Annual Report | 2012

Center for Neuroscience and Cell Biology
Associate Laboratory

A New Culture Through Scientific Research

CNC
CENTER FOR NEUROSCIENCE AND CELL BIOLOGY
UNIVERSITY OF COIMBRA, PORTUGAL

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INTRODUCTION

General Objectives

CNC is a multidisciplinary research Institute, which brings together researchers from various Faculties and affiliated hospitals in the University of Coimbra. In 1990 CNC was recognized by FCT as a *Laboratório Associado* with the major mission of fostering fundamental and translational research and advanced training in biomedical sciences with a particular focus in neurosciences.

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 to provide specialized services to the community; 4) Outreach Programme (science and society).

The scientific productivity of CNC is demonstrated by an annual average of 1677 publications in peer reviewed journals in the last eleven years, an effort supported by 486 grant projects achieved in competitive calls. In 2012, 237 scientific papers were published and 28 new research projects were financed (22 FCT projects, 2 national projects and 5 international projects).

The core scientific activity of CNC is the study of the molecular basis of degenerative processes common to aging and neurodegenerative disorders. In parallel, several groups explore mechanisms of neuroprotection and regeneration, which may be future candidates for the development of potential therapeutic strategies. 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.

Post-graduate education is a major goal at CNC. The Doctoral Programme in Experimental Biology and Biomedicine (PDBEB) and the participation in the MIT/Portugal Doctoral Programme provide Master and PhD students with a multi-faceted education in molecular life sciences related to disease and contribute to international scientific networking.
Development of new technologies routed 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 research institute in the field of biotechnology, the CNC-Biotech Institute at BIOCANT. Research performed in this Institute is crucial to promote technology transfer and the creation of novel biomedical and biotechnology enterprises, which is one of the aims of CNC at BIOCANT Park.

The Outreach programme, the fourth current aim of CNC, aims at society scientific education and public perception of the importance of science for human health. To reach this goal, specific scientific programmes continued to be implemented in collaboration with schools and several social and cultural associations.

Future plans of CNC for the next two coming years include the reinforcement and expansion of the ongoing competitive basic research focused on the molecular mechanisms of neurodegeneration, neuroprotection, neurogenesis and brain repair, from the cellular level to in vivo animal models, as specified in each group research plan in this Annual Report. Perform high quality research, with international impact in fundamental cellular and molecular neuroscience and mechanisms of brain disease, is a common goal of most of the groups, some of which are currently working in the borderline between basic and applied research. Pushing forward some translational research approach to boost the development of high quality translational research in Neuroscience is one of the aims in a near future. Promoting internal collaborations between groups working in different areas at CNC will allow using biocompatible carriers for drug and gene delivery, such as viral vectors, molecular biology and proteomics approaches and the use of new sensors and electrodes to study brain function. Simultaneously, in the area of Biotechnology, the development of cutting-edge research projects, namely in the areas of stem cells and computational biology, allowing interdisciplinary approaches, will lead to innovation and to the increase of research projects of excellence. Post-graduate programmes will continue in the next coming years. Besides the CNC PhD Programme (PDBEB), CNC is a partner in the European Master Program (Neurasmus) and the European PhD Programme developed under the scope of ENC Network, as well as the MIT-Portugal Programme.

Technology transfer programme will strongly benefit with the "CNC Biotech – Investigação em Biotecnologia e capacitação do sector empresarial" project, which will be carried out in the Biotechnology unit at Biocant-Park. The scientific activity of this unit is established to start on the last 2013 trimester.

In what it concerns the Outreach Programme, the strong collaboration that exists with "Ciência Viva", "Instituto de Educação e Cidadania"(IEC) and several high schools will be maintained, and steady extended to other institutions.

CNC will pursue its involvement as a partner of MIT-Portugal and HMS-Portugal programs and a founder member of Health Cluster Portugal (HCP).

The 2012 Annual Report highlights the CNC accomplishments and the contribution of its dedicated researchers, students, support teams and administrative staff to achieve the main scientific goals of this research Center.

Facts & Figures (2012)

RESEARCH STAFF

Members holding Ph.D.	209
Ph.D.Students	200
MSc Students	12
Grant Technicians	25

PUBLICATIONS

Scientific papers published	237
Scientific papers *In Press*	33

THESIS CONCLUDED

Ph.D. thesis	36
MSc thesis	33

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. The 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 comercial firms – Reagente 5 and ILC.

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"	A. Rodrigues, Leal e Carreira, A. Mourão
"Revisor Oficial de Contas"	Leal e Carreira, Sociedade Revisora de Contas

External Advisory Commitee: Enrique Cadenas (USA); Roberta Brinton (USA); George Perry (USA); Mark Smith (USA); Helmut Sies (Germany); Stephen Zinder (USA).

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 his field of study. In 2012, 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 Morais Cardoso*)

Neuroendocrinology and Neurogenesis Group (*Head: Claudia Cavadas*)

Chronic Inflamation Group (*Head: Mª Margarida Carneiro*)

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*)

Farmacometrics Group (*Head: Amílcar Falcão*)

Bioorganic and Medicinal Chemistry Group (*Head: Maria Luísa Sá e Melo*)

Cell and Molecular Toxicology | *Rui Carvalho*

Mitochondrial Toxicology and Disease Group (*Head: Anabela Pinto Rolo*)

Redox Biology in Health and DiseaseGroup (*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 Grifith Jones)*

Cell and Development Biology | *Celeste Lopes and 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)*

Insuline Resistance and Adipocyte Group (*Head: Eugénia Carvalho)*

RESEARCH ACTIVITY

NEUROSCIENCE AND DISEASE

Coordinator: Catarina Resende de Oliveira

The core research activity of this area is focused on three main issues: 1. understanding of synapses formation and modulation; 2. deciphering the cellular and molecular mechanisms underlying selective neurodegeneration associated to brain disorders; 3. development of neuroprotective and neuroregenerative strategies. The seven groups in this area have been accomplishing these aims: The Glutamatergic Synapses Group,by analysing glutamatergic synaptic modification during development and in response to neuronal activity and the role of mRNA translation in presynaptic differentiation ; The Purines Group focused not only on the modulation of synaptic activity by purines, particularly involving A2A receptors, but also the control of endocannabinoids signalling and CB1 receptor; The Molecular Mechanisms of Disease Group focused on mitochondria dysfunction and inter-organelle cross-talk as a common link in neurodegeneration occurring in Alzheimer's and Parkinson's disease either associated to protein aggregation, microtubule depolymerisation or to type 1 and type 2 diabetes; The Mitochondrial Dysfunction and Signalling in Neurodegeneration Group exploring transcription deregulation linked to mitochondria-driven apoptosis in Huntington's disease; The Neuronal Cell Death and Neuroprotection Group studying excitotoxic neuronal damage in hippocampus and the expression of neurotrophic factors upon neuronal injury; The Neuroendocrinology and Neurogenesis Group devoted to the study of the hypothalamic-adrenal axis and adipose tissue on healthy lifespan and the regulation of endogenous neural progenitor cells proliferation; The Neuroprotection and Neurogenesis in Brain Repair Group centred on the endogenous factors promoting neurogenesis from SVZ neural stem cells.

Major Achievements

The Groups in this Area by using cellular and animal models of disease obtained a vast range of research data, as reported in their individual reports, showing the importance of glutamate receptors modulation in maintaining synaptic integrity and the signalling pathways involving purines, endocannabinoids, growth factors and mitochondria in the identification of new targets for neuroprotection The pro-neurogenic effect of soluble factors secreted by endothelial cells , of growth hormone , NO and inflammatory molecules is also a key issue.

Neuromodulation Group

Rodrigo A. Cunha	PhD – *head of group*
Paula G. Agostinho	PhD
Ângelo José Ribeiro Tomé	PhD
Attila Köfalvi	PhD
Geanne Matos de Andrade	PhD
Ricardo Jorge A. Rodrigues	PhD
Henrique Bernardo Silva	PhD
Rui Daniel Prediger	PhD
Lisiane O. Porciúncula	PhD
Catarina Alexandra Gomes	PhD
Daniela Pochmann	PhD
Ana Margarida Q F Nunes	PhD
Paula M. Canas	PhD
Ana Patrícia Simões	PhD
João Pedro O S P Lopes	PhD
Carolina Melo de Souza	PhD
Daniel Rial	PhD Student
Pedro Manuel V. Garção	PhD Student
Elisabete O. Augusto	PhD Student
Sílvia Viana da Silva	PhD Student
Marco António P. Matos	PhD Student
Nélio da Mota Gonçalves	PhD Student
Samira C. Ferreira	PhD Student
Sílvia Viana da Silva	PhD Student
Tiago Manuel P. Alfaro	PhD Student
Francisco M. Gonçalves	PhD Student
Ana Cristina F. Lemos	PhD Student
Eszter Szabó	PhD Student
Joana Medeiros Marques	PhD Student
Joana Isabel E. B. Real	PhD Student
Marta Regina S. do Carmo	PhD Student
António Manuel C. da Silva	PhD Student
Gonçalo Filipe P. Cristóvão	MSc Student
Anna V. Pliássova	MSc Student
Tiago Emanuel S. Silva	MSc Student
Rui Oliveira Beleza	MSc Student
Caroline Delgado Veloso	Technician

Glutamatergic Synapses Group

Ana Luísa Carvalho	PhD – *head of group*
Ramiro Almeida	PhD
Sandra Santos	PhD
Rui Costa	PhD
Tatiana Catarino	PhD
Joana Ferreira	PhD
Susana Louros	PhD
João Miguel Peça L. Novo	PhD
Luís Ribeiro	PhD Student
Dominique Fernandes	PhD Student
Mariline Silva	PhD Student
Carlos Adriano A. Matos	PhD Student
Joana Pedro	PhD Student
Joni Leeuwen	MSc Student
Maria Joana Pinto	MSc Student
Rodolfo Águas	MSc Student
Pedro Alves	MSc Student
Luís Martins	MSc Student

Neuroprotection and Neurogenesis in Brain Repair Group

João O. Malva	PhD – *head of group*
Jorge Valero	PhD
Francisca Eiriz	PhD

Neuronal Cell Death and Neuroprotection Group

Carlos B. Duarte	PhD – h*ead of group*
Armanda E. Santos	PhD
Emília P. Duarte	PhD
Margarida Vaz Caldeira	PhD
Ana Rita A. Santos	PhD
Graciano Leal	PhD Student
Joana F. C. Fernandes	PhD Student
Pedro João Afonso	PhD Student
João T. Costa	PhD Student
Marta Dias M. Vieira	PhD Student
Miranda Mele	PhD Student
Ivan Salazar	PhD Student
Sara Oliveira	PhD Student

Diogo O. Comprido	PhD Student
Michele Curcio	PhD Student
Luís Miguel Rodrigues	MSc Student

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Ana Cristina Rego	PhD – *head of group*
Ildete Luisa Ferreira	PhD
Mª Teresa Cunha Oliveira	Post-Doctoral Fellow
Tatiana R. Rosenstock	Post-Doctoral Fellow
Elisabete Ferreiro	Post-Doctoral Fellow
Mário Laço	PhD
Rita Perfeito	PhD
Sandra Mota	PhD Student
Márcio Ribeiro	PhD Student
Luana Carvalho Naia	PhD Student
Carla Maria Nunes Lopes	PhD Student
António M. Silva	PhD Student
Ana Catarina H. Oliveira	PhD Student
Ana Cristina Silva	PhD Student
Rita Perfeito	PhD Student
Carolina Noronha	MSc Student
Gladys Caldeira	MSc Student
Joana C Rodrigues	MSc Student
Ana Margarida Oliveira	MSc Student
Paulo André Santos	MSc Student
Susana Cardoso	Collabotator
Sofia Sousa	Collabotator

Molecular Mechanisms of Disease Group

Sandra Morais Cardoso	PhD – *head of group*
Cláudia Mª F. Pereira	PhD
Paula Isabel Moreira	PhD
Ana Isabel Duarte	PhD
Ana Raquel Esteves	PhD
Rosa M. Matos Resende	PhD
Daniela M. Arduíno	PhD
Sónia Correia	PhD
Ana Catarina Fonseca	PhD Student
Cristina Carvalho	PhD Student
Daniel Santos	PhD Student
Diana F.F. Silva	PhD Student
Renato Xavier Santos	PhD Student
Susana Cardoso	PhD Student
Ana Isabel Fernandes	PhD Student
Emanuel Candeias	PhD Student
Diogo Martins Branco	MD

Neuroendocrinology and Neurogenesis Group

Cláudia Cavadas	PhD – *head of group*
Ana Rita Álvaro	PhD
António F. Ambrósio	PhD (Collaborator)
Armando Cristóvão	PhD
Bruno Carreira	PhD
Caetana Carvalho	PhD
Célia Aveleira	PhD
Paulo F. Santos	PhD
Joana R. 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
Ana Patricia Marques	PhD Student

Chronic Inflamation Group

Mª Margarida Carneiro	PhD – *head of group*
Helena Mª Carvalheiro	PhD Student
Mónica Teresa P. Abreu	MSc Student
Tiago R. Sousa	MSc Student
Milene Gonçalves	MSc Student
Andreia Angelo	MSc Student
Ana Xavier	MSc Student
Joana Gomes	MSc Student
Paula Pereira	MSc Student

Neuromodulation Group

Head: Rodrigo A. Cunha

Objectives

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 are committed to exploring the basic properties and function of the neuromodulation systems operated by adenosine and ATP in the nervous system, namely: different features of adenosine and ATP receptors (expression, binding characteristics, coupling to transducing systems, desensitisation), formation and inactivation of ATP and adenosine and physiological roles (control of neurotransmitter release, of ion channels and of synaptic transmission and plasticity). We are now fostering the understanding the role of these neuromodulation systems in physio-pathological conditions using animal models of aging, epilepsy, diabetic neuropathies, Alzheimer's and Parkinson's diseases and, more recently, of psychiatric conditions such as chronic stress, depression and ADHD. 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) and an emergent group is under constitution led by Ricardo Rodrigues.

Main Achievements

1-We first identified and characterised adenosine A2A receptors (A2AR) in the limbic and neocortex and their selective role in the control of synaptic plasticity. We proposed the concept that the coordinated action of A2AR and A1R assist sharpening the salience of information encoding in neuronal circuits. We identified A2AR as the targets for caffeine to prevent memory impairment and afford neuroprotection in animal models of Alzheimer's disease, diabetes, attention deficit and hyperactivity disorder, epilepsy, chronic stress and depression.

2-Another major achievement was the identification of key mechanisms associated with the ability of adenosine A2A 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 A2A receptor antagonists as novel neuroprotective drugs.

3-We advanced the role of ATP as a danger signal in brain diseases since the extracellular levels of ATP are increased upon noxious brain insults and neurodegeneration is attenuated by antagonists of (ATP) P2R namely of P2Y1R, in animal models of epilepsy, ischemia and Alzheimer's disease.

4-We described for the first time that CB1R control mitochondrial bioenergetics. We also found that in human Alzheimer's patients, the endocannabinoid signalling machinery is overactive contributing to synaptic dysfunction and that CB1R are tightly controlled by A2AR in synapses.

Glutamatergic Synapses Group

Head: Ana L. Carvalho

Objectives

Our main objective is to understand at the molecular and cellular level the mechanisms of synapse formation, function and plasticity. We use a combination of primary neuronal cultures, organotypic brain slice cultures and acute slices, molecular biology, biochemistry and cell biology tools to identify novel molecules and processes involved in synaptogenesis, in synaptic activity and in synaptic plasticity. Synaptic dysfunction is thought to underlie disorders such as schizophrenia, autism, mental retardation and addiction. Therefore, strengthening our understanding of the molecular and cellular processes regulating synaptic biology will potentially translate to novel therapeutical approaches for intervention in the diseased brain. Specifically, we are investigating: (i) The role of local protein synthesis in synaptic function/formation and the consequences to synaptic transmission when local protein synthesis is perturbed in the presynaptic site. (ii) The molecular and cellular mechanisms that regulate synaptic strength during synaptic plasticity.

Regulation of glutamatergic neurotransmission; PI: Ana Luísa Carvalho

Glutamate receptors of the AMPA (AMPAR) 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 AMPARs (Santos el al. J. Proteome Res. 2010), whose function we are addressing. One of these proteins is Contactin associated protein1 (Caspr1), which regulates the cell surface and synaptic expression of AMPARs (Santos et al., J. Biol. Chem. 2012). We have novel evidence suggesting that this protein may also be involved in post-transcriptional regulation of AMPAR subunits, which we are currently addressing.

The appetite-regulating hormone ghrelin regulates cognitive functions, by acting on the hippocampus. Given the role of AMPAR traffic in the expression of the synaptic plasticity mechanisms, which are cellular correlates for learning and memory, we are presently testing whether ghrelin affects hippocampal AMPAR traffic and 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 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.

Protein acetylation has recently emerged as an important mechanism in the regulation of synaptic plasticity and learning and memory. We have studied how protein acetylation affects the molecular composition of synapses, and found that the synapse-enriched protein cortactin is acetylated in hippocampal neurons. We are currently addressing how cortactin acetylation affects synapse maturation and stability (Catarino et al., J. Cell Sci. 2012).

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.

One goal of our research is to establish the "hot spots" of axonal mRNA translation. For that purpose our objectives are to determine if local mRNA translation is required for presynaptogenesis and if local protein synthesis occurs at the sites of nascent synapses.

Main Achievements

We are interested in how AMPAR function is regulated in the brain, since the mechanisms for changing synaptic strength depend on the regulation of AMPAR 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 have focused on the cell adhesion molecule Caspr1, which interacts with AMPAR subunits and localizes to excitatory synapses. Its overexpression promotes the synaptic localization of GluA1-containing AMPARs, whereas its downregulation decreases it. Additionally, Caspr1 increases the amplitude of glutamate-evoked currents. Our data identify Caspr1 as a novel regulator of AMPAR 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. 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 (Ferreira et al. J. Biol. Chem. 2011). In addition, we have contributed to the description that glutamate binding to GluN2B is required for its cell surface and synaptic expression (She et al. J. Biol. Chem., 2012).

Synapse maturation and stabilization require structural reorganization of the spine actin-based cytoskeleton. We have found that acetylation of the F-actin-binding protein cortactin, which can be triggered either by BDNF or neuronal activity, promotes the synaptic accumulation of the postsynaptic scaffold protein PSD95, in a transcription-independent manner. Acetylation of cortactin promotes its translocation away from synapses, and its detachment from synaptic binding partners such as Shank. This is correlated with increased clustering of PSD95 in synapses (Catarino et al., J. Cell Sci. 2012). This evidence indicates that protein acetylation can affect synaptic function through other mechanisms besides transcription regulation.

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 preventes 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.

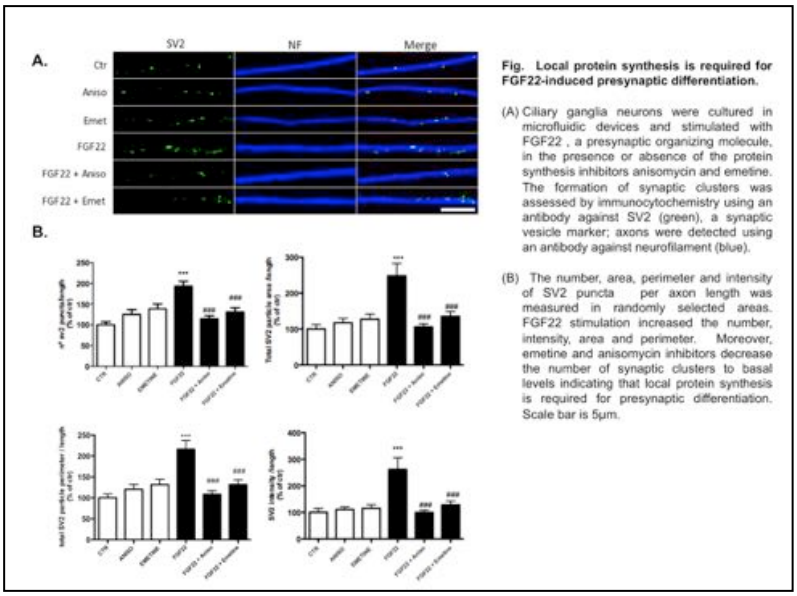


Fig. Local protein synthesis is required for FGF22-induced presynaptic differentiation.

(A) Ciliary ganglia neurons were cultured in microfluidic devices and stimulated with FGF22 , a presynaptic organizing molecule, in the presence or absence of the protein synthesis inhibitors anisomycin and emetine. The formation of synaptic clusters was assessed by immunocytochemistry using an antibody against SV2 (green), a synaptic vesicle marker; axons were detected using an antibody against neurofilament (blue).

(B) The number, area, perimeter and intensity of SV2 puncta per axon length was measured in randomly selected areas. FGF22 stimulation increased the number, intensity, area and perimeter. Moreover, emetine and anisomycin inhibitors decrease the number of synaptic clusters to basal levels indicating that local protein synthesis is required for presynaptic differentiation. Scale bar is 5μm.

We are interested in the biologic function of ataxin-3, the protein whose polyQ expansion causes Machado-Joseph disease (MJD) (Matos et al., Prog. Neurobiol. 2011). In collaboration with Sandra Macedo-Ribeiro at IBMC (University of Porto) we have characterized ataxin-3 sumoylation as well as 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) we are currently addressing the role of ataxin-3 phosphorylation in the pathogenesis of MJD.

Neuroprotection and Neurogenesis in Brain Repair Group

Head: João Malva

Objectives

Our main aim is to contribute with fundamental knowledge, new tools and innovative strategies to treat brain diseases and promote brain repair. Our scientific niche resides on coordinated investigation crosslinking neuroprotection, neruoinflammation and neural stem cells.

In 2012, the main specific aims of our group included the following:

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

2. To revela a role of fractalkine as a modulator of microglial function.

3. To identify novel factors responsible for endothelial cell-neural stem cells communication in the neural stem cell niche.

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

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

6. To study the proneurogenic effect of histamine in the SVZ stem cell niche.

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

8. To reveal a role for endothelial BDNF in rerouting neuroblasts from the SVZ into the ischemic striatum of the mice.

9. To study changes on adult neurogenesis in a mouse model of Alzheimer's disease.

10. To analyze the effects of neuroinflammatory events on hippocampal dependent memory and adult neurogenesis in a mouse model of Alzheimer's disease.

We took advantage of an excellent network of national and interantional collaborators to develop new interdisciplinary projects. These collaborative and innovative strategies were a pilar for the internationalization of our group.

Main Achievements

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

2. We identified, and pharmacologically dissected, a role for peptides, such as galanin and somatostatin, in SVZ neual stem cells differentiation of new neurons.

3. We develop (in close collaboration with Lino Ferreira at CNC/Biocant) new biocompatible nanoparticles able to release retinoic acid in the stem cell niche.

4. We could identify a new progenurogenic action of histamine (via H1 receptor) in SVZ stem cells.

5. We conclude the delopment of a strategy to functional identify neural stem cell-derived oligodendrocytes.

6. We revealed proneurogenic properties of CX546 AMPAkine in SVZ neural stem cells cultures.

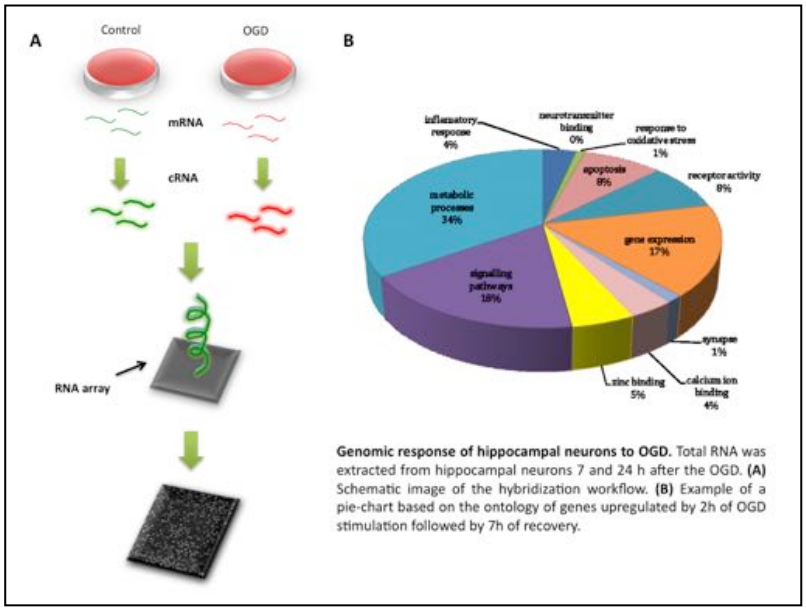
A key objective of the research group has been the training of new scientist at the master, PhD and postdoctoral level. Thus, in 2012, Sofia Grade was awarded with a PhD degree and Ismael Neiva with a Ms degree.

Finally, consistently with our commitment with science awareness in society, we also contirbuted to Brain Awareness Week and other activitites approaching the laboratory to the society.

Neuronal Cell Death and Neuroprotection Group

Head: Carlos B. Duarte

Objectives



Genomic response of hippocampal neurons to OGD. Total RNA was extracted from hippocampal neurons 7 and 24 h after the OGD. **(A)** Schematic image of the hybridization workflow. **(B)** Example of a pie-chart based on the ontology of genes upregulated by 2h of OGD stimulation followed by 7h of recovery.

Numerous disorders of the CNS are characterized by neuronal cell death, which may arise from the deregulation of the activity of neurotransmitter systems and/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). The activity of glutamatergic synapses in the hippocampus is normally regulated by the neurotrophin BDNF (brain-derived neurotrophic factor), which is also an endogenous neuroprotectant, counteracting to some extent the effects of glutamate as a toxin. The two major research interests of this group are: i) the study of molecular mechanisms contributing to excitotoxic neuronal damage, particularly in the hippocampus, a brain region highly vulnerable to glutamate toxicity, and ii) the molecular mechanisms of neuroprotection by BDNF.

The $[Ca^{2+}]_i$ overload under excitotoxic conditions 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 which may contribute to neuronal demise and how they may be affected by activation of neurotrophin receptors, thereby contributing to neuroprotection.

Overactivation of Ca^{2+} permeable glutamate receptors also induces Ca^{2+}-dependent signalling pathways that are coupled to neuronal death through regulation of transcriptional activity. This group also addresses the changes in the pattern of gene expression upon ischemic or excitotoxic stimuli in order to possibly identify new genes involved in neuronal death or survival, thus broadening the set of therapeutic targets in brain ischemia. Moreover, we are interested in excitotoxicity-induced mechanisms of AMPA receptors trafficking to the cell surface.

Main Achievements

The $[Ca^{2+}]_i$ overload in neurons subjected to excitotoxic stimulation and in brain ischemia activates calpains, and our recent studies identified several targets of this protease and the functional consequences (J Neurosci 32:4610-22 [2012]; J Neurosci 31:4622-35 [2011]; Neurobiol Dis. 44:292-303 [2011]). In brain ischemia there is also a downregulation of the ubiquitin-proteasome system (USP), but the molecular mechanisms involved are not fully understood. We now showed a transient downregulation of the ubiquitin-proteasome system in hippocampal neurons subjected to excitotoxic conditions and following oxygen-glucose deprivation (OGD), a model of global ischemia.

Downregulation of the proteasome activity, leading to an accumulation of ubiquitinated proteins, was specifically mediated by calcium entry through extrasynaptic NMDA receptors, and was only observed in the nuclear compartment. Furthermore, glutamate stimulation was found to decrease the total deubiquitinase activity in hippocampal neurons, but was without effect on the activity of Uch-L1, showing that not all deubiquitinases are affected. These results indicate that excitotoxic stimulation with glutamate has multiple effects on the ubiquitin-proteasome system which may contribute to the demise process in brain ischemia and in other neurological disorders.

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 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. Other interesting genes that came up on our analysis code for synaptic scaffolds, proteins involved in synaptic translation, and ionic channels. To confirm the DNA microarray results, we looked at the mRNA levels of selected genes by real time PCR while the level of corresponding proteins is being evaluated by Western Blotting. We will further investigate the role of some selected genes in the hippocampal neuronal death in in vitro and in vivo models of cerebral ischemia as this will pave the way to identify new therapeutic targets in cerebral ischemia.

We and others have shown that pre-incubation of hippocampal neurons with BDNF prevents neuronal death as determined by analysis of nuclear morphology (e.g. Cell Death Differ. 12:1329-43 [2005]). However, it was still unknown whether BDNF also prevents the degeneration of axons and dendrites, and the functional demise of synapses, which would be required to preserve neuronal activity. We have now studied the time-dependent changes in several neurobiological markers, and the regulation of proteolytic mechanisms in cultured rat hippocampal neurons subjected to excitotoxic stimulation.

Proteasome and calpain inhibition did not reproduce the protective effect of BDNF and caspase inhibition in preventing chromatin condensation. However, proteasome and calpain inhibition did protect the neuronal markers for dendrites (MAP-2), axons (Neurofilament-H) and the vesicular glutamate transporters (VGLUT1-2), whereas caspase inhibition was unable to mimic the protective effect of BDNF on neurites and synaptic markers. Importantly, BDNF partially prevented the downregulation of synaptic activity measured by the KCl-evoked glutamate release using a FRET glutamate nanosensor, showing that the neurotrophin also provides functional protection to hippocampal neurons.

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Head: A. Cristina Rego

Objectives

Our group studies defective intracellular signaling pathways underlying mitochondrial dysfunction, oxidative stress and excitotoxicity in distinct neurodegenerative conditions. These include the polyglutamine expansion disorders Huntington's disease (HD) and Machado-Joseph disease (MJD), Parkinson's disease and Alzheimer's disease (AD). In 2012 we produced *in vitro* and *ex-vivo* experimental data focusing on AD, MJD and HD.

AD is the most common age-related neurodegenerative disorder among the elderly, affecting both the hippocampus and the cerebral cortex and leading to progressive debilitating cognitive deficits. Recent evidence demonstrates that glutamate receptors are dysregulated by amyloid beta peptide (Aβ) oligomers, resulting in disruption of glutamatergic synaptic transmission which parallels early cognitive deficits. Indeed, overactivation of *N*-methyl-D-aspartate receptor (NMDAR) has been implicated in early synaptic dysfunction that precedes late neurodegeneration in AD. Although it is well accepted that neuronal death in AD is related to disturbed intracellular Ca2+ homeostasis, little is known about the contribution of NMDARs containing GluN2A or GluN2B subunits on Aβ-induced intracellular Ca2+ rise and neuronal dysfunction. In addition, oligomers of Aβ1-42 are considered the most synaptotoxic forms, responsible for early cognitive deficits in AD.

Taking this into account we developed research work in AD with the following aims:

1. To evaluate the role of NMDAR subunits in dysregulation of intracellular Ca2+ homeostasis induced by Aβ1–42 preparation containing a high percentage of oligomers in cerebral cortical neurons.

2. To define role of NMDARs on Aβ-evoked neuronal dysfunction and cell death through changes in microtubule polymerization in mature hippocampal cultures.

3. To investigate whether Aβ oligomers trigger endoplasmic reticulum (ER) stress by an NMDAR-dependent mechanism, leading to neuronal dysfunction, and further analyze the contribution of GluN2A and GluN2B subunits in hippocampal cultures.

Research developed in the context of polyglutamine diseases aimed at exploring cellular pathological mechanisms, namely mitochondrial dysfunction and oxidative stress, occurring in MJD and in HD.

MJD, also known as Spinocerebellar Ataxia type 3, is an inherited dominant autosomal neurodegenerative disorder. An expansion of CAG repeats in the ATXN3 gene is translated as an expanded polyglutamine domain in the disease protein, ataxin-3. Selective neurodegeneration in MJD is evident in several subcortical brain regions including the cerebellum. Mitochondrial dysfunction has been proposed as a mechanism of neurodegeneration in polyglutamine disorders. Thus, we used different cell models and transgenic mice to assess the importance of mitochondria on cytotoxicity observed in MJD.

Moreover, considering that alterations in the ubiquitin-proteosome system have been reported in several neurodegenerative disorders characterized by protein misfolding and aggregation, including the MJD, and that this nis caused by a CAG expansion in the ATXN3 gene. Encoding a deubiquitinating enzyme, ataxin-3, we also investigated ataxin-3 deubiquitinating activity and the functional relevance of ataxin-3 interactions with two proteins previously described to interact with ataxin-3, hHR23A and valosin-containing protein (VCP / p97).

HD is another CAG repeat disorder affecting the HD gene, which encodes for huntingtin (Htt) and is characterized by prominent cell death in the striatum. Oxidative stress was previously implicated in HD neurodegeneration, but the role of the major endogenous antioxidant system, the glutathione redox cycle, has been less studied following expression of full-length mutant Htt (FL-mHtt). Thus, we also aimed to analyze the glutathione system in striatal cells derived from HD knock-in mice expressing mutant Htt *versus* wild-type cells.

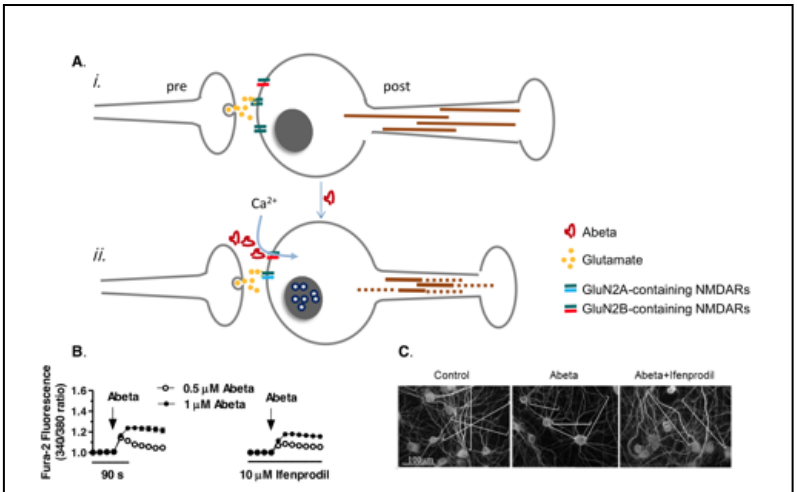


Illustration of amyloid-beta peptide (Abeta) interaction with NMDA receptors containing GluN2B subunits, DNA fragmentation and microtubule depolymerization (**A**/*i*). Representative tracings showing the influence of GluN2B-composed NMDAR antagonist ifenprodil on Abeta-induced Ca^{2+}, changes (**B**) and neurite length (**C**) in cultured neurons. Based on Costa et al. (*Aging Cell*, 2012), Ferreira et al. (*Cell Calcium* ,2012) and Mota et al. (*Curr. Alzh. Res.*, 2012).

Main Achievements

In the context of AD, we showed that Aβ increases intracellular Ca2+ (Ca2+i) through activation of GluN2B-containing NMDARs. Moreover, pre-exposure to Aβ decreased NMDA-evoked Ca2+ rise and pre-exposure to NMDA decreased Aβ response. Interestingly, simultaneous addition of Aβ and NMDA potentiated Ca2+i. This study contributed for the understanding of the molecular basis of early AD pathogenesis, by exploring the role of GluN2A and GluN2B subunits in Aβ toxicity (Ferreira et al., *Cell Calcium*, 2012).

Moreover, we found that Aβ1-42 causes a decrease in total and polymerized beta-III and alpha-tubulin, suggesting microtubule disassembly. The effects of Aβ on beta-III tubulin polymerization were correlated with reduced neurite length and neuronal DNA fragmentation. These Aβ effects were suggested to involve extrasynaptic GluN2B-containing NMDARs. Data suggested that Aβ-induced hippocampal neuronal dysfunction occurs through NMDAR-dependent microtubule disassembly associated to neurite retraction and DNA fragmentation in mature hippocampal cells (Mota et al., *Curr. Alzh. Res*., 2012).

We also showed that Aβ oligomers induce NADPH oxidase-mediated superoxide production downstream of GluN2B subunits and impaired ER and cytosolic Ca^{2+} homeostasis. These events preceded changes in cell viability and activation of ER stress-mediated apoptotic pathway, associated with translocation of the transcription factor GADD153/CHOP to the nucleus. Significantly, ER stress occurred after interaction of Aβ oligomers with GluN2B subunits. Data further highlighted the role of NMDAR GluN2B subunits on ER stress-mediated hippocampal dysfunction caused by Aβ oligomers (Costa et al., *Aging Cell*, 2012).

In the context of MJD, we found that cells expressing expanded ataxin-3 exhibited higher susceptibility to 3-nitropropionic acid (3-NP), an irreversible inhibitor of mitochondrial complex II. Moreover, cerebellar granule cells from MJD transgenic mice were more sensitive to 3-NP inhibition than wild-type cerebellar neurons. Ataxin-3 mutant PC6-3 cells differentiated into a neuronal phenotype exhibited a decrease in complex II activity. Mitochondria from MJD transgenic mouse model and MJD patient's lymphoblast cells also showed a trend toward reduced complex II activity. These data suggest that mitochondrial complex II activity is moderately compromised in MJD, which may designate a common feature in polyglutamine toxicity (Laço et al., *Biochim. Biophys. Acta*, 2012). When evaluating ataxin-3 interactors, we were able to confirmed ataxin-3 affinity for both hHR23A and VCP/p97. VCP/p97 was shown to be an activator of wild-type ataxin-3, exhibiting no effect on expanded ataxin-3. In contrast, we observed no significant alterations in ataxin-3 enzyme kinetics or substrate preference in the presence of hHR23A alone or in combination with VCP. Based on these results we proposed a model where ataxin-3 normally functions with its interactors to specify the cellular fate of ubiquitinated proteins (Laço et al., PLoS One, 2012).

In addition, HD mutant striatal cells exhibited increased intracellular reactive oxygen species (ROS), despite increased levels of both reduced and oxidized glutathione, and enhanced activities of glutathione-related enzymes. Nevertheless, glutamate-cysteine ligase (GCL) and glutathione synthetase activities and levels of GCL catalytic subunit were decreased in cells expressing mHtt, suggesting decreased *de novo* synthesis of glutathione. Enhanced intracellular glutathione in mutant cells was explained by decreased extracellular glutathione, which occurred concomitantly with decreased mRNA levels and activity of the multidrug resistance protein 1 (Mrp1), a transport protein that mediates cellular export of glutathione. Data suggest that boosting GSH-related antioxidant defense mechanisms induced by mHtt is insufficient to counterbalance increased ROS and emergent apoptotic features in HD striatal cells (Ribeiro et al., *Free Radic. Biol. Med*., 2012).

Molecular Mechanisms of Disease Group

Head: Sandra Cardoso

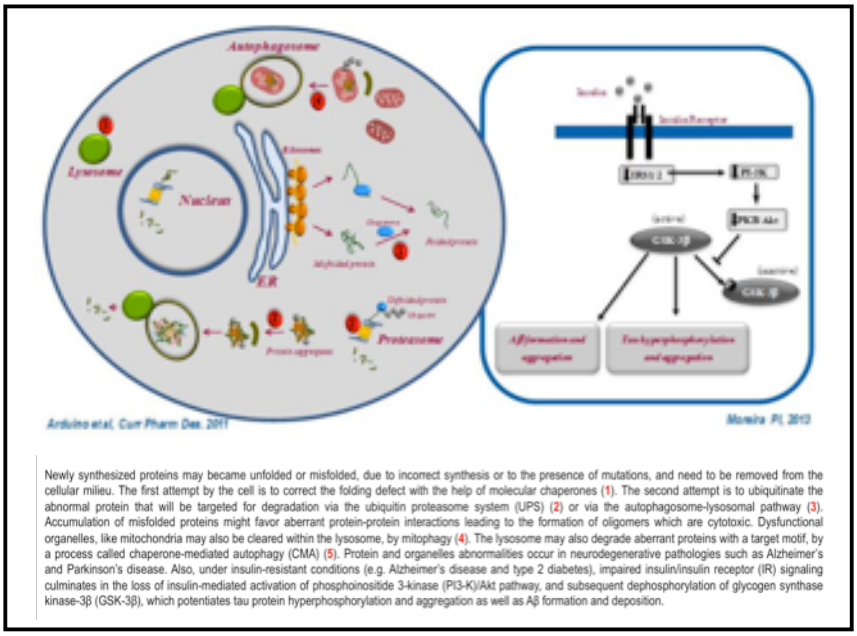
Objectives

Interventions aiming to treat neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) diseases, and numerous other pathological states (e.g. type 2 diabetes) may spring from a detailed understanding of the pathways underlying proteostasis.

One aim of our research group is to investigate how the decline in proteostasis capacity allows the manifestation of protein-aggregation diseases, including AD and PD. In particular, we are interested in understanding the impact of mitochondria dysfunction on protein quality control mechanisms, such as ER-associated unfolded protein response, autophagy and the ubiquitin-proteasome system, and how these mechanisms contribute to changes in cell metabolism, synaptic remodeling/function and survival/death pathways in an AD and PD context. Based on these, we also intend to explore new therapeutic targets and risk factors, namely aging and type 2 diabetes.

Main Achievements

We demonstrated for the first time that Aβ, namely oligomeric Aβ, activates an ER stress-mediated apoptotic pathway that occurs by a mitochondria-dependent mechanism. In addition, we found that GluN2A and GluN2B subunits of N-methyl-D-aspartate receptors (NMDARs) are differentially implicated in oligomeric Aβ-induced neuronal ER stress. We also 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 autophagic-lysosomal pathway alteration. Finally, we showed that brain mitochondria are a functional bridge between type 2 diabetes and AD, two age-related pathologies. Moreover, we found that mitochondrial preconditioning protects against glucotoxicity; this protective effect being mediated by mitochondrial reactive oxygen species and hypoxia inducible factor 1alpha (HIF-1a).



Newly synthesized proteins may became unfolded or misfolded, due to incorrect synthesis or to the presence of mutations, and 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 to ubiquitinate the abnormal protein that will be targeted for degradation via the ubiquitin proteasome system (UPS) (2) or via the autophagosome-lysosomal pathway (3). Accumulation of misfolded proteins might favor aberrant protein-protein interactions leading to the formation of oligomers which are cytotoxic. Dysfunctional organelles, like mitochondria may also be cleared within the lysosome, by mitophagy (4). The lysosome may also degrade aberrant proteins with a target motif, by a process called chaperone-mediated autophagy (CMA) (5). Protein and organelles abnormalities occur in neurodegenerative pathologies such as Alzheimer's and Parkinson's disease. Also, under insulin-resistant conditions (e.g. Alzheimer's disease and type 2 diabetes), impaired insulin/insulin receptor (IR) signaling culminates in the loss of insulin-mediated activation of phosphoinositide 3-kinase (PI3-K)/Akt pathway, and subsequent dephosphorylation of glycogen synthase kinase-3β (GSK-3β), which potentiates tau protein hyperphosphorylation and aggregation as well as Aβ formation and deposition.

Neuroendocrinology and Neurogenesis Group

Head: Cláudia Cavadas

Objectives

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 and cortical 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. 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. We are continuing to investigate the effect of diabetes or hyperglycemia on neuronal dysfunction and retina microglia changes, and especially 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.

4. Our group aims at investigating the adipose tissue regulation upon conditions mimicking chronic diseases with increased prevalence, such as obesity. One of the conditions known to occur on adipose tissue during obesity is hypoxia. We aim at clarifying the mechanisms of adipose tissue changes upon hypoxia.

5. The dysfunction of sympathoadrenal system has been proposed to explain the dysfunction of the cardiovascular system in depression; however, the adrenal medulla function in depressed patients is poorly investigated. Therefore, we are studying the adrenal medullary system in a depression model induced by chronic stress.

Main Achievements

1- 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 (Sousa-Ferreira et al., *submitted*). 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- We have shown that the retinal adenosinergic system is affected by diabetes/hyperglycemia (specialy on the levels of adenosine receptors A_{1A}, A_{2A}, A_3) and may play a potential role in cell protection against the hyperglycemic environment (Vindeirinho et al., *in revision*). Moreover TNF receptor 1 contributes to retinal neural cell death induced hyperglycemic environment (Costa et al., 2012). We observed that NPY Y1 and Y2 receptors are present in the rat retinal cells (Santos-Carvalho et., al, 2012). Targeting neuropeptide Y (NPY) system as a neuroprotective strategy in the retina. Moreover, we observed that NPY inhibited glutamate-induced cell death mediated by the activation of NPY Y_2, Y_4 or Y_5 receptors (Santos-Carvalho et al., *under revision*); therefore, these results suggest that NPY system is a potential neuroprotective target in retinal degenerative diseases, such as glaucoma.

3- We investigated the effect hypoxia on adipose tissue formation, namely on adipocytes differentiation from pre-adipocytes. Hypoxia induced lipid accumulation without PPARγ2 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 (Rosmaninho-Salgado et al., under revision). Moreover we observed that dipeptidyl-peptidase-IV (DPPIV) by cleaving neuropeptide Y (NPY) induces lipid accumulation and PPAR-γ expression (Rosmaninho-Salgado et al., 2012a,b).

4- We are also investigating the involvement of hypothalamic NPY on caloric restriction induced autophagy. Our results using in vitro and in vivo models show that NPY stimulates autophagy in hypothalamic and cortical neurons, and is involved on autophagy induced by caloric restriction.. (Aveleira et al., *in revision*).

5. We are investigating the effect of depression induced by unpredictable chronic stress on mouse adrenal medulla, and we observed an increase of adrenal medulla volume with a decrease of adrenal medulla function markers and progenitor cell markers (Santana et al., *in preparation*). we recently observed that human adrenal medulla contain progenitor cells that, in part, may contribute to the adrenal medulla changes upon depression (Santana et al., 2012).

Emerging Group- Chronic Inflammation Group

Head: Margarida Carneiro

LRRK2 role on auto-antibody production by human B Cells

B cells are cells of the immune system that have a dual function: they produce antibodies to fight disease causing agents, and they participate in the regulation of the immune response by interacting with other cells. However, genetic mutations might cause alterations in these functions, and B cells end up have a role in disease exacerbation by reacting against the person's own organs and/or cells. In this project we studied how the LRRK2 mutations had an influence on the function of B cells. For that purpose we analyzed blood samples from Parkinson's patients with and without the LRRK2-G2019S mutation and matched healthy volunteers. We found out that:

1. LRRK2-G2019S mutation seems to reduce B cell interaction with T cells. Since the MAPK-pathway is associated with cytokine production, it is plausible that the reduction in MKK6 expression we observed in the LRRK2-G2019S B cells is intimately related to their lower production of TGF-b and IL-10. These two cytokines are crucial for the differentiation of naïve CD4+ T cells into Treg cells. Furthermore, the interaction of CD40 on B cells with CD154 on T cells is fundamental for proper B cell maturation and B-T communication. Therefore, by reducing all these molecules, LRRK2-G2019S mutation seems to impair normal B-T cell cross-regulation.

2. LRRK2-G2019S mutation seems to promote B cell apoptosis by impairing the expression of MAPK-pathway-associated proteins and IL-6receptor which are involved in cell proliferation, while apparently promoting the CD95/FADD/caspase-8 apoptotic pathway;

3. LRRK2-G2019S mutation is more frequently associated with autoantibody production. Usually, in "classical" autoimmune diseases the peripheral memory B cell pool is reduced, there is an accumulation of plasmacytes and of BAFF-R+ B cells. Apparently, LRRK2-G2019S mutation seems to promote –more efficiently than in the non-LRRK2 mutated PD patients- the entrance of self-antigens released during neurodegeneration in a pathogenic antigen-processing pathway, leading to autoantibody production. Moreover, as is typical in chronic autoimmune inflammation there is the production of auto-reactive antibodies against modified self-antigens in LRRK2-G2019S PD patients. The significantly more frequent presence of ASCA antibodies in LRRK2-G2019S PD patients suggests that there is an underlying colonic inflammation. Even though no patient had clinical signs of colitis, our data support the findings that LRRK2 is associated with colitis and Crohn's disease. Whether this sub-clinical colitis is solely the result of enteric dopaminergic-neurons loss or a combination of that neuronal loss with impaired Treg-induction (Treg cells are essential in preventing chronic colitis) remains to be assessed. Finally, the autoimmune phenomena which seem to be promoted by the presence of LRRK2-G2019S mutation do not seem to lead B cells to become autoreactive against self-antigens usually linked to systemic autoimmune diseases, thus restricting this auto-reactivity to the by-products of the neurodegenerative process.

Peripheral blood B cell subsets alterations in Alzheimer's Disease and Mild Cognitive Impairment

There is emerging evidence supporting an impact of the immune system in the process of neurodegradation in Alzheimer's disease (AD), corroborated by recent genome-wide association studies linking pro-inflammatory TREM2 and other proinflammatory mediators with a significant increase in AD risk. This involvement of the systemic immune response in AD has been equally supported by several studies showing that AD patients present imbalanced peripheral blood immune cell subsets when compared to matched healthy controls. Further, as vaccination is at the forefront of new therapeutic strategies for AD, a deeper understanding of the systemic immuneresponse in neurodegeneration is of utmost importance. In particular, focusing on functional alterations of different immune cells allows the identification of potential immunoregulatory mechanisms that might be impaired in neurodegenerative processes leading to their aggravation and perpetuation. Moreover, recognizing and defining systemic immune system changes associated to AD can open new perspectives to identify novel biomarkers useful in the early diagnosis of AD and new pharmacological targets.

B lymphocytes and plasma cells (terminally-differentiated antibody-producing B lymphocytes) play a central role in the immune response both as regulators (cytokine production; antigen presentation; cell-cell interaction) and as antibody-producing cells. Dramatic alterations in peripheral blood B lymphocytes subsets are a hallmark of chronic inflammatory diseases, in particular those with an autoimmune basis. In autoimmune chronic inflammation B lymphocytes exhibit abnormal proliferation, maturation/differentiation and apoptosis, while producing auto-reactive antibodies against self-antigens, which have been made available after pathogenic tissue and/or cell destruction. Hitherto existing data on peripheral blood B lymphocytes in neurodegeneration are scarce, data on the presence of autoantibodies in AD are contradictory, and investigations developed in the earliest pre-dementia stage of AD, designated as Mild Cognitive Impairment (MCI) are even more scarce. Consequently, the potential to recognize a B lymphocyte profile of alterations from MCI to AD and the presence of autoantibodies against central nervous system-derived self-antigens as biomarkers of early-AD remain largely unexplored.

Data from our studies on a cohort of AD, MCI and healthy age-matched individuals have shown that the peripheral blood B lymphocytes in AD and MCI patients present clear abnormalities reminiscent of chronic autoimmune inflammation. In addition, we detected the presence of IgG+ autoantibodies against central nervous system proteins (alpha-synuclein; Tau protein and phosphorylated Tau) in the sera from AD and MCI patients. Therefore, it is likely that systemic physiological changes in AD promote altered functionality of B lymphocytes and plasma cells leading to their autoreactivity and concomitant autoantibody production, contributing to and exacerbating neurological deterioration.

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BIOTECHNOLOGY AND HEALTH

Coordinator: Euclides Pires

The general objectives of this area are: 1) unveal and understand normal interactions that occur in living organisms from a molecular up to a system level; 2) design vectors to deliver drugs and nucleic acids aiming to modulate or correct abnormal interactions; 3) develop new biomaterials for stem cell differentiation, tracking and transplantation as well biomaterials with anti-microbial properties.This programme encompasses basic and translational research approaches which are conducted by five research groups. The research performed by the Molecular Systems Biology, by the Structural and Computational and by the Molecular Biotechnology groups is concerned with the first objective (unveal and understand interactions); the research performed by the Vectors and Gene Therapy Group is concerned with the second objective (design of vectors for drug and nucleic acid delivery);whereas the work performed by the Biomaterials and Stem Cell –Based Therapeutics group is concerned with the third objective (development of biomaterials).

Major Achievements

Enzymes involved in moiety-transfer cycles (MTC) was shown to have a Km (NAD+) significantly higher than the Km (NADH), indicating that the design principal of NAD redox cycles are distinct from those for NADP.

Studies on theinterplay amoung the three main defenses of human erythrocytes against hydrogen peroxide (glutathione peroxide, catalise and peroxiredoxin) proved that peroxiredoxin is the main defence of erythrocyte against H2O2, under most physiological circunstances.

Structural and enzymatic characterization of 6 recombinat aspartic protein from Arabidopsis revealed unusual properties like a strong dependence of redox conditions and a surprising insensitivity to pepstatin.

Transepithelial permeability measurements upon pollen studies treatment, complement the picture previously set for the pollen effects on lung airways which was based on degradation of intercellular adhesion properties on cell detachment studies.

A set of 50 compounds was selected by virtual screening of 11 milion putative inhibitors of transthyretin-dependent amyloidoses. 3 of which represent families of potential therapeutic drugs.

Silencing of miR-21 with anti-miRNA-LNA oligonucleotides formulated with cationic liposomes decrease tumour cell viability.

Lipid base monocarrierswith the ability to specifically bind to receptors are expressed were further developed to simultaneously encapsulate anti-BCR-ARL S,RNA aiming at specific delivery to CML cells.

Gene silencing systems suggest that therapeutic strategies involving non-allele-specific silencing may be safe and effective in Machado Joseph Disease

Development of a new set of nanomaterials to control cell differention of stem cells as well as devellopment of synthetic niches to potentiate in vivo stem cells engrattment proceeds with very promising results.

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Molecular Biotechnology Group

Head: Carlos Faro

The Molecular Biotechnology group has a long time interest on the study of proteases and their biotechnological applications. Initially, the research was focused on the biochemistry and biology of the aspartic proteases from the flowers of cardoon, whose milk-clotting activity has been exploited since the Roman times for the manufacture of the most appreciated cheeses in Portugal. These enzymes, named cardosins, were purified and characterized from the flowers, their genes cloned and a 3D structure-based engineered form was produced in K.lactis to be used as a substitute of the cardoon flower extract at the industrial scale.

With the sequencing of the Arabidopsis genome the research interest shifted towards the aspartic proteases of this model plant. A first bioinformatic study revealed that the Arabidopsis genome contains at least 51 genes encoding pepsin-like aspartic proteases and subsequently a systematic analysis of the biology and biochemistry of these genes/enzymes was launched. Selective representative members, shown to be involved in defensive mechanisms against biotic and abiotic stresses, were cloned and expressed in heterologous systems for further characterization. The results suggested an important role for aspartic proteases in cell death mechanisms and support the hypothesis that they might be the so far elusive caspase homologues in plants.

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. 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 recombinant enzyme was shown to be active and its activity was significantly inhibited by therapeutic HIV protease inhibitors such as indinavir, nelfinavir, amprenavir and atazanavir, suggesting that they might be an alternative therapeutic strategy if resistance to traditional antibiotics emerges as a serious problem in the future.

Pollen allergy has a remarkable clinical impact all over Europe. Pollens with distinct allergenic activity contain high molecular weight proteases displaying serine and/or aminopeptidase activity. These proteases were purified and shown to increase transepithelial permeability through disruption of transmembrane proteins and to degrade airways bioactive peptides that can disturb the balance between the anti- and pro-inflammatory effects in the lung. The results suggest a model for their involvement in the sensitization to a range of airborne allergens by facilitating allergen delivery across the epithelium, and through a direct contribution to the inflammation process characteristic of allergic diseases. So far, primary focus has been given to proteases derived from high allergenic species, but our work suggests that even less allergenic pollens are likely to be involved in the allergic sensitization and in chronic respiratory inflammation. Their presence may also explain the extent of respiratory symptoms in subjects with non-IgE mediated rhinitis or asthma that takes place at the peak of pollen season. It remains to be studied whether these proteases can be a specific therapeutic target in the prevention and treatment of allergic disorders related to pollinosis.

Finally, a degradomics platform started to be implemented using pollen proteases and specific targets, aiming to identify protease cleavage sites. This theme is important because proteases are involved in many diseases, ranging from allergic diseases to viral infections. However, not all proteases' targets are known and improving our knowledge in this field will identify potential biomarkers, proteases' targets, and point towards potential therapeutic targets.

Molecular Systems Biology Group

Head: Armindo Salvador

Objectives

Finding general organization principles connecting design and function in metabolism, in protection against reactive chemical species (RS) and in RS-mediated signaling. Ongoing projects address the following questions:

a. What are the design principles of the most prevalent elementary circuits in metabolic networks? Namely, moiety transfer cycles (MTC). Previously we focused on MTC that are involved in the transfer of redox equivalents. We are now focusing in those involved in the transfer of other moieties.

b. Is the overall architecture of moiety transfer in metabolism phylogenetically conserved? If so, why?

c. How does the naturally evolved design of antioxidant defense systems relate to the function of these systems (which are largely composed of interconnected MTC) and what design features are key for their effectiveness?

d. How do cells integrate signaling through and protection against H2O2? Do any general principles apply?

e. How does protein aminoacid sequence and structure evolutionarily adapt to oxidative stress?

f. How are reactive scission products from lipid autoxidation generated in vivo and how are the concentrations of these products and of similar reactive metabolic intermediates and side products regulated?

2. Developing improved computational approaches to profile the performance of biochemical circuits and to design circuits with prescribed performance characteristics.

3. Developing a rule-based approach to achieve semi-quantitative predictions of product profiles in complex reaction networks involved in lipid autoxidation and metabolism. Application towards improving fundamental understanding about how these processes occur in vivo, towards multiplexed early diagnostic of chronic diseases, and to food biotechnology.

4. Developing a method for profiling mitotic-cycle-dependent metabolism without having to synchronize cells. Application to demonstrate eventual metabolic heterogeneity of eukaryotic cells across the mitotic cycle.

Main Achievements

Previously, in collaboration with Dr. Marta Piñeiro (U. of Coimbra) we developed a computational approach to generate and analyze the reaction networks involved in the autoxidation of polyunsaturated fatty acyl chains (PUFA). We showed that our approach was able to explain the formation of all products from linoleic acid autoxidation hitherto detected in experiments in vitro under mild conditions, except for pyranosic, furanosic and branched products. We also selected markers for the occurrence of specific scission mechanisms in vivo. Over 2012 we extended this approach to consider the stereochemistry of the compounds, which will allow to pinpoint suitable markers for additional scission mechanisms. We have also established a collaboration with the Max Planck Institute for Molecular Cell Biology and Genetics (Simmons' lab, Germany) towards identifying the mechanism markers in yeast cells genetically modified to produce linoleic (18:2) and palmitoleic (16:2) acids as the sole PUFA, which is ongoing. This approach seeks to clarify the main open problem about the generation of reactive autoxidation products such as 4-hydroxynon-2-enal.

In collaboration with Dr. Marta Piñeiro, Sílvia Gramacho (U. of Coimbra) and Dr. Elmar Heinzle (U. of Saarland, Germany) we developed a GC-MS-based method for determining 13C enrichment in bases, (deoxy)ribose and (deoxy)nucleotides from DNA and RNA. Development of a complementary LC-MS-based method is ongoing in collaboration with Dr. Marta Piñeiro, Sílvia Gramacho, Dr. Bruno Manadas (Center for Neuroscience and Cell Biology, CNC) and Dr. Vera Mendes (CNC). These methods are being applied to accomplish objective 4 above.

Previously, we set up a mathematical model of H2O2 metabolism in human erythrocytes based on kinetic data obtained in vitro for purified enzymes. Over 2012 we systematically compared the predictions of this model with experimental observations of the behavior of intact erythrocytes exposed to various treatments. Many observations agreed with the predictions from our model. However, there were two critical discrepancies. First, the contribution of peroxiredoxin 2 (Prx2) for H2O2 consumption in intact erythrocytes at low to oxidative loads is comparable or to that of catalase, whereas the model predicted that Prx2 should reduce >99% of the H2O2 under these conditions. Second, Prx2 is much more susceptible to sulfinylation in intact erythrocytes than predicted based on the kinetic parameters for the purified protein. Based on a systematic analysis of the discrepancies we proposed a model for Prx2 action in human erythrocytes in which a hitherto unidentified inhibitor reversibly binds >99% of the Prx2, simultaneously making it more susceptible to sulfinylation. This model proved able to resolve the discrepancies. We are currently investigating its functional implications. The proposed inhibition may solve one of the most important open problems in H2O2-mediated signaling. Namely, how can H2O2 oxidize the thiol groups of the phosphatases and kinases it is known to modulate in vivo if these thiols are both much less H2O2-reactive and much less abundant than peroxiredoxins?

We have recently established a collaboration with Dr. Carla Real (University of Lisbon) to test our hypothesis in vivo using fluorescent probes in genetically modified zebrafish.

We have implemented a first working version of a multiobjective non-linear optimization method for finding designs in which simple biochemical circuits perform robustly.

We developed an incremental computational technique to avoid the computation of nonbonded interactions in the molecular dynamics software GROMACS. Validated it computationally for proteins and small molecules. Performed extensive free energy studies on amino acid analogues and determined that the results were statistically equivalent to those of the standard GROMACS distribution. Our approach permits 2- to 12-fold faster simulations depending on the CPU model.

Structural and Computational Biology Group

Head: Rui M. M. Brito

Objectives

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, monitoring changes in intrinsic tryptophan fluorescence at different urea and protein concentrations.

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 and intrinsic tryptophan fluorescence to follow the TTR conformational changes accompanying amyloid formation.

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 APBS-electrostatics calculations were applied to characterize in detail the structural and energetic determinants of known TTR binders.

IIb. Searching for COX-1 inhibitors and their counterparts

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics. Conventional NSAIDs inhibit the catalytic function of cyclooxygenase-1 and -2 (COX-1 and COX-2), which may lead to serious side effects. Because of these problems, when working on the development of new putative drugs it is important to understand their interaction with COX.

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

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

The objective is to use bioinformatic tools for comparative sequence and structure analysis, for selected set of proteins within serotypes H1-H3 and H5, and N1 and N2, with the goal of highlighting and discussing the conserved regions within the different protein domains and, conversely, identify within the defined active site regions any critical position of frequent mutations.

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

Within the Ibercivis project, one of the main objectives pursued throughout 2012 was to involve Portuguese researchers and citizens in Ibercivis.

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 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 intermediate state constituted by 8 to 10 TTR monomers. After the initial conformational changes, TTR aggregation proceeds via a nucleation-and-growth mechanism.

II. Rational design of inhibitors of amyloid formation

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

From ITC experiments is possible the characterization of the binding properties (association constants and cooperativity effects) and the thermodynamic profiles of binding between WT-TTR and the four ligands under study. 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. Searching for COX-1 inhibitors and their counterparts

Preliminary results on the docking of COX-1 with a set of known ligands and decoys reveal that the available scoring functions are not suitably distinguishing between them which poses a major problem. We are presently exploiting machine learning methodologies such as regression and classification under a Support Vector Machine framework to improve available scoring functions.

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

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

ClustalW2 was employed to analyse the sequence alignments of the sets of selected 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

To promote the involvement of Portuguese researchers and citizens in Ibercivis, several dissemination and promotion actions were taken such the organization of worskhops:

* Rui M. M. Brito, Elsa S. Henriques, Cândida G. Silva, Carlos J. V. Simões. (2012) "A Matemática ajuda-nos a ver como as moléculas mexem. Introdução aos métodos da dinâmica molecular", in "Curso em Matemática Aplicada", Instituto de Educação e Cidadania, May 25, Mamarrosa, Portugal. (URL: http://www.educacao-e-cidadania.pt/?q=node/90)* Rui M. M. Brito, Cândida G. Silva, Paulo Gama Mota, Fermín Serrano Sanz. (2012) "Computação Voluntária e Ciência Cidadã", in "Chá das 3", Museu da Ciência da Universidade de Coimbra, September 22, Coimbra, Portugal. (URL: http://www.museudaciencia.org/index.php?iAction=Actividades&iArea=4&iId=421)

Also, two posters promoting the Ibercivis platform were prepared and sent to research institutions and secondary schools.

Vectors and Gene Therapy Group

Head: Mª Conceição P. Lima

Objectives

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 approaches for cancer and neurodegenerative disorders and of prophylactic strategies for infectious diseases.

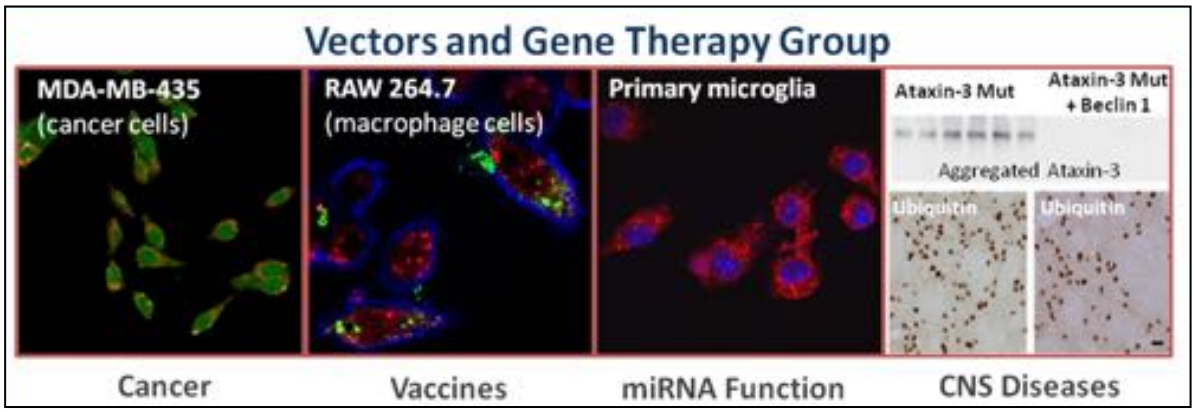
Our studies on non-viral vectors have been mainly focused on the evaluation of the potential of novel lipid-based nanosystems and polymeric nanoparticles in gene therapy strategies for the treatment of both cancer and neurodegenerative disorders, and for the development of vaccines for infectious diseases.

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.

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/spinocerebellar ataxia type 3 (MJD). This knowledge is expected to allow the generation of disease-modifying approaches for MJD therapy.

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. In this regard, new chitosan-based delivery systems able to simultaneously encapsulate antigens and a second adjuvant have been developed and we aim to evaluate possible synergistic effects between chitosan and the second adjuvant (mast cell activator c48/80 and aluminum compounds).



Main Achievements

Regarding non-viral-mediated gene delivery, biophysical studies on membrane interactions of Gemini surfactant and cell penetrating peptide-based vector formulations have provided interesting insights into the mechanisms through which vectors modulate membrane physical properties and contribute to the delivery of nucleic acid molecules. 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.

Concerning the design and development of nanocarriers, we have generated a novel lipid-based system exhibiting the ability to specifically and efficiently deliver genetic

material into hepatocellular carcinoma cells through its specific binding to the asialoglycoprotein receptor.

Moreover, a new anti-tumoral strategy was developed involving silencing of the oncomir miR-21, overexpressed in glioblastoma (GBM), through delivery of anti-miRNA LNA oligonucleotides via tumor-targeted stabilized nucleic-acid lipid particles (SNALPs) followed by cell exposure to sunitinib. We have shown that SNALP-mediated miR-21 silencing enhances the cytotoxic effect of sunitinib in different glioma cell lines, thus revealing the therapeutical potential associated with the combination of miRNA-based gene therapy with anti-angiogenic activity towards GBM.

In a different approach, proprietary targeted lipid-based nanoplatforms for drug and siRNA delivery towards cancer cells and the tumor microenvironment were developed. They successfully targeted breast cancer cells harvested from patients submitted to mastectomy. In addition, in a murine model of human breast cancer, targeted delivery of doxorubicin completely suppressed tumor invasion.

We have also investigated the role of miR-155 in Alzheimer's disease development and observed an upregulation of this miRNA in a transgenic mouse model of this disease, which preceded plaque and tangle formation and contributed to the establishment of a pro-inflammatory environment.

Regarding viral-mediated gene delivery, we have generated lentiviral and adeno-associated viral vectors, to study the pathogenesis of neurodegenerative diseases, with a focus on Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD). Lentiviral-based in vivo models of MJD allowed fruitful investigation of disease-modifying approaches involving the strategies of gene silencing, autophagy activation and proteolysis inhibition. It is expected that these studies will contribute to the finding of new therapies for this devastating disorder for which no effective therapy is available.

Immunization with the protein antigens or pDNA encapsulated in polymeric nanovectors to target the lymphoid structures of the mucosal immune system was also addressed by our group through the development of immunopotentiators/ chitosan-based nanovectors able to stimulate the innate immune system. Mechanistic studies of the adjuvants, as well as vaccination studies with hepatitis B and anthrax antigens are under way.

Biomaterials and Stem Cell-Based Therapeutics Group

Head: Lino Ferreira

Objectives

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 and (ii) development of nanomaterials to manipulate stem cells and control their differentiation.

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

One of the main objectives of the Biomaterials and Stem Cell-Based Therapeutic research group is to develop biomaterials for the efficient differentiation/maturation 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 research in this topic aims at generating new platforms to modulate (stem) cell activity. We have running projects in the use of nanomaterials to track stem cells, to reprogram somatic cells, and to control the differentiation of stem cells.

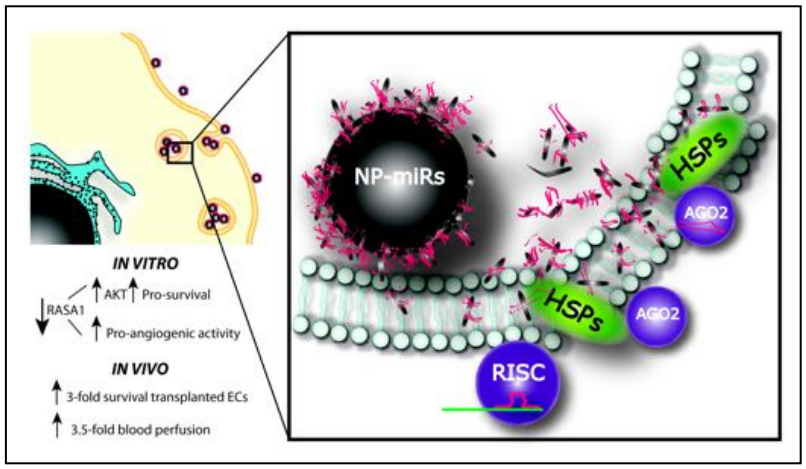
Main Achievements

Four major achievements have arisen from our recent work: first, the development of a new set of nano- and microparticles to control the differentiation of stem by the efficient spatio-temporal delivery of biomolecules (Tiago *et al.*, ACS Nano 2012; Bernardino et al., Stem Cells 2012); second, the development of synthetic niches to modulate cell activity (Maia *et al.*, Biomacromolecules 2012); third, the development of new antimicrobial nanomaterials and coatings (Botequim *et al.*, Langmuir 2012) and forth, the development of cardiac patches (Pereira *et al.*, Advanced Materials 2012).

Our recent work shows that VEGF functionalized dextran (dexOx-VEGF) is comparatively superior to free VEGF in prolonging the phosphorylation of VEGF receptor 2 (VEGFR-2). Both dexOx-VEGF and free VEGF activate VEGFR-2 and the complexes are internalized into early endosomes ($EEA1^+$ vesicles) and then transported to lysosomes ($Rab7^+$ vesicles). However, after cell activation dexOx-VEGF is preferentially co-localized in early endosomes where VEGF signaling is still active while free VEGF is preferentially transported to late endosomes or lysosomes. We further show that dexOx-VEGF after phosphorylation of VEGF receptor 2 induces an increase of intracellular Ca^{2+} and activates VEGF downstream effectors such as Akt and extracellular signal-regulated kinase (ERK½) proteins. Under specific conditions the activation level is different from the one observed for free VEGF, thus suggesting mechanistic differences, which is illustrated by cell migration and cord-like formation studies. DexOx-VEGF can be crosslinked with adipic acid dihydrazide to form a degradable gel, which in turn can be incorporated in a fibrin gel containing endothelial cells (ECs) to modulate their activity. We envision that these constructs might be beneficial to extend the pro-angiogenic activity of VEGF in ischemic tissues and to modulate the biological activity of vascular cells.

In collaboration with João Malva and Liliana Bernardino research groups, we demonstrated the differentiation mechanism mediated by RA released from a polymeric NP within neural stem cells. NPs were used to facilitate the cellular internalization, intracellular positioning and concentration of RA above its solubility limit (approximately 63 ng/mL) at physiologic pH.[19] Our results showed that RA released from NPs interacted with RA receptor (RAR), activated SAPK/JNK signaling pathway, and induced the methylation of histone lysine residues which in turn associated with the promoter regions of proneurogenic genes such as Ngn1 and Mash1. Further, we have demonstrated successfully that a NP formulation could be used *in vivo* to control the differentiation of neural stem cells. To the best of our knowledge no study has demonstrated so far the ability of controlling *in vivo* the neuronal differentiation of SVZ cells by small molecules delivered from NPs. Notably, our formulation offers a significant advantage over free solubilized RA, either by avoiding the use of solvents like DMSO, and by achieving a proneurogenic effect with a RA concentration ~2500-fold lower than the one needed with free RA. Finally, our study was the first to show the dynamics of the initial stages of stem cell differentiation either *in vitro* or *in vivo* after exposure to a formulation of NPs containing a biomolecule or a solubilized biomolecule. The study of the differentiation profile of stem cells in both conditions is important to design more effective formulations to modulate the stem cell niche. Our results show that RA^+-NPs were more robust in maintaining the signature of gene expression (although with different kinetics) either *in vitro* or *in vivo* than solubilized RA.

Also in collaboration with João Malva and Liliana Bernardino research groups, we have described a unique role of histamine in inducing functional neuronal differentiation from cultured mouse SVZ stem/progenitors cells. This proneurogenic effect depends on histamine 1 receptor activation and involves epigenetic modifications and increased expression of *Mash1*, *Dlx2* and *Ngn1* genes. Biocompatible PLGA microparticles engineered to release histamine in a controlled/prolonged manner also triggered robust neuronal differentiation *in vitro*. Preconditioning with histamine-loaded microparticles facilitated neuronal differentiation of SVZ-GFP cells grafted in hippocampal slices or in several brain regions *in vivo*.



In collaboration with Jeffrey Karp research group, we have described a novel biocompatible and mechanically tunable elastomer, poly(glycerol sebacate urethane) (PGSU), suitable for efficient encapsulation and controlled delivery of bioactive macromolecules and with the potential to be applied to cardiac drug delivery

Pharmacometrics Group

Head: Amílcar Celta Falcão

Objectives

Pharmacometrics is the science of developing and applying mathematical and statistical methods to characterize and predict the pharmacokinetics and pharmacodynamics of drugs and biomarker-outcomes behavior. Currently, its integration as an applied science in drug discovery and development processes is considerably increasing.

The principal aim of the Pharmacometrics Group is to early predict the kinetics of drug candidates since this area has been recently regarded as one of the major reasons for the failure of new drug candidates in vivo. Drugs and drug candidates that act at the Central Nervous System, including antiepileptic drugs and antiparkinsonian drugs, are particularly under investigation within our group.

Moreover the Pharmacometrics group also performs the pharmacokinetic analysis of those compounds during clinical studies. This information is extremely important as 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.

Main Achievements

In vitro and in vivo methodologies developed within our group and internationally accepted in the year of 2011 were applied for a set of compounds with anticonvulsant activity including the recently marketed, eslicarbazepine acetate, in order to in deep characterize their pharmacokinetics in plasma and brain (biophase). Moreover pharmacostatistical models were developed in order to foresee brain concentrations based on those found in plasma.

It is also important to highlight that our expertise in in vivo studies and pharmacokinetic analysis allowed us to demonstrate relevant in vivo drug-drug interactions between herbal extracts and amiodarone, a narrow therapeutic index drug, in rats. The new approach integrating the in vitro/in vivo pharmacokinetic analysis referred in the previous paragraph are also being carried out in order to identify the mechanisms involved in such herb-drug interactions.

In parallel, bioanalytical methodologies have been developed and fully validated in order to quantify the compounds under investigation in plasma, erythrocytes, brain, liver and other relevant biological samples by HPLC. At this field, the Pharmacometrics group clearly demonstrates an evident increase which is internationally well-recognized.

Bioorganic and Medicinal Chemistry Group

Head: Maria Luísa Sá e Melo

Objectives

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 exert profound biological effects in cholesterol and fatty-acid metabolism, immune regulation, neurodegenerative mechanisms and cell differentiation and proliferation. Based on previous results on the structure requirements for oxysterols to display cytotoxicity (J. Med. Chem. 2009, 4007; ibid, 2010, 7632; ibid, 2011, 6375) and aiming to push forward potency and selectivity, a focused library of oxygenated sterols in rings A and B has been prepared and evaluated for cytotoxicity against human cancer and non-cancer cells. A SAR analysis 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 SAR analyses. The naturally occurring lupane-type triterpenoids betulin and betulinic acid and ursane-type ursolic acid have been thoroughly investigated for their promising chemopreventive and antitumor activities.We focused on the synthesis of lupane-type imidazole carbamates and N-acylimidazole bearing derivatives. The promising results prompted us to extend our study to 2′-methylimidazole, triazole and fluorolactone derivatives, to establish meaningful SAR. The compounds with better cytotoxicy were tested for their ability to induce apoptosis and cell cycle arrest.

The understanding of the GPR30 receptor, concerning specific ligands, their structure and type of action, in vitro and in vivo, is another objective. Through SAR studies we will search for more effective ligands. We will explore the selective modifications on the estradiol scaffold and relative binding affinity of each compound towards the nuclear and membrane-associated ERs in vitro by pharmacologic approaches and selective assays in cell lines differentially expressing those receptors. SAR studies will give information about the receptor, which will be incorporated into a 3D model of GPR30 to direct future syntheses.

The rapid growth of high quality X-ray crystal structures and online publicly available chemical databases with annotated activity for thousands of small molecules creates an opportunity to develop accurate computational models for fast in silico virtual screening. We are currently interested in deriving high quality benchmarking test sets for docking and scoring, as well as developing and validating new algorithms for induced-fit docking.

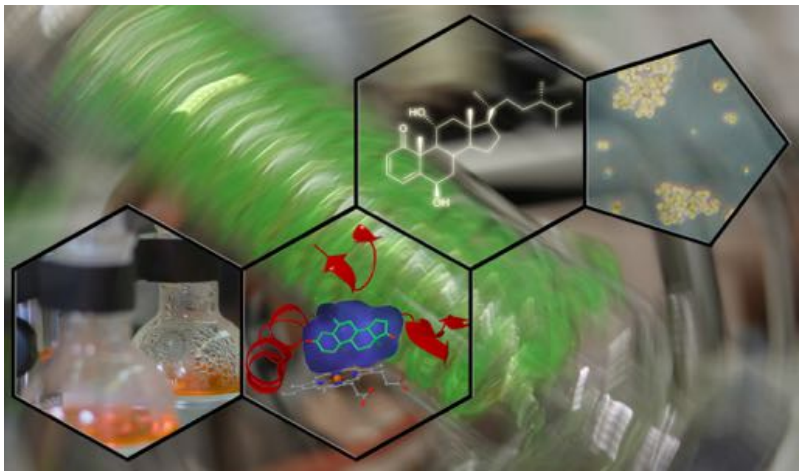
The research activities of the group are supported by the following expertise:

a) 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.

b) Synthesis in drug discovery: asymmetric synthesis for chiral drugs; biocatalysis; chemo-enzymatic methods; clean processes.

c) Biological evaluation in vitro.

d) Analysis of structure-activity relationships (SAR) to predict potency and improve “hits” to “lead candidates” by optimizing their selectivity against the target and pharmacokinetics.



Main Achievements

The chemical diversity of oxysterols has been enlarged through the preparation of cyclic and acyclic acetals. The reactions under use followed a synthetic methodology previously studied by us (Tetrahedron, 2010, 66, 2455-2462). Three series of four different types of acetals were produced, having in common heavily oxygenated moieties, either in the A ring or in the A and B rings simultaneously of the sterols. Further evaluation in vitro in the cancer human cell line A549 (from lung adenocarcinoma epithelium) and in the non-cancer ARPE-19, using the Alamar Blue assay revealed an antiproliferative activity for the oxysterol acetals in a low micromolar range. For the 3 series studied, one of

the most heavily oxygenated, with the acetals on the ring B showed the best cytotoxic profiles, in terms of IC50 values towards the cancer line. The molecular variations studied encompassed homologous series and analogical approaches by ring closure. The best selective cytotoxicity was found for a member of the acetal cyclic series. This study will contribute to correlate the structural features with the biological chemistry of the oxysterols.

Recently, we focused on the synthesis of ursane-type imidazole carbamates, N-acylimidazole bearing derivatives, 2′-methylimidazole and triazole derivatives (Bioorg. Med. Chem., 2012, 5774) and fluorolactone derivatives (ChemMedChem, 2012, 1635), in order to establish meaningful SAR and study their ability to induce apoptosis and cell cycle arrest in pancreatic cancer cells. The overall findings suggest that some of the new ursane-type derivatives are strong regulators of tumor cells proliferation, inducing cell cycle arrest and apoptosis.

Addressing the GPR30 receptor, synthetic modifications of the steroid skeleton were performed, such as on OH groups at key positions of the estradiol framework, C-3 and C-17. Diols and beta-hydroxy ether derivatives were obtained from selective opening of epoxides. Ecologically natural catalysts, as lipases in organic media have been used to prepare the corresponding monoacylated derivatives. The relative binding affinity and intrinsic activity of each test compound towards the nuclear and membrane-associated ERs are now under investigation.

Flexible docking and scoring with the Internal Coordinate Mechanics method was benchmarked against 40 targets of critical importance for structure-based drug design. 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 (J. Comp.-Aided Mol. Des., 2012, 675). The in silico models for noncovalent inhibitors of AmpC β-lactamase were tested prospectively in the virtual screening of about 6 million commercially available compounds. Sixty-one chemically diverse top-scoring compounds were experimentally tested, which led to the identification of seven previously unknown inhibitors (J. Chem. Inf. Model., 2012, 1367).

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CELL AND MOLECULAR TOXICOLOGY

Coordinator: Rui Carvalho

The area is mainly concerned with the study of cellular and molecular basis of drug- and disease-related cell dysfunction, in which mitochondria, lipid membranes or free radicals could be involved, for the purpose of translating this knowledge into disease treatment and prevention. Three groups have been accomplished such goals: Mitochondrial Toxicology and Disease, focused on exploration of the interplay between mitochondria, metabolism, disease and human toxicology; Redox Biology in Health and Disease, centered on mechanisms inherent to neuromodulation and aging involving NO, and to the protective role of polyphenols in peroxynitrite-induced endothelial dysfunction and in nitrite-driven regulatory processes; Membrane Toxicity, with a focus on the study of the role of membrane lipids in modulating drug-mediated cell dysfunction. The recent Pharmacometrics Group brings an insight into optimization of drug efficacy and safety to prevent costly and life-threatening drug-induced toxicity.

Major Achievements

The groups in this Area, by using in vitro and in vivo approaches, obtained a vast range of results, as indicated in their individual reports. In brief:

The role of mitochondria as a mediator of xenobiotics toxicity, including doxorubicin cardiotoxicity and hepatotoxicity of both ecstasy and a related amphetamine was established and the underlying mechanisms clarified.

Acute exercise or sub-chronic administration of doxorubicin to wistar rats protected against heart mitochondrial dysfunction induced by this drug.

The malignant transformation of a human bronchial epithelial cell line by exposition to Cr(VI) was associated with altered mitochondria and bioenergetic phenotype.

NMR analysis of metabolic changes in rat heart or in steatotic liver, upon treatments with doxorubicine or ursodeoxycholic acid, respectively, gave new insights into the interplay between mitochondria, metabolism and human toxicology.

The manipulation of hepatic mitochondrial lipids by diet led to changes in mitochondrial bioenergetics and susceptibility to hepatotoxicants.

NO scavenging by circulating erythrocytes was established as a major NO-inactivation pathway in the rat brain, as assessed in vivo.

A novel microarray approach, upon stereotaxic insertion into the rat brain, permitted to study, in real-time, the dynamics of blood and oxygen oscillations during changes in neuronal activity (neurovascular coupling).

A hypothesis of a diet-dependent NO, able to modulate signaling pathways in gastrointestinal tract was formulated.

Beyond their antioxidant properties, anthocyanins counteracted peroxynitrite-induced apoptotic effects in endothelial cells by interfering in crucial signaling pathways upstream and downstream of mithocondria.

A bioanalytical framework was developed to support pharmacokinetics studies involving a novel antiepileptic drug, eslicarbazepine acetate, and its association with carbamazepine and oxcarbazepine, bringing insights into future rational evaluation of polytherapy in epilepsy.

Mitochondrial Toxicology and Disease Group

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Filipe Duarte	PhD
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Nuno Machado	PhD student
Ana Branco	PhD student
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Susana Pereira	PhD student
Claudia Pereira	PhD student
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Katia Mesquita	PhD student
Ana Patrícia Gomes	PhD student
Sofia Marques Ribeiro	PhD student
Ana Carolina Moreira	PhD student
Ana Maria Silva	PhD student
Mariana Ribeiro	PhD student
Filomena da Silva	PhD student
Ana Francisca Soares	PhD student
Ludgero Tavares	PhD student,
Paulo Guerreiro	PhD student
Carlos Rodrigues	PhD student
Mariana Val	Master student
João Fonseca	Master student
Ana Cristina Lemos	Technician
Cláudia Deus	Technician
Rute Loureiro	Technician

Redox Biology in Health and Disease Group

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Teresa Carmo Silva	PhD
Rui Barbosa	PhD
Carla Nunes	PhD
Ana Ledo	PhD
Cátia Lourenço Marques	PhD
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João Frade	PhD
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Cassilda Pereira	PhD student
Nuno Ferreira	PhD student
Diana Serra	PhD student
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Mitochondrial Toxicology and Disease Group

Head: Anabela Rolo

Objectives

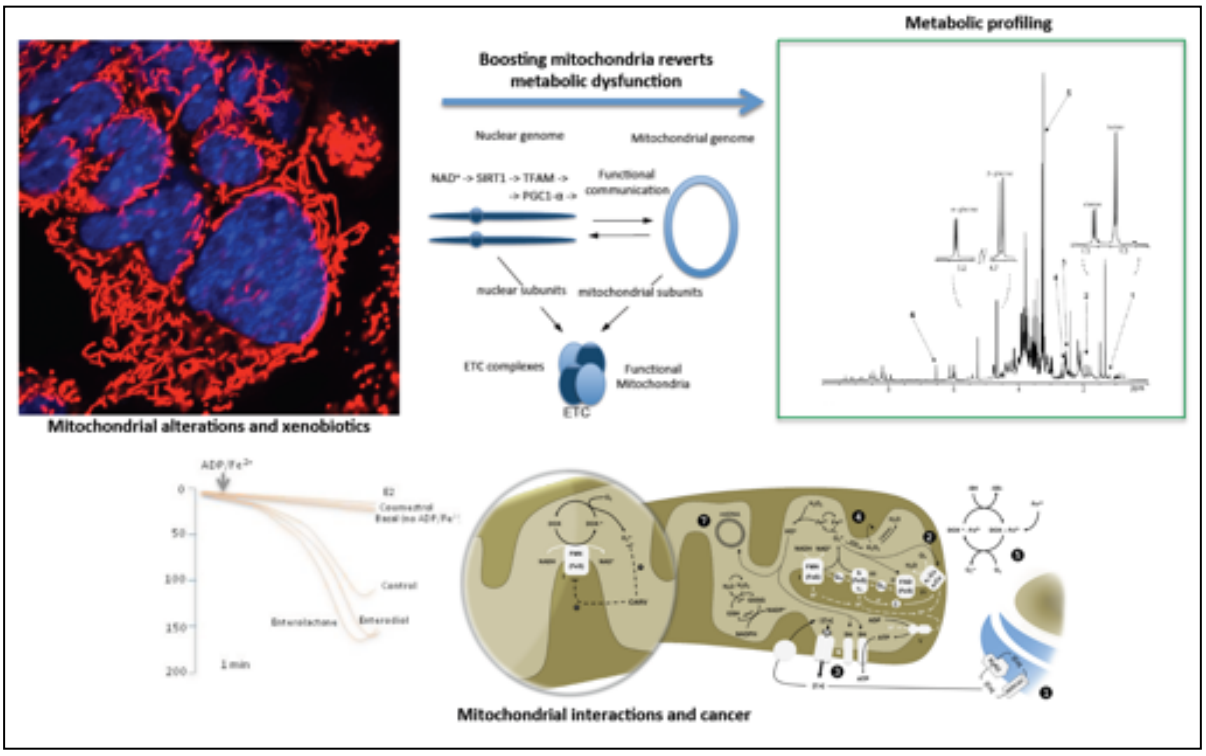
Perturbation of metabolic equilibrium due to mitochondrial dysfunction is implicated in the development of several human pathologies such as insulin resistance and diabetes, cardiovascular diseases and cancer. Also, due to its central role in cell biology (calcium homeostasis, redox regulation and intermediate metabolism), drug-mediated mitochondrial liability is nowadays a critical hinder factor in the safety of many pharmaceuticals. The main objective of our group is to determine which pathways, involving mitochondria, are disturbed/activated by physiologic and pathologic stress and how this influences energetic metabolism. Our ultimate goal is to identify potential therapeutic targets in order to normalize cell function in diseases such as diabetes, cancer and menopause. 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 and metabolism. From polarographic, spectrophotometric and fluorimetric techniques to NMR spectroscopy, the objective of our group is to explore the interplay between mitochondria, metabolism, and human pathology, identifying strategies that can increase mitochondrial capacity in order to prevent mitochondrial degeneration and restore the normal phenotype.

Main Achievements

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

Concerning metabolism alteration and mitochondrial toxicity-induced by therapies against menopause-related physiological, we observed that for higher concentrations, some phytoestrogens can cause alterations on mitochondrial function in different target tissues, most likely by targeting the oxidative phosphorylation process. Resveratrol decreases oxygen consumption by direct effects on Complex I activity in brain and liver mitochondria from. In contrast to enterodiol and enterolactone, both resveratrol and coumestrol exhibit antioxidant capacity. Coumestrol increases the expression of GLUT-1 at the BBB and decreases temperature variation in OVX rats, being a potential alternative to the hormonal therapy in menopause.

By using an in vitro system composed of four strains (C57BL/6J, MOLF/EiJ, CZECHII/EiJ and PERA/EiJ) of mouse embryonic fibroblasts (MEFs) with mtDNA polymorphisms, we showed strain-dependent differences in the response to drug-induced toxicity. Our results also showed that there were clear differences among the four strains of MEFs at passages 3 and 10, with CZECHII/EiJ having a lower mitochondrial robustness when compared to C57BL/6J, followed by MOLF/EiJ and PERA/EiJ. We proposed that this model is a useful starting point to study compounds that may cause mitochondrial off-target



toxicity in early stages of drug development, thus decreasing the number of experimental animals used.

We investigated doxorubicin (DOX) cardiac, hepatic and renal toxicity in Wistar rats, with a special focus on alterations of mitochondrial bioenergetics in a sub-chronic and acute treatment models. The results confirm that alterations of mitochondrial function, which are more evident in the heart, are an early marker of DOX-induced toxicity, existing even in the absence of cardiac functional alterations. We also obtained evidences in an *in vitro* model (H9c2 cardiomyoblasts) that DOX toxicity is dependent on the cell differentiation state, with more adult muscle cells being more susceptible to DOX-induced cell death.

We also showed that the effects on mitochondrial homeostasis of two widely used natural compounds, resveratrol and berberine, are largely mediated by SIRT1 and add the knowledge of a different mechanism of action, centered on SIRT1 activation, for both of this compounds when used at physiological doses. We showed that SIRT1 is a metabolic sensor that integrates a variety of pathways to maintain mitochondrial homeostasis and therefore is extremely important for the development of age-related diseases like insulin resistance, and is an important target for the development of new drugs to treat diseases where mitochondrial homeostasis is disrupted. We have also demonstrated that chenodeoxycholic acid promotes a total reversal of obesity-associated complications in a prolonged model of high fat feeding, while not encountering any side effects. We also demonstrate a regulatory role of CDCA in the metabolism of several tissues, associated with stimulation of UCP1 expression in both white and brown adipose tissue. Also, regulation of mitochondrial permeability transition via cyclophilin D acetylation/phosphorylation is a key protective strategy in the prevention of damage by ischemia/reperfusion.

Redox Biology in Health and Disease Group

Head: João Laranjinha

Objectives

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 (NO) 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.

Main Achievements

a) We have provided support to the concept that the vascular network is critical for the regulation of nitric oxide-mediated volume signaling in the brain. This was possible by demonstrating in vivo, in the rat brain, that nitric oxide (•NO) scavenging by circulating red blood cells constitutes the major •NO inactivation pathway in the brain. Data also supports an inverse relationship between vascular density and •NO diffusion radius.

Thus, the vascular network seems critical for the regulation of •NO-mediated volume signaling in the brain. The understanding of the major mechanism that switches off nitric oxide actions in the brain is critical for the general understanding of its activity, including the modulation of neurovascular and neurometabolic coupling and, more specifically, its protective effect during ischemia.

We have addressed these issues in vivo on basis of state-of-the-art microelectrode technology that allows a fine tuned spatial and temporal measurement •NO concentration dynamics in the brain.

b) We have uncovered functional implications of the pathway nitrate:nitrite:nitric oxide following nitrate intake in the diet and revealed a novel activity for pepsin as an anti-ulcerogenic compound in vivo. In particular: (i) Dietary nitrite triggers pepsinogen nitration in the stomach by dietary nitrite, (ii) Nitration decreases pepsin function preventing the progression of gastric ulcers under inflammatory conditions.

We have also established the notion that protein nitration is found physiologically in the stomach of healthy animals.

c) The ongoing study of the molecular mechanisms involved in the vascular cytoprotection afforded by anthocyainins supported the benefits of these compounds as as nutraceuticals, in the context of inflammatory diseases.

More specifically, malvidin-3-glucoside, a major dietary anthocyanin, exerts a protective role in NO balance and in inhibition of proinflammatory signaling pathways in injured endothelial cells, such as NF-kB, supressing proinflammatory mediators. Also, cyanidin-3-glucoside, showed a much higher anti-inflammatory efficiency in human intestinal HT-29 cell line, under cytokine injury, than 5-aminosalicylic acid, a well established drug in therapeutics of this disease, as evaluated by NO, PG2 and IL8 production and iNOS and COX-2 expressions.

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MICROBIOLOGY

Coordinator: Milton Costa

The Microbiology Area strives to understand several aspects of the taxonomy, systematics, biodiversity, molecular biology, biotechnology and pathogenesis of archaea, bacteria and eukaryotic microorganisms. There is no unifying topic, as it could not be from such a diverse group of people with different backgrounds. There is no common thread to the research, but each group achieves results and, in some cases, achieves important results.

The Microbiology Area comprises two working groups; The Microbiology of Extreme Environments (which will soon change its name) is primarily devoted to the microbiology of extremophiles, particularly bacteria and archaea that are extremely ionizing radiation-resistant, bacteria and archaea that live in thermal and hypersaline environments (particularly from beep Mediterranean brines) and deep sub-surface environments. This group has been very successful in studying the metabolic pathways for the synthesis of compatible solutes in hyper/thermophiles and co-authored four patents. This group has also been active in the study of the ecology and evolution of pathogenesis genes in *Legionella* spp.

Another group named Medical Microbiology focuses on the biological traits of two groups of microbes (mycobacteria and fungi) that drive their success as facultative intracellular infectious agents and as opportunistic agents of human infection, together with the host response according to changes in the surface characteristics of microbial cells on yeast pathogenesis and the synthesis of mycobacterial polysaccharides that are regulate and aid in the synthesis of mycolic acids.

Major Achievements

Isolation and characterization of novel organisms from extreme environments for basic studies and for their biotechnological potential.

Identification new compatible solutes in hyper/thermophilic bacteria and archaea, elucidation their biosynthetic pathways and their role in environmental stress tolerance. The pathway for the synthesis of compatible solutes responsible for osmotic stress adaptation of the members of the deep-rooted lineage of bacteria *Planctomycetes* was recently elucidated. New compatible solutes were identified in one extremely radiation-resistant bacterium.

Determination of the contribution of natural environmental *Legionella pneumophila* strains in the molecular evolution of genes crucial for infection under distinct environmental conditions.

Achievement of a detailed profile of the microbial diversity of a deep groundwater of a natural mineral water and the population dynamics that occur before and after bottling and storage.

A novel mechanism for *C. albicans* to subtly manipulate extracellular levels of ATP, thus avoiding avoiding the overt activation of macrophages was deciphered.

Genes that code for the enzymes responsible for the synthesis of *A. infectoria* cell wall components were recently identified.

A novel chromogenic yeast identification procedure (CandiDetect) was developed.

Mycobacteria synthesize unique intracellular methylglucose polysaccharides to modulate fatty acid synthesis. We have identified most of the genes in this novel pathway and characterized the enzymes, two of which had their 3D structures solved by our collaborators.

Microbiology of Extreme Environments Group

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Ana Catarina M. Ferreira	PhD Student
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Tânia de Jesus Leandro	Grant technician
Ana Filipa d'Avó	Grant technician

Medical Mycology – Yeast Research Group

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Rui Manuel Costa Soares	PhD student
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Diogo Reis	Master student
Mariana Almeida	Master student
Marta Ereira Mota	Master student
Mafalda Costa	Master student
Nelson Cunha	Master student
Tomé Cardoso	Master student
Branca Margarida Silva	MPharmSc FCT BI
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Microbiology of Extreme Environments Group

Head: Milton Costa

Objectives

1) 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.

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

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

4) The study the biodiversity of the brine and brine-seawater interface of Lake Medee, with high sodium and chloride levels, and Lake Kryos that contains extremely high levels of magnesium and chloride, to obtain enzymes of biotechnology value.

5) The identification of lead natural extracts with proven potential for subsequent fractionation towards the isolation of active compounds that can be further developed into future therapies for Q fever.

6) To determine if distinct constrains exerted by different niches and hosts shaped the evolution and the ability of Legionella pneumophila strains to infect protozoan and mammalian cells and to identify the underlying mechanisms, aiming to correlate the L. pneumophila lifestyle with their virulence.

7) To unreavel the microbial phylogenetic diversity and community structure of two unknown and hydrochemically distinct Deep hypersaline anoxic basins, located in Mediterranean Sea, using massively parallel 454 pyrosequencing of the 16S rRNA gene. Using a microarray approach, we will identify the genomic and transcriptomic events occurring during cell adjustment to different hydrochemical conditions by comparing microbial communities and linking then to 16S diversity and gene expression. With the application of these methodologies, we expect to obtain a deep description of the structure and function of these extreme and strange environments. The results could support us with valuable data suitable for biotechnological applications.

Main Achievements

We have completed the genome sequence of Dehalogenimonas lykanthroporepellens type strain (BL-DC-9(T)) and Mycobacterium hassiacum DSM 44199.

We have isolated and characterized one genus: Oceanicella and six novel bacterial species': Oceanicella actignis, Dehalogenimonas alkenigignens, Cecembia calidifontis, Natrinema salaciae, Nevskia aquatilis and Nevskia persephonica.

We detected a complex but not stable autochthonous structure on groundwater samples between different replicas. We observed that the bottling procedures and storage time induced profound modifications on groundwater diversity. We concluded that the same relative composition pattern was replicated for the same time of storage between different collection samples, indicating that the population dynamics that occur in the bottle were reproducible. A high diverse bacterial composition and low archaeal diversity were detected in groundwater and in bottled water samples. The majority of the sequences collected from groundwater were from autotrophic populations, mainly Gram-positive organisms. On the other hand, bottle environments were dominated by Gram-negative heterotrophic organisms.

We have described a new bacterial hydrolase specific for the compatible solutes α-D-mannopyranosyl-(1→2)-D-glycerate and α-D-glucopyranosyl-(1→2)-D-glycerate.

We determined that ehe plant Selaginella moellendorffii possesses enzymes for synthesis and hydrolysis of the compatible solutes mannosylglycerate and glucosylglycerate.

Medical Mycology – Yeast Research Group

Head: Teresa Gonçalves

Objectives

"*Alternaria infectoria* FKS, CHS and melanin synthesis genes: the combination to oportunism":

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. Macrophage in vitro infection by A. infectoria spores – effect of caspofungin treatment

4. Alternaria infectoria extracellular vesicles

5. Identification of the melanin synthesis pathway

"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 2A gene expression

3. Sinergistic effect with TLR2 and Dectin1

3. C. albicans infection of A2A knockout mice peritoneal macrophages

4. Impact of ectophosphatases in C. albicans internalisation

"Elucidation of the biosynthesis of mycobacterial virulence factors as targets for new tuberculosis therapies":

1. Identification of genes of the mycobacterial MGLP pathway

2. Characterization of the key-enzymes

3. Construction of mutants for target validation towards drug development

4. Crystallization and three-dimensional structure determination

5. The role of polymethylated polysaccharides in mycobacterial physiology

Main Achievements

"*Alternaria infectoria* FKS, CHS and melanin synthesis genes: the combination to oportunism"

1. 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 fumigatus. 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.

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BIOPHYSICS AND BIOMEDICAL NMR

Coordinator: Carlos Geraldes

This Area consists mainly of two subareas: 1) Metal based agents for diagnosis and therapy: to develop and study new metal-based compounds for medical diagnostic imaging (in particular MRI contrast agents and nuclear imaging 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 Ln3+-based paramagnetic complexes of Gd3+ and paramagnetic nanoparticles and liposomes with interesting photoluminescent properties for optical imaging (OI), and/or high 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 r1 and r2 relaxivities of these agents as efficient reporters for Molecular Imaging has been pursued. A series of 67Ga and 68Ga-labeled with targeting capacities are also 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. 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 and in vitro interaction with serum components have/will be studied. The mechanism of action of the vanadium complexes at the molecular level have/will be investigated, in particular the effect on target proteins of the insulin signaling cascade, complemented by MRI and in vivo/ex vivo 1H MRS in animal models. 2) Intermediate metabolism studies: 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. Besides human and rodent metabolism, we are also applying our methods to study glucose and amino acid metabolism in fish, for applications in aquaculture.

Inorganic Biochemistry and Molecular Imaging Group

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Intermediary Metabolism Group

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Fátima Martins	PhD student
Cristiano Santos	Master student
Margarida Coelho	Master student
Paula da Silva	Master student
Ana Rita Gonçalves	Technician
João Rito	Technician
Filipa Simões	Technician

Inorganic Biochemistry and Molecular Imaging Group

Head: Carlos Geraldes

Objectives

Our general objective is the study of inorganic compounds for medical diagnostic imaging (in particular MRI contrast agents), inorganic drugs for medical therapy, and the study of environmental and toxicological effects of inorganic species. 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 nanoparticles with interesting photoluminescence properties for optical imaging (OI), and high r_2 relaxivities, especially at high fields, yielding negative contrast in T2-weighted MRI images. The r1 relaxivity of new lanthanide chelates will be increased by designing new chelating agents which increase the number of inner sphere water molecules and optimize the water exchange rates. Second-sphere water relaxation contributions should also be optimized.

We also study the molecular and cellular mechanisms of action of lithium salts in the therapy of the bipolar disorder, using cell and animal systems. Several types of new inorganic vanadium(IV/V) complexes were synthesised, chemically characterized in aqueous solution and their potential use as efficient oral insulin-enhancing agents for type II diabetes and toxicity effects was investigated in different cell systems.

Other projects in Inorganic Chemistry include the study of the impact of metal ions on human health and environment and the development of novel materials and methods for on-line detection of environmentally hazardous metals. Systems for the detection and quantification of Zn(II), Cd(II), Hg(II), Al(III) and Ga(III) are being studied, using multinuclear NMR spectroscopy, DFT calculations and luminescence techniques. In addition, there is also interest on the study of polymers as biosensors for nucleic acids, sugars and proteins.

Main Achievements

A) New Gd(III)/Mn(II) complexes as MRI contrast agents (CA):

1) Supramolecular Adducts of Gd(III) DOTP and Cyclodextrins with Ammonium Groups were characterized by relaxometry and NMR.

2) The Gd(III) complexes of Tris(phosphonomethyl) cyclen derivative ligands were studied - thermodynamic stability, kinetics, solution structure, and relaxivity of Gd3+ complexes.

3) The Gd(III) complexes of the Tris Amide conjugates of the DO3A-N-(a-amino)propionate ligand were studied as stable, high relaxivity CAs for MRI

4) New Tris-3,4-HOPO lanthanide complexes were studied as potential imaging probes

5) Thermodynamic stability and relaxation studies of small triaza-macrocylic Mn(II) chelates as MRI agents.

B) Nanoparticulate molecular imaging agents:

1) Supramolecular Protamine/Gd-loaded Liposomes Adductswere studied as as Relaxometric Protease Responsive Probes

2) Yeast Cell Wall Particles: promising class of nature-inspired microcarriers for multimodal imaging

3) Lanthanide-DTPA Grafted Silica Nanoparticles are Bimodal-Imaging Contrast Agents

4) The effects of the the silica coating on the relaxometric behavior of γ-Fe2O3@SiO2 core shell nanoparticles as MRI CAs.

5) Gold nanoparticles functionalised with stable, fast water exchanging Gd3+ chelates are high relaxivity Contrast Agents for MRI

6) Silica Nanoparticles for Bimodal MRI-Optical Imaging were obatained via grafting of Gd3+ and Eu3+/Tb3+ Complexes

7) (Gd,Yb,Tb)PO4 Up-Conversion Nanocrystals for Bimodal Luminescence-MR Imaging

8) MRI Tracking of Macrophages with Glucan Particles Entrapping a Paramagnetic Agent

C) Radiolabeled compounds for nuclear imaging:

1) A Gallium Complex with a New Tripodal Tris-Hydroxypyridinone for Potential Nuclear Diagnostic Imaging studied in solution and in vivo of the 67Ga-labeled species

2) Different phosphorus-containing ligands complexing 68Ga for PET-imaging of bone metabolism were compared

3) Ga(III) chelates of amphiphilic DOTA-based ligands were synthetized and the 67Ga-radioabeled ligands were studied in vitro and in vivo.

4) Spectroscopic, radiochemical, and theoretical studies of the Ga3+-HEPES buffer system were undertaken, giving evidence for the formation of Ga3+- HEPES complexes in 68Ga labeling reactions

D) NMR studies of protein structure and dynamics and protein- ligand interactions:

1) The Interaction of La3+ Complexes of DOTA/DTPA-Glycoconjugates with the RCA120 lectin was studied by Saturation Transfer Difference (STD) NMR Spectroscopy.

2) The Enantioselective binding of a chiral lanthanide(III) complex to human serum albumin was studied by 1H STD NMR techniques

3) Gd(III) Chelates were studied as NMR Probes of Protein-Protein Interactions for the case of Rubredoxin and Cytochrome c3

4) The structure and dynamics of the catalytic domain of MMP-1 was studied by NMR through tagged lanthanides.

E) NMR studies of micelles and membrane models:

1) The Interaction of the uncupler carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP) with lipid membrane systems was studied by 31P NMR.

2) The Nimesulide interaction with membrane model systems was studied by 31P NMR.

3) A 13C NMR method for the Quantification of Cholesterol Solubilized in Bile Salt Micellar Aqueous Solutions was described.

F) Metal based drugs:

1) Vanadium-based insulin-mimetic agents: proof that VO(dmpp)2 normalizes pre-diabetic parameters as assessed by in vivo magnetic resonance imaging and spectroscopy

2) Lithium compounds for bipolar disorder therapy: 23Na MQF NMR characterization of Na+ Binding and Dynamics in Animal Cells, effects of Na+/Li+ Competition

G) Small complexes and polymers:

1) Structural and photophysical studies on Gallium(III) 8-hydroxyquiniline-5-sulphonates, effect of the excited state decay in ligand photolabilization.

2) The driving effect of cation dehydration on the binding of metal ions to polyelectrolytes in water

3) NMR, DFT and luminescence studies of the complexes of Al(III)-8-hydroxyquinoline-5-sulfonate

4) Spectroscopic Properties, Excitation, and Electron Transfer in an Anionic Water-Soluble Poly(fluorine alt-phenylene)-perylenediimide Copolymer

H) Human MRI studies of liver desease:

1) Fat deposition decreases diffusion parameters at diffusion weighted MRI (DTI) in phantoms and patients with liver steatosis.

Intermediary Metabolism Group

Head: John G. Jones

Objectives

The overall objective of my lab 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.

Main Achievements

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 Research Laboratories in both USA and Europe. This has resulted in productive collaborations and has secured ~100,000 euros in International funding during 2011-2012. The group head is also one of the founder members of the APDP-RC, a Clinical Research Center established in 2011 at the Portuguese Diabetes Association in Lisbon - one of the largest Diabetes Outpatient Clinics in Europe. This will provide unique opportunities for further development of our tracer metabolic and metabonomic studies in both Type 1 and Type 2 diabetes patients and integration with other leading Diabetes Research Laboratories in Portugal and beyond. APDP-RC was awarded an FCT "Center of Excellence" Grant in the last funding cycle and developing stable isotope tracer studies of glucose, fructose and lipid metabolism for early diagnosis of insulin resistance and diabetes is one of the key aims of this project.

Our collaborative clinical studies with Mayo Clinic and the German Diabetes Foundation during 2011-2012 results in five papers accepted for publication or published in medium to high-impact Journals and we have a further three that are in preparation. In animal models, we extended the deuterated water tracer method to determine the contribution of dietary fructose to postprandial plasma glucose and glycogen (Delgado et al (2013) *Am. J. Physiol.* **304,** 384-391), as well as probing the enrichment of hepatic acetyl-CoA from deuterated water and 13C-substrates in order to determine the precursors of *de novo* lipogenesis. These methods are in the process of being translated into noninvasive human studies.

We are making progress 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. A clinical project was initiated in late 2012 at APDP-RC and is ongoing. LC-MS has also been successfully applied to study postprandial glucose kinetics in mice and rats in collaboration with Paula Macedo´s Lab in the New University of Lisbon. We are co-investigators of their project that was approved by FCT and will involve applying our technology to inform glucose kinetics in mouse models of obesity and insulin resistance.

We have also built upon our collaboration with Prof Baanantes´s Group at the University of Barcelona in the study of fish Nutrition and are Co-investigators in a Project funded by the Spanish Ministry of Science. Our group also gained an FCT Postdoctoral Fellowship (Ivan Viegas) and an FCT Ph.D. fellowship (Joao Rito). We have published 3 papers demonstrating the application of deuterated water to follow glucose, glycogen and amino acid metabolism and we have unpublished data that demonstrate the that contribution of dietary carbohydrate to blood glucose and liver glycogen can be increased by supplementation of fishmeal with cooked starch.

Publications

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CELL AND DEVELOPMENT BIOLOGY

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 School of Medicine (FMUC) and University Hospitals Center of Coimbra (CHUC), both in terms of basic research with human samples (biochemistry, cell and molecular biology), setting up novel clinically-relevant services and trials, and furthering translational research. Partnerships in place include: Immunology, Oncobiology, Genetics, Neurology, Dermatology, Reproduction, Endocrionology (Obesity, Diabetes) and Cardiology. The main goal of this line of research is to create added value in translational research, not only in terms of basic and clinical knowledge, but also patents and industry-based contracts. As noted in the group reports in the previous two years of the CNC project major steps have been taken in that direction.

One of the major strengths of the groups in this area is the strong collaboration with clinical departments and MDs, allowing the collection of human tissues and samples for the development of translational investigation.This has been achieved in the past as the publication record for the various groups in this area demonstrates, with increased quality of publications, funding, and quality of young researchers in the past two years. Furthermore national and international collaborations are in place, which are paramount for the success of clinical-based research.

The groups in this area will continue to develop the research lines in which they are engaged, further strengthening existing clinical-based translational collaborations and seeking new ones, to create networks that may serve for future projects. This is evident from each of the groups' individual research proposals. Namely, the Reproduction group proposes to develop established solid research in the field of gamete quality and stem cell biology to issues related to treating infertility and preserving germline tissue, with the cryopreservation of oocytes and ovarian and testicular tissue for cancer patients, and on the managing of diabetes in reproductive matters. New postdocs with independent funding have been hired, and further funding is being negotiated with two pharmaceutical companies for service-based research. Funding from the German National funding Agency has also been obtained for collaborative work, and joint projects will be submitted. Furthermore the group has embarked on an "omics" approach to male gametes (proteomics, metabolomics) and stem cells (metabolomics) that hold promise for future clinical applications. The Cellular Immunology and Oncobiology group proposes to carry out novel research in cartilage repair and on the immunological basis of cancer, and has taken major steps in terms of patent and industry-based contract developments, which they will continue to pursue. The main advantage of an extensive collection of human samples (e.g. tumors) will developed in collaborations focused on genetic and epigenetic regulation of cancer. The Phagocytosis and Pathogens group has achieved good financing and publication status, has hired experienced researchers (including a Marie Curie postdoc) and will further its basic research in pathogen biology in part by participating in Portugal-Harvard Medical School initiatives and collaborating with other CNC groups, namely in the Micobiology and Vector areas. The strength of this group is the extensive expertise in intracellular membrane trafficking at the cellular level, which is being translated into pathogen management, as the pathogens under study (causal agents for tuberculosis and AIDS among others) highjack the cellular trafficking machinery for successful infection. The Metabolism, Insulin Resistance and Complications group has also increased its funding and critical research mass and proposes to enhance collaborations with CHUC services and CNC groups in the area of diabetes, obesity and wound healing, and has further established collaborations in cardiology to analyze surgically removed human samples. The analysis focuses on a comprehensive approach, involving biochemistry, gene expression and epigenetics. Collaborations with other groups with a focus on Metabolism (for sample analysis) and stem cells (wound healing) are ongoing, and participation in projects with funding outside of Portugal is a welcome development, which will be continued in the current project.

There is an enormous wealth of expertise in terms of healthcare, medical know-how, sample collection and patient groups at CHUC/FMUC, which will explored further during this proposal, provided there are common interests and the partnerships are mutually beneficial. Besides ongoing efforts, novel collaborations will be attempted in gynecology, histocompatibility and diagnosis counseling.

During the previous proposal we tried to stimulate University Hospitals to consider the hiring of CNC personnel, which could further partnerships, and introduce researchers into positions where they can help develop new projects and collaborations. Encouragingly this has happened in a few cases. Some funds from CHUC have also been obtained, either in terms of consumables or equipment use (such as flow cytometry, cell sorting or gene arrays) that would not be otherwise available.

Additionally alternative funding and wealth-producing initiatives are being actively sought since the previous proposal and have included the establishment of patents (2), industry contracts (2 current, 2 to start soon), and direct support from pharmaceutical companies. The goal is to help CNC reach alternative sources of funding, and bridge basic and clinical research on a more direct basis than is traditionally the case.

Cellular Immunology and Oncobiology Group

Celeste Lopes	PhD – *head of group*
Ana Bela Sarmento	PhD
Fernando Monteiro Judas	PhD
Alexandrina Mendes	PhD
Maria Teresa Rosete	PhD
Teresa Martins	PhD
Anália do Carmo	PhD
Sílvia Neves	PhD
Ana Luísa Vital	PhD
Marta da Silv	PhD
Margarida Gonçalo	MD
Hermínio Espírito Santo	MD
Maria Olinda Rebelo	MD
Ana Cristina Gonçalves	PhD student
Joana Costa e Silva	PhD student
Mariana Freitas	PhD student
Ana Teresa Rufino	PhD student
Diana Dinis Azenha	PhD student
Patrícia Domingues	PhD student
João Boto Martins	PhD student
Raquel Alves	PhD student
Ana Inês Crespo	PhD student
Diana Carvalho	PhD student
Carlos Melo	PhD student
Humberto Ferreira	PhD student
Sara Lima	PhD student
Vera Francisco	PhD student
Joana Liberal	PhD student
Vera Patrícia Gonçalves	Msc
Isabel Ferreira	Master student
Diana Matias	Master student
Patrícia Guarino	Master student

Biology of Reproduction, Stem Cells and Human Fertility Group

João Ramalho de S. Santos	PhD – *head of group*
Alexandra Amaral	PhD
Sandra Amaral	PhD
Ana Paula Sousa	PhD
Paula Mota	PhD
Marta Baptista	PhD Student
Renata Tavares	PhD Student
Ana Sofia Rodrigues	PhD Student
Beatriz Sousa	PhD Student
Marília Cordeiro	PhD Student
Carla Paiva	PhD Student
Tânia Perestrelo	PhD Student
Marcelo Correia	PhD Student
Maria Inês Sousa	PhD Student
Ângela Crespo	PhD Student
Rodrigo Santos	PhD Student
Mónica Marques	Master Student
Bárbara Lourenço	Volunteer

Infection, Phagocytosis and Pathogens Group

Otília V. Vieira	PhD – *head of group*
Luís Estronca	PhD
Marisa Rego	PhD
Faraj Barah	PhD
Ângela Inácio	Post-Doctoral Fellow
Michelle Viegas	Post-Doctoral Fellow
Elda Bonifácio	Research Technician

Insuline Resistance and Adipocyte Group

Eugénia Carvalho	PhD – *head of group*
Ermelindo Leal	Post-Doctoral Fellow
Daniel Espinoza	Post-Doctoral Fellow
Susana Guerreiro	Post-Doctoral Fellow
Marta Santos	Post-Doctoral Fellow
Pedro Gonçalves	Post-Doctoral Fellow
Maria J Pereira	PhD Student
Liane Moura	PhD Student
Patrícia Lopes	PhD Student
Roksana Pirzgalska	PhD Student
Amelia Fuhrmann	Msc
Carlos Moura	Msc

Cellular Immunology and Oncobiology Group

Head: Mª Celeste Lopes

Objectives

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 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 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.

Main Achievements

RESEARCH ON CELLULAR IMMUNOLOGY:

• Immunobiology of antigen presenting cells:

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

Cymbopogon citratus has anti-inflammatory properties by inhibiting TNF-α and CCL5 production in macrophages through NF-κB, p38 MAPK and JNK pathways modulation. The suppression of NF-κB pathway by *Cymbopogon citratus* is partially 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. Active components of that essential oil have been identified and characterized pharmacologically.

• 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.

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. The results also demonstrate that the farnesyltransferase inhibitor, α-HFPA, is effective independently of Ras mutations and that epigenetic modulators have a synergistic effect dependent on the schedule of administration.

• 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 extracelular microenvironment; 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:

Cytogenetic heterogeneity and distinct clonal pathways of glioma evolution was revealed by iFISH analysis. The 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 the results showed that PI3k/Akt, ERK1/2 and PKC contribute to glioma survival, proliferation and chemoresistence to temozolomide therapy. We have also identified changes in Claspin in gliomas and are studying the functional implications of these mutations in gliomagenesis.

Biology of Reproduction, Stem Cells and Human Fertility Group

Head: João Ramalho-Santos

Objectives

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 metabolic-based methods to improve stem cell propagation and differentiation into specific fates.

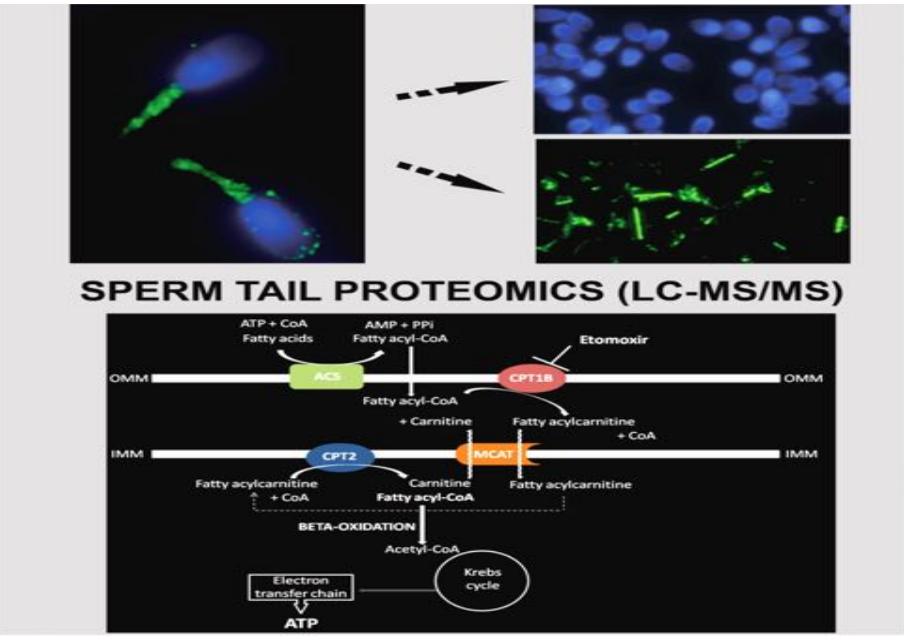
Current projects include research to characterize the most viable human gametes, both in terms of basic science and for application in Assisted Reproduction. In this regard more functional sub-populations of sperm from a heterogeneous ejaculate are being isolated and

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. Besides classic sperm functional parameters characterization of these subpopulations includes mitochondrial activity, reactive oxygen species (ROS) formation, ATP production, sperm proteomics and metabolomics.

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 culture and 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, and by conditions such as aging and diabetes. 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. The action of endocrine disruptors and other pharmacological agents at physiological concentrations is of particular interest, as some well-known substances seem to affect sperm function via novel non-genomic mechanisms.

Finally the group is pursuing the modulation of stem cell pluripotency and differentiation using metabolic cues, and this work is being expanded to also included the generation, propagation and differentiation of induced pluripotent cells (iPS cells). In this work we have identified possible metabolic switches that might be involved in the control of pluripotency, including mitochondrial activity and enzymes related to glycolysis and the Krebs cycle. We are currently testing these switches functionally. Importantly, these metabolic characteristics peculiar to stem cells seem to also be present in cancer cells, and this has led to parallel interests in the common metabolic interests that might modulate both pluripotency and oncogenicity.



Main Achievements

Recently concluded research includes:

1- Publishing the first characterization of the human sperm tail proteome, which showed that sperm uses previously unidentified metabolic pathways for motility, such as the oxidation of fatty acids (collaboration with the University of Barcelona, Spain).

2- Continuing use of the simple assay to monitor human sperm chromatin status we developed in recent years to monitor this parameter in a clinical setting (University

Hospitals of Coimbra), in order to ascertain its possible introduction in routine analysis. Aspects being studied include the outcome of Assisted Reproduction Techniques, male age and several urological pathologies.

3- Using flow cytometry, fluorescence assisted cell sorting (FACS) and swim-up techniques to determine that the production of reactive oxygen species (ROS) is extremely variable in human sperm, with several distinct subpopulations being identified. Given that low amounts of ROS are necessary for sperm function, while high amounts are deleterious, we are trying to identify the more functional subpopulation in terms of ROS production.

4- Using a simple system that allows for the maintenance of viable and motile human sperm in culture for about two weeks, developed last year, to study the long-term effects of different substances on human sperm function. We have found that physiologically low (nano and picomolar) concentrations of endocrine disruptors such as DDE, and pharmacological agents such as sildenafil/Viagra compromise sperm function. We have also used this system to test potential new spermicides, discovering that early sperm responses in terms of calcium signaling modulate spermicidal properties.

5- Discovery that mitochondrial inhibition curtains embryonic stem cell differentiation towards a neuronal phenotype, indicating that mitochondrial activity is crucial for cellular differentiation, thus confirming that mitochondria play an important role in stem cell biology.

6- Previous research determined that all pluripotent stem cells share a preference for aerobic glycolysis and activate metabolic switches that favor this pathway, characteristics that are also common in cancer cells. We have found that pharmacological modulation of some of these metabolic switches (namely those related to hexokinase 2 and pyruvate dehydrogenase) negatively influences stem cell pluripotency, by pushing cells towards differentiation. These results again stress metabolism as a key factor in understanding cellular status.

7- Publication of a bioethics paper on the use of pluripotent stem cells, which tackles both embryo-derived cells, and induced pluripotent stem cells (iPSC).

8- Establishment of the basic conditions for testicular organ cultures for in vitro modulation of testis function in rodents and marmosets (collaboration with the University of Munster, Germany), which will be translated to the domestic cat as a model for endangered felids.

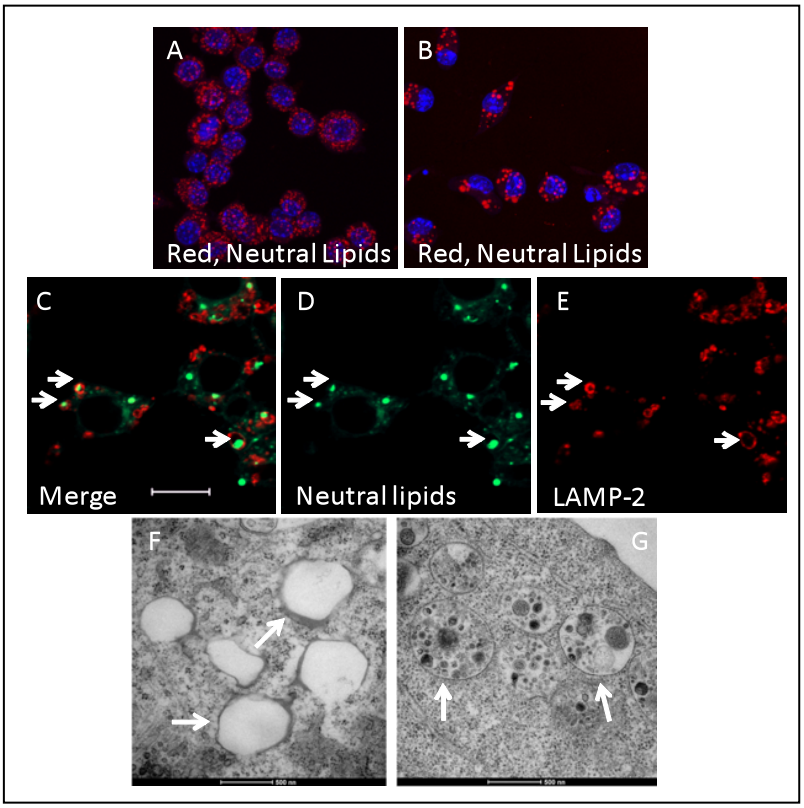
9- Funding for 4 FCT Projects was obtained during this period.

Infection, Phagocytosis and Pathogens Group

Head: Mª Otília Vieira

Objectives

The main objectives of my laboratory are to apply concepts and methodologies of Cell Membrane Traffic to study two types of pathologies: 1) Host-pathogen interactions that disrupt normal membrane traffic, specifically we are focused on *Mycobacterium tuberculosis*/macrophages interactions; and 2) Pathologies that disrupt normal membrane traffic via perturbations of cellular cholesterol homeostasis, specifically we are interested in the etiology of atherogenesis and in the reasons for the failure of apoptotic cells clearance in the arterial *intima*.



Main Achievements

1. We have performed a high-throughput lentiviral trafficking library and have found novel factors involved in plasma membrane re-sealing in macrophages infected with *Mycobacterium tuberculosis*.

2. We have generated a methodology that permits delivery of specific chemical products of Low Density Lipoproteins (LDL) oxidation to macrophages. This methodology allowed the identification of a compound, a cholesteryl-hemiester. This biological compound is sufficient to cause pathological lysosomal lipid accumulation (lipidosis) and massive apoptosis in macrophages (both outcomes occur in atherogenesis).

3. Efferocytosis (phagocytosis of apoptotic cells) is an important concern in the progression of lesions in atherogenesis. Foam cells dying from lipidosis recruit macrophages and smooth muscle cells to the lesion, an inflammatory process, and the recruited cells convert to foam cells and also die setting up a chronic inflammation. Exactly why this happens is not known. Thus, my laboratory has been studying the kinetics of phagocytosis of apoptotic cells by non-professional phagocytes, smooth muscle cells, that are also recruited to atheromatous lesions. We find that phagosomes containing IgG-opsonized particles fuse faster with lysosomes than phagosomes containing apoptotic cells in smooth muscle cells. Understanding this system may help us consider ways to accelerate phagosome-lysosome fusion as a possible route to resolve atherosclerotic lesions.

Insuline Resistance and Adipocyte Group

Head: Eugénia Carvalho

Objectives

a) Immunosuppressive agents, such as cyclosporine and rapamycin cause dyslipidemia and diabetes in solid organ-transplantation. We aimed to investigate whether adipose tissue plays a role in the perturbations of glucose and lipid metabolism caused by these agents. We used adipose tissue from healthy volunteers and from *in vivo* treated Wistar rats.

b) Diabetes is one of the most widespread and costly diseases in the world. It may cause diabetic foot ulcers, decreasing the welfare of patients. Peripheral neuropathy impairs wound healing. We have used different cellular and animal models to unveil the molecular mechanisms of wound healing.

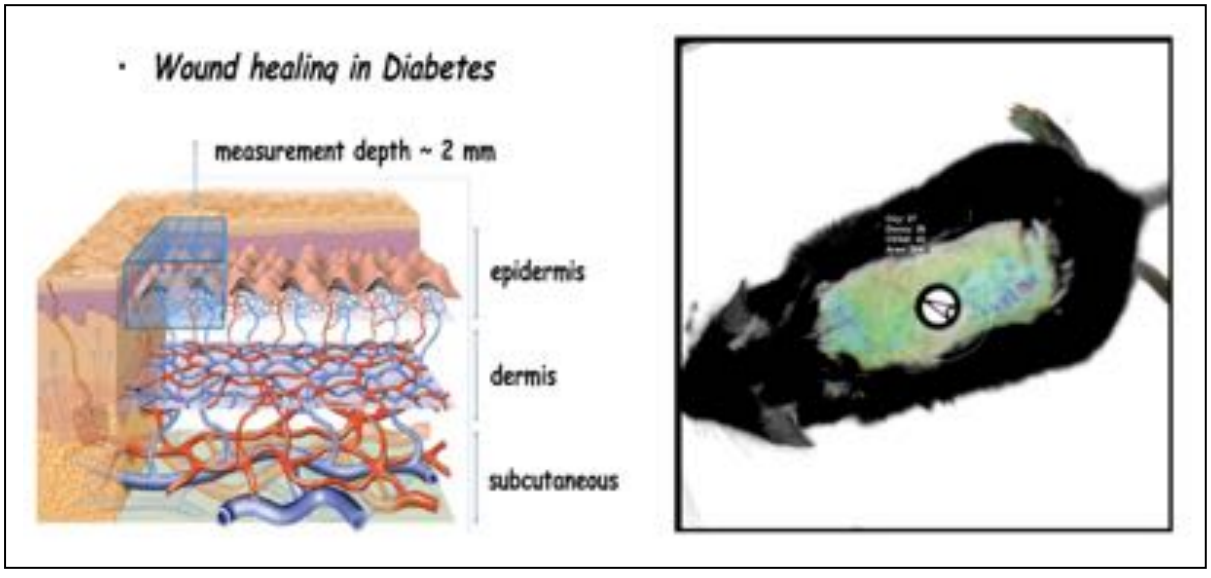
Recent studies suggest that neuropeptides and mast cells participate in wound healing but the mechanisms of their action are not clear. Our main hypothesis is that skin mast cells are dysfunctional in diabetes due to neuropeptide deficiency, contributing to impaired wound healing. We assessed wound healing 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.

Furthermore, 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.

c) Congestive heart failure (HF) is a major health care burden and life-threatening condition. Insulin resistance, impaired glucose tolerance and overt diabetes are associated with the disease, which is accompanied by inflammation and oxidative stress. Epicardial adipose tissue (EAT) has been related to HF and myocardial dysfunction through unidentified mechanisms. We aim at understanding the role of EAT in HF conditions. Our objective is to study the role of EAT on the heart muscle, not only at the metabolic and inflammatory levels, but also to assess oxidative and ER stress, autophagy, apoptosis and mitochondrial dysfunction in these tissues derived from patients with diabetes and the association of these factors with the presence of CVD.

Main Achievements

a) We shave hown that rapamycin and the calcineurin inhibitors, cyclosporin A and tacrolimus, at therapeutic concentrations, had a concentration-dependent inhibitory effect on basal and insulin-stimulated glucose uptake in human subcutaneous and omental adipocytes. Rapamycin inhibited mammalian target of rapamycin complex 2 (mTORC2) assembly, protein kinase B (PKB) Ser473 phosphorylation and phosphorylation of the PKB substrate AS160, and this leads to impaired insulin signalling (*Paper I, published*). On the other hand, cyclosporin A and tacrolimus have no effects on expression or phosphorylation of upstream insulin signalling proteins (insulin receptor substrate 1 and 2, PKB, AS160), as well as the glucose transport proteins, GLUT4 and GLUT1 (*Paper II, submitted*). Instead, removal of GLUT4 from the cell surface was observed, probably mediated through increased endocytosis, as shown in L6 cells. These studies suggest a different mechanism for cyclosporin A and tacrolimus, in comparations to rapamycin, with respect to impairment of glucose uptake in adipocytes. In In addition, we have shown that all three IAs increased isoproterenol-stimulated lipolysis and enhanced isoproterenol-stimulated phosphorylation of one of the main lipases involved in lipolysis, hormone-sensitive lipase. They also inhibited lipid storage and tacrolimus and rapamycin down-regulated gene expression of lipogenic genes in adipose tissue, and all three IAs increased interleukin-6 (IL-6), but not tumor



necrosis factor α (TNF-a) or adiponectin, gene expression and secretion (*Paper III, published*).

b) Diabetic foot ulceration (DFU) and associated impaired healing, is a major problem that significantly impairs the quality of life of diabetic patients, leads to prolonged hospitalization and may result in lower extremity amputations. DFU occurs almost exclusively in the presence of diabetic neuropathy. Recent studies suggest that neuropeptides and mast cells participate in wound repair but the mechanisms of their action are not clear. Our hypothesis is that skin mast cells are dysfunctional in diabetes due to neuropeptide deficiency, contributing to abnormal wound healing. Using animal models we have shown that mast cell deficiency severely impairs wound healing in both diabetic and non-diabetic settings, that mast cell deficiency leads to skin hypoxia and aberrant expression of growth factor and cytokines. In addition, STZ-induced diabetes affects skin mast cell abundance and function and SP exerts its beneficial effect on wound healing, at least partly, through mast cells *(in preparation*).

Moreover, we have also developed chitosan derivatives (MPC foams) for neurotensin (NT) delivery into diabetic and non-diabetic wounds and evaluated the *in vivo* responses to these chitosan derivatives with or without neurotensin. The *in vivo* results demonstrated that NT loaded MPC foams induced a faster healing (50%) in the early phases of wound healing in diabetic mice, decreasing inflammatory infiltrate and increasing migration of fibroblasts. The results of this study suggest that NT released from MPC dressings might have the potential to modulate wound healing (*submitted*).

c) Epicardial Adipose Tissue (EAT) is an active endocrine and paracrine organ located on the surface of the heart surrounding the large coronary arteries that may influence the development of CVD and it has been implicated in the pathogenesis of coronary artery disease. Our main preliminary findings are that in the groups we have studies, in non-diabetic patients, insulin-stimulated glucose transport is significantly lower in EAT cells, compared to sabcutaneous adipose tissue (SAT) cells of the same patients, highlighting the possible physiologic, metabolic, endocrine and inflammatory differences present between both types of adipose tissue. In diabetic patients with congestive heart failure, the insulin-stimulated glucose uptake was impaired in either SAT or EAT. This impairment in activation of glucose transport by insulin could possibly be due to a reduced GLUT4 protein expression. In fact, at the mRNA level, GLUT4 gene expression was significantly decreased in EAT of diabetic patients. In addition, various cardiovascular conditions are characterized by an enhanced vascular inflammation, in which IL-1 signaling may be an essential mediator in the pathogenesis of CHF by suppressing cardiac contractility, promoting myocardial hypertrophy, and inducing cardiomyocyte apoptosis. In fact, IL-α gene was significantly increased in EAT of diabetic patients (*in preparation*).

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BIOMEDICAL INTER-INSTITUTIONAL RESEARCH PROGRAMME

Psychiatry Research

António Ferreira de Macedo, Ana Telma Pereira (FMUC, CNC)

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 inpute 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 $P < 1 \times 10\text{-}4$. 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 $p<10\text{-}4$ (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 familiality 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). Few months after, this funding has been canceled due to FMUC financial constraints. However, we have developed a new diagnostic interview entitled EP-GENE (Entrevista Psiquiátrica para Estudos Genéticos 1.0; 2011) and assessed 50 SZ/BP patients used it and OPCRIT.

EP-GENE is a new semi-structured diagnostic interview developed by the Researh Group on Psychiatric Genetics (Grupo de Estudos de Genética Psiquiátrica-GEGP/FMUC). Its construction was based on (a) the Diagnostic Interview for Genetic Studies (DIGS; Nurnberger et al., 1994), which were translated to Portuguese by present investigators, who use it for two decades, reporting excellent inter-rater reliability (Azevedo et al., 1993); and on (b) the Diagnostic Interview for Psychoses and Affective Disorders (DIPAD) developed by Genomic Psychiatry Cohort lead by the Dr C. Pato (University of Southern California), with whom the GEGP/FMUC collaborates for more than twenty years. The EP-GENE collect and record information regarding a research subject's functioning and psychopathology with primary emphasis on information relevant to the study of the affective disorders and schizophrenia. The organization of the interview and the item coverage are designed to elicit information necessary for making rigorous diagnoses based on multiple diagnostic classification systems. Unlike the DI-PAD, the EP-GENE allows to collect information not only oriented for the completion of the OPCRIT, but also other relevant information to achieve to a better clinical characterization and phenotypic refinement of major psychiatric disorders.

Recently, in the first trimester of 2013 we have we have obtained limited funding from the "Gabinete de Apoio à Investigação" (Office of Research Support) from Faculty of Medicine-University of Coimbra, to continue developin the research project entitled "Phenotypic Dimensions in Psychosis" (Pereira04.01.13). Our aims include: 1. Assess 200 SZ/BD/SzA probands (from multiplex families and unrelated cases) – diagnostic classification and lifetime-ever occurrence of symptoms using all available clinical information; 2. Deposit the 200 Blood/DNA samples in the FMUC (Laboratório de Citogenética) repository for future studies; 3. Contribute to phenotypic refinement and formulation of alternative phenotypes: symptom dimensions and subphenotypes. 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 in other cities/hospitals, such as in Oporto and Aveiro.

Clinical research – 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 developing 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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Neurology Research

Studies on neurodegenerative disorders

Luis Cunha, Isabel Santana (FMUC, CHUC); Inês Baldeiras, Catarina Oliveira (FMUC, CNC)

Biomarkers for the early diagnosis of Alzheimer´s Disease (AD) is one of our main areas of interest. In this context, under the scope of the project PIC/IC/83206/2007, we have determined the Cerebrospinal Fluid (CSF) levels of Aβ42, t-tau and p-tau in a group of 170 AD patients, 135 controls (100 FTLD patients and 35 controls without cognitive impairment) and 90 MCI patients.

First, a cross-sectional study involving non-demented controls and AD patients was done in order to establish cut-off values for the CSF biomarkers and sensitivity and specificity figures, to differentiate between AD and controls were calculated. All markers, and ratios between them (t-tau/Aβ42 and Aβ42/p-tau), exhibited areas under the ROC curves (AUC) above 0.80, and the ratio Aβ42/p-tau resulted in sensitivity and specificity levels above 85%. Based on the above analysis, we established criteria for CSF profile classification, assuming that a typical CSF-AD profile would represent a high-risk of progression to AD in MCI patients. Out of the 90 patients diagnosed with MCI, 46 had a CSF profile typical of AD (MCI-AD, $p>0.05$ for all CSF markers), presented with lower MMSE scores and higher ADAS-Cog scores than MCI-nonAD patients and ApoE-ε4 allele distribution was significantly increased in the MCI-AD group (Baldeiras I, Santana I, Garrucho MH, Pascoal R, Lemos R, Santiago B, Oliveira CR. Sinapse 2012;12 (2):14-22).

Out of the 90 patients diagnosed with MCI at baseline, 63 were followed for more than 2 years and were considered for the following analysis: 46 (73%) were cognitively stable and 17 (27%) developed AD during follow-up. We then applied the same cut-off values previously established in the AD and control groups to the MCI cohort and evaluated the ability of the CSF markers to predict AD development in MCI patients. The ratio t-tau/Aβ42 was the best predictor of future development of AD in MCI patients with a sensitivity of 88%, specificity of 69%, positive predictive value (PPV) of 50% and negative predictive value (NPV) of 94%. Kaplan-Meyer estimates of the probability of conversion to AD in patients with MCI showed that higher baseline t-tau/Aβ42 levels were significantly associated with conversion to AD.

Regarding Frontotemporal Lobar Degeneration (FTLD), the second most frequent type of neurodegenerative dementia, the accuracy of CSF markers to distinguish FTLD from AD and controls is still unsatisfactory. We therefore determined the levels of CSF Aβ40, along with the other conventional biomarkers (Aβ42, t-tau and p-tau), to see if there was an additional value in descriminating between FTLD, AD and non-demented subjects. As expected, t-tau and p-tau were significantly elevated and Aβ42 was lower in AD compared to FTLD and controls. In FTLD, t-tau and p-tau were also significantly increased in relation to controls. Reduced Aβ40 levels were also seen in both patients groups relative to controls. Logistic regression analysis identified Aβ42, Aβ40 and p-tau as the best combination of biomarkers to differentiate between AD and controls (sensitivity 99%, specificity 97%). To descriminate FTLD from controls, Aβ40 and p-tau were the best combination (sensitivity 81%, specificity 90%). Finally, to differentiate AD from FTLD, the best biomarker subset was Aβ42 and t-tau (sensitivity 86%, specificity 75%). Although CSF Aβ40 does not appear to have an additional value in the distinction between AD and FTLD, it seems to be usefull in the descrimination between FTLD and non-demented subjects (Baldeiras I, Santana I, Garrucho MH, Santiago B, Duro D, Lemos R, Oliveira CR. Oral presentation for the "25ª Reunião do Grupo de Estudos de Envelhecimento Cerebral e Demência", Tomar 29-30 June 2012).

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Research in neurodegenerative diseases: C9orf72 repeat expansion (GGGGCC) in a series of Portuguese FTLD individuals

Maria Rosário Almeida, Isabel Santana, Beatriz Santiago

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative condition characterized by extensive clinical, pathological and genetic heterogeneity. Positive family history is observed in 25-50% of the cases with an autosomal dominant inheritance. In some FTLD families, mutations in MAPT and PGRN genes have been identified and were associated to tau and TDP43 pathology, respectively. Interestingly, some relatives of these families also developed MND with TDP43 pathology. Recently a pathological hexanucleotide repeat expansion in C9orf72 on chromosome 9p21 has been identified as the major cause of these FTLD/MND forms. Therefore in the present work we aimed to assess the frequency of the C9orf72 repeat expansion (GGGGCC) in a series of Portuguese FTLD individuals and their associated phenotypic characteristics.

Eighty three patients with clinical diagnosis of FTLD assisted in the Dementia outpatient clinic of CHUC or with genetic investigation at the CNC have been enrolled in the study. Of these, 42 sporadic FTLD and 37 familial and 4 with concomitant FTLD and MND patients were tested for the C9orf72 hexanucleotide repeat expansion in the framework of the Early-Onset Dementia (EOD)-Consortium. The expansion has been identified in 7 patients, one (2%) of 42 sporadic FTLD, four (18%) of 37 familial FTLD and two (50%) of 4 with FTLD/MND. All these patients have been previously tested for MAPT and PGRN genes with no mutations found. As a result, the pathogenic expansion in C9orf72 was present in a significant proportion of cases, unexplained by the available recognized genetic causative defects. Although few patients with FTLD and MND were analyzed, the expansion accounts for half of the cases and 18% of familial FTLD. Detailed phenotypic evaluation of the seven C9orf72 expansion carriers revealed a high clinical heterogeneity, intra and inter-familial. The location of this expansion within C9orf72 intronic region suggests the pathogenic involvement of the mutant RNA in the underlying FTLD mechanism.

Research in neurodegenerative diseases: progranulin peripheral levels as a screening tool for the identification of subjects with PGRN mutations in a Portuguese cohort

Maria Rosário Almeida, Inês Baldeiras, Maria Helena Ribeiro, Beatriz Santiago, Cristina Machado, João Massano, Joana Guimarães, Catarina Resende Oliveira, Isabel Santana

Progranulin (PGRN) mutations are associated with different clinical phenotypes, including Frontotemporal Lobar Degeneration (FTLD), Corticobasal Syndrome (CBS) and Alzheimer's Disease (AD). As all pathogenic PGRN mutations identified so far cause disease through haploinsufficiency, progranulin levels determination has been proposed as a reliable method to identify mutation carriers. The aim of the present study concerns the evaluation of the accuracy of peripheral progranulin levels in the identification of the PGRN mutation carriers detected thus far in our Portuguese cohort.

Serum progranulin levels were measured in 244 subjects (124 patients in the spectrum of FTLD, 2 asymptomatic descendents of a FTLD patient, 56 AD patients and 64 controls) by a novel commercial ELISA kit. Low progranulin levels were detected in 7 individuals (five bvFTD, one CBS and one still clinically unaffected) that constituted the group of the null progranulin mutation carriers previously identified in our molecular diagnosis laboratory. The pathogenic mutations found, consisted of four insertion-deletions, causing frameshifts resulting in premature stop codons, three of which were novel. In addition, a normal progranulin level was found in a patient harbouring a novel missense variant. For this novel ELISA kit, we established a progranulin cut-off level that identified with 100% accuracy the pathogenic mutation carriers. This study supports the use of a novel assay for the determination of progranulin levels as a screening procedure to identify patients harboring null PGRN mutations. This approach would significantly decrease the required PGRN mutation analysis workload, and should be extended to other clinical phenotypes than bvFTD, and to apparently sporadic cases.

Research in gliomas: diagnostic and prognostic value of the IDH1 codon 132 mutation and MGMT promoter methylation in gliomas

Maria Rosário Almeida, Olinda Rebelo, Herminio Tão

Gliomas are the most common primary brain tumors and their major representative forms are astrocytomas, oligodendrogliomas and ependymomas. Grade IV astrocytomas, usually referred as glioblastoma multiforme, is the most frequent and lethal type. Despite advances in therapeutic approaches, the prognosis of most patients is still extremely poor. Therefore, the identification of molecular markers to improve the clinical outcome of these patients represents an important challenge. This present study aimed to assess the frequency and the value of diagnostic and/or prognostic of two

markers: (i) somatic mutations in isocitrate dehydrogensase 1 and 2 genes (IDH1 and

IDH2) and (ii) the methylation of O6-methylguanine DNA methyltransferase gene

(MGMT), in a series of Portuguese patients with gliomas. One hundred and twenty eight patients assisted in the University Hospital of Coimbra were enrolled. Of these, 103 patients with astrocitomas, 15 with oligodendrogliomas, 6 with mixed gliomas and 4 with ependymomas were screened for the presence of somatic mutations in IDH1 and IDH2 genes using directed sequencing. Hypermethylation of the promoter region of the MGMT gene was evaluated using Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) in 30 glioblastoma patients. We found seventeen patients with known missense mutations in codon 132 of the IDH1 gene, including Arg132His (most frequent), Arg132Ser and Arg132Leu. No mutations were found in IDH2 gene. Our data revealed that IDH1 mutations are frequent in secondary glioblastomas but rare in primary glioblastomas (100% vs 1%, $p<0,0001$).

This mutation seems to be associated with a more favorable prognosis. The MGMT promoter was methylated in 67% and unmethylated in 33% of glioblastoma patients. The patients who had the MGMT methylated and underwent chemotherapy/radiotherapy showed longer survival ($p<0,001$). In conclusion, our data suggested the importance of these markers evaluation, in a routine basis, due to their diagnostic and prognostic value.

Translational Bi-Genomics and Pharmacogenomics

Manuela Grazina

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 uses 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.

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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 mitocondrial 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.

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.

Dermatology Research

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

Allergic contact dermatitis (ACD) and delayed exanthematic drug eruptions are T-cell mediated skin reactions. Pathomechanisms are not fully understood, namely in which considers antigen presentation.

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 facilitate antigen presentation and 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 the antigen presentation of systemic drugs. It is our objective to evaluate if DC suffer the same activation and maturation process in the presence of systemic drugs as they do in the presence of contact allergens.

We have exposed THP-1 cells to the main systemic drugs causing drug eruptions (amoxicillin, ampicillin, carbamazepine, valproic acid, allopurinol and oxypurinol). We studied phenotypic and genotypic changes in these cells by RT-PCR and WB.

We confirm that, like contact sensitizers, systemic drugs directly activate THP-1 cells, namely through p38MAPKinase signalling pathway, and the expression of mRNA for pro-inflammatory cytokines, like IL-8, and genes dependent on Nfr2–ARE pathway, like the hemeoxygenase HMOX-1.

The activation of innate pro-inflammatory pathways in dendritic cells can contribute to the initiation of the immune response and therefore justify the frequency of drug eruptions observed with these drugs.

Publications

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Serra D, Ramos L, Brinca A, Gonçalo M .(2012). Acute generalized exanthematous pustulosis associated with acyclovir, confirmed by patch testing. Dermatitis: 23:99-10.

Serra D, Amaro P, Gonçalo M, Silva M, Ferrando B, Pasini B, Figueiredo A .(2012). Gastric leiomyoma and hyperplastic polyposis coli in a patient with multiple cutaneous and uterine leiomyomatosis. J Cutan Med Surg.:16(3):20811.

Svedman C et al. (2012). Follow-up of the monitored levels of preservative sensitivity in Europe. Overview of the years 2001-2008. Contact Dermatitis, 67:312-4.

Arthritis Research

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

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. Pharmacological characterization identified some monoterpene compounds as the active ingredients of those essential oils. Further pharmacological characterization is underway to establish the relative potency of the active compounds.

Publications

Mobasheri A, Lewis R, Ferreira-Mendes A, Rufino A, Dart C, Barrett-Jolley R. (2012). Potassium channels in articular chondrocytes. Channels; 6:1-10.

Rufino AT, Cavaleiro C, Judas F, Salgueiro L, Lopes MC, Mendes AF. (2012) The essential oil of Eryngium duriaei subsp. juresianum inhibits IL-1β induced NF-kB and MAPK activation in human chondrocytes. Osteoarthritis Cartilage.; 20 (Suppl. 1): S290 (P580).

Research in brain tumors

Alberto Orfão (CSIC, University Salamanca), Maria Dolores Tabernero (University Hospital, Salamanca), Hermínio Tão (HUC), Olinda Rebelo (HUC), Marcos Barbosa (FMUC, HUC), Anália do Carmo (CNC), M. Celeste Lopes (FFUC, CNC)

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

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Domingues PH , Teodósio C , Ortiz J , Sousa P , Otero A , Maillo A, Bárcena P, García-Macias MC, Lopes MC , Oliveira CR, Orfao A, Tabernero MD .(2012). Immunophenotypic identification and characterization of tumor cells and infiltrating cell populations in meningiomas. Am J Pathol.;181:1749-61.

Matias D, Balça-Silva J, Carmo A, Sarmento-Ribeiro A, Lopes MC. (2012). Glioma cell motility is modulated by CXCL12/CXCR4. Acta Méd Port. January PP-17.

Tabernero MD, Maíllo A, Nieto AB, Diez-Tascón C, Lara M, Sousa P, Otero A, Castrillo A, Patino-Alonso Mdel C, Espinosa A, Mackintosh C, de Alava E, Orfao A. (2012). Delineation of commonly deleted chromosomal regions in meningiomas by high-density single nucleotide polymorphism genotyping arrays. Genes, Chromosomes and Cancer: 51: 606–617

Yeast nosocomial infections

HIV-1 Vpr variants in mother-child pairs. Using a yeast model to predict AIDS progression

Rui Soares1,2, Graça Rocha1, Andrea Speigel3, Marta Mota1, António Meliço-Silvestre1,3, António Vieira3 , Teresa Gonçalves1,2. 1Faculty of Medicine, University of Coimbra, Portugal; 2Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal; 3CHUC, Coimbra, Portugal

We pretend to characterize HIV-°©-1 Vpr variants in two subpopulations of HIV-°©-1 infected patients, Long term non progressors (LTNP) and Fast Progressors (FP). This clinical classification is based on several parameters measured by clinicians in charge. This study, partially completed with the LTNP patients characterization, will enable to detect the most frequent mutations in Portuguese-°©-infected population. A collaboration between the research group and the Serviço de Infecciosas do Hospital dos Covões, CHUC from Coimbra allows gathering, until now, blood samples from 60 HIV1-°©-infected individuals, with no symptoms that have not yet initiated therapy, classified as LTNP.; 30 HIV1-°©-infected patients with primary AIDS symptoms that initiated a year-°©-ago HAART. A group of patients, considered Fast Progressors will also be studied. In the viruses carried by these patients it will be characterized the Vpr genomic variants

Novel techniques for the diagnosis and treatment of human Infertility

Teresa Almeida Santos (HUC, FMUC), Ana Paula Sousa (HUC, CNC), Alexandra Amaral (CNC), Renata Tavares (CNC), Marta Baptista (CNC), Raquel Brito (HUC), 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.

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 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

Amaral A, Ramalho-Santos J. Assessment of mitochondrial potential: Implications for a correct monitoring of human sperm function. *Int. J. Andrology* (*in press*)

INTERNATIONALIZATION

Internationalization has been a permanent concern of the CNC strategy. To attain this goal the researchers have been encouraged to establish colaborations and joint projects with laboratories abroad, and to colaborate in the organizaton 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 in collaboration

Neuroscience and Disease

Neuromodulation Group

Activities:

-The group was selected to receive students from the European Network 'European Neuroscience Campus'.

-The PI is a member of the Steering Committee of the European Network 'European Neuroscience Campus'.

-The PI is the Portuguese representative at the European Network 'European Neurosciences Institutes Network (ENI-Net)'

-The PI participated in several post-graduation courses in Brazil (Universidade federal do Ceará, Universidade Federal de Santa Catarina, Universidade Federal do Rio Grande do Sul).

-The PI was selected for the program Ciência sem Fronteiras (CAPES, Brazil).

Receiving students:

-Jimmy George (PhD student from the European Neuroscience Campus) – carried out the experimental work for his PhD in the lab from September 2011 until October 2012

-Stefania Zappettini (Department of Experimental Medicine, University of Genoa, Italy) - carried out experimental work in the lab for her PhD from August 2011 until April 2012.

-Jessié Martins Gutierres (Department of Biochemsitry, Federal University of Santa Maria, Brazil) - carried out experimental work in the lab for his PhD from September 2011 until October 2012.

-Adalberto Alves de Castro (Department of Pharmacology, Federal University of Santa Catarina, Brazil) - carried out experimental work in the lab for his PhD from October 2011 until October 2012.

-Ana Carla Lima Nunes (Department of Physiology and Pharmacology, Federal University of Ceará, Brazil) - carried out experimental work in the lab for her PhD from June 2012 until December 2012.

-Eszter Szabó (Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary) - carried out experimental work in the lab for her PhD from September 2012 until present.

Glutamatergic Synapses Group

There is active collaboration with several international research groups listed below, which includes exchange of students, joint research projects and joint publications:

Chinfei Chen, Harvard Medical School, Boston, USA

Ann Marie Craig, University of British Columbia, Vancouver, Canada

José A Esteban, Centro de Biologia Molecular Severo Ochoa, Madrid, Spain

Laurent Groc, Interdisciplinary Neuroscience Institute, University of Bordeaux, France

Ka Wan Li, VU University Amsterdam, The Netherlands

Noo Li Jeon, WCU Multiscale Mechanical Design, Seoul National University, Seoul, Korea

Ulrich Hengst, Columbia University, New York, USA

Samie R. Jaffrey, Weill Medical College of Cornell University, New York, USA

International Graduate Training Networks:

The group has actively participated in teaching within the European Neurasmus Joint Master Program (http://www.neurasmus.u-bordeaux2.fr/). The group participated in teaching formal courses (Cell Regulation, Molecular and Cellular Neuroscience and Neuroscience and Disease) and in providing laboratory rotations to international students.

The group also hosted international master students through the Erasmus Program (Olga Iuliano, Italy, Marilisa Vigorita, Italy), as well as international PhD students (Fabiano Carvalho, Brazil) and undergraduate students (Daniela Mandrone, Italy) for short-term internships.

Neuronal Cell Death and Neuroprotection Group

Carlos B. Duarte and Emília P. Duarte are members of the Education Board of Neurasmus (European Erasmus Mundus MSc program in Neuroscience).

Collaborative publications

J.R. Gomes, J.T. Costa, C.V. Melo, F. Felizzi, P. Monteiro, M.J. Pinto, A.R. Inácio, T. Wieloch, R.D. Almeida, M. Grãos, C.B. Duarte (2012). Excitotoxicity downregulates TrkB.FL signaling and upregulates the neuroprotective truncated TrkB receptors in cultured hippocampal and striatal neurons. J Neurosci 32, 4610-4622.

E.P. Duarte, M. Curcio, L.M. Canzoniero, C.B. Duarte (2012). Neuroprotection by GDNF in the ischemic brain. Growth Factors 30, 242-257.

International Collaborators

F. Felizzi [ETH Zurich, Department of Biosystems Science and Engineering (DBSSE), Zurich, Switzerland], T. Wieloch [Wallenberg Neuroscience Center, Lund University, Lund, Sweeden], A.R. Inácio [Wallenberg Neuroscience Center, Lund University, Lund, Sweeden], J. Takano [RIKEN Brain Science Institute, Hirosawa, Wako-shi, Saitama, Japan], N. Iwata [RIKEN Brain Science Institute, Hirosawa, Wako-shi, Saitama, Japan], T.C. Saido [RIKEN Brain Science Institute, Hirosawa, Wako-shi, Saitama, Japan], B.A. Bahr [Biotechnology Research and Training Center, University of North Carolina-Pembroke, Pembroke, NC], L.M.T. Canzoniero [University of Sannio, Italy])

Carlos B. Duarte is co-supervisor of the PhD student Michele Curcio, from the University of Sannio, Italy.

Other research collaborations:

Lorella M.T. Canzoniero, University of Sannio, Italy (Study of the alterations in GDNF signaling in brain ischemia). PI: Carlos B. Duarte

Clive Bramham, University of Bergen, Norway (In vivo study of the role of BDNF in synaptic plasticity in the hippocampus). PI: Carlos B. Duarte

Duan-Wu Zhang and Jiahuai Han, Key Laboratory of the Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Xiamen, Fujian 361005 China. (Neuronal death by necroptosis: towards the molecular signaling upon an ischemic stimulus). PI: Armanda Santos

Arsénio Fernández-López, Área de Biología Celular, Instituto de Biomedicina, Universidad de Léon, 24071 Léon, Spain (Neuronal death by necroptosis: towards the molecular signaling upon an ischemic stimulus). PI: Armanda Santos

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Organization of 2 international PhD courses:

"Neurodegenerative diseases: From molecules to clinics and beyond" course, PhD programme in 'Biologia Experimental e Biomedicina' (BEB), integrated at the European Neuroscience Campus (ENC) Network courses (29th Oct – 2nd Nov, 2012).

"Brain plasticity and cognition: the ever-changing brain", PhD programme in 'Biologia Experimental e Biomedicina' (BEB) (8-10th Oct, 2012).

Published abstracts - participation in international meetings (2012):

FENS meeting, held in Barcelona, Spain (7 abstracts)

Society for Neuroscience (SfN) meeting, held in New Orleans, LA, USA (4 abstracts)

Poster (only) presentations in international meetings:

Neurodegenerative Diseases Meeting - Cold Spring Harbor, 28 November-1 December, New York, USA.

Redox Signaling and Oxidative Stress in Health and Disease – IV Spanish and Portuguese Meeting on Free Radicals. 5-7th June, Valencia, Spain.

S (AC Rego) in international meeting: Redox Signaling and Oxidative Stress in Health and Disease – IV Spanish and Portuguese Meeting on Free Radicals. 5-7th June, Valencia, Spain.

Research collaboration with:

Henry L. Paulson (MD, PhD), University of Michigan Health System, Michigan, U.S.A. _ doctoral work of Mário Laço.

Sandrine Humbert (PhD), Institut Curie, Orsay, France _ study of phosphorylated huntingtin; doctoral work of Carla Lopes.

Ernest Arenas (MD, PhD), Karolinska Institutet, Stockholm, Sweden _ doctoral work of Ana Catarina Oliveira.

Frederic Saudou (PhD), Institut Curie, Orsay, France _ study of phosphorylated huntingtin.

Michael Hayden (MD, PhD), The University of British Columbia, Vancouver, Canada _ use of YAC128 mice.

Tiago Fleming Outeiro (PhD), University Medizin Goettingen, Goettingen, Germany _ study of phosphorylated alpha-synuclein.

Molecular Mechanisms of Disease Group

William L. Klein (Cognitive Neurology and Alzheimer's Disease Center, Northwestern University Institute for Neuroscience, Northwestern University, Evanston, IL, USA)

Tiago Fleming Outeiro (Göttingen University, Germany & Instituto de Medicina Molecular, IMM, Lisbon)

Sueli Cristina Marques (October 2006-2012)

"Chromatin remodeling in Alzheimer's disease pathogenesis" (June 2012).

Ana Maria Cuervo (Albert Einstein College of Medicine, New York, USA) Hosted Daniela Arduino

Russel Swerdlow (University of Kansas, Kansas City, USA) Hosted Ana Raquel Esteves and Diana F Silva

Marcia Haigis (Harvard University, Boston, USA) Hosts Daniel Santos

Arduíno DM, Esteves AR, Cortes L, Silva DFF, Grazina MM, Swerdlow RH., Oliveira, CR, Cardoso SM. (2012) Mitochondrial Metabolism in Parkinson's Disease Impairs Quality Control Autophagy by Hampering Microtubule-Dependent Traffic. Human Molecular Genetics. 21(21):4680-702 (Impact factor 2011: 7.636)

Branco DM, Esteves AR, Arduino DM, Santos D; Swerdlow RH, Oliveira CR, Januario C, Cardoso SM (2012) Ubiquitin Proteasome System in Parkinson Disease: a keeper or a witness? Experimental Neurology 238(2):89-99.(Impact factor 2011: 4.699)

Costa R.O., Lacor P.N., Ferreira I.L., Resende R., Auberson Y.P., Klein W.L., Oliveira C.R., Rego A.C., Pereira C.F. (2012) Endoplasmic reticulum stress occurs downstream of GluN2B subunit of N-methyl-D-aspartate receptor in mature hippocampal cultures treated with amyloid-β oligomers. Aging Cell 11, 823-833.

Marques SC, Lemos R, Ferreiro E, Martins M, de Mendonça A, Santana I, Outeiro TF, Pereira CM. (2012) Epigenetic regulation of BACE1 in Alzheimer's disease patients and in transgenic mice. Neuroscience. 220, 256-266.

Silva DF, Selfridge JE, Lu J, E L, Cardoso SM, Swerdlow RH. (2012) Mitochondrial abnormalities in Alzheimer's disease: possible targets for therapeutic intervention. Adv Pharmacol. 64:83-126. Rev

Neuroendocrinology and Neurogenesis Group

Participation in graduate Training Networks:

European Neuroscience Campus (ENC) network;

Organization of 1 core advanced courses in Neuroscience, at the Center for Neuroscience and Cell Biology, offered within the scope of the PhD Programme in Experimental Biology and Biomedicine and the ENC Network: "Advanced Course Neuroendocrinology, Aging and Obesity; 3-7 December 2012, as part of the PhD Programme in Experimental Biology and Biomedicine (PDBEB), hosted by the Center for Neuroscience and Cell Biology, Coimbra.

Collaborators that are formally co-supervisors of PhD students of our group:

Monika Ehrhart-Bornstein, Molecular Endocrinology Group, Department of Medicine, Carl Gustav Carus University of Dresden, Germany (Co-supervisor of Magda Matos Santana).

Tamas Horvath, Section of Comparative Medicine, Yale University School of Medicine (EUA), (Co-supervisor of Mariana Botelho da Rocha).

Collaborators in Research Projects:

Tamas Horvath, Section of Comparative Medicine; Yale School of Medicine PO Box 208016, New Haven, USA. Project "Caloric restriction increases lifespan: role of neuropeptide Y on autophagy regulation".

Carlos Lopez Otin - Departamento de Bioquímica y Biología Molecular Facultad de Medicina, Universidad de Oviedo, Oviedo, Spain. Project "Caloric restriction increases lifespan: role of neuropeptide Y on autophagy regulation".

Biotechnology and Health

Molecular Systems Biology Group

Active collaborations:

Max Planck Institute for Molecular Cell Biology and Genetics (Germany):
Researchers: Sophie Ayciriex, Julio Sampaio, Michal Surma, Andrej Shevchenko
Project: Identification of mechanisms of chain scission in in vivo autoxidation of polyunsaturated fatty acids through computational selection of mechanism markers and shotgun lipidomics analysis

University of Saarland (Germany):
Researchers: Elmar Heinzle
Project: Development and application of a method for profiling mitotic-cycle-dependent metabolism without having to synchronize cells

University of California – Merced (USA):
Researchers: Fabian Filipp, Rohit Gupta
Project: Application of rule-based modeling and lipid profiling to clarify the regulation of fatty acids biosynthesis

University of Lleida (Spain)
Researchers: Rui Alves
Project: Uncovering the evolutionary adaptations of protein aminoacid sequence and structure to O2-rich environments

National Institute of Food Technology Entrepreneurship & Management (India)
Researchers: Chakkaravarthi Saravanan
Project: Clarification of the mechanisms of polyunsaturated fatty acid autoxidation through rule-based modeling

VIT University (India)
Cooperation in research training of B. Tech. and M. Sc. students

Vectors and Gene Therapy Group

Gerrit Borchard, University of Genève, Switzerland and Centre Pharmapeptides, Archamps, France.

- Jeffrey Bruce and Peter Cannol, Department of Neurosurgery, Gabriele Bartoli Brain Tumor Research Laboratory, Columbia University, New York, USA

Veronica Colomer, John Hopkins, School of Medicine, Baltimore, USA.

Nicole Déglon & Philipe Hantraye, Service Hospitalier Frederic Joliot, Departement de Recherches Medicales, Direction des Sciences du Vivant, Commissariat a l'Energie Atomique (CEA), Orsay, France.

Nejat Duzgunes, University of the Pacific, San Francisco, USA.

Mauro Giacca, Laboratory of Molecular Medicine, ICGEB - International Centre for Genetic Engineering and Biotechnology, Trieste, Italy.

Hirokazu Hirai, Department of Neurophysiology, Gunma University, Gunma, Japan

Hans Junginger, Former Professor at Leiden University, Netherlands and visiting Professor at Naresuan University, Phitsanulok, Thailand.

Raghu Kalluri, MD Anderson Cancer, Houston, USA/Center for Neuroscience and Cell Biology, UC.

Arnulf Koeppen, Veteran's Hospital, Albany, USA.

Sebastian Kugler, Department of Neurology, Faculty of Medicine, University of Göttingen, University of Gottingen, Gottingen, Germany.

Ed Lavelle, Trinity College of Dublin, Ireland

Bernard Lebleu, University of Montpellier, Montpellier, France.

- Faustino Mollinedo, Instituto de Biología Molecular y Celular del Cáncer, Centro de Investigación del Cáncer, CSIC-Universidad de Salamanca, Campus Miguel de Unamuno, E-37007 Salamanca, Spain.

- Bo Nyström, Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, N-0315 Oslo, Norway

Henry Paulson, University of Michigan , Ann Harbor, USA.

Valérie Pierrefite-Carle, Unity INSERM, Faculty of Medicine, Nice, France,

- Teresa J. T. Pinheiro, School of Life Sciences, University of Warwick, Coventry, United Kingdom

Margus Pooga, Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia.

Simo Schwarz Jr, CIBBIM-Nanomedicine Drug Delivery and Targeting, Vall d´Hebron Institut de Recerca, Barcelona, Spain

Herman Staats, Duke University Medical Center, USA

María Jesús Vicent, Centro de Investigación Príncipe Felipe, Medicinal Chemistry Unit, Polymer Therapeutics Laboratory, Valencia, Spain

Ernst Wagner, Department of Pharmacy, University of Munich, Germany.

Industrial Academic Initial Training Network towards treatment of Polyglutamine Diseases. Grant agreement no.: 264508. SEVENTH FRAMEWORK PROGRAMME; THE PEOPLE PROGRAMME.

Seventh Framework Programme The People Programme; Cancer associated fibroblasts function in tumor expansion and invasion; Reference: FP7-PEOPLE-2012-ITN (2013 - 2016).

Biomaterials and Stem Cell-Based Therapeutics

Participation at the international program MIT-Portugal, focus area of bioengineering. Lino Ferreira is contributing for the "Cell and Tissue Engineering" module with Robert Langer (MIT) and Joaquim Cabral/Cláudia Lobato (IST).

Lino Ferreira is associate PI of the European Federation for Systematic Stem Cell Biology (EuroSyStem) since 2009. The EuroSyStem Federation brings together elite European research teams to create a unique and world-leading programme in fundamental stem cell biology. The ambition is to interlink complementary biological and computational expertise so as to drive the generation of new knowledge on the characteristics of normal and abnormal stem cells.

During 2012, several networks involving international researchers have been established or continued:

1-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).

2-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).

3-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), Carolyn Carr (University of Oxford), Lino Ferreira (CNC, Portugal).

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

5- 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).

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

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

8- iPSC disease models. Xavier Nissam (I-Stem, France), Patrícia Pereira (CNC, Portugal), Lino Ferreira (CNC, Portugal).

9- In vitro blood-brain barrier models. Romeo Cechelli (University of Lille, France), Sezin Aday (CNC, Portugal), Catarina Almeira (CNC, Portugal), Susana Rosa (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Cell and Molecular Toxicology

Mitochondrial Toxicology and Disease Group

Networks or other forms of participation of the Research Group at the international level):

Edward Perkins (Mercer U., USA) Cancer Stem Cell Responses to DNA Damage (P. Oliveira)

Faustino Mollinedo (CSIC, Spain), Apoptosis Signaling in Melanoma (P. Oliveira)

Jon Holy (U. Minnesota, USA), Anticancer Effects of of Phytochemicals (P. Oliveira)

Kendall Wallace (U. Minnesota, USA), Doxorubicin-induced Mitochondrionopathy (P. Oliveira)

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

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

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

Yvonne Will (Pfizer R&D, USA), SIRT3 and drug-induced cardiac mitochondrial toxicity (P. Oliveira)

Michael Sack (NHLBI, USA), SIRT3 and drug-induced cardiac mitochondrial toxicity (P. Oliveira)

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

Piero Portincasa (U. Bari, Italy), Mitochondrial role in metabolic diseases (P. Oliveira)

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

David Sinclair (Harvard Medical School, USA), Sirtuins, Mitochondrial Biogenesis and Metabolic Regulation (A. Rolo/C.Palmeira).

Joan Rosseló (CSIC, Spain), Mitochondrial Tolerance and Liver Ischemic Preconditioning (A. Rolo/C.Palmeira)

Saber Hussain (Wright State Univ., USA), Evaluation of Mitochondrial Toxicity of Silver and Gold Nanoparticles: (C. Palmeira)

Jan Kopecky (Academy of Sciences, Czech Republic), FXR receptor: a target to prevent systemic metabolic disease (A. Rolo/C.Palmeira)

Nika Danial (Dana-Farber Cancer Institute, USA), Metabolic checkpoints: cellular bioenergetics and cellular responses to stress (C.Palmeira)

Bjoern Bauer and Anika Hartz, School of Pharmacy, University of Minnesota-Duluth, USA (V. Sardão)

Redox Biology in Health and Disease Group

Enrique Cadenas - Dept. Pharmaceutical Sciences, University of Southern California, USA. Nitric oxide in neurodegeneration and aging.

Greg Gerhardt - Dept. Anatomy and Neurobiology, and Center for Microlelectrode Technology (CenMet) University of Kentucky, Lexington, Kentucky, USA. Development of microsensors for nitric oxide measurement in tissues.

Rafael Radi - Facultad de Medicina, Universidad de la República, Montevideo, Uruguay. New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite.

Homero Rubbo - Facultad de Medicina, Universidad de la República, Montevideo, Uruguay. New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite.

Jon O. Lundberg - Department of Physiology and Pharmacology, Karolinska Institutet, Sweden. New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite.

Eduardo Weruaga - Departmento de Biologìa Celular y Patología, Instituto de Neurociencias de Castilla y León, Universidade de Salamanca, Spain. Transgenic mice for nNOS and the impact in neurovascular coupling.

Nadezda Lukacova - Institute of Neurobiology, Centrum of Excellence, Slovak Academy of Sciences, Košice, Slovak Republic. Immunolocalization of nNOS in the barin and the correlation with nitric oxide dynamics.

Juan Sastre – Faculty of Pharmacy, University of Valencia, Spain. Prevention of inflammatory processes in the gastrointestinal epithelia by dietary flavonoids.

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

Microbiology

Microbiology of Extreme Environments Group

Anjos J, C Fernandes, A Abrunheiro, C Quintas, B Silva, A, N Gow, Teresa Gonçalves (2012). "Beta-(1,3) – Glucan synthase complex from Alternaria infectoria, a rare dematiaceous human pathogen" Medical Mycology 50(7):716-25.

Carolina Coelho, Lydia Tesfa, Jinghang Zhang, Johanna Rivera, Teresa Gonçalves, and Arturo Casadevall. Analysis of Cell cycle and Replication of Mouse Macrophages after in vivo and in vitro Cryptococcus neoformans infection using Laser Scanning Cytometry. Infection Immunity, 80: 1467-1478.

Tiago I, Maranha A, Mendes V, Alarico S, Moynihan PJ, Clarke AJ, Macedo-Ribeiro S, Pereira PJB, Empadinhas N (2012) Genome sequence of Mycobacterium hassiacum, a rare source of heat stable mycobacterial proteins. Journal of Bacteriology, 194(24):7010-1.

Biophysics and Biomedical NMR

Inorganic Biochemistry and Molecular Imaging Group

Sebastian Cerdán and Pilar Lopez-Larrubia, IIB "Alberto Sols", CSIC, Universidade Autónoma de Madrid, Espanha: "Non-invasive NMR studies of organ function with stable isotope tracers and contrast agents"

Claudio Luchinat, CERM, Universidade de Florença, Itália: "Lanthanide binding tags for NMR of proteins: exploiting paramagnetic shifts and residual dipolar couplings"

European Union COST D38 Action "Metal-based systems for Molecular Imaging applications": network of about 50 European Universities, with active collaboration with the following groups:

Silvio Aime, Center of Molecular Imaging, University of Torino, Italy: Functionalized liposomes and nanoparticles as responsive multimodal molecular imaging agents for image guided therpy (Teranostics).

Ivan Lukes, Charles University of Prague, Chech Republic: NMR and relaxometric characterization Gd-based MRI Contrast Agents

Lothar Helm, EPFL, Lausanne, Switzerland: relaxometric studies of MRI contrast agents

Joop Peters, TUDelft, Netherlands: NMR and relaxometry of Gd- complexes and nanoparticles as MRI CAs.

Eva Tóth, Centre de Biophysique Moléculaire, CNRS, University of Orleans, France: Chemical and in vivo animal characterization of MRI CAs for Alzheimer's disease.

Frank Roesch, Institute of Nuclear Chemistry, Johannes Gutenberg Universitaet, Mainz, Germany: characterization of Ga-based chelates as tracers for PET imaging

Robert Muller, University of Mons-Hainaut, Belgium: Functionalized Iron oxide and silica nanoparticles as targeted MRI contrast Agents.

European Union COST TD1004 Action "Theragnostic agents: imaging and Theray": network of about 40 European Universities, with active collaboration with several groups:

European Union TD1103 Action "Hyperpolarization: Physics and applications": network of about 35 European Universities, with active collaboration with a group in University of Barcelona.

C.F.G.C. Geraldes gave classes in European Master on Molecular Imaging (EMMI) Intensive Program Course "Design, Synthesis and Validation of Imaging Probes", Torino, in September 2011.

Intermediary Metabolism Group

Our collaborative clinical studies with Mayo Clinic and the German Diabetes Foundation during 2011-2012 results in five papers accepted for publication or published in medium to high-impact Journals and we have a further three that are in preparation. We have secured~100,000 € in funds from these Centers directly or as Collaborators in approved Projects.

For the fish studies, we have continued our productive collaboration with Prof Baanantes´s Group at the University of Barcelona in the study of fish Nutrition and are Co-investigators in a Project funded by the Spanish Ministry of Science. We have also submitted an application for an European Research Council Synergy Grant involving our Institutions plus the University of Aveiro. This collaboration has resulted in three papers accepted for publication or published and a Book Chapter.

Cell and Development Biology

Cellular Immunology and Oncobiology Group

Alberto Orfão from Center for Cancer Investigation, University of Salamanca, Spain. Assessment of genetic heterogeneity in gliomas. Co-supervision of two PhD student.

Maria Dolores Tabernero Redondo, from University Hospital, Salamanca, Spain. Chromosomal, genetic and immunophenotypic characterization of brain tumors. Co-supervision of two PhD student.

Raimundo Freire from University Hospital of Canarias, Tenerife, Spain. Implications of Claspin mutations in DNA replication, cell cycle checkpoints and oncogenesis. Co-supervision of two PhD student.

Fran Lund from Rochester University. CD38 and immune regulation. Co-supervision of one PhD student.

Carmen García-Rodriguez from Institute of Biology and Molecular Genetic. CSIC-University of Valladolid, Spain. Study of the cytokine release profile, by protein arrays, of dendritic cells. Co-supervision of two PhD student.

Ali Mobasheri from School of Veterinary Science and Medicine, University of Nottingham, England. Collaborative projects: a) Metabolic activity and viability of chondrocytes in cryopreserved human osteochondral allografts and b) Mechanisms of chondrocyte resistance to hyperglycemia: modulation of ATP-dependent K+ channels and causes of failure in osteoarthritis. Co-supervision of one PhD student.

Francisco Blanco from CIBER-BBN, Centro de Investigación Biomédica, Centro Hospitalario Universitario A Coruña, Spain. Modulation of the chondrogenic potential of adipose tissue derived mesenchymal stem cells. Co-supervision of one PhD student.

Biology of Reproduction, Stem Cells and Human Fertility Group

Collaboration with the University of Pittsburgh, USA

Energy metabolism of human pluripotent stem cells and their differentiated counterparts

Sandra Varum, Ana Sofia Rodrigues, Michelle B. Moura, Olga Momcilovic, Charles Easley, João Ramalho-Santos, Bennett Van Houten & Gerald Schatten

PLoS ONE 6 (6): e20914.

Collaboration with the University of Birmingham, UK

Ca2+ signalling through CatSper and Ca2+ stores generate different behaviours in human sperm

Wardah Alasmari, Sarah Costello, João Correia, Senga Oxenham, Jennifer Morris, Leonor Fernandes, João Ramalho-Santos, Jackson Kirkman-Brown, Francesco Michelangeli, Stephen Publicover & Christopher Barratt

In vitro effects of cationic compounds on functional human sperm parameters

Marta Baptista, Stephen J. Publicover & João Ramalho-Santos

Fertility and Sterility 10.1016/j.fertnstert.2012.11.008

Collaboration with the University of Barcelona, Spain

The human sperm proteome: a review of proteomic studies from basic science to clinical applications

Alexandra Amaral, Judit Castillo, João Ramalho-Santos & Rafael Oliva

Collaboration with the University of Munster, Germany

Xenografting as a tool to preserve endangered species: Outcomes and challenges in model systems

Paula C Mota, João Ramalho-Santos & Stefan Schlatt

Infection, Phagocytosis and Pathogens Group

Since Jan. 2012, and within the framework of the Harvard Medical School-Portugal Program, Dr. Otilia Viaira is the PI of a consortium (which includes Profs. Michael Brenner, Heinz Remold and Victor Hsu, Harvard University; Prof. R. Appelberg, University of Porto; and Dr. D. Barral, Gulbenkian Institute of Science) that studies "New Approaches to Fight Tuberculosis". The aim of this collaborative project is to understand the mechanism and regulation of resealing of the macrophage plasma membrane during Mycobacterium infection.

Also in 2012, one of our publications was the product of a collaborative project with Prof. Paul Verkade (University of Bristol, UK) and Prof. A. Shevchenko from the Max-Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany (L.M.B.B. Estronca, J. Silva, J. Sampaio, A. Shevchenko, P. Verkade, W.L.C. Vaz and O.V. Vieira1 (2012). Molecular Etiology of Atherogenesis - In Vitro Induction of Lipidosis in Macrophages with a New LDL Model. PLoS One. 2012;7(4):e34822.)

Insuline Resistance and Adipocyte Group

The group, haded by Eugenia Carvalho has a broad range of international active collaborations in the different fields of research, we collaborate with Dr. A. Veves & Dr J. Zabolotny, at Harvard Medical School, USA, for the study of inflammation and wound healing and to gain experience working with transgenic animal models. Dr Veves is Research Director at the Beth Israel Deaconess Medical Center Foot Center and Microcirculation Lab Harvard Medical School, his particular interest is in wound healing in diabetes and is involved in both basic science research in animal models and particularly in translational research that involves human subjects. With him we learn techniques in the field of wound healing in human subjects, particularly, the Doppler and laser Doppler imaging technique to evaluate the microvascular function of diabetic patients and the Medical Hyperspectral Imaging technique to evaluate the skin oxigenation in patients. Dr. Zabolotny's laboratory is in the Division of Endocrinology, Diabetes, and Metabolism at Beth Israel Deaconess Medical Center and Harvard Medical School. Dr. Zabolotny's group is focused on understanding the molecular mediators of insulin and leptin resistance in obesity, and impaired wound healing in diabetes and inflammatory bowel disease, with a particular focus on the role of inflammation in the pathogenesis of these disorders. Her group has significant experience in generating and studying transgenic and knockout mouse models. We have several students perform part of their studies in their laboratories, and some of their travel expenses have been paid by fellowships from the European Foundation for the Study of Diabetes.

In addition we also collaborate with Prof. J. Eriksson, Global Medical Science Director (executive level) Global Medicines Development, Cardiovascular/Gastrointestinal, Clinical Discovery, AstraZeneca R&D in Sweden, a specialist in Internal medicine and in Endocrinology (including diabetology). With him we have been investigating the role of the immunosuppressive agents, rapamycin, cyclosporin A and tacrolimus in lipolysis and their effects in altering the expression

of genes involved in lipid metabolism in human adipose tissue. In his laboratory we have had a PhD student, Maria Joao Pereira, who has just defended her thesis.

Moreover, our collaboration with Prof A. Valverde, at the Instituto de Investigaciones Biomedicas Alberto Sols, Spain, is related to insulin action, insulin resistance and brown adipocytes. We presently have a Master student at her lab to perform part of his studies on brown adipocytes regarding their modulation by immunosuppressive agents. Finally with Prof G. Lopaschuk, at the University of Alberta, Canada, who is an expert on the heart, we are performing heart studies on human epicardial fat tissue. We have recently published a review together "Cherian S, Lupaschuk DG and Carvalho E. Cellular cross-talk between epicardial adipose tissue and myocardium in relation to the pathogenesis of cardiovascular disease. Am J Physiol Endocrinol Metab. 2012 Oct;303(8):E937-49: IF: 4.7".

Participation in the organization of scientific meetings

January 2012

"International Course in Reproduction & Pluripotency"

Date: January – one week

CNC members involved in the organization: João Ramalho Santos

"XI Jornadas de Bioinformática"

Date: 23 -25 January – Barcelona

CNC members involved in the organization: Armindo Salvador

"Advanced course on Principles and Practice in Drug Development, Doctoral Program on Biomedicine and Experimental Biology (CNC/UC) and doctoral program MIT-Portugal."

Date: 23 -30 January – Coimbra

CNC members involved in the organization: Conceição Pedroso Lima, João Nuno Moreira, Lino Ferreira

"Gene & Cell Therapy of CNS: from microRNAs to IPS Cells"

January 30 - February 3 2012

Date: 30 January - 3 February, Coimbra

CNC members involved in the organization: Conceição Pedroso Lima

February 2012

"Metabolic epigenetics and the progression of câncer"

Date: 2 nd February - Coimbra

CNC members involved in the organization: Conceição Pedroso Lima

"Neurobiology and Disease"

Course of the Doctoral Programme in Health Sciences from the Faculty of Medicine, University of Coimbra,

Date: 20 - 24 February – Coimbra

CNC members involved in the organization: Ana Cristina Rego & Cláudia MF Pereira

"Developing therapeutic strategies for Machado-Joseph disease: from worm to mouse (to human?)"

Date: 24th February - Coimbra

CNC members involved in the organization: Ana Cristina Rego

"Advanced course on Oncobiology, Doctoral Program on Biomedicine and Experimental Biology (CNC/UC)"

Date: 26th February- 1st March - Coimbra

CNC members involved in the organization: Conceição Pedroso Lima, João Nuno Moreira

March 2012

"Chondrocyte senescence: implications in osteoarthritis and cartilage tissue engineering"

Date: 5 - 7th March – Coimbra

CNC members involved in the organization: Alexandrina Mendes

"Mitochondria in Health and Disease",Annual Meeting of the European Society of Clinical Investigation

Date: 22 - 23th March – Budapest, Hungary

CNC members involved in the organization: Anabela Rolo

April 2012

"6th National Meeting on Cell Signaling (SINAL2012)"

Date: 13 - 14th April – Braga

CNC members involved in the organization: Ana Cristina Rego

June 2012

"NMR Basics: Theory, Processing and Applications"

Date: 11 - 15th June– Coimbra

CNC members involved in the organization: Maria Luísa Ramos

"International Symposium on Metal complexes (ISMEC 2012)"

Date: 18 - 22th June – Lisboa

CNC members involved in the organization: Carlos Geraldes

July 2012

"9th European Biophysics Congress"

Date: 13 - 17th July – Coimbra

CNC members involved in the organization: Armindo Salvador

"EUROMAR 2012 Magnetic Resonance Conference"

Date: 1 - 5th July – Dublin, Ireland

CNC members involved in the organization: Carlos Geraldes

"XXIV IUPAC Symposium on Photochemistry"

Date: 15- 20th July – Coimbra

CNC members involved in the organization: Maria Luísa Ramos

September 2012

"Developmental programming of offspring obesity"

Date: 4th September – Coimbra

CNC members involved in the organization: Cláudia Cavadas

" IV Ibero-American NMR Meeting"

Date: 25 - 28th September – Aveiro

CNC members involved in the organization: Mª Margarida Castro & Carlos Geraldes

October 2012

"European Conference of Magnetic Resonance in Biology and Medicine (ESMRMB 2012)

Date: 4-6 October-2 November – Lisboa

CNC members involved in the organization: Carlos Geraldes

"Preventive and therapeutical non-pharmacological strategies against Alzheimer's disease. Studies in 3xTg-AD mice"

Date: 8th October - Coimbra

CNC members involved in the organization: Ana Cristina Rego

"Cognitive brain reserve"

Alexandre Castro Caldas

Institute of Health Sciences, Catholic University of Portugal, Lisbon, Portugal

Date: 10th October - Coimbra

CNC members involved in the organization: Ana Cristina Rego

"Neurodegenerative diseases- From molecules to clinics and beyond"

Course of the Doctoral Programme in Experimental Biology and Biomedicine

Date: 29 October-2 November – Coimbra

CNC members involved in the organization: Ana Cristina Rego, Cláudia MF Pereira, Luís Pereira de Almeida, Paula Agostinho

"Neurodegenerative diseases: From molecules to clinics and beyond"

Date: October 29 - November 2- Coimbra

CNC members involved in the organization: Conceição Pedroso Lima

"The ubiquitin-proteasome system in Huntington's disease"

Date: 31st October - Coimbra

CNC members involved in the organization: Ana Cristina Rego

December 2012

"Prostaglandin profiling reveals a role for haematopoietic prostaglandin D synthase in adipose tissue macrophage polarisation"

Date: 5th December- Coimbra

CNC members involved in the organization: Cláudia cavadas

"Biology of Proteolysis in Pathology"

Date: 10 – 14 December- Coimbra

CNC members involved in the organization: Paula Moreira, Raquel Esteves, Sandra Cardoso

GRADUATE STUDIES PROGRAMME

During 2012, CNC organized 20 Advanced Courses and hosted 34 seminars. The seminars were attended by local graduate students and researchers, whereas the advanced courses also met the interest of people from other Portuguese Universities. Besides the organization of courses and seminars, CNC also supported the ongoing research work for Ph.D. and M.Sc. thesis. Throughout this year, 36 Ph.D. and 33 M.Sc. thesis were concluded.

In October 2002 CNC, with the financial support of FCT, launched an International Doctoral Programme in Experimental Biology and Biomedicine to provide advanced, multidisciplinary, research-oriented training in emerging areas of modern Biology and Biomedicine. The programme included advanced courses in top research areas, taught by forein scientists in collaboration with local investigators, laboratory rotations and research work o be carried out within international networks organized by CNC. The programme provided fellowships to 12 students.

Advanced Courses 2012

January

MIT – Neuroscience module
January 3 - 13

Rodrigo Cunha

Biomedical Magnetic Resonance: Molecular Imaging & Metabolism
January 4 - 6
Carlos Geraldes, John Jones

Pluripotency & Reproduction
January 9 - 13
João Ramalho-Santos

MIT – Drug Development
January 16 - 27
João Nuno Moreira, Luís Almeida, Sérgio Simões

Gene & Cell Therapy of CNS: from microRNAs to IPS Cells
January 30 - February 3
Luis Almeida

February

Metabolic Remodeling in Cancer
February 6 - 10
Paulo Oliveira, John Jones

Cell & Tissue Engineering
February 14 - 17
Lino Ferreira

Oncobiology
February 28 - March 2
João Nuno-Moreira

March

Chondrocyte Senescence: Implications in Osteoarthritis and Cartilage Tissue Engineering
March 5 - 7
Alexandrina Mendes

Vaccine Immunology with Exploration of Immune Response to Mucosal Vaccines
March 12 - 16
Olga Borges

September

CNC Cores
September 18 - 21, 2012
Microscopy - Luísa Cortes

Flow Cytometry - Isabel Nunes

Mass Spectrometry - Bruno Manadas

Molecular Systems Biology
September 24 - 28, 2012
Armindo Salvador

October

Fundamentals of Neuroscience
October 1 - 4, 2012
Rodrigo A. Cunha, Paula M. Agostinho, Paula M. Canas, Catarina V. Gomes, Ricardo J. Rodrigues, Angelo R. Tomé

Brain plasticity and cognition: the ever-changing brain
October 8 - 10, 2012
Jorge Valero, Ana Cristina Rego, Elisabete Ferreiro

Molecular Neuroscience
October 22 - 26, 2012
Ana Luísa Carvalho, Carlos Duarte, Emília Duarte, Ramiro Almeida

Neurodegenerative diseases: From molecules to clinics and beyond
October 29 - November 2, 2012
Ana Cristina Rego, Paula Agostinho, Luís Pereira de Almeida, Cláudia MF Pereira

November

Trends in Molecular Biotechnology
November 12 - 16, 2012
Isaura Simões, Pedro Castanheira

Medical Microbiology
November 26 - 30, 2012
Teresa Gonçalves, Nuno Empadinhas, Susana Alarico, Vítor Mendes, Chantal Fernandes

December

Neuroendocrinology, Aging and Obesity
December 3 - 7, 2012
Cláudia Cavadas, Célia Aveleira, Joana Salgado, Lígia Ferreira

Biology of proteolysis in pathology
December 10 - 14, 2012
Sandra Cardoso, Paula Moreira, Ana Raquel Esteves

Seminars

January

Magnetic Resonance Molecular Imaging
2012.1.6
Prof. Robert N. Muller
Department of General, Organic and Biomedical Chemistry and Faculty of Medicine and Pharmacy
University of Mons, Belgium

Stress, Memory and the Brain
2012.1.6
Nuno Sousa
Escola de Ciências da Saúde, Univ. do Minho

Sperm Cell Proteomics
2012.1.13
Rafael Oliva
Faculty of Medicine, University of Barcelona, Spain

RNA therapeutics to the CNS
2012.1.30
Beverly L. Davidson
Roy J. and Lucille A. Carver College of Medicine
University of Iowa, Iowa City, USA

February

IPS cells: Programming and Reprogramming
2012.2.1
Niels Geijsen
Utrecht University Veterinary School
Utrecht, The Netherlands

Metabolic epigenetics and the progression of cancer
2012.2.9
Frederick Domann
Department of Radiation Oncology, Holden Comprehensive Cancer Center
The University of Iowa, Iowa City, USA

Gene transfer with viral vectors: From basic science to clinical applications
2012.2.17
Seppo Ylä-Herttuala
A.I. Virtanen Research Institute for Molecular Sciences
University of Kuopio, Finland

Mr Hyde revealed: IL-7/IL-7R signaling in T-cell leukemia
2012.2.28
João Taborda Barata
Institute of Molecular Medicine, Faculty of Medicine
University of Lisbon, Portugal

March

Metabolic osteoarthritis
2012.3.7
Francisco Javier Blanco
Centro de Investigación Biomédica, Centro Hospitalario Universitário A Coruña
Instituto de Investigación Biomédica de A Coruña
A Coruña, Spain

Construção de uma política pública para a doença de Alzheimer
2012.3.9
Marisa Matias
Centro de Estudos Sociais, Faculdade de Economia
Universidade de Coimbra

Biomechanics and Signalling Networks in Collective Cell Migration
2012.3.12
Enrique Martín-Blanco
Instituto de Biología Molecular de Barcelona, CSIC
Barcelona, Spain

Activation of innate and adaptive immunity by particulate vaccine adjuvants
2012.3.14
Ed Lavelle
School of Biochemistry and Immunology, Trinity College Dublin
University of Dublin, Ireland

QSAR Approach to Perfluoroalkyl Acid Toxicity
2012.3.19
Kendall B. Wallace
Department of Biomedical Sciences
University of Minnesota - Duluth, USA

Imaging signal transduction in single dendritic spines
2012.3.26
Ryohei Yasuda
Duke University Medical Center
Durham, USA

Unidentified bright objects and multiple sclerosis copycats
2012.3.30
Mónica Marta
Neuroimmunology Unit - Neurosciences & Trauma
Blizard Institute, Barts and The London School of Medicine and Dentistry
London, UK

April

Signaling pathways that control muscle cell mass in health and disease: focus on autophagy-lysosome and ubiquitin-proteasome systems
2012.4.11
Marco Sandri
Dulbecco Telethon Institute
Venetian Institute of Molecular Medicine
Padova, Italy

The birth and post-natal development of purinergic signaling
2012.4.27
Geoffrey Burnstock
Emeritus Professor, University College Medical School
London, England

June

Drug discovery from natural products in a lab of immunopharmacology
2012.6.13
Jian-Ping Zuo
Lab.of Immunopharmacology
Chief, 1st Dept.of Pharmacology
Deputy Director, Academic Committee of SIMM, Shanghai Institute of Materia Medica
Chinese Academy of Sciences

Structure and function in the dendritic integration of protein synthesis dependent synaptic plasticity
2012.6.19
Inbal Israely
Neuronal Structure and Function Lab
Champalimaud Foundation
Lisboa, Portugal

September

Developmental programming of offspring obesity
2012.9.4
Michael G. Ross
Geffen School of Medicine, UCLA
UCLA School of Public Health
USA

Self-organization mechanisms in Myxococcus xanthus biofilms
2012.9.28
Oleg Igoshin
Rice University
Houston, USA

October

Preventive and therapeutical non-pharmacological strategies against Alzheimer´s disease. Studies in 3xTg-AD mice
2012.10.8
Lydia Giménez-Llort
Department of Psychiatry and Forensic Medicine, Institute of Neuroscience
Autonomous University of Barcelona
Spain

Cognitive brain reserve
2012.10.10
Alexandre Castro Caldas
Institute of Health Sciences
Catholic University of Portugal
Lisbon, Portugal

hTERT Promoter Methylation: An Epigenetic Cancer Biomarker
2012.10.15
Pedro Castelo Branco
The Hospital for Sick Children
University of Toronto
Toronto, Canada

The cerebellar circuit: from synapse to behavior
2012.10.25
Megan R. Carey
Champalimaud Neuroscience Programme
Champalimaud Centre for the Unknown
Lisbon, Portugal

How early life sensory experience controls synaptic and circuit maturation in the cortex
2012.10.25
Michael Ashby
Bristol Neuroscience, University of Bristol
Bristol, UK

Functional importance of mitochondrial diaphorases in maintaining phosphorylation potential during inhibition of electron transport chain
2012.10.26
Christos Chinopoulos
Semmelweis University, Department of Medical Biochemistry
Budapest, Hungary

The ubiquitin-proteasome system in HD
2012.10.31
José Lucas
Centro de Biología Molecular Severo Ochoa (CBM/SO)
CSIC/UAM
Madrid, Spain

November

Adaptation of Bird Flu to Humans via Mutations of the RNA Polymerase
2012.11.14
Darren Hart
EMBL Grenoble
France

Industrial "white" Biotechnology: From Biodiversity to "Designer Bugs"
2012.11.16
Juergen Eck
B.R.A.I.N
Biotechnology Research And Information Network AG
Zwingenberg, Germany

Fungal cell wall remodeling, drug tolerance mechanisms and virulence
2012.11.30
Carol Munro
University of Aberdeen
Aberdeen, Scotland

Molecular mechanisms of insulin action in the liver: opposite role of role IRS2 and PTP1B
2012.11.30
Angela Valverde
Consejo Superior de Investigaciones Cientificas
Instituto de Investigaciones Biomedicas Alberto Sols
Madrid, Spain

December

Prostaglandin profiling reveals a role for haematopoietic prostaglandin D synthase in adipose tissue macrophage polarisation
2012.12.5
Sam Virtue
University of Cambridge Metabolic Research Laboratories
Cambridge
United Kingdom

Molecular mechanisms of neurodegeneration in Parkinsons disease
2012.12.14
Miguel Vila
Vall d Hebron Research Institute
Center for Networked Biomedical Research on Neurodegenerative Diseases
Barcelona, Spain

PhD thesis concluded in 2012

Ana Patrícia Figueiredo Rocha Simões
Purinergic control of neuroinflammation and neuroprotection by the blockade of P2 receptors under excitotoxic conditions in the hippocampus
Supervisor: Rodrigo A. Cunha

Ana Teresa Inácio Ferreira Varela
Regulation of mitochondrial function in ischemia / reperfusion: looking for therapeutic strategies in fatty livers
Supervisor: Carlos M. Palmeira & Rodrigo A. Cunha

Samira Cardoso Lopes Ferreira
Presynaptic A_2A adenosine receptors control CB_1 cannabinoid receptor-mediated effects at the corticostriatal nerve terminals
Supervisor: Attila Kofalvi & Rodrigo A. Cunha

Susana Louros
The Role of Transmembrane AMPAR Regulatory Proteins (TARPs) in Synapse Remodeling and Homeostatic Plasticity
Supervisor: Ana Luísa Carvalho

João Noutel
Synapse Development in a Mouse Model for an Autism Spectrum Disorder
Supervisor: Ana Luísa Carvalho

Joana Ferreira
Molecular Mechanisms of Synaptic Traffic of Glutamate Receptors of the NMDA Type: Implications in Synaptic Plasticity
Supervisor: Ana Luísa Carvalho

Tatiana Catarino
Regulation of Synapse Composition by Protein Acetylation: the Role of Acetylated Cortactin
Supervisor: Ana Luísa Carvalho

Sofia Grade
Strategies for the use of neural stem cells in brain repair
Supervisor: João Malva

Ana Rita A. Santos
Regulation of the proteome by brain-derived neurotrophic factor in hippocampal neurons: protein synthesis vs protein degradation
Supervisor: Carlos Duarte

Rita Catarina Gonçalves Perfeito
Interplay between alpha-synuclein and oxidative stress in Parkinson's disease cell models
Supervisor: Ana Cristina Rego

Mário Luís Nôro Laço
Title of the thesis: "Studies on the activity of ataxin-3 and mitochondrial dysfunction in models of Machado-Joseph disease".
Supervisor: Ana Cristina Rego

Daniela Moniz Arduíno
The cross-talk between endoplasmic reticulum and mitochondria in Parkinson's disease: relevance to autophagic cell death
Supervisor: Sandra M Cardoso

Ana Raquel Esteves
Mitochondrial dysfunction towards protein aggregation in Parkinson disease: contribution of cytoskeletal disorganization
Supervisor: Sandra M Cardoso

Sueli Cristina Marques
Chromatin remodeling in Alzheimer's disease pathogenesis
Supervisor: Cláudia Pereira

Rui Miguel Oliveira Costa
Endoplasmic reticulum stress during amyloid β peptide-induced cell death: role of mitochondria and glutamatergic N-methyl-D-aspartate receptors
Supervisor: Cláudia Pereira

Sónia Catarina de Sousa Correia
Mitochondrial preconditioning-triggered brain tolerance: Implications for Alzheimer's disease and Diabetes
Supervisor: Paula I Moreira

Gabriel Costa
The role of tumor necrosis factor receptor 1 in diabetic retinopathy: contribution to the early neuronal cell death
Supervisor: Paulo Santos

Carlos José Vieira Simões
Virtual Screening with Sense and Sensibility in the Search of New Amyloid Inhibitors
Supervisor: Rui Brito

Sónia Patrícia Dias Duarte
On the development of anti-tumoral therapeutic strategies: suicide gene therapy mediated by lipid-based systems and cancer stem-cell based vaccination
Supervisor: Conceição Pedroso Lima

Lígia Silva
A novel multifunctional lipid-based nanoparticle for the delivery of siRNA to cancer cells and the tumor microenvironment
Supervisor: Conceição Pedroso Lima

Ana Teresa Simões
Calpain-mediated proteolysis ofataxin-3 in Machado-Joseph disease
Supervisor: Conceição Pedroso Lima

Cristiana da Silva Oliveira Paulo.
Permanent antifungal materials and coatings: bioactivity and cytotoxicity characterization
Supervisor: Lino Ferreira

Helena Sofia Esmeraldo de Campos Vazão
Approaches to improve the differentiation, maturation and biological activity of vascular cells derived from stem cells
Supervisor: Lino Ferreira

António João Sales Mano
Avaliação da utilidade de parâmetros cinéticos derivados do CA-125 no acompanhamento do cancro epitelial do ovário
Supervisor: Amílcar Falcão

Gonçalo de Castro Pereira
Mitochondrial Physiology During Doxorubicin-induced Selective Cardiotoxicity
Supervisors: Paulo Oliveira, António Moreno

João Paulo Soeiro Terra Teodoro
Physiologic pathways and molecular mechanisms regulating energy homeostasis in diabetes
Supervisors: Carlos Palmeira, Anabela Rolo

Filipe Valente Duarte
Dibenzofuran exposure: cellular and mitochondrial damage
Supervisors: Carlos Palmeira, Anabela Rolo

Ana Teresa Inácio Ferreira Varela
Regulation of mitochondrial function in ischemia/reperfusion: looking for therapeutic strategies in fatty livers
Supervisors: Carlos Palmeira, Anabela Rolo

Bárbara da Silva Rocha
Biological role of nitrite and nitric oxide in the stomach: cellular dysfunction and production of physiologically-active molecules
Supervisor: João Laranjinha

Joana Isabel Félix Paixão
Papel das antocianinas no contexto da prevenção da aterosclerose: mecanismos moleculares de protecção contra a apoptose e inflamação em células endoteliais
Supervisor: João Laranjinha

Vitor Gonçalo Mendes
New Insights into the Biosynthesis of the Mycobacterial Methylglucose Lipopolysaccharide
Supervisor: Teresa Gonçalves

Sara Carvalhal Figueiredo
Innovative Platforms for MRI-based Applications
Supervisor: Carlos F. G.C. Geraldes

Inês Ribeiro Violante
The neurobiological basis of Neurofibromastosis type 1: new insights into brain structure, function and neurochemistry
Supervisors: Prof. Dr. Miguel Castelo Branco and Carlos F.G.C.Geraldes

Cristina Barosa
Noninvasive Measurements of Human Hepatic Metabolism Using Deuterated Water
Supervisor: Jonh Jones

Ivan Viegas
Sources of Blood Glucose and Liver Glycogen in the Seabass: Implications for Carbohydrate Metabolism in Fish
Supervisor: Jonh Jones

Maria Joao Pereira
Effects of immunosuppressive drugs on human adipose tissue metabolism
Supervisor: Eugenia Carvalho

Master Thesis

Marilisa Vigorita
Role of ataxin3 aggregation and phosphorylation in the pathogenesis of Machado-Joseph disease
Supervisor: Ana Luísa Carvalho

Gladys Tarcila Lima Caldeira
Changes in transcription factors related to mitochondrial biogenesis and antioxidant defenses in Alzheimer's disease models
Supervisor: Ana Cristina Rego

Joana Cristina Pedro Rodrigues
Pyruvate dehydrogenase and mitochondrial function in Huntington's disease – influence of insulin/IGF and histone deacetylase inhibitors
Supervisor: Ana Cristina Rego

Ana Margarida Alves de Oliveira
Análise protetora de derivados de luteolina num modelo de Huntington
Supervisor: Ana Cristina Rego

Diogo Martins- Branco
Ubiquitin proteasomal system impairment potentiates alpha-synuclein oligomerization
Supervisor: Sandra Morais Cardoso

Milene Gonçalves (Mestrado em Bioquímica, FCTUC)
Definição da Resposta Imune Periférica na Doença de Alzheimer
Supervisor: Margarida Carneiro

Tiago Rodrigues Sousa
Imune response in Ncf1 deficiency mice with DSS induced colitis
Supervisor: Margarida Carneiro

Geema Kondandaraman
B cell phenotype studies and expression of ganglioside GM1 in Osteosarcoma
Supervisor: Margarida Carneiro

Marco Heestermans
Peripheral B lymphocytes express lower amounts of TGF-β in Parkinson patients, possibly affecting peripheral regulatory T cell differentiation
Supervisor: Margarida Carneiro

Célia Sofia Silva Bidarra Vaz
Medicamentos Potencialmente Inapropriados em Idosos – a realidade de um serviço de Medicina
Supervisor: Amílcar Falcão e Ana Fortuna

Sónia Alexandra Simões Sousa
Narcolepsia: efeito adverso da vacina contra o vírus H1N1
Supervisor: Olga Borges

Isabel Cristina Pinto
Rotavírus: uma abordagem à vacinação por vacinas derivadas de VLP´s
Supervisor: Olga Borges

Daniela Pereira Alves
Vacinas para a hepatite B:novos adjuvantes
Supervisor: Olga Borges

Sara Margarida Quaresma
Imunização passiva na doença de Alzheimer
Supervisor: Olga Borges

Cláudia Saraiva
Development of lipoplex formulations for specific genetic material delivery to pancreatic cancer cells
Supervisor: Henrique Faneca & Conceição Pedroso Lima

Catarina Rebelo
Development of liposome formulations for drug delivery to breast cancer cells
Supervisor: Henrique Faneca

Pedro Miguel Serrano Germano Calado Carreiras
Uma estratégia para a discriminação entre compostos activos e inactivos em experiências de rastreio virtual: COX-1 como caso de estudo
Supervisor: Rui M. M. Brito

Diana dos Santos Mota
Doença Fúngica Invasiva na Imunodeficiência Primária
Supervisor: Teresa Gonçalves

Carla Cristina Veríssimo Dias
Candida albicans OPY2 gene complementation in Saccharomyces cerevisiae
Supervisor: Teresa Gonçalves

Patrícia Guarino
Avaliação de alterações genéticas e epigenéticas envolvidas na susceptibilidade a cancro na 122aematol de Down
Supervisors: Profs Ana Bela Sarmento Ribeiro e Isabel Marques Carreira

Diana Isabel Lourenço Matias
Study of Integrin contribution to Glioblastoma cell motility
Supervisors: Profs Maria Celeste Lopes and Ana Bela Sarmento

Amelia Fuhrmann
Effects of cyclosporine A and sirolimus on glucose and lipid metabolism - an in vivo rat model
Supervisor: Eugenia Carvalho

João Carlos Pinho da Silva
Hemisuccinato de colesterol na etiologia da aterogénese.
Supervisor: Otília Vieira

Patrícia Sofia Alçada Morais
Propriedades de subpopulações de espermatozóides humanos: Sexo e bioenergética
Supervisor: João Ramalho

Bárbara Sofia Leitão Pinhão Lourenço
Selecção de ovócitos para técnicas de Reprodução Assistida mediante o estudo das células do cumulus
Supervisor: João Ramalho

Maria Inês Ramalho de Almeida e Sousa
Effects of nicotine, anandamide and sildenafil citrate on sperm function: The Role of Mitochondria
Supervisor: João Ramalho

Andreia Filipa Marques Silva
Avaliação da integridade da cromatina em espermatozóides humanos: relevância clínica
Supervisor: João Ramalho

Sónia Catarina Simões
Biomarcadores em tumores das células germinativas
Supervisor: João Ramalho

Andreia Marques Gomes
Efeito do 3-bromopiruvato na pluripotência de mESC
Supervisor: João Ramalho

M. Manuela Coutinho Alves de Azevedo
Control of migration of neural stem cells by calpain signaling
Supervisor: Inês Araujo

Mariana Monteiro Loureiro Val
Cr(VI) carcinogenesis: Understanding the "lethal" drivers of epithelial-stromal co-evolution
Supervisor: Mº Cármen Alpoim

Susana Filipa Pereira Sampaio
Role of p66Shc signaling on Doxorubicin-induced cardiac mitochondrial dysfunction.
Supervisor: Paulo Oliveira

Tania Sofia Silva Sousa
Investigação do cross-talk genómico na neuropatia ótica hereditária de Leber
Supervisor: Manuela Grazina

TECHNOLOGY TRANSFER

Translational research and technology transfer have been progressivly 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 envolving 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 technology transfer unit (Biocant) in colaboration with Cantanhede Municipal Council.

This unit became the anchor of Biocant Park a Biotechnology Park that is rapidly growing by atracting new Biotechnology companies.

BIOCANT

Biocant is a private, non-profit, innovation centre created by CNCB together with the municipality of Cantanhede for technology transfer in biotechnology.

Founded 8 years ago, Biocant has grown to become a reference in the field and the catalyst of Biocant Park, the first Portuguese biotechnology park.

Biocant is organized into seven main functional units with highly qualified teams and state of art equipment: 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.

Throughout the past year Biocant has filed four patent applications and its researchers published papers in journals such as PNAS and JBC.

Companies operating in Biocant Park

At the present 20 companies operate in Biocant Park. 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.

OUTREACH PROGRAMME

Coordinator: Maria Teresa Girão da Cruz

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.

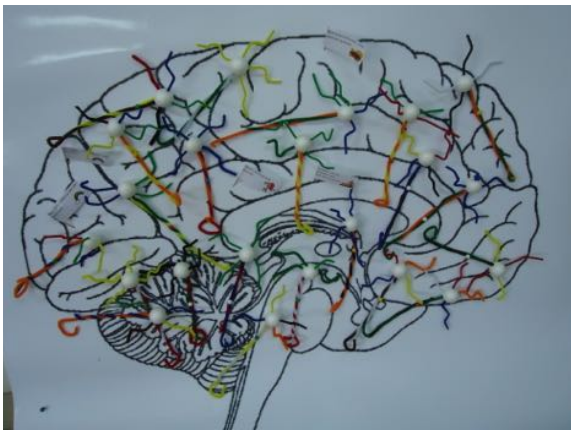
The Center yearly participates in various activities exclusively planned to the lay public, namely during the Brain Awareness Week, Science and Technology Week, European Researchers Night, and Science Fairs. Elementary to high school students are also a committed public of all CNC's outreach actions. 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 and outreach activities have been recognized throughnumerous media articles and broadcasts (over 400 in 2012), and important awards – namely two "Artigo Destaque" awards by the Portuguese Society for Neuroscience, a Silver Certificate on SET for Britain by the British Parliament House of Commons, and the public award for best activity during Science and Technology Week.

Brain Awareness Week (BAW), March 12-18

In Portugal, BAW 2012 focused on the theme "Brain and Health". Initiatives were intended both for the general public and for the students, and were designed to increase community awareness of the potential for



improving the long term health of the brain through lifestyle changes and risk reduction strategies: 1) a public debate about neurodegenerative diseases, 2) the exhibition "Brain in colors", including works by CNC researchers; 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 (Ocupação Científica de Jovens nas Férias), July 09-20

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 28

Together with the Science Museum of the University of Coimbra, CNC took part for the fourth 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 "Science and Sports", participated in a theatre play, and took part in the "speed-dating" event.

Science and Technology Week, November 19-25

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. As a result of the programme "Artists in Residence – Art, Science and Technology" (that took place in 2010), in 2012 the MARIONET theatre company produced the play "MIM – My Inner Mind", that was on stage at CNC from November 21st to December 01st 2012. This event won the public award for best activity during Science and Technology Week.

I Want More and Better Cells! Stem Cells: What are they? Where are they? What can they be used for?

This CNC project is supported by "COMPETE-Media Ciência" and included the development and production of different materials intended to facilitate the communication with the public on the stem cells subject: radio interviews, newspaper illustrated chronicles, animated videos, and a cartoon book.

Novel Social and Scientific Dialogues for Neurodegenerative Diseases

This public engagement project is carried out in collaboration with the Center for Social Studies (CES), and is part of the BIOSENSE science shop project. The close interaction between two research centers on the biomedical and social sciences fields – CNC and CES –, and the neurodegenerative diseases' patient associations – Alzheimer Portugal, and Associação Portuguesa de Doentes de Huntington – aims to address science and technology-based concerns related to the neurodegenerative diseases raised by the citizens themselves, by promoting science-society dialogues and collaborations.

CORE FACILITIES

ANIMAL HOUSE

Head of Unit: Alexandre Pires

The Animal House is a shared resource that provides services in laboratory animal experimentation and husbrandy, 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 22ºC 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.

Staff: Carmen Semião (caretaker)
Fátima Graça (assistant technician)
Maria Eugénia Campos (assistant technician)

Animal room – IVC cages (type I)

Laminar flow chamber

FLOW CYTOMETRY UNIT

Head of Unit: Isabel Nunes Correia

The flow cytometry unit provides scientific and technical support both to CNC and external researchers. Currently, it is equipped with a Becton Dickinson FACSCalibur cell analyser and a Partec CyFlow® Space cell sorter. For researchers wishing to use flow cytometry, the unit offer 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.

Since 2007, when the unit was created, the number of users is increasing every year, and presently flow cytometry is an important and central technique for the fulfilment of many CNC investigation projects.

FACSCalibur cell analyzer

MICROSCOPY UNIT

Head of Unit: Luísa Cortes

The Microscopy Unit, at the Center for Neuroscience and Cell Biology (MU-CNC), is a centralized facility where users receive the support needed to carry out conventional and advanced imaging techniques, based on Light Microscopy. The unit has combined resources to provide state-of-the-art equipment that is open to all researchers. We offer the same services to outside CNC groups or companies.

The primary goal of the MU-CNC is to enhance the research and teaching environment for the CNC scientific community. To meet these goals, the MU-CNC:

- provides technical training to local users and visiting researchers;

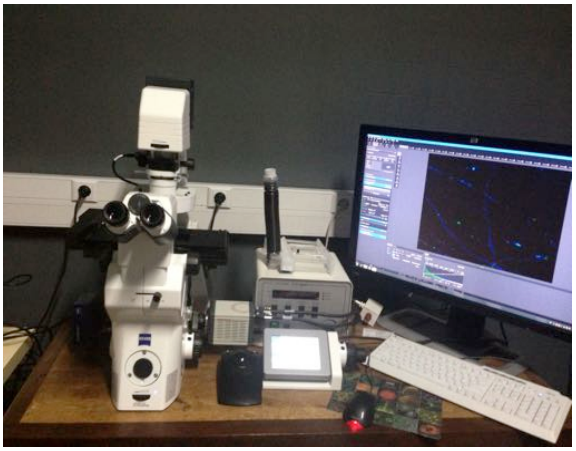
- offers consultation on experimental design and image analysis;

- evaluates new methods and fluorescence tools and communicates acquired knowledge to users;

- implements advances in hardware and software relevant for biomedical sciences;

- provides ongoing education in theory and practice by organizing training courses and workshops.

Presently, the unit manages a laser scanning confocal microscopy, a P.A.L.M. laser microdissecting microscope, a single cell calcium imaging system, two widefield systems (one of them fully motorized) and other brightfield microscopes. The systems are prepared for advanced applications, including live cell imaging and single cell calcium measurements, enabling the researchers to image 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 it full characterization.



Laser scanning confocal microscope

P.A.L.M. laser microdissecting microscope

MASS SPECTROSCOPY UNIT

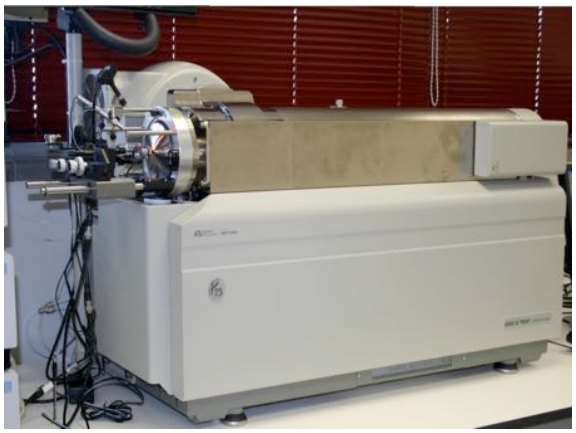
Head of Unit: Bruno Manadas

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.

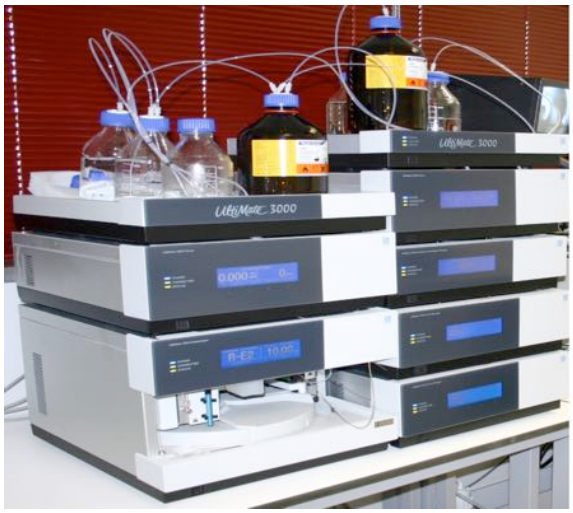
Presently, the Mass Spectrometry Unit is equipped with state of the art technology, namely: a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex), hybrid triple quadrupole/ion-trap mass spectrometer with capacity of MS3, and a two-dimensional liquid chromatography system Ultimate 3000 (Dionex/LCPackings). The unit also contains several software packages for data processing, including 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).

Staff: Vera Mendes (technician)



4000 QTRAP mass spectrometer



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

NMR SPECTROSCOPY UNIT

Head of Unit: Prof. Carlos Geraldes

The Unit currently stands with a 600 MHz NMR Spectrometer (Varian VNMRS 600) equipped for liquid state NMR as HR-MAS NMR, a narrow bore 500 MHz NMR Spectrometer (Varian Unity 500) equipped for liquid state NMR, a 20 MHz NMR relaxometer (Bruker Minispec 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 is part of the Portuguese Nuclear Magnetic Resonance Network (PTNMR).

Staff: Emeric Wasielewski

Varian 600 NMR Spectrometer

SERVICES

LABORATORY OF BIOCHEMICAL GENETICS

Coordinator: Manuela Grazina

Mitochondrial Respiratory Chain (MRC) and Krebs cycle enzymes

Certification – "Sistema de gestão da qualidade, SGQ, iso 9001" at CNC-Laboratório Associado

The certification process continued and, after Audit in June 2012, the certificate was maintained (APCER, Certificate ISO 9001, reg. PT-2011/CEP.3971). This represents a step forward in the future of Services' Laboratories.

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 (MRC Mitochondrial Biology Unit, Cambridge, UK), 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" (March 2012), and the III Advanced Course & Workshop on Clinical Case Reports: the second genome: mitochondrial bigenomics – from genotype to phenotype and clinical expression" (January 2012), allowing the visit of Prof. Lee-Jun Wong, Doctor Fernando Scaglia (Baylor College of Medicine, Houston – Texas, USA), Prof. Massimo Zeviani (MRC Mitochondrial Biology Unit, Cambridge, UK), 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 finishing the set up of screening key 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 60 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 72 samples (some patients had 2 or more tissues analysed), in 720 assays, including 28 lymphocytes isolated of peripheral blood, 38 muscular biopsies, 3 liver, 1 heart and 2 other samples. A MRC deficiency was detected in 29 patients.

The number of Hospitals asking for our Services increased.

The validation of the Krebs cycle enzymes (fumarase, alfa-ketoglutarate dehydrogenase, malate dehydrogenase, aconitase, isocytrate dehydrogenase) is under final validation and 174 samples were analysed (1218 assays). These tests represent an important set up for improving diagnostic of mitochondrial bioenergetic defects.

Concerning the analysis of Coenzyme Q10 (collaboration with Dr. Rafael Artuch, Hospital San Juan de Dios- Barcelona, Spain), we have analysed 36 samples (plasma, muscle, liver), in 180 assays. Detection of Coenzyme Q10 deficiency represents a huge improvement in diagnosis of MRCD, since this is the only treatable deficiency in this group of inherited errors of metabolism.

Amino Acid Analysis

Our laboratory received 256 samples (211 - plasma, 36 - urine and 9 - cerebrospinal fluid) of physiological fluids for amino acid analysis, corresponding to 768 assays. 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.

Mitochondrial DNA (mtDNA) and nuclear (nDNA) genomes studies

We have received 191 samples of 175 patients (blood - 137, muscle -34, liver - 3, heart – 1 and other tissues - 11), for DNA extraction, representing a 108% increase in the number of patients, compared to last year. It is noteworthy that, given the fact that we are now offering a more extensive series of genetic assays, we received some requests for analysing samples already existing in the Laboratory.

Molecular differential analysis of mitochondrial cytopathies, as a highthrouput 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 156 samples, comprising a total of 5,761 assays for mtDNA point mutations, deletions and gene panels' analysis. Further PCR-RFLP analyses were performed to validate point mutations in 56 samples (168 assays). Deletions have been detected in 13 samples and a total of 228 mtDNA sequence variations, 4 of which are novel variants, under characterization.

Concerning **mtDNA copy** number assays for depletion screening, we investigated 42 samples of 37 patients, including blood (13), muscle (22), liver (3) and other (4) tissues, comprising a total of 1176 real time PCR assays.

Implementation of analysis for other genes, such as ANT, TP, TK and twinkle has continued, in the attempt of finding the cause for mtDNA depletion or multiple deletions, but limitations in personnel available did not allow finishing the accomplishment of this objective.

Concerning the **screening of nDNA related to MRCD**, we have screened 256 samples, comprising a total of 18,300 assays.

POLG1,2 genes were screened in 29 samples of 29 patients (3,190 DNA sequencing assays). We have identified 244 sequence variations in 29 patients. 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 22 samples of 17 patients and 5 index cases (1,210 assays) and identified 58 sequence variations, 5 of which are probable pathogenic related to mtDNA depletion, relevant for genetic diagnosis and genetic counselling.

Screening of SURF1 gene (35 samples of 33 patients, 2590 assays) allowed detection of 70 sequence variations, including 4 possibly pathogenic mutations, relevant for genetic diagnosis and genetic counselling that are under confirmation.

We have also analysed 40 samples of 40 patients for implementation of TP, MPV17 and twinkle genes (3060 assays) and identified 93 sequence variations (2 different), but no pathogenic mutations were identified so far.

Staff: Marta Simões; Cândida Mendes; Carla Veríssimo; João Pratas; Maria João Santos, Carolina Ribeiro; Mónica Vaz

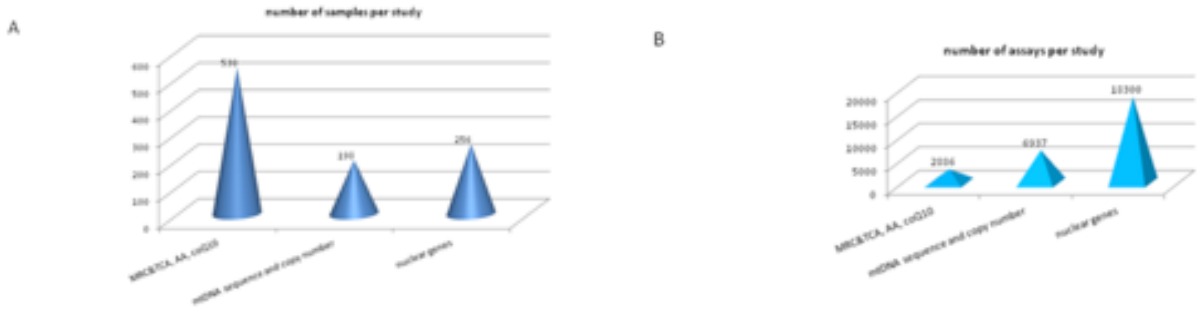


Figure 1- Number of samples analysed and assays performed in LBG, according to the study, for biochemical and genetic diagnosis, in the year of 2012.

LABORATORY OF NEUROCHEMISTRY

Coordinators: Catarina Resende Oliveira, Inês Baldeiras

The Neurochemistry Unit is integrated in the Neurology Department of the University Hospitals of Coimbra (CHUC) 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, demielinizing, 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 neutralyzing antibodies (NABs) against Interferon-β (IFN-β) in multiple sclerosis patients undergoing treatment with IFN-β.

Early and differential diagnosis of dementias is a particular important area of work of this laboratory. The Neurochemistry unit is, in the framework of the Portuguese Epidemiological Surveillance Program for Human Prion Diseases, the national reference laboratory for Cerebrospinal Fluid (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 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.

Staff: Inês Baldeiras
Maria Helena Ribeiro
Rui Pascoal

	Blood (Serum/Plasma)	CSF	Urine	Brain extracts	Other extracts
Cytochemistry and electrophoresis	286	286			
IgG Oligoclonal bands	157	157			
Vitamin A/E	160				
Redox Satus	60				
Purines & Pyrimidines			5		
Arylsulfatase A			0		
Anti-neuronal antibodies	62				
Antiepileptic drugs	3				
NABs against INFβ	56				
CSF Tau, p-Tau and Aβ42		183			
CSF 14-3-3 protein		87			
Prion protein isoforms				2	
Oxidative Stress	26				117

Table 1: During the year 2012, the Neurochemistry Unit has recieved arround 650 blood and 400 CSF samples and has performed the analysis presented.

LABORATORY OF MOLECULAR GENETICS CARDIOPATHIES

Coordinator: Isabel Marques Carreira

Screening of mutations in 31 genes associated with cardiopathies

In the laboratory of Molecular Genetics of Cardiopathies (LGMC) the main study area is the Hypertrophic Cardiomyopathy(HCM).

It is inherited as an autossomal dominant disease and the most common heart disease affecting 1 in 500 people worldwide. This disease is characterized by left ventricular hypertrophy, with the predominant involvement of the interventricular septum, and of unexplained etiopathogeny

HCM can present at any age and is highly variable. Patients can remain asymptomatic throughout their life, but is also associated with adverse clinical events, like heart failure, stroke and sudden cardiac death.

In about half of the HCM patients a disease causing mutation can be detected in one of the genes encoding for sarcomeric proteins. More than 1000 distinct sarcomere protein gene mutations have been identified to cause HCM. Identification of a disease causing mutation in a HCM patient (the proband) implies the opportunity of screening by means of predictive DNA testing in relatives, and can thus better identify the relatives at risk for HCM and associated death.

In our lab (LGMC), the method of molecular diagnostics implemented, is the MassARRAY, Iplex Gold (Sequenom) - W35_SpectroCHIP, which analyzes 540 mutations in 31 genes associated with the development of cardiopathies.

In the year 2012, the method, was adjusted and consolidated. The procedure involves collaboration, for the high throughput analysis, with a laboratory in Lisbon. Validation and interpretation of the results as well as the familial studies are done in the LGMC.

Thirty one cases were refereed in 2012 from the cardio-genetic consultation in the Pediatric Hospital of Coimbra. Of these cases, 25 were index cases in which the 540 mutations of the 31 genes were evaluated. The remaining 6 cases were familial and therefore targeted studies were done.

In 2012, the LGMC was revalidated the quality certificate (APCER), continuing to be a certified laboratory for the "Research of mutations in genes associated with cardiopathies".

Staff: Ana Cristina Santos

LABORATORY OF NEUROGENETICS AND INHERITED EYE DISORDERS

Coordinator: Maria do Rosário Almeida

Molecular testing of Neurodegenerative and Vision related diseases

The molecular diagnosis of several Neurodegenerative diseases such as, Frontotemporal Lobar degeneration, Familial Alzheimer Disease and Parkinson´s Disease has been already available in our laboratory for a few years. Therefore, during 2012 the group has continuously acquired experience through the increased number of referrals from different Neurology departments in order to become a reference centre in this area. During this year, more than one hundred genetic testing referrals came along with the patients' samples to be tested in our laboratory. In addition, several initiatives have been performed to promote the available genetic testing, in particularly, an updated of the CNC website concerning the "Services and Cores" has been made during 2012 to allow the download of the referrals by the clinicians and also the instructions to collect and send the samples to

the laboratory. Importantly, continuous efforts have been made to ensure that the methodologies and diagnostic strategies used are compatible with current scientific knowledge. Therefore, during 2012, additional genetic tests have been implemented to increase the informativeness of the molecular diagnosis in neurodegenerative diseases (eg. detection of hexanucleotide expansion mutation in C9orf72 gene and the mutation analysis in the SQSTM1 gene). Also, a few genetic tests have been implemented to test patients followed in dementia outpatient´s clinic of the University Hospital of Coimbra (HUC) who developed rare types of dementia, such as: Fatal familial insomnia and dementia in hereditary cerebral hemorrhage with amyloidosis - Dutch type. Finally, the group was also involved in taking part of European Consortiums initiatives in order to tackle early onset dementia in a more efficient manner. Thus, research Projects have been outline and submitted to get financial support.

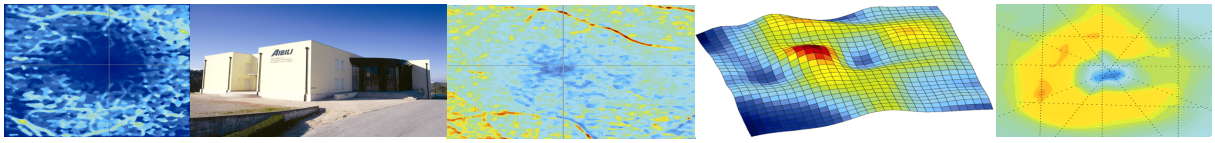
In addition, a new genetic test has been also implemented in order to evaluate the presence of somatic mutations in isocitrate dehydrogensase 1 gene (IDH1) in patients with gliomas which was demonstrated to have an important diagnostic value.

The ophthalmology area is other area in which our laboratory offers genetic tests, in particularly to inherited vision related diseases such as: Retinitis pigmentosa, Nanophthalmia and Anophthalmia / Microphthalmia (A/M). Therefore, during 2012, the molecular analysis of known genes related with these conditions has also been expanded.

Staff: Maria do Rosário Almeida
Maria Helena Ribeiro
Ana Cristina Santos

AIBILI REPORT OF ACTIVITIES

association for innovation and biomedical research on light and image



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 complemment 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 Trials (CEC) and the Coimbra Coordinating Centre for Clinical Research (4C).

Centre for Clinical Trials

Investigator-Driven Clinical Trials

1. Correlation phenotype/genotype in diabetic retinopathy

ClinicalTrials.gov n. NCT01228981

Protocol n. CEC/120

Principal Investigator: Conceição Lobo

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 nonproliferative 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 ά) and Group C 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 454 sequencing 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).

2. Identifying progression of retinal disease in eyes with NPDR in diabetes type 2 using noninvasive

procedures

ClinicalTrials.gov n. NCT01145599

Protocol n. ECR-RET-2010-02

Coordinating Investigator: José Cunha-Vaz

Participating Centres (19): Amsterdam, Antwerp, Barcelona, Bonn, Coimbra, Glostrup, Leipzig, Lisbon, London (2), Milan, Padova, Paris (3), Val.ncia, Rome, Rotherdam, Surrey.

Support: EVICR.net

The rate of progression of diabetic retinopathy varies widely between different patients, even with similar metabolic control. It is becoming clear that a large percentage of patients with mild NPDR will take a long time to develop any sight-threatening complication. The inclusion of eyes/patients in a clinical trial that do not show any significant worsening during the period of the trial masks any beneficial effect of the drug being tested. It appears that the only option is to identify the eyes/patients

that show progression of retinopathy during a pre-trial run-in period and only include such patients. Characterization of progressor phenotypes in the early stages of diabetic retinopathy and identification of biomarkers of disease progression are also objectives of major interest.

The purpose of this study is to identify eyes that show worsening and disease progression (progressor phenotypes) allowing improved patient care.

3. 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

EudraCT n. 2009-016760-36

ClinicalTrials.gov n. NCT01281098

Protocol n. CC-02-2009

Principal Investigator: José Cunha-Vaz

Grant: Pfizer

Panretinal photocoagulation (PRP) can cause regression of retinal neovascularization and reduce the risk of severe vision loss in people with proliferative diabetic retinopathy (PDR).

However, this destructive treatment may be associated with side effects (e.g. pain, transient blurring, loss of peripheral and/or night vision, increased risk of macular edema and central vision loss) and it is not always efficient in the regression of the neovascularization. Vascular endothelial growth factor (VEGF) has been shown to play a role in retinal neovascularization and retinal vascular leakage related with PDR and diabetic macular edema. Anti-VEGF treatments have been hypothesized as an adjunctive treatment for the management of retinal neovascularization and macular edema related with diabetic retinopathy (DR).

The combination of intravitreal anti-VEGF treatment with pegaptanib, where a series of 3 injections are injected to reverse the neovascularization, while maintaining the macula dry will be complemented by the more long term effect of the panretinal photocoagulation. The peripheral photocoagulation proposed is expected to eliminate the chronic VEGF stimulus by eliminating the chronic ischemic factor, while maintaining the visual fields useful for daily activities such as driving, etc.

The purpose of this trial is to evaluate safety and to compare the efficacy of a combination of intravitreous injection of pegaptanib (0.3 mg) plus PRP versus PRP alone in the regression of retinal neovascularization in eyes with high-risk PDR.

4. Prospective, randomized, multicenter, open label phase II study to access 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

ClinicalTrials.gov n. NCT01280929

Protocol n. CRFB002DPT04T

Principal Investigator: João Figueira

Grant: Novartis

Panretinal photocoagulation (PRP) can cause regression of retinal neovascularization and reduce the risk of severe vision loss in people with proliferative diabetic retinopathy (PDR).

This destructive treatment is associated with side effects and it is not always efficient in the regression of the neovascularization. Anti-VEGF treatments have been hypothesized as an alternative adjunctive treatment for the management of retinal neovascularization and macular edema related with diabetic retinopathy (DR).

The purpose of this trial is to evaluate safety and to compare the efficacy of intravitreous injection of ranibizumab alone (0.5 mg), versus combination of intravitreous injection of ranibizumab (0.5 mg) plus PRP, versus PRP alone in the regression of retinal neovascularization in eyes with high-risk PDR.

5. 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 n. NCT01440660

Protocol n. 4C-2011-01

Principal Investigator: Luisa Ribeiro

Financial Support: DoIT – Ag.ncia de Inova..o, Portugal - Project n. 13853

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.

6. 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

EudraCT Number: 2012-001200-38

ClinicalTrials.gov n. NCT01726075

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: European Union 7th Framework Programme – Call Health 2011 - Project n. 278040-2

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, intravitreous 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 (Sim. & Hern.ndez. 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 intravitreous 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.

7. C-TRACER Project nº 1 - Biomarkers of Diabetic Retinopathy Progression

ClinicalTrials.gov n. NCT01607190

Protocol n. 4C-2012-02

Coordinating Investigator: Jos. Cunha-Vaz

Participating Centres (2): Coimbra, Hyderabad (India).

Grant: Champalimaud Foundation

Work published by one of the research groups involved (Arch. Ophthalmol. 2004; 122: 211-217) proposed 3 distinct phenotypes of diabetic retinopathy progression. In another study, a group of 52 patients with type 2 diabetes and non-proliferative diabetic retinopathy was

followed over 7 years. In this study, 15% of patients from phenotype B, 30% of patients from phenotype C and no patients from phenotype A developed clinically significantly macular edema (CSME) needing photocoagulation. This

project aims to validate this predictive model of diabetic retinopathy progression to CSME in an independent and larger population. Data for validation will be collected from a prospective observational study, that will include an initial period of three visits (V0, V6 and V12), performed at six-month interval, of 200 patients (1 eye per patient) with diabetes type 2 and mild nonproliferative diabetic retinopathy, followed by another examination 1 year later. The initial visits (V0, V6 and V12) are mandatory to classify patients into one of the 3 phenotypes (A, B and C) shown to be present in type 2 diabetes in an expected relative proportion of 50%, 30% and 20%, respectively. This will allow obtaining 100 phenotype A, 60 phenotype B and 40 phenotype C, of which 0-5, 9-10 and 12, respectively, are expected to develop CSME within the next one year period. Baseline and follow-up examinations (V0, V6, V12 and V24) will include bestcorrected visual acuity, fundus photography analysed by automated microaneurysm counting and turnover using Retmarker DR software, HD-OCT retinal thickness measurements, HbA1C and lipid blood levels. The outcomes to be tested will be the occurrence of need for photocoagulation for CSME according to ETDRS Guidelines and/or vision loss of at least 2 lines documented in ETDRS charts. The performance of the predictive model will be analysed with the ROC analysis and index of discrimination. The findings are expected to have a major impact on diabetic retinopathy management in the European Union and India.

8. Early Markers of choroidal neovascularization (CNV) in fellow eyes of patients with agerelated Macular Degeneration (AMD) and CNV in one eye

ClinicalTrials.gov n. NCT00801541

Protocol n. A9010002

Principal Investigator: Rufino Silva

Participating Centres (3): Coimbra, Belfast and Milan

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.

9. Epidemiological study of the prevalence of Age-Related Macular Degeneration in Portugal

ClinicalTrials.gov n. NCT01298674

Protocol n. CC-01-2009

Coordinating Investigator: Rufino Silva

Grant: Novartis

The objective of the study is the determination of prevalence of age-related macular degeneration in well defined population over 55 years of age.

It will involve population based epidemiological studies focused on two well defined locations in Portugal with distinct geographic characteristics and different levels of sun exposure in the Central Region of Portugal: Mira, near the Atlantic Ocean and Lous., amountainous area away of the sea.

10. STRONG – Early onset of Neovascular Glaucoma

Coordinating Investigator: Norbert Pfeiffer (Mainz, Germany)

Financial Support: European Union 7th Framework Programme – Call Health 2012 - Project n.305321

The aim of the STRONG Study is to study the natural course of the disease, to determine risk factors for the occurrence of neovascular glaucoma and to identify biomarkers that better characterize the course of the disease. At the same time we aim to test the efficacy of

aganirsen for the treatment of neovascular glaucoma in about three hundred patients in 35 clinical centres across Europe. Should results be positive, this would potentially be the first non-invasive treatment for this disease. In addition, so far, the natural cause of CRVO and NVG is still not known in greater enough detail and STRONG will also provide important data on this issue in a very large and well-

investigated cohort of patients. By analysing patient

data, the time frame of the disease and the optimization of treatment time points are also an objective and are going to be of value to patients in the future. As the detection with today's clinical standards of the disease is often deferred, there is a need for more sensitive methods. This could be possible with innovative detection methods, e.g. serum-biomarkers.

By analysing patient's samples, it may be possible to detect biomarkers for the earlier stagesof the disease as well as detection of patients who are more likely to develop neovascular glaucoma.

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 (POSURDEX)

EudraCT n. 2004-004996-12

Sponsor: Allergan

Principal Investigator: João Figueira

2. 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 (RETAIN)

EudraCT n. 2010-019795-74

Sponsor: Novartis

Principal Investigator: João Figueira

3. A Multicenter, Open-label, Randomized Study Comparing the Efficacy and Safety of 700 µg Dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS) to Ranibizumab in Patients with Diabetic Macular Edema (POSURDEX)

EudraCT n. 2011-005631-20

Sponsor: Allergan

Principal Investigator: João Figueira

Age-Related Macular Degeneration

4. A multicenter, patient-masked, safety extension study to evaluate the biodegradation of the brimonidine tartrate posterior segment drug delivery system (Brimo 33)

EudraCT n. 2010-019079-32

Sponsor: Allergan

Principal Investigator: Eduardo Silva

5. The safety and efficacy of AL-8309B ophthalmic solution for the treatment of geographic atrophy (GA) secondary to age-related macular degeneration (AMD) (C-08-36)

EudraCT n. 2008-007706-37

Sponsor: Alcon

Principal Investigator: Rufino Silva

6. Investigational Observational Portuguese Project with Lucentis in Age Macular Degeneration (AMD) among 50 ophthalmologic Centers for 12 months (PICO)

Sponsor: Novartis

Principal Investigator: Rufino Silva

7. Safety and Efficacy Study of ESBA1008 versus LUCENTIS. for the Treatment of Exudative Age-Related Macular Degeneration (SEE)

EudraCT n. 2011-000536-28

Sponsor: Alcon

Principal Investigator: Rufino Silva

8. Study to observe the effectiveness and safety of ranibizumab through individualized patient treatment and associated outcomes (Luminous)

ClinicalTrials.gov n. NCT01318941

Sponsor: Novartis

Principal Investigator: José Cunha-Vaz

9. A Retrospective non-interventional study to assess the effectiveness of existing antivascular endothelial growth factor (anti-VEGF) treatment regimens in patients with wet age-related macular degeneration (Review)

Clinical Trials.gov n. NCT01447043

Sponsor: Bayer

Principal Investigator: Rufino Silva

Glaucoma

10. 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 (N.201051)

EudraCT n. 2010-022984-36

Sponsor: Santen

Principal Investigator: Luísa Ribeiro

11. 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 (C-10-40)

EudraCT n. 2010-024512-34

Sponsor: Alcon

Principal Investigator: Luísa Ribeiro

12. Prospective, Non-Intervencional, Longitudinal Cohort Study to evaluate the long-term safety of XALATAN. Treatment in Pediatric Populations (Xalatan Pediatrico)

Sponsor: Pfizer

Principal Investigator: Pedro Faria

Retinal Vein Occlusion

13. A 24-month, phase IIIb, open-label, randomized, active-controlled, 3-arm, multicenter study assessing the efficacy and safety of an individualized, stabilization-criteria-driven PRN dosing regimen with 0,5mg ranibizumab intravitreal injections applied as monotheraphy or with adjunctive laser photocoagulation in comparison to laser photocoagulation in patients with visual impairment due to macular edema secondary to branch vein occlusion (BRVO)

EudraCT n. 2011-002859-34

Sponsor: Novartis

Principal Investigator: Rufino Silva

14. A 24-month, phase IIIb, open-label, single arm, multicenter study assessing the efficacy and safety of an individualized, stabilization criteria-driven PRN dosing regimen with 0.5- mg ranibizumab intravitreal injections applied as monotherapy in patients with visual impairment due to macular edema secondary to central retinal vein occlusion (CRVO)

EudraCT n. 2011-002350-31

Sponsor: Novartis

Principal Investigator: Rufino Silva

Other

15. Randomized, open label multi-center study comparing cabazitaxel at 25 mg/m2 in combination with prednisone every 3 weeks to Docexatel in combination with prednisone in patients with metastatic castration resistant prostate cancer not pre-treated with chemotherapy (Firstana)

EudraCT n. 2010-022064-12

Sponsor:Sanofi

Principal Investigator: Luisa Ribeiro

16. 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 (Ivabradina)

EudraCT n. 2006-005475-17

Sponsor: Servier

Principal Investigator: Luisa Ribeiro

17. 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 (FTY 720 2399)

EudraCT n. 2010-020515-37

Sponsor: Novartis

Principal Investigator: Luisa Ribeiro

Neurological Disorders

18. 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 n. 2007-003035-22

Sponsor: Acadia

Principal Investigator: Luísa Cunha

19. Pharmacokinetic assessment of ceftriaxone (Betasporina®) or clavulanic acid or ceftriaxone plus clavulanic acid administered by the endovenous route

EudraCT n. 2011-005089-39

Sponsor: Laborat.rios Atral

Principal Investigator: Carlos Fontes Ribeiro

20. Efficacy and safety of Eslicarbazepine acetate (BIA 2-093) as monotheraphy for patients with newly diagnosed partial-onset seizures: a double- blind, double-dummy, randomized, active-controlled, parallel- group, multicenter clinical study

EudraCT n. 2009-011135-13

Sponsor: Bial

Principal Investigator: Francisco Sales

21. Safety and efficacy of eslicarbazepine acetate (ESL) as adjunctive therapy for partial

seizures in elderly patients.

EudraCT n. 2009-012587-14

Sponsor: Bial

Principal Investigator: Luís Cunha

22. 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

23. A multinational, multicenter, randomized, double-blind, parallel-group, placebocontrolled 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

24. 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

25. 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

26. A Phase 3, 40-Week, Active-Controlled, Double-Blind, Double Dummy Extension Study of Preladenant in Subjects with Moderate to Severe Parkinson's Disease.

EudraCT n. 2009-015162-57

Sponsor: Schering-Plough

Principal Investigator: Luís Cunha

27. Efficacy and safety of 3 doses of S 38093 (2, 5 and 20 mg/day) versus placebo in patients with mild to moderate Alzheimer's disease. A 24-week international, multi-centre, randomised, double blind, placebo-controlled phase IIb study followed by a 24-week extension period.

EudraCT n. 2010-024626-37

Sponsor: Servier

Principal Investigator: Luís Cunha

28. A multicenter, double-blind, double-dummy, follow-up study evaluating the long-term safety of lacosamide (200 to 600mg/day) in comparison with carbamazepine (400 to 1200mg/day), used as monotherapy in subjects with partial-onset or generalized tonicclonic seizures ≥16 years of age coming from the SP0993 study.

EudraCT n. 2010-021238-74

Sponsor: UCB

Principal Investigator: Luís Cunha

29. Efficacy and safety of 3 doses of S 38093 (2, 5 and 20 mg/day) versus placebo, in coadministration with donepezil (10 mg/day) in patients with moderate Alzheimer's Disease.A 24-week international, multi-centre, randomised, double-blind, placebocontrolled phase IIb study.

EudraCT n. 2011-005862-40

Sponsor: Servier

Principal Investigator: António Freire

30. 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

Multinational Studies

1. ClinicalTrials.gov nº NCT01173614

Project Gullstrand - European Project for the Determination of Average Biometric Values of Human Eyes.

Protocol n. ECR-COR-2010-01

Coordinating Investigator: Jos Rozema

Participating Centres (13): Alicante, Antwerp, Barcelona, Chieti, Coimbra, Crete, Girona,

Leipzig, Mainz, Milan, Rome, Tel Aviv, Valencia.

Support: EVICR.net

4C Services: Coordination.

2. ClinicalTrials.gov nº NCT01145599

Identifying progression of retinal disease in eyes with NPDR in diabetes type 2 using noninvasive procedures

Protocol n. ECR-RET-2010-02

Coordinating Investigator: José Cunha-Vaz

Participating Centres (19): Amsterdam, Antwerp,

Barcelona, Bonn, Coimbra, Glostrup,

Leipzig, Lisbon, London (2), Milan, Padova, Paris (3), Val.ncia, Rome, Rotherdam, Surrey.

Support: EVICR.net

4C Services: Protocol design, coordination, monitoring, data management and statistical

analysis/final report.

3. EudraCT nº 2012-001200-38 / ClinicalTrials.gov nº NCT01726075

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: European Union 7th Framework Programme – Call Health 2011 - Project n. 278040-2

4C Services: Protocol design, coordination, data management and statistical analysis/final report.

4. STRONG - European Consortium for the Study of a Topical Treatment of Neovascular

Glaucoma

Project Coordinator: Norbert Pfeiffer

Participating Centres (4): Coimbra, Koln, London, Mainz.

Financial Support: European Union 7th Framework Programme – Call Health 2012 - Project n. 305321

4C Services: Coordination.

5. ClinicalTrials.gov nº NCT01607190

Biomarkers of Diabetic Retinopathy Progression

Project Coordinator: José Cunha-Vaz Participating Centres (2): Coimbra, Hyderabad (India)

Financial Support: Champalimaud Foundation

4C Services: Protocol design, coordination, data management and publication.

6. ClinicalTrials.gov nº NCT01771081

POLARIS - A Prospective non-interventional study to assess the effectiveness of existing antivascuLar endothelial growth factor (Anti-VEGF) treatment regimens in patients with diabetic macular edema (DME) with central involvement

Project Coordinator: Cecilia Martinho / Sandrina Nunes

Participating Centres (27): Alicante, Amiens, Barcelona (5), Berlin, Coimbra, Creteil, Dijon, Giessen, Girona, Hamburg, Leipzig, Lisbon, Milano (3), Mainz, Munich, Padova, Paris (2), Rome, Tuebingen, Udine.

Sponsor: Bayer

4C Services: Coordination.

7. EUR-USH - European young investigators network for Usher syndrome

Project Coordinator: Kerstin Nagel-Wolfrum

Participating Centres (5): Coimbra, Paris, Mainz Montpellier, Nijmegen.

Financial Support: Project n. 12-058 – European Union 7th Framework Programme – Call E-RARE 2

4C Services: Protocol design and dissemination.

8. PROTEUS - Prospective, randomized, multicenter, open label, phase II / III study to assess

efficacy and safety of ranibizumab 0.5 mg intravitreal injections plus panretinal

photocoagulation (PRP) versus PRP in monotherapy in the treatment of subjects with

high risk proliferative diabetic retinopathy

Project Coordinator: José CunhaVaz / Sandrina Nunes

Participating Centres (12): Alicante, Cheltenham, Coimbra, Dijon, Liverpool, London, Milan, Padova, Paris, Porto, Rome, Valencia.

Financial Support: Novartis

4C Services: Protocol design, study development, coordination, data management, statistical analysis, final report.

9. ClinicalTrials.gov nº NCT01745263

VitaminD3 – Omega3 – Home Exercise HeALTHy Ageing and Longevity Trial (DO-HEALTH)

Principal Investigator: Heike A. Biscchoff-Ferrari

Financial Support: European Union 7th Framework Programme – Call Health 2011 - Project n.

278588-2

4C Services: IMP management.

National Studies - IDCTs

1. ClinicalTrials.gov nº NCT01298674

Epidemiological study of the prevalence of Age-Related Macular Degeneration in Portugal

Coordinating Investigator: Rufino Silva

Grant: Novartis

4C Services: Protocol design, coordination, data management and statistical analysis/final

report.

2. ClinicalTrials.gov nº NCT01715870

Life style and food habits questionnaire in the Portuguese population aged 55 or more

Coordinating Investigator: Rufino Silva

Grant: Novartis

4C Services: Protocol design, coordination, data management and statistical analysis/final

report.

3. ClinicalTrials.gov nº NCT01281098; EudraCT nº 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

Grant: Pfizer

4C Services: Protocol design, study submission, coordination and monitoring, data

management and statistical analysis/final report.

4. ClinicalTrials.gov nº NCT01280929

Prospective, randomized, multicenter, open label phase II study to access 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.

5. 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 n. 4C-2011-01

Principal Investigator: Luísa Ribeiro

Financial Support: Project n. 13853 – DoIT – Agência de Inovação, Portugal

4C Services: Protocol design, study submission, coordination and monitoring, data

management and statistical analysis/final report.

6. Pharmacokinetic assessment of ceftriaxone (Betasporina) or clavulanic acid or ceftriaxone plus clavulanic acid administered by the endovenous route

Protocol n. ATRAL/CFC/273/11

Principal Investigator: Carlos Fontes Ribeiro

Sponsor: Atral

4C Services: Monitoring.

FUNDING

In 2012 funding of "Laboratório Associado – Centro de Neurociências e Biologia Celular" reached the amount of 10.320.377,39€.

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 5.568.806,48€ distribuited as follows:

Strategical Project (PEst-C/SAU/LA0001/2011)	2.369.968,79€
Projects	2.603.501,13€
Science Program	561.115,32€
Doctoral Program	34.221,24€

The related items supported the main expenses of Center for Neuroscience and Cell Biology during 2012.

Besides Center for Neuroscience is financed by other national and international agencies. In 2012 Center for Neuroscience received the amount of 163.400,12€ concerning other national projects, and 707.686,43€ concerning international projects. Funding of CNC-Biotech ascended 3.806.658,52€.

FCT ongoing projects as well as other national and international projects are listed in the following table.

The amount of other remaining funds, which are not listed ascends to a value of 73.825,84€.

ONGOING PROJECTS

Title	Financing Agency	Duration	Budget (CNC)	Expenditure 2012
National Projects:				
"Rede Nacional de Ressonância Magnética Nuclear." Coordinator: Carlos Geraldes	FCT Refª:REDE/1517/RMN/2005	01/01/2010 to 31/12/2013	216.528,43	58.044,46€
"Caracterização de alterações genéticas em gliomas humanos por arrays de polimorfismos de necleótido único (SNP): correlação com as características clínicas e biológicas e citogenéticas da doença." Coordinator: Catarina de Oliveira	FCT Refª: PIC/IC/83108/2007	05/01/2009 to 04/10/2013	150.440,00	36.488,64€
"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 Refª: PTDC/SAU-NEU/108668/2008	18/03/2010 to 29/02/2012	100.000,00	3.963,52€
"Micro e nano design de materiais com funcionalidades especificas para promover a regeneração de tecido ósseo usando células estaminais adultas." Coordinator: João Nuno Moreira Proponent: Universidade do Minho	FCT Refª:MIT/ECE/0047/2009	01/06/2010 To 30/11/2013	32.880,00	18.532,65€
"Benefícios do controlo metabólico precoce: prevenção da formação de memória hiperglicémica através da estimulação da bioenergética." Coordinator: Carlos Palmeira	FCT Refª: PTDC/QUI-BIQ/103514/2008	01/03/2010 to 31/03/2013	126.667,00	39.019,20€
"O Neuropeptídeo Y (NPY) e a dipeptidil-peptidase IV (DPPIV) como novos alvos terapêuticos na regulação do tecido adipose na obesidade." Coordinator: Joana Salgado	FCT Refª: PTDC/SAU-FCF/102415/2008	05/02/2010 to 04/09/2012	96.184,00	38.025,98€
"NPwhY - Inervação e angiogénese para o benefício da osteogénese: envolvimento do NPY na regeneração óssea." Coordinator: João Malva Proponent: Instituto de Engenharia Biomédica -INEB	FCT Refª: PTDC/SAU-OSM/101469/2008	05/02/2010 to 31/07/2013	9.000,00	190,31€
"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." Coordinator: Leonor de Almeida	FCT Refª: PTDC/SAU-OSM/102907/2008	01/05/2010 to 31/10/2013	122.336,00	24.167,34€
"Vida e morte das células ganglionares da retina: neuromodulação e neuroprotecção pelo Neuropeptídeo Y." Coordinator: Francisco Ambrósio Proponent: Faculdade de Medicina da Universidade de Coimbra	FCT Refª: PTDC/SAU-NEU/099075/2008	01/04/2010 to 30/09/2013	51.897,00	7.270,94€
"A restrição calórica aumenta a esperança de vida: papel do neuropeptídeo Y na autofagia." Coordinator: Cláudia Cavadas	FCT Refª: PTDC/SAU-FCF/099082/2008	01/04/2010 to 15/07/2013	153.150,00	47.428,59€
"Efeito da cafeína e dos receptores da adenosina A2A na resposta ao stress: papel da regulação da supra-renal." Coordinator: Cláudia Cavadas	FCT Refª: PTDC/SAU-NEU/108110/2008	01/04/2010 to 15/07/2013	90.000,00	11.268,65€
"A Abertura da Caixa Pandora Para uma Terapia Activa Anti-cancro da Mama - O Papel do Direccionamento Selectivo da Mitocôndria." Coordinator: Paulo Oliveira Participants: Faculdade de Farmácia da Universidade de Coimbra	FCT Refª: PTDC/QUI-QUI/101409/2008	01/04/2010 to 31/03/2013	170.976,00	30.702,63€
"Impacto da metanfetamina na barreira hemato-encefálica: estudo dos mecanismos envolvidos e do papel de neuroinflamação." Coordinator: Ana Paula Silva Proponent: Faculdade de Medicina da Universidade de Coimbra	FCT Refª: PTDC/SAU-FCF/098685/2008	01/04/2010 to 30/09/2013	68.490,00	9.915,19€
"Papel da Comunicação intercelular entre células endoteliais e celulas estaminais neurais na "stemness" e a neurogénese: novos alvos terapeuticos para a reparação cerebral." Coordinator: Fabienne Agasse	FCT Refª: PTDC/SAU-NEU/101783/2008	01/04/2010 to 30/06/2013	86.000,00	10.635,70€

"São os Fitoestrogénios Aditivos "Alimentares Seguros e Eficazes para Mulheres em Menopausa? Uma Aproximação In Vitro e In Vivo para este Problema." Coordinator: Mª Sancha Santos	FCT Refª: PTDC/AGR-ALI/108326/2008	01/04/2010 to 30/09/2013	168.716,00	62.820,04€
"Mecanismos moleculares de insuficiência cardíaca: o papel do adipócito como orgão endócrino." Coordinator: Daniel Espinoza	FCT Refª: PTDC/SAU-OSM/104124/2008	22/03/2010 to 30/12/2013	191.757,00	22.986,10€
"Análise do proteome do hipocampo de ratinhos expostos a medicação psicotrópica." Coordinator: Bruno Manadas	FCT Refª: PTDC/SAU-NEU/103728/2008	15/03/2010 to 31/07/2013	120.000,00	32.082,57€
"Design de sensores químicos e biossensores compósitos para a monitorização em tempo-real e em simultâneo de óxido nítrico e oxigénio in vivo no cérebro." Coordinator: Rui Barbosa Participants: Faculdade de Farmácia da Universidade de Coimbra	FCT Refª: PTDC/SAU-BEB/103228/2008	01/05/2010 to 31/10/2013	50.800,00	12.728,01€
"Caracterização dos princípios de design de circuitos metabólicos prevalentes." Coordinator: Armindo Salvador Participants: Universidade de Coimbra; Universidade do Minho	FCT Refª: PTDC/QUI-BIQ/119657/2010	01/04/2012 to 31/03/2015	117.226,00	24.988,63€
"Terapia génica Não invasiva e Não viral da doença de Machado-Joseph" Coordinator: Luis Almeida	FCT Refª: PTDC/SAU-FAR/116535/2010	01/04/2012 to 31/03/2015	108.280,00	19.548,41€
"Estudo do mecanismo patogénico da Doença de Machado-Joseph num novo modelo de células estaminais pluripotentes induzidas." Coordinator: Luis Almeida	FCT Refª: PTDC/SAU-NMC/116512/2010	24/01/2012 to 23/01/2015	145.360,00	62.135,75€
"Avaliação Neuropsicológica e Investigação Bigenómica nas Demência Frontotemporal." Coordinator: Maria Manuela Grazina	FCT Refª: PTDC/SAU-EPI/121811/2010	01/01/2012 to 31/12/2014	199.699,00	45.187,38€
"Impacto da terapia com exendina-4 nos mecanismos moleculares subjacentes à disfunção cerebral associada à diabetes tipo 2 a longo prazo." Coordinator: Ana Isabel Duarte	FCT Refª: PTDC/SAU-TOX/117481/2010	01/05/2012 to 30/04/2014	144.305,00	47.162,09€
"Papel da proteína p66Shc na Persistência de Danos Mitocondriais Induzidos por Fármacos." Coordinator: Ignacio Vega Naredo	FCT Refª: PTDC/SAU-TOX/117912/2010	01/03/2012 to 28/02/2014	79.291,00	26.936,89€
"TranstirRetina é uma metaloprotease: possíveis implicações em doenças do sistem nervoso." Coordinator: Sukalian Chaterjee Proponent: Instituto de Biologia Molecular e Celular (IBMC)	FCT Refª: PTDC/SAU-ORG/118863/2010	01/05/2012 to 30/04/2015	56.152,00	0,00€
"DEMTEST: Diagnóstico de demencias rapidamente progressivas baseado em biomarcadores - optimização de protocolos de diagnóstico." Coordinator: Catarina Oliveira	FCT Refª: JPND/0001/2011	01/06/2012 to 31/05/2015	35.000,00	4.935,98€
"O Metabolismo enquanto modelador da pluripotência e diferenciação de células estaminais." Coordinator: João Ramalho	FCT Refª: PTDC/EBB-EBI/101114/2008	15/04/2010 to 14/10/2013	147.656,00	44.541,55€
"Derivados de Benzazolo Marcados com Fluor - 18 e Tecnécio - 99m para visualização In Vivo de depósitos de Amilóide." Coordinator: Catarina Oliveira Proponent: Instituto Técnológico e Nuclear (ITN) Participants: Faculdade de Medicina da Universidade de Coimbra; Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/QUI-QUI/102049/2008	01/01/2010 to 30/06/2013	4.800,00	0,00€
"Planctomyces - uma linhagem filogenéticamente 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 31/10/2013	189.624,00	54.858,06€
"Análise dos mecanismos moleculares que determinam disfunção da alfa-sinucleína e a citoxicidade na doença de Parkinson - o papel do GDNF." Coordinator: Ana Cristina Rego Participants: ; Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/SAU-NEU/101928/2008	05/02/2010to 31/07/2013	134.400,00	43.280,11€

"Optimização da utilização de hidratos de carbono em robalo de aquacultura através de perfis metabólicos." Coordinator: John Jones Participants: FCTUC	FCT Refª: PTDC/EBB-BIO/098111/2008	01/04/2010 to 30/09/2013	175.292,00	43.810,54€
"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 31/10/2013	195.000,00	79.920,61
"Mapeamento do papel metabólico e neuromodulador da insulina no hipocampo." Coordinator: Attila Kõfalvi	FCT Refª: PTDC/SAU-OSM/105663/2008	17/03/2010 to 31/07/2012	100.000,00	20.710,71€
"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 Kõfalvi	FCT Refª: PTDC/SAU-NEU/100729/2008	17/03/2010 to 16/06/2012	91.000,00	20.499,13€
"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 02/11/2013	122.562,00	19.705,30€
"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	42.453,11€
"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	33.108,36€
"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 Proponent: IMAR- Instituto do MAR	FCT Refª: PTDC/SAU-OSM/104731/2008	01/05/2010 to 30/10/2013	65.200,00	9.721,55€
"Alimentos Fucncionais para Neuroprotecção: um papel para o Hypericum perforatum." Coordinator: João Malva Proponent: Universidade Minho (UM) Participants: Instituto de Ciências Biomédicas Abel Salazar (ICBAS/UP); Universidade Católica Portuguesa (UCP)	FCT Refª: PTDC/AGR-ALI/105169/2008	01/05/2010 to 31/10/2013	6.000,00	622,94€
"Skingineering - 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 31/03/2013	44.748,00	23.752,74€
"Análise sistemática de proteínas Rab na fagocitose e na maturação do fagossoma do Mycobacterium tuberculosis." Coordinator: Maria Otilia Vieira Participants: Instituto de Biologia Molecular e Celular - IBMC/UP	FCT Refª: PTDC/BIA-BCM/112138/2009	01/01/2011 to 31/12/2013	171.993,00	53.208,23€
"Actividade Protectora da SIRT3 na Disfunção Mitocondrial Induzida por Fármacos." Coordinator: Paulo Oliveira	FCT Refª: PTDC/SAU-TOX/110952/2009	01/03/2011 to 28/02/2014	128.800,00	43.031,95€
"A enigmática maltocinase de micobactérias." Coordinator: Nuno Empadinhas	FCT Refª: PTDC/BIA-BCM/112459/2009	01/04/2011 to 30/09/2013	113.058,00	60.997,88€
"Transporte entre células da alfa-sinucleina na doença de Parkinson. O factor de progressão?" Coordinator: Manuel Garrido	FCT Refª: PTDC/SAU-NMC/109955/2009	01/04/2011 to 31/03/2014	144.738,00	38.845,72€
"Uma nova formulação de nanopartículas para aplicação de terapia génica em tumores sólidos." Coordinator: Henrique Faneca	FCT Refª: PTDC/QUI-BIQ/116080/2009	01/04/2011 to 31/03/2014	94.000,00	18.679,89€

"Simugrowth-Desenvolvimento de um modelo computacional para a simulação das propriedades biomecânicas de cartilagem desenvolvida in-vitro em função do estimulo mecânico em bioreactor." Coordinator: Alexandrina Mendes Proponent: Universidade de Aveiro Participants: Universidade do Minho (UM)	FCT Refª: PTDC/EME-TME/113039/2009	03/04/2011 to 02/10/2013	28.830,00	3.587,23€
"O papel do intestino no desenvolvimento da esteatose hepatica induzida pela frutose." Coordinator: John Jones	FCT Refª: PTDC/SAU-MET/111398/2009	01/07/2011 to 30/06/2014	139.476,00	36.910,10€
"Nitrato:nitrito:óxido nítrico: uma via crítica que suporta o impacto benéfico do vinho e do azeite na fisiologia gastrointestinal e cardiovascular." Coordinator: João Laranjinha	FCT Refª: PTDC/AGR-ALI/115744/2009	01/03/2011 to 28/02/2014	142.474,00	1.831,72€
"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." Coordinator: Ricardo Das Neves	FCT Refª: PTDC/SAU-ENB/113696/2009	01/04/2011 to 31/03/2014	135.649,00	55.332,10€
"Targets - TARgeted GEne Therapy Strategies to treat nerve injury." Coordinator: Sérgio Paulo de Magalhães Simões Proponent: INEB Participants: Instituto de Biologia Molecular e Celular - IBMC/UP; ADFC/FC/UP	FCT Refª: PTDC/CPM-NAN/115124/2009	01/04/2011 to 31/03/2014	3.060,00	0,00€
"O papel da adenosina e do receptor A2A na resposta imunitária a Candida albicans." Coordinator: Teresa Maria Gonçalves	FCT Refª: PTDC/SAU-MIC/115598/2009	01/06/2011 to 31/05/2013	49.832,00	21.886,46€
"Regulação do sistema ubiquitina-proteassoma pelo BDNF nas sinapses do hipocampo: importância na plasticidade sináptica." Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-NMC/120144/2010	10/02/2012 to 09/02/2015	154.678,00	51.712,08€
"Fibrilas Interrompidas: Inibição de interacções aberrantes proteína-proteína em Amilóides." Coordinator: Rui Brito	FCT Refª: PTDC/QUI-QUI/122900/2010	01/03/2012 to 28/02/2015	113.768,00	17.285,14€
"Nova Abordagem na Luta Contra a Tuberculose." Coordinator: Maria Otília Vieira	FCT Refª: HMSP-ICT/0024/2010	01/01/2012 to 31/12/2014	206.610,00	58.278,61€
"Libertação de neuropeptídeos em feridas: uma nova terapêutica para o tratamento do pé diabético." Coordinator: Ermelindo Leal	FCT Refª: PTDC/SAU-FAR/121109/2010	01/04/2012 to 31/03/2014	106.872,00	21.804,88€
"Contribuição para a erradicação da malária. Uma nova abordagem para atingir multi-alvos no ciclo de vida do parasita." Coordinator: Luísa Melo Proponent: Faculade de Farmácia da Universidade de Coimbra; Participants: Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/SAU-FAR/118459/2010	01/03/2013 to 28/02/2015	5.500,00	635,40€
"O Óxido Nítrico na Doença de Alzheimer - Molécula Sinalizadora e Mediador de Patogénese." Coordinator: Ana Ledo	FCT Refª: PTDC/BIA-BCM/116576/2010	01/04/2012 to 31/03/2015	81.698,00	17.416,72€
"Desenvolvimento de nanoparticulas multifuncionais inovadoras para o tratamento do cancro de mama." Coordinator: João Nuno Moreira Proponent: Universidade do Minho	FCT Refª: PTDC/SAU-DMA/121028/2010	20/04/2012 to 19/04/2015	76.857,00	201,63€
"Proteases de Polens, relevância nas doenças alérgicas." Coordinator: Paula Verissimo	FCT Refª: PTDC/SAU-ESA/72571/2006	01/05/2008 to 22/05/2012	199.850,00	20.997,27€
"Papel do ATP e dos seus receptores P2Y1 na neuroprotecção." Coordinator: Rodrigo Cunha	FCT Refª: PTDC/SAU-FCF/100423/2008	18/03/2010 to 17/09/2012	189.697,00	43.957,30€
"Acoplamento neurovascular entre a actividade neuronal e o fluxo sanguineo no encéfalo mediado pelo óxido nítrico." Coordinator: João Laranjinha	FCT Refª: PTDC/SAU-NEU/108992/2008	01/05/2010 to 31/10/2013	100.000,00	15.300,17€
"Perfis dinâmicos do óxido nítrico no cérebro: regulação da respiração celular com implicações para a doença de Alzeheimer e para o envelhecimento." Coordinator: João Laranjinha	FCT Refª: PTDC/SAU-NEU/103538/2008	01/06/2010 to 30/06/2013	100.000,00	24.236,44€

"HotMetal-Estratégias de resistência a metais pesados e disseminação de resistências a antibióticos nas fontes marinhas hidrotermais." Coordinator: Milton Costa Proponant: IMAR-Instituto do Mar	FCT Refª: PTDC/MAR/109057/2008	01/06/2010 to 30/11/2013	9.000,00	0,00€
"Análise das alterações da transcrição em modelos cerebrais e periféricos da doença de Huntington - influência da modulação das desacetilases das histonas." Coordinator: Ana Cristina Rego	FCT Refª: PTDC/SAU-FCF/108056/2008	05/02/2010 to 04/08/2013	199.999,00	75.056,34€
"Papel da proteólise da ataxina-3 mediada por calpaínas na doença de Machado-Joseph: terapia molecular com vectores virais." Coordinator: Luis de Almeida	FCT Refª: PTDC/SAU-NEU/099307/2008	05/02/2010 to 30/04/2013	107.000,00	39.920,36€
"Genes FKS, CHS e de sintese de melanina em Alternaria infectoria: a combinação para o oportunismo." Coordinator: Teresa Gonçalves	FCT Refª: PTDC/SAU-ESA/108636/2008	01/03/2010 to 31/08/2012	100.000,00	19.871,40€
"Papel da Fisiologia Mitocondrial na Resistência das Células Estaminais Tumorais à Quimioterapia." Coordinator: Paulo Oliveira	FCT Refª: PTDC/QUI-BIQ/101052/2008	01/04/2010 to 30/06/2013	143.016,00	23.161,74€
"Biorstimul - Desenvolvimento e construção de um novo conceito de bioreactor para a caracterização biomecânica e bioquíma de tecidos de cartilagem desenvolvidos in-vitro." Coordinator: Celeste Lopes Proponent: Universidade Aveiro	FCT Refª: PTDC/EME-PME/103578/2008	16/03/2010 to 15/03/2013	41.600,00	10.968,38€
"Detecção do potencial sensibizante de quimicos através de um teste in vitro alternativo: uma imposição da nova legislação da União Europeia." Coordinator: Maria Rosete Participants: Universidade Aveiro	FCT Refª: PTDC/SAU-OSM/099762/2008	01/04/2010 to 30/09/2013	128.200,00	37.616,17€
"Mecanismos e propriedades anti-inflamatórias de plantas medicinais: investigação multidisciplinar para a sua validação e utilização como fonte de fitofármacos." Coordinator: Maria Rosete Proponent:: Universidade Coimbra Participants: Universidade Aveiro	FCT Refª: PTDC/SAU-FCF/105429/2008	01/05/2010 to 31/10/2013	55.800,00	16.208,90€
"Alteração do tráfego intracelular mediado pela mitocôndria na doença de Parkinson." Coordinator: Sandra Cardoso Participants: Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/SAU-NEU/102710/2008	05/02/2010 to 31/05/2013	102.600,00	26.884,01€
"Regeneração cardiaca com células vasculares embrionárias e uma matriz biomimética." Coordinator: Lino Ferreira	FCT Refª: PTDC/SAU-BEB/098468/2008	01/04/2010 to 30/06/2013	180.000,00	59.188,25€
"Nanomateriais para detecção de células." Coordinator: Lino Ferreira Participants: Biocant-Associação de transferência de Tecnologia	FCT Refª: PTDC/CTM/099659/2008	01/04/2010 to 30/06/2013	76.000,00	21.988,16€
"Mecanismos responsáveis pelos efeitos do óxido nitrico na proliferação de células estaminais neurais após lesão cerebral." Coordinator: Caetana Carvalho	FCT Refª: PTDC/SAU-NEU/102612/2008	01/04/2010 to 30/09/2013	120.000,00	13.345,98€
"O papel da tradução localizada de mRNAna formação da junção neuromuscular." Coordinator: Ramiro Almeida	FCT Refª: PTDC/SAU-NEU/104100/2008	01/05/2010 to 31/08/2013	120.000,00	26.228,65€
"Mecanismos Moleculares do Tráfego Sináptico de Receptores do Glutamato do Tipo NMDA." Coordinator: Ana Luísa Carvalho	FCT Refª: PTDC/SAU-NEU/099440/2008	15/09/2010 to 14/09/2013	164.424,00	32.559,31€
"Regulação das proteínas hnRNP pela neurotrofina BDNF: importância da plasticidade sináptica." Coordinador: Carlos Duarte	FCT Refª: PTDC/SAU-NEU/104297/2008	15/09/2010 to 14/09/2013	120.000,00	34.069,59€
"Parametrização do metabolismo e crescimento tumorais através da análise de fluxos metabólicos e engenharia metabólica." Coordinador: Rui Carvalho	FCT Refª: PTDC/EBB-EBI/115810/2009	01/01/2011 to 31/12/2013	169.578,00	27.417,85€
"Localização e metabolismo da APP e relação com o controlo da degeneração sináptica em modelos animais da doença de Alzheimer." Coordinador: Catarina Oliveira	FCT Refª: PTDC/SAU-NMC/114810/2009	23/08/2011 to 22/08/2013	89.493,00	16.092,57€

"Caracterização da interacção Proteína - Carbohidrato da Laforina - Poteína humana envolvida na Doença de Lafora." Coordinador: Carlos Geraldes Proponent: Biocant	FCT Refª: PTDC/BIA-PRO/111141/2009	01/03/2011 to 28/02/2014	31.140,00	4.058,58€
"Regulação por fosforilação da ataxina-3, a proteína mutada na Doença de Machado Joseph." Coordinador: Ana Luísa Carvalho Participants: UM; IBMC	FCT Refª: PTDC/SAU-NMC/110602/2009	01/01/2011 to 31/12/2013	123.777,00	24.316,43€
"Regulação da estabilidade do RNA mensageiro para a subunidade GluR1 dos receptores do glutamato." Coordinador: Ana Luísa Carvalho	FCT Refª: PTDC/BIA-BCM/113738/2009	01/04/2011 to 30/06/2013	108.001,00	45.656,87€
"Pré-condicionamento via mitocôndria:potencial efeito neuroprotector na doença Alzheimer." Coordinador: Paula Moreira	FCT Refª: PTDC/SAU-NMC/110990/2009	03/01/2011 to 31/08/2013	93.735,00	20.925,55€
"Via para a síntese do MGLP de micobactérias. Caracterização bioquímica e estrutural das enzimas envolvidas." Coordinador: Nuno Empadinhas Participants: IBMC	FCT Refª: PTDC/BIA-PRO/110523/2009	01/01/2011 to 31/12/2013	130.624,00	35.642,39€
"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	31.586,14€
"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	12.031,64€
"iCALP - Identificação das funções fisiológicas das calpaínas no controlo da proliferação e migração celulares no sistema nervoso central." Coordinador: Inês Araujo	FCT Refª: PTDC/SAU-NMC/112183/2009	01/03/2011 to 28/02/2014	142.560,00	18.019,84€
Programa MIT Coordinator: Catarina Oliveira, Lino Ferreira	FCT Refª: MIT-Portugal 2011/2012	01/09/2006 to 31/12/2012	89.100,00	48.561,65€
"Novas estratégias para a recuperação da fertilidade e potencial genético de felídeos selvagens: desenvolvimento do xenotransplante e da transplantação de células espermatogoniais estaminais em gato doméstico como modelo para felídeos selvagens." Coordinator: Paula Mota	FCT Refª: PTDC/CVT/119477/2010	01/05/2012 to 30/04/2015	62.813,00	8.382,80€
"Modulação da actividade de células estaminais hematopoiéticas por acção de nanopartículas capazes de libertar factores de transcrição – STEMCELLMODULATORS." Coordinator: Ricardo Pires das Neves	FCT Refª: PTDC/CTM-NAN/120552/2010	01/05/2012 to 30/04/2015	115.884,00	22.254,15€
"Modulação da piruvato desidrogenase cinase e pluripotência: Implicações para cancro e biologia de células estaminais." Coordinator: João Ramalho	FCT Refª: PTDC/QUI-BIQ/120652/2010	06/05/2012 to 05/05/2015	130.000,00	23.795,68€
"Produção e propagação de linhas de células estaminais pluripotentes usando modulação metabólica." Coordinator: João Ramalho	FCT Refª: PTDC/EBB-EBI/120634/2010	06/05/2012 to 05/05/2015	94.000,00	17.713,74€
"Papel fisio-patológico da ecto-5'-nucleotidase - um novo alvo para neuroprotecção." Coordinator: Rodrigo Cunha	FCT Refª: PTDC/SAU-TOX/122005/2010	01/05/2012 to 31/08/2014	147.605,00	28.721,49€
"Papel do accumbens e amigdala no controlo da neuropatologia causada por stress crónico." Coordinator:Rodrigo Cunha	FCT Refª: PTDC/SAU-NSC/122254/2010	01/04/2012 to 30/09/2014	148.080,00	35.084,63€
"BIOMARKAPD: Biomarcadores para Doença de Alzheimer e Doença de Parkinson." Coordinator:Catarina Oliveira	FCT Refª: JPND/0005/2011	01/06/2012 to 31/05/2015	48.500,00	5.884,61€
			Sub – Total FCT	**2.603.501,13€**

Other National Projects				
"Ibercivis.pt - Uma plataforma de computação voluntária para a Península Ibérica." Coordinator: Rui Manuel Pontes M. F. Brito	UMIC - Agência para a Sociedade do Conhecimento	16/06/2010 to 15/06/2013	87.380,00	23.262,89€
"Quero mais e melhores células! (células estaminais: o que são? Onde estão? Para que servem?)" Coordinator: Cláudia Cavadas	Ciência Viva – Agência Nacional para a Cultura Científica e Tecnológica	01/11/2011 to 31/01/2013	83.040,00	33.487,06€
DoIT – projeto nº 013853	Agência da Inovação, S.A.	01/07/2010 to 30/08/2014	378.154,38	94.993,51€
QREN-Amilotera: 021622 Coordinator: Rui Brito	Agência da Inovação, S.A	01/09/2011 to 31/08/2014	85.804.45	11.656,66€
			Sub – Total Other	**163.400,12€**
			Total National Projects	**2.766.901,25€**
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 1/05/2012	45.000,00	0,00€
"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	24.450,14€
"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	42.902,79€
"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 28/02/2015	202.332,86	36.743,03€
Novel nanoparticles for drug delivery to the skin Coordinator: Lino Ferreira	Queen Mary – 289454 Ref.ª: FP7-PEOPLE-2011-ITN	01/11/2011 to 31/10/2015	471.627,60	82.550,40€
"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	21.701,89€
"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	2.306,95€
"Award NIH_5R01DK029953-30" Coordinator: John Jones	NIH – 5Ro1DK029953-30	01/08/2011 to 31/07/2012	8.203,65	8.761,63€
DDZ – Research Collaboration Agreement Coordinator: John Jones	DDZ – Research Collaboration Agreement	01/11/2011 to 31/10/2012	14.309,00	12.766,69€
"Role of the autophagy-related protein Beclin-l in Machado-Joseph disease." Coordinator: Luís Pereira de Almeida	Association Française contre les Myopathies Ref.ª: SB/NF/2010/2008	30/10/2010 to 10/04/2013	110.000,00	44.512,42€
"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 30/11/2014	944.680,00	260.538,08€
"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	36.404,05€
"LRRK2 role on auto-antibody production by human B cells" Coordinator: Margarida Carneiro	Michael J. Fox Foundation for Parkinson's Research	01/11/2011 to 14/12/2012	103.009,00	64.458,71€
"DFRH/WIIA/51/2011 - Welcome II" Coordinator: Catarina Oliveira/Otília Vieira	DFRH/WIIA/51/2011 - Welcome II	01/02/2012 to 31/01/2014	119.740,50	54.611,27€
"Unravelling the early steps in the biosynthesis of the mycobacterial MGLP." Coordinator: Nuno Empadinhas	Mycobacterial MGLP	01/04/2012 to 31/03/2014	19.758,57	11.735,91€

"CAFFEIN-Cancer Associated Fibroblasts (CAF) Function in Tumor Expansion and Invasion". Coordinator: João Nuno Moreira	Marie Curie grant 316610 Refª FP7-People-2012-ITN	01/10/2012 to 30/09/2014	209.781,00	697,70€
"DDZ II - Research Collaboration Agreement". Coordinator: John Jones	DDZ II - Research Collaboration Agreement	01/11/2012 to 31/10/2013	14.112,00	1.898,72€
ERC-2012-StG 307384-NanoTrigger Coordinator:Lino Ferreira	ERC-2012-StG 307384-NanoTrigger	01/11/2012 to 30/10/2017	1.699.320,00	646,05€
		Total International Projects		**707.686,43€**
		TOTAL		**3.474.587,68€**

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 Sarmento 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 Monteiro de 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 A. Francisco	(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
Ângelo R. Tomé	(Assistant Prof., FCTUC)	60
António F. Ambrósio	(Assistant Inv., FMUC)	Collaborator
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	60
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	30
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Arsélio P. Carvalho	(Full Prof., FCTUC)	100
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Attila Köfalvi	(Assistant Inv., CNC)	100
Bruno Miguel Neves	(Assistant Prof., Univ. Aveiro)	50
Bruno José F. Manadas	(Assistant Inv., CNC)	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
Catarina R. Oliveira	(Full Prof., FMUC)	60
Célia M. Antunes	(Assistant Prof., FCTUC)	80
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	40

Daniela Cipestre Vaz	(Assistant Prof., Inst. Polit. Leiria)	100
Elsa Henriques	(Assistant Inv., CNC)	100
Emília P. Duarte	(Assistant Prof., FCTUC)	60
Euclides Pires	(Associate Prof., FCTUC)	80
Eugénia Carvalho	(Assistant Inv., CNC)	100
Faraj Barah	(Principal Inv., CNC)	100
Fernando Monteiro Judas	(Assistant Prof., FMUC)	25
Frances Lund	(Investigator, Rochester Univ, EUA)	Collaborator
Geanne Matos de Andrade	(Associate Prof., Brasil)	40
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 Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Araújo	(Assistant Prof., UALG)	20
Inês Esteves Baldeiras	(Investigator, FMUC)	35
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)	50
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
José Dionisio	(Assistant Prof., FFUC)	75
Leonor Almeida	(Full Prof., FFUC)	50
Lino Ferreira	(Assistant Inv., CNC)	100
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Luís M. Rosário	(Associate Prof., FCTUC)	60
Luís 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
Mª Carmen Alpoim	(Associate Prof., FCTUC)	60
Mª Celeste Lopes	(Full Prof., FFUC)	80

Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Dolores T. Redondo	(Investigator, Univ. Salamanca)	Collaborator
Mª do Rosário Almeida	(Assistant Inv., CNC)	100
Mª Emilia O. Quinta Ferreira	(Associate Prof., FCTUC)	60
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Mª Helena Ribeiro	(Investigator, FMUC)	20
Mª Isabel J. Santana	(Associate Prof., FMUC)	30
Mª Luisa D. Ramos	(Investigator, FCTUC)	80
Mª Luisa Sá e Melo	(Full Prof., FFUC)	60
Mª Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	20
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Mª Otilia Vieira	(Assistant Inv., CNC)	100
Mª Sancha Santos	(Investigator, FCTUC)	100
Mª Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
Mª 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)	60
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
Ricardo Rodrigues	(Assistant Inv., CNC)	100
Renata Silva	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	75
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)	60
Rui Pinto	(Assistant Prof., FMUC)	30
Rui Prediger	(Inv. Assistant Prof., FCTUC)	80

Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	40
Sandra Maria R. Carvalho Bós	(Assistant Inv., FMUC)	60
Sérgio Simões	(Assistant Prof., FFUC)	60
Sílvia 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	**Time % at CNC**
Alessandro Bolli	
Akhilesh Rai	100
Ana Isabel Duarte	100
Ana Luísa Cardoso	100
Ana Patricia Simões	100
Ana Raquel Esteves	100
Ana Rita Araújo Santos	50
Ana Teresa Simões	100
Ângela Inácio	100
Bharathi Pandurangan	100
Bruno Carreira	100
Cândida Gonçalves da Silva	100
Carla Nunes	100
Carolina Melo Souza	100
Catarina Alexandra Gomes	100
Catarina Miranda	100
Célia Aveleira	100
Chantal Fernandes	100
Clévio Nóbrega	100
Cristiana Paulo	100
Cristina Barosa	100
Daniel Espinoza	100
Daniela Pochmann	100
Elisabete Baptista Ferreiro	100
Ermelindo Leal	100
Francisca Eiriz	100
Giovannia Pereira	40
Helena Vazão	100

Igor Tiago	100
Ivan Viegas	100
Joana Ferreira	100
Joana Rosmaninho-Salgado	100
João Alves	100
João Fernando S. Carvalho	10
João Gonçalo Oliveira Frade	100
João Miguel Neves Duarte	100
João Pedro Lopes	100
João Reina Silva	10
Jorge Valero Gomez-Lobo	100
Licinia J. Simões	100
Lígia Maria S. Ferreira	100
Liliana Bernardino	30
Liliana Mendonça	100
Luis Miguel Estronca	100
Luisa Jordão	50
Mª Alexandra B. Amaral	100
Mª Isabel N. Ferreira	50
Mª Teresa Cunha Oliveira	100
Marco André Coelho das Neves	100
Marina Marques Pinto	20
Mário Laço	100
Marisa A. Rego Encarnação	100
Marta Filipa Lima	10
Marta Santos	100
Paula M. Canas	100
Paula Mota	100
Pedro Gonçalves	100
Raquel Ferreira	100
Ricardo Santos	100
Rita Perfeito	100
Rosa M. B. Matos Resende	100
Rui Nobre	100
Rui Oliveira Costa	100
Rui Pinto	100
Sandra Catarina G. Amaral	100
Sónia Duarte	100
Susana Guerreiro	100
Susana Isabel E. Alarico	100
Susana Ribeiro Louros	100
Susana Rosa	100
Tatiana Catarino	100

Tatiana R. Rosenstock	100
Teresa Delgado	100
Teresa Serafim	100
Vilma Sardão Oliveira	100
Vitor Mendes	100

PhD Students	**Time % at CNC**
Adalberto Alves de Castro	100
Ana Branco M. Tiago	100
Ana Cristina F. Lemos	100
Ana Cristina Gonçalves	40
Ana Cristina Gregório	100
Ana Carolina Moreira	100
Ana Cristina R. Silva	100
Ana Catarina Ferreira	50
Ana Catarina R. Graça Fonseca	100
Ana Santos Carvalho	100
Ana Filipa Branco	100
Ana Francisca Lima	100
Ana Inês R. Crespo	100
Ana Isabel Serralheiro	100
Ana Isabel Plácido Fernandes	100
Ana M. Metelo	100
Ana Mª Sequeira Cardoso	100
Ana Marisa Simões	100
Ana Patricia B Marques	100
Ana Patricia S. Gomes	100
Ana Sofia C. Valdeira	100
Ana Sofia Carvalho	100
Ana Sofia M. Leal	100
Ana Sofia V. Cunha	100
Ana Sofia Rodrigues	100
Ana Teresa I. Varela	100
Ana Teresa Rufino	100
André Ferreira Martins	100
André Filipe M. Soares	100
Ângela Fernandes	100
Ângela Pascoal Crespo	100
António Silva	100
Bárbara Rocha	100
Beatriz Lacerda de Sousa	100
Bruno Miguel F. Gonçalves	100

Carla Mª 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 Praça de Almeida	100
Catarina Sofia H. Jesus	50
Cátia Diogo	100
Cátia Moreira de Sousa	100
Carolina de Souza	100
Cláudia Maria C. Deus	100
Cláudia Sofia Alves Pereira	100
Cristina Carvalho	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
Diogo Comprido	100
Dominique Fernandes	100
Dulce Bento	100
Elisabete Oliveira Augusto	100
Eszter Szabó	100
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
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 Gutierres	100
Jimmy George	100
Joana Balça Silva	100
Joana Bicker	100
Joana Domingues Vindeirinho	100
Joana Filipa C. Fernandes	100
Joana Filipa D. Neves	100
Joana I. Real	100
Joana Liberal	50
Joana Marques	100
Joana Paixão	100
Joana Pedro	100
Joana Ribeiro Guedes	100
Joana Sousa	100
João Abrantes	100
João André Duarte	15
João Carlos Almeida	100
João Demétrio B. Martins	100
João Filipe Martins	10
João Correia Teixeira	100
João Teodoro	100
João Manuel Rito	100
João Trigueiro Costa	100
Jorge Manuel Ruivo	100
Josephine Blerch	100
Júlia Valente	100
Kátia Mesquita	100
Liane Moura	100
Ligia Gomes da Silva	100
Luana Naia	100
Ludgero C. Tavares	100

Luís André A. França	100
Luís Ribeiro	100
Luís Rodrigues	100
Lisa Rodrigues	90
Magda Santana	100
Marcelo Correia	100
Márcio José C. Ribeiro	100
Márcio Rodrigues	100
Marco António P. Matos	100
Mª Inês Morte	100
Mª Joana G. Pinto	100
Mª João Rodrigues Pereira	50
Mª la Salete J. Baptista	100
Mariana Botelho da Rocha	100
Mariana Freitas	100
Mariana Oliveira Conceição	100
Mariana Ponte C. Ribeiro	100
Marília Henriques Cordeiro	100
Mariline Silva	100
Marta Daniela Passadouro Caetano	100
Marta Isabel D. Mota Vieira	100
Marta Isabel Rodrigues Baptista	100
Marta Pereira	100
Marta Regina Santos do Carmo	100
Michela Comune	100
Michele Curcio	100
Miguel António Caetano	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
Nuno Silva	100
Patrícia Henriques Domingues	100
Patrícia Lopes	100
Patrícia Raquel Pereira	100
Paulo Gameiro Guerreiro	100
Paulo Magalhães	100
Pedro Alves	50
Pedro Alexandre Martins	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 Santos Tavares	100
Raquel Trindade	100
Renato Xavier C. Santos	100
Ricardo Romão Leão	25
Rodrigo Luiz Santos	100
Roksana Pirzgalska	100
Rui Benfeitas Vicente	100
Rui M. Costa Soares	5
Rui Cruz	100
Sandra Figueiredo	100
Sandra Isabel F. Mota	100
Sandra Cristina Jesus	100
Sandra M. Almeida Santos	100
Sandro Pereira	100
Samira C. Ferreira	100
Sara Raquel Oliveira	100
Sara Rute C. Figueiredo	100
Sara Tavares M. Lima	100
Sezin Aday	100
Sílvia Viana Silva	60
Sofia Marques Ribeiro	100
Sofia Romano	100
Sónia Correia	100
Susana Cardoso	100
Susana Patrícia S. Pereira	100
Swarna Pandian	100
Tiago Alexandre Sousa Santos	100
Tiago Alfaro	100
Udaya Geetha Vijayakumar	100
Vanessa Isabel S. Mendes	100
Vera Lúcia G. Francisco	100
Vitor Manuel Carmona	100
Zaida Almeida	75

MSc Students	Time % at CNC
Ana Filipa Cruz	100
David Bowman	100
Dina Farinha	100
Isabel Ferreira	100
Ivan Lalanda Salazar	100
Luís Filipe Martins	100
Joni Leeuwen	100
Mª Inês R. Sousa	100
Mariana Val	100
Rodolfo Águas	100
Tânia Jesus Leandro	100
Tânia Marisa Perestrelo	100

Grant Technicians	Time % at CNC
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
Andreia Lamaroso	100
Branca M. Silva	100
Caroline Delgado Veloso	100
Elda Bonifácio	100
Emanuel Candeias	100
José Miguel J. Paiva	100
Liliana Freitas Antunes	100
Mafalda Matos	100
Marcelo Francisco R. Rodrigues	100
Pedro Joaquim Cruz	15
Pedro Tiago C. Curto	100
Ricardo Jorge Pais	25
Rute Marisa A. Loureiro	100
Sara Monteiro Lopes	100
Sofia Isabel Oliveira Sousa	100
Sónia Neto R. Pereira	100
Vanessa R. Anjos	100
Vera Patricia Gonçalves	50

MD	Time % at CNC
António Macedo	Collaborator
Cristina Januário	Collaborator
Diogo Branco	Collaborator
Hermínio José T. Espírito Santo	30
Maria Isabel Santana	30
João André Freitas	Collaborator
Luís Cunha	Collaborator
Luísa Diogo	Collaborator
Mª Margarida Martins Gonçalo	40
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 Veríssimo	(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
Mª 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)	20

TECHNICAL STAFF

		Time % at CNC
Cármen Lídia Graça Semião	(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, CNC)	100
Isabel Dantas Fernandes	(Graduate Technician, CNC)	100
Luisa Leitão Cortes	(PhD, Graduate Technician, CNC)	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, CNC)	100
Sara da Costa Jordão A. Lopes	(Technician, CNC)	100
Sandra Freire	(Technician, CNC)	100
Telma Patrícia Simões Graça	(Technician, CNC)	100
Vera Oliveira	(Graduate 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 Luís	(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 AREA

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)	Collaborator
Ana Rita Costa Álvaro	(Inv. Assistant Prof., UTAD)	60
Ângelo Tomé	(Assistant Prof., FCTUC)	60
António F. Ambrósio	(Investigator, FMUC)	Collaborator
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	30
Arsélio P. Carvalho	(Full Prof., FCTUC)	100
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)	60
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	40
Emília P. Duarte	(Assistant Prof., FCTUC)	60
Geanne Matos de Andrade	(Associate Prof., Brasil)	40
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)	35
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	30
João O. Malva	(Principal Inv., FMUC)	50
João Peça-Silvestre	(Post-Doc Fellow, MIT)	35
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Margarida Vaz Caldeira	(Inv. Assistant Prof., FCTUC)	80
Mª do Rosário Almeida	(Assistant Inv., CNC)	100
Mª Isabel J. Santana	(Associate Prof., FMUC)	30
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Mª 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

Ricardo Rodrigues	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	75
Rui Prediger	(Assistant Prof., FMUC)	80
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Investigator, FMUC)	60

Post-Doc Members	**Time % at CNC**
Ana Isabel Duarte	100
Ana Patricia Simões	100
Ana Raquel Esteves	100
Ana Rita Araújo Santos	50
Bruno Carreira	100
Carolina Melo Souza	100
Catarina Alexandra Gomes	100
Célia Aveleira	100
Daniela Pochmann	100
Elisabete Baptista Ferreiro	100
Francisca Eiriz	100
Joana Ferreira	100
Joana Rosmaninho-Salgado	100
João Pedro Lopes	100
Jorge Valero Gomez-Lobo	100
Liliana Bernardino	30
Mª Teresa Cunha Oliveira	100
Marina Marques Pinto	20
Mário Laço	100
Paula M. Canas	100
Rita Perfeito	100
Rosa M. B. Matos Resende	100
Rui Oliveira Costa	100
Susana Ribeiro Louros	100
Tatiana Catarino	100
Susana Ribeiro Louros	100
Tatiana Catarino	100
Tatiana R. Rosenstock	100

PhD Students	**Time % at CNC**
Ana Catarina Fonseca	100
Ana Catarina Oliveira	100
Ana Cristina F Lemos	100
Ana Cristina Silva	100
Ana Isabel Fernandes	100

Ana Patricia Marques	100
Ana S. Carvalho	100
António M. Silva	100
António Manuel C da Silva	100
Carla Maria Nunes Lopes	100
Carlos Adriano A. Matos	100
Cristina Carvalho	100
Daniel Rial	100
Daniel Santos	100
Diana FF Silva	100
Diogo O. Comprido	100
Dominique Fernandes	100
Elisabete O. Augusto	100
Emanuel Candeias	100
Eszter Szabó	100
Francisco Manuel Q Gonçalves	100
Gabriel Costa	100
Graciano Leal	100
Helena Mª Carvalheiro	100
Ivan Salazar	100
Joana F. C. Fernandes	100
Joana Isabel E B Real	100
Joana Medeiros V Marques	100
Joana Pedro	100
Joana Vindeirinho	100
João T. Costa	100
Luana Carvalho Naia	100
Luís Ribeiro	100
Magda Santana	100
Márcio Ribeiro	100
Marco António P. Matos	100
Maria Inês Morte	100
Mariana Botelho Rocha	100
Mariline Silva	100
Marta Dias M. Vieira	100
Marta Regina Santos do Carmo	100
Michele Curcio	100
Miranda Mele	100
Nélio da Mota Gonçalves	100
Pedro João Afonso	100
Pedro Manuel V. Garção	100
Renato Xavier Santos	100
Rui Gonçalo Cruz	100
Samira C. Ferreira	100
Sandra Mota	100
Sara Oliveira	100
Sílvia Viana da Silva	100
Susana Cardoso	100
Tiago Manuel P. Alfaro	100

MSc Students	Time % at CNC
Ana Margarida Oliveira	100
Ana xavier	100
Andreia Ângelo	100
Anna Pliássova	100
Gladys Caldeira	100
Gonçalo P Cristovão	100
Joana Rdrigues	100
Joana Gomes	100
Joni Leeuwen	100
Luís Martins	100
Luís Rodrigues	100
Maria Joana Pinto	100
Milene Gonçalves	100
Mónica Teresa Abreu	100
Paula Pereira	100
Paulo André Santos	100
Pedro Alves	100
Rodolfo Águas	100
Rui Beleza	100
Tiago Silva	100
Tiago Sousa	

Undergraduate Students	Time % at CNC
Sofia Sousa	100
Susana Cardoso	100

Grant Technicians	Time % at CNC
Caroline Veloso	100

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
Ana Simões	(Assistant Inv., CNC)	100
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
Cristiana Paulo	(Assistant Inv., CNC)	100
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
Helena Vazão	(Assistant Inv., CNC)	100
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
Mª Amália Jurado	(Assistant Prof., FCTUC)	80
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Luísa Sá e Melo	(Full Prof., FFUC)	60
Mª Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Marília Rocha	(Investigator, HUC)	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
Samuel Silvestre	(Assistant Inv., CNC)	100
Sérgio Simões	(Assistant Prof., FFUC)	80
Sónia Duarte	(Assistant Inv., CNC)	100
Tiago Quininha Faria	(Assistant Inv., CNC)	100
Vera Lúcia Dantas Moura	(Manager Science & Tech., UC)	50

Post-Doc Members	**Time % at CNC**
Alessandro Boli	100
Akhilesh Rai	10
Ana Luísa Cardoso	100
Bruno Manadas	100
Bharathi Pandurangan	100
Cândida Gonçalves da Silva	100
Catarina Miranda	100
Clévio Nóbrega	100
João Fernando S. Carvalho	10

João Reina Silva	10
Lígia Maria S. Ferreira	100
Liliana Mendonça	100
Mª Isabel N. Ferreira	50
Marco André Coelho das Neves	100
Marta Filipa Lima	10
Rui Nobre	100
Susana Rosa	100

PhD Students	**Time % at CNC**
Ana Cristina Gregório	100
Ana Isabel Serralheiro	100
Ana Maria Cardoso	100
Ana Sofia C. Valdeira	100
André Filipe M. Soares	100
Ângela Valério-Fernandes	100
Bruno Miguel F. Gonçalves	100
Carlos Samuel M. Boto	100
Carlos José Vieira Simões	25
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
Filipa Lebre	100
Graciana Tribuna	50
Inês Vasconcelos Miranda Santos	100
Isabel Maria Santos Onofre	100
Ivana Kostic	100
João Abrantes	100
Joana Bicker	100
Joana Filipa Neves	100
Joana Ribeiro Guedes	100
Joana Sousa	100
Ligia Gomes da Silva	100
Márcio Rodrigues	100
Mª 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
Sónia Patricia Duarte	100
Udaya Geetha Vijayakumar	100
Vitor Carmona	100

MSc Students	**Time % at CNC**
Ana Cristina V. Ferreira	100
Angelo Serani	100
Carlos Custódio	100
Catarina Jesus	100
Catarina Rebelo	100
Cláudia Saraiva	100
Gabriela Leão	100
Joana Sousa	100
Mariana Magalhães	100
Patricia Rosado	100
Raquel Trindade	100
Ricardo Gaspar	100
Ricardo Silva	100
Sara Matias C. Silva	100

Grant Technicians	**Time % at CNC**
Ana Rita M. Leal	100
Ana Sofia L. Coelho	100
David Bowman	10
Dina Farinha	100
José Paiva	100

Liliana Freitas Antunes	100
Mafalda Santos	100
Pedro Branco	15
Pedro Coelho	100
Ricardo Jorge Pais	25
Sandra Pinto	100
Vanessa Rebelo Anjos	100

Cell and Molecular Toxicology

Rui Carvalho, 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
Mª 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	**Time % at CNC**
Ana Teresa Varela	100
Carla Nunes	100
João Gonçalo Oliveira Frade	100
João Miguel Neves Duarte	100
Vilma Sardão Oliveira	100

PhD Students	**Time % at CNC**
Ana Carolina Moreira	100
Ana Filipe Branco	100
Ana Francisca Soares	100
Ana Maria Silva	100
Ana Patricia S. Gomes	100
Bárbara Rocha	100

Carlos Rodrigues	100
Cassilda Pereira	100
Cátia Diogo	100
Cláudia Sofia Alves Pereira	100
Diana Jurado S. Serra	100
Filipa Libório Carvalho	100
Filomena Grilo da Silva	100
Gonçalo Pereira	100
Inês Biscaia Barbosa	100
Joana Paixão	100
Kátia Almeida Mesquita	100
Ludgero Tavares	100
Marcelo Rodrigues	100
Mariana Ponte Cardoso Ribeiro	100
Miguel Caetano	100
Nuno Ferreira	100
Nuno Gabriel Machado	100
Paulo Gameiro Guerreiro	100
Sandro Pereira	100
Sofia Marques Ribeiro	100
Sónia Pereira	100
Susana Pereira	100

MSc Students	**Time % at CNC**
Mariana Val	100
João Fonseca	100

Grant Technicians	**Time % at CNC**
Ana Cristina Lemos	100
Joana Mª Ramalho A. Sousa	100
Ludgero Canário Tavares	100
Manuel Joaquim G. Matos	100
Rute Loureiro	100
Susana Pereira	100

Microbiology

Milton Costa, PhD, Coordinator

Members holding PhD		**Time % at CNC**
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	60
Joana Cardoso Costa	(Inv. Assistant Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	60
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Teresa Gonçalves	(Assistant Prof., FMUC)	50

Post-Doc Members	**Time % at CNC**
Chantal Fernandes	100
Igor Tiago	100
Joana Cardoso da Costa	100
Susana Isabel E. Alarico	100
Vitor Mendes	

PhD Students	**Time % at CNC**
Ana Catarina Ferreira	100
Ana Luísa N. Gomes Nobre	100
Ana Maranha	100
Ana Sofia V. Cunha	100
Carolina Coelho	50
Igor Clemente Tiago	100
Lisa Rodrigues	100
Luis André A. França	100
Rui Soares	100

MSc Students	**Time % at CNC**
Andreia Lamaroso	100
Diogo Reis	100
Mafalda Costa	100
Mariana Almeida	100
Marta Mota	100
Tomé Cardoso	100

Grant Technicians	**Time % at CNC**
Branca Silva	20
Diana dos Santos Mota	10
Nelson Alexandre C. Cunha	10
Tânea Leandro	100
Ana Filipa dÁvó	100

Biophysics and Biomedical NMR

Carlos Geraldes, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Carlos G. Geraldes	(Full Prof., FCTUC)	80
Ivana Jarak	(Assistant Inv., CNC)	100
John Griffith Jones	(Principal Inv., CNC)	100
Luís M. Rosário	(Associate Prof., FCTUC)	60
Manuela Carvalheiro	(MD, HUC)	15
Mª Luisa D. Ramos	(Investigator, FCTUC)	80
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	80
Rosa M. Santos	(Assistant Prof., FCTUC)	60

Post-Doc Members	**Time % at CNC**
Cristina Barosa	100
Giovannia Araujo de Lima Pereira	40
Ivan Viegas	100
Licinia J. Simões	100

PhD Students	**Time % at CNC**
André Martins	100
Fátima Martins	100
Filipe Coreta Gomes	40
Helena Leitão	100
Inês Violante	10
Joana I. Real	100
João André Duarte	25
João Teixeira	100
Sara Figueiredo	100

MSc Students	**Time % at CNC**
Ana Marguerita Metelo	100
Cristiano Santos	100
David Gaspar Dias	100
Margarida Coelho	100
Paulo da Silva	60

Grant Technicians	**Time % at CNC**
Ana Rita Gonçalves	100
Filipa Simões	100
João Rito	100

Cell and Development Biology

Mª Celeste Lopes, PhD, João Ramalho Santos, PhD, Coordinators

Members holding PhD		**Time % at CNC**
Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Ana Bela Sarmento 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
João Ramalho Santos	(Associate Prof., FCTUC)	80
José Alberto Correia e Vale	(MD, Univ. Salamanca)	Collaborator
Manuel Aureliano Alves	(Associate Prof., Univ. Algarve)	Collaborator
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Dolores T. Redondo	(Investigator, Univ. Salamanca)	Collaborator
Mª Otilia Vieira	(Assistant Inv., CNC)	100
Mª 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
Sílvia Sousa Neves	Assistant Prof., FMUC)	40
Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Maria C. Martins	(Assistant Inv., IPO)	80

Post-Doc Members	**Time % at CNC**
Ana Luísa Vital	100
Ângela Inácio	100
Daniel Spinoza	100
Ermelindo Leal	100
Luis Miguel Estronca	100
Luisa Jordão	50
Mª Alexandra B. Amaral	100
Marisa Rego	100
Marta Santos	100
Michelle Viegas	100
Paula Mota	100

Pedro Gonçalves	100
Sandra Catarina G. Amaral	100

PhD Students	**Time % at CNC**
Ana Cristina Gonçalves	40
Ana Inês R. Crespo	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ângela Pascoal Crespo	100
Beatriz Lacerda de Sousa	100
Carla Patrícia R. Paiva	100
Carlos Manuel Melo	100
Diana Margarida Carvalho	100
Diana Dinis Azenha	100
Humberto Gomes Ferreira	100
Joana Balça Silva	100
Joana Liberal	50
João Demétrio B. Martins	100

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