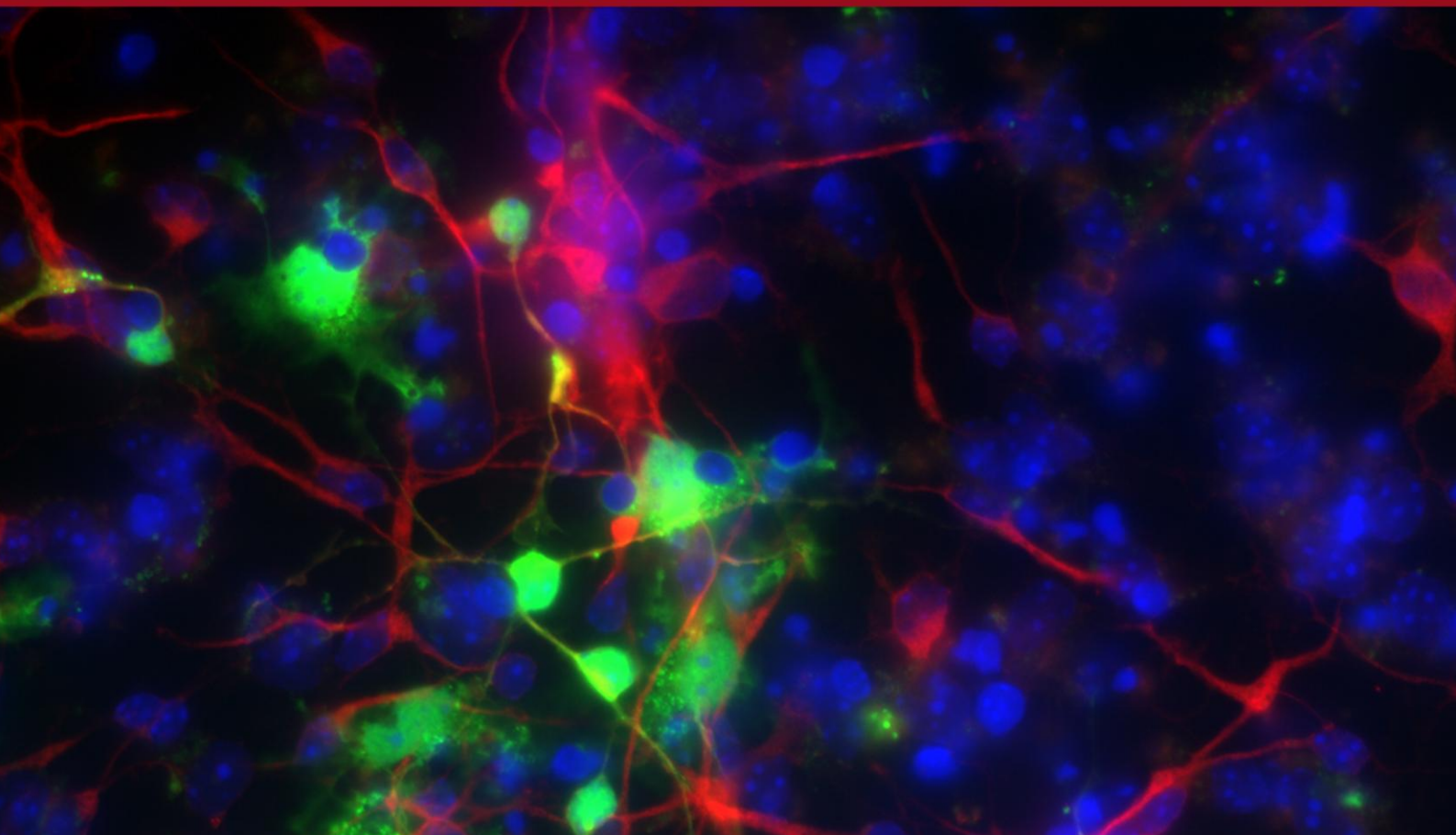
Center for Neuroscience and Cell Biology

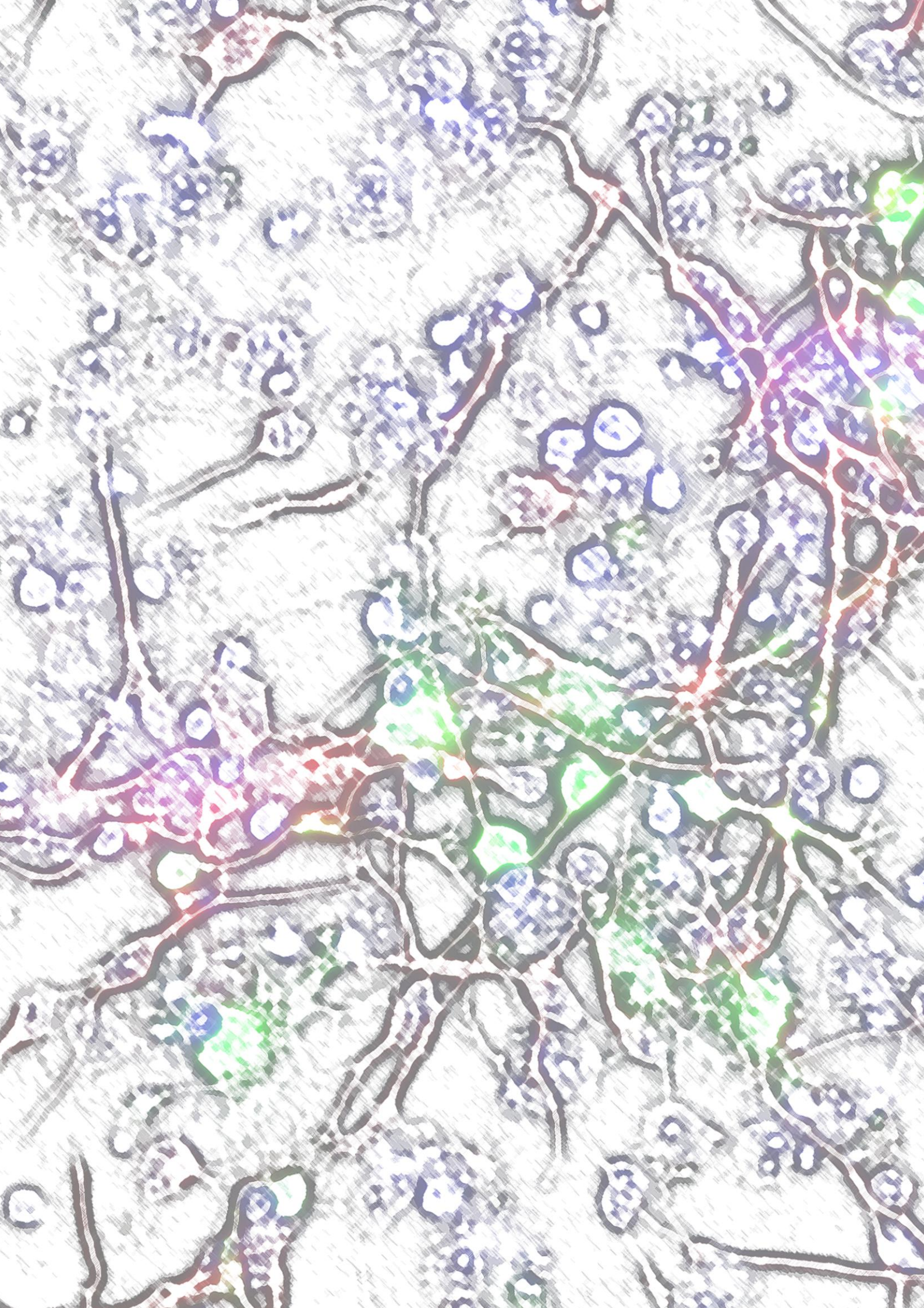
ASSOCIATE LABORATORY

Experimental Biology and Biomedicine | A new culture through Scientific Research

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

Annual Report | 2010





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Introduction
General Objectives

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

The scientific productivity of CNC in 2010 is demonstrated by 227 publications (56 in press), an effort supported by 96 grant projects (87 FCT Projects, 2 national projects, 7 international projects).

In 2010 CNC also supported the training of 186 PhD Students, (18 PhD Thesis concluded) and 62 MSc Students (24 MSc Thesis concluded).

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

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

Graduated training includes a Doctoral Programme in Experimental Biology and Biomedicine and the participation in the MIT-Portugal Doctoral Program. Since 2009 CNC is a partner of the European Neuroscience Campus Network, which offers an uniform PhD training in Neuroscience. CNC integrates international networks such as the European Excellence Neuroscience Institutes Network (ENI-NET), the MIT-Portugal and Harvard Medical School-Portugal Programs, and is a founder of Health Cluster Portugal (HCP).

CNC major mission is to foster fundamental and translational research and training in biomedical science with a particular focus on neurosciences.

Facts & Figures

2010

RESEARCH STAFF

Members holding Ph.D.	148
Ph.D.Students	186
MSc Students	62
Grant Technicians	25

Scientific papers published	171
Scientific papers published *In Press*	56

THESIS CONCLUDED

Ph.D. thesis	18
MSc thesis	24

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%), Hospitais da Universidade de Coimbra, Fundação para a Ciência e Tecnologia, AIBILI, Fundação Bissaya Barreto and two comercial firms – Reagente 5 and ILC.

1- Governing Body

President:	*Catarina Resende de Oliveira*
Vice Presidents:	*Euclides Pires*
	Carlos Faro
	Leonor Almeida
***Honorary President**:*	*Arsélio Pato de Carvalho*
Executive Council	Directors of the Departments
Research Council	CNC members holding PhD
"Conselho Fiscal"	T. Macedo, A. Rodrigues, Leal e Carreira
"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).

2- Scientific Areas and Research Groups

At present, research programmes and projects are organized in 6 scientific areas, each coordinated by a senior scientist. The programme for each area is implemented by small research groups each headed by a research leader in his field of study. In 20010, 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: Claudia Pereira*)

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

Molecular Biotechnology and Health | *Euclides Pires*

Molecular Biotechnology Group (*Head: Carlos Faro)*

Molecular Systems Biology Group (*Head: Armindo Salvador)*

Structural and Computational Biology Group (*Head: Rui Brito*)

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

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

Pharmacometrics Group (*Head: Amilcar Falcão*)

Biorganic and Medicinal Chemistry Group (*Head: Mª Luisa Sá e Melo*)

Cell and Molecular Toxicology | *Leonor Almeida*

Mitochondrial Toxicology and Disease Group (*Head: Paulo Oliveira*)

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)*

Insulin, Resistance and Adipocite Group (*Head: Eugénia Carvalho)*

Emerging Group

Chronic Inflammation Group (*Head: Margarida Carneiro)*

Neuroscience and Disease Area

Coordinator: Catarina Resende Oliveira

The core research activity of this area is focused on three main issues: 1. understanding synapses formation and modulation; 2. deciphering cellular and molecular mechanisms underlying selective neurodegeneration; 3. development of neuroprotective and neuroregenerative strategies. These aims have been accomplished by: analysing glutamatergic synaptic modification and mRNA translation in presynaptic differentiation; modulation of synaptic activity by purines, and control of endocannabinoids signalling; mitochondria dysfunction and inter-organelle cross-talk in neurodegeneration in Alzheimer's and Parkinson's diseases and diabetes; exploring transcription deregulation linked to mitochondria-driven apoptosis in Huntington's disease; studying excitotoxic neuronal damage and the expression of neurotrophic factors upon neuronal injury; studying the hypothalamic-adrenal axis and adipose tissue on healthy lifespan and endogenous factors promoting neurogenesis from SVZ neural stem cells.

The mais achievments in this area can summarized:

- a proteomic screening for interactors of long-form AMPA receptor subunits identified novel proteins that bind to GluA1/GluA4-containing receptors. Presynaptic assembly requires axonal translation.

- Caffeine and adenosine A2A receptors revert memory impairment caused by early life convulsions. They regulate brain inflammation and control presynaptic cannabinoid system. Astrocytic CB1Rs control glucose uptake and metabolism and CB1R ligands normalize cortical glucoregulation.

- Aβ oligomers, induce ER stress in mature hippocampal cultures through NR2B subunits of NMDARs. Impaired ER Ca2+ homeostasis and enhanced ER stress was observed during AD progression. Diabetes induces mitochondrial abnormalities and an oxidative imbalance similar to those found in AD brains. Mitochondrial modulators protect brain endothelial and neuronal cells against high-glucose levels and promote a mild increase in reactive oxygen species levels. Mitochondrial alterations induced by $A\beta_{1-42}$ affect the microtubular network. Taxol, a microtubule stabilizer, ameliorated cell death induced by Aβ and reduced tau hyperphosphorylation.

- In HD, BDNF prevents detrimental changes in transcription factors and histone acetylation states in cortical neurons exposed to selective mitochondrial inhibition. FK506 protects neurons against apoptosis and necrosis under mild cell death stimulus in the presence of FL-mHtt.

- Excitotoxicity down-regulates full-length VGAT, with a concomitant generation of tVGAT, which is likely to affect GABAergic neurotransmission and may influence cell death during ischemia. Dopaminergic injury caused intense astrocyte proliferation in lesioned striatum and upregulation of $A_{2A}R$.

- Hypothalamic neurospheres express feeding-related neuropeptides, and differentiate to functional neurons that are affected in obesity. The retinal adenosinergic system is affected by diabetes/hyperglycemia. Gliptins inhibit basal and insulin-induced lipid accumulation and low levels of hypoxia induced adipogenesis.

- Neuronal differentiation in SVZ stem/progenitor cell cultures is promoted by soluble factors secreted by endothelial cells , growth hormone , NO and inflammatory molecules.

Neuromodulation Group

Rodrigo A. Cunha	PhD – *Head of group*
Ângelo José R.Tomé	PhD
Attila Köfalvi	PhD
Geanne M. Andrade	PhD
Henrique B. Silva	PhD
Lisiane O. Porciúncula	PhD
Paula G. Agostinho	PhD
Rui Prediger	PhD
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Catarina Alexandra Gomes	Post-Doc Fellow
Daniela Pochmann	Post-Doc Fellow
Manuella P. Kaster	Post-Doc Fellow
Pablo Pandolfo	Post-Doc Fellow
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Ana Paula Ardais	PhD Student
Ângela V. Fernandes	PhD Student
Carla Sofia G. Silva	PhD Student
Elisabete O. Augusto	PhD Student
Marco António P. Matos	PhD Student
Nuno Miguel J. Machado	PhD Student
Pedro Manuel V. Garção	PhD Student
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Glutamatergic Synapses Group

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Joana Ferreira	PhD Student
Luís Ribeiro	PhD Student
Maria Inês Coelho	PhD Student
Maria Joana Pinto	PhD Student
Sandra Sofia Rebelo	PhD Student
Susana Louros	PhD Student
Tatiana Catarino	PhD Student
Dominique Fernendes	MSc Student
Luís Pedro Leitão	MSc Student
Pedro Daniel Rio	MSc Student
Tânia Marisa Perestrelo	MSc Student
Joana Pedro	Grant Technician

Neuroprotection and Neurogenesis in Brain Repair Group

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Maria Franciaca Eiriz	PhD Student
Raquel Ferreira	PhD Student
Tiago Alexandre Santos	PhD Student

Neuronal Cell Death and Neuroprotection Group

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Emília P. Duarte	PhD
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Joana F. C. Fernandes	PhD Student
João T. Costa	PhD Student
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Miranda Mele	PhD Student
Diogo O. Comprido	MSc Student
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Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

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Mª João Rodrigues Ribeiro	PhD Student
Mário Laço	PhD Student
Rita Perfeito	PhD Student
Sandra Mota	PhD Student
Carla Maria Nunes Lopes	PhD Student
Carolina Noronha	MSc Student
Luis M. Bajouco	MSc Student
Sofia Isabel Sousa	MSc Student

Molecular Mechanisms of Disease Group

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Daniel Santos	MSc Student
Diana Raposo	MSc Student
Diogo Martins Branco	MSc Student
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Sílvia Catarina F. Gomes	MSc Student

Neuroendocrinology and Neurogenesis Group

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Paulo Santos	PhD
Caetana Carvalho	PhD
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João Filipe Martins	PhD Student
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Ana Sofia Lourenço	MSc Student
Fábia Sofia Vicente	MSc Student
Jorge Pascoal	MSc Student
Marta Maria Estrada	MSc Student
Vanessa Machado	MSc Student
Ana Patricia Marques	Grant Technician
Célia Aveleira	Grant Technician
Vera Raquel Cortez	Grant Technician

Neuromodulation Group | *Head: Rodrigo A. Cunha*

The group 'Purines at CNC' pursued the following main objectives in 2010:

Expand the wealth of brain disorders where the chronic consumption of moderate does of caffeine affords neuroprotection;

Consolidate the involvement of adenosine A2A receptors in the control of neurodegeneration and explore their underlying mechanisms of action;

Advance the hypothesis that synaptotoxicity is an initial key event and neuro-inflammation a main process responsible for amplification of neurodegeneration;

Define if extracellular ATP is a danger signal in brain disorders and if the manipulation of its receptors (P2 receptors) afford neuroprotection.

The Laboratory of Neuromo-dulation and Metabolism group has two main lines of research interest: Mapping the pre- and post-synaptic neuromodulation in brain areas involved in neurological and psychiatric disorders. Since the endo-cannabinoid system appears to be the major effect system of presynaptic neuromodulation, the CB1 cannabinoid receptor is the major target of our interest. The adenosinergic and mono-aminergic systems tightly control the endo-cannabinoid signalling and this control is impaired in all neuro-logical and psychiatric disorders. Thus, we also aim at disentangling the control on endocannabinoid signalling by several other neuromodulator systems.

Endocannabinoids are released in an activity-dependent manner in the brain, but also, the endocannabinoid system represents a major anabolic homeostatic regulator system in the body, controlling the release as well as the action of insulin and IGFs. Hence, our second line of investigation tackles this putative role of CB1Rs in cerebral gluco-regulation, and the impairment of such putative control in disease models and in neurological patients. We also explore the putative glucoregulator role insulin and IGFs in the brain since this question is largely unanswered.

The group 'Purines at CNC' completed the following achievements in 2010:

Editing a special issue of Journal of Alzheimer's Disease dedicated to the therapeutic prospects of caffeine in different brain disorders;

Showing that caffeine and adenosine A2A receptors are valid therapeutic options to revert memory impairment caused by early life convulsions;

Define the key enabling role of adenosine A2A receptors in triggering brain inflammation;

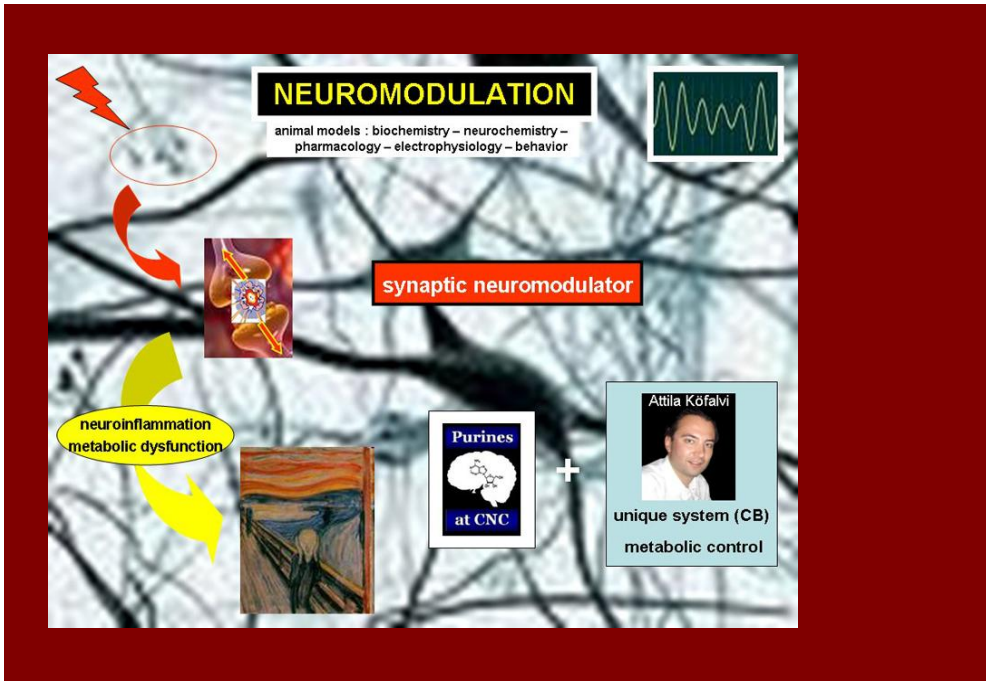
Provide the first evidence that adenosine A2A receptors are a main switch controlling other presynaptic inhibitory neuromodulation system.

The Laboratory of Neuromodulation and Metabolism group has pioneered several novel techniques in 2009 and 2010 in the area of immunochemistry and neuro-chemistry to answer our scientific questions which has been unanswered by previous attempts of others. As a first step, we had to characterize these novel tools to master them, allowing thus a rapid execution of each tasks under optimized condition.

We revealed that the A2A adenosine receptors co-localize with CB1 cannabinoid receptors in both VGLUT-1- and VGLUT-2-positive synapses in the striatum, as assessed by flow cytometry analysis of nerve terminals (a novel protocol invented and pioneered by us, allowing the rapid bulk assessment of the distribution and co-localization of synaptic markers), as well as with confocal microscopy. Furthermore, we found evidence for that post-synaptic A2ARs inhibit the release of endocannabinoids, while apparent presynaptic A2ARs inhibit the function of presynaptic CB1Rs in corticostriatal glutamatergic terminals (1 in press publication for 2011 and at least one more to submit). This fits in our major hypothesis that the adenosinergic system is an antagonistic modulator of the endocannabinoid system, and that ATP, co-released with glutamate under strong depolarization of the nerve terminal, degrades into adenosine in the synapse, and by antagonizing the endocannabinoid signalling, rescues the given terminal from being silenced. We also found evidence for the presence of functional CB1Rs in noradrenergic, serotonergic and glutamatergic terminals in the frontal cortex, where they inhibit the release of the neuro-transmitter/ neuromodulator in question.

As for the metabolic investigations, we are the second group which reveals functional CB1Rs in astrocytes. We found that these astrocytic CB1Rs can control glucose uptake and metabolism through the control of the GSK3alpha and the consequent glycogen synthesis. We used several techniques pioneered by us allowing the assessment of subregional differences in the uptake and metabolism of glucose. We discovered that beta-amyloid decreases the level of a major endocannabinoid molecule, anandamide, thereby impairs CB1R-mediated glucoregulation in the hippocampus, which is also a major feature of the disease in humans (1 submitted publication). Additionally, we found and we will report for the first time that type-1 diabetes induces early impairment in cortical glucose uptake and metabolism, and at later time-points, untreated type-1

diabetes maintains the impairment of the metabolism but not of the uptake of glucose (1 publication soon to be submitted). We found furthermore that CB1R ligands can normalize the early impairment in cortical glucoregulation. Indeed, this is accompanied with our novel observations that high concentrations of insulin (>30 nM) are capable of inducing large increases in the uptake and the metabolism of glucose, both in the cortex and in the hippocampus – under CB1R blockade. This is in accordance with novel publications of others showing that CB1R blockade facilitates while CB1R activation impairs insulin's action in peripheral tissues. We then also found that the presynaptic components of amino acid signalling in the hippocampus and the retina are affected by diabetes, although, these changes appeared subtle.



Glutamatergic Synapses Group | *Head: Ana L. Carvalho*

Our research group is interested in studying synapses, the contacts between neurons where communication occurs. We are interested in understanding how these connections are formed and modified during development, and in response to neuronal activity. Changes in the efficiency of synapses are the cellular correlate of learning and memory, and synaptic dysfunction is an initial event in many neurodegenerative diseases. We use a combination of primary neuronal cultures, molecular cell biology and biochemistry to study excitatory glutamatergic synapses. Below is a brief description of some of the ongoing projects in the laboratory.

Regulation of glutamatergic neurotransmission; PI: Ana Luísa Carvalho

Glutamate receptors of the AMPA type mediate the fast excitatory neurotransmission in the CNS, and play key roles in synaptic plasticity. A proteomic screening performed in our laboratory identified novel binding partners for AMPA receptors (Santos el al. J. Proteome Res 2010), whose function we are currently addressing. Additionally, we are interested in stargazin, a member of the transmembrane AMPA receptor regulatory protein (TARP) family, which interacts with AMPA receptor subunits to facilitate their targeting to synapses. Stargazin-mediated synaptic targeting of AMPA receptors requires the interaction of stargazin with PSD95, and phosphorylation of stargazin. Based on preliminary data from our laboratory we are testing the hypothesis that stargazin and its phosphorylation may play a role in synaptic scaling, a form of homeostatic synaptic plasticity whereby neurons calibrate synaptic activity by adjusting their overall synaptic strength up or down to compensate for excessive excitation or inhibition.

NMDA receptors are the Ca^{2+}-permeable ionotropic glutamate receptors that act as coincidence detectors in the induction of synaptic plasticity. There is much controversy in the field regarding the differential role of NMDA receptors composed of different subunits, both in what concerns their localization to synapses and their involvement in synaptic plasticity. In order to directly tackle this question, we are studying the mechanism of synaptic accumulation of NMDARs using neuronal cultures from knock-out mice for NMDAR subunits, and reintroducing 1) the individual splice variants for the GluN1 subunit; 2) mutated subunits of the NMDAR complex, to identify molecular determinants involved in NMDAR trafficking.

Appetite-regulating hormones such as leptin and ghrelin target the hypothalamus to mediate their effects, but have recently been described to affect hipopcampal-dependent memory processes. A collaborative study with our laboratory showed that leptin regulates AMPA receptor traffic in the hippocampus (Moult et al. J Neurosci 2010). We are currently interested in understanding how ghrelin, an appetite-stimulating hormone which was shown to enhance memory processes and synaptic plasticity in the hippocampus, regulates glutamatergic transmission.

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

It has been known for many years that axons are capable of "locally responding" to guidance cues but only now are the mechanisms responsible for these phenomena starting to be understood. Recent data has shown that local translation is required for other neurodevelopmental mechanisms like neuronal survival and axonal pathfinding. Also, the observation that distal axons have a diverse mRNA composition leads us to ask if local mRNA translation may play an important role in other neurodevelopmental processes like presynaptic differentiation.

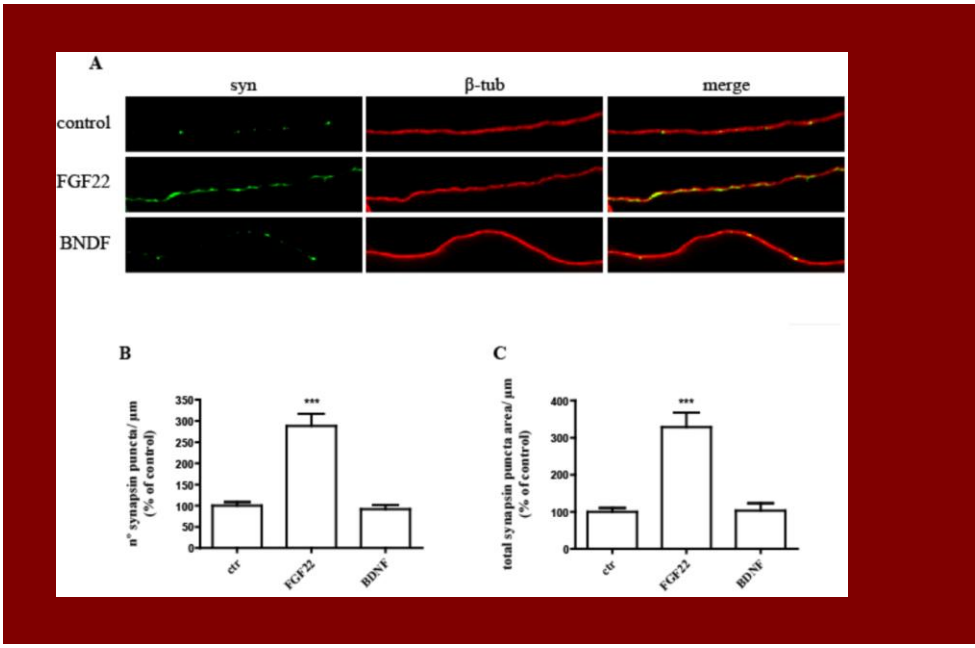
The first goal of our research was to establish the "hot spots" of axonal mRNA translation. For that purpose our objectives were to determine if local mRNA translation is required for presynaptogenesis and if local protein synthesis occurs at the sites of nascent synapses.

We are interested in the binding partners of AMPA receptor subunits, and in how they change the receptor function, since the regulation of AMPA receptor function and traffic is at the basis for long-lasting changes in synaptic strength that underlie higher brain functions such as learning and memory (Santos et al. Neuroscience 2009). We performed a proteomic screening for interactors of long-form AMPA receptor subunits (Santos et al. J Proteome Res 2010), and identified, along with known interactors, novel proteins that bind to GluA1/GluA4-containing receptors. We have focused our attention on the cell adhesion molecule Caspr1, which interacts with AMPA receptor subunits. This protein partially localizes to excitatory synapses, and its overexpression in hippocampal neurons promotes the synaptic localization of GluA1, whereas its

downregulation using shRNA decreases the synaptic clustering of GluA1-containing AMPA receptors. Co-expression of Caspr1 refractory to the shRNA molecules can rescue basal levels of synaptic AMPA receptors in hippocampal neurons in culture. Additionally, in an heterologous system, co-transfection of GluA1 with Caspr1 increases the amplitude of glutamate-evoked currents. These data suggest that Caspr1 regulates AMPA receptor function (Santos et al. in preparation).

Another goal of the research in the group is to understand the mechanisms of synaptic accumulation of NMDA receptors. NMDA receptors in hippocampal neurons are tetramers comprised of two GluN1 subunits combined with two GluN2A and/or GluN2B subunits. GluN1 is essential for channel function and for trafficking GluN2 to synapses, and is alternatively spliced. To address 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 we developed a lentiviral expression system to rescue expression of GluN1 in hippocampal cultures from GluN1-/- mice. Contrary to predictions in the literature, we found that GluN1 splice variants accumulate at the synapse at similar levels, all exhibit increased synaptic accumulation with chronic blockade of NMDA receptor activity, and all disperse to extrasynaptic membrane upon activation of PKC. These results indicate that the major mechanisms mediating homeostatic synaptic accumulation and PKC dispersal of NMDA receptors occur independently of GluN1 splice isoform (Ferreira et al. under revision in J. Biol. Chem.).

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. For that purpose we need to physically isolate axons from cell bodies. In order to achieve this objective, we made use of microfluidic chambers, small multi-compartment devices capable of creating physical and fluidical independent areas. We 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. Using a reporter system we are currently evaluating if mRNA translation occurs locally in the axons at pre-existing synaptic sites or, as an alternative, if induced presynaptogenesis requires the formation of new synaptic sites.



Local axonal application of FGF22 induces presynaptic assembly in hippocampal neurons cultured in microfluidic chambers.

Neuroprotection and Neurogenesis in Brain Repair Group | *Head: João O. Malva*

Project nº PTDC/SAU-NEU/101783/2008

The general aim of the project is to elucidate, at least in part, how the endothelial cells may influence stemness and neurogenesis from the neural stem cells of the subventricular zone (SVZ) neurogenic niche. During the past year, we studied 2 types of heterocellular cell interactions: one based on the secretion of soluble factors by endothelial cells and we decided to focus our attention on the Angiopoietin-1 (Ang-1), and the other one involving contact *via* laminin-1 binding to α6β1 integrin. These studies correspond to task 1 and 2 of the project.

Project nº PTDC/SAU-NEU/104415/2008

We are unraveling the effects of histamine and its receptors in SVZ cell proliferation, survival and differentiation/maturation using both in vitro and in vivo experimental models. To promote an efficient delivery of histamine into the neurogenic niche we are using poly (lactic acid-co-glycolic acid) (PLGA) microparticles as a biomaterial engineered to incorporate and release histamine in a controlled way, under physiologic conditions.

Project "Endocannabinoids as modulators of neurogenesis"

We are investigating the capacities of cannabinoids to modulate neurogenesis in SVZ cell cultures via stimulation of the type 1 cannabinoid receptor (CB1R). The presence of CB1R in SVZ cells was assessed by immunocytochemistry and western blotting and CB1R-immunoreactivity was detected in nestin- and in GFAP-positive cells.

Project "Differentiation of neural stem cells by a novel retinoic acid-loaded nanoparticles delivery system"

In this project, we aim to better understand how retinoic acid (RA) regulates SVZ neurogenesis and the respective intracellular pathways and cellular processes that can be involved in the neuronal differentiation. As a strategy to improve intracellular delivery of RA, taking into consideration difficulties raised by its poor solubility in water, RA-loaded nanoparticles were developed. Nanoparticles revealed to be an excellent platform to ensure RA delivery and were able to induce neuronal differentiation.

Project "Contribution of microglia to neural inflammation: Neuropeptide Y modulates IL-1β-induced microglia activation"

We are interested in investigating the effect of neuropeptide Y (NPY) on the production of nitric oxide (NO) and IL-1β in microglia.

Project "Functional identification of subventricular zone-derived oligodendrocytes"

The project aimed at developing a functional methodology, based on single cell calcium imaging, to study oligodendroglial differentiation from SVZ cultures. Using a sequence of cell type-specific stimulus under real time calcium levels recordings, we intended to achieve a unique calcium signature for each cell type present on a SVZ culture. Accordingly, neurogenic, astrogenic and oligodendrogenic compounds were used to validate our method based on the verification of a shift on the calcium pattern of the whole cell population.

Project "Growth hormone (GH)-mediated actions on dentate gyrus neural stem cells"

In this study we are investigating the role of growth hormone (GH) on cultures of neural stem cells. Results indicate that GH is present in the cytoplasm of newborn neural cells and that the hippocampal production of these neural precursors is significantly increased by the hormone, suggesting that GH plays an important role on brain stem cells proliferation.

Main Achievements

Project nº PTDC/SAU-NEU/101783/2008

We showed that Ang-1 promote proliferation and neuronal differentiation in SVZ stem/progenitor cell cultures. This work has been published in "The Journal of Neuroscience", a journal of high impact factor in the field of Neuroscience. We also showed that physical contact with endothelial cells sustains stemness in SVZ cells. This work will be ready for publication in 2011.

Project nº PTDC/SAU-NEU/104415/2008

Exposure of SVZ neurospheres to histamine or histamine-releasing microparticles increased the percentage of NeuN-neurons as well as the percentage of cells displaying neuronal-like profile of intracellular calcium calcium ([Ca2+]i) responses detected by single-cell calcium imaging. This proneurogenic effect was mediated via activation of histamine 1 receptor (H1R). Moreover, histamine induced phospho-JNK-labeling in tau-positive axons. Using an ex vivo approach consisting in co-culturing SVZ-GFP neurospheres and hippocampal slices, we showed that histamine-releasing microparticles promoted an increase of doublecortin/GFP positive cells after 1 week.

Project "Endocannabinoids as modulators of neurogenesis"

Proliferation is increased in SVZ cultures exposed to 100 nM, 300 nM and 1 µM of (R)-(+)-Methanandamide (CB1 receptor agonist) for 48 h as assessed using the BrdU incorporation assay. Moreover, (R)-(+)-Methanandamide induces neuronal differentiation in 7 day-treated culture as functionally and morphologically evaluated using single cell calcium imaging and NeuN+ cells counting. This proneurogenic effect was mediated via activation CB1R, since a CB1R antagonist (AM251 1µM) blocked this effect.

Project: "Differentiation of neural stem cells by a novel retinoic acid-loaded nanoparticles delivery system"

Nanoparticles were able to efficiently deliver RA within mouse SVZ cells and consequently promoted neuronal differentiation. In fact, exposure of SVZ cells to 0.1µg/mL RA-nanoparticles for 7 days induced a higher expression of neuronal markers like NeuN (~165%), β-III tubulin (~135%) and NR1 (~150%), as compared to control cultures (100%). This phenotypic neuronal differentiation occurred in parallel with increased functional neuronal activity, detected by Single Cell Calcium Imaging. The work developed during this period culminated in one published paper.

Project "Contribution of microglia to neural inflammation: Neuropeptide Y modulates IL-1ß induced microglia activation"

Our results showed that upon LPS challenge (100 ng/ml), microglial cells release IL-1β, which promotes the NO production through a NF-κB-dependent pathway. Moreover, 1 µM NPY inhibits NO synthesis through Y1 receptor activation, which prevents IL-1 β release and thus inhibits nuclear translocation of NF-κB. The role of NPY in key inflammatory events may contribute to unravel novel gateways to modulate inflammation associated with brain pathology.

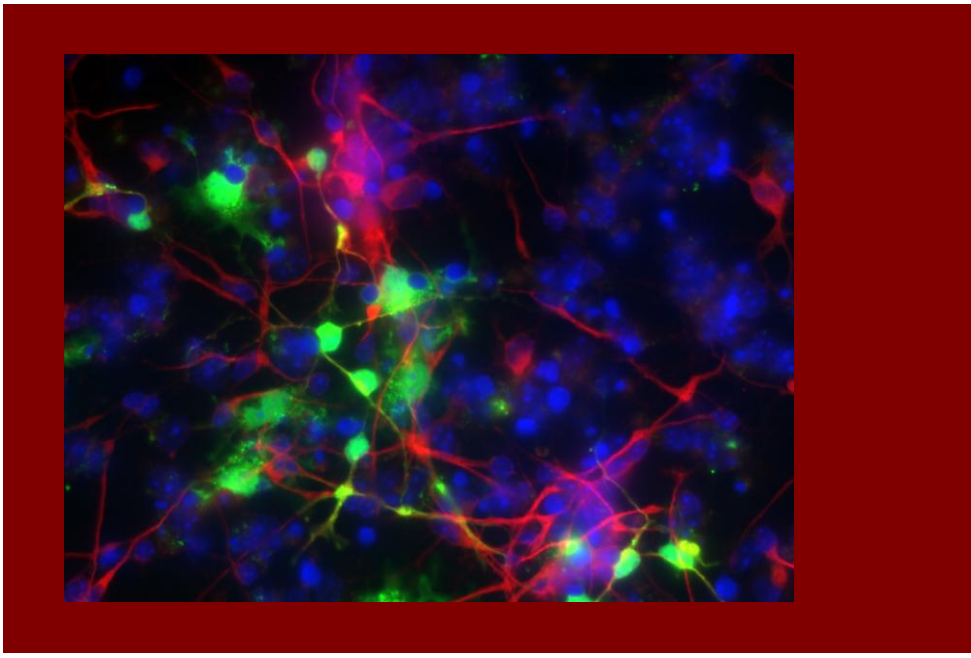
Project "Functional identification of subventricular zone-derived oligodendrocytes"

By applying a rational sequence of three stimuli – KCl, histamine and thrombin - to the heterogeneous cell population we were able to identify each cell phenotype according to its calcium signature.

Oligodendrocytes are the thrombin-responsive cells in a SVZ cell culture, and exert no intracellular calcium increase upon KCl or histamine administration, which on their turn, stimulate neurons and immature cells respectively. Astrocytes, on the other hand, are non-responsive to any of the stimuli.

Project "Growth hormone (GH)-mediated actions on dentate gyrus neural stem cells"

There is a cooperative neuro-regenerative effect between endogenous and exogenous GH. Our results demonstrate that GH is a potent factor for inducing proliferation and survival of these neural stem cells. We demonstrated here that the hormone activates various anti-apoptotic pathways. In summary, our data show that GH may be a powerful weapon for the repair of central nervous system injuries.



MAP2 in red, GFP in green, Hoechst 33342 in blue

Neuronal Cell Death and Neuroprotection Group | *Head: Carlos B. Duarte*

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

The $[Ca^{2+}]_i$ overload resulting from overactivation of Ca^{2+} permeable glutamate receptors activates Ca^{2+}-dependent signalling pathways that are coupled to neuronal death through regulation of transcription activity. This group is particularly interested in the contribution of the JNK signalling pathway in excitotoxic cell death, focusing on the role of JNK scaffold proteins and on transcription regulation.

In addition to the activation of toxic signalling pathways, the $[Ca^{2+}]_i$ overload under excitotoxic conditions also upregulates the activity of calpains, which cleave several neuronal proteins. Many of these proteins are not degraded after cleavage, but their subcellular distribution and/or activity may be affected. This group is investigating changes in proteolysis under excitotoxic conditions and the impact on the activity of GABAergic and glutamatergic neurons. Changes in the glutamatergic and GABAergic systems in brain ischemia should affect the balance between excitatory and inhibitory activity, thereby modulating the demise process.

Another goal of this group is to understand the mechanisms controlling the expression of neurotrophic factors upon neuronal injury. We have previously reported that selective injury to dopaminergic neurons in substantia nigra cell cultures triggers the expression of glial cell line-derived neurotrophic factor (GDNF), a trophic factor involved in the protection and repair of nigrostriatal dopaminergic neurons in animal models of Parkinson's disease (*Neurobiol Dis* 23, 533-542 [2006]). We have shown that GDNF upregulation by astrocytes is triggered by diffusible factors released upon neuronal injury (*Neurobiol Dis* 25, 92-104 [2007]). A potential interaction between adenosine and the neuroprotective actions of GDNF is under investigation since adenosine agonists have been shown to increase the expression of neurotrophic factors. However, adenosine A_{2A} antagonists are emerging as promising therapeutic agents to control motor symptoms in Parkinson's disease.

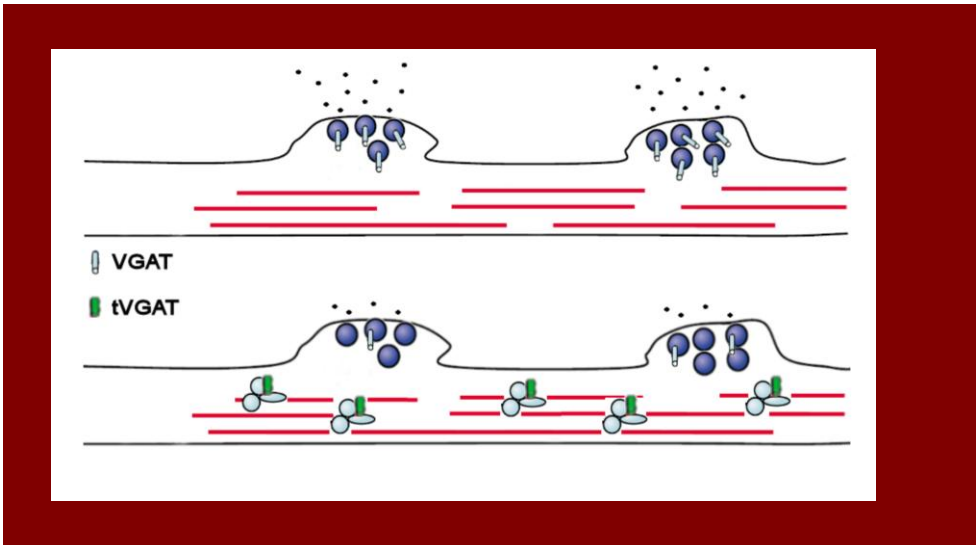
In transient global cerebral ischemia the activation of Ca^{2+}-permeable AMPA receptors (Ca-AMPARs) is crucial to prime hippocampal neuronal death. We investigated the role of the JNK signaling pathway in the propagation of the excitotoxic signal downstream Ca-AMPARs. Using HEK293 cells expressing GluA4 containing Ca-AMPARs we showed that excitotoxicity mediated by these receptors is partially regulated by the Ca^{2+}-dependent activation of JNK. In vivo studies in rats injected (i.p.) with kainate, which induces hippocampal neuron death upon activation of AMPARs, showed an increased interaction between the endogenous GluA4 subunits and the N-terminal region of the JNK scaffold protein JIP-1 in the rat brain hippocampus. The results suggest that JIP-1 might propagate the toxic signal mediated by GluA4-containing Ca-AMPARs (Vieira et al., 2010).

Under excitotoxic conditions there is also a deregulation of proteolytic systems, and abnormal cleavage of key proteins. Despite the downregulation of the proteasome under these conditions we found that excitotoxic stimulation of hippocampal neurons leads to cleavage of GAD65 and GAD67 by a mechanism sensitive to inhibitors of the ubiquitin-proteasome system. However, since none of the proteins are directly ubiquitinated the results suggest that GADs are cleaved after ubiquitination and degradation of an unknown binding partner by the proteasome. The characteristic punctate distribution of GAD65 along neurites of differentiated hippocampal neurons was lost after excitotoxic injury, and the total GAD activity measured in brain extracts at 24h postmortem (when there is a partial cleavage of GADs) was also decreased (Baptista et al., 2010).

In additional studies we investigated the changes in VGAT protein levels during ischemia and in excitotoxic conditions, which may also affect the demise process. We found that VGAT is cleaved by calpains following excitotoxic stimulation of hippocampal neurons with glutamate, giving rise to a stable cleavage product (tVGAT). VGAT cleavage was also observed after transient cerebral ischemia and following intrahippocampal injection of kainate. Calpain cleaves the transporter in the N-terminal region, and VGAT cleavage induces a loss of synaptic delivery. Our results show that excitotoxicity down-regulates full-length VGAT, with a concomitant generation of tVGAT, which is likely to affect GABAergic neurotransmission and may influence cell death during ischemia (Gomes et

al., J Neurosci 31, 4622-4635 [2011]).

In the 6-hydroxydopamine rat model of Parkinson's disease, the degeneration of dopaminergic neurons was associated with a marked increase in the expression of A_{2A} receptors ($A_{2A}R$) in striatal astrocytes. A novel finding of this work was that although dopaminergic injury caused intense astrocyte proliferation all over the lesioned striatum, upregulation of $A_{2A}R$ was mostly observed in astrocytes in areas of spared dopaminergic terminals, in the transition between lesioned tissue, completely devoid of dopaminergic markers, and areas with surviving dopaminergic terminals. In the transition areas, thicker axons and hypertrophy of dopaminergic terminals suggest an attempt of neuronal repair, which does not support the idea that stimulation of neuroinflammatory mechanisms by $A_{2A}R$ contributes to the demise of dopaminergic neurons. No upregulation of the potent dopaminotrophic factor GDNF was observed in these conditions. In addition, neuronal cells bodies stained for tyrosine hydroxylase were observed in the transition areas, suggesting that some striatal neurons might undergo a change in neurotransmitter phenotype as a compensatory reaction to degeneration of dopaminergic terminals. In vivo administration of A_{2A} agonists or antagonists is currently underway to test the role of $A_{2A}R$ in the survival and regeneration of dopaminergic terminals and in toxin-induced proliferation of glial cells in the striatum.



VGAT cleavage under excitotoxic conditions changes the distribution of the protein along axons. (A) and (B) represent the distribution of the protein under control conditions and after excitotoxic stimulation, respectively. VGAT cleavage at the N-terminal region by calpain may eliminate trafficking signals that determine the subcellular distribution of the protein. Therefore, tVGAT is not accumulated in the vesicles present in synaptic regions. This is likely to affect the amount of GABA stored in synaptic vesicles accumulated at the synapse and may decrease the synaptic release of the neurotransmitter (from J Neurosci 31, 4622-4635 [2011]).

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group | *Head: A. Cristina Rego*

In 2010 the research group 'Mitochondrial dysfunction and signaling in neurodegeneration' explored transcription deregulation linked to mito-chondrial inhibition and mitochondrial-driven apoptosis in human Huntington's disease (HD) *versus* control cybrid lines exposed to stress stimuli affecting the mitochondria. In this context we determined the protective role of brain-derived neurotrophic factor (BDNF), an important neurotrophin that seems to be affected in HD brains, and FK506, a calcineurin inhibitor. We further analysed neuronal intrinsic apoptotic pathway upon cocaine and heroin combination, relevant for understanding the neurotoxic consequences of speedball abusers.

HD is a genetic neuro-degenerative disorder characterized by striatal neuro-degeneration and involving apoptosis. In Ferreira et al (*Exp. Neurol*. 222, 243-255, 2010) we aimed to investigate the involvement of mitochondrial-dependent apoptosis in Huntington's disease (HD) *versus* control cybrids, obtained from the fusion of human platelets with mitochondrial DNA-depleted NT2 cells, and further exposed to 3-nitropropionic acid (3-NP, an irreversible inhibitor of succinate dehydrogenase) or staurosporine (STS, a classic inducer of intrinsic apoptosis).

3-NP that has been used to explore the primary mechanisms of cell death associated with mitochondrial dysfunction, metabolic impairment and neurodegeneration in Huntington's disease. Previously we showed that BDNF protected cortical neurons against apoptotic cell death caused by inhibition of mitochondrial complex II (with 3-NP) by regulating the degradation of the pro-apoptotic protein Bim (Almeida et al., *Neurobiol. Dis.* 35, 448-456, 2009). In Almeida et al. (*Neurotox. Res.* 17, 399-405, 2010), we investigated the roles of the neurotrophins BDNF and NGF in the dysregulation of transcription factors and histone modifying enzymes induced by 3-NP in primary cortical neurons.

FK506 is an inhibitor of calcineurin (or protein phosphatase 3, formerly known as protein phosphatase 2B) which has shown neuroprotective effects in several cellular and animal models of HD. In previous studies we showed that FK506 prevented mitochondrial-dependent apoptotic cell death induced by 3-NP in rat primary cortical cultures through redistribution of Bcl-2 and Bax in the mitochondrial membrane, namely a decrease in mitochondrial Bax and an increase in mitochondrial Bcl-2 levels upon exposure to FK506 and 3-NP (Almeida et al., *Neurobiol. Dis.* 17, 435-444, 2004). Thus, we examined the protective effects of FK506 in two striatal HD models, primary rat striatal neurons treated with 3-NP and immortalized striatal ST*Hdh* cells derived from HD knock-in mice expressing normal (ST$Hdh^{7/7}$) or full-length mutant huntingtin (FL-mHtt) with 111 glutamines (ST$Hdh^{111/111}$), under basal conditions or after exposure to 3-NP or STS (Rosenstock et al., *Neurochem. Int., in press*).

Cocaine and heroin are frequently co-abused by humans, in a combination known as speedball. Recently, chemical interactions between heroin (Her) or its metabolite morphine (Mor) and cocaine (Coc) were described, resulting in the formation of strong adducts. In Cunha-Oliveira et al. (*Toxicology* 276, 11-17, 2010), we evaluated whether combinations of Coc and Her affect the neurotoxicity of these drugs, using rat cortical neurons incubated with Coc, Her, Her followed by Coc (Her+Coc) and Her plus Coc (Her:Coc).

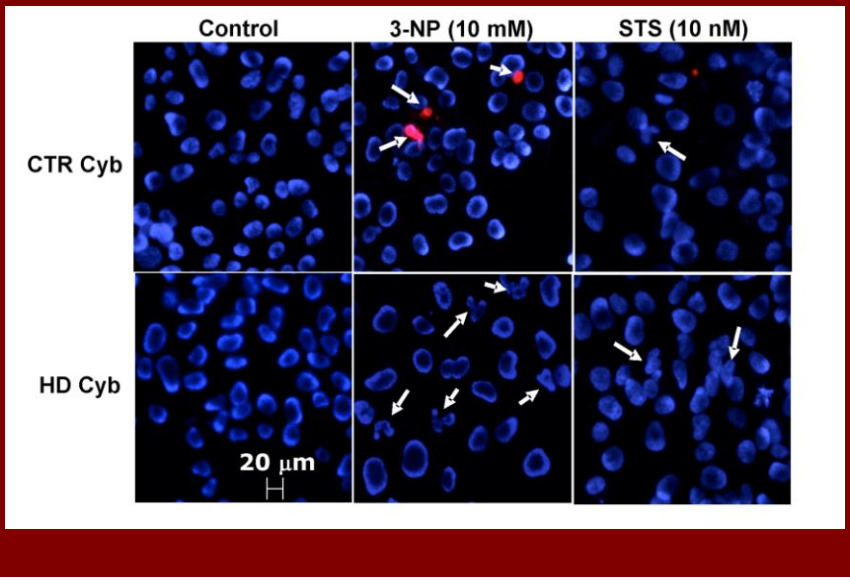
In Ferreira et al (*Exp. Neurol*. 222, 243-255, 2010) we showed that HD cybrids did not exhibit significant modifications in the activity of mitochondrial respiratory chain complexes I-IV or in mtDNA sequence variations suggestive of a primary role in mitochondrial susceptibility in the subpopulation of HD carriers studied. However, a slight decrease in mitochondrial membrane potential and increased formation of intracellular hydroperoxides was observed in HD cybrids under basal conditions. Furthermore, apoptotic nuclei morphology and a moderate increase in caspase-3 activation, as well as increased levels of superoxide ions and hydroperoxides were observed in HD cybrids upon 3-NP or STS treatment. 3-NP-evoked apoptosis in HD cybrids involved cytochrome c and AIF release from mitochondria, which was associated with mitochondrial Bax translocation. Control cybrids subjected to 3-NP showed increased mitochondrial Bax and Bim levels and the release of AIF, but not cytochrome c, suggesting a different mode of cell death, linked to the loss of membrane integrity. Additionally, increased mitochondrial Bim and Bak levels, and a slight release of cytochrome c in untreated HD cybrids may help to explain their moderate susceptibility to mitochondrial-dependent apoptosis.

In Almeida et al. (*Neurotox. Res.* 17, 399-405, 2010) we further showed that BDNF prevented the 3-NP-induced decrease in cAMP response-element binding protein (CREB) phosphorylation and CREB-binding protein levels. Both NGF and BDNF counteracted the increase in the levels of histone H3 and H4 acetylations and

reduced histone deacetylase (HDAC) activity induced by 3-NP. BDNF further led to hyperphosphorylation of HDAC2. Our results support an important role for neurotrophins, particularly BDNF, in preventing detrimental changes in transcription factors and histone acetylation states in cortical neurons that have been subjected to selective mitochondrial inhibition.

In Rosenstock et al. (*Neurochem. Int., in press*) we showed that FK506 ameliorates cell death features in Huntington's disease striatal cell models. In rat striatal neurons, FK506 abolished 3-NP-induced increase in caspase-3 activation, DNA fragmentation/condensation and necrosis. Nevertheless, in ST*Hdh*$^{111/111}$ cells under basal conditions, FK506 could not prevent in a significant way the release of cytochrome c and apoptosis inducing factor (AIF) from mitochondria, or alter Bax/Bcl-2 ratio, but significantly reverted caspase-3 activation. In ST*Hdh*$^{111/111}$ cells treated with 0.3 mM 3-NP or 25 nM STS, linked to high necrosis, exposure to FK506 exerted no significant effects on caspase-3 activation. However, effects of FK506 on ST*Hdh*$^{111/111}$ cells exposed to 10 nM STS showed that this compound can effectively prevent cell death by apoptosis and moderate necrosis. The results suggested that FK506 may be neuroprotective against apoptosis and necrosis under mild cell death stimulus in the presence of FL-mHtt.

In Cunha-Oliveira et al. (*Toxicology* 276, 11-17, 2010) we demonstrated that neurons exposed to Her, Her followed by Coc (Her+Coc) and Her plus Coc (Her:Coc, 1:1) exhibited a decrease in cell viability, which was more pronounced in neurons exposed to Her and Her+Coc, in comparison with neurons exposed to the mixture (Her:Coc). Cells exposed to the mixture showed increased intracellular calcium and mitochondrial dysfunction, as determined by a decrease in intracellular ATP levels and in mitochondrial membrane potential, displaying both apoptotic and necrotic characteristics. Conversely, a major increase in cytochrome c release, caspase 3-dependent apoptosis, and decreased metabolic neuronal viability were observed upon sequential exposure to Her and Coc. The data show that drug combinations potentiate cortical neurotoxicity and that the mode of co-exposure changes cellular death pathways activated by the drugs, strongly suggesting that chemical interactions occurring in Her:Coc, such as adduct formation, shift cell death mechanisms towards necrosis. Since impairment of the prefrontal cortex is involved in the loss of impulse control observed in drug addicts, the data presented may contribute to explain the increase in treatment failure observed in speedball abusers.



Representative image of apoptotic and necrotic cell death induced by 3-nitropropionic acid (3-NP) or staurosporine (STS) in Huntington's disease (HD) and control (CTR) cybrids. Cells were incubated with Hoechst 33342 plus propidium iodide and observed under fluorescence microscopy for nuclei morphology.
In: Ferreira IL, Nascimento MV, Ribeiro M, Almeida S, Cardoso SM, Grazina M, Pratas J, Santos MJ, Januário C, Oliveira CR, Rego AC (2010) Mitochondrial-dependent apoptosis in Huntington's disease human cybrids. Exp Neurol. 222: 243-255.

Molecular Mechanisms of Disease Group | *Head: Cláudia Pereira*

Alzheimer's disease (AD) and Parkinson's disease (PD) are progressive neurodegenerative diseases characterized by the extracellular deposition of amyloid-beta peptide (Aβ) and alpha-synuclein (α-syn), respectively. Although the aberrant peptide accumulation is recognized as an important common feature in these neurodegenerative diseases, the mechanisms of pathogenesis remain an important subject of competing hypothesis and debate. The major goal of the Molecular Mechanisms of Disease group during 2010 was to identify novel strategies for therapeutic intervention that may delay or even stop the neurodegenerative process in these brain disorders.

The involvement of mitochondria dysfunction, which has emerged as a potential 'lowest common denominator' linking several neurodegenerative disorders, was investigated using in vitro and in vivo AD models. We further explored the mitochondria/endoplasmic reticulum (ER) cross-talk as a primary molecular mechanism leading to neuronal loss triggered in AD by Aβ, namely by Aβ oligomers (the main neurotoxins in AD). Furthermore, we analyzed the role of different subunits of N-methyl-D-aspartate receptors (NMDARs) in oligomeric Aβ-induced ER stress.

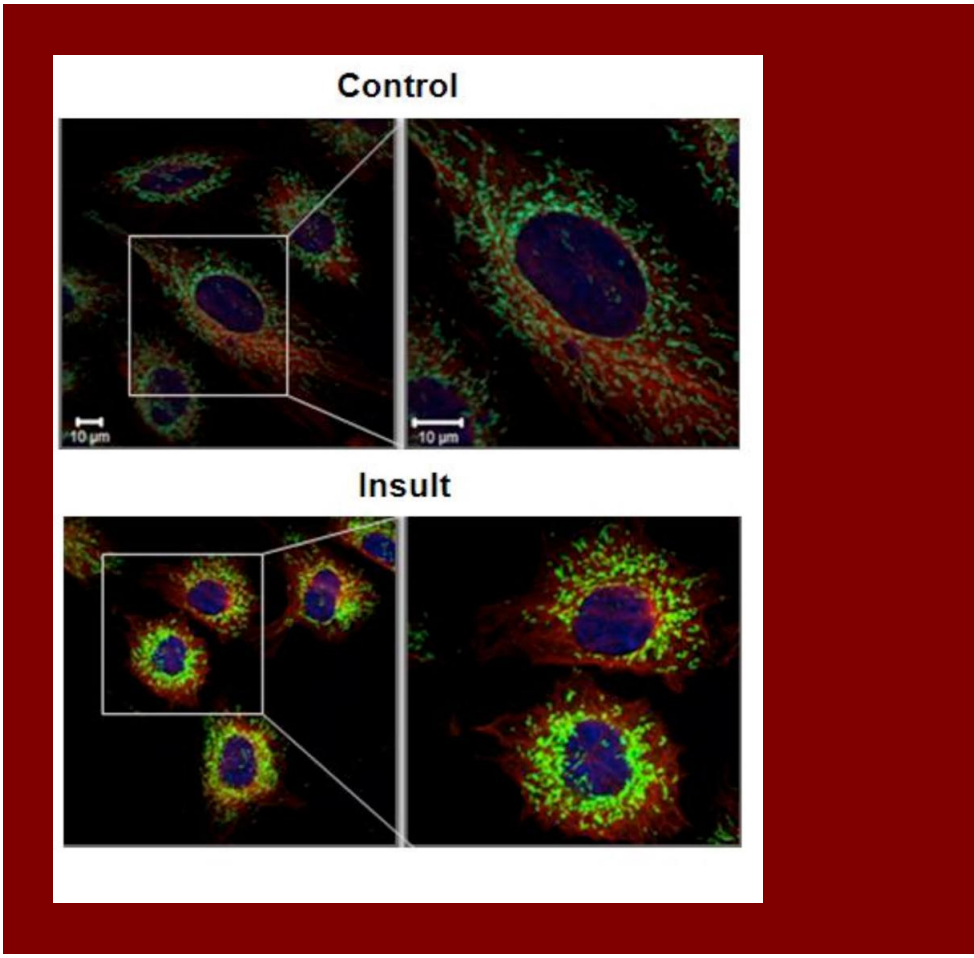
Another goal of our research group is to clarify the impact of type 1 and type 2 diabetes and associated complications on brain function (including cognitive performance) and AD development. The focus of our studies has been the analysis of mitochondrial (dys)function and their signaling pathways particularly in neuronal and brain endothelial cells. The realization that mitochondria are at the intersection of cells' life and death has made them a promising target for drug discovery and therapeutic interventions. In this line, we are interested in clarifying if mitochondria or their signaling pathways can be manipulated to promote preconditioning-induced brain tolerance (an adaptive response that protects the brain against the same stress or against a different stress).

Another main research interest is to evaluate the role of mitochondrial dysfunction on microtubule depolymerization, proteassomal impairment and protein aggregation, and its relevance to the neurodegenerative pathway in AD and PD. The role of mitochondria-mediated autophagic up-regulation on the control of cell death/survival in PD and AD is one of our main goals.

Aβ activates an ER stress-mediated apoptotic pathway by a mitochondrial-dependent process. Aβ, namely Aβ oligomers, induce ER stress in mature hippocampal cultures upon interaction with NR2B subunits of NMDARs by a mechanism involving NADPH oxidase-mediated superoxide production. Increased levels of ER stress markers were detected in the cerebral cortex and hippocampus of triple transgenic mice (3xTg-AD) and were shown to be more pronounced in the hippocampus. Furthermore, using peripheral blood mononuclear cells obtained from control and mild cognitive impairment (MCI) subjects and mild or moderate-severe AD patients we obtained evidences of impaired ER Ca2+ homeostasis and enhanced ER stress during disease progression. In mitDNA-depleted rho0 cells we described that a functional mitochondria is required for Aβ-induced apoptosis occurring through an ER stress-mediated process. The role of mitochondrial dysfunction in AD was supported by data obtained in cultured neurons from the 3xTg-AD mice which exhibited changes in mitochondrial density and morphology as well as in mitochondria axonal transport. To get a better insight into the role of mitochondrial dysfunction in AD we have used cybrid cells (that recapitulate the focal mitochondrial deficits of AD patients), which have a compromised ability to cope with Aβ-induced ER stress.

We showed that an insulin-induced acute hypoglycemic episode potentiates the effects of type 1 diabetes having detrimental effects in mitochondrial bioenergetics and antioxidant defenses. Moreover, metabolic alterations associated to type 2 diabetes induce mitochondrial abnormalities and an oxidative imbalance similar to those found in AD brains. These results support the idea that brain mitochondria are a functional link between AD and (pre)diabetes. Additionally, brain vessels and synaptosomes are differently affected by aging and/or chronic hypoxia and the compensatory mechanisms developed during aging are overcome by chronic hypoxia justifying the high prevalence of neurodegenerative conditions in elderly people especially in those that have chronic vascular diseases. Concerning preconditioning studies, mitochondrial modulators protect brain endothelial and neuronal cells against high-glucose levels. Mechanistically, these mitochondrial modulators promote a mild increase in reactive oxygen species levels followed by the induction of hypoxia inducible factor (HIF)-1α signaling pathway. Therefore, mitochondria and HIF-1α signaling pathway could represent feasible therapeutic targets to counteract neuronal and vascular dysfunction promoted by diabetes and associated complications.

Mitochondria assume the control of a wide range of cellular processes and the up-stream position of this organelle in the cascade of events leading to neurodegeneration in AD has been recognized. It is generally accepted that Aβ added extracellulary is up-taken by cells and induces cytotoxicity. In this regard we used a clonal human neuroblastoma cell line, SHSY-5Y, which was exposed to $A\beta_{1-42}$. Mitochondrial alterations induced by $A\beta_{1-42}$ affect the microtubular network by inducing an increase in free tubulin and Tau post-translational modifications. Moreover, we observed that tubulin acetylation levels where decreased in cells exposed to Aβ which we attribute to SIRT2 activity. In consequence of microtubule cytoskeleton collapse axonal transport becomes compromised. Since higher LC3II levels are indicator of activated macroautophagy, we could conclude that Aβ was able to simultaneously activate and impair autophagy. Taxol, a microtubule stabilizer drug, ameliorated cell death induced by Aβ and, most interesting, mitochondrial and cytosolic Aβ oligomers content were significantly reduced. In accordance, tau hyperphosphorylation was reduced upon taxol exposure. These observations point out that an intervention at a microtubule level may be effective as a disease modifying therapy.



Mitochondrial network. Green – TOM 20 (mitochondrial marker); Red – α-tubulin.
One interest of our group is to explore mitochondrial dynamics under several protective and deleterious conditions. Diverse in vitro and in vivo models of Alzheimer's and Parkinson's diseases and diabetes-related neurodegeneration are being used.

Neuroendocrinology and Neurogenesis Group | *Head: Claudia Cavadas*

Our group continues studying the contribution of hypothalamic-adrenal axis, other brain areas and adipose tissue on healthy lifespan.

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

2. The understanding of pathophysiological and exogenous conditions that regulate proliferation and differentiation of endogenous neural progenitor cells is strategy to achieve neuronal repair by using neural stem cells. In this context our group is studying the role and mechanisms of inflammation and antiepileptic drugs in regulating rat neural stem cells proliferation and differentiation. In more detail, we are interested on the study of the the role of nitric oxide (NO) in post-injury neurogenesis and characterize the mechanisms underlying the proneurogenic effect of NO.

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. The identification of retinal targets to promote neuronal protection and repair is another goal of our group. The background of the group is strong in retina research; therefore we will continue to investigate the effect of diabetes or hyperglycemia on neuronal dysfunction and retina microglia changes, and specially the changes induced on adenosinergic system. The potential of neuropeptide Y (NPY) system as a neuroprotective strategy in the retina will be also investigated.

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

1- Our studies on proliferation of endogenous neural progenitor cells, as a strategy to promote neuronal repair, show that nitric oxide (NO) promotes proliferation of neural stem cells by activating the ERK pathway and promoting translocation of of the cyclin-dependent kinase inhibitor p27KIP1 from the nucleus to the cytosol, which, in turn, promotes cell cycle progression. We further observed that in a mouse model of seizure-induced injury, NO from inducible NO synthase is mandatory for seizure-induced neurogenesis. Also in the context of developing new tools to neuronal repair, we isolated the progenitor cells from rat hypothalamus that grows in neuropheres.

Hypothalamic neurospheres express feeding-related neuropeptides, including Neuropeptide Y (NPY) (Fig. 1), Agouti-related protein (AGRP), pro-opiomelanocortin (POMC), cocaine-amphetamine-responsive transcript (CART) and Orexin-A. Moreover, hypothalamic progenitor cells differentiate to functional neurons that are affected by hypothalamic neurodegeneration that occurs in obesity. In conclusion, our results show that hypothalamic progenitor cells have a neuronal lineage and are a source for new feeding-related neurons. These evidences open new perspectives to study hypothalamic neurogenesis in energy balance regulation and feeding dysfunctions.

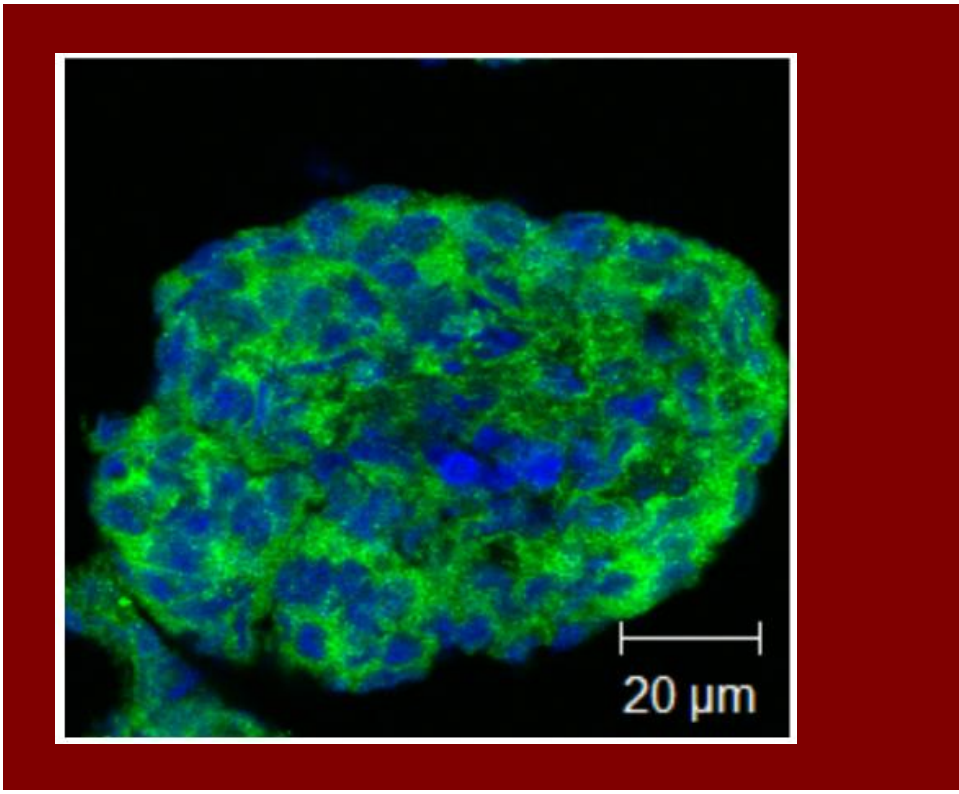
2- We evaluated the effect diabetes or hyperglycemia on adenosinergic system of retina. And we observed that diabetes does affect the adenosinergic system in rat retina). Overall, we have shown that the retinal adenosinergic system is affected by diabetes/hyperglycemia (specialy on the levels of adenosine receptors A_{1A}, A_{2A}, A_3) and may play a potential role in cell protection against the hyperglycemic environment. Targeting neuropeptide Y (NPY) system as a neuroprotective strategy in the retina, we observed that NPY protects necrotic and apoptotic cell death induced by glutamate in rat retina. NPY Y_2, Y_4 and Y_5 receptors activation inhibit necrotic glutamate toxicity although glutamate induced apoptotic cell death is prevented by the activation of Y_5 receptor. Therefore, these NPY receptors may be viewed as potential neuroprotective target in retinal degenerative diseases, such as glaucoma.

3- We investigated the role of the anti-diabetic drugs – gliptins (DPPIV inhibitors) - on adipose tissue regulation. The murine pre-adipocyte cell line, 3T3-L1, was used as a cell model. In these adipocytes, gliptins (vildagliptin, sitagliptin, or saxagliptin)

inhibited basal lipid accumulation and lipid accumulation induced by insulin. In “in vivo” studies showed that the administration of mice with sitagliptin (10 mg/kg) induced a decrease on the epididymal fat weight concomitant with a decrease on adipocyte diameter. These results may explain the lack of weight gain in patients treated with DPPIV inhibitors, suggesting that gliptins are putative pharmacological strategies to prevent adipose tissue increase in obese overweight patients and may increase life expectancy of diabetic patients.

We also investigated the effect hypoxia on adipose tissue formation, namely on adipocytes differentiation from pre-adipocytes. By using the widely used $CoCl_2$ as a hypoxia mimetic agent, our results suggest that low levels of hypoxia induced adipogenesis, but high levels of hypoxia inhibit adipogenesis with lipid accumulation in adipocytes contributing to obesity.

4- We are also investigating the involvement of hypothalamic NPY on caloric restriction induced autophagy and the mechanisms underlying this process. Interestingly, NPY induced the activation of autophagy in hypothalamic neurons (cell line N42) and in primary cell cultures of rat hypothalamic neurons. In both models, NPY induced an increase in the autophagy through NPY Y1, Y2 and Y5 receptors activation. A better understanding of the role of NPY in the regulation of autophagy will provide a new putative therapeutic strategy to extend longevity and ameliorate age-related deteriorations in combination with caloric restriction.



Rat hypothalamic neurospheres contain Neuropeptide Y (NPY)

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Molecular Biotechnology and Health Area

Coordinator: Euclides Pires

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

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

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Molecular Biotechnology Group | *Head: Carlos Faro*

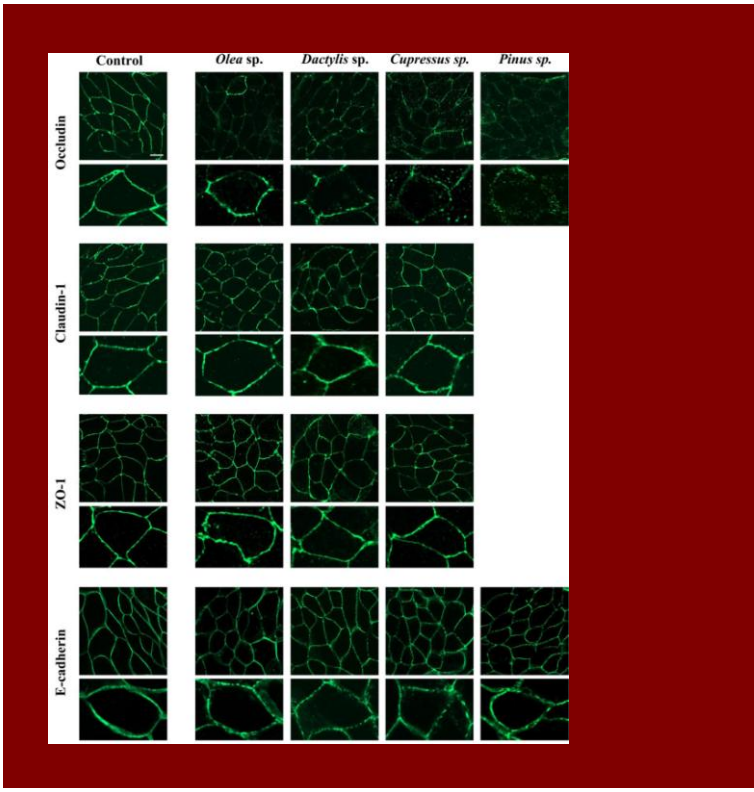
The Molecular Biotechnology group has a long-time interest in studying biotechnologically and/or biomedically relevant plant proteases. Understanding the structure-function relationship of proteases. Plant aspartic proteases has been the main research objective. Initial studies used cardosins, the milk-clotting enzymes from the flowers of cardoon, as working models. Since the sequencing of Arabidopsis genome our interest shifted towards the study of aspartic proteases from this model organism, involved in disease resistance and stress responses. The goal is to understand the possible biological functions of this family of enzymes.

Another line of research is devoted to study serine proteases from allergenic pollens. The enzymes have been purified and caracterized in our laboratory and seem to play an important role in allergy. The overall goal is to understand the molecular mechanism underlying the possible involvement of these proteases in eliciting the allergenic response as well as to assess whether or not they can be good therapeutic targets.

In order to study the structure-function relationship of Arabidopsis aspartic proteinases there is a need to set out a reproducible and efficient method to produce the recombinant forms of these enzymes. Throughout 2010, over 50 Arabidopsis genes encoding protease aspartic proteinase of the pepsin-type were amplified and cloned into several E.coli expression vectors. Expression trails for six of the genes were successful and the recombinant enzymes were purified and characterized both at the structural and enzymatic level. The preliminary study revealed common enzymatic properties among them such a strong dependence of the redox conditions and a very surprising insensitivity to pepstatin A. In contrast, the Arabidopsis aspartic proteinases differ significantly on their specificity suggesting a very define and specific role in plant cells.

Allergic disorders, such as seasonal rhinitis and asthma, are increasing causes of morbidity worldwide and result often from exposure to airborne pollen. Over the past year we evaluated the presence of protease activity in several allergenic pollens and we assess the action of these proteases on the immunologic and inflammatory response to airborne allergens.

All pollen diffusates were shown to have high molecular proteases with low pI and predominant serine and/or aminopeptidase activity. These proteases were involved in the degradation of airways bioactive peptides. Moreover all pollen extracts, with distinct allergic potential, were able of increasing transepithelial permeability and cell detachment in vitro by degrading intercellular adhesion proteins. These results suggested that the proteases normally presented in the pollen grains might be involved in the sensitization to a range of airborne allergens by facilitating their contact to subepithelial immune cells. The identification and characterization of identical proteases within the majority of pollen types, accountable for the disruption of intercellular complexes, constitutes an important step to come across a therapeutic target in the treatment of allergic disorders.



Molecular Systems Biology Group | *Head: Armindo Salvador*

The principal objectives of the group are:

1. Discovering and understanding the naturally evolved principles of quantitative design of the most prevalent elementary circuits in metabolic networks. These design principles are rules associating quantitative design (*e. g.*, relationships among enzyme kinetic parameters or among these and effector concentrations) to function.

2. Kinetic modeling and systems analysis of the biogenesis, fate and regulation of reactive metabolic intermediates. Reactive intermediates are involved in many pathologies and, much for the same reasons that make them toxic, also participate in important regulatory mechanisms. We seek a better grasp of the trade-offs involved in the metabolic processes that generate these species and to understand how these trade-offs inpend on the regulatory design of these processes.

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

4. Developing an Internet-based platform for distributed colaboration in kinetic modeling of biochemical processes. Current solutions for archival and communication of kinetic models just store "frozen" versions of the models and do not promote discussion and further development. This is a major limitation beacuse model development should be viewed as a dynamic process reflecting the evolving knowledge about biochemical processes. We seek to develop a platform — WikiModels — for developing models as a comunity activity through constant open peer-review of modeling decisions, recording successive states of a model and tracking credit for contributions.

Our main Achievements are:

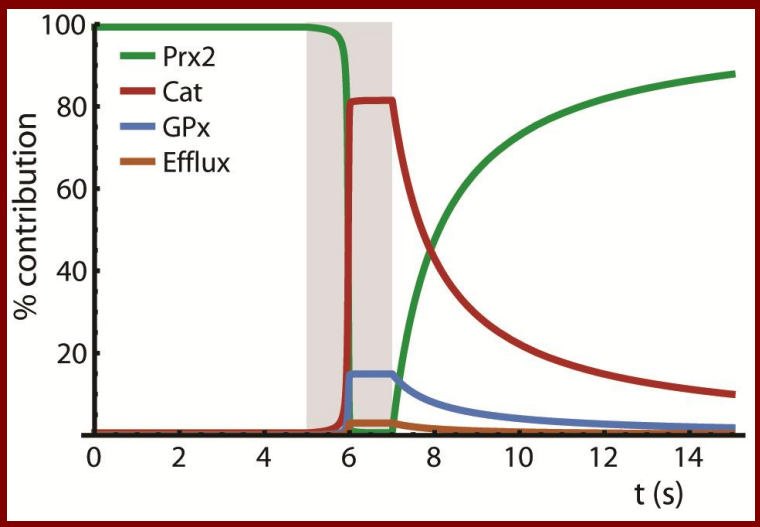
Seeking to characterize the design principles of moiety-transfer cycles (MTC) we collected and curated data for NAD redox cycles in various model organisms. These data revealed that for the enzymes involved in these cycles the $K_M(NAD^+)$ are significantly higher than the $K_M(NADH)$, indicating that the design principles of NAD redox cycles are distinct from those for NADP redox cycles. The analysis of the functional requirements that might mediate such an evolutionary outcome is ongoing.

Seeking to clarify the functional interplay among the three main defenses of human erythrocytes against hydrogen peroxide — the glutathione peroxidase (GPx1), catalase and peroxiredoxin 2 (Prx2) systems — we set up a kinetic model based on published data. Analysis of this model highlighted the following. (i) Under basal conditions, assuming a sustained extra-cellular H_2O_2 (eH_2O_2) concentration <30 nM and <25 nM/s intracellular H_2O_2 (iH_2O_2) production, Prx2 is fully reduced. Prx2, catalase and GPx1 would then eliminate 99%, 0.6% and 0.1% of the H_2O_2, respectively. (ii) Doses >0.4 mM H_2O_2 entering the erythrocyte over <1 min, or sustained eH_2O_2 concentrations >200 nM fully oxidize Prx2 to its disulfide form. Catalase then becomes the main defense against H_2O_2. (iii) Once all Prx2 is oxidized, erythrocytes can regenerate the reduced form in minutes if eH_2O_2 concentrations and iH_2O_2 production return to basal values. These results have the following implications. (a) Crossing of inflammation sites may plausibly cause substantial but transient Prx2 oxidation, as the following reasoning shows. The residence time of a circulating erythrocyte in an inflammation site is ~2 s. A dose >0.4 mM H_2O_2 will enter the erythrocyte in 2 s if the local eH_2O_2 concentration is >16 mM, which is in the scale of experimentally determined values for inflammatory foci in other organisms. (b) A stepwise increase in Prx2 oxidation through repeated crossing of inflammation sites is very unlikely. This because such crossings are expected to occur many hours in between; time enough for Prx2 to be fully reduced. (c) High enough sustained eH_2O_2 concentrations sufficient to fully oxidize Prx2 are only conceivable in systemic inflammation. Altogether, our results show that Prx2 is the main defense of erythrocytes against H_2O_2 under most physiological circumstances.

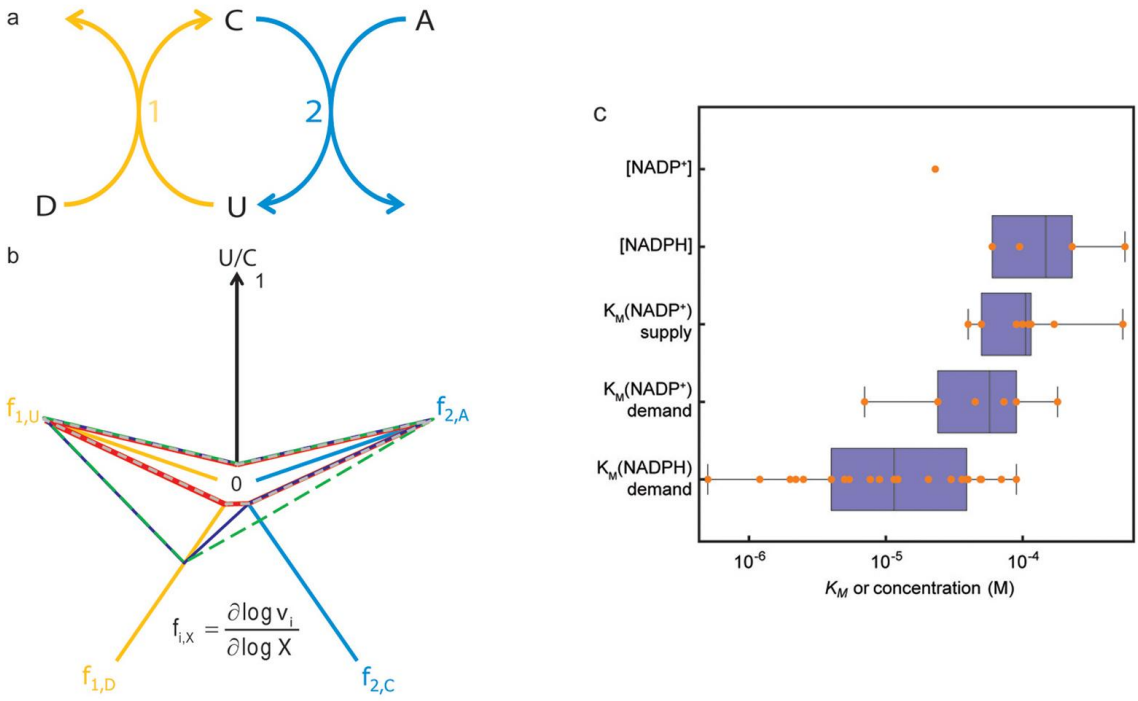
Seeking to clarify the pathways through which reactive scission products of the autoxidation of polyunsaturated fatty acyl chains (PUFA) we are using a computational approach to generate and analyze the corresponding reaction networks. This approach accounts near-comprehensively for the autoxidation chemistry and generates reaction networks including tens of thousands of compounds. Applying various graph theoretical algorithms we can then trace pathways of formation of individual compounds, find minimal pathways, and identify products that can serve as markers for relevant pathways. Applying this approach to the autoxidation of linoleyl chains we identified several products whose detection *in vivo* would demonstrate the occurrence of specific pathways for scission of the carbon chain.

We demonstrated that the patterns of proteome aminoacid usage in aerobic organisms undergo evolutionary adaptation towards minimizing the proteins' surface damage by reactive oxygen species. This adaptation occurs across all the three domains of Life: Bacteria, Archaea and Eukarya.

We have also shown that, contrary to the prevailing view, antioxidant defenses such as catalase and glutathione evolved long before photosynthesis, in response to high production rates of reactive oxygen species by water photolysis in the photic zone of the Archean ocean.



Contribution of peroxiredoxin 2 (Prx2), catalase (Cat), glutathione peroxidase (GPx) and efflux for H_2O_2 removal from human erythrocytes before, during and after a 2 s pulse (shaded region) of 50 µM extracelular H_2O_2, which is sufficiet to cause full Prx2 autoxidation within 1s. Note the reversion of the relative contributions of Cat and Prx2. The simulation assumes a basal 1 nM extracellular H_2O_2 concentration and kinetic parameters estimated from experimental data in the literature.



Design principles for moiety-transfer cycles.

Structural and Computational Biology Group | *Head: Rui M. M. Brito*

The group is strategically focused on the use of experimental and computational methodologies to study the molecular basis of human and animal pathologies, in particular amyloid diseases. Combining the reach of experimental and computational approaches, the group has been working in four main inter-related topics:

1. The characterization of the kinetics and molecular species involved in the initial stages of amyloid formation by the protein Transthyretin (TTR), the causative agent of Familial Amyloid Polyneuropathy (FAP), a mostly fatal human disease with some incidence and social relevance in Portugal;

2. Virtual screening and rational design of inhibitors of TTR amyloidosis. The experience gained with TTR will also be used to model inhibitors of amyloid formation by the A-beta-peptide of Alzheimer´s, a project in collaboration with Doctor Claudia Pereira of CNBC;

3. Extension to Portugal and support of the volunteer computing network Ibercivis (*www.ibercivis.pt*) and development of the project AMILOIDE to run in this platform;

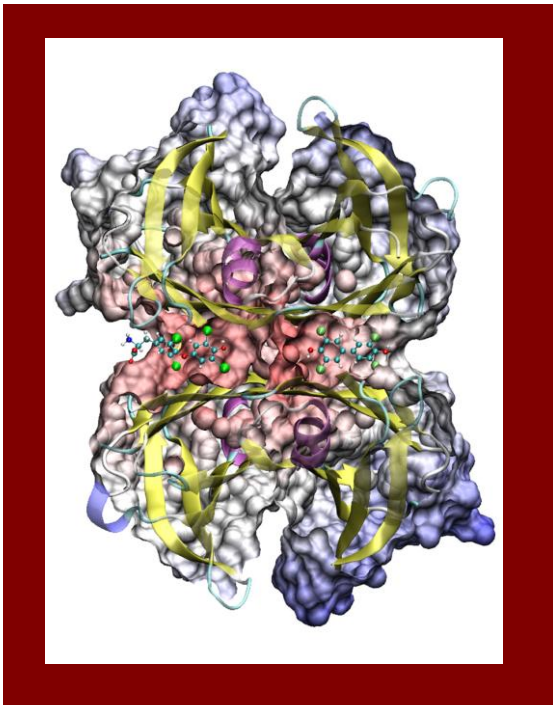
4. Development of computational tools for the storage and management (project P-found: www.p-found.org) and data mining of large data sets produced in protein folding and unfolding simulations.

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.

During 2010, an important milestone was reached by the group in the area of drug design: a large virtual screening campaign to find effective inhibitors of transthyretin-dependent amyloidoses was completed. From an initial library of 11 million compounds, and after computational screening involving multiple methodologies and large computational resources, a set of 50 compounds was selected, purchased and experimentally assayed, with 4 of them revealing significant anti-amyloid activity. Moreover, 3 out of these 4 compounds represent structural families different from those known to date, and present better physico-chemical properties from a pharmacological poin of view. As a consequence of these results, the group is now initiating procedures to file patents to protect the commercial exploitation of these innovations.

In parallel with the scientific work, the group has also payed particular attention to issues of Science communication. In particular, a member of the group was awarded the prize "Connecting Science to People" by the Sociedade Portuguesa de Bioquímica and the company Take-the-Wind, for the pro-duction of the movie AMILOIDE:

(*http://www.youtube.com/user/1967SPB#p/u/2/L-aKaWHfawo*) (see also the trailler “AMILOIDE, inception style” at *http://www.youtube.com/watch?v=LBm1Rnsw2-Y*).



Structural representation of the binding modes of the natural and synthetic TTR ligands: thyroxine (T4) (left pocket) and PCB (right pocket). The contour surface clipped view, exposing the binding sites interior, is radial-colored from the center (red) to the edge (blue) of the protein.

Vectors and Gene Therapy Group | *Head: Mª Conceição P. Lima*

The CNC laboratory of vectors and gene therapy is 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 the establishment of disease models, study of disease mechanisms and development of new molecular therapeutic strategies. Our studies have been focused on the evaluation of the potential of these novel nanosystems for the treatment of both cancer and neurodegenerative disorders, and for the development of vaccines for infectious diseases.

Non-viral vectors, including cationic liposomes, targeted SNALPs 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 LNAs, aiming at promoting silencing of known oncogene proteins and cancer-related miRNAs (oncomirs). The anti-tumoral effect of gene therapy strategies, either *per se* or in combination with chemotherapeutic agents, has been evaluated both *in vitro* and in animal models for different types of cancer. In addition, non-viral vectors have been developed to target Central Nervous System (CNS) disorders, aiming at promoting neuronal survival by targeting inflammatory and apoptotic pathways.

Viral vectors, particularly lentiviral and adeno-associated viruses are powerful technological platforms for gene delivery to the CNS, which we have been using for investigating the pathogenesis and modeling of neurodegenerative diseases, with a focus on Machado-Joseph disease (MJD). This knowledge is expected to allow the generation of disease-modifying approaches for MJD therapy. Encouraging results have already been obtained with the strategies of gene silencing, proteolysis inhibition, autophagy activation, adenosine receptor blockage and modulation of the interaction of ataxin-3 with other polyglutamine proteins. It is expected that these studies will contribute to the finding of new therapies for this fatal disorder for which no effective therapy is available.

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

Regarding the development of non-viral gene delivery and gene silencing approaches for the treatment of cancer, important achievements were made in 2010.

A splicing correction strategy was developed using the $S4_{13}PV$ cell penetrating peptide, which was shown to efficiently mediate intracellular delivery of splice-switching oligonucleotides and promote modulation of the splice pattern of a target gene. Another therapeutic strategy was explored towards glioblastoma multiforme (GBM), which involved silencing of the oncomir miR-21. We demonstrated that miR-21 expression is markedly altered in a retrovirally-induced mouse model of GBM as well as in human GBM specimens and cell lines, compared with control brain tissues. We have shown that silencing of miR-21 expression levels mediated by anti-miRNA LNA oligonucleotides formulated with cationic liposomes resulted in a decrease in tumor cell viability.

In a different approach, a proprietary lipid-based nanoparticle containing doxorubicin was developed. It combines dual targeting towards breast cancer and endothelial cells from angiogenic blood vessels and intracellular triggered release of the encapsulated payload. The 180-fold enhancement in doxorubicin cytotoxicity compared to the commercially available non-targeted formulation, along with an active tumor accumulation, translated to a significant increase of the therapeutic efficiency, as the nanoparticle completely suppressed tumor invasiveness into the surrounding healthy tissues *in vivo*. The clinical potential of such strategy was reinforced by successful ex-vivo targeting studies on breast tumor cells harvested from patients submitted to mastectomy.

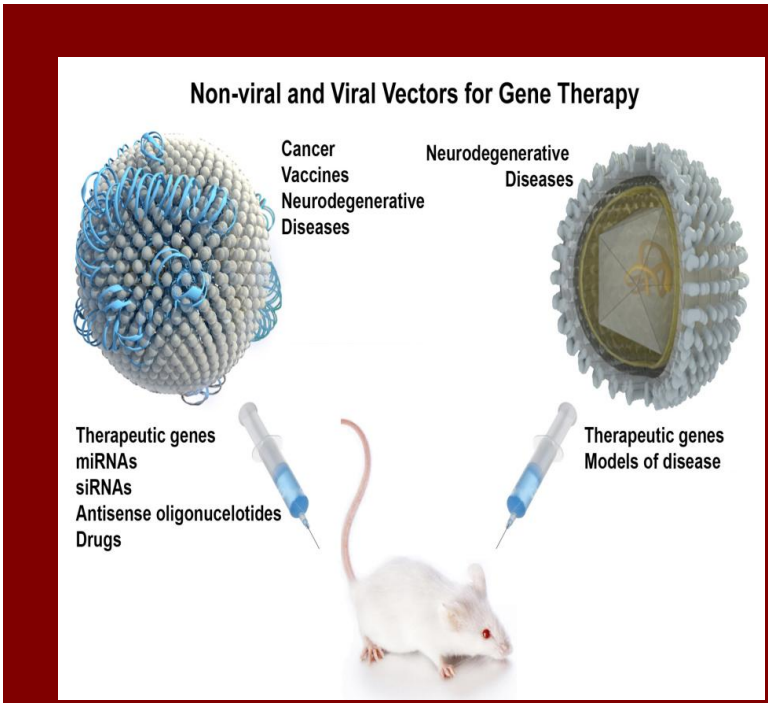
In another line of work, lipid-based nanoparticles were engineered to exhibit small size, high stability, high encapsulation yields of nucleic acids and ability to specifically bind to receptors overexpressed at the surface of chronic myeloid leukemia cells (CML). Aiming at their use for CML treatment, nanocarriers were further developed to simultaneously encapsulate anti-*BCR-ABL* siRNA and imatinib allowing both molecules to be used in lower therapeutic doses. This approach allowed specific delivery to CML cells and to address two specific molecular targets: *BCR-*

ABL oncogene and Bcr-Abl oncoprotein.

Concerning brain-related gene therapy strategies, Tf-lipoplex-mediated knockdown of the transcription factor c-Jun was found to improve neuronal survival and decrease inflammation in an animal model of excitotoxic injury, leading to reduction of seizure activity and neuronal loss.

Regarding mucosal vaccine projects, several polymeric delivery systems were developed and evaluated. One objective was to encapsulate not only antigens but also immunopotentiators. In this regard, we were able to produce aluminum chitosan-based particles that were then used to prepare complexes with a plasmid encoding the hepatitis B antigen. These complexes presented appropriate size and zeta potential to transport and protect DNA, forming a stable suspension. Finally, complexes showed to protect DNA in the presence of DNAses. In the second project, mast cell activator c48/80 was encapsulated in 3 different delivery systems: poly-e-caprolactone, chitosan and chitosan-alginate nanoparticles.

Regarding the use of viral-mediated gene delivery and gene silencing systems, new results on the use of RNA interference to Machado-Joseph disease were obtained. Using a rat model of MJD we showed that (i) overexpression of wild-type ataxin-3 did not protect against MJD pathology, (ii) knockdown of wild-type ataxin-3 did not aggravate MJD pathology and (iii) non-allele-specific silencing of ataxin-3 strongly reduced neuropathology in a rat model of MJD. Our findings indicate that therapeutic strategies involving non-allele-specific silencing may be safe and effective in MJD. We also showed that the autophagic pathway is impaired in MJD and that its activation by Beclin-1 overexpression alleviates the disease.



Biomaterials and Stem Cell-Based Therapeutics Group | *Head: Lino Ferreira*

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

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

Cells do not only connect to each other, but also to a support structure called the extracellular matrix (ECM). The ECM has multiple functions in cell growth, differentiation, and cell maintenance, as well as in tissue morphogenesis. It functions via a number of cell-surface receptors, including integrins, which are involved in anchoring the cells to the ECM, and in transmitting and controlling information across cell membranes, which regulates processes such as migration and differentiation among others. To mimic the 3D architecture and biological role of the ECM, researchers have developed biomaterials capable of modulating the biological activity of stem cells. The 3D scaffolds are important to overcome some of the limitations of 2D culture systems. First, 2D cell culture systems promote unnatural interaction with soluble factors. In 2D culture systems only a part of the cell (the basal region) interacts with the ECM and the neighboring cells, while the apical region of the cell is exposed to culture media. Therefore, it is conceivable that the distribution of integrins at the cell surface and the organization of the intracellular machinery is affected by this unnatural polarization, and thus changing the cellular response. Second, the efficiency of the differentiation process varies, depending in the final cellular lineage. Some experimental data indicates that 2D cell culture may not adequately reproduce features important for the differentiation of stem cells into specific cell lineages. Third, 2D culture systems are not exact models of *in vivo* embryonic development, and therefore, many aspects of human development might be underrepresented. 3D culture systems might be important to study the regulatory mechanisms in morphogenesis- the development of form in embryo.

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

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

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

3- Development of biomaterials with antimicrobial properties

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

Three major achievements have arisen from our recent work: first, the development of a new set of nanomaterials to control the differentiation of stem cells (Maia *et al.*, ACS Nano 2011) by the efficient spatio-temporal delivery of biomolecules; second, the development of synthetic niches to potentiate in vivo stem cell engraftment and therapeutic effect (Pedroso *et al.*, PLoS One 2011; Kraehenbuehl *et al.,* Biomaterials 2011; Vazão *et al.*, PLoS One 2011); and third, the development of new antimicrobial nanomaterials and coatings (Paulo *et al.*, Biomacromolecules 2010).

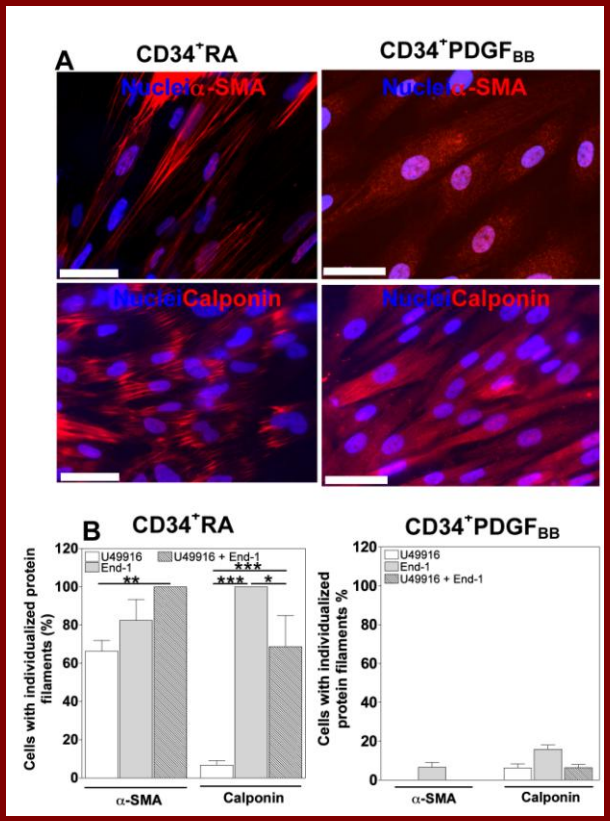
Our recent work shows that the 3D culture of human embryonic stem cell-derived smooth muscle precursor cells (hESC-derived SMPCs) modulates gene expression towards the expression observed on complete differentiated smooth muscle cells (Vazão *et al.,* PLoS One 2011). SMPCs were encapsulated in fibrin gels for 3 days after which the cells were characterized at protein and gene levels. Gene expression of SMPCs was compared to human vascular smooth muscle cells (hVSMCs) under the same culture conditions. The culture of SMPCs in 3D gels modulated the expression of smooth muscle cell (SMC) genes (a-SMA, SM-MHC or SMa-22) towards the one observed for hVSMCs cultured in 3D gels. We complemented these studies by evaluating the expression of

extracellular matrix and adhesion molecules by a quantitative real-time PCR array. This array evaluated the expression of 84 genes involved in cell-cell and cell-matrix interactions. Again, the 3D culture of SMPCs modulated extracellular matrix and adhesion molecule genes towards the expression observed in hVSMCs. The number of genes with similar expression increased from 9 to 53 when SMPCs were cultured in 2D or 3D, respectively.

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

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

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



*Maturation of hESC-derived smooth muscle progenitor cells (SMPCs). (A and B) Expression and organization of smooth muscle cell proteins on hESC-derived SMPCs treated with vasoactive agents for 3 days. Bar corresponds to 50 µm. Results are Mean ± SEM (n=8); *, **, *** denote statistical significance (P<0.05, P<0.01, P<0.001, respectively).*

Pharmacometrics Group | *Head: Amílcar Falcão*

Pharmacometrics is the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drugs's pharmacokinetic, pharmacodynamic, and biomarker-outcomes behaviour. In effect, pharmacometrics is the science of interpreting and describing pharmacology in a quantitative fashion.

We explored methods to predict early in the drug development the ADME (Absorption, Distribution, Metabolism and Excretion) as well as drug-drug interactions of new chemical entities (NCEs). Model-based drug development is characterised by the development and application of pharmacostatistical models of drug efficacy and safety from non-clinical and clinical data to improve drug development knowledge management and decision making.

The Pharmacometrics Group as a new group (less than 4 years of existence), has no much experience to share at the present time. Therefore, some individual ongoing projects were incorporated in the group and some of our achievements can be easily found analysing the productivity section. Nevertheless, our preclinical research studies in new drug development process focused on eslicarbazepine acetate (ESL) could be the considered the main structured achievement in 2008.

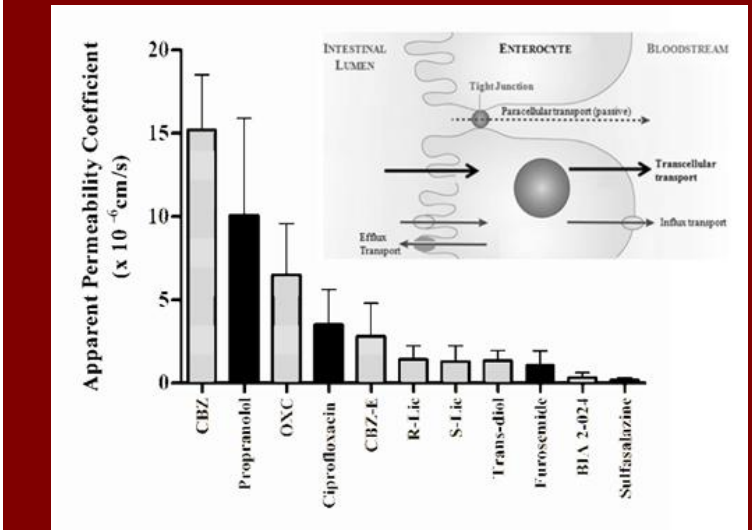
Recently, we have developed and validated new bioanalytical methods to support new pharmacokinetics studies involving not only ESL but also Carbamazepine (CBZ) and Oxcarbazepine (OXC). These bioanalytical tools are required to support a future rational evaluation of the interest in polytherapy with ESL and CBZ.

Some findings obtained from clinical trials indicate an improved efficacy in seizure control when ESL is administered as add-on to CBZ, but the scientific rational for this clinical observation is unknown.

In addition, other projects involving the ESL and other structurally related compounds have been in course in order to understand the reasons of the success of ESL and the failure of other compounds chemically very similar. The knowledge of such reasons may be useful to the design of new molecules as drug candidates to epilepsy. In this context, a series of dibenz[b,f]azepine-5-carboxamide derivatives have been compared using in vitro models to predict intestinal permeability (PAMPA and Ussing Chamber).

Other ongoing projects involve the development of appropriate methodologies to explore the potential of herb-drug and drug-drug interactions with drugs of narrow therapeutic index (for example, amiodarone).

On the other hand, the pharmacometrics evaluation of conventional drugs (fluoroquinolones) by alternative routes to oral administration is in course; at the moment we have already developed a methodology to quantify ciprofloxacin, norfloxacin and lomefloxacin.



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

Our aims are in drug discovery and development and have the support of Medicinal Chemistry and Bioorganic Chemistry as core knowledge. At present, the main focus of the group is on drug discovery in oncology.

Among several biological activities, oxysterols are promising anticancer drugs. The creation of a large library of sterols to establish comprehensive structure-cytotoxic correlations was another goal to pursue. To mimic nature and following our work on epoxysterols (*J. Med. Chem*. 2009, 52, 4007-41019), the synthesis and cytotoxic evaluation of ring-B oxygenated steroid derivatives has been an objective in 2010. The synthesis of a library of sterols to further study the influence of chemical modifications on their ability to induce cell death in tumour cell lines was planned.

Pentacyclic triterpenoids are a class of pharmacologically active and structurally rich natural products with privileged motifs for further modifications and structure-activity relationship (SAR) analyses.The naturally occurring lupane-type triterpenoids betulin and betulinic acid have been thoroughly investigated during the past years for their promising medical properties, and particularly their chemopreventive and antitumor activities. We focused our attention on the synthesis of lupane-type imidazole carbamates and *N*-acylimidazole bearing derivatives (Bioorg. & Med. Chem., 2009, 17, 6241-6250). The promising results obtained, prompted us to extend our study to 2′-methylimidazole and triazole derivatives, in order to establish meaningful SAR.

After new potent aromatase inhibitors were identified in silico using a fast high-throughput screening (HTS) methodology and their inhibition activity confirmed in vitro using a biochemical assay (*J. Med. Chem.*, 2009, 52, 143-150; *Eur. J. Med. Chem.* 2009, 44, 4121-4127; *J. Steroid Biochem. Mol. Biol*., 2008, 110: 10-17; *Chem Med Chem ,* 2007, 2, 1750-1762), their accurate structure determination from X-ray diffraction and molecular quantum mechanical calculations has been one of our goals.

Another aim was to develop new structure- and ligand-based strategies for rational anticancer drug design. Our studies focused on predicting the nature and the degree of binding pocket plasticity upon ligand binding, as well as ligand-induced multi-domain allosteric movements. The flexible target approach uses molecular dynamics, Monte Carlo and normal mode analysis to sample binding modes with biological relevance. This method has been optimized in our group for homology modeling and virtual ligand screening purposes. A homology model for chemokine receptor CXCR4 was developed based on a new ligand-guided approach. Moreover, molecular dynamics and 3D pharmacophore searches, useful to identify new FGF-2 inhibitors with antiangiogenic activity and novel allosteric modulators of Hsp90 with antiproliferative activity in tumor cell lines have been pursued.

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

- Computational approaches in drug discovery: 4D (pocket ensemble) molecular docking; pharmacophore- and structure-based drug design; virtual screening; focused library design based on hit and target.

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

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

Main Achievements :

a) A library of steroids was prepared and evaluated toward human cancer and non cancer cell lines and a SAR analysis performed to understand the influence of chemical modifications at C17, the oxygenation pattern on ring B and the esterification of key hydroxyl groups with acidic chains on the ability to induce cell death (J Med. Chem., 2010, 53, 7632). In this work, the cytotoxicity was evaluated in a colon cell line and selected oxysterols were further tested in five human cancer cell lines and three non cancer. From the large number of oxygenated sterols prepared a selective cytotoxic profile could be withdrawn. Moreover, the combination of a potent oxysterol with the largely used anticancer agents, cisplatin and doxorubicin led to the conclusion that such combination may reduce the overall clinical dose of Doxo to treat colon cancers. From the syntheses undertaken, some pharmaceutical green processes have been disclosed, including a clean and efficient method to prepare 5alpha, 6beta-dihydroxy sterols from available Delta5-steroids (Tetrahedron, 2010, 66, 2455). Furthermore, a new direct method to yield 6beta-hemiphtalate cholesteryl derivatives from the corresponding Delta5-olefins was also reported (J Med Chem, 2010, 53, 7632).

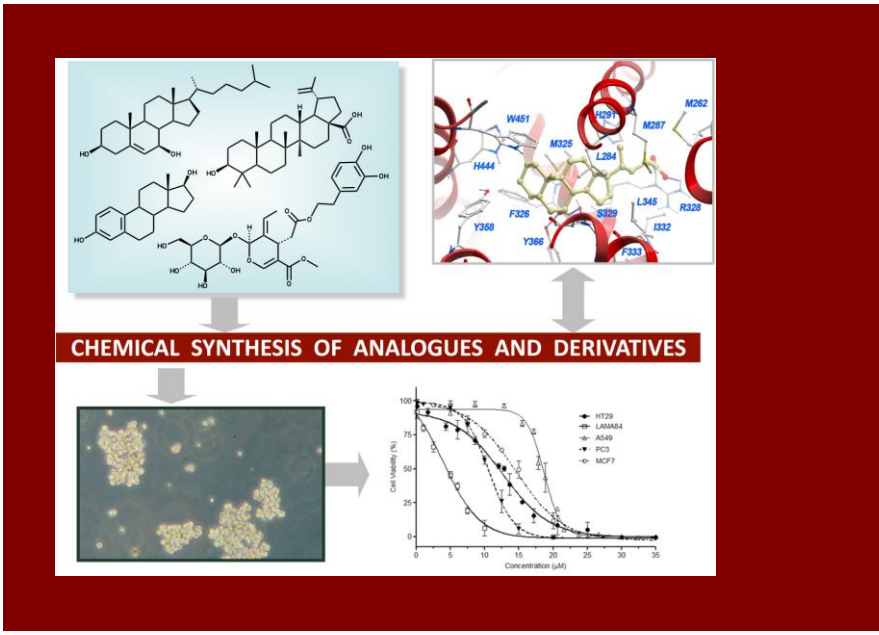
b) Recently, we focused our attention on the synthesis of lupane-type imidazole carbamates and *N*-acylimidazole bearing derivatives. Our results showed that addition of an imidazolyl moiety at the C-3 and/or C-

28 positions of betulin and betulinic acid can result in more potent *in vitro* anticancer agents than betulinic acid in human cancer cell lines of different tumor types. The promising results prompted us to extend our study to 2´-methylimidazole and triazole derivatives, in order to establish meaningful SAR (Bioorg. & Med. Chem., 2010, 18, 4385-4396). The overall findings suggest that these new lupane-type derivatives can inhibit the growth of various cancer cell lines at micromolar concentrations and are promising new experimental anticancer agents. Moreover the cytotoxicity of these compounds appears to be selective seeing that non-tumoral BJ cells tolerated substantially higher doses of these compounds than tumor cells.

c) X-ray diffraction and molecular quantum mechanical calculations has explained the high anti-aromatase potency of two non-steroid compounds identified by high throughput screening, HTS (Acta Cryst., 2010, C66, 499). A B-norsteroid has been also identified as a structurally unique potent inhibitor and X-ray diffraction results allowed to correlate the active site geometry of the molecule with the aromatase inhibition power (Acta Cryst., 2010, C66, 185).

d) We are also currently interested in developing new computer-assisted techniques for rational drug design. An intrinsic complexity in the structure-based identification of new active compounds is related to the flexibility of the protein binding pockets. Our results using ligand binding pocket ensembles suggest that the flexible receptor representation (4D docking) dramatically improves ligand discrimination of homology models generated in the low sequence identity region. The ligand-guided optimization of CXCR4 homology models used Monte Carlo and normal mode analysis to re-shape initial unrefined structures into molecular recognition devices useful for structure-based drug design and virtual ligand screening of new CXCR4 antagonists (J Comput. Aided Mol. Des., 2010, 24, 1023). Moreover, we have successfully demonstrated that molecular dynamics simulations can be used to incorporate protein flexibility into 3D-pharmacophore searches of new active compounds. This approach can be particularly useful to design small molecules that block flexible protein-protein interactions such as FGF-2 inhibitors.

Our results expanded the chemical diversity space of FGF-2 inhibitors with potent inhibitors of FGF-2-induced cell proliferation and angiogenesis (J. Biol. Chem., 2010, 285, 8733-8742). Another important application of flexible protein-ligand modeling is related to the allosteric modulation of proteins such as Hsp90. Dynamic pharmacophore-based models were used to screen large compound databases and this approach proved useful for the discovery of new small molecule allosteric inhibitors (J Chem Theory and Computation, 2010, 6, 2978-2989).



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Cell and Molecular Toxicology Area

Coordinator: Leonor Almeida

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.

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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Redox Biology in Health and Disease Group

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Mitochondrial Toxicology and Disease Group | *Head: Paulo J. Oliveira*

Mitochondria are the energy powerplants of cells by producing the majority of the chemical energy cell use for their processes. One major breakthrough occurred with the discovery that mitochondria play an important role in cell death processes. Together with the fact that mitochondria also are active players in cytosolic calcium homeostasis and in intermediate metabolism, it is pertinent to question if different molecules, which can interact with living systems, or even disease conditions, promote their biological effects through mitochondrial-mediated effects. In fact, numerous examples of mitochondria-mediated cell injury can be found in the literature; not only chemicals can negatively affect mitochondrial function but also the origin and progression of several pathologies is closely related with disruption of mitochondrial homeostasis. The main and general objective of the "Mitochondrial Toxicology and Disease Group" is to provide an insight into the role of mitochondria as a primary intracellular target in the initiation of drug- and disease-induced cell dysfunction, as well as the modulation of mitochondrial bioenergetics and cell metabolism during carcinogenesis. One particular objective is to understand how mitochondria are involved in the pathophysiology of several diseases, including diabetes, cholestasis, cancer and how mitochondrial function can be altered by chemotherapy, not only to decrease the side effects of agents commonly used in oncology, but also to specifically identify new mitochondrial targets in tumor cells.

We use different in vitro (isolated mitochondrial fractions, cultured cell lines) and in vivo models (animal models of drug or disease-induced mitochondrial alterations) in order to analyze mitochondrial function, toxicology and metabolism. From polarographic, spectrophotometric and fluorimetric techniques to NMR spectroscopy, the objective of our group is to explore the interplay between mitochondria, metabolism, disease, and human toxicology.

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

1) Anti-cancer mitochondrial toxicology: Following previous studies, we have investigated the mechanisms involved in the mitochondrial toxicity of doxorubicin, including detecting early alterations of cardiac mitochondrial function without corresponding alteration of hemodynamic parameters. We also demonstrated that acute exercise protects against induction of the mitochondrial permeability transition induced by DOX in Wistar rats. Still regarding DOX toxicity, we concluded that sub-chronic administration of DOX to Wistar rats results in oxidative stress but no induction of apoptotic signalling. We have also demonstrated that dimethylaminopyridine derivatives of lupane triterpenoids are potent disruptors of mitochondrial structure and function in cancer cells.

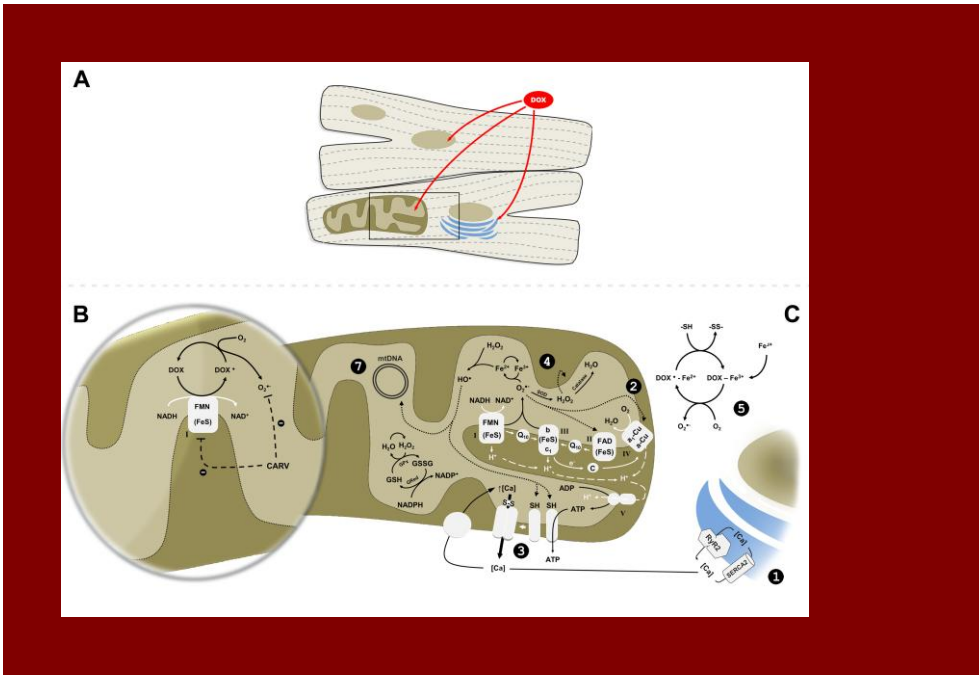
2) Modulation of mitochondrial function and dynamics: We have established that AMPK activation by berberine is dependent on SIRT1 activation and that the consequent stimulation of mitochondrial biogenesis mediates the preventive effects of berberine on diet-induced insulin resistance. We also observed that the perpetuation of hyperglycemic memory is associated with increased ROS generation, oxidative damage and decreased protein content in regulators of mitochondrial biogenesis (PGC-1alpha, SIRT1, TFAM). Regarding ischemia/reperfusion damage, we observed that the increased susceptibility of fatty livers to MPT induction is associated with increased acetylation of cyclophilin D, caused by alterations on SIRT3 activity and NAD^+ content.

3) Mitochondrial alterations induced by xenobiotics: We have investigated the protective effect of nitrogen-based compounds against the cytotoxic effects of oxidative stress on cardiomyoblasts, which can be predictive of their utility in cardiovascular diseases with an oxidative aetiology. We also evaluated the toxicity of selected phytoestrogens (coumestrol, enterodiol, enterolactone and resveratrol) on isolated mitochondria from brain, heart and liver from male and female adult Wistar-Han rats, using 17beta-Estradiol (E2) as a positive control. Some of the phytoestrogens were shown to have intrinsic antioxidant activity and to present gender-specific mitochondrial alterations. We have also performed seminal studies on the toxicity of Ecstasy and a related amphetamine on hepatic mitochondria, showing a multitude of effects that can explain their hepatotoxicity.

4) Mitochondria, carcinogenesis and chromium toxicity: Following the successful implementation of a methodology to obtain a malignant epithelial cell line (RenG2) by exposing a bronchial epithelial cell line (BEAS-2B) to Cr(VI), other cell lines with increasing malignant phenotype were established. The results revealed that as the cell's malignant phenotype increased, mitochondrial membrane potential decreased simultaneously with a extensive remodelling of the mitochondrial network, which involved increased mitochondrial mass.

The presence of sub-populations with stem-like characteristics in the more malignant cell lines, suggests that the pathway to a more malignant phenotype involves de-differentiation of malignant epithelial cells to cancer stem cells.

5) Metabolic profiling and toxicology: Intermediary metabolism has been analyzed from the cell to whole organism levels using stable isotope tracers and NMR isotopomer analysis. Particular emphasis has been given to metabolic alterations in the heart accompanying doxorubicin treatment, in the steatotic liver upon treatment with ursodeoxycholic acid and in glucose whole body homeostasis in streptozotocin treated rats as a function of insulin administration by distinct routes. Methodologies for monitoring de novo lipogenesis where also developed using deuterated water and 2H NMR analysis of liver and plasma lipids.



The figure demonstrates how doxorubicin, an anti-cancer agent that causes a dose-dependent and cumulative cardiotoxicity, interferes with mitochondrial function. We have demonstrated that doxorubicin interferes with specific pathways of ATP generation and calcium accumulation in mitochondria.

Redox Biology in Health and Disease Group | *Head: João Laranjinha*

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

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

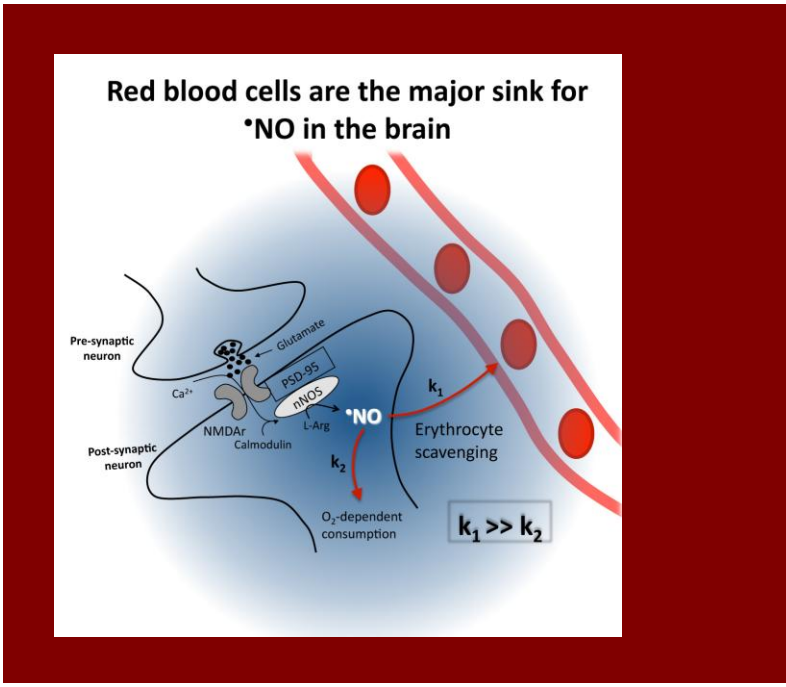
We have established the major pathway for nitric oxide (NO) scavenging in the brain. This has been done in vivo in the rat brain. The mechanisms underlying NO synthesis and inactivation in the brain are essential determinants of NO neuroactivity. Although NO production (via its synthase) is well characterized, the pathways of inactivation in vivo remain largely unknown. By showing that NO scavenging by circulating erythrocytes constitutes the major NO-inactivation pathway in the brain we provide critical insights for the understanding of the biological impact of such a diffusional messenger that conveys information associated with its concentration dynamics.

We have described a differential concentration dynamics of NO along the trisynaptic loop in hippocampus in vivo with stronger and longer lasting effects of glutamate activation on NO overflow seen in the A1 region as compared with the dentate gyrus. These results provide a quantitative and temporal basis for a better understanding of NO activity in the rat hippocampus namely in plastic phenomena (memory, learning).

We have formulated the hypothesis of a diet-dependent production of the ubiquitous cell modulator, nitric oxide, implying that dietary nitrate/nitrite may be a key modulator of critical signaling pathways in gastrointestinal tract with implications for health and disease, namely via the posttranslational modifications of proteins with critical local functions. Preliminary data encompassing the nitration of local proteases have been forwarded.

We have developed a three component microarray consisting of a Laser Doppler flow probe, and ejection pipette and a nitric oxide selective electrode that, upon stereotaxic insertion into the rat brain, will permit to study, in real-time, the neurovascular coupling phenomenon. The dynamics of blood and oxygen oscillations during changes in neuronal activity (neurovascular coupling) are not well known and have been addressed for more than 100 years.

We demonstrated that, beyond their antioxidant properties, anthocyanins possessing either catecholic or monophenolic structures, counteract peroxynitrite-induced apoptotic effects in endothelial cells by interfering in crucial signaling death pathways upstream and downstream of mithocondria. Of note, they prevent the inactivation of PI3K/Akt pathway, the increase in cytoplasmatic Bax levels and its translocation into nucleus. These results implicate dietary polyphenols in the modulation of signaling cascades and mechanistically support their beneficial effects, in the context of atherosclerosis prevention, beyond the controversial antioxidant activity in vivo.



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Microbiology Area

Coordinator: Milton Costa

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

We will identify new compatible solutes; elucidate their biosynthetic pathways and role in stress tolerance.

We also assess the contribution of natural environmental Legionella pneumophila strains into the molecular evolution of crucial genes in host infection.

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

The Yeast Research Group is unravelling the role of adenosine and adenosine receptors in the resistance of Candida albicans to macrophage attack. We will identify *Alternaria infectoria* FKS, CHS and melanin synthesis genes, as well as the biodiversity and oral yeast load in Type 1 diabetes children.

The Extreme Environments Group has participated in the first Portuguese exploration of the Atlantic sea-floor and in the international expedition Middle and Mamba 09 to the deep brine basins of the Mediterranean Sea, retrieving a large number of samples from those unexplored environments. We have isolated strains from several environments leading to the description of 3 new Genera and 5 new Species of bacteria. The performed analyses of alkaline groundwater showed the presence of a diverse and very sable microbial community.

We discovered of a unique MpgS gene from the spikemoss Selaginella moellendorffii, and identified the gene for glycosyl hydrolases, crucial for MG and GG hydrolysis in organisms that accumulate these solutes as response to stress conditions. We also identified the first MGPG hydrolase, catalysing the final step in the synthesis of MGG in Rhodopirellula baltica.

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

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

The Yeast Research group has identified 9 Alternaria infectoria AiCHS genes, 5 of which involved on melanin synthesis. Echinocandin resistance mutations in A. infectoria strains were also obtained. A full characterisation of the yeast flora of children T1D and control subjects was performed, and has identified host and environmental factors impacting in yeast load and biodiversity.

The presence of Candida albicans cells inside macrophages decreases the number of A2A receptors, and does not triggers the expression of Adora gene, opposed to what happens when macrophages were exposed to LPS.

Microbiology of Extreme Environments Group

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António Veríssimo Pires	PhD
Maria F. Gomes Nobre	PhD
Nuno Silva Empadinhas	PhD
Joana Cardoso da Costa	Post-Doc Fellow
Susana Isabel Alarico	Post-Doc Fellow
Igor Clemente Tiago	PhD Student
Ana Luísa Gomes Nobre	PhD Student
Vitor Gonçalo Mendes	PhD Student
Ana Sofia Ventura Cunha	PhD Student
Luís André Antunes França	PhD Student
Gabriel Paiva	MSc Student
Ana Branco M. Tiago	MSc Student
Ana Catarina M. Ferreira	MSc Student
Luísa Silva Lopes	MSc Student
Ana Filipa d'Avó	MSc Student
Filipa Passos	MSc Student
Tânia Leandro	MSc Student

Medical Mycology – Yeast Research Group

Teresa Gonçalves	PhD – *Head of group*
Chantal V. Fernandes	Post-Doc Fellow
Carolina Paiva Coelho	PhD Student
Lisa Rodrigues	PhD Student
Rui Costa Soares	PhD Student
Cindy Rodrigues	MSc Student
Alexandra Abrunheiro	Grant Technician
Branca Margarida Silva	Grant Technician

Microbiology of Extreme Environments Group | *Head: Milton Costa*

The objectives for 2010 were:

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

b. 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.

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

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

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

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

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

During 2010:

1. We have participated in the first Portuguese exploration of the Atlantic sea-floor and in the international expedition Middle and Mamba 09 to the deep brine basins of the Mediterranean Sea and the retrieved a large number of samples from those unexplored environments. We have also isolated strains from thermal springs, saline ecosystems and other environments leading to the description of 3 new Genera and 5 new Species of bacteria.

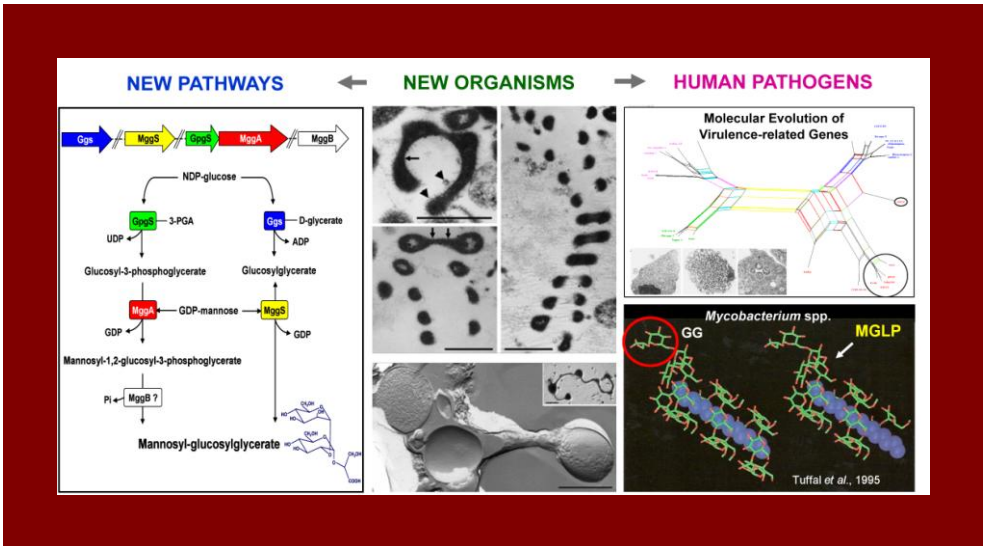
2. We have expressed a unique mannosylglycerate synthase (MgS) gene from the spikemoss *Selaginella moellendorffii*, and found that the corresponding enzyme has unique catalytic features, as it efficiently synthesizes both mannosyl-glycerate (MG) and glucosylglycerate (GG). We have also identified the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

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

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

5. We have determined the allelic diversity of five *Legionella pneumophila* type II Lsp-related genes, in strains isolated from natural and man-made environments and disease-related, suggesting an ancient divergence for each group, indicating that the operating selective pressures have equally affected the evolution of the five Lsp-related genes. The observed allelic diversity could be attributed to the substrate playing a specific role in a subset of niches, and not related with more virulence-related phenotypes within mammalian cells.

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



Medical Mycology – Yeast Research Group | *Head: Teresa Gonçalves*

Projects and objectives

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

Objectives 2010:

1. Identification of the CHS genes of Alternaria infectoria

2. Identification of the genes involved in the melanin synthesis pathway

3. Macrophage In vitro infection by A infectoria spores

4. Enzymatic protocol for viable protoplast of A infectoria (conidia and hyphae)

"Type 1 diabetes children oral yeast colonization"

Objectives 2010:

1. Biodiversity and oral yeast load in children with T1D

2. Immunological markers

3. Oral care and oral hygiene in control and T1d subjects aged 2-15 years.

"Role of adenosine and adenosine receptors in the resistance of Candida albicans to macrophage attack"

Objectives 2010:

1. Role of A2A in C albicans infection

2. Adora gene expression

Projects and achievements

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

During the first 6months of project the main achievements were:

1. Identification of 9 AiCHS genes

2. Identification of 5 of the genes coding for enzymes of the melanin synthesis pathway

3. Echinocandin resistance mutations in A infectoria strains

4. Phagocytosis rate of A infectoria spores by RAW264.7 macrophage cells. Effect of Caspofungin.

A paper is being submitted

J Anjos, C Fernandes, C Quintas, P Gonçalves, A, N Gow, Teresa Gonçalves. "β(1,3) – Glucan synthase complex from Alternaria infectoria, a rare dematiaceous human pathogen"

"Type 1 diabetes children oral yeast colonization"

During 2010 it were:

1. Full characterisation (species and load) of the yeast commensal flora of children in the centre of Portugal, T1D and control subjects

2. Host and environmental factors that impact in the yeast load and in the biodiversity

A paper is being submitted:

Ana Luísa Costa, Branca Silva, Rui Soares, Diana Mota, Vera Alves, Alice Mirante, João Carlos Ramos, João Maló de Abreu, Manuel Santos Rosa, Teresa Gonçalves. Type 1 diabetic children oral yeast carriage: a question of metabolic control?

"Role of adenosine and adenosine receptors in the resistance of Candida albicans to macrophage attack"

2010 achievements:

1. An immunocytochemistry assay was performed to assess the relative density of A2A adenosine receptors in macrophage cells either infected with C. albicans cells or stimulated by LPS. The results clearly show that the presence of yeast cells inside the macrophage decreases the number of A2A receptors in the cell membrane

2. Expression of Adora gene

Using a real time RT-PCR assay it was possible to prove that, in macrophages, C. albicans is not able to trigger an increase in the expression of Adora gene, the gene that codes for A2A adenosine receptor, opposed to what happens when macrophages were exposed to LPS.

Publications

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Empadinhas N, Pereira PJB, Albuquerque L, Costa J, Sá-Moura B, Marques AT, Macedo-Ribeiro S, and da Costa MS. Functional and structural characterization of a novel mannosyl-3-phosphoglycerate synthase from Rubrobacter xylanophilus reveals its dual substrate specificity. *Molecular Microbiology* (*in press*).

Empadinhas N, and da Costa MS. Diversity, biological roles and biosynthetic pathways for sugar-glycerate containing compatible solutes in bacteria and archaea. *Environmental Microbiology* (*in press*).

Ricardo E, Silva AP, Gonçalves T, Costa de Oliveira S, Granato C, Martins J, Rodrigues AG, Pina-Vaz C. Candida krusei reservoir in a neutropaenia unit: molecular evidence of a foe? *Clin. Microbiol. Infect.* (*in press*).

Biophysics and Biomedical NMR Area

Coordinator: Carlos Geraldes

Our Objectives are:

1. Developing integrated measurements of hepatic glycogen and lipid metabolism in spontaneously feeding animals using deuterated water.

2. Quantifying hepatic transaldolase exchange activity and its effects on estimates of gluconeogensis in subjects with normal and impaired glucose tolerance.

3. Develop and study new diagnostic imaging tools - metal based nanoparticles and chelates as multimodal (MRI, nuclear imaging,optical) targeted agents – *in vitro* and in animal models.

4. Study inorganic-based drugs for therapy –Vanadium complexes as oral insulin-mimetic agents–mechanisms of action in adipocytes and animal models.

5. NMR and DFT theoretical studies of ion-polymer complexes, of complexes of transition metal ions with hydoxyacids and their phosphorylated derivatives and of metal ion interactions with polyelectrolytes.

Main Achievements

1. We developed a novel measurement of lipogenesis based on a true precursor-product analysis of hepatic acetyl-CoA and triglyceride methyl enrichments from $^{2}H_2O$. Our method reveals that acetyl-CoA enrichment is significantly less than body water both for *in situ* and perfused livers. The difference between acetyl-CoA and body water enrichments is sensitive to the experimental setting.

2. The $^{2}H_2O$ method is used to measure gluconeogenesis in humans. It assumes negligible exchange of the lower three carbons of fructose-6-phosphate via transaldolase (TA). Using a glucose enrichment from [1-^{13}C]acetate to assess TA we established that ~35-45% of the labeling of 5th carbon of glucose by deuterium was due to transaldolase exchange rather than gluconeogenesis.

3. The Gd(III) complex of the macrocyclic ligand *trans*-H_6do2a2p can be a useful candidate as MRI CA.

4. The ability of a series of simple Eu^{3+} complexes with different charges and lipophilicities to enter cells overexpressing or not the ABCB1 (P-gp or P-glycoprotein) protein, which is expressed in human embryonic astrocytes, was studied.

5. Three new classes of nanoparticulate (NP) molecular imaging agents were developed and studied: a) NPs of dextrin dex-C16 and dextrin-PEG functionalized with $^{153}Sm^{3+}$- DOTA-monoamide for γ scintigraphy and the fluorescent probes FITC and rhodamin-123; b) g-Fe2O3@SiO2 core-shell NPs; c) NP metal organic frameworks (MOF) containing different Ln(III) ions with small r_1 and large r_2 relaxivities.

6. ^{67}Ga-radiolabeled compounds for nuclear imaging: a) three model Ga-NOTA/DOTA-GRGDG peptide conjugates targeted to the integrin a_vb_3 receptor; b) amphiphilic NOTA and DOTA-type chelates of Ga(III), studied ex vivo and in Wistar rats.

7. Non-toxic concentrations of $V^{IV}(dmpp)_2$ significantly increase glucose uptake by primary adipocytes in the absence of insulin and inhibit free fatty acid release. This compound promotes the phosphorylation of Akt1 in the insulin signaling cascade.

Inorganic Biochemistry and Molecular Imaging Group

Carlos Campos Geraldes	PhD – *Head of group*
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Sara Rute C. Figueiredo	PhD Student
André Ferreira Martins	PhD Student
João Miguel C. Teixeira	PhD Student
Helena Santos Leitão	PhD Student
Adriana Branco	MSc Student
Ana M. Martins Metelo	MSc Student
Andreia Sousa	MSc Student
Cátia Marisa Melo	MSc Student
David M. Gaspar Dias	MSc Student
Henrique F. Carvalho	MSc Student
Neuza luisa Domingues	MSc Student
Rui Filipe Silva Carvalho	MSc Student
Rui Pedro Lopes	MSc Student

Intermediary Metabolism Group

John Jones	PhD – *Head of group*
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Manuela Carvalheiro	MD, PhD
Teresa Delgado	Post-Doc Fellow
Pedro Coxito	PhD Student
Cristina Barosa	PhD Student
Joao Andre Duarte	PhD Student
Filipa Simoes	MSc Student
Ana Rita Gonçalves	Grant Technician
Joana Barra	Grant Technician

Inorganic Biochemistry and Molecular Imaging Group | *Head: Carlos Geraldes*

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

The design and development of metal based agents for multimodal targeted molecular imaging agents is followed by *in vitro* cell studies and animal model evaluation using MRI and nuclear imaging techniques. These agents include Ln^{3+}-based paramagnetic complexes of Gd^{3+} and nanoparticles with interesting photoluminescent properties for optical imaging (OI), and high r_1/r_2 relaxivities, especially at high fields, yielding negative contrast in T1/T2-weighted MRI imaging and as bimodal molecular imaging agents. The r_1 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 will also be optimized. Ga^{3+}-binding macrocyclic complexes conjugated or not with targeting agents such as peptides, will also be designed, obtained and studied. The solution chemistry of the complexes will be studied and the ^{67}Ga and ^{68}Ga-labeled complexes will be analysed in vitro and in animal models as potential nuclear imaging (gamma imaging and PET) agents.

Several types of new inorganic vanadium(IV/V) complexes are synthesised, chemically characterized in aqueous solution and their potential use as efficient oral insulin-enhancing agents for type II diabetes and toxicity effects is investigated in different cell systems and in animal models. This will include studies of the mechanism of the effects of the vanadium complexes in adipocytes, in particular on the insulin signaling cascade target proteins (using western blot analysis) and in vivo MRS and MRI studies of the effects of the vanadium complexes in animal models of type II diabetes. These will include in vivo ^1H MRS and MRI and metabolic studies using ^1H NMR in cell extracts and in animal biopsies using HR-MAS NMR techniques , as well as 13C NMR of ^{13}C-labeled substrates in tissue extracts.

Inorganic Chemistry projects include a) NMR structural and DFT theoretical studies: conjugated oligomers and polymers for molecular electronic device applications, such as of poly(9,9-dialkylfluorene)s; complexes of transition metal ions with hydoxyacids and their phosphorylated derivatives; b) studies of metal ion interactions with polyelectrolytes.

Main Achievments:

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

1) A thorough investigation of transition and Ln(III) ion complexes of the macrocyclic ligand *trans*-H_6do2a2p (H_6L) showed that the Gd(III) complex can be a useful candidate for MRI contrast agent (high thermodynamic stability and kinetic inertness in solution.). The structures of the Ln(III) complexes were determined by X-ray crystal diffraction and paramagnetic NMR in solution. A structural change occurs at Sm with the loss of an inner-sphere water molecule.

2) An overview of the use of glycoconjugates either as probes or as targets in molecular imaging using MRI was published as a review in a Medicinal Chemistry journal.

3) We have studied by atomic absorption spectrometry the ability of a series of simple Eu^{3+} complexes with different charges and lipophilicities to enter cells overexpressing or not the ABCB1 (P-gp or P-glycoprotein) protein, which is expressed in and human embryonic astrocytes. Lipophilic contrast agents can be efficiently taken up by cells and accumulate inside mitochondria when they are positively charged. They are not P-gp substrates, which is one of the major obstacles for them to cross the BBB. This may be useful to tune the capacity of b-amyloid targeted Gd-complexes to pass the blood-brain barrier (BBB) and be useful Molecular Imaging agents of Alzheimer's disease.

B) Nanoparticulate molecular imaging agents:

4) Self-assembled nanoparticles (NPs) of dextrin Dex-C16 e dextrin-PEG functionalized with a DOTA-monoamide-type metal chelator, were labeled with the γ-emitting $^{153}Sm^{3+}$ radioisotope and the fluorescent probes FITC and rhodamin-123. Their labeling of macrophages was studies by confocal microscopy. The blood clearance rate and organ biodistribution of the NPs was studied in mice using gamma scintigraphy. The effect of PEG surface coating was also studied.

5) Changing the thickness of the coated silica layer of g-Fe2O3@SiO2 core-shell NPs has a significant impact on the *r*1, *r*2, and *r*2 * relaxivities of their aqueous suspensions. The silica layer has regions that are porous to water and others that are not. The viability and the mitochondrial dehydrogenase expression of the microglial cells do not appear to be sensitive to the vesicular load with these core-shell nanoparticles. The silica shell thickness can be tuned to allow for both a sufficiently high response as CA, and adequate grafting of targeted biomolecules.

6) Aqueous suspensions of NP metal organic frameworks (MOF) containing different Ln(III) ions have small r1 and large r2 relaxivities, which are proportional to μeff^2, indicating their high negative contrast ability, specially at high magnetic fields. Magnetometry of the Dy(III), Ho(III), and Gd(III) NPs showed a typical paramagnetic behaviour.

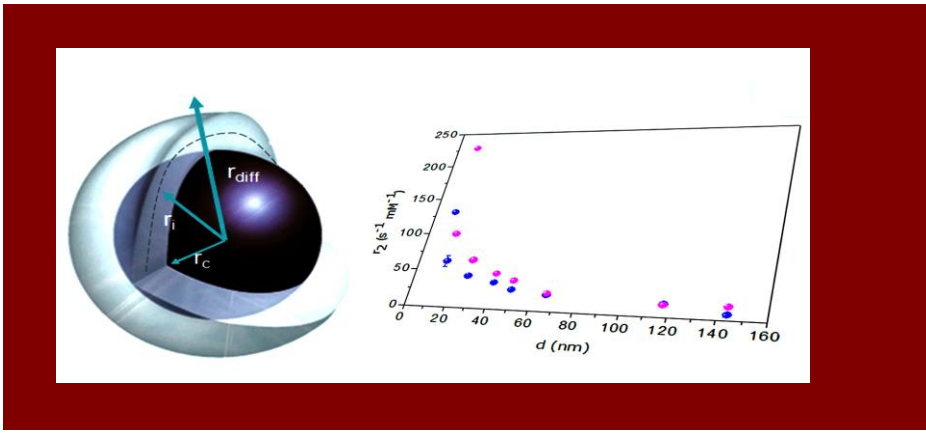
C) Radiolabeld compounds for nuclear imaging:

7) We reported the synthesis of three model glycine-arginine- glycine-aspartic acid-glycine (GRGDG) conjugates based on derivatives of NOTA and of their Ga(III) complexes targeted to the integrin avb3 receptor. ^{71}Ga NMR showed that the Ga(III)-labeled conjugates are highly stable in aqueous solution. The ^{67}Ga-labeled conjugates have high kinetic stability and show a weak but specific binding to the receptors in a U87MG-glioblastoma cell line.

8) Several amphiphilic NOTA and DOTA-type macrocyclic chelates of Ga(III) e Al(III) were characterized chemically and radiolabeled with ^{67}Ga, and their stability in human serum was studied, as well as their biodistribution and scintigraphis imaging in Wistar rats, showing hepatic and renal uptake compatible with their lipophilicities and total elimination at 24 h.

D) Vanadium-based insulin-mimetic agents:

9) Non-toxic concentrations of $V^{IV}(dmpp)_2$ significantly increase glucose uptake by primary adipocytes in the absence of insulin and inhibit free fatty acid release. This compound promotes the phosphorylation of Akt1 in the insulin signaling cascade, being a promising candidate as antidiabetic drug.



Intermediary Metabolism Group | *Head: John G. Jones*

Objectives:

1. Quantifying transaldolase exchange and its effects on measurements of gluconeogenesis in humans: The deuterated water (^2H_2O) method is extensively used to measure gluconeogenesis in humans. This method assumes negligible exchange of the 4-5-6 triose fragment of fructose-6-phosphate and glyceraldehyde-3-phosphate via transaldolase exchange. If such an exchange does occur, then glucose can be labeled with deuterium by simple exchange with a labeled three carbon precursor without net hexose synthesis. As a result of this process, gluconeogenic rates are overestimated. The present studies were undertaken to determine if transaldolase exchange occurs in humans and if so, does it alter rates of gluconeogenesis measured using the deuterated water method. In order to address this question, ^2H_2O was given by mouth and [1-^{13}C]acetate was infused intravenously in non-diabetic humans. [1-^{13}C]acetate is metabolized to yield [1-^{13}C]glyceraldehyde-3-phosphate and [1-^{13}C]dihydroxyacetone phosphate via Krebs cycle, gluconeogenic and triose phosphate isomerase reactions. These precursors combine to form fructose-1,6 phosphate then subsequently [4-^{13}C]glucose or [3-^{13}C]glucose. The ratio of [3-^{13}C]- to [4-^{13}C] glucose enrichment allows calculation of the fraction of unlabeled glucose-6-phosphate that has participated in transaldolase exchange. Therefore, in the present experiments, enrichment of glucose from [1-^{13}C]acetate and ^2H_2O was simultaneously measured using ^{13}C and ^{2}H nuclear magnetic resonance (NMR) spectroscopy allowing calculation of the contribution of transaldolase exchange to incorporation of deuterium on the position 5 of glucose following ^2H_2O ingestion.

2. Quantifying the contribution of galactose to hepatic glycogen synthesis in animal models and in humans: The liver is capable of synthesizing glycogen both from glucose and 3-carbon gluconeogenic precursors - the so-called “direct” and “indirect pathways”. Competition between these pathways for sustaining postprandial hepatic glycogen synthesis following a mixed meal has been well studied in humans. Direct and indirect pathway contributions have been measured with labeled glucose tracers, as well as with deuterated water (^2H_2O). To date, these approaches have not accounted for contributions from dietary galactose. A 200 ml glass of skimmed milk that is typically included in such meals contains sufficient galactose equivalents to contribute significantly to net hepatic glycogen synthesis. Hence, our aims are to develop tracer methods for resolving the contribution of galactose from those of direct and indirect pathway fluxes.

3. Developing a labeled meal-tolerance test for quantifying peripheral and hepatic insulin sensitivity in human subjects and animal models: The P.I. is now affiliated with the Associação Protectora dos Diabéticos de Portugal (APDP) and is involved in a 3-way collaboration with APDP and the Laboratory of Prof. Paula Macedo in characterizing glucose intolerance in pre-diabetic subjects. One of the key objectives of this study is developing measurements of glucose and insulin interactions under “real-life” conditions such as following ingestion of a mixed meal. Determining the relationship between mealtime insulin excursions and whole-body glucose kinetics requires modeling the dynamic changes in glucose appearance and disposal fluxes against a background of meal-induced endogenous glucose and insulin excursions. This is a significant departure from conventional clamp studies of insulin-stimulated glucose kinetics which are performed in the basal fasting state where fixed plasma insulin and glucose levels are imposed. Effective modeling of glucose appearance and disposal fluxes during the meal requires frequent sampling of plasma glucose levels and tracer enrichments. The sampling method has to be sufficiently sensitive for analysis of small blood volumes and the sampling throughput needs to be fast in order to accommodate the large number of samples.

Main Achievements:

Transaldolase exchange activity accounts for a significant fraction of glucose enrichment from ^2H_2O in healthy subjects, therefore gluconeogenesis is overestimated with ^2H_2O and other tracer methods: Eleven healthy 16hr-fasted subjects were studied before and during a 0.35 mU/kgFFM/min insulin infusion with glucose clamped at 110 mg/dl. Transaldolase exchange activity was assessed via glucose carbon 3 and 4 enrichments from [1-^{13}C]acetate. Gluconeogenesis was measured via glucose position 5 enrichment from ^2H_2O.

Plasma [3-^{13}C] to [4-^{13}C]glucose ratios were 0.66 ± 0.04 before and 0.59 ± 0.05 during clamp indicating that transaldolase exchange accounted for ~35% of position 5 enrichment from ^2H_2O. After correction for transaldolase exchange, rates of gluconeogenesis were lower ($p < 0.001$) and glycogenolysis higher ($p<0.001$) than uncorrected rates both before and during the clamp.

In conclusion, there is significant transaldolase exchange activity resulting in an overestimation of gluconeogenesis measured by ^2H_2O, or indeed any gluconeogenic tracer. The effects of transaldolase exchange can be corrected by measuring

enrichments of the triose moieties of glucose from a gluconeogenic ^{13}C-tracer. Future studies are ongoing to determine if the extent of transaldolase exchange is influenced by differing experimental conditions and disease states.

Quantifying the contribution of galactose to hepatic glycogen synthesis in animal models and human subjects:

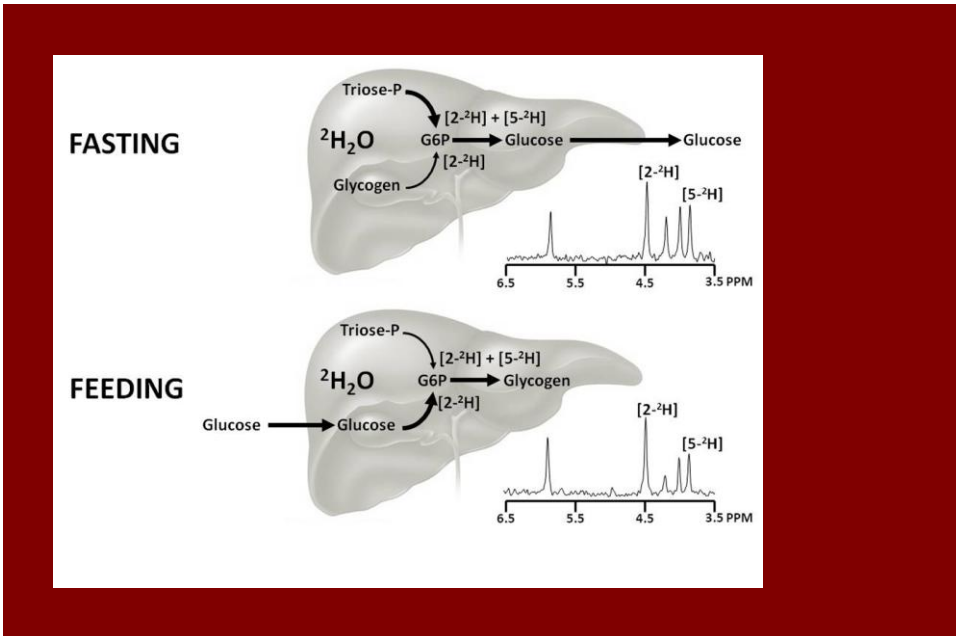
1. *Animal model:*

The effects of galactose on direct and indirect pathway measurements of hepatic glycogen fluxes were studied. 24-hr fasted rats were gavaged with 2 g/kg 100% glucose (GLU, n = 6) or 90% glucose-10% galactose loads (GLU+GAL, n = 6). Direct/indirect pathway contributions were analyzed with $^{2}H_2O$ and [U-^{13}C]glucose while galactose utilization was independently followed with [1-^{13}C]galactose. For GLU, the direct pathway contributed 30 ± 3% and indirect pathway supplied 70 ± 3% of glycogen. For GLU+GAL, 20 ± 3% was derived via direct ($P < 0.05$ vs. GLU), 57 ± 4% via indirect ($P < 0.05$ vs GLU) and 23 ± 4% from galactose. Thus, galactose reduced the apparent direct/indirect pathway ratio read by [U-^{13}C]glucose.

2. *Determining the galactose contribution to hepatic glycogen synthesis in humans:*

Glucuronide enrichment from $^{2}H_2O$ was used to quantify direct and indirect pathway contributions to hepatic glycogen synthesis following a milk-containing breakfast meal. Under these conditions, glucuronide position 2 ^{2}H-enrichment (G2) was significantly less than that of body water. We hypothesized that incomplete glucose-6-P-fructose-6-P (G6P-F6P) exchange during direct pathway metabolism of glucose and/or inflow of unlabeled UDP-glucose from galactose were responsible. G6P-F6P exchange in 6 healthy subjects was quantified by supplementing a milk-containing breakfast meal with 10 grams of [U-$^{2}H_7$]glucose and quantifying the depletion of position 2 enrichment in urinary menthol glucuronide. In a separate study, six subjects ingested $^{2}H_2O$ and Acetaminophen followed by an identical breakfast meal with 10 grams of [1-^{13}C]glucose to resolve direct/indirect pathways and galactose contributions to glycogen synthesis. Glucuronide, glucose and body water $^{2}H/^{13}C$-enrichments were determined by ^{2}H- and ^{13}C-NMR.

From the [U-$^{2}H_7$]glucose study, G6P-F6P exchange was found to be ~95% complete, therefore the difference between G2 and body water enrichments in the $^{2}H_2O$ study (0.20 ± 0.03% versus 0.27 ± 0.03%, $p < 0.005$) was attributed to galactose glycogenesis. Dietary galactose contributed 19 ± 3% to hepatic glycogen synthesis. Of the remainder, 58 ± 5% was derived from the direct pathway and 22 ± 4 % via the indirect pathway. In conclusion, the contribution of galactose to hepatic glycogen synthesis was resolved from that of direct and indirect pathways using a combination of $^{2}H_2O$ and [1-^{13}C]glucose tracers.



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Cell and Development Biology Area

Coordinator: João R. Santos & Celeste Lopes

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

One of the major strengths of the groups, included in the "Cell and Development Biology" area, is the strong collaboration with clinical departments, allowing the collection of human tissues and samples for the development of translational investigation in several distinct, yet interconnected research lines. In line with this, the major goal in 2009 was the consolidation of the research projects being carried out, which was achieved as the publication record for the various groups in this area demonstrates.

As mentioned in the previous report, the main purpose for this area was to continue the consolidation of the research carried out, as well as the recruitment of new researchers to address specific needs. The quality of publications has clearly increased, and this promises to continue in 2011.

In 2010, the Reproduction group has now established solid grounds in the field of stem cell biology, namely n the metabolic regulation of pluripotency. This work has been extended to induced pluripotent cells.

The Cellular Immunology and Oncobiology group was able to strengthen national and international collaborations established in previous years, which will become more apparent in the near future when collaboration manuscripts already submitted become published.

The Chronic Inflammation group was continued its expansion in line with the process of new recruitments and solidified its national and international cooperation networks. Publica-tions completely produced at the CNC were published in high impact journals. The group has also set up an excellent core service facility for diagnosis related to immunological-based disorders.

The Phagocytosis and Pathogens group reached a significant dimension in line with the process of new recruitments initiated in the previous year, and has now achieve good financing status with good publications coming out this year in high impact journals

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

Cellular Immunology and Oncobiology Group

Celeste Lopes	PhD – *Head of group*
Alexandrina Mendes	PhD
Ana Bela Sarmento	MD, PhD
Fernando Judas	MD, PhD
Maria Teresa C. Rosete	PhD
Teresa Maria C. Martins	PhD
Silvia Neves	PhD
Anália do Carmo	Post-Doc Fellow
Ana Luisa Vital	PhD Student
Ana Cristina Gonçalves	PhD Student
Ana Inês Crespo	PhD Student
Ana Teresa Rufino	PhD Student
Bruno Miguel das Neves	PhD Student
Carlos Manuel Melo	PhD Student
Diana M. Carvalho	PhD Student
Humberto Ferreira	PhD Student
João Martins	PhD Student
Marta Viegas da Silva	PhD Student
Patrícia Domingues	PhD Student
Sara Tavares Melo Lima	PhD Student
Susana Carvalho Rosa	PhD Student
Vera Lúcia G. Francisco	PhD Student
Ana Raquel Fonseca	MSc Student
Isabel Ferreira	MSc Student
Liliana Correia	MSc Student
Raquel Alves	MSc Student
Vera Gonçalves	Grant Technician

Biology of Reproduction, Stem Cells and Human Fertility Group

João Ramalho Santos	PhD – *Head of group*
Maria Alexandra B. Amaral	Post-Doc Fellow
Sandra Catarina do Amaral	Post-Doc Fellow
Ana Paula M. de Sousa	PhD Student
Paula Cristina Mota	PhD Student
Marta Isabel R. Baptista	PhD Student
Renata Tavares	PhD Student
Ana Sofia Rodrigues	PhD Student
Beatriz L. de Sousa	PhD Student
Marília H.Cordeiro	PhD Student
Carla Patrícia R. Paiva	PhD Student

Infection, Phagocytosis and Pathogens Group

Otília V. Vieira	PhD – *Head of group*
Luís Estronca	Post-Doc Fellow
Luísa Jordão	Post-Doc Fellow
Ângela Inácio	PhD Student
Michelle Stump	PhD Student
Katia Mesquita	PhD Student

Insulin Resistance and Adipocyte Group

Eugenia Carvalho	PhD – *Head of group*
Ermelindo Leal	Post-Doc Fellow
Ana Tellechea	PhD student
Maria Joao Pereira	PhD student
Liane Moura	PhD student
Patrícia Lopes	PhD student
Daniel Espinoza	Grant Technician

Emerging Group

Chronic Inflammation Group

Mª Margarida S.Carneiro	PhD – *Head of group*
Helena Mª L. Carvalheiro	PhD Student
Paulo Jorge R. dos Santos	PhD Student
Milene Vieira Gonçalves	MSc Student
Mónica Teresa P. Abreu	MSc Student
Tiago R. Sousa	MSc Student

Cellular Immunology and Oncobiology Group | *Head: Mª Celeste Lopes*

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

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

Research on cellular immunology focused in:

a) Immunobiology of antigen presenting cells:

1) modifications of the proteomic, lipidomic and intracellular signalling profiles of skin dendritic cells differentially induced by chemical sensitizers and irritants to establish *in vitro* tests that can predict the sensitizing potential of chemicals;

2) mechanisms of *Leishmania infantum* immune evasion to explore the potential of dendritic cell-based vaccination against this parasite;

3) identification of potential new anti-inflammatory compounds in plant aqueous and volatile extracts;

4) signal transduction profile triggered by neuropeptides in dendritic cells in inflammatory conditions.

b) Chondrocyte biology and osteoarthritis:

1) identifying the role of the extracellular glucose concentration in modulating the anabolic and catabolic functions of normal and osteoarthritic chondrocytes

2) characterizing the role of ATP-dependent K^+ channels in regulating glucose transport and its specific transporters in aging and osteoarthritic human chondrocytes

3) identifying new compounds in essential oils with anti-osteoarthritic activity

c) CD38 in immune function: studying the role of CD38 in immune regulation, namely during mycobacterial infections and development of systemic autoimmunity.

Research on oncobiology focused in:

3. Cell signalling pathways involved in cancer and chemoresistance: evaluating the cell signalling pathways involved in cancer and chemoresistance, namely the role of oxidative stress, the deregulation of apoptotic pathways and mitochondrial dysfunction, that can allow the identification of new molecular therapeutic targets.

4. Pathways involved in thyroid and breast cancer: to investigate new players in thyroid and breast carcinogenesis, based on previously obtained clinical data which highlighted the possible role of LRP1B and Claspin as tumour suppressors in these two types of cancer, respectively.

5. Signaling pathways and genetic abnormalities of gliomas: identification of chromosomal and genetic abnormalities involved in human gliomas and cell signalling pathways involved in their progression and migration.

Main Achievements

Cellular immunology

a) Immunobiology of antigen presenting cells:

1) Skin sensitizers differentially modulate metabolic and signaling pathways in dendritic cells; the differential pattern of effects identified is being translated into an in vitro test that predicts with 95% accuracy the sensitizing potential of chemicals.

2) *L. infantum* promastigotes successfully infect mouse bone marrow-derived dendritic cells and subvert their immunogenicity through cleavage of the p65 NF-kB protein.

3) Polyphenols from *Cymbopogon citratus* are proteasome inhibitors that prevent NF-kB activation and NF-kB-dependent gene expression induced by inflammatory and infectious stimuli in macrophages.

4) Neurotensin downregulates the pro-inflammatory properties of skin-dendritic cells and increases epidermal growth factor expression.

b) Chondrocyte biology and osteoarthritis:

1) Hyperglycemia-like conditions shift normal and more significantly OA chondrocytes towards a pro-catabolic and anti-anabolic phenotype, providing a link between metabolic disorders and OA development and progression.

2) Activation (opening) of K(ATP) channels in aged/OA chondrocytes can restore their ability to cope with high extracellular glucose concentrations.

3) Pinane-derived oxygenated compounds are effective inhibitors of NF-kB activation induced by inflammatory stimuli in human chondrocytes.

c) 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.

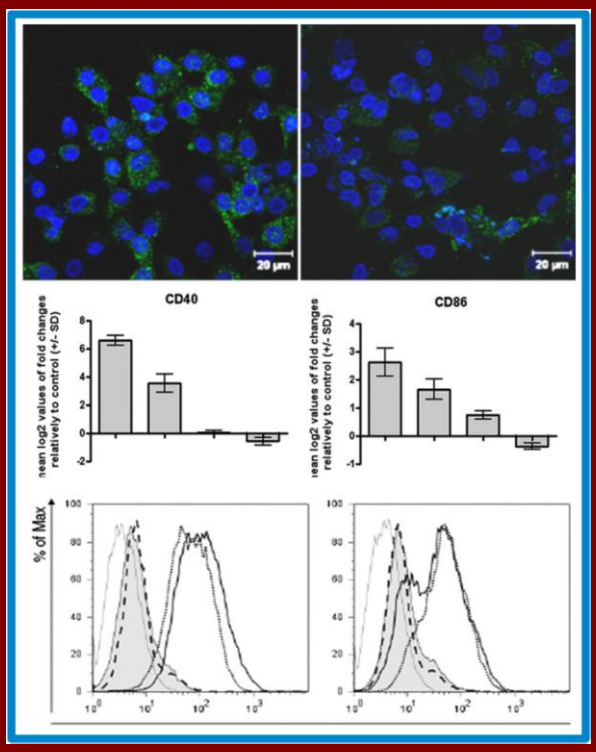
Oncobiology

a) Cell signalling pathways involved in cancer and chemoresistance: we found the involvement of oxidative stress and mitochondrial dysfunction in neoplastic development as well as in the levels of apoptotic modulators that may be related with the resistance to cell death. Our results also demonstrate that the farnesyltransferase inhibitor, α-HFPA, is effective independently of Ras mutations. Besides that, resistance to conventional chemotherapy and new targeted therapies is a problem and contribute to disease relapse.

b) 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; identified and characterized a new probable-high risk HPV (HPV108); and identified changes in claspin associated with increased susceptibility to breast cancer.

c) Signaling pathways and genetic abnormalities of gliomas: iFISH evaluation revealed a complex cytogenetic heterogeneity and distinct clonal pathways of glioma evolution.

In addition, the study of gene expression profile (GEP) demonstrated i) a clear association between the GEP of gliomas and tumor histopathology and ii) among grade IV astrocytoma, GEP are significantly associated with the cytogenetic profile of the ancestral tumor cell clone. Regarding the cell signalling pathways our results seem to indicate that the activation of PI3K/Akt, MAP kinase and CXCR4 signaling pathways may contribute to the chemoresistance that characterizes gliomas.



Biology of Reproduction and Human Fertility Group | *Head: João Ramalho Santos*

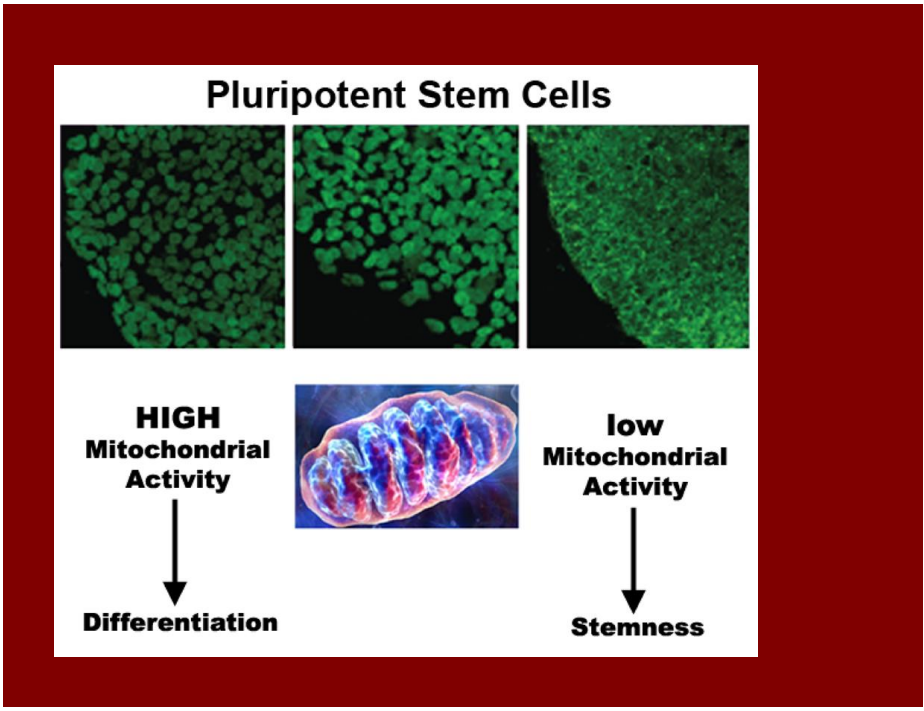
The main goals involve determining the metabolic and mitochondrial bioenergetic cues that govern gonad homeostasis, proper mammalian gamete function, and pluripotent stem cell status. The general goal is understanding the basic biology of spermatogenesis and sperm function, increasing the success rates of Assisted Reproduction in humans and endangered species, and establishing better reproductive toxicology model systems. In addition we aim to develop efficient methods to improve stem cell generation, propagation and differentiation into specific fates.

Recently concluded research includes:

1- Characterization of testicular mitochondrial bioenergetics and the finding that they are very distinct from that of mitochondria from other tissues, both in terms of basic function and how it is modulated by different substances. Furthermore, recent research shows that liver mitochondria are not appropriate to monitor the reproductive effects of certain toxic substances such as DDE, as they do not adequately convey the putative effects of these substances in spermatogenesis. Testicular are therefore suggested as a more suitable alternative for this purpose.

2- Development of novel assays to improve the analysis of human sperm function and the diagnosis of male infertility, given that the methodology currently employed is unreliable. Namely the analysis of mitochondrial functionality in mature human sperm, and how it can be efficiently monitored routinely, was also perfected and we were able to determine that it can be used to identify a subpopulation of sperm within a heterogeneous ejaculate with better fertilization potential, potentially leading to improved success rates in fertilization.

3- Discovery of a role for mitochondria in maintaining human embryonic stem cell pluripotency and in inhibiting stem cell differentiation into specific fates. Previous data described last year in human embryonic stem cell was extended to induced pluripotent stem cells, suggesting that metabolic modulation may play an important role for all pluripotent stem cells. This research is currently underway.



Infection and Pathogens Group | *Head: Mª Otília Vieira*

The Main objectives of our research are:

1. Systematic quantitative functional analysis of Rab proteins in the regulation of phagocytosis and phagosomal maturation of inert particles and *Mycobacterium tuberculosis* variant bovis (BCG).

2. Design and synthesis of new surfactants capable of prevent sexually transmitted infections.

3. Identification of the initial biochemical/biophysical process in atheroma formation.

In the Project 1 the main achievement was:

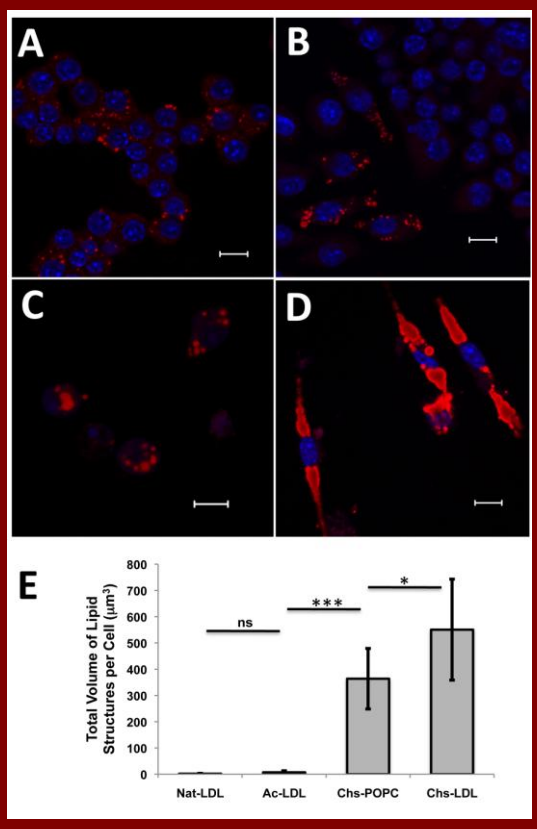
Rab8 (a and b isoforms), that like Rab10 is a member of the group V Rabs, are required for the internalization and maturation of IgG-opsonised phagocytic particles. Now, we are investigating the involvement of Rab8 in Mycobacterium-containing phagosomes.

In the Project 2 the main achievement was:

The *in vitro* effects of surfactant concentration, exposure time and structure on the viability of different mammalian cell types typically encountered in the vagina, namely, fully polarized and confluent epithelial cells, confluent but non-polarized epithelial-like cells, dendritic cells, and human sperm. Representatives of the different families of commercially available surfactants – nonionic, zwitterionic, anionic, and cationic – were examined. The toxicity results obtained contributed to the understanding of the mechanisms involved in surfactant toxicity and may be used to design and synthesize more effective and less harmful surfactants for use in vaginal gels for sexually transmitted infections prophylaxis

In the Project 3 the main achievement was:

The identification of a critical component of oxidized LDL, cholesteryl hemiesters, that could be responsible for lipid over-accumulation in endolysosomal structures and macrophages death. We also got some hints about the mechanism of action.



Lysosomal accumulation of cholesterylesters induced by CHEMS-LDL is irreversible.
Raw cells were pulsed with 300 µg/ml of acLDL or CHEMS-LDL for 48 h and then chased for 96 h. Cells pulsed with ac-LDL (A) and then chased (B). Cells pulsed with CHEMS-LDL (C) and then chased (D). In red, lipid droplets visualized by Oil-red staining. In blue, DAPI staining. All are merged images. Bars, 10 µm.
*E, Quantification of the total volume of lipid structures per cell after 96 chase. The results are the mean ± SD of three independent experiments. In every experiment 20 individual cells were analyzed. ***, p<0.0001; *, p<0.05; ns, not significant.*

Insulin Resistance and Adipocyte Group| *Head: Eugenia Carvalho*

Objectives:

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

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

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

d. Natural biopolymers like chitosan, collagen and their derivatives, are presently receiving the 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.

e. In the last decades some reports reveal the neuropeptide neurotensin (NT) as an immune mediator in the Central Nervous System and in the gastrointestinal tract, however its effects on skin immunity were not identified. Our present studies investigated the effect of NT on signal transduction and on pro/anti-inflammatory function of skin dendritic cells. Furthermore, we investigated how neurotensin can modulate the inflammatory responses triggered by LPS in skin dendritic cells.

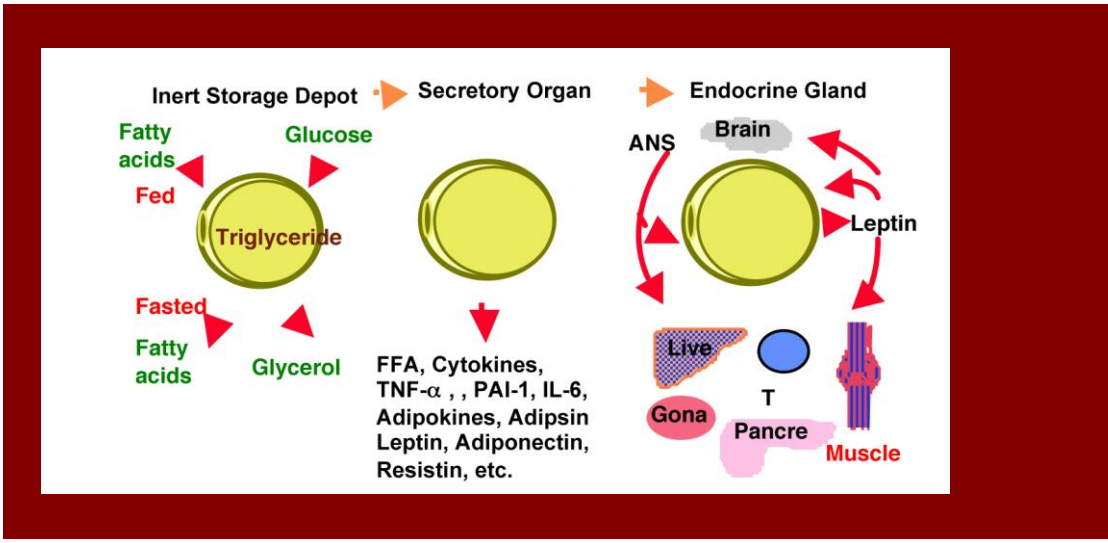
Some of the main achievements during this past year were: In collaboration with Prof. Jan Eriksson, Gothenburg University, Maria Joao Pereira, one of my PhD students, started her PhD project on the role of glucocorticoids (GCs) and immunosuppressive agents (IA) as important players in the impairment of glucose and lipid metabolism in the metabolic syndrome in isolated rat and human adipocytes. The induction of insulin resistance by GCs and IA is a process that is still poorly understood. The main hypothesis is that GCs and IA are associated with insulin resistance, causing major metabolic changes in adipocytes leading to impaired insulin sensitivity. Our preliminary results indicate that the treatment of isolated rat fat cells with IA (cyclosporin A, tacrolimus, Prednisolone and Dexamethasone) causes a significant decrease in the insulin stimulated glucose uptake. These results demonstrate that these compounds can inhibit insulin stimulated glucose uptake ex-vivo, promoting insulin resistance, causing major metabolic changes in adipocytes. Increased knowledge on the mechanisms responsible for the development of insulin resistance caused by GCs and IA is of great importance to find new and more efficient treatments for post-transplant diabetes. One paper is being submitted shortly as a result.

Furthermore, we studied the effects of various vanadium compounds, which have been shown to have promising anti-diabetic activity in isolated primary rat adipocytes. These studies originated two papers in collaboration with Dr Aureliano Alves from the UALG and Dr Margarida Castro from the Faculty of Sciences and Technology at the University of Coimbra. In these studies, the effects of different vanadium compounds namely pyridine-2,6-dicarboxylatedioxovanadium(V) (V5-dipic), bis(maltolato) oxovanadium(IV) (BMOV) and amavadine, and oligovanadates namely metavanadate and decavanadate were analysed on basal and insulin stimulated glucose uptake in rat adipocytes (Pereira et al. 2009). In addition, we reported in biochemical ex vivo studies with isolated adipocytes that a vanadium compound containing a pyridinone ligand, the bis(1,2-dimethyl-3-hydroxy-4-pyridinonate)oxovanadium (IV), VIVO(dmpp)2, has promising antidiabetic activity (Passadouro et al. 2010).

In addition, in 2007 I started another collaboration that is still ongoing with Dr A. Veves from the Department of Surgery at the Beth Israel Deaconess Medical Center and Harvard Medical School. I started obtaining preliminary data on studies concerning the complications of diabetes, namely the diabetic food ulcer. Peripheral neuropathy is an important contributing factor to the development of diabetic foot problems. We studied the role of diabetic neuropathy-related Substance P (SP) deficiency in the development of impaired wound healing and the associated mechanisms in experimental diabetes. We described the effect of the neuropeptide Substance P topical administration on the skin and the reduction of wounds size in control

and diabetic mice. We found that SP has healing effects on the skin of control and STZ diabetic mice and that administration of the NK-1R pharmacologic inhibitor CJ 012,255 counteracts wound healing effect observed by SP. In addition, we observed similar impairment in wound healing in NK-1R KO mice. At least two manuscripts are under preparation for publication.

Last but not least, in collaboration on studies from Dr Lino Ferreira's lab at the CNC/Biocant we have a manuscript that has just been submitted, looking at the improved survival, vascular differentiation and wound healing potential of umbilical cord blood stem cells co-cultured with endothelial cells in a biomimetic gel. In the present study, we investigated the use of UCB-derived $CD34^+$ cells to promote the healing of diabetic wounds when administered topically in a fibrin gel. The co-transplantation of CD34+ cells with CD34+-derived ECs improved the wound healing relatively to controls, by decreasing the inflammatory reaction and increasing the neovascularization of the wound. Collectively, our data show that the therapeutic potential of hematopoietic stem cells derived from cord blood is enhanced when they are co-cultured with their vascular progenies. The crosstalk between hematopoietic stem cells and vascular cells is beneficial for their adhesion, survival, differentiation and therapeutic effect. To improve the therapeutic benefit of the cell coculture, multiple applications, rather than a single application of the cells encapsulated in fibrin gel, could be performed. Further studies are needed to address the potential of this approach. We postulate that this co-culture system may have potential therapeutic applications, not only in chronic wounds, but also in other vascular and ischemic problems.



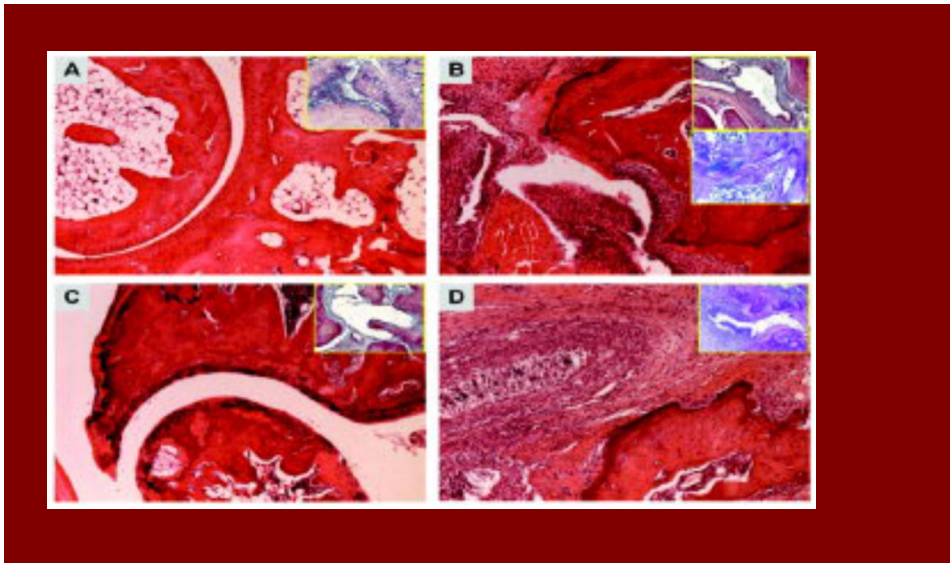
Emerging Group

Immunology Group | *Head: Mª Margarida Carneiro*

CD8+ T cells are part of the T cell pool infiltrating the synovium in rheumatoid arthritis (RA). However, their role in the pathogenesis of RA has not been fully delineated. Using the K/BxN mouse model of spontaneous chronic arthritis, which shares many similarities with RA, we studied the potential of CD8+ T cell depletion with monoclonal antibodies (mAb) to stop and reverse the progression of experimental arthritis.

CD8+ T cells from the peripheral blood and joints of K/BxN mice were mainly CD69+ and CD62L-CD27+ T cells expressing proinflammatory cytokines (interferon-γ [IFNγ], tumor necrosis factor α [TNFα], interleukin-17a [IL-17A], and IL-4), and granzyme B. In mice receiving anti-CD8 mAb, the arthritis score improved 5 days after treatment. Recovery of the CD8+ T cells was associated with a new increase in the arthritis score after 20 days. In thymectomized and anti-CD8 mAb-treated mice, the arthritis score improved permanently. Histologic analysis showed an absence of inflammatory infiltrate in the anti-CD8 mAb-treated mice. In anti-CD8 mAb-treated mice, the serologic levels of TNFα, IFNγ, IL-6, and IL-5 normalized. The levels of the disease-related anti-glucose-6-phosphate isomerase antibodies did not change.

These results indicate that synovial activated effector CD8+ T cells locally synthesize proinflammatory cytokines (IFNγ, TNFα, IL-17, IL-6) and granzyme B in the arthritic joint, thus playing a pivotal role in maintaining chronic synovitis in the K/BxN mouse model of arthritis.



Monoclonal anti-CD8 therapy induces disease amelioration in the K/BxN mouse model of spontaneous chronic polyarthritis

Publications

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Biomedical Inter-Institutional Research programme

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

This programme involves the close interaction between both clinicians and basic researchers in translational research projects.

1. Psychiatry Research

Carlos Pato, Michele Pato (University of Southern California.), Maria Helena Azevedo (FMUC), António Ferreira de Macedo (FMUC)

1.1. Molecular Genetics Studies of Complex Disorders

Our team has over 20 years experience in population studies of schizophrenia (Sz) and Bipolar Disorder (BP) focusing on the identification of susceptibility genes for these disorders through the use of linkage and the more recent state-of-the art association analysis with genome wide association studies (GWAS) and whole genome and exome sequencing. For this purpose several populations have been analyzed: a relatively homogenous population from Azores, augmented by a similarly homogenous subsample from Madeira, and a mainland Portuguese population. To date we have collected over 3000 DNA samples, including 700 schizophrenic patients, 500 bipolar patients, and 1400 unaffected family members. Additionally, 350 unaffected (i.e. no history of psychiatric disorder) subjects of Azorean descent have been collected as a control group. The schizophrenic sample includes 100 multiplex (2 or more affected members) families, and the bipolar sample includes 120 multiplex families. This sample is being expanded by Dr Pato at The University of Southern California (USC-Center for Genomic Psychiatry), with a project integrating a US- wide network of academic medical centers that have created the Genomic Psychiatry Cohort (GPC). The aims of this project are to assemble a cohort of 10,000 patients with schizophrenia and 10,000 controls without schizophrenia or a family history of schizophrenia, from 8 sites and in the future, assemble a similar sample of bipolar patients. The cohort from the USA and Portugal has reached 28,000 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.

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

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

However, the problem of the phenotypic heterogeneity in the area of psychosis still remains to be solved and we have to face the possibility that it could even be increased in samples of the magnitude used in GWAS. It is necessary, in parallel with these large GWAS, to implement nested studies, using clinical covariates that shows high 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 our central objectives will be to investigate some key phenotypic measures/symptom dimensions selected for their heritabilities in order to better characterize the genetic architecture of psychosis.

1.2. Phenotypic Studies of Complex Disorders

In parallel with the genetic studies of schizophrenia and bipolar disorder, we have developed a range of clinical investigations in areas in which a more clear understanding of the phenotypic definitions and boundaries were needed. These studies have focused in the area of personality, namely studying the perfectionism and the relationship between this trait and some disorders of the obsessive-compulsive spectrum (eating disorders and OCD) and sleep problems. Another important area of interest is the study of affective disorders in the perinatal period, a topic of research which have been relatively neglected.

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

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2. Neurology Research

Luis Cunha (FMUC, HUC), Isabel Santana (FMUC, HUC), Maria do Rosário Almeida (CNC), Maria Helena Ribeiro (FMUC), Inês Baldeiras (FMUC), Catarina Oliveira (CNC, FMUC)

2.1. Biochemical studies in neurodegenerative disorders

Cerebrospinal fluid (CSF) biomarker identification in Alzheimer´s Disease (AD) has been one of our main areas of interest. In this context, three CSF biomarkers have been found to have the highest diagnostic potential: amyloid b(1-42) protein (Ab42), total tau (t-tau) and phosphorylated tau protein (p-tau). Recently, we have assessed the performance of CSF markers in Mild Cognitive Impairment (MCI) cases, evaluating the utility of these protein markers in identifying MCI cases that latter will progress to AD. We have studied a group of 42 MCI patients, followed for at

least 2 years, 145 AD patients and 33 neurological controls without cognitive impairment.

MCI patients who developed AD during follow-up (MCI-AD; n = 11) had significantly lower baseline CSF levels of Ab42 and higher t-tau and p-tau levels than MCI patients who remained stable (MCI-St; n = 31). Optimal cut-off values for each biomarker or combination of biomarkers were defined in the AD and control group, resulting in sensitivity and specificity levels over 85% for the differentiation of AD from controls. Using the previously established cut-offs, a combination of Ab42, t-tau and p-tau could predict future development of AD in MCI patients with a sensitivity of 91% and a specificity of 70%. CSF biomarkers may therefore help stratify MCI patients into those with very low or high risk for future development of AD.

Oxidative stress in Mild Cognitive Impairment and Alzheimer´s disease is also a specific research interest of our laboratory. Recently we conducted a longitudinal study on peripheral oxidative changes in amnestic-MCI patients. We found that cellular levels of lipid peroxidation markers increased in both patients who remained cognitively stable (MCI-St) and those who progressed to AD (MCI-AD) during the study. However, only in MCI-AD patients, this was accompained by a significant decrease in intracellular antioxidant defenses (oxidyzed / reduced gluthatione ratio and vitamin E). Moreover, in MCI-AD patients, the longitudinal decrease in intracellular vitamin E was associated with the deterioration in cognitive performance. These results suggest that accumulation of oxidative damage may start in pre-symptomatic phases of AD pathology and that progression to AD might be related to deplection of antioxidant defenses.

PUBLICATIONS

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3. Translacional Bi-Genomics and Pharmacogenomics

Luísa Diogo (CHC-HP), Catarina Oliveira (CNC, FMUC), Manuela Grazina (CNC, FMUC), Isabel Santana (FMUC, HUC), Beatriz Santiago (HUC), Carmo Macário (HUC), Paula Garcia (CHC-HP), Guiomar Oliveira (CHC-HP), Lina Carvalho (FMUC, HUC), Beatriz Costa (HUC), Mariana Vide Tavares (HUC)

3.1. Biochemical genetics study in Metabolic and proliferation disorders

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 the factor involved in mitochondrial biogenesis or mtDNA replication, transcription or stability.

In the last few years the involvement of mtDNA alterations in proliferation disorders has been suggested and sequence variations have been identified.

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.

A collaborative project was established with Dr. Fernando Scaglia and Prof. Lee-Jun Wong (Baylor College of Medicine, Houston, Texas, USA) for the study of autism patients. We have screened mtDNA copy number, total mtDNA sequence, *POLG1,2* and *DGOUK* genes. Additionally, we have screened plasma ATP and aminoacid levels as possible biomarkers in autistic patients. The study is preliminary, but an increase of ATP plasma levels and mtDNA content has been detected. The results are being gathered for publication.

A research project to evaluate the prenatal history of the cases with mtDNA mutations identified in LBG has been started and we believe that it represents a valuable contribution for the investigation of prenatal manifestations of MRCD.

We have also started a new project aiming to evaluate the role of mtDNA content as a possible biomarker in lung cancer.

Additionally, we have continued to evaluate amino-acid plasma levels in patients who underwent bowel resection

due to cancer, in order to evaluate maintenance of absorption capacity. This investigation will possibly allow correlating the length of intestine remaining with the absorption capacity and contribute for a better nutritional management of these patients.

PUBLICATIONS

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Mendes C, Simões M, Silva E, Reis A, Castelo Branco M, Oliveira CR, Grazina M (2010). Mitochondrial energy impairment in Kjer-type optic atrophy. *Journal of Inherited Metabolic Disease*, 33 (S91): 270-.P.

3.2. Biogenomic investigation in Neurodegenerative disorders

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 continued, 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). So far, the sequencing of nucleotide regions corresponding to genes coding for remaining ND genes (2, 3, 4, 6, 7) has been initiated. The MRC complexes activity was also evaluated in more 14 FTD patients. We have found 20 sequence variations in 40% of patients, pointing 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.

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), aims to analyse the coding exons and the flanking intronic regions of 5HTR2A gene, in 92 samples from FTD patients were initiated The first results (3 PCR reactions and 6 sequencing reactions per sample) allowed the identification of a total of 866 sequence variations, either intronic or within exonic coding sequences. This variations are under characterization, namely by searching in genetic databases.

We have performed the evaluation of homocysteine in plasma samples, in vascular and neurological disorders. Accordingly, we have analyzed 47/40 plasma samples for homocysteine/BDNF evaluation, as a possible risk factor in vascular and neurodegenerative disorders, concerning patients suffering from stroke, dementia and mild cognitive impairment. The results are being prepared for publication.

Additionally, a collaboration has been established with the group of A. C. Rego for the analysis of mtDNA in Huntington cybrids. The results are heterogeneous, revealing different patterns of mtDNA variations, both in controls and HD patients. One pathogenic mutation, 3394A>G, with status "unclear", according to MITOMAP (www.mitomap.org) was found in one (HD-5) of 5 patients (20%) with 38 years old and having 25/44 CAG repeats genotype, together with other polymorphic variants. We have found 3 novel sequence variations in the control subjects, occurring in genetic regions that are phylogenetically conserved. The results were gathered and submitted for publication

We have continued the genetic studies in eye disorders, in collaboration with IBILI and Serviço de Oftalmologia dos HUC.

PUBLICATIONS

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3.3 Pharmacogenomics

We have implemented 3 lines of reserach aiming to identify genetic variants that will contribute for either identification of susceptibility factors or to support the development of more rationale therapies, including the implementation of pharmacogenetic approach.

In Alzheimer's disease, patients CYP2D6 polymorphism has been shown to be involved in the oxidative metabolism of many different classes of commonly used drugs including donepezil. The data obtained in 51 AD patients indicates that a genetic variant associated to a slower pattern of metabolization is associated with later onset of the disease, suggesting that the pattern of metabolization of xenobiotics and drugs influences the risk for the disease onset.

In women undergoing epidural after labouring, we have performed the evaluation of 40 DNA samples. Genetic analysis of polymorphisms 118A>G, gene OPRM1, and val150met, gene COMT, has shown allele frequencies of 0,538 and 0,463 for val158 and 158met; and 0,837 and 0,162 for A118 and 118G variants, respectively. This is a preliminary, but original study that observed the frequency variation according to secondary effects such as "pruritus".

The MRC activity and mtDNA copy number following the incubation of human cells with the anaesthetic propofol have been evaluated. The results show that propofol does not inhibit OXPHOS pathway or induce decrease of cell viability. But elevated concentrations of propofol have an impact on mitochondrial genome replication, inducing a significant reduction of mtDNA content.

A new line of research related to pharmacogenomics approach in lung cancer and chronic pain, was also initiated.

4. Dermatology Research

Margarida Gonçalo (HUC), Américo Figueiredo (FMUC, HUC), Teresa Cruz (FFUC, CNC), Rosário Domingues (UA), Pedro Domingues (UA), Celeste Lopes (FFUC, CNC)

4.1. Contact dermatitis

Allergic contact dermatitis, which can affect more than 15% of the population, and delayed exanthematic drug eruptions, frequently observed in Dermatology clinics, are T-cell mediated skin reactions that present some diagnostic and therapeutic problems. The pathomechanism of these reactions is not fully understood, namely in which considers the sensitizing phase that involves antigen presentation by dendritic cells and which is absolutely necessary for further specific T cell recognition and effector function.

In allergic contact dermatitis the knowledge of how the different contact allergens interact with skin dendritic cells, which receptors/molecules they use for being recognized, which intracellular signalling pathways, genes and nuclear transcription factors are activated and how they modify the membranar expression of adhesion and activation molecules, cytokine and chemokine receptors and how they modify the release of cytokines and chemokines that promote DC maturation and migration to induce T cell sensitization, is extremely important to find pathways to prevent this process and therefore reduce sensitization to chemicals that regularly contact the skin. In collaboration with the Dermatology Department of the University Hospital of Coimbra and the Chemistry Department of the University of Aveiro, we are investigating the effect of chemical sensitizers and irritants on different intracellular signalling pathways. We intend to disclose whether sensitizers could be differentiated from irritants based on a specific signalling pathway profile and we observed that p38MAPK and JNK are triggered only by sensitizers and not irritants. Furthermore, we are also studding the modulation of the lipid profile of DC during maturation. Our results indicate that LPS and chemical sensitizers increased ceramides and decreased the expression of the sphingosine 1 phosphate receptor 1.

PUBLICATIONS

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5. Arthritis research

Fernando Judas (HUC, FMUC), Alexandrina Mendes (FFUC, CNC), Ali Mobasheri (U.Nottingham,U.K.), Celeste Lopes (FFUC, CNC), José António Pereira da Silva (HUC, FMUC), Luis Graça (IMM), João Eurico da Fonseca (IMM), Margarida Carneiro (CNC)

5.1 Inflammation

In collaboration with the Orthopedic and Bone Bank Departments of HUC, we are using normal and osteoarthritic (OA) human articular cartilage and chondrocytes to 1) establish cryopreservation protocols that improve the clinical outcome of implanted osteochondral allografts; 2) identify cellular and molecular mechanisms relevant in OA pathogenesis that can be translated into new therapeutic strategies; and 3) identify compounds in essential oils with potential anti-osteoarthritic activity.

The results of our studies show that 1) arbutin is more effective as a cryoprotective agent than DMSO or glycerol and that combination with these agents does not further increase chondrocyte cryoprotection; 2) hyperglycemia-like conditions shift OA chondrocytes towards a pro-catabolic and anti-anabolic phenotype, providing a link between metabolic disorders and OA development and progression, 3) in aged/OA chondrocytes, regulation of glucose transporters by ATP-dependent K^+ channels is defective, suggesting that pharmacological modulation of these channels may restore the ability of aged/OA chondrocytes to cope with hyperglycemia-like conditions; and 4) pinane-derived oxygenated compounds are potent NF-kB inhibitors. Current work is underway to further elucidate their mechanism of action and their potential as disease-modifying osteoarthritis drugs.

PUBLICATIONS

Neves A, Rosa SC, Gonçalves J, Rufino A, Judas F, Lopes MC, Salgueiro L, Cavaleiro C, Mendes AF. (2010) Screening of five essential oils for identification of potential inhibitors of IL-1-induced NF-kB activation and NO production in human chondrocytes: characterization of the inhibitory activity of a-pinene. *Planta Med.* 76(3):303-308.

5.2. Studies on rheumatoid arthritis

In 2010 our team, in collaboration with the Department of Rheumatology of the Hospitais da Universidade de Coimbra and the Cellular Immunology Group at IMM has published a new therapeutic strategy for chronic polyarthritis by depleting circulating CD8 T lymphocytes in arthritic K/BxN mice. Additionally, we carried out an extensive study of the CD8+ T lymphocytes in the peripheral and synovial fluid of rheumatoid arthritis patients with different stages of disease activity.

Together with the Rheumatology Group of IMM we have published several studies addressing the role of cytokines, neutrophils and B lymphocytes in early stages of rheumatoid arthritis.

Overall these collaborations created a dynamic exchange of ideas and patient samples, allowing a multifaceted view into the pathogenesis of rheumatoid arthritis, and paving the ground for further collaborative projects.

PUBLICATIONS

Cascão R, Moura RA, Perpétuo I, Canhão H, Vieira-Sousa E, Mourão AF, Rodrigues AM, Polido-Pereira J, Queiroz MV, Rosário HS, Souto-Carneiro MM, Graca L, Fonseca JE. (2010) Identification of a cytokine network sustaining neutrophil and Th17 activation in untreated early rheumatoid arthritis. *Arthritis Res Ther*. 12(5):R196.

Raposo BR, Rodrigues-Santos P, Carvalheiro H, Agua-Doce AM, Carvalho L, Pereira da Silva JA, Graça L, Souto-Carneiro MM. (2010) Monoclonal anti-CD8 therapy induces disease amelioration in the K/BxN mouse model of spontaneous chronic polyarthritis. *Arthritis Rheum.* 62(10):2953-62.

Moura RA, Weinmann P, Pereira PA, Caetano-Lopes J, Canhão H, Sousa E, Mourão AF, Rodrigues AM, Queiroz MV, Souto-Carneiro MM, Graça L, Fonseca JE. (2010) Alterations on peripheral blood B-cell subpopulations in very early arthritis patients. *Rheumatology (Oxford)*. 49(6):1082-92.

Cascão R, Rosário HS, Souto-Carneiro MM, Fonseca JE. (2010) Neutrophils in rheumatoid arthritis: More than simple final effectors. *Autoimmun Rev*. 9(8):531-5.

6. Research in brain cancer

Alberto Orfão (CSIC, Univ. Salamanca), Fernando Gomes (HUC), Hermínio Tão (HUC), Olinda Rebelo (HUC), Celeste Lopes (FFUC, CNC)

6.1. Studies on genetic heterogeneity of gliomas

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

The project entitled "Intratumoral 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 seem to indicate that the activation of PI3K/Akt, MAP kinase and CXCR4 signaling pathways may contribute to the chemoresistance that characterizes gliomas.

Presently, genome-wide allelotyping is being performed in gliomas and this analysis will facilitate the identification of new genetic/chromosomal changes, relevant for the understanding of the pathogenesis of the disease.

PUBLICATIONS

Vital AL, Tabernero MD, Crespo I, Rebelo O, Tão H, Gomes F, Lopes MC, Orfão A. (2010) Intratumoral patterns of clonal evolution in gliomas. *Neurogenetics*. 11(2):227-39.

Vital AL, Tabernero MD, Crespo I, Rebelo O, Tão H, Gomes F, Oliveira CR, Lopes MC, Orfão A. (2010) Gene expression profiles of human glioblastomas are associated with both tumor cytogenetics and histopathology. *Neuro-Oncology* 12(9):991–1003.

Carmo A, Patricio I, Cruz MT, Carvalheiro H, Oliveira CR, Lopes MC. (2010) CXCL12/CXCR4 promotes motility and proliferation of glioma cells. *Cancer Biol Ther*. 9(1):56-65.

7. Yeast nosocomial infections

Cidália Pina-Vaz (FMUP, Hospital S. João), Acácio Gonçalves Rodrigues (FMUP, Hospital S. João), Elizabete Ricardo (FMUP), Teresa Gonçalves (FMUC, CNC)

Worldwide, in the last two decades, invasive fungal infections in hospitalized patients have increased significantly. According to data obtained from USA and Europe Candida species are, respectively, the 4th and 6th cause of systemic infections related to healthcare, representing 8 to 15% of the hospital-acquired sepsis. Associated to this type of infections are high morbidity and mortality rates. Although *C. albicans* is the most prominent agent of these infections, other species assume particular importance due to the inneficency of the available therapeutic tools. From 2008 until 2010 a study was undertaken under the leadership of the Faculty of Medicine of the University of Porto and the Hospital de S. João, in Porto. The main objective of this research programme was to type yeast isolates using estriction endonuclease analysis (REA) of mtDNA, in order to trace and prevent possible yeast infection outbreaks.

Eighteen C. krusei strains were isolated from 3 different patients, from several biological products, admitted at a neutropenic unit, within a short period of time (2 months). Patients B and C were admitted consecutively in the same room. All patients were submitted to fluconazole prophylaxis. Additionally, two environmental C. krusei strains were collected from the two room surfaces (patient's 1 and 2 rooms) and one C. krusei isolate was recovered from the blood of patient A, two years before this study. The susceptibility profile of the C. krusei isolates was evaluated. Total DNA was extracted and digested with the restriction enzyme Hinf I. All strains were fluconazole resistant, as expected. Isolates from different patients were unrelated. Isolates from different biological products of the same patient showed indistinguishable Hinf I restriction patterns. Isolates obtained from surrounding environment were similar to the one obtained from the respective patient. Using this strategy it was possible to exclude the hypothesis of an outbreak; endogenous reservoir strains could invade, and contaminate the environment or even last for long periods of time in the patient which should be taken into account when planning antifungal therapy.

PUBLICATIONS

Ricardo E, Silva AP, Gonçalves T, Costa de Oliveira S, Granato C, Martins J, Rodrigues AG, Pina-Vaz C (2010). Candida krusei reservoir in a neutropaenia unit: molecular evidence of a foe? Clin Microbiol Infect (DOI:10.1111/j.1469-0691.2010.03223) *Accepted for publication*

7.1 Oral yeast carriage in type I diabetic children

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

Diabetes is a condition that favors the occurrence of oral yeast infections, usually due to elements of the normal flora of patients. This collaboration, under the leadership of Faculty of Medicine (FMUC), aims to characterize the yeast species of normal and type I diabetic children, together with the yeast load in each individual. In this study, undertaken from 2009 until 2010, the Medical Mycology Yeast Research Group was responsible for the identification of the yeast isolates obtained from saliva and mucosal specimens from 200 patients. In these specimens it was quantified the yeast load, using a CFU based methodology. The identification procedures used were molecular biology based.

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

Forty percent of the children had no yeasts either in the saliva or in the mucosal surface; only 40% were colonized by yeasts in the mucosa. The number of diabetic patients with no yeasts in the oral mucosa was higher than that of control subjects. Diabetics with a poor metabolic control had a higher number of yeast cells in the mucosa. The most prevalent yeast was Candida albicans; the biodiversity was higher in saliva than in mucosa. Patients inhabiting rural areas exhibited a higher yeast biodiversity. Oral hygiene was found to be determinant on the clearance of non-C. albicans colonizing the mucosa. The T1D children had higher levels of CD4+T-cells in their saliva than control subjects. The higher level of CD4+ cells in the saliva of these patients was correlated with a lower passive colonization by yeast cells.

PUBLICATIONS

Ana Luísa Costa, Branca Silva, Rui Soares, Diana Mota, Vera Alves, Alice Mirante, João Carlos Ramos, João Maló de Abreu, Manuel Santos Rosa, Teresa Gonçalves (2010) Type 1 diabetic children oral yeast carriage: a question of metabolic control? *Submitted*

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

Graça Rocha (CHC, FMUC), A. Meliço-Silvestre (HUC, FMUC), Teresa Gonçalves (FMUC, CNC)

The biological functions of HIV-1 Vpr have been involved in the replication and pathogenesis of the virus. Infants with perinatal acquired HIV-1 infection have widely variable courses, the long-term non-progressors and fast progressors. The aim of this ongoing work is to study the correlation, in a population of perinatal infected children, between the Vpr variant present and disease progression. The HIV prevention requires an efficient HIV testing and counselling. Once a patient is declared infected, the monitoring of the virus is required in order to initiate antiretroviral (ARV). This clinical decision is based mainly on the CD4+ T-cell counts, even in low resource settings. However, a CD4 cut-off has not been established and normal CD4 cell counts may differ based on race, gender ethnicity and geography. People newly infected with HIV have widely variable courses. The long-term non-progressors, should not need Highly Active Antiretroviral Therapy (HAART), and remain asymptomatic for over 5 years, while in the fast progressors the therapy should immediately be initiated, once the HIV infection is detected. ARV therapy, particularly HAART, results in noxious side effects, especially in children, since their bodies are still developing and they are likely to be exposed to HAART for prolonged periods of time, increasing the vulnerability to colateral complications. In order to avoid HAART side effects, the ideal diagnosis test would include not only the HIV screening but also a tool to recognize the virus variant in what regards the potential disease progression.

In this study 31 perinatal HIV-infected children have been included, since 2005. The HIVvpr gene was amplified and sequenced. The children were clinically evaluated the disease progression evaluated by several clinical markers such as haematological parameters, viral load, development delay, opportunistic infections, other pathophysiological conditions.

The analysis of Vpr sequences in 31 patients showed that 11 carried the mutation R77Q. At the time of first medical appointment the children infected with the Vpr variant carrying the mutation showed lower viral load than children with no mutation. During the period considered (2005-2010) these children remained with no clinical signs of disease and with no need of aggressive therapy.

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

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

Teresa Almeida Santos (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 and use it to select the best sperm have been published (below).

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

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

PUBLICATIONS

Amaral A, Ramalho-Santos J. (2010) Assessment of mitochondrial potential: Implications for a correct monitoring of human sperm function*. Int. J. Andrology* 33: e180-e186.

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

9. Research in Retinitis pigmentosa: Identification and recruitment of patients with retinitis pigmentosa associated with mutations in RHO

Maria do Rosário Almeida (CNC), Eduardo Silva (IBILI-FMUC, HUC)

Retinitis pigmentosa (RP) is included in the group of inherited retinal diseases characterized by photoreceptores (cones and rods) and retinal pigment epithelium degeneration. Clinically this disease is characterised by retinal pigments accumulation and progressive loss of peripheral vision. It is mainly inherited by an autosomal dominant manner, and this form is responsible for 25% of all cases of RP. Mutations in the rhodopsin gene (*RHO*) are the most frequent cause of this form of RP. Therefore, the molecular diagnosis of these patients appeared to be crucial to understand the evolution of the disease and to establish genotype-phenotype correlations. Thus, the main aim of the present work was the implementation of the molecular diagnosis of patients with RP, associated with *RHO* mutations. This work, was part of a research project designated *"Integrated analysis of the degeneration of rods and cones associated with mutations in the rhodopsin gene,"* funded by the Foundation for Science and Technology (FCT) (E-Rare2/SAU/0001/2008). This project gathers six groups composed of clinicians and scientists specializing in inherited retinal diseases from different countries who are organized in five different and complementing workpackages. The workpackage 1 was carried out by the group and concerns the *"Identification and recruitment of Rod-cone dystrophies (RCD) patients with mutations in the major gene RHO leading to dominant forms of RCD"*. In the present work, 50 patients have been studied for mutations analysis in *RHO* by amplification of the entire coding region of this gene, followed by direct sequencing. Four variants have been identified in this sample set. Two of them, 5'UTR-26A> G and IVS3+4 C> T, have been previously described in literature and have been considered common polymorphisms. The two remaining variants, p.Tyr60His and p.Arg69Cys located in exon 1, are new variants, never described before. Consequently, additional studies have been initiated in order to confirm the pathogenic nature of these variants. It should be noted that, the identification of mutations in this gene contributes to an early diagnosis of the patients as well as their family members at high-risk to develop RP. In addition, it also contributes to the establishment of genotype-phenotype correlations in order to improve the prognosis and *follow-up* of these patients.

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 jointly with laboratories abroad

Neuroscience and Disease

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

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

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

Cognitive function in animal models of Alzheimer's disease. Gemma Casadesus (Department of Neurosciences, Case Western Reserve University, Ohio, USA); Paula Moreira (CNC, Portugal).

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. Pablo Pandolfo, Reinaldo Takahashi (Universidade Federal de Santa Catarina Florianópolis, Brazil); Attila Köfalvi, Rodrigo Cunha (CNC, Portugal).

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

Effect of hypoxia in the rodent brain. Joseph C. LaManna (Department of Physiology & Biophysics, Case Western Reserve University, Ohio, USA); Paula Moreira (CNC, Portugal).

Endocannabinoid signalling in Alzheimer's disease. European Neuroscience Institute (University of Aberdeen, United Kingdom, Department of Medical Biochemistry and Biophysics, Division of Molecular Neurobiology, Karolinska Institutet, Sweden); Attila Köfalvi (CNC, Portugal).

Endovanilloids controlling striatal input terminals. László Köles (Dept. Pharmacology and Pharmacotherapy, Semmelweis Medical University, Budapest, Hungary); Attila Köfalvi (CNC, Portugal).

Genetic modification of CB1 cannabinoid receptors. Catherine Ledent (IRIBHN, Université libre de Bruxelles, Brussels B-1070, Belgium); Attila Köfalvi (CNC, Portugal).

Glucocorticoids trigger frontocortical endocannabinoid release inducing the extinction of aversive memory. European Neuroscience Institute (University of Aberdeen, United Kingdom, Department of Medical Biochemistry and Biophysics, Division of Molecular Neurobiology, Karolinska Institutet, Sweden); Reinaldo Takahashi (Department of Pharmacology, Center of Biological Sciences, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil); Attila Köfalvi (CNC, Portugal).

Mapeamento do papel metabólico e neuromodulador da insulina no hipocampo. Lisiane Porciuncúla, Gabriela Ghisleni, Jean-Pierre Oses, (Universidade Federal do Rio Grande do Sul, Brazil); Attila Köfalvi, Rodrigo Cunha (CNC, Portugal).

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

Mitochondrial dysfunction and oxidative stress in Alzheimer's disease. George Perry (College of Sciences, University of Texas at San António, USA); Paula Moreira (CNC, Portugal).

Mitochondrial fission and fusion in Alzheimer's disease. Mark A. Smith, Xiongwei Zhu (Department of Pathology, Case Western Reserve University, Ohio, USA); Paula Moreira (CNC, Portugal).

Mitochondrial metabolism in Parkinson's disease impairs quality control autophagy. Ana Maria Cuervo (Department of Developmental and Molecular Biology at the Albert Einstein College of Medicine, New York, USA); Sandra Morais Cardoso (CNC, Portugal).

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

Modulation of norepinephrine and acetylcholine release in the prefrontal cortex by CB1 cannabinoid receptors and its interaction with α2-adrenoceptors. Beáta Sperlágh (Institute of Experimental Medicine, Budapest, Hungary); Attila Köfalvi (CNC, Portugal).

NAP rescue mitochondria function in Parkinson's disease. Illana Goze (Tel Aviv University, Israel); Sandra Morais Cardoso (CNC, Portugal).

Neuroprotective role of insulin and IGF-1 against Huntington's disease-associated diabetes in vitro and in vivo. Patrik Brundin, Jia-Yi Li, Maria Björkqvist (Wallenberg Neuroscience Center, Section for Neuronal Survival Department of Physiological Sciences, University of Lund, Sweden); Ana I. Duarte (CNC, Portugal).

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

Nurr1 and GDNF genetic modification of mice adult neural stem cells and human cells derived from the umbilical cord – replacement cell therapy in a Parkinson's disease mouse model Research collaboration. Ernest Arenas (Stem Cell Neurobiology Unit, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden) Ana C. Rego (CNC, Portugal).

Papel da Comunicação intercelular entre células endoteliais e celulas estaminais neurais na "stemness" e a neurogénese: novos alvos terapeuticos para a reparação cerebral. Florence Hofman, Thomas Chen (University of Southern California, Los Angeles, USA); Alexandra Rosa, Fabienne Agasse (CNC, Portugal).

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

PGC-1alpha role in mitochondrial function and its contribution to AD neurodegeneration. Russell H Swerdlow (University of Kansas, Neurology, Kansas City, USA); Sandra Morais Cardoso (CNC, Portugal).

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

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

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

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

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

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

Role of nucleus ataxin-3 on mitochondrial function – implication for neurodegeneration in Machado-Joseph disease. Henry L. Paulson (Department of Neurology, University of Michigan Health System, Biomedical Sciences Research Building, Ann Arbor, Michigan, U.S.A.); Mário Laço (CNC, Portugal).

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

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

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

The neuronal ischemic response through Ca^{2+}-permeable AMPA receptors: genetic expression profile and mechanisms of receptor trafficking. Luís Miguel Martins (Cell Death Regulation Laboratory, MRC Toxicology Unit, Leicester LE1 9HN, UK); Armanda Santos (CNC, Portugal).

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

The therapeutic role of the non-psychoactive CB2 receptor agonists and COX-2 inhibitors in metabolic alterations in Alzeimer's disorder. María L. de Ceballos (Instituto Cajal, CSIC, Doctor Arce, 37, 28002 Madrid, Spain, CIBERNED): Attila Köfalvi (CNC, Portugal).

Toxic pathways triggered by activation of Ca^{2+}-permeable AMPA receptors. Lloyd Greene (Dept. of Pathology, Columbia University Medical Center, New York, USA); Jonhatan Ham (Institute of Child Health, University College of London, London, UK); Armanda Santos (CNC, Portugal).

Unraveling the role of SIRT3 in Parkinson´s disease. Marcia Haigis (Harvard Medical School, Boston, USA); Sandra Morais Cardoso (CNC, Portugal).

Molecular Biotechnology and Health

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

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

Advancing the field of drug delivery – combined targeted treatments against human breast cancer and human leukemia (The OncotargetNanoMed network. María Jesús Vicent (Centro de Investigación Príncipe Felipe, Medicinal Chemistry Unit, Polymer Therapeutics Laboratory, Valencia, Spain); João Nuno-Moreira (CNC, Portugal).

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

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

Cell and Tissue Engineering. Robert Langer (MIT); Joaquim Cabral, Cláudia Lobato (IST); Lino Ferreira (CNC, Portugal).

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

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

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

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

Development of a mucosal hepatitis B vaccine: Design and mechanisic studies of a prototypic multi-component delivery. Gerrit Borchard (University of Genève, Switzerlan and Centre Pharmapeptides, Archamps, France); Olga Borges (CNC, Portugal).

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

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

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

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

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

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

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

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

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

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

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

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

Cell and Molecular Toxicology

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

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

Cancer Stem Cell Responses to DNA Damage. Edward Perkins (Mercer Univ., USA); Paulo Oliveira (CNC, Portugal).

Cadmium and carcinogenesis. Marc Chanson (Foundation for Medical Research, Switzerland); Mª Carmen Alpoim (CNC, Portugal).

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

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

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

Effects of caffeine consumption on streptozotocin-induced diabetic rats. Rolf Gruetter (EPFL, Lausanne); Rui Carvalho (CNC, Portugal).

Electrochemical identification of reactive oxygen and nitrogen species in cellular and animal models of vascular injury. (Luso-Spanish co-joint action) with Santiago Lamas (Centro de Biología Molecular "Severo Ochoa", Madrid, Spain); João Laranjinha (CNC, Portugal).

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

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

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

Metabolic profile of the reperfused working heart. Gary Lopaschuk (Univ. Alberta, Canada); Rui Carvalho (CNC, Portugal).

Mitochondrial Dynamics and Metabolic Diseases. Luca Scorrano (Univ. Padova, Italy); Carlos Palmeira, Anabela Rolo (CNC, Portugal).

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

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

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

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

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

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

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

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

Sirtuins and Nitric Oxide in Mitochondrial Biogenesis. David Sinclair (Harvard Medical School, USA); Carlos Palmeira, Anabela Rolo (CNC, Portugal).

Microbiology

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

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

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

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

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

Biophysics and Biomedical NMR

Characterization of Ga-based chelates as tracers for PET imaging. Frank Roesch (Institute of Nuclear Chemistry, Johannes Gutenberg Universitaet, Mainz, Germany); Carlos Geraldes (CNC, Portugal).

Chemical and in vivo animal characterization of MRI CAs for Alzheimer's disease. Eva Tóth (Centre de Biophysique Moléculaire, CNRS, University of Orleans, France); Carlos Geraldes (CNC, Portugal).

Effect of Transaldolase Enzyme Pathway on Gluconeogenesis in People with Prediabetes. Robert Rizza, Rita Basu (Rizza Laboratory at Mayo Clinic); John Jones (CNC, Portugal).

Functionalized Iron oxide and silica nanoparticles as targeted MRI contrast Agents. Robert Muller (University of Mons-Hainaut, Belgium); Carlos Geraldes (CNC, Portugal).

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

In vitro and in-vivo effects of vanadium-based insulin-mimmetic agents – sties using MRS and MRI. Sebastian Cerdán, Pilar Lopez-Larrubia (CSIC, Universidade Autónoma de Madrid, Espanha); Carlos Geraldes (CNC, Portugal).

Lanthanide binding tags for NMR of proteins: exploiting paramagnetic shifts and residual dipolar couplings. Claudio Luchinat (CERM, Universidade de Florença, Itália); Carlos Geraldes (CNC, Portugal).

NMR and relaxometric characterization Gd-based MRI Contrast Agents. Ivan Lukes (Charles University of Prague, Chech Republic); Carlos Geraldes (CNC, Portugal).

NMR and relaxometry of Gd- complexes and nanoparticles as MRI CAs. Joop Peters (TUDelft, Netherlands); Carlos Geraldes (CNC, Portugal).

Relaxometric studies of nanoparticulate MRI contrast agents. Lothar Helm (EPFL, Lausanne, Switzerland); Carlos Geraldes (CNC, Portugal).

Cell and Development Biology

Assessment of genetic heterogeneity in gliomas: impact on the clinical and biological behaviour of the disease. Alberto Orfão (Center for Cancer Investigation, University of Salamanca, Spain); Mª Celeste Lopes (CNC, Portugal).

CD38 and immune regulation. Fran Lund (Rochester University); Mª Celeste Lopes (CNC, Portugal).

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

Characterization of a new mucosatropic HPV type: HPV 108. Ethel de Villiers (DKFZ, Heidelberg, Germany); Mª Celeste Lopes (CNC, Portugal).

Effect of aging and diabetes on testicular organization. Stefan Schlatt (University of Muenster, Germany); João Ramalho-Santos (CNC, Portugal).

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

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

Inflammation and the adipocyte. Janice Zabolotny (Harvard Medical School); Eugenia Carvalho (CNC; Portugal).

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

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

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

Ovarian dynamics and cryopreservation. Teresa K. Woodruff (Northwestern University, USA); João Ramalho- Santos (CNC, Portugal).

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

Sperm metablism and proteomics. Rafael Oliiva (University of Barcelona, Spain); João Ramalho- Santos (CNC, Portugal).

Testicular organization and xenotransplanting of testicular tissue in cats. Stefan Schlatt (University of Muenster, Germany); João Ramalho- Santos (CNC, Portugal).

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

Participation in the organization of scientific meetings

January 2010

Oxidative Stress and Neurodegenerative Diseases. Joint Workshop of the Cost Actions D34 and B35.
Dates: January 15-17, 2010, Lisbon
CNC members involved in the organization: João Laranjinha

Neurosciences Module of the Bio-Engeneering MIT-Portugal Doctoral Programme
Dates: January 18 -26, 2010
CNC members involved in the organization: Rodrigo Cunha

February 2010

Principles and Practice in Drug Development, MIT-Portugal Doctoral Programme
Dates: February 1-12, 2010
CNC members involved in the organization: João Nuno Moreira

May 2010

Annual Meeting of the ENI-NET, Coimbra, Portugal
Dates: May 6-7, 2010, Center for Neuroscience and Cell Biology, Coimbra,
CNC members involved in the organization: Rodrigo Cunha, Attila Kofalvi, Inês Araújo

International Course on Toxicology "Survival or Death as a Matter of Fat"
Dates: 11-14 May , 2010, Coimbra
CNC members involved in the organization: Several Investigators of the Toxicology area at the Centre for Neurosciences and Cell Biology, University of Coimbra, Portugal (Mª Amália Jurado, João laranjinha, Eugenia Carvalho)

IV Luso-Spanish meeting on Free Radicals in Biomedicine and International Symposium on the pathophysiology of reactive oxygen and nitrogen species
Dates: 19-21 May , 2010, Salamanca
CNC members involved in the organization: João Laranjinha

June 2010

Personalized Medicine - MIT-Portugal Program
Dates: June 18, 2010, Biocant Park, Cantanhede
CNC members involved in the organization: Lino Ferreira

Purines 2010 - Adenine Nucleosides and Nucleotides in Biomedicine
Dates: Junho de 2010, Tarragona, Spain
CNC members involved in the organization: Rodrigo Cunha

July 2010

Post-graduate course on "Molecular Mechanisms in Neurodegeneration: From Molecules to Cures", of Harvard Medical School HMS - Portugal Educational Program
Dates: 15-21 July , 2010, Sesimbra
CNC members involved in the organization: Ana Cristina Rego

Molecular Mechanisms in Neurodegeneration: From Molecules to Cures - Harvard Medical School – Portugal Educational Program
Dates: 15-21 July, 2010, Sesimbra.
CNC members involved in the organization: Ana Cristina Rego

September 2010

1st Ibercivis Workshop
Dates: 8-9 September 2010, Institute of Biocomputation and Physics of Complex Systems, Zaragoza, Spain
CNC members involved in the organization: Rui Brito

16th International Charles Heidelberger Symposium on Cancer Research
Dates: September 26-29, 2010.
CNC members involved in the organization: João Nuno Moreira

XXXIV Annual Congress of the Sociedade Brasileira de Neurociências e Comportamento
Dates: 8-12 September, 2010, Caxambú, Brasil
CNC members involved in the organization: Rodrigo Cunha

October 2010

European Neuroscience Campus (ENC) Network post-graduate course at BEB, PhD Programme in Experimental Biology and Biomedicine: "Neurodegenerative disorders – Clinical and neuropathological aspects"
Dates: 11-15 October, 2010, CNC, University of Coimbra.
CNC members involved in the organization: Ana Cristina Rego

November 2010

2nd National Meeting on Medicinal Chemistry
Dates: 28-30 November, 2010, Coimbra, Portugal
Patronage: European Federation of Medicinal Chemistry and Group of Medicinal Chemistry of the Portuguese Chemical Society
CNC members involved in the organization: AJ Leitão, JAR Salvador, JFS Carvalho, MAC Neves, ML Sá e Melo, MM Cruz Silva

Graduate Studies Programme

The Center has continued with its PhD Program in Experimental Biology and Biomedicine (PDBEB), funding for which was renewed by FCT until 2013. The Program includes 12 scholarships for students selected by CNC and funding to organize advanced courses taught by CNC and foreign faculty. Previous efforts to include other graduate students have continued, and in 2009/2010 courses were attended by an average of 24 students, including students enrolled in advanced PhD and Masters Programs at the Faculties of Medicine, Pharmacy and Science & Technology, as well as some students from other Portuguese Institutes. Despite this increase in attendance the focus has continued to be on quality teaching and career mentoring to ensure the best possible results in terms of ongoing student projects. CNC researchers have also taught courses in other Masters and PhD Programs at the University of Coimbra, with which the CNC collaborates either formally or informally. Results of the PDBEB Program have been outstanding in terms of track record, with students publishing in top journals in 2010, including first authorship manuscripts in both Nature Neuroscience and Current Biology. In 2010 joined the European Neuroscience Campus (ENC), together with top institutions in Amsterdam (The Netherlands), Bordeaux (France), Gottingen (Germany) and Zurich (Switzerland). The ENC provides 10 scholarships plus training funds, and each student must work in at least two of the partner institutes. ENC students that choose the CNC will use the PDBEB training framework already in place. For this purpose 5 advanced courses on Neuroscience were organized.

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

Advanced Courses 2010

JANUARY 2010

Immunology
January 4 - 8
Margarida Carneiro
mmargarida.carneiro@gmail.com

Gene Therapy and Nuclear Biology
January 11 - 15
Luis Almeida
luispa@ci.uc.pt

Neurodegenerative Disorders
January 25 - 29
Ana Cristina Rego (*acrego@cnc.cj.uc.pt*), Paula Agostinho (*pagost@cnc.cj.uc.pt*), Cláudia Pereira (*cpereira@cnc.cj.uc.pt*), Luis Pereira Almeida (*luispa@ci.uc.pt*)

FEBRUARY 2010

Drug Development (MIT-Portugal)
February 1 - 12
Joao Nuno Moreira

Molecular Neuroscience
February 22 - 26
Ana Luisa Carvalho (*alc@ibili.uc.pt*), Carlos B. Duarte (*cbduarte@ci.uc.pt*), Cláudia Cavadas (*ccavadas@gmail.com*), Ramiro D. Almeida (*rda2001@med.cornell.edu*)

MARCH 2010

Biomedical Magnetic Resonance: Molecular Imaging and Metabolism
March 2 - 5
Carlos Geraldes (geraldes@bioq.uc.pt), John Jones

Oncobiology
March 8 - 12
Analia Carmo (*analiacarmo@gmail.com*), Joao Nuno Moreira (*jmoreira@ff.uc.pt*)

Lab Rotations
March 15 - 26

SEPTEMBER 2010

Basic introduction to Neurosciences
September 22 - 24
Responsible: Rodrigo Cunha and Cláudia Cavadas

OCTOBER 2010

Neurodegenerative disorders: clinical and neuropathological aspects
October 11 - 15
Responsible: Ana Cristina Rego

Brain Repair – Tinkering with brain damage
October 18 - 22
Rodrigo Cunha, Inês Araújo (*araujo_ines@netcabo.pt*), Attila Köfalvi

Cores CNC
October 25 - 29
Responsible: Bruno Manadas (Mass Spectrometry); Carlos Geraldes (RMN); Isabel Nunes-Correia (Flow Cytometry); Luisa Cortes (Miscroscopy)

NOVEMBER 2010

Molecular and Cellular Neurosciences - From synapse formation to function
November 1 - 5
Ana Luisa Carvalho, Carlos Duarte, Emília Duarte, Ramiro Almeida

Cell Biology of Infection
November 8 - 12
Responsible: Otília Vieira

Biology of Aging
November 15 - 19
Responsible: Sandra Cardoso

Lab Rotations
November 22 - 26

Lab Rotations
November 29 - December 3

DECEMBER 2010

The Complex World of RNA: RNA Regulation in Neuronal Development, Function and Dysfunction
December 9 - 10
Ramiro de Almeida, Ana Luísa Carvalho, Luís Pereira de Almeida, Carlos B. Duarte

Molecular Biotechnology: from molecules to market
December 13 - 17
Responsible: Carlos Faro (*cfaro@biocant.pt*)

Seminars

2010 Series | CNC Auditorium 16:00 h

JANUARY

29.1.2010
A new function of huntingtin in mitosis and neurogenesis.
Sandrine HUMBERT
Institut Curie
Centre Universitaire
Orsay, France

15.1.2010
Gene Therapy Progress: Prospects for moving to Clinic in Neuromuscular Disorders
Brian Kaspar
The Research Institute at Nationwide Children s Hospital
Center for Gene Therapy
700 Children s Drive, WA3010
Columbus, OH 43205, USA
CNC Auditorium, 2nd floor

20.1.2010
Plasticity genes and circuit remodelling in the adult brain
Elly Nedivi
The Picower Institute for learning & Memory
Dept of Brain & Cognitive sciences
Massachussetts Institute of Technology
Cambridge, USA

FEBRUARY

25.2.2010
Intracellular machinery and signaling mechanisms for synaptic plasticity
José Esteban
Centro de Bíologia Molecular Severo Ochoa - CSIC
Universidad Autómona de Madrid
Madrid, Spain
Thursday, February 25 th, 14:00 h

26.2.2010
The Arc of synaptic memory
Clive Bramham
Department of Biomedicine
University of Bergen
Bergen, Norway

26.2.2010
Studies on the Tumour Metabolome: the Warburg Effect and the HIF-1 Pathway
John Griffiths
Cancer Research
UK Cambridge Research Institute
Friday, 11:00 h, BEB Room

MARCH

5.3.2010
The multi-faceted role of creatine in energy metabolism: studies in mouse models
Arend Heerchap
Bergen, Norway
Department of Radiology

12.3.2010
E-cadherin and cancer
Raquel Seruca
IPATIMUP
Cancer Genetics Unit
CNC Auditorium, 2nd floor, March 12th, 11:00h

19.3.2010
Cancer Prevention and Therapy in Mouse Models of Breast Cancer
Teresa Rose-Hellekant
University of Minesota
Medicall School
Duluth, MN, USA
CNC Auditorium, 2nd floor, 14:00h

APRIL

13.4.2010
Selective Targeting of Pediatric Neural Cancer Stem Cells by Telomerase Inhibition
Pedro Castelo-Branco
The Arthur and Sonia Labatt
Brain Tumor Research Center
The Hospital for Sick Children
University of Toronto, ON, Canada
CNC auditorium, 2nd floor, Tuesday, April 13th, 12:00h

19.4.2010
New therapeutic approaches for metastatic colon carcinoma
Dr. Valerie Pierrefite-Carle
Principal Investigator
GéPITOs, UMR 6235 CNRS/UNSA
Faculté de Médecine
France

MAY

7.5.2010
Endocytic Recycling Trafficking and the Primary Cilium
Duarte C. Barral
Faculdade de Ciências Medicas
Universidade Nova de Lisboa

14.5.2010
Fatal redox functions of cytochrome c in mitochondria
Valerian Kagan
University of Pittsburgh, USA

JUNE

4.6.2010
From microarrays to novel mechanisms of brain development and disease
Elva Diaz
Department of Pharmacology
University of California at Davis, School of Medicine, USA

9.6.2010
The Role of Systems Modeling in Drug Discovery and Predictive Health
Eberhard O. Voit
Department of Biomedical Engineering,
Georgia Institute of Technology, USA

JULY

5.7.2010
New therapeutic approaches for metastatic colon carcinoma
Dr. Valérie Pierrefite-Carle
GéPITOs, UMR 6235 CNRS/UNSA
Faculty of Medicine
University of Nice, FRANCE

1.7.2010
Biological properties of organonoselenium compounds
Andreza de Bem
Universidade Federal de Santa Catarina,
Centro de Ciências Biológicas
Florianópolis, SC – Brazil
Thursday, 1st July 12:00 h CNC Auditorium

16.7.2010
Spectraplakins: gigantic cytoskeletal linker molecules as key players in neuronal development
Andreas Prokop
Faculty of Life Sciences
University of Manchester, UK
July 16th, CNC auditorium, 11:30 h

22.7.2010
Targeted delivery of nanocarriers: from diagnosis to therapeutics
Gert Storm
Department of pharmaceutical sciences
University of Utrecht
Netherlands
Thursday, July 22nd, 17:00 h, CNC Auditorium

OCTOBER

15.10.2010
Analysing the molecular pathogenesis of Spinocerebellar ataxia type 3
Thorsten Schmid
University of Tuebingen,
Medical Genetics, Tuebingen,
Germany

1.10.2010
Nitric oxide in pathogenesis of spinal cord injury
N. Lukáčová
Institute of neurobiology, Slovak Academy of Sciences

NOVEMBER

5.11.2010
Mechanisms of Developmental Remodeling at a Thalamic Synapse
Chinfei Chen
Children s Hospital, Boston
Harvard Medical School
Boston, MA, USA

10.11.2010
Coat Proteins – Mechanisms and Physiology
Victor Hsu
Harvard Medical School,
Boston, MA

19.11.2010
Sirtuins as regulators of cellular switches
Prof Dra Marcia Haigis
Department of Pathology
Harvard Medical School

DECEMBER

6.12.2010
Redox signaling and redox-induced post-translational modifications in the vascular endothelium
Santiago Lamas
Centro de Biología Molecular "Severo Ochoa"
campus UAM
Madrid-SPAIN

10.12.2010
Intra-axonal protein synthesis in neurodegenerative disorders / Deciphering the role of local translation in Trisomy 21 mental retardation
Ulrich Hengst
Intra-axonal protein synthesis in neurodegenerative disorders
Columbia University Medical Center
New York, USA
CNC auditorium, 2nd floor, 14:30 h

&

Maria Luz Montesinos
Deciphering the role of local translation in Trisomy 21 mental retardation
Dpto. de Fisiología Médica y Biofísica
Facultad de Medicina. Universidad de Sevilla
SPAIN

17.12.2010
The mistery of MAM fraction
Mariusz R. Wieckowsk
Laboratory of Bioenergetics and Biomembranes,
Department of Biochemistry,
Nencki Institute of Experimental Biology,
Polish Academy of Sciences

14.12.2010
Alterations in Adult Hippocampal Neurogenesis in Transgenic Models of Huntington's Disease
Dr. Joana Gil Mohapel
University of Victoria, Victoria, CANADA

Thesis concludes in 2010

Alexandre Gaspar-Maia
Role of Chd-1 in stem cell pluripotency
28-01-2010
Supervisor: João Ramalho-Santos

Ana Clara Braz Cristóvão
NADPH oxidase 1: a Key player in paraquat induced toxicity in Parkinson's disease
21-09-2010
Supervisor: Emília Duarte, Yoon Seong Kim, Graça Baltazar

Ana Filipa da Costa Simões
Exploring the relationship between the cardiac and the anterior blood/endothelial populations in zebrafish embryos
09-03- 2010
Supervisor: Roger Patient
Co-Supervisor: Ana Cristina Rego

Ana Rita Araújo Santos
Regulation of the proteome by brain-derived neurotrophic factor in hippocampal neurons: protein synthesis vs protein degradation
2010
Supervisor: Carlos Duarte

Ana Sofia Almeida
Conformational and functional alterations on a Aspartic Proteinase promoted by Trifluoroethanol
28-09-2010
Supervisor: Euclides Pires

Cândida Susana Gonçalves da Silva
Knowledge Extraction and Data Mining Strategies for the Analysis of Protein Folding and Unfolding Simulation Data. Transthyretin as a case study.
27-04-2010
Supervisor: Rui Brito

Catarina Pimentel
Investigations of mechanisms underlying growth cone advance and guidance in the model organism Drosophila melanogaster
16-07-2010
Supervisor: Ana Luísa Carvalho, Andreas Prokop

Daniela Maria Barroso de Moura Cipreste Vaz
The Role of Disulphides on Structure, Packing and Stability of Human Interleukin-4
11-10-2010
Supervisor: Rui Brito

Helena Sofia de Azevedo Domingues
Role of microglia in the modulation of T cell immune responses in the pathogenesis of EAE
27-09- 2010
Supervisors: João Malva; Hartmut Wekerle

João Carlos Rodrigues Gomes
VGAT and TrkB cleavage under excitotoxic conditions and in vivo cerebral ischemia: functional implications
2010
Supervisor: Carlos Duarte

João Fernando dos Santos Carvalho
Bioactive sterols. Synthesis, Antitumoral Evaluation and Structure-Activity Studies
14-12-2010
Supervisors: Mª Luisa Sá e Melo, Sérgio Simões

Liliana Simões Mendonça
Novel nanotechnological approaches for Chronic Myeloid Leukemia treatment: Molecular and cellular targeting for gene silencing of overexpressed genes
2010
Supervisor: João Nuno Moreira

Maria Alexandra Barreto Amaral
Human Sperm Mitochondrial Function: Implications for Male (In)Fertility
04-01-2010
Supervisor: João Ramalho-Santos

Marta Susana de Almeida Viegas da Silva
CD38 and immune function: role in infections by intracellular bacteria and systemic autoimmunity.
2010
Supervisors: Celeste Lopes

Nuno Ricardo Santos Loureiro da Silva Ferreira
Protein Conformational Plasticity and Aggregation. Transthyretin and d-Toxin as two case studies.
15-11-2010
Supervisor: Rui Brito

Nuno Penacho Pereira
Development of new cationic liposome-based systems to promote gene delivery: physicochemical characterization and evaluation of biological activity
29-06-2010
Supervisor: Mª Conceição Pedroso Lima

Sandra Filipa Tavares Varum
Role of Mitochondria in stem cell pluripotency
19-05-2010
Supervisor: João Ramalho-Santos

Sílvia Sousa Neves
Suicide gene therapy for oral carcinoma: anti-tumoral activity and cellular and molecular mechanisms
19-04-2010
Supervisor: Mª Conceição Pedroso Lima

Tiago Cardoso Alves
NMR studies on the regulation of glucose and fatty acid oxidation in liver, heart and skeletal muscle in awake rats
24-09-2010
Supervisors: Rui Carvalho, Carlos Palmeira

Vera Lúcia Dantas Nunes Caldeira de Moura
Combining targeted and triggered drug delivery to tumor cells and the tumor microenvironment: a novel strategy on cancer therapeutics
2010
Supervisor: João Nuno Moreira

Master Thesis

Ana Santos
Clonagem, expressão heteróloga e caracterização de uma nova subtilisina microbiana
2010
Supervisor: Carlos Faro

André Soares
Optimizing an expression system to study the intracellular targeting of Chlapsin in Chlamydomonas reinhardtii
2010
Supervisor: Isaura Simões

Rodrigo Santos
Intracellular localization of Promotion of Cell Survival 1, an Arabidopsis thaliana aspartic proteinase – Evidences for its targeting beyond ER
2010
Supervisor: Isaura Simões

Sofia Ribeiro
Insights on the Interaction between Constitutive Disease Susceptibility 1A, an atypical plant aspartic proteinase, and its putative partner NADPH-dependent Thioredoxin Reductase A
2010
Supervisor: Isaura Simões

Patricia Ribeiro
The Prostate Cancer Metabolome.Non-invasive detection and progostic evaluation of cancer
2010
Supervisor: Euclides Pires

Inês Cardoso
Actividade proteolítica em pólenes e relação com doenças alérgicas
2010
Supervisor: Paula Verissimo

Joana Serodio
Purificação e caracterização parcial de um inibidor de tripsina de Allium sativa
2010
Supervisor: Paula Verissimo

Maria Joana Guimarães Pinto
The role of local protein synthesis in FGF22-induced presynaptogenesis
2010
Supervisor: Ana Luísa Carvalho

Luís Miguel Morais Calado de Oliveira Bajouco
Amyloid beta peptide 1-42 oligomers disturb intracellular calcium homeostasis through activation of N-methyl-D-aspartate receptors in Alzheimer's disease – a role for NR2A and NR2B subunits
23-11-2010
Supervisor: Ana Cristina Rego

Carla Maria Nunes Lopes
Peripheral and cerebral metabolic features in an animal model of Huntington's disease expressing full-length mutant huntingtin
29-07-2010
Supervisor: Ana Cristina Rego

Luís Maria Marques dos Santos Bimbo
Targeted delivery of gene silencing molecules to breast cancer Cells
2010
Supervisor: João Nuno Moreira

Sara Varela Amaral
Development of protein and lipid-based systems to promote gene delivery
18-07-2010
Supervisor: Henrique Faneca

João Carlos Ribas de Almeida
Ataxin-3 aggregation and its impact on membrane destabilization
14-09-2010
Supervisor: Conceição P. Lima

Patrícia Celeste Soares Rebelo
Alterações dos receptores A_1 e A_{2a} da adenosina num modelo animal da doença de Parkinson: função neuroprotectora?
2010
Supervisor: Carlos Duarte

Joana Rita Domingues Vindeirinho
Uncovering the neuroprotective role of adenosine in diabetic retinopathy: Alterations in the adenosinergic system
09-09-2010
Supervisor: Paulo Santos

Ana Patrícia Marques
Diferentiation and proliferation of mouse pre-adipocytes: role of neuropeptide Y, DPPIV and hypoxia
10-09-2010
Supervisors: Claudia Cavadas; Joana Rosmaninho-Salgado

Lucilia Silva
Neurotensin effects on skin dendritic cells and fibroblasts: repercussions in wound healing and diabetic wound healning
2010
Supervisor: Eugenia Carvalho

Steve François Carvalho
Effects of Abeta and estradiol on brain mitochondrial oxidative stress and damage
Setembro 2010
Supervisor: Paula Moreira

Diana F.F. Silva
Mitochondria: the common up-stream driver of Abeta and Tau pathology in Alzheimer´s disease
Julho 2010
Supervisor: Sandra Morais Cardoso

Ana Isabel Plácido
Role of endothelial cells on survival of mature neurons. Implications for the etiopathogenesis of Alzheimer's disease
Julho 2010
Supervisor: Claudia Pereira

Isaura Vanessa Antunes Martins
Neuroprotection by leptin and ghrelin against amyloid-beta toxicity in hippocampal neurons. Relevance for therapeutic intervention in Alzheimer's disease
Julho 2010
Supervisor: Claudia Pereira

Sílvia CF Gomes
Toxicity of amyloid-beta peptide in hypothalamic cells and protection by leptin and ghrelin. Implications for the etiopathogenesis of Alzheimer's disease
Julho 2010
Supervisor: Claudia Pereira

Daniel Andrade
From cerebrovascular alterations to therapeutic options in Alzheimer's disease
Março 2010
Supervisor: Claudia Pereira

Outreach Programme

Coordinator: Maria Teresa Girão da Cruz

The Outreach Programme developed by CNC 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 acces to science. The dissemination of scientific information equally contributes to the appreciation of the research activity performed at the CNC. The creation of a Science Communication Office by the CNC is the outcome of the successful outreach programme developed in the past years and the recognition of the importance of an appropriate communication strategy.

In 2010 CNC pursued developing research and training activities relying on national and international networking. Joint projects sharing of equipment and services have been established at local national and international levels.

At local level Biomedical inter-institutional research programmes involve close partnerships with clinicians at HUC, CHC /Paediatric Hospital and IPO, contributing to set up novel clinically relevant services and trials furthering translational research.

Partnerships with neurology, neurosurgery, ophthalmology, dermatology, rheumatology, reproduction, endocrinology, orthopaedics, and psychiatry, at HUC are already established. A close interaction with the development and metabolic disorders department at Paediatric Hospital has been the driving force of specialized services in the field of mitochondrial cytopathies.

At a national level the Neuroscience and Disease, Biotechnology and Health, Microbiology and Biomedical NMR research groups participate in networking activities with IMM, IBMC and ITQB respectively. Networking activities with groups from the Universities of Minho and Porto, involve the Cell and Molecular Toxicology and the Neuroscience and Disease lines. CNC is a member of the National Spectrometry Network (RNEM) and the Portuguese Nuclear Magnetic Resonance Network (PNMR).

At an international level, Networking activities involving several CNC research lines where established with groups from USA, Brasil, and Uruguai.

CNC is a partner of the Network of European Institutes (ENI-NET - *www.eni-net.org*), MIT-Portugal Programme, and since 2009 is a partner of the Harvard Medical School-Portugal Programme and the European Neurosciences Campus Network (ENC-Network - *www.enc-network.eu*).

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.

Brain Awareness Week, March 14-20

BAW 2010 focused on the theme "Art and the Brain". Initiatives were intended both for the general public: 1) "A Scientific Photo Exhibition", which attracted hundreds of visitors, 2) a Baroque music concert commented by experts in neurosciences, psychology, and music; and for the students: 3) "Neuroscientists go to Schools" and 4) "Art and the Brain at the Science Museum", where neuroscientists visited schools and the Science Museum in Coimbra and gave lectures on brain related subjects to high school students; elementary and middle school students performed hands on activities related to the five senses, and 5) "Open Laboratories" where students visited CNC's laboratories and took part in talks about neuroscience research. The total audience was 1625 students.

"Science in the Holidays" Programme, July 12-23

Portuguese high-school students participated in a 10 day programme during Summer Holidays, promoted by Ciência Viva Agency. Adding to visits to facilities and laboratories, students were included in different research groups and had the opportunity to run several molecular/cell biology techniques as part of short projects. The end results were presented publicly at CNC and published at the Ciência Viva web site.

European Researchers' Night, September 24

Together with the Science Museum of the University of Coimbra, CNC took part for the second time in the organization of the activities of the European Researchers' Night. This "edutainement" initiative is promoted by the European Commission in order to bring the public closer to the researchers in a non-scientific environment. The motto for this Night was again "Scientists to the Stage". The researchers were dared to write a play about their daily lives, and to get on stage and perform before a live audience. CNC researchers also took part in the conferences intended for high-school students.

Science and Technology Week, November 22-28

During the Science and Technology week and the National Day for Scientific Culture CNC organized activities in order to promote the direct contact with the public. These activities were intended for high-school and undergraduate students, and the general public who had the opportunity to visit CNC's laboratories on the several open days (five) and listen to conferences about CNC research. The major goal of these activities is to contribute to the public understanding of the science being carried out in Portugal. Approximately 150 people participated in the open days.

Artists in Residence, January - July

CNC joined a national program called "Artists in Residence – Art, Science and Technology". For nine months CNC housed the MARIONET theatre company, who have been doing extensive work on the Art and Science interconnection. The major goal of this initiative is the creation of new ways of communicating with the public, by using the artistic language to explore scientific subjects.

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.

1. BIOCANT

Biocant is a private, non-profit, innovation centre created by CNCB together with the municipality of Cantanhede for technology transfer in biotechnology. Founded 3 years ago, Biocant has grown to become a reference in the field and the catalyst of Biocant Park, the first Portuguese biotechnology park.

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. Biocant expects to spin-out its first company by the end of 2008.

2. Companies operating in Biocant Park

At the present 8 companies operate in Biocant Park: Crioestaminal, GeneBox, GenePrediT, GeneLab, Novexem, Hematos, 4Health and Biocant Ventures. Along with Biocant they form a biotech cluster of excellence, bringing together over 100 researchers, in a unique enabling environment. Linking basic and applied research more closely to successful innovation, Biocant paved the way for a new paradigm of economic development in the Center Region of Portugal.

Core Facilities

At present CNC groups its research Core Facilities under five well defined platforms: Animal House, Flow Cytometry Unit, Microscopy Unit, Mass Spectometry Unit and NMR Spectoscopy Unit.

ANIMAL HOUSE

Head of Unit: Alexandre Pires | *Graduate in Agricultural Engineering and Animal Production, Msc in Laboratory Animal Science*

Head of Facility since 2006

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

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

Animal Room – IVC cages (type II)

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.

Laminar flow chamber

FLOW CYTOMETRY UNIT

Head of Unit: Isabel Nunes Correia | *PhD in Biochemistry Technology (2007) at University of Coimbra*

Head of Facility since 2007

The Flow cytometry Unit provides technical support on flow cytometry both to CNC and external researchers. Currently, it is equipped with a FACSCalibur cell analyser and a separate computer and software to enable researchers to fully analyse their flow cytometry data. For researchers wishing to use flow cytometry in their studies, the unit provides assistance in planning projects, choosing fluorochromes, analyzing experimental results and presenting data.

The Unit organizes annual flow cytometry seminars with the purpose to initiate new users and make this powerful technology known to all researchers, endeavouring to deepen CNC research. Even though the unit has started to operate recently, several CNC research groups are already taking advantage of this facility, performing apoptose, receptor expression and siRNAs intracellular delivery studies, among others.

FACSCalibur cell analyzer

MICROSCOPY UNIT

Head of Unit: Luísa Cortes | *PhD in Enzymology (2006) at University of Coimbra*

Head of Facility since 2007

The Microscopy Unit provides technical support on the investigation made using Light Microscopy. Besides managing the resources, the unit assists in planning microscopy oriented projects, analysing experimental results, processing acquired images and presenting data.

Presently, the unit manages a laser scanning confocal microscope (Zeiss LSM 510 Meta), a P.A.L.M. laser microdissecting microscope, a single cell calcium imaging system, 2 widefield systems and other brightfield microscopes. The systems are prepared for advanced applications which include live cell imaging and single cell calcium measurements, enabling the researchers of imaging dynamic events and molecular interactions.

P.A.L.M. laser microdissecting microscope

The P.A.L.M. laser dissecting microscope is a perfect tool for the isolation of different cell populations within a sample, allowing its full characterization. Using this technology, collaboration has been established, with the service of Anatomical Pathology from the FMUC, with the aim of studying the differences of gene expression between tumour cells at diverse stages

Laser scanning confocal microscope

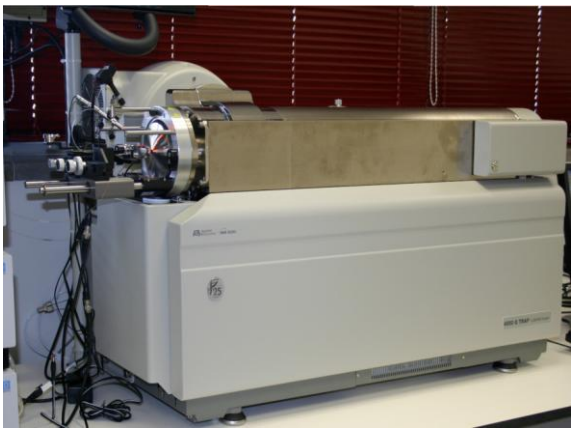
MASS SPECTROSCOPY UNIT

Head of Unit: Bruno Manadas | *Post-Doc, PhD in Cellular Biology (2008) at University of Coimbra*

Head of Facility since 2008

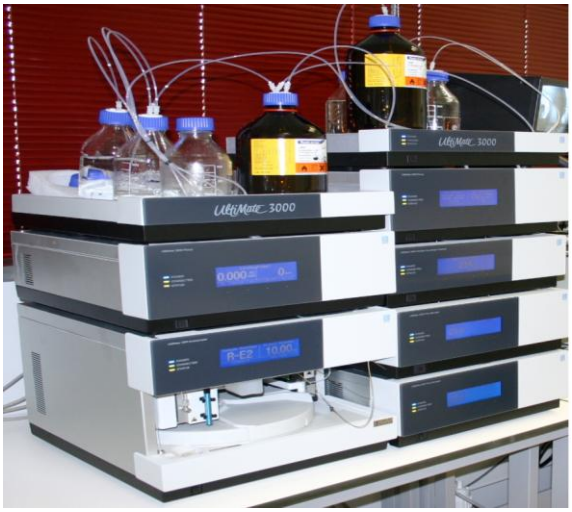
Staff: Vera Mendes (technician)

The Mass Spectrometry Unit is specialized in identification and quantification of proteins from simple and complex samples; identification and quantification of post-translational modifications, and identification and quantification of metabolites. The Unit is also involved in the identification of biomarkers through proteomics and metabolomics techniques with the purpose of developing new prognosis and diagnosis methods, in collaboration with other R&D units at CNC, Biocant, and external partners.



4000 QTRAP mass spectrometer

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 MS^3, a two-dimensional liquid chromatography system Ultimate 3000 (Dionex/LCPackings), a ExQuest (Bio-Rad) – image acquisition and spot picking robot and a data processing station (connected to two data acquisition stations). The unit also contains several software packages for data processing, including PDQuest and ProteomeWeaver for 2D gel analysis, Protein Pilot and PEAKS for protein identification, post-translational modifications and de novo sequencing.



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

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).

NMR SPECTROSCOPY UNIT

Head of Unit: Prof. Carlos Geraldes | *PhD in Inorganic Chemistry (1976) at Oxford University, UK*

Head of Facility since 2008

Staff: Emeric Wasielewski, PhD in Biophysics

The Nuclear Magnetic Resonance Spectroscopy Laboratory provides technical support on analysis of liquid and semi-solid samples by Nuclear Magnetic Resonance (NMR) Spectroscopy and Electron Spin Resonance (EPR) Spectroscopy.

The Unit currently stands with a 600 MHz NMR Spectrometer (Varian VNMR 600), a narrow bore 500 MHz NMR Spectrometer (Varian Unity 500), a 20 MHz NMR relaxometer (Bruker mq20) and an X-band EPR Spectrometer (Bruker ESP 300 E).

The state-of-the art equipment comprise unique package of features that can provide information for NMR structural studies, metabolic studies in ex-vivo biosamples and biopsies. The unit also performs 1D, most 2D and some 3D NMR experiments on small-to-medium sized molecules and characterizes aqueous or non-aqueous samples, like paramagnetic and diamagnetic solutions, and biological tissues. Determine the quality control of various samples of industrial interest, such as water contents in oils, study small paramagnetic complexes and paramagnetic metalloproteins, and execute spin label and spin trap research, are also main areas of significance in our Unit.

This Unit integrates the Portuguese Nuclear Magnetic Resonance Network (PTNMR).

Varian 600 NMR Spectrometer

Services

CNC Laboratório Associado provides the following specialized services to the community particularly to Hospitals and Pharmaceutical industries:

Mitochondrial Respiratory chain (MCR) and Krebs cycle enzymes; Mitochondrial DNA and Nuclear Genome studies in mitochondrial cytopathies; Amino Acid analysis for metabolic disorders diagnosis; Molecular testing of neurodegenerative and vision related genetic diseases; Biomarkers studies in Alzheimer's disease; Mutation screening of the genes *MYH7, MYBPC3, TNNT2, TNNI3, MYL2* in Hypertrophic and Dilated Cardiomyopathy

As a member of the National Spectometry Network and of the Portuguese Nuclear Magnetic Resonance Network, CNC provides yet Mass Spectrometry and NMR services to research and high education institutions and industries.

Biochemical Genetics Laboratory

Coordinators: Catarina Resende Oliveira, Manuela Grazina

Team: Cândida Mendes, Carla Veríssimo, João Pratas, Maria João Santos, Marta Simões

The coordinator of LBG (Manuela Grazina) has established international collaborations, allowing significant developments in the assays performed, namely with Prof. Lee-Jun Wong and Doctor Fernando Scaglia (Baylor College of Medicine, Houston – Texas, USA), Prof. Massimo Zeviani (National Neurological Institute "C. Besta", Milan, Italy), Prof. Robert Taylor (Mitochondrial Pathology, University of Newcastle upon Tyne, UK) and Dr. Rafael Artuch (Hospital San Juan de Dios- Barcelona, Spain).

Additionally, she organized an advanced Course on "Translational bigenomics – from the bench to the bedside", allowing the visit of Prof. Lee-Jun Wong to LBG, which was a valuable step forward for improving genetic diagnosis in LBG.

The visit to the Laboratory of Dr. Rafael Artuch, in Barcelona, allowed the start of implementation of Coenzyme Q10 assay at LBG, as an essential tool for diagnostic of Mitochondrial Respiratory Chain Diseases in Portugal.

Mitochondrial Respiratory Chain (MRC) and Krebs cycle enzymes

There were studied 86 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 98 samples (some patients had 2 or more tissues analysed), in 726 assays, including 52 lymphocytes isolated of peripheral blood, 39 muscular biopsies, 5 liver, 1 heart and 1 kidney samples. A MRC deficiency was detected in 33 patients.

The number of samples decreased, compared to last year, but the number of assays for each sample has augmented.

The analysis of aconitase and alfa-ketoglutarate dehydrogenase was implemented and the screening of Coenzyme Q10 and PDH was initiated.

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

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 is also performed in selected samples, according to clinic manifestations and results from previous screening. Copy number (mtDNA) assays are now part of the genetic mitochondrial genome screening.

We have received 393 samples from 371 patients suspected of Mitochondrial Cytopathy, that represent an increase compared to year 2009, for DNA extraction, including blood (313), muscle (27), liver (5), heart (1), kidney (1) and other (46) tissues, comprising a total of 3771 assays for point mutations and deletions analysis. Deletions have been detected in 23 samples and a total of 590 (166 different) known mtDNA sequence variations and 42 novel variants have been detected in 91/112 samples analysed of 79/96 patients investigated. Further 67 PCR-RFLP analyses were performed to validate point mutations in 38 samples of 30 patients.

We have analysed 3 samples of RNA, extracted from blood, in order to perform reverse transcriptase PCR, for confirming a novel alteration (18 assays).

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.

Concerning mtDNA copy number assays for depletion screening, we investigated 143 samples (162% increase, compared to year 2009) of 42 patients, including blood (64), muscle (46), liver (14), heart (18) and kidney (1) tissues, comprising a total of 2070 real time PCR assays. We have confirmed diagnosis of mtDNA depletion in 8 patients.

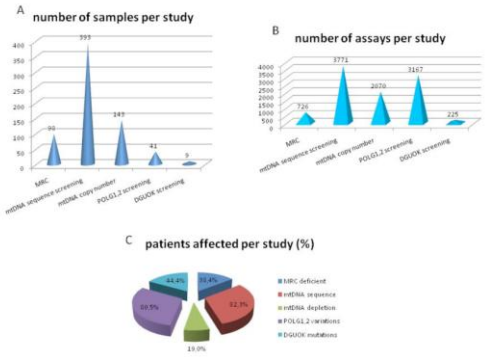


Fig. 1- Number of samples analysed, assays performed and patients affected, according to the study, for biochemical and genetic diagnosis of mitochondrial cytopathies

We have found that mtDNA depletion is about 5 times more frequent than mtDNA pathogenic point mutations in children, allowing us to suggest that mtDNA copy number investigation in children is mandatory relatively to mtDNA point mutation genetic screening. The acquisition of equipment has allowed the screening of a higher number of samples, allowing improvement in genetic diagnosis. Presently, to our knowledge, LBG is the only Laboratory with this assay available in Portugal.

Polymerase gamma (*POLG*) is the only polymerase existing in mitochondria, and is responsible for the constant and exact replication of mitochondrial genome, so that mitochondria have the adequate number of copies of the mitochondrial genome to maintain mitochondrial respiratory chain structure and functions. This polymerase is heterodimeric, and is encoded by 2 different genes, *POLG1* (15q25) and *POLG2* (17q24.1). Currently, it is estimated that 25% of mitochondrial diseases are related to *POLG* genes.

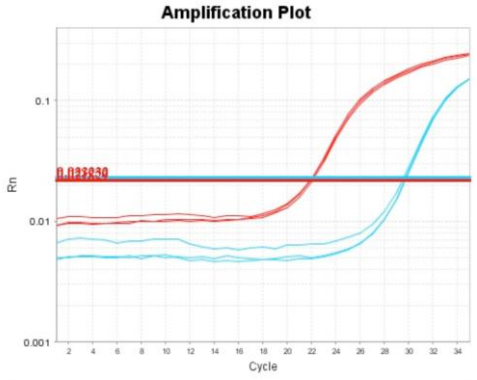


Fig. 2- Diagram of mtDNA depletion detection by real time-PCR

Concerning the screening of nDNA, related to mitochondrial diseases, we have continued *POLG1,2* genes screening in 41 samples of 41 patients, comprising a total of 3167 DNA sequencing assays. We have identified 122 sequence variations (22 different) in 33 patients, that are in characterization for pathogenicity. The acquisition of a sequencer was essential for the improvement of the analysis, but limitations in the personnel did not allow to screen all the samples with requests for this assays, given the huge size of *POLG1* gene.

Deoxyguanosine kinase (*DGUOK*) gene (2p13) encodes a mitochondrial nucleoside kinase, an enzyme responsible for phosphorylation of purine deoxyribonucleosides, therefore mutations in *DGUOK* gene have been shown to cause mtDNA depletion, particularly in cases with hepatic failure and nistagmus. Our aim was to sequence the DGUOK gene exons and splicing regions, in patients diagnosed with MSD in our laboratory, searching for pathogenic causative mutations.

We have implemented DGUOK gene screening, performed in 9 samples of 9 patients (225 assays) and identified the first 4 Portuguese patients (unrelated) with probable pathogenic mutations related to mtDNA depletion, allowing genetic diagnosis and genetic counselling in 4 families.

We have implemented *OPA1* and *OPA3* genetic screening, but no samples were analysed due to limitations in personnel available.

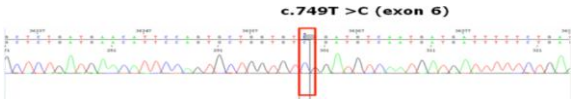


Fig. 3- Electropherogram of pathogenic mutation in DGUOK gene

Amino Acid Analysis

Our laboratory received 426 samples (338 - plasma, 74 - urine and 14 - cerebrospinal fluid) of physiological fluids for amino acid analysis. The patients investigated (children, adolescents adults) were categorized in three clinical conditions: (1) selective screening of metabolic disorder, characterized by either primary or secondary abnormalities in the amino acid profile (2) amino acid profile changes secondary to proximal renal tubular or hepatic dysfunction of any origin; (3) nutritional evaluation of patients with protein restrictive diets. The majority of samples are from children, although less frequently, adults and adolescents are also monitored. Amino acids analysis is a very important approach in early metabolic disorder diagnosis, and frequently helps to prevent mental retardation or even death.

Molecular Genetics of Cardiopathies Laboratory

Coordinators: Catarina Resende Oliveira, Isabel Marques Carreira

Team: Ana Cristina Santos

***Screening* of 540 mutations of 31 genes associated with cardiopathies**

Cardiomyopathies are associated with myocardial dysfunction which varies from an asymptomatic course to heart failure (HF). Hypertrophic cardiomyopathy (HCM) is an autosomal dominant genetic disease which may affect 1 in 500 individuals. It is an important risk factor for sudden death (SD) or HF at any age. Genetic diagnosis is important in all patients with suspected HCM for several reasons: (i) counseling on professional and/or leisure activities; (ii) for genetic counseling; and (iii) to facilitate genetic diagnosis in family members.

During 2010 the name of the laboratory was changed to: "Laboratório de Genética Molecular de Cardiopatias" (LGMC) and a new methodology of diagnosis was introduced to allow the diagnosis of a larger number of cardio disorders besides hypertrophic cardiomyopathy (HCM). The new method studies 540 mutations of 31 genes associated with a cardiopathies instead of the study of the 5 genes previously done. This methodology allows a better genotype-phenotype correlation which brings great benefit for the clinical management of patients.

All *índex* cases are run in a Chip studied *by* MassARRAY. Results of all the mutations are validated by sequencing. In order to determine whether the mutation is *de novo* or familial the study of the parents is always suggested. Extended family members are evaluated in a cardiogenetics consultation and whenever a family member at risk is identified it is referred for study. During 2010, 24 índex cases were studied as well as 17 relatives. All cases were referred after genetic counseling by the consultation of "Cardio-Genética do Serviço de Genética Médica do Hospital Pediátrico de Coimbra".

Neuro-Ophthalmology Genetics Laboratory

Coordinators: Maria do Rosário Almeida

Team: Maria do Rosário Almeida, Maria Helena Ribeiro, Ana Cristina Santos

Molecular testing of Neurodegenerative and Vision related genetic diseases

The molecular genetic diagnostic tests previously established in the laboratory regarding neurodegenerative disorders such as: Frontotemporal Dementia, Familial Alzheimer Disease and Parkinson Disease, have been carried out in a routine basis in a close functional interaction between the laboratory and the clinicians at the Neurology Department of University Hospital of Coimbra (HUC). It is important to mention that during this year the clinical applicability of the provided tests not only contributed to an accurate diagnosis but also to identify the relatives at high risk to develop the disease, in the context of formal genetic counselling. In addition, a new test has been developed by the group and involved the Glucocerebrosidase gene (GBA), in which mutations have been reported to modify risk for Parkinson Disease and Dementia with Lewy bodies (DLB). Another challenge that faced this group was the increasing demand for genetic services within the ophthalmology field, which is also one main interest of this group.

Therefore, the molecular genetic tests to vision inherited diseases, in particularly to Nanophthalmia and Retinitis Pigmentar associated with mutations in the *MFRP* and *RHO* genes, respectively, have been implemented this year. It is important to emphasise that the availability of these genetic tests, gave a significant contribution to an early diagnosis which seems to be essential for successful treatments, when it is still possible to prevent irreversible damages.

10. Neurochemistry Laboratory

Inês Baldeiras (FMUC, CNC), Mª Helena Garruncho (FMUC, CNC)

The Neurochemistry Unit is integrated in the Neurology Department of the University Hospitals of Coimbra (HUC) and develops its activity in essentially two areas: laboratorial support of diagnosis and follow-up of neurological and metabolic diseases and clinical research of neurodegenerative disorders.

In what concerns the immediate support to the patient, the Neurochemistry Unit provides several test that help in the diagnosis and control of progression of neurodegenerative, 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

Cerebrospinal fluid biomarker identification in neurodegenerative disorders, mainly in dementias, has been one of our main areas of research interest. Early and differential diagnosis of dementias is of particular importance as this type of disorders remains, in the present, fatal and without effective treatment.

Accordingly, interventions with curative or stabilization potential would have a huge impact in human health and life expectancy. The Neurochemistry unit is, in the framework of the Portuguese Epidemiological Surveillance Program for Human Prion Diseases, the national reference laboratory for CSF analysis, and it performs:

- Quantification of CSF levels of total-Tau protein, phosphorylated-Tau protein and β-amyloid$_{1-42}$ peptide for dementia diagnosis
- Detection of 14-3-3 protein in CSF in suspected cases of Creutzfeldt-Jakob Disease (CJD)
- Immunodetection of Prion protein isoforms in brain extracts of CJD patients

Characterization of oxidative status in neurodegenerative disorders is also a specific research interest of this unit. In this context, we perform, either in patients blood or in several cellular extracts, the:

- Evaluation of plasma and cellular oxidative stress

This includes the determination of a broad spectrum of non-enzymatic (uric acid, vitamin E, oxidized and reduced glutathione) and enzymatic antioxidants (glutathione reductase and peroxidase), nitrogen oxidative species and lipid (malondialdehyde) and protein (carbonyls) oxidation markers.

During the year 2010, the Neurochemistry Unit has recieved nearly 700 blood and 400 CSF samples and has performed the following analysis:

	Blood (Serum/Plasma)	CSF	Urine	Brain extracts	Other extracts
Cytochemistry and electrophoresis	304	304			
IgG Oligoclonal bands	223	223			
Vitamin A/E	259				
Redox Satus	116	87			
Purines & Pyrimidines			7		
Arylsulfatase A			0		
Anti-neuronal antibodies	73				
Antiepileptic drugs	1				
NABs against INFβ	72				
Tau, p-Tau and Aβ42		160			
14-3-3 protein		75			
Prion protein isoforms				2	
Oxidative Stress	48				333

Funding

In 2010 funding of CNC/LA amounts to 5.522.593, 75 Euros.

This value is based on the certified expenditure and excludes both the salaries of the Academic staff and the individual PhD grants which are paid directly to the students by financing agencies.

The main financing contribution comes from FCT (5.155.253, 40€).

The other contribution are concerned with international projects (242.428, 93€) other national projects (30.564, 48€) and other contribution (services, prizes, etc – 94.292,94 €).

The main financing contribution was made by “Fundação para a Ciência e Tecnologia (FCT)”, concerning global institution programs and national projects, namely amount of 5 155 253,40€ distribuited as follows:

Plurianual 2010:	2 214 274,47€
Projects:	2 045 015,79€
Infrastructures:	206 076,52€
Science Program:	640 000,95€
Doctoral Program:	34 124,95€
Integration Fellowship:	15 760,72€

The related items supported the main part of Center for Neuroscience and Cell Biology costs during 2010.

Besides Center for Neuroscience is financed by other national and international agencies. In 2010 Center for Neuroscience received the amount of 30 564,48€ concerning other national projects and 242 482,93€ concerning international projects.

In the following are listed FCT ongoing projects as well as other national and international projects.

ONGOING PROJECT

Title	Financing Agency	Duration	Budget (CNC)	Expenditure 2010
National Projects:				
"Diagnóstico precoce de Doença de Alzheimer: avaliação de critérios de classificação recente e exploração de novos instrumentos de estudo" Coordinator: Sandra Cardoso	FCT Refª : PIC/IC/83206/2007	01/01/2009 to 31/12/2011	20.280,00	5.123,29
"Avanço na área de entrega de fármacos: terapias combinadas no tratamento do cancro da mama e leucemia (a rede Onco Target Nano Med)" Coordinator: Mª da Conceição Lima	FCT Refª: NANO/NMed-At/0042/2007	01/07/2009 to 31/12/2011	72.069,00	25.395,42
"Rede Nacional de Espectrometria de Massa" Coordinator: Euclides Pires	FCT Refª: REDE/1506/REM/2005	01/01/2009 to 31/12/2011	138.960,42	46.468,99
"Rede Nacional de Ressonância Magnética Nuclear" Coordinator: Carlos Geraldes	FCT Refª: REDE/1517/RMN/2005	01/01/2010 to 31/12/2012	158.237,00	60.243,26
"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 04/05/2012	150.440,00	52.528,21

"Alterações nas vias fisiológicas e mecanismos moleculares reguladores da homeostase energética na obesidade e síndrome metabólico: identificação de novas estratégias e alvos terapeuticos" Coordinator: Carlos Palmeira	FCT Refª: PTDC/SAU-OSM/72443/2006	01/09/2007 to 31/08/2010	156.000,00	43.759,75
"Regulação dos receptores AMPA pela hiperglicémia na retina" Coordinator: Francisco Ambrósio Participants: Faculdade de Medicina da Universidade de Coimbra (FMUC)	FCT Refª: PTDC/SAU-NEU/71228/2006	01/06/2007 to 31/05/2010	40.064,00	8.750,23
"Desenvolvimento de novas estratégias para terapia anti-tumoral baseadas na utilização do peptídeo permeante S413)-PV com o objectivo de potenciar a entrega intracelular de ácidos nucleicos e proteínas com actividade terapêutica" Coordinator: : Mª da Conceição Lima	FCT Refª: PTDC/BIO/65627/2006	01/05/2007 to 30/04/2010	136.000,00	32.976,56
"Nanostructured photoluminescent rare-earth nonotubes and microporous silicates" Coordinator: Carlos Geraldes Participants: Universidade de Aveiro;	FCT Refª: PTDC/CTM/73243/2006	01/12/2007 to 30/11/2010	14.544,00	6.967,12
"Contribuição de subunidades dos receptores N-metil-D-aspartato na disfunção neuronal na doença de Alzheimer" Coordinator: Ana Cristina Rego	FCT Refª: PTDC/SAU-NEU/71675/2006	01/09/2007 to 31/08/2010	99.944,00	31.984,60
"Silenciamento da doença de Machado-Joseph: interferencia de RNA para a ataxina-3 mediada por vectores lentivirais" Coordinator: Luis de Almeida	FCT Refª: PTDC/SAU-FCF/70384/2006	01/07/2007 to 30/06/2010	170.000,00	46.558,54
"Alterações do metabolismo da glicose e lipído por agentes imunossupressores implicações no diagnóstico tratamento da diabetes pós-transplante" Coordinator: John Jones	FCT Refª: PTDC/SAU-OSM/65140/2006	01/10/2007 to 31/12/2010	152.223,00	28.055,88

"Alterações na Microglia e Neurónios do Hipocampo Induzidas por Me-tanfetamina: Papel das Citocinas Pró-inflamatórias e do Neuropeptídeo y " Coordinator: Ana Paula Martins Participants: AIBILI; Faculdade de Farmácia; IBILI;	FCT Refª: PTDC/SAU-FCF/67053/2006	01/05/2007 to 30/04/2010	88.000,00	16.565,79
"Interacção entre a nicotina e a cafeína no núcleo estriado. Relevância na doença de Parkinson" Coordinator: Rodrigo Cunha	FCT Refª: PTDC/SAU-NEU/81064/2006	01/05/2007 to 30/04/2010	94.378,00	5.836,08
"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	17/03/2010 to 17/09/2012	100.000,00	52.969,85
"Mecanismos de plasticidade sináptica e de neuroprotecção pelo BDNF no hipocampo: inibição da neurodegemeração vs. regeneração." Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-FCF/72283/2006	01/05/2007 to 30/04/2010	136.000,00	8.273,35
"Células estaminais da região subventricular na reparação cerebral em epilepsia do lobo temporal." Coordinator: João Malva	FCT Refª: PTDC/SAU-NEU/68465/2006	01/05/2007 to 30/04/2010	148.828,00	15.870,66
"Micro e nano design de materiais com funcionalidades especificas para promover a regeneração de tecido ósseo usando células estaminais adultas." Coordinator: João Nuno Moreira Participants: Universidade do Minho	FCT Refª: MIT/ECE/0047/2009	01/06/2010 To 31/05/2013	32.880,00	0,00
"Novos Mecanismos Mitocondriais Para a Toxicidade Cardioselectiva da Doxorubicina" Coordinator: Paulo Oliveira	FCT Refª: PTDC/SAU-OSM/64084/2006	15/09/2007 to 13/03/2010	115.800,00	5.243,47

“Influência do Estado de Diferenciação Celular na Apoptose Induzida por Isoproterenol em Células Ventriculares Embrionárias H9c2-Vias de Sinalização Envolvidas” Coordinator: Paulo Oliveira	FCT Refª: PTDC/QUI/64358/2006	01/11/2007 to 31/04/2010	85.000,00	2.786,78
“Modelação Quantitativa da Difusão Passiva Trans-Citótica de Moléculas Anfifílicas através da Barreira Hemato-Encefálica “ Coordinator: Armindo Salvador Participants: Faculdade de Ciências e Tecnologia da Universidade de Coimbra; Instituto de Tecnologia Química e Biológica	FCT Refª: PTDC/SAU-FCF/69072/2006	01/07/2007 to 30/06/2010	18.720,00	8.679,03
"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 31/08/2012	126.667,00	26.194,23
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 04/02/2012	96.184,00	15.532,75
“Função da cortactina no tráfego celular dos receptores do glutamato do tipo do tipo AMPA” Coordinator: Ana Luísa Carvalho	FCT Refª: PTDC/BIA-BCM/71789/2006	01/04/2008 to 31/03/2011	89.000,00	20.378,31
“Acções troficas dos factores neuro-tróficos: dependência da coactivação de receptores A2A da adenosina.” Coordinator: Emilia Duarte Participants: Instituto de Medicina Molecular; Fac. Farmácia Univ. Lisboa	FCT Refª: PTDC/SAU-NEU/64126/2006	01/07/2007 to 30/06/2010	29.907,00	17.679,00
“Neuroprotecção pela insulina e IGF-1 na diabetes associada à doença de Huntington” Coordinator: Ana Cristina Rego	FCT Refª: PTDC/SAU-FCF/66421/2006	22/08/2007 to 21/08/2010	124.000,00	31.725,23

"NPwhY - Inervação e angiogénese para o benefício da osteogénese: envolvimento do NPY na regeneração óssea" Coordinator: João Malva Participants: INEB	FCT Refª: PTDC/SAU-OSM/101469/2008	05/02/2010 to 04/02/2013	9.000,00	0,00
"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 30/04/2013	122.336,00	11.809,01
"Vida e morte das células ganglionares da retina: neuromodulação e neuroprotecção pelo Neuropeptídeo Y" Coordinator: Francisco Ambrósio Participants: Faculdade de Medicina da Universidade de Coimbra	FCT Refª: PTDC/SAU-NEU/099075/2008	01/04/2010 to 31/03/2013	51.897,00	10.414,04
"A restrição calórica aumenta a esperança de vida: papel do neuropeptídeo Y na autofagia" Coordinator: Claudia Cavadas	FCT Refª: PTDC/SAU-FCF/099082/2008	01/04/2010 to 31/03/2013	153.150,00	33.079.02
"Efeito da cafeína e dos receptores da adenosina A2A na resposta ao stress: papel da regulação da supra-renal" Coordinator: Claudia Cavadas	FCT Refª: PTDC/SAU-NEU/108110/2008	01/04/2010 to 31/03/2013	90.000,00	19.827,12
"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	193.776,00	72.013,03

“Impacto da metanfetamina na barreira hemato-encefálica: estudo dos mecanismos envolvidos e do papel de neuroinflamação” Coordinator: Ana Paula Silva Participants: Faculdade de Medicina da Universidade de Coimbra	FCT Refª: PTDC/SAU-FCF/098685/2008	01/04/2010 to 31/03/2013	68.490,00	7.529,06
“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 31/03/2013	86.000,00	30.153,28
“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 31/03/2013	168.716,00	20.134,13
“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 21/03/2013	191.757,00	83.930,08
“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 14/03/2013	120.000,00	55.290,53
“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: Fac. Farmácia Universidade de Coimbra	FCT Refª: PTDC/SAU-BEB/103228/2008	01/05/2010 to 30/04/2013	100.000,00	6.235,41

“Clivagem dos transportadores vesiculares do glutamato (VGLUT) e do GABA (VGAT) em condições de excitotoxicidade: identificação dos locais de clivagem e implicações funcionais” Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-NEU/65846/2006	17/04/2007 to 16/04/2010	115.256,00	4.527,64
“Neuropeptídeo Y na retina: porquê? E para quê?” Coordinator: Cláudia Cavadas	FCT Refª: PTDC/SAU-NEU/73119/2006	01/05/2007 to 30/11/2010	123.668,00	30.753,85
"P-found: computação GRID e armazenamento distribuido de dados de simulações de dobragem de proteínas." Coordinator: Rui Brito Participants: Univ. Minho, Faculdade Ciências Coimbra, Faculdade Ciências Tecnologia Univ. Coimbra, Critical Software	FCT Refª: GRID/GRI/81809/2006	01/06/2007 to 31/12/2010	27.545,00	7.939,73
“Estudo de processos de bioluminescência.” Coordinator: Rui Brito Participants: ADDF	FCT Refª: PTDC/FIS/73578/2006	01/07/2007 to 31/12/2010	50.928,00	12.457,64
“Novas funções biológicas de compostos fenólicos do vinho: regulação celular e acção anti-infla-matória via formação de óxido nítrico a partir de nitrito contido na dieta.” Coordinator: João Laranjinha	FCT Refª: PTDC/AGR-ALI/71262/2006	15/05/2007 to 14/05/2010	123.478,00	26.702,41
“Papel e mecanismos moleculares do receptor CD36 na fagocitose de célulasapoptóticas: implicações para a aterosclerose” Coordinator: Otília Vieira	FCT Refª: PTDC/SAU-MII/66285/2006	01/09/2007 to 31/08/2010	159.936,00	45.778,83

“Actividade metabólica e viabilidade do condrócito em enxertos osteocartilagíneos humanos criopreservados.” Coordinator: Celeste Lopes	FCT Refª: PTDC/SAU-OSM/67936/2006	01/09/2007 to 28/02/2011	32.648,83	19.314,71
“Design, synthesis and biological assessment of multifunctional compounds as anti-Alzheimer drugs” Coordinator: Paula Agostinho Participants: Faculdade de Farmácia, Univ. Lisboa	FCT Refª: PTDC/SAU-NEU/64151/2006	01/08/2007 to 31/07/2011	12.740,00	5.973,02
"Modulação das vias metabólicas envolvidas no stress oxidativo mitocondrial em condições de hiperglicémia: sua relevância na prevenção da diabetes." Coordinator: Carlos Palmeira	FCT Refª: PTDC/QUI/72826/2006	01/01/2008 to 31/03/2010	36.000,00	4.483,36
"Searching for high level rules in protein folding and unfolding: from amyloid diseases to protein structure prediction", Coordinator: Rui Brito Participants: Universidade do Minho	FCT Refª: PTDC/BIA-PRO/72838/2006	01/01/2008 to 31/12/2010	39.556,00	12.409,44
"Nanoestruturas endereçadas para imagem molecular médica multimodal." Coordinator: Carlos Geraldes Participants: Universidade do Minho, Faculdade de Medicina Universidade de Coimbra	FCT Refª: PTDC/QUI/70063/2006	01/01/2008 to 31/12/2011	32.352,00	20.813,48
"O Metabolismo enquanto modelador da pluripotência e diferenciação de células estaminais." Coordinator: João Ramalho	FCT Refª: PTDC/EBB-EBI/101114/2008	15/04/2011 to 14/04/2013	147.656,00	20.390,15

"Derivados de Benzazolo Marcados com Fluor - 18 e Tecnécio - 99m para visualização In Vivo de depósitos de Amilóide." Coordinator: Catarina Oliveira Proponente: ITN, IP	FCT Refª: PTDC/QUI-QUI/102049/2008	01/01/2010 to 31/12/2012	4.800,00	3.065,79
"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 30/04/2013	189.624,00	33.211,73
"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: IMM	FCT Refª: PTDC/SAU-NEU/101928/2008	05/02/2010to 04/02/2013	160.000,00	29.603,57
"Optimização da utilização de hidratos de carbono em robalo de aquacultura através de perfis metabólicos." Coordinator: John Jones Participants: Faculdade de Ciências e Tecnologia	FCT Refª: PTDC/EBB-BIO/098111/2008	01/04/2010 to 31/03/2013	179.000,00	32.463,40
"Mechanismos moleculares envolvidos na cicatrização cutânea na diabetes - a importancia de neuropeptídeos." Coordinator: Eugénia Carvalho	FCT Refª: PTDC/SAU-MII/098567/2008	01/05/2010 to 30/04/2013	195.000,00	31.964,85
"Mapeamento do papel metabólico e neuromodulador da insulina no hipocampo." Coordinator: Attila Köfalvi	FCT Refª: PTDC/SAU-OSM/105663/2008	17/03/2010 to 16/03/2012	100.000,00	32.047,10
"Demonstração de que os receptores de adenosina A2A controlam a plasticidade sináptica glutamatergica via dos receptores de canabinóide CB1 no corpo estriado, fornecendo assim alvos terapêuticos atrativos." Coordinator: Attila Köfalvi	FCT Refª: PTDC/SAU-NEU/100729/2008	17/03/2010 to 16/03/2012	91.000,00	29.697,91

"Interacção de Lipoplexos com Membranas Celulares: uma Abordagem Biofísica da Terapia Génica." Coordinator: Amália Jurado	FCT Refª: PTDC/QUI-BIQ/103001/2008	03/05/2010 to 02/05/2013	122.562,00	9.852,08
"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	19.549,04
"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	11.631,76
"Clarificação do Papel Mitocondrial na Cardiotoxicidade da Doxorubicina Usando um Sistema de Perfusão de Corações Intactos - Papel de Diferentes Calendários de Tratamento com Doxorubicina." Coordinator: António Moreno Proponente: Faculdade de Ciências e Tecnologia	FCT Refª: PTDC/SAU-OSM/104731/2008	01/05/2010 to 30/04/2013	65.200,00	24.449,88
"Alimentos Fucncionais para Neuroprotecção: um papel para o Hypericum perforatum." Coordinator: João Malva Proponente: Universidade Minho	FCT Refª: PTDC/AGR-ALI/105169/2008	01/05/2010 to 30/04/2012	6.000,00	0,00
"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 31/13/2013	44.748,00	0,00

"Nanoquimica de compósitos magnéticos/luminescentes para aplicações de diagnóstico médico in vitro" Coordinator: António Guiomar Participants: Universidade de Aveiro	FCT Refª: PTDC/QUI/67712/2006	01/01/2008 to 31/12/2010	15.300,00	6.107,24
"Reconstrução e análise sistémica da rede reaccional de espécies reactivas de oxigénio, azoto e enxofre em sistemas fisiológicos representativos." Coordinator: Armindo Salvador Participants: Fundação da Faculdade de Ciências, Universitat de Lleida.	FCT Refª: PTDC/QUI/70523/2006	01/01/2008 to 31/12/2010	162.752,00	34.302,03
"BIOINK - Aprendizagem incremental de Kernel Machines para análise de dados em bioinformática" Coordinator: Paula Verissimo Participants: Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Instituto Superior de Engenharia de Coimbra.	FCT Refª: PTDC/EIA/71770/2006	01/01/2008 to 31/12/2010	4.200,00	1.144,71
"Proteases de Polens, relevância nas doenças alérgicas." Coordinator: Paula Verissimo	FCT Refª: PTDC/SAU-ESA/72571/2006	01/05/2008 to 30/04/2011	199.850,00	44.638,35
“AspectGrid: Aspectos Grid para Aplicações Científicas” Coordinator: Rui Brito Participants: Universidade do Minho	FCT Refª: GRID/GRI/81880/2006	01/07/2007 to 30/06/2010	10.446,00	9.122,36
“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	75.467,04
“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 30/04/2013	100.000,00	12.410,81

"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 31/05/2013	100.000,00	17.059,88
"HotMetal-Estratégias de resistência a metais pesados e disseminação de resistências a antibióticos nas fontes marinhas hidrotermais" Coordinator: Milton Costa Participants: IMAR	FCT Refª: PTDC/MAR/109057/2008	01/06/2010 to 31/05/2013	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 04/02/2013	199.999,00	39.454,63
"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 04/02/2013	107.000,00	15.437,51
"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 28/02/2012	100.000,00	39.161,50
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 31/03/2013	143.016,00	49.736,35
"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 Participants: Universidade Aveiro	FCT Refª: PTDC/EME-PME/103578/2008	16/03/2010 to 15/03/2013	41.600,00	11.194,56

“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 31/03/2013	175.000,00	20.488,31	
“Mecanismos e propriedades anti-inflamatórias de plantas medicinais: investigação multidisciplinar para a sua validação e utilização como fonte de fitofármacos” Coordinator: Maria Rosete Participants: Universidade Coimbra e Universidade Aveiro	FCT Refª: PTDC/SAU-FCF/105429/2008	01/05/2010 to 30/04/2013	55.800,00	5.637,60	
“Alteração do tráfego intracelular mediado pela mitocôndria na doença de Parkinson” Coordinator: Sandra Cardoso Participants: Instituto de Medicina Molecular	FCT Refª: PTDC/SAU-NEU/102710/2008	05/02/2010 to 02/04/2013	137.589,00	24.222,70	
"Regeneração cardiaca com células vasculares embrionárias e uma matriz biomimética." Coordinator: Lino Ferreira	FCT Refª: PTDC/SAU-BEB/098468/2008	01/04/2010 to 31/03/2013	180.000,00	28.517,30	
“Nanomateriais para detecção de células” Coordinator: Lino Ferreira Participants: Biocant	FCT Refª: PTDC/CTM/099659/2008	01/04/2010 to 31/03/2013	136.000,00	11.328,59	
“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 31/03/2013	120.000,00	39.491,98	

"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 30/04/2013	120.000,00	19.541,84	
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	1.527,98	
"Regulação das proteínas hnRNP pela neurotrofina BDNF: importância da plasticidade sináptica" Coordenador: Carlos Duarte	FCT Refª: PTDC/SAU-NEU/104297/2008	15/09/2010 to 14/09/2013	120.000,00	4.552,88	
Programa MIT Coordinator: Catarina Oliveira, Lino Ferreira	FCT Refª: MIT-Portugal	01/09/2006 to 31/08/2011	854.145,02	98.427,18	
Sub – Total FCT				**2 045 015,79**	
"Histamine in the neural and cancerstem cell niche: a role inglioblastoma ontogeny". Coordinator: Liliana Bernardino e Fabienne Agasse	Fundação Calouste Gulbenkian Refª: 96542	10/11/2008 to 09/11/2011	50.000,00	19.702,02	
"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	10.844,46	
Sub – Total Outros				**30 564,48**	
Total National Projects				**2 075 580,27**	

International Projects:				
"Dissecting mechanisms of neuronal dysfunction in wound healing in diabetes type 1" Coordinator: Eugenia Carvalho	EFSD/JDRF	01/01/2009 to 31/12/2010	100.000,00	47.259,56
"BNOX – The role of reactive oxygen species in B cell tolerization and immune memory". Coordinator: Margarida Carneiro	Marie Curie Actions - 239422 Ref .ª: FP7- PEOPLE– ERG-2008	01/06/2009 to 31/05/2012	45.000,00	33.290,53
"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	13.500,05
"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	25.805,94
"The role of local mRNA translation in synapse formation" Coordinator: Ramiro Daniel Carvalho de Almeida	Marie Curie Actions Refª: PIRG-GA-2009-249288	01/04/2010 to 31/03/2014	100.000,00	30.889,14
"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 29/10/2011	60.000	1.664,66
"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 31/01/2012	376.016,20	90.073,05
Total International Projects				**245 585,68**
TOTAL				**2 318 063,20**

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
Amílcar Falcão	(Full Prof., FFUC)	80
Ana Bela Sarmento Ribeiro	(Assistant Prof., FMUC)	40
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Ledo	(Assistant Inv., CNC)	100
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Silva Martins	(Assistant Inv., FMUC)	Collaborator
Ana Rita Costa Álvaro	(Invited Assistant Prof., UTAD)	60
Anabela Maduro Almeida	(Assistant Prof., Univ. Vasco Gama)	80
Anabela P. Rolo	(Assistant Inv., CNC)	100
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)	80
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	80
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Attila Köfalvi	(Assistant Inv., CNC)	100
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)	80
Célia M. Antunes	(Assistant Prof., FCTUC)	80
Cláudia Cavadas	(Assistant Prof., FFUC)	60
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Daniela Cipestre Vaz	(Assistant Prof., Inst. Polit. Leiria)	70
Elsa Henriques	(Assistant Inv., CNC)	100
Emília P. Duarte	(Assistant Prof., FCTUC)	80

Euclides Pires	(Associate Prof., FCTUC)	60
Eugénia Carvalho	(Assistant Inv., CNC)	100
Fabienne Agasse	(Assistant Inv., CNC)	100
Fernando Monteiro Judas	(Assistant Prof., FMUC)	25
Geanne Matos de Andrade	(Associate Prof., Brasil)	40
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	Collaborator
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 Inv., CNC)	100
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
Isaura Simões	(Assistant Inv., CNC)	100
Ivana Jarak	(Assistant Inv., CNC)	100
João Laranjinha	(Associate Prof., FFUC)	60
João Nuno Moreira	(Assistant Prof., FFUC)	80
João O. Malva	(Principal Inv., FMUC)	80
João Ramalho Santos	(Associate Prof., FCTUC)	80
John Griffith Jones	(Principal Inv., CNC)	100
Jorge António R. Salvador	(Associate Prof., FFUC)	60
José Alberto Correia e Vale	(MD, Univ. Salamanca)	Collaborator
José Custódio	(Associate Prof., FFUC)	80
Leonor Almeida	(Full Prof., FFUC)	60
Lino Ferreira	(Assistant Inv., CNC)	100
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Luís M. Rosário	(Associate Prof., FCTUC)	80
Luís Pereira Almeida	(Assistant Prof., FFUC)	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 Emilia O. Quinta Ferreira	(Associate Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Mª Isabel J. Santana	(Associate Prof., FMUC)	40
Mª Luisa D. Ramos	(Investigator, FCTUC)	80

Mª Luisa Sá e Melo	(Full Prof., FFUC)	60
Mª Madalena Caldeira Santos	(Associate Prof., FCTUC)	80
Mª Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Manuela Carvalheiro	(MD, HUC)	15
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	80
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Mª Otilia Vieira	(Assistant Inv., CNC)	100
Mª Rosário Almeida	(Assistant Inv., CNC)	100
Mª Sancha Santos	(Investigator, FCTUC)	80
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
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
Ramiro Almeida	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Renata Silva	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	80
Rosa M. Santos	(Assistant Prof., FCTUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	30
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui Daniel Schroder Prediger	(Assistant Prof., Brasil)	80
Rui Manuel Reis	(Investigator, Univ. Minho)	Collaborator
Rui M. M. Brito	(Associate Prof., FCTUC)	60
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Assistant Inv., FMUC)	60
Sérgio Simões	(Assistant Prof., FFUC)	80
Sílvia Sousa Neves	(Assistant Prof., FMUC)	40

Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Dinis Silva	(Associate Prof., FFUC)	60
Teresa Gonçalves	(Assistant Prof., FMUC)	40
Teresa Maria C. Martins	(Assistant Investigator, IPO)	80
Tiago Quininha Faria	(Assistant Inv., CNC)	100
Vitor Manuel C. Madeira	(Full Prof., FCTUC)	80

Post-Doc Members

Ana Isabel Duarte	100
Ana Luísa Cardoso	100
Ana Margarida Quaresma Nunes	100
Anália do Carmo	100
Bharathi Pandurangan	100
Bruno O. Manadas	100
Cândida Gonçalves da Silva	70
Carla Nunes	100
Catarina Alexandra Gomes	90
Chakkaravarthi Saravanan	100
Chantal Fernandes	100
Clévio Nóbrega	100
Daniela Pochmann	100
Elisabete Baptista Ferreiro	100
Ermelindo Leal	100
Joana Cardoso da Costa	100
Joana Salgado	100
João Gonçalo Silva Frade	100
João Miguel Neves Duarte	100
Licinia J. Simões	100
Liliana Bernardino	100
Luis Miguel Estronca	100
Luisa Jordão	50
Manuella P. Kaster	100
Marco André Coelho das Neves	100
Margarida Vaz Caldeira	100

Mª Alexandra B. Amaral	100
Mª Teresa Cunha Oliveira	100
Pablo Pandolfo	80
Rosa M. B. Matos Resende	100
Rui Nobre	100
Sandra Catarina G. Amaral	100
Sara Xapelli	100
Susana Isabel E. Alarico	100
Tatiana R. Rosenstock	100
Teresa Delgado	100
Vilma A. Oliveira	100

PhD Students

Alexandra Rosa	100
Alexandre S. Rodrigues	80
Ana Burgeiro	100
Ana C. Fortuna	100
Ana Cristina Gonçalves	40
Ana Carolina Moreira	100
Ana Catarina R. Graça Fonseca	100
Ana Cristina R. Silva	100
Ana Filipe Branco	100
Ana Francisca Lima	100
Ana Francisca Soares	100
Ana Inês R. Crespo	100
Ana Isabel Serralheiro	100
Ana Luísa N. Gomes Nobre	100
Ana Luísa Vital	100
Ana M. Metelo	100
Ana Mª Sequeira Cardoso	100
Ana Paula Ardais	80
Ana Paula Marques de Sousa	100
Ana Patricia S. Gomes	100
Ana Patrícia Simões	100

Ana V. Rafael	100
Ana Raquel Esteves	100
Ana Rita A. Santos	100
Ana Santos Carvalho	100
Ana Sofia M. Leal	100
Ana Sofia V. Cunha	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa I. Varela	100
Ana Teresa Rufino	100
Ana Teresa Simões	100
André F. Martins	100
Ângela Fernades	100
Ângela Inácio	100
António Sales Mano	100
Bárbara Rocha	100
Beatriz Lacerda de Sousa	100
Bruno Carreira	100
Bruno Miguel das Neves	100
Bruno Miguel F. Alves	100
Camile Woitiski	100
Carla Lopes	100
Carla Sofia G. Silva	100
Carla Patrícia R. Paiva	100
Carlos Adriano Matos	100
Carlos Manuel Melo	100
Carlos José Vieira Simões	70
Carlos Samuel M. Boto	100
Carlos Rodrigues	100
Carolina Coelho	100
Cassilda Pereira	100
Catarina Sofia H. Jesus	50
Cátia Diogo	100
Cátia Marques	100
Cláudia Sofia Alves Pereira	100
Cristina Carvalho	100

Cristiana Paulo	100
Cristina Barosa	100
Daniela Gonçalves	100
Daniela M. Arduíno	100
Daniela Pereira S. Alho	100
Diana Jurado S. Serra	100
Diana F. Silva	100
Diana Margarida Carvalho	100
Dulce Bento	100
Elisabete Oliveira Augusto	100
Eva Serrão	30
Filipa L. Carvalho	100
Filipa Lebre	100
Filipe Coreta Gomes	40
Filipe Duarte	100
Filomena Grilo da Silva	100
Gabriel Nascimento Costa	100
Gonçalo Pereira	100
Graciana Tribuna	50
Graciano da Silva Leal	100
Helena Carvalheiro	100
Helena Leitão	100
Helena Sofia Domingues	10
Helena Vazão	100
Hugo Alves Figueiredo	100
Humberto Gomes Ferreira	100
Inês Biscaia Barbosa	100
Inês Vasconcelos M. Santos	100
Inês Violante	10
Igor Clemente Tiago	100
Isabel Maria Santos Onofre	100
Joana Filipa C. Fernandes	100
Joana Ferreira	100
Joana I. Real	100
Joana Paixão	100
Joana Santos Barbosa	10

Joana Sousa	100
João André Duarte	15
João Demétrio B. Martins	100
João Filipe Martins	10
João Carlos R. Gomes	100
João Correia Teixeira	100
João Pedro S. Monteiro	100
João Teodoro	100
João Trigueiro Costa	100
Kátia Mesquita	100
Liana Moura	100
Ligia Gomes da Silva	100
Lígia Maria Ferreira	100
Liliana Mendonça	100
Luís André A. França	100
Luís Ribeiro	100
Lisa Rodrigues	100
Luana Naia	100
Magda Santana	100
Márcio José C. Ribeiro	100
Márcio José Abreu M. Rodrigues	100
Marco António P. Matos	100
Marco Aurélio G. Alves	100
Mª Francisca Eiriz	100
Mª Inês Pinto Coelho	100
Mª Inês Morte	100
Mª Isabel Nascimento Ferreira	100
Mª Joana G. Pinto	100
Mª João Ferreira Ribeiro	100
Mª João Rodrigues Pereira	100
Maria Nunes Pereira	100
Mariana Oliveira Conceição	100
Mariana Ponte C. Ribeiro	100
Marília Henriques Cordeiro	100
Mário Laço	100
Marta Daniela Passadouro Caetano	100

Marta Isabel D. Mota Vieira	100
Marta Isabel Rodrigues Baptista	100
Marta Viegas da Silva	100
Michelle Stumpf Viegas	100
Miranda Melle	100
Nélio Gonçalves	100
Nuno Ferreira	100
Nuno Fonseca	100
Nuno Machado	100
Pablo Devessa Peleteiro	70
Patrícia Henriques Domingues	100
Patrícia Lopes	100
Paula Mota	100
Paulo Jorge Rodrigues dos Santos	50
Paulo Gameiro Guerreiro	100
Pedro Alexandre Martins	100
Pedro Coxito	100
Pedro Manuel Batista Branco	100
Pedro Manuel V. Garção	100
Pedro Miguel Costa	100
Pedro Cruz	100
Raquel Ferreira	100
Renata Gomes	100
Renata Santos Tavares	100
Renato Xavier C. Santos	100
Ricardo Santos	100
Rita Perfeito	100
Rui Benfeitas Vicente	100
Rui M. Costa Soares	5
Rui Oliveira Costa	100
Rui Cruz	60
Rui Sanches	100
Sandra Isabel F. Mota	100
Sandra Jesus	100
Sandra M. Almeida Santos	100
Sandra Sofia Rebelo	100

Sandro Pereira	100
Samira C. Ferreira	100
Sara C. Figueiredo	100
Sara Tavares M. Lima	100
Sara Trabulo	100
Sezin Aday	100
Sílvia Margarida Silva	100
Sónia Correia	100
Sónia Duarte	100
Sueli Cristina Marques	100
Susana Carvalho Rosa	100
Susana Cardoso	100
Susana Patrícia S. Pereira	100
Susana Ribeiro Louros	100
Tatiana Catarino	100
Teresa Serafim	100
Tiago Alfaro	80
Vera Lúcia G. Francisco	100
Vera Moura	100
Vitor Gonçalo Silva C. Mendes	100
Zaida Almeida	100

MSc Students

Adriana Branco	100
Ana Branco M. Tiago	100
Ana Filipa Neves d'Avó	100
Ana Catarina M. Ferreira	50
Ana Daniela Sampaio	100
Ana Raquel Fonseca	100
Ana Isabel Plácido Fernandes	100
Ana Isabel Reis Santos	100
Ana Rita Leal	50
Ana Sofia Lourenço	100
Andreia Esteves Sousa	100

Carla Marina Gomes	100
Carolina Noronha	100
Cátia Machado Melo	100
Cátia Moreira de Sousa	20
Cindy Rodrigues	5
Cláudia Vanessa Moniz	100
Daniel Santos	100
David G. Dias	100
Diana Raposo	100
Diana Rodrigues	100
Dina Farinha	100
Diogo Comprido	100
Diogo Martins-Branco	50
Diogo Natario	20
Dominique Fernandes	100
Fábia Sofia Vicente	100
Filipa C. Passos	100
Filipe Marques Teixeira	100
Francisco Gonçalves	100
Gabriel Paiva	100
Henrique Carvalho	100
Isabel Ferreira	100
Isaura Vanessa Martins	100
Joana Ribeiro Guedes	100
Jorge Filipe Pascoal	100
José Paiva	100
Liliana Correia	100
Liliana Freitas Antunes	50
Luís Bajouco	30
Luís Bimbo	50
Luís Gama Mendes	100
Luís Pedro Leitão	100
Luísa Silva Lopes	100
Mª la Salete J. Baptista	20
Mariana Vagos Ribeiro	100
Marta Falcão Estrada	100

Milene Vieira Gonçalves	25
Mónica Teresa P. Abreu	50
Neuza Silva Domingues	100
Pedro Curto	50
Pedro Daniel Rio	100
Raquel Alves	100
Ricardo Poeta	20
Rui Filipe Carvalho	100
Rui Pedro Lopes	60
Sílvia Catarina F. Gomes	100
Sofia Oliveira Sousa	80
Tânia Jesus Leandro	100
Tânia Marisa Perestrelo	100
Tiago Alexandre Sousa Santos	100
Tiago Rodrigues Sousa	40
Vanessa Machado	100

Grant Technician

Alexandra Isabel Abrunheiro	100
Alexandra Sofia T. Silva Moura	50
Ana Maria P. Silva	100
Ana Patrícia Marques	100
Ana Rita Gonçalves	100
Anabela M. Simões	100
André Soares	100
Branca M. Silva	100
Bruno Gago	100
Caroline Delgado Veloso	100
Catarina Mendes Morais	100
Célia Aveleira	100
Daniel Espinoza	100
Joana Barra	100
Joana Pedro	100
Ludgero Tavares	100

Manuel Garrido	100
Nidia Duarte Moreira	100
Nuno Gabriel Machado	100
Patrícia Celeste S. Rebelo	100
Raquel Vinhas	100
Sara Monteiro Lopes	100
Sónia Neto R. Pereira	100
Vera Grandão Cortez	100
Vera Patricia Gonçalves	100

MD

António Macedo	Collaborator
Cristina Januário	Collaborator
Hermínio José T. Espírito Santo	30
Luís Cunha	Collaborator
Luísa Diogo	Collaborator
Mª Helena Azevedo	Collaborator
Mª Margarida Martins Gonçalo	20
Maria Olinda R. Rebelo	30

SERVICE STAFF

		Time % at CNC
Sandra Manuela Domingues dos Santos	(PhD, 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	(PhD, Graduate Technician, CNC)	100
João Miguel Pratas	(Graduate Technician, CNC)	100
Luciana C. Albuquerque Pinto	(Graduate Technician, CNC)	100
Maria Helena Garruncho	(Graduate Technician, FMUC)	Collaborator
Maria João Ferreira Canas dos Santos	(Graduate Technician, CNC)	100
Marta Sofia Marques Simões	(Graduate Technician, CNC)	100
Vera Mendes	(Graduate Technician, CNC)	100

TECHNICAL STAFF

		Time % at CNC
Alexandre Simão Vieira Pires	(Graduate Technician, CNC)	100
Cármen Lídia Graça Semeão	(Graduate Technician, CNC)	100
Diana dos Santos Simões Graça	(Technician, CNC)	100
Fátima Cristina dos S. Carvalho Graça	(Technician, CNC)	100
Filomena Maria F. Pereira dos Santos	(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
Mónica Serrano	(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
Sara da Costa Jordão A. Lopes	(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
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 Área

Neuroscience and Disease

Catarina Resende Oliveira, MD, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Silva Martins	(Assistant Inv., FMUC)	Collaborator
Ana Rita Costa Álvaro	(Invited 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)	80
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Attila Köfalvi	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	80
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	80
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Emília P. Duarte	(Assistant Prof., FCTUC)	80
Fabienne Agasse	(Assistant Inv., CNC)	100
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 Inv., CNC)	100
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
João O. Malva	(Principal Inv., FMUC)	80
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Mª Isabel J. Santana	(Associate Prof., FMUC)	40
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60

Paula G. Agostinho	(Investigator, FMUC)	60
Paula Isabel Moreira	(Assistant Prof., FMUC)	60
Paulo Santos	(Assistant Prof., FCTUC)	80
Ramiro Almeida	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	80
Rui Daniel Schroder Prediger	(Assistant Prof., Brasil)	80
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Investigator, FMUC)	60

Post-Doc Members

Ana Isabel Duarte	100
Ana Margarida Quaresma Nunes	100
Catarina Alexandra Gomes	100
Daniela Pochmann	100
Elisabete Baptista Ferreiro	100
Joana Salgado	100
Liliana Bernardino	100
Manuella P. Kaster	100
Margarida Alexandra Vaz Caldeira	100
Mª Teresa Cunha Oliveira	100
Pablo Pandolfo	80
Rosa M. B. Matos Resende	100
Sara Xapelli	100
Tatiana R. Rosenstock	100

PhD Students

Alexandra Rosa	100
Alexandre S. Rodrigues	80
Ana Catarina Ribeiro G. Fonseca	100
Ana Cristina R. Silva	100
Ana Patrícia Simões	100
Ana Paula Ardais	80
Ana Raquel Esteves	100
Ana Rita A. Santos	100
Ana Santos Carvalho	100

Ângela Fernandes	100
Ângela Inácio	100
Bruno Carreira	100
Carla Lopes	100
Carla Sofia G. Silva	100
Carlos Adriano Matos	100
Cristina Carvalho	100
Daniela M. Arduíno	100
Diana F. Silva	100
Elisabete Oliveira Augusto	100
Gabriel Nascimento Costa	100
Graciano da Silva Leal	100
Helena Sofia Azevedo Domingues	10
Joana Ferreira	100
Joana Filipa C. Fernandes	100
Joana Santos Barbosa	10
João Filipe da Costa Martins	10
João Trigueiro Costa	100
Luana Naia	100
Luís Filipe da Silva Ribeiro	100
Magda Santana	100
Márcio José C. Ribeiro	100
Marco António P. Matos	100
Mª Francisca Eiriz	100
Mª Inês Coelho	100
Mª Inês Morte	100
Mª Joana Guimarães Pinto	100
Mª João Ferreira Ribeiro	100
Mário Laço	100
Marta Isabel D. Mota Vieira	100
Miranda Melle	100
Nuno Machado	100
Pablo Devessa Peleteiro	70
Pedro Manuel V. Garção	100
Raquel Ferreira	100
Renato Santos	100

Rita Perfeito	100
Rui Oliveira Costa	100
Rui Sanches	100
Samira C. Ferreira	100
Sandra Isabel F. Mota	100
Sandra Sofia Rebelo	100
Sílvia Margarida Viana Silva	100
Sónia Correia	100
Sueli Cristina Marques	100
Susana Cardoso	100
Susana Ribeiro Louros	100
Tatiana Catarino	100
Tiago Alfaro	80

MSc Students

Ana Isabel Plácido Fernades	100
Ana Isabel Reis Santos	100
Ana Sofia Lourenço	100
Carolina Noronha	100
Daniel Santos	100
Diana Raposo	100
Diana Rodrigues	100
Diogo Martins-Branco	5
Diogo Oliveira Comprido	100
Dominique Moreira Fernandes	100
Fábia Sofia B. Vicente	100
Filipe Marques Teixeira	100
Francisco Gonçalves	100
Isaura Vanessa Martins	100
Jorge Filipe Pascoal	100
Luís Pedro Leitão	100
Luís Bajouco	30
Marta Falcão Estrada	100
Pedro Daniel Rio	100
Sílvia Catarina F. Gomes	100
Sofia Oliveira Sousa	80

Tânia Perestrelo	100
Tiago Alexandre Sousa Santos	100
Vanessa Machado	100

Grant Technician

Ana Patrícia Marques	100
Caroline Delgado Veloso	100
Célia Aveleira	100
Joana Pedro	100
Patrícia Celeste S. Rebelo	100
Vera Grandão Cortez	100

MD

António Macedo	Collaborator
Cristina Januário	Collaborator
Luís Cunha	Collaborator
Luísa Diogo	Collaborator
Mª Helena Azevedo	Collaborator

Molecular Biotechnology and Health

Euclides Pires, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Alcino Jorge Lopes Leitão	(Assistant Prof., FFUC)	60
Amílcar Falcão	(Full Prof., FFUC)	80
Anabela Maduro de Almeida	(Assistant Prof., Univ. Vasco Gama)	80
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Carlos Faro	(Associate Prof., FCTUC)	60
Daniela Cipestre Vaz	(Assistant Prof., Inst. Polit. Leiria)	70
Elsa Henriques	(Investigator, CNC)	100
Euclides Pires	(Associate Prof., FCTUC)	60
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	Collaborator
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
Mª Amália Jurado	(Assistant Prof., FCTUC)	60
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
Marlene Maria Tourais Barros	(Assistant Prof., FCTUC)	60
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	60
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Renata Dias da Silva	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Rui M. M. Brito	(Associate Prof., FCTUC)	60
Sérgio Simões	(Assistant Prof., FFUC)	80
Tiago Quininha Faria	(Assistant Inv., CNC)	100

Post-Doc Members

Ana Luísa Cardoso	100
Bharathi Pandurangan	100
Cândida Gonçalves da Silva	70
Chakkaravarthi Pandurangan	100
Clévio Nóbrega	100
Marco André Coelho das Neves	100
Rui Nobre	100

PhD Students

Ana C. Fortuna	100
Ana Francisca Lima	100
Ana Isabel A. Serralheiro	100
Ana Maria S. Cardoso	100
Ana Sofia Mendes Leal	100
Ana Teresa Simões	100
António Sales Mano	100
Bruno Miguel F. Alves	100
Carlos José Vieira Simões	70
Carlos Samuel M. Boto	100
Catarina Sofia H. Jesus	50
Cristiana Paulo	100
Daniela Gonçalves	100
Daniela Pereira S. Alho	100
Dulce Bento	100
Eva Serrão	30
Filipa Lebre	100
Graciana Tribuna	50
Helena Vazão	100
Inês Vasconcelos Miranda Santos	100
Isabel Maria Santos Onofre	100
Joana Sousa	100
João Pedro Monteiro	100
Lígia Maria Ferreira	100
Ligia Gomes da Silva	100
Liliana Mendonça	100

Márcio José M. Rodrigues	100
Maria Nunes Pereira	100
Mª Isabel Nascimento Ferreira	100
Mariana Conceição	100
Marta Daniela Passadouro Caetano	100
Nélio Gonçalves	100
Nuno Fonseca	100
Pedro Alexandre Martins	100
Pedro Manuel Batista Branco	100
Pedro Miguel Costa	100
Pedro Cruz	100
Renata Gomes	100
Rui Cruz	60
Rui Benfeitas Vicente	100
Sandra Jesus	100
Sandra M. Almeida Santos	100
Sara Trabulo	100
Sezin Aday	100
Sónia Duarte	100
Vera Moura	100
Zaida Catarina L. Almeida	100

MSc Students

Ana Rita Mendes Leal	50
Carla Gomes	100
Cátia Moreira de Sousa	20
Cláudia Vanessa Moniz	100
Dina Farinha	100
Diogo Natario	20
Joana Ribeiro Guedes	100
Liliana Freitas Antunes	50
Luís Bimbo	50
Mª la Saleta J. Baptista	20
Pedro Curto	50
Ricardo Poeta	20

Grant Technician

Alexandra Sofia Silva Moura	50
André Soares	50
Catarina Mendes Morais	100
José Paiva	100
Manuel Garrido	100
Nidia Duarte Moreira	100
Raquel Vinhas	100

Cell and Molecular Toxicology

Leonor Almeida, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ana Ledo	(Assistant Inv., CNC)	100
Anabela P. Rolo	(Assistant Inv., CNC)	100
Carlos M. Palmeira	(Associate Prof., FCTUC)	80
Ignacio Vega-Naredo	(Assistant Inv., CNC)	100
João Laranjinha	(Associate Prof., FFUC)	60
José Custódio	(Associate Prof., FFUC)	80
Leonor Almeida	(Full Prof., FFUC)	60
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	
Carla Nunes	100
João Gonçalo Oliveira Frade	100
João Miguel Neves Duarte	100
Vilma Sardão Oliveira	100

PhD Students	
Ana Burgeiro	100
Ana Carolina Moreira	100
Ana Filipe Branco	100
Ana Francisca Soares	100
Ana Patricia S. Gomes	100
Ana V. Rafael	100
Ana Teresa I. Varela	100
Bárbara Rocha	100
Camile Woitiski	100
Carlos Rodrigues	100

Cassilda Pereira	100
Cátia Diogo	100
Cátia Marques	100
Cláudia Sofia Alves Pereira	100
Diana Jurado S. Serra	100
Filipa Libório Carvalho	100
Filipe Duarte	100
Filomena Grilo da Silva	100
Gonçalo Pereira	100
Inês Biscaia Barbosa	100
Joana Paixão	100
João Teodoro	100
Marco Aurélio Alves	100
Mariana Ponte Cardoso Ribeiro	100
Nuno Ferreira	100
Paulo Gameiro Guerreiro	100
Ricardo Santos	100
Sandro Pereira	100
Susana S. Pereira	100
Teresa Serafim	100

MSc Students

Luís Gama Mendes	100
Mariana Vagos Ribeiro	100

Grant Technician

Ana Maria P. Silva	100
Anabela M. Simões	100
Bruno Gago	100
Ludgero Canário Tavares	100
Nuno Gabriel Machado	100
Sara Monteiro Lopes	100
Sónia Neto R. Pereira	100

Microbiology

Milton Costa, PhD, Coordinator

Members holding PhD		**Time % at CNC**
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Teresa Gonçalves	(Assistant Prof., FMUC)	40
Post-Doc Members		
Chantal V. Fernandes		100
Joana Cardoso da Costa		100
Susana Isabel E. Alarico		100
PhD Students		
Ana Luísa N. Gomes Nobre		100
Ana Sofia V. Cunha		100
Carolina Coelho		100
Igor Clemente Tiago		100
Lisa Oliveira Rodrigues		100
Luis André A. França		100
Rui Costa Soares		5
Vitor Gonçalo Silva C. Mendes		100
MSc Students		
Ana Filipa Neves d'Avó		100
Cindy Rodrigues		5
Gabriel Paiva		100
Ana Branco M. Tiago		100
Ana Catarina M. Ferreira		50
Filipa Calçada de Passos		100
Luísa Silva Lopes		100
Tânia Jesus Leandro		100
Grant Technician		
Alexandra M. Abrunheiro		100
Branca Silva		100

Biophysics and Biomedical NMR

Carlos Geraldes, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Carlos G. Geraldes	(Full Prof., FCTUC)	80
Célia M. Antunes	(Assistant Prof., FCTUC)	80
Ivana Jarak	(Assistant Inv., CNC)	100
John Griffith Jones	(Principal Inv., CNC)	100
Luís M. Rosário	(Associate Prof., FCTUC)	80
Manuela Carvalheiro	(MD, HUC)	15
Mª Luisa D. Ramos	(Investigator, FCTUC)	80
Mª Madalena Caldeira Santos	(Associate Prof., FCTUC)	80
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	80
Rosa M. Santos	(Assistant Prof., FCTUC)	60

Post-Doc Members	
Licinia J. Simões	100
Teresa Delgado	100

PhD Students	
Ana M. Metelo	100
André Martins	100
Cristina Barosa	100
Filipe Coreta Gomes	40
Helena Leitão	100
Hugo Alves Figueiredo	100
Inês Violante	10
Joana I. Real	100
João André Duarte	15
João Correia Teixeira	100
Pedro Coxito	100
Sara Figueiredo	100

MSc Students

Adriana Branco	100
Ana Rita Gonçalves	100
Andreia Raquel Sousa	100
Cátia M. Melo	100
David Gaspar Dias	100
Henrique Carvalho	100
Joana Barra	100
Neuza Silva Domingues	100
Rui Silva Carvalho	100
Rui Pedro Lopes	60

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
Artur Augusto Paiva	(Graduate Technician, HUC)	50
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
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Dolores T. Redondo	(Investigator, Univ. Salamanca)	Collaborator
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Mª Otilia Vieira	(Assistant Inv., CNC)	100
Mª Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
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	
Anália do Carmo	100
Ermelindo Leal	100
Luis Miguel Estronca	100
Luisa Jordão	50
Mª Alexandra B. Amaral	100
Sandra Catarina G. Amaral	100

PhD Students	
Ana Cristina Gonçalves	40
Ana Inês R. Crespo	100
Ana Luísa Vital	100
Ana Paula Marques de Sousa	100

Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ângela Inácio	100
Beatriz Lacerda de Sousa	100
Bruno Miguel das Neves	100
Carla Patrícia R. Paiva	100
Carlos Manuel Melo	100
Diana Margarida Carvalho	100
Helena Maria Carvalheiro	100
Humberto Gomes Ferreira	100
João Demétrio B. Martins	100
Kátia Mesquita	100
Liane Moura	100
Mª João R. Pereira	100
Marília Henriques Cordeiro	100
Marta Isabel Rodrigues Baptista	100
Marta Viegas da Silva	100
Michelle Stumpf Viegas	100
Patrícia Henriques Domingues	100
Patrícia Lopes	100
Paula Mota	100
Paulo Jorge Rodrigues dos Santos	50
Sara Tavares M. Lima	100
Susana Carvalho Rosa	100
Renata Santos Tavares	100
Vera Lúcia G. Francisco	100

MSc Students

Ana Raquel Fonseca	100
Isabel Ferreira	100
Liliana Correia	100
Milene Vieira Gonçalves	25
Mónica Teresa P. Abreu	50
Raquel Alves	100
Tiago Rodrigues Sousa	40

Grant Technician

Daniel Espinoza	100
Vera Patrícia Gonçalves	100

MD

Hermínio José T. Espírito Santo	30
Maria Olinda R. Rebelo	30
Mª Margarida Martins Gonçalo	20

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