School, Boston, MA, USA), Ana Luisa Carvalho (CNC, Portugal).

Structure-function analysis of the NMDA receptor domains involved in synaptic delivery under basal conditions and during synaptic plasticity. Ann Marie Craig (Brain Research Centre, University of British Columbia, Vancouver, BC, Canada), Ana Luisa Carvalho (CNC, Portugal).

The neuronal ischemic response through Ca²⁺-permeable AMPA receptors: genetic expression profile and mechanisms of receptor trafficking. Luís Miguel Martins (Cell Death Regulation Laboratory, MRC Toxicology Unit, Leicester LE1 9HN, UK), Armanda Santos (CNC, Portugal).

The Neuropeptide Y (NPY) and Dipeptidyl-peptidase IV (DPP-IV) as new promising targets on the adipose tissue regulation in obesity. Eric Grouzmann (Division of Clinical Pharmacology and Toxicology, Lausanne University Medical School, Switzerland), Claudia Cavadas (CNC, Portugal).

The pathological interaction between diabetes and Alzheimer's disease: exploring the role of brain endothelial mitochondria and uncoupling proteins. George Perry (College of Sciences, University of Texas at San Antonio, USA), Paula Moreira (CNC, Portugal).

The role of mRNA local translation in presynaptogenesis. Samie R. Jaffrey (Weill Cornell Medical College, New York, USA), Noo Li Jeon (WCU Multiscale Mechanical Design, Seoul National University, Seoul, Korea), Ana Luisa Carvalho (CNC, Portugal).

The role of OPA1 proteolytic processing in mitochondrial fission/fusion and mitophagy in Alzheimer's disease. Xiongwei Zhu (Institute of Pathology, Case Western Reserve University, USA), Paula Moreira (CNC, Portugal).

Trafficking of mRNAs in hippocampal neurons. Enrico Tongiorgi (BRAIN Centre for Neuroscience, University of Trieste, Italy), Carlos Duarte (CNC, Portugal).

Toxic pathways triggered by activation of Ca²⁺-permeable AMPA receptors. Lloyd Greene (Dept. of Pathology, Columbia University Medical Center, New York, USA), Jonathan Ham (Institute of Child Health, University College of London, London, UK), Armanda Santos (CNC, Portugal).

Unraveling Sirtuin 3 role in mitochondrial dynamics: implications in Parkinson's disease. Marcia Haigis (Harvard Medical School, USA), Sandra Morais Cardoso (CNC, Portugal).

Biotechnology and Health

AAV vectors-mediated gene therapy. Sebastian Kugler (Department of Neurology, Faculty of Medicine, University of Göttingen, Göttingen, Germany); Luis Pereira de Almeida (CNC, Portugal).

A biophysical approach to the role of lipids in hepatic mitochondrial toxicity. Teresa Pinheiro (Department of Biological Sciences, University of Warwick, UK); M^ª Amália Jurado (CNC, Portugal).

Advancing the field of drug delivery – combined targeted treatments against human breast cancer and human leukemia (The OncotargetNanoMed network). María Jesús Vicent (Centro de Investigación Príncipe Felipe, Medicinal Chemistry Unit, Polymer Therapeutics Laboratory, Valencia, Spain), Simo Schwarz Jr (CIBBIM-Nanomedicine Drug Delivery and Targeting, Vall d'Hebron Institut de Recerca, Barcelona, Spain), João Nuno-Moreira (CNC, Portugal).

Analysis of the hippocampal proteome in mice exposed to psychotropic medication. Mike Dunn and David Cotter (UCD Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland), Carlos Faro (CNC, Portugal).

Antimicrobial coatings. Andreas Zumbuehl (Department of organic Chemistry, University of Geneva, Switzerland), Cristiana Paulo (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Application of non-viral suicide gene therapy approaches in animal models for cancer and mechanisms associated with the antitumor response. Valérie Pierrefite-Carle (Unity INSERM, Faculty of Medicine, Nice, France); Conceição Pedroso Lima (CNC, Portugal).

Biological applications of new formulations based on synthetic polymers, in the context of gene therapies. Bo Nyström (Department of Chemistry, University of Oslo, Norway), M^a Amália Jurado (CNC, Portugal).

Cell internalization mechanisms of anti-HIV peptides. Abraham Loyer (Department of Biological Chemistry, Institute of Life Sciences, Hebrew University of Jerusalem, Israel); Conceição Pedroso Lima (CNC, Portugal).

Cardiac kit. Christine Mummery (University of Leiden, Netherlands), Pedro Gouveia (CNC, Portugal), Ricardo Neves (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Cardiac regeneration. Jeffrey Karp (Harvard-MIT Division of Health Science and Technology, USA), Ivana Kostic (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Cell reprogramming. Tariq Enver (University College of London, UK), Carlos Boto (CNC, Portugal), Ana Lima (CNC, Portugal), Lino Ferreira (CNC, Portugal), Ricardo Neves (CNC, Portugal).

Design principles of biochemical circuits, mathematical methods for systems analysis of biochemical networks. Michael Savageau (U.C. Davis, U.S.A.), Armindo Salvador (CNC, Portugal).

Design of chitosan-based particles as adjuvant for mucosal Hepatitis B vaccine. Gerrit Borchard (University of Genève, Switzerland and Centre Pharmapeptides, Archamps, France), Hans Junginger (Former Professor at Leiden University, Netherlands and visiting Professor at Naresuan University, Phitsanulok, Thailand), Olga Borges (CNC, Portugal).

Development of a method for profiling mitotic-cycle-dependent metabolism without having to synchronize cells. Elmar Heinze (University of Saarland, Germany), Armindo Salvador (CNC, Portugal).

Development of a tissue engineered intestine. Jeffrey Karp (Harvard-MIT Division of Health Science and Technology, USA), Patrícia Pereira (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Development of lipid-based nucleic acid delivery systems for application in gene therapy. Nejat Duzgunes (University of the Pacific, San Francisco, USA); Conceição Pedroso Lima (CNC, Portugal).

Development of non-viral vectors for siRNA delivery to the central nervous system. Ernst Wagner (Department of Pharmacy, University of Munich, Germany); Conceição Pedroso Lima (CNC, Portugal).

Dissecting the pathogenesis of Machado-Joseph disease. Henry Paulson (University of Michigan, Ann Harbor, USA); Luis Pereira de Almeida (CNC, Portugal).

Encapsulation of viral vectors into targeted nanolipid-based carriers: evaluation of therapeutic activity in animal models of ischemia. Mauro Giacca (Laboratory of Molecular Medicine, ICGEB - International Centre for Genetic Engineering and Biotechnology, Trieste, Italy); Sergio Simões (CNC, Portugal).

Gecko-inspired tissue adhesives. Robert Langer (Department of Chemical Engineering, Massachusetts Institute of Technology, MIT, EUA), Jeffrey Karp (Harvard-MIT Division of Health Science and Technology, USA), Maria Pereira (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Lentiviral vectors-mediated ataxin-3 gene silencing. Nicole Déglon & Philippe Hantraye (Service Hospitalier Frederic Joliot, MIRcen Program, Departement de Recherches Medicales, Direction des Sciences du Vivant, Commissariat a l'Energie Atomique (CEA), Orsay, France); Luis P. Almeida (CNC, Portugal).

Lipid-based therapeutic strategies for cancer diseases. Faustino Mollinedo (Center for Cancer Research, Institute of Molecular and Cell Biology of Cancer, CSIC - University of Salamanca, Spain), M^a Amália Jurado (CNC, Portugal).

Lipid-protein interactions addressed by a number of biophysical techniques and model systems. Teresa Pinheiro (School of Life Sciences, University of Warwick, Coventry, United Kingdom), M^a Amália Jurado (CNC, Portugal).

Lipoplex- and cell penetrating peptide-based delivery of steric-block oligonucleotides and application in splice correction. Bernard Lebleu (University of Montpellier, Montpellier, France); Conceição P. Lima (CNC, Portugal).

Methods and software for kinetic modeling, factors shaping proteins' aminoacid usage. Rui Alves (University of Lleida, Spain), Armindo Salvador (CNC, Portugal).

Models of Machado-Joseph disease. Veronica Colomer, John Hopkins (School of Medicine, Baltimore, USA); Luis P. Almeida (CNC, Portugal).

Nanomaterials for cell tracking. John Martin (Centre for Cardiovascular Biology and Medicine, University College of London, UK), Renata Gomes (CNC, Portugal), Jorge Ruivo (UCL, Portugal), Lino Ferreira (CNC, Portugal).

Retroviral-like membrane-associated aspartic proteinases from Rickettsiae: potential new therapeutic targets for rickettsioses. Alexander Wlodawer (Macromolecular Crystallography Laboratory, NCI-Frederick, USA), Juan J. Martinez (The University of Chicago, Department of Microbiology, Chicago, USA), Carlos Faro (CNC, Portugal).

Silencing Machado-Joseph disease e Autophagy in Machado-Joseph disease. Arnulf Koeppen (University of Michigan, Albany, USA); Conceição P. Lima (CNC, Portugal).

Three-dimensional matrices for cell culture and transplantation. Robert Langer (Department of Chemical Engineering, Massachusetts Institute of Technology, MIT, EUA), Ali Khademhosseini (Harvard-MIT Division of Health Science and Technology, USA), Helena Vazão (CNC, Portugal), Sezin Aday (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Ultrastructural and biophysical studies of the interaction of cell penetrating peptides with cellular membranes. Margus Pooga, (Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia); Conceição P. Lima (CNC, Portugal).

Toxicology

Anticancer Effects of Phytochemicals. Jon Holy (U. Minnesota, USA), Paulo Oliveira (CNC, Portugal).

Apoptosis Signaling in Melanoma. Faustino Mollinedo (CSIC, Spain), Paulo Oliveira (CNC, Portugal).

Development of microsensors for nitric oxide measurement in tissues. Greg Gerhardt (Dept. Anatomy and Neurobiology, and Center for Microelectrode Technology (CenMet) University of Kentucky, Lexington, Kentucky, USA) , João Laranjinha (CNC, Portugal).

Diet Modulation During Pregnancy and Mitochondrial Function. Mark Nijland (U. Texas, USA), Paulo Oliveira (CNC, Portugal).

Doxorubicin-induced Mitochondrionopathy. Kendall Wallace (U. Minnesota, USA), Paulo Oliveira (CNC, Portugal).

Evaluation of Mitochondrial Toxicity of Silver and Gold Nanoparticles. Saber Hussain (Wright State Univ., USA), Carlos Palmeira (CNC, Portugal).

FXR receptor: a target to prevent systemic metabolic disease. Jan Kopecky (Academy of Sciences, Czech Republic), Anabela Pinto Rolo, Carlos Palmeira (CNC, Portugal).

Immunolocalization of nNOS in the brain and the correlation with nitric oxide dynamics. Nadezda Lukacova (Institute of Neurobiology, Centrum of Excellence, Slovak Academy of Sciences, Košice, Slovak Republic), João Laranjinha (CNC, Portugal).

Mesenchymal Stem Cells as Anti-Cancer Weapons. Teresa Rose-Hellekant (Univ. Minnesota, USA), Vilma Sardão (CNC, Portugal).

Metabolic checkpoints: cellular bioenergetics and cellular responses to stress. Nika Danial (Dana-Farber Cancer Institute, USA), Anabela Pinto Rolo, Carlos Palmeira (CNC, Portugal).

Mitochondrial Biogenesis and Metabolic Regulation. David Sinclair (Harvard Medical School, USA), Sirtuins, Anabela Pinto Rolo, Carlos Palmeira (CNC, Portugal).

Mitochondrial Dynamics and Disease. Luca Scorrano (Univ. Padova, Italy), Anabela Pinto Rolo, Carlos Palmeira (CNC, Portugal).

Mitochondrial role in metabolic diseases. Piero Portincasa (U. Bari, Italy), Paulo Oliveira (CNC, Portugal).

Mitochondrial-sirtuins in the context of exercise. Jose Vina (U. Valencia, Spain), Paulo Oliveira (CNC, Portugal).

Mitochondrial Tolerance and Liver Ischemic Preconditioning. Joan Rosseló (CSIC, Spain), Anabela Pinto Rolo, Carlos Palmeira (CNC, Portugal).

New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Rafael Radi (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay), Homero Rubbo (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay), Jon O. Lundberg (Department of Physiology and Pharmacology, Karolinska Institutet, Sweden), João Laranjinha (CNC, Portugal).

Nitric oxide in neurodegeneration and aging. Enrique Cadenas (Dept. Pharmaceutical Sciences, University of Southern California, USA), João Laranjinha (CNC, Portugal).

p66Shc/oxidative stress and hyperglycaemia induced myoblast apoptosis. Mariusz Wieckowski (Nemki Institute, Poland), Paulo Oliveira (CNC, Portugal).

Phytoestrogens and Blood-brain Barrier. Anika Hartz, Bjorn Bauer (University of Minnesota, USA), Vilma Sardão (CNC, Portugal).

Polyphenols and vascular cells redox signaling. Anne Nègre-Salvayre (INSERM-U, Institut Louis Bugnard CHU Rangueil, Toulouse, France), João Laranjinha (CNC, Portugal).

Prevention of inflammatory processes in the gastrointestinal epithelia by dietary flavonoids. Juan Sastre (Faculty of Pharmacy, University of Valencia, Spain), João Laranjinha (CNC, Portugal).

Redox modulation of autophagy processes. Ana Coto-Montes (U. Oviedo, Spain), Ignacio Vega-Naredo (CNC, Portugal).

Role of Mitochondrial TRAP-1 on Carcinogenesis. Patricia Scott (U. Minnesota, USA), Paulo Oliveira (CNC, Portugal).

SIRT3 and drug-induced cardiac mitochondrial toxicity. Yvonne Will (Pfizer R&D, USA), Michael Sack (NHLBI, USA), Paulo Oliveira (CNC, Portugal).

Transgenic mice for nNOS and the impact in neurovascular coupling. Eduardo Weruaga (Departamento de Biología Celular y Patología, Instituto de Neurociencias de Castilla y León, Universidade de Salamanca, Spain), João Laranjinha (CNC, Portugal).

Microbiology

Cloning, expression and regulation of genes for the synthesis of compatible solutes in Thermus thermophilus. José Berenguer (Universidad Autónoma de Madrid, Spain), Milton Costa (CNC, Portugal).

Extremophilic enzymes. Garo Antranikian (Institute of Technical Microbiology, Hamburg University of Technology, Hamburg, Germany), Milton Costa (CNC, Portugal).

Gamma radiation-resistant bacteria: taxonomy, diversity and physiology. Fred Rainey (Louisiana State University, Baton Rouge LA, USA), Milton Costa (CNC, Portugal).

Legionella genetics and modulation of host cell biology. Yousek Abu Kawaik (Department of Microbiology and Immunology, University of Louisville Medical Center, Louisville, USA), Joana Costa (CNC, Portugal).

Mediterranean deep-sea brines biodiversity. Michail M. Yakimov (Consiglio Nazionale delle Ricerche - Istituto per l'Ambiente Marino Costiero (CNR-IAMC), Messina, Sicilia, Italy), Milton Costa (CNC, Portugal).

NMR

Agentes de Imagem Multimodal para detecção de placas A-beta amiloide na doença de Alzheimer. Eva Tóth (CBM, CNRS, Orleans, França), Carlos Geraldes (CNC, Portugal).

Metal-based systems for Molecular Imaging applications and TD1004. Theragnostics Imaging and Therapy: An Action to Develop Novel nanosized systems for Imaging-guided drug delivery. EU COST actions, D38, Carlos Geraldes (CNC, Portugal).

Cell Biology

Assessment of genetic heterogeneity in gliomas. Alberto Orfão (Center for Cancer Investigation, University of Salamanca, Spain), M^a Celeste Lopes (CNC, Portugal).

Biologia Celular e tráfico membranar. Victor W. Hsu (Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and Harvard Medical School), M^a Otília Vieira (CNC, Portugal).

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Espectrometria de massa e "lipidomics". Andrej Shevchenko (Max-Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany), M^a Otília Vieira (CNC, Portugal).

Chromosomal, genetic and immunophenotypic characterization of brain tumors. Maria Dolores Tabernero Redondo (University Hospital, Salamanca, Spain), M^a Celeste Lopes (CNC, Portugal).

CD38 and immune regulation. Fran Lund (Rochester University), M^a Celeste Lopes (CNC, Portugal).

CD38 and immune responses against Mycobacterium tuberculosis. Andrea Cooper (Trudeau Institute, Saranac Lake, USA), M^a Celeste Lopes (CNC, Portugal).

Desenho e síntese de compostos químicos. Alfin Vaz, Pharmacokinetics (Dynamics & Metabolism, Pfizer Global Research and Development, Groton, U.S.A), M^a Otília Vieira (CNC, Portugal).

Immunosuppressive therapy and insulin resistance. Jan Eriksson (Gothenburg University), Eugenia Carvalho (CNC, Portugal)

Implications of Claspin mutations in DNA replication, cell cycle checkpoints and oncogenesis. Raimundo Freire (University Hospital of Canarias, Tenerife, Spain), M^a Celeste Lopes (CNC, Portugal).

Imunologia. Michael B. Brenner (Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and

Harvard Medical School), M^a Otilia Vieira (CNC, Portugal).

Inflammation and the adipocyte and PTP1b in wound healing. Janice Zabolotny (Harvard Medical School), Eugenia Carvalho (CNC, Portugal)

Mechanisms of chondrocyte resistance to hyperglycemia: modulation of ATP-dependent K⁺ channels and causes of failure in osteoarthritis. Ali Mobasheri (School of Veterinary Science and Medicine, University of Nottingham, England), M^a Celeste Lopes (CNC, Portugal).

Metabolic activity and viability of chondrocytes in cryopreserved human osteochondral allografts. Ali Mobasheri (School of Veterinary Science and Medicine, University of Nottingham, England), M^a Celeste Lopes (CNC, Portugal).

Microbiologia e Mycobacterium tuberculosis. Heinz G. Remold (Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and Harvard Medical School), M^a Otilia Vieira (CNC, Portugal).

Microscopia electrónica. Paul Verkade (Schools of Biochemistry, and Physiology and Pharmacology, Medical Sciences, University of Bristol, University Walk, United Kingdom), M^a Otilia Vieira (CNC, Portugal).

Modulation of the chondrogenic potential of adipose tissue derived mesenchymal stem cells. Francisco Blanco (CIBER-BBN, Centro de Investigación Biomédica, Centro Hospitalario Universitario A Coruña, Spain), M^a Celeste Lopes (CNC, Portugal).

The role of neuropeptides in wound healing. Aristides Veves (Harvard Medical School), Eugenia Carvalho (CNC, Portugal).

Mitochondria and metabolism in pluripotent embryonic and induced stem cells. Gerald Schatten (University of Pittsburgh, USA), Miguel Ramalho-Santos (University of California, San Francisco, USA), João Ramalho-Santos (CNC, Portugal).

Study of the cytokine release profile, by protein arrays, of dendritic cells. Carmen García-Rodríguez (Institute of Biology and Molecular Genetic. CSIC-University of Valladolid, Spain), M^a Celeste Lopes (CNC, Portugal).

Testicular organization, testicular stem cell biology and xenotransplanting of testicular tissue in felines. Stefan Schlatt (University of Muenster, Germany), João Ramalho-Santos (CNC, Portugal).

Participation in the organization of scientific meetings

January, 2011

Reproduction & Pluripotency - PDBEB Advanced course

Dates: January 10-14, 2011, Coimbra

CNC Members involved in the organization: João Ramalho-Santos

Principles and practice in Drug Development - PDBEB/MIT-Portugal program Advanced course

Dates: January 17- 28, 2011, Coimbra

CNC Members involved in the organization: João Nuno Moreira, Luís Pereira de Almeida, Sérgio Simões

Adult Stem Cells – from basal biology to cell replacement therapies - PDBEB Advanced course

Dates: January 31-February 4, 2011, Coimbra

CNC Members involved in the organization: Cláudia Cavadas and Inês Araújo.

February, 2011

Oncobiology - PDBEB Advanced course

Dates: February 21-25, 2011, Coimbra

CNC Members involved in the organization: João Nuno Moreira

March, 2011

Metabolic Remodeling - PDBEB Advanced course

Dates: 7-11 March 2011, Coimbra

CNC Members involved in the organization: John Jones

31st European Winter Conference of Brain Research - Caffeine control of brain function – role of adenosine receptors

Dates: March 12-19, 2011, Les Deux Alpes, França,

CNC Members involved in the organization: Rodrigo Cunha

April, 2011

6th International Meeting of the Portuguese Society of Stem Cells and Cell Therapy

Dates: April 27-29, 2011, Biocant, Cantanhede

CNC Members involved in the organization: Inês Araújo, Cláudia Cavadas, Lino Ferreira

5th Annual Meeting on Cell Signalling (SINAL)

Dates: April 28-30, 2011, Coimbra

CNC Members involved in the organization: Ana Luisa Carvalho, Carlos B. Duarte

May, 2011

XII International Congress on Molecular Systems Biology (ICMSB2011)

Dates: 8-12 May 2011, Lleida (Spain)

CNC Members involved in the organization: Armino alvador

XII Meeting of the Portuguese Society for Neuroscience - Functional Neurosciences Symposium

Dates: May 26-28, 2011, Lisboa

CNC Members involved in the organization: Rodrigo Cunha

July, 2011

2011 FENS/IBRO Summer School: Metabolic Aspects of Chronic Brain Diseases- FENS-IBRO European Neuroscience Schools Programme

Dates: July 20-26, 2011, Reisenburg Castle, Günzburg, Germany

CNC Members involved in the organization: Ana Cristina Rego

August, 2011

Annual congress of EUROMAR

Dates: 21-25 August 2011, Ireland

CNC Members involved in the organization: Carlos F. Geraldés

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October, 2011

Excitotoxic Signaling - PhD Programme BEB (PDBEB) and European Neuroscience Campus (ENC) Network

Dates: October 19-21, 2011, Coimbra

CNC Members involved in the organization: Armanda Emanuela Santos, Carlos Bandeira Duarte, Ana Cristina Rego

Neurodegenerative diseases: From molecules to clinics and beyond - PhD Programme BEB (PDBEB) and European Neuroscience Campus (ENC) Network

Dates: October 24-28, 2011, Coimbra

CNC Members involved in the organization: Ana Cristina Rego, Paula Agostinho, Cláudia Pereira, Inês Araújo, Luís Pereira de Almeida

Regenerative Medicine – PhD Program on Health Sciences, FMUC

Dates: October 24-28, 2011, Coimbra

CNC Members involved in the organization: João O. Malva

November, 2011

10th short course of the Portuguese Biophysical Society - Nanosciences for Life

Dates: November 17-19, 2011, Santarém

CNC Members involved in the organization: Armindo Salvador

December, 2011

The Complex World of RNA: RNA Regulation in Neuronal Development, Function and Dysfunction - PDBEB Advanced course

Dates: December 9-10, 2011, Coimbra

CNC Members involved in the organization: Ramiro de Almeida, Ana Luísa Carvalho, Luís Pereira de Almeida, Carlos B. Duarte

Graduate Studies Programme



The Center has continued with its PhD Program in Experimental Biology and Biomedicine (PDBEB), funding for which was renewed by FCT until 2013. The Program includes 12 scholarships for students selected by CNC and funding to organize advanced courses taught by CNC and foreign faculty. Previous efforts to include other graduate students have continued, and in 2010/2011 courses were attended by an average of 24 students, including students enrolled in advanced PhD and Masters Programs at the Faculties of Medicine, Pharmacy and Science & Technology, as well as some students from other Portuguese Institutes. Despite this increase in attendance the focus has continued to be on quality teaching and career mentoring to ensure the best possible results in terms of ongoing student projects. CNC researchers have also taught courses in other Masters and PhD Programs at the University of Coimbra, with which the CNC collaborates either formally or informally. Results of the PDBEB Program have been outstanding in terms of track record, with students publishing in top journals in 2010/11, including first authorship manuscripts in both Nature Neuroscience and Current Biology. In 2010 joined the European Neuroscience Campus (ENC), together with top institutions in Amsterdam (The Netherlands), Bordeaux (France), Gottingen (Germany) and Zurich (Switzerland). The ENC provides 10 scholarships plus training funds, and each student must work in at least two of the partner institutes. ENC students that choose the CNC will use the PDBEB training framework already in place. For this purpose 5 advanced courses on Neuroscience were organized.

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In 2010/11 CNC was also involved in the organization of the advanced courses of neurosciences and drug development, which are run in the frame of the MIT-Portugal agreement.

Advanced Courses

JANUARY 2011

Pluripotency & Reproduction

January 10 - 14

João Ramalho-Santos

MIT- Drug Development

January 17 - 28

João Nuno Moreira (Optional Course for BEB students, open to Other Programs)

Adult Stem Cells: from basic biology to cell replacement therapies

January 31 - February 4

Inês Araújo

FEBRUARY 2011

Metabolic Remodeling

February 7 - 11

Rui Carvalho

Mitochondrial Dynamics and Metabolic Diseases

February 14 - 18

Anabela Rolo

Oncobiology

February 21 - 25

João Nuno Moreira

MAY 2011

Biostatistics

May 23 - 26

Pedro Oliveira (ICBAS)

SEPTEMBER 2011

CNC Cores (Mass Spectrometry, Flow Cytometry and Microscopy)

September 20 - 23

Bruno Manadas, Isabel Nunes Correia, Luísa Cortes

Introduction to Neurosciences

September 26 - 30

Core CNC Neuroscience Courses, Offered to the European Neuroscience Campus (ENC)

Cláudia Cavadas, Inês Araújo

OCTOBER 2011

Molecular Neurosciences (from synapse structure to function)

October 3 - 7

Core CNC Neuroscience Courses, Offered to the European Neuroscience Campus (ENC)

Ana Luisa Carvalho, Carlos Duarte, Ramiro Almeida, Emília Duarte

System Neurosciences + Optogenetics

October 10 - 14

Core CNC Neuroscience Courses, Offered to the European Neuroscience Campus (ENC)

Rodrigo Cunha, Ramiro Almeida, Henrique Silva

Excitotoxic Signaling

October 19 - 21

Core CNC Neuroscience Courses, Offered to the European Neuroscience Campus (ENC)
Armanda Santos, Carlos Duarte, Ana Cristina Rego

Neurodegenerative disorders: From molecules to clinics and beyond

October 24 - 28

Core CNC Neuroscience Courses, Offered to the European Neuroscience Campus (ENC)
Ana Cristina Rego, Paula Agostinho, Inês Araújo, Cláudia Pereira, Luís Pereira de Almeida

Integrating Biology – The “Omics” World

October 31 - November 4

Isabel Marques Carreira , Joana Barbosa de Melo

NOVEMBER 2011**Immunology**

November 7 - 11

Margarida Carneiro

Molecular Systems Biology

November 14 - 18

Armindo Salvador

Lab Rotations

November 21 - December 2

DECEMBER 2011**Neuroendocrinology & Neuromodulation**

December 5 - 9

Paula Moreira, Ana Duarte, Rosa Resende

Microbiology

December 12 - 16

Milton Costa, Nuno Empadinhas

CNC Meeting

December 19 - 20

Seminars

2011 Series | CNC Auditorium, 16:00h

JANUARY

14.1.2011

Ca²⁺ stores and store-operated channels and regulation of motility in human sperm

Stephen Publicover
University of Birmingham, UK

17.1.2011

Decoding olfactory perception in mice

Jean-Francois Cloutier, Ph.D.
Associate Professor Dept. Neurology & Neurosurgery
Canada Research Chair in Developmental Neurobiology
Montreal Neurological Institute
McGill University
Montreal, Quebec
Canada

25.1.2011

Microengineered hydrogels for stem cell bioengineering and tissue regeneration

Ali Khadhemosseini
Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School,
Cambridge,
Harvard-MIT, USA

27.1.2011

Unravelling gene function by high-throughput screening using genome-wide siRNA and microRNA libraries

Miguel Mano
International Centre for Genetic Engineering and Biotechnology (ICGEB)
Trieste, Italy

28.1.2011

MicroRNA-mediated gene silencing: from mechanisms to function

Ana Eulálio
International Centre for Genetic Engineering and Biotechnology (ICGEB)
Trieste, Italy

28.1.2011

MicroRNA-mediated gene silencing: from mechanisms to function

Ana Eulálio
International Centre for Genetic Engineering and Biotechnology (ICGEB)
Trieste, Italy

13.1.2011

Mitochondria in Alzheimers Disease

Russel H Swerdlow
University of Kansas School of Medicine,
Departments of Neurology and Molecular and Integrative Physiology,
Kansas City, Kansas, USA

FEBRUARY

3.2.2011

Peripheral nervous system stem cells as a treatment for Parkinson disease

Ricardo Pardal
Instituto de Biomedicina de Sevilla,
Depto. de Fisiología Médica e Biofísica,
Universidad de Sevilla

4.2.2011

Stem cell for drug discovery and cell therapy: bioprocess challenges

Paula Marques Alves
Animal Cell Technology Unit,
ITQB-UNL/IBET, Lisboa

10.2.2011

Metabolic epigenetics and the progression of cancer

Frederik Doman
Department of Radiation Oncology,
Holden Comprehensive Cancer Center,
The University of Iowa, Iowa City, USA

17.2.2011

Exploring the role of mitochondrial dynamics in T cells : movement, AICD, and..

Silvia Campello
Department of Cell Physiology and Metabolism,
University of Geneva, Switzerland

25.2.2011

Breast Cancer Stem Cells: its importance in cancer biology

Joana Paredes
Institute of Molecular Pathology and Immunology
University of Porto

7.2.2011

Regulation of the Gating Mechanisms in Potassium Channels by RNA Editing

Miguel Holmgren
National Institute of Neurological Disorders and Stroke,
National Institutes of Health, USA

14.2.2011

Subcellular structures and signaling pathways involved in the mechanism of action of antitumor ether lipids

Faustino Molido
Centro de Investigación del Cáncer,
Instituto de Biología Molecular y Celular del Cáncer, C.S.I.C.,
Universidad de Salamanca, Spain

17.2.2011

Müller cells in identity crisis: to be or not to be a dopaminergic neuron

Fernando Garcia de Mello
Laboratório de Neuroquímica e de Biologia da Retina
Instituto de Biofísica Carlos Chagas Filho
Universidade Federal do Rio de Janeiro
Brasil

25.2.2011

Tumor Microenvironment Controls the Rate of Cancer Progression and Metastasis

Raghu Kalluri
Harvard Medical School and Beth
Israel Deaconess Medical Center

MARCH

11.3.2011

Probing Synaptic and Circuitry Mechanisms of Psychiatric Disorders

Guoping Feng
McGovern Institute Poitras,
Department of Brain and Cognitive Sciences,
MIT, Cambridge, USA

4.3.2011

Are developmental genes co-opted in cancer? The unfolding story of the mammary developmental gene

Teresa Rose-Hellekant
University of Minnesota Medical School
Duluth, MN, USA

MAY

6.5.2011

The Role of PPAR γ in pancreatic beta-cells

Hannah Welters
Peninsula College of Medicine and Dentistry,
Universities of Exeter and Plymouth,
UK

OCTOBER

26.10.2011

Cell transplantation in Parkinson s disease: from clinical trials to benchside

Mariah Lelos
Brain Repair Group,
School of Biosciences,
Cardiff University, UK

17.10.2011

Optogenetics

10h30m – 12h: Foundations of optogenetics – current possibilities and hurdles
14h30m – 16h: Optogenetic exploitation of attention circuitry in the prefrontal cortex

Huib Mansvelder
Professor of Neurophysiology at VU University Amsterdam
Director of Biomedical Sciences Education at VU University Amsterdam
Head department of Integrative Neurophysiology at VU University Amsterdam

20.10.2011

Delineation of Neurodegenerative Signalling Pathways

Michael Courtney
Dept. of Neurobiology
A. I. Virtanen Institute,
University of Kuopio, Kuopio, Finland

NOVEMBER

4.11.2011

Personalized health; studies and methods

Jildau Bouwman
TNO Quality of Life,
the Netherlands

11.11.2011

Keeping mitochondria in shape: a matter of life and death

Luca Scorrano

Dept. of Cell Physiology and Metabolism

University of Geneva Medical School

Genève - Switzerland

11.11.2011

The relay molecules in mammalian autophagy: Ambra1 and the Beclin/Vps34 complex?

Francesco Cecconi

Dulbecco Telethon Institute, Department of Biology

University of Rome Tor Vergata

Rome - Italy

18.11.2011

Stochastic and ultrasensitivity in bacterial networks

Oleg Igoshin

Rice University,

Houston, TX, USA

DECEMBER

9.12.2011

Neurobiology of IGF-1 in health and disease

Ignacio Torres-Aleman

Cajal Institute and CIBERNED,

Madrid, Spain

14.12.2011

Bacteria: the good the bad and the ugly

Eliora Ron

Tel Aviv University

Israel

13.12.2011

Biological imaging in the visible and in the near infrared with luminescent polymetallic lanthanide complexes: real-time oxygen sensing and in vivo tumour detection

Stéphane Petoud

CNRS, Orleans,

France

PhD thesis concluded in 2011

Alexandra Isabel Freitas Rosa

Role of intercellular communication between endothelial and stem cells in stemness and neurogenesis.

18-05-2011

Supervisor: João Malva

Ana Burgeiro

Apoptosis Signaling as a Therapeutic in Melanoma.

14-04-2011

Supervisor: Paulo Oliveira

Co-Supervisor: Maria S. Santos

Ana Fortuna

Pharmacometric analysis of carbamazepine derivatives: in vitro, in vivo and in silico evaluation of pharmacological properties.

04-10-2011

Supervisor: Carlos Duarte

Ana Francisca Soares

Profiling the control of hepatic glucose and lipid metabolism for evaluating novel strategies of insulin delivery.

30-06-2011

Supervisor: Rui A. Carvalho,

Co-Supervisors: John G. Jones and Francisco J. Veiga

Ana Luisa Vital Castanheira de Carvalho

Assessment of Intratumoral Genetic Heterogeneity in Gliomas: as assessed by interphase FISH and cDNA micro-arrays: impact on the clinical and biological behaviour of the disease.

29-06-2011

Supervisors: Alberto Orfão and M. Celeste Lopes

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Ana Paula Marques de Sousa

Functional characterization of the human sperm: Implications for fertility.

06-04-2011

Supervisor: João Ramalho-Santos

Ana Raquel Esteves

Mitochondrial dysfunction towards protein aggregation in Parkinson disease: contribution of cytoskeletal disorganization.

12-01-2011

Supervisor: Sandra Cardoso

Ana Raquel Santos Calhãa Mano Soares

Zebrafish small RNAs: unraveling expression and function of novel microRNAs and tRNAs in a vertebrate model.

16-09-2011

Supervisors: Manuel Santos and M. Celeste Lopes

Ana Rita Araújo dos Santos

Regulation of the proteome by brain-derived neurotrophic factor in hippocampal neurons: protein synthesis vs protein degradation.

24-01-2011

Supervisor: Carlos Duarte

Andrea Catarina Amaro de Campos Lobo

VGLUT1 and VGLUT2 cleavage under excitotoxic conditions and in cerebral ischemia.

19-05-2011

Supervisor: Ana L. Carvalho and Carlos Duarte

Bruno Pereira Carreira

Proliferative mechanisms controlled by nitric oxide in neural stem cells.

6-07-2011

Supervisors: Inês Araújo and Caetana Carvalho

Bruno Miguel Neves

Modulação das Células Dendríticas por Estímulos Alergênicos e Infeciosos.

11-01-2011

Supervisors: M. Teresa Cruz and M. Celeste Lopes

Camile Woitiski

Oral delivery of bioactive insulin through design and development of a polymeric nanoparticulate carrier.

08-07-2011

Supervisor: Francisco J. Veiga,

Co-Supervisor: Rui A. Carvalho

Carla Sofia Gomes da Silva

Effect of purines in the developing hippocampus: consequences for the establishment of circuits related to learning and memory.

13-12-2011

Supervisor: Rodrigo A. Cunha

Cátia Filipa Lourenço Marques.

In vivo nitric oxide concentration dynamics induced by glutamatergic neuronal activation in rat brain and its role in neurovascular coupling.

14-11-2011

Supervisor: João Laranjinha

Hugo João Marques Prazeres

Molecular and functional changes in familial thyroid cancer.

27-01-2011

Supervisors: Teresa Martins and Paula Soares

Igor Clemente Tiago

Microbial Diversity in a Low Saline Alkaline Environment. The heterotrophic aerobic populations, the uncultivable populations and the autotrophic carbon fixation.

24-03-2011

Supervisor: António Veríssimo

Isabel Ferreira

Gene transfer approaches for Machado-Joseph Disease: Targeting the autophagy pathway.

26-07-2011

Supervisor: Luis Pereira de Almeida

João Carlos Rodrigues Gomes

VGAT and TrkB cleavage under excitotoxic conditions and in vivo cerebral ischemia: functional implications.

10-02-2011

Supervisor: Carlos Duarte

Ligia Maria de Sousa Ferreira

Terapia génica para o NPY, a nível central e periférico, como estratégia para regulação do peso corporal e do apetite.

14-07-2011

Supervisors: Cláudia Cavadas and Luís Pereira de Almeida

Marco Alves

Mitochondrial function and cell death in cardioplegic preserved hearts: effects of Ischemia and Ischemia-reperfusion.

12-01-2011

Supervisors: Rui A. Carvalho and Paulo J. Oliveira

Paula Cristina Cardoso Ramos Mota

The domestic cat as a model for endangered felids: From isolated mitochondria as a screening tool, to testis tissue xenografting for fertility recovery.

03-02-2011

Supervisor: João Ramalho-Santos

Raquel Margarida da Silva Ferreira

Contribution of microglia to neural inflammation: neuropeptide Y modulates interleukin-1beta-induced microglia activation.

28-06-2011

Supervisor: João Malva

Ricardo Miguel Oliveira dos Santos

Nitric oxide production, diffusion and inactivation in the rat brain: In vivo studies using microelectrodes.

04-11-2011

Supervisor: João Laranjinha

Rita Catarina Mendes dos Santos

Síntese e avaliação da citotoxicidade de novos compostos triterpénicos pentacíclicos do tipo lupano.

14-05-2011

Supervisor: Jorge António Ribeiro Salvador

Rui O. Costa

Endoplasmic reticulum stress during amyloid β peptide-induced cell death: role of mitochondria and glutamatergic N-methyl-D-aspartate receptors.

06-12-2011

Supervisor: Claudia Pereira

Sara Maria David Trabulo

Development of new approaches based on the S4(13)-PV cell penetrating peptide to improve the intracellular delivery of nucleic acids aiming at anti-tumor therapy.

14-01-2011

Supervisor: M^a. Conceição Pedroso Lima

Susana Carvalho Rosa

Regulação das funções dos condrócitos articulares humanos por estímulos catabólicos e anabólicos: implicações no desenvolvimento e progressão da osteoartrite.

19-01- 2011

Supervisors: Alexandrina F. Mendes and M. Celeste Lopes

Tatiana Catarino

Regulation of Synapse Composition by Protein Acetylation: the Role of Acetylated Cortactin.

December 2011

Supervisor: Ana L. Carvalho

Teresa Serafim

Mitochondria and Cancer: Opening Pandora's Box.

26-12-2011

Supervisor: Paulo Oliveira

Co-Supervisor: Maria Paula Marques

Vitor Gonçalo Silva e Costa Mendes

New insights into the biosynthesis of the Mycobacterial Methylglucose Lipopolysaccharide.

09-12-2011

Supervisor: Milton Costa

Co-Supervisor: Nuno Empadinhas

Master thesis

Ana Isabel Reis Santos

Stimulation of neural stem cell proliferation by nitric oxide is dependent on S-nitrosylation of p21Ras.

09-09-2011

Supervisors: Inês Araújo and Caetana Carvalho

Ana Rita Leal

Production and characterization of recombinant shewasin D, a pepsin homologue from "Shewanelladenitrificans". Evidences for its in vivo expression.

14-07-2011

Supervisor: Isaura Simões

Ana Sofia Lourenço

Regulation of mitochondrial biogenesis by nitric oxide during neural stem cell proliferation.

04-09-2011

Supervisors: Inês Araújo and Caetana Carvalho.

Diana Isabel Queirós Guedes Rodrigues

Synaptic localization of the amyloid precursor protein in the rat hippocampus

September 2011

Supervisor: Paula M. Agostinho

Co-Supervisor: Rodrigo A. Cunha

Dominique Fernandes

Post-transcriptional mechanisms of regulation of AMPA receptors: Regulation of GluA1 expression by the Contactin associated protein 1.

September 2011

Supervisor: Ana Luisa Carvalho

Fábia Vicente

Role of A_{2A} adenosine receptors on catecholamine release from mouse adrenal gland using a perfusion system.

14-09-2011

Supervisor: Cláudia Cavadas

Filipe Alberto Marques Teixeira

Presynaptic CB1 cannabinoid receptors control serotonin release in the rodent frontal cortex

July, 2011

Supervisor: Attila Köfalvi

Francisco Manuel Queiroz Gonçalves

ATP - Mediated Neurotransmission and Purinergic Receptors Relevance

July, 2011

Supervisor: Ângelo R. Tomé

Inês Castanheira da Costa

Título: Effect of caffeine consumption on the evolution of sarcoidosis

July, 2011

Supervisor: Carlos Robalo Cordeiro

Co-Supervisor: Rodrigo A. Cunha

Jorge Filipe da Conceição Pascoal

Autophagy in hypothalamic cells: role of neuropeptide Y.

03 -12-2011

Supervisor: Cláudia Cavadas

Liliana Freitas Antunes

Heterologous overexpression and purification of the membrane retroviral-like aspartic proteinase homologue from *Rickettsia conorii*.

18-07-2011

Supervisor: Isaura Simões

Luana Carvalho Naia

Role of insulin and IGF-1 in energy metabolism in Huntington's disease: Studies in cortical and striatal neurons from YAC128 transgenic mice and human lymphoblasts

13-01-2011

Supervisor: Ana Cristina Rego

Luís Leitão

Role of β -actin local translation in axonal outgrowth.

September 2011

Supervisor: Ana Luisa Carvalho

Maria João Rodrigues Ferreira Ribeiro

Creation and characterization of mitochondrial DNA-depleted human Huntington's disease and control derived lymphoblasts.

13-01-2011

Supervisor: Ana Cristina Rego

Marta Maria Vieira Matutino Falcão Estrada

The role of gliptins on adipogenesis.

02-11-2011

Supervisors: Cláudia Cavadas and Joana Rosmaninho-Salgado

Olga Iuliano

Contactin associated protein 1 (Caspr1) as a regulator of AMPA receptors.

January 2011

Supervisor: Ana Luisa Carvalho

Pedro Rio

The GluN2B subunit of NMDA receptors and its role in glutamatergic synapses: Towards the characterization of the proteome of postsynaptic densities from wild-type and GluN2B-null cultured neurons.

September 2011

Supervisor: Ana Luisa Carvalho

Pedro Tiago Cardoso Curto

Production of Phytase(s) in *Chlamydomonas reinhardtii*: a proof of concept study.

14-07-2011

Supervisor: Isaura Simões

Rui Benfeitas

The physiological role of peroxiredoxin 2 in human erythrocytes: a kinetic analysis.

Supervisor: Armindo Salvador

Sofia Isabel Oliveira Sousa

Excitotoxicidade em células granulares do cerebelo que expressam ataxina-3 mutante – relevância para a patogénese da doença de Machado-Joseph.

12-09-2011

Supervisor: Ana Cristina Rego

Tânia Perestelo,

Retrograde-induced cell death: role of proneurotrophins.

September 2011

Supervisor: Ramiro Almeida

Tiago Jorge Bento Cardoso

Neuroprotection mediated by P2 receptors of NMDA-dependent toxicity in hippocampal slices.

September 2011

Supervisor: Ângelo R. Tomé

Vanessa Mendes Machado

Modulation of neural stem cell proliferation and migration by calpains.

02-07-2011

Supervisors: Inês Araújo and Caetana Carvalho.

Outreach Programme



The Outreach Programme developed by CNC, and coordinated by the investigador Teresa Girão da Cruz, offers opportunities to develop partnerships with schools and to extend our scientific resources to the community. The programme is designed to engage students in their science studies and potential careers related to the life sciences, and to broaden the public's access to science. The dissemination of scientific information equally contributes to the appreciation of the research activity performed at the CNC. The creation of a Science Communication Office by the CNC is the outcome of the successful outreach programme developed in the past years and the recognition of the importance of an appropriate communication strategy.

The Outreach Programme developed by CNC under the coordination of the Science Communication Office offers opportunities to develop partnerships with schools and to extend our scientific resources to the community. The programme is designed to engage students in their science studies and potential careers related to the life sciences, and to broaden the public's access to science. The dissemination of scientific information equally contributes to the appreciation of the research activity performed at the CNC. Our outreach efforts have the enthusiastic involvement of the Center's research staff, graduate and undergraduate students.

The Center yearly participates in various activities exclusively planned to the lay public, namely during the Brain Awareness Week, Science and Technology Week, and European Researchers Night. Elementary to high school students are also a committed public of all CNC's outreach actions. CNC intensively collaborates with the Ciência Viva Agency, the Portuguese Society for Neuroscience, the Science Museum (University of Coimbra), and Exploratório (Centro Ciência Viva, Coimbra) for the organization of science communication actions.

Some of our outreach activities are also carried out through the "Instituto de Educação e Cidadania" (IEC, Mamarrosa), a non-profit institution, dedicated to education and to promoting science and knowledge in schools, and among the rural populations in underprivileged areas. The IEC is housed in a modern building, provided with modern equipment, and includes classrooms and laboratories for students and teachers. The IEC has established protocols with several schools, and the CNC channels some of its outreach activities through IEC and the schools it is linked to.

The Science Communication Office is also in charge of liaising with the media, providing the necessary information for the communication of important achievements by CNC researchers. Our research activity has been recognized through numerous media articles and broadcasts (over 150 in 2011), and important awards – namely the L'Oréal Prize for Women in Science, the Bluepharma Award, the Seeds of Science Award, the MIT Award for Innovation in Education, the best research project in Aging and Brain Dementia-Alzheimer's disease and "Artigo Destaque" award, both by the Portuguese Society for Neuroscience.

Brain Awareness Week, March 13-19

In Portugal, BAW 2011 focused on the theme "Art and the Brain". Initiatives were intended both for the general public: 1) a conference and music concert, 2) the exhibition "Painting the brain", including works by students on; and for the students: 3) "Neuroscientists go to Schools", where neuroscientists visited schools in the region and gave lectures on brain related subjects to high school students; elementary and middle school students performed hands on activities related to the brain awareness week subject, and 4) "Open Laboratories" where students visited CNC's laboratories and took part in talks about neuroscience research.

"Science in the Holidays" Programme, July 11-22

Portuguese high-school students participated in a 10 day programme during Summer Holidays, promoted by Ciência Viva Agency. Students were tutored by CNC researchers

and were included in different research groups. They had the opportunity to run several molecular/cell biology techniques as part of short projects, adding to visits to facilities and laboratories. The end results were presented publicly at CNC and published at the Ciência Viva web site.

European Researchers' Night, September 23

Together with the Science Museum of the University of Coimbra, CNC took part for the third time in the organization of the activities of the European Researchers' Night. This initiative is promoted by the European Commission in order to bring the public closer to the researchers in a non-scientific environment. CNC researchers organized experiments and demonstrations for the public under the theme "Coloured Science", participated in public interviews, and took part in the "speed-dating" event.

Science and Technology Week, November 21-27

During the Science and Technology week and the National Day for Scientific Culture CNC traditionally organizes activities in order to promote the direct contact with the public. This year the activities were mainly intended for high-school students and the general public. CNC researchers organized conferences and visits to the laboratories on the several open days (five). The major goal of these activities is to contribute to the public understanding of the science being carried out in Portugal, of the subjects of research, and of the results obtained.

CNC at the Instituto de Educação e Cidadania (IEC)

The activities of the IEC carried out in 2011 with the participation of researchers from the CNC were of various types: 1) Lectures for the general public at IEC; 2) Lectures at schools; 3) Experimental Science Courses for high school students and teachers, as indicated below:

- 1) Lectures for the general public at IEC:
The status of the Education (May 6)
What is diabetes (March 5)
What is obesity (March 12)
Evaluation of health condition (September 12)
VIH-SIDA (October 1)
Human infertility (October 22)
The human papillomavirus (November 5)
- 2) Lectures at schools:
Genetic therapy (February 23)
The Brain; Brain repair (March 16)
Chronic diseases (March 9)
Brain and drugs (April 16)
In vitro fertilization (April 13)
Stem cells (November 16)
- 3) Experimental science courses for high school students and teachers (each course lasts 10 weeks, three hours per week and accepts 10 high school students and 2 teachers):
From gene to protein (October to December)
Microbiology (October to December)

Technology Transfer



Translational research and technology transfer have been progressively developed in CNC leading to a promising interaction with Industry and local authorities. The outcome of this interaction was the participation of CNC as a founding member of ABAP (Association involving seven Municipal Councils of the Center Region of Portugal aiming at knowledge based development). The main contribution of CNC for that goal was the creation of a technology transfer unit, Biocant, in coloboration with Cantanhede Municipal Council. This unit became the anchor of Biocant Park a Biotechnology Park that is rapidly growing by atracting new Biotechnology companies.



1. BIOCANT

Biocant is a private, non-profit, innovation centre created by CNCB together with the municipality of Cantanhede for technology transfer in biotechnology. Founded 3 years ago, Biocant has grown to become a reference in the field and the catalyst of Biocant Park, the first Portuguese biotechnology park.

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Biocant is organized into seven main functional units with highly qualified teams: Genomics, Cellular Biology, Molecular Biotechnology, Microbiology, Bioinformatics, System Biology, Tissue Engineering, and Advanced Services. Biocant provides services and R&D activities based on post-genomic platforms such as whole-genome sequencing, DNA chips, proteomics, interactomics and metabolomics.

Several research projects are currently in progress, some in collaboration with national or international research institutions, hospitals and companies.

2. Companies operating in Biocant Park

At the present 20 companies operate in Biocant Park: AP-Bio, Biocant Ventures, Biotrend, Converde/CEV, Crioestaminal, Equigerminial, Hittag Biotecnology, Interactome, GeneBox, GenePrediT, GeneLab, Matera, Vetdiagnos, 4Health, Bioalvo, Cell2B, Hematos, Lab. Vidaurre, NMT and Treat U . Along with Biocant they form a biotech cluster of excellence, bringing together over 100 researchers, in a unique enabling environment. Linking basic and applied research more closely to successful innovation, Biocant paved the way for a new paradigm of economic development in the Center Region of Portugal.

Core Facilities



CNC research Core Facilities: Animal House, Flow Cytometry Unit, Microscopy Unit, Mass Spectrometry Unit and NMR Spectroscopy Unit.

ANIMAL HOUSE

Head of Unit: Alexandre Pires | Graduate in Agricultural Engineering and Animal Production, Msc in Laboratory Animal Science

Head of Facility since 2006

Staff: Carmen Semião (caretaker), Fátima Graça (assistant technician); Maria Eugénia Campos (assistant technician)

The Animal House is a shared resource that provides services in laboratory animal experimentation and husbandry, for all CNC and FMUC scientists using animals in their research.

The present facility has a capacity to house about 3000 animals (rats/mice). This facility offers the following services: complete husbandry, including feeding, watering, daily cage changing, as well as routine procurement, inventory and care. In 2007, the facility started to provide specialized animal services, namely: breeding and housing of transgenic/knockout strains of mice as well as wild type colonies, production of rats/mice embryos and litters and maintenance of athymic nude mice.

The Animal House contains a barrier maintained facility, with 8 positive pressurised rooms, which are kept at 22°C with a relative humidity of 55%. The rodents are breed in individually ventilated cages and a 12-hour light-dark cycle is maintained with an automatic timer. The facility has an animal identification system and software to monitor animal records.



Animal Room – IVC cages (type II)



Laminar flow chamber

FLOW CYTOMETRY UNIT

Head of Unit: Isabel Nunes Correia | PhD in Biochemistry Technology (2007) at University of Coimbra
Head of Facility since 2007

The Flow cytometry Unit provides technical support on flow cytometry both to CNC and external researchers. Currently, it is equipped with a FACSCalibur cell analyser and a separate computer and software to enable researchers to fully analyse their flow cytometry data. For researchers wishing to use flow cytometry in their studies, the unit provides assistance in planning projects, choosing fluorochromes, analyzing experimental results and presenting data.

The Unit organizes annual flow cytometry seminars with the purpose to initiate new users and make this powerful technology known to all researchers, endeavouring to deepen CNC research. Even though the unit has started to operate recently, several CNC research groups are already taking advantage of this facility, performing apoptose, receptor expression and siRNAs intracellular delivery studies, among others.



FACSCalibur cell analyzer



MICROSCOPY UNIT

Head of Unit: Luísa Cortes | PhD in Enzymology (2006) at University of Coimbra

Head of Facility since 2007

The Microscopy Unit provides technical support on the investigation made using Light Microscopy. Besides managing the resources, the unit assists in planning microscopy oriented projects, analysing experimental results, processing acquired images and presenting data.

Presently, the unit manages a laser scanning confocal microscope (Zeiss LSM 510 Meta), a P.A.L.M. laser microdissecting microscope, a single cell calcium imaging system, 2 widefield systems and other brightfield microscopes. The systems are prepared for advanced applications which include live cell imaging and single cell calcium measurements, enabling the researchers of imaging dynamic events and molecular interactions.

The P.A.L.M. laser dissecting microscope is a perfect tool for the isolation of different cell populations within a sample, allowing its full characterization. Using this technology, collaboration has been established, with the service of Anatomical Pathology from the FMUC, with the aim of studying the differences of gene expression between tumour cells at diverse stages



P.A.L.M. laser microdissecting microscope



Laser scanning confocal microscope

MASS SPECTROSCOPY UNIT

Head of Unit: Bruno Manadas | Post-Doc, PhD in Cellular Biology (2008) at University of Coimbra

Head of Facility since 2008

Staff: Vera Mendes (technician)

The Mass Spectrometry Unit is specialized in identification and quantification of proteins from simple and complex samples; identification and quantification of post-translational modifications, and identification and quantification of metabolites. The Unit is also involved in the identification of biomarkers through proteomics and metabolomics techniques with the purpose of developing new prognosis and diagnosis methods, in collaboration with other R&D units at CNC, Biocant, and external partners.

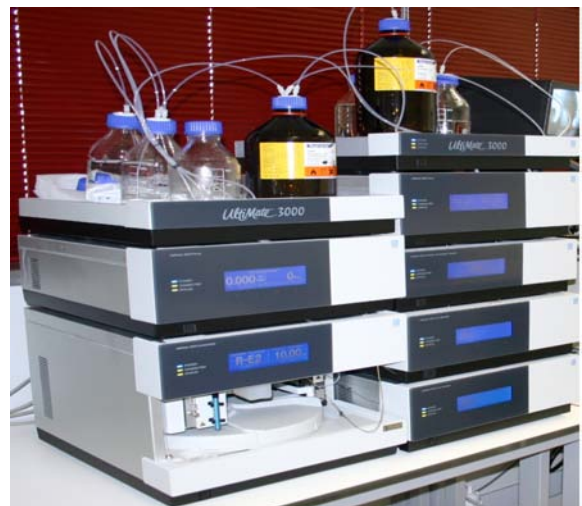
Presently, the Mass Spectrometry Unit is equipped with state of the art technology, namely: a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex), hybrid triple quadrupole/ion-trap mass spectrometer with capacity of MS³, a two-dimensional liquid chromatography system Ultimate 3000 (Dionex/LCPackings), a ExQuest (Bio-Rad) – image acquisition and spot picking robot and a data processing station (connected to two data acquisition stations). The unit also contains several software packages for data processing, including PDQuest and ProteomeWeaver for 2D gel analysis, Protein Pilot and PEAKS for protein identification, post-translational modifications and de novo sequencing.

By combining the high resolving power of the LC system with the structure elucidation from the mass spectrometer, the Mass Spectrometry Unit is able to identify peptides, metabolites, drugs, pesticides, among others, from complex mixtures.

The Unit integrates the National Mass Spectrometry Network (RNEM).



4000 QTRAP mass spectrometer



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

NMR SPECTROSCOPY UNIT

Head of Unit: Prof. Carlos Geraldes | PhD in Inorganic Chemistry (1976) at Oxford University, UK
Head of Facility since 2008

Staff: Emeric Wasielewski, PhD in Biophysics

The Nuclear Magnetic Resonance Spectroscopy Laboratory provides technical support on analysis of liquid and semi-solid samples by Nuclear Magnetic Resonance (NMR) Spectroscopy and Electron Spin Resonance (EPR) Spectroscopy.

The Unit currently stands with a 600 MHz NMR Spectrometer (Varian VNMR 600), a narrow bore 500 MHz NMR Spectrometer (Varian Unity 500), a 20 MHz NMR relaxometer (Bruker mq20) and an X-band EPR Spectrometer (Bruker ESP 300 E).

The state-of-the-art equipment comprise unique package of features that can provide information for NMR structural studies, metabolic studies in ex-vivo biosamples and biopsies. The unit also performs 1D, most 2D and some 3D NMR experiments on small-to-medium sized molecules and characterizes aqueous or non-aqueous samples, like paramagnetic and diamagnetic solutions, and biological tissues. Determine the quality control of various samples of industrial interest, such as water contents in oils, study small paramagnetic complexes and paramagnetic metalloproteins, and execute spin label and spin trap research, are also main areas of significance in our Unit.

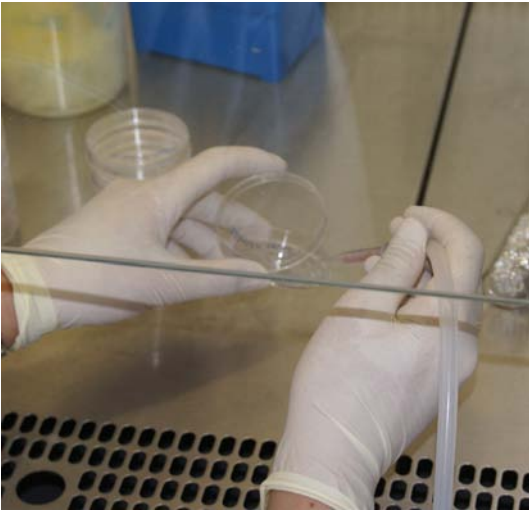
This Unit integrates the Portuguese Nuclear Magnetic Resonance Network (PTNMR).



Varian 600 NMR Spectrometer



Services



CNC Laboratório Associado provides the following specialized services to the community particularly to Hospitals and Pharmaceutical industries:

Mitochondrial Respiratory chain (MCR) and Krebs cycle enzymes; Mitochondrial DNA and Nuclear Genome studies in mitochondrial cytopathies; Amino Acid analysis for metabolic disorders diagnosis; Molecular testing of neurodegenerative and vision related genetic diseases; Biomarkers studies in Alzheimer's disease; Mutation screening of the genes *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *MYL2* in Hypertrophic and Dilated Cardiomyopathy

As a member of the National Spectrometry Network and of the Portuguese Nuclear Magnetic Resonance Network, CNC provides yet Mass Spectrometry and NMR services to research and high education institutions and industries.

Services

IMPLEMENTATION OF CERTIFICATION – “SISTEMA DE GESTÃO DA QUALIDADE, SGQ, ISO 9001” AT CNC-LABORATÓRIO ASSOCIADO

The certification process at the CNC started a few years ago with the preparation of basic procedures and the implementation started in 2009 with the integration of four Services' Laboratories (Biochemical Genetics Laboratory – Coordinator Manuela Grazina; Neurochemistry Laboratory – Coordinator Inês Baldeiras; Molecular Genetics of Cardiopathies Laboratory – Coordinator Isabel Marques Carreira; Neurogenetics and Genetics of Vision Laboratory – Coordinator Maria do Rosário Almeida).

The first step for achievement of certificate was the “Auditoria externa de 1ª fase” in July 2010.

The implementation of a SGQ requires a deep reorganization of all documents and procedures usually in use.

A Department of “Gestão da Qualidade” (Director Manuela Grazina; Assistant Rosa Fernandes) was created to build up the structure of the system and manage all the procedures and elaborate all documents necessary. All the steps, from the contacts of clinicians, to samples entrance, calibrations and verifications of all equipments involved in the assays, contacts with companies that sell reagents, and registration of all the actions, problems, results, reports, inquiries, etc, must be controlled.

It is a complex laborious process that requires specific formation and a special commitment of all members involved, including Director of CNC (Catarina Resende de Oliveira), Director of Quality (Manuela Grazina), Assessor for the Director of Quality (Rosa Fernandes) and all Laboratory's members. We have been certified by APCER after Audit in June 2011 (Certificate ISO 9001, reg. PT-2011/CEP.3971). This represents a step forward in the future of Services' Laboratories.

Biochemical Genetics Laboratory

Coordinator: Manuela Grazina

Team: Marta Simões; Cândida Mendes; Carla Veríssimo; João Pratas; M^a João Santos; Carolina Ribeiro; Mónica Vaz

The coordinator of LBG (Manuela Grazina) maintains international collaborations, allowing significant developments in the assays performed, namely with Prof. Lee-Jun Wong and Doctor Fernando Scaglia (Baylor College of Medicine, Houston – Texas, USA), Prof. Massimo Zeviani (National Neurological Institute “C. Besta”, Milan, Italy), Prof. Robert Taylor (Mitochondrial Pathology, University of Newcastle upon Tyne, UK) and Dr. Rafael Artuch (Hospital San Juan de Dios- Barcelona, Spain).

Additionally, she organized the III Advanced Course on “Translational bigenomics – from the bedside to the bench and back again” (May 2011), and the III Advanced Course & Workshop on Clinical Case Reports: the second genome: mitochondrial bigenomics – from genotype to phenotype and clinical expression” (January 2011), allowing the visit of Prof. Lee-Jun Wong, Doctor Fernando Scaglia (Baylor College of Medicine, Houston – Texas, USA), Prof. Massimo Zeviani (National Neurological Institute “C. Besta”, Milan, Italy), Prof. Robert Taylor (Mitochondrial Pathology, University of Newcastle upon Tyne, UK), to LBG, which was a valuable step forward for improving genetic diagnosis in LBG. A significant effort has been put on the set up of screening new genes for allowing diagnosis and genetic counselling.

Mitochondrial Respiratory Chain (MRC) and Krebs cycle enzymes

Biochemical assays related to energetic function are an important issue for probable diagnosis of Mitochondrial Respiratory Chain Diseases.

There were studied 66 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 79 samples (some patients had 2 or more tissues analysed), in 790 assays, including 39 lymphocytes isolated of peripheral blood, 33 muscular biopsies, 2 liver, 1 heart, 1 and 4 other samples. A MRC deficiency was detected in 40 patients.

The number of samples decreased, but the number of assays for each sample increased, compared to last year.

The validation of the Krebs cycle enzymes (fumarase, alfa-ketoglutarate dehydrogenase, malate dehydrogenase, aconitase, isocitrate dehydrogenase) is under validation and 65 samples were analysed (195 assays). These tests represent an important set up for improving diagnostic, particularly in cases with normal MRC.

The analysis of Coenzyme Q10 was implemented, in collaboration with Dr. Rafael Artuch (Hospital San Juan de Dios- Barcelona, Spain), after validation with 95 samples. We have analysed 42 samples (plasma, muscle, liver) of 34 patients, in 168 assays. A Coenzyme Q10 deficiency was detected in 14 samples, representing a huge improvement in diagnosis of MRCD, since this is the only treatable deficiency.

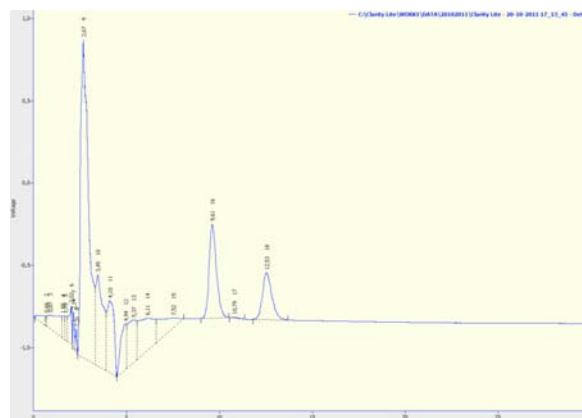


Fig.1. Chromatogram of Coenzyme Q10 detection

Mitochondrial DNA (mtDNA) and nuclear (nDNA) genomes studies

We have received 159 samples of 92 patients (blood - 106, muscle -37, liver - 3, heart - 1, kidney - 1 and other tissues - 11), for DNA extraction. However, given the fact that we are now offering a more extensive series of genetic assays, we received a high number of requests for analysing samples already existing in the Laboratory.

Molecular differential analysis of mitochondrial cytopathies, as a highthroughput screening, has been performed by sequencing analysis, of 11 mtDNA regions, covering a total of 424 mtDNA sequence variations that include 31 confirmed pathogenic mutations associated to MRC associated diseases. We have continued to screen deletions by flanking PCR of 6 hot-spot regions. Total mtDNA sequencing or gene panel analysis is also performed in selected samples, according to clinic manifestations and results from previous biochemical and/or genetic screening.

Mitochondrial DNA depletion syndrome (MDS), a mitochondrial cytopathy, comprises a heterogeneous group of diseases, caused by defects in intergenomic communication, namely due to nuclear genes mutations causing severe reduction of mtDNA content, with energy production impairment. That mtDNA reduction copies has been implicated as a major cause of mitochondrial disease in children. Copy number (mtDNA) assays are now part of the genetic mitochondrial genome screening. Nuclear genes screening includes 9 genes related to MRC function and or mtDNA biogenesis.

We have analysed 176 samples, comprising a total of 5194 assays for mtDNA point mutations and deletions analysis. Deletions have been detected in 4 samples and a total of 633 (327 different) known mtDNA sequence variations and 45 novel variants have been detected in 103 samples analysed of 99 patients investigated. Further PCR-RFLP analyses were performed to validate point mutations in 49 samples of 38 patients. We have analysed 200 control samples to verify the absence of novel alterations found in genetic screening. Two pathogenic mutations were found and 14 novel possible pathogenic variations are under characterization.

Concerning mtDNA copy number assays for depletion screening, we investigated 151 samples of 115 patients, including blood (42), muscle (38), liver (68) and other (3) tissues, comprising a total of 4228 real time PCR assays. We have confirmed diagnosis of mtDNA depletion in 20 samples of 12 patients. We have identified this type of mutation as the main mtDNA-related cause for MRC and the number of these assays is still increasing, compared to 2010.

Polymerase gamma (*POLG*) is the only polymerase existing in mitochondria, and is responsible for the constant and exact replication of mitochondrial genome, so that mitochondria have the adequate number of copies of the mitochondrial genome to maintain mitochondrial respiratory chain structure and functions. This polymerase is heterodimeric, and is encoded by 2 different genes, *POLG1* (15q25) and *POLG2* (17q24.1). Currently, it is estimated that 25% of mitochondrial diseases are related to *POLG* genes. Deoxyguanosine kinase (*DGUOK*) gene (2p13) encodes a mitochondrial nucleoside kinase, an enzyme responsible for phosphorylation of purine deoxyribonucleosides, therefore mutations in *DGUOK* gene have been shown to cause mtDNA depletion, particularly in

cases with hepatic failure and nistagmus. Our aim was to sequence the *DGUOK* gene exons and splicing regions, in patients diagnosed with MSD in our laboratory, searching for pathogenic causative mutations. *SURF1* gene (9q34.2) encodes a 30kDa hydrophobic inner mitochondrial membrane protein, involved in assembly of cytochrome c oxidase. Mutations in this gene have been described as the genetic cause for MRC. *OPA1* gene (3q28) encodes a mitochondrial protein responsible for cristae and mtDNA maintenance and involved in mitochondrial fusion. More than 200 pathogenic mutations have been described in MRC phenotypes, being responsible for 60-70% of the genetic cause of optic atrophy. *OPA2* gene (19q13.2-q13.3) encodes a mitochondrial protein with unknown function with assigned mutations causing several disease phenotypes. Implementation of analysis for other genes, such as *ANT*, *TK* and *twinkle* has started, in the attempt of finding the cause for mtDNA depletion or multiple deletions, but limitations in personnel available did not allow the accomplishment of this objective.

Concerning the screening of nDNA related to MRC, we have continued *POLG1,2* genes screening in 57 samples of 57 patients, comprising a total of 6270 DNA sequencing assays. We have identified 282 sequence variations (23 different) in 39 patients, which are in characterization for pathogenicity. Limitations in the personnel did not allow screening entire gene for all the samples, given the huge size of *POLG1* gene.

We have continued *DGUOK* gene screening, performed in 10 samples of 10 patients (555 assays) and identified 21 sequence variations (8 different); 3 cases (unrelated) have probable pathogenic mutations related to mtDNA depletion, relevant for genetic diagnosis and genetic counselling. We have implemented genetic screening of *OPA1,3* genes (44 samples of 44 patients, 3080 assays) and identified 2 sequence variations (2 different), but no pathogenic mutations were found so far. Screening of *SURF1* gene (5 samples of 5 patients, 975 assays) allowed detection of 7 sequence variations (6 different) and 4 possibly pathogenic mutations, relevant for genetic diagnosis and genetic counselling that are under characterization.

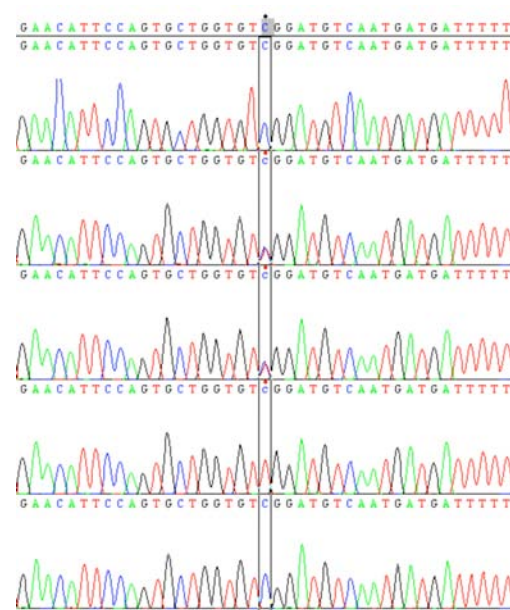


Fig. 2. Electropherogram showing detection of a pathogenic mutation in *DGUOK* gene allowing identification of the genetic cause for the disease – diagnosis – and genetic counselling in one family

Amino Acid Analysis

Our laboratory received 295 samples (231 - plasma, 55 - urine and 9 - cerebrospinal fluid) of physiological fluids for amino acid analysis. The patients investigated (children, adolescents adults) were categorized in three clinical conditions: (1) selective screening of metabolic disorder, characterized by either primary or secondary abnormalities in the amino acid profile (2) amino acid profile changes secondary to proximal renal tubular or hepatic dysfunction of any origin; (3) nutritional evaluation of patients with protein restrictive diets. The majority of samples are from children, although less frequently, adults and adolescents are also monitored. Amino acids analysis is a very important approach in early metabolic disorder diagnosis, and frequently helps to prevent mental retardation or even death.

Molecular Genetics of Cardiopathies Laboratory
 Coordinator: Isabel Marques Carreira

Team: Ana Cristina Santos

Screening of 540 mutations in 31 genes associated with cardiopathies

The method of molecular genetics diagnosis of cardiopathies, implemented in the laboratory in 2010 - MassARRAY, Iplex Gold (Sequenom) - W35_SpectroCHIP where they are analyzed 540 mutations in 31 genes associated with the development of cardiopathies, was consolidated during the year 2011. The major objective of the laboratory was to integrate this new method in routine laboratory and the establishment of a protocol with a laboratory in Lisbon that integrates the project.

This new approach requires a permanent interaction with the clinic, not only to establish the diagnosis but also for the counseling of affected patients and their families as well as to identify family members at risk to be studied.

The cases received in 2011 came from the cardio-genetic consultation in the Medical Genetics department of the

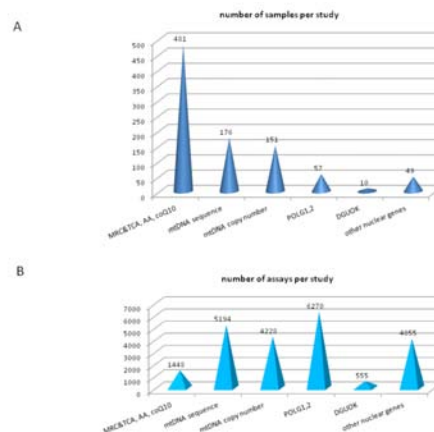


Fig. 3. Number of samples analysed and assays performed in LBG, according to the study, for biochemical and genetic diagnosis

Pediatric Hospital of Coimbra. 27 cases were received, 24 were index cases when the study was done of all 540 mutations and three familial cases for targeted studies.

All results were sent for genetics counseling by the cardio-geneticist and in the report of the positive cases it was mentioned the need for the patient to be followed by a cardiologist.

Another area developed in 2011 was the implementation of all documents and records necessary to the process of certification of the laboratory that was successfully achieved in July 2011. The lab is now certified for "Research of mutations in genes associated with cardiopathies".

Neuro-Ophthalmology Genetics Laboratory

Coordinator: Maria do Rosário Almeida

Team: Maria do Rosário Almeida, Maria Helena Ribeiro, Ana Cristina Santos e Ana Cristina Pinheiro

Molecular testing of Neurodegenerative and Vision related genetic diseases

Genetic testing for several Neurodegenerative diseases such as, Frontotemporal Lobar degeneration (*MAPT* and *PGRN* genes), Familial Alzheimer Disease (Presenilin1, Presenilin2, Amyloid precursor protein genes) and Parkinson Disease (Parkin and LRRK2 genes) has been available in our laboratory. Therefore, during 2010, more than one hundred molecular diagnosis tests have been performed and the respective reports have been issued and sent to the different clinicians who had requested them. In addition, the apolipoprotein E genotyping, in particular APOE-e4, have been set up in a routine basis, as a risk factor to develop Alzheimer Disease. Due to the potential risk factor of Glucocerebrosidase gene (GBA) mutations in Parkinson Disease, an increase number of referrals had been observed to test the two most common mutations in this gene, N370S and L444P in patients with Parkinson Disease.

The ophthalmology area is other area in which our laboratory has genetic tests available, in particularly to two *inherited* vision related diseases such as: Nanophthalmia and Retinitis pigmentosa with the mutation analysis of *MFRP* and *RHO* genes, respectively. In order to rule out the pathogenic nature of the new variants found, 50 healthy individuals without any history of ocular disease was used as controls.

Finally, the group was also involved in promoting and organizing quality assessment schemes at National level involving the molecular genetic laboratories where such genetic tests are performed towards the establishment of laboratory recommendations / guidelines to test these different genetic diseases. Therefore, a questionnaire was prepared and the feedback data was analyzed in a survey on genetic testing activity.

To achieve this, a close functional interaction between the laboratory and the clinicians at the Neurology Department of HUC has been established in order to improve the patient's diagnostics, follow-up and management. Since genetic diagnostic tests are playing an increasingly important role in clinical practice, the clinical referrals have increased in many specialities within medicine. In Neurology, its clinical applicability not only contributes to an accurate diagnosis but also to identify the relatives at high risk to develop the disease, in the context of formal genetic counselling. During 2009, ninety three referrals have been sent to our laboratory with the clinical diagnosis of Alzheimer Disease (9 cases), Parkinson Disease (63 cases) and Frontotemporal dementia (21 cases). The molecular strategy used to perform the molecular diagnosis, involved the mutation search of the genes associated with these disorders using different techniques such as: PCR, RFLP, direct sequencing and dosage. Another challenge that faced this group was the increasing demand for genetic services within the ophthalmology field, which is also one main interest of the Institute of Biomedical Research in Light and Image (IBILI). Therefore, the implementation of molecular genetic tests to vision inherited diseases such as: Nanophthalmia also took place and mutations in *MFRP* gene have been identified for the most of the cases.

At the same time, the group aimed to house both research and diagnostic activity which is fundamental to establish not only the new research findings that are of relevance in a clinical setting, but also to find out the best way to move these quickly from a research setting to diagnostic service in a timely and efficient manner. Therefore, research Projects have been outline and submitted to get financial support.

Neurochemistry Laboratory

Coordinator: Inês Baldeiras

Team: Inês Baldeiras, M^a Helena Garruncho, Rui Pascoal

The Neurochemistry Unit is integrated in the Neurology Department of the University Hospitals of Coimbra (HUC) and develops its activity in essentially two areas: laboratorial support of diagnosis and follow-up of neurological and metabolic diseases and clinical research of neurodegenerative disorders.

In what concerns the immediate support to the patient, the Neurochemistry Unit provides several test that help in the diagnosis and control of progression of neurodegenerative, demyelinating, neuromuscular and metabolic disorders:

- Cerebrospinal Fluid (CSF) cell count and chemical analysis

- Electrophoresis of CSF/serum proteins

- Detection of Immunoglobulin G Oligoclonal Bands in CSF/serum by Isoelectrical Focusing

- Determination of plasma Vitamin A and E levels by high-performance-liquid chromatography (HPLC)

- Evaluation of plasma and CSF redox status

- Quantification of urinary levels of purines and pyrimidines by HPLC

- Evaluation of the urinary activity of Arylsulfatase A

- Seric evaluation of anti-neuronal antibodies in patients with polineuropathies
- Quantification of serum levels of antiepileptic drugs in patients under therapy
- Determination of serum neutralizing antibodies (NABs) against Interferon- β (IFN- β) in multiple sclerosis patients undergoing treatment with IFN- β

Cerebrospinal fluid biomarker identification in neurodegenerative disorders, mainly in dementias, has been one of our main areas of research interest. Early and differential diagnosis of dementias is of particular importance as this type of disorders remains, in the present, fatal and without effective treatment. Accordingly, interventions with curative or stabilization potential would have a huge impact in human health and life expectancy. The Neurochemistry unit is, in the framework of the Portuguese Epidemiological Surveillance Program for Human Prion Diseases, the national reference laboratory for CSF analysis, and it performs:

- Quantification of CSF levels of total-Tau protein, phosphorylated-Tau protein and β -amyloid₁₋₄₂ peptide for dementia diagnosis

- Detection of 14-3-3 protein in CSF in suspected cases of Creutzfeldt-Jakob Disease (CJD)

- Immunodetection of Prion protein isoforms in brain extracts of CJD patients

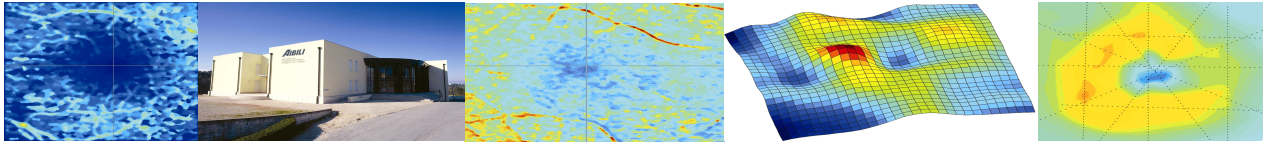
Characterization of oxidative status in neurodegenerative disorders is also a specific research interest of this unit. In this context, we perform, either in patients blood or in several cellular extracts, the:

- Evaluation of plasma and cellular oxidative stress

This includes the determination of a broad spectrum of non-enzymatic (uric acid, vitamin E, oxidized and reduced glutathione) and enzymatic antioxidants (glutathione reductase and peroxidase), nitrogen oxidative species and lipid (malondialdehyde) and protein (carbonyls) oxidation markers.

During the year 2011, the Neurochemistry Unit has received around 700 blood and 500 CSF samples and has performed the following analysis:

	Blood (Serum/Plasma)	CSF	Urine	Brain extracts	Other extracts
Cytochemistry and electrophoresis	233	233			
IgG Oligoclonal bands	220	220			
Vitamin A/E	174				
Redox Satus	143	82			
Purines & Pyrimidines			5		
Arylsulfatase A			1		
Anti-neuronal antibodies	55				
Antiepileptic drugs	0				
NABs against INF β	109				
Tau, p-Tau and A β 42		189			
14-3-3 protein		108			
Prion protein isoforms				1	
Oxidative Stress	20				214



AIBILI Report of Activities

2011

1. Introduction

Health research should be patient-oriented, i.e., addressing the needs of the patients, and to achieve this goal it is necessary to strengthen the clinical research process and to have the infrastructure and organization necessary to translate basic laboratory discoveries into the reality of improved patient care.

It is these competencies that AIBILI brings to its association with CNC positioning itself as a natural complement to the predominantly laboratory activities of CNC.

AIBILI is dedicated to clinical research and technology transfer in the area vision neurosciences. AIBILI is certified by ISO 9001 since 2004 to perform clinical research.

Two units of AIBILI integrate particularly well with the activities of CNC namely: the Centre for Clinical Research (CEC) and the Coimbra Coordinating Centre for Clinical Research (4C).

2. Centre for Clinical Trials

Investigator-Driven Clinical Trials

1. Early Markers of choroidal neovascularization (CNV) in fellow eyes of patients with age-related macular degeneration (AMD) and CNV in one eye

ClinicalTrials.gov nº NCT00801541

Protocol nº A9010002

Principal Investigator: Rufino Silva

Age-related macular degeneration (AMD) causes loss of visual acuity by progressive destruction of macular photoreceptor cells and retinal pigment epithelial cell function. The

characteristic early features are pigment clumping, formation of yellow drusen deposits under the retinal pigment epithelium (RPE), patchy atrophy of the underlying choriocapillaris, overlying RPE and photoreceptor complex. These features are commonly referred to as dry AMD or age related maculopathy (ARM). Dry AMD affects ~ 6% of Caucasian individuals aged 65 – 74 and rises to 20% of those aged > 75.

In some people neovascularization is stimulated from the choriocapillaris, perhaps by

vascular endothelial growth factor (VEGF) and/or other local inflammatory cytokines, to

grow through a fragmented Bruch's membrane under the RPE and/or under the retina.

When neovascularisation is present the condition is termed wet, exudative or neovascular

AMD. Neovascular AMD occurs in 10 – 20% of people with

dry AMD and causes accelerated and severe visual loss by leakage of serum and blood and then scarring under the macula.

Data from the Macular Photocoagulation Study Group show that 42-58% of patients with dry AMD features in one eye and CNV in the fellow eye will develop bilateral CNV within 5 years.

It is crucial to understand the natural history of the conversion from dry to neovascular AMD and to identify markers of this conversion.

It is proposed that longitudinal monitoring of patients with unilateral CNV with the new imaging techniques such as Optical Coherence Tomography (OCT) and automated analysis of digital fundus images will permit a better understanding of the development of CNV and of the progression of dry to neovascular AMD.

An observational, non-interventional multinational study is in progress involving 160 patients from three Centres (Coimbra, Belfast and Milan) followed for 4 years.

2. Correlation phenotype/genotype in diabetic retinopathy

ClinicalTrials.gov nº NCT01228981

Principal Investigator: Conceição Lobo

Protocol nº CEC/120

Financial Support: PTDC/SAU-OSM/103226/2008 - Foundation for Science and Technology, Portugal

Our research team has assembled a large group of diabetic patients (400) in the early stages of nonproliferative retinopathy (level 20 to 35 ETDRS) and is in the process of following them for a period of two years to identify retinal biomarkers of retinopathy progression (Project PTDC/SAU OSM/72635/2006). Blood samples will be collected also to perform a genetic analysis.

It is a unique opportunity to match candidate genes for retinopathy progression with the different phenotypes and patterns of progression identified in this particularly well characterized large population of patients/eyes with non proliferative retinopathy in diabetes type 2.

A list of candidate genes has been identified and classified in three groups based on gene organization and Single Nucleotide Polymorphisms (SNPs) density. Group 1 includes Aldose Reductase (ARL), Receptor for Advanced Glycation End Products (RAGE) and Vascular Endothelial Growth Factors (VEGF) and are by far the most important ones constituting a group that deserves a more thoroughly analysis. The Group 2 includes Intracellular Adhesion Molecule (ICAM 1) and Tumour Necrosis Factor (TNF α) and Group 3 includes Nitric Oxide Synthase 1 (NOS1) and Angiotensin Converting Enzyme (ACE). The group 1 and 2 genes will be analysed by high throughput gene wide sequencing using the 454sequencing technology. The group

3 genes will be analysed by DNA chips covering all SNPs described in NCBI SNP database. The patients/eyes that showed slow progression (expected 200) will serve as controls in comparison with the patients/eyes showing rapid progression of the retinopathy (expected 200).

3. Validation of a predictive model to estimate the risk of conversion to clinically significant macular edema and/or vision loss in mild nonproliferative retinopathy in diabetes type 2

ClinicalTrials.gov nº NCT00763802

Protocol nº CNTM/330

Principal Investigator: José Cunha-Vaz

Financial Support: PDTC/SAU-OSM/72635/2007 - Foundation for Science and Technology, Portugal

Work recently published by our research group (Arch. Ophthalmol. 2004; 122: 211-217) proposed the existence of 3 distinct patterns of diabetic retinopathy progression. In a subsequent work, we were able to mathematically confirm the existence of 3 phenotypes of diabetic retinopathy (Invest. Ophthalmol. Vis. Scis.) using a different and larger group of patients. Our group has now followed another group of 52 patients with type 2 diabetes and non-proliferative diabetic retinopathy, over a 5-year period, after an initial 2-year period of tight control (in a total of 7-year of follow-up), checking for an end-point of clinically significant macular edema (CSME) needing photocoagulation. We were also able to retrospectively classify each of these patients into one of the proposed phenotypes. In the first 2 years, while no end-point was found on patients from phenotype A, 11% of patients from phenotype B (1 patient) and 30% of patients from phenotype C (4 patients) got to the end-point needing photocoagulation. Although we had only a small number of end-point cases, this work permitted us to propose a predictive model of diabetic retinopathy progression to CSME. This project aims to validate our predictive model of diabetic retinopathy progression to CSME needing photocoagulation and/or vision loss (CPM - Coimbra Predictive Model), in an independent and larger population. Data for validation of the CPM is being collected for a prospective observational study, cohort study, that includes an initial selection period of two visits (V0 and V6), performed at six-month interval, of 400 patients (1 eye per patient) with diabetes type 2 and mild nonproliferative diabetic retinopathy and 20/25 Best Corrected Visual Acuity (BCVA) or better (reexamined 2 years later).

The incorporation of this model into the clinical practice is expected to increase the consistency in patterns of treatment and improve the quality of care for patients with diabetic retinal diseases.

4. Phenotypes of Nonproliferative Diabetic Retinopathy in Diabetes type 2 patients identified by Optical Coherence Tomography, Colour Fundus Photography, Fluorescein Leakage and Multifocal Electrophysiology (DIAMARKER Project: Genetic susceptibility for multi-systemic

complications in diabetes type-2: New biomarkers for diagnostic and therapeutic monitoring)

ClinicalTrials.gov nº NCT01440660

Protocol nº 4C-2011-01

Principal Investigator: Luisa Ribeiro

Financial Support: Project nº 13853 – DoIT – Agência de Inovação, Portugal

The rate of progression of Diabetic Retinopathy (DR) varies widely between different patients, even with similar metabolic control [Cunha-Vaz, Prog Retin. Eye Res. 2005]. It is becoming clear that a large percentage of patients with mild Non Proliferative Diabetic Retinopathy (NPDR) will take longer time to develop any sight-threatening complication than the rest of the mild NPDR patients. The full characterization of phenotypes of DR progression in the early stages of DR and the identification of biomarkers of disease progression are of major interest for patients' management in clinical trials and also in the clinical practice. Taking into account patients' phenotype, it may contribute to the development of new personalized medicine for DR, in type 2 diabetic patients.

The purpose of this study is to characterise phenotypes of NPDR progression using multimodal testing/imaging procedures.

5. EUROCONDOR - Neurodegeneration as an early event in the pathogenesis of Diabetic Retinopathy: A multicentric, prospective, phase II-III, double-blind randomized controlled trial to assess the efficacy of neuroprotective drugs administered topically to prevent or arrest Diabetic Retinopathy

Project Coordinator: Rafael Simó

Clinical Trial Principal Investigator: José Cunha-Vaz

Participating Centres (11): Barcelona, Birmingham, Cheltenham, Coimbra, Liverpool, London, Milan, Odense, Padova, Paris, Ulm.

Financial Support: Project nº 278040-2 – European Union 7th Framework Programme – Call Health 2011

Diabetic Retinopathy (DR) remains the leading cause of blindness among working-age individuals in developed countries (Congdon et al. JAMA 2003; 290:2057-60; Fong et al. Diabetes Care 2004; 27:2540-53). Current treatments for DR such as laser photocoagulation, intravitreal injections of corticosteroids or anti-VEGF agents are indicated only in advanced stages of the disease and are associated with significant adverse effects and increased costs. Therefore, new pharmacological treatments for the early stages of the disease are needed (Simó & Hernández. Diabetes Care 2009; 32:1556-62, Cheung et al. Lancet 2010; 376:124-36).

DR has been classically considered to be a microcirculatory disease of the retina. However, there is evidence suggesting that retinal neurodegeneration is an early event in the pathogenesis of DR which antedates and participates in the

microcirculatory abnormalities that occur in DR. For this reason, it is reasonable to hypothesize that therapeutic strategies based on neuroprotection will be effective not only in preventing or arresting retinal neurodegeneration but also in preventing the development and progression of DR in its early stages (i.e. microaneurysms and/or retinal thickness). In fact, several neuroprotective drugs have been successfully used in experimental models of DR (Imai et al. Dev Ophthalmol 2009; 44:56-68).

When the early stages of DR are the therapeutic target, it would be inconceivable to recommend an aggressive treatment such as intravitreal injections. The use of eye drops has not been considered a good route for the administration of drugs addressed to prevent or arrest DR. This is because it is generally assumed that they do not reach the posterior chamber of the eye (i.e. the vitreous and the retina). However, this is a misleading concept and there is emerging evidence that eye drops are useful in several diseases of the retina including DR (Aiello LP N Engl J Med 2008; 359:967-9; Cheung et al. Lancet 2010; 376:124-36). For this clinical trial the drugs Brimonidine and Somatostatin were selected since they have an adequate penetration into the vitreous and induce neuroprotection by means of receptors expressed in the retina.

The purpose of this study is to assess whether neuroprotective drugs administered topically (Brimonidine and Somatostatin) are able to prevent or arrest neurodegeneration as well as the development and progression of DR in its early stages.

Industry-Sponsored Clinical Trials

Diabetic Retinopathy

1. A 3-year, phase 3, multicenter, masked, randomized, sham-controlled trial to assess the safety and efficacy of 700 µg and 350 µg Dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS) applicator system in the treatment of patients with diabetic macular edema

EudraCT nº 2004-004996-12

Sponsor: Allergan

Principal Investigator: João Figueira

2. A phase 2/3 randomized, controlled, double-masked, multi-center, comparative dose-finding trial, in parallel groups, to compare the safety and efficacy of intravitreal injections of 0.3, 0.03 or 0.003mg Pegaptanib Sodium (Macugen®), given as often as every 6 weeks for 3 years, to sham injections, in subjects with diabetic macular edema (DME) involving the center of the macula

EudraCT nº 2005-001460-32

Sponsor: EyeTech

Principal Investigator: João Figueira

3. Prospective, randomized, open label phase II study to assess efficacy and safety of Macugen® (pegaptanib 0.3mg intravitreal injections) plus panretinal photocoagulation (PRP) and PRP (monotherapy) in the treatment of patient with high risk proliferative diabetic retinopathy

EudraCT nº 2009-016760-36

Sponsor: Pfizer

Principal Investigator: José Cunha-Vaz

4. Prospective, randomized, multicenter, open label phase II study to assess efficacy and safety of Lucentis® monotherapy (ranibizumab 0.5mg intravitreal injections) compared with Lucentis® plus panretinal photocoagulation (PRP) and PRP (monotherapy) in the treatment of patients with high risk proliferative diabetic retinopathy

EudraCT nº 2009-014409-15

Sponsor: Novartis

Principal Investigator: João Figueira

5. A 2 year Randomized, single-masked, multicenter, controlled phase IIIb trial assessing the Efficacy and safety of 0.5 mg ranibizumab in two "treat and extend" Treatment algorithms vs. 0.5 mg ranibizumab as needed in patients with macular edema and visual impairment secondary to diabetes mellitus

EudraCT nº 2010-019795-74

Sponsor: Novartis

Principal Investigator: João Figueira

6. A single arm, open-label, multicenter study evaluating the long-term safety and tolerability of 0.5mg fingolimod (FTY720) administered orally once daily in patients with relapsing forms of multiple sclerosis

EudraCT nº 2010-020515-37

Sponsor: Novartis

Principal Investigator: Luisa Ribeiro

Age-Related Macular Degeneration

7. A randomized, double-masked, active controlled, phase 3 study of the efficacy, safety, and tolerability of repeated doses of intravitreal VEGF Trap-Eye in subjects with neovascular age-related

EudraCT nº 2007-000583-25

Sponsor: Bayer

Principal Investigator: Rufino Silva

8. A multicenter, masked, randomized, sham-controlled, paired-eye comparison, 12-month (plus 12-month extension) study to evaluate the safety and effects on retinal structure and visual function of Brimonidine tartrate posterior segment drug delivery system (Brimonidine Tartrate PS DDS) applicator system in patients with geographic atrophy from age-related macular degeneration

EudraCT nº 2008-004187-37

Sponsor: Allergan

Principal Investigator: Rufino Silva

9. A multicenter, patient-masked, safety extension study to evaluate the biodegradation of the brimonidine tartrate posterior segment drug delivery system

EudraCT nº 2010-019079-32

Sponsor: Allergan

Principal Investigator: Eduardo Silva

10. The safety and efficacy of AL-8309B ophthalmic solution for the treatment of geographic atrophy (GA) secondary to age-related macular degeneration (AMD)

EudraCT nº 2008-007706-37

Sponsor: Alcon

Principal Investigator: Rufino Silva

11. Investigational Observacional Portuguese Project with Lucentis in Age Macular Degeneration (AMD) among 50 ophthalmologic Centers for 12 months

Sponsor: Novartis

Principal Investigator: Rufino Silva

12. Safety and Efficacy Study of ESBA1008 versus LUCENTIS® for the Treatment of Exudative Age-Related Macular Degeneration

EudraCT nº 2011-000536-28

Sponsor: Alcon

Principal Investigator: Rufino Silva

Retinal Toxicity

13. Long term (3 years) ophthalmic safety and cardiac efficacy and safety of ivabradine administered at the therapeutic recommended doses (2.5/5/7.5 mg b.i.d.) on top of anti anginal background therapy, to patients with chronic stable angina pectoris. An international, double-blind placebo controlled study

EudraCT nº 2006-005475-17

Sponsor: Servier

Principal Investigator: Luísa Ribeiro

Glaucoma

14. An evaluation of the ocular surface health in subjects using DuoTrav APS eye drops solution versus Xalacom® eye drops solution

EudraCT nº 2009-010604-29

Sponsor: Alcon

Principal Investigator: Luisa Ribeiro

15. A 3-month, multicenter, investigator-masked, pilot study to evaluate the efficacy and safety of Bimatoprost/Timolol fixed combination vs Latanoprost in treatment-Naive patients with open-angle glaucoma at high risk of glaucomatous progression

EudraCT nº 2009-012799-28

Sponsor: Allergan

Principal Investigator: Pedro Faria

16. A phase III, randomized, double-masked 6-month clinical study to compare the efficacy and safety of the preservative-free fixed dose combination of tafluprost 0.0015% and timolol 0.5% eye drops to those of tafluprost 0.0015% and timolol 0.5% eye drops given concomitantly in patients with open angle glaucoma or ocular hypertension

EudraCT nº 2010-022984-36

Sponsor: Santen

Principal Investigator: Luísa Ribeiro

17. Safety and IOP-Lowering Efficacy of Brinzolamide 10 mg/mL / Brimonidine 2 mg/mL Fixed Combination Eye Drops, Suspension compared to Brinzolamide 10 mg/mL Eye Drops, Suspension and Brimonidine 2 mg/mL Eye Drops, Solution in Patients with Open-Angle Glaucoma or Ocular Hypertension

EudraCT nº 2010-024512-34

Sponsor: Alcon

Principal Investigator: Luísa Ribeiro

18. Prospective, Non-Intervencional, Longitudinal Cohort Study to evaluate the long-term safety of XALATAN® Treatment in Pediatric Populations

Sponsor: Pfizer

Principal Investigator: Pedro Faria

Myopia

19. A 12 month, phase III, randomized, double-masked, multi-center, active-controlled study to evaluate the efficacy and safety of two different dosing regimens of 0.5 mg ranibizumab vs. verteporfin PDT in patients with visual impairment due to choroidal neovascularization secondary to pathologic myopia.

EudraCT nº 2010-021662-30

Sponsor: Novartis

Principal Investigator: Rufino Silva

Uveitis

20. A Multicenter Study of the Efficacy and Safety of the Human Anti-TNF Monoclonal Antibody Adalimumab as Maintenance Therapy in Subjects Requiring High Dose Corticosteroids for Active Non-infectious Intermediate-, Posterior-, or Pan-uveitis

EudraCT nº 2009-016095-68

Sponsor: Abbott

Principal Investigator: Rui Proença

21. A Multicenter Study of the Efficacy and Safety of the Human Anti-TNF Monoclonal Antibody Adalimumab in Subjects with Inactive Non-infectious Intermediate-Posterior-, or Pan-uveitis

EudraCT nº 2009-016008-22

Sponsor: Abbott

Principal Investigator: Rui Proença

22. A Multicenter Open-Label Study of the Long-term Safety and Efficacy of the Human Anti-TNF Monoclonal Antibody Adalimumab in Subjects with Non-infectious Intermediate-, Posterior-, or Pan-uveitis

EudraCT nº 2009-016196-29

Sponsor: Abbott

Principal Investigator: Rui Proença

Cataract

23. Efficacy and safety assessment of intracameral T2380 (Fixed combination of G146phenylephrine and tropicamide) for mydriasis and anaesthesia in phacoemulsification cataract surgery

EudraCT nº 2010-021188-34

Sponsor: Théa

Principal Investigator: Conceição Lobo

Neurological Disorders

24. A Multi-Center, Open-Label Extension Study to Examine the Safety and Tolerability of ACP-103 in the Treatment of Psychosis in Parkinson's Disease

EudraCT nº 2007-003035-22

Sponsor: Acadia

Principal Investigator: Luís Cunha

25. Pharmacokinetic assessment of ceftriaxone (Betasporina®) or clavulanic acid or ceftriaxone plus clavulanic acid administered by the endovenous route

EudraCT nº 2011-005089-39

Sponsor: Laboratórios Atral

Principal Investigator: Carlos Fontes Ribeiro

26. Efficacy and safety of agomelatine oral administration (25 to 50 mg/day) in elderly patients suffering from Major Depressive Disorder

EudraCT nº 2009-011795-29

Sponsor: Servier

Principal Investigator: Carlos Fontes Ribeiro

27. Efficacy and safety of Eslicarbazepine acetate (BIA 2-093) as monotherapy for patients with newly diagnosed partial-onset seizures: a double-blind, double-dummy, randomized, active-controlled, parallel-group, multicenter clinical study

EudraCT nº 2009-011135-13

Sponsor: Bial

Principal Investigator: Francisco Sales

28. Safety and efficacy of eslicarbazepine acetate (ESL) as adjunctive therapy for partial seizures in elderly patients.

EudraCT nº 2009-012587-14

Sponsor: Bial

Principal Investigator: Luís Cunha

29. Efficacy and safety of BIA 9-1067 in idiopathic Parkinson's disease patients with "wearing-off" phenomenon treated with levodopa plus a dopa

decarboxylase inhibitor (DDCI): a double-blind, randomised, placebo- and active-controlled, parallel-group, multicentre clinical study.

EudraCT n° 2010-021860-13

Sponsor: Bial

Principal Investigator: Luís Cunha

30. A multinational, multicenter, randomized, double-blind, parallel-group, placebo-controlled study of the effect on cognitive performance, safety, and tolerability of SAR110894D at the doses of 0.5 mg, 2 mg, and 5 mg/day for 24 weeks in patients with mild to moderate Alzheimer's Disease on stable donepezil therapy.

EudraCT n° 2010-022596-64

Sponsor: Sanofi

Principal Investigator: Luís Cunha

31. A Phase 3, 12-Week, Double-Blind, Double-Dummy, Placebo- and Active-Controlled Efficacy and Safety Study of Preladenant in Subjects with Moderate to Severe Parkinson's Disease

EudraCT n° 2009-015161-31

Sponsor: Shering-Plough

Principal Investigator: Luís Cunha

32. A multicenter, double-blind, double-dummy, randomized, positive-controlled study comparing the efficacy and safety of lacosamide (200 to 600 mg/day) to controlled release carbamazepine (400 to 1200 mg/day), used as monotherapy in subjects (≥ 16 years) newly or recently diagnosed with epilepsy and experiencing partial-onset or generalized tonic-clonic seizures.

EudraCT n° 2010-019765-28

Sponsor: UCB

Principal Investigator: Luís Cunha

3. Coimbra Coordinating Centre for Clinical Research

The Coimbra Coordinating Centre for Clinical Research (4C) is a platform/structure qualified to support Investigator-Initiated and Industry-Sponsored Clinical Trials by providing the following services:

- Protocol design and Statistical planning
- Study documents elaboration
- Submission to the regulatory authorities
- Coordination and Study implementation

- Monitoring and Quality control
- Data management and Electronic data capture solutions
- Periodical reports to the sponsor and/or regulatory authorities
- Statistical analysis and Final study report
- Medical writing and Publication support

Clinical Trial Coordination

National Studies – Investigator-Driven Clinical Trials

1. ClinicalTrials.gov n° NCT01298674

Epidemiological study of the prevalence of Age-Related Macular Degeneration in Portugal.

Protocol n° CC-01-2009

Coordinating Investigator: Rufino Silva

4C Services: Protocol design, coordination, data management and statistical analysis/final report.

2. ClinicalTrials.gov n° NCT01281098; EudraCT n° 2009-016760-36

Prospective, randomized, open label phase II study to assess efficacy and safety of Macugen® (pegaptanib 0.3 mg intravitreal injections) plus panretinal photocoagulation (PRP) and PRP (monotherapy) in the treatment of patients with high risk proliferative diabetic retinopathy.

Protocol n° CC-02-2009

Principal Investigator: José Cunha-Vaz

Financial Support: Pfizer

4C Services: Protocol design, study submission, coordination and monitoring, data management and statistical analysis/final report.

3. ClinicalTrials.gov n° NCT01280929

Prospective, randomized, multicenter, open label phase II study to assess efficacy and safety of Lucentis® monotherapy (ranibizumab 0.5 mg intravitreal injections) compared with Lucentis® plus panretinal photocoagulation (PRP) and PRP (monotherapy) in the treatment of patients with high risk proliferative diabetic retinopathy.

Protocol n° CRFB002DPT04T

Coordinating Investigator: José Cunha-Vaz

Financial Support: Novartis

4C Services: Protocol design, study submission, coordination and monitoring, data management and statistical analysis/final report.

4. ClinicalTrials.gov nº NCT01440660

Phenotypes of Nonproliferative Diabetic Retinopathy in Diabetes type 2 patients identified by Optical Coherence Tomography, Colour Fundus Photography, Fluorescein Leakage and Multifocal Electrophysiology (DIAMARKER Project: Genetic susceptibility for multi-systemic complications in diabetes type-2: New biomarkers for diagnostic and therapeutic monitoring).

Protocol nº 4C-2011-01

Principal Investigator: Luísa Ribeiro

Financial Support: Project nº 13853 – DoIT – Agência de Inovação, Portugal

4C Services: Protocol design, study submission, coordination and monitoring, data management and statistical analysis/final report.

5. EudraCT nº 2009-014612-34

Prospective, multicenter, open-label study to evaluate the safety and efficacy of intravitreal ranibizumab in patients with subfoveal or juxtafoveal choroidal neovascularization (CNV) secondary to other causes than Age-related Macular Degeneration and Pathological Myopia (angioid streaks, pseudohistoplasmosis, inflammatory chorioretinal diseases and idiopathic lesions)

Protocol nº CRFB002APT02T

Coordinating Investigator: José Cunha-Vaz

4C Services: Protocol design, study submission, coordination and monitoring, data management and statistical analysis/final report.

EVICR.net Coordinating Centre

AIBILI is the Coordinating Centre of the European Vision Institute Clinical Research Network, that is a network of European Ophthalmological Clinical Research Sites, dedicated to perform clinical research in ophthalmology with the highest standards of quality, following the European and International Directives for Clinical Research according to harmonized Standard Operating Procedures (SOPs).

It is a platform for clinical trial research in ophthalmology in Europe and a useful Industry resource in order to promote the development of new drugs and medical devices.

EVICR.net is an independent European Economic Interest Grouping (EEIG), established in 2010 in accordance with the Council Regulation (EEC) n.º 2137/85.

This Network has an infrastructure for management of multicenter clinical trials located in the Coordinating Center at AIBILI, Coimbra, Portugal. It has common and harmonized organizational and technical SOPs, quality

control and staff training according to ICH GCP-Guidelines.

EVICR.net serves as a fundamental resource for the development of translational research and particularly Pharmaceutical and Medical Devices Innovation in the European Union.

At present, EVICR.net has 78 Centres members from 16 European Countries that are either certified or in the process of certification.

Investigator-Driven Clinical Trials

1. ClinicalTrials.gov nº NCT01173614

Project Gullstrand - European Project for the Determination of Average Biometric Values of Human Eyes

Protocol nº ECR-COR-2010-01

Coordinating Investigator: Jos Rozema

Participating Centres (7): Alicante, Antwerp, Leipzig, Mainz, Rome, Tel Aviv, Valência.

2. ClinicalTrials.gov nº NCT01145599

Identifying progression of retinal disease in eyes with NPDR in diabetes type 2 using non-invasive procedures

Protocol nº ECR-RET-2010-02

Coordinating Investigator: José Cunha-Vaz

Participating Centres (13): Amsterdam, Antwerp, Bonn, Coimbra, Leipzig, Lisboa, London (2), Milan, Padova, Paris, Valência, Rome.

3. EUROCONDOR - Neurodegeneration as an early event in the pathogenesis of Diabetic Retinopathy: A multicentric, prospective, phase II-III, double-blind randomized controlled trial to assess the efficacy of neuroprotective drugs administered topically to prevent or arrest Diabetic Retinopathy

Project Coordinator: Rafael Simó

Clinical Trial Principal Investigator: José Cunha-Vaz

Participating Centres (11): Barcelona, Birmingham, Cheltenham, Coimbra, Liverpool, London, Milan, Odense, Padova, Paris, Ulm.

Financial Support: Project nº 278040-2 – European Union 7th Framework Programme – Call Health 2011

4. Publications (2011)

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12. Bernardes R, Cunha-Vaz J: Evaluation of the blood-retinal barrier with optical coherence tomography. In: *Optical Coherence Tomography: A Clinical and Technical Update*. Rui Bernardes et al., Ed. Springer. 2012: Chapter 8. . *In press*.
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Funding



Introduction

In 2011 funding of “Laboratório Associado – Centro de Neurociências e Biologia Celular” reached 6 292 845,30€.

The main financing contribution was made by “Fundação para a Ciência e Tecnologia (FCT)”, concerning global institution programs and national projects, in a total amount of 5 267.869,56€ distributed as follows:

Strategical Project (Plurianual) 2011:	1 863 007,00€
Projects:	2 735 925,77€
Science Program:	633 468,00€
Doctoral Program:	35 468,79€

The Center for Neuroscience is also financed by other national and international agencies. In 2011 the Center for Neuroscience received the amount of 75.046,29€ concerning other national projects and 558.542,95€ concerning international projects. QREN-Biotech funding was 412.430,42€

FCT ongoing projects, as well as, other national and international projects, are listed in the next section.

Other funding (not listed) was 47.466,22€ (prizes, supports, benefits).

ONGOING PROJECTS

Title	Financing Agency	Duration	Budget (CNC)	Expenditure 2011
National Projects:				
“Avanço na área de entrega de fármacos: terapias combinadas no tratamento do cancro da mama e leucemia (a rede Onco Target Nano Med)” Coordinator: M ^a da Conceição Lima	FCT Ref ^a : NANO/NMed-At/0042/2007	01/07/2009 to 31/12/2011	72.069,00	39.500,78
“Rede Nacional de Espectrometria de Massa” Coordinator: Euclides Pires	FCT Ref ^a : REDE/1506/REM/2005	01/01/2009 to 31/12/2011	138.960,42	49.655,99
“Rede Nacional de Ressonância Magnética Nuclear” Coordinator: Carlos Gerales	FCT Ref ^a : REDE/1517/RMN/2005	01/01/2010 to 31/12/2012	158.237,00	60.235,49
“Caracterização de alterações genéticas em gliomas humanos por arrays de polimorfismos de nucleótido único (SNP): correlação com as características clínicas e biológicas e citogenéticas da doença” Coordinator: Catarina de Oliveira	FCT Ref ^a : PIC/IC/83108/2007	05/01/2009 to 04/05/2012	150.440,00	49.200,22
“Nanostructured photoluminescent rare-earth nonotubes and microporous silicates” Coordinator: Carlos Gerales Participants: Universidade de Aveiro;	FCT Ref ^a : PTDC/CTM/73243/2006	01/12/2007 to 30/11/2010	14.544,00	215.96
“Papel dos receptores A2A da adenosina localizados na microglia e em terminais glutamatérgicos no controlo da plasticidade sináptica e dano cerebral” Coordinator: Rodrigo Cunha	FCT Ref ^a : PTDC/SAU-NEU/108668/2008	17/03/2010 to 17/09/2012	100.000,00	30.783,36

<p>"Micro e nano design de materiais com funcionalidades específicas para promover a regeneração de tecido ósseo usando células estaminais adultas."</p> <p>Coordinator: João Nuno Moreira</p> <p>Participants: Universidade do Minho</p>	<p>FCT</p> <p>Refª:</p> <p>MIT/ECE/0047/2009</p>	<p>01/06/2010</p> <p>To</p> <p>31/05/2013</p>	<p>32.880,00</p>	<p>3.686,86</p>
<p>"Benefícios do controlo metabólico precoce: prevenção da formação de memória hiperglicémica através da estimulação da bioenergética"</p> <p>Coordinator: Carlos Palmeira</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI-BIQ/103514/2008</p>	<p>01/03/2010</p> <p>to</p> <p>31/08/2012</p>	<p>126.667,00</p>	<p>54.823,59</p>
<p>O Neuropeptídeo Y (NPY) e a dipeptidil-peptidase IV (DPPIV) como novos alvos terapêuticos na regulação do tecido adiposo na obesidade</p> <p>Coordinator: Joana Salgado</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-FCF/102415/2008</p>	<p>05/02/2010</p> <p>to</p> <p>04/09/2012</p>	<p>96.184,00</p>	<p>38.393,33</p>
<p>"Função da cortactina no tráfego celular dos receptores do glutamato do tipo do tipo AMPA"</p> <p>Coordinator: Ana Luísa Carvalho</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/BIA-BCM/71789/2006</p>	<p>01/04/2008</p> <p>to</p> <p>31/03/2011</p>	<p>89.000,00</p>	<p>704,37</p>
<p>"NPwhY - Inervação e angiogénese para o benefício da osteogénese: envolvimento do NPY na regeneração óssea"</p> <p>Coordinator: João Malva</p> <p>Participants: INEB</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-OSM/101469/2008</p>	<p>05/02/2010</p> <p>to</p> <p>04/02/2013</p>	<p>9.000,00</p>	<p>8.553,87</p>
<p>"Acção de polifenóis da dieta no processo inflamatório intestinal quer como agentes simples quer em combinação com fármacos anti-inflamatórios: utilização de modelos in vitro e in vivo"</p> <p>Coordinator: Leonor de Almeida</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-OSM/102907/2008</p>	<p>01/05/2010</p> <p>to</p> <p>30/04/2013</p>	<p>122.336,00</p>	<p>28.509,76</p>
<p>"Vida e morte das células ganglionares da retina: neuromodulação e neuroprotecção pelo Neuropeptídeo Y"</p> <p>Coordinator: Francisco Ambrósio</p> <p>Participants: Faculdade de Medicina da Universidade de Coimbra</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/099075/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>51.897,00</p>	<p>17.031,60</p>

<p>“A restrição calórica aumenta a esperança de vida: papel do neuropeptídeo Y na autofagia”</p> <p>Coordinator: Cláudia Cavadas</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-FCF/099082/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>153.150,00</p>	<p>51.268,02</p>
<p>“Efeito da cafeína e dos receptores da adenosina A2A na resposta ao stress: papel da regulação da supra-renal”</p> <p>Coordinator: Cláudia Cavadas</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/108110/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>90.000,00</p>	<p>56.565,66</p>
<p>“A Abertura da Caixa Pandora Para uma Terapia Activa Anti-cancro da Mama - O Papel do Direccionamento Selectivo da Mitocôndria”</p> <p>Coordinator: Paulo Oliveira</p> <p>Participants: Faculdade de Farmácia da Universidade de Coimbra</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI-QUI/101409/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>170.976,00</p>	<p>45.774,16</p>
<p>“Impacto da metanfetamina na barreira hemato-encefálica: estudo dos mecanismos envolvidos e do papel de neuroinflamação”</p> <p>Coordinator: Ana Paula Silva</p> <p>Participants: Faculdade de Medicina da Universidade de Coimbra</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-FCF/098685/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>68.490,00</p>	<p>23.971,43</p>
<p>“Papel da Comunicação intercelular entre células endoteliais e células estaminais neurais na "stemness" e a neurogênese: novos alvos terapêuticos para a reparação cerebral”</p> <p>Coordinator: Fabienne Agasse</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/101783/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>86.000,00</p>	<p>37.236,77</p>
<p>“São os Fitoestrogénios Aditivos Alimentares Seguros e Eficazes para Mulheres em Menopausa? Uma Aproximação In Vitro e In Vivo para este Problema”</p> <p>Coordinator: Mª Sancha Santos</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/AGR-ALI/108326/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>168.716,00</p>	<p>51.233,59</p>
<p>“Mecanismos moleculares de insuficiência cardíaca: o papel do adipócito como órgão endócrino”</p> <p>Coordinator: Daniel Espinoza</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-OSM/104124/2008</p>	<p>22/03/2010</p> <p>to</p> <p>21/03/2013</p>	<p>191.757,00</p>	<p>20.137,17</p>

<p>"Análise do proteome do hipocampo de ratinhos expostos a medicação psicotrópica"</p> <p>Coordinator: Bruno Manadas</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/103728/2008</p>	<p>15/03/2010</p> <p>to</p> <p>14/03/2013</p>	<p>120.000,00</p>	<p>12.885,85</p>
<p>"Design de sensores químicos e biosensores compósitos para a monitorização em tempo-real e em simultâneo de óxido nítrico e oxigénio in vivo no cérebro"</p> <p>Coordinator: Rui Barbosa</p> <p>Participants: Faculdade de Farmácia da Universidade de Coimbra</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-BEB/103228/2008</p>	<p>01/05/2010</p> <p>to</p> <p>30/04/2013</p>	<p>100.000,00</p>	<p>15.115,09</p>
<p>"Actividade metabólica e viabilidade do condrócito em enxertos osteocartilagíneos humanos criopreservados."</p> <p>Coordinator: Celeste Lopes</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-OSM/67936/2006</p>	<p>01/09/2007</p> <p>to</p> <p>28/02/2011</p>	<p>32.648,83</p>	<p>15.475,94</p>
<p>"Design, synthesis and biological assessment of multifunctional compounds as anti-Alzheimer drugs"</p> <p>Coordinator: Paula Agostinho</p> <p>Proponente: Faculdade de Farmácia, Univ. Lisboa</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/64151/2006</p>	<p>01/08/2007</p> <p>to</p> <p>31/07/2011</p>	<p>12.740,00</p>	<p>5.821,99</p>
<p>"Nanoestruturas endereçadas para imagem molecular médica multimodal."</p> <p>Coordinator: Carlos Gerales</p> <p>Participants: Universidade do Minho,</p> <p>Faculdade de Medicina Universidade de Coimbra</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI/70063/2006</p>	<p>01/01/2008</p> <p>to</p> <p>31/12/2011</p>	<p>32.352,00</p>	<p>5.530,23</p>
<p>"O Metabolismo enquanto modelador da pluripotência e diferenciação de células estaminais."</p> <p>Coordinator: João Ramalho</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/EBB-EBI/101114/2008</p>	<p>15/04/2011</p> <p>to</p> <p>14/04/2013</p>	<p>147.656,00</p>	<p>44.148,09</p>
<p>"Derivados de Benzazolo Marcados com Fluor - 18 e Tecnécio - 99m para visualização In Vivo de depósitos de Amilóide."</p> <p>Coordinator: Catarina Oliveira</p> <p>Proponente: ITN, IP</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI-QUI/102049/2008</p>	<p>01/01/2010</p> <p>to</p> <p>31/12/2012</p>	<p>4.800,00</p>	<p>981,42</p>

"Planctomyces - uma linhagem filogeneticamente profunda. Decifrando os mecanismos envolvidos na adaptação a condições de stress." Coordinator: Milton Costa	FCT Refª: PTDC/BIA- MIC/105247/2008	01/05/2010 to 30/04/2013	189.624,00	60.253,94
"Análise dos mecanismos moleculares que determinam disfunção da alfa-sinucleína e a citotoxicidade na doença de Parkinson - o papel do GDNF." Coordinator: Ana Cristina Rego Participants: IMM	FCT Refª: PTDC/SAU- NEU/101928/2008	05/02/2010to 04/02/2013	160.000,00	50.727,31
"Optimização da utilização de hidratos de carbono em robalo de aquacultura através de perfis metabólicos." Coordinator: John Jones Participants: Faculdade de Ciências e Tecnologia	FCT Refª: PTDC/EBB- BIO/098111/2008	01/04/2010 to 31/03/2013	179.000,00	81.758,49
"Mechanismos moleculares envolvidos na cicatrização cutânea na diabetes - a importancia de neuropeptídeos." Coordinator: Eugénia Carvalho	FCT Refª: PTDC/SAU- MII/098567/2008	01/05/2010 to 30/04/2013	195.000,00	26.040,99
"Mapeamento do papel metabólico e neuromodulador da insulina no hipocampo." Coordinator: Attila Köfalvi	FCT Refª: PTDC/SAU- OSM/105663/2008	17/03/2010 to 16/03/2012	100.000,00	45.495,01
"Demonstração de que os receptores de adenosina A2A controlam a plasticidade sináptica glutamatergica via dos receptores de canabinóide CB1 no corpo estriado, fornecendo assim alvos terapêuticos atrativos." Coordinator: Attila Köfalvi	FCT Refª: PTDC/SAU- NEU/100729/2008	17/03/2010 to 16/03/2012	91.000,00	36.595,45
"Interacção de Lipoplexos com Membranas Celulares: uma Abordagem Biofísica da Terapia Génica." Coordinator: Amália Jurado	FCT Refª: PTDC/QUI- BIQ/103001/2008	03/05/2010 to 02/05/2013	122.562,00	30.031,71

"A interacção patológica entre a diabetes e a doença de Alzheimer: explorando o papel das mitocôndrias do endotélio cerebral e das suas proteínas desacopladoras." Coordinator: Paula Moreira	FCT Refª: PTDC/SAU- NEU/103325/2008	01/04/2010 to 31/03/2013	120.000,00	41.439,92
"Histamina versus anti-histamínicos: novos moduladores da neurogénese?" Coordinator: Liliana Bernardino	FCT Refª: PTDC/SAU- NEU/104415/2008	01/04/2010 to 31/03/2013	91.000,00	38.791,36
"Clarificação do Papel Mitocondrial na Cardiotoxicidade da Doxorubicina Usando um Sistema de Perfusão de Corações Intactos - Papel de Diferentes Calendários de Tratamento com Doxorubicina." Coordinator: António Moreno Proponente: Faculdade de Ciências e Tecnologia	FCT Refª: PTDC/SAU- OSM/104731/2008	01/05/2010 to 30/04/2013	65.200,00	22.882,43
"Alimentos Funcionais para Neuroprotecção: um papel para o Hypericum perforatum." Coordinator: João Malva Proponente: Universidade Minho	FCT Refª: PTDC/AGR- ALI/105169/2008	01/05/2010 to 30/04/2012	6.000,00	4.792,76
"Skineering - Engenharia de análogos de pele recorrendo à tecnologia de cell sheets." Coordinator: João Ramalho Proponente: Universidade Minho	FCT Refª: PTDC/SAU- OSM/099422/2008	01/04/2010 to 31/13/2013	44.748,00	4.260,27
Análise sistemática de proteínas Rab na fagocitose e na maturação do fagossoma do Mycobacterium tuberculosis Coordinator: Maria Otilia Vieira Participants: IBMC/UP	FCT Refª: PTDC/BIA- BCM/112138/2009	01/01/2011 to 31/2012/2013	171.993,00	50.254,52
"Actividade Protectora da SIRT3 na Disfunção Mitocondrial Induzida por Fármacos." Coordinator: Paulo Oliveira	FCT Refª: PTDC/SAU- TOX/110952/2009	01/03/2011 to 28/02/2012	128.800,00	53.776,08

<p>“A enigmática maltocinase de micobactérias.”</p> <p>Coordinator: Nuno Empadinhas</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/BIA-BCM/112459/2009</p>	<p>01/04/2011</p> <p>to</p> <p>31/03/2012</p>	<p>113.058,00</p>	<p>25.572,55</p>
<p>“Transporte entre células da alfa-sinucleína na doença de Parkinson. O factor de progressão?”</p> <p>Coordinator: Manuel Garrido</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NMC/109955/2009</p>	<p>01/04/2011</p> <p>To</p> <p>31/03/2014</p>	<p>144.738,00</p>	<p>21.351,74</p>
<p>“Uma nova formulação de nanopartículas para aplicação de terapia génica em tumores sólidos.”</p> <p>Coordinator: Henrique Faneca</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI-BIQ/116080/2009</p>	<p>01/04/2011</p> <p>To</p> <p>31/03/2014</p>	<p>94.000,00</p>	<p>7.481,84</p>
<p>“Simugrowth-Desenvolvimento de um modelo computacional para a simulação das propriedades biomecânicas de cartilagem desenvolvida in-vitro em função do estímulo mecânico em bioreactor.”</p> <p>Coordinator: Alexandrina Mendes</p> <p>Proponente: Universidade de Aveiro</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/EME-TME/113039/2009</p>	<p>03/04/2011</p> <p>To</p> <p>02/10/2013</p>	<p>28.830,00</p>	<p>1.785,22</p>
<p>“O papel do intestino no desenvolvimento da esteatose hepática induzida pela frutose.”</p> <p>Coordinator: John Jones</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-MET/111398/2009</p>	<p>01/07/2011</p> <p>To</p> <p>30/06/2014</p>	<p>139.476,00</p>	<p>16.118,51</p>
<p>“Nitrato:nitrito:óxido nítrico: uma via crítica que suporta o impacto benéfico do vinho e do azeite na fisiologia gastrointestinal e cardiovascular.”</p> <p>Coordinator: João Laranjinha</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/AGR-ALI/115744/2009</p>	<p>01/03/2011</p> <p>To</p> <p>28/02/2014</p>	<p>142.474,00</p>	<p>45.778,51</p>
<p>“Indução de células estaminais pluripotentes a partir de células do sangue do cordão umbilical através de metodologia não-viral e a sua diferenciação em cardiomiócitos – iPSCardio.”</p> <p>Coordinator: Ricardo Das Neves</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-ENB/113696/2009</p>	<p>01/04/2011</p> <p>To</p> <p>31/03/2014</p>	<p>135.649,00</p>	<p>6.093,06</p>
<p>“Targets - Targeted Gene Therapy Strategies to treat nerve injury.”</p> <p>Coordinator: Conceição P. Lima</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/CPM-NAN/115124/2009</p>	<p>01/04/2011</p> <p>To</p> <p>31/03/2014</p>	<p>3.060,00</p>	<p>0,00</p>

<p>"O papel da adenosina e do receptor A2A na resposta imunitária a Candida albicans."</p> <p>Coordinator: Teresa Maria Gonçalves</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-MIC/115598/2009</p>	<p>01/06/2011</p> <p>To</p> <p>31/05/2013</p>	<p>49.832,00</p>	<p>19.943,81</p>
<p>"Nanoquímica de compósitos magnéticos/luminescentes para aplicações de diagnóstico médico in vitro"</p> <p>Coordinator: António Guiomar</p> <p>Participants: Universidade de Aveiro</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI/67712/2006</p>	<p>01/01/2008</p> <p>to</p> <p>31/03/2011</p>	<p>15.300,00</p>	<p>2558,03</p>
<p>"Reconstrução e análise sistémica da rede reaccional de espécies reactivas de oxigénio, azoto e enxofre em sistemas fisiológicos representativos."</p> <p>Coordinator: Armindo Salvador</p> <p>Participants: Fundação da Faculdade de Ciências, Universitat de Lleida.</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI/70523/2006</p>	<p>01/01/2008</p> <p>to</p> <p>31/12/2010</p>	<p>162.752,00</p>	<p>17.954,88</p>
<p>"BIOINK - Aprendizagem incremental de Kernel Machines para análise de dados em bioinformática"</p> <p>Coordinator: Paula Verissimo</p> <p>Participants: Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Instituto Superior de Engenharia de Coimbra.</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/EIA/71770/2006</p>	<p>01/01/2008</p> <p>to</p> <p>31/12/2011</p>	<p>4.200,00</p>	<p>838,85</p>
<p>"Proteases de Polens, relevância nas doenças alérgicas."</p> <p>Coordinator: Paula Verissimo</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-ESA/72571/2006</p>	<p>01/05/2008</p> <p>to</p> <p>22/05/2012</p>	<p>199.850,00</p>	<p>68.656,83</p>
<p>"Papel do ATP e dos seus receptores P2Y1 na neuroprotecção"</p> <p>Coordinator: Rodrigo Cunha</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-FCF/100423/2008</p>	<p>18/03/2010</p> <p>to</p> <p>17/09/2012</p>	<p>189.697,00</p>	<p>66.660,79</p>
<p>"Acoplamento neurovascular entre a actividade neuronal e o fluxo sanguíneo no encéfalo mediado pelo óxido nítrico"</p> <p>Coordinator: João Laranjinha</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/108992/2008</p>	<p>01/05/2010</p> <p>to</p> <p>30/04/2013</p>	<p>100.000,00</p>	<p>42.435,51</p>

<p>“Perfis dinâmicos do óxido nítrico no cérebro: regulação da respiração celular com implicações para a doença de Alzheimer e para o envelhecimento”</p> <p>Coordinator: João Laranjinha</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/103538/2008</p>	<p>01/06/2010</p> <p>to</p> <p>31/05/2013</p>	<p>100.000,00</p>	<p>51.676,66</p>
<p>“HotMetal-Estratégias de resistência a metais pesados e disseminação de resistências a antibióticos nas fontes marinhas hidrotermais”</p> <p>Coordinator: Milton Costa</p> <p>Participants: IMAR</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/MAR/109057/2008</p>	<p>01/06/2010</p> <p>to</p> <p>31/05/2013</p>	<p>9.000,00</p>	<p>1.200,44</p>
<p>“Análise das alterações da transcrição em modelos cerebrais e periféricos da doença de Huntington - influência da modulação das desacetilases das histonas”</p> <p>Coordinator: Ana Cristina Rego</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-FCF/108056/2008</p>	<p>05/02/2010</p> <p>to</p> <p>04/02/2013</p>	<p>199.999,00</p>	<p>74.350,77</p>
<p>“Papel da proteólise da ataxina-3 mediada por calpaínas na doença de Machado-Joseph: terapia molecular com vectores virais”</p> <p>Coordinator: Luis de Almeida</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/099307/2008</p>	<p>05/02/2010</p> <p>to</p> <p>04/02/2013</p>	<p>107.000,00</p>	<p>38.660,68</p>
<p>“Genes FKS, CHS e de síntese de melanina em Alternaria infectoria: a combinação para o oportunismo”</p> <p>Coordinator: Teresa Gonçalves</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-ESA/108636/2008</p>	<p>01/03/2010</p> <p>to</p> <p>28/02/2012</p>	<p>100.000,00</p>	<p>39.051,96</p>
<p>Papel da Fisiologia Mitocondrial na Resistência das Células Estaminais Tumorais à Quimioterapia</p> <p>Coordinator: Paulo Oliveira</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/QUI-BIQ/101052/2008</p>	<p>01/04/2010</p> <p>to</p> <p>31/03/2013</p>	<p>143.016,00</p>	<p>33.473,16</p>
<p>“Biorstimul - Desenvolvimento e construção de um novo conceito de bioreactor para a caracterização biomecânica e bioquímica de tecidos de cartilagem desenvolvidos in-vitro”</p> <p>Coordinator: Celeste Lopes</p> <p>Participants: Universidade Aveiro</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/EME-PME/103578/2008</p>	<p>16/03/2010</p> <p>to</p> <p>15/03/2013</p>	<p>41.600,00</p>	<p>7.324,54</p>

<p>“Detecção do potencial sensibilizante de químicos através de um teste in vitro alternativo: uma imposição da nova legislação da União Europeia”</p> <p>Coordinator: Maria Rosete</p> <p>Participants: Universidade Aveiro</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-OSM/099762/2008</p>	<p>01/04/2010 to 31/03/2013</p>	<p>175.000,00</p>	<p>21.177,80</p>
<p>“Mecanismos e propriedades anti-inflamatórias de plantas medicinais: investigação multidisciplinar para a sua validação e utilização como fonte de fitofármacos”</p> <p>Coordinator: Maria Rosete</p> <p>Participants: Universidade Coimbra e Universidade Aveiro</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-FCF/105429/2008</p>	<p>01/05/2010 to 30/04/2013</p>	<p>55.800,00</p>	<p>15.818,30</p>
<p>“Alteração do tráfego intracelular mediado pela mitocôndria na doença de Parkinson”</p> <p>Coordinator: Sandra Cardoso</p> <p>Participants: Instituto de Medicina Molecular</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/102710/2008</p>	<p>05/02/2010 to 02/04/2013</p>	<p>137.589,00</p>	<p>39.258,12</p>
<p>"Regeneração cardíaca com células vasculares embrionárias e uma matriz biomimética."</p> <p>Coordinator: Lino Ferreira</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-BEB/098468/2008</p>	<p>01/04/2010 to 31/03/2013</p>	<p>180.000,00</p>	<p>79.513,20</p>
<p>“Nanomateriais para detecção de células”</p> <p>Coordinator: Lino Ferreira</p> <p>Participants: Biocant</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/CTM/099659/2008</p>	<p>01/04/2010 to 31/03/2013</p>	<p>136.000,00</p>	<p>29.114,41</p>
<p>“Mecanismos responsáveis pelos efeitos do óxido nítrico na proliferação de células estaminais neurais após lesão cerebral”</p> <p>Coordinator: Caetana Carvalho</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/102612/2008</p>	<p>01/04/2010 to 31/03/2013</p>	<p>120.000,00</p>	<p>48.251,04</p>
<p>“O papel da tradução localizada de mRNAs na formação da junção neuromuscular”</p> <p>Coordinator: Ramiro Almeida</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/104100/2008</p>	<p>01/05/2010 to 30/04/2013</p>	<p>120.000,00</p>	<p>40.809,87</p>

<p>“Mecanismos Moleculares do Tráfego Sináptico de Receptores do Glutamato do Tipo NMDA”</p> <p>Coordinator: Ana Luísa Carvalho</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/099440/2008</p>	<p>15/09/2010</p> <p>to</p> <p>14/09/2013</p>	<p>164.424,00</p>	<p>67.636,29</p>
<p>“Regulação das proteínas hnRNP pela neurotrofina BDNF: importância da plasticidade sináptica”</p> <p>Coordinator: Carlos Duarte</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NEU/104297/2008</p>	<p>15/09/2010</p> <p>to</p> <p>14/09/2013</p>	<p>120.000,00</p>	<p>67.512,29</p>
<p>“Parametrização do metabolismo e crescimento tumorais através da análise de fluxos metabólicos e engenharia metabólica”</p> <p>Coordinator: Rui Carvalho</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/EBB-EBI/115810/2009</p>	<p>01/01/2011</p> <p>to</p> <p>31/12/2013</p>	<p>169.578,00</p>	<p>43.700,91</p>
<p>“Localização e metabolismo da APP e relação com o controlo da degeneração sináptica em modelos animais da doença de Alzheimer”</p> <p>Coordinator: Catarina Oliveira</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NMC/114810/2009</p>	<p>23/08/2011</p> <p>to</p> <p>22/08/2012</p>	<p>89.493,00</p>	<p>18.105,98</p>
<p>“Caracterização da interacção Proteína - Carbohidrato da Laforina - Proteína humana envolvida na Doença de Lafora”</p> <p>Coordinator: Carlos Gerales</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/BIA-PRO/111141/2009</p>	<p>01/03/2011</p> <p>to</p> <p>28/02/2014</p>	<p>31.140,00</p>	<p>5.040,28</p>
<p>“Regulação por fosforilação da ataxina-3, a proteína mutada na Doença de Machado Joseph”</p> <p>Coordinator: Ana Luísa Carvalho</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NMC/110602/2009</p>	<p>01/01/2011</p> <p>to</p> <p>31/12/2013</p>	<p>123.777,00</p>	<p>31.905,26</p>
<p>“Regulação da estabilidade do RNA mensageiro para a subunidade GluR1 dos receptores do glutamato”</p> <p>Coordinator: Ana Luísa Carvalho</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/BIA-BCM/113738/2009</p>	<p>01/04/2011</p> <p>to</p> <p>31/03/2013</p>	<p>108.001,00</p>	<p>31.749,90</p>
<p>“Pré-condicionamento via mitocôndria: potencial efeito neuroprotector na doença Alzheimer”</p> <p>Coordinator: Paula Moreira</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/SAU-NMC/110990/2009</p>	<p>03/01/2011</p> <p>To</p> <p>28/02/2013</p>	<p>93.735,00</p>	<p>22.742,84</p>
<p>“Via para a síntese do MGLP de micobactérias. Caracterização bioquímica e estrutural das enzimas envolvidas”</p> <p>Coordinator: Nuno Empadinhas</p>	<p>FCT</p> <p>Refª:</p> <p>PTDC/BIA-PRO/110523/2009</p>	<p>01/01/2011</p> <p>to</p> <p>31/12/2013</p>	<p>130.624,00</p>	<p>20.227,48</p>

"Desenvolvimento de uma vacina contra a hepatite B para ser administrada através das mucosas: Desenho e estudos mecanísticos de um protótipo de um sistema de libertação multicomponente nanoparticular" Coordinador: Olga Ribeiro	FCT Refª: PTDC/SAU-FAR/115044/2009	01/01/2011 to 31/12/2013	122.060,00	27.999,05
"Desenvolvimento de uma nova estratégia terapêutica para o cancro do pâncreas envolvendo uma acção concertada de terapia génica e quimioterapia" Coordinador: Henrique Faneca	FCT Refª: PTDC/SAU-BMA/114482/2009	01/01/2011 to 31/12/2013	100.000,00	20.014,04
"iCALP - Identificação das funções fisiológicas das calpains no controlo da proliferação e migração celulares no sistema nervoso central" Coordinador: Inês Araújo	FCT Refª: PTDC/SAU-NMC/112183/2009	01/03/2011 to 28/02/2014	142.560,00	20.377,72
Programa MIT Coordinador: Catarina Oliveira, Lino Ferreira	FCT Refª: MIT-Portugal 2011/2012	01/09/2006 to 31/08/2012	89.100,00	36.461,82
Sub – Total FCT				2.735.925,77
Other National Projects				
"Histamine in the neural and cancerstem cell niche: a role in glioblastoma ontogeny". Coordinador: Liliana Bernardino e Fabienne Agasse	Fundação Calouste Gulbenkian Refª: 96542	10/11/2008 to 09/11/2011	50.000,00	21.875,95
"Ibercivis.pt - Uma plataforma de computação voluntária para a Península Ibérica" Coordinador: Rui Manuel Pontes M. F. Brito	UMIC - Agência para a Sociedade do Conhecimento	16/06/2010 to 15/06/2013	87.380,00	20.384,71
"Quero mais e melhores células! (células estaminais: o que são? Onde estão? Para que servem?) Coordinador: Cláudia Cavadas	Ciência Viva – Agência Nacional para a cultura científica e tecnológica	01/11/2011 to 31/12/2012	83.040,00	2.509,86
DoIT – projeto nº 013853	Agência da Inovação, S.A.	01/07/2010 to 30/06/2013	378.154,38	30.275,77

QREN-Amiloterá: 021622 Coordinator: Rui Brito	Agência da Inovação, S.A	26/09/2011	228533,09	0,00
Sub – Total Other				75.046,29
Total National Projects				2.810.972,06
International Projects:				
“BNOX – The role of reactive oxygen species in B cell tolerization and immune memory”. Coordinator: Margarida Carneiro	Marie Curie Actions - 239422 Ref.ª: FP7- PEOPLE–ERG-2008	01/06/2009 to 31/05/2012	45.000,00	10.463,67
“Transplantation of magnetic – labelled vascular cells and cardiomyocytes isolated from human embryonic stem cells in a bioactive injectable gel for myocardium regeneration after infarct”. Coordinator: Lino Ferreira	Marie Curie Actions – 230929 Refª: FP7-PEOPLE-2007-4-3-IRG	01/04/2009 to 31/03/2013	100.000,00	29.256,56
“Role of Mitochondrial Physiology in Tumor Stem Cell Resistance to Chemotherapeutics” Coordinator: Paulo Oliveira	Marie Curie Actions – 251850 Ref.ª: FP7-PEOPLE-2009-IEF	01/08/2010 to 30/07/2012	147.283,60	67.535,09
"Industrial Academic Initial Network towards treatment of Polyglutamine diseases" Coordinator: Luís Almeida	Marie-Curie-264508 Ref.ª FP7-PEOPLE-ITN-2010	01/03/2011 To 28/02/2014	211.441,00	889,38
Novel nanoparticles for drug delivery to the skin Coordinator: Lino Ferreira	Queen Mary - 289454 Ref.ª: FP7-PEOPLE-2011-ITN	01/11/2011 To 30/10/2015	471.627,64	3.095,73
"Docotral Candidate Agreement 159302-1-2009-NL-Era Mundus-EMJD" Coordinator: Rodrigo Cunha	Marie-Curie-Cycle 2-2011-PT	21/06/2011 To 30/08/2014	89.680,00	6.804,88
“The role of local mRNA translation in synapse formation” Coordinator: Ramiro Daniel Carvalho de Almeida	Marie Curie Actions Refª: PIRG-GA-2009-249288	01/04/2010 to 31/03/2014	100.000,00	30.422,18

"Award NIH_5R01DK029953-30" Coordinator: John Jones	NIH – 5R01DK029953-30	01/08/2011 To 31/07/2012	9.470,98	123,90
DDZ – Research Collaboration Agreement Coordinator: John Jones	DDZ – Research Collaboration Agreement	01/11/2011 To 31/10/2012	14.309,00	1.788,85
“Role of the autophagy-related protein Beclin-I in Machado-Joseph disease.” Coordinator: Luís Pereira de Almeida	Association Française contre les Myopathies Ref.ª: SB/NF/2010/2008	30/10/2010 to 29/10/2012	110.000	6.0481,57
“New Treatments for Stress-induced Dysregulation of Circuits Regulating Reward, Fear, and Habit Learning”. Coordinator: Rodrigo Cunha	Massachusetts Institute of Technology Ref.ª: DARPA-BAA-009-68	01/04/2010 To 30/06/2012	531.712,00	314.446,04
“Ability of a Mitochondria improver, berberine, to attenuate Parkinson’s disease” Coordinator: Carlos Palmeira	Michael J. Fox Foundation for Parkinson’s Research	01/09/2011 to 31/08/2012	40.602,31	1.909,43
“LRRK2 role on auto-antibody production by human B cells” Coordinator: Margarida Carneiro	Michael J. Fox Foundation for Parkinson’s Research	01/11/2011 a 31/10/2012	103.009,00	31.325,67
Total International Projects				558.542,95
TOTAL				3.369.515,01

List of Staff and Research Students



List of Staff and Research Students | General List

Members holding PhD		Time % at CNC
Alcino Jorge Lopes Leitão	(Assistant Prof., FFUC)	60
Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Ali Mobasheri	(Investigator, Univ Nottingham, UK)	Collaborator
Amílcar Falcão	(Full Prof., FFUC)	80
Ana Bela Sarmiento Ribeiro	(Assistant Prof., FMUC)	40
Ana Cristina Fortuna	(Inv. Assistant Prof., FFUC)	80
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Ledo	(Assistant Inv., CNC)	100
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Luísa Vital Carvalho	(Health Tech. HUC)	40
Ana Paula M. Sousa	(Investigator, HUC)	50
Ana Paula Silva Martins	(Assistant Inv., FMUC)	Collaborator
Ana Rita Costa Álvaro	(Inv. Assistant Prof., UTAD)	60
Anabela Maduro Almeida	(Assistant Prof., Univ. Vasco Gama)	80
Anabela P. Rolo	(Assistant Inv., CNC)	100
Anália do Carmo	(Assistant Prof., Univ. Vasco da Gama)	80
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Andrea Cooper	(Investigator, Trudeau Inst., EUA)	Collaborator
Ângelo R. Tomé	(Assistant Prof., FCTUC)	60
António F. Ambrósio	(Assistant Inv., FMUC)	Collaborator
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	60
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Attila Köfalvi	(Assistant Inv., CNC)	100
Bruno Miguel Neves	(Assistant Prof., Univ. Aveiro)	50
Caetana Carvalho	(Full Prof., FCTUC)	80
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Carlos G. Geraldes	(Full Prof., FCTUC)	80
Carlos Manuel Matias	(Assistant Inv., FCTUC)	60
Carlos Faro	(Associate Prof., FCTUC)	60
Carlos M. Palmeira	(Associate Prof., FCTUC)	80
Cármen Garcia-Rodriguez	(Investigator, Univ. Valladolid, Spain)	Collaborator
Catarina R. Oliveira	(Full Prof., FMUC)	80

Célia M. Antunes	(Assistant Prof., FCTUC)	80
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	40
Daniela Cipreste Vaz	(Assistant Prof., Inst. Polit. Leiria)	100
Elsa Henriques	(Assistant Inv., CNC)	100
Emília P. Duarte	(Assistant Prof., FCTUC)	80
Euclides Pires	(Associate Prof., FCTUC)	60
Eugénia Carvalho	(Assistant Inv., CNC)	100
Fabienne Agasse	(Assistant Inv., CNC)	60
Fernando Monteiro Judas	(Assistant Prof., FMUC)	25
Fran Lund	(Investigator, Rochester Univ, EUA)	Collaborator
Geanne Matos de Andrade	(Associate Prof., Brasil)	30
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	10
Henrique Faneca	(Assistant Inv., CNC)	100
Henrique Bernardo Silva	(Assistant Inv., CNC)	100
Ignacio Vega-Naredo	(Assistant Inv., CNC)	100
Ildete Luisa Ferreira	(Assistant Inv., CNC)	100
Inês Araújo	(Assistant Prof., UALG)	20
Inês Esteves Baldeiras	(Investigator, FMUC)	20
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	30
Isaura Simões	(Assistant Inv., CNC)	100
Ivana Jarak	(Assistant Inv., CNC)	100
Joana Cardoso Costa	(Inv. Assistant Professor, FCTUC)	80
João Laranjinha	(Associate Prof., FFUC)	60
João Nuno Moreira	(Assistant Prof., FFUC)	80
João O. Malva	(Principal Inv., FMUC)	100
João Peça-Silvestre	(Post-Doc Fellow, MIT)	35
João Ramalho Santos	(Associate Prof., FCTUC)	80
John Griffith Jones	(Principal Inv., CNC)	100
Jorge António R. Salvador	(Associate Prof., FFUC)	60
José Alberto Correia e Vale	(MD, Univ. Salamanca)	Collaborator
José Custódio	(Associate Prof., FFUC)	80
Leonor Almeida	(Full Prof., FFUC)	60
Lino Ferreira	(Assistant Inv., CNC)	100
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Luis M. Rosário	(Associate Prof., FCTUC)	60
Luis Pereira Almeida	(Assistant Prof., FFUC)	80
Manuel Aureliano Alves	(Associate Prof., Univ Algarve)	Collaborator
Manuel Garrido	(Investigator, Genibet)	80
Manuela Carvalheiro	(MD, HUC)	15
Margarida Vaz Caldeira	(Inv. Assistant Prof., FCTUC)	80
Mª Amália Jurado	(Assistant Prof., FCTUC)	60

M ^a Carmen Alpoim	(Associate Prof., FCTUC)	60
M ^a Celeste Lopes	(Full Prof., FFUC)	80
M ^a Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
M ^a Cristina Januário dos Santos	(Inv., Assistant Prof., FMUC)	20
M ^a Dolores T. Redondo	(Investigator, Univ. Salamanca)	Collaborator
M ^a do Rosário Almeida	(Assistant Inv., CNC)	100
M ^a Emília O. Quinta Ferreira	(Associate Prof., FCTUC)	80
M ^a Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
M ^a Isabel J. Santana	(Associate Prof., FMUC)	40
M ^a Luisa D. Ramos	(Investigator, FCTUC)	80
M ^a Luisa Sá e Melo	(Full Prof., FFUC)	60
M ^a Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
M ^a Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
M ^a Margarida Catalão Castro	(Assistant Prof., FCTUC)	80
M ^a Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
M ^a Otilia Vieira	(Assistant Inv., CNC)	100
M ^a Sancha Santos	(Investigator, FCTUC)	100
M ^a Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
M ^a Teresa Girão da Cruz	(Assistant Inv., CNC)	100
Marília Rocha	(Investigator, HUC)	60
Marlene Maria Tourais Barros	(Assistant Prof., FCTUC)	60
Marta Susana Silva	(Health Tech. IPO, Coimbra)	50
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	60
Paula G. Agostinho	(Investigator, FMUC)	60
Paula Isabel Moreira	(Assistant Prof., FMUC)	40
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Paulo Santos	(Assistant Prof., FCTUC)	80
Raghu Kalluri	(Investigator, HMS)	35
Raimundo Freire	(Investigator, H. U. Canárias, Spain)	Collaborator
Ramiro Almeida	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Renata Silva	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	80
Rosa M. Santos	(Assistant Prof., FCTUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	30
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui Manuel Reis	(Investigator, Univ. Minho)	Collaborator
Rui M. M. Brito	(Associate Prof., FCTUC)	75
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	40

Sandra Maria R. Carvalho Bós	(Assistant Inv., FMUC)	60
Sérgio Simões	(Assistant Prof., FFUC)	80
Silvia Sousa Neves	(Inv. Assistant Prof., FMUC)	40
Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Dinis Silva	(Associate Prof., FFUC)	60
Teresa Gonçalves	(Assistant Prof., FMUC)	50
Teresa Maria C. Martins	(Assistant Investigator, IPO)	80
Tiago Quininha Faria	(Assistant Inv., CNC)	100
Vera Lúcia Dantas Moura	(Manager Science & Tech., UC)	50
Vitor Manuel C. Madeira	(Full Prof., FCTUC)	80

Post-Doc Members

Akhilesh Rai		10
Alexandra Rosa		40
Ana Isabel Duarte		100
Ana Luísa Cardoso		100
Ana Raquel Esteves		100
Ana Rita Araujo dos Santos		100
Bharathi Pandurangan		100
Bruno Manadas		100
Cândida Gonçalves da Silva		100
Carla Nunes		100
Catarina Alexandra Gomes		90
Chakkaravarthi Saravanan		100
Chantal Fernandes		100
Clévio Nóbrega		100
Daniela Pochmann		100
Elisabete Baptista Ferreiro		100
Ermelindo Leal		100
Igor Tiago		100
Joana Salgado		100
João Fernando S. Carvalho		10
João Reina Silva		10
Jorge Valero Gomez-Lobo		100
Licinia J. Simões		100
Ligia Ferreira		100
Liliana Bernardino		30
Liliana Mendonça		100
Luis Miguel Estronca		100
Luisa F. Jordão		50

Manuella Kaster	30
Marco André Coelho das Neves	100
M ^a Alexandra B. Amaral	100
M ^a Isabel Nascimento Ferreira	50
M ^a Teresa Cunha Oliveira	100
Marina Marques Pinto	20
Marta Lima	10
Paula Mota	100
Rosa M. B. Matos Resende	100
Rui Nobre	100
Rui Prediger	100
Sandra Catarina G. Amaral	100
Sara Xapelli	75
Susana Isabel E. Alarico	100
Susana Rosa	100
Tatiana R. Rosenstock	100
Teresa Delgado	100
Vilma A. Oliveira	100

PhD Students

Adalberto Alves de Castro	100
Ana Branco M. Tiago	100
Ana Catarina Morouço Ferreira	50
Ana Cristina F. Lemos	100
Ana Cristina Gonçalves	40
Ana Cristina Gregório	100
Ana Carolina Moreira	100
Ana Catarina R. Graça Fonseca	100
Ana Cristina R. Silva	100
Ana Filipe Branco	100
Ana Francisca Lima	100
Ana Francisca Soares	100
Ana Inês R. Crespo	100
Ana Isabel Serralheiro	100
Ana M. Metelo	100
Ana M ^a Sequeira Cardoso	100
Ana Patrícia S. Gomes	100
Ana Patrícia Simões	100
Ana Santos Carvalho	100
Ana Sofia C. Valdeira	100

Ana Sofia M. Leal	100
Ana Sofia V. Cunha	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa I. Varela	100
Ana Teresa Rufino	100
Ana Teresa Simões	100
André Ferreira Martins	100
André Filipe M. Soares	100
Ângela Fernandes	100
Ângela Inácio	100
Ângela Pascoal Crespo	100
António Sales Mano	100
Bárbara Rocha	100
Beatriz Lacerda de Sousa	100
Bruno Miguel F. Gonçalves	100
Carla M ^a Nunes Lopes	100
Carla Patrícia R. Paiva	100
Carlos Adriano Matos	100
Carlos Fernando D. Rodrigues	100
Carlos José Vieira Simões	25
Carlos Manuel Melo	100
Carlos Samuel M. Boto	100
Carolina Coelho	15
Cassilda Pereira	100
Catarina Mendes Morais	100
Catarina Sofia H. Jesus	50
Cátia Diogo	100
Cátia Marques	100
Cátia Moreira de Sousa	100
Clarissa Schitine	25
178 Cláudia Sofia Alves Pereira	100
Cristina Carvalho	100
Cristiana Paulo	80
Cristina Barosa	100
Daniel Rial	100
Daniel F. Santos	100
Daniela Gonçalves	100
Daniela Luis	100
Daniela M. Arduíno	100
Daniela Pereira S. Alho	100
David Dias	100

Diana Dinis Azenha	100
Diana Jurado S. Serra	100
Diana F. Silva	100
Diana Margarida Carvalho	100
Dulce Bento	100
Elisabete Oliveira Augusto	100
Eva Carolina Serrão	40
Fátima Martins	100
Filipa L. Carvalho	100
Filipa Lebre	100
Filipe Coreta Gomes	40
Filipe Duarte	100
Filomena Grilo da Silva	100
Francisco Manuel Queiroz	100
Gabriel Nascimento Costa	100
Gonçalo Pereira	100
Graciana Tribuna	50
Graciano da Silva Leal	100
Helena Carvalheiro	100
Helena Leitão	100
Helena Vazão	100
Hugo Alves Figueiredo	100
Humberto Gomes Ferreira	100
Inês Biscaia Barbosa	100
Inês Vasconcelos M. Santos	100
Inês Violante	10
Isabel Maria Santos Onofre	100
Ivana Kostic	100
Jessié Martins Gutierrez	100
Jimmy George	100
Joana Balsa Silva	100
Joana Bicker	100
Joana Domingues Vindeirinho	100
Joana Filipa C. Fernandes	100
Joana Filipa D. Neves	100
Joana Isabel Ferreira	100
Joana I. Real	100
Joana Liberal	50
Joana Paixão	100
Joana Pedro	100
Joana Ribeiro Guedes	100
Joana Sousa	100

Joana Teresa Gonçalves	20
João Abrantes	100
João André Duarte	15
João André Freitas	10
João Demétrio B. Martins	100
João Filipe Martins	10
João Correia Teixeira	100
João Teodoro	100
João Trigueiro Costa	100
Jorge Manuel Ruivo	100
Kátia Mesquita	100
Liane Moura	100
Ligia Gomes da Silva	100
Ludgero C. Tavares	100
Luís André A. França	100
Luís Ribeiro	100
Lisa Rodrigues	90
Luana Naia	100
Magda Santana	100
Márcio José C. Ribeiro	100
Márcio Rodrigues	100
Marco António P. Matos	100
M ^a Francisca Eiriz	100
M ^a Inês Pinto Coelho	100
M ^a Inês Morte	100
M ^a Joana G. Pinto	100
M ^a João Rodrigues Pereira	50
M ^a Ia Salete J. Baptista	100
Maria Nunes Pereira	100
Mariana Botelho da Rocha	100
Mariana Freitas	100
Mariana Oliveira Conceição	100
Mariana Ponte C. Ribeiro	100
Marília Henriques Cordeiro	100
Mário Laço	100
Marta Daniela Passadouro Caetano	100
Marta Isabel D. Mota Vieira	100
Marta Isabel Rodrigues Baptista	100
Marta Regina Santos do Carmo	100
Michela Comune	100
Michelle Stumpf Viegas	100
Miguel Maria Lino	100

Miranda Melle	100
Nélio Gonçalves	100
Nuno Ferreira	100
Nuno Fonseca	100
Nuno Gabriel Machado	100
Nuno Miguel Jesus Machado	100
Patricia Henriques Domingues	100
Patricia Lopes	100
Patricia Raquel Pereira	100
Paulo Santos	100
Paulo Gameiro Guerreiro	100
Pedro Alexandre Martins	100
Pedro Coxito	100
Pedro João Madeira Afonso	100
Pedro José Gouveia	100
Pedro Manuel Batista Branco	100
Pedro Manuel V. Garção	100
Pedro Miguel Costa	100
Raquel Alves	100
Renata Gomes	100
Renata Santos Tavares	100
Renato Xavier C. Santos	100
Ricardo Romão Leão	25
Ricardo Santos	100
Rita Perfeito	100
Rodrigo Luiz Santos	100
Rui Benfeitas Vicente	100
Rui M. Costa Soares	5
Rui Cruz	100
Sandra Isabel F. Mota	100
Sandra Cristina Jesus	100
Sandra M. Almeida Santos	100
Sandra Sofia Rebelo	100
Sandro Pereira	100
Samira C. Ferreira	100
Sara Rute C. Figueiredo	100
Sara Tavares M. Lima	100
Sara Trabulo	50
Sezin Aday	100
Silvia Viana Silva	60
Stefania Zappettini	100
Sofia Marques Ribeiro	100

Sofia Morais Grade	100
Sónia Correia	100
Sónia Patricia Duarte	100
Sueli Cristina Marques	100
Susana Cardoso	100
Susana Patrícia S. Pereira	100
Susana Ribeiro Louros	100
Swarna Pandian	100
Tiago Alexandre Sousa Santos	100
Tatiana Catarino	100
Teresa Serafim	100
Tiago Alfaro	100
Vanessa Isabel S. Mendes	100
Vera Lúcia G. Francisco	100
Vitor Gonçalo Silva C. Mendes	100
Zaida Almeida	75

MSc Students

Adriana Branco	100
Albert Rafels Ybern	30
Ana Cristina V. Ferreira	100
Ana Isabel Plácido Fernandes	100
Ana Isabel Reis Santos	100
Ana Margarida Alves de Oliveira	100
Ana Rita Fonseca	100
Ana Sofia Lourenço	100
Andreia Lamaroso	100
Andreia Marques Gomes	100
Andreia Sousa	100
Anna Vladimirovna Pliássova	100
Bárbara Sofia Lourenço	100
Carla Marina Gomes	100
Carolina Noronha	100
Catarina Rebelo	100
Cátia Machado Melo	100
Cindy Rodrigues	5
Cláudia Maria C. Deus	100
Cláudia Saraiva	100
Deolinda Santinho	50
Diana Raposo	100

Diana Matias	100
Dina Farinha	100
Diogo Martins Branco	10
Dominique Fernandes	100
Elisabete Priscila Ferreira	100
Fábia Sofia Vicente	100
Filipa C. Passos	100
Filipa Isabel F. Barradas	100
Gladys Tarcilia Lima Caldeira	100
Hélia Filipa B. Jeremias	100
Henrique Carvalho	100
Isabel Ferreira	100
Isaura Vanessa Martins	100
Ismael Neiva	25
Ivan Lalanda Salazar	100
Joana Cristina J. Viegas	100
Joana Cristina Pedro Rodrigues	100
João Fonseca	100
Jorge Filipe Pascoal	100
João Silva	100
Luís Pedro Leitão	100
Margaux Laura Matias	100
M ^a Inês R. Sousa	100
Mariana Val	100
Marta Ereira Mota	15
Marta Falcão Estrada	100
Neuza Silva Domingues	100
Patrícia Guarino	100
Patricia Sofia Morais	100
Pedro Miguel S. Carreiras	75
Pedro Daniel Rio	100
Ricardo Gaspar	100
Rui Filipe Carvalho	100
Rui Pedro Lopes	60
Sara Matias C. Silva	100
Sílvia Catarina F. Gomes	100
Susana Filipa P. Sampaio	100
Tânia Jesus Leandro	100
Tânia Marisa Perestrelo	100
Tiago Soares Silva	100
Vanessa Machado	100

Grant Technician

Alexandra Isabel Abrunheiro	100
Ana Cristina F. Lemos	100
Ana Filipa d'Avó	100
Ana Maria P. Silva	100
Ana Rita Gonçalves	100
Ana Rita M. Leal	100
Ana Sofia L. Coelho	100
Branca M. Silva	100
Bruno Carreira	100
Carla Sofia G. Silva	100
Caroline Delgado Veloso	100
Catarina Sofia Pimentel	25
Célia Aveleira	100
Daniel Espinoza	50
David José Botequim	10
Elda Bonifácio	100
Emanuel Candeias	100
Filipe Alberto Teixeira	100
Joana Barra	100
José Miguel J. Paiva	100
Liliana Freitas Antunes	100
Mafalda Matos	100
Marcelo Francisco R. Rodrigues	100
Patrícia Celeste S. Rebelo	100
Pedro Joaquim Cruz	15
Pedro Tiago C. Curto	100
Raquel Ferreira	100
Ricardo Jorge Pais	25
Rui Oliveira Costa	100
Rute Marisa A. Loureiro	100
Sara Monteiro Lopes	100
Sofia Isabel Oliveira Sousa	100
Sónia Neto R. Pereira	100
Telma Sofia Bernardo	100
Vanessa R. Anjos	100
Vera Grandão Cortez	100
Vera Patricia Gonçalves	50

MD

António Macedo

Collaborator

Cristina Januário

Collaborator

Herminio José T. Espírito Santo

30

Luis Cunha

Collaborator

Luisa Diogo

Collaborator

M^a Margarida Martins Gonçalo

20

Maria Olinda R. Rebelo

30

SERVICE STAFF

		Time % at CNC
Ana Cristina F. Barbosa Soares	(Graduate Technician, CNC)	100
Ana Cristina Franco dos Santos	(Graduate Technician, CNC)	100
António José Azinhaga Teles Grilo	(Graduate Technician, CNC)	100
Cândida Elsa Frias Mendes	(Graduate Technician, CNC)	100
Carla Margarida dos Santos Verissimo	(Graduate Technician, CNC)	100
Emeric Wasielewski	(Graduate Technician, CNC)	100
João Miguel Pratas	(Graduate Technician, CNC)	100
Luciana C. Albuquerque Pinto	(Graduate Technician, CNC)	100
Magno Delmiro Garcia	(Graduate Technician, CNC)	100
M ^ª Helena Garrucho Ribeiro	(Graduate Technician, HUC)	20
Maria João Ferreira Canas dos Santos	(Graduate Technician, CNC)	100
Marta Sofia Marques Simões	(Graduate Technician, CNC)	100
Mónica Alexandra V. Serrano	(Graduate Technician, CNC)	100
Paulo Rodrigues-Santos	(Graduate Technician)	50

TECHNICAL STAFF

		Time % at CNC
Alexandre Simão Vieira Pires	(Graduate Technician, CNC)	100
Cármén Lídia Graça Semeão	(Graduate Technician, CNC)	100
Diana dos Santos Simões Graça	(Technician, CNC)	100
Fátima Cristina dos S. Carvalho Graça	(Technician, CNC)	100
Isabel Conceição Calado Esteves Costa	(Technician, CNC)	100
Isabel Nunes Correia	(PhD, Graduate Technician,	100
Isabel Dantas Fernandes	(Graduate Technician, CNC)	100
Luisa Leitão Cortes	(PhD, Graduate Technician,	100
Maria do Céu Mendes Gomes	(Technician, CNC)	100
Maria Isabel Gonçalves	(Technician, CNC)	100
Maria Eugénia A. Silva Lopes Campos	(Technician, CNC)	100
Vera Mónica M. Mendes	(Technician, CNC)	100
Virginia Maria R. Ferreira Fonseca	(Technician, CNC)	100
Maria da Rosário da Costa Faro	(Graduate Technician, CNC)	100
Odete Pereira Cabanelas	(Graduate Technician, CNC)	100
Sandra Manuela Domingues dos Santos	(PhD, Graduate Technician,	100
Sara da Costa Jordão A. Lopes	(Technician, CNC)	100
Telma Patrícia Simões Graça	(Technician, CNC)	100

ADMINISTRATIVE STAFF

		Time % at CNC
Carla Lopes Rodrigues	(Administrative Assistant, CNC)	100
Catarina Alexandra Ferreira Gomes	(Graduate Administrative, CNC)	100
Elisabete Cosmos dos Santos Machado	(Graduate Administrative, CNC)	100
Heidi Maria da Silva Lopes Gonçalves	(Graduate Administrative, CNC)	100
Lia da Costa Jordão Aparício Lopes	(Graduate Administrative, CNC)	100
Mónica Alexandra Rodrigues Morais	(Graduate Administrative, CNC)	100
Nilza Clara F. Marques Manadas	(Administrative Assistant, CNC)	100
Rosa Alexandra Folhas Fernandes	(Graduate Administrative, CNC)	100
Sandra Cristina Santos Luis	(Graduate Administrative, CNC)	100
Sílvia Marisa Esteves de Sousa	(Graduate Administrative, CNC)	100
Susana Adelaide Rocha da Silva	(Administrative Assistant, CNC)	100
Tatiana de Azevedo Paula	(Graduate Administrative, CNC)	100

Research Staff and Students | Research Área

Neuroscience and Disease

Catarina Resende Oliveira, MD, PhD, Coordinator

Members holding PhD		Time % at CNC
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Silva Martins	(Assistant Inv., FMUC)	10
Ana Rita Costa Álvaro	(Inv. Assistant Prof., UTAD)	60
Ângelo Tomé	(Assistant Prof., FCTUC)	60
António F. Ambrósio	(Investigator, FMUC)	10
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	60
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Attila Köfalvi	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	80
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	80
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	40
Emília P. Duarte	(Assistant Prof., FCTUC)	80
Fabienne Agasse	(Assistant Inv., CNC)	60
Geanne Matos de Andrade	(Associate Prof., Brasil)	30
Henrique Bernardo Silva	(Assistant Inv., CNC)	100
Ildete Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Araújo	(Assistant Prof., UALG)	20
Inês Esteves Baldeiras	(Investigator, FMUC)	20
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	30
João O. Malva	(Principal Inv., FMUC)	100
João Peça-Silvestre	(Post-Doc Fellow, MIT)	35
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Margarida Vaz Caldeira	(Inv. Assistant Prof., FCTUC)	80
M ^a do Rosário Almeida	(Assistant Inv., CNC)	100
M ^a Cristina Januário dos Santos	(Inv., Assistant Prof., FMUC)	20
M ^a Isabel J. Santana	(Associate Prof., FMUC)	40

M ^a Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
M ^a Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Paula G. Agostinho	(Investigator, FMUC)	60
Paula Isabel Moreira	(Assistant Prof., FMUC)	40
Paulo Santos	(Assistant Prof., FCTUC)	80
Ramiro Almeida	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	80
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	40
Sandra Maria R. Carvalho Bós	(Investigator, FMUC)	60

Post-Doc Members

Alexandra Rosa		40
Ana Isabel Duarte		100
Ana Raquel Esteves		100
Ana Rita Araújo Santos		100
Catarina Alexandra Gomes		100
Daniela Pochmann		100
Elisabete Baptista Ferreiro		100
Elsa Lamy		50
Joana Rosmaninho-Salgado		100
Jorge Valero Gomez-Lobo		100
Liliana Bernardino		30
Manuella P. Kaster		30
M ^a Teresa Cunha Oliveira		100
Marina Marques Pinto		20
Rosa M. B. Matos Resende		100
Rui Prediger		100
Sara Xapelli		75
188 Tatiana R. Rosenstock		100

PhD Students

Adalberto Alves de Castro		100
Ana Catarina Ribeiro G. Fonseca		100
Ana Cristina Figueiredo Lemos		100
Ana Cristina R. Silva		100
Ana Patrícia Simões		100
Ana Santos Carvalho		100

Carla Maria Nunes Lopes	100
Carlos Adriano Matos	100
Clarissa Schitine	25
Cristina Carvalho	100
Daniel F. Santos	100
Daniel Rial	100
Daniela Luis	100
Daniela M. Arduino	100
Diana F. Silva	100
Elisabete Oliveira Augusto	100
Francisco Manuel Queiroz	100
Gabriel Nascimento Costa	100
Graciano da Silva Leal	100
Helena Maria Carvalheiro	100
Jessié Gutierrez	100
Jimmy George	100
Joana Domingues Vindeirinho	100
Joana Isabel Ferreira	100
Joana Filipa C. Fernandes	100
Joana Pedro	100
Joana Teresa Gonçalves	20
João Filipe da Costa Martins	10
João Trigueiro Costa	100
Luana Naia	100
Luis Filipe da Silva Ribeiro	100
Magda Santana	100
Márcio José C. Ribeiro	100
Marco António P. Matos	100
M ^a Francisca Eiriz	100
M ^a Inês Coelho	100
M ^a Inês Morte	100
M ^a Joana Guimarães Pinto	100
Mariana Botelho Rocha	100
Mário Laço	100
Marta Isabel D. Mota Vieira	100
Marta Regina Santos Carmo	100
Miranda Melle	100
Nuno Miguel Jesus Machado	100
Pedro João Madeira Afonso	100
Paulo Santos	100
Pedro Manuel V. Garção	100
Renato Santos	100

Rita Perfeito	100
Samira C. Ferreira	100
Sandra Isabel F. Mota	100
Sandra Sofia Rebelo	100
Silvia Viana Silva	60
Sofia Morais Grade	100
Sónia Correia	100
Stefania Zappettini	100
Sueli Cristina Marques	100
Susana Cardoso	100
Susana Ribeiro Louros	100
Swarna Pandian	100
Tatiana Catarino	100
Tiago Alexandre Sousa Santos	100
Tiago Alfaro	100

MSc Students

Ana Isabel Plácido Fernades	100
Ana Isabel Reis Santos	100
Ana Margarida Alves de Oliveira	100
Ana Sofia Lourenço	100
Anna Vladimirovna Pilássova	100
Diana Raposo	100
Diogo Martins Branco	10
Dominique Moreira Fernandes	100
Fábia Sofia B. Vicente	100
Gladys Tarcilia Lima Caldeira	100
Isaura Vanessa Martins	100
Ismael Neiva	25
Ivan Lalanda Salazar	100
Joana Cristina Pedro Rodrigues	100
Jorge Filipe Pascoal	100
Luis Pedro Leitão	100
Marta Falcão Estrada	100
Pedro Daniel Rio	100
Silvia Catarina F. Gomes	100
Tânia Perestrelo	100
Tiago Soares Silva	100
Vanessa Machado	100

Grant Technician

Bruno Carreira	100
Carla Sofia Gomes Silva	100
Caroline Delgado Veloso	100
Catarina Sofia Pimentel	25
Célia Avelreira	100
Emanuel Candeias	100
Filipe Alberto Teixeira	100
Patricia Celeste S. Rebelo	100
Raquel Ferreira	100
Rui Oliveira Costa	100
Sofia Isabel Oliveira Sousa	100
Vera Grandão Cortez	100

MD

António Macedo	Collaborator
Luís Cunha	Collaborator
Lúisa Diogo	Collaborator

Molecular Biotechnology and Health

Euclides Pires, PhD, Coordinator

Members holding PhD		Time % at CNC
Alcino Jorge Lopes Leitão	(Assistant Prof., FFUC)	60
Amílcar Falcão	(Full Prof., FFUC)	80
Ana Cristina Fortuna	(Inv. Assistant Prof., FFUC)	80
Anabela Maduro de Almeida	(Assistant Prof., Univ. Vasco Gama)	80
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Carlos Faro	(Associate Prof., FCTUC)	60
Daniela Cipestre Vaz	(Assistant Prof., Inst. Polit. Leiria)	100
Elsa Henriques	(Investigator, CNC)	100
Euclides Pires	(Associate Prof., FCTUC)	60
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	10
Henrique Faneca	(Assistant Inv., CNC)	100
Isaura Simões	(Assistant Inv., CNC)	100
João Nuno Moreira	(Assistant Prof., FFUC)	80
Jorge António R. Salvador	(Associate Prof., FFUC)	60
Lino Ferreira	(Assistant Inv., CNC)	100
Luís Pereira Almeida	(Assistant Prof., FFUC)	80
Manuel Garrido	(Investigator, Genibet)	80
M ^a Amália Jurado	(Assistant Prof., FCTUC)	80
M ^a Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
M ^a Luísa Sá e Melo	(Full Prof., FFUC)	60
M ^a Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Marília Rocha	(Investigator, HUC)	60
Marlene Maria Tourais Barros	(Assistant Prof., FCTUC)	60
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	60
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Raghu Kalluri	(Investigator, HMS)	35
Renata Dias da Silva	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Rui M. M. Brito	(Associate Prof., FCTUC)	60
Sérgio Simões	(Assistant Prof., FFUC)	80
Tiago Quinhina Faria	(Assistant Inv., CNC)	100
Vera Lúcia Dantas Moura	(Manager Science & Tech., UC)	50

Post-Doc Members

Akhilesh Rai	10
Ana Luísa Cardoso	100
Bruno Manadas	100
Bharathi Pandurangan	100
Cândida Gonçalves da Silva	100
Chakkaravarthi Pandurangan	100
Clévio Nóbrega	100
João Fernando S. Carvalho	10
João Reina Silva	10
Lígia Maria S. Ferreira	100
Liliana Mendonça	100
M ^a Isabel N. Ferreira	50
Marco André Coelho das Neves	100
Marta Filipa Lima	10
Rui Nobre	100
Susana Rosa	100

PhD Students

Ana Cristina Gregório	100
Ana Francisca Lima	100
Ana Isabel Serralheiro	100
Ana Maria Cardoso	100
Ana Sofia C. Valdeira	100
Ana Sofia Mendes Leal	100
Ana Teresa Simões	100
André Filipe M. Soares	100
193 Ângela Valério-Fernandes	100
António Sales Mano	100
Bruno Miguel F. Gonçalves	100
Carlos José Vieira Simões	25
Carlos Samuel M. Boto	100
Catarina Mendes Morais	100
Catarina Sofia H. Jesus	50
Cátia Moreira de Sousa	100
Cristiana Paulo	100
Daniela Gonçalves	100
Daniela Pereira S. Alho	100

Dulce Marisa Bento	100
Eva Carolina Serrão	40
Filipa Lebre	100
Graciana Tribuna	50
Helena Vazão	100
Inês Vasconcelos Miranda Santos	100
Isabel Maria Santos Onofre	100
Ivana Kostic	100
João André Freitas	10
João Abrantes	100
Joana Bicker	100
Joana Filipa Neves	100
Joana Ribeiro Guedes	100
Joana Sousa	100
Jorge Manuel Ruivo	10
Ligia Gomes da Silva	100
Márcio Rodrigues	100
M ^a de la Salete J. Baptista	100
Maria Nunes Pereira	100
Mariana Conceição	100
Marta Daniela Passadouro Caetano	100
Michela Comune	100
Miguel Maria Lino	100
Nélio Gonçalves	100
Nuno Fonseca	100
Patrícia Raquel Pereira	100
Pedro Alexandre Martins	100
Pedro José Gouveia	100
Pedro Manuel Batista Branco	100
Pedro Miguel Costa	100
Renata Gomes	100
Ricardo Romão Leão	25
Rui Cruz	100
Rui Benfeitas Vicente	100
Sandra Cristina Jesus	100
Sandra Marina A. Santos	100
Sara Trabulo	50
Sezin Aday	100
Sónia Patricia Duarte	100
Vanessa Isabel S. Mendes	100
Zaida Catarina L. Almeida	75

MSc Students

Albert Rafels Ybern	30
Ana Cristina V. Ferreira	100
Carla Marina Gomes	100
Catarina Rebelo	100
Cláudia Saraiva	100
Dina Farinha	100
Filipa Isabel F. Barradas	100
Hélia Filipa B. Jeremias	100
Maragaux Laura Matias	100
Pedro Miguel S. Carreiras	75
Ricardo Gaspar	100
Sara Matias C. Silva	100

Grant Technician

Ana Rita M. Leal	100
Ana Sofia L. Coelho	100
David José Botequim	10
José Paiva	100
Liliana Freitas Antunes	100
Mafalda Santos	100
Pedro Joaquim Cruz	15
Pedro Curto	100
Ricardo Jorge Pais	25
Vanessa Rebelo Anjos	100

Cell and Molecular Toxicology

Leonor Almeida, PhD, Coordinator

Members holding PhD Time % at CNC

Ana Ledo	(Assistant Inv., CNC)	100
Anabela P. Rolo	(Assistant Inv., CNC)	100
Carlos M. Palmeira	(Associate Prof., FCTUC)	80
Ignacio Vega-Naredo	(Assistant Inv., CNC)	100
João Laranjinha	(Associate Prof., FFUC)	60
José Custódio	(Associate Prof., FFUC)	80
Leonor Almeida	(Full Prof., FFUC)	60
M ^a Carmen Alpoim	(Associate Prof., FCTUC)	60
Maria S. Santos	(Investigator, FCTUC)	80
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	30
Teresa Dinis Silva	(Associate Prof., FFUC)	60

Post-Doc Members

Carla Nunes	100
Vilma Sardão Oliveira	100

PhD Students

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Ana Carolina Moreira	100
Ana Filipe Branco	100
Ana Francisca Soares	100
Ana Patricia S. Gomes	100
Ana Teresa I. Varela	100
Bárbara Rocha	100
Carlos Rodrigues	100
Cassilda Pereira	100
Cátia Diogo	100
Cátia Marques	100
Cláudia Sofia Alves Pereira	100

Diana Jurado S. Serra	100
Filipa Libório Carvalho	100
Filipe Duarte	100
Filomena Grilo da Silva	100
Gonçalo Pereira	100
Inês Biscaia Barbosa	100
Joana Paixão	100
João Teodoro	100
Kátia Almeida Mesquita	100
Ludgero Tavares	100
Mariana Ponte Cardoso Ribeiro	100
Nuno Ferreira	100
Nuno Gabriel Machado	100
Paulo Gameiro Guerreiro	100
Ricardo Santos	100
Sandro Pereira	100
Sofia Marques Ribeiro	100
Susana S. Pereira	100
Teresa Serafim	100

MSc Students

Ana Rita Fonseca	100
Cláudia Maria Loureiro	100
Elisabete Priscila Ferreira	100
Joana Cristina Viegas	100
João Fonseca	100
Mariana Val	100
Susana Filipa Sampaio	100

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Grant Technician

Ana Cristina Lemos	100
Ana Maria P. Silva	100
Marcelo Francisco Rodrigues	100
Rute Marisa A. Loureiro	100
Sara Monteiro Lopes	100
Sónia Neto R. Pereira	100
Telma Bernardo	100

Microbiology

Milton Costa, PhD, Coordinator

Members holding PhD Time % at CNC

António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Joana Cardoso Costa	(Inv. Assistant Prof., FCTUC)	80
M ^a Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Teresa Gonçalves	(Assistant Prof., FMUC)	40

Post-Doc Members

Chantal V. Fernandes	100
Igor Clemente Tiago	100
Susana Isabel E. Alarico	100

PhD Students

Ana Branco M. Tiago	100
Ana Catarina M. Ferreira	50
Ana Luisa N. Gomes Nobre	100
Ana Sofia V. Cunha	100
Carolina Coelho	15
Lisa Oliveira Rodrigues	90
Luis André A. França	100
Rui Costa Soares	5
Vitor Gonçalo Silva C. Mendes	100

MSc Students

Andreia Lamarouso	100
Cindy Rodrigues	5
Filipa Calçada de Passos	100
Marta Ereira Mota	15
Tânia Jesus Leandro	100

Grant Technician

Alexandra M. Abrunheiro	100
Ana Filipa Neves d'Avó	100
Branca Silva	100

Biophysics and Biomedical NMR

Carlos Geraldes, PhD, Coordinator

Members holding PhD Time % at CNC

Carlos G. Geraldes	(Full Prof., FCTUC)	80
Célia M. Antunes	(Assistant Prof., FCTUC)	80
Ivana Jarak	(Assistant Inv., CNC)	100
John Griffith Jones	(Principal Inv., CNC)	100
Luis M. Rosário	(Associate Prof., FCTUC)	80
Manuela Carvalheiro	(MD, HUC)	15
M ^a Luisa D. Ramos	(Investigator, FCTUC)	80
M ^a Margarida Catalão Castro	(Assistant Prof., FCTUC)	80
Rosa M. Santos	(Assistant Prof., FCTUC)	60

Post-Doc Members

Elsa Lamy	50
Licinia J. Simões	100
Teresa Delgado	100

PhD Students

Ana M. Metelo	100
André Martins	100
Cristina Barosa	100
David Gaspar Dias	100
Fátima Martins	100
Filipe Coreta Gomes	40
Helena Leitão	100
Hugo Alves Figueiredo	100
Inês Violante	10
Joana I. Real	100
João André Duarte	15
João Miguel C. Teixeira	100
Pedro Coxito	100
Sara Figueiredo	100

MSc Students

Adriana Branco	100
Andreia Raquel Sousa	100
Cátia M. Melo	100
Henrique Carvalho	100
Neuza Silva Domingues	100
Rui Silva Carvalho	100
Rui Pedro Lopes	60

Grant Technician

Ana Rita Gonçalves	100
Joana Barra	100

Cell and Development Biology

M^a Celeste Lopes, PhD, João Ramalho Santos, PhD, Coordinators

Members holding PhD Time % at CNC

Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Ali Mobasher	(Investigator, Univ. Nottingham, UK)	Collaborator
Ana Bela Sarmiento Ribeiro	(Assistant Prof., FMUC)	40
Ana Luísa Carvalho Vital	(Health Tech., HUC)	40
Ana Paula Marques de Sousa	(Investigator, HUC)	50
Anália do Carmo	(Assistant Prof., Univ. Vasco Gama)	100
Andrea Cooper	(Investigator, Trudeau Inst., EUA)	Collaborator
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Bruno Miguel das Neves	(Assistant Prof., Univ Aveiro)	50
Cármen Garcia-Rodriguez	(Investigator, Univ. Valladolid, Spain)	Collaborator
Eugénia Carvalho	(Assistant Inv., CNC)	100
Fernando Monteiro Judas	(Assistant Prof., FMUC)	25
Fran Lund	(Investigator, Rochester Univ., EUA)	Collaborator
João Ramalho Santos	(Associate Prof., FCTUC)	80
José Alberto Correia e Vale	(MD, Univ. Salamanca)	Collaborator
Manuel Aureliano Alves	(Associate Prof., Univ. Algarve)	Collaborator
M ^a Celeste Lopes	(Full Prof., FFUC)	80
M ^a Dolores T. Redondo	(Investigator, Univ. Salamanca)	Collaborator
M ^a Otilia Vieira	(Assistant Inv., CNC)	100
M ^a Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
Marta Susana Silva	(Health Tech., IPO, Coimbra)	50
Raimundo Freire	(Investigator, H. U. Canárias, Spain)	Collaborator
Rui Manuel Reis	(Investigator, Univ. Minho)	Collaborator
202 Silvia Sousa Neves	Assistant Prof., FMUC)	40
Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Maria C. Martins	(Assistant Inv., IPO)	80

Post-Doc Members

Ermelindo Leal	100
Luis Miguel Estronca	100
Luisa Jordão	50
M ^a Alexandra B. Amaral	100

Paula Mota	100
Sandra Catarina G. Amaral	100

PhD Students

Ana Cristina Gonçalves	40
Ana Inês R. Crespo	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ângela Inácio	100
Ângela Pascoal Crespo	100
Beatriz Lacerda de Sousa	100
Carla Patrícia R. Paiva	100
Carlos Manuel Melo	100
Diana Diniz Azenha	100
Diana Margarida Carvalho	100
Humberto Gomes Ferreira	100
Joana Balça Silva	100
Joana Liberal	50
João Demétrio B. Martins	100
Liane Moura	100
M ^a João R. Pereira	100
Mariana Freitas	100
Marília Henriques Cordeiro	100
Marta Isabel Rodrigues Baptista	100
Michelle Stumpf Viegas	100
Patrícia Henriques Domingues	100
Patrícia Lopes	100
Raquel Alves	50
Renata Santos Tavares	100
Rodrigo Luiz Santos	100
Sara Tavares M. Lima	100
Vera Lúcia G. Francisco	100

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MSc Students

Andreia Marques Gomes	100
Bárbara Sofia Lourenço	100
Deolinda Santinho	100

Diana Isabel Matias	100
Isabel Ferreira	100
João Silva	100
M ^a Inês R. Sousa	100
Patrícia Guarino	100
Patrícia Sofia Morais	100

Grant Technician

Daniel Espinoza	100
Elda Bonifácio	100
Vera Patrícia Gonçalves	100

MD

Herminio José T. Espírito Santo	30
Maria Olinda R. Rebelo	30
M ^a Margarida Martins Gonçalo	40



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