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

Center for Neuroscience and Cell Biology

UNIVERSITY OF COIMBRA

Associate Laboratory

A new culture through Scientific Research

Annual Report | 2013

Experimental Biology and Biomedicine | Research Programmes

Biology | Neurosciences | Health and Disease | Biotechnology

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INTRODUCTION

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

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

The scientific productivity of CNC is demonstrated by an annual average of 1913 publications in peer reviewed journals in the last twelve years, an effort supported by 526 grant projects achieved in competitive calls. In 2013, 236 scientific papers were published and 40 new research projects were financed (29 FCT projects, 6 national projects and 5 international projects).

The core scientific activity of CNC is the study of the molecular basis of degenerative processes common to aging and neurodegenerative disorders. In parallel, several groups explore mechanisms of neuroprotection and regeneration, which may be future candidates for the development of potential therapeutic strategies. This core activity is complemented by supporting areas which also develop their own research activity, opening the scope of intervention of CNC in the biomedical field, while providing novel lines of research applicable to Neuroscience.

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

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

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

Technology transfer programme will strongly benefit with the “CNC Biotech – Investigação em Biotecnologia e capacitação do sector empresarial” project, which will be carried out in the Biotechnology unit at Biocant-Park, UC-Biotech. The scientific activity of this unit will be initiated in the first trimester of 2014.

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

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

The 2013 Annual Report highlights the CNC accomplishments and the contribution of its dedicated researchers, students, support teams and administrative staff to achieve the main scientific goals of this research Center.

Facts & Figures (2013)

RESEARCH STAFF

Members holding Ph.D.	206
Ph.D.Students	192
MSc Students	68
Grant Technicians	40

PUBLICATIONS

Scientific papers published	236
Scientific papers *In Press*	44

THESIS CONCLUDED

Ph.D. thesis	42
MSc thesis	44

Organization

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

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

GOVERNING BODY

President	*Catarina Resende de Oliveira*
Vice Presidents	*Euclides Pires*
	Carlos Faro
	João Ramalho Santos
Honorary President	*Arsélio Pato de Carvalho*
Executive Council	Directors of the Departments
Research Council	CNC members holding PhD
"Conselho Fiscal"	A. Rodrigues, Leal e Carreira, A. Mourão
"Revisor Oficial de Contas"	Leal e Carreira, Sociedade Revisora de Contas

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

SCIENTIFIC AREAS AND RESEARCH GROUPS

At present, research programmes and projects are organized in 6 scientific areas, each coordinated by a senior scientist. The programme for each area is implemented by small research groups each headed by a research leader in his field of study. In 2013, 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*)

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

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

Molecular Mechanisms of Disease Group (*Head: Sandra Morais Cardoso*)

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

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

Biotechnology and Health | *Euclides Pires*

Molecular Biotechnology Group (*Head: Carlos Faro)*

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

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

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

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

Farmacometrics Group (*Head: Amílcar Falcão*)

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

Cell and Molecular Toxicology | *Rui Carvalho*

Mitochondrial Toxicology and Disease Group (*Head: Anabela P. Rolo & 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 | *João Ramalho Santos*

Cellular Immunology and Oncobiology Group (*Head: Celeste Lopes*)

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

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

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

NEUROSCIENCE AND DISEASE AREA

Coordinator: Catarina Resende de Oliveira

This area pursued its research activity centered on three main issues: 1. understanding of synapses formation and modulation; 2. deciphering the cellular and molecular mechanisms underlying selective neurodegeneration associated to brain disorders; 3. development of neuroprotective and neuroregenerative strategies. The groups in this area have been achieved important research results as indicated in their individual reports wich can be summurized as follows.
We hypothesize that brain dysfunction involves a modification of glutamate synapses, aberrant synaptic plasticity, as well as a deregulated synaptic wiring. This might involve abnormal dynamics of glutamate receptors, and the mechanisms of glutamate receptor traffic and regulation of the postsynaptic composition were explored. Furthermore, optogenetic tools were created, as well as animal models, to study the synaptic circuits involved in neuropsychiatric disorders.

Several candidate targets to manipulate synaptic function were explored, namely caffeine acting through adenosine A2A receptors prevents memory dysfunction upon brain diseases and neuropeptide Y over-expression displays a neuroprotective and anti-aging effect, strengthening their potential therapeutic use. Neuronal loss and regeneration were also addressed by exploring the pro-neurogenic action of endogenous peptides and BDNF.

The mechanisms of neurodegeneration were dissected to unravel novel therapeutic targets. A novel microarray approach was developed, allowing to study, in vivo and in real-time, the dynamics of blood and oxygen oscillations during neuronal activity. Mitochondria dysfunction and impairment of cellular bioenergetics were shown to be a common feature in neurodegenerative disorders, involving autophagic-lysosomal pathways and a cross talk with the endoplasmic reticulum. The capacity to modulate mitochondria function opens new perspectives to treat brain diseases.

Neuromodulation Group

Rodrigo A. Cunha	PhD – *head of group*
Paula G. Agostinho	PhD
Ângelo José Ribeiro Tomé	PhD
Attila Köfalvi	PhD
Geanne Matos de Andrade	PhD
Ricardo Jorge A. Rodrigues	PhD
Henrique Bernardo Silva	PhD
Lisiane O. Porciúncula	PhD
Manuella Kaster	PhD
Rui Daniel Prediger	PhD
Ana Patrícia Simões	Post-Doctoral Fellow
Carolina Melo de Souza	Post-Doctoral Fellow
Catarina Alexandra Gomes	Post-Doctoral Fellow
Daniel Rial	Post-Doctoral Fellow
Joana Isabel Real	Post-Doctoral Fellow
João Pedro O. S P Lopes	Post-Doctoral Fellow
Joana Marques	Post-Doctoral Fellow
Nélio da Mota Gonçalves	Post-Doctoral Fellow
Paula M. Canas	Post-Doctoral Fellow
Samira Ferreira	Post-Doctoral Fellow
Amber Kherkoffs	PhD Student
Ana Cristina Lemos	PhD Student
Anna Pliassova	PhD Student
*António Manuel C. da Silva	PhD Student
Elisabete O. Augusto	PhD Student
Eszter Szabó	PhD Student
Francisco M. Gonçalves	PhD Student
Jimmy George	PhD Student
Marco António P. Matos	PhD Student
Marta Regina C. Oliveira	PhD Student
Nuno Jesus Machado	PhD Student
Sílvia Viana da Silva	PhD Student
Sofia Alexandra Ferreira	PhD Student
Patrícia Sofia Morais	PhD Student
Pedro Manuel V. Garção	PhD Student
Tiago Manuel P. Alfaro	PhD Student
Xinli Xu	PhD Student
Ana Carolina Xavier	MSc Student
Gonçalo Filipe P. Cristóvão	MSc Student
João Filipe Amorim	MSc Student
Liliana Caetano	MSc Student
Paula Silva	MSc Student
Rui Oliveira Beleza	MSc Student
Tiago Emanuel S. Silva	MSc Student
Caroline Delgado Veloso	Grant Technician

Glutamatergic Synapses Group

Ana Luísa Carvalho	PhD – *head of group*
João Miguel Peça Silvestre	PhD
Paulo Pinheiro	PhD
Sandra Santos	PhD
Luís Ribeiro	Post-Doctoral Fellow
Susana Louros	Post-Doctoral Fellow
Tatiana Catarino	Post-Doctoral Fellow
Carlos Adriano A. Matos	PhD Student
Dominique Fernandes	PhD Student
Gladys Caldeira	PhD Student
Jeannette Schmidt	PhD Student
Lara Franco	PhD Student
Mariline Silva	PhD Student
Mohamed Hussien	PhD Student
Bruno Cruz	MSc Student
Mário Carvalho	MSc Student

Neuronal Cell Death and Neuroprotection Group

Carlos B. Duarte	PhD – h*ead of group*
Armanda E. Santos	PhD
Emília P. Duarte	PhD
João T. Costa	PhD
Michele Curcio	PhD
Ramiro Almeida	PhD
Margarida Vaz Caldeira	Post-Doctoral Fellow
Miranda Mele	Post-Doctoral Fellow
Rui Costa	Post-Doctoral Fellow
Graciano Leal	PhD Student
Ivan Salazar	PhD Student
Joana F. C. Fernandes	PhD Student
Joana Pedro	PhD Student
Maria Joana Pinto	PhD Student
Marta Dias M. Vieira	PhD Student
Pedro João Afonso	PhD Student
Sara Oliveira	PhD Student
Susana Sampaio	PhD Student

Eduardo Morais	MSc Student
Helena Martins	MSc Student
Mª Cristina Aspromonte	MSc Student
Luís Martins	Grant Technician
Pedro Alves	Grant Technician

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Ana Cristina Rego	PhD – *head of group*
Ildete Luisa Ferreira	PhD
Elisabete Ferreiro	Post-Doctoral Fellow
Jorge Valero	Post-Doctoral Fellow
Mário Laço	Post-Doctoral Fellow
Rita Perfeito	Post-Doctoral Fellow
Sandra Mota	Post-Doctoral Fellow
Tatiana R. Rosenstock	Post-Doctoral Fellow
*António M. Silva	PhD Student
Carla Maria Nunes Lopes	PhD Student
Luana Carvalho Naia	PhD Student
Márcio Ribeiro	PhD Student
Ana Raquel Fontes	MSc Student
Carolina Noronha	MSc Student
Catarina Vaz	MSc Student
Giorgia Mastrella	MSc Student
Valeria de Rosa	MSc Student

Molecular Mechanisms of Disease Group

Sandra Morais Cardoso	PhD – *head of group*
Cláudia Mª F. Pereira	PhD
Paula Isabel Moreira	PhD
Ana Isabel Duarte	Post-Doctoral Fellow
Ana Raquel Esteves	Post-Doctoral Fellow
Rosa M. Matos Resende	Post-Doctoral Fellow
Sónia Correia	Post-Doctoral Fellow
Ana Catarina Fonseca	PhD Student
Ana Plácido	PhD Student
Emanuel Candeias	PhD Student
Daniel Santos	PhD Student
Diana F.F. Silva	PhD Student
Renato Xavier Santos	PhD Student
Andreia Palma	MSc Student
Catarina Xavier	MSc Student
Guilherme Loureiro	MSc Student
Inês Sebastião	MSc Student
Rui Simões	MSc Student
Cristina Carvalho	Grant Technician
Susana Cardoso	Grant Technician

Neuroendocrinology and Neurogenesis Group

Cláudia Cavadas	PhD – *head of group*
Ana Rita Álvaro	PhD
António F. Ambrósio	PhD (Collaborator)
Armando Cristóvão	PhD
Caetana Carvalho	PhD
Joana R. Salgado	PhD
Paulo F. Santos	PhD
Bruno Carreira	Post-Doc Fellow
Célia Aveleira	Post-Doc Fellow
Ligia Ferreira	Post-Doc Fellow
Ana Patricia Marques	PhD Student
Ana S. Carvalho	PhD Student
Joana Vindeirinho	PhD Student
Magda Santana	PhD Student
Maria Inês Morte	PhD Student
Mariana Botelho Rocha	PhD Student
Janete Cunha Santos	PhD Student
Sara Matias Silva	PhD Student

Chronic Inflamation Group

Mª Margarida Carneiro	PhD – *head of group*
Helena Mª Carvalheiro	PhD Student
Mónica Teresa P. Abreu	PhD Student
Tiago R. Sousa	PhD Student
Ana Xavier	MSc Student
Joana Gomes	MSc Student
Fábio Paiva	Grant Technician

Neuromodulation Group

Head: Rodrigo A. Cunha

Objectives

The general objective of the group is to identify modulation systems that can be targeted to interfere with the evolution of neurodegenerative diseases, with a central focus on purines (adenosine and ATP). We mostly focus on the initial stages of neurodegenerative disorders, under the working hypothesis that one of the key early features transversal to different such diseases is the dysfunction of synapses. This involves both neuronal and glial (astrocytes and microglia) maladaptive changes, with alterations of receptors, metabolic support and neuroinflammatory status, leading to abnormal synaptic plasticity and synaptic pruning that recapitulates features of neurodevelopment.

Our efforts over the years have identified a key role of adenosine A2A receptors (A2AR) in the control of neurodegenerative disorders; A2AR selectively control synaptic plasticity and they are up-regulated in afflicted areas upon brain diseases. We have shown that their blockade prophylactically prevents alterations in animal models of Alzheimer's disease, epilepsy or diabetic encephalopathy; this is in remarkable agreement with the prophylactic benefit afforded by the regular consumption of caffeine (an adenosine receptor antagonist) against diseases such Alzheimer's or Parkinson's. We are currently engaged in consolidating this concept that caffeine and selective A2AR antagonists can effectively control brain damage in different neuropsychiatric conditions. Additionally, we are exploring the mechanisms of action of A2AR in different brain areas (hippocampus, prefrontal cortex, amygdala and striatum) mingling the use of different A2AR-selective drugs, transgenic mice with tissue selective deletions of A2AR, virus designed to over-express or down-regulate A2AR and opto-genetic tools to selectively manipulate A2AR-containing cells combined with parallel behavioral, electrophysiological, morphological and neurochemical approaches exploiting subcellular fractionation techniques.

We now post that A2AR up-regulation may actually be a causative factor of aberrant synaptic plasticity underlying abnormal phenotypic changes, through a combination of direct neuronal control of synaptic plasticity, and glial control of synaptic function involving altered astrocyte-to-neuron communication and modified microglia-dependent neuro-inflammatory context.

In parallel, two emergent lines within the group are exploring the role of purines and of cannabinoids in the control of brain metabolism (Attila Kofalvi) and the role of purines, namely of extracellular ATP, in different processes characteristic of neurodevelopment (Ricardo Rodrigues).

Main Achievements

1-We have detailed the role of caffeine and A2AR in the control of memory impairment in animal models of dementia. We developed and validated two metabolic-based models of sporadic dementia, one based on the consumption on a high sucrose diet and the other on the intracerebroventricular administration of streptozotocin, and we showed in the later that caffeine affords a robust neuroprotection through up-regulated A2AR in cortical synapses.

2-We documented our working hypothesis that Alzheimer's disease might be associated with an early alteration of glutamatergic synapses, where the amyloid precursor protein is most abundantly located.

3-We expanded the proof-of-concept that caffeine and selective A2AR antagonists are effective controllers of brain damage in different neuropsychiatric diseases, namely in animal models of Machado-Joseph's disease or of attention deficits and hyperactivity disorders.

4-We studied the impact of cell type-selective genetic deletions of A2AR on different emotional responses. This showed that A2AR control fear memory prompting a novel research line to understand the role of A2AR in plastic changes in amygdalar circuits and the potential of caffeine and A2AR antagonists to manage chronic stress and post-traumatic stress disorders.

5-We continued exploring the interaction of A2AR with different modulator systems. We reported interactions of A2AR with nicotinic receptors controlling striatal dopamine release, which provides a tentative explanation for coffee and tobacco co-abuse, and may help design novel strategies to help quitting smoking. We also found a novel key role of A2AR controlling the processing and release of BDNF from microglia cells, as a tentative mechanism to understand the ability of A2AR to control microglia proliferation and neuro-inflammatory reactions.

6-We began tackling the role of A2AR in astrocytes showing that they play a major role in the control of Na+/K+-ATPase, the main energizing system driving astrocytic metabolism and function, namely neuron-glia communication.

7-We unraveled a novel role for A2AR in the control of the migration of interneurons during neurodevelopment, associated with a mis-wiring of hippocampal circuits and persistent long-term behavioral deficits associated with caffeine consumption during pregnancy in rodents.

8-We identified the likely source of the adenosine that selectively activates A2AR as ATP-derived adenosine; this paves the way to consider ecto-nucleotidases (which extracellularly convert ATP into adenosine) as novel candidate targets to control neuropsychiatric disorders.

9-Since ATP is a well-established danger signal related to the recruitment of the immune-inflammatory system, we explored the role of ATP (P2) receptors in the control of brain damage and found neuroprotective actions of P2Y1 and P2X7 receptor antagonists in animal models of ischemia and Parkinson's disease.

Glutamatergic Synapses Group

Head: Ana L. Carvalho

Objectives

Synapses are neuronal specializations that transduce information between cells and mediate the precise flow of information between neuronal circuits. Memories and behaviors are encoded and shaped by changes in the structure and efficacy of synapses. As such, a current hypothesis is that the etiology of brain disorders either stems from, or gives rise to, synaptic malfunction. The Synapse Biology group focuses on understanding the molecular and cellular processes regulating synaptic biology, contributing to a deeper understanding of information processing in the healthy brain and potentially to identifying novel therapeutic avenues for intervention in the diseased brain.

The following questions related to synaptic function/dysfunction are currently pursued:

(i) The cell biology of synaptic plasticity (PI: Ana Luisa Carvalho)

Long-term alterations in the structure and function of synapses underlie at the cellular level higher cognitive functions. Glutamate receptors of the AMPA and NMDA types convert specific patterns of neuronal activity into long-term synaptic plasticity. We are interested in the mechanisms that control the cellular traffic of AMPAR and NMDAR; in particular we have focused on their modulator proteins and on hormonal systems that have an impact on the regulation of synaptic plasticity through the regulation of receptor traffic and synaptic structure.

(ii) Synaptic circuits of neuropsychiatric disorders (PI: João Peça)

Several lines of evidence have implicated postsynaptic scaffolding protein in the etiology of neuropsychiatric disorders such as schizophrenia, autism and anxiety-disorders. Targeted disruption of the SAPAP- and Shank-family of proteins has helped identified some of the specific abnormalities in synaptic signaling and the behavioral deficits arising from modeling these conditions in mice. Presently, we are interested in using novel mutant animals to grasp the molecular and circuit defects giving rise to abnormal social behaviors in autism and schizophrenia.

Main Achievements

(i) The cell biology of synaptic plasticity (PI: Ana Luisa Carvalho)

Synapse maturation and plasticity requires structural reorganization of the spine actin cytoskeleton. We found that activity-regulated acetylation of the F-actin-binding protein cortactin promotes synaptic maturation and the accumulation of the postsynaptic scaffold protein PSD95 (J. Cell Sci. 126: 149-62 [2013]). This evidence indicates that protein acetylation can affect synaptic function through transcription-independent mechanisms.

Activity-dependent changes in synapse strength are considered the cellular basis of behavior, but this plasticity tends to destabilize the neuronal circuits leading to runaway excitation or inhibition. There is evidence in several systems for synaptic homeostatic control, important to maintain neuronal activity within a dynamic range. We have investigated the molecular mechanisms that underlie synaptic scaling, one form of homeostatic plasticity, and found a role for the Transmembrane AMPA receptor interacting protein stargazin, and its phosphorylation, in mediating synaptic upscaling in cortical neurons, in response to chronic activity blockade. In collaboration with Chinfei Chen at Harvard Medical School we found that in the absence of stargazin the refinement of the retinogeniculate synapse, between the retina ganglion cells and the lateral geniculate nucleus in the thalamus, is specifically disrupted during the experience-dependent phase. Importantly, we found that stargazin expression and phosphorylation are regulated by visual experience, and correlate with AMPAR rectification at the retinogeniculate synapse (Louros et al., in revision). Altogether these data suggest a role for stargazin in homeostatic and experience-dependent plasticity.

Hormones that regulate energy metabolism also affect higher brain function, and the orexigenic hormone ghrelin in particular enhances hippocampal-dependent memory retention. We found that the cognitive benefits of ghrelin are associated with increased glutamatergic transmission and enhanced synaptic plasticity in the hippocampus (PNAS 111(1):E149-58 [2014]). Our results establish a framework to understand a possible link between the regulation of energy metabolism and learning.

(ii) Synaptic circuits of neuropsychiatric disorders (PI: João Peça)

We succeeded in establishing a work group with a core of 2 Msc students and 2 PhD students (plus 1 additional PhD student as part of a close collaboration). Another key focus was the integration and setting up of partnerships with groups sharing similar interests, particularly of Drs. Carlos Duarte, Ana Luisa Carvalho and Ramiro Almeida.

One main achievement was the success in capturing competitive grants support from Marie Curie Actions and a NARSAD Young Investigator Award.

Neuronal Cell Death and Neuroprotection Group

Head: Carlos B. Duarte

Objectives

Neurotrophic factors play numerous roles in the nervous system, including the regulation of neuronal development, long-term modulation of synaptic transmission and in neuronal survival and neuroprotection under several different injury conditions. These effects are mediated by activation of specific receptors with tyrosine kinase activity, thereby inducing several parallel intracellular signaling cascades. Alterations in these signaling mechanisms have been associated with various disorders of the central and peripheral nervous systems. This group focuses on i) understanding the molecular mechanisms induced locally by neurotrophic factors to regulate neuronal development and ii) on the alterations in neurotrophic factor signaling in brain ischemia. Another major interest of the group is the understanding of the neurotoxic signaling mechanisms activated in brain ischemia.

Three core questions related to neurotrophic factor function/dysfunction and neurotoxic signaling mechanisms are currently pursued:

(i) Local protein regulation in neuronal development (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. In fact, a significant number of mRNAs has been found in pure preparations of distal axons and growth cones and its composition is far more complex than initially thought. This observation leads us to ask if local mRNA translation may play an important role in other neurodevelopmental processes like presynaptic differentiation. One goal of our research is to identify which mRNA(s) are required for presynaptic differentiation in response to neurotrophic factor stimulation. For that purpose we developed reporter assays to address this objective and monitor local translation in live cells.

(ii) Synaptic dysregulation in brain ischemia (PIs: Carlos Duarte and Emília Duarte)

In brain ischemia, the decrease in blood supply to the brain leads to the extracellular accumulation of glutamate. The resulting increase in glutamate receptor activity plays a key role in neuronal death (excitotoxicity) in brain ischemia by activating an excitotoxic signaling cascade. The $[Ca^{2+}]_i$ overload resulting from the overactivation of glutamate receptors leads to an abnormal stimulation of calpains (Ca^{2+}-dependent proteases), with consequent cleavage and downregulation of different proteins, including neurotrophic factor receptors and synaptic proteins. We are interested in 1) the downregulation of neurotrophic factor signaling in brain ischemia and 2) the changes in the synaptic proteome and neuronal connectivity under the same conditions.

(iii) Alterations in gene expression in brain ischemia and neuronal cell death (PI: Armanda Santos)

Ischemia may induce delayed responses due to alterations in gene expression. We have been investigating the changes in the pattern of gene expression upon ischemic or excitotoxic stimuli in order to possibly identify new genes involved in neuronal

Main Achievements

Our main contributions are to the understanding of the formation and function of synapses, as well as to the characterization of deregulated synaptic processes in brain ischemia.

(i) Local protein regulation in neuronal development (PI: Ramiro Almeida)

Our goal is to detect if local mRNA translation is required upon induction of presynaptogenesis. We have successfully established a microfluidic culture system and using this new platform we were able to specifically induced axonal differentiation. We observed that presynaptic assembly requires axonal translation, indicating that local protein translation can regulate the formation of new synapses. To assess the role of β-actin in presynaptic differentiation we developed a reporter assay which is mimics the endogenous mRNA (β-actin reporter). We first asked if the endogenous mRNA is present in axons. Using pure axonal lysates we observed that β-actin mRNA is present in distal axons and growth cones. Moreover, FGF22 stimulation induces a significant increase in the levels of the β-actin reporter, and in the number of F-actin rich puncta suggesting that local translation of β-actin mRNA regulates presynaptic differentiation.

(ii) Neurotrophic factor dysregulation in brain ischemia (PIs: Carlos Duarte and Emília Duarte)

Glial cell line-derived neurotrophic factor (GDNF) plays an important role in neuronal survival through binding to the GFRα1 receptor and activation of the receptor tyrosine kinase Ret. Brain ischemia alters the expression of the GDNF signaling machinery but the molecular mechanisms involved and the functional implications are not yet elucidated. We found that excitotoxic stimulation with glutamate as well as in vivo and in vitro (oxygen-glucose deprivation [OGD] in cultured hippocampal neurons) ischemia downregulate Ret protein levels via a calpain-dependent mechanism. Although calpain inhibitors prevented the downregulation of Ret receptors following

excitotoxic stimulation, they did not fully prevent the downregulation of GDNF-induced intracellular signaling activity, suggesting that additional mechanisms may be involved. This alteration of the neuroprotective GDNF support to neurons may contribute to neuronal death in brain ischemia.

(iii) Dysregulation of GABAergic synapses in brain ischemia (PI: Carlos Duarte)

The dysregulation of GABAergic synapses in the ischemic brain contributes to the imbalance of the excitatory/inhibitory equilibrium and to neuronal death (Neurobiol Dis 65: 220–232 [2014]). We reported a downregulation of $GABA_A$ receptor ($GABA_AR$) expression, affecting both mRNA and protein levels of $GABA_AR$ subunits, in cultured hippocampal neurons subjected to OGD. Similar alterations in the abundance of $GABA_AR$ subunits were observed in in vivo brain ischemia. OGD reduced the interaction of surface $GABA_AR$ with the scaffold protein gephrin, followed by clathrin-dependent receptor internalization. Internalization of $GABA_AR$ was dependent on glutamate receptor activation and mediated by dephosphorylation of β3 subunits. The results showed a key role for β3 $GABA_AR$ subunit dephosphorylation in the downregulation of GABAergic synaptic transmission in brain ischemia, contributing to neuronal death.

(iv) Alterations in gene expression associated with neuronal death in brain ischemia (PI: Armanda Santos)

To identify molecular changes elicited by ischemic insults, we subjected hippocampal primary cultures to OGD, which resulted in delayed neuronal death with an excitotoxic component. We observed that at 7h after OGD there was a general repression of genes, whereas at 24h there was a general induction of gene expression. Genes related with functions such as transcription and RNA biosynthesis were highly regulated at both periods of incubation after OGD, confirming that the response to ischemia is a dynamic and coordinated process. Furthermore, our results indicate that OGD activates a transcriptional program leading to a downregulation in the expression of genes coding for synaptic proteins, suggesting that the synaptic proteome may change after ischemia.

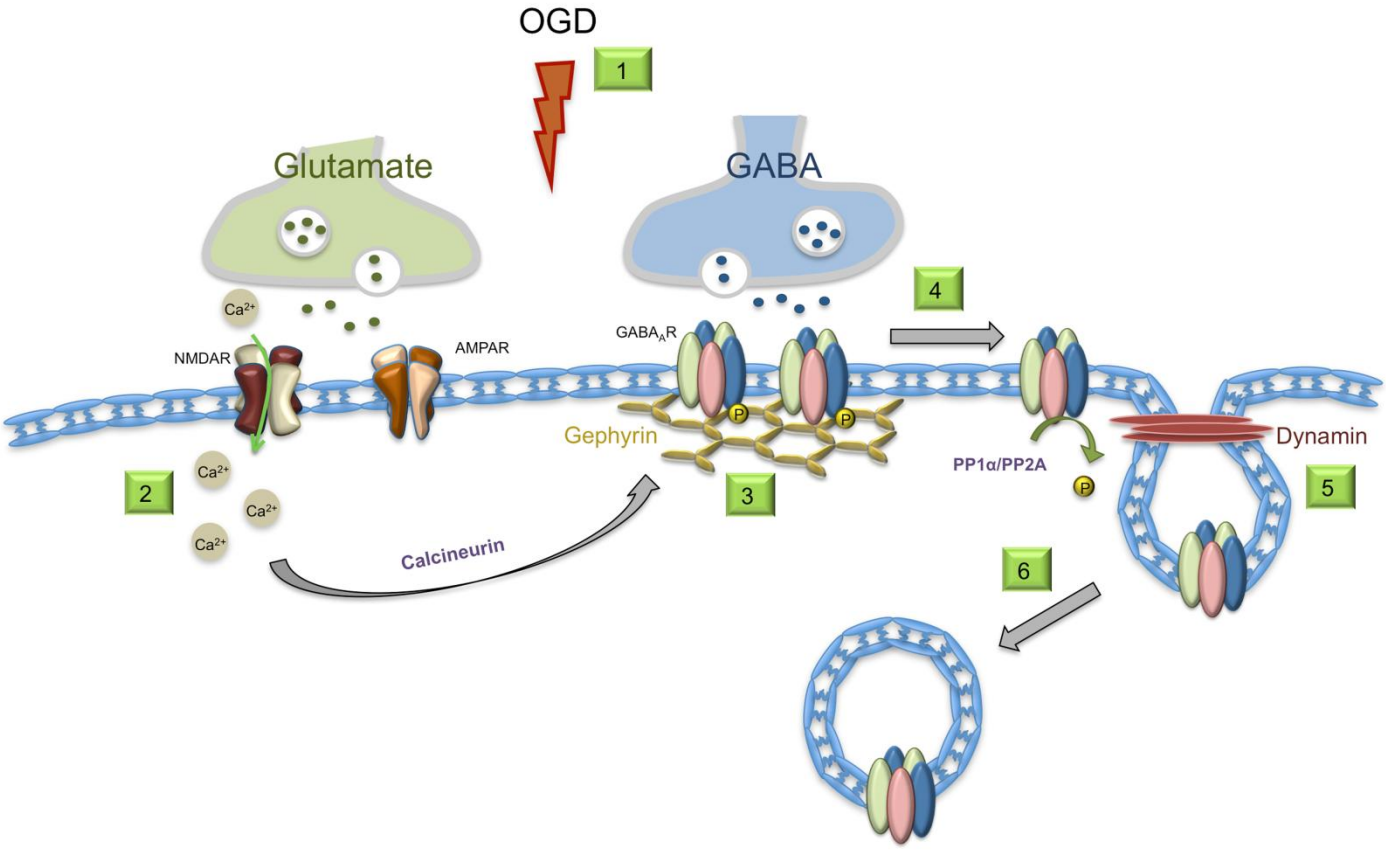


Fig. 1 Model of $GABA_AR$ internationalization during cerebral ischemia

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Head: A. Cristina Rego

Objectives

Neurodegenerative diseases are chronic, irreversible and debilitating disorders of the central nervous system, characterized by cognitive decline and selective brain neurodegeneration. The latter has been largely attributed to mitochondrial dysfunction and protein misfolding. However, how modified or mutant proteins interfere with neuronal and mitochondrial function is not completely clear. Our research sheds light on the characterization and identification of molecular targets for therapeutic intervention by focusing on mitochondrial dysfunction and interrelated signaling pathways in distinct neurodegenerative disorders, namely Alzheimer's disease (AD), Huntington's disease (HD), a polyglutamine-expansion disorder, and Parkinson's disease. These are a group of chronic neurodegenerative brain disorders that usually strike in mid-life and along aging, causing progressive loss of motor and cognitive functions. Although clinical manifestations vary, the outcome is the same: patients become incapacitated over a period of years and finally die. In particular, AD is the most common age-related neurodegenerative disorder among the elderly, affecting both the hippocampus and the cerebral cortex and leading to progressive debilitating cognitive deficits. HD is an autosomal dominant CAG repeat disorder affecting the *HD* gene, which encodes for huntingtin (Htt), and is characterized by prominent cell death in the striatum and involuntary movements. PD is the most common age-related movement neurodegenerative disorder, characterized by a progressive degeneration of dopaminergic neurons in the *substantia nigra pars compacta* and the formation of intracytoplasmic inclusions, mainly composed of alpha-synuclein.

Our group has been using complementary molecular, cellular (including peripheral blood cells from human subjects and primary neuronal cultures) and *in vivo* animal experimental approaches to examine defective intracellular signaling pathways underlying mitochondrial dysfunction and deregulated bioenergetics. Evaluation of mitochondrial-related mechanisms of neurodegeneration, including oxidative stress, excitotoxicity and calcium deregulation, linked to synaptic deregulation, and lately their correlation with transcriptional dysfunction have been also a matter of interest. Moreover, several neuroprotective strategies have been tested, including neurotrophic factors (e.g. IGF-1), NMDA receptor antagonists, histone deacetylase inhibitors or sirtuin modulators to counterbalance mitochondrial and neuronal dysfunction. These studies are intended to shed light on the mechanisms of neurodegeneration directly or indirectly affecting mitochondrial function in several neurodegenerative diseases.

In 2013 we mainly focused our research in AD and HD pathological mechanisms

Main Achievements

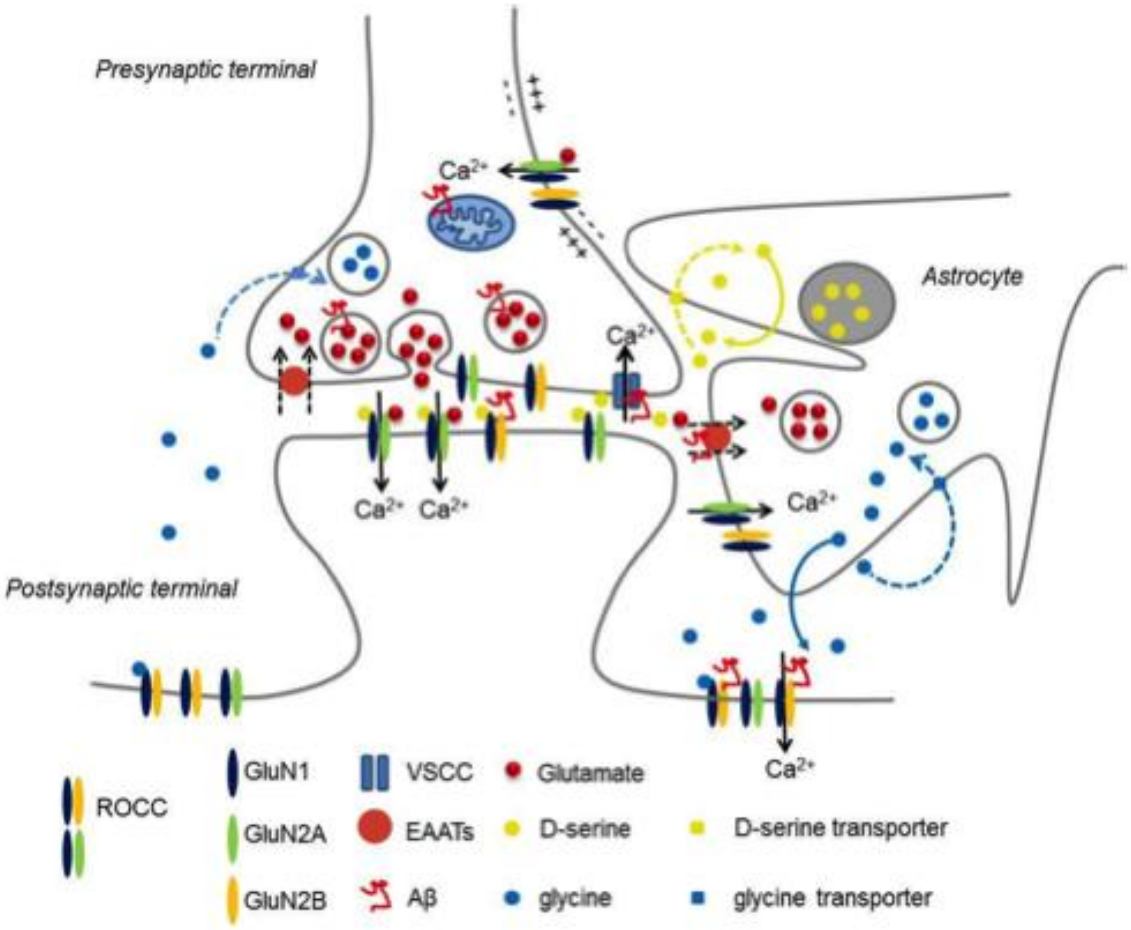
Recent evidence demonstrated dysregulation of glutamatergic synaptic transmission by amyloid-beta peptide (Abeta) oligomers in AD. Our group showed that Abeta1-42 oligomers disturb intracellular Ca2+ homeostasis, causes microtubule deregulation and endoplasmic reticulum (ER) stress by selectively activating *N*-methyl-D-aspartate receptors (NMDARs) composed by GluN2B subunits. These and other data were described in the review by Mota and Ferreira et al. (*Neuropharmacology*, 2013) where we explored the importance of targeting the tripartite glutamatergic synapse in asymptomatic and possible reversible stages of AD. In collaboration with members of the 'Cell Metabolism and Quality Control' group, we also described the mechanisms underlying Abeta toxicity, namely the involvement of Abeta-induced ER stress in brain endothelial cell death (Fonseca et al., *Biochim Biophys Acta*, 2013) and the contribution of mitochondrial dysfunction for ER stress in neurons (Costa et al., *Mol Cell Neurosci*, 2013). Moreover, endogenous or exogenous alpha-synuclein was demonstrated to be neuroprotective against Abeta toxicity in neurons, which may occur in early stages of the Lewy body variant of AD (Resende et al., *Neurochem Res*, 2013).

Mitochondrial dysfunction and metabolic changes caused by mutant Htt have been a matter of highly interest in HD progression. By analysing platelet mitochondria from pre-symptomatic *versus* symptomatic HD human carriers and age-matched control individuals, we showed that mitochondrial platelets exhibited reduced activity of citrate synthase and complex (Cx)-I in pre-symptomatic and symptomatic HD carriers. Positive correlation between Cx activity and protein subunits was observed for Cx-I in symptomatic HD patient's mitochondria. Results highlighted mitochondrial changes occurring before the onset of HD clinical symptoms (Silva et al., *Mitochondrion*, 2013).

Previously we showed that oxidative stress occurs in HD knock-in striatal cells, but little was known regarding cell antioxidant response against exogenous stimuli. Therefore, we analyzed cellular antioxidant profile following hydrogen peroxide (H2O2) and staurosporine (STS) exposure and tested the protective effect of cystamine and creatine in striatal cells expressing mutant Htt. Mutant cells displayed increased mitochondrial reactive oxygen species (ROS), along with increased superoxide dismutases (SODs) and components of glutathione redox cycle. Exposure to H2O2 and STS enhanced ROS in mutant cells and largely increased XO activity. Both stimuli decreased glutathione reductase

with consequent rise in oxidized glutathione or glutathione disulfide in mutant cells. Additionally, creatine and cystamine increased mutant cells viability and prevented ROS formation in HD cells subjected to H2O2 and STS. Data indicated that exposure to noxious stimuli induces a higher susceptibility to oxidative stress. Furthermore, creatine and cystamine were shown to prevent H2O2- and STS-evoked ROS formation in HD striatal cells (Ribeiro et al., *Toxicol. Sci.*, 2013).

Insulin growth factor-1 (IGF-1) peripheral administration in R6/2 HD mice was previously demonstrated to protect against HD-associated impaired glucose tolerance by enhancing blood insulin levels (Duarte et al., *Exp Neurol*, 2011). Thus, we investigated intranasal administration of recombinant human IGF-1 (rhIGF-1), in order to promote IGF-1 delivery to the brain, in YAC128 mice. We showed that IGF-1 supplementation enhanced IGF-1 cortical levels and improved motor activity and metabolic abnormalities in YAC128 mice. Moreover, decreased Akt activation in HD mice brain was ameliorated following IGF-1 administration. Upregulation of Akt following rhIGF-1 treatment occurred concomitantly with increased phosphorylation of mutant Htt at Ser421. Data suggested that intranasal administration of rhIGF-1 ameliorates HD-associated glucose metabolic brain abnormalities and mice phenotype (Lopes et al., *Mol Neurobiol*, in press).



The tripartite glutamatergic synapse - a target for amyloid-beta peptide
(Mota and Ferreira et al., *Neuropharmacology*, 2013)

Molecular Mechanisms of Disease Group

Head: Sandra Cardoso

Objectives

We were interested in understanding how pathways that control aging, such mitochondrial metabolism, impact neuronal degeneration and synaptic loss. The identification of such regulatory network provides a therapeutic window to treat a broad spectrum of diseases associated with mitochondrial deregulation, including neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's (PD) diseases. Furthermore, we aimed to identify potential molecular targets that could be intervened in order to halt the degenerative pathways occurring in brain pathologies.

One major focus of our research was to investigate ER stress as a crucial molecular mechanism implicated in neuronal, glial and endothelial dysfunction through the deregulation of calcium and redox homeostasis, excitotoxicity, inflammation, mitochondrial dysfunction and impairment of protein homeostasis during aging and in brain pathologies, in particular in age-related neurodegenerative disorders such as AD.

Another goal of our research was to elucidate the role of mitochondria and insulin signaling pathways in neuronal and endothelial (dys)function occurring in AD and diabetes-associated neurodegeneration. The influence of gender on the molecular mechanisms underlying aging-related changes in the diabetic brain is another goal of our group. We also seek to clarify the potential protective role of antidiabetic agents, mitochondrial antioxidants, uncoupling protein 2 (UCP2) and preconditioning in the aforesaid pathological conditions.

Main Achievements

We have been depicting the role of mitochondrial metabolism signaling in the regulation of cellular quality control mechanisms, such as: the ubiquitin proteasomal system and the autophagic lysosomal pathway, in sporadic models of age-related AD and PD. We provided evidence that mitochondrial impairments cause the loss of microtubule network, culminating in intracellular trafficking deficits, which enhanced α-synuclein aggregation, due to disturbances in the autophagic-lysosomal pathway.

In cultured cortical and hippocampal neurons, we demonstrated that the AD-associated Aβ peptide, namely oligomeric Aβ, activates an ER stress-mediated apoptotic pathway and a deleterious ER-mitochondria crosstalk and that Aβ-induced activation of GluN2B subunits of N-methyl-D-aspartate receptors (NMDARs) is an upstream event of neuronal ER stress. The role of ER stress in the vascular alterations occurring in the AD brain was further supported by data obtained in Aβ-treated endothelial cells from cerebral microvasculature.

We also showed that brain mitochondria are a functional bridge between type 2 diabetes (T2D) and AD. Additionally, we found that T2D and AD animals present similar behavioral, cognitive and vascular anomalies. These findings support the idea that T2D increases the risk of developing AD. It was also observed that type 1 diabetes and insulin-induced hypoglycemia impact differently mitochondria from cortex and hippocampus, brain areas associated with learning and memory. Moreover, we saw that mitochondrial preconditioning protects against glucotoxicity, this protective effect being mediated by mitochondrial reactive oxygen species and hypoxia inducible factor 1alpha (HIF-1a).

Neuroendocrinology and Neurogenesis Group

Head: Cláudia Cavadas

Objectives

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

2. The role of hypothalamic NPY modulation will be investigated in a mouse model of premature and accelerated aging of *Hutchinson Gilford progeria syndrome* (HGPS).

3. We are also investigating the microRNA maestro in the central regulation of food intake, obesity and aging.

4. 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 mechanisms underlying the effects of NO of microglial origin on the proliferation of neural stem cells the in co-cultures of SVZ with microglia isolated from wild-type or iNOS knockout mice. Moreover, the hypothalamic neurogenesis will be also investigated.

5. We aim at studding the role of intermittent hypoxia induced by sleep apnea on two regulator systems of energy balance: the hypothalamus and the white adipose tissue. It is known that sleep apnea prevalence is very high in obese patients, and that sleep apnea promotes obesity – a risk factor of aging progression. In our group we will study the changes induced by intermittent hypoxia on rodent hypothalamus and white adipose tissue, using in vitro and in vivo models.

6. Since retina is highly susceptible to eye diseases, somehow related with aging, we are interested on the identification of new strategies and targets to promote neuronal retinal protection and repair. We are continuing to investigate the effect of diabetes or hyperglycemia on neuronal dysfunction and retina microglia changes, and especially the changes induced on adenosinergic system. The potential of neuropeptide Y (NPY) system and adenosinergic systems as a neuroprotective strategy in the retina will be also investigated.

Main Achievements

1. NPY and NPY receptors are present in the retina and have neuroprotective role in retinal cell death (Santos-Carvalho et al 2013a, 2013b, 2013c). The rat retinal adenosinergic system is affected by diabetes and high glucose conditions, and the modulation observed may uncover a possible mechanism for the alleviation of the inflammatory and excitotoxic conditions observed in diabetic retinas (Vindeirinho et al 2013).

2. We show for the first time that NO from inflammatory origin leads to a decreased function of the EGF receptor, which compromised proliferation of NSC. We also demonstrated that NO-mediated nitration of the EGF receptor caused a decrease in its phosphorylation, thus preventing regular proliferation signaling through the ERK/MAPK pathway (Carreira et al., 2013 and submitted).

3. Neurogenesis also occurs in the hypothalamus and we showed that rat hypothalamic progenitor cells have a neuronal lineage and are a source for new feeding-related neurons. These results contribute to consider that hypothalamic neurogenesis is a possible mechanism to remodel feeding circuits in obesity and hypothalamic dysfunctions (see review Sousa-Ferreira et al 2013).

1. Caloric restriction (CR), a non-genetic intervention that has consistently been found to extend life span across a variety of species, increases NPY in the critical brain region for maintaining metabolic homeostasis – the hypothalamus. On the other hand, CR increases autophagy, which has a role in preventing neurodegeneration, and has been related to longevity increase. Therefore, we investigated the involvement of hypothalamic NPY on autophagy induced by CR. The results show that NPY and CR induced the activation of autophagy in rodent hypothalamic neurons. Moreover, NPY receptor antagonists blocked the autophagy induced by CR in hypothalamic neurons. Overall, these results show that NPY directly induces autophagy and mediates autophagy induced by CR in hypothalamic neurons (Aveleira et al 2013, in 2nd revision). Also in cortical neurons, CR and ghrelin activated autophagy through NPY system (Marques 2013, Master Thesis).

2. The anti-aging effect of hypothalamic NPY modulation was investigated in a mouse model of premature and accelerated aging of *Hutchinson Gilford progeria syndrome* (HGPS), the *Zmpste24*$^{-/-}$ mice. Interestingly, the modulation of NPY in the hypothalamus rescued some aging phenotype features of HGPS mice, such as the low body weight, lipodystrophy, alopecia, memory impairment and the increase of aging brain markers (Cavadas et al., provisional 2013).

Chronic Inflammation Group

Head: Margarida Carneiro

Objectives

The Immunology Group has two main research areas: 1) systemic immune alterations in neurodegenerative diseases (Parkinson's disease and Alzheimer's disease) and 2) chronic autoimmune inflammation (rheumatoid arthritis and colitis). Within these two main topics we focus on the following aspects:

1. Understand the mechanisms underlying autoantibody production and B lymphocyte deficiencies in neurodegenerative diseases.
2. Characterize functional defects of CD8 T lymphocytes in rheumatoid arthritis, and clarify their role in disease pathogenesis.
3. Study how defective reactive oxygen species production induces chronic colitis.

Main Achievements

1) In a project funded by the Michael J Fox Foundation we have identified major changes in peripheral blood B lymphocytes from PD and AD patients, and unveiled potential mechanisms underlying the production of auto-reactive antibodies against CNC-derived proteins specific for these neurodegenerative diseases.

2) We have identified CD8 T lymphocytes as potential targets for anti-arthritic therapy in a mouse model of chronic polyarthritis. In a study funded by Abbott we have identified major changes in circulating and synovial fluid CD8 T lymphocytes in rheumatoid arthritis patients, which are currently submitted to a peer-reviewed journal. Moreover, in collaboration with the BioCant-based biotech company H-Tag we are currently developing a new drug to modulate CD8 T lymphocytes in arthritis.

3) In a project partially funded by a Marie Curie Grant and in collaboration with teams at the University of Turku; the Karolinska Institute; the University of Erlangen and the Oporto Hospital Center, we have submitted a manuscript which has been returned with the editor's request for corrections in which we report that defective reactive oxygen species production alters the STAT1 pathway and induces autoantibody production in systemic lupus erythematous and chronic granulomatous disease. The results of another study, within this same consortium, have been accepted for publication in PLoSOne, and show that deficient production of reactive oxygen species leads to exacerbated chronic colitis due to local hyper-inflammation.

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BIOTECHNOLOGY AND HEALTH AREA

Coordinator: Euclides Pires

Biotechnology exists as a research area (line) in CNC from the beginning of the Center. However, since then, several changes occurred, groups moved to other areas whereas some came in or were created. In 2013 there were 7 research groups in this area: Molecular Biotechnology Group; Molecular Systems Biology Group; Structural and Computational Biology Group; Vectors and Gene Therapy Group; Biomaterials & Stem Cell-Based Therapeutics Group; Pharmacometrics Group and Bioorganic and Medicinal Chemistry Group. The objectives and the main achievements of each groups area detailed ahead in the report. The general objectives of the area were: 1) unveal and understand normal interactions that occur in living organisms, from molecular up to system level; 2) design vectors to deliver drugs and nucleic acids aiming to modulate or correct abnormal interactions; 3) develop new biomaterials for stem cell differentiation, tracking and transplation as well as biomaterials with anti-microbial properties.

The year of 2013 was a hall-mark for Associate Laboratories (LA).FCT (main Portuguese govern financing agency) challenged LAs to submit a Strategic Plan in line with 2020 European Program. The strategic plan proposed by CNC required a profound reorganization of the existing scientific areas (lines). Scientific activity was then organized under two "Domains": Neurosciences and Biotechnology. In the frame of this Strategic Program, Neuroscience emerged as the main fundamental research Domain in CNC.

Present definitions (concepts) of biotechnology encompass several aspects that were not considered when the term was coined. For instance in the UN Convention on Biological Diversity, Biotechnology is defined as:" The use of living systems and organisms to develop or make useful products, or any technological application that uses biological systems, living organisms or derivatives to make or modify products or processes for specific uses." This definition fits the Biotechnology activity develop by the aforementioned CNC Biotech groups whose aims are in line with those of 2020 Program. The groups will proceed with fundamental research topics closely associated with the development of applications for health, agriculture and environment. This is not too presumptive since CNC Biotech groups have the fundamental know-how, the laboratory expertise, access to the most of the required technologies and a growing net of contacts with industry.

Thus success of this area will depend in a great deal on the ability "to focus" on the resolution of specific problems and on the creation of products of utility.

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Molecular Biotechnology Group

Head: Carlos Faro

Objectives

The research group has a main interest on proteolytic enzymes and their role in regulating complex and highly dynamic protein cascades, networks and signaling pathways, in addition to their degradative function and biotechnological potential. Our research activities can be subdivided into two main focus areas:

1. Biochemistry, biology and biotechnology potential of plant aspartic proteases

Among the proteolytic machinery of plants, APs represent the second largest class of proteases. Strikingly, whereas mammals contain fewer APs coded in their genomes, a large representation of AP genes is found in land plants. This clearly suggests that the overrepresentation of APs in plants may represent an important role in diversification of protein functions. Indeed, some functions are starting to be uncovered, with proposed roles in highly regulated processes like resistance to biotic and abiotic stresses, programmed cell death, plastid homeostasis, reproduction or hybrid sterility and wide compatibility. These studies provide strong implications for these proteases as important players in developmental processes and stress responses. Therefore, deciphering the biology/structure-function relationships of plant APs are crucial and challenging tasks that deserve focused studies. On-going activities in the lab include heterologous expression, biochemical, structural and functional characterization of a selected group of atypical as well as classical plant APs, combined with state-of-the-art high-throughput proteomics approaches to outline their specificity profiles and substrate repertoire (degradome).

Supported by an extended knowledge on cardosins – APs responsible for the milk-clotting activity of Cynara cardunculus flowers aqueous extracts used as coagulants in cheese production – ongoing activities also involve the development of a fermentation-derived engineered form of cardosin with potential for scalability of production and commercial applications.

2. Biochemistry and biology of prokaryotic aspartic proteases and their role as potential therapeutic targets in pathogenic Bacteria

The relevance of proteolytic events for bacterial pathogenicity has been described for different pathogenic bacteria. Given the lack of effective therapeutics and/or the progressive increase in antimicrobial resistance, the search for alternative therapeutic strategies using proteases as candidate targets for intervention represents a challenging topic for future research. Strikingly, the presence of APs of both pepsin and retropepsin-type in prokaryotes has always been a matter of debate and our work has provided the first unequivocal documentation of these types of activities in prokaryotes. On-going activities include a detailed biochemical, structural and functional characterization of selected prokaryotic pepsin- and retroviral-like enzymes to further understand the structural features, molecular evolution, "targetability" or potential applicability of these ancestral forms of APs.

3. The role of pollen proteases in allergic respiratory diseases

Allergic disorders, such as seasonal rhinitis and asthma, are increasing causes of morbidity worldwide and regularly result from exposure to airborne pollen. In the past we have established that pollen grains, with distinct allergenic abilities, release proteases that might be involved in the sensitization process facilitating allergen delivery across the epithelium. On-going activities include purification and functional characterization of pollen proteases with the ability to compromise epithelium barrier integrity to recognize their contribution on immunologic and inflammatory response, typical of the allergic diseases.

Main Achievements

1) Biochemistry, biology and biotechnology potential of plant aspartic proteases

In this context, innovative plant aspartic protease-based products were developed targeting cheese and dermocosmetic markets. Our research group reinforced our position as a reference in the area of biotechnological applications of aspartic proteases, translated in one publication in international peer-review journal in Q1 quartile in the category of Biotechnology & Applied Microbiology and in the submission of 2 patent applications.

- We developed and optimized a eukaryotic-based expression platform for a saposin-like protein of plant origin in the generally regarded as safe (GRAS) yeast Kluyveromyces lactis and the highest reported yield for a nontagged PSI domain was obtained. Also, we confirmed the bioactivity of cirsin PSI domain towards different phytopathogenic fungi.
- We pursued our work with cardosins by implementing a stepwise optimization strategy for the heterologous production in a GRAS yeast. This resulted in the successful production of an engineered form of cardosin in K. lactis. The improved production yields allowed the development of a rennet preparation to be used as a rennet substitute in cheese production at industrial scale (patent application: GB1305025.7 and paper in preparation). Moreover, this synthetic form of cardosin was purified, enzymatically characterized and its three-dimensional structure determined by X-Ray crystallography (paper in preparation). In parallel, we focused on alternative applications for fermentation-derived plant APs which resulted in the development of a new desquamation agent for cosmetics applications (patent application GB1305023.2).
- We also proceeded with the expression and characterization of different atypical APs from Arabidopsis suggested to be involved in programmed cell death events

during pollen maturation. We implemented a multi-tiered expression platform which includes a novel pro-viral plant-based expression system for production of these proteases and we have determined the first specificity profile of an atypical AP by a high-throughput proteomics approach (Proteomic Identification of Protease Cleavage Sites -PICS).

2) Biochemistry and biology of prokaryotic aspartic proteases and their role as potential therapeutic targets in pathogenic Bacteria

In this context, we have been focused in understanding evolution and function of pepsins and retropepsins in prokaryotes. Our findings on prokaryotic aspartic proteases place our research group at the forefront of research in this field as we were the firsts to characterize this group of enzymes in bacteria.

- We extended our studies to the pepsin-like enzyme from Shewanella denitrificans (shewasin D), showing that this protease accumulates preferentially in the cytoplasm (highly uncommon for APs). Additionally, we determined the specificity profile with PICS for both shewasin A and D and confirmed the resemblances with pepsin-like APs (paper in preparation).
- In parallel, we have been working on novel APs of the retropepsin-type present in different pathogenic bacteria (e.g. Rickettsia and Legionella). Rickettsiae are gram-negative strict intracellular bacteria and many of them are pathogenic to humans causing severe infections like Mediterranean spotted fever. We have identified and characterized a novel membrane embedded AP of the retropepsin-type (APRc) conserved in 51 sequenced Rickettsia genomes. We demonstrated that APRc shares several enzymatic properties with retropepsins, and that it is expressed, at least, in two pathogenic species of Rickettsia. We determined the specificity profile by PICS (HIV-1 protease is the only retropepsin for which this type of analysis has been reported) and our results showed that APRc shares similar specificity preferences with both retropepsin and pepsin-type APs. Additionally, we provided evidence that APRc is inhibited by specific HIV-1 protease inhibitors, a novel finding which to our knowledge has not been reported for prokaryotic retropepsin-like enzymes. Finally, we provided experimental evidence for its potential role as a modulator of rickettsial major surface antigen and virulence determinant (OmpB/Sca5) (paper in preparation). Also, diffraction data at a 2.6 Å resolution was obtained for APRc and structure determination is currently ongoing.

3) The role of pollen proteases in allergic respiratory diseases

Serine and metalloproteinase were purified from pollen diffusates. These proteases caused disruption of transmembrane adhesion proteins resulting in a time-dependent increase of transepithelial permeability.

Some proteases were able to activate Protease-actived receptor 2 leading to an increase of intracellular calcium. Additionally, all proteases showed to induce proinflamatory cytokine IL-6 and IL-8 production.

Molecular Systems Biology Group

Head: Armindo Salvador

Objectives

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

a. What are the design principles of the most prevalent elementary circuits in metabolic networks? Namely, moiety transfer cycles (MTC).

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

c. How does the naturally evolved design of antioxidant defense systems relate to the function of these systems and what design features are key for their effectiveness?

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

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

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

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

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

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

Main Achievements

Previously, we set up a mathematical model of H_2O_2 metabolism in human erythrocytes. Over 2013 we further updated and validated this model based on recent experimental data. This validation has shown that the *effective* rate constant for H_2O_2 reduction by Prx2 in intact erythrocytes is two orders of magnitude lower than the rate constant determined for the purified protein. This might be due to an experimental artifact in the latter determinations or, more likely, to a strong reversible inhibition of Prx2's peroxidase activity. The updated model accounting for the low peroxidase activity is in near quantitative agreement with a large set of experimental results without requiring any adjustment of other parameters. The functional consequences of the low effective peroxidase activity are also similar irrespective of how it obtains. Namely, it permits an effective transduction of H_2O_2–mediated signals while sparing NADPH in H_2O_2 elimination. [Benfeitas, Selvaggio, Antunes, Coelho, Salvador (2014), submitted]

H_2O_2 elimination can be metabolically costly and requires a substantial investment in protein defenses. These defenses cannot be rapidly upregulated upon a sudden increase in H_2O_2 exposure, thus leaving the protein thiols exposed to oxidative damage for long periods. We hypothesize that the Peroxiredoxin/Thioredoxin/Thioredoxin Reductase system (PTTRs) solves this conundrum by "blocking" the thiols through reversible covalent modification once H_2O_2 concentrations begin to increase. We termed this mechanism "anticipatory blocking control" (ABC). To assess this hypothesis, we started by developing a generic mathematical model that captures those features of the PTTRs that are common to most cells. We used this model to deduce the generic design requirements for effective integration between H_2O_2 elimination, H_2O_2 signaling, anticipatory blocking and NADPH management. Finally, we found that the H_2O_2 metabolism in human erythrocytes satisfies these design requirements. [Selvaggio & Salvador (2014), manuscript in preparation]

Fast-growing microorganisms are more susceptible to acute environmental stresses than slow-growing ones. Mechanistically, this phenomenon arises because at higher growth rates expression of stress defenses is disfavored over the expression of growth-promoting enzymes (Fig. 1A). But why does natural selection favor such a growth-robustness reciprocity? Using idealized models of self-replicating cells we found that these transcriptional profiles can be explained by the interplay among three fundamental principles (Fig. 1B): (a) maximization of growth rate, (b) unavoidability of damage to cellular components, and (c) growth-related damage dilution. Thus, at high substrate availability the high growth rates attainable are sufficient to quickly dilute damage, and the expression of defenses would decrease growth. In contrast, at low substrate the attainable growth rates are insufficient to effectively dilute damage, and growth under stress is maximized when defenses are expressed. As result, slow growing cells become pre-adapted to acute environmental stresses. [Bolli & Salvador (2014), manuscript in preparation]

Previously, we developed the first GC-MS method to determine ^{13}C enrichment in (deoxy)nucleosides with positional isotopomeric resolution. In 2013 we applied it to profile the metabolism of *S. cerevisiae* cells at mitotic-cycle S phase without requiring cell cycle synchronization or cell sorting. A preliminary analysis demonstrates that the mitotic cycle of these cells is metabolically heterogeneous even when the cell population is not undergoing macroscopic oscillations. A quantitative analysis of the results is ongoing. Future experiments will determine if proliferating Mammalian cells also show metabolic heterogeneity over the mitotic cycle, with implications for cancer therapy. [Miranda Santos, Gramacho, Pineiro, Martínez-Gómez, Fritz, Hollemeyer, Salvador, Heinzle (2014), under submission]

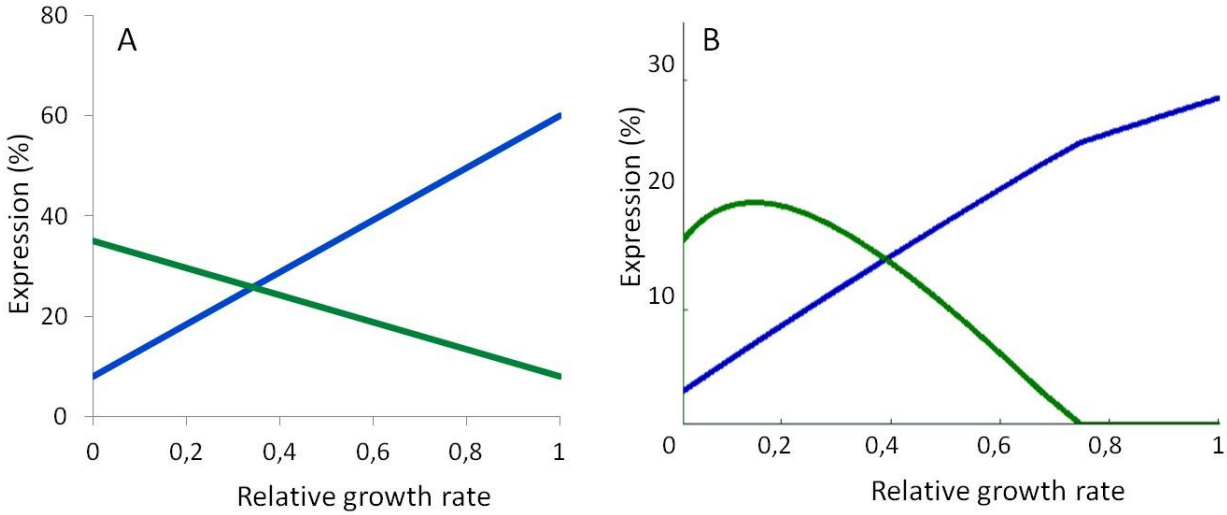


Fig. 1. Comparison between experimental and model generated profiles of expression of stress defense and growth-associated genes. A. Dependence from normalized growth rate of the relative expression of growth associated (blue line) and stress defense (green line) genes in Escherichia coli (modified from Shoval et al., (2012) Science. 336:1157). Panel B shows corresponding transcriptional profiles generated by our cellular models.

Structural and Computational Biology Group

Head: Rui M. M. Brito

Objectives

I. Rational design of inhibitors of amyloid formation

The objective is to use an up-to-dated local copy of ChEMBL (https://www.ebi.ac.uk/chembl/) - a database of bioactive drug-like small molecules Its current version (chembl_17) contains the 2D structures of 1,324,941 distinct compounds. Overall, for these compounds are reported 12,077,491 activities values associated to 9,356 target proteins. We will make use of our local copy of the ChEMBL to: i) locate any compound record associated with anti-amyloid activity; ii) generate chemical hashed and pharmacophore fingerprints, along with hundreds of molecular descriptors, for every compound stored in the database; iii) retrieve compounds with high chemical, shape and/or electrostatic similarity using 2D and 3D similarity searches; and iv) retrieve "high-activity AND low-affinity" entries from the significant fraction of the SAR and discovery data on modern drugs.

II. In vitro assessment of hit compound for A-beta and TTR amyloid inhibition activity

The identification of compounds with the ability to bind to amyloid fibrils is a crucial step in the development of new probes for the detection of amyloid deposits in medical imaging. The aim of this study is to establish a methodology to quantify the association constants and binding mode of small molecules towards TTR amyloid fibrils.

We have been exploring the use of STD-NMR experiments to characterize the interaction of TTR fibrils with compounds with two action profiles: (i) clinic diagnostics and (ii) fibril disruption. The setup of the experiment is being validated using a compound known to bind to TTR fibrils – ThT. These experiments will be employed to determine the ligand mapping and dissociation constant.

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

Within the Ibercivis project, one of the main objectives pursued throughout 2013 was to involve Portuguese researchers and citizens in Ibercivis.

Main Achievements

The main results achieved are described below:

I. Rational design of inhibitors of amyloid formation

Ia. Retrieval of experimental information on amyloid inhibitors

ChEMBL includes bioactivity data for 97817 compounds against 23 amyloid targets. Two preprocessing protocols guarantee that (i) 2D structures follow the same representation and include a single fragment; and (ii) a 3D structure is calculated for all compounds.

Although our database is built on top of the ChEMBL, it stores the values on around 1400 molecular descriptors on each compound, thus shifting its focus of the database for the bioactivity data to the compounds data. The molecular descriptors calculation packages used include the following: ChemAxon (academic license), CDK (open source), Mold^2 (open source; FDA), and OpenEye (restricted academic license).

Ib. Molecular Modeling: construction and refinement of receptor and pharmacophore models

Given the wide structural diversity found amongst amyloid inhibitors reported in the literature and annotated in ChEMBL, structural alignments have been a demanding challenge. While attempts to identify common structural features amongst inhibitors, through the use of clustering methods, are underway, we have taken an alternative approach to the definition of a "pharmacophore" based on the design of concatamers. Three virtual, composite ligands that combine structural features proven critical for inhibitory activity have been built as queries for ligand-based virtual screening, in particular, 3D similarity searches based on shape similarity, chemical complementarity and electrostatics overlap.

Ic. Validation of the molecular docking program AutoDock Vina for virtual screening against amyloid fibrils

Using a co-crystal structure of Thioflavin T (ThT) with an amyloid-like oligomer of beta2-microglobulin (b2m; PDB 3MZT), we evaluated AutoDock Vina – a fast molecular docking program – in terms of pose prediction accuracy using the RMSD value between its predicted poses for the binding of ThT to b2m and the available experimental conformations. Results show that Vina correctly predicts the binding modes of ThT to b2m, thus making it a suitable candidate for future high-throughput docking.

Id. Ligand-based virtual screening

Virtual ligand screening has been conducted using 3D similarity search methods against all compounds deposited in ChEMBL (approximately 1.7 million compounds) and two chemical libraries filtered from the ZINC dabatase. Following definitions in the FILTER program by OpenEye (OE), a “drug-like” subset containing 5.9 million ZINC compounds and a “blockbuster” subset containing 13.1 million ZINC compounds have been assembled. All chemical libraries have been screened using OE’s ROCS approach to measure/compare shape overlap and chemical complementarity, and OE’s EON approach to measure similarity in electrostatics. Fifteen query molecules have been used as template for the comparisons.

II. In vitro assessment of hit compound for A-beta and TTR amyloid inhibition activity

We investigated the binding of Thioflavin-T (ThT), a probe known to interact with amyloid fibrils, using saturation transfer difference (STD) NMR to set up an experimental protocol useful to detect the binding mode of new compounds to TTR amyloid fibrils. In addition, we used fluorescence spectroscopy and took advantage of the large fluorescence enhancement of ThT upon binding to amyloid fibrils to develop fluorescence competition assays to quantify the association of non-fluorescent ligands to these fibrils.

III. Ibercivis – A volunteer computing platform for the Iberian Peninsula

Throughout 2013, Ibercivis has increased its scope of action. The scientific projects supported include not only volunteer computing projects but also other citizen science projects requiring for a large group of volunteers to actively contribute in the collection or analysis of data. To promote the involvement of Portuguese researchers and citizens in Ibercivis, several dissemination activities were promoted in public events.

Vectors and Gene Therapy Group

Head: Mª Conceição P. Lima

Objectives

The research in the Group of Vectors and Gene Therapy has been devoted to the design and development of carriers, including viral and non-viral vectors, for nucleic acid and drug delivery aiming at their application as technological platforms for 1) establishment of disease models, 2) study of disease mechanisms and 3) development of new molecular therapeutic approaches for cancer and neurodegenerative disorders and of prophylactic strategies.
Our studies on non-viral vectors have been mainly focused on the evaluation of the potential of novel lipid-based nanosystems and polymeric nanoparticles in gene therapy strategies for the treatment of both cancer and neurodegenerative disorders, and for the development of vaccines.
Non-viral vectors, such as cationic liposomes, stable nucleic acid lipid particles and cell-penetrating peptides have been explored as carrier systems to deliver nucleic acids, including plasmid DNA encoding therapeutic proteins, as well as antisense oligonucleotides, siRNAs and anti-miRNA locked nucleic acids, aiming at promoting silencing of known oncogene proteins and both cancer-related and pro-inflammatory miRNAs. The group is interested in investigating the anti-tumoral effect of gene therapy strategies, either *per se* or in combination with chemotherapeutic agents, both *in vitro* and in animal models for different types of cancer. In addition, non-viral vectors are currently being developed to study the role of miRNAs in neuroinflammation, aiming at promoting neuronal survival by targeting the inflammatory pathways associated with neurodegenerative diseases.
Fundamental research work addressing the development and physicochemical characterization of new nucleic acid delivery systems has also deserved the attention of our group. Research efforts have been developed to define through a biophysical approach the architecture parameters that endow vectors with the ability to transpose membranes and efficiently deliver their cargo into the cell.

In addition, the fact that tumor survival and proliferation are largely dependent on the microenvironment, represents an opportunity to engineer novel therapeutic strategies to address unmet medical needs, upon choosing more than one target from the pool of tumor–stroma interactions. Therefore, the study of the functional contribution of tumor microenvironment on cancer progression and metastasis, aiming at identifying novel therapeutic targets is becoming an emergent area of research in our group. This is aligned with the design and understanding of the mechanistic basis of non-viral carriers aiming at targeting drugs and nucleic acids to the tumor microenvironment, in orthotopic murine models of cancer. These lines of research have included a component of translational research, following the collaboration with the Portuguese Institute of Oncology from Coimbra and the Faculty of Medicine and the Hospital of the University of Coimbra. Viral vectors, particularly lentiviral and adeno-associated viruses are powerful technological platforms for gene delivery to the CNS, which we have been using for investigating the pathogenesis and modeling of neurodegenerative diseases, with a focus on Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD). This knowledge is expected to allow the generation of disease-modifying approaches for MJD therapy.
The group also addresses mucosal vaccination (oral and nasal) using antigens (protein or DNA) encapsulated in polymeric nanovectors, to target the lymphoid structures of the mucosal immune system. In this regard, new chitosan-based delivery systems able to simultaneously encapsulate antigens and an immunopotentiator (mast cell activator c48/80, aluminum compounds and exosomes) have been developed and tested (*in vitro* and *in vivo*) with the purpose of improving immune response modulation.

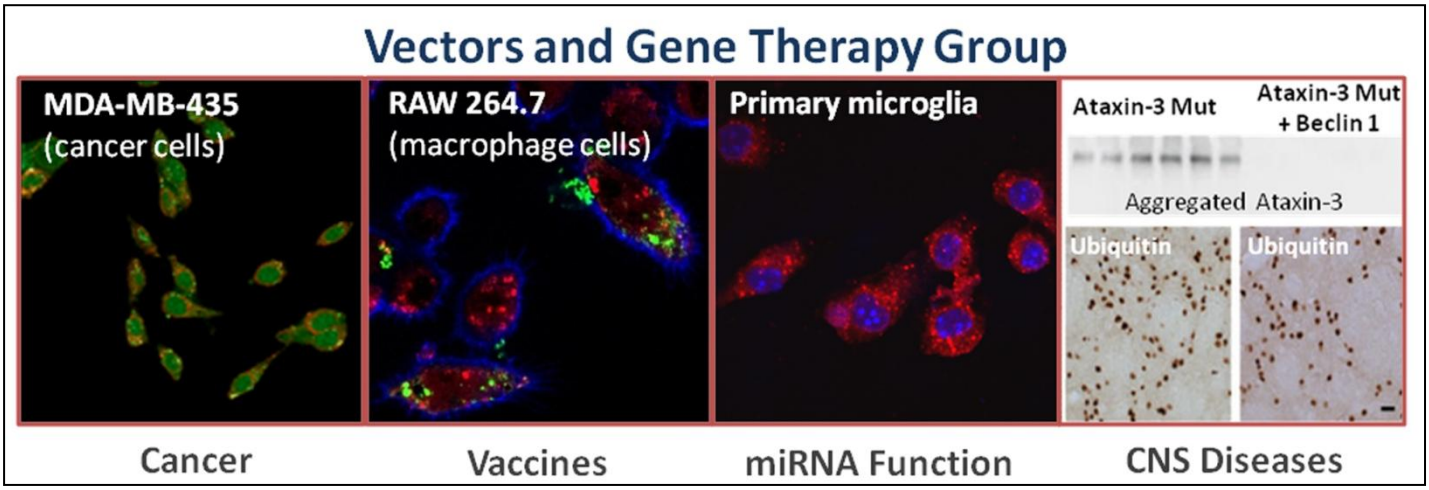
Main Achievements

Regarding non-viral-mediated gene delivery, an extensive screening of a variety of molecules (gemini surfactants, copolymers, cell penetrating peptides and fullerene nanoparticles) for their capacity to produce efficient nucleic acid delivery systems has been carried out and structure-activity relationships, established. Several characteristics susceptible of modulation emerged as critical to improve vector performance, e.g. hydrocarbon chain length and spacer chemical nature in gemini surfactants; combinatory proportions of copolymer components and the corresponding cloud point; amino-acid sequence, presence of specific amino acid residues (e.g. histidine) and acylation in cell penetrating peptides; surface chemistry in fullerenes.
Regarding targeted cancer gene therapy, we have generated a novel lipid-based system exhibiting the ability to specifically and efficiently deliver DNA into hepatocellular carcinoma cells through its specific binding to the asialoglycoprotein receptor. A new anti-tumoral strategy was also developed involving silencing of the oncomir miR-21, overexpressed in glioblastoma (GBM), through delivery of anti-miRNA LNA oligonucleotides via tumor-targeted stabilized nucleic-acid lipid particles (SNALPs) followed by cell exposure to sunitinib. We have shown that SNALP-mediated miR-21 silencing enhances the cytotoxic effect of sunitinib in different glioma cell lines, thus revealing the therapeutical potential associated with the combination of miRNA-based gene therapy with anti-angiogenic activity towards GBM. We have also developed a novel ligand-mediated targeted lipid-based nanoplatform for siRNA delivery towards cancer cells and endothelial cells from angiogenic blood vessels. Following a marked improvement on siRNA internalization into the target cells, along with destabilization in mildly acidic endosomes, an effective downregulation of eGFP has been achieved. This strategy was further validated against *PLK1*, following demonstrating that *PLK1*-silencing can impact multiple cellular players of tumor aggressiveness, thus enabling the opportunity to interfere with different hallmarks of cancer, in tumors of diverse histological origin. In addition, the developed targeted liposomes revealed to be nonimmunogenic, even in a multi-administration schedule,

thus constituting a valuable tool for the specific and safe systemic delivery of siRNA to solid tumors.

Regarding neurodegenerative diseases, we have generated lentiviral and adeno-associated viral vectors to study their pathogenesis focusing on Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD). Development of lentiviral-based in vivo models of MJD, in which we are experts, allowed fruitful investigation of disease-modifying strategies involving gene silencing, autophagy activation and proteolysis inhibition. We have also investigated the contribution of immune-related miRNAs to cell migration and phagocytosis in the context of Alzheimer's disease (AD) and have identified specific miRNAs whose levels are deregulated in AD patients with respect to healthy controls. It is expected that these studies contribute to the finding of new therapies for these devastating disorders for which no effective therapy is available.

Regarding DNA-based vaccination, we clarified the adjuvanticity mechanisms of chitosan nanoparticles, which increased antigen nasal residence, induced the production of IL-1β by DC cells, via a NLRP3 inflammasome-dependent pathway and promoted mast cell activation. The *in vivo* immunogenicity of antigens was considerably increased.



Biomaterials and Stem Cell-Based Therapeutics Group

Head: Lino Ferreira

Objectives

The research group has two main avenues of research: (i) development of bioengineering platforms to modulate the differentiation and maturation of stem cells, (ii) development of nanomedicine platforms to modulate the activity of stem cells and their progenies.

1- Bioengineering platforms to modulate the differentiation and maturation of stem cells. One of the main objectives of the research group is to develop biomaterials and bioengineering platforms for the efficient differentiation, maturation and engraftment of stem cells and their progenies (focus: cardiovascular lineages). We are primarily working with human pluripotent stem cells (induced pluripotent stem cells and human embryonic stem cells) and fetal hematopoietic stem cells (human cord blood). The group is developing scaffolds capable of retaining the cells at the desired location, while serving as a template for cell assembly, survival, differentiation and engraftment. The group is also designing biomaterials that provide several different types of information to stem cells, with the purpose of controlling their differentiation. New strategies based on topography and fluid shear stress to modulate the differentiation of mesoderm cells such as vascular cells and cardiomyocytes derived from human pluripotent stem cells are under development.

2- Nanomedicine platforms to modulate the activity of stem cells and their progenies. 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 to control exogenous and endogenous stem cells for regenerative medicine. Our group is particularly interested to use these tools to induce *in vivo* stem cell differentiation and to mobilize stem cells from their niches to treat cardiovascular diseases. For this purpose, we are developing nanomaterials that release efficiently small molecules or non-coding RNA (miRNAs) to manipulate stem cells or their progenies.

The 2 avenues of research of the group target cardiovascular diseases. Cardiovascular diseases (CVDs), a group of disorders of the heart and blood vessels, are the number one cause of death globally. More people die annually from CVDs than from any other cause. Stem cells are an important source of cells for regenerative medicine applications. Several clinical trials are underway to investigate their therapeutic effects. Yet, it is of utmost importance to understand the bioactivity of stem cells and eventually to control it. The paracrine effect of stem cells remains to be elucidated as well as new platforms to improve stem cell survival after transplantation. Stem cells are also an important source of cardiac and vascular cells for drug screening and toxicological assessment. They can be an useful *in vitro* model to study specific diseases and to find new therapeutic targets.

The research group uses many approaches to answer research questions, including the design of new biomaterials with relevant biological information, molecular and cell biology, microfluidic systems, high content analysis, and animal experimentation. The group uses molecular analysis to identify signaling pathways that are activated when cells are exposed to specific signals or extracellular matrixes. The group has strong experience in the differentiation of pluripotent stem cells into vascular lineages.

Main Achievements

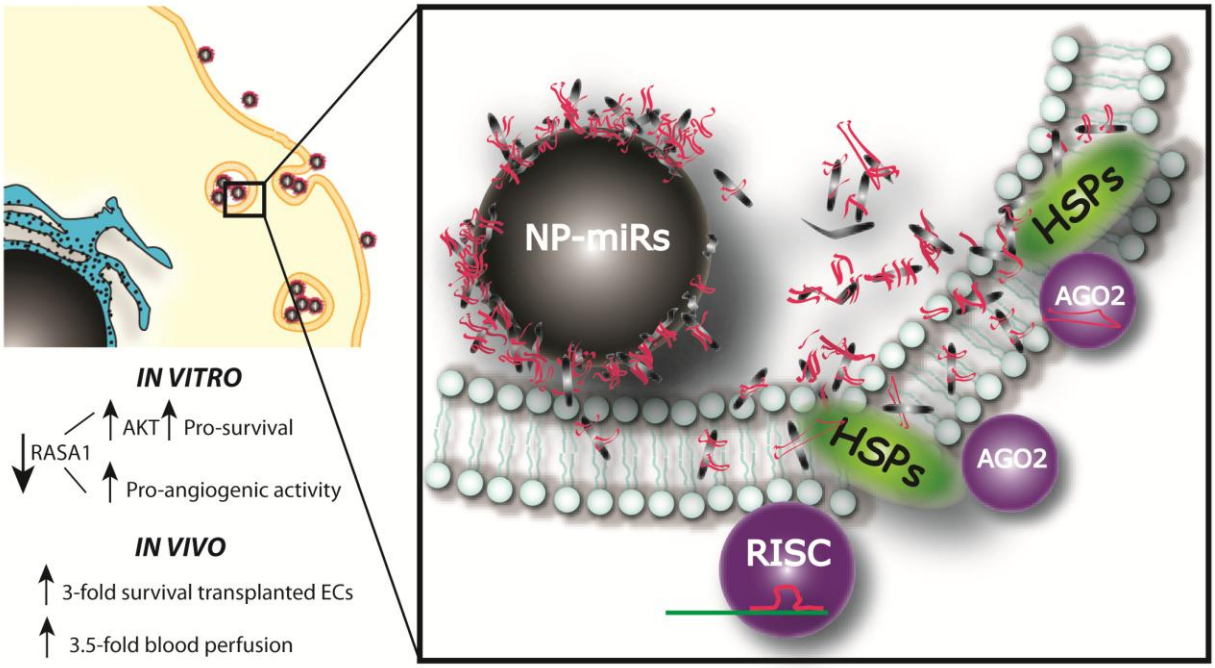
During the last year, the group has done significant progresses to address the following scientific questions: **(i) how to improve the *in vivo* engraftment of stem cells and to enhance their differentiation? (ii) can we use stem cells to generate in vitro models for drug screening? (iii) how to design biomaterials for cardiac applications?** To tackle the first question we developed a new set of nanomaterials to monitor and improve the engraftment of stem cells and their progenies (Gomes et al., ACS Nano 2013). We reported the use of biodegradable nanoparticles (NPs) containing perfluoro-1,5-crown ether (PFCE), a fluorine-based compound (NP170-PFCE), with the capacity to track cells *in vivo* by Magnetic Ressonance Imaging (MRI) and efficiently release miRNA. NP170-PFCE complexed with miRNAs accumulated whitin the cell's endolysosomal compartment and interacted with higher frequency with Argonaute 2 (Ago2) and GW182 proteins, which are involved in the biological action of miRNAs, than commercial complexes formed by commercial reagents and miRNA, which in turn accumulated in the cell cytoplasm. The release of miRNA132 (miR132) from the NPs increased 3-fold the survival of endothelial cells (ECs) transplanted *in vivo* and 3.5-fold the blood perfusion in ischemic limbs relatively to control.

To tackle the second question we have developed a novel blood vessel on a chip, combining vascular cells differentiated from induced pluripotent stem cells and microfluidic systems (manuscript submitted). We have differentiated human pluripotent stem cells into embryonic arterial endothelial cells (ECs), which were then cultivated under static or flow conditions to screen compounds that affect specifically embryonic vasculature. Using this platform, we have identified a compound from a library of 1,200 chemical compounds that is toxic for embryonic ECs. The vascular toxicity of the compound was further validated in prenatal mouse ECs and in mice embryos. In a separate work, we have generated a human BBB model using cord blood-derived hematopoietic stem cells. The cells were initially differentiated into ECs followed by the induction of BBB properties by co-culture with pericytes. The brain-like endothelial cells (BLECs) express tight junctions and

transporters typically observed in brain endothelium and maintain expression of most *in vivo* BBB properties for at least 20 days. The model shows a good correlation with human BBB permeability data.

To tackle the third question we have developed a novel biocompatible and mechanically tunable elastomer, poly(glycerol sebacate urethane) (PGSU), suitable for efficient encapsulation and controlled delivery of bioactive macromolecules and with the potential to be applied to cardiac drug delivery (Pereira *et al*, Advanced Materials 2013). In a separate study we have engineered a bioinspired elastic and biocompatible hydrophobic light-activated adhesive (HLAA) that achieves a strong level of adhesion to wet tissue and is not compromised by pre-exposure to blood (Lang *et al.*, Science Translational Medicine 2014). The HLAA provided an on-demand hemostatic seal, within 5 seconds of light application, when applied to high-pressure large blood vessels and cardiac wall defects in pigs. HLAA-coated patches attached to the interventricular septum in a beating porcine heart and resisted supraphysiologic pressures by remaining attached for 24 hours, which is relevant to intracardiac interventions in humans.

During 2013, the group has filled 2 patents, published 7 publications in international journals (being 4 publications in journals with impact factor above 7) and 2 book chapters and submitted 2 publications. The group has attracted additional funding from FCT (EXPL/BIM-MED/2267/2013; Portugal-China joint innovation centre for advanced materials project). In addition, the group trained three PhD students that have defended their PhD thesis during 2013.



Pharmacometrics Group

Head: Amílcar Celta Falcão

Objectives

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

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

Moreover the Pharmacometrics group also performs the pharmacokinetic analysis of those compounds during clinical studies. This information is extremely important as Pharmacometrics aims to assess quantitatively the pharmacokinetics and pharmacodynamics of drugs, using data from various phases of drug development which are then linked together and quantitatively related to each other.

Main Achievements

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

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

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

Bioorganic and Medicinal Chemistry Group

Head: Maria Luísa Sá e Melo

Objectives

The main focus of the Bioorganic and Medicinal Chemistry Group research is on drug discovery.

Steroids comprise a wide range of structurally related compounds with important functions *in vivo* and have shown a great therapeutic value due to anticancer, antiviral and antimicrobial activities. Recently, the link between malaria and steroids, as testosterone and dexamethasone, has been associated to the immune response of the human organism to the disease. Moreover, reports on the use of cholic acids as carriers of synthetic peroxides, which mimic the natural product artemisin, are quite encouraging. In the last decade our group has generated a large library of oxysterols with a vast array of structural variations and diverse biological activities. Noteworthy, oxysterols were recently reported to increase the sensitivity of tumor cells to other chemotherapeutic agents, including by ourselves. With this in mind and aware of the importance of multitarget therapies in malaria as a promising approach to circumvent drug resistance, the aim has been to evaluate the potential of our library of sterols for malaria treatment and to synthesize new hybrid antimalarials for drug development, to contribute to the ultimate goal of eradicating malaria.

Pentacyclic triterpenoids are a class of pharmacologically active and structurally rich natural products with privileged motifs for further modifications and SAR analyses. The naturally occurring oleanane-type triterpenoids, oleanolic acid and glicirretinic acid and ursane-type ursolic acid have been thoroughly investigated for their promising chemopreventive and antitumor activities.We focused on the synthesis of oleanane-type imidazole carbamates and *N*-acylimidazole bearing derivatives. The promising results prompted us to extend our study to 2´-methylimidazole and triazole derivatives, to establish meaningful SAR. The compounds with better cytotoxicy were tested for their ability to induce apoptosis and cell cycle arrest.

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

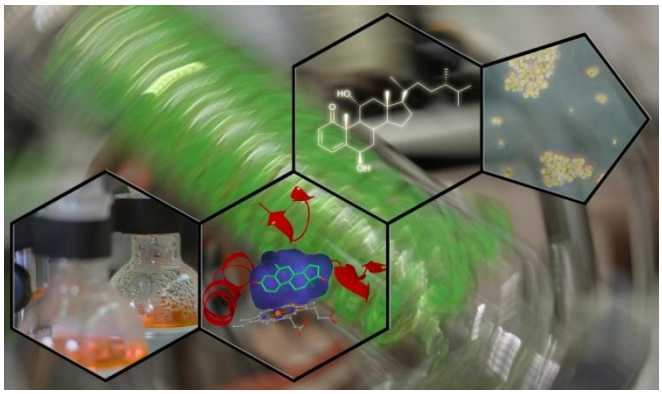
The research activities of the group are supported by the following expertise:

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

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

c) Biological evaluation *in vitro*.

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



Main Achievements

The library of oxysterols, synthesized in the group, has been screened for antiplasmodial activity against *P. falciparum* W2 (chloroquine resistance) and some of them presented low micromolar IC_{50} values. The found antimalarial activity is very representative of their rich structural molecular diversity (unpublished results in collaboration with Malaria Group, iMed). Knowing that drug resistance requires new drugs, the syntheses of hybrid antimalarials based on the most potent oxysterol scaffolds and stable tetraoxanes, synthetic analogues of artemisin, have been performed. Four new chemical entities were prepared and structurally characterized to be evaluated in vitro and in vivo screens for antimalarial drug discovery and further drug development.

- Recently, we focused on the synthesis of oleanane-type imidazole carbamates, N-acylimidazole bearing derivatives, 2´-methylimidazole and triazole derivatives (*Org. Biomol. Chem., 2013, 11, 1726*) and oleanane-type pentacyclic triterpenoids bearing a boronate ester moiety at C3 (*Eur. J. Med. Chem. 2013, 46*), in order to establish meaningful SAR and study their ability to induce apoptosis and cell cycle arrest in cancer cells. The overall findings suggest that some of the new oleanane-type derivatives are strong regulators of tumor cells proliferation, inducing cell cycle arrest and apoptosis.

- Adressing the GPR30 receptor, synthetic modifications of the steroid skeleton were performed, and evaluated for their interactions with the receptor. Two of the synthetized compounds seem to show some agonist behavior. In order to confirm the results, the studies will be extended to the T47 -D cell line, a breast cancer cell line that express GPR 30 receptors. On the other hand, considering the pharmacological activity of the compounds as agonists, we start to determine its potential therapeutic interest in diseases in which activation of GPR30 can have beneficial effects, particularly in the endothelial dysfunction associated with menopause.

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CELL AND MOLECULAR TOXICOLOGY AREA

Coordinator: Rui Carvalho

The general objective of this research area is to understand fundamental mechanisms of cellular toxicity caused by chemical agents or by different disease processes. We consider that several stress responses resulting from exposure to foreign molecules are identical to what is observed in the context of several diseases, creating a phenotype that is deleterious to the tissue and whole organism. Specifically, we focus our research in a variety of cellular responses ranging from metabolic, including mitochondrial, remodeling, production of reactive oxygen and nitrogen species and antioxidant modulation, cell death, autophagy/mitophagy and cell transformation in the context of a carcinogenic process. By understanding the mechanisms behind these responses, new cellular targets can be identified in order to pursue pharmacological and non-pharmacological strategies to improve the tissue phenotype.

Major Achievements

Work performed in our research line has achieved a series of important objectives:

1) The basis for gut-brain mechanisms of communication based on redox chemistry of nitric oxide

2) The mechanisms behind the cytoprotection afforded by anthocyainins and wine polyphenols

3) Development of micro-sensors for in vivo use in the brain, specifically to detect microvascular perfusion in the brain

4) The role of stress protein p66Shc on mitochondrial diseases

5) The mechanisms involved in the anticancer activity of phytoalkaloids, and dimethylaminopyridine derivatives of lupane triterpenoids

6) Showing the role of antiestrogens on cancer cells and mitochondria

7) Mechanisms underlying hexavalent chromium [Cr(VI)]induced malignant transformation and establishing a new in vitro model for the carcinogenesis induced by Cr(VI)

8) The role of mitochondria in hyperglycemic memory and role of SIRT1/AMPK activation in stress responses

9) Effects of dietary modification on liver mitochondrial metabolism and resistance to hepatotoxic agents

10) Early description of the metabolic profile of bone cells in estrogen-deprived rodent models

11) Modulation by bile acids of farnesoid X receptor and thermogenesis in brown fat

12) Use of NMR to fingerprint cancer stem cell differentiation and lung cancer cell metabolism

13) Organization of research seminars and meetings

14) Completion of Master and Ph.D. thesis

15) Growing internationalization of the area

16) Obtaining new funding to secure the development of projects.

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: Anabela Rolo | Paulo Oliveira

Objectives

Mitochondria are critical organelles in the context of cell physiology. Mitochondria are the cell energy powerplants by producing the majority of the chemical energy, and play an important role in cell death and quality control processes. Since mitochondria are also active players in cellular redox and calcium homeostasis, as well as in intermediate metabolism, the general objective of our research group is to provide insights into the role of mitochondrial alterations in metabolism, redox signaling and stress responses in chemical toxicology, cancer, cardiovascular and hepatic diseases, aging, and stem cell differentiation.

We are particularly interested in finding out whether intrinsic, pharmacological or non-pharmacological (e.g. by exercise) regulation of mitochondrial biogenesis/metabolism and quality control alters (cancer) reduces organ injury during distinct pathologies or as caused by the toxicity of different xenobiotics. The mechanisms of mitochondrial biogenesis and regulation by molecules such as resveratrol or proteins such as sirtuins are important scientific questions. In the same context, another important aim was to investigate how regulation of mitochondrial activity impacts (cancer) stem cell differentiation.

Still in the context of cancer stem cells biology, another of our objectives was to establish an *in vitro* model to study Cr(VI)-induced carcinogenesis and the role of cancer stem cells and the microenvironment in the process.

Different molecules or pathologies activate mitochondrial stress/toxicity responses, affecting the cell phenotype and often organ survival. Among the different xenobiotics tested by our research group in the context of mitochondrial toxicology, anti-cancer agents such as anthracyclines or retinoids were among the chosen. Similarly, we developed a library of distinct mitochondrial-directed molecules which we tested against cancer cell lines of different origins to investigate their potential as future anti-neoplastic agents.

In the framework of mitochondrial alterations during the aging process, another objective of our group was to investigate the role of bone mitochondrial bioenergetics impairment and mitochondrial/peroxisomal fatty acid beta-oxidation unbalance on estrogen-deprivation-induced menopause.

A metabolic approach to some of the biological problems described above has been performed by using nuclear magnetic resonance (NMR)-based metabolomics, which allows for a precise fingerprinting of metabolite fluxes in each condition. Our objective is to use this approach to couple mitochondrial alterations to overall cell metabolism in the context of disease and toxicology.

Main Achievements

We have produced a series of high-impact achievements of which we select the following:

a) In an international, multi-institutional effort, we demonstrated that disrupted ATP synthase activity and mitochondrial hyperpolarization-dependent oxidative stress is associated with p66Shc phosphorylation in fibroblasts of neuropathy, ataxia and retinitis pigmentosa (NARP) patients. In this context, oxidative stress and p66Shc phosphorylation were mitigated by antioxidant treatment, which may be important in the management of that genetic disease.

b) We showed that the alkaloid sanguinary causes very fast death of human melanoma cell lines by inducing oxidative stress, with a powerful inhibitory effect demonstrated by the antioxidant N-acetyl-l-cysteine (NAC). We also showed that dimethylaminopyridine derivatives of lupane triterpenoids cause mitochondrial disruption and inhibit the proliferation of human breast cancer cells. Some of the tested compounds were in fact potent inducers of the mitochondrial permeability transition (MPT) pore.

c) The effects of endoxifen (EDX) were demonstrated to be less toxic on liver mitochondria than its pro-drug tamoxifen (TAM). Furthermore, similarly to TAM, EDX prevented and reversed the MPT. EDX combined with retinoic acid significantly potentiated the antiproliferative effect of the drugs alone and decreased cell migration at concentrations that did not affect the proliferation of non-neoplasic cells. Additionally, the antiestrogens acted synergistically with the NMDA receptor antagonist MK-801 to decrease melanoma cell proliferation.

d) Aiming at understanding the mechanism underlying hexavalent chromium [Cr(VI)]induced malignant transformation, we succeeded at establishing an in vitro model of carcinogenesis induced by Cr(VI). Cell sorter analysis allowed the establishment of a dendogram correlating hierarchically the diverse cellular sub-populations, as well as the identification of cellular sub-populations with stem-like properties (CSCs), with a more malignant phenotype in spite of being more quiescent. Co-culture experiments revealed that the isolated CSCs sub-populations were obtained following a process of dedifferentiation as result of a paracrine crosstalk between the mouse stroma and the epithelial transformed cells.

e) We showed that a rapeseed oil-rich diet, when administrated to Wistar-Han rats, caused fast alterations of liver mitochondrial bioenergetics and membrane composition as well as altered *in vitro* susceptibility to mitochondrial toxicants. Also, we were first to demonstrate that the dioxin TCDD altered the regulation of the ATP-sensitive potassium channels in cardiac mitochondria, which is a downstream stress response triggered by that pollutant.

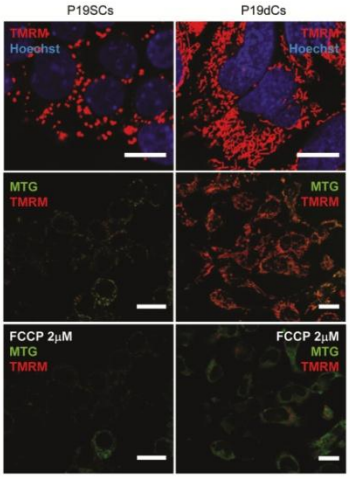
f) We analyzed for the first time *in vivo* bone cell metabolites in sham and ovariectomized twelve-week old

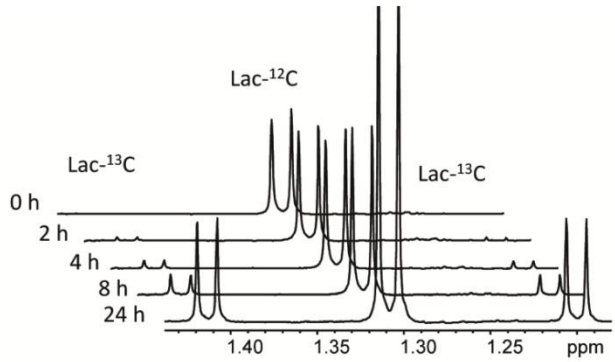
female Wistar-Han rats. Our results suggest metabolic alterations in osteocytes have shown a high repercussion on metabolic profile, which may be associated with the decline in estrogens.

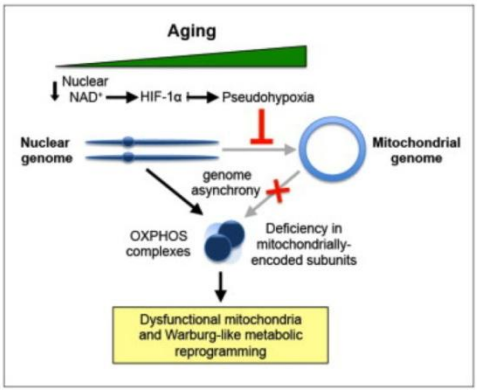
g) When investigating the role of mitochondria as main driver of hyperglycemic memory, transforming a transient insult in permanent cellular damage, we concluded that SIRT1 and AMPK activation are able to counteract metabolic dysfunction by stimulating mitochondrial activity.

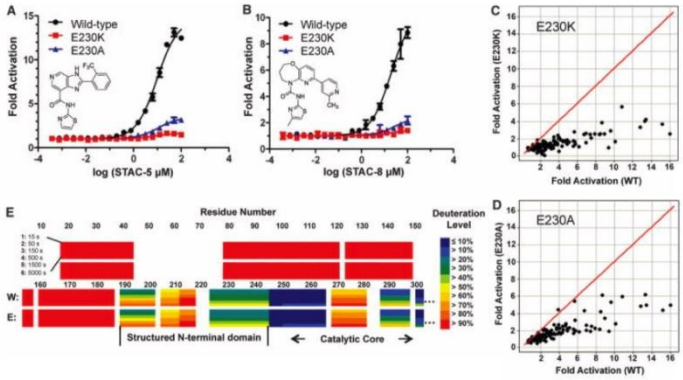
h) We also demonstrated that activation of farnesoid X receptor by bile acids, as well as enhancement of brown fat thermogenesis using chenodeoxycholic acid and stimulation of autophagy by dibenzofuran induce alterations on the cellular energetic status.

i) Finally, by using NMR we were able to obtain a metabolic fingerprinting of cancer stem cell differentiation and understand how increase of mitochondrial capacity directs differentiation of P19 embryonal carcinoma cells. The same technique has been used to fingerprint the oxidative and reductive metabolism of lung cancer cells.









Redox Biology in Health and Disease Group

Head: João Laranjinha

Objectives

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

We are interested in: (a) the study of the molecular mechanisms inherent in neuromodulation and aging that critically involve nitric oxide, connecting the dynamic profiles of nitric oxide (NO) in the brain with its role as a neuromodulator and as the mediator of neurovascular and neurometabolic coupling; (b) the analysis of the mechanisms of action of plant-derived dietary phenolic compounds, particularly those present in wine, in terms of protection against vascular endothelial dysfunction, anti-inflammatory properties, as well as their impact on nitrite-driven regulatory processes, encompassing the non-enzymatic production of nitric oxide from dietary nitrite in the gastric compartment.

Main Achievements

1) Development and construction of novel microsensors for in vivo use in the brain, namely: a) A biomimetic sensor based on hemin/carbon nanotubes/chitosan modified microelectrode for nitric oxide measurement in the brain and b) A self-mixing microprobe for monitoring microvascular perfusion in rat brain.

2) We have described a novel interaction between dietary nitrate and gut proteins with physiological impact. The nitration of pepsin by dietary nitrite in the stomach affords this protein with antiulcerogenic activity. This novel pathway mechanistically also supports the view that green leafy vegetables (major sources of nitrite and nitrate) are beneficial to patients suffering from peptic ulcer.

3) We have identified anti-inflammatory actions for red wine polyphenols that are mechanistically supported by the modulation of inflammatory cascades orchestrated by NFkB, suppression of cycloxigenase and inducible nitric oxide synthase expression as well as inhibition of oxidant-mediated tyrosine nitration. These results support the view that red wine polyphenols may represent a simple and inexpensive therapeutic strategy in the context of intestinal inflammation.

4) The study of the molecular mechanisms involved in the vascular cytoprotection afforded by anthocyainins, supporting the benefits of these compounds as as nutraceuticals, revealed that cyanidin-3-glucoside (Cy3G), a major dietary anthocyanin, against cytokine-triggered inflammatory response in the human intestinal HT-29 cell line, reduced cellular inflammation, in terms of NO, PGE2 and IL-8 production and of iNOS and COX-2 expressions, at a much lower concentration than 5-aminosalicylic acid (5-ASA), suggesting a higher anti-inflammatory efficiency. Interestingly, Cy3G and 5-ASA neither prevented IkB-a degradation nor the activation of NF-kB, but significantly reduced the levels of activated STAT1 accumulated in the cell nucleus. Similar results were obtained in activated macrophages (RAW 264.7 cells), where the combination of Cy3glc with 5-ASA lead also to an increase in the anti-inflammatory action of this drug. *In vivo* experiences, in a rat model of intestinal inflammation, treated with an anthocyanin rich extract obtained from blueberries (*Vaccinium corymbosum L.*), confirmed the high anti-inflammatory action of anthocyanins and their benefits in the inflammed intestinal lumen, together with 5-ASA. Taking into account the high concentrations of dietary anthocyanins potentially reached in the gastrointestinal tract, they may be envisaged as a promising nutraceutical, giving complementary benefits in the context of inflammatory bowel disease.

5) We have proposed a gut-brain communication on basis of redox chemistry on nitric oxide. Data points to implications of the redox conversion of nitrite to nitric oxide in the gut that in turn may signal from the digestive to the central nervous system, influencing brain function.

6) We have suggested the putative occurrence of a ascorbate-driven nitrite/nitric oxide pathway in the brain. Thus, the redox interplay of nitrite and nitric oxide might participate in the regulation of brain homeostasis in a process that may be facilitated by ascorbate. The challenging hypothesis of a nitrite/nitric oxide/ascorbate redox interplay with functional consequences in the neurovascular coupling and neurometabolism still requires further refinement.

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Teodoro JS, Zouhar P, Flachs P, Bardova K, Janovska P, Gomes AP, Duarte FV, Varela AT, Rolo AP, Palmeira CM, Kopecký J. Enhancement of brown fat thermogenesis using chenodeoxycholic acid in mice. *Int. J. Obes. (Lond*). *(In press)*

MICROBIOLOGY AREA

Coordinator: Milton Costa

The Microbiology of Extreme Environments Group continues to examine the microbiological diversity of extreme environments, namely organisms that grow at extremely high temperature, low pH, extremely high salinity (deep sea anoxic brines) and extremely gamma-radiation resistant organisms. This is group has isolated and characterized many extremophiles some of which are quite novel. These organisms are important for fundamental research and especially for biotechnological applications.

Microorganisms capable of osmotic adjustment accumulate low-molecular-weight organic compounds, designated compatible solutes (CS), which can be taken up from the environment or synthesized de novo. Knowledge of the biosynthetic pathways for CS in prokaryotes has increased significantly in recent years due to the high biotechnological potential of CS. These CS accumulate in bacteria, archaea and eucarya. Our group has, in the past several years focused on the osmotic adaptation of thermophilic bacteria. This work is primarily related to the elucidation of the pathways for the biosynthesis of mannosylglycerate (MG), glucosylglycerate (GG), mannosyl-glycosylglucerate (MGG) and trehalose, and the molecular biology of osmoadaptation in Thermus thermophilus. These studies have the objective of using some of the compatible solutes for biotechnological purposes. Just recently we cloned and expressed genes for the synthesis of MG in a plant of the genus Sellaginella sp.

Legionella pneumophila (LP) is a ubiquitous bacterium in natural and water distribution systems that causes pneumonia in humans. Most studies on infection mechanisms of LP have focused mainly on isolates from man-made environments and on clinical related strains. Using LP strains from distinct environments allowed us to determine if particular conditions and specific host/pathogen interactions have influenced the evolution of LP virulence determinants, and resolve if certain LP strains are predominant in human infections. To our knowledge, this is the first time a culture collection of natural environmental LP strains will be tested for their relative ability for environmental persistence and for infect and survival within distinct host cells. We will also assess the contribution of natural environmental LP strains into the molecular evolution of crucial genes in host infection.

Medical Microbiology Group is involved in three major projects. Namely, unravelling the role of adenosine and adenosine receptors in the resistance of Candida albicans to macrophage attack. To accomplish it, we will determined the role of A2A in C albicans infection and express the Adora gene.

In another project: “Alternaria infectoria FKS, CHS and melanin synthesis genes: the combination to oportunism", we will identify Alternaria infectoria FKS, CHS and melanin synthesis genes. Furthermore, A infectoria spores will be used to promote macrophage infection n vitro.

In the project “Type 1 diabetes children oral yeast colonization” the main objectives are to determine the biodiversity and oral yeast load in Type 1 diabetes children, identification of immunological markers and compare the oral care and oral hygiene in control and Type 1 diabetes children subjects aged 2-15 years.

Tuberculosis has killed humans for millennia and infects a third of the human population. Despite over a century of research, it is still the leading cause of death by a single pathogen. New emerging strains resistant to multiple drugs are spreading at the expenses of debilitated immune systems and synergy with HIV/AIDS epidemic, representing a worldwide threat. To halt the progression of TB, basic research is mandatory especially the identification of new drug targets against which new, fast-acting drugs can be designed. To modulate fatty acids synthesis for cell wall assembly, mycobacteria synthesize unique methylglucose lipopolysaccharides (MGLP) but the genes and enzymes involved remain largely unknown. Our ongoing enzymatic, genetic and structural studies will provide a comprehensive understanding of the enzymes in this pathway, paving the way for the validation of new targets to halt the progression of tuberculosis.

Microbiology of Extreme Environments Group

Milton Simões da Costa	PhD – head of group
António Veríssimo Pires	PhD
Joana Cardoso da Costa	PhD
Maria Fernanda Nobre	PhD
Igor Clemente Tiago	Post-Doctoral Fellow
Ana Luísa Gomes Nobre	PhD Student
Ana Sofia Ventura Cunha	PhD Student
Ana Catarina M. Ferreira	PhD Student
Luís André A. França	PhD Student
Tânia de Jesus Leandro	PhD Student
Ana Filipa d'Avó	Grant Technician
Luciana Pinto	Grant Technician

Medical Mycology – Yeast Research Group

Teresa Gonçalves	PhD – *head of group*
Célia Nogueira	PhD
Nuno Empadinhas	PhD
Chantal Fernandes	PhD
Carolina Isabel P. Coelho	Post-Doctoral Fellow
Susana Alarico	Post-Doctoral Fellow
Vítor Mendes	Post-Doctoral Fellow
Ana Maranha Tiago	PhD student
Lisa Catarina O. Rodrigues	PhD student
Mª Graça Rocha	PhD student
Rui Manuel Costa Soares	PhD student
Diogo Reis	MSc student
Vânia Moreira	MSc student
Alexandra Abrunheiro	Grant Technician
Mafalda Costa	Grant Technician
Mariana Almeida	Grant Technician
Marta Mota	Grant Technician
Marta Sousa	Grant Technician

Microbiology of Extreme Environments Group

Head: Milton Costa

Objectives

The objectives for 2013 were:

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

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

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

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

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

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

7) To unravel the microbial diversity and community structure of a deep mineral water aquifer and the bottled water produced from said water using massively parallel 454 pyrosequencing of the 16S rRNA gene, DGGE, FISH and cultivation.

8) To determine the microbiome composition of terrestrial crustacean *Porcellio dilatatus* (Crustacea:Isopoda) hindgut.

9) To construct metagenome libraries derived from the microbial populations associated with the digestive system (comprising the stomach, hindgut and hepatopancreas) of *Porcellio dilatatus* (Crustacea:Isopoda). Those libraries will be screened for plant cellulosic biomass degrading microbial enzymes.

10) To determine the functional diversity in continental serpentinization-driven deep aquifers.

Main Achievements

During 2013:

1) We have completed the genome sequence of *Dehalogenimonas lykanthroporepellens* type strain (BL-DC-9(T)) and published a paper on this research

2) We completed the genome sequence Rubrobacter radiotolerans and have had a paper accepted for publication.

3) We have isolated and characterized several novel bacterial species: *Natrinema salacieae*, *Heliimonas saccharivorans, Rhodopirellula lusitana* and *Rhodopirellula rubra*, *Rubrobacter calidifluminis* and *Rubrobacter naiadicus*.

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

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

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

7) We produced shotgun metagenome sequencing data from a serpentinization-driven deep aquifer.

8) We extracted and purifieded total microbial DNA from Porcellio dilatatus (Crustacea:Isopoda) hindgut. The 16S rRNA gene amplicons were massively parallel sequenced using Illumina platform, and we began the construction of metagenomic libraries derived from the microbial populations associated with the digestive system of Porcellio dilatatus .

Medical Mycology – Yeast Research Group

Head: Teresa Gonçalves

Objectives

A. *Alternaria infectoria* an opportunistic agent of human infection and of severe allergies"

Objectives 2013:

1. Cell wall directed antifungals efficiency in the eradication of Alternaria infection. Modulation of CHS and FKS gene expression and of the regulation of cell wall chitin and glucan synthesis by Caspofungin and Nikkomycin. Macrophage in vitro infection by A. infectoria spores – effect of caspofungin treatment.

2. Extracellular vesicles as a delivery platform for virulence factors: *A. infectoria* extracellular vesicles production

3. Characterization of the macrophage response to *in vitro* infection by *A. infectoria* spores

4. Hyphal cell wall nanoparticles

B. Role of adenosine and adenosine receptors in *Candida albicans* infection

Objectives 2013:

1. Involvement of adenosine and adenosine A_{2A} receptor in *C albicans* infection

2. *C. albicans* infection of A_{2A} knockout mice peritoneal macrophages

3. Differential gut infection of C. albicans in aged mice. Involvement of A_{2A} receptors

3. Characterisation of ectophosphatases and ectonucleotidase activity of *C. albicans*.

C. Validation of Chromogenic media for the identification of pathogenic yeasts

D. Identification of novel gene functions in pathogenic mycobacteria with focus on those involved in the biosynthesis of mycobacterial virulence factors

Objectives 2013:

1) Identification of genes of the mycobacterial MGLP pathway and biochemical characterization of key-enzymes.

2) Protein crystallization and three-dimensional structure determination

Main Achievements

A. *Alternaria infectoria* an opportunistic agent of human infection and of severe allergies

1. Susceptibility to caspofungin and nikkomycin Z. Collaboration with Professor Neil Gow of the Institute of Medical Sciences of Aberdeen, UK.

Papers

C Fernandes, J Anjos, LA Walker, BMA Silva, L Cortes, M Mota, CA Munro, NAR Gow, T Gonçalves (2014). Modulation of *Alternaria infectoria* cell wall chitin and glucan synthesis by cell wall synthase inhibitors. Submitted to Antimicrobial Agents and Chemotherapy.

Gene sequences deposited in the NCBI database:

Accession numbers JX436211 to JX436224, JX443517, and JX443518

2. Extracellular vesicles of *A. infectoria*. Together with Professor Arturo Casadevall at the Einstein School of Medicine, NY, USA, we identified and characterised, morphologically and proteomically the extracellular vesicles.

Papers:

BMA Silva, R Prados-Rosales, J Espadas-Moreno, JM Wolf, JL Luque-Garcia, T Gonçalves, A Casadevall (2014). Characterization of *Alternaria infectoria* extracellular vesicles. Medical Mycology. 52 (2): 202-210

B. Role of adenosine and adenosine receptors in *C. albicans* infection

1. During 2013 we continued tackling the involvement of purines and of the adenosine A2A receptor in *C. albicans* infection of macrophages.

MS submitted to mBio Journal

L Rodrigues; F Curado; C Coelho; V Cabral; L Cortes, RA. Cunha; T Gonçalves. INVOLVMENT OF ADENOSINE A2A RECEPTORS IN MACROPHAGE INFECTION BY *CANDIDA ALBICANS*.

2. Impact of caffeine treatment in *C. albicans* skin infection model.

We studied how *C. albicans* infection of keratynocytes proceeds in the presence of caffeine.

MS under preparation

M Mota, L Cortes, F Queiróz, T Gonçalves. IMPACT OF CAFFEINE IN THE INTERNALIZATION OF *C. ALBICANS* BY HUMAN KERATINOCYTES.

3. *In vivo* infection of *C. albicans*. Mice of different age groups were orally infected with yeasts. This is an ongoing work; data is being gathered to characterise the differential yeast infection in several organs (stomach, intestine, cecum, liver). Multicentric study including CNC, FMUC, FMUP and Hospital de S. João, Porto.

C. Chromogenic media for yeasts identification

A prototype is being developed for the rapid identification of a group of yeasts. This is an ongoing work. A provisional patent application is being prepared.

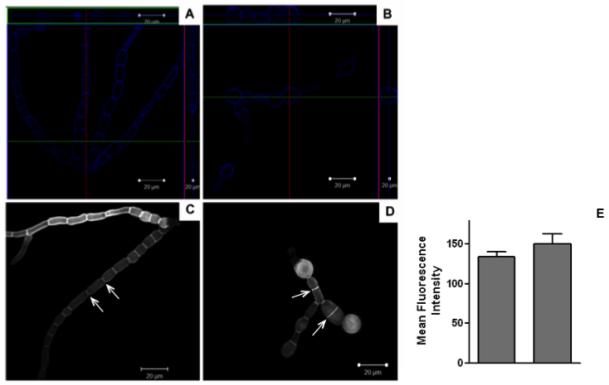
D. Identifying the genes for MGLP biosynthesis and characterization of enzymes.

We expressed recombinantly and characterized biochemically 3 novel mycobacterial enzymes that had unknown functions or were misannotated in mycobacterial genomes:

1) A glycoside hydrolase restricted to nontuberculous mycobacteria that is critically involved in mycobacterial recovery from nitrogen stress. This work was carried out in collaboration with Rita Ventura at ITQB, Oeiras (Costa et al, unpublished).

2) An atypical GPG phosphatase of a novel protein family, which is the second type in mycobacteria and the third version found in nature (Alarico et al, unpublished).

3) A rare acyltransferase considered essential for M. tuberculosis growth was found to catalyze the third step in MGLP biosynthesis in collaboration with Anthony Clarke, University of Guelph, Canada (Maranha et al, unpublished).



Publications:

Alarico S, Empadinhas N, da Costa MS (2013) A new bacterial hydrolase specific for the compatible solutes alpha-D-mannopyranosyl-(1→2)-D-glycerate and alpha-D-glucopyranosyl-(1→2)-D-glycerate. Enzyme and Microbial Technology 52(2):77-83.

E. Crystallization and determination of the three-dimensional structures of mycobacterial proteins representing potential targets for drug design.

The three-dimensional structure of an essential mycobacterial maltokinase was solved in collaboration with Sandra Macedo-Ribeiro at IBMC, Porto (Fraga et al, unpublished).

The three-dimensional structure of an essential maltosyltransferase was solved in collaboration with Tom L. Blundell, University of Cambridge, UK and fragment-based drug design trials are in progress (Mendes et al, unpublished).

The three-dimensional structure of a mycobacterial thermostable GpgS was solved in collaboration with Pedro J. Pereira at IBMC, Porto. This structure allowed crucial insights instrumental for drug design and screening strategies (Silva et al, unpublished).

Publications

Alarico S, Empadinhas N, da Costa MS. (2013) A new bacterial hydrolase specific for the compatible solutes alpha-D-mannopyranosyl-(1→2)-D-glycerate and alpha-D-glucopyranosyl-(1→2)-D-glycerate. *Enzyme and Microbial Technology* 52(2):77-83. *IF: 2.59, Q1*

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Albuquerque L, Taborda M, La Cono V, Yakimov M & da Costa MS. (2013) *Natrinema salaciae* sp. nov., a halophilic archaeon isolated from the deep, hypersaline anoxic Lake Medee in the Eastern Mediterranean Sea. *Systematic and Applied Microbiology*, 35:368-373.

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Leandro T, França L, Nobre MF, Rainey FA, & da Costa MS. (2013) *Heliimonas saccharivorans* gen. nov., sp. nov., a member of the family *Chitinophagaceae* isolated from a mineral water aquifer and emended description of *Filimonas lacunae*. *International Journal of Systematic and Evolutionary Microbiology*, 63: 3793-3799.

Marques J, Paula A, Gonçalves T, Ferreira M, Carrilho E. (2013) Ozone action on Streptococcus mutans and Lactobacillus fermentum: A pilot study. *World J. Stomatol*. 2(1): 18-23. doi:10.5321/wjs.v2.i1.18.

Miranda I, Silva-Dias A, Rocha R, Teixeira-Santos R, Coelho C, Gonçalves T, Santos MAS, Pina-Vaz C, Solis NV, Filler SG, Rodrigues AG. (2013) Candida albicans CUG Mistranslation Is a Mechanism To Create Cell Surface Variation. *mBio* 4(4). pii: e00285-13. doi: 10.1128/mBio.00285-13. *IF: 5.6, Q1*

Nobre A, Empadinhas N, Nobre MF, Lourenço EC, Maycock CD, Ventura MR, Mingote A, & da Costa MS. (2013) The plant *Selaginella moellendorffii* possesses enzymes for the synthesis and hydrolysis of the compatible solutes mannosylglycerate and glucosylglycerate. *Planta,* 237:891-901

Pereira SG, Albuquerque L, Nobre MF, Tiago I, Veríssimo A, Pereira A, and da Costa MS. (2013). *Pullulanibacillus uraniitolerans* sp. nov., a new acidophilic, U (VI)-resistant species isolated from an acid uranium mill tailing effluent and emended description of the genus *Pullulanibacillus*. *Int. J. Syst. Evol. Microbiol*. 63:158-162.

Silva BMA, Prados-Rosales R, Espadas-Moreno J, Wolf JM, Luque-Garcia JL, Gonçalves T, Casadevall A. (2013) Characterization of Alternaria infectoria extracellular vesicles. *Medical Mycology* 52 (2): 202-210. doi: 10.1093/mmy/myt003. *IF: 2.168, Q1*

Tiago I and Verissimo A. (2013) Microbial and functional diversity of a subterrestrial high pH groundwater associated to serpentinization. Environ. *Microbiology* 15(6), 1687–1706.

BIOPHYSICS AND BIOMEDICAL NMR AREA

Coordinator: Carlos Geraldes

The General Objectives of this area are:

a) Study of inorganic compounds (chelates and nanosystems) for medical diagnostic imaging, in particular MRI contrast agents and multimodal systems

b) Structure and dynamics of proteins and protein-ligand interactions using NMR techniques.

c) MRI studies of liver steatosis in humans

d) The effects of high fructose feeding on hepatic lipid and carbohydrate fluxes.

e) Characterizing dietary carbohydrate utilization by farmed fish.

The Main Achievements of this area are:

1) A series of new Gd(III) and Mn(II) chelates were studied in solution and their properties relevant for efficient MRI agents (in particular relaxivity) were obtained.

2) Studies of Ga^{3+} complexes for PET Imaging

3) In vitro/in vivo MRI agents studies

4) Human in vivo MRI studies of liver in steatosis

5) NMR in Cell Biophysics

6) NMR studies of protein structure and dynamics in solution – use of paramagnetic tags in MMP-1.

7) 1. ^{2}H-enrichment distribution of hepatic glycogen from $^{2}H_2O$ reveals the contribution of dietary fructose to glycogen synthesis.

8. Determining the effects of transaldolase exchange on estimates of gluconeogenesis in type 2 diabetes:

9. Noninvasive measurement of murine hepatic acetyl-CoA ^{13}C-enrichment following overnight feeding with ^{13}C-enriched fructose and glucose.

Inorganic Biochemistry and Molecular Imaging Group

Carlos F. Campos Geraldes	PhD – *head of group*
Maria Margarida Castro	PhD
Ana Marguerita Metelo	PhD student
Filipe Manuel C. Gomes	PhD student
André Ferreira Martins	PhD student
Helena Santos Leitão	PhD student
David Miguel Dias	PhD student

Intermediary Metabolism Group

John Jones	PhD – *head of group*
Cristina Barosa	Post-Doctoral Fellow
Ivan Viegas	Post-Doctoral Fellow
Fátima Martins	PhD student
João Rito	PhD student
João Silva	PhD student
Catia Marques	MSc student
Paula da Silva	MSc student
Filipa Simões	Grant Technician
Margarida Coelho	Grant Technician

Inorganic Biochemistry and Molecular Imaging Group

Head: Carlos Geraldes

Objectives

Our general objective is the study of inorganic compounds for medical diagnostic imaging (in particular MRI contrast agents), inorganic drugs for medical therapy, and the study of environmental and toxicological effects of inorganic species. The design and development of metal based agents for multimodal targeted molecular imaging agents is followed by *in vitro* cell studies and animal model evaluation using MRI and nuclear imaging techniques. These agents include Ln^{3+}-based paramagnetic nanoparticles with interesting photoluminescence properties for optical imaging (OI), and high r_2 relaxivities, especially at high fields, yielding negative contrast in T2-weighted MRI images. The r1 relaxivity of new lanthanide chelates will be increased by designing new chelating agents which increase the number of inner sphere water molecules and optimize the water exchange rates. Second-sphere water relaxation contributions should also be optimized. We also study the structure and dynamics of proteins and protein-ligand interactions using NMR techniques.

Main Achievements

1) A series of new Gd(III) and Mn(II) chelates were studied in solution and their properties relevant for efficient MRI agents (in particular relaxivity) were obtained.

In vitro evaluation of new small Gd(III) and Mn(II) complexes as potential MRI CAs:

a) In vitro evaluation of amide conjugates of the DO3A-N-(a--amino)propionate ligand as potential MRI CAs.

b) Studies of new Tris-3,4-HOPO lanthanide complexes as potential MR imaging probes.

c) In vitro studies of new small, triaza-macrocylic Mn(II) chelates as potential MRI CAs.

2) Studies of Ga^{3+} complexes for PET Imahging

a) Studies of efficiency of ^{68}Ga radiolabeling reaction conditions: Spectroscopic, radiochemical, and theoretical studies of Ga^{3+}- HEPES - evidence for the formation of Ga^{3+}-HEPES complexes in ^{68}Ga labelling reactions. The efficiency of ^{68}Ga radiolabeling reaction conditions in HEPES buffer was rationalized.

b) Structural and photophysical studies on Gallium(III) 8-hydroxyquiniline-5-sulphonates.

3) In vitro/in vivo MRI agents

a) Cell labeling and in vivo MRI cell tracking using a positive MRI contrast agent - MRI Tracking of Macrophages using Glucan Particles Entrapping a Paramagnetic Agent. A new, very efficient positive MRI Agent for macrophage labeling and in vivo MRI tracking was developed and evaluated.

b) In vitro/in vivo studies of new PIB conjugates for Abeta amyloid MR/PET Imaging. New PIB conjugates for Abeta amyloid for MR/PET Imaging were studied in vitro.

c) New dextrin covered iron oxide nanoparticles as MRI contrast agents were studied in vitro and in rodents.

4) Human in vivo MRI studies of liver in steatosis

a) Fat deposition decreases diffusion parameters at MRI: a study in phantoms and patients with liver steatosis.

b) MR fat fraction mapping: a simple biomarker for liver steatosis quantification in nonalcoholic fatty liver disease patients.

5) NMR in Cell Biophysics:

a) Biophysical studies of drug-membrane interactions using NMR. A biophysical approach to menadione membrane interactions: relevance for menadione-induced mitochondria dysfunction and related deleterious/therapeutic effects

b) ^{23}Na Multiple Quantum Filtered NMR Characterization of Na^+ Binding and Dynamics in Animal Cells – a Comparative Study and Effect of Na^+/Li^+ Competition

6) NMR studies of protein structure and dynamics in solution – use of paramagnetic tags.

a) Examination of matrix metalloproteinase-1 (MMP-1) in solution: a preference for the pre- collagenolysis state

Intermediary Metabolism Group

Head: John G. Jones

Objectives

a) The effects of high fructose feeding on hepatic lipid and carbohydrate fluxes: The Western diet is characterized by high intake of refined sugar and high-fructose corn syrup and is implicated in the soaring rates of diabetes and non-alcoholic fatty liver disease. Fructose is a carbohydrate that is solely metabolized by liver, hence diets high in fructose present the liver with a substantial nutritional challenge. The immediate fate of fructose is phosphorylation and conversion to triose phosphates. Triose phosphates may in turn be metabolized to pyruvate and acetyl-CoA via glycolysis and pyruvate dehydrogenase activities. This acetyl-CoA in turn can be recruited for *de novo* lipogenesis. Triose phosphates can be also converted to glucose and glycogen *via* gluconeogenic pathways resulting in elevated hepatic glucose production and glycogen synthesis. Since high fructose feeding is associated with both excessive hepatic lipid levels (possibly related to increased rates of *de novo* lipogenesis) and impaired control of hepatic glucose production (possibly related to increased rates of gluconeogenesis), determining the flux of fructose carbons into glucose/glycogen and into hepatic triglyceride is a key objective. To this end, we have been developing novel noninvasive stable isotope tracer methods to determine the contribution of dietary fructose to the synthesis of hepatic glucose, glycogen and triglyceride. This approach will allow us to determine if fructose is directly contributing carbons for *de novo* lipogenesis and/or facilitating *de novo* lipogenesis from all acetyl-CoA sources, possibly by upregulation of *de novo* lipogenesis enzymes. These methodologies are being currently applied to animal models but we are also translating to human studies where they will be applied to characterize hepatic metabolic fluxes during high sugar feeding.

b) Effect of oral medium-chain triglyceride on cerebral substrate utilization in rodent disease models: Diseases such as Alzheimers and epilepsy are characterized by a decrease in cerebral glucose oxidation. In the initial stages, restricted glucose conversion to acetyl-CoA is hypothesized to be an important contributory factor. In this setting, the neurons are believed to intact but in a hypometabolic state, which may compromise their energetic and functional capacities. If this is the case, provision of alternative oxidizable substrates to generate acetyl-CoA may restore cellular Krebs cycle flux and energetic state. While glucose is the principal oxidizable substrate for brain metabolism, ketone bodies can also be efficiently utilized as a source of acetyl-CoA. Therefore, the initial objectives are to quantify competition of glucose and ketone bodies to cerebral acetyl-CoA synthesis in isolated brain slices. This will be initially applied to healthy rodents in order to optimize experimental protocols and methodologies. When this is accomplished, the protocol may then be applied to appropriate disease models.

c) Characterizinf glycerol utilization by seabass: The European seabass is an important farmed marine fish species. As carnivorous fish, their metabolism is adapted to high levels of dietary protein, thus their efficiency in utilizing dietary carbohydrates is poor. Increased carbohydrate utilization would be both economically and environmentally beneficial, since high-cost fish meal could be substituted in part by lower cost substrates while at the same time the conversion of dietary amino acids to glucose and generation of waste ammonia would be spared. Glycerol is a by-product of biodiesel synthesis and it has been evaluated as a feed supplement in rainbow trout and channel catfish. In mammals, it is efficiently converted to glucose via gluconeogenesis, but its metabolism by fish is not known. We hypothesize that glycerol effectively competes with dietary amino acids for gluconeogenic carbons thereby sparing their conversion to glucose.

Main Achievements

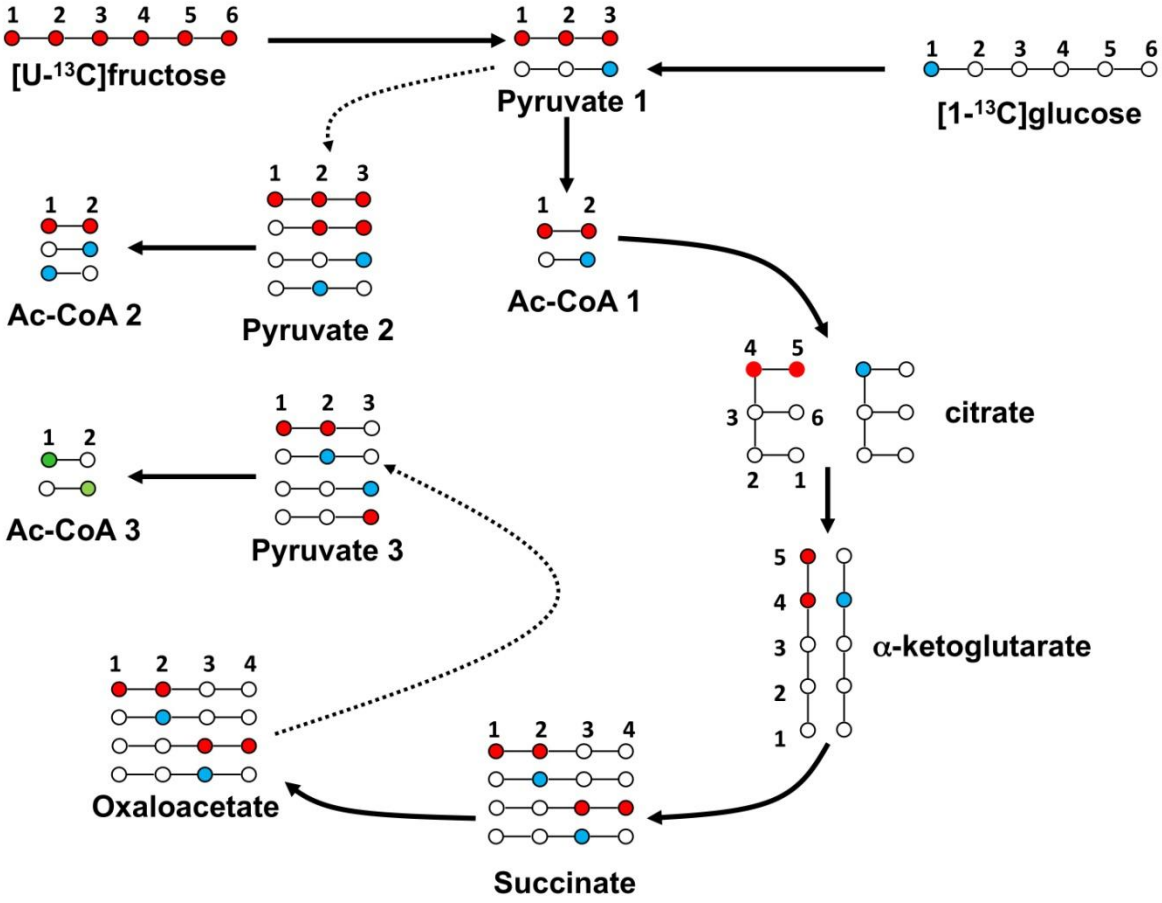
1. ^{2}H-enrichment distribution of hepatic glycogen from $^{2}H_2O$ reveals the contribution of dietary fructose to glycogen synthesis: ^{2}H-enrichment of glycogen positions 5 and 2 from $^{2}H_2O$ informs direct and indirect pathway contributions to glycogenesis. Inclusion of position 6S ^{2}H-enrichment data allows indirect pathway sources to be resolved into triose-phosphate and Krebs cycle precursors. This analysis was applied to 6 rats that had fed on standard chow (SC), and 6 fed on SC plus 35% sucrose in the drinking water, all of which were also given $^{2}H_2O$. Overnight net hepatic glycogen synthesis was similar between HS and SC rodents. Direct pathway contributions were also similar (403 ± 71 vs. 578 ± 76 mmol/gdw), but triose-phosphate contributions were significantly higher for HS (382 ± 61 vs. 87 ± 24 mmol/gdw, $p<0.01$) while Krebs cycle inputs were lower for HS (110 ± 9 mmol/gdw vs. 197 ± 32 mmol/gdw, $p<0.05$). Hence, the ^{2}H-enrichment distributions of hepatic glycogen and glucose from $^{2}H_2O$ informs the contribution of dietary fructose to hepatic glycogen and glucose synthesis.

2. Effects of transaldolase exchange on estimates of gluconeogenesis in type 2 diabetes: Transaldolase exchange (TA) overestimates gluconeogenesis measured with $^{2}H_2O$. However, it is unknown if TA differs in people with type 2 diabetes (T2DM). $^{2}H_2O$ was ingested and [1-^{13}C]acetate and [3-^{3}H]glucose infused in T2DM (n=10) and healthy nondiabetic (ND, n=8) subjects. TA was assessed from the ratio of ^{13}C3 to ^{13}C4 glucose enrichment (^{13}C3/^{13}C4) measured by ^{13}C NMR. Glucose turnover was measured before (~16hr fast) and during hyperglycemic (~10mM) moderate dose insulin (~0.35 mU/kg/min) clamp.

^{13}C3/^{13}C4 in T2DM vs. ND was no different at baseline and clamp indicating equivalent TA. To determine if incomplete triose-phosphate isomerase exchange (TPI) contributed to asymmetric ^{13}C3/^{13}C4, [U-^{13}C]glycerol was infused in lieu of [1-^{13}C]acetate at a separate visit in a subset of ND (n=7) subjects. Both tracers yielded ^{13}C3/^{13}C4

< 1.0 at baseline and at clamp conditions indicating that TPI exchange was essentially complete and did not contribute to asymmetric glucose enrichment. Uncorrected and corrected rates of gluconeogenesis were no different in T2DM vs. ND both at baseline and during clamp. TA correction resulted in equivalent estimates of corrected gluconeogenesis in T2DM and ND that were ~25-35% lower than uncorrected gluconeogenesis both at baseline and during the clamp. In conclusion, TA exchange does not differ between T2DM and ND under these conditions and the $^{2}H_2O$ method provides an accurate comparison of gluconeogenic fluxes in subjects with and without diabetes.

3. Noninvasive measurement of murine hepatic acetyl-CoA ^{13}C-enrichment following overnight feeding with ^{13}C-enriched fructose and glucose. The ^{13}C-isotopomer enrichment of hepatic cytosolic acetyl-CoA of overnight-fed mice whose drinking water was supplemented with [U-^{13}C]fructose, and [1-^{13}C]glucose and *p*-amino benzoic acid (PABA) was quantified by ^{13}C NMR analysis of urinary *N*-acetyl- PABA. Four mice were given normal chow plus drinking water supplemented with 5% [1-^{13}C]glucose, 2.5% [U-^{13}C]fructose, and 2.5% fructose (Solution 1) overnight. Four were given chow and water containing 17.5% [1-^{13}C]glucose, 8.75% [U-^{13}C]fructose and 8.75% fructose (Solution 2). PABA (0.25%) was present in both studies. Urinary *N*-acetyl-PABA was analyzed by ^{13}C NMR. In addition to [2-^{13}C]- and [1,2-$^{13}C_2$]acetyl isotopomers from catabolism of [U-^{13}C]fructose and [1-^{13}C]glucose to acetyl-CoA, [1-^{13}C]acetyl was also found indicating pyruvate recycling activity. This precluded precise estimates of [1-^{13}C]glucose contribution to acetyl-CoA while that of [U-^{13}C]fructose was unaffected. The fructose contribution to acetyl-CoA from Solutions 1 and 2 was 4.0 ± 0.4% and 10.6 ± 0.6%, respectively, indicating that it contributed to a minor fraction of lipogenic acetyl-CoA under these conditions.



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CELL AND DEVELOPMENT BIOLOGY AREA

Coordinator: João Ramalho Santos

The main goal of the Groups in this Research Line is to strengthen CNC involvement in translational aspects of biomedical research, working in close collaboration with medical partners. Indeed, one of the major strengths of the groups in this 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 topics, including Immunology, Oncobiology, Dermatology, Reproduction, Endocrionology (Obesity, Diabetes) and Cardiology.

This has been achieved in the past as the publication record for the various groups in this area demonstrates, with increased quality of publications in the past four years.

This Research Line includes groups active in (non-neuroscience related) clinical collaborations. All groups are active, have appropriate funding and are publishing adequately at different levels. In fact a significant increase both in competitive funding and productivity throughout the line as a whole are noteworthy in terms of the previous report. Also of note 20% of the published research manuscripts produced by this Research Line during the period under evaluation, plus 3 completed PhD Theses, involved extensive collaborations with other CNC groups, and a total of 80% of total publications were collaborative in nature, notably in translational aspects.

Outputs of clinical significance include:

1- The distinct effects of immunosuppressive therapy on lipid and glucose metabolism.

2- The role of inflammation in diabetic wound healing and cartilage damage in diabetes-associated osteoarthritis and how it can be used for therapeutic purposes.

3- The possible paracrine and endocrine roles of Epicardial Adipose Tissue in heart failure potentiated by diabetes.

4- The use of calcium oscillations and proteomics data to identify novel markers for sperm function.

5- The identification of specific targets for endocrine disruptors in human sperm.

6- The validation of a novel cost-effective diagnostic tool for Assisted Reproduction.

7- The identification of mechanisms involved in macrophage lipidosis with relevance for the development of atherosclerosis.

8- The development of an in vitro/in silico/in chemico method for quantifying the potency of skin allergens (patent in preparation).

9- The unveiling a new pathway involved in non-medullary thyroid cancer.

10- The characterization of the complex heterogeneity and distinct clonal pathways of glioma evolution with a clear association between the gene expression profile (GEP) of gliomas and tumor histopathology.

Cellular Immunology and Oncobiology Group

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Sara Rebelo	MSc Student
Solange Machado	MSc Student
Marta Baptista	Grant Technician
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Infection, Phagocytosis and Pathogens Group

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Insuline Resistance and Adipocyte Group

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Patrícia Lopes	PhD Student
Roksana Pirzgalska	PhD Student
Carlos Moura	Msc Student
Fábio Carvalho	Msc Student

Cellular Immunology and Oncobiology Group

Head: Mª Celeste Lopes

Objectives

Immunobiology of antigen presenting cells:

1) development of non-animal cell-based approaches to detect skin and respiratory allergens, as demanded by the new European policy

2) screening of lead molecules with anti-inflammatory and anti-tumoral properties obtained from medicinal plants

3) evaluation of the cross-talk between autophagy and inflammasome in antigen presenting cells

Chondrocyte biology and osteoarthritis:

1) elucidate the mechanisms by which hyperglycemia can favour the development and progression of osteoarthritis to identify target specific strategies for prevention and treatment of diabetes mellitus-associated osteoarthritis.
2) identify new compounds in plant volatile extracts with potential anti-osteoarthritic activity, as well as with potential activity against other diseases with a chronic inflammatory component, namely inflammatory bowel disease.
3) validate the use of a new concept bioreactor, developed in collaboration with researchers from the University of Aveiro, for cartilage tissue engineering.

Oncobiology

To evaluate the cell signaling pathways involved in cancer (haematologic cancer, breast cancer and brain tumors), namely the role of oxidative stress and mitochondrial dysfunction, the deregulation of apoptotic, checkpoint and DNA repair pathways, as wells as chromosomal, genetic and epigenetic abnormalities, aiming at identifying new genes and cell signaling pathways potentially relevant for cancer development and progression

Main Achievements

•Immunobiology of antigen presenting cells:

We developed an in vitro/in silico/in chemico method for quantifying the potency of skin allergens that is of uttermost importance for the Globally Harmonized System of Classification and Labeling (GHS) (Provisional Patent Application nº 20121000088462).

• **Chondrocyte biology and osteoarthritis**:

1) We found that exposure to hyperglycemia-like glucose concentrations is sufficient to induce inflammatory responses and impair autophagy in human chondrocytes. These mechanisms can contribute to the development and progression of diabetes-associated osteoarthritis and represent potential targets for the development of directed therapies and preventive strategies.
2) The bioreactor was optimized and validated. The results obtained show that the mechanical stimulation of the chondrocyte constructs favors cartilage matrix production.

•Oncobiology:

We found the involvement of oxidative stress and mitochondrial dysfunction in neoplasic development; changes in the levels of apoptotic modulators which may be related with resistance to cell death; and alterations in checkpoint responses (e.g., Claspin mutations that alter Chk1 activation).

We unravelled a new pathway involved in non-medullary thyroid cancer involving LRP1B and the modulation of the extracelular microenvironment.

The study of human brain tumor samples revealed a complex heterogeneity and distinct clonal pathways of glioma evolution and a clear association between the gene expression profile (GEP) of gliomas and the tumor histopathology.

Biology of Reproduction, Stem Cells and Human Fertility Group

Head: João Ramalho-Santos

Objectives

The main Objectives of the group are the characterization of metabolic pathways focusing on mitochondrial activity, and how they can be used to both determine and modify human gamete functionality; and as cues to modulate pluripotent stem cell fate.

In terms of research in reproduction the goals are always two-fold: to decipher basic molecular mechanisms, and to translate those finding into clinically relevant deliverables and novel methodologies for Assisted Reproduction, both as diagnostic and as interventions. In the past year basic research focused on novel projects on sperm proteomics and metabolomics, and the former study has suggested novel metabolic pathways relevant for human sperm function that might be actively used to increase fertilization rates. Research has also focused on how a heterogeneous population of sperm may be separated into subpopulations, thus allowing the use of only the more functional gametes in Assisted Reproduction.

In terms of applied research the group has pioneered a simple and cost-effective assay to analyze sperm chromatin status, and this assay has been further validated in a large study. We also were able to pinpoint novel ways in which environmentally relevant contaminants may affect human sperm function at a non-genomic level, by interfering with sperm metabolism. Additionally, the group has collaborated with the Coimbra University Health System (CHUC) in order to preserve germinal tissues from oncological patients that may have their fertility potential compromised following chemotherapy and radiotherapy. This project, named Oncofertility, is ongoing. In terms of preserving the germline of rare individuals, similar strategies are being implemented to conserve gonadal tissue of animals from at-risk species, namely wild felids, using the domestic cat as a model.

The expertise in metabolic studies in Reproduction has been expanded in a novel approach to modulate the fate of pluripotent stem cells (both embryonic and induced). The Group has successfully implemented changes in metabolic cues in order to control the pluripotency or differentiation ability of stem cells, with relevance for tissue engineering. In essence mitochondrial quiescence is related to pluripotency, while differentiation is keyed by an increase in mitochondrial oxidative phosphorylation activity. Additionally, in the course of these experiments unexpected parallels between pluripotent stem cells and cancer cells were discovered at the metabolic level, showing that both cell types control mitochondrial activity in a similar manner. This novel potential will be explored in the near future, focusing on cancers in the reproductive system, while maintaining previous research lines.

Main Achievements

In 2013 the main group achievements were

1- Validation in a large multi-year study of a novel simple diagnostic technique to assess human sperm chromatin damage that can be clinically implemented, and that provides cost-effective data in terms of determining the potential of a given semen sample for Assisted Reproduction (Publication 11)

2- Discovery that the endocrine disruptor DDE (the main metabolite of the pesticide DDT) can act on human sperm in a non-genomic manner and at environmentally relevant concentrations (at the picomolar level), by affecting the sperm-specific ion channel CatSper and causing functional changes in intracellular calcium concentrations and sperm metabolism. These results suggest a new possible mechanism to explain the negative role of these compounds on male infertility (Publication 12).

3- Establishment of mitochondrial complex III as a gateway controlling pluripotent stem cell differentiation into a neuronal phenotype, by affecting differentiation initiation, reactive oxygen species levels, and the cell cycle (Publication 13)

4- Characterization for the first time of the human sperm tail proteome, identifying over 1000 novel proteins in the male gamete. The data suggests novel metabolic pathways that may be important for human sperm function, and thus represent putative targets for both contraception and infertility interventions (Publication 2).

5- Deciphering basic mechanisms of functionally relevant calcium homeostasis in human sperm that use different types of channels and reservoirs, and how they may be important to control sperm metabolism and function (Publication 6).

6- Implementing novel methods to sort human sperm and to assess damage in the male gamete at the mitochondrial level (Publications 7 and 8).

7- Determining the molecular metabolic and mechanisms by which novel molecules suggested to function as spermicides act on human sperm (Publication 1). This research led to an industry contract to assess spermicides for the company Innotech Pharmaceuticals.

Infection, Phagocytosis and Pathogens Group

Head: Mª Otília Vieira

Objectives

The research in my lab is focused in tuberculosis (TB) and in atherogenesis. We addressed our scientific questions by a combination of cell biology, lipidomic analysis, lentiviral shRNA libraries screenings, confocal and electron microscopies, etc.

The main goal of applied research on Mycobacterium is to produce a vaccine that is effective. Understanding the "life cycle" of Mycobacterium within macrophages is at the very center of its pathogenesis and immune evasive strategies. For example, realizing that BCG does not evoke significant MHC class I immune responses has led to new vaccine strategies now being tested in which BCG is engineered to escape the phagosome and elicit MHC I restricted T cell responses. During last year we started to define new directions in the cell biology of TB. Namely how it infects and kills the cells and the mechanisms that are involved in the membrane repair process that is a key part of how the organism kills its host cells. We hope that our research will allow manipulation of the outcome of macrophage death and explain differences in antigen presentation and induction of adaptive immunity. We predict that the mechanistic understanding of the process of membrane resealing of the macrophage and its regulation will be an important step towards the identification of new therapeutic targets and better designed vaccine strain characteristics against TB. In this context we want to stress that multidrug resistant TB infections have become a serious global health threat. The only vaccine, a disarmed strain of a bovine form of the bacterium, is largely ineffective in preventing infection. Thus, our research will help to point the ways to developing better vaccine strain characteristics.

Within the frame of our second scientific project on the etiology of atherogenesis, I should stress that our view of atherogenesis subscribes the etiological role of LDL oxidation and the idea that inefficient efferocytosis is a fundamental problem in atherogenesis. My laboratory has initiated work on both fronts and we are addressing these two issues. Admittedly, a problem as complex as atherogenesis may have a multiparametric etiology and there may be synergies between different causes. However, we believe that each of these putative causes needs to be examined individually and in systematic detail, both *in vitro* and *in vivo*, and this is the goal for the next years. The results obtained so far by my group are extremely promising and our present perspective of the problem of atherogenic etiology is, as far as I know, refreshingly new. In the end we want to elucidate the molecular etiology of atherogenesis and identify potential targets for diagnostic and therapeutic intervention in atherosclerosis. We cannot ignore that despite the incredible progress in cardiology research, cardiovascular disease remains the leading cause of death in the world!

Gene family	# of members	# in trafficking library	# Hits in primary screening	# verified hits
Rabs	62	58	24	6
Snares	36	23	6	0
Arf/Arl	29	23	3	1
Tetraspanins	33	15	1	0
Synaptotagmins	29	26	2	0
ESCRTs	29	10	1	1
Snare regulators	42	18	2	1
Coat complexes and Annexins	41	36	2	1
Sorting Nexins	33	26	5	2

Table 1 – Screening of Lentiviral shRNA Trafficking Library for molecules required for Plasma Membrane Repair (1,900 shRNAs)
The first column shows the families of proteins screened. The second column displays the number of proteins of each family identified in the human genome. The third column shows the number of proteins of each family present in the library. The fourth column has the number of positive hits. The fifth column contains the number of validated hits.

Main Achievements

Within the framework of the Harvard Medical School-Portugal Program, I am the PI of a consortium (which includes Profs. M. Brenner, H. Remold and V. Hsu, Harvard University; Prof. R. Appelberg, University of Porto; and Dr. D. Barral, CEDOC-FCM-New University of Lisbon) that studies "New Approaches to Fight Tuberculosis". This collaborative project is based on the finding that plasma membrane repair during Mycobacterium infection that culminates with apoptosis of the host macrophage is crucial for enhancing innate and adaptive immunity. In contrast, necrosis of the host macrophage takes place when plasma membrane repair does not occur and this outcome leads to evasion of defense mechanisms. Plasma membrane repair requires translocation of lysosomal - and Golgi apparatus-derived vesicles to the damaged membrane. We screened a lentiviral shRNA Traffic Library and we have identified several host effectors required for resealing of the macrophage plasma membrane.

My second subject of interest, connected with my previous experience in atherosclerosis research, aims to identify the molecular etiology and cellular mechanisms leading to pathological lysosomal lipid accumulation (lipidosis) in diseases like atherosclerosis and the causes of inefficient efferocytosis (phagocytosis) of apoptotic cells. We have generated a methodology that permits delivery of specific chemical products of cholesteryl linoleate oxidation via native LDL presented to macrophages. This model was useful in studying induction of lipidosis in macrophages *in vitro*. We are now screening a wide range of cholesteryl ester oxidation products and attempting to elucidate the detailed mechanisms involved. Some of the molecules we have studied evoke a progressive, uncontrolled, and irreversible lipidosis over chronic exposure to sublethal concentrations making this a good laboratory model for atherogenesis. The process seems to result from intracellular accumulation of non-degradable cholesterol derivatives that impair normal cholesterol homeostasis in macrophages and lead to lipidosis.

Last year we have published 2 papers and at the moment we are preparing 5 new manuscripts and a patent.

Insuline Resistance and Adipocyte Group

Head: Eugénia Carvalho

Objectives

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

b) Diabetes is one of the most widespread and costly diseases in the world. It may cause diabetic foot ulcers, decreasing the welfare of patients. Peripheral neuropathy impairs wound healing. We have used different cellular and animal models to unveil the molecular mechanisms of wound healing. Recent studies suggest that neuropeptides and mast cells participate in wound healing but the mechanisms of their action are not clear. Our main hypothesis is that skin mast cells are dysfunctional in diabetes due to neuropeptide deficiency, contributing to impaired wound healing. We assessed wound healing in both streptozotocin-induced diabetic (STZ-DM) and non-diabetic (non-DM) mast cell deficient mice (KitW/KitW-v) and their wild type (WT) littermates. Furthermore, natural biopolymers like chitosan, collagen and their derivatives, are presently receiving greatest attention as wound dressing materials for wound healing applications. Employing these chitosan derivatives simultaneously as dressings and as platforms for the delivery of a neuropeptide, neurotensin (NT) has not yet been evaluated and it is being addressed in our work.

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

Main Achievements

a) We shave shown that rapamycin and the calcineurin inhibitors, cyclosporin A and tacrolimus, at therapeutic concentrations, had a concentration-dependent inhibitory effect on basal and insulin-stimulated glucose uptake in both human subcutaneous and omental adipocytes, as well as in vivo rat models. In addition, we have shown that all three IAs increased isoproterenol-stimulated lipolysis and enhanced isoproterenol-stimulated phosphorylation of one of the main lipases involved in lipolysis, hormone-sensitive lipase. Furthermore, we used a higher dose of CsA (15mg/kg/day) *in vivo* for 15 days, in order to evaluate CsA effects in glucose and lipid metabolism. This was done through quantification of ^{2}H-enrichment of glucose, glycogen and TG after $^{2}H_2O$ administration by ^{2}H NMR. Although we determine that CsA at this dose affects body weight and glucose tolerance, we could not see differences in glycogen synthesis or *de novo* lipogenesis, under these conditions.In conclusion, the molecular and metabolic changes observed contributes to a better understanding of the mechanisms involved in the development of NODAT and dyslipidemia after immunosuppressive therapy.

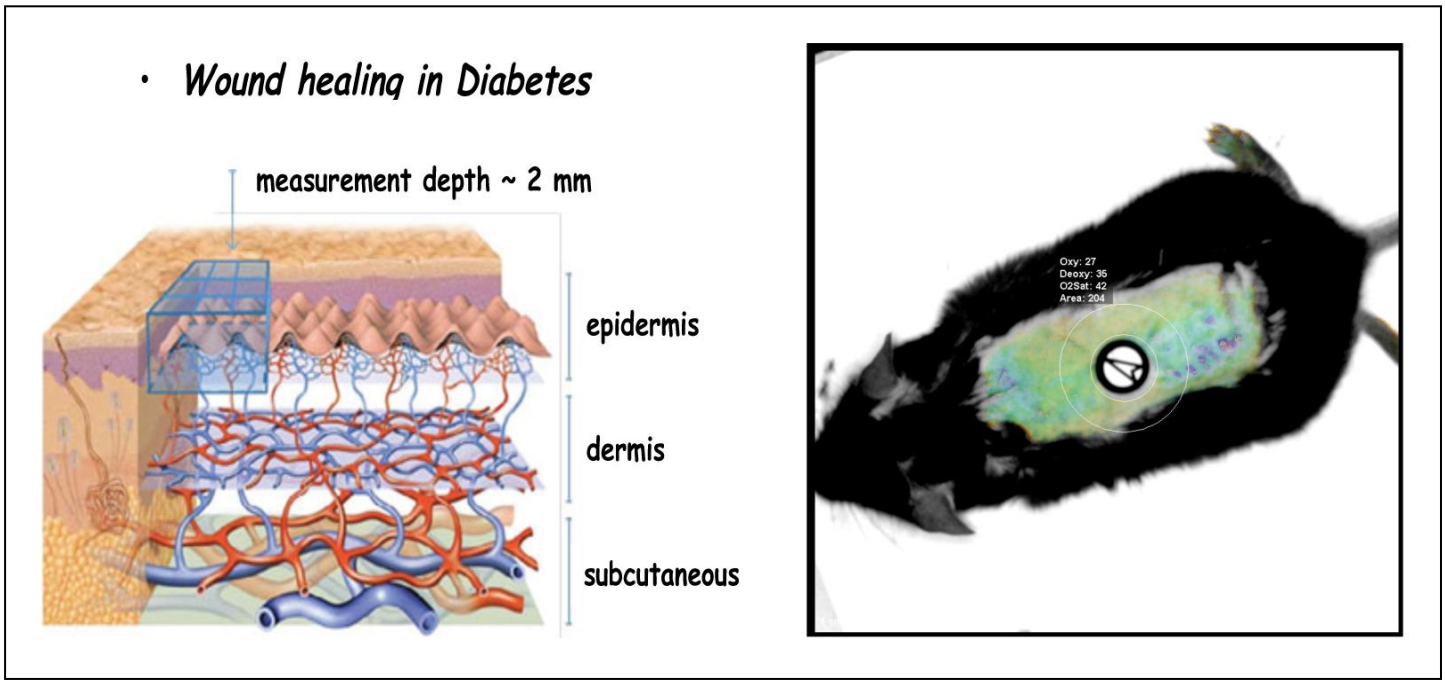
b) Diabetic foot ulceration (DFU) and associated impaired healing, is a major problem that significantly impairs the quality of life of diabetic patients, leads to prolonged hospitalization and may result in lower extremity amputations. DFU occurs almost exclusively in the presence of diabetic neuropathy. The *in vitro* effects of NT in the migration, proliferation and regulation of cytokine expression of skin cells, namely in macrophages and keratinocytes, under hyperglycemic and/or inflammatory conditions were studied. From *in vitro* results, it was concluded that NT impairs macrophage migration under hyperglycemic conditions as well as it decreases their pro-inflammatory cytokines (IL-1β and IL-12) expression under hyperglycemic and inflammatory conditions. In addition, it was also found that hyperglycemia modulates NT and NT receptor expression in both tested conditions. On the other hand and for human keratinocytes, the presence of NT strongly stimulated NT and NTR2 expression. However, results also showed that NT did not affect cell proliferation and migration, as well as the expression of some inflammatory cytokines (IL-1β and IL-8) and growth factors (EGF, VEGF and PDGF) under hyperglycemic conditions. These results thus suggest that NT did not exert a direct effect on keratinocytes function, but it seems to present a paracrine effect on other skin cells such as fibroblasts, macrophages and dendritic cells.

In addition, the development and characterization of three chitosan derivatives (*N*-carboxymethyl chitosan (CMC), 5-methyl pyrrolidinone chitosan (MPC) and *N*-succinyl chitosan (SC)) and of type I mice collagen-based dressings as supports for the topical delivery of NT into diabetic wounds were performed. The evaluation of the progression of wound healing and of modulation of inflammatory, angiogenic and re-epithelializating factors were performed (*in vivo*) using MPC and collagen-based dressings (with or without the release of NT) in a full-thickness wound healing model in diabetic mice.

From *in vivo* tests, it was found that NT alone induced faster healing in either control (22%) or diabetic (29%) wounds at day 3 (if compared to non-treated wounds). MPC alone and NT-loaded MPC dressings presented different wound healing profiles either in control or in diabetic mice, at day 1 post-wounding, leading to significant reductions in wound sizes (48% and 43%, respectively, in control, and 35% and 50%, respectively in diabetic animals). RT-PCR analysis showed that NT-loaded MPC dressings reduced inflammatory cytokines expression (TNF-α) and

decreased the inflammatory infiltrate at day 3. At day 10, the MMP-9 expression was also reduced in diabetic mouse skin, and led to increased fibroblast migration and to a higher collagen (COL1A1, COL1A2 and COL3A1) expression and deposition in wound sites. Results obtained when using NT-loaded collagen dressings showed that, in diabetic mice, a faster healing was achieved (17% wound area reduction). In addition, this strategy significantly reduced the inflammatory cytokine expression (TNF-α and IL-1β) as well as the inflammatory infiltrate, at day 3 post-wounding. After complete healing (fd), the MMP-9 expression was also reduced in diabetic mouse skin. Once again, this probably led to fibroblast migration and to higher collagen (COL1A2 and COL3A1) expression and deposition. Finally and in conclusion, NT may enhance diabetic wound healing and its activity can be further improved when it is loaded into MPC or collagen based dressings. The results show that NT is a promising neuropeptide that can be used for the treatment of diabetic wounds, either alone or, preferably, combined with biocompatible and biodegradable wound dressings.

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



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BIOMEDICAL INTER-INSTITUTIONAL RESEARCH PROGRAMME

Psychiatry Research

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

Molecular genetics studies of complex disorders

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

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

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

copy number variants (CNVs) led to the identification of 22q11.2, 15q13.2 and 1q21.1 as regions with excess CNVs in Sz.

An exploratory WGA study in the Portuguese Sz probands was carried out on the Affymetrix GeneChip® Mapping 500K Assay. We identified a total of 55 SNPs that showed nominally significant associations with schizophrenia at a threshold of $P < 1 \times 10^{-4}$. 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, 2012). However, the problem of the phenotypic heterogeneity in the area of psychosis still remains to be solved and we have to face the possibility that it could even be increased in samples of the magnitude used in GWAS. It is necessary, in parallel with these large GWAS, to implement nested studies, using clinical covariates that shows high familiality and are potentially under the control of a smaller set of genes, defining more homogeneous sub-samples. One of the areas of expertise of our team is phenotypic definition, and in this context, we intend to use phenotypic measures potentially more adequate to dissect the underlying pathologic mechanisms.

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

In the last trimester of 2011 we have obtained limited funding from the "Programa de Estímulo à Investigação" (Program to Incentive Research) from Faculty of Medicine-University of Coimbra, to develop a research project entitled "*Phenotypic Dimensions in Psychosis*" (PHEDIP/PEI-FMUC, 2011). Few months after, this funding has been canceled due to FMUC financial constraints. However, we have developed a new diagnostic interview entitled EP-GENE (Entrevista Psiquiátrica para Estudos Genéticos 1.0; 2011) and assessed 50 SZ/BP patients used it and OPCRIT.

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

In May 2013 we have obtained limited funding from the "Gabinete de Apoio à Investigação" (Office of Research Support) from Faculty of Medicine-University of Coimbra, to continue developing the research project entitled "*Phenotypic Dimensions in Psychosis*" (Pereira04.01.13). The project duration is 12 months. Our aims include: 1. Assess 200 SZ/BD/SzA probands (from multiplex families and unrelated cases) – diagnostic classification and lifetime-ever occurrence of symptoms using all available clinical information; 2. Deposit the 200 Blood/DNA samples in the FMUC (Laboratório de Citogenética) repository for future studies; 3. Contribute to phenotypic refinement and formulation of alternative phenotypes: symptom dimensions and subphenotypes.

During 2013 we have progressed mainly in aims 1 and 2 – aproximately 100 patients were evaluated for the presence of psychopathological signs and symptoms and of lifetime diagnosis. The extraction of their DNA, performed in the Cytogenetics Laboratory of FMUC (partner of this project) was carried out in 90% of patients - all who gave their informed consent for this procedure.

Three doctoral thesis, which we are supervising, are in progress within this project "Phenotypic Dimensions in Psychosis": 1-Schizophrenia - Subphenotypes and dimensions (Dra. Raquel Alexandra da Silva Correia, FMUP); 2-Subphenotypes in Bipolar Disorders (Dr. José Valente, FMUC); 3-Social cognition in bipolar disorder and schizophrenia: Clinical phenotypes and neural basis (Dr. Nuno Madeira, FMUC).

Clinical research – phenotypic studies of complex disorders

In parallel with the genetic studies of schizophrenia and bipolar disorder, we have developed a range of clinical investigations in areas in which a more clear understanding of the phenotypic definitions and boundaries were needed. These studies have focused in the area of personality, namely studying the perfectionism and the relationship

between this trait and psychopathology. Our correlational studies have established an association between the maladaptive aspects of perfectionism and a broad range of psychopathological conditions and health problems (e.g. sleep problems). However, the cognitive mechanisms that mediate this association are not fully understood, and the main cognitive processes and cognitions underlying perfectionist behavior and its negative emotional consequences wait for further clarification. We are now developing a project to investigate the role of multilevel cognitive processes in the relationship between psychological distress (PD) and perfectionism in a non-clinical sample of undergraduate students and a clinical sample of depressive and anxiety disorders. The first data wave (transversal study) was completely collected and inputed in 2013. It was also in 2013 that the first preliminary results were presented/published, namely the Portuguese validation of several relevant self-reported questionnaires. The second data wave (prospective study) collection also begun in the past year.

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

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

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Neurology Research

Studies on neurodegenerative disorders

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

Biomarkers for the early differential diagnosis of Dementia is one of our main areas of interest.

Established cerebrospinal fluid (CSF) biomarkers exist for early Alzheimer's Disease (AD): total and hyperphosphorylated tau (tau and p-tau) that reflect AD-type axonal degeneration, and the 42 amino acid isoform of amyloidβ (Aβ42) that reflects senile plaque pathology. These biomarkers have recently been incorporated in the new proposed revised criteria for AD. However, large variations in all biomarker measurements have been reported between studies, both between and within centres and laboratories. Such variations seriously jeopardize the introduction of biomarkers in clinical routine and trials around the world. In this context, we are currently participating in an EU Joint Programme - Neurodegenerative Disease Research (JPND) project, supported by FCT through JPND/0005/2011, aimed at the standardization of the established and new fluid biomarkers for AD.

During the first year of the project, our group has been particularly focused on performing studies on possible pre-analytical confounders that might influence CSF biomarkers stability. We are leading a sub-task on in vitro pre-analytical confounders regarding CSF manipulation and storage, focusing on the influence of spinning conditions of the CSF samples, blood contaminatin, aliquots volume during short and long-term storage and freeze and thaw cycles. The results are currently being analysed and will contribute to the understanding of how sample manipulation influences the final result and to the development of consensus-based recommendations for CSF manipulation and storage that ensure biomarkers stability overtime.

Clinical diagnosis of rapidly progressive dementias, namely sporadic Creutzfeldt-Jakob (sCJD), can be supported by the cerebrospinal fluid (CSF) biomarker 14-3-3 protein.

However, this protein is usually analyzed in a qualitative manner (Negative, Positive or Weak Positive), lacking standardization and unequivocal standard, leading to a subjective interpretation of borderline results. To overcome these difficulties, alternative protein markers have been proposed for the diagnosis of sCJD. We have evaluated the added diagnostic value of CSF Tau and phosphorylated tau (pTau) in cases of suspected sCJD, for whom a final diagnosis of definite sCJD (n=70) or an alternative diagnosis of non-prion disease (Non-CJD; n=209) was reached. Taking into account all cases (sCJD vs non-CJD), qualitative 14-3-3 protein revealed an overall accuracy of 78.1% with a sensitivity of 97.1% and specificity of 71.8%. By adding Tau protein evaluation, a significantly increase in discriminating power to 95.2% with a sensitivity of 94.2% and specificity of 95.6% (P<0.0001) was found. Further inclusion of pTau/Tau ratio in the model, significantly increased specificity to 97.1% (P=0.0178). When just considering 14-3-3 protein Positive results, no added value was observed for Tau and pTau/Tau ratio. On the other hand, when considering 14-3-3 Weak Positive results, Tau protein significantly improved the sensitivity of the combined model from 70 to 87.5% (P<0.0001). In light of these results, we strongly believe that Tau protein assay is of utmost importance in clarifying 14-3-3 borderline results in sCJD suspected cases. This work was supported by FCT through JPND/0001/2011 under the aegis of an EU Joint Programme - Neurodegenerative Disease Research (JPND) project, and was presented orally at the 2nd Iberian Congress on Prion Diseases, Faro 2-3 December 2013 (M.J.Leitao, I.Baldeiras, M.H.Ribeiro, I.Santana and C.R.Oliveira. Added value of CSF Tau proteins in the diagnosis of suspected sCJD cases with a borderline 14-3-3 result).

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Research in neurodegenerative diseases: Frequency of *SQSTM1* mutations in sporadic and familial Frontotemporal lobar degeneration

Maria Rosário Almeida Beatriz Santiago João Massano, Maria Helena Ribeiro, Catarina Resende Oliveira, Julie van der Zee, Christine Van Broeckhoven, Isabel Santana

There is increasing evidence that Frontotemporal Lobar Degeneration (FTLD) and Amyotrophic Lateral Sclerosis (ALS) are closely related clinical conditions with a significant proportion of patients harboring common genetic defects. In particular, a pathogenic expansion of hexanucleotide (G_4C_2) repeat in *C9orf72* gene was recently identified as a major cause of familial ALS and FTLD in several patients' cohorts from different geographical regions. Recently, mutations in the sequestosome 1 (*SQSTM1*) gene, which encodes p62 protein, have been reported in patients with ALS. Furthermore, p62 colocalizes with TDP-43 in brains of FTLD patients with ALS, suggesting its role in the pathogenesis of both FTLD and ALS. In the present study we aim to assess the frequency of the *SQSTM1* mutations in a series of Portuguese FTLD individuals and their associated phenotypic characteristics. One hundred and ten patients with clinical diagnosis of FTLD assisted in the Dementia outpatient clinic of CHUC or with genetic investigation at the CNC have been enrolled in the study. All patients recruited were tested for mutations in the *SQSTM1* gene in the framework of the Early-Onset Dementia (EOD)-Consortium. Three missense mutations have been identified in three patients, none of which were found in the healthy controls. *In silico* analysis predicts that these rare variants will have a pathogenic role. Curiously, one of these patients carries also the *C9orf72* hexanucleotide repeat expansion. In addition, the most common Paget mutation, p.P392L, was identified in three patients, of whom only one had a concomitant clinical diagnosis of FTLD and Paget disease previously explained by the presence of the C9orf72 pathogenic expansion. The presence of the Paget mutation in two remain FTLD cases suggested that these patients should be monitored for altered bone metabolism. Two additional common variants were also observed in both patients and controls (rs199854262 and rs150470670).

SQSTM1 mutations were present in our FTLD cohort in approximately 3% of the patients. Although this frequency

needs to be confirmed in larger cohorts, it seems that mutations in this gene only explain a small proportion of FTLD patients. However, due to the fact that p62 is a multifunction protein mainly involved in clearance of ubiquitinated proteins via autophagy and/or proteosomal degradation, it is predictable its involvement in various neurodegenerative diseases. In addition the co-occurance of more than one gene mutation in some of the patients requires additional studies to determine these mutations penetrance and also to rule out whether SQSTM1 is a causative gene or a modifier gene for FTLD.

Research in neurodegenerative diseases: Glucocerebrosidase mutation search in Parkinson disease patients

Maria Rosário Almeida, Fradique Moreira, Cristina Januário

Parkinson's disease is characterized by the appearance of motor manifestations, such as, bradykinesia, resting tremor, rigidity and postural instability; however, the majority of patients also have non-motor manifestations, including cognitive impairment. Cognitive impairment includes deficits in executive functions, impaired memory, attention deficits and changes in visual-spatial abilities. Recently, the presence of heterozygous mutations in the glucocerebrosidase (*GBA*) gene was identified as a genetic risk factor for the development of Parkinson's disease. Apart from being a risk factor for the development of Parkinson's disease, individuals harboring glucocerebrosidase mutations have, tendentiously, an early age at onset, as well as, an higher incidence of cognitive impairment than the non-carriers for glucocerebrosidase gene mutations. The homozygous or compound heterozygous mutations on the glucocerebrosidase gene are responsible for Gaucher disease, the most prevalent lysosomal disease worldwide. In the present work we aimed to ascertain the frequency of *GBA* mutations in a cohort of sixty six patients with Parkinson´s Disease followed in the Movement Disorder outpatient clinic of University Hospital of Coimbra. Of these, one patient was homozygous for N370S mutation, three patients harbor a heterozygous L444P and one patient was heterozygous for N370S mutation. Therefore, in our cohort, heterozygous known mutations in *GBA* were found in (4/66) 6% of the patients and a homozygous mutation was found in one case, resulting in Gaucher clinical diagnosis. Although the pathophysiological mechanisms responsible for the relationship between the *GBA* mutations and the onset of Parkinson's disease are not fully understood, several theories have been proposed, including protein aggregation due to impairment of the mechanisms involved in protein degradation and lipid deregulation. Our findings support the role of *GBA* in the development of Parkinson Disease.

Publications

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Translational Bi-Genomics and Pharmacogenomics

Manuela Grazina

Biochemical genetics study in Metabolic and proliferation disorders

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

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

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

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

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

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

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

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

Publications

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Bigenomic investigation in Neurodegenerative disorders

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

Neurodegenerative disorders are complex and the mechanisms underlying the phenotypic expression of this group of diseases are not clearly understood. Finding genetic risk factors, either from nuclear or mitochondrial genome origin, will contribute to identify new tools for early diagnosis. Our aim is to search for genetic risk factors in our population and identify disease risk groups.
We have finished, in collaboration with Neurology Department of University Hospitals, a Research Project for Medical Students, concerning the evaluation of mtDNA *ND1* sequence variations in a larger sample of FTD patients, following the evidences of the involvement of MRC complex I in FTD, reported in 2004 (Grazina M, Silva F, Santana I, Santiago B, Oliveira M, Cunha L, Oliveira C. Frontotemporal dementia and mitochondrial DNA transitions. *Neurobiol. Dis.* 2004; 15-2: 306-311). Our results point to the involvement of mtDNA and MRC in FTD. The role of mtDNA needs further examination, but our results support mitochondrial cascade hypothesis in FTD etiopathogeny.

One of the most complex neurodegenerative diseases is Multiple Sclerosis, and we aimed to investigate the role of mitocondrial respiratory chain (MRC) and mtDNA genetic variations, including haplogroups, in this disease and we have found that 48% of patients have MRC deficiency correlating with haplogroup J and with the presence of mtDNA sequence variations (3 fold higher).
Additionally we have continued the genetic characterization of dementias related to 5HTR2A. Accordingly, the project of the PhD student Daniela Luís entitled "Genetic Regulation of 5HT2A receptor in Frontotemporal Dementia", assigned by FCT in 2008 (SFRH/BD/45387/2008), aiming to analyse the coding exons and the flanking intronic regions of 5HTR2A gene, in 92 samples from FTD patients was concluded. We have found 174 sequence variations, 3 of which are novel, 2 in the coding region (no aminoacid alteration) and 1 intronic (does not affect splicing), undergoing *in silico* characterization, to evaluating possible pathogenicity and selection for further functional studies.

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

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

Pharmacogenomics

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

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

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

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

The aim of this study was to investigate the association between four CYP2D6 alleles:* 2, *3,*4 and *10 in a group of 96 patients with probable diagnosis of Alzheimer's disease and their clinical characteristics. Our results reveal a positive association with the age, age of onset and depression features with alleles *4 and *10. suggesting that genetic variations previously associated to decreased CYP2D6 activity may be a protective factor on the manifestation and progression of Alzheimer's disease.

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

Other projects applying pharmacogenomics approaches in pain are in progress.

Dermatology Research

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

Contact sensitizers induce an innate immune response in dendritic cells (DC) that enhances their antigen presentation and T cell response. Little is known concerning a similar effect of systemic drugs that cause T-cell mediated cutaneous adverse drug reactions (CARD). We have shown that, *in vitro,* some of these drugs have effects on THP-1 cells that are very similar to contact sensitizers. Systemic drugs, particularly allopurinol/oxypurinol and carbamazepine, exert cytotoxicity on THP-1 cells, with an intensity that seems to correlate with the severity of the CADR they cause. Although in a somehow divergent way, systemic drugs, at concentrations that reduce 30% cell viability, activate p38 MAPK activation and upregulate the expression of genes coding for DC maturation markers (CD40/CD83), pro-inflammatory cytokine/chemokines (IL-8) and the detoxifying intracellular enzyme, hemeoxygenase 1 (HMOX-1). Similarly to contact sensitizers that induce allergic contact dermatitis, a direct activation of monocytic or dendritic cells that participate in antigen presentation may be an important step in the pathophysiology of delayed immune mediated CADR.
At present, we are evaluating if concomitant factors that *in vivo* have shown to enhance drug presentation (multiple drug exposure or exposure to other DC stimulus – ROS, LPS and other microbial or viral products or increased temperature) modify the response of THP-1 cells to systemic drugs.

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Arthritis Research

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

Inflammation and osteoarthritis

In collaboration with the Orthopedic and Bone Bank Departments of CHUC, we are using normal and osteoarthritic (OA) human articular cartilage and chondrocytes to identify molecular mechanisms relevant for the development of target- and pathway-specific drugs to halt the development and/or progression of distinct osteoarthritis (OA) phenotypes. For this, we are studying i) the role of mitochondria and quality control mechanisms in mediating high glucose-induced inflammatory and catabolic processes that contribute to chondrocyte aging and the development and progression of diabetes-associated OA, ii) the role of hyperinsulinemia in modulating chondrocyte functions and its implications for diabetes-associated OA development and progression and iii) identification and pharmacological characterization of compounds with potential anti-osteoarthritic activity.

Publications

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Research in brain tumors

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

The project entitled "brain tumors: gliomas and meningiomas" 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.

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

Regarding the cell signalling transduction pathways, our results performed in glioma cell lines indicate that the activation of PI3K/Akt and MAP kinase signaling pathways contribute to the chemoresistance that characterizes glioma cells.

In recent years, evidences have accumulated which show an association among histologically benign/grade I meningiomas, between complex tumour karyotypes (≥2 genetic alterations), particularly those that include monosomy 14, and a shorter patient relapse-free survival. We have analyzed the pattern of expression of a broad panel of proteins in meningiomas to determine whether the immunophenotypic profile of single cells from individual tumours is associated with the most relevant features of the disease, including tumour histopathology and cytogenetics, as well as patient outcome. We have shown that multiparameter flow cytometry (MFC) immunophenotyping is a well-suited technique for the evaluation of the pattern of (quantitative) expression of relatively large numbers of tumour-associated proteins in individual tumour cells, when an appropriate marker combination is used for exclusion of other types of non-neoplastic cells (e.g. inflammatory cells) infiltrating the tumour.

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Tabernero MD, Jara-Acevedo M, Nieto AB, Caballero AR, Otero A, Sousa P, Gonçalves J, Domingues PH, Orfao A. (2013) Association between mutation of the NF2 gene and monosomy 22 in menopausal women with sporadic meningiomas. *BMC Med. Genet*. ; 14(1):114.

Domingues PH, Sousa P, Otero A, Gonçalves JM, Ruiz L, Oliveira C, Maia Celeste Lopes MC, Orfao A, Tabernero MD. Proposal for a new risk stratification classification for meningioma based on patient age, WHO tumor grade, size, localization, and karyotype. *Neuro-Oncology*. *(In press)*

Domingues PH, Teodósio C, Otero A, Sousa P, Gonçalves JM, Nieto AB, Lopes MC, Oliveira C, Orfao A, Tabernero MD. The protein expression profile of meningioma cells is associated with distinct cytogenetic tumor subgroups. *Neuropathology and Applied Neurobiology*. *(In press)*

Yeast nosocomial infections

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

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

The biological functions of HIV-1 Vpr have been involved in the replication and pathogenesis of the virus. Part of this collaboration is an ongoing work aimed to study the correlation, in a population of infected subjects, between the Vpr variant present and disease progression.

During the period considered we gathered samples and clinical data of 167 patients belonging to the following groups: HIV infected, assyntomatic, no therapy needed; HIV infected, assyntomatic, that initiated therapy; HIV infected, under different therapeutic programs. The analysis of Vpr sequences in 80 patients is completed and characterised in terms of the mutation R77Q.

During 2013 the collaborative protocol CHC and FMUC/CNC was transferred to CHUC due to the hospital fusion.

A poster was presented at the 2013 ESPID, Milan, Italy:

Rui Soares, Graça Rocha, Andrea Speigel, Marta Mota, António Meliço-Silvestre, Dr. Vieira, Teresa Gonçalves. HIV1 VPR POLYMORPHYSMS ASSOCIATED WITH AA 77: A VIRUS HOTSPOT? ESPID 2013. Milan, Italy.

Novel techniques for the diagnosis and treatment of human Infertility

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

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

For sperm analysis the focus has been on complementing traditional analysis by including new parameters with a higher predictive value in terms of defining proper sperm function. These parameters include sperm viability, sperm mitochondrial activity, and sperm chromatin status, monitored using simple, easy and quick assays that can be implemented clinically with minimal effort. The collaboration has recently been extended to two other Portuguese labs (University of Oporto and Gaia Hospital) and one in France (Clinique Pasteur, Brest) for a multi-center evaluation and validation of procedures. Papers describing a novel methodology to assess sperm chromatin routinely, and how to correctly determine sperm mitochondrial function have been published.

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.

INTERNATIONALIZATION

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

Projects in collaboration

Neuroscience and Disease

Neuromodulation Group

Networks:

Member of the Steering Committee of the European Neuroscience Campus (with Univ. Amsterdan, The Netherlands; Univ. Bordeaux, France; Univ. Zurich, Switzerland; Univ. Gottingen, Germany)

Member of the European Network of Neurosciences Institutes (ENI-Net)

EU Joint Programme – Neurodegenerative Disease Research (JPND, BIOMARKAPD) with Alexandre de Mendonça (Inst. Molecular Medicine, Univ. Lisbon), Magda Tsolaki (Univ. Thessaloniki, Greece), Sermin Genc (Univ. Izmir, Turkey), Anja Simonsen (Univ.Copenhagen, Denmark), Elisabeth Kapaki (Univ. Athens, Greece)

Member of the Coffee and Health Forum managed by the Institute for Scientific Information of Coffee

Research grants:

Joint research project with Ki Ann Goosens and Ann Graybiel (McGovern Institute, MIT, USA)

Ciência sem Fronteiras program with Lisiane Porciúncula (Univ. Federal Rio Grande do Sul, Brazil)

Graduate training:

Co-supervision of a post-doctoral student (Samira Ferreira) with Nuno Sousa (Univ. Minho)

Co-supervision of a PhD student (Silvia Sousa) with Christophe Mulle (Univ. Bordeaux, France)

Co-supervision of a PhD student (Marta Carmo) with Geanne Matos (Univ. Federal Ceará, Brazil)

Co-supervison of a PhD student (Filipe Matheus) with Rui Prediger (Univ. Federal Santa Catarina, Brazil)

Co-supervision of a PhD student (Jimmy George) with Thierry Amédée (Univ. Bordeaux, France)

Co-supervision of a PhD student (Amber Kerkhofs) with Huibert Manvelder (Univ.Amsterdam, The Netherlands)

Co-supervision of a PhD student (Xu Xinliu) with Nelson Rebola (Univ. Bordeaux, France)

Graduate teaching:

Course entitled ‘Fronteiras da Ciência’, PhD program in Biochemistry, Univ. Federal Rio Grande do Sul, Brazil

Glutamatergic Synapses Group

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

Carlos Pato, University of Southern California, Los Angeles, USA

Chinfei Chen, Harvard Medical School, Boston, USA

Daniel Choquet, Bordeaux Neuroscience Institute, France

Hey-Kyoung Lee, Johns Hopkins University, Baltimore, USA

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

Laurent Groc, Bordeaux Neuroscience Institute, France

Guoping Feng, MIT, Cambridge, USA

Neuronal Cell Death and Neuroprotection Group

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

Collaborative Research:

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

Ulrich Hengst, Columbia University, New York, USA

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

Eduardo Aguade, Center for Genomic Research, Barcelona, Spain

Ben Bahr, Biotechnology Research and Training Center, William C. Friday Laboratory, University of North Carolina Pembroke, NC, USA

Clive Bramham, Department of Biomedicine, University of Bergen, Norway

Enrico Tongiorgi, BRAIN Center for Neuroscience, Department of Biology, University of Trieste, Italy

Lorella M.T. Canzoniero, University of Sannio, Benevento, Italy

Tadeusz Wieloch, Wallenberg Neuroscience Center, University of Lund, Sweden

Duan-Wu Zhang and Jiahuai Han, Key Laboratory of the Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Xiamen, Fuijan 361005 China.

Arsénio Fernández-López, Área de Biología Celular, Instituto de Biomedicina, Universidad de Léon, 24071 Léon, Spain

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Participation in international meetings:

- 47th Annual Scientific Meeting of the European Society for Clinical Investigation (ESCI), 17-20th April 2013, Albufeira, Portugal (3 abstracts).

- Cell Symposia "*Mitochondria: From Signaling to Disease*", 5-7th May, Lisboa, Portugal (1 abstract).

Invited speaker (AC Rego) **in international meeting:**

- NEURASMUS Annual Meeting and Workshop*: 'The changing brain: from plasticity to brain diseases and brain repair… looking into the future',* 1-5th July 2013, Coimbra, Portugal.

Research collaboration with:

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

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

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

- Michael Hayden (MD, PhD), The University of British Columbia, Vancouver, Canada _ studies in the YAC128 mice.

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

Collaborative publication:

Lopes C., Ribeiro M., Duarte A. I., Humbert S., Saudou F., Pereira de Almeida L., Hayden M., Rego A. C. IGF-1 intranasal administration rescues Huntington's disease phenotypes in YAC128 mice. *Mol. Neurobiol.* (*in press*).

Molecular Mechanisms of Disease Group

Collaborative Research:

Russell H Swerdlow (Kansas University, USA);

Illana Gozes (Tel Aviv University, Israel, and Allon Therapeutics, Vancouver, Canada);

Marcia Haigis (Harvard Medical School, USA);

Merari F.R. Ferrari (Instituto de Biociências – USP, Brasil);

Edelmiro Moman (Luxembourg);

Isidre Ferrer Abizanda (Barcelona, Spain);

David Busija Department of Pharmacology, Tulane University School of Medicine, USA);

Gemma Casadesus, Joseph LaManna, Xiongwei Zhu (Institute of Pathology, Case Western Reserve University, USA);

George Perry (College of Sciences, University of Texas at San Antonio, USA);

James Bennett (VCU Parkinson's Disease Center, Virginia Commonwealth University, USA);

Maria Björkqvist (Wallenberg Neuroscience Center, Neuronal Survival Unit, Lund Medical School, Lund, Sweden);

Jorge Busciglio (School of Biological Sciences, University of California, Irvine, USA);

Laszlo Otvos (Department of Biology, Temple University, Philadelphia, USA); Catherine Lawrence (Faculty of Life Sciences, University of Manchester, UK).

Neuroendocrinology and Neurogenesis Group

Carlos Lopez Otin - Departamento de Bioquímica y Biología Molecular Facultad de Medicina, Universidad de Oviedo, Oviedo, Spain. (FCT project collaborator).

Leonard Guarente - Glenn Laboratory for the Science of Aging at MIT ; USA - (FCT project collaborator)

Licio Velloso - University of Campinas, Brasil (FCT-Capes Project)

Monika Ehrhart-Bornstein - Molecular Endocrinology Group, Department of Medicine, Carl Gustav Carus University of Dresden, Germany (Co-supervisor of PhD student)

Tamas Horvath - Section of Comparative Medicine; Yale School of Medicine PO Box 208016, New Haven, USA (Co-supervisor of PhD student; FCT project collaborator)

Biotechnology and Health

Molecular Biotechnology Group

Dr. Alexander Wlodawer, Macromolecular Crystallography Laboratory, NCI-Frederick, USA,

Dr. Alice Y. Cheung, University of Massachusetts at Amherst, Amherst, USA.

Dr. Christopher Overall, Centre for Blood Research, University of British Columbia, Vancouver, Canada

Dr. Herta Steinkellner, Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria

Dr. Juan J. Martinez, Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, USA

Dr. Pitter Huesgen, Central Institute for Engineering, Electronics and Analytics (ZEA-3), Forschungszentrum Jülich, Germany

Dr. Sandra Vairo-Cavalli, LIPROVE Universidad Nacional de La Plata, La Plata, Argentina.

Molecular Systems Biology Group

Max Planck Institute for Molecular Cell Biology and Genetics (Germany):

Researchers: Sophie Ayciriex, Julio Sampaio, Michal Surma, Andrej Shevchenko

Project: Identification of mechanisms of chain scission in *in vivo* autoxidation of polyunsaturated fatty acids through computational selection of mechanism markers and shotgun lipidomics analysis

University of Saarland (Germany):

Researchers: Elmar Heinzle

Project: Development and application of a method for profiling mitotic-cycle-dependent metabolism without having to synchronize cells

University of California – Merced (USA):

Researchers: Fabian Filipp, Rohit Gupta

Project: Application of rule-based modeling and lipid profiling to clarify the regulation of fatty acids biosynthesis

University of Lleida (Spain)

Researchers: Rui Alves

Project: Uncovering the evolutionary adaptations of protein aminoacid sequence and structure to O2-rich environments

VIT University (India)

Cooperation in research training of B. Tech. and M. Sc. students

Vectors and Gene Therapy Group

Research:
Eranet E-Rare4/0003/2012, €141581; Mar 2013 – Feb 2016. European network with german, dutch and israeli groups.

Graduate Training:

Treat PolyQ Marie Curie Innovative Training Network ITN Network 264508 SEVENTH FRAMEWORK PROGRAMME; €211441; Mar 2011 - Mar 2015.
2. Cancer associated fibroblasts function in tumor expansion and invasion - Seventh Framework Programme The People Programme. Reference: FP7-PEOPLE-2012-ITN; €209781.

Biomaterials and Stem Cell-Based Therapeutics

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

During 2013, several networks involving international researchers have been established or continued:

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

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

3-Nanomaterials for cell tracking. Seppo Hertualla (A.I. Virtanen institute, Department of Biotechnology and Molecular Medicine, University of Eastern Finland, Finland), Renata Gomes (CNC, Portugal), Jorge Ruivo (UCL, Portugal), Carolyn Carr (University of Oxford), Lino Ferreira (CNC, Portugal).

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

5- Unraveling the effect of arterial flow in smooth muscle cells derived from induced pluripotent stem cells containing Hutchinson-Gilford Progeria Syndrome (HGPS). Xavier Nissam/ Marc Peschanski (i-Stem, France), Patrícia Pereira (CNC, Portugal), Helena Vazão (CNC, Portugal), Lino Ferreira (CNC, Portugal).

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

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

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

Cell and Molecular Toxicology

Mitochondrial Toxicology and Disease Group

Research collaboration:

Edward Perkins (Mercer U., USA) Cancer stem cell responses to DNA damage (P. Oliveira)

Faustino Mollinedo (CSIC, Spain), Apoptosis signaling in melanoma (P. Oliveira)

Jon Holy (U. Minnesota, USA), Anticancer effects of phytochemicals (P. Oliveira)

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

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

Mark Nijland (U.Texas, USA), *In utero* modulation of mitochondrial function in non-human primates (P. Oliveira)

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

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

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

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

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

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

Anika Hartz, Bjorn Bauer (U. Minnesota, USA), Phytoestrogen modulation of blood-brain barrier permeability (V. Sardão)

Anatoly Zhitkovich (Brown U., USA), Origin of cancer stem cells (C. Alpoim)

Gregory Stephanopoulos (MIT, USA), Cancer metabolismo by ^{2}H and ^{13}C isotopomer analysis (R. Carvalho)

Gary Lopaschuk (U. Alberta, Canada), Cardiac metabolic remodeling by ^{13}C NMR isotopomer analysis (R. Carvalho)

Rolf Gruetter (Ecole Polytechnique Fédérale, Lausanne, Switzerland), Metabolic compartmentation in the brain (R. Carvalho)

Clemens Steegborn (U. Bayreuth, Germany), Structural and functional features of Sirtuins (C. Palmeira, A. Rolo)

David Sinclair (Harvard Medical School, USA), Sirtuins, mitochondrial biogenesis and metabolic regulation (C. Palmeira/A. Rolo)

Joan Rosselo (CSIC, Spain), Mitochondrial tolerance and liver ischemic preconditioning (C. Palmeira/A. Rolo)

Saber Hussain (Wright State U., USA), Evaluation of mitochondrial toxicity of silver and gold particles (C. Palmeira)

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

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

Visits from foreign students:

Cheryl Zehowski, U. Minnesota, USA

Soumia Lassed, U. Mentouri, Algeria

Krzysztof Kochel, U. Lodz, Poland

Visits from foreign researchers:

Fernando Nogueira, U. São Paulo, Brazil

Redox Biology in Health and Disease Group

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

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

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

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

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

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

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

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

Biophysics and Biomedical NMR

Inorganic Biochemistry and Molecular Imaging Group

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

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

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

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

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

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

Intermediary Metabolism Group

1) Contract and collaboration with Prof Michael Roden of the German Diabetes Foundation (DDZ) for ^{2}H NMR analysis of plasma and urine samples to quantify hepatic gluconeogenesis from a study of healthy subjects infused with different lipid mixtures and administered with deuterated water.

2) Collaboration with Radboud University Medical Center, Nijmegen on *in vivo* measurement of hepatic lipid fluxes during high fructose feeding resulted in one publication during 2013 a newly accepted paper: Nunes. P.M., Wright, A.J., Veltien, A., van Asten, J.J.A., Tack, C.J., Jones, J.G. and Heerschap, A. 2014. Dietary lipids do not contribute to the higher hepatic triglyceride levels of fructose compared to glucose fed mice. FASEB J. (*in press*).

3) Collaboration with Drs Rita Basu and Adrian Vella at Mayo Clinic. Three publications with Basu et al. (see Papers 3, 7 and 8 in the publication list) and collaboration in a funded NIH project with Dr Vella (see Funding section).

4) Collaboration with Prof Isabel Baanante of University of Barcelona that has resulted in one published paper (see Paper 1 in the publication list) and collaboration in a funded project (see Funding section).

Cell and Development Biology

Cellular Immunology and Oncobiology Group

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

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

Carmen García-Rodriguez from Institute of Biology and Molecular Genetic. CSIC-University of Valladolid, Spain. Co-supervision of 2 PhD students.

Maurício Sforcin, Departamento de Microbiologia e Imunologia, Instituto de Biociências, UNESP,18618-970, Botucatu, SP, Brasil. Graduate Training Networks.

Alberto Orfão from Center for Cancer Investigation, University of Salamanca, Spain. Assessment of genetic heterogeneity in gliomas.

Maria Dolores Tabernero Redondo, from University Hospital, Salamanca, Spain. Chromosomal, genetic and immunophenotypic characterization of brain tumors.

Fran Lund from Rochester University. CD38 and immune regulation

Raimundo Freire from University Hospital of Canarias, Tenerife, Spain. Implications of Claspin mutations in DNA replication, cell cycle checkpoints and oncogenesis

Biology of Reproduction, Stem Cells and Human Fertility Group

Ongoing International collaborations include:

Sperm Proteomics and Metabolomics (University of Barcelona, Spain). Collaboration with Rafael Oliva. Group Members involved: Alexandra Amaral, Carla Paiva.

Novel methods for sperm quality assessment (University of Muenster, Germany). Collaboration with Stefan Schlatt/Con Mallidis. Group Member involved: Sandra Amaral.

Xenografting and Male fertility preservation in humans and endangered species (University of Muenster, Germany). Collaboration with Stefan Schlatt. Group Member involved: Paula Mota.

Moninoring intracellular calcium movements in human sperm (University of Birmingham, UK). Collaboration with Stephen Publicover. Group Members involved: Marta Baptista, Renata Tavares.

Effect of environmental disruptors in sperm channel conductance as monitored by patch-clamp (University of Dundee, UK). Collaboration with Christopher Barratt. Group Member involved: Renata Tavares.

Internationalization also involves PhD students doing collaborative work and/or being co-supervised with other Researchers, that do a large part of their work abroad:

Beatriz Lacerda: Regulation of stem cell pluripotency by NRF-1 (University of California-San Francisco, USA). Collaboration with Miguel Ramalho-Santos.

Marília Cordeiro: Ovarian follicle dynamics (Northwestern University, USA). Collaboration with Teresa Woodruff.

Carla Paiva: Comparative sperm proteomics and relation to metabolism and movement (University of Barcelona, Spain). Collaboration with Rafael Oliva.

Tânia Perestrelo: Physical properties and their role in stem cell pluripotency (John Hopkins University, USA). Collaboration with Denis Wirtz.

Ângela Crespo: NK cells and maternal-fetal immunity (Harvard University, USA). Co-supervision with Jack Strominger.

Rodrigo Santos: Molecular regulation of stem cell pluripotency (Cambridge University, UK). Co-supervision with José Silva.

Infection, Phagocytosis and Pathogens Group

Since 2012-Present: Coordinator of a consortium formed by Portuguese and Harvard Medical School (HMS) laboratories. This consortium is funded within the frame of the HMS-Portugal Program. This program aims at promoting new inter-institutional Translational Medicine projects.

Insuline Resistance and Adipocyte Group

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

In addition we also collaborate with Prof. J. Eriksson, Global Medical Science Director (executive level) Global Medicines Development, Cardiovascular/Gastrointestinal, Clinical Discovery, AstraZeneca R&D in Sweden, a specialist in Internal medicine and in Endocrinology (including diabetology). With him we have been investigating the role of the immunosuppressive agents, rapamycin, cyclosporin A and tacrolimus in lipolysis and their effects in altering the expression of genes involved in lipid metabolism in human adipose tissue. In his laboratory we have had a PhD student, Maria Joao Pereira, who has just defended her thesis.

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

More recently we have initiated a collaboration with the research group of Dr Louise Torp Dalgaard at Roskilde University, Roskilde, Denmark, who's specialties are in depth knowledge of metabolism, type 2 diabetes, obesity, beta-cell dysfunction, gene-expression, microRNAs and uncoupling proteins. With her laboratory we are studying the role of microRNAs in wound healing in our models.

Participation in the organization of scientific meetings

January 2013

Course on Principles and Practice in Drug Development
Date: January 21 - February 1
CNC members involved in the organization: João Nuno Moreira; Luís Almeida, Sérgio Simões

February 2013

Course in Synaptic and mitochondrial dysfunction in Parkinson's disease
Date: 27th February 2013
CNC members involved in the organization: Ana Cristina Rego

Course in Interactions of Nutrients with Mitochondria and Gene Expression
Date: February 11 - 15, 2013
CNC members involved in the organization: *John Jones, Carlos Palmeira, Paulo Oliveira, Anabela Rolo*

4th International Conference on Bioinformatics Models, Methods and Algorithms – Bioinformatics / BIOSTEC 2013, Barcelona (Spain)
Date: February 11-14
CNC members involved in the organization: Armindo Salvador

Seminar Stimulation of mitochondrial oxidative capacity in white fat independent of UCP1: A key to lean phenotype
Date: February 14th 2013
CNC members involved in the organization: Carlos Palmeira/Anabela Rolo

Course in Membrane Traffic and Disease
Date: February 14th 2013
CNC members involved in the organization: Otilia Vieira

International PhD course in Neurobiology and Disease"
Date: 25th February – 1st March, 2013
CNC members involved in the organization: Ana Cristina Rego

Course in Regenerative medicine for Parkinson's disease
Date: 27th February 2013
CNC members involved in the organization: Ana Cristina Rego

April 2013

47th Annual Meeting of the European Society for Clinical Investigation and Organization of the workshop "Mitochondrial Physiology: From Basic Research to the Clinic", Albufeira
Date: April 17-20, 2013
CNC members involved in the organization: Paulo Oliveira

May 2013

18th International Society of Magnetic Resonance (ISMAR) Meeting/14th NMR Users (AUREMN) Meeting/Vth Iberoamerican NMR Meeting, Rio de Janeiro, Brasil
Date: May 19-24
CNC members involved in the organization: Carlos Geraldes

June 2013

XIII Meeting of the Portuguese Neuroscience Society (SPN), Luso, Portugal
Date: June 1
CNC members involved in the organization: Ana Luisa Carvalho, Sandra Morais Cardoso, Paula I Moreira and Cláudia Pereira

July 2013

Annual meeting of Neurasmus (Erasmus-Mundus MSc program in Neuroscience), Coimbra
Date: July 1-5, 2013.
CNC members involved in the organization: Emília P. Duarte

1st International Summer School on Principles-Oriented Systems Biology
Date: July 1-12
CNC members involved in the organization: Armindo Salvador

9th European Biophysics Congress, Lisbon (Portugal)
Date: July 13-17
CNC members involved in the organization: Armindo Salvador

21st Annual International Conference on Intelligent Systems for Molecular Biology / 12th European Conference on Computational Biology, Berlin (Germany)
Date: July 19-20
CNC members involved in the organization: Armindo Salvador

First International Conference on Stem Cells for Drug Screening and Regenerative Medicine
Date: July 19
CNC members involved in the organization: Lino Ferreira, João Nuno Moreira

GRADUATE STUDIES PROGRAMME

During 2013 CNC organized 8 Advanced Courses and hosted 58 seminars. Local graduate students and researchers attended the seminars, whereas the advanced courses also met the interest of people from other Portuguese Universities. Besides the organization of courses and seminars, CNC also supported ongoing research work for Ph.D. and M.Sc. theses. Throughout this year 43Ph.D. and 44 M.Sc. theses were concluded.

In October 2002 CNC, with the financial support of FCT, launched an International Doctoral Programme in Experimental Biology and Biomedicine to provide advanced, multidisciplinary, research-oriented training in emerging areas of modern Biology and Biomedicine. The programme included advanced courses in top research areas, taught by foreign scientists in collaboration with local investigators, laboratory rotations and research work to be carried out within international networks organized by CNC. Students from the European Neuroscience Campus (ENC) Erasmus Mundus PhD and PhD students from several Marie Curie International Training Networks (ITNs) in which CNC is a partner, and who perform part of their work at the Institute, are also enrolled in PDBEB. In 2013 the Programme was under evaluation for renewal by FCT.

Advanced Courses 2013

Gene and Cell therapy of CNS: from microRNAs to iPS cells and gene repair
January 7 - 11
Luís Almeida , Clévio Nóbrega, Rui Nobre, Liliana Mendonça, Lígia Ferreira, Ana Luísa Cardoso, Catarina Miranda

Reproductive Biology, Pluripotent Stem Cells and Human Fertility
January 14 - 18
João Ramalho, M.ª Alexandra Amaral, Sandra Amaral, Paula Mota, Ana Paula Sousa

MIT - Principles and Practice in Drug Development
January 21 - February 1
João Nuno Moreira, Luís Almeida, Sérgio Simões

Lab Rotations 4
February 4 - 8

Interactions of Nutrients with Mitochondria and Gene Expression
February 11 - 15
John Jones, Carlos M. Palmeira, Paulo J. Oliveira, Anabela P. Rolo

Membrane Traffic and Disease
February 18 - 22
Otília Vieira, Henrique Girão, Winchil Vaz

Oncobiology
February 26 - March 1
João Nuno Moreira, Henrique Faneca, Vera Moura, Raghu Kalluri

Real-Time Electrochemical Measurements in the brain of living animals
March 4 - 8
João Laranjinha, Rui Barbosa

Seminars

January

Translational research of dementia. Why biomarkers matter
2013.1.4
Catarina Resende de Oliveira
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Role of Bri2 in early pathology of Alzheimer's Disease
2013.1.7
Charlotte Teunissen
Department of Molecular Cell Biology and Immunology
VU University Amsterdam
Amsterdam, Netherlands

Splice isoform-specific suppression of the Cav2.1 variant underlying Spinocerebellar ataxia type 6
2013.1.9
Edgardo Rodriguez
Departments of Molecular Physiology and Biophysics, Internal Medicine and Neurology
University of Iowa
Iowa City, USA

Using TALENs to model neurological disease in human pluripotent stem cells
2013.1.10
Neville Sanjana
Broad Institute
Cambridge, USA

Discovery of new anti-inflammatory drugs from medicinal plants using bio-guided assays
2013.1.11
Vera Francisco
Cellular Immunology and Oncobiology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

The endocannabinoid system in charge of neuromodulation and glucose metabolism in the brain
2013.1.18
Attila Köfalvi
Neuromodulation Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

February

Presynaptic A2A adenosine receptors control CB1 cannabinoid receptors-mediated effects at corticostriatal nerve terminals
2013.2.1
Samira Ferreira
Neuromodulation Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Reconstruction of mycobacterial pathways from missing enzyme links: playing hide and seek
2013.2.8
Nuno Empadinhas
Molecular Mycobacteriology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Stimulation of mitochondrial oxidative capacity in white fat independent of UCP1: A key to lean phenotype
2013.2.14
Jan Kopeky
Department of Adipose Tissue Biology and Center for Applied Genomics
Academy of Sciences of the Czech Republic
Prague, Czech Republic

Nanotoxicity: challenges, research gaps, and progress beyond traditional toxicology
2013.2.14
Saber M. Hussain
Nanobiotechnology Group Lead Molecular Bioeffects Branch
Bioeffects Division Human Effectiveness Directorate
Air Force Research Laboratory, Wright Patterson Air Force Base
Ohio, USA

Phytoestrogens as alternative to hormone replacement therapy during menopause – The heroes, the villains or the useless?
2013.2.15
Vilma Sardão
Mitochondrial Toxicology and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Missorting of Lysosomal proteins in neurodegeneration
2013.2.20
Thomas Braulke
Dept. of Biochemistry, Childrens Hospital
University Medical Center Hamburg-Eppendorf
Hamburg, Germany

How sweet is our fish? Insights on carbohydrate metabolism in sea bass (Dicentrarchus labrax L.)
2013.2.22
Ivan Viegas
Intermediary Metabolism Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Notch signaling in the regulation of tumour angiogenesis
2013.2.28
António Duarte
Faculty of Veterinary Medicine
Technical University of Lisbon
Lisbon, Portugal

March

Neurotensin and chitosan-based dressings: new approaches for diabetic wound healing treatment
2013.3.1
Liane Moura
Molecular and Translational Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Development of Intraoperative Chemical Diagnostics Using Microelectrode Arrays for the Treatment of Neurological Disorders
2013.3.7
Greg Gerhardt
Parkinson´s Disease Translational Research Center of Excellence
Center for Microelectrode Technology
University of Kentucky Medical Center
Lexington, Kentucky, USA

Early alterations in dopamine neurotransmission in progressive models of Parkinson´s disease
2013.3.8
Martin Lundblad
Develop. Neurobiology, Faculty of Medicine
Lund University
Lund, Sweden

Antioxidant defense in human erythrocytes: understanding the role of peroxiredoxin 2
2013.3.8
Rui Benfeitas
Molecular Systems Biology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Use of induced pluripotent stem cells to explore molecular mechanisms of accelerated aging disorders
2013.3.11
Xavier Nissan
I-STEM, France

Deficient production of reactive oxygen species leads to severe chronic DSS-induced colitis in Ncf1/p47phox-mutant B10.Q mice
2013.3.15
Tiago Sousa
Immunology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Development of brain circuitry regulating innate and social behaviours
2013.3.15
Jean François Cloutier
Montreal Neurological Institute
McGill University
Montréal, Canada

Lupane triterpenoids as breast cancer mitocans
2013.3.22
Teresa Serafim
Mitochondrial Toxicology and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

April

Impaired wound healing and peripheral neuropathy in diabetes: from mechanistic insights to potential therapeutic targets
2013.4.5
Ermelindo Leal
Molecular and Translational Medicine Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Monoaminergic regulation of spatial learning and memory: Effects of selective lesions and restoration by grafted neural precursors
2013.4.5
Giampiero Leanza
BRAIN Center for Neuroscience
University of Trieste
Trieste, Italy

In utero renal mitochondrial adjustments to moderate maternal nutrient restriction
2013.4.12
Susana Pereira
Mitochondrial Toxicology and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

The role of telomeres in cancer and ageing
2013.4.19
Miguel Godinho Ferreira
Telomeres and Genome Stability Lab
Gulbenkian Institute of Science
Oeiras, Portugal

Kainate receptors and neuronal development: novel roles for the non-canonical signaling
2013.4.19
Ricardo Rodrigues
Neuromodulation Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Membrane traffic in host-pathogen interactions and in cholesterol homeostasis
2013.4.26
Otília Vieira
Membrane Traffic and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

May

Hexavalent Chromium and Cancer Stem Cells: a view to a kill!
2013.5.3
Carlos Rodrigues
Mitochondrial Toxicology and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Endoplasmic reticulum stress response in Alzheimer´s disease
2013.5.10
Cláudia Pereira
Molecular Mechanisms of Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Bioengineering strategies to modulate stem cell differentiation and improve cell engraftment
2013.5.17
Lino Ferreira
Biomaterials and Stem Cell-Based Therapeutics Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Post-translational Control of Metabolic and Mitochondrial Homeostasis in Response to Nutrient Stress
2013.5.21
Michael N. Sack
National Heart, Lung and Blood Institute
National Institutes of Health
Bethesda, MD, USA

Improving the Performance of Molecular Dynamics Simulations - A Non-computational Approach
2013.5.24
David Bowman
Molecular Systems Biology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Mitochondrial bigenomics: from health to translation for disease
2013.5.31
Manuela Grazina
Laboratory of Biochemical Genetics
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

June

Peripheral immune response in Alzheimer´s and Parkinson´s disease: B cells; autoimmunity and LRRK2
2013.6.7
Margarida Carneiro
Immunology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Neuropeptide Y in the hypothalamus: is it more than a food intake mechanism?
2013.6.14
Cláudia Cavadas
Neuroendocrinology and Neurogenesis Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Regulation of synapse assembly through local protein dynamics
2013.6.21
Ramiro Almeida
Glutamatergic Synapses Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Chitosan Nanoparticles: more than a delivery system?
2013.6.28
Filipa Lebre
Vectors and Gene Therapy Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

July

Metabolic studies with deuterated water from mice to men
2013.7.5
John Jones
Intermediary Metabolism Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

NMDA receptors and Nrf2 - initial targets in Alzheimer´s disease
2013.7.12
Cristina Rego
Mitochondrial Dysfunction and Signaling in Neurodegeneration Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Novel nanosystems for cancer gene therapy
2013.7.19
Henrique Faneca
Vectors and Gene Therapy Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Towards interpreting lipid autoxidation -omic profiles and controlling lipid autoxidation: A near-comprehensive approach to poli-unsaturated fatty acyl autoxidation
2013.7.26
Armindo Salvador
Molecular Systems Biology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

September

Understanding the Neurobiology of Nitric Oxide: Concentration Dynamics in the Rodent Brain
2013.9.6
Ana Ledo
Redox Biology in Health and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal
Bisfenol A effects in thyroid: a toxicogenomic approach
2013.9.11
Concetta Ambrosino
Universita degli Studi dei Sannio, Benevento
and Istituto di Ricerche Genetiche Gaetano Salvatore - Biogem, Ariano
Irpino, Italy

Effects of immunosupressive drugs - Cyclosporine A and Sirolimus - in glucose and lipid metabolism
2013.9.13
Patrícia Lopes
Molecular and Translational Medicine Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Neuro-mass spectrometry: searching for the (un)known
2013.9.20
Bruno Manadas
Mass Spectrometry Unit
Center for Neuroscience and Cell Biology (CNC)
Biocant
Cantanhede, Portugal

Challenging the use of anticancer drugs with targeted nanotechnologies-based strategies
2013.9.27
João Nuno Moreira
Vectors and Gene Therapy Research Group
Center for Neuroscience and Cell Biology (CNC)
and Faculty of Pharmacy
University of Coimbra
Coimbra, Portugal

October

MICC - What is it?
2013.10.4
Luísa Cortes
Microscopy Imaging Center of Coimbra - MICC
Center for Neuroscience and Cell Biology
University of Coimbra
Coimbra, Portugal

Good laboratory practices
2013.10.9
Isabel Nunes
Center for Neuroscience and Cell Biology
University of Coimbra
Coimbra, Portugal

Principles of Two-Photon Microscopy and Applications
2013.10.11
Ana Isabel Oliveira
Microscopy Imaging Center of Coimbra - MICC
Center for Neuroscience and Cell Biology
University of Coimbra
Coimbra, Portugal

Is cysteine a feeding signal to trigger meal-induced insulin sensitization?
2013.10.18
Joana Gaspar
Chronic Diseases Research Center (CEDOC)
Faculty of Medical Sciences
University of Lisbon
Lisbon, Portugal

Male fertility preservation in extreme situations: alternative approaches to gamete production
2013.10.25
Paula Mota
Biology of Reproduction and Stem Cells Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

November

Low estradiol, weak bones: the meta´´bone´´lomics of the post-menopausal osteoporosis
2013.11.1
Ana Maria Silva
Mitochondria, Metabolism and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Mitochondrial regulation of molecular mechanisms involved in cellular degeneration
2013.11.8
Sandra Morais Cardoso
Molecular Mechanisms of Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Inhibition of DPP-IV: a new therapeutic approach for diabetic retinopathy?
2013.11.15
Rosa Fernandes
Laboratory of Pharmacology and Experimental Therapeutics
Institute of Biomedical Research in Light and Image (IBILI)
Faculty of Medicine
University of Coimbra
Coimbra, Portugal

Assessment of the biological effects of oxidized LDL products: a systematic in vitro study
2013.11.29
Luís Estronca
Membrane Traffic and Disease Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra

December

The role of adenosine receptors in suicide
2013.12.6
Paula Canas
Neuromodulation Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Diabetes-Associated Osteoarthritis: Unraveling pathological mechanisms and pharmacological targets
2013.12.13
Ana Rufino
Chondrocyte Biology and Osteoarthritis Research Group
Faculty of Pharmacy and Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

PhD thesis concluded in 2013

Ana Inês Rebelo Crespo
Genetic imbalances and numerical chromosomal alterations in glioblastomas as assessed by single-nucleotide polymorphism (SNP)-arrays and their impact on gene expression
January 9th, 2013
Supervisor: Mª Celeste Lopes, Alberto Órfão, Mª Dolores Tabernero

António João Sales Mano
Avaliação da utilidade de parâmetros cinéticos derivados do CA-125 no acompanhamento do cancro epitelial do ovário
January 18th, 2013
Supervisor: Amilcar Falcão

Ana Patrícia Gomes
Unraveling new roles for SIRT1 in mitochondrial biology
January 18th, 2013
Supervisors: Anabela Rolo, Carlos Palmeira

João Monteiro
A lipidomic approach to hepatic mitochondrial function and toxicology: role of diet-induced modifications
January 21th, 2013
Supervisors: Paulo Oliveira, Mª Amália Jurado

Ana Sofia Mendes Leal
Preparation and biological evaluation of new triterpene derivatives of ursolic and oleanolic acids
February 19th, 2013
Supervisor: Jorge António Ribeiro Salvador

Célia Laurinda dos Santos Nogueira
Biomarkers and etiopathogeny of gastric carcinoma
February 26th, 2013
Supervisor: Teresa Gonçalves

Ana Catarina Henriques Oliveira
Molecular cascades in midbrain dopaminergic neuron development: emphasis on Wnts
February 27th, 2013
Co-supervisor: Ana Cristina Rego

Joana Paixão
Role of anthocyanins in the context of atherosclerosis prevention: molecular mechanisms of protection against apoptosis and inflammation in endothelial cells
March 2013
Supervisor: João Laranjinha

Ângela Rosalina Sanches Inácio
A systematic investigation of the potential use of surfactants as microbicides: implications for surfactant use in the prophylaxis of sexually transmitted infections
March 14th, 2013
Supervisor: Mª Otilia Vieira

João Manuel Trigueiro Costa
The role of calpains on TrkB and gephyrin cleavage under excitotoxic conditions: characterization and functional implications
March 26th, 2013
Supervisor: Carlos Duarte

Ana Cristina Rosa da Silva
Role of brain-derived neurotrophic factor and mitochondrial function in Huntington's disease
April 11th, 2013
Supervisor: Ana Cristina Rego
Co-supervisor: Prof. Doutor Luís Pereira de Almeida

Ana Santos Carvalho
Neuropeptide Y system in the retina: Why? and What for?
April 16th, 2013
Supervisors: Cláudia Cavadas and António Francisco Ambrósio

Sandra Isabel Freitas Mota
NMDA receptors-associated events and oxidative stress in models of Alzheimer's disease".
May 9th, 2013
Supervisor: Ana Cristina Rego
Co-supervisor: Doutora Cláudia Maria Fragão Pereira

Claudia Pereira
Idiosyncrasy of drug induced mitochondrial liabilities : from mitochondrial DNA single nucleotide polymorphisms to mitochondrial sirtuins
May 21th, 2013
supervisors: Paulo Oliveira, António Moreno

Mariana Freitas
Ação do tabaco e stresse oxidativo na carcinogénese da próstata - Implicações prognósticas e terapêuticas
May 22th, 2013
Supervisor: Ana Bela Sarmento Ribeiro

Carlos Henrique Vieira Melo
Molecular and Cellular Mechanisms of Neuroprotection and Plasticity induced by Brain-Derived Neurotrophic Factor May
May 28th, 2013
Supervisor: Carlos Duarte

Maria José Maio Nunes Pereira
Platforms for tissue reconstruction: compliant biomaterials for local drug delivery and tissue adhesion
July 2013
Supervisor: Lino Ferreira

Cátia Diogo
Oxidative stress, mitochondrial dysfunction and cellular pathology in experimental models of hyperglycaemia and high fat diet
July, 13th 2013
Supervisors: Paulo Oliveira, António Moreno

Maria Inês Frade Marquez Varela Morte
Effects of exposure to eslicarbazepine acetate and to other antiepileptic drugs on neurotoxicity and hippocampal development
July, 15th, 2013
Supervisors: Caetana Carvalho and Inês Araújo

Michele Curcio
Excitotoxic Stimulation as ON/OFF Switch of the Proteolytic Systems in Hippocampal Neurons
July 16th, 2013
Co-supervisor: Carlos Duarte

Susana Maria Batiste Tieres Tomé Cardoso
Exploring the role of mitochondria and uncoupling proteins in hypoglycemia and/or hyperglycemia-induced brain injury
July 16th, 2013
Supervisor: Paula Moreira

Magda Matos Santana
Stress, depression and adrenal gland: an insight into the adrenal medullary catecholaminergic system
July 26th, 2013
Supervisor: Cláudia Cavadas

Vera Lúcia Francisco
Anti-inflammatory mechanism and properties of plants used in traditional medicine: evaluation of their potential use as source for new anti-inflammatory drugs
July 31th, 2013
Supervisor: Celeste Lopes

Carolina Isabel Paiva Coelho
Murine macrophage response to Cryptococcus neoformans phagocytosis
September 9th, 2013
Supervisor: Teresa Gonçalves

Pedro Costa
MicroRNAs as molecular targets for non-viral gene therapy of glioblastoma: development of a new lipid-based nanosystem for nucleic acid delivery to brain tumor cells
July 25th, 2013
Supervisor: Conceição P. Lima

Luís Filipe da Silva Ribeiro
A link between metabolic signaling and cognition: the hippocampal function of ghrelin
September 11th, 2013
Supervisor: Ana Luísa Carvalho

Sandro Pereira
A Metabolic Switch for Cell Differentiation
September 12th, 2013
Supervisors: Paulo Oliveira, Rui Carvalho, João Ramalho-Santos

Cristina Isabel Marques Maurício de Carvalho
Diabetes-associated endotelial dysfunction. A highway to Alzheimer's disease? The role of brain endothelial mitochondria
October 9th, 2013
Supervisor: Paula Moreira

Joana Medeiros Vieira Marques
Role of kainate receptors in neuronal development
October 31th, 2013
Supervisor: Juan Lerma
Co-Supervisor: Carlos Palmeira

Liane Moura
Development of novel therapeutic approaches for wound healing in diabetes
November 5th, 2013
Supervisor: Eugenia Carvalho

Marco António Paisana de Matos
Role of adenosine A_{2A} receptors in astrocytes – implications for glutamatergic activity
November 19th, 2013
Supervisor: Paula Agostinho, Rodrigo Cunha

Ana Margarida Abrantes
Hipóxia Tumoral - Metabonómica e Imagem: Estudo Experimental
November 28th, 2013
Supervisors: Rui Carvalho, Filomena Botelho

Nélio Gonçalves
Gene transfer approaches for the study of the adenosine A2A receptors role in Machado-Joseph disease
November 29th, 2013
Supervisors: Luis Pereira de Almeida, Rodrigo Cunha

Renata Sofia Mota Gomes
Nanomaterials for miRNA delivery and non-invasive imaging in cardiovascular regeneration
December 2013
Supervisor: Lino Ferreira

Sezin Aday
Platforms to modulate the activity of hematopoietic stem cells and their progenies
December 2013
Supervisor: Lino Ferreira

Pedro Manuel Venâncio Garção
Functional interaction between presynaptic nicotinic and adenosine receptors in the control of dopamine release in the striatum
December 10th, 2013
Supervisor: Paula Agostinho, Catarina Oliveira

Luis França
Microbial Diversity and Dynamics of a Groundwater and a Bottled Natural Mineral Water
December 12th, 2013
Supervisor: Milton Costa

Ana Carolina Moreira
Phytoestrogens as Alternative to the Hormone Replacement Therapy: Mitochondrial and Cellular Interactions
December 18th, 2013
Supervisors: Vilma Sardão, Mª Sancha Santos

Márcio José de Abreu Marques Rodrigues
Avaliação do efeito de extratos vegetais usados em regimes de emagrecimento no perfil cinético de fármacos de estreita margem terapêutica utilizados para patologias do foro cardiovascular: a amiodarona
December 27th, 2013
Supervisor: Amilcar Falcão

Miranda Mele
Modulation of $GABA_A$ receptors in cerebral ischemia: alterations in receptor trafficking coupled to neuronal death after oxygen/glucose deprivation
December 30th, 2013
Supervisor: Carlos Duarte

Ana Branco
Impact of H9c2 Cardiomyoblast Differentiation on Isoproterenol Toxicity: Different Modulation of Signaling Pathways
Supervisors: Paulo Oliveira, Maria Santos

Sofia Cunha
Insights on the Accumulation and Biosynthetic Pathway for Mannosylglucosylglycerate in the Deep-Branching Phylum Plantomyces
2013
Supervisor: Milton Costa

Master Thesis

Nelson Cunha
Elderly: Are Your Defenses Ready For Fungal Infections?
March 2013
Supervisor: Teresa Gonçalves

Marta Isabel Ereira Mota
Influência do receptor A_{2A} na internalização de Candida albicans por queratinócitos
March 2013
Supervisor: Teresa Gonçalves

Carolina Helena de Freitas Noronha
Role of alpha-synuclein in neurodegeneration
June 4th, 2013
Supervisor: Ana Cristina Rego

Rui Soares
Implicações clinicas das mutações da proteína viral R na progressão da infeção HIV
June 2013
Supervisor: Teresa Gonçalves

Joni Fiona van Leeuwen
Effects of ghrelin on hippocampal glutamate receptors and neuronal morphology
July 2013
Supervisor: Ana Luísa Carvalho

Luís Martins
The role of local protein synthesis in presynaptogenesis
July 2013
Supervisor: Carlos Duarte

Mariana Cruz Almeida
Characterization of the innate imune response to Alternaria infectoria
July 2013
Supervisor: Teresa Gonçalves

Tomé Cardoso
Papel do ATP na infeção de macrófagos por Candida albicans
July 2013
Supervisor: Teresa Gonçalves

Mafalda Costa
Biosynthesis of rare methylglucose lipopolysaccharides in rapidly-growing mycobacteria: characterization of a key hydrolase
July 2013
Supervisor: Teresa Gonçalves

Luís Miguel Sousa Rodrigues
BDNF-induced local protein synthesis at the synapse: a regulatory role for hnRNPK
September 2013
Supervisor: Carlos Duarte

Cristiano Santos
Quantifying the effects of high fructose feeding on the intestinal permeability of endotoxins
September 2nd, 2013
Supervisor: John Jones

Paulo André Ribeiro dos Santos
Role of selective kinases and GDNF on iron-mediated alpha-synuclein phosphorylation – relevance to Parkinson's disease
September 6th, 2013
Supervisor: Ana Cristina Rego

Tiago André Ferreira Henriques
High-resolution respirometry for metabolic profiling of acute rat hippocampal slices.
September 13th, 2013
Supervisor: João Laranjinha

Valeria de Rosa
Aβ-mediated changes in CREB and ERK activity in cultured cortical neurons: involvement of NMDA receptors
September 17th, 2013
Supervisor: Ana Cristina Rego

Carlos Moura
Mechanisms of insulin resistance after immunosuppressive therapy in brown adipose tissue
September 20th, 2013
Supervisor: Eugenia Carvalho

Ana Carolina Nobre Torres
Steroids in a multitarget approach for malaria eradication. Development of hybrid antimalarials
October 31th, 2013
Supervisor: Maria Luisa Sá e Melo

André F. Martins
Multimodal imaging probes for the diagnostics of Alzheimer's disease
November 2013
Supervisor: Carlos Geraldes

Helena Cristina Gil Cardeira dos Santos Leitão
Non-invasive imaging biomarkers for liver steatosis, inflammation and fibrosis
November 2013
Supervisor: Carlos Geraldes

Ana Bárbara Silva Pinheiro
The multifaceted role of the endocannabinoid system in the regulation of cerebral glucose uptake
Supervisor: Rodrigo Cunha

Anna Vladímirovna Pliássova
Localization of secretases involved in the processing of β-amyloid precursor protein related to Alzheimer's disease
Supervisor: Rodrigo Cunha

Andreia Luís
Role of ER stress in sensitization induced DC maturation/toxicity
Supervisor: Mª Celeste Lopes

Angelo Serani
The role of PDGF in the regulation of the intracellular pool of MMP-2: MMP-2 contribution to SNALP internalization in glioma cells
Supervisor: Conceição P. Lima

Bruno Peixoto
The Plant Specific Insert (PSI) and its Molecular Role in Protein Sorting
Co-Supervisor: Paula Veríssimo

Carlos Custódia
Role of miR-21 in the regulation of microglia immune response to glioma
Supervisor: Conceição P. Lima

Daniela Patrícia Martins Dias Pedroso
A Chloroplastidial Atypical Aspartic Protease from Arabidopsis thaliana: Optimization of heterologous expression, Purification and Biochemical characterization
Supervisor: Carlos Faro

Denis Brito
Purificação e Caracterização de uma Protease do Pólen de Chenopodium sp.
Supervisor: Paula Veríssimo

Gabriela Leão Santos
Development of a novel therapeutic strategy for breast cancer involving a concerted action of gene therapy and chemotherapy
Supervisor: Conceição P. Lima

Gonçalo Filipe Pires Cristóvão
A_{2A} receptor blockade in the control of microglia impact upon neurons during early development
Supervisor: Rodrigo Cunha

Inês Mahú
AUTOPHAGY AND INFLAMMSOME: HOW ARE THEY RELATED?
Supervisor: Mª Celeste Lopes

Joana Filipa Monteiro de Sousa
Rastreio Virtual na descoberta de possíveis inibidores da 5-alpha reductase
2013
Supervisor: Cândida G. Silva e Jorge António Ribeiro Salvador

Mariana Magalhães
Development of a gene delivery system for therapeutic application on hepatocarcinoma
Supervisor: Conceição P. Lima

Marisa Ferreira Marques
Autophagy in cortical neurons: role of caloric restriction and neuropeptide Y
2013
Supervisor: Célia Aveleira

Mónica Marques
Supervisor: João Ramalho

Paulo Alexandre Gonçalves Teixeira
Bacterial Retropepsin-Like Proteases: The Evidence from Legionella pneumophila
Supervisor: Carlos Faro

Paulo Filipe Espírito Santo
Screening of Saccharomyces cerevisiae strains for recombinant protein expression
Supervisor: Carlos Faro

Pedro Miguel Ribeiro Oliveira Lopes
Cafeína e frequência das exacerbações na doença pulmonar obstrutiva crónica
Supervisor: Rodrigo Cunha

Ricardo Cleto de Sousa Marinho
Efeitos do metilglioxal e da piridoxamina na bioenergética e no estado redox de mitocôndrias de cérebro de rato
2013
Supervisor: Paula Moreira

Ricardo Silva
MiRNA contribution to APP metabolism and Aβ production in Alzheimer's disease: Identification of new miRNA-related SNPs in the 3'UTR of human APP and APOE genes
Supervisor: Conceição P. Lima

Rita Pereira
Supervisors: Rui Carvalho, Isabel Vitória

Rui O. Beleza
Role of P2Y1 receptors on neuronal polarity and axonal growth
Supervisor: Rodrigo Cunha

Sara Hadem
Inibidores de Proteinases Aspárticas com Actividade Antimicrobiana
Supervisor: Paula Verissimo

Sarah Beatriz de Oliveira Pagliaro
Cellular and molecular effects on prostate cancer stem cells of anti-prostate cancer therapeutics
Supervisor: Conceição P. Lima

Tiago Emanuel Soares Silva
Interaction between ecto-5'-nucleotidase and adenosine A_{2A} receptors in nerve terminals of mice prefrontal cortex
Supervisor: Rodrigo Cunha

Vanessa Filipa Florêncio Monteiro
On the formulation of targeted drug combinations
Supervisor: Conceição P. Lima

TECHNOLOGY TRANSFER

Translational research and technology transfer have been progressively developed in CNC leading to a promising interaction with Industry and local authorities.

The main contribution of CNC for that goal was the creation of a technology transfer unit, Biocant, in collaboration with Cantanhede Municipal Council. This unit became the anchor of Biocant Park, a Biotechnology Park that is rapidly growing by atracting new Biotechnology companies.

BIOCANT

Biocant is a private, non-profit, innovation centre created by CNCB together with the municipality of Cantanhede for technology transfer in biotechnology. Founded 8 years ago, Biocant 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 involving research institutions, hospitals and companies.

Companies operating in Biocant Park

At the present 20 companies operate in Biocant Park: AP-Bio, Biocant Ventures, Biotrend, Converde/CEV, Crioestaminal, Equigerminal, Hittag Biotecnology, Interactome, GenePrediT, Genebox, GeneLab, Matera, Vetdiagnos, 4Health, Cell2B, Klon, NutriAdd, Treat U, Reg4Life and Coimbra Genomics. Along with Biocant they form a biotech cluster of excellence that attracted altogether over 70M€ euros investment (50% is private) and generated 400 highly qualified jobs.

OUTREACH PROGRAMME

Outreach / Science and Society

Coordinator: Maria Teresa Girão da Cruz

The Outreach Programme developed by CNC under the coordination of the Science Communication Office offers opportunities to develop partnerships with schools and to extend our scientific resources to the community. The programme is designed to engage students in their science studies and potential careers related to the life sciences, and to broaden the public's access to science. The dissemination of scientific information equally contributes to the appreciation of the research activity performed at the CNC. Our outreach efforts have the enthusiastic involvement of the Center's research staff, graduate and undergraduate students.

The Center yearly participates in various activities exclusively planned to the lay public, namely during the Brain Awareness Week, Science and Technology Week, and European Researchers Night. Elementary to high school students are also a committed public of all CNC's outreach actions. CNC intensively collaborates with the Ciência Viva Agency, the Portuguese Society for Neuroscience, the Science Museum (University of Coimbra), and Exploratório (Centro Ciência Viva, Coimbra) for the organization of science communication actions. Some of our outreach activities are also carried out through the "Instituto de Educação e Cidadania" (IEC, Mamarrosa), a non-profit institution, dedicated to education and to promoting science and knowledge in schools, and among the rural populations in underprivileged areas. The IEC is housed in a modern building, provided with modern equipment, and includes classrooms and laboratories for students and teachers. The IEC has established protocols with several schools, and the CNC channels some of its outreach activities through IEC and the schools it is linked to.

The Science Communication Office is also in charge of liaising with the media, providing the necessary information for the communication of important achievements by CNC researchers. Our research and outreach activities have been recognized through numerous media articles and broadcasts (over 500 in 2013), and important awards – namely the Santa Casa Award for Neuroscience, the Alice and Albert Netter Award by the European Society of Gynecology, and the Merit Award by the Portuguese Health Ministry.

Brain Awareness Week (BAW), March 11-17

In Portugal, BAW 2013 focused on the theme "Creative Brain". Initiatives were intended both for the general public and for the students, and were designed to explore the recent scientific research into how and why the brain allows us moments of insight and creativity and how the ability to think creatively plays an important role in almost all areas of our life, to produce new ideas, and to think flexibly.: 1) a Café Scientifique about creativity, the brain and mental disorders, 2) the exhibition "Brain in colors", including works by CNC researchers; 3) "Neuroscientists go to Schools", where neuroscientists visited schools in the region and gave lectures on brain related subjects to high school students; elementary and middle school students performed hands on activities related to the brain awareness week subject, and 4) "Open Laboratories" where students visited CNC's laboratories and took part in talks about neuroscience research.

"Science in the Holidays" Programme (Ocupação Científica de Jovens nas Férias), July 08-19

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

European Researchers' Night, September 2t

Together with the Science Museum of the University of Coimbra, CNC took part for the fifth time in the organization of the activities of the European Researchers' Night. This initiative is promoted by the European Commission in order to bring the public closer to the researchers in a non-scientific environment. CNC researchers organized experiments and demonstrations for the public under the theme "The world in 2020", participated in a theatre play, and took part in the "speed-dating" event.

Science and Technology Week, November 18-24

During the Science and Technology week and the National Day for Scientific Culture CNC traditionally organizes

activities in order to promote the direct contact with the public. The activities were mainly intended for high-school students and the general public. CNC researchers organized conferences at local schools and visits to the laboratories on the several open days (five). Also, in collaboration with the Science Museum an interactive theatre play - and mystery dinner – was organized under the theme "Who killed Schrodinger's cat", where the public had the opportunity to "meet" many world famous scientists. The major goal of these activities is to contribute to the public understanding of the science being carried out in Portugal, of the subjects of research, and of the results obtained.

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

This CNC project supported by "COMPETE-Media Ciência", and intended to facilitate the communication with the public on the stem cells subject, was concluded in 2013 with the production of six animated videos and the publication and distribution of the cartoon book.

Novel Social and Scientific Dialogues for Neurodegenerative Diseases

This public engagement project is carried out in collaboration with the Center for Social Studies (CES), and is part of the BIOSENSE science shop project. Together with CES researchers, we started a pilot activity with CNC researchers, collaborator physicians and patient associations (Alzheimer, Parkinson, and Huntington) in order to create new channels for communication and exchange of knowledge. The series of debates "Alzheimer à Conversa" resulted from this collaboration.

C3 – Children With Sciences

CNC has long been a partner of Exploratório, the Science Centre of Coimbra, for the training of high school Biology teachers and for the establishment of partnerships with participant schools for cooperation in science education projects. We have also started a new collaborative project supported by Ciência Viva, "C3 – Crianças com Ciências", together with the parents association of local kindergarten and elementary schools, aiming to promote science awareness and experimental teaching. Included in this project, several outreach activities have taken place at the schools and at our laboratories.

Ask me Science

"Ask me Science" (Pergunta-me Ciência) is a project supported by Ciência Viva involving CNC researchers and high school students and teachers. Under the motto "The world looks so different after learning science", the project aims to bring closer researchers and the school population, promoting the awareness to experimental research and current biomedical research. The website perguntameciencia.cnc.uc.pt was created to support the access of participating students and teachers to the project contents and agenda. Being a pilot project with the collaboration of Quinta das Flores School, we aim to expand it to lasting outreach actions in local and regional schools.

Science Communication Workshops

Several science communication workshops have been promoted by CNC in order to provide scientists with the tools to make their work public even more effectively (either to peers, students, the media, the general public, funding agencies and others); to promote the public interest and participation in science; to deliver outreach activities to schools and the community. All workshops had the collaboration of scientists, science communicators and journalists. The 2013 edition was organized in collaboration with the Center for Social Studies (University of Coimbra).

CORE FACILITIES

ANIMAL HOUSE

Head of Unit: Prof. João Laranjinha

The Animal House is a shared resource that provides services in laboratory animal experimentation and husbrandy, for all CNC and FMUC scientists using animals in their research.

The present facility has a capacity to house about 3000 animals (rats/mice). This facility offers the following services: complete husbandry, including feeding, watering, daily cage changing, as well as routine procurement, inventory and care. In 2007, the facility started to provide specialized animal services, namely: breeding and housing of transgenic/knockout strains of mice as well as wild type colonies, production of rats/mice embryos and litters and maintenance of athymic nude mice.

The Animal House contains a barrier maintained facility, with 8 positive pressurised rooms, which are kept at 22ºC with a relative humidity of 55%. The rodents are breed in individually ventilated cages and a 12-hour light-dark cycle is maintained with an automatic timer. The facility has an animal identification system and software to monitor animal records.

Staff: Carmen Semião (caretaker)
Fátima Graça (assistant technician)
Maria Eugénia Campos (assistant technician)
Patrícia Ribeiro (Veterinary Doctor)

Animal room – IVC cages (type I)

Laminar flow chamber

FLOW CYTOMETRY UNIT

Head of Unit: Isabel Nunes Correia

The flow cytometry unit provides scientific and technical support both to CNC and external researchers. Currently, it is equipped with a Becton Dickinson FACSCalibur cell analyser and a Partec CyFlow® Space cell sorter. For researchers wishing to use flow cytometry, the unit offer assistance in planning projects, choosing fluorochromes, analyzing experimental results and presenting data.

The unit organizes annual flow cytometry seminars with the purpose to initiate new users and make this powerful technology known to all researchers, endeavouring to deepen CNC research.

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

FACSCalibur cell analyzer

MICROSCOPY UNIT

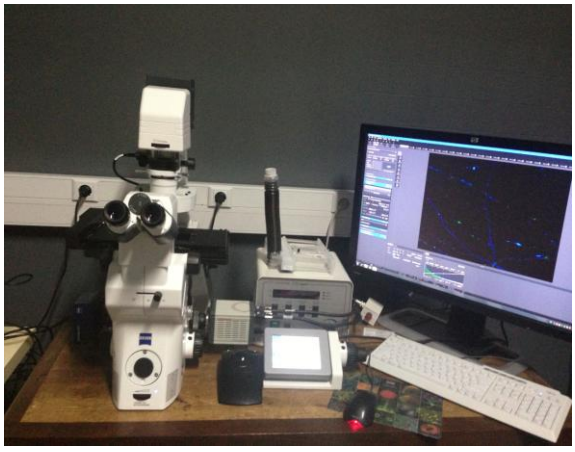
Head of Unit: Luísa Cortes

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

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

- provides technical training to local users and visiting researchers;
- offers consultation on experimental design and image analysis;
- evaluates new methods and fluorescence tools and communicates acquired knowledge to users;
- implements advances in hardware and software relevant for biomedical sciences;
- provides ongoing education in theory and practice by organizing training courses and workshops.

Presently, the unit manages a laser scanning confocal microscopy, a P.A.L.M. laser microdissecting microscope, a single cell calcium imaging system, two widefield systems (one of them fully motorized) and other brightfield microscopes. The systems are prepared for advanced applications, including live cell imaging and single cell calcium measurements, enabling the researchers to image dynamic events and molecular interactions. The P.A.L.M. laser dissecting microscope is a perfect tool for the isolation of different cell populations within a sample, allowing it full characterization.



Laser scanning confocal microscope

P.A.L.M. laser microdissecting microscope

MASS SPECTROSCOPY UNIT

Head of Unit: Bruno Manadas

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

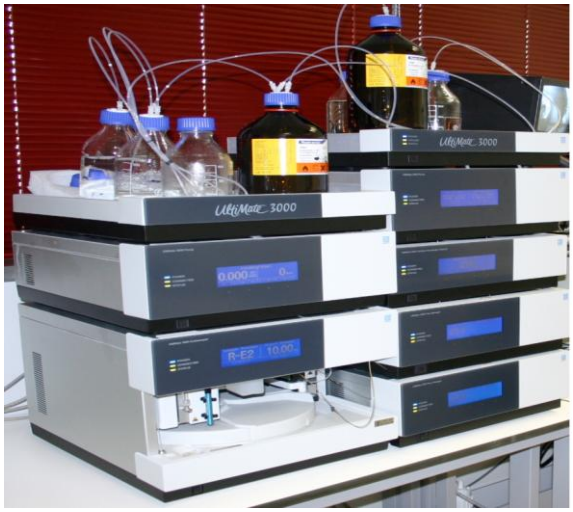
Presently, the Mass Spectrometry Unit is equipped with state of the art technology, namely: a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex), hybrid triple quadrupole/ion-trap mass spectrometer with capacity of MS3, and a two-dimensional liquid chromatography system Ultimate 3000 (Dionex/LCPackings).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

By combining the high resolving power of the LC system with the structure elucidation from the mass spectrometer, the Mass Spectrometry Unit is able to identify peptides, metabolites, drugs, pesticides, among others, from complex mixtures.

The Unit integrates the National Mass Spectrometry Network (RNEM).

Staff: Vera Mendes (technician)

4000 QTRAP mass spectrometer



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

Services

LABORATORY OF BIOCHEMICAL GENETICS

Coordinator: Manuela Grazina

Mitochondrial Respiratory Chain (MRC) and Krebs cycle enzymes

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

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

The coordinator of LBG (Manuela Grazina) maintains international collaborations, allowing significant developments in the assays performed, namely with Prof. Lee-Jun Wong and Doctor Fernando Scaglia (Baylor College of Medicine, Houston – Texas, USA), Prof. Massimo Zeviani (MRC Mitochondrial Biology Unit, Cambridge, UK), Prof. Robert Taylor (Mitochondrial Pathology, University of Newcastle upon Tyne, UK) and Dr. Rafael Artuch (Hospital San Juan de Dios- Barcelona, Spain).

Additionally, she organized the III Advanced Course on "Translational bigenomics – from the bedside to the bench and back again" (March 2012), and the III Advanced Course & Workshop on Clinical Case Reports: the second genome: mitochondrial bigenomics – from genotype to phenotype and clinical expression" (January 2012), allowing the visit of Prof. Lee-Jun Wong, Doctor Fernando Scaglia (Baylor College of Medicine, Houston – Texas, USA), Prof. Massimo Zeviani (MRC Mitochondrial Biology Unit, Cambridge, UK), Prof. Robert Taylor (Mitochondrial Pathology, University of Newcastle upon Tyne, UK), to LBG, which was a valuable step forward for improving genetic diagnosis in LBG. A significant effort has been put on finishing the set up of screening key genes for allowing diagnosis and genetic counselling.

Mitochondrial Respiratory Chain (MRC) and Krebs cycle enzymes

Biochemical assays related to energetic function are an important issue for probable diagnosis of Mitochondrial Respiratory Chain Diseases.

There were studied 60 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 72 samples (some patients had 2 or more tissues analysed), in 720 assays, including 28 lymphocytes isolated of peripheral blood, 38 muscular biopsies, 3 liver, 1 heart and 2 other samples. A MRC deficiency was detected in 29 patients.

The number of Hospitals asking for our Services increased.

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

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

Amino Acid Analysis

Our laboratory received 256 samples (211 - plasma, 36 - urine and 9 - cerebrospinal fluid) of physiological fluids for amino acid analysis, corresponding to 768 assays. The patients investigated (children, adolescents adults) were categorized in three clinical conditions: (1) selective screening of metabolic disorder, characterized by either primary or secondary abnormalities in the amino acid profile (2) amino acid profile changes secondary to proximal renal tubular or hepatic dysfunction of any origin; (3) nutritional evaluation of patients with protein restrictive diets. The majority of samples are from children, although less frequently, adults and adolescents are also monitored. Amino acids analysis is a very important approach in early metabolic disorder diagnosis, and frequently helps to prevent mental retardation or even death.

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

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

Molecular differential analysis of mitochondrial cytopathies, as a highthrouput screening, has been performed by sequencing analysis, of 11 mtDNA regions, covering a total of 424 mtDNA sequence variations that include 31 confirmed pathogenic mutations associated to MRC associated diseases. We have continued to screen deletions by flanking PCR of 6 hot-spot regions. Total mtDNA sequencing or gene panel analysis is also performed in selected samples, according to clinic manifestations and results from previous biochemical and/or genetic screening.

Mitochondrial **DNA depletion syndrome** (MDS), a mitochondrial cytopathy, comprises a heterogeneous group of diseases, caused by defects in intergenomic communication, namely due to nuclear genes mutations causing severe reduction of mtDNA content, with energy production impairment. That mtDNA reduction copies has been implicated as a major cause of mitochondrial disease in children. Copy number (mtDNA) assays are now part of the genetic mitochondrial genome screening. Nuclear genes screening includes 9 genes related to MRC function and or mtDNA biogenesis.

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

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

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

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

POLG1,2 genes were screened in 29 samples of 29 patients (3,190 DNA sequencing assays). We have identified 244 sequence variations in 29 patients. Limitations in the personnel did not allow screening entire gene for all the samples, given the huge size of POLG1 gene.

We have continued **DGUOK gene** screening, performed in 22 samples of 17 patients and 5 index cases (1,210 assays) and identified 58 sequence variations, 5 of which are probable pathogenic related to mtDNA depletion, relevant for genetic diagnosis and genetic counselling.

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

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

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

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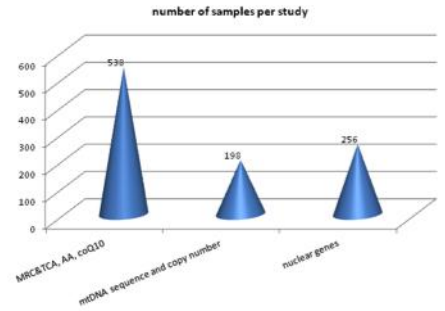

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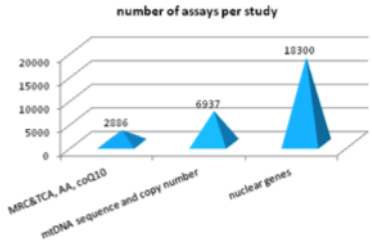

LABORATORY OF NEUROCHEMISTRY

Coordinators: Catarina Resende Oliveira, Inês Baldeiras

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

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

Cerebrospinal Fluid (CSF) cell count and chemical analysis

Electrophoresis of CSF/serum proteins

Detection of Immunoglobulin G Oligoclonal Bands in CSF/serum by Isoelectrical Focusing

Determination of plasma Vitamin A and E levels by high-performance-liquid chromatography (HPLC)

Evaluation of plasma and CSF redox status

- Quantification of urinary levels of purines and pyrimidines by HPLC
- Evaluation of the urinary activity of Arylsulfatase A -
- Seric evaluation of anti-neuronal antibodies in patients with polineuropathies
- Quantification of serum levels of antiepileptic drugs in patients under therapy
- Determination of serum neutralyzing antibodies (NABs) against Interferon-β (IFN-β) in multiple sclerosis patients undergoing treatment with IFN-β.

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

- Quantification of CSF levels of total-Tau protein, phosphorylated-Tau protein and β-amyloid1-42 peptide for dementia diagnosis

Detection of 14-3-3 protein in CSF in suspected cases of Creutzfeldt-Jakob Disease (CJD)

Immunodetection of Prion protein isoforms in brain extracts of CJD patients

Characterization of oxidative status in neurodegenerative disorders is also a specific interest of this unit. In this context, we perform, either in patient's 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 of 2013, the Neurochemistry Unit has received around 650 blood and 500 CSF samples and has performed the following analysis:

	Blood (Serum/Plasma)	CSF	Urine	Brain extracts	Other extracts
Cytochemistry and electrophoresis	364	364			
IgG Oligoclonal bands	220	220			
Vitamin A/E	129				
Redox Satus	60				
Purines & Pyrimidines			0		
Arylsulfatase A			4		
Anti-neuronal antibodies	65				
Antiepileptic drugs	5				
NABs against INFβ	9				
CSF Tau, p-Tau and Aβ42		218			
CSF 14-3-3 protein		101			
Prion protein isoforms				5	
Oxidative Stress	28				191

LABORATORY OF MOLECULAR GENETICS CARDIOPATHIES

Coordinator: Isabel Marques Carreira

Screening of mutations in 53 genes associated with cardiopathies

In the laboratory of Molecular Genetics of Cardiopathies (LGMC) the main study area is the Hypertrophic Cardiomyopathy(HCM) and Sudden Death (SD)

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

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

In our lab (LGMC), genotyping is achieved through a high-throughput and high accurate DNA Microchip platform optimized for genetic analysis using an iPlex MassArray system, which analyzes mutations in 53 genes associated with the development of cardiopathies. The procedure involves collaboration with a laboratory in Lisbon. Validation and interpretation of the results as well as the familial studies are done in the LGMC.

Forty three cases were refereed in 2013 of these cases, 26 were index cases and the remaining cases were *familial*. A genotype-phenotype correlation was established in some cases which triggered family studies and genetic counseling.

In 2013, the LGMC was revalidated the quality certificate (APCER), continuing to be a certified laboratory for the "Research of mutations in genes associated with cardiopathies".

Team: Ana Cristina Santos

LABORATORY OF NEUROGENETICS

Coordinator: Maria do Rosário Almeida

Molecular testing of Neurodegenerative diseases

The Neurogenetics Laboratory is now both nationally and internationally recognized centre for genetic testing of various Neurodegenerative diseases such as: Frontotemporal Lobar degeneration (FTLD), Familial Alzheimer Disease (AD) and Parkinson´s Disease (PD). During 2013, more than three hundred genetic testing referrals were ordered, some of them concerning mutation search in genes very recently discovered. Moreover, due to the close functional relation with the outpatient clinics of dementia and movement disorders of the University Hospital of Coimbra, rare dementia cases have been also diagnosed. It is important to emphasise that additional relatives of Fatal insomnia family, previously identified, have been studied in the current year in a genetic counselling context and after provided an informed written consent. Importantly, continuous efforts have been made to ensure that the methodologies and diagnostic strategies used are in accordance with current scientific knowledge and several initiatives have been performed to promote the molecular diagnostic tests available in the Lab. The group also took part of the two joint research Projects of the European - Early onset dementia (EOD) consortium in order to improve diagnostic and prognostic tools. Other research Proposals have been prepared and submitted to the evaluation of independent experts in order to get funding.

FUNDING

In 2013 funding of “Laboratório Associado – Centro de Neurociências e Biologia Celular” ascended the amount of 8.969.000,00€.

The main financing contribution was made by “Fundação para a Ciência e Tecnologia (FCT)”, concerning global institution programs and national projects, namely amount of 5.112.710,32€ distribuited as follows:

Strategical Project_ PEst-C/SAU/LA0001/2013	1.621.145,16€
Incentivo/SAU/LA0001/2013	14.486,00€
Projects:	2.941.378,92€
Science Program:	523.449,80€
Doctoral Program:	12.250,44€

The related items supported the main part of Center for Neuroscience and Cell Biology expenses during 2013.

Besides Center for Neuroscience is financed by other national and international agencies. In 2013 Center for Neuroscience received the amount of 236.716,48€ concerning other national projects and 936.253,49€ concerning international projects. Funding of CNC-Biotech ascended 2.601.256,07€.

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

The amount of other resting funds, which are not listed ascends a value of 82.063,64€.

Note: Financing values are based on expenditure values 2013

ONGOING PROJECTS

Title	Financing Agency	Duration	Budget (CNC)	Expenditure 2013
National Projects:				
"Rede Nacional de Espectrometria de Massa" Coordinator: Euclides Pires	FCT Refª: REDE/1506/REM/2005	01/01/2009 to 30/06/2014	138.960,42	40.295.43€
"Rede Nacional de Ressonância Magnética Nuclear." Coordinator: Carlos Geraldes	FCT Refª: REDE/1517/RMN/2005	01/01/2010 to 31/12/2013	216.528,43	41.470,93€
"Micro e nano design de materiais com funcionalidades especificas para promover a regeneração de tecido ósseo usando células estaminais adultas." Coordinator: João Nuno Moreira Proponent: Universidade do Minho	FCT Refª: MIT/ECE/0047/2009	01/06/2010 To 30/11/2013	32.880,00	10.610,58€
"Benefícios do controlo metabólico precoce: prevenção da formação de memória hiperglicémica através da estimulação da bioenergética." Coordinator: Carlos Palmeira	FCT Refª: PTDC/QUI-BIQ/103514/2008	01/03/2010 to 31/03/2013	126.667,00	6.044,75€
"NPwhY - Inervação e angiogénese para o benefício da osteogénese: envolvimento do NPY na regeneração óssea." Coordinator: João Malva Proponent: Instituto de Engenharia Biomédica - INEB	FCT Refª: PTDC/SAU-OSM/101469/2008	05/02/2010 to 31/07/2013	9.000,00	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 31/10/2013	122.336,00	57.849,85€
"Vida e morte das células ganglionares da retina: neuromodulação e neuroprotecção pelo Neuropeptídeo Y." Coordinator: Francisco Ambrósio Proponent: Faculdade de Medicina da Universidade de Coimbra	FCT Refª: PTDC/SAU-NEU/099075/2008	01/04/2010 to 30/09/2013	51.897,00	16.671,27€

“A restrição calórica aumenta a esperança de vida: papel do neuropeptídeo Y na autofagia.” Coordinator: Cláudia Cavadas	FCT Refª: PTDC/SAU-FCF/099082/2008	01/04/2010 to 15/07/2013	153.150,00	22.272,93€
“Efeito da cafeína e dos receptores da adenosina A2A na resposta ao stress: papel da regulação da supra-renal.” Coordinator: Cláudia Cavadas	FCT Refª: PTDC/SAU-NEU/108110/2008	01/04/2010 to 15/07/2013	90.000,00	1.967,07€
“A Abertura da Caixa Pandora Para uma Terapia Activa Anti-cancro da Mama - O Papel do Direccionamento Selectivo da Mitocôndria.” Coordinator: Paulo Oliveira Participants: Faculdade de Farmácia da Universidade de Coimbra	FCT Refª: PTDC/QUI-QUI/101409/2008	01/04/2010 to 31/03/2013	170.976,00	22.478,02€
“Impacto da metanfetamina na barreira hemato-encefálica: estudo dos mecanismos envolvidos e do papel de neuroinflamação.” Coordinator: Ana Paula Silva Proponent: Faculdade de Medicina da Universidade de Coimbra	FCT Refª: PTDC/SAU-FCF/098685/2008	01/04/2010 to 30/09/2013	68.490,00	27.074,18€
“Papel da Comunicação intercelular entre células endoteliais e celulas estaminais neurais na "stemness" e a neurogénese: novos alvos terapeuticos para a reparação cerebral.” Coordinator: Fabienne Agasse	FCT Refª: PTDC/SAU-NEU/101783/2008	01/04/2010 to 30/06/2013	86.000,00	7.285,33€
“São os Fitoestrogénios Aditivos “Alimentares Seguros e Eficazes para Mulheres em Menopausa? Uma Aproximação In Vitro e In Vivo para este Problema.” Coordinator: Mª Sancha Santos	FCT Refª: PTDC/AGR-ALI/108326/2008	01/04/2010 to 30/09/2013	168.716,00	33.972,02€
“Mecanismos moleculares de insuficiência cardíaca: o papel do adipócito como orgão endócrino.” Coordinator: Daniel Espinoza	FCT Refª: PTDC/SAU-OSM/104124/2008	22/03/2010 to 30/12/2013	191.757,00	62.950,40€
“Análise do proteome do hipocampo de ratinhos expostos a medicação psicotrópica.” Coordinator: Bruno Manadas	FCT Refª: PTDC/SAU-NEU/103728/2008	15/03/2010 to 31/07/2013	120.000,00	19.280,04€
“Design de sensores químicos e biossensores compósitos para a monitorização em tempo-real e em simultâneo de óxido nítrico e oxigénio in vivo no cérebro.” Coordinator: Rui Barbosa Participants: Faculdade de Farmácia da Universidade de Coimbra	FCT Refª: PTDC/SAU-BEB/103228/2008	01/05/2010 to 31/10/2013	50.800,00	11.387,63€
“Caracterização dos princípios de design de circuitos metabólicos prevalentes.” Coordinator: Armindo Salvador Participants: Universidade de Coimbra; Universidade do Minho	FCT Refª: PTDC/QUI-BIQ/119657/2010	01/04/2012 to 31/03/2015	117.226,00	21.970,10€
“Terapia génica Não invasiva e Não viral da doença de Machado-Joseph” Coordinator: Luis Almeida	FCT Refª: PTDC/SAU-FAR/116535/2010	01/04/2012 to 31/03/2015	108.280,00	28.562,13€

“Estudo do mecanismo patogénico da Doença de Machado-Joseph num novo modelo de células estaminais pluripotentes induzidas.” Coordinator: Luis Almeida	FCT Refª: PTDC/SAU-NMC/116512/2010	24/01/2012 to 23/01/2015	145.360,00	16.481,95€
“Avaliação Neuropsicológica e Investigação Bigenómica nas Demência Frontotemporal.” Coordinator: Maria Manuela Grazina	FCT Refª: PTDC/SAU-EPI/121811/2010	01/01/2012 to 31/12/2014	199.699,00	40.098,37€
“Impacto da terapia com exendina-4 nos mecanismos moleculares subjacentes à disfunção cerebral associada à diabetes tipo 2 a longo prazo.” Coordinator: Ana Isabel Duarte	FCT Refª: PTDC/SAU-TOX/117481/2010	01/05/2012 to 31/10/2014	144.305,00	30.202,32€
“Papel da proteína p66Shc na Persistência de Danos Mitocondriais Induzidos por Fármacos.” Coordinator: Ignacio Vega Naredo	FCT Refª: PTDC/SAU-TOX/117912/2010	01/03/2012 to 28/02/2014	79.291,00	33.672,88€
“TranstirRetina é uma metaloprotease: possíveis implicações em doenças do sistem nervoso.” Coordinator: Sukalian Chaterjee Proponent: Instituto de Biologia Molecular e Celular (IBMC)	FCT Refª: PTDC/SAU-ORG/118863/2010	01/05/2012 to 30/04/2015	56.152,00	11.364,01€
“DEMTEST: Diagnóstico de demencias rapidamente progressivas baseado em biomarcadores - optimização de protocolos de diagnóstico.” Coordinator: Catarina Oliveira	FCT Refª: JPND/0001/2011	01/06/2012 to 31/05/2015	35.000,00	20.949,45€
“Alterações na transmissão sináptica GABAérgica na isquemia cerebral - mecanismos moleculares responsáveis pela internalização dos receptores GABAA.” Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-NMC/0198/2012	01/07/2013 to 30/06/2015	141.136,00	26.608,44€
“Regulação do metabolismo energético no cérebro pelo óxido nítrico: solução para a glicólise aeróbia” Coordinator: João Laranjinha	FCT Refª: PTDC/BBB-BQB/3217/2012	03/07/2013 to 02/07/2015	134.938,00	5.191,85€
“Previsão da diabetes e feridas em familiares em primeiro grau de diabéticos tipo 2” Coordinator: John Jones Proponent: Associação Protectora dos Diabéticos de Portugal (APDP)	FCT Refª: EXCL/DTP-PIC/0069/2012	01/06/2013 to 31/05/2016	173.264,00	8.609,13€
“Estudo da contribuição dos miRNAs para o metabolismo do peptídeo b-amilóide: desenvolvimento de uma plataforma lentiviral para expressão de múltiplos miRNAs no contexto da doença de Alzheimer” Coordinator: Ana Luisa Colaço Cardoso	FCT Refª: EXCL/DTP-PIC/0069/2012	01/03/2013 to 28/02/2015	100.800,00	34.036,85€
“Silenciamento da Doença de Machado-Joseph pela via sistémica” Coordinator: Rui Jorge Gonçalves Pereira Nobre	FCT Refª: EXPL/NEU-NMC/0331/2012	01/03/2013 to 30/04/2014	48.900,00	42.352,26€

“Do controlo da neuroinflamação à neuroproteção: bloqueio dos reptores A2A para o tratamento do glaucoma” Coordinator: Ana Raquel Sarabando Santiago	FCT Refª: PTDC/BIM-MEC/0913/2012	01/06/2013 to 31/05/2015	32.401,00	1.253,93€
“Tecido cardíaco humano para a avaliação de toxicidade – CARDIOTOX” Coordinator: Susana Carvalho Rosa	FCT Refª: EXPL/DTP-FTO/0570/2012	01/07/2013 to 30/06/2014	36.800,00	5.132,05€
“Efeitos do peptídeo orexigénico grelina na transmissão sináptica glutamatérgica” Coordinator: Sandra Manuela Domingues dos Santos	FCT Refª: PTDC/NEU-NMC/1098/2012	01/07/2013 To 30/06/2015	199.975,00	41.124,84€
"O Metabolismo enquanto modelador da pluripotência e diferenciação de células estaminais." Coordinator: João Ramalho	FCT Refª: PTDC/EBB-EBI/101114/2008	15/04/2010 to 14/10/2013	147.656,00	24.055,12€
"Derivados de Benzazolo Marcados com Fluor - 18 e Tecnécio - 99m para visualização In Vivo de depósitos de Amilóide." Coordinator: Catarina Oliveira Proponent: Instituto Técnológico e Nuclear (ITN) Participants: Faculdade de Medicina da Universidade de Coimbra; Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/QUI-QUI/102049/2008	01/01/2010 to 30/06/2013	4.800,00	352,60€
"Planctomyces - uma linhagem filogenéticamente profunda. Decifrando os mecanismos envolvidos na adaptação a condições de stress." Coordinator: Milton Costa	FCT Refª: PTDC/BIA-MIC/105247/2008	01/05/2010 to 31/10/2013	189.624,00	37.031,35€
"Análise dos mecanismos moleculares que determinam disfunção da alfa-sinucleína e a citoxicidade na doença de Parkinson - o papel do GDNF." Coordinator: Ana Cristina Rego Participants: ; Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/SAU-NEU/101928/2008	05/02/2010 to 31/07/2013	134.400,00	10.480,64€
"Optimização da utilização de hidratos de carbono em robalo de aquacultura através de perfis metabólicos." Coordinator: John Jones Participants: FCTUC	FCT Refª: PTDC/EBB-BIO/098111/2008	01/04/2010 to 30/09/2013	175.292,00	11.615,61€
"Mechanismos moleculares envolvidos na cicatrização cutânea na diabetes - a importancia de neuropeptídeos." Coordinator: Eugénia Carvalho	FCT Refª: PTDC/SAU-MII/098567/2008	01/05/2010 to 31/10/2013	195.000,00	52.268,26€
"Interacção de Lipoplexos com Membranas Celulares: uma Abordagem Biofísica da Terapia Génica." Coordinator: Amália Jurado	FCT Refª: PTDC/QUI-BIQ/103001/2008	03/05/2010 to 02/11/2013	122.562,00	48.329,83€

"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	15.234,70€
"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	5.727,48€
"Clarificação do Papel Mitocondrial na Cardiotoxicidade da Doxorubicina Usando um Sistema de Perfusão de Corações Intactos - Papel de Diferentes Calendários de Tratamento com Doxorubicina." Coordinator: António Moreno Proponent: IMAR- Instituto do MAR	FCT Refª: PTDC/SAU-OSM/104731/2008	01/05/2010 to 30/10/2013	65.200,00	8.134,30€
"Alimentos Fucncionais para Neuroprotecção: um papel para o Hypericum perforatum." Coordinator: João Malva Proponent: Universidade Minho (UM) Participants: Instituto de Ciências Biomédicas Abel Salazar (ICBAS/UP); Universidade Católica Portuguesa (UCP)	FCT Refª: PTDC/AGR-ALI/105169/2008	01/05/2010 to 31/12/2013	6.000,00	509,87€
"Skingineering - Engenharia de análogos de pele recorrendo à tecnologia de cell sheets." Coordinator: João Ramalho Proponente: Universidade Minho	FCT Refª: PTDC/SAU-OSM/099422/2008	01/04/2010 to 31/03/2013	44.748,00	0,00€
"Análise sistemática de proteínas Rab na fagocitose e na maturação do fagossoma do Mycobacterium tuberculosis." Coordinator: Maria Otilia Vieira Participants: Instituto de Biologia Molecular e Celular - IBMC/UP	FCT Refª: PTDC/BIA-BCM/112138/2009	01/01/2011 to 30/06/2014	171.993,00	48.732,09€
"Actividade Protectora da SIRT3 na Disfunção Mitocondrial Induzida por Fármacos." Coordinator: Paulo Oliveira	FCT Refª: PTDC/SAU-TOX/110952/2009	01/03/2011 to 30/08/2014	128.800,00	20.222,99€
"A enigmática maltocinase de micobactérias." Coordinator: Nuno Empadinhas	FCT Refª: PTDC/BIA-BCM/112459/2009	01/04/2011 to 30/09/2013	113.058,00	25.635,75€
"Transporte entre células da alfa-sinucleina na doença de Parkinson. O factor de progressão?" Coordinator: Manuel Garrido	FCT Refª: PTDC/SAU-NMC/109955/2009	01/04/2011 to 30/09/2014	144.738,00	28.481,01€
"Uma nova formulação de nanopartículas para aplicação de terapia génica em tumores sólidos." Coordinator: Henrique Faneca	FCT Refª: PTDC/QUI-BIQ/116080/2009	01/04/2011 to 30/09/2014	94.000,00	22.535,70€
"Simugrowth-Desenvolvimento de um modelo computacional para a simulação das propriedades biomecânicas de cartilagem desenvolvida in-vitro em função do estimulo mecânico em bioreactor." Coordinator: Alexandrina Mendes Proponent: Universidade de Aveiro Participants: Universidade do Minho (UM)	FCT Refª: PTDC/EME-TME/113039/2009	03/04/2011 to 31/03/2014	28.830,00	19879,23€

“O papel do intestino no desenvolvimento da esteatose hepatica induzida pela frutose.” Coordinator: John Jones	FCT Refª: PTDC/SAU-MET/111398/2009	01/07/2011 to 30/06/2014	139.476,00	40.329,26€
“Nitrato:nitrito:óxido nítrico: uma via crítica que suporta o impacto benéfico do vinho e do azeite na fisiologia gastrointestinal e cardiovascular.” Coordinator: João Laranjinha	FCT Refª: PTDC/AGR-ALI/115744/2009	01/03/2011 to 31/08/2014	142.474,00	25.338,74€
“Indução de células estaminais pluripotentes a partir de células do sangue do cordão umbilical através de metodologia não-viral e a sua diferenciação em cardiomiócitos – iPSCardio.” Coordinator: Ricardo Das Neves	FCT Refª: PTDC/SAU-ENB/113696/2009	01/04/2011 to 31/12/2014	135.649,00	43.368,21€
“Targets - TARgeted GEne Therapy Strategies to treat nerve injury.” Coordinator: Sérgio Paulo de Magalhães Simões Proponent: INEB Participants: Instituto de Biologia Molecular e Celular - IBMC/UP; ADFC/FC/UP	FCT Refª: PTDC/CPM-NAN/115124/2009	01/04/2011 to 30/09/2014	3.060,00	0,00€
“O papel da adenosina e do receptor A2A na resposta imunitária a Candida albicans.” Coordinator: Teresa Maria Gonçalves	FCT Refª: PTDC/SAU-MIC/115598/2009	01/06/2011 to 31/05/2013	49.832,00	7.420,33€
“Regulação do sistema ubiquitina-proteassoma pelo BDNF nas sinapses do hipocampo: importância na plasticidade sináptica.” Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-NMC/120144/2010	10/02/2012 to 09/02/2015	154.678,00	52.549,87€
“Fibrilas Interrompidas: Inibição de interacções aberrantes proteína-proteína em Amilóides.” Coordinator: Rui Brito	FCT Refª: PTDC/QUI-QUI/122900/2010	01/03/2012 to 28/02/2015	113.768,00	19.267,98€
“Nova Abordagem na Luta Contra a Tuberculose.” Coordinator: Maria Otília Vieira	FCT Refª: HMSP-ICT/0024/2010	01/01/2012 to 31/12/2014	206.610,00	72.624,41€
“Libertação de neuropeptídeos em feridas: uma nova terapêutica para o tratamento do pé diabético.” Coordinator: Ermelindo Leal	FCT Refª: PTDC/SAU-FAR/121109/2010	01/04/2012 to 30/09/2014	106.872,00	23.596,11€
“Contribuição para a erradicação da malária. Uma nova abordagem para atingir multi-alvos no ciclo de vida do parasita.” Coordinator: Luísa Melo Proponent: Faculade de Farmácia da Universidade de Coimbra; Participants: Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/SAU-FAR/118459/2010	01/03/2013 to 28/02/2015	5.500,00	350,29€
“O Óxido Nítrico na Doença de Alzheimer - Molécula Sinalizadora e Mediador de Patogénese.” Coordinator: Ana Ledo	FCT Refª: PTDC/BIA-BCM/116576/2010	01/04/2012 to 31/03/2015	81.698,00	26.803,78€

“Desenvolvimento de nanoparticulas multifuncionais inovadoras para o tratamento do cancro de mama.” Coordinator: João Nuno Moreira Proponent: Universidade do Minho	FCT Refª: PTDC/SAU-DMA/121028/2010	20/04/2012 to 19/04/2015	76.857,00	18.776,69€
“O sistema neuropeptídeo Y: potencial novo alvo terapêutico na retinopatia diabética” Coordinator: Francisco Ambrósio Proponent: Universidade de Coimbra	FCT Refª: PTDC/NEU-OSD/1113/2012	01/05/2013 to 30/04/2015	36.000,00	1.102,21€
“Estratégia terapêutica combinada baseada na modulação de miRNAs direcionada para glioblastoma multiforme: um novo nanosistema de base lipídica para entrega sistémica.” Coordinator: Maria Conceição Pedroso Lima	FCT Refª: PTDC/DTP-FTO/0265/2012	02/03/2013 to 01/03/2015	99.768,00	25.159,13€
“Um Novo Modelo para a Esquizofrenia: Defeitos na Plasticidade Homeostática Mediada por Stargazina.” Coordinator: Ana Luísa Carvalho	FCT Refª: PTDC/NEU-NMC/0750/2012	01/07/2013 to 30/06/2015	117.262,00	3.712,51€
“Doença de Machado-Joseph, agregação e degradação proteicas, biologia de células estaminais, proteostase, neurodegeneração.” Coordinator: Luís Almeida	E-RARE4/0003/2012	01/03/2013 to 29/02/2016	141.581,00	25.816,60€
“Ambiguidade e virulência em patogénios humanos.” Coordinator: Nuno Empadinhas Proponent: IBMC Instituto de Biologia Molecular e Celular - IBMC/UP	FCT Refª: PTDC/BBB-BEP/0695/2012	01/07/2013 to 30/06/2015	69.840,00	22.744,99€
“Papel dos receptores P2Y1 na polaridade neuronial e no crescimento axonial: implicações na proliferação das fibras musgosas na epilepsia.” Coordinator: Ricardo Rodrigues	FCT Refª: EXPL/NEU-NMC/0671/2012	10/03/2013 to 09/04/2014	48.240,00	40.750,23€
“Tratamento da doença de Alzheimer com um novo peptídeo inibidor da BACE1.” Coordinator: Armanda Santos	FCT Refª: PTDC/SAU-SCC/1351/2012	15/06/2013 to 14/06/2015	177.611,00	18.443,59€
“Plataformas combinatoriais para promover a sobrevivência celular- PROSURVIVAL.” Coordinator: Hugo Fernandes	FCT Refª: PTDC/BIM-MED/1118/2012	01/07/2013 to 30/06/2015	130.000,00	27.549,42€
“Mecanismos associados à regulação ribosomal durante o desenvolvimento axonal.” Coordinator: Rui da Costa	FCT Refª: EXPL/NEU-NMC/0541/2012	01/07/2013 to 30/06/2014	49.998,00	28.386,58€
“Acoplamento neurovascular entre a actividade neuronal e o fluxo sanguineo no encéfalo mediado pelo óxido nítrico.” Coordinator: João Laranjinha	FCT Refª: PTDC/SAU-NEU/108992/2008	01/05/2010 to 31/10/2013	100.000,00	27.444,97€
“Perfis dinâmicos do óxido nítrico no cérebro: regulação da respiração celular com implicações para a doença de Alzeheimer e para o envelhecimento.” Coordinator: João Laranjinha	FCT Refª: PTDC/SAU-NEU/103538/2008	01/06/2010 to 30/06/2013	100.000,00	7.810,88€

“HotMetal-Estratégias de resistência a metais pesados e disseminação de resistências a antibióticos nas fontes marinhas hidrotermais.” Coordinator: Milton Costa Proponant: IMAR-Instituto do Mar	FCT Refª: PTDC/MAR/109057/2008	01/06/2010 to 30/11/2013	9.000,00	7.790,95€
“Análise das alterações da transcrição em modelos cerebrais e periféricos da doença de Huntington - influência da modulação das desacetilases das histonas.” Coordinator: Ana Cristina Rego	FCT Refª: PTDC/SAU-FCF/108056/2008	05/02/2010 to 04/08/2013	199.999,00	11.110,00€
“Papel da proteólise da ataxina-3 mediada por calpaínas na doença de Machado-Joseph: terapia molecular com vectores virais.” Coordinator: Luis de Almeida	FCT Refª: PTDC/SAU-NEU/099307/2008	05/02/2010 to 30/04/2013	107.000,00	12.913,33€
“Papel da Fisiologia Mitocondrial na Resistência das Células Estaminais Tumorais à Quimioterapia.” Coordinator: Paulo Oliveira	FCT Refª: PTDC/QUI-BIQ/101052/2008	01/04/2010 to 30/06/2013	143.016,00	34.870,77€
“Biorstimul - Desenvolvimento e construção de um novo conceito de bioreactor para a caracterização biomecânica e bioquíma de tecidos de cartilagem desenvolvidos in-vitro.” Coordinator: Celeste Lopes Proponent: Universidade Aveiro	FCT Refª: PTDC/EME-PME/103578/2008	16/03/2010 to 15/03/2013	41.600,00	12.105,72€
“Detecção do potencial sensibizante de quimicos através de um teste in vitro alternativo: uma imposição da nova legislação da União Europeia.” Coordinator: Maria Rosete Participants: Universidade Aveiro	FCT Refª: PTDC/SAU-OSM/099762/2008	01/04/2010 to 30/09/2013	128.200,00	48.142,94€
“Mecanismos e propriedades anti-inflamatórias de plantas medicinais: investigação multidisciplinar para a sua validação e utilização como fonte de fitofármacos.” Coordinator: Maria Rosete Proponent:: Universidade Coimbra Participants: Universidade Aveiro	FCT Refª: PTDC/SAU-FCF/105429/2008	01/05/2010 to 31/10/2013	55.800,00	18.053,21€
“Alteração do tráfego intracelular mediado pela mitocôndria na doença de Parkinson.” Coordinator: Sandra Cardoso Participants: Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/SAU-NEU/102710/2008	05/02/2010 to 31/05/2013	102.600,00	12.162,47€
"Regeneração cardiaca com células vasculares embrionárias e uma matriz biomimética." Coordinator: Lino Ferreira	FCT Refª: PTDC/SAU-BEB/098468/2008	01/04/2010 to 30/06/2013	180.000,00	12.665,82€
“Nanomateriais para detecção de células.” Coordinator: Lino Ferreira Participants: Biocant-Associação de transferência de Tecnologia	FCT Refª: PTDC/CTM/099659/2008	01/04/2010 to 30/06/2013	76.000,00	13.354,21€

“Mecanismos responsáveis pelos efeitos do óxido nitrico na proliferação de células estaminais neurais após lesão cerebral.” Coordinator: Caetana Carvalho	FCT Refª: PTDC/SAU-NEU/102612/2008	01/04/2010 to 30/09/2013	120.000,00	18.947,92€
“O papel da tradução localizada de mRNAna formação da junção neuromuscular.” Coordinator: Ramiro Almeida	FCT Refª: PTDC/SAU-NEU/104100/2008	01/05/2010 to 31/08/2013	120.000,00	33.665,41€
“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 31/12/2013	164.424,00	60.548,28€
“Regulação das proteínas hnRNP pela neurotrofina BDNF: importância da plasticidade sináptica.” Coordinador: Carlos Duarte	FCT Refª: PTDC/SAU-NEU/104297/2008	15/09/2010 to 14/03/2014	120.000,00	13.120,24€
"Parametrização do metabolismo e crescimento tumorais através da análise de fluxos metabólicos e engenharia metabólica." Coordinador: Rui Carvalho	FCT Refª: PTDC/EBB-EBI/115810/2009	01/01/2011 to 30/06/2014	169.578,00	64.386,07€
“Localização e metabolismo da APP e relação com o controlo da degeneração sináptica em modelos animais da doença de Alzheimer.” Coordinador: Catarina Oliveira	FCT Refª: PTDC/SAU-NMC/114810/2009	23/08/2011 to 31/12/2013	89.493,00	55.294,45
“Caracterização da interacção Proteína - Carbohidrato da Laforina - Poteína humana envolvida na Doença de Lafora.” Coordinador: Carlos Geraldes Proponent: Biocant	FCT Refª: PTDC/BIA-PRO/111141/2009	01/03/2011 to 30/08/2014	31.140,00	7.340,84€
"Regulação por fosforilação da ataxina-3, a proteína mutada na Doença de Machado Joseph." Coordinador: Ana Luísa Carvalho Participants: UM; IBMC	FCT Refª: PTDC/SAU-NMC/110602/2009	01/01/2011 to 30/06/2014	123.777,00	26.870,55€
“Regulação da estabilidade do RNA mensageiro para a subunidade GluR1 dos receptores do glutamato.” Coordinador: Ana Luísa Carvalho	FCT Refª: PTDC/BIA-BCM/113738/2009	01/04/2011 to 30/06/2013	108.001,00	29.893,15€
"Pré-condicionamento via mitocôndria:potencial efeito neuroprotector na doença Alzheimer." Coordinador: Paula Moreira	FCT Refª: PTDC/SAU-NMC/110990/2009	03/01/2011 to 31/08/2013	93.735,00	46.390,34€
“Via para a síntese do MGLP de micobactérias. Caracterização bioquímica e estrutural das enzimas envolvidas.” Coordinador: Nuno Empadinhas Participants: IBMC	FCT Refª: PTDC/BIA-PRO/110523/2009	01/01/2011 to 30/06/2014	130.624,00	54.014,57€
"Desenvolvimento de uma vacina contra a hepatite B para ser administrada através das mucosas: Desenho e estudos mecanísticos de um protótipo de um sistema de libertação multicomponente nanoparticular." Coordinador: Olga Ribeiro	FCT Refª: PTDC/SAU-FAR/115044/2009	01/01/2011 to 31/03/2014	122.060,00	45.521,59€

"Desenvolvimento de uma nova estratégia terapêutica para o cancro do pâncreas envolvendo uma acção concertada de terapia génica e quimioterapia." Coordinador: Henrique Faneca	FCT Refª: PTDC/SAU-BMA/114482/2009	01/01/2011 to 30/06/2014	100.000,00	41.733,03€
"iCALP - Identificação das funções fisiológicas das calpaínas no controlo da proliferação e migração celulares no sistema nervoso central." Coordinador: Inês Araújo	FCT Refª: PTDC/SAU-NMC/112183/2009	01/03/2011 to 31/08/2014	142.560,00	31.875,91€
Programa MIT Coordinator: Catarina Oliveira, Lino Ferreira	FCT Refª: MIT-Portugal 2013	01/01/2013 to 31/12/2013	16.765,00	15.658,01€
"Novas estratégias para a recuperação da fertilidade e potencial genético de felídeos selvagens: desenvolvimento do xenotransplante e da transplantação de células espermatogoniais estaminais em gato doméstico como modelo para felídeos selvagens." Coordinator: Paula Mota	FCT Refª: PTDC/CVT/119477/2010	01/05/2012 to 30/04/2015	62.813,00	24.085,42€
"Modulação da actividade de células estaminais hematopoiéticas por acção de nanopartículas capazes de libertar factores de transcrição – STEMCELLMODULATORS." Coordinator: Ricardo Pires das Neves	FCT Refª: PTDC/CTM-NAN/120552/2010	01/05/2012 to 30/04/2015	115.884,00	43.557,34€
"Modulação da piruvato desidrogenase cinase e pluripotência: Implicações para cancro e biologia de células estaminais." Coordinator: João Ramalho	FCT Refª: PTDC/QUI-BIQ/120652/2010	06/05/2012 to 05/05/2015	130.000,00	42.131,54€
"Produção e propagação de linhas de células estaminais pluripotentes usando modulação metabólica." Coordinator: João Ramalho	FCT Refª: PTDC/EBB-EBI/120634/2010	06/05/2012 to 05/05/2015	94.000,00	41.116,84€
"Papel fisio-patológico da ecto-5`-nucleotidase - um novo alvo para neuroprotecção." Coordinator: Rodrigo Cunha	FCT Refª: PTDC/SAU-TOX/122005/2010	01/05/2012 to 31/08/2014	147.605,00	73.020,52€
"Papel do accumbens e amigdala no controlo da neuropatologia causada por stress crónico." Coordinator:Rodrigo Cunha	FCT Refª: PTDC/SAU-NSC/122254/2010	01/04/2012 to 30/09/2014	148.080,00	61.583,48€
"BIOMARKAPD: Biomarcadores para Doença de Alzheimer e Doença de Parkinson." Coordinator:Catarina Oliveira	FCT Refª: JPND/0005/2011	01/06/2012 to 31/05/2015	48.500,00	7.086,23€
"Bioprospecção de enzimas com capacidade de degradar biomassa vegetal no metagenoma do sistema divestivo de Porcellio dilatatus (Crustacea,Isopoda)." Coordinator:Antonio Veríssimo	FCT Refª: PTDC/AGR-TEC/3789/2012	01/05/2013 to 30/04/2015	90.000,00	11.204,06€
"Patofisiologia da Toxicidade Cardíaca Persistente da Doxorubicina: Uma ligação entre Mitocôndria e Epigenetica" Coordinator:Paulo Oliveira	FCT Refª: PTDC/DTP-FTO/1180/2012	01/05/2013 to 30/04/2015	175.000,00	82.155,10€

“O metilfenidato e as alterações na barreira hemato-encefálica numa situação fisiológica e na perturbação de hiperatividade com défice de atenção” Coordinator:Ana Paula Silva Proponent:Universidade de Coimbra	FCT Refª: PTDC/NEU-OSD/0312/2012	01/06/2013 to 31/05/2015	60.336,00	3.462,35€
“Mecanismos de protecção neuronal contra stress oxidativo mediados pela DJ-1: implicações na doença de Parkinson” Coordinator:Bruno Manadas Participant:Biocant, Univ.Minho, U.Beira Interior	FCT Refª: PTDC/NEU-NMC/0205/2012	01/05/2013 to 30/04/2015	113.870,00	15.641,46€
“Biossíntese de polissacáridos raros de metilmanose em micobactérias não tuberculosas” Coordinator:Nuno Empadinhas Participant:IBMC,ITQB	FCT Refª PTDC/BIA-MIC/2779/2012	01/07/2013 to 30/06/2015	100.360,00	15.544,96€
“Investigação bigenómica translacional na Neuropatia Ótica Hereditária de Leber: Correlação Genótipo-Fenótipo” Coordinator:Manuela Grazina Participant:CCMAR-Alg	FCT Refª: PTDC/DTP-EPI/0929/2012	01/04/2013 to 31/03/2015	192.780,00	34.900,75€
"Células estaminais tumorais e progressão tumoral: dos mecanismos moleculares às consequencias clínicas" Coordinator:Maria Carmen Alpoim	FCT Refª: PTDC/BBB-BQB/2450/2012	01/05/2013 to 30/04/2015	132.248,00	44.094,27€
“Mecanismos e estrategias de tratamento da deficiência da cicatrização cutânea na diabetes” Coordinator:Susana Gerreiro	FCT Refª: PTDC/BIM-MED/0492/2012	01/07/2013 to 30/06/2014	50.000,00	11.706.94€
“Nova abordagem da disfunção reprodutora na diabetes:análise 3D da espermatogénese e microscopia confocal Raman para análise da função mitocondrial” Coordinator:Sandra Amaral	FCT Refª: PTDC/BEX-BCM/0224/2012	03/07/2013 to 02/07/2014	48132,00	13.863,58€
Sub – Total FCT				**2.941.378,92€**
Other National Projects				
"Ibercivis.pt - Uma plataforma de computação voluntária para a Península Ibérica.” Coordinator: Rui Manuel Pontes M. F. Brito	UMIC - Agência para a Sociedade do Conhecimento	16/06/2010 to 31/12/2013	87.380,00	22.199,46€
“Quero mais e melhores células! (células estaminais: o que são? Onde estão? Para que servem?)” Coordinator: Cláudia Cavadas	Ciência Viva – Agência Nacional para a cultura científica e tecnológica	01/11/2011 to 31/01/2013	83.040,00	35.540,06€

DoIT – projeto nº 013853 Coordinator: Catarina Oliveira	Agência da Inovação, S.A.	01/07/2010 to 30/08/2014	378.154,38	102.424,60€
"Aging,Stress and Chronic Diseases:From mecanisms to therapeutics" Coordinator:Luis Almeida Proponent:Universidade de Coimbra	Mais Centro-Programa Operacional Regional do Centro Refª: SCT_2011_02_006_4819	01/06/2013 to 30/06/2015	128.093.92	13.056,47€
"New Strategies do manage Brain Diseases." Coordinator: Luís Almeida Proponent: Universidade de Coimbra	Mais Centro-Programa Operacional Regional do Centro Refª:SCT_2011_02_002_4756	01/06/2013 To 30/06/2015	305.220,15	11.075,72€
QREN-Amilotera: 021622 Coordinator: Rui Brito	Agência da Inovação, S.A	01/09/2011 to 31/08/2014	85.804.45	20.817,57€
"Stemcell based platforms for Regenerative and Therapeutic Medicine" Coordinator: Carlos José Fialho da Costa Faro Proponent: Universidade de Coimbra	Mais Centro-Programa Operacional Regional do Centro Refª: SCT_2011_02_008_4832	01/02/2013 To 28/02/2015	682.875,01	31.602,60€
Sub – Total Other				**236.716,48€**
Total National Projects				**3.178.095,40€**
International Projects:				
"Transplantation of magnetic – labelled vascular cells and cardiomyocytes isolated from human embryonic stem cells in a bioactive injectable gel for myocardium regeneration after infarct". Coordinator: Lino Ferreira	Marie Curie Actions – 230929 Refª: FP7-PEOPLE-2007-4-3-IRG	01/04/2009 to 31/03/2013	100.000,00	24.620,92€
"Industrial Academic Initial Network towards treatment of Polyglutamine diseases" Coordinator: Luís Almeida	Marie-Curie-264508 Ref.ª FP7-PEOPLE-ITN-2010	01/03/2011 to 28/02/2015	202.332,86	52.682,52€
Novel nanoparticles for drug delivery to the skin Coordinator: Lino Ferreira	Queen Mary - 289454 Ref.ª: FP7-PEOPLE-2011-ITN	01/11/2011 to 31/10/2015	471.627,60	133.226,49€
"Docotral Candidate Agreement 159302-1-2009-NL-Era Mundus-EMJD" Coordinator: Rodrigo Cunha	Marie-Curie-Cycle 2-2011-PT	21/06/2011 to 30/08/2014	89.680,00	30.268,18€
"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	12.679,41€
"The effect of TCF7L2 on Glucose Metabolism" Coordinator: John Jones	Mayo Clinic 5Ro1DK078646-07	01/08/2013 to 31/07/2014	15.701,04	5.313,44€
"Role of the autophagy-related protein Beclin-l in Machado-Joseph disease." Coordinator: Luís Pereira de Almeida	Association Française contre les Myopathies Ref.ª: SB/NF/2010/2008	30/10/2010 to 10/04/2013	110.000,00	6.673,39€

“New Treatments for Stress-induced Dysregulation of Circuits Regulating Reward, Fear, and Habit Learning”. Coordinator: Rodrigo Cunha	Massachusetts Institute of Technology Ref.ª: DARPA-BAA-009-68	01/04/2010 to 30/11/2014	944.680,00	205.591,28€
“DFRH/WIIA/51/2011 - Welcome II” Coordinator: Catarina Oliveira/Otília Vieira	Marie Curie Actions DFRH/WIIA/51/2011 - Welcome II	01/02/2012 to 31/01/2014	119.740,50	68.167,20€
“Unravelling the early steps in the biosynthesis of the mycobacterial MGLP.” Coordinator: Nuno Empadinhas	Mycobacterial MGLP	01/04/2012 to 31/03/2014	19.758,57	5.573,23€
“CAFFEIN-Cancer Associated Fibroblasts (CAF) Function in Tumor Expansion and Invasion”. Coordinator: João Nuno Moreira	Marie Curie grant 316610 Refª FP7-People-2012-ITN	01/10/2012 to 30/09/2014	209.781,00	20.711,64€
“DDZ II - Research Collaboration Agreement”. Coordinator: John Jones	DDZ II - Research Collaboration Agreement	01/11/2012 to 31/10/2013	14.112,00	12.213,28€
"Trigerralde nanomaterials to modulate cell activity" Coordinator:Lino Ferreira	European Research council executive agency" ERC-2012-StG 307384-NanoTrigger	01/11/2012 to 30/10/2017	1.699.320,00	248.168,11€
“Caffeine alleviation of MJD/SCA3” Coordinator: Luís Almeida	National Ataxia Foundation	01/01/2013 to 31/12/2014	11.186,27€	46,90€
“LRRK2 role on auto-antibody production by human B cells.” Coordinator: Margarida Carneiro	The Michael J. Fox Foundation for Parkinson’s Research	16/05/2013 to 16/03/2014	81.325,76	54.066,34€
“Mitochondrial Trafficking In Alzheimer Disease: Revealing the Role of Hummr.”	Alzheimer Association NIRG-13-282387	01/11/2013 to 31/10/2014	71.495,56	1.210,10€
ENC Network Cycle 4-2013 - PT - 04 -Amber Kerkhofs Coordinator: Rodrigo Cunha	ENC Network Cycle-04-2013-PT	01/10/2013 to 30/09/2015	121.900,00	9.505,03€
ENC Network Cycle 4-2013 - PT - 07 - Xin-Li Xu Coordinator: Rodrigo Cunha	ENC Network Cycle-04-2013-PT	01/10/2013 to 30/09/2015	126.400,00	10.580,03€
"Cellular and synaptic dissection of the neuronal circuits of social and autistic behavior" Coordinator: João Peça	Marie Curie FP7-People-20123-CIG PCIG13-GA-2013-618525	01/08/2013 To 31/07/2017	100.000,00	25.202,91€
"Chronic effects of silver nanoparticles (AgNPs) on rat liver, kidney and heart mitochondrial function" Coordinator: Carlos Manuel M. Palmeira	DFAS_Indianopolis Center EOARD FA8655-13-1-3036	25/02/2013 To 28/02/2014	18.461,44	9.753,09
Total International Projects				**936.253,49€**
TOTAL				**4.114.348,89€**

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)	50
Ana Bela Sarmento Ribeiro	(Assistant Prof., FMUC)	40
Ana Cristina Fortuna	(Inv. Assistant Prof., FFUC)	50
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Ledo	(Assistant Inv., CNC)	100
Ana Luísa Cardoso	(Assistant Inv., CNC)	100
Ana Luísa Monteiro de Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula M. Sousa	(Investigator, HUC)	50
Ana Rita Costa Álvaro	(Inv. Assistant Prof., UTAD)	60
Anabela Maduro A. Francisco	(Assistant Prof., Univ. Vasco Gama)	50
Anabela P. Rolo	(Assistant Prof., Univ. Aveiro)	60
Anália do Carmo	(Assistant Prof., Univ. Vasco da Gama)	80
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Ângela Inácio	(Project Investigator, CNC)	100
Ângelo R. Tomé	(Assistant Prof., FCTUC)	70
António F. Ambrósio	(Assistant Inv., FMUC)	Collaborator
António Macedo Santos	(Assistant Prof., FMUC)	30
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	60
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	30
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Arsélio P. Carvalho	(Full Prof., FCTUC)	100
Attila Köfalvi	(Assistant Inv., CNC)	100
Bruno José F. Manadas	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	100
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Carlos G. Geraldes	(Full Prof., FCTUC)	70
Carlos José V. Simões		30
Carlos Manuel Matias	(Assistant Inv., FCTUC)	60
Carlos Faro	(Associate Prof., FCTUC)	80
Carlos M. Palmeira	(Full Professor, FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	60
Célia Laurinda Nogueira	(Assistant Prof., FMUC)	40
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Daniela Cipestre Vaz	(Assistant Prof., Inst. Polit. Leiria)	30
Emília P. Duarte	(Assistant Prof., FCTUC)	80

Euclides Pires	(Associate Prof., FCTUC)	80
Eugénia Carvalho	(Assistant Inv., CNC)	100
Faraj Barah	(Investigator, CNC)	100
Fernando Monteiro Judas	(Assistant Prof., FMUC)	20
Gabriela Silva	(Assistant Prof., FFUC)	10
Geanne Matos de Andrade	(Associate Prof., Brasil)	30
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	10
Henrique Faneca	(Assistant Inv., CNC)	100
Henrique Bernardo Silva	(Assistant Inv., CNC)	100
Hugo Fernandes	(Assistant Inv., CNC)	100
Ildete Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Esteves Baldeiras	(Investigator, FMUC)	30
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	30
Isaura Simões	(Assistant Inv., CNC)	100
Joana Cardoso Costa	(Inv. Assistant Professor, FCTUC)	60
Joana Rosmaninho-Salgado	(Intern, CHUC)	80
João Laranjinha	(Associate Prof., FFUC)	60
João Moura Alves	(Assistant Prof., Inst. Pol. Viana Castelo)	50
João Nuno Moreira	(Assistant Prof., FFUC)	80
João Peça-Silvestre	(Assistant Inv., CNC)	100
João Ramalho Santos	(Associate Prof., FCTUC)	80
John Griffith Jones	(Principal Inv., CNC)	100
Jorge António R. Salvador	(Full Prof., FFUC)	60
José Alberto Correia e Vale	(MD, Univ. Salamanca)	Collaborator
José Custódio	(Associate Prof., FFUC)	80
José Dionisio	(Assistant Prof., FFUC)	75
Leonor Almeida	(Full Prof., FFUC)	50
Lino Ferreira	(Assistant Inv., CNC)	100
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Luís M. Rosário	(Associate Prof., FCTUC)	60
Luís Pereira Almeida	(Assistant Prof., FFUC)	80
Manuel Garrido	(Investigator, Genibet)	30
Manuella Pinto Kaster	(Associate Professor, Brasil)	40
Mª Amália Jurado	(Assistant Prof., FCTUC)	60
Mª Carmen Alpoim	(Associate Prof., FCTUC)	45
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Dolores T. Redondo	(Investigator, Univ. Salamanca)	Collaborator
Mª do Rosário Almeida	(Assistant Inv., CNC)	100
Mª Emilia O. Quinta Ferreira	(Associate Prof., FCTUC)	60
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Mª Helena Ribeiro	(Investigator, FMUC)	20
Mª Isabel J. Santana	(Associate Prof., FMUC)	30

Mª Luisa Sá e Melo	(Full Prof., FFUC)	60
Mª Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	20
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Mª Otilia Vieira	(Assistant Inv., CNC)	100
Mª Sancha Santos	(Principal Inv., FCTUC)	100
Mª Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
Mª Teresa Girão da Cruz	(Assistant Inv., CNC)	100
Marília Rocha	(Investigator, HUC)	50
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	60
Paula G. Agostinho	(Investigator, FMUC)	60
Paula Isabel Moreira	(Assistant Prof., FMUC)	60
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Paulo Pinheiro	(Assistant Inv., CNC)	100
Paulo Santos	(Assistant Prof., FCTUC)	60
Pedro Castanheira	(Investigator, Biocant)	Collaborator
Raghu Kalluri	(Investigator, HMS)	35
Ramiro Almeida	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Ricardo Rodrigues	(Assistant Inv., CNC)	100
Renata Silva	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	75
Rosa M. Santos	(Assistant Prof., FCTUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	60
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui M. M. Brito	(Associate Prof., FCTUC)	30
Rui Pinto	(Assistant Prof., EUVG)	30
Rui Prediger	(Assistant Prof., Brasil)	40
Samuel Silvestre	(Assistant Prof., UBI)	Collaborator
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Assistant Inv., FMUC)	60
Sara Domingues	(Assistant Prof., FFUC)	60
Sérgio Simões	(Associate Prof., FFUC)	60
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
Vera Lúcia Dantas Moura	(Manager Science & Tech., UC)	50
Vitor Manuel C. Madeira	(Full Prof., FCTUC)	80

Post-Doc Members

	Time % at CNC
Adrian Balsa	100
Akhilesh Rai	100
Alessandro Boli	100
Ana Isabel Duarte	100
Ana Burgeiro	100
Ana Oliveira	100
Ana Patricia Simões	100
Ana Raquel Esteves	100
Ana Silva	100
Ana Teresa Simões	100
Ana Teresa Varela	100
Bruno Carreira	100
Cândida Gonçalves da Silva	35
Carla Nunes	100
Carolina Coelho	40
Carolina Melo Souza	100
Catarina Alexandra Gomes	100
Catarina Miranda	100
Cátia Marques	100
Célia Aveleira	100
Chantal Fernandes	100
Clévio Nóbrega	100
Cristiana Paulo	100
Cristina Barosa	100
Daniel Rial	100
Denisa Daud Mateus	100
Elsa Henriques	100
Elisabete Baptista Ferreiro	100
Ermelindo Leal	100
Filipe Valente Duarte	100
Helena Vazão	100
Ignacio Vega-Naredo	100
Igor Tiago	60
Ivan Viegas	100
Joana Isabel Real	100
Joana Marques	100
João Fernando S. Carvalho	5
João M. Trigueiro Costa	100
João Paulo Teodoro	100

João Pedro Lopes	100
Jorge Valero Gomez-Lobo	100
Lígia Maria S. Ferreira	100
Liliana Mendonça	100
Luis Miguel Estronca	100
Luis Ribeiro	100
Margarida Caldeira	100
Mª Alexandra B. Amaral	100
Mª Teresa Cunha Oliveira	100
Mário Laço	100
Marisa A. Rego Encarnação	100
Michele Curcio	100
Miranda Mele	100
Marta Santos	100
Nelio Gonçalves	100
Patricia Ribeiro	100
Paula M. Canas	100
Paula Mota	100
Pedro Miguel Coelho	100
Ricardo Santos	100
Rita Perfeito	100
Rosa M. B. Matos Resende	100
Rui Nobre	100
Rui Oliveira Costa	100
Samira Ferreira	100
Sandra Catarina G. Amaral	100
Sandra Isabel F. Mota	100
Sezin Aday	100
Sónia Correia	100
Sónia Duarte	100
Sonia Luzia Pinho	100
Susana Guerreiro	100
Susana Isabel E. Alarico	100
Susana Ribeiro Louros	100
Susana Rosa	100
Pedro Gonçalves	100
Pedro Miguel Costa	100
Tatiana Catarino	100
Tatiana R. Rosenstock	100
Teresa Serafim	100
Vilma Sardão Oliveira	100
Vitor Mendes	100

PhD Students	Time % at CNC
Amber Kherkoffs	100
Ana Branco M. Tiago	100
*Ana Cristina F. Lemos	100
Ana Cristina Gonçalves	100
Ana Cristina Gregório	100
Ana Carolina Moreira	100
Ana Catarina Ferreira	100
Ana Catarina R. Graça Fonseca	100
Ana Cristina Ferreira	100
Ana Filipa Cruz	100
Ana Francisca Lima	100
Ana Isabel Serralheiro	100
Ana Luísa Nobre	100
Ana M. Metelo	20
Ana Mª Sequeira Cardoso	100
Ana Mª Silva	100
Ana Patricia Marques	100
Ana Plácido	100
Ana Santos Carvalho	100
Ana Sofia Lourenço	100
Ana Sofia C. Valdeira	100
Ana Sofia V. Cunha	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ana Teresa Viegas	100
André Ferreira Martins	100
André Filipe M. Soares	100
Andreia Gomes	100
Ângela Valério-Fernandes	100
Ângela Pascoal Crespo	100
Anna Vladimirovna Pliassova	100
António Silva	100
Bárbara Rocha	100
Beatriz Lacerda de Sousa	100
Bruno Miguel F. Gonçalves	100
Carla Mª Nunes Lopes	100
Carla Patrícia R. Paiva	100
Carlos Adriano Matos	100
Carlos Fernando D. Rodrigues	100

Carlos Manuel Melo	100
Carlos Samuel M. Boto	100
Cassilda Pereira	100
Catarina Mendes Morais	100
Catarina Praça de Almeida	100
Catarina Sofia H. Jesus	50
*Cátia Moreira de Sousa	100
Daniel F. Santos	100
Daniela Gonçalves	100
Daniela Pereira S. Alho	100
David Bowman	25
David Dias	100
Diana Dinis Azenha	100
Diana Jurado S. Serra	100
Diana F. Silva	100
Diana Margarida Carvalho	100
Dina Pereira	100
Dominique Fernandes	100
Dulce Bento	100
Edna Filipa Soares	100
Elda Bonifácio	100
Elisabete Oliveira Augusto	100
Emanuel Candeias	100
Emanuel Costa	100
Eszter Szabó	100
Fátima Martins	100
Filipa L. Carvalho	100
Filipa Lebre	100
Filipe Coreta Gomes	20
Filipe Duarte	100
Filomena Grilo da Silva	100
Francisco Manuel Queiroz	100
Geetha Vijayakumar	100
Gianluca Selvaggio	100
Gladys Caldeira	100
Graciana Tribuna	50
Graciano da Silva Leal	100
Helena Carvalheiro	100
Helena Leitão	100
Henrique Miguel Alexandrino	100
Humberto Gomes Ferreira	100
Inês Biscaia Barbosa	100
Inês Honório	100

Inês Santarino	100
Inês Vasconcelos M. Santos	75
Isabel Maria Santos Onofre	100
Ivan Salazar	100
Ivana Kostic	100
Janete Cunha Santos	100
Jeannette Schmidt	100
Jimmy George	100
Joana Balça Silva	100
Joana Bicker	100
Joana Domingues Vindeirinho	100
Joana Filipa C. Fernandes	100
Joana Filipa D. Neves	100
Joana Liberal	100
Joana Paixão	100
Joana Pedro	100
Joana Ribeiro Guedes	100
Joana Sousa	100
João Abrantes	100
João André Freitas	50
João Carlos Almeida	100
João Demétrio B. Martins	100
João Manuel Rito	50
João Silva	80
Jorge Manuel Ruivo	50
Josephine Blerch	100
Júlia Valente	50
Kátia Mesquita	100
Lara Franco	100
Liane Moura	100
Lisa Rodrigues	100
Luana Naia	100
Ludgero C. Tavares	100
Luís André A. França	100
Magda Santana	100
Marcelo Correia	100
Márcio José C. Ribeiro	100
Marco António P. Matos	100
Mª Graça Rocha	40
Mª Inês Morte	100
Mª Inês Almeida Sousa	100
Mª Joana G. Pinto	100
Mª la Salete J. Baptista	100

Mª Madalena Ribeiro	100
Mariana Botelho da Rocha	100
Mariana Oliveira Conceição	100
Mariana Ponte C. Ribeiro	100
Marília Henriques Cordeiro	100
Mariline Silva	100
Marta Daniela Passadouro Caetano	100
Marta Isabel D. Mota Vieira	100
Marta Pereira	100
Marta Regina S. Carmo Oliveira	100
Michela Comune	100
Michelle Stumpf Viegas	100
Miguel Maria Lino	100
Mohamed Hussien	100
Mónica Abreu	100
Nuno Ferreira	100
Nuno André Fonseca	100
Nuno Gabriel Machado	100
Nuno Miguel Jesus Machado	100
Nuno Mendonça Silva	100
Patrícia Henriques Domingues	100
Patrícia Lopes	100
Patrícia Raquel Pereira	100
Patrícia Rosado	100
Patrícia Sofia Morais	100
Paulo Gameiro Guerreiro	100
Pedro Alexandre Martins	100
Pedro João Madeira Afonso	100
Pedro José Gouveia	100
Pedro Manuel Batista Branco	100
Pedro Manuel V. Garção	100
Raquel Alves	100
Ravi Adusumalli	100
Rakikumar Kapavarapu	85
Renato Xavier C. Santos	100
Ricardo Romão Leão	25
Rodrigo Luiz Santos	100
Roksana Pirzgalska	100
Rui Benfeitas Vicente	100
Rui Miguel Martins	50
Rui M. Costa Soares	40
Rui Cruz	100
Rui Figueiredo	100

Sandra Figueiredo	100
Sandra Cristina Jesus	100
Sandra Marina A. Santos	100
Sara Amaral	100
Sara Lopes	100
Sara Matias Silva	100
Sara Raquel Oliveira	100
Sara Tavares M. Lima	100
Sílvia Viana Silva	60
Sofia Anastácio	100
Sofia Alexandra Ferreira	100
Sofia Romano	100
Susana Patrícia S. Pereira	100
Susana Sampaio	100
Tânia Leandro	100
Tânia Perestrelo	100
Tiago Rodrigues Sousa	100
Tiago Alfaro	75
Vanessa Isabel S. Mendes	100
Vera Calhau	100
Vera Lúcia G. Francisco	100
Vitor Manuel Carmona	100
Xinli Xu	100

MSc Students	**Time % at CNC**
Ana Marta Silva	100
Ana Pica-Milho	100
Ana Raquel Fontes	100
Ana Raquel Coelho	100
Ana Torres	100
*Ana Xavier	100
Andreia Luís	50
Andreia Palma	30
Ângelo Serani	100
Bruno Cruz	100
Carlos Custodia	100
Carlos Moura	100
Carolina Helena Noronha	50
Catarina Vaz	50
Catarina Xavier	30
Cátia Marques	100
Daniela Pedroso	100

Denis Brito	100
Diogo Reis	100
Edmilson Semedo	100
Eduardo Morais	100
Fábio Carvalho	50
Gabriela Leão	100
Giorgia Mastrella	100
Gonçalo Cristóvão	100
Guilherme Loureiro	30
Helena Martins	100
Inês Mahú	50
Inês Sebastião	100
Inês Simões	100
Joana Gomes	25
Joana Portela	100
Joana Filipa Sousa	50
João Filipe Amorim	100
José Miguel Codesso	100
Liliana Caetano	100
João Filipe Amorim	100
Mª Cristina Aspromonte	100
Mª Helena Silva	100
Mariana Magalhães	100
Mário Carvalho	100
Mário Correia	100
Mónica Marques	100
Paula Silva	100
Paulo Espírito Santo	100
Paulo Teixeira	100
Pedro Cunha	100
Pedro Miguel Fernandes	50
Pedro Rafael Reis	50
Renata Couto	100
Ricardo Silva	100
Ruben Branco	100
Rui Beleza	100
Rui Silva	100
Rui Simões	100
Sara Dias	100
Sara Handem	100
Sara Rebelo	100
Sarah Pagliaro	100
Sílvia Magalhães Novais	100

Susana Cecílio	100
Solange Machado	100
Tatiana Isabel Martins	100
Teresa Silva	100
Tiago Henriques	100
Tiago Silva	100
Valeria de Rosa	100
Vanessa Monteiro	100
Vânia Moreira	50

Grant Technicians	**Time % at CNC**
Alexandra Isabel Abrunheiro	100
Ana Marisa Simões	100
Ana Filipa d'Avó	100
Ana Rita M. Leal	60
Ana Sofia L. Coelho	100
Cândida Dias	100
Caroline Delgado Veloso	100
Catarina Rebelo	100
Cláudia Maria C. Deus	100
Cristina Carvalho	100
Dina Farinha	100
Diogo Maio	100
Fabio Paiva	100
Fatima Nunes	25
Filipa Simões	100
Luís Martins	100
Isabel Ferreira	50
Joana Furtado	100
João Ferreira	50
José Miguel J. Paiva	100
Luciana Pinto	100
Mafalda Costa	100
Margarida Coelho	100
Mariana Almeida	100
Mariana Val	100
Marta Baptista	100
Marta Mota	30
Marta Sousa	100
Miguel Caetano	100
Neuza Domingues	100
Pedro Alves	50
Pedro Tiago C. Curto	100

Raquel Marisa Trindade	100
Renata Tavares	100
Rita Pereira	100
Sandra Pinto	100
Sónia Neto R. Pereira	100
Susana Cardoso	100
Vanessa R. Anjos	100
Zaida Catarina Almeida	100

MD	**Time % at CNC**
Hermínio José T. Espírito Santo	30
Maria Isabel Santana	30
Luís Cunha	Collaborator
Luísa Diogo	Collaborator
Mª Margarida Martins Gonçalo	40
Maria Olinda R. Rebelo	Collaborator

SERVICE STAFF

		Time % at CNC
Ana Cristina F. Barbosa Soares	(Graduate Technician, CNC)	100
Ana Cristina Franco dos Santos	(Graduate Technician, CNC)	100
António José Azinhaga Teles Grilo	(Graduate Technician, CNC)	100
Cândida Elsa Frias Mendes	(Graduate Technician, CNC)	100
Carla Margarida dos Santos Veríssimo	(Graduate Technician, CNC)	100
João Miguel Pratas	(Graduate Technician, CNC)	100
Luciana C. Albuquerque Pinto	(Graduate Technician, CNC)	100
Mª Helena Garrucho Ribeiro	(Graduate Technician, HUC	20
Maria João Ferreira Canas dos Santos	(Graduate Technician, CNC)	100
Marta Sofia Marques Simões	(Graduate Technician, CNC)	100
Mónica Alexandra V. Serrano	(Graduate Technician, CNC)	100
Paulo Rodrigues-Santos	(Graduate Technician)	20

TECHNICAL STAFF

		Time % at CNC
Cármen Lídia Graça Semião	(Graduate Technician, CNC)	100
Diana dos Santos Simões Graça	(Technician, CNC)	100
Fátima Cristina dos S. Carvalho Graça	(Technician, CNC)	100
Isabel Conceição Calado Esteves Costa	(Technician, CNC)	100
Isabel Nunes Correia	(PhD, Graduate Technician, CNC)	100
Isabel Dantas Fernandes	(Graduate Technician, CNC)	100
Luisa Leitão Cortes	(PhD, Graduate Technician, CNC)	100
Maria do Céu Mendes Gomes	(Technician, CNC)	100
Maria Isabel Gonçalves	(Technician, CNC)	100
Maria Eugénia A. Silva Lopes Campos	(Technician, CNC)	100
Vera Mónica M. Mendes	(Technician, CNC)	100
Virginia Maria R. Ferreira Fonseca	(Technician, CNC)	100
Maria da Rosário da Costa Faro	(Graduate Technician, CNC)	100
Odete Pereira Cabanelas	(Graduate Technician, CNC)	100
Sandra Manuela Domingues dos Santos	(PhD, Graduate Technician, CNC)	100
Sara da Costa Jordão A. Lopes	(Technician, CNC)	100
Sandra Freire	(Technician, CNC)	100
Vera Oliveira	(Graduate Technician, CNC)	100

ADMINISTRATIVE STAFF

		Time % at CNC
Carla Lopes Rodrigues	(Administrative Assistant, CNC)	100
Catarina Alexandra Ferreira Gomes	(Graduate Administrative, CNC)	100
Heidi Maria da Silva Lopes Gonçalves	(Graduate Administrative, CNC)	100
Lia da Costa Jordão Aparício Lopes	(Graduate Administrative, CNC)	100
Mª Luísa R. Caldeira Bonito	(Graduate Administrative, CNC)	100
Mónica Alexandra Rodrigues Morais	(Graduate Administrative, CNC)	100
Nilza Clara F. Marques Manadas	(Administrative Assistant, CNC)	100
Rosa Alexandra Folhas Fernandes	(Graduate Administrative, CNC)	100
Sandra Cristina Santos Luís	(Graduate Administrative, CNC)	100
Sílvia Marisa Esteves de Sousa	(Graduate Administrative, CNC)	100
Susana Adelaide Rocha da Silva	(Administrative Assistant, CNC)	100
Tatiana de Azevedo Paula	(Graduate Administrative, CNC)	100

RESEARCH STAFF AND STUDENTS / RESEARCH AREA

Neuroscience and Disease

Catarina Resende Oliveira, MD, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Rita Costa Álvaro	(Inv. Assistant Prof., UTAD)	60
Ângelo Tomé	(Assistant Prof., FCTUC)	70
António F. Ambrósio	(Investigator, FMUC)	Collaborator
António Macedo Santos	(Assistant Prof., FMUC)	30
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	30
Arsélio P. Carvalho	(Full Prof., FCTUC)	100
Attila Köfalvi	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	100
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	60
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Emília P. Duarte	(Assistant Prof., FCTUC)	80
Geanne Matos de Andrade	(Associate Prof., Brasil)	30
Henrique Bernardo Silva	(Assistant Inv., CNC)	100
Ildete Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Esteves Baldeiras	(Investigator, FMUC)	35
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	30
Joana Rosmaninho-Salgado	(Intern, CHUC)	80
João Peça-Silvestre	(Assistant Inv., CNC)	100
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Manuella Kaster	(Assistant Professor, Brasil)	40
Mª do Rosário Almeida	(Assistant Inv., CNC)	100
Mª Isabel J. Santana	(Associate Prof., FMUC)	30
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Paula G. Agostinho	(Investigator, FMUC)	60
Paula Isabel Moreira	(Assistant Prof., FMUC)	60
Paulo Pinheiro	(Assistant Inv., CNC)	100
Paulo Santos	(Assistant Prof., FCTUC)	60
Ramiro Almeida	(Assistant Inv., CNC)	100
Ricardo Rodrigues	(Assistant Inv., CNC)	100

Rodrigo A. Cunha	(Associate Prof., FMUC)	75
Rui Prediger	(Associate Prof., Brasil)	80
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Investigator, FMUC)	60

Post-Doc Members	**Time % at CNC**
Ana Isabel Duarte	100
Ana Oliveira	100
Ana Patricia Simões	100
Ana Raquel Esteves	100
Bruno Carreira	100
Carolina Melo Souza	100
Catarina Alexandra Gomes	100
Célia Aveleira	100
Daniel Rial	100
Elisabete Baptista Ferreiro	100
Joana Isabel Real	100
Joana Marques	100
João Pedro Lopes	100
João T. Costa	100
Jorge Valero Gomez-Lobo	100
Lígia Maria Ferreira	100
Luis Ribeiro	100
Margarida Vaz Caldeira	100
Mário Laço	100
Michele Curcio	100
Miranda Mele	100
Nélio Gonçalves	100
Paula M. Canas	100
Rita Perfeito	100
Rosa M. B. Matos Resende	100
Rui Oliveira Costa	100
Samira Ferreira	100
Sandra Mota	100
Sónia Correia	100
Susana Ribeiro Louros	100
Tatiana Catarino	100
Tatiana R. Rosenstock	100

PhD Students	Time % at CNC
Amber Kherkoffs	100
Ana Catarina Fonseca	100
Ana Cristina F Lemos	100
Ana Patricia Marques	100
Ana Placido	100
Ana S. Carvalho	100
Anna Vladimirovna Pliassova	100
António Manuel C da Silva	100
Carla Maria Nunes Lopes	100
Carlos Adriano A. Matos	100
Daniel Santos	100
Diana FF Silva	100
Dominique Fernandes	100
Elisabete O. Augusto	100
Emanuel Candeias	100
Eszter Szabó	100
Francisco Manuel Q Gonçalves	100
Gladys Caldeira	100
Graciano Leal	100
Helena Mª Carvalheiro	100
Ivan Salazar	100
Janete Cunha Santos	100
Jeannette Schmidt	100
Jimmy George	100
Joana F. C. Fernandes	100
Joana Pedro	100
Joana Vindeirinho	100
Lara Franco	100
Luana Carvalho Naia	100
Magda Santana	100
Mª Joana Pinto	100
Márcio Ribeiro	100
Marco António P. Matos	100
Maria Inês Morte	100
Mariana Botelho Rocha	100
Mariline Silva	100
Marta Dias M. Vieira	100
Marta Regina S. Carmo Oliveira	100
Mohamed Hussien	100
Mónica Abreu	100
Nuno Jesus Machado	100
Patrícia Sofia Morais	100
Pedro João Afonso	100
Pedro Manuel V. Garção	100
Renato Xavier Santos	100
Sara Matias Silva	100
Sara Oliveira	100
Sílvia Viana da Silva	100
Sofia Alexandra Ferreira	100

Susana Sampaio	100
Tiago Manuel P. Alfaro	75
Tiago Sousa	100
Xinli Xu	100

MSc Students	**Time % at CNC**
Ana Raquel Fontes	100
*Ana Xavier	100
Andreia Palma	30
Bruno Cruz	100
Catarina Vaz	50
Catarina Xavier	30
Carolina Helena Noronha	50
Eduardo Morais	100
Giorgia Mastrella	100
Gonçalo P Cristovão	100
Guilherme Loureiro	30
Helena Martins	100
Inês Sebastião	100
Joana Gomes	25
João Filipe Amorim	100
Liliana Caetano	100
Mª Cristina Aspromonte	100
Mário Carvalho	100
Paula Silva	100
Rui Beleza	100
Rui Simões	100
Tiago Silva	100
Valeria de Rosa	100

Grant Technicians	**Time % at CNC**
Caroline Veloso	100
Cristina Carvalho	100
Fábio Paiva	100
Luis Martins	100
Pedro Alves	50
Susana Cardoso	100

Biotechnology and Health

Euclides Pires, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Alcino Jorge Lopes Leitão	(Assistant Prof., FFUC)	60
Amílcar Falcão	(Full Prof., FFUC)	50
Ana Cristina Fortuna	(Inv. Assistant Prof., FFUC)	50
Ana Luísa Cardoso	(Assistant Inv., CNC)	100
Anabela Maduro de Almeida	(Assistant Prof., Univ. Vasco Gama)	50
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Bruno Manadas	(Investigator, CNC)	100
Carlos Faro	(Associate Prof., FCTUC)	80
Carlos José Vieira Simões		30
Daniela Cipestre Vaz	(Assistant Prof., Inst. Polit. Leiria)	30
Euclides Pires	(Associate Prof., FCTUC)	60
Gabriela Silva	(Assistant Prof., FFUC)	10
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	10
Henrique Faneca	(Assistant Inv., CNC)	100
Hugo Fernandes	(Assistant Inv., CNC)	100
Isaura Simões	(Assistant Inv., CNC)	100
João Nuno Moreira	(Assistant Prof., FFUC)	80
Jorge António R. Salvador	(Full Prof, FFUC)	60
Lino Ferreira	(Assistant Inv., CNC)	100
Luís Pereira Almeida	(Assistant Prof., FFUC)	80
Manuel Garrido	(Investigator, Genibet)	30
Mª Amália Jurado	(Assistant Prof., FCTUC)	80
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Luísa Sá e Melo	(Full Prof., FFUC)	60
Mª Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Marília Rocha	(Investigator, HUC)	50
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	60
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Pedro Castanheira	(Investigator, Biocant)	Collaborator
Raghu Kalluri	(Investigator, HMS)	35
Renata Dias da Silva	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Rui M. M. Brito	(Associate Prof., FCTUC)	30
Rui Miguel Pinto	(Assistant Prof., EUVG)	30
Samuel Silvestre	(Assistant Prof., UBI)	Collaborator
Sara Domigues	(Assistant Prof., FFUC)	60
Sérgio Simões	(Assistant Prof., FFUC)	80

Tiago Quininha Faria	(Assistant Inv., CNC)	100
Vera Lúcia Dantas Moura	(Manager Science & Tech., UC)	50

Post-Doc Members	**Time % at CNC**
Adrian Balsa	100
Akhilesh Rai	100
Alessandro Boli	100
Ana Teresa Simões	100
Cândida Gonçalves da Silva	35
Catarina Miranda	100
Cristiana Paulo	100
Clévio Nóbrega	100
Elsa Henriques	100
Helena Vazão	100
João Fernando S. Carvalho	15
Lígia Maria S. Ferreira	100
Liliana Mendonça	100
Patrícia Ribeiro	100
Pedro Miguel Coelho	100
Pedro Miguel Costa	100
Rui Nobre	100
Sezin Aday	100
Sónia Luzia Pinho	100
Sónia Patricia Duarte	100
Susana Rosa	100

PhD Students	**Time % at CNC**
Ana Cristina Gregório	100
Ana Cristina Ferreira	100
Ana Filipa Cruz	100
Ana Francisca Lima	100
Ana Isabel Serralheiro	100
Ana Maria Cardoso	100
Ana Sofia Lourenço	100
Ana Sofia C. Valdeira	100
Ana Teresa Viegas	100
André Filipe M. Soares	100
Andreia Gomes	100
Ângela Valério-Fernandes	100
Bruno Miguel F. Gonçalves	100
Carlos Samuel M. Boto	100
Catarina Mendes Morais	100

Catarina Oliveira Almeida	100
Catarina Sofia H. Jesus	50
Cátia Moreira de Sousa	100
Daniela Gonçalves	100
Daniela Pereira S. Alho	100
David Bowman	25
Dina Pereira	100
Dulce Marisa Bento	100
Emanuel Costa	100
Edna Filipa Soares	100
Filipa Lebre	100
Geetha Vijayakumar	100
Gianluca Selvaggio	100
Graciana Tribuna	50
Inês Honório	100
Inês Vasconcelos Miranda Santos	75
Isabel Maria Santos Onofre	100
Ivana Kostic	100
João Abrantes	100
João Carlos Almeida	100
João Freitas	50
Joana Bicker	100
Joana Filipa Neves	100
Joana Ribeiro Guedes	100
Joana Sousa	100
Jorge Ruivo	50
Josephine Blersch	100
Mª de la Salete J. Baptista	100
Mariana Conceição	100
Marta Daniela Passadouro Caetano	100
Michela Comune	100
Miguel Maria Lino	100
Nuno Fonseca	100
Nuno Mendonça Silva	100
Patrícia Raquel Pereira	100
Patrícia Rosado	100
Pedro Alexandre Martins	100
Pedro José Gouveia	100
Pedro Manuel Batista Branco	100
Ravi Adusumalli	100
Ravikumar Kapavarapu	85
Ricardo Romão Leão	25
Rui Cruz	100

Rui Figueiredo	100
Rui Benfeitas Vicente	100
Sandra Cristina Jesus	100
Sandra Figueiredo	100
Sandra Marina A. Santos	100
Sara Lopes	100
Sofia Anastácio	100
Sofia Pereira Romano	100
Vanessa Mendes	100
Vera Calhau	100
Vitor Carmona	100

MSc Students	**Time % at CNC**
Ana Pica-Milho	100
Ana Torres	100
Angelo Serani	100
Carlos Custódio	100
Daniela Pedroso	100
Denis Brito	100
Diogo Maio	100
Edmilson Semedo	100
Gabriela Leão	100
Joana Filipa Sousa	50
José Miguel Codeso	100
Mariana Magalhães	100
Paulo Espírito Santo	100
Paulo Teixeira	100
Pedro Cunha	100
Pedro Miguel Fernandes	50
Pedro Rafael Reis	50
Ricardo Silva	100
Ruben Branco	100
Sara Dias	100
Sara Handem	100
Sarah Pagliaro	100
Susana Cecílio	100
Teresa Silva	100
Vanessa Monteiro	100

Grant Technicians	**Time % at CNC**
Ana Marisa Simões	100
Ana Rita M. Leal	60

Ana Sofia L. Coelho	100
Catarina Rebelo	100
Dina Farinha	100
Fátima Nunes	25
Joana Furtado	100
José Paiva	100
Pedro Curto	100
Raquel Marisa Trindade	100
Sandra Pinto	100
Vanessa Rebelo Anjos	100
Zaida Catarina Almeida	100

Cell and Molecular Toxicology

Rui Carvalho, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ana Ledo	(Assistant Inv., CNC)	100
Anabela P. Rolo	(Assistant Prof., Univ Aveiro)	60
Carlos M. Palmeira	(Full Professor., FCTUC)	80
João Laranjinha	(Associate Prof., FFUC)	60
José Custódio	(Associate Prof., FFUC)	80
Leonor Almeida	(Full Prof., FFUC)	50
Mª Carmen Alpoim	(Associate Prof., FCTUC)	45
Maria S. Santos	(Principal Inv., FCTUC)	100
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	60
Teresa Dinis Silva	(Associate Prof., FFUC)	60

Post-Doc Members	**Time % at CNC**
Carla Nunes	100
Catia Marques	100
Filipe Valente Duarte	100
Ignacio Vega-Naredo	100
João Paulo Teodoro	100
Mª Teresa Cunha Oliveira	100
Ricardo Santos	100
Teresa Laura Serafim	100
Vilma Sardão Oliveira	100

PhD Students	**Time % at CNC**
Ana Carolina Moreira	100
Ana Maria Silva	100
Bárbara Rocha	100
Carlos Rodrigues	100
Cassilda Pereira	100
Diana Jurado S. Serra	100
Filipa Libório Carvalho	100
Henrique Miguel Alexandrino	50
Inês Biscaia Barbosa	100
Joana Paixão	100
Kátia Almeida Mesquita	100
Ludgero Tavares	100

Mariana Ponte Cardoso Ribeiro	100
Nuno Ferreira	100
Nuno Gabriel Machado	100
Paulo Gameiro Guerreiro	100
Rui Miguel Martins	50
Susana Pereira	100

MSc Students	**Time % at CNC**
Ana Marta Silva	100
Ana Raquel Coelho	100
Inês Simões	100
João Amorim	100
Mª Helena Silva	100
Mário Correia	100
Renata Couto	100
Rui Gonçalo Silva	100
Sílvia Magalhães Novais	100
Tatiana Martins	100
Tiago Henriques	100

Grant Technicians	**Time % at CNC**
*Ana Cristina Lemos	100
Cândida Dias	100
Cláudia Deus	100
Miguel Caetano	100
Rita Pereira	100
Sónia Pereira	100
Mariana Monteiro Val	100

Microbiology

Milton Costa, PhD, Coordinator

Members holding PhD		**Time % at CNC**
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	60
Célia Laurinda Nogueira	(Assistant Prof., FMUC)	40
Joana Cardoso Costa	(Inv. Assistant Prof., FCTUC)	60
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	60
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Teresa Gonçalves	(Assistant Prof., FMUC)	40

Post-Doc Members	**Time % at CNC**
Carolina Coelho	40
Chantal Fernandes	100
Igor Tiago	60
Susana Isabel E. Alarico	100
Vitor Mendes	

PhD Students	**Time % at CNC**
Ana Catarina Ferreira	100
Ana Luísa N. Gomes Nobre	100
Ana Maranha Tiago	100
Ana Sofia V. Cunha	100
Lisa Rodrigues	100
Luis André A. França	100
Mª Graça Rocha	40
Tânia Leandro	100
Rui Soares	40

MSc Students	**Time % at CNC**
Diogo Reis	100
Vânia Moreira	50

Grant Technicians	**Time % at CNC**
Alexandra Abrunheiro	100
Luciana Pinto	100
Mafalda Costa	100
Mariana Almeida	100
Marta Mota	30
Marta Sousa	100
Ana Filipa dÁvó	100

Biophysics and Biomedical NMR

Carlos Geraldes, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Carlos G. Geraldes	(Full Prof., FCTUC)	70
John Griffith Jones	(Principal Inv., CNC)	100
Luís M. Rosário	(Associate Prof., FCTUC)	60
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	20

Post-Doc Members	**Time % at CNC**
Cristina Barosa	100
Ivan Viegas	100

PhD Students	**Time % at CNC**
Ana Marguerita Metelo	20
André Martins	100
David Miguel Dias	100
Fátima Martins	100
Filipe Coreta Gomes	20
Helena Leitão	100
João Rito	50
João Silva	80

MSc Students	**Time % at CNC**
Cátia Marques	100
Paula da Silva	100

Grant Technicians	**Time % at CNC**
Margarida Coelho	100
Filipa Simões	100

Cell and Development Biology

João Ramalho Santos, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Ana Bela Sarmento Ribeiro	(Assistant Prof., FMUC)	40
Ana Paula Marques de Sousa	(Investigator, HUC)	50
Anália do Carmo	(Assistant Prof., Univ. Vasco Gama)	80
Ângela Inácio	(Project Investigator, CNC)	100
Eugénia Carvalho	(Assistant Inv., CNC)	100
Fernando Monteiro Judas	(Assistant Prof., FMUC)	20
João Moura Alves	(Assistant Prof., Inst Pol. Viana Castelo)	50
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 Otilia Vieira	(Assistant Inv., CNC)	100
Mª Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
Teresa Maria C. Martins	(Assistant Inv., IPO)	80

Post-Doc Members	**Time % at CNC**
Ana Burgeiro	100
Ana Silva	100
Denisa Daud Mateus	100
Ermelindo Leal	100
Luis Miguel Estronca	100
Mª Alexandra B. Amaral	100
Marisa Rego Encarnação	100
Marta Santos	100
Paula Mota	100
Pedro Gonçalves	100
Sandra Catarina G. Amaral	100
Susana Guerreiro	100

PhD Students	**Time % at CNC**
Ana Cristina Gonçalves	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ângela Pascoal Crespo	100
Beatriz Lacerda de Sousa	100
Carla Patrícia R. Paiva	100

Carlos Manuel Melo	100
*Cátia Moreira Sousa	100
Diana Margarida Carvalho	100
Diana Dinis Azenha	100
Elda Bonifácio	100
Humberto Gomes Ferreira	100
Inês Santarino	100
Joana Balça Silva	100
Joana Liberal	100
João Demétrio B. Martins	100
Júlia Valente	50
Liane Moura	100
Marcelo Correia	100
Mª Inês Almeida Sousa	100
Mª Madalena Ribeiro	100
Marília Cordeiro	100
Michelle Stumpf Viegas	100
Patrícia Domingues	100
Patrícia Lopes	100
Raquel Alves	100
Rodrigo Santos	100
Roksana Pirzgalska	100
Sara Lima	100
Tânia Perestrelo	100
Vera Francisco	100

MSc Students	**Time % at CNC**
Andreia Luis	50
Carlos Moura	100
Fabio Carvalho	50
Inês Mahú	50
Joana Portela	100
Mónica Marques	100
Solange Machado	100

Grant Technicians	**Time % at CNC**
Isabel Ferreira	50
João Ferreira	50
Marta Baptista	100
Neuza Domingues	100
Renata Tavares	100

MD Members

Herminio Espirito Santo	Collaborator
Mª Margarida Gonçalo	40

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