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Introduction

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INTRODUCTION

In the 2006 annual report of activities, an overview of the achievements of the Center for Neuroscience and Cell Biology (CNC), in both science and education, is provided.

In the year 2006, the CNC pursued its commitment to excellence in research in the biomedical area, trying to achieve greater insights into the complexity of living cells and obtain a better knowledge of both normal and pathological processes. The CNC brings together researchers in several groups from the Faculties of Medicine, Pharmacy and Science and Technology, at the University of Coimbra. The diverse scientific background of the CNC staff has been crucial to advance innovation in fundamental and translational research based on the integration of diverse scientific expertise in molecular and medical sciences.

The CNC focuses on six thematic areas of research, which are further divided into sub-themes: Neuroscience and Disease, Molecular Biotechnology and Health, Cell and Molecular Toxicology, Microbiology, Biophysics and Biomedical NMR, Cell and Development Biology.

The organization of research in each of these areas, being carried out under the leadership of usually a young accomplished scientist, has contributed to establish a dynamic research structure, which can be evaluated and optimized on the basis of performance. The recruitment of novel group leaders or potential group leaders, which are fostered to create their research teams to perform independent work, bringing new competences and strengthening some of the research areas at CNC, started to be implemented.

In 2006 CNC was recognized as a Center of the Network of European Neuroscience Institutes (ENI), which dedicates to the promotion of the independent work of young investigators and encourages the interaction with similar Neuroscience Centers in Europe and the development of research projects in an European context. Under the scope of the collaboration established by the Portuguese Government with the Massachusetts Institute of Technology (MIT), focusing on basic research and education, CNC was included as a partner in the focus area of Bio-Engineering Systems. This partnership is expected to build on the research capacity existing in the Center and develop emerging aspects of cell and tissue engineering and computational biology.

Education at CNC is focused on the domain of molecular life sciences related to disease, in the fields of Cellular and Molecular Biology, Neuroscience and Biotechnology. The aim of the CNC graduate studies programme is to provide Master and PhD students with a multi-faceted education in those scientific fields, through an international doctoral programme.

During 2006 the CNC continued involved in the promotion of science outside the scientific community, through a strong commitment in the "Brain Awareness Week", in the organization of high school students visits to CNC Laboratories, involving the students in research activities being carried out by the young group leaders.

In the interface between CNC and Society diverse activities continued to be developed, under the scope of an Outreach Programme, that fosters translational research involving Hospitals and Pharmaceutical Industries. As a founding partner of the biotechnology association Biocant, the Center has also contributed to create a biotech cluster, to transfer technology and human resources, in the center region of Portugal.

For 2007, the CNC will pursue on its major mission, the understanding of the cellular and molecular basis of disease, trying to identify new therapeutical targets, with a particular emphasis on the relationship between fundamental and translational research.

The annual report for 2006 highlights the young researchers within the various research themes being developed at CNC and their contribution to achive the main scientific goals of the Center.

Facts and Figures

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II. FACTS AND FIGURES (2006)

RESEARCH STAFF

Members and collaborators holding Ph.D.	109
Post-Doc Members	25
Ph.D.Students	112
MSc Students	38

PUBLICATIONS IN 2006

Publications in press	139
	61

THESIS CONCLUDED – 2006

Ph.D. thesis	17
MSc thesis	5

FUNDING - 2006

Pluriannual	1.660.729,10 €
International Projects	32.003,49 €
National Projects	2.038.982,46 €
Others	28.639,00 €

Organization of CNC

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ORGANIZATION

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

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

1- Governing Body

President: ……... *Catarina Resende de Oliveira*

Vice Presidents: ……. *Euclides Pires*
……. *Carlos Faro*
……. *Leonor Almeida*

Honorary President: … *Arsélio Pato de Carvalho*

Executive Council …….. Directors of the Departments
Research Council …….. CNC members holding PhD
" **Conselho Fiscal** " ……. T. Macedo, A. Rodrigues, Leal e Carreira
" **Revisor Oficial de Contas** " ……. Leal e Carreira, Sociedade Revisora de Contas

External Advisory Commitee ……. Bertil Fredholm (Sweden); Enrique Cadenas (USA); Roberta Brinton (USA); George Perry (USA); Helmut Sies (Germany); Stephen Zinder (USA).

2- Scientific Areas and Research Groups

At present, research programmes and projects are organized in 6 scientific areas, each coordinated by a senior scientist. The programme for each area is implemented by small research groups each headed by a research leader in his field of study. In 2006, the research groups for each area can be identified, according to the following organization:

Neuroscience and Disease | *Catarina Oliveira*
Neuromodulation Group (*Head: Rodrigo Cunha*)
Molecular Biology of Glutamate Receptors Group (*Head: Ana Luísa Carvalho*)
Neurotoxicity and Epilepsy Group (*Head: João Malva*)
Neuroprotection Group *(Head: Carlos B. Duarte*)
Mitochondrial Dysfunction and Cell Death Group (*Head: A. Cristina Rego*)
Molecular Mechanisms of Disease Group (*Head: Claudia Pereira*)
Retinal Dysfunction and Neurogenesis Group (*Head: Francisco Ambrosio*)

Molecular Biotechnology and Health | *Euclides Pires*
Molecular Biotechnology Group (*Head: Carlos Faro)*
Molecular Systems Biology Group (*Head: Armindo Salvador)*
Protein Stability and Folding Group (*Head: Rui Brito*)
Macromolecular Crystallography Group (*Head: Sandra Ribeiro*)
Vectors and Gene Therapy Group (*Head: M. Conceição Pedroso Lima*)

Cell and Molecular Toxicology | *Leonor Almeida*
Mitochondrial Toxicology and Pharmacology Group (*Head: Paulo Oliveira*)
Free Radicals and Antioxidants Group (*Head: João Laranjinha*)
Membrane Toxicity Group (*Head: Amália Jurado*)

Microbiology | *Milton Costa*

Microbiology of Extreme Environments Group (*Head: Milton Costa*)
Yeast Research Group (*Head: Teresa Gonçalves*)

Biophysics and Biomedical NMR | *Carlos Geraldes*

Intermediate Metabolism Group *(Head: John Grifith Jones)*
Inorganic Biochemistry Group *(Head: Carlos Geraldes)*

Cell and Development Biology | *Celeste Lopes and João Ramalho Santos*

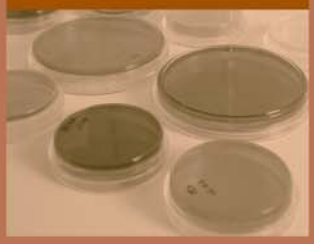
Cellular and Molecular Immunology Group (*Head: Celeste Lopes*)
Endothelial Dysfunction and Diabetes Group (*Head: Raquel Maria Fino Seiça)*
Biology of Reproduction and Human Fertility Group (*Head: João Ramalho Santos)*

Emerging Groups

Mechanisms of Insulin Resistance – The Role of the Adipocite (*Head: Eugénia Carvalho)*
Infection, Phagocytosis and Pathogens Group (*Head: Otilia Vieira)*

3- Departments

Fundamental know how, education programmes and specific equipment (core facilities-like arrangements) are organized in Departments chaired by senior scientists. At present there are 10 Departments: Cell Biology (Arsélio Carvalho), Biophysics (Luis Rosário), Biochemistry (Leonor Almeida), Neurochemistry (Catarina Oliveira), Neuropharmacology (Rodrigo Cunha), Molecular Biotechnology (Euclides Pires), Neurobiology (Carlos Duarte), Microbiology (Milton Costa), Vectors and Gene Therapy (C. Pedroso Lima), Graduate Studies (Luis Pereira de Almeida) and Advanced Strategic Studies (Arsélio Carvalho).



Research Activity

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AREA A: NEUROSCIENCE AND DISEASE

Coordinator-Catarina Oliveira

Introduction

Research in the area of Neuroscience and Disease focuses not only on the understanding of normal brain function but also on the causes leading to the failure of function occurring in age-related and neurodegenerative diseases.

The major questions that confront the seven groups integrated in this area are the molecular mechanisms of cellular and synaptic plasticity, particularly those involving glutamate receptors, and its endogenous modulation by purines, NPY, NO, cytokines and neurotrophins. The demonstration of the pro-neurogenic role of some of these factors, namely NPY and TNFα, opens new strategies for brain repair through the manipulation of neurogenesis. The contribution of mitochondria impairment and energy failure to neuronal cell death induced by amyloidogenic peptides, α-synuclein or polyglutamine expansion is also a major interest of research.

The most recent data obtained by the research groups in the Neuroscience and Disease Area, led to a better understanding of the mechanisms of neurodegeneration and to a greater insight into the mechanisms of neuroprotection, which promise to shed light on the therapeutical strategies in a wide range of neurodegenerative disorders.

The research groups have established fruitful collaborations not only with national and international laboratories, but also with other areas at CNC, namely the Cell and Molecular Toxicology, the Cell and Development Biology and the Biophysics and Biomedical NMR areas. This strong interaction with other groups, both at national and international levels, can be stated by the co-authorship of published scientific papers and the organization of advanced courses integrated in the CNC Graduate Studies Programme. In 2006, under the supervision of researchers of the Neuroscience and Disease area 4 Ph.D. thesis were concluded.

The research highlights and ongoing and future work for each group are briefly described in the following pages.

Neuromodulation Group:

Rodrigo A. Cunha	(*Ph.D. - head of group*)
Attila Köfalvi	(Post-Doctoral Fellow)
Paula M. Canas	(Ph.D. Student)
Ricardo J Rodrigues	(Ph.D. Student)
Nelson Rebola	(Ph.D. Student)
João M.N. Duarte	(Ph.D. Student)
M.Fátima Pereira	(Ph.D. Student)
Rui Sanches	(MSc Student)
Carla G. Silva	(Research Grantee)
Ana Patrícia Simões	(Research Grantee)
Tiago Alfaro	(Junior Researcher)

Glutamatergic Synapses Group:

Ana Luísa Carvalho	(*Ph.D. - head of group*)
*Carlos Duarte	(Ph.D.)
*Armanda Santos	(Ph.D.)
Margarida Caldeira	(Ph.D. Student)
Sandra D. Santos	(Ph.D. Student)
Andrea Lobo	(Ph.D. Student)
Tatiana Catarino	(Ph.D. Student)
Joana Ferreira	(Research Assistant)
Marta Vieira	(Trainee)

Neuroprotection and Neurogenesis in Brain Repair Group:

João Malva	(*Ph.D. - head of group)*
*Armando J. Cristovão	(Ph.D.)
Ana Paula Silva	(Ph.D.)
*Cláudia Cavadas	(Ph.D.)
Fabienne Agasse	(Ph.D.)
Paulo César Silva Pinheiro	(Ph.D.)
Bruno Silva	(Ph.D. Student)
Joana Catarina C. Lourenço	(Ph.D. Student)
Liliana Inácio Bernardino	(Ph.D. Student)
Raquel Ferreira	(Ph.D. Student)
Sara Alves Xapelli	(Ph.D. Student)
Sofia Domingos	(Undergraduate Student)
Sofia Grade	(Undergraduate Student)

Neuronal Cell Death and Neuroprotection Group:

*Carlos B. Duarte	(*Ph.D. - head of group*)
Emília Pedrosa Duarte	(*Ph.D. - head of group*)
Ana Rita Araújo Santos	(PhD. Student)
Andrea Catarina A. C. Lobo	(PhD. Student)
Bruno José O. Manadas	(Ph.D. Student)
Ana C. Saavedra Martins	(Ph.D. Student)
João Carlos R. Gomes	(Ph.D. Student)
Carlos Henrique V. Melo	(MSc Student)

Mitochondrial Dysfunction and Cell Death Group

A. Cristina Rego	(*Ph.D.- head of group*)
*Catarina R. Oliveira	(M.D., Ph.D.)
A. Cristina Rego	(Ph.D.)
Ildete Luisa Ferreira	(Ph.D.)
Ana Catarina Oliveira	(Ph.D. Student)
Ana Duarte	(Ph.D. Student)
Jorge Oliveira	(Ph.D. Student)
Mário Laço	(Ph.D. Student)
Joana Gil	(Ph.D. Student)
Sandra Almeida	(Ph.D. Student)
Teresa Oliveira	(Ph.D. Student)
Rita Perfeito	(Ph.D. Student)
Ana Cristina Silva	(MSc Student)
Marcio Ribeiro	(MSc Student)
Maria Viegas Nascimento	(MSc Student)
Susana Louros	(Undergraduate Student)

Molecular Mechanisms of Disease Group

Cláudia M. Fragão Pereira	(*Ph.D.- head of group*)
*Catarina R. Oliveira	(M.D., Ph.D.)
Paula Maria G. Agostinho	(Ph.D.)
Sandra Isabel M. Cardoso	(Ph.D.)
*Paula Isabel Moreira	(Ph.D. Student)
Rosa Maria B. Resende	(Ph.D. Student)
Rui Miguel Oliveira Costa	(Ph.D. Student)
Elisabete Baptista Ferreiro	(Ph.D. Student)
João Pedro S. Oliveira Lopes	(Ph.D. Student)
Pedro Manuel V. Garção	(Ph.D. Student)
Ana Raquel Esteves	(Ph.D. Student)
Daniela Arduíno	(Ph.D. Student)
Ana Filipa Domingues	(MSc Student)
Marco António Matos	(MSc Student)
Ana Catarina Fonseca	(MSc Student)
Sueli Cristina F. Marques	(Undergraduate Student)
Carla Jesus Cardoso Sousa	(Undergraduate Student)
Cristina Carvalho	(Undergraduate Student)
Sónia Correia	(Undergraduate Student)

Retinal Dysfunction and Neurogenesis Group

Francisco Ambrósio	(*Ph.D. – head of group*)
*Cláudia Cavadas	(Ph.D.)
Paulo Santos	(Ph.D.)
*Armando Cristóvão	(Ph.D.)
Caetana Carvalho	(Ph.D.)
Inês Araújo	(Ph.D.)
Raquel Santiago	(Ph.D.Student)
Ermelindo Leal	(Ph.D. Student)
Joana Salgado	(Ph.D. Student)
Ana Rita Álvaro	(Ph.D. Student)
Célia Aveleira	(Ph.D. Student)
Bruno Carreira	(PhD. Student)
Lígia Ferreira	(Ph.D. Student)
Gabriel Costa	(Ph.D. Student)
Joana Gaspar	(Ph.D. Student)

Tiago Pereira	(MSc Student)
Áurea Castilho	(MSc Student)
João Martins	(MSc Student)

**Investigator doing collaborative research involving different groups at CNC.*

NEUROMODULATION GROUP (*Head: Rodrigo A. Cunha*)

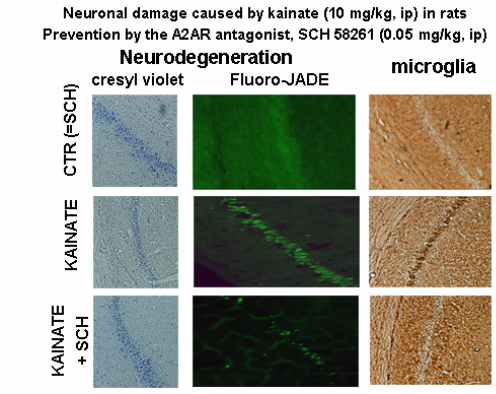
Evidence is accumulating suggesting that some neurodegenerative diseases start with an early synaptic dysfunction and elimination that precedes neuronal death. Our aim is to investigate synaptic modulation systems preventing the earlier events in neurodegeneration. Our primary focus is adenosine receptors, inhibitory A_1 receptors (A_1Rs) and facilitatory A_{2A}Rs, and we also explore other modulatory system such as ATP P2Rs and cannabinoid CB1Rs. We grasp their roles in controlling glutamate release and synaptic transmission in vitro and their neuroprotective role in *in vitro* and *in vivo* models of chronic brain diseases such as epilepsy, Alzheimer disease, diabetes or stress.

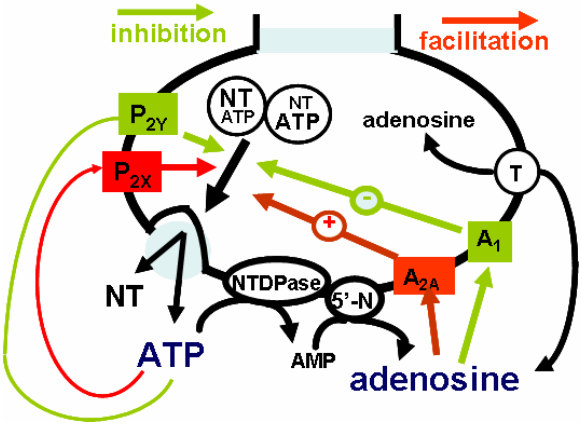
Research Highlights

A_{2A}Rs control the transition between plasticity and degeneration: We found that A_{2A}Rs facilitate NMDA receptors. This provides a crucial control to trigger the ignition of synaptic plastic changes. However, in accordance with the role of the hyper-activation of NMDA receptors to trigger neuronal damage, A_{2A}R antagonists confer robust protection against noxious brain stimuli involving NMDA receptor-induced toxicity.

Dimers between A1 and A2A receptors define if adenosine inhibits or facilitates neuronal activity: We defined the existence of heterodimers between A1 and A2A receptors, which allow the preferential activation of A1 receptors at lower concentrations of adenosine and a predominant A2AR-mediated effect at higher concentrations of adenosine.

ATP is a danger signal and P2Y1R blockade affords neuroprotection: Either β-amyloid peptides or glutamate cause neuronal damage. This is accompanied by increased extracellular levels of ATP and damage is prevented by ATP hydrolysis or blockade of P2Y1RS.





Ongoing and Future Work

1-Use of cell-specific A_{2A}R knockouts to evaluate the role of pre- and post-synaptic A_{2A}Rs on synaptic plasticity related to learning & memory and to addiction. 2-Explore the potential of A_{2A}Rs in controlling memory impairment caused by stress and by type II diabetes; 4-Explore if ATP and P2Y1Rs are key elements in different models of brain damage; 5-Explore the potential of chronic consumption of caffeine (antagonist of adenosine receptors) in neurodegenerative diseases; 6-Explore if the effect of caffeine is additive to that of nicotine and cannabinoid receptor antagonists.

Key References

Ciruela, F., Casadó, V., Rodrigues, R.J., Lujan, R., Burgueño, J., Canals, M., Borycz, J., Rebola, N., Goldberg, S.R., Mallol, J., Cortés, A., Canela, E.I., Lopez-Gimenez, J.F., Milligan, G., Lluis, C., Cunha, R.A., Ferré, S., Franco, R. (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A_1-A_{2A} receptor heteromers. *J Neurosci* 26, 2080-2087.

Cunha, G.M.A., Canas, P.M., Oliveira, C.R., Cunha, R.A. (2006) Increased density and synapto-protective effect of adenosine A_{2A} receptors upon sub-chronic restraint stress. *Neuroscience* 141, 1775-1781.

GLUTAMATERGIC SYNAPSES (*Head: Ana Luísa Carvalho*)

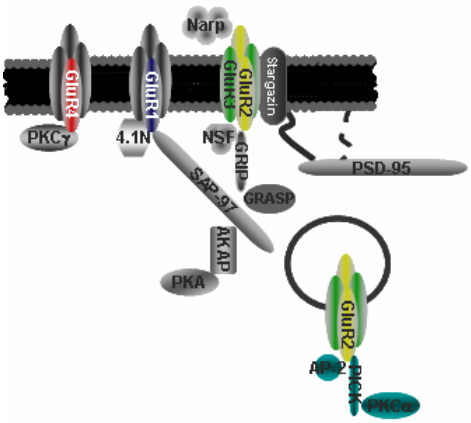
Glutamate is the major excitatory neurotransmitter in the central nervous system, and changes in the characteristics and cellular localization of glutamate receptors are thought to underlie the mechanisms for synaptic plasticity. Our group focuses on investigating the mechanisms that regulate glutamate receptor activity and cellular traffic, and on excitotoxicity mediated by Ca^{2+}-permeable AMPA-type glutamate receptors.

Research Highlights

Cellular traffic of AMPA-type glutamate receptors

One of the major interests of the group concerns the cellular traffic of AMPA receptors. We have recently characterized the interaction of GluR4 AMPA receptor subunit with PKCγ, and shown that it is necessary for efficient GluR4 phosphorylation and cell surface expression (Gomes et al., 2007). Using proteomic approaches, we have now identified novel binding partners for AMPA receptors, and are presently characterizing the role of these interaction partners on receptor stability and cellular traffic.

The cytoskeleton remodelling that takes place during glutamate receptor traffic is another of the interests of this group. A strong candidate for linking actin polymerization in dendritic spines and clathrin-mediated endocytosis is cortactin. We are currently investigating the role of cortactin in AMPA receptor traffic.



Schematic diagram of AMPA receptor bonding partners

Diversity of AMPA-type glutamate receptors

We have detected splice isoforms of AMPA receptor subunits that encode truncated receptors. The truncated receptor subunits heteroligomerize with full-length AMPA receptor subunits and play a dominant negative role. The receptor complexes containing truncated subunits reach the cell surface neurons, but are not delivered to synapses. Recently, we have found that expression of truncated AMPA receptor subunits in hippocampal neurons is protective against cell death caused by excessive glutamate, and that the excitotoxic stimulation itself promotes the expression of the truncated isoform of GluR1 AMPA receptor subunit. Moreover, truncated GluR3 is upregulated in the cortex and hippocampus of epileptic rats. These observations suggest that the expression of truncated AMPA receptor subunits may constitute an intrinsic neuroprotective mechanism.

Regulation of glutamate receptors by neurotrophins

We have found that BDNF regulates the transcription and traffic of AMPA and NMDA-types glutamate receptors. Moreover, BDNF promotes the surface expression of GluR1 in hippocampal neurons and synaptic accumulation of GluR1-containing AMPA receptors in hippocampal slices. We have now extended this study and shown that chelation of endogenous extracellular BDNF with TrkB-IgG fusion protein results in decreased levels of GluR1 in hippocampal cultured neurons, suggesting that endogenous BDNF tonically regulates GluR1 expression. Additionally, BDNF increases NR1 and NR2B at the plasma membrane of neurons. This effect is correlated with a BDNF-induced upregulation of the $[Ca^{2+}]_I$ response to NMDA receptor activation in hippocampal neurons. The data on the effect of BDNF on NMDA receptors are now in press in Molecular and Cellular Neuroscience (Caldeira et al., 2007).

Cell death pathways triggered by Ca^{2+}-permeable AMPA receptors.

Another interest of this group concerns the toxic pathways triggered by activation of Ca^{2+}-permeable AMPA receptors. We have found that the AP-1 transcription factor is activated by toxic glutamate stimuli to homomeric GluR4 AMPA receptors, and that activation of this transcription factor in necessary for cell death to occur (Santos et al., 2006). We are now investigating the involvement of the JNK pathway in the toxic process.

Ongoing and Future Work

At the moment we are launching a new project that concerns NMDA receptor traffic. We will use hippocampal cultures from knock-out mice for the various NMDA receptor subunits and imaging techniques in live and fixed cells to investigate the subunit-specific rules that govern NMDA receptor traffic, and to explore the selective involvement of the different NMDA receptor subunits in synaptic plasticity.

Key References

Santos A.E., Duarte C.B., Iizuka M., Barsoumian E.L., Ham J., Lopes M.C., Carvalho A.P., and Carvalho A.L. (2006) Excitotoxicity mediated by Ca^{2+}-permeable GluR4-containing AMPA receptors involves the AP-1 transcription factor. *Cell Death Diff.* Cell Apr;13(4):652-60.

Gomes A.R., Correia S.S., Esteban J.A., Duarte C.B., and Carvalho A.L. PKC Anchoring to GluR4 AMPA Receptor Subunit Modulates PKC-Driven Receptor Phosphorylation and Surface Expression. *Traffic*. (*in press*).

NEUROPROTECTION AND NEUROGENESIS IN BRAIN REPAIR *(Head: João O. Malva)*

Hyperexcitability of the hippocampal neuronal network can cause hyperactivation of glutamate receptors, neuronal death, neurogenesis and inflammation. The search for new antiepileptogenic and neuroprotective compounds is of critical relevance for the treatment of the pharmaco-resistant forms of temporal lobe epilepsy, and, at the long-term, will open new possibilities for brain repair.

The major research areas of the "Neuroprotection and Neurogenesis in Brain Repair" group are: 1) *Synaptic modulation* - Modulation of glutamatergic synapses by kainate and NPY receptors; 2) *Neuroprotection* – Neuroprotective properties of NPY and cytokines; 3) *Inflammation* – The role of inflammation in excitotoxic insults; 4) *Brain Repair* – Manipulation of neurogenesis and development of new strategies for brain repair.

Research Highlights

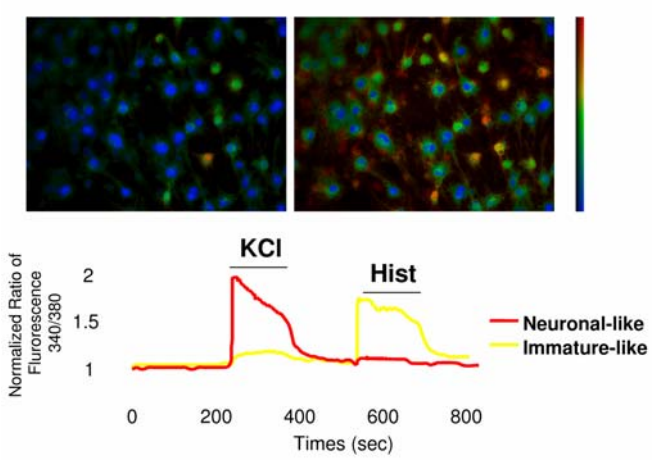
A functional interaction between NPY Y2 receptors and PKC activity was identified following status epilepticus in rats. The common target is downstream Y2 receptors or its coupled G-protein and it includes N-P/Q-type calcium channels.

Excitotoxic damage of mice organotypic cultures of hippocampal slices induces overexpression of BDNF in activated microglial cells and strong up-regulation of TrKB receptors in neurons.

NPY and TNF-alpha are strong pro-neurogenic factors in stem/progenitor cell cultures derived from the mice subventricular zone (SVZ). Ex vivo treatment of SVZ cultures with NPY and TNF alpha increases the proliferation and differentiation of neurons – evaluated functionally by single cell calcium imaging and by immunocytochemistry (doublecortin, MAP2 and NeuN).

Ongoing and Future Work

The role of inflammation in temporal lobe epilepsy seizure activity, with or without secondary generalization, is under investigation.



Role of interleukin 1-beta in the potentiation of excitotoxic lesions in mice organotypic slice cultures.
Functional binding of NPY receptors in the mice subventricular zone.
Grafting of SVZ neurospheres-EGFP+ (pre-treated with neurogenic factors) in wild-type organotypic hippocampal slice cultures, following excitotoxic lesions.
In vivo grafting of SVZ neurospheres-EGFP+ (pre-treated with neurogenic factors) in epileptic mice hippocampus.

Key References

Silva AP, Lourenço J, Xapelli S, Ferreira R, Kristiansen H, Woldbye DP, Oliveira CR and Malva JO. Protein kinase C activity blocks neuropeptide Y-mediated inhibition of glutamate release and contributes to excitability of the hippocampus in status epilepticus. *FASEB J* (*in press*).

Xapelli S, Agasse F, Ferreira R, Silva AP and Malva JO (2006) Neuropeptide Y as an endogenous antiepileptic, neuroprotective and pro-neurogenic peptide. Recent Patents on *CNS Drug Discovery* 1: 315-324.

NEURONAL CELL DEATH AND NEUROPROTECTION GROUP (*Head: Carlos Duarte and Emília Duarte*)

In addition to promoting the survival and differentiation of developing neurons, neurotrophic factors protect neurons from toxic injury. This group investigates two aspects of neurotrophic factor biology: i) control of endogenous expression upon neuronal injury; ii) excitotoxic cell damage and neuroprotection by neurotrophins.

We have previously shown that selective injury to dopaminergic neurons in substantia nigra cell cultures increases the expression of the neurotrophic factor GDNF, which in turn down-regulates heme oxygenase-1 (*Free Radic. Biol. Med.* 39, 1611-1619, 2005). Our goal is to identify the mechanisms controlling the expression of neuroprotective factors upon neuronal injury, both in cell cultures and in a rat model of Parkinson's disease.

The neurotrophin BDNF protects hippocampal neurons from excitotoxicity-induced changes in nuclear morphology, in vivo and in vitro. We have shown that this effect requires protein synthesis (Cell Death Different 12, 1329-1343 [2005]). One of the goals of this group is to understand the molecular mechanisms leading to neural death under excitotoxic conditions, and to elucidate the mechanisms of neuroprotection by BDNF under the same conditions.

Research Highlights

In the 6-OHDA rat model of Parkinson's disease, we found that the administration of L-DOPA decreased the expression of HO-1, an indicator of oxidative stress. This might be related to the up-regulation of GDNF, as observed in cell culture models (*Saavedra et al*, *Free Radic. Biol. Med.* 39, 1611-1619, 2005), and suggests that L-DOPA might have protective effects *in vivo* as opposed to its toxic effects in cell culture models.

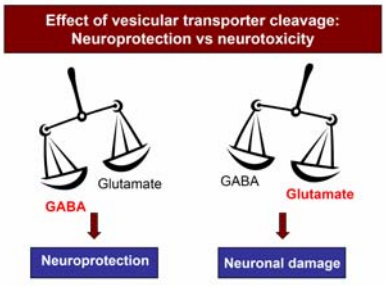
We have also shown that GDNF up-regulation by astrocytes upon toxic injury to substantia nigra cell cultures is dependent on dopaminergic cell damage and is mediated by a neuron-glia crosstalk involving soluble mediators (*Saavedra et al*, *Neurob. Dis.* 23, 533-542, 2006). IL-1β is involved but other factors, still unidentified, are required to trigger GDNF expression upon dopaminergic injury (*Saavedra et al*, *Neurobiol Dis* 25, 92-104, 2007).

Previous studies from our laboratory showed that excitotoxic stimulation of cultured hippocampal neurons with glutamate leads to the cleavage of the vesicular glutamate (VGLUTs) and GABA (VGAT) transporters. Calpains play an important role of the cleavage of the transporters, giving rise to a stable product. Since the vesicular glutamate and GABA transporters play key roles in the excitatory and inhibitory activity in the hippocampus, their cleavage may affect the overall neuronal activity, thereby modulating the demise process.

Given the role played by protein synthesis in neuroprotection by BDNF we carried out a large scale proteomics study aiming at identifying the effect of the neurotrophin on the proteome of cultured hippocampal neurons. This study was concluded and showed effects of BDNF on proteins with various functions: protein folding, protein synthesis, proteolysis, RNA metabolism, cytoskeleton regulators and signal transduction mediators.

Ongoing and Future Work

Nigrostriatal slices in culture will be used to investigate the neuron-glia crosstalk and the control of GDNF expression both at the level of the neuronal cell bodies and in the area of the terminals. Intercellular mediators triggering GDNF up-regulation will be identified using a proteomic approach to compare conditioned media from control and injured cultures. The components showing changes upon injury will be tested for their effect on GDNF expression both i*n vitro* and *in vivo*.



The VGLUT and VGAT cleavage sites under excitotoxic conditions and the functional consequences of this cleavage will be investigated, focusing on the effects on the activity of the transporters and on their intracellular trafficking. The role of calpains in the cleavage of the transporters will also be investigated in vivo, using transgenic mice overexpressing calpastatin, an endogenous calpain inhibitor. The recent characterization of the BDNF-induced changes in the proteome of cultured hippocampal neurons will allow us to further investigate the molecular mechanisms of neuroprotection by BDNF under excitotoxic conditions.

Key References:

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MITOCHONDRIAL DYSFUNCTION AND CELL DEATH GROUP (*Head: A. Cristina Rego*)

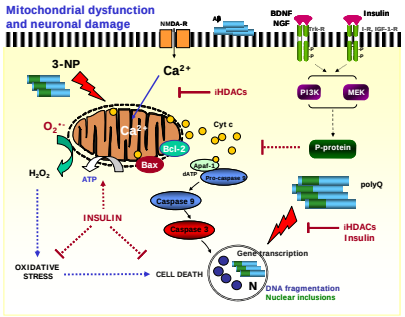
Mitochondrial impairment and energy deficits are common features of many neurodegenerative diseases of the central nervous system, which are known to affect selective brain areas and distinct cellular proteins. Neuronal dysfunction associate with deficits in mitochondrial function frequently occurring due to excessive activation of *N*-methyl-D-aspartate (NMDA) receptors (excitotoxicity), which may involve deregulation of intracellular calcium homeostasis and oxidative stress, and lead to neuronal death by apoptosis, necrosis and/or autophagy. Centering on mitochondria, the main objective of our group is to identify molecular/cell targets for therapeutic intervention and evaluate the efficacy of neuroprotective strategies aimed at recovering mitochondrial function and neuronal survival in distinct neurodegenerative conditions, namely polyglutamine expansion (Huntington's and Machado-Joseph's disorders), Parkinson's and Alzheimer's diseases.

Research Highlights

During the past year, our group investigated: i) mitochondrial (dys)function and neuroprotection induced by histone deacetylase (HDAC) inhibitors and neurotrophins in Huntington's disease (HD) cellular models; and ii) the role of insulin on metabolic dysfunction, cell death and insulin receptor-mediated intracellular signaling pathways in cortical neurons subjected to oxidative stress, a deleterious condition associated with age-related neurodegenerative diseases.

We determined the relationship between mitochondrial dysfunction and Ca2+-handling in intact HD striatal cells expressing full-length mutant huntingtin by real-time functional imaging of intracellular Ca^{2+} and mitochondrial membrane potential, in collaboration with Dr Lisa Ellerby and Dr. David Nicholls (Buck Institute, Novato, CA, USA). Treatment with HDAC inhibitors induced more efficient Ca2+-handling, thus improving the neuronal ability to cope with excitotoxic stimuli (*Oliveira et al*, *J Neurosci*, 2006). We also compared the bioenergetic behavior of mitochondria isolated from different transgenic HD and wild-type mice with *in-situ* respiratory parameters in intact HD striatal neurons, stressing the importance of assessing HD mitochondrial function in the cellular context (*Oliveira et al*, *J Neurochem*, in press). Furthermore, we studied the effect of brain-derived neurotrophic factor (BDNF), compared to nerve growth factor (NGF), as inhibitors of neuronal death induced by 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase, largely used to study mitochondrial dysfunction in HD. 3-NP was shown to induce both caspase-dependent and -independent cell death pathways (*Almeida et al*, *J Cell Biochem*, 2006). Neurotrophins (in particular BDNF)-mediated intracellular signaling pathways prevented against mitochondrial-dependent neuronal death and regulated transcription, protecting against selective mitochondrial inhibition (*Almeida et al*, submitted for publication).

In previous studies we showed that insulin prevented from oxidative stress-induced cortical neuronal death through an increase in antioxidants uric acid and GSH/GSSG (the latter resulting from changes in the glutathione redox cycle) (*Duarte et al*, *Free Radic Biol Med*, 2005). We further showed that insulin stimulated glucose uptake and metabolism under oxidative stress conditions by restoring intracellular ATP, phosphocreatine and adenosine (*Duarte et al*, *Diabetes*, 2006). Also, insulin decreased extracellular adenosine that preferentially resulted from ATP release (*Duarte et al*, *Diabetes*, 2006). These changes were recently linked to the stimulation of neuronal insulin and IGF-1 receptors by insulin and the preferential activation of PI-3K/Akt and inhibition of GSK-3β signaling pathways. Furthermore, insulin appears to regulate the expression of 'candidate' proteins involved in glucose metabolism, antioxidant defense and prevention against apoptosis (*Duarte et al*, submitted for publication).



Ongoing and Future Work

Currently we are exploring mitochondrial-dependent toxicity and oxidative stress in HD striatal cell lines and in cybrids - cytoplasmic hybrid systems obtained from the fusion of HD patient (HD cybrids) or control (control cybrids) platelets with mitochondrial DNA-depleted human teratocarcinoma cells (ρ0 NT2 cells). In HD cybrids and HD striatal cell lines we will analyse the protective effect of FK506, BDNF, as well as compounds proposed for HD clinical trials and shown to interfere with oxidative stress. By using the retina as a neuronal model, we are also examining apoptotic features, energy parameters and oxidative stress during aging in normal Wistar and Goto-Kakizaki rats, a model of type-2 diabetes.

Furthermore, we will analyse mitochondrial dysfunction in Machado-Joseph's disease, a polyglutamine disorder with a high incidence in the Portuguese population. Analysis of mitochondrial dysfunction and posttranslational modifications of alpha-synuclein will be also examined in Parkinson's disease (PD). We will further explore the contribution of NMDA receptor subtypes on mitochondrial function and the interplay with endoplasmic reticulum in the hippocampus of Alzheimer' disease animal models. In the near future, we will investigate the use of neural stem cells on brain neuronal recovery by cell replacement therapies in HD and PD animal models.

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MOLECULAR MECHANISMS OF DISEASE GROUP (*Head: Cláudia Pereira*)

The prion (PrPSc isoform), amyloid-β (Aβ) and α-synuclein (α-syn) peptides are now considered crucial in the pathogenesis, respectively, of prion-related encephalopathies, Alzheimer disease (AD) and Parkinson disease (PD), which are characterized by selective synaptic and neuronal loss. Although the aberrant peptide accumulation is recognized as an important common feature in these neurodegenerative diseases, the mechanisms of pathogenesis remain an important subject of competing hypothesis and debate. Our general aims are: i) to investigate the primary molecular and cellular events induced by these disease-related peptides and their causal relationships; ii) to identify and test potential therapeutic agents that target the underlying disease mechanisms.

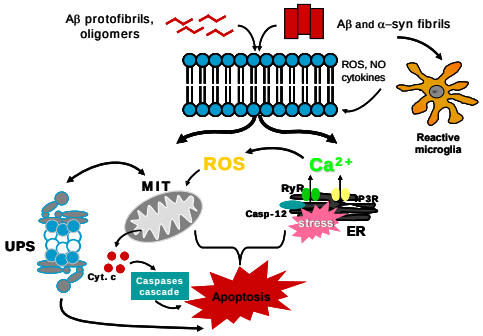
Research Highlights

One of the major interests of the group concerns the investigation of endoplasmic reticulum (ER)/mitochondria cross-talk as a primary molecular mechanism leading to neuronal loss triggered by Aβ or PrP. In cultured cortical neurons, these peptides induced ER Ca^{2+} release and activated the ER stress-mediated apoptotic pathway by a mitochondrial-dependent process. The role of mitochondria into this apoptotic pathway was confirmed using mtDNA-depleted Rho0 cells, without functional mitochondria. Evidence from literature demonstrates that oligomeric and fibrillar assemblies of Aβ peptide differentially affect neuronal survival. We showed that Aβ oligomers are more toxic than fibrils to cortical neurons, leading to GSK3β-mediated tau phosphorylation and apoptotic death through a mechanism involving ER Ca^{2+} release and ER stress. In addition, cdk5, which is indirectly activated by Ca^{2+}, was also involved in tau phosphorylation triggered by Aβ and PrP.

Another focus of research of our group is the role of neuroinflammation, in particular of microglia activation, on Aβ- or PrP-induced neuronal death. Using co-cultures of microglia/cortical neurons, Aβ- and PrP-activated microglia was observed to trigger neuronal death through the release of interleukin-6.

Ongoing and Future Work

Our main focus of interest continues to be the clarification of the cellular and molecular mechanisms involved in peptides-induced neuronal dysfunction occurring in neurodegenerative diseases. We are now extending our studies of Aβ-induced ER stress to an animal model of AD in order to investigate how ER stress correlates with Aβ and tau pathology and also with cognitive deficits. Using AD cybrids, we demonstrated that a defect in the mitochondrial respiratory chain potentiates Aβ-induced ER stress. The mechanisms by which mitochondrial impairment affects ER function leading to ER stress-mediated apoptotic death are now being explored.



Brain accumulation of α-syn and Aβ peptides represents an early event in the pathogenesis of the most common subtype of AD, the Lewy body variant of AD. We are currently investigating the intracellular signalling pathways triggered by either intracytoplasmic α-syn or from α-syn released from damaged presynaptic termini on the neuronal response to Aβ, namely on ER and mitochondrial dysfunction.

Mitochondrial deficits are intimately correlated with PD ethiopathogenesis, and proteasomal dysfunction is described as an initial event in some PD familial forms. We are presently addressing the role of α–-syn on mitochondria/proteasome cross-talk in PD. Moreover, the involvement of the autophagic pathway will be studied.

The contribution of two additional cellular systems in AD pathogenesis is now being addressed. The cholinergic system is particularly affected in AD. Work currently in progress aims to investigate how presynaptic hippocampal nAChRs subtypes are affected in early AD and how correlate with cognitive deficits and synaptic loss. Moreover, the consequences of chronic nicotine consumption will be studied. Evidence has been presented that dysfunctional brain endothelial cells contribute to the neuronal cell loss characteristic of AD. Our goal at this point is to look at the role of Aβ, aging and diabetes on neurovascular dysfunction in AD, focusing on the potential involvement of mitochondrial impairment and angiogenesis growth factors signaling, and the synergy between the dual vascular/neuronal toxicity of Aβ.

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RETINAL DYSFUNCTION AND NEUROGENESIS GROUP *(Head: António F. Ambrósio)*

Age-related retinal diseases, such as diabetic retinopathy (DR) and age-related macular degeneration (AMD), are leading causes of blindness in developed countries. At present, they have no effective treatment, which has been hampered because the pathogenic processes are not clearly elucidated yet. Our main goal is to give insight on the molecular and cellular mechanisms underlying neuronal, glial and endothelial cell dysfunction in diabetic retinopathy. We are particularly interested in the role played by glutamate, ATP, oxidative/nitrosative stress, and by some inflammatory mediators, namely nitric oxide, interleukin 1-beta and TNF-alpha.

Neuropeptide Y (NPY) is expressed in the retina, but its physiological role is unknown. We are investigating the modulatory role of NPY in neurotransmitter release and Ca2+ regulation in the retina, as well as its potential neuroprotective and proliferation/differentiation properties, that might be useful to treat retinal disorders.

Finally, we are also interested in the role played by nitric oxide in neurodegeneration and neurogenesis, in models comprising injury/inflammation in CNS.

Research Highlights

We found that high glucose levels 1) increase the release of excitatory neurotransmitters; 2) impair Ca2+ homeostasis; and 3) affect the expression of ionotropic glutamate receptor subunits in retinal neural cells. Of particular relevance is the upregulation of GluR2 subunit expression, which seems to act as a protective mechanism. 4) We also demonstrated that high glucose-induced apoptosis in retinal cells is independent of caspase activation and is mediated by the translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus.

Ongoing and Future Work

1. We will proceed investigating the molecular and cellular mechanisms underlying retinal dysfunction and degeneration induced by hyperglycemia, as follows:

a) AMPA receptors have a major role in retinal physiology. We will investigate the impact of hyperglycemia in the physiology of AMPA receptors, and the molecular mechanisms underlying the alteration of AMPA receptor subunits expression in retinal cells.

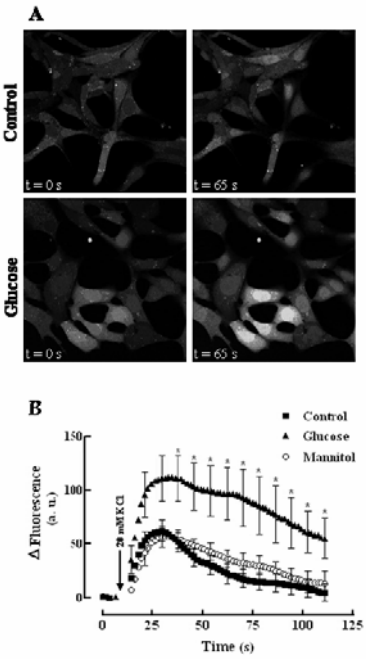
b) We will study the impact hyperglycemia has on neurotransmission in the retina, and in the hippocampus.

c) The role of inflammatory mediators, particularly IL-1 beta and nitric oxide, in the breakdown of blood-retinal barrier, and the potential use of anti-inflammatory drugs to treat DR, will be investigated.

d) We intend to elucidate the role of ATP in the activation of microglial cells and in the production of inflammatory mediators, and the possible involvement of these mediators in retinal degeneration and in blood-retinal barrier breakdown.

2. We will proceed investigating the role of NPY in retinal physiology and in neurogenesis and cell differentiation.

3. We will evaluate the toxic effect of drugs of abuse in the retina, namely ecstasy, but also other amphetamines.



High glucose affects $[Ca^{2+}]_i$ regulation in retinal cells.

4. We intend to clarify the role of nitric oxide, particularly from inflammatory origin, on neurogenesis and cell death in CNS.

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AREA B: MOLECULAR BIOTECHNOLOGY AND HEALTH

Coordinator - Euclides Pires

Introduction

The scope of the scientific activities developed by the groups of the Molecular Biotechnology and Health Area is the understanding of the function displayed by macromolecules as individual entities and as components of more complex systems. Relevant information obtained at this fundamental level is then incorporated into translational projects aiming at novel biotechnological applications, in particular drug design and design of new drug carriers.

A common feature of the groups of this area is a deep know how of a particular set of technologies which is used to tackle specific problems investigated by these groups, but that is also made available to other groups of CNC.

In 2006 there are five groups in this area:

Molecular Biotechnology Group – This group focuses its research on the structure function relationship of biotechnological and biomedical relevant proteases. The group has developed expertise on screening, purification and primary structure determination of new proteins as well on the production of recombinant proteins.

Structural and Computational Biology Group – This group has a research track record on folding and stability of amyloid proteins. The group has developed expertise on spectroscopy and computacional analysis to study protein secondary structure changes. At present the group is widening the scope of its scientific activity to modelling of protein-protein and protein lingand interaction, to virtual screening and rational drug design.

Macromolecular Crystallography Group – This group focuses considerable part of its activity on the structure and characterization of proteases associated with human neurological diseases. Protein tertiary structure determinations are conducted and supervised at CNC by this group.

Molecular Systems Biology Group – This group is particularly interested in the discovery, explanation and exploitation of the principles that underlie the design or the reconfiguration of metabolic networks upon environment or "specific signal" changes. This new group brought into CNC the expertise on system theoretic analysis which is of crucial importance for the design of complex biotechnological developments.

Vectors and Gene Therapy Group – This group has focused its activity on the design and assay, both in vivo and in vitro, of viral and non viral drug carriers. The expertise of the group on the design and production of carriers and on the generation of specific animal models has contributed to the success of several projects at CNC.

Research highlights, ongoing and future work of these groups are briefly described in following pages. Part of the work carried out was incorporated in Ph.D. thesis (5) and Master thesis (1) which were concluded in 2006 under the supervision of researchers of this area.

Collaboration of the groups of this area with Biocant – (Biotechnology Transfer Unit at Cantanhede) was resumed and strengthed during 2006.

Molecular Biotechnology Group

Carlos Faro	(*Ph.D. - head of group*)
Euclides Pires	(Ph.D.)
Paula Veríssimo	(Ph.D.)
Fernando J. S. Delgado	(Ph.D.)
Isaura Simões	(Ph.D.)
Pedro Castanheira	(Ph.D.)
Maria Conceição Egas	(Ph.D.)
Ana Sofia F. de Almeida	(Ph.D. Student)
Catarina Pimentel	(Ph.D. Student)
*Luísa Cortes Bastos	(Ph.D. Student)
Maria José Simões	(Ph.D. Student)
Rita Videira	(Ph.D. Student)
Ana Cristina Gomes	(MSc Student)
Rui Cruz	(MSc Student)
Raquel Vinhas	(MSc Student)
Paulo Barracosa	(MSc Student)
Maria do Rosário F.C. Faro	(Graduate Technician)

Molecular Systems Biology Group

Armindo J. A. S. Salvador	(*Ph.D.- head of group)*
Fernando Nogueira	(Ph.D.)
Miguel A. L. Marques	(Ph.D.)
Daniela Cipreste Vaz	(Ph.D. Student)
Nuno Ricardo Ferreira	(Ph.D. Student)
Cândida Susana G. Silva	(Ph.D. Student)
Carlos José Vieira Simões	(Ph.D. Student)
Joaquim Rodrigues	(Graduate)

Structural and Computational Biology Group

Rui Brito	(*Ph.D.- head of group*)
Cândida Susana G. da Silva	(Ph.D. Student)
Daniela Cipreste Vaz	(Ph.D. Student)
Nuno Ricardo Ferreira	(Ph.D. Student)
Joaquim Rui C. Rodrigues	(Research Student – Project Fellowship)
Bárbara Joana Henriques	(Research Student)

Macromolecular Crystallography Group

Sandra Ribeiro	(*Ph.D.- head of group)*
Ricardo L. Tomé	(Ph.D. Student)
Luísa Cortes Bastos	(MSc)
Rui Abreu	(MSc)
Aurea Filipa A. Castilho	(Research Student – Project Fellowship)
Bruna Moreira	(Undergraduate Student)

Vectors and Gene Therapy Group

M. Conceição Pedroso Lima	*(Ph.D.- head of group)*
Sérgio Simões	(Ph.D.)
João Nuno Moreira	(Ph.D.)
Luís Almeida	(Ph.D.)
Henrique Faneca	(Ph.D.)
Ana Eulálio	(Ph.D.)
Cristina Fonseca	(Ph.D.)
Miguel Mano	(Ph.D.)

Ana Filipe	(Ph.D. Student)
Ana Luísa Cardoso	(Ph.D. Student)
Adriana Santos	(Ph.D. Student)
Ana Catarina Pinto	(Ph.D. Student)
Isabel Ferreira	(Ph.D. Student)
Lígia Ferreira	(Ph.D. Student)
Isabel Nunes	(Ph.D. Student)
Liliana Mendonça	(Ph.D. Student)
Nuno Penacho	(Ph.D. Student)
Sandro Alves	(Ph.D. Student)
Sílvia Sousa Neves	(Ph.D. Student)
Vera Moura	(Ph.D. Student)
Luís Bimbo	(MSc Student)
João Paulo Pereira	(Undergraduate Student)
Alexandra Faustino	(Undergraduate Student)

**Investigator doing collaborative research involving different groups at CNC.*

MOLECULAR BIOTECHNOLOGY GROUP (*Head: Carlos Faro*)

The Molecular Biotechnology group is interested in studying the structure and function of some biotechnology and/or biomedically relevant proteases such as i) the aminopeptidase from the allergenic pollen of *Parietaria judaica* , ii) hepsin, a membrane serine protease involved in prostate cancer, and iii) CDR1, an aspartic protease from *Arabidopsis thaliana* implicated in plant defence mechanisms against bacterial pathogens.

Research Highlights

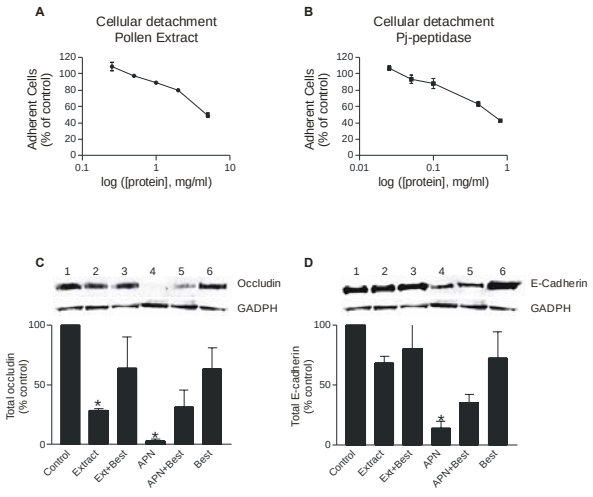
In the past year we showed that the Pj-peptidase, a aminopeptidase isolated from *Parietaria judaica* pollen, is able of degrading bioactive peptides involved in the maintenance and recovery of the bronchomotor tone. Furthermore, we proved that Pj-peptidase is also capable to cause cellular detachment of airway epithelial cells in culture by degrading intercellular adhesion proteins like occludin, and E-cadherin. The damaging induced by the Pj-peptidase on the airway epithelium is likely to promote an increased access of *P. judaica* allergenic proteins to subepithelial antigen-presenting dendritic cells, and could therefore play a major role in potentiating the allergic response.

Previous results suggest that a haplotype found in the 3'UTR of the hepsin gene is associated with prostate cancer. Subsequent analysis demonstrated that the altered haplotype reduces the mRNA stability of a reporter gene in prostate cell lines and prostate biopsies. Our results clearly indicate that the 3'UTR haplotype leads to a down-regulation of hepsin expression through a specific interaction between the specific 3?UTR cis element and a regulatory protein. The identification of this mRNA binding protein is currently in progress.

The *Arabidopsis thaliana* Constitutive Disease Resistance 1 (CDR1) gene product is an aspartic proteinase that has been implicated in disease resistance signalling. During last year, the recombinant form of the enzyme was produced in E.coli and its structural and enzymatic properties were characterised in detail. CDR1 displays rather unusual properties that make it unique among aspartic proteinases. The findings unveil a pattern of unprecedented functional complexity for Arabidopsis CDR1 consistent with a very specific role in the defense mechanism against pathogens.

Ongoing and Future Work

Work currently in progress aims to study the molecular mechanisms underlying the involvement of hepsin in prostate cancer and the structure-function relationship of a particular subset of aspartic proteinases with anti-apoptotic properties.



*Dose-dependent effect of P. judaica pollen extract (**A**) and Pj-peptidase (**B**) on cell detachment of A549 cell line. Immunoblots of junctional proteins from A549 cell monolayers treated with P. judaica pollen extract or Pj-peptidase. Occludin (**C**) and E-cadherin (**D**) were degraded after exposure of A549 cells to P. judaica pollen extract (lanes 2) as well as Pj-peptidase (lanes 4). In both cases,this effect was reverted by the addition of bestatin (lanes 3 and 5).*

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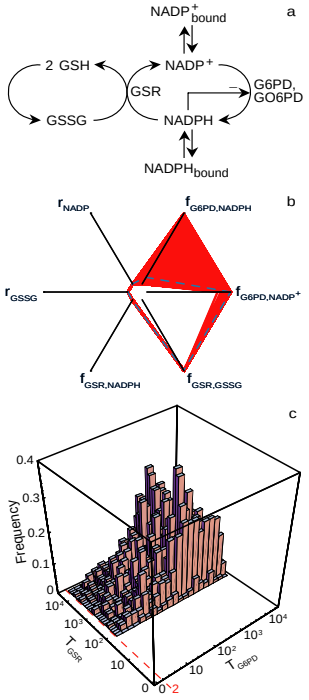
MOLECULAR SYSTEMS BIOLOGY GROUP (*Head: Armindo Salvador*)

Natural selection places strong demands on extant organisms as regards effective operation in their environment. In turn, these demands translate into strong constraints on the design of biochemical networks. As result, the naturally evolved networks obey to a number of design principles — rules relating their reaction/interaction structure to the function they perform —, which can be inferred through systems-theoretic analyses informed by knowledge of pertinent physical-chemical constraints and performance requirements. Our group's main interest is the discovery, explanation and exploitation of such principles in metabolic networks.

Research Highlights

Moiety-transfer cycles, whereby a moiety-donor metabolite transfers a moiety to a cycled intermediate carrier, which in turn transfers it to a moiety acceptor, are arguably the most prevalent elementary circuits in biochemical networks. Their role is analogous to that of power-supply units in electronic circuits: they couple supply to demand, ensuring that the moiety in point is transferred to metabolic acceptors at a rate that is proportional to demand and insensitive to fluctuations in the outside supply. Our previous work on two well-characterized moiety-transfer cycles — the coupled NADPH and GSH redox cycles in human erythrocytes (Fig. 1a) — showed that: (a) flux capacity is not always (and perhaps not often) a major factor mediating the evolutionary maintenance of high enzyme activities, and (b) the activities of consecutive enzymes in a pathway may be evolutionarily tuned to different functional requirements (Salvador & Savageau, 2006). Recently, we showed that the level of performance displayed by this circuit in normal human erythrocytes is only achievable under a strict design. Namely (Fig. 1b), the kinetic orders of glucose 6-phosphate dehydrogenase and glutathione reductase for the respective substrates $NADP^+$ and GSSG must approach 1, the kinetic order of GSR for NADPH must be small, *and* the ratios of oxidized to reduced glutathione and $NADP^+$/NADPH both must be small. We have also identified the functional requirements that pose the strongest constraints on the values of most parameters and shown that natural selection for fulfilling these requirements leads, as side effect, to the evolution of large tolerances of flux with respect to enzyme deficiencies (Fig. 1c). The latter finding, suggests a long-sought explanation for the evolution of genetic dominance of the wild type over null mutant alleles.



Analysis of the naturally evolved design of the coupled NADPH and GSH redox cycles in human erythrocytes.

Ongoing and Future Work

We are currently extending the studies above to address the following questions. Do the design rules that characterize the NADPH and GSH redox cycles in human erythrocytes apply in general to moiety-transfer cycles where there is one master moiety-supplying process and one master moiety-consuming process? (That is, do those rules qualify as design *principles*?) What rules apply where there are several equally important moiety-supplying processes and/or several equally important moiety-consuming processes? What, if any, is the functional role of intermediate sequestration and product inhibition found in various moiety-transfer cycles? Are there functional reasons for some bi-bi enzyme mechanisms being selected over others in the context of these circuits? Is the evolution of large tolerances for enzyme deficiencies a very frequent outcome in moiety-transfer cycles?

Our studies have identified the intrinsic non-enzymatic reactivity of many metabolites as a potentially very important consideration in the design of biochemical networks. So as to further examine this issue, we are conducting a theoretic-experimental analysis of the covalent modification of hemoglobin by reactive metabolites. We aim to clarify (a) the extent to which different intermediates and byproducts of the glycolytic, pentose phosphates and polyols pathways covalently modify hemoglobin, and (b) whether these pathways are designed to minimize damage to hemoglobin by their intermediates.

We have also started modeling the metabolism of proliferating cells in the context of a theoretic-experimental collaboration aiming to clarify the metabolic reconfiguration of metabolism induced by Akt activation in tumor cells. We have already developed a stoichiometric model of 13C isotopomer conversion that contemplates the most relevant pathways and that will serve as basis for flux estimation form 13C NMR tracer studies being performed by our collaborators at the University of Pennsylvania. Our future aim for this project will be do develop constraints-based and kinetic models that will help understanding the regulatory mechanisms involved in the reconfiguration of metabolism that occurs when cells transit from a quiescent to a proliferating mode.

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STRUCTURAL AND COMPUTATIONAL BIOLOGY GROUP (*Head: Rui Brito*)

During 2006, and in accordance with the CNC strategic goals, the "Protein Stability and Folding Group" changed its designation in order to reflect the integration of new collaborators and the widened scope of its scientific activity. Building on the previous experience on the use of spectroscopic and computational methodologies to study protein folding and stability in amyloid diseases, the group is now setting its goals on a wider approach to the study of human disorders, going from the detailed modelling of protein-protein and protein-ligand interactions using mixed molecular dynamics and quantum mechanics approaches, to virtual screening and rational drug design, computer modelling of protein interaction networks and simulation of metabolic pathways.

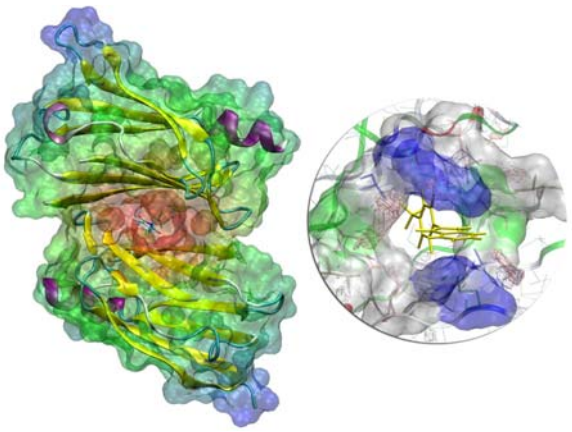
Research Highlights

Following up on the efforts of previous years setting up computational methodologies to study biologically relevant problems, the group concentrated its efforts on: *i)* computational modelling of the initial stages of amyloid formation by Transthyretin (TTR), the protein responsible for the development of FAP (Familial Amyloid Polyneuropathy), a neurodegenerative disease of very poor outcome in most patients; *ii)* virtual screening and molecular modelling of inhibitors of TTR amyloid formation; *iii)* unfolding simulations of amyloidogenic and non-amyloidogenic proteins; *iv)* quantum mechanics simulations of light emission processes in bioluminescent organisms.

Ongoing and Future Work

Combining the reach of experimental and computational methodologies, we are working on the characterization of the molecular species involved on the initial stages of amyloid formation by the protein transthyretin. Additionally, a significant effort is being made in the area of virtual screening and rational design of TTR amyloidosis inhibitors. Docking and Molecular Dynamics simulations in a massive parallel computer (*Centopeia*, UC) are being routinely used. Ongoing collaborations with computer scientists are allowing us to develop tools for data mining of large data sets produced in protein folding and unfolding computer simulations.

Additionally, using time-dependent density functional theory (TDDFT) we are currently studying the process of light emission in two systems present in bioluminescent organisms: *i)* the luciferin/luciferase present in fireflies and some beetles, and *ii)* mutants of the green fluorescent protein (GFP), namely the variant Y66W. These systems are of great importance, both from a fundamental and from a technological point of view.



In silico screening of TTR amyloidosis inhibitors. The images were produced with the programs VMD (University of Illinois) and CheVi (SimBioSys).

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Varsano, D., Felice, R. Di, Marques, M.A.L. and Rubio A. (2006). A time-dependent density functional theory (TDDFT) study of the excited states of DNA bases and their assemblies. *J. Phys. Chem.* B 110, 7129-7138.

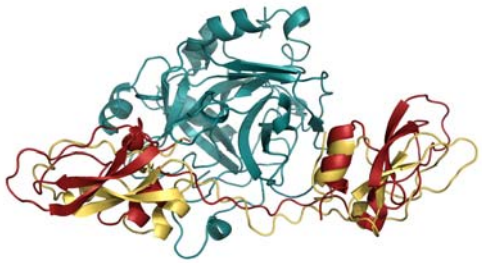
MACROMOLECULAR CRYSTALLOGRAPHY (*Head- Sandra Macedo-Ribeiro*)

Understanding the physiological functions displayed by biological macromolecules implies the knowledge of their three-dimensional structures. Macromolecular crystallography uses single crystal X-ray diffraction techniques to establish general principles for protein and nucleic acid architecture. Such studies provide a powerful route towards understanding protein function, also contributing towards the rational design of new drugs, ultimately leading to the development of new therapeutic agents.

Research Highlights

The group is focused on the structural characterization of proteins, particularly proteases associated with human diseases. The major research topic concerns the molecular and structural characterization of the ubiquitin hydrolase mutated in Machado-Joseph´s disease – ataxin-3. Ataxin-3 is a modular protein composed of a catalytic cysteine-protease domain – the Josephin domain, followed by two or three ubiquitin interaction motifs (UIM). –the polyglutamine tract, expanded in Machado-Joseph's disease patients is located at the C-terminus of the protein, immediately after a putative nuclear localization signal.

Aiming at the rational design of novel anticoagulants, we are also investigating the mechanisms of inhibition displayed by new proteinaceous thrombin inhibitors. We have recently solved the three-dimensional structure of the complex formed between thrombin and boophilin, a dual-head Kunitz inhibitor isolated from the cattle tick *Boophilus microplus*.



Ongoing and Future Work

We have identified early oligomeric species of non-expanded ataxin-3 and started the characterization of its aggregation pathway (Gales *et al.* 2005). Characterization of the aggregation mechanisms of non-expanded ataxin-3 is ongoing in collaboration with the Protein Stability and Folding Group at the CNC. Although focused on the structural characterization of the mechanism of ataxin-3 oligomerization and amyloid formation, our group is closely collaborating with other groups at CNC (e.g. Molecular Biology of Glutamate Receptors Group) with strong expertise in cellular biology with the goal of identifying the toxicity and intracellular dynamics of ataxin-3. Together, those results will provide a more detailed picture of normal ataxin-3 function and aggregation, and give further clues for identifying how expansion of the polyglutamine tract could lead to neuron-specific degeneration in Machado-Joseph´s disease.

As a result of the close interplay with the Molecular Biotechnology Group, we are expanding our research goals and crystallizing novel aspartic proteases identified in *Arabidopsis* genome as well as other proteases characterized and purified in that group.

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VECTORS AND GENE THERAPY GROUP (*Head: Mª Conceição Pedroso Lima*)

The CNC laboratory of vectors and gene therapy is devoted to the design and development of carriers, including viral and non-viral vectors, for nucleic acid and drug delivery aiming at their application in gene therapy and gene silencing approaches.

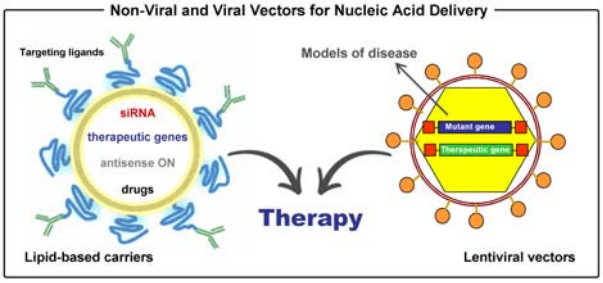
Research Highlights

Regarding the development of non-viral vectors our specific aims are to generate lipid-based nanosystems capable of efficiently deliver the carried material to target tissues or cells and to specific molecular targets. Our studies have been focused on the evaluation of the potential of the developed nanosystems for the treatment of two major diseases: cancer and neurodegenerative disorders. Cancer has been the main target disease in which approaches dealing with delivery of both gene silencing agents (antisense oligonucleotides or siRNAs) and plasmids encoding therapeutic genes have been evaluated. Mechanistic studies on the interaction of the developed systems with target cells, including cell internalization and intracellular trafficking, have also been addressed aiming at their optimization for specific therapeutic applications. We have provided evidence for the ability of a breast tumor vasculature-homing peptide to function as a targeting agent of poly(ethylene glycol) sterically stabilized liposomes to human breast tumor cells, which may be an advantageous complement to treatments for breast cancer. Several cancer animal models have been generated, including murine models, both orthotopic and subcutaneous xenografts, as well as a human leukaemia model in SCID mice for exploring the potential of the generated nanosystems. Important findings were achieved regarding the therapeutic effects observed upon application of both protein-associated lipoplexes in suicide gene therapy and immuno gene therapy approaches in murine models (both for oral squamous cell carcinoma and mammary adenocarcinoma) and sterically stabilized pH-sensitive liposomes in gene silencing approaches in human leukaemia–bearing SCID mice.

Our studies on the potential of the developed nanosystems in gene silencing approaches for neurodegenerative disorders demonstrated that protein-associated lipoplexes can be successfully applied to mediate downregulation of reporter and therapeutic genes in both neuronal cell lines and primary cultures. Moreover, lentiviral vectors-mediated expression of polyglutamine-expanded ataxin-3 in the rat brain was shown to induce motor and neuropathological abnormalities which replicate Machado-Joseph disease / Spinocerebellar ataxia type 3, demonstrating that this strategy can be employed to produce a new genetic animal model of this disorder.

Ongoing and Future Work

Our current and future work aims at further improving the different nucleic acid delivery systems, namely by enhancing their efficiency and targeting specificity. To this end, mechanistic studies on the process of nucleic acid delivery mediated by the developed lipid based-nanosystems, including their interaction with the cell surface, route of internalization and intracellular trafficking, are being addressed. Regarding cancer, significant efforts are currently devoted to promote specific delivery to different cells or tissues through functionalization of the developed systems with targeting agents (peptides, proteins or mAbs), whose selection will be made based on the tumor cell surface marker specificity. Regarding neurodegenerative disorders, we aim at evaluating the potential of protein-associated lipoplexes to mediate in vivo delivery of siRNAs and downregulation of specific pro-apoptotic proteins. Moreover, we have recently generated evidences that lentiviral vectors mediate efficient silencing of mutant ataxin-3 in the rat brain. Further studies are ongoing to demonstrate that this approach can be used for preventing neuropathological changes associated with Machado/Joseph disease.



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AREA C: CELL AND MOLECULAR TOXICOLOGY

Coordinator – Leonor Almeida

Introduction

This area is mainly concerned withd the study of cellular and molecular basis of drug- and disease-induced cell toxicity and has been organized in three research groups:

The *Mitochondrial Toxicology and Pharmacolgy Group*, particularly focused on the role of mitochondria as a primary intracellular target in initiation of drug- and disease-induced cell dysfunction. Anticancer agents, namely, tamoxifen and its metabolite hydroxytamoxifen, doxorubicin and newly synthesized phenolic compounds, are the most relevant drugs that have been studied by using *in vitro* and *in vivo* approaches. On the other hand, the relevance of mitochondrial dysfunction to the pathogenesis of some diseases, such as cholestasis, steatosis and metabolic syndrome in experimental rat models, and diabetes in Goto-Kakizaki and streptozotocin-injected rat models, has also been demonstrated and underlying mechanisms have been indicated.

The *Free Radicals and Antioxidants Group*, centred on studies of free radicals and oxidants, either as messenger molecules relevant to physiological functions, or as promoters of toxic pathways in the pathogenesis of chronic degenerative processes. This group has been particularly active in exploring the dynamics of NO production in hippocampal brain slices and the biological determinants of NO activity in this tissue and in cultured PC12 cells, in the context of molecular mechanisms inherent to neuromodulation and aging. Also, it has been involved in the molecular mechanisms underlying the health-promoting role of dietary polyphenol antioxidants, in particular those present in red wine, mainly in connection with the protection against vascular endothelial dysfunction and the non-enzymatic production of nitric oxide in the gastric compartment.

The *Membrane Toxicity Group*, focused on the study of membrane changes (biophysical approach) upon the interaction with xenobiotic molecules, in particular, insecticides and herbicides, by using bacterial models and membrane models, aimed to elucidate the molecular mechanisms underlying their toxicological effects upon biological systems.

The specific research highlights and ongoing and future work are indicated for each group.

Recently, a new research group is emerging focused on “Pharmacometry” that should be individualized next year. The originality of research conducted by the leader of this novel area at CNC (A.C. Falcão) led to the awarding of the *Eminent Scientist of the Year 2006 - Europe* (Clinical Pharmacology), an award conferred by the International Research Promotion Council (IRPC).

Within the scientific activities of this area, 2 Ph.D. and 2 MSc thesis were concluded, and more 8 Ph.D. and 4 MSc thesis are ongoing. The groups have interacted with other areas within CNC, namely Neuroscience and Disease and Cell and Development Biology, as indicated by some joint publications. In addition, they have established other strong collaborations, either with national or foreign researchers, as evidenced mainly by authorship of published papers and organization of scientific events. Worthy of notice was the 2006 edition of the “International Courses on Toxicology at the CNC", organized by this Area on a yearly basis, intitled "Toxicology: Subcellular Targets of Anticancer Therapy", which had the participation of highly recognized scientists (see Graduate Studies Programme).

Mitochondrial Toxicity Group

Paulo J. Oliveira	(*Ph.D. – head of group*)
Amílcar Falcão	(Ph.D.)
José Custódio	(Ph.D.)
*Maria Sancha Santos	(Ph.D.)
Anabela Pinto Rolo	(Ph.D.)
Vilma A. Sardão Oliveira	(Ph.D. Student)
*Paula Moreira	(Ph.D. Student)
Filomena Silva	(Ph.D. Student)
*Marco Alves	(Ph.D. Student)
Ricardo Marques	(Master Student)
Teresa Serafim	(Pre-doctoral Student)
Sandro Pereira	(Pre-doctoral Student)
Júlio Matos	(Undergraduated Student)
Gonçalo Pereira	(Undergraduated Student)
Ana Filipa Branco	(Undergraduated Student)
*Sónia Correia	(Undergraduated Student)
*Cristina Carvalho	(Undergraduated Student)
Cláudia Pereira	(Undergraduated Student)

Free Radicals and Antioxidants Group

João Laranjinha	(*Ph.D. – head of group*)
Leonor Martins de Almeida	(Ph.D.)
Teresa do Carmo P. D. Silva	(Ph.D.)
*Rui Manuel S. G. Barbosa	(Ph.D.)
Ana Margarida Ledo	(Ph.D. Student)
Bruno Miguel A. F. Gago	(Ph.D. Student)
João Gonçalo Frade	(Ph.D. Student)
Carla Maria dos S. Nunes	(Ph.D. Student)
Paula Matos de Brito	(Ph.D. Student)
Ana Carina Fernandes Pais	(MSc Student)
Nuno Ricardo E. Ferreira	(MSc Student)
Cátia Filipa L. Marques	(MSc Student)
Nuno Alexandre Gonzalez	(Undergraduate Student)
Ricardo Santos	(Undergraduate Student)
Tânia Laranjeiro	(Undergraduate Student)
Núria Filipa Serrador Simões	(BI Fellowships)

Membrane Toxicity Group

Amália Jurado	(*Ph.D. – head of group*)
Romeu Videira	(Ph.D.)
Manuel Joaquim G. Matos	(Ph.D. Student)
João Demétrio Martins	(MSc Student)
João Pedro Monteiro	(MSc Student)
Sandra Marina A. Santos	(MSc Student)
Ana Isabel Martins da Cruz	(Undergraduate Student)
João Miguel Bento	(Undergraduate Student)
Susana Sofia Gaspar Capela	(Undergraduate Student)
Carla A. P. Lourenço	(Undergraduate Student)
Vera Soraia M. Sampaio	(Undergraduate Student)

**Investigator doing collaborative research involving different groups at CNC.*

MITOCHONDRIAL TOXICOLOGY AND PHARMACOLOGY GROUP (*Head: Paulo Oliveira*)

Mitochondria play a pivotal role in cellular metabolism and in energy production. Research over the years has demonstrated that mitochondria, besides being the powerhouses of cells, have also important roles on cell calcium homeostasis and on the induction of cell death.

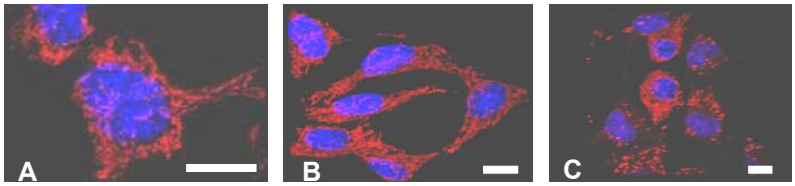
Numerous examples of mitochondria-mediated cell injury can be found in the literature; not only chemicals can negatively affect mitocondrial function but also the origin and progression of several pathologies is closely related with disruption of mitochondrial homeostasis. The main and general objective of the Mitochondrial Toxicology Group is to provide an insight into the role of mitochondria as a primary intracellular target in the initiation of drug- and disease-induced cell dysfunction.

Research Highlights

1) Cholestasis, steatosis and hepatic ischemia: bile acids therapy. Cellular mechanisms with relevance to mitochondrial dysfunction: Mechanisms of action of hepatoprotective compounds; mitochondrion and its implication in pathologies such as cholestasis, steatosis and metabolic syndrome; mitochondrial biogenesis; interference with the regulation of expression of the mitochondrial genome and with the coordinated expression of the mitochondrial and nuclear genomes; bile acid receptors and regulation of carbohydrate and lipid metabolism.

2) Drug-Induced Mitochondrial Dysfunction and Cytotoxicity: Mechanisms of drug-induced mitochondrial dysfunction are also studied in the group. In a different approach, we observed that all deleterious effects induced by Tamoxifen, an anti-neoplastic agent, were highly exacerbated in the presence of 17β-estradiol, especially regarding direct effects on Complex I. We also investigate the mechanisms by which doxorubicin (DOX), another potent anti-neoplastic agent, is toxic to the cardiac tissue, with a special focus on the role of mitochondria. In vivo studies showed that DOX alters the regulation of the mitochondrial permeability transition through thiol oxidation and alteration of protein expression. Endurance exercise but not vitamin E-succinate was demonstrated to prevent DOX-induced cardiac mitochondrial dysfunction. Embryonic ventricular H9c2 cells are also being used as a model to study DOX cardiotoxicity and the role of mitochondria in the mechanisms of cell death. On the other hand, we also showed that tert-butylhydroperoxide causes mitochondrial dysfunction and oxidative stress-induced apoptosis on the same cell line. The group has also investigated the effects of other compounds such as ecstasy, carbaryl (a pesticide) or cerebrocast, among others, on isolated mitochondrial fractions. Newly synthesized compounds or phytochemicals are being tested as possible anti-proliferative agents in several tumor cell lines. Results obtained *in situ* are compared with data obtained from experiments in isolated mitochondrial fractions.

3) Diabetes-induced mitochondrial dysfunction: The group has demonstrated how diabetes affects the mitochondrial function of brain, kidney, heart, testis and liver mitochondria. Insulin was shown to prevent some of those alterations. Goto-Kakizaki and streptozotocin-injected rats were used to pinpoint precise mitochondrial sites affected by hyperglycaemia.



K1735-M2 cells observed by confocal microscopy after labeling with tetramethylrhodamine-methylester (red, a fluorescent probe for mitochondria) and Hoechst (nuclei, blue). A) and B) Control cells showing filamentous mitochondria, C) The same cells after treatment with a compound extracted from birch bark. Cell showed mitochondrial fragmentation and overall decrease in fluorescence, previous to other morphological changes. Bar represents 10 μm.

Ongoing and Future Work

Synthetic polyphenols are being tested in different tumour lines to identify powerful and novel anti-cancer agents. The same compounds are being characterized in terms of structure/activity relationships (in collaboration with external groups). As an example, polyamines complexed with platinum or palladium are also being used to distinguish mitochondrial vs. nuclear effects. Mitochondrial effects of phytochemicals have been also characterized in both intact cells and isolated mitochondrial fractions. We are also investigating the role of mitochondrial dependent and independent mechanisms of cell death induced by DOX on H9c2 myoblasts or adult muscle cells. New molecular approaches are and will be used to measure end-points of mitochondrial and cellular dysfunction, including techniques aimed at measuring changes in gene expression and cell cycle alterations, and their relation with mitochondrial dysfunction.

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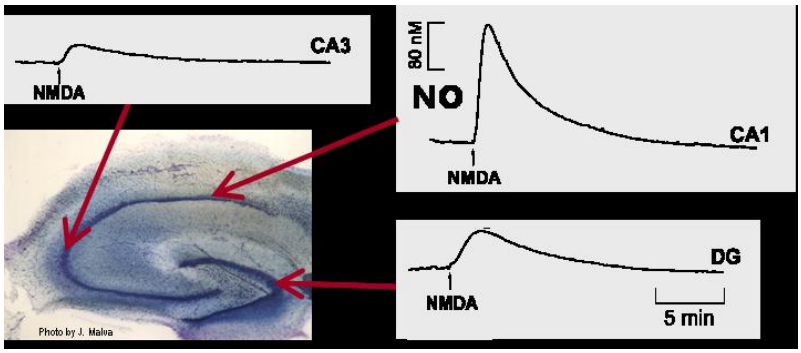
FREE RADICALS AND ANTIOXIDANTS GROUP *(Head: João Laranjinha)*

Reactive oxygen and nitrogen species play a pivotal in the modulation of critical cellular functions but extensive oxidative damage to biomolecules (oxidative stress) can lead to cell death by a variety of different mechanisms, either by turning off vital processes or by upregulating toxic cascades. Long term objectives of this group involve: 1) the study of molecular mechanisms inherent to neuromodulation, and aging that critically involve free radicals and oxidants, particularly nitric oxide (•NO); 2) To establish molecular mechanisms underlying the health-promoting role of plant-derived dietary phenolic compounds, particularly those present in wine and olive oil, in connection with the protection against vascular endothelial dysfunction and the non-enzymatic production of nitric oxide from nitrite in the gastric compartment

Research Highlights

Distinct patterns of •NO concentration dynamics were identified in rat hippocampal slices upon stimulation of two AMPA and NMDA glutamate receptors, substantiating the hypothesis that AMPA receptors, conversely to NMDA receptors, which produce •NO at high fluxes, are involved in the fine tuning of glutamate-dependent •NO production. In this regard, at high extracellular glutamate astrocytes increased GSH release, helping neurons to maintain levels of GSH under conditions of glutamate toxicity. We have further supported the notion that mitochondria is likely to play a center stage in the mechanisms of •NO toxicity in the presence of DOPAC and that the presence of the later shifts cell death from a classic apoptotic pathway to a programmed death mechanism still to be characterized.



Wine polyphenols positively affect vascular function beyond their antioxidant properties, in particular resveratrol protected endothelial cells from peroxynitrite-triggered cell death, by increasing intracellular GSH and by preventing the activation of caspases-9 and -3, interrupting the mitochondrial cell death pathway. Also, it inhibited the proliferation of cultured vascular smooth cells, induced by minimally oxidized LDL, by interfering with the PI3K/Akt/mTOR cellular survival pathway. Finally, *in vivo* evidences for a vitamin E redox activity of wine polyphenols were described.

Ongoing and Future Work

To measure the patterns of •NO change in rat brain *in vivo* in connection with brain activities in which •NO plays a significant role.

To study the non-enzymatic production of •NO in the gastric compartment derived from dietary nitrate/nitrite and its pathophysiological impact.

To elucidate the cardioprotective mechanisms of phenolic compounds from red wines beyond their antioxidant properties, particularly those related with anti-inflammatory properties and protection of vascular dysfunction, by using cell models and apolipoprotein-E deficient mice submitted to a high cholesterol diet.

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MEMBRANE TOXICITY GROUP (*Head: Amália Jurado*)

A growing body of evidence has shown that lipid-bilayer structure and dynamics play a pivotal role for membrane functionality. It is more and more apparent that biological membranes show a small-scale lateral structure and lipid microdomains enriched in sphingolipids and cholesterol (lipid rafts) have been recently identified as excellent platforms for spatial control of cellular signalling processes. The particular physical properties of lipid rafts make them attractive regions of the membrane to favour infection and intoxication and to modulate signal transduction.

The main purpose of the Membrane Toxicity Group is to find out more about the particular role played by lipids and the lipid-bilayer of cell membranes in health and disease conditions. The emphasis is on biophysical properties of the lipid-bilayer and on the way they affect membrane functions, that is a lipidomics approach. Advances in the elucidation of the aspects of lipid-bilayer structure and dynamics potentially involved in abnormal membrane functioning and disease have been built upon experimental approaches focusing on lipid-bilayer interactions of lipophilic, membrane-active compounds, namely drugs and pesticides.

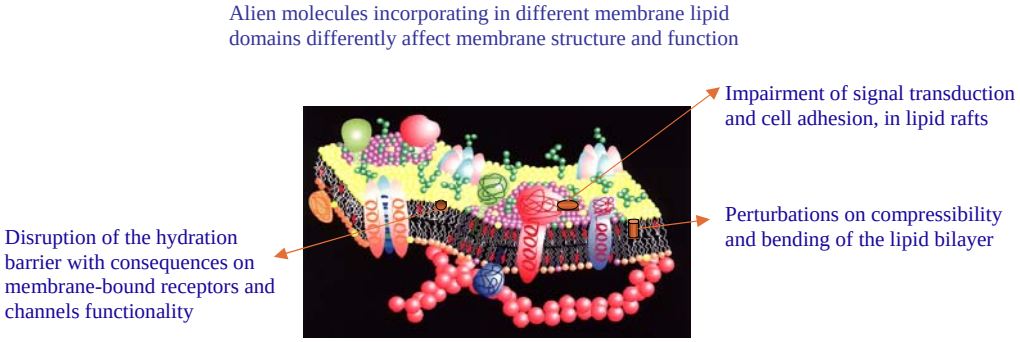
Research Highlights

The cellular effects of a variety of molecular compounds interacting with membranes have been correlated to their ability to affect and modulate lipid-membrane organisation. The compounds under study, either drugs (cytostatics, anti-arrhythmics) or environmental pollutants (insecticides, herbicides, organometals), were chosen taking into account their physico-chemical characteristics making them presumable disturbers of membrane properties. Different biological systems, from dispersions of synthetic and native membrane lipids, to subcellular fractions, protoplasts and prokaryotic and eukaryotic cells in culture, were used to unravel the impact of perturbations of the organisational principles that govern molecular assemblies of proteins and lipids in membranes, as consequence of xenobiotics membrane incorporation, on the structure and function of whole cells. Alterations induced in the structural order and organisation of lipid membranes have been correlated with adverse effects on bioenergetics, cell growth and viability, and suggested as being involved in the action mechanisms of xenobiotics focused on the target cells and/or on their non-selective side-effects.

Ongoing and Future Work

In collaboration with other research groups, we are planning to carry out a systematic study to assess the potential risks of the exposure to carbon nanomaterials, for human health and the environment. Profiting the large experience in developing toxicological screening strategies for pesticides and environmental pollutants, by using a serial stepwise increase of biological complexity, from lipid membranes to functional organelles and whole cells, we will evaluate the suitability of these toxicological methods when applied to nanoparticles. The interaction of nanoparticles with lipid membranes will be studied to evaluate the relevance of the lipid-bilayer as a toxicological target and the role of membrane lipids in nanoparticles delivery.

On the other hand, our research group, due to its adequate experience on lipids biochemistry and biophysics and membrane modelling, is expecting to co-operate with other groups carrying out protein-centred works on membrane mediated cellular processes, contributing with an experimental approach in a lipid context.



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AREA D: MICROBIOLOGY

Coordinator-Milton Costa

Introduction

The Microbiology research at CNC has, for several years, been dedicated to the study of microbial diversity and ecology in a wide variety of "extreme" environments, mainly characterized by high temperature, high salinity or both, extremely dry or highly alkaline, to name a few. Our efforts have also been directed to the study of the metabolic versatility that continues to expand as new species are isolated from those environments. We keep probing the mechanisms that allow microbes to thrive under such conditions, namely the synthesis and accumulation of "stress" molecules, namely compatible solutes. The identification of the genes and enzymes for some of these solutes, namely mannosylglycerate and glucosylglycerate, as well as their biosynthesis in different organisms, has increased our knowledge of their contribution to stress adaptation in extremophiles.

Our studies on compatible solutes biosynthesis allowed us to detect genes involved in polysaccharide biogenesis and glycolipid assembly in some actinobacteria, namely in the medically important genera *Mycobacterium* and the antibiotic producers *Streptomyces*. Some of these genes are essential for the growth of *M. tuberculosis* and the corresponding enzymes are currently under biochemical and structural investigation as future targets for anti-mycobacterial therapies.

A different line of research concerns the ecology of *Legionella* spp. in natural environments; we are characterizing their population genetics by diverse molecular approaches. The characterization of pathogenic isolates plays a central role in their epidemiology, therefore, it is crucial to generate the information for identifying, tracking, and intervening against disease outbreaks.

Another line of research deals with the interaction that yeasts and molds establish with other organisms namely their hosts or with bacteria. The understanding of the molecular responses of yeasts and molds to other organisms or to antifungals is of medical relevance specially if we consider the increasing incidence of opportunistic fungal infections in immuno-compromised patients.

Microbiology of Extreme Environments Group

Milton Simões da Costa	(*Ph.D. – head of group*)
António Manuel V. Pires	(Ph.D.)
Maria Fernanda P. N. Nobre	(Ph.D.)
Nuno Empadinhas	(Ph.D.)
André Antunes	(Ph.D.)
Igor Clemente Tiago	(Ph.D. Student)
Chantal Fernandes	(Ph.D. Student)
Joana Cardoso da Costa	(Ph.D. Student)
Susana Isabel Elias Alarico	(Ph.D. Student)
Ana Luísa Nabais G.Nobre	(Ph.D. Student)
Sofia Ramos	(MSc Student)

Yeast Research Group

Teresa Gonçalves	(*Ph.D. – head of group*)
Jorge Fernandes dos Anjos	(Ph.D.)
Cristiana da Silva O. Paulo	(MSc Student)
João Paulo Carvalho	(Pre-Graduation Student)
Alexandra Abrunheira	(Pre-Graduation Student)

**Investigator doing collaborative research involving different groups at CNC.*

MICROBIOLOGY OF EXTREME ENVIRONMENTS GROUP (*Head: Milton da Costa*)

During 2006 we obtained several important results on the biosynthesis of several compatible solutes of thermophilic and mesophilic organisms described several new species of bacteria and showed that specific Legionella spp. and clones persist in particular boreholes or aquifers over a period of years. These research projects are a continuation of programs that we started several years ago. New results have been obtained from the microbial diversity of samples obtained from deep salt layers in the Red Sea at a dept of about 1300 meters. This will, no doubt, turn into an exciting new area of research.

Research Highlights

In a separate study we also knockout the genes for the synthesis of mannosylglycerate in *Thermus thermophilus* and conclusively proved the role of both compatible solutes in the osmotic adaptation of this organism. We also examined the transport of trehalose in this species and elucidated two pathways for the synthesis of the very rare compatible solute glucosylglycerate in *Persephonella marina*.

During the last year we described or began the description of novel thermophilic and mesophilic organisms, namely *Tepidicella xavieri* and others. Among these species we have obtained all the necessary data to show that a new *Deinococcus* sp. is, unlike all other species of this genus non-radiation resistant. The implications to our knowledge of radiation resistance is immense and the complete genome sequence will begin in early 2007.

We evaluated and demonstrated the persistence of *Legionella* spp. in groundwater, used as water source in different thermal spas. *Legionella* strains were isolated from 176 water samples, collected from four boreholes, in two distinct geographical areas. FAME profiles analysis were used to identify *Legionella* species detected and the isolates were typed by Random Amplifed Polymorphic DNA; PCR technique - RAPD.

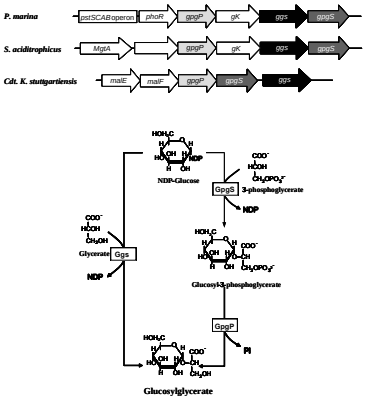
The biodiversity of a rare natural alkaline groundwater environment continues to be evaluated. The alkalinity in this particular environment is probably generated by active serpentinization results in an $Ca(OH)_2$ enriched, extremely diluted groundwater with pH 11.4, making it one of the most alkaline environment of earth.

The ISO Certified Microbiological Quality Control Lab for water and food was established with four fulltime technicians and researchers. It has since moved to the BIOCANT research campus in Cantanhede but is still associated with the CNCBC.

Ongoing and Future Work

We will continue to work on several aspects of osmotic adaptation of thermophiles and the biosynthesis of compatible solutes, particularly related to the synthesis of glucosylglycerate which serves other functions, namely as a precursor of a lipopolisccharide in mycobacteria (including *M. tuberculosis*) and a glycolipid in pathogenic *Nocardia* spp.

A large number of new extremophilic species have been isolated from hot springs, the Red Sea deep salt brines and elsewhere and are being characterized.



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MEDICAL MICOLOGY – YEAST Group (*Head: Teresa Gonçalves*)

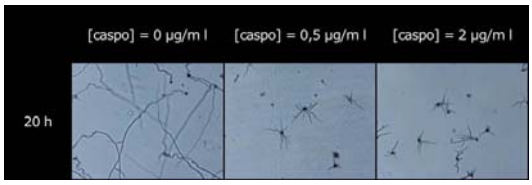
The outbreak of patients bearing immunitary deficiencies has led to an increased incidence of opportunistic fungal infections, difficult to diagnose, treat and with poor outcome. Our main goal is to understand how yeasts and molds respond to the presence of other organisms (the host or bacteria) and to antifungals.

Research Highlights

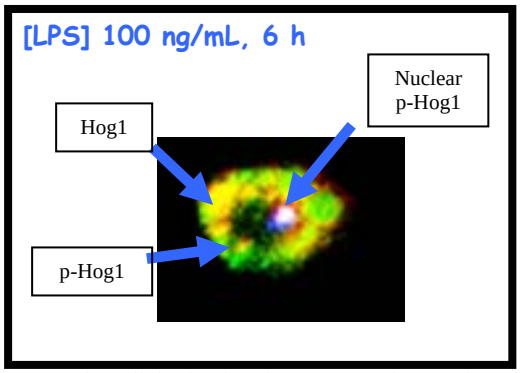
The research activity during 2006 was focused in topics regarding fungal infections, and the response of yeasts to environmental adversities such as bacterial components.

a) Clinical isolates, obtained from several hospitals and health centers, allowed us to construct a collection (1,000 different strains) of pathogenic yeasts, used in the validation of a chromogenic kit for the automatic identification of yeasts.

b) A case of cerebral phaeohyphomycosis in a child, caused by *Alternaria infectoria,* a dematiaceous fungus, prompt us to project, developed during 2006, allowing the identification, isolation and cloning of the AiFKS gene, and of caspofungin susceptibility of this mold, opening new perspectives in what regards the antifungal therapy of these infections, difficult to treat and with high mortality.



c) The activation of the yeast Hog1p, the homologue of the mammalian p38, upon exposure to bacterial endotoxin, as published by our group, may be an important pathway for the optimisation of fungal survival. The recognition that the ability of yeasts in sensing the presence of bacteria (through endotoxin detection) supports the idea of more robust yeast cells, with a potential impact in mixed infections where yeasts are both exposed to live and lysed bacteria (due to macrophage attack), such a LPS-induced stress/sensing mechanism would be an advantage for yeasts. New insights in what regards the evolution of the inflammatory system in eukaryotes are also expected with these studies, opening the possibility of considering the use of yeasts as a basic model to study the signal transducing mechanisms underlying inflammatory responses at the cellular level and to assist in the search and screening of new potential anti-inflammatory drugs.



d) Vpr1 is an HIV protein involved in the progression of AIDS. A study was initiated, together with clinical partners, with the final goal of constructing an yeast expression model that allows the prediction of mitochondrial disfunction as a marker of fast progressors related to different Vpr1 variants.

Ongoing and Future Work

Yeast response to bacterial endotoxin. The Hog1p activation due to bacterial LPS and concomitant increase in the robustness of *Candida albicans* under a situation of mixed infection, is being performed in collaboration with the Institute of Medical Sciences of the University of Aberdeen, UK. In *Saccharomyces cerevisiae* was initiated a study aimed to characterise the impact of LPS in the whole cell regulation.

Caspofungin susceptibility of Alternaria infectoria. The AiFKS gene expression in the presence of inhibitory caspofungin concentrations is being studied, using a real-time RT-PCR approach. The morphological impact of this antifungal on the mold is being studied using fluorescent probes.

Vpr1 expression in *Saccharomyces cerevisiae*. After the characterisation of the Vpr1 variants in mother-child pairs, finished during 2006, two variants were chosen, one from a fast progressor and another from a long-term survival patient. The two sequences, tagged the peptide coding sequence FLAG, are used to express the protein in the *yeast S. cerevisiae*, using a pYEX-BX plasmid. In this model a study will be conducted aimed to characterise the protein expression, i. e. Vpr1 localization, degree of mitochondrial disfunction, and, finally, the construction of a prediction kit.

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AREA E: BIOPHYSICS AND BIOMEDICAL NMR

Coordinator – Carlos Geraldes

Introduction

The research activity of this area is made up of two sub-areas:

1) Molecular Imaging and Inorganic Biochemistry, whose goal is the study of inorganic drugs for medical diagnostics and therapy, in particular, new Gd(III)-based chelates as targeted contrast agents for MRI, targeted agents for gamma scintigraphy in Nuclear Medicine, the mechanism of action of lithium salts in bipolar disease and development of new vanadium complexes as oral insulin-mimetic agents; the study of environmental and toxicological effects of inorganic species such as chromium(VI) compounds is also undertaken;

2) Biomedical NMR, involving studies of intermediate metabolism using stable isotope tracers and NMR, from the cellular level to perfused organs and humans with clinical applications.

The last two sub areas are a result of several years of collaboration between researchers from the CNC and from the NMR Center of the University of Coimbra. This collaboration, and the sharing of many facilities at the University of Coimbra in these subareas has enriched both research centers and the result have appeared in previous annual reports of CNC.

The present report includes only the work that is beeing performed in close colaborations and that is expected to lead to joint publications, leaving out many others fruitful interactions between the two research centers.

Intermediary Metabolism Group (*Head: John G. Jones*)

John G. Jones	(*Ph.D. – head of group*)
• Rui Carvalho	(Ph.D.)
• Madalena Caldeira	(Ph.D.)
• Ana Francisca Soares	(Ph.D. Student)
* João Duarte	(Ph.D. Student)
• Marco Alves	(Ph.D. Student)
• Sara Gonçalves	(Ph.D. Student)
• Tiago Alves	(Ph.D. Student)
• Claudia Silva	(Postgraduate Research Fellow)
• Daniela Ribeiro	(Undergraduate Student)
• Manuela Almeida	(Undergraduate Student)
• Cristina Barosa	(Technician)
• Patrícia Nunes	(Technician)

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

• Carlos G. Geraldes	(*Ph.D. – head of group*)
• Maria M. Catalão Castro	(Ph.D.)
• Maria Carmen Alpoim	(Ph.D.)
• Duarte A. Marques	(Ph.D.)
• Tiago Brandão Rodrigues	(Ph.D. Student)
• Giovannia Araujo Pereira	(Ph.D. Student)
• Teresa Jesus Delgado	(Ph.D. Student)
• Romeu Miranda Francisco	(Ph.D. Student)
• Rita Susana Rosa Branco	(Ph.D.Student)
• Ester Escribano Aranda	(MSc Student)
• Ana Isabel Rafael	(MSc Student)
• Maria João Silva Gonçalves	(MSc Student)
• Inês Violante	(Undergraduate Student)
• Sara Figueiredo	(Undergraduate Student)
• Tiago Rito	(Undergraduate Student)

• *Collaboration NMR Spectroscopy Unit (61/94)/CNC*

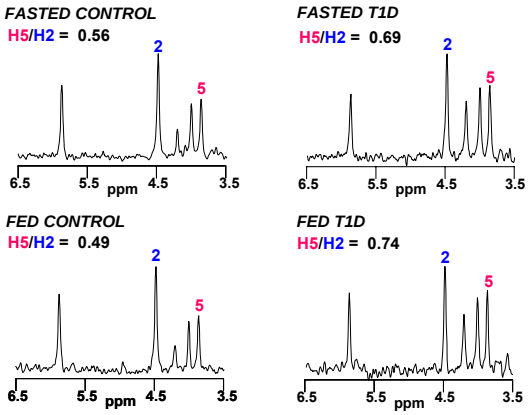
**Investigator doing collaborative research involving different groups at CNC.*

INTERMEDIARY METABOLISM GROUP *(Head: John G. Jones)*

In diabetes, the loss of glucose homeostasis results in secondary complications such as heart disease and blindness. To understand the precise effects of this on the function of liver, heart and brain, we are developing safe and practical stable-isotope tracer measurements of glucose metabolism in humans and in animal models of diabetes. These measurements are providing new insights about how glucose metabolism is modified in the liver, heart and brain in the setting of insulin resistance, hyperglycemia and diabetes.

Research Highlights

$^{2}H_2O$ and noninvasive biopsy of hepatic UDP-glucose by Paracetamol was used to study hepatic glycogen synthesis during feeding and its hydrolysis to glucose during fasting in a group of Type 1 Diabetic (T1D) patients and healthy controls. In TID patients compared to controls, a significantly higher fraction of liver glycogen was synthesized by the less efficient indirect pathway (glucose → G6P → C3 compounds →G6P→ → glycogen) during feeding, while during fasting, hepatic glycogen hydrolysis sustained a significantly smaller fraction of endogenous glucose production.



Metabolism in the hippocampus was studied using acutely dissociated hippocampal slices superfused in the presence of ^{13}C tracers and both ^{1}H- and ^{13}C-NMR spectroscopy. The adenosine A1 receptor was found to be involved in the regulation of hippocampal metabolism after hypoxia, being important to afford neuroprotection. Intermediary metabolism in hippocampal slices from STZ-treated rats was studied and was not different from controls. Learning and memory was evaluated in the Y-maze. Due to a locomotor deficit of the STZ-treated animals (evaluated in the open field arena) we were not able to evaluate mnemonic alterations in this model. In another study, profiles of gene expression in heart tissue were monitored for hearts made ischemic for various periods in the presence of two distinct cardioplegic solutions, Celsior and HBS (histidine buffered solution). The two solutions seem to have similar cardioprotective effects. However, the HBS solution seems to be more effective in relation to the inhibition of the apoptotic process and to the maintenance of cardiac metabolism while the Celsior seems to be more effective in protecting from the inflammatory process.

Ongoing and Future Work

We will develop new methods and paradigms for studying the interaction of glucose and lipid metabolism in the setting of insulin resistant and Type-2 diabetes conditions. Methodologies will include integrating high field human magnetic resonance studies with tracer measurements of glucose and fat oxidation/synthesis in individual organs, and developing novel LC-MS assays of lipogenesis and gluconeogenesis from deuterated water and other stable isotope tracers. The LC-MS methodology will facilitate the design of longitudinal metabolic studies in small animal models of diabetes.

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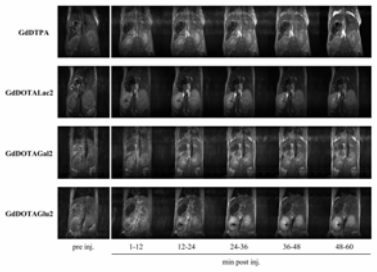
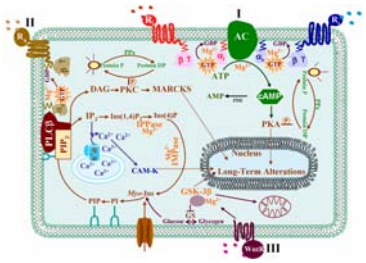
INORGANIC BIOCHEMISTRY AND MOLECULAR IMAGING GROUP *(Head: Carlos Geraldes)*

The general objectives are: 1) Design and development of metal based agents for multimodal targeted medical imaging agents, followed by *in vitro* and animal model evaluation using MRI and nuclear imaging techniques; 2) Studies of inorganic compounds for therapy – bipolar disorder and insulin-mimmetic agents – mechanisms of action using cell and animal studies; 3) Study of environmental and toxicological effects of inorganic species of Cr(VI) using cell studies.

Research Highlights

In 2006, several research projects were developed, focused on: *a) Synthesis and in vitro characterization of metal-based agents for Molecular Imaging (MI):* New targeted metal chelates useful as contrast agents for Magnetic Resonance Imaging (MRI) were developed, which have higher relaxivities and specific tissue targeting capacities. Gd^{3+} complexes with bifunctional ligands derived from DOTA or DTPA, bound to chemical functions which recognize cell markers, such as Gd^{3+}-glycoconjugates targeted to the internalizable liver asyaloglycoprotein membrane receptor (ASGPR), or micellar Gd^{3+}-amphyphilic chelates, recognized by the liver reticuloendotelial system, were synthetised and chemically and physically characterized *in vitro*, in particular their r_1 relaxivity (efficacy) and. These compounds, as well as a self-assembling Gd^{3+}-chelate metallostar were assessed by *in vivo* MRI of mice, while the *in vivo* pharmacokinetics and biodistribution of the $^{153}Sm^{3+}$- labeled compounds was studied in Wistar rats using gamma scintigraphy, yielding, respectively, very efficient hepatobiliary agents and very efficient and rapidly excreted kidney imaging compounds. *b) Metal Compounds for Therapy:* The molecular and cellular mechanisms of the therapeutic action of Li^+ in bipolar disease were reviewed and studied using neuronal cell models (rat cortical and hippocampal neurons and astrocytes) extracts of rat brain and rat models. The effect of Li^+ and other mood stabilizing agents, such as carbamazepine and valproate, on dopamine D2 receptor mediated inhibition of adenylate cyclase in cell models, and the interaction of dopamine D2 and beta-adrenergic receptors in the prefrontal cortex of rats was studied using microdialysis. New vanadium compounds were developed and chemically characterized in water solution in order to optimize their efficiency and minimize their toxicity as as oral insulin mimetic agents for type II diabetes. *c) Toxicology of metal ions:* The mechanism of Cr(VI)-induced carcinogenesis was studied using *in vitro* cell models, in particular by characterizing its effect on the signalling mechanisms downstream of genetic lesions.





Ongoing and Future Work

We are and will develop new metal based multimodal molecular imaging systems targeted to molecular markers of various pathologies (eg. integrins in neo-angiogenesis, amyloid deposits), including reporter groups based on quantum dots and other luminescent systems, targeted lipossomes or LDL particles containing Gd(III) chelates, inorganic nanoparticles, or micellar chelates specific for Ga(III) or Gd(III), or their radioactive isotopes (eg. ^{68}Ga for PET). Such agents will be assessed by cell and animal model (MRI, imaging studies. Insulin mimmetic inorganic agents will be further studied, as well as their toxicity and mechanism of action on target cells (eg. adipocytes). The effects of Li^+ on G proteins will also be further investigated. With the installation of a new high field (600 Mz) NMR spectrometer, integrated in the national NMR network and part of the national facility, with CP-MAS nanoprobe, will provide an opportunity to the development of metabolomic studies in intact pathological tissue biopsies and their extracts. If support and collaboration is established with the Molecular Biology and Biotechnology groups in the CNC, a much needed NMR based Structural Biology core of studies will be implemented.

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AREA F: CELL AND DEVELOPMENT BIOLOGY

Coordinators – Maria Celeste Lopes and João Ramalho Santos

Introduction

The main goal established for this Area in 2005 has begun to be fulfilled in 2006, both by strengthening existing research groups with new investigators, collaborations and areas of interest, and by recruiting young and successful Scientists to establish their own independent programs.

The investigators in this area are organized in three groups identified in the next pages. More recently, two new research groups have emerged focused on the: 1) Mechanisms of Insulin Resistance–the role of the adipocyte (Head: Eugénia Carvalho) and 2) Infection, Phagocytosis and Pathogens (Head: Otília Vieira)

The next step in the development of this area will be the continuing recruitment of collaborators and researchers (for example in the form of graduate students, or Post-Doctoral Fellows). The new research teams will join the three previously established Groups: Diabetes and Obesity, Cellular Immunology and Oncobiology, and Biology of Reproduction and Human Fertility.

Besides basic research in their respective fields, all the Groups in this Area have a common strong medical component, and projects have involved clinicians and clinical samples (both nationally and internationally), as can be quickly ascertained from their Research Highlights and Publication records. Therefore, during 2006 researchers continued to create and expand synergies with the University Hospitals and other Medical Centers in Portugal and abroad, both in terms of research projects and funding sources, an aspect that is essential for the future.

If possible, we hope to recruit more scientists in the upcoming year to address specific needs, or to further existing collaborations. We therefore expect to expand the trend of more medically relevant research. It should also be noted that some of the groups are already collaborating, namely the Diabetes and the Reproduction groups, that have a continuing joint project on the influence of Diabetes on mammalian fertility, and the Reproduction and Infection Groups, with a budding collaboration on the effects of surfactants in both infection and human sperm function. More importantly, all the groups listed have active collaborations with groups in other areas of the CNC (Neuroscience and Disease, Cellular and Molecular Toxicology and Molecular Biotechnology and Health).

The work performed by each group is briefly described in the following pages.

Cellular Immunology and Oncobiology Group

Celeste Lopes	(*Ph. D. – head of group*)
Alexandrina F. Mendes	(Ph.D.)
Anália do Carmo	(Ph.D.)
Maria Teresa Cruz Rosete	(Ph.D.)
Teresa Maria C. Martins	(Ph.D.)
*Carlos Bandeira Duarte	(Ph.D.)
Ana Bela Sarmento	(M.D., Ph.D.)
Adriana Teixeira	(M.D, HUC)
Alberto Orfao	(M.D., Ph.D., CIC)
Ana Cristina Gonçalves	(MSc Student, FMUC)
Américo Costa Figueiredo	(M.D., Ph.D., HUC)
Emília Cortesão	(M.D., MSc Student, FMUC)
Fernando Judas	(M.D., Ph.D., HUC)
Fernando Gomes	(M.D., HUC)
Hermínio José Tão E. Santo	(M.D., HUC)
Isabel Sousa	(M.D., HUC)
Manuela Lacerda	(M.D., Ph.D., IPO)
Marília Dourado	(M.D., Ph.D., FMUC and CIMAGO)
Maria Dolores Tabernero	(M.D., Ph.D., CIC)
Maria Margarida Gonçalo	(M.D., HUC)
Olinda Rebelo	(M.D., HUC)
Paulo Figueiredo	(M.D., IPO)
Ana Luisa Vital	(Ph.D. Student)
Artur Augusto Paiva	(Ph.D. Student)
Bruno Miguel das Neves	(Ph.D. Student)
Hugo Prazeres	(Ph.D. Student)
Inês Crespo	(Ph.D. Student)
José Mário Tenera Morgado	(Ph.D. Student)
Maria Teresa Matos	(Ph.D. Student)
Marta Viegas da Silva	(Ph.D. Student)
Rui Nobre	(Ph.D. Student)
Susana Carvalho Rosa	(Ph.D. Student)
Andreia Madeira	(Fellowship)

Diabetes and Obesity Group

Raquel Maria Fino Seiça	*(Ph.D.- head of group)*
Cristina Maria Tristão Sena	(Ph.D.)
Eugénia Maria L. Carvalho	(Ph.D.)
*Maria Sancha V. Santos	(Ph.D.)
*Paula Isabel S. Moreira	(Ph.D. Student)
Elsa Cristina Gomes Nunes	(MSc Student)
Teresa Margarida M. Louro	(MSc Student)
Paulo Nuno C. Matafome	(MSc Student)

Biology of Reproduction and Human Fertility Group

João Ramalho Santos	(*Ph.D.- head of group*)
Alexandra Amaral	(Ph.D. Student)
Sandra Amaral	(Ph.D. Student)
Sandra Varum	(Ph.D. Student)
Ana Paula Sousa	(Ph.D. Student)
Paula Mota	(Ph.D. Student)
Sandra Gamboa	(Researcher, Ph.D. Student)
Maria Inês Morte	(Masters Student)
Sara Diniz	(Masters Student)
Ana Sofia Rodrigues	(Masters Student)
Raquel Brito	(Masters Student)
Renata Tavares	(Student)
Marta Baptista	(Student)
Luís Martins	(Student)

Emerging Groups

Mechanisms of Insulin Resistance–the role of the adipocyte

Eugénia Carvalho (*Ph.D. - head of group*)

Infection, Phagocytosis and Pathogens Group

Otilia Vieira (*Ph.D. - head of group*)

Carla Cardoso (Post-doc)

**Investigator doing collaborative research involving different groups at CNC.*

CELLULAR IMMUNOLOGY AND ONCOBIOLOGY GROUP *(Head: Maria Celeste Lopes)*

The cellular immunology and the oncobiology sub-groups share common interests on the identification of the cellular mechanisms that regulate the function of normal human cells, trying to understand how disruption of these processes lead to disease.

The main objectives of this group are to identify: 1) cellular mechanisms involved in the development and progression of inflammatory and allergic diseases; 2) genetic abnormalities, gene expression profiles and new prognostic markers in tumours; and 3) molecular targets for new therapeutic strategies in prevention and/or treatment of chronic inflammation, allergy and cancer.

Research Highlights

In 2006, the research projects of the cellular immunology sub-group focused on studying: i) the efficacy of new putative cryoprotective agents to preserve chondrocyte functions, namely the viability and metabolism of articular chondrocytes and their ability to maintain cartilage integrity, and the modulation of articular chondrocyte functions by high and low glucose concentrations; ii) how skin sensitizers and irritants modulate the expression of dendritic cell surface molecules (chemokine and cytokine receptors), cytokine production and transcription factors activation; and iii) the role of the CD38 on regulation of immune responses

The research projects of the oncobiology sub-group focused on evaluating: i) chromosomal and genetic abnormalities of human glioma tumors; ii) the role of CD26/DPPIV expression and 8-azaguanine response in acute leukaemia, and proteasome inhibitors in chronic lymphocytic leukemia; and iii) the molecular changes relevant to thyroid carcinogenesis.

Ongoing and Future Work

We are developing a research program that involves several clinical units:

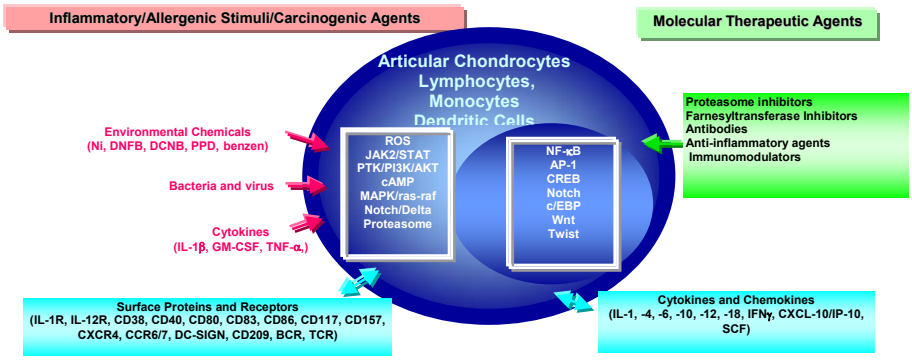
In collaboration with the Orthopaedic and Bone Bank Departments of the University Hospital of Coimbra, we are using 1) normal and osteoarthritic human articular cartilage and chondrocytes, from diabetic and non-diabetic patients, and a human chondrocytic cell line, to compare chondrocyte viability, dynamics of glucose transport and expression of glucose transporters; and 2) normal articular cartilage from cadaver tissue to identify new effective cryoprotective agents that may be used with large human osteochondral pieces at tissue banks.

In collaboration with the Dermatology Department of the University Hospital of Coimbra, we are using skin dendritic cells in culture to investigate the effect of skin sensitizers and irritants on the: 1) activation of intracellular signaling pathways and 2) expression of membrane-associated proteins and cytokine production. We are looking for the cellular events suitable for the development of in vitro tests to predict the sensitizing potential of chemicals.

In collaboration with the Neuropathology Laboratory and Neurosurgery Service of the University Hospital, Coimbra, and with the Center for Cancer Research of Salamanca, we are evaluating the chromosomal abnormalities, using iFISH, and the gene expression profile, using cDNA micro-arrays and SNP-array analysis, in samples of human brain tumors, previously diagnosed as gliomas.

In collaboration with the Portuguese Oncology Institute of Coimbra, we are studying: 1) the role of multifunctional ectoenzymes CD38 and CD157 in prostate cancer, 2) the genetic factors modifying BRCA1/2-associated breast and ovarian cancer risk and 3) the molecular changes relevant for thyroid carcinogenesis.

In collaboration with the Clinical Hematology Department of the University Hospital of Coimbra, we are studying haematological neoplasias to clarify the prognostic significance of point mutations involving N-Ras gene, and their correlation with the response to treatment.



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Matos TJ, Duarte CB, Gonçalo M and Lopes MC. 2005. Role of oxidative stress in ERK and p38 MAPK activation induced by the chemical sensitizer DNFB in a fetal skin dendritic cell line. *Immunol. and Cell Biol.* 83:607-614.

Cruz MT, Gonçalo M, Paiva A, Morgado JM, Figueiredo A, Duarte CB and Lopes MC. 2005. Contact sensitizers downregulate the expression of the chemokine receptors CCR6 and CXCR4 in a skin dendritic cell line. *Arch Dermatol Res*, 297: 43-47.

DIABETES AND OBESITY GROUP (*Head: Raquel Maria Fino Seiça*)

Type 2 diabetes has reached epidemic proportions. The disease often results in long-term micro and macrovascular complications including retinopathy, neuropathy, nephropathy and increased risk of cardiovascular disease.

The ongoing research in this group seeks to understand the cellular and molecular mechanisms of type 2 diabetes and its chronic complications.

The view of the adipocyte as a passive reservoir for energy storage is no longer valid; it is emerging as participant in regulating physiologic and pathologic processes, including immunity and inflammation, as a secretory and endocrine organ.

Thus, to discover the molecular links between obesity, insulin resistance and type 2 diabetes as a foundation for effective treatments and prevention strategies of diabetic complications are major goals.

Research Highlights

Diabetes is associated with long-term vascular complications. It is critical to improve treatment and prevention of the microvascular and macrovascular complications of diabetes because these complications account for the excessive morbidity and mortality associated with this disease. Our research work aimed to examine the action of new anti-diabetic, hipolipidemic and antioxidant drugs that prevent diabetic complications and characterize their cellular and molecular mechanisms of action on metabolic, oxidative and inflammatory status and vascular function of diabetic animal models.

There is a close interplay between adipose tissue, muscle, liver and pancreas, even in the early course of development of insulin resistance and diabetes. Better understanding of the mechanisms of adipose tissue regulation and identification of the molecular basis of the deregulated adipose tissue may provide new insights into the causes of insulin resistance, diabetes and the associated complications.

Ongoing and Future Work

The research studies are in five general categories:

1. Testing anti-diabetic and cholesterol-lowering drugs, when used alone and in combination, in animal models with type 2 diabetes and dyslipidemia for alteration in metabolic, oxidative and inflammatory markers and endothelial function.

2. Characterizing vascular endothelium dysfunction in diabetic animal models with atherogenic diet and trying to address novel therapeutical approaches that may improve endothelial dysfunction.

3. Evaluating signalling pathways within liver, for their role in the regulation of glucose metabolism in the animal models previously mentioned.

4. Studying the role of the adipocyte as an endocrine organ in congestive heart failure patients. We want to see if increased plasma levels of inflammatory cytokines, which seems to be directly related to the deterioration of cardiac performance, have an impact on insulin resistance in these patients.

5. Studying the molecular mechanisms by which glucocorticoids, important regulators of glucose, protein and lipid metabolism, acting mainly in the liver, muscle and adipose tissue, cause insulin resistance at the level of the adipocyte and their impact on the molecular mechanisms of insulin resistance in vivo.

Key References

Moreira PI, Rolo AP, Sena C, Seiça R, Oliveira CR, Santos MS (2006). Insulin attenuates diabetes-related mitochondrial alterations: a comparative study. *Med. Chem.,* 2: 299-308.

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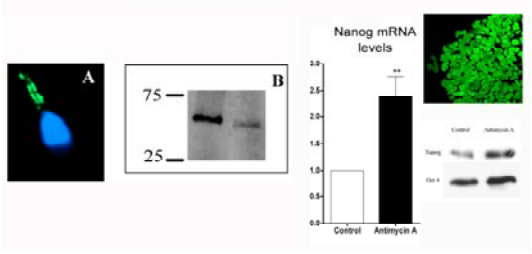
BIOLOGY OF REPRODUCTION AND HUMAN FERTILITY GROUP (*Head: João Ramalho-Santos*)

The main goal consists in determining what makes a good sperm, from a cellular, biochemical and molecular standpoints. Several animal models are used (horse, rat, cat, human), as well as wild populations of felids. We are currently researching changes in sperm that may correlate with fertility (mitochondrial DNA mutations, abnormal mitochondrial DNA replication, ATP production, antioxidant defenses), and the effect of diabetes on sperm metabolism and physiology. These studies are being carried out both in bulk populations of sperm from males with different semen characteristics, as well as in populations that have been sorted by either classical methods or flow cytometry, using a variety of markers. Furthermore, we are also studying the particularities of testicular mitochondrial bioenergetics, as well as the effect of mitochondrial bioenergetics on human embryonic stem cell pluripotency. Interestingly, fertile human sperm have many functional mitochondrial markers (see below, left), when compared with infertile samples. However, mitochondrial inhibition using Antimycin A results in an up-regulation of pluripotency markers such as Nanog in stem cells (below, right), suggesting a diverse role for mitochondria in different cellular processes.



Research Highlights

1- Novel sperm markers to assess human fertility (this work has been published, see Key References). 2- Detailed analysis of ATP production and antioxidant defenses in equine sperm, and impact on stallion fertility. The idea is to come up with the best possible indicators for stallion fertility, with relevance for animal breeding (part of this work has been submitted). 3-Discovery of proteins related to mitochondrial DNA replication in sperm, the presence of which varies with semen characteristics, determines mitochondrial DNA copy number in mature sperm, and may thus have implications for human fertility (this work is in press). 4- Characterization of metabolic and antioxidant defense adaptations that take place in the testis of animal models for diabetics, including a decrease in sperm and ATP production (leading to lower motility), as well as an up-regulation in the glutathione system (this work has been published). 5- Characterization of nuclear and mitocondrial genome cross-talk in mammalian somatic cell nuclear transfer embryos and pluripotent and differentiating embryonic stem cells (this work has been published, see Key References).

Ongoing and Future Work

1- Development of a novel simple assay to monitor human sperm quality with direct application to the clinical practice. This assay was derived from previous work carried out in the cat (Mota & Ramalho-Santos, 2006). 2- Employment of different strategies to create and analyze homogeneous subpopulations of sperm from the heterogeneous initial ejaculate using flow cytometry with a variety of functional markers, as well as more classical methodologies. The goal is to determine if the differences we have found between individuals in terms of mitochondrial DNA, functional markers etc, also apply to distinct subpopulations within the ejaculate of the same individual. 3-Analysis of the energetic competency of discarded human oocytes and failed fertilizations using the vital dye Brilliant Cresyl Blue. This will allow further analysis of the same samples by immunocytochemistry or RT-PCR, in search of changes that may be related to successful human fertilization. 4- Implementation and optimization of protocols to cryopreserve human ovarian tissue for future transplantation following oncological interventions. We will also study the influence of cryopreservation on ovarian cell apoptosis.5- Characterization of testicular apoptosis and mitochondrial bioenergetics, and how it compares with other tissues. 6- Dissect the role of mitochondria on embryonic stem cell pluripotency.

Key References

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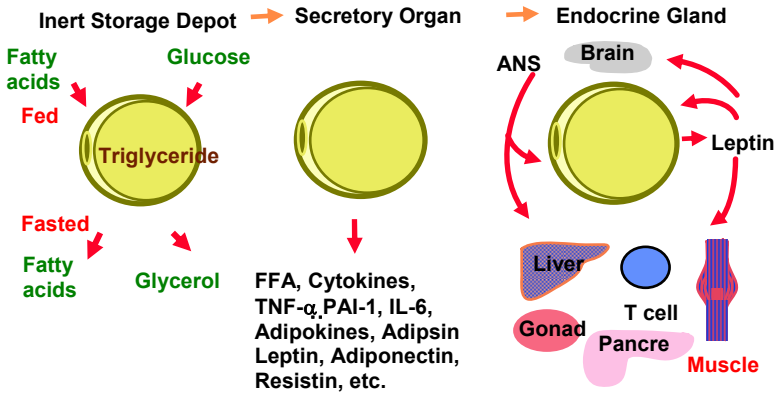
EMERGING GROUP

MECHANISMS OF INSULIN RESISTANCE – THE ROLE OF THE ADIPOCITE (*Head: Eugénia Carvalho*)

Research Highlights

- Mechanisms of insulin resistance, pathogenesis of type 2 diabetes and obesity
- Signal transduction pathways in the cardiovascular system
- The adipocyte as a source and target for inflammation and atherosclerosis
- Glucocorticoid effects on insulin action, glucose transport and metabolism

The view of the adipocyte as a passive reservoir for energy storage is no longer valid it is emerging as participant in regulating physiologic and pathologic processes, including immunity and inflammation, as a secretory and endocrine organ, modulating appetite, energy expenditure, insulin sensitivity, endocrine, reproductive systems and bone metabolism. It stores excess energy in the form of lipids and is able to dramatically change its size in agreement with changing metabolic needs, with obesity as the result, increasing in both adipocyte number and size. An excess of adipose tissue increases the risk for obesity, coronary artery disease, hypertension, lipid abnormalities, type 2 diabetes and even cancer. The obese state has been characterized by a deregulation of the adipose tissue that can cause a state of low-grade, chronic, systemic inflammation which can link both the metabolic and vascular pathologies. There is a close interplay between adipose tissue, muscle, liver and pancreas, even in the early course of development of insulin resistance and diabetes. Studies in healthy first-degree relatives of type 2 diabetic patients have shown that abdominal obesity, waist-hip ratio, and fat cell size are important markers of insulin resistance and already show deregulated adipose tissue at the molecular level. Better understanding of the mechanisms of adipose tissue regulation and identification of the molecular basis of the deregulated adipose tissue may provide new insights into the causes of insulin resistance, diabetes and the associated complications.



Ongoing and Future Work

1-We are studying the role of the adipocyte as an endocrine organ in congestive heart failure (CHF) patients. We want to see if increased plasma levels of inflammatory cytokines, which seems to be directly related to the deterioration of cardiac performance, have an impact on insulin resistance in these patients. We will show whether the insulin signaling pathway leading to Glut4 translocation in pericardial fat is defect, by measuring the protein/gene expression of insulin signaling molecules, such as IRS-1, Glut4 and adipokines in muscle, fat cells lysates and in the stromal-vascular fraction. We will assess the difference pro-inflammatory and/or anti-inflammatory signaling pathways in fat cells and macrophages, i.e. NF-kB, JNK, AMPK and MAP-K, differences/similarities with the insulin signaling pathway and evaluation of insulin resistance and degrees of inflammation in humans with CHF.

2-Glucocorticoids (GC) are important regulators of glucose, protein and lipid metabolism, acting mainly in the liver, muscle and adipose tissue. We want to investigate the molecular mechanisms by which these drugs cause insulin resistance at the level of the adipocyte and their impact on the molecular mechanisms of insulin resistance in vivo. Our main aim in this study is to compare the effects of prenatal exposure to different GC receptor ligands. We will be interested in monitoring the somatic changes of body weight at various post natal intervals, age at eye opening, and thymus and adrenal weights at sacrifice, as well as, various parameters reflecting metabolic activity and inflammation.

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Carvalho E. (2006) O adipócito: vítima ou vilão. *Endoc Metab & Nutrição*. 15(5):249-251

Carvalho E, Kotani K, Peroni O, Kahn BB. (2005) Adipose-specific overexpression of Glut4 reverses insulin resistance and diabetes in mice lacking Glut4 selectively in muscle. *Am J Physiol Endocrinol Metab.*289(4):E551-61

EMERGING GROUP

INFECTION, PHAGOCYTOSIS AND PATHOGENS GROUP (*Head: Otilia Vieira*)

The **Prevention** and **Treatment** of infection is becoming a big challenge for three main reasons:

1. More and more pathogens are developing multi-drug resistance;
2. Effective vaccines do not exist for the majority of them;
3. In poor regions around the world (Asia, Africa, Latin America, etc.) there is an urgent need for effective, safe, and affordable microbicides that can be used in combating transmission of contact-transmitted viral disease.

These limitations argue strongly for a better understanding of the mechanistic basis of infection in terms of the molecular and cellular mechanisms that regulate host-pathogen interactions on one hand, and for a real and urgent need to expand the range of preventive interventions for viral disease transmission on the other. Such knowledge may eventually lead to better weapons for preventing and combating infectious processes that will ultimately benefit us all.

Research Highlights

In 2006 we initiated two research projects entitled:

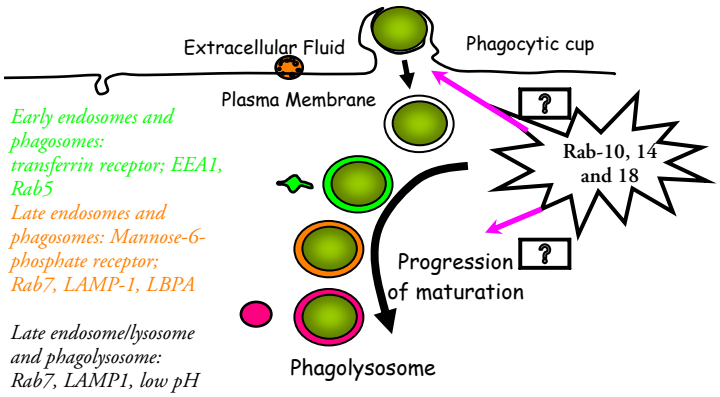
1. Role of Rab10, 14 and 18 on Fc-mediated phagocytosis and phagosomal maturation of inert latex particles;
2. Use of surfactants in disinfection and in prevention of viral disease transmission.

In the first research project we are using professional (Raw 264.7) and engineered phagocytes (HeLa and A431 cells stably transfected with the human FcγRIIA) and IgG-opsonized latex-beads. The cells are tranfected transiently with wild-type and truncated forms of Rab-10, -14 and –18.

In the second project we are performing a systematic study on the effect of surfactants on bacterial and fungus growth (*Escherichia coli* and *Candida albicans*), viral infection (adenovirus), and viability of mammalian epithelial cells and human sperm.

Ongoing and Future Work

At the moment we are investigating the time-course of the association between wild-type Rabs and phagosomes and the involvement of the same Rabs in the internalization and maturation (Scheme) of IgG-opsonized latex-particles. The latter experiments are being performed in cells that transiently overexpress the dominant-negative or consitutively-active forms of our Rabs of interest. Once this set of experiments is done the goal is to knockdown by RNA interference the endogenous Rabs and confirm the results obtained with the approach described above.



In the second research project, we are looking at the effect of anionic, cationic, zwitterionic and non-ionic surfactants on growth of E. coli, adenovirus infection and toxicity towards an epithelial cell line. The goal is to find a surfactant with bacteriostatic, bactericidal and antiviral activity and innocuos to the mammalian epithelial cells.

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Vieira OV, Gaus K, Verkade P, Vaz WL, and Simons K (2006). FAPP2, cilium formation and the compartmentalization of the apical membrane in polarized MDCK cells. *Proc. Nat. Acad. Sc. USA* 103(49):18556-18561.

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Amaral A, Ramalho-Santos J, St John JC The expression of polymerase gamma and mitochondrial transcription factor A and the regulation of mitochondrial DNA content in mature human sperm. *Hum Reprod.* (*in press*)

Cruz MT, Gonçalo M, Figueiredo A, Duarte CB and Lopes MC Effect of skin sensitizers on inducible nitric oxide synthase expression and nitric oxide production in skin dendritic cells: role of different immunosuppressive drugs. *Immunopharmacology and Immunotoxicolog.* (*in press*)

Dourado M, Sarmento AB, Pereira SP, Alves V, Silva T, Mota Pinto A, Rosa MS CD26/DPPIV expression and 8-azaguanine response in T-Acute Lymphoblastic Leukaemia Cell lines in Culture. *Pathophysiology.* (*in press*)

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Internationalization

5

Internationalization

Projects jointly with laboratories abroad and other international collaborations

Neuroscience and Disease

ATP and P2 receptors in epilepsy. B.Frenguelli (Dept.Neurosciences, Univ. Dundee, UK), Rodrigo A. Cunha (CNC-Portugal).

Axonal transport of mitochondria in the triple transgenic mouse model of Alzheimer disease. Jorge Busciglio (Department of Neurobiology and Behavior, University of California, Irvine, USA), Claudia Pereira (CNC-Portugal).

Biochemical and functional characterization of adenosine receptor dimmers. R.Franco, F.Ciruela (Dept.Biochemistry and Molecular Biology., Univ. Barcelona, Spain), Rodrigo A. Cunha (CNC-Portugal).

Cannabinoids and kainate receptors in the mice hippocampus. Giovanni Marsicano (Bordeaux), Ana Paula Silva and Joana Lourenço (CNC-Portugal).

Cell cycle reactivation in the triple transgenic mouse model of Alzheimer disease. Salvatore Oddo / Frank LaFerla (Department of Neurobiology and Behavior, University of California, Irvine, USA) , Claudia Pereira (CNC-Portugal).

Characterization of the BDNF-induced changes in the proteome of cultured hippocampal neurons. Michael Fountoulakis, (Foundation for Biomedical Research of the Academy of Athens, Greece), Carlos Duarte (CNC-Portugal).

Characterization of presynaptic P2 receptors. M.T.Miras-Portugal (Dept.Biochemistry and Molecular Biology, Complutense Univ., Spain), Rodrigo A. Cunha (CNC-Portugal).

Dysfunctional mitochondria recruits oligomeric and fibrillar α-syn to shut them down. Russell Swerdlow (Department of Neurology, University of Virginia Health System, Charlottesville, USA), Claudia Pereira (CNC-Portugal).

Effect of BDNF on the synaptic targeting of AMPA receptors. José A. Esteban (University of Michigan Medical School, Ann Arbor, USA), Ana Luisa Carvalho (CNC-Portugal).

Effect of dopaminergic injury and L-DOPA administration on GDNF expression in the nigrostriatal system in a rat model of Parkinson's disease. Jordi Alberch. (Department of Cell Biology and Pathologic Anatomy, School of Medicine, University of Barcelona, Spain), Carlos Duarte (CNC-Portugal)

Effect of long-term caffeine consumption on the adenosinergic system in the brain. A. de Mendonça (Lab. Neurosciences, Fac.Medicine, Univ. Lisbon, Portugal), Rodrigo A. Cunha (CNC-Portugal).

Electrophysiology of Layer V neurons and Neuropeptide Y. William Colmers (Edmonton), João O. Malva and Sara Xapelli (CNC-Portugal).

Functional binding of Neuropeptide Y receptors in epilepsy. David Woldbye (Copenhagen), João Malva, Ana Paula Silva and Joana Lourenço (CNC-Portugal).

Functional neuroprotection by BDNF. Wolf Frommer, (Carnegie Institution, Stanford, California, USA), Carlos Duarte (CNC-Portugal).

Growth factors and extracellular matrix regulate the survival and differentiation of mice neural stem/progenitor cells. Prof. João Bettencourt-Relvas (Institute of Cell Biology, Federal Institute of Technology Zurich, Zurich, Switzerland), A. C. Rego (CNC-Portugal).

Interaction between adenosine A_{2A} and GDNF receptors in the basal ganglia. J.A.Ribeiro (lab.Neurosciences, Fac. Medicine, Univ. Lisboa, Portugal), Rodrigo A. Cunha (CNC-Portugal).

Interactions between cannabinoids and adenosine in the control of memory. R.Takahashi (Dept.Pharmacology, Univ.Fed.Santa Catarina, Brazil), Rodrigo A. Cunha (CNC-Portugal).

Interaction between P2 and NMDA receptors. J.Lerma (Cajal Institute, CSIS, Spain), Rodrigo A. Cunha (CNC-Portugal).

Localization and role of P2 receptors in the cerebellum. F.A.Edwards (University College London, UK), Rodrigo A. Cunha (CNC-Portugal).

Mitochondrial dysfunction and changes in alpha-synuclein phosphorylation and toxicity in a human dopaminergic model of Parkinson's disease – a protective role for GDNF. Dr Tiago Fleming Outeiro (Harvard Medical School, MA, USA), A. C. Rego (CNC-Portugal).

Mitochondrial protective effects of HDAC inhibitors. Prof. David G. Nicholls and Prof. Lisa Ellerby (Buck Institute for Age Research, Novato, California, USA), A. C. Rego (CNC-Portugal).

Neuroprotection by adenosine receptors focusing on their influence in neuro-inflammation. J.F.Chen (Dept.Neurology, University of Boston, USA), Rodrigo A. Cunha (CNC-Portugal).

NMDA receptor traffic and synaptic plasticity. Ann Marie Craig (Brain Research Centre, University of British Columbia, Vancouver, Canada), Ana Luisa Carvalho (CNC-Portugal).

Organotypic hippocampal slice cultures. Jens Zimmer (Odense), João O. Malva, Liliana Bernardino and Sara Xapelli (CNC-Portugal).

Pharmacological characterization of adenosine A_{2A} receptors in the brain. B.B. Fredholm (Lab. Pharmacology, Karolinska Institute, Sweden), Rodrigo A. Cunha (CNC-Portugal).

Presynaptic kainate receptors in the mossy fiber CA3 synapses. Christophe Mulle (Bordeaux), João O. Malva and Paulo Pinheiro (CNC-Portugal).

Protein cleavage in the ischemic rat brain. Robert Gabriel, (Department of Experimental Zoology and Neurobiology, University of Pecs, Pecs, Hungary), Carlos Duarte (CNC-Portugal).

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

Retinal gene expression profiles in an experimental model for diabetic retinopathy. Dr. Willem Kamphuis, (Netherlands Ophthalmic Research Institute, Amsterdam, Gelderse Blinden Stichting, Netherlands), Landelijke Stichting voor Blinden en Slechtzienden, (Netherlands), Rotterdamse Vereniging Blindenbelangen, (Netherlands), Francisco Ambrosio (CNC-Portugal).

Role of presynaptic adenosine receptors in the basal ganglia. S.Ferré (National institute of Drug Abuse, Bethesada, USA), Rodrigo A. Cunha (CNC-Portugal).

Role of adenosine A_{2A} receptors in the control of LPS-induced neuro-inflammation. M.A.Lynch (Dept.Physiology, Trinity College, Dublin, Ireland), Rodrigo A. Cunha (CNC-Portugal).

Role of adenosine A_{2A} receptors in the control of mossy fibers. C.Mulle (UMR 5291 CNRS, Univ. Bordeaux, France), Rodrigo A. Cunha (CNC-Portugal).

Role of adenosine A_{2A} receptors in the control of memory dysfunction. D.O.Souza (Inst.Biochemistry, Univ.Fed.Rio Grande do Sul, Brazil), Rodrigo A. Cunha (CNC-Portugal).

Role of cortactin in the trafficking of AMPA-type glutamate receptors. Andras Kapus (The St. Michael's Hospital Research Institute, Toronto, Canada)., Ana Luisa Carvalho (CNC-Portugal).

Role of nucleus ataxin-3 on mitochondrial function – implication for neurodegeneration in Machado-Joseph disease. Prof. Henry Paulson (Department of Neurology, University of Iowa Carver College of Medicine, IA, USA), A. C. Rego (CNC-Portugal).

Role of pro-inflammatory cytokines and microglia in excitotoxic neuronal death. Annamaria Vezzani (Milan), João O. Malva and Liliana Bernardino (CNC-Portugal).

T cells and Experimental Autoimmune Encephalitis. Hartmut Wekerle (Munich), João Malva and Sofia Domingues (CNC-Portugal).

The involvement of oxidative stress in Alzheimer disease. George Perry (College of Sciences, University of Texas at San Antonio, Texas, USA) and Mark Smith (Institute of Pathology, Case Western Reserve University, Cleveland, USA), Claudia Pereira (CNC-Portugal).

Molecular Biotechnology and Health

Application of non-viral suicide gene therapy approaches in animal models for cancer: molecular and cellular events associated with the antitumor response. Valérie Pierrefite-Carle (Unité INSERM, Faculté de Medicine, Nice, France), M. Conceição Pedroso de Lima (CNC-Portugal).

Data Mining of Protein Unfolding Simulations. Paulo Azevedo (Universidade do Minho, Portugal), Rui Brito (CNC-Portugal).

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

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

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

Grid Warehousing and Data Mining of Protein Unfolding Simulations. Werner Dubitzky (University of Ulster, UK), Rui Brito (CNC-Portugal).

Lentiviral vectors-mediated ataxin-3 gene silencing. Nicole Déglon & Philipe Hentraye (URA CEA-CNRS 2210, Service Hospitalier Frederic Joliot, MIRCen Program, Departement de Recherches Medicales, Direction des Sciences du Vivant, Commissariat a l'Energie Atomique (CEA), 91401 Orsay Cedex, France), Luís Pereira de Almeida (CNC-Portugal).

Mechanisms of nuclear import and export of ASFV genome. Maria Salas (Centro de Biologia Molecular, Universidad Autonoma de Madrid, Spain), M. Conceição Pedroso de Lima (CNC-Portugal).

Molecular mechanisms of Amyloid formation by Transthyretin. Maria João Saraiva and Ana Margarida Damas (Universidade do Porto), Rui Brito (CNC-Portugal).

Protein docking and drug design. Richard Michael Jackson (Leeds University, UK), Rui Brito (CNC-Portugal).

Protein stability and amyloidogenesis in Ataxin-3. Sandra Macedo Ribeiro (Universidade do Porto), Rui Brito (CNC-Portugal).

Protein structure and dynamics using high field, multidimensional solution NMR. Christina Redfield (Oxford University, UK), Rui Brito (CNC-Portugal).

Protein structure using high field solid state NMR. Hartmut Oschkinat (Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany), Rui Brito (CNC-Portugal).

Regulation of mRNA stability by 3'UTR binding proteins. Matthias Hentze (EMBL Heidelberg), Carlos Faro (CNC-Portugal).

Serine protease inhibitors. Maria Luiza Oliva (Escola Paulista de Medicina, S. Paulo, Brasil), Carlos Faro (CNC-Portugal).

Structural characterization of cell penetrating peptides. Francisco Gavilanes (Departamento de Bioquímica y Biologia Molecular I, Facultad de Ciencias Quimicas, Universidad Complutense de Madrid, Spain), M. Conceição Pedroso de Lima (CNC-Portugal).

Structure-function relationship of aspartic proteases. Daniel Bur (Actelion, Switzerland), Carlos Faro (CNC-Portugal).
Substrate specificy and inhibition of aspartic proteases. Ben Dunn (University of Florida, Gainesville, USA), Carlos Faro (CNC-Portugal).

Targeted antisense therapy: a novel approach for the treatment of human small cell lung cancer. Uwe Zangemeister-Wittke (Department of Pharmacology, University of Bern, Switzerland), João Nuno Moreira (CNC-Portugal).

Cell and Molecular Toxicology

Bile acids and mitochondrial dysfunction; Nucleoside analogue reverse transcriptase inhibitors (NRTI)-induced mitochondrial depletion and cardiomyopathy. Prof. Kendall Wallace (University of Minnesota-Duluth, USA), Anabela Rolo (CNC-Portugal).

Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Rafael Radi and Homero Rubbo (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay), João Laranjinha (CNC-Portugal).

Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Jon O. Lundberg (Department of Physiology and Pharmacology, Karolinska Institutet, Sweden), João Laranjinha (CNC-Portugal).

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

Doxorubicin-induced mitochondrial cardiomyopathy, Prof. Kendall Wallace (University of Minnesota-Duluth, USA), Paulo J. Oliveira (CNC-Portugal).

Mitochondrial and nuclear targets of novel natural and synthetic anti-proliferative agents, Prof. Jon Holy and Prof. Edward Perkins (University of Minnesota-Duluth, USA), Paulo J. Oliveira (CNC-Portugal).

Mitochondrial genetics and biochemistry; Mitochondrial genetic disease; Mitochondrial involvement in apoptosis; Mitochondrial degeneration in aging. Prof. Gino Cortopassi (University of California, Davis, USA), Anabela Rolo (CNC, Portugal).

Mitochondrial tolerance and liver ischemic preconditioning: pathophysiological mechanisms. Prof. Joan Rosseló (Barcelona, Spain), Anabela Rolo (CNC-Portugal).

Nitric oxide and excitotoxicity. The role of astrocytes. Simon Heales (Institute of Neurology, University College London), João Laranjinha (CNC-Portugal).

Nitric oxide in neurodegeneration and aging. Enrique Cadenas (Dept. Molecular Pharmacology & Toxicology, University of Southern California, USA), João Laranjinha (CNC-Portugal).

Role of oxidized LDL in PI3K/Akt/mTOR signaling pathway in vascular smooth cells and putative counteracting effects of resveratrol. Anne Nègre-Salvayre (INSERM Unit 466, Biochemistry Department, CHU Rangueil,Toulouse, France), João Laranjinha (CNC-Portugal).

Microbiology

Biodiversity of extreme environments. Frederick Rainey (Louisiana State University,USA), Milton Costa (CNC-Portugal).

Biodiversity of extreme environments. Robert Huber (University of Regensburg andKommunale Berufsfachschule für Biologisch-technische Assistenten, Germany), MiltonCosta and André Antunes (CNC-Portugal).

Biotechnology of extremophiles. Garo Antranikian (Hamburg University of Technology,Germany), Milton Costa (CNC-Portugal).

Expression of compatible solute genes from metagenomes from abyssal environments. Edward DeLong (MIT, USA), Milton Costa (CNC-Portugal).

Genetic tools for thermophilic bacteria. José Berenguer (Universidad Autónoma de Madrid, Spain), Milton Costa and Nuno Empadinhas (CNC-Portugal).

Radiation-resistant organisms. John Battista (Louisiana Sate University, USA), Milton Costa (CNC-Portugal).

Biophysics and Biomedical NMR

European Molecular Imaging Laboratory. Bernard Tavitian (CEA, Orsay, Paris), (Network of 56 European Universities), Carlos Geraldes (CNC-Portugal).

Metal-based systems for Molecular Imaging applications. (Network of about 50 European Universities), Carlos Geraldes (CNC-Portugal).

Non-invasive NMR studies of organ function with stable isotope tracers and contrast agents. Sebastian Cerdan (Laboratorio de RMN, Instituto de Investigaciones Biomédicas), Alberto Sols (CSIC, Univerisdade Autónoma de Madrid, Espanha), Carlos Geraldes (CNC-Portugal).

Cytotoxic and Therapeutic Effects of Transition Metal Ions, Virtudes Moreno Martinez (Universidade de Barcelona, Espanha). Carlos Geraldes (CNC-Portugal).

Vanadium Complexes with Ligands derived from Pyrimidinones with Potential Insulin Mimetic Properties, Fernando Avecilla Porto (Universidade da Coruna, Espanha), João Pessoa (Universidade Técnica de Lisboa). Carlos Geraldes (CNC-Portugal).

Hepatic intermediary metabolism of glucose in children with and without hepatic glucose-6-phosphatase activity. Shawn Burgess (Dallas), John Jones (CNC-Portugal).

Noninvasive measurement of hepatic glycogen kinetics in Type 1 diabetics. Michael Roden (Vienna), Matthew Merritt (Dallas), Michel Beylot (Lyon), John Jones (CNC-Portugal).

Noninvasive NMR studies of organ function with stable isotope tracers and contrast agents. Sebastian Cerdan (Madrid), John Jones (CNC-Portugal).

Influence of Transplant Preservation Solutions in Cardiac Metabolic and Immunological Profiles. Rui Carvalho(CNC-Portugal).

Relation Between Metabolic Alterations in the Hippocampus and Memory Deficits Induced by Diabetes. Rui Carvalho (CNC-Portugal).

Prevenção pela cafeína do deficit de memória causado pela diabetes. Rodrigo Cunha (CNC Portugal).

Os recursos pesqueiros no âmbito da politica europeia de desenvolvimento sustentável: Uma abordagem interdisciplinar para o caso do robalo (Dicentrarchus labrax), Miguel Pardal (FCTUC-Portugal), John Jones (CNC Portugal).

Oxidative Stress in Heart Disease: Role of Catecholamines. John Jones (CNC Portugal).

Improvement of Insulin Oral Availability Through Encapsulation in Polyelectrolyte Complex Nanoparticles. John Jones (CNC Portugal).

Cell and Development Biology

Assessment of intratumoral genetic heterogeneity in gliomas: impact on the clinical and biological behaviour of the disease. Alberto Orfão, (Center for Cancer Research Universitiy of Salamanca), Maria Dolores Tabernero (Research Unit of the University Hospital, Salamanca), Maria Celeste Lopes (CNC-Portugal).

Impact of diabetes on articular chondrocyte functions: identification of pharmacological targets. Ali Mobasheri (Division of Veterinary Medicine, School of Veterinary Science and Medicine, University of Nottingham, Leicestershire, UK), Maria Celeste Lopes (CNC-Portugal).

Multifunctional ectoenzymes CD38and CD157: role in solid tumors and lymphoid malignancies. Fran Lund (Trundeau Institute, Saranac Lake, USA), Maria Celeste Lopes (CNC-Portugal).

Mitochondrial DNA alterations in sperm that may relate to human fertility. Justin St John (University of Birmingham, UK), João Ramalho Santos (CNC-Portugal).

Metabolism and organelle dynamics in embryonic stem cells. Christopher Navara, Gerald Schatten and Paul Sammak (University of Pittsburgh, USA), João Ramalho Santos (CNC-Portugal).

Reproductive potential of freeze-dried sperm in primates. Gabriel Sanchez-Partida (Monash University, Australia), João Ramalho Santos (CNC-Portugal).

PARTICIPATION IN THE ORGANIZATION OF SCIENTIFIC MEETINGS

March 2006

“Smart Toxicology: Subcellular Targets of Anti-cancer Therapy”. 2nd Edition of the International courses of Toxicology at Center for Neurosciences and Cell Biology.
Date: 22-24 Março 2006, Coimbra
CNC members involved in the organization: João Laranjinha, Paulo Oliveira, Leonor Almeida,Carlos Palmeira

April 2006

“Toxicology of nitric oxide and oxygen free radicals”. Curso integrado no Programa Doctoral em Biomedicina e Biología Experimental do Centro de Neurociências e Biologia Celular de Coimbra.
Date: 10-13 Abril 2006 Coimbra
CNC members involved in the organization: João Laranjinha

“Then and Now. Diversity and Physiology”. Organização de palestra na Fundación Ramon Areces, National Academy of Catalonia.
Date: April, 27- 28 2006 Barcelona, Espanha.
CNC members involved in the organization: Milton Costa

"From Mad Cows to Neurotic Yeast, and Back: Molecular Approaches for Studying Neurodegenerative Diseases"
Date: April 28, 2006, Coimbra
CNC members involved in the organization: Cristina Rego

July 2006

"CAG triplet repeat disorders" - 5th Forum of European Neuroscience
Date: July 8-12 2006, Viena Austria
CNC members involved in the organization: Cristina Rego

"Life at the Limits". Membro da Comissão Científica do 2nd Congress of European Microbiologists e coordenador do simpósio.
Date: July 4-8 2006. Madrid
CNC members involved in the organization: Milton Costa

“The synthesis of sugar derivatives and their role in osmotic adaptation in bacteria and archaea”. 2nd FEMS Congress of European Microbiologists.
Date: July 4-8 2006. Madrid
CNC members involved in the organization: Milton Costa

September 2006

"Protein Folding". VI Ibero-American Congress of Biophysics
Date: September, 24 - 27 2006, Madrid, Spain
CNC members involved in the organization: Rui Brito

November 2006

"Die or Survive? Molecular Mechanisms of Bile Acid" Faculdade de Farmácia, Universidade deLisboa.
Date: November,16 2006 Coimbra
CNC members involved in the organization: Cristina Rego

December 2006

“Aging and Cell Death” 15th National Congress of Biochemistry
Date: 8-10 December 2006 Aveiro
CNC members involved in the organization: Carlos Duarte

Graduate Studies Programme

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GRADUATE STUDIES PROGRAMME

During 2006, CNC organized 18 Advanced Courses and hosted 27 seminars. The seminars were attended by local graduate students and researchers, whereas the advanced courses also met the interest of people from other Portuguese Universities. Besides the organization of courses and seminars, CNC also supported the ongoing research work for Ph.D. and M.Sc. thesis. Throughout this year, 17 Ph.D. and 5 M.Sc. thesis were concluded.

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

Advanced Courses 2006

January 2006

Neurosystems
January 9-13
Miguel Castelo-Branco (Portugal), Louis Toth (USA), Elia Formisano (Netherlands), Rui Costa (USA)

Neuromodulation
January 16-20
João O. Malva (Portugal), Ana P. Silva (Portugal), Armando Cristóvão (Portugal), Christophe Mulle (France), William Colmers (Canada), Rosa Cossart (France)

Mini-Course: Biostatistics
January 23-25
Pedro Anastácio (Portugal)

February 2006

Structural Biology: Amyloidosis
February 6-10
Rui Brito (Portugal), Sandra Ribeiro (Portugal), Daniel A. Kirschner (USA), Sheena E. Radford (UK), Steven Finkbeiner (USA)

Immunology
February 13-17
Celeste Lopes (Portugal), Teresa Cruz (Portugal), Alexandrina F. Mendes (Portugal), Kai Zacharowski (Germany), Anabela Cordeiro da Silva (Portugal), Carmen Garcia-Rodriguez (Spain)

Genetics of the Nucleus
February 20-24
Isabel Marques Carreira (Portugal) Catarina Resende de Oliveira (Portugal), Maj A. Hultén (UK), Thomas Liehr (Germany)

March 2006

Biology of Proteolysis
March 6-10
Paulo Pereira (Portugal), David J Katzmann (USA), Randy Hampton (USA), Robert C Piper (USA)

Cancer Gene Therapy
March 14-17
Conceição Pedroso-Lima (Portugal), Sérgio Simões (Portugal), João Nuno Moreira (Portugal), Esther Chang(USA) , Mirco Ponzoni (Italy)

Toxicology: Anticancer therapy
March 22-24
Paulo Oliveira (Portugal), Leonor Almeida (Portugal), Carlos Palmeira (Portugal), João Laranjinha (Portugal), Sten Orrenius(Sweden), Catherine Brenner (France), Ulf Rapp (Germany), Jon Holy (USA)

Diabetes and Vascular Disease
March 27-31
Raquel Seiça (Portugal), Cristina Sena (Portugal), María Molsalve (Spain), Fausta Natella (Italy), Michael Boarder (UK), Franscisco Ambrósio (Portugal), Sérgio Lemos (Portugal)

April 2006

Toxicology of Nitric Oxide
April 10-13
João Laranjinha (Portugal), Rafael Radi (Uruguay), Ana Denicola (URUGUAY), Homero Rubbo (Uruguay)

Functional Genomics
April 17-21
Paulo Santos (Portugal), Stephen Bustin (UK), Jo Vandesompele (Belgium), Katja Kotsch (Germany), José Cabeda (Portugal)

October 2006

Structural Biology
October 9-13
Ulrich Baumann (Switzerland), Christina Redfield (UK), Rui Brito (Portugal), Sandra Ribeiro (Portugal)

Lab Rotations
October 16 - November 03
Rodrigo Cunha (Portugal), João Malva (Portugal), Cláudia Pereira (Portugal), João Nuno Moreira (Portugal), Sérgio Simões (Portugal), Milton Costa (Portugal), João Gomes (Portugal), Carlos Duarte (Portugal), Sandra Santos (Portugal), Joana Ferreira (Portugal), Ana Luísa Carvalho (Portugal), Claudia Cavadas (Portugal), Paulo Santos (Portugal), Inês Araujo (Portugal), Francisco Ambrósio (Portugal), Paulo Oliveira (Portugal), Teresa Martins (Portugal), Maria Celeste Lopes (Portugal), Cristina Rego (Portugal)

November 2006

Lab Rotations
November 13-24
Rodrigo Cunha (Portugal), João Malva (Portugal), Cláudia Pereira (Portugal), João Nuno Moreira (Portugal), Sérgio Simões (Portugal), Milton Costa (Portugal), João Gomes (Portugal), Carlos Duarte (Portugal), Sandra Santos (Portugal), Joana Ferreira (Portugal), Ana Luísa Carvalho (Portugal), Claudia Cavadas (Portugal), Paulo Santos (Portugal), Inês Araujo (Portugal), Francisco Ambrósio (Portugal), Paulo Oliveira (Portugal), Teresa Martins (Portugal), Maria Celeste Lopes (Portugal), Cristina Rego (Portugal)

Molecular Cell Biology
November 27 - December 01
Carlos Duarte (Portugal) Gregg G. Gundersen (USA) , Geri Kreitzer (USA), Edgar R. Gomes (USA), Helder Maiato (Portugal)

December 2006

Development Biology
December 04-07
Pedro Domingos (USA), Rui Martinho (Portugal), Antonio Jacinto (Portugal), Josh Brickman (Scotland), Leonor Saude (Portugal), José António Belo (Portugal), Moises Mallo (Portugal), Guillermina López-Bendito (Spain)

Reproductive Biology
December 11-15
João Ramalho-Santos (Portugal), Christopher Navara (USA), António Granado (Portugal)

Seminars
2006 Series | IBILI 16:00 h

January

13 Pharmacogenetic approaches to study cognitive and sensorimotor dysfunction
Rui M. Costa | Dept. of Neurobiology, Duke University Medical Center, Durham NC, USA

20 Spikes and Snacks: The Electrophysiology of Obesity
William Colmers | Department of Pharmacology University of Alberta, Edmonton, Canada

February

17 Negative regulation of Toll-like receptors. Implications in atherogenesis
Carmen García-Rodríguez | Instituto de Biología y Genética Molecular (CSIC-UVA), Facultad de Medicina, Universidad de Valladolid, Espana

24 When are chromosomes, chromosomes?
Thomas Liehr | Institut fur Humangenetik und Anthropologie, Jena, Germany

March

10 Regulation of multivesicular body cargo recognition and sorting
David J. Katzmann | Mayo Clinic Colege of Medicine, Rochester, MN, USA

17 Tumor vascular technology: a new therapeutic strategy for cancer
Mirco Ponzoni | Differentiation Therapy Unit Laboratory of Oncology G. Gaslini Children's Hospital Genoa, Italy

24 Mitochondria, oxidative stress and apoptosis
Sten Orrenius | Karolinska Institute, Sweden

30 Regulation of vascular endothelial cells by receptors (15:00 h)
Michael Boarder | Leicester School of Pharmacy De Montfort University Leicester, England

31 Molecular and cellular cognition: memory and how it fails
Alcino J. Silva | Departments of Neurobiology, Psychiatry and Biobehavioral Sciences, Psychology and Brain Research Institute, UCLA Los Angeles, USA

April

7 Real-time detection of fatty acid synthesis and TCA-cycle anaplerosis in cultured human glioma cells with 13C NMR spectroscopy
Anthony Mancuso | The University of Pennsylvania, USA

13 Nitric Oxide-derived oxidants, mitochondria and apoptotic signalling
Rafel Radi | School Medicine, Univ. Mentevideo, Uruguay

21 Laser capture microdissection and real-time PCR analysis in biomedical sciences
Stephen A. Bustin | Barts and the London Queen Mary's School of Medicine and Dentistry, University of London, UK.

28 From Mad Cows to Neurotic Yeast, and Back: Molecular Approaches for Studying Neurodegenerative Diseases
Tiago Fleming Outeiro | Mass GeneralÊInstitute for Neurodegenerative DiseaseÊ- MIND Harvard Medical School Charlestown, USA

May

3 Plasmodium - Hepatocyte interactions: implications for protection against Malaria
Local: Sala de Higiene; 3rd. Floor; Faculty of Medicine | Time: 12:00 hrs
Patricia R.S. Leirião | Instituto Gulbenkian de Ciência / Instituto de Medicina Molecular

4 The protective/toxic effects of four flavonoids on hepatic mitochondria - Possible relevance for cancer development
Local: Amphitheater of the Department of Zoology | Time: 16:00 hrs
Daniel J. Dorta | Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brasil

5 Embryoid bodies as models for studying embryonic hematopoiesis
Leonor Parreira | Instituto de Medicina Molecular Faculdade de Medicina de Lisboa, Lisboa, Portugal

October

13 Using NMR to study calcium binding in modular proteins containing epidermal growth factor-like (EGF) domains
Christina Redfield | Department of Biochemistry, University of Oxford , U.K.

20 Plasmodium and its host - how good is their interaction?
Maria Mota | Institute of Molecular Medicine, Lisbon

27 Metabolomics and Biomarkers in the Context of Inborn Errors of Metabolism
Isabel Tavares de Almeida | Centro de Patogénese Molecular, Faculdade de Farmácia, Universidade de Lisboa

November

3 Cardiac Mitochondrial Toxicity of Antineoplastic Agents
Paulo Oliveira | Centro de Neurociências e Biologia Celular, Coimbra

10 Stem cells from the subventricular zone (SVZ) of the adult brain: source of new neurons for brain repair?
Fabienne Agasse | Centre for Neuroscience and Cell Biology | Instituto de Bioquimica, Universidade de Coimbra

16 Die or Survive? Molecular Mechanisms of Bile Acid Effects
Cecília Rodrigues | Centro de Patogénese Molecular, Faculdade de Farmácia, Universidade de Lisboa

24 The apical membrane and ciliogenesis in polarized epithelial cells: is there a connection?
Otília Vieira | Centro de Neurociências e Biologia Celular, Coimbra

29 Functional Analysis of Kinesins during Epithelial Polarization
Geri Kreitzer | Dept. of Cell and Developmental Biology, Weill Medical College of Cornell University, New York, USA

December

6 Mechanisms of Axon Guidance: How to get to the right place [10:00 h]
Gulillermina Lopez Bendito | Instituto de NeurocienciasCSIC-Universidad Miguel Hernández, Campus de San Juan, San Juan de Alicante, Spain

14 Modulation of ion channels by changes in lipid composition , exogenous compounds, and osmotic pressure gradients [Anfiteatro Quim. Fisiológica, Rés-do-chão, Fac. Medicina, 15:00 h]
Sid Simon | Department of Neurobiology, Duke University Medical Center Durham, U.S.A.

15 Do human embryonic stem cells disregulate the cell cycle, similar to early cancer cells?
Christopher Navara | Pittsburgh Development Center, University of Pittsburgh, USA

THESIS CONCLUDED IN 2006

Ph.D. Thesis

Ana Cristina Saavedra Martins, “Neuroprotective response to dopaminergic injury: role of neuronglia crosstalk”.
22 de Dezembro de 2006
Orientadores: Maria Celeste Lopes e Jordi Alberch

Ana Paula Kuan Yon Chung, “Caracterização de Espécies dos Géneros meiothermus e Thermus, e Diversidade Filogenética de Estirpes de Thermus Globalmente Distribuídas”.
3 de Novembro de 2006
Orientadores: Milton Costa e Frederick Rainey

Ana Sofia Bregieiro Eulálio, "Transporte Núcleo-Citoplasmático de Proteínas Estruturais do Vírus da Peste Suína Africana: Mecanismos e Relevância Virológica da Proteína".
Janeiro de 2006
Orientador: Conceição Pedroso Lima
Co-Orientador: Sérgio Simões

André Guimarães Lemos Antunes, "Microbiology of the Northern brine-filled pools of the Red Sea".
13 de Janeiro de 2006
Orientadores: Robert Huber
Co-Orientador: Milton Costa

Catarina Isabel Ribeiro Pimentel, “Caracterização Molecular e Funcional da Região Promotora dos Genes das Cardosinas”.
20 de Novembro de 2006
Orientadores: Carlos Faro e Dominique Van Der Stracken

Constança Sofia Ferreira de Figueiredo, “Regulating HLA class I Expression to Decrease Immunogenicity of Cellular Therapeutics”.
21 de Dezembro de 2006
Orientadores: Alberto Órfão e Carlos Duarte

Cristina Isabel dos Santos Fonseca, "Nanovesículas de Base Lipídica para Vectorização de Oligonucleótidos *Antisense*: Desenvolvimento, Caracterização Fisico-Química e Avaliação da Actividade Anti-Tumoral em Modelos Celulares e Animal de Leucemia Humana”
Novembro de 2006
Orientador: Sérgio Simões
Co-Orientador: Conceição Pedroso Lima

Fernanda Maria Lopes Ferreira, “Alterações no Metabolismo Mitocondrial em Modelos Animais de Diabetes Mellitus”.
17 de Janeiro de 2006
Orientadores: Maria Sancha Santos e Carlos Palmeira

Jorge Miguel de Ascenção Oliveira, “Mitochondrial function and Ca^{2+} homeostasis in experimental models of Huntington’s disease: Understanding disease pathogenesis and pharmacological interventions”.
12 de Dezembro de 2006
Orientador: Prof. Doutor Jorge Gonçalves
Co-Orientador: Prof. Doutora Ana Cristina Rego

Liliana Cristina Pereira Montezinho, “Efeitos do Lítio nos processos de transdução de Sinal Acoplados À Adenilato Ciclase – Importância para o Tratamento da Doença Bipolar”.
10 de Março de 2006
Orientadores: Maria Margarida C. A. Castro e Carlos F. G. C. Geraldes

Luísa Maria Oliveira Pinheiro Leitão Cortes Bastos, “Peptidase, uma nova Aminopeptidase N do Pólen de Parietaria Judaica: Efeito na integridade e na função do epitélio pulmunar”.
19 de Dezembro de 2006
Coordenadores: Euclides Pires e Paula Verissimo

Maria Manuela Monteiro Grazina, "Genoma Mitocondrial e Défice Energético no Diagnóstico das Doenças da Cadeia Respiratória mitocondrial."
20 de Junho de 2006
Orientadores: Catarina Resende de Oliveira

Marília João da Silva Pereira Rocha, “Caracterização do perfil cinético da gentamicina e da vancomicina em recém-nascidos prematuros”.
24 de Novembro de 2006
Orientador: Amílcar Falcão

Miguel Luís Cunha Mano, "Péptidos Permeantes como Sistemas Promissores para Transporte de Ácidos Nucleicos: Mecanismos de Internalização Celular e Actividade Biológica do Péptido S4(13)PV"
Fevereiro de 2006
Orientador: Conceição Pedroso Lima
Co-Orientador: Sérgio Simões

Paulo César da Silva Pinheiro, “Presynaptic kainate receptors in the hippocampus: a critical role for GluR7 at the mossy fiber synapses”.
19 de Janeiro de 2006
Orientadores; João O. Malva
Co-Orientadores: Christophe Mulle

Teresa Margarida Torcato da Conceição Proença de Almeida, “Interaction between purinergic and nicotinic receptors”.
21 de Março de 2006
Orientador: Rodrigo A. Cunha

Zélia Maria Cordeiro da Silva, "Biological Significance of Trehalose for Thermus SPP - Biochemical Characterization of trehalose Synthesis and Transport mechanisms in Thermus thermpphilus".
16 de Janeiro de 2006
Orientadores: Milton Costa e Frederick Rainey

Master Thesis

Ana Cristina Gomes "Polen. proteases e alergia"
12 de Dezembro de 2006
Orientadores: Doutora Paula Verissimo e Doutor António Veríssimo

Helena Sofia Nogueira Correia, “*Agrimonia eupatoria* L. *e Equisetum telmateia* Ehrh. Perfil Polifenólico e Capacidade de Captação de Espécies Reactivas de Oxigénio”.
23 de Janeiro de 2006.
Orientadores: Teresa do Carmo Pimenta Dinis e Maria Teresa Batista

Joana Medeiros Vieira Marques,"Envolvimento da proteína Hog1 de Saccharonyces cerevisiae na resposta da levedura lippolissalarido bacteriana".
2 de Novembro de 2006
Orientadores: Doutora Teresa Gonçalves e Doutor António Veríssimo

Nuno Ricardo Esteves Ferreira, “Detecção Electroquímica de Óxido Nítrico em Fatias de Hipocampo de Rato com Microeléctrodos de Fibra de Carbono”.
Maio 2006.
Orientadores: Rui M. Barbosa e João Laranjinha

Paula Cristina Ramos Mota "A qualidade espermática em felídeos: desenvolvimento e comparação de diferentes marcadores no gato doméstico".
3 de Fevereiro de 2006
Orientador: Doutor João Ramalho

Outreach Programme

7

OUTREACH PROGRAMME

The CNC Outreach Programme aims at fostering research through interaction with clinical faculty and industry, as well as, society education through specific programmes.

1-INTERACTION WITH CLINICAL FACULTIES

1.1 Biomedical Inter-Institutional Research Programme (CNC, HUC, CHC, IPO)

1.1.1. Psychiatry Research: Molecular Genetics Studies of Complex Disorders

(Carlos Pato, Michele Pato (University of Southern California.), M.H. Azevedo, C.R. Oliveira (CNC))

These studies are focused on the identification of candidate genes for Schizophrenia and Bipolar Disorder through the use of linkage and association analysis. For this purpose two 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 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.

In 2006, these studies have utilized "state of the art" DNA and RNA microarray technology to identify chromosomal regions of linkage to each disorder, as well as areas of increased expression in the presence of illness. This convergent genetic-genomic approach has led to the identification of several areas in the human genome to target for follow-up study, most notably on Chromosome 5 for schizophrenia and psychosis, and on Chromosome 6 for Bipolar Disorder. Additionally, collaborators from the Broad Institute in the United States and from the Centre on Addiction and Mental Health (CAMH) in Toronto, Canada have been using the sample, collectively known as the "Portuguese Island Collection," to investigate specific candidate genes for schizophrenia, including neuregulin 1, syntaxin 1A, and genes from the dopaminergic and serotonergic systems. We have also formed the International Shizophrenia collaborative to use whole genome approaches to define the genomics of this disorder.

PUBLICATIONS

Pato CN, Middleton FA, Gentile KL, Morley CP, Medeiros HM, Macedo A, Azevedo MH, Pato MT, (2005) "Genetic linkage to chromosome 6q22 is a consistent finding in Portuguese subpopulations and may generalize to broader populations. *Am J Med Genet B Neuropsychiatr Genet*. Apr 5;134(1):119-21.

Middleton FA, Pato MT,Gentile KL, McGann L, Brown AM, Trauzzi M, Diab H, Morley CP, Medeiros HM, Macedo A, Azevedo MH, Pato CN, (2005) Jul 5 "Gene expression analysis of peripheral blood leukocytes from discordant sib-pairs with schizophrenia and bipolar disorder reveals points of convergence between genetic and functional genomic approaches". Am *J Med Genet B Neuropsychiatr Genet*.; 136(1):12-25.

Petryshen TL, Middleton FA, Kirby A, Aldinger KA, Purcell S, Tahl AR, Morley CP, McGann L, Gentile KL, Rockwell GN, Medeiros HM, Carvalho C, Macedo A, Dourado A, Valente J, Ferreira CP, Patterson NJ, Azevedo MH, Daly5 MJ, Pato CN, Pato MT, Sklar P, (2005) Apr "Support for involvement of neuregulin 1 in schizophrenia pathophysiology", *Mol Psychiatry*.; 10(4):366-74, 328.

Albert HC Wong, Likhodi O, Trakalo J, Yusuf M, Sinha A, Pato CN, Pato MT, Van Tol HHM, and Kennedy JL, (2005) Jul 26 "Genetics and transcription of 14-3-3 Isoforms in schizophrenia and bipolar disorder" *Schizophrenia Research*, [Epub ahead of print].

Petryshen TL, Middleton FA, Tahl AR, Rockwell GN, Purcell S, Aldinger KA, Kirby A, Morley CP, McGann L, Gentile KL, Medeiros HM, Carvalho C, Macedo A, Albus M, Maier W, Trixler M, Eichhammer P, Azevedo MH, Schwab SG, Wildenauer DB, Pato MT, Pato CN, Daly MJ, Sklar P, (2005) Dec "Genetic Investigation of chromosome 5q $GABA_a$ Receptor subunit genes in schizophrenia", *Mol Psychiatry*. 10(12):105.

Matthew B. McQueen, B. Devlin, Stephen V. Faraone, Vishwajit L. Nimgaonkar, Pamela Sklar, Jordan W. Smoller, Rami Abou Jamra, Margot Albus, Silviu-Alin Bacanu, Miron Baron, Thomas B. Barrett, Wade Berrettini, Deborah Blacker, William Byerley, Sven Cichon, William Coryell, Nick Craddock, Mark Daly, J. Raymond DePaulo, Howard J. Edenberg, Tatiana Foroud, Michael Gill, T. Conrad Gilliam, Marian Hamshere, Ian Jones, Lisa Jones, Suh-Hang Juo, John R. Kelsoe, David Lambert, Christoph Lange, Bernard Lerer, Jianjun Liu, Wolfgang Maier, James D. MacKinnon, Melvin G. McInnis, Francis J. McMahon, Dennis L. Murphy, Markus M. Nöthen, John I. Nurnberger, Jr., Carlos N. Pato, Michele T. Pato, James B. Potash, Peter Propping, Ann E. Pulver, John P. Rice, Marcella Rietschel, William Scheftner, Johannes Schumacher, Ricardo Segurado, Kristel Van Steen, Weiting Xie, Peter Zandi, and Nan M. Laird, (2005) Oct; "Combined Analysis from Eleven Linkage Studies of Bipolar Disorder Provides Strong Evidence for Susceptibility Loci on Chromosomes 6q and 8q" *Am J Hum Genet*. 77(4):582-95. Epub 2005 Aug 15.

Ni X, Trakalo J, Valente J, Azevedo MH, Pato MT, Pato CN, Kennedy JL, (2005) Jul 19 "Human p53 tumor suppressor gene (TP53) and schizophrenia: Case-control and family studies". *Neurosci Lett*. [Epub ahead of print]

Middleton FA, Trauzzi MG, Shrimpton AE, Gentile KL, Morley CP, Medeiros H, Pato MT, Pato CN,Complete maternal uniparental isodisomy of chromosome 4 in a subject with major depressive disorder detected by high density SNP genotyping arrays. Am J Med Genet B Neuropsychiatr Genet. (2006) Jan 5;141(1):28-32

1.1.2. Neurology Research: studies on neurodegenerative disorders
(*Luis Cunha (H.U.C.), Catarina Oliveira (CNC)*)

Cerebrospinal fluid (CSF) biomarkers identification in Alzheimer´s Disease (AD) has been one of our areas of interest. CSF biomarkers would be of great value in the early diagnosis of AD and in increasing diagnostic accuracy.
We studied a group of 74 patients with Dementia (54 with AD (NINCDS-ADRDA), 20 with other types of Dementia non-AD type (NAD)), 14 individuals with Mild Cognitive Impairment (MCI) and a group of 55 non-demented age-matched controls. CSF levels of tau protein, amyloid $\beta_{(1-42)}$ protein (Aβ42) and tau protein phosphorylated at threonine-181 (p-tau181) were determined. Apolipoprotein E (ApoE) genotyping was performed in peripheral blood.
We have found significantly increased levels of tau protein and p-tau181 and decreased levels of Aβ42 in AD patients relative to NAD patients and controls. MCI patients showed tau protein and p-tau181 levels similar to AD patients, while Aβ42 were significantly higher than in AD patients, similar to NAD patients and controls. In the group of AD patients, the presence of at least one ApoE-ε4 allele resulted in higher tau protein and p-tau181 levels and lower Aβ42 levels.

We have continued working on the biochemical characterization of MCI and early AD, and assessment of peripheral markers with predictive value for the evolution of the disease (MCI to AD).
Three subject groups were recruited: 35 cognitively healthy age-matched controls, 84 patients with MCI (Petersen criteria) and 39 with mild AD (NINCDS-ADRDA criteria/CDR=1). Antioxidant defenses, lipid/protein oxidation markers and nitric oxide metabolites were measured. The activity of glutathione peroxidase and reductase was assessed in erithrocytes.
The longitudinal study of MCI patients, (3 years follow up) has been acomplished in 29 patients and has showed a significant decrease in erithrocytes antioxidant defenses (vitamin E and reduced glutathione), while the levels of lipid oxidation markers were increased. The activity of glutathione peroxidase and reductase did not change and an increase in plasmatic levels of antioxidant defenses uric acid and reduced glutathione were found. The results were independent of the presence of ApoE-ε4 alleles.

1.1.3 Pediatric Research: metabolic disorders
(Luísa Diogo (CHC); Catarina Oliveira (CNC); Manuela Grazina (CNC))

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. Inherited defects causing mitochondrial dysfunction can be due to mutations either in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA). Each mitochondrion contains its own DNA that codes for 13 peptides of the mitochondrial respiratory chain (MRC) system, where the oxidative phosphorylation (OXPHOS) occurs, plus the two structural rRNAs and 22 tRNAs necessary for mtDNA genes expression.

We have continued the approach implemented last year for the molecular differential analysis of mitochondrial cytopathies, as a high-throughput screening. The quantification of mtDNA mutation, by real time PCR, is being implemented and a collaboration with University of Newcastle upon Tyne has been established. The evaluation of complex I activity in cell suspensions, namely fibroblasts, and the study of Krebs cycle enzymes both in fibroblasts and peripheral blood leukocytes is now being applied in the differential diagnosis with metabolic disorders.

In 2006 the experimental work leading to the MSc Thesis intitled "Determination of the frequency of mtDNA haplogroups in patients suspected of Mitochondrial Cytopathy" was concluded. The study was performed in 350 subjects, including 83 subjects with definite diagnosis based on mtDNA analysis positive result (PDD), and 216 control subjects. A statistically significant difference was detected between haplogrup H1 frequencies of PDD and controls, (29% and 15%, respectively; $p<0.008$). The results suggest that macro-haplogrups JT, UK and L may influence clinical phenotype manifestation, but a higher phylogenetic resolution in a higher magnitude sample, is required to confirm these data. Accordingly, the accumulation of polymorphisms in the internal branches of human phylogeny could affect the fixation of potentially deleterious mtDNA mutations and influence the phenotype expression.

A PhD Thesis focused on the MRC activity and mtDNA investigation, during a period of 7 years (1997-2003), was also concluded. The study included the evaluation of 577 individuals, including 46 family members and 531 patients, followed at several Portuguese hospitals, mainly Paediatric Hospital (346) and Neurology Service of University Hospitals (160) of Coimbra. Our data show that MRC deficiency can be detected in leukocytes isolated from peripheral blood (LEU) with high sensibility, but with a low specificity, as compared to evaluation in other(s) tissue(s). Additionally, we have clearly confirmed that analysis of isolated mitochondria and fresh frozen muscle homogenate (HM2) provide different results concerning MRC analysis. These results highlight additional information contributing to a more accurate biochemical study of muscle tissue, taking into account the differences observed for the fractions obtained during mitochondria isolation. The genetic analysis of mtDNA has allowed the identification of mtDNA sequence variations in 120 individuals, including six new alterations probably pathogenic. These results have contributed to the definite diagnosis of MRC defect in 89 patients (17.5%), including 56 adults and 28 children. The estimated frequency for pathogenic mtDNA mutations in children and adults is 8.3:100,000 (1:12,048) and 2.8:100,000 (1:35,714), respectively, in the center region of Portugal.

As expected, we have found high heterogeneous results, concerning all data presented, both at genetic, or biochemical level, as well as clinical information. Nevertheless, a positive correlation, for the same tissue, for both biochemical and

genetic data could be demonstrated in some cases. In subjects with multiple CRM deficiencies, diverse mtDNA alterations were found, essentially deletions (isolated point mutation was identified only in two individuals). In addition, a positive correlation was observed, taking into account mtDNA analysis and the CRM enzymatic activity evaluation.

Taking into consideration the total number of cases evaluated, both adults and children, it was possible to establish the diagnosis of a significant number of cases. According to demographic data for Centre Portugal region population results (CENSOS 2001; source: INE), a frequency of 1:20,000 for DCRM in the population of Centre Portugal could be estimated.

PUBLICATIONS

Grazina MMM (2006). Mitochondrial Genome and Energetic Deficiency in Diagnosis of Mitochondrial Respiratory chain Diseases (*PhD Thesis*).

Correia C, Coutinho AM, Diogo L, Grazina M, Marques C, Miguel TS, Ataíde A, Borges L, Oliveira C, Oliveira G, Vicente AM (2006). High frequency of biochemical markers for mitochondrial dysfunction in autism: no association with the mitochondrial aspartate/glutamate carrier SLC25A12 gene. *J Autism Dev Disord.* 36(8): 1137-1140.

Cordeiro M, Rodrigues D, Garcia P, Lopes da Silva S, Macário MC, Vilarinho L, Grazina M, Oliveira CR, Diogo L (2006). Sindrome de Leigh: revisão de oito casos. *Sinapse*, 6 (1): 118.

Grazina MM, Diogo L, Garcia P, Silva E, Garci T, Robalo C, Oliveira C. Atypical presentation of Leber`s hereditary optic neuropathy associated to mtDNA 11778G > A point mutation – A case report. *Eur J Paediatr Neurol* (*in Press*).

1.1.4 DNA investigation in Neurodegenerative disorders

(Catarina Oliveira (CNC); Manuela Grazina (CNC))

Neurodegenerative disorders are complex disorders 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, as well as to support the development of more rationale therapies, including the implementation of pharmacogenetic approach.

We have continued the mtDNA screening analysis in Alzheimer's disease (AD) and Frontotemporal dementia (FTD) patients and age matched controls. Evidences that mtDNA mutations/ polymorphisms may contribute to the risk of disease expression, coul be demonstrated. The evaluation of mtDNA *ND1* sequence variations in a larger sample of FTD patients started to be performed, 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).

A strategy to improve the study of the mtDNA haplogroups, to be applied in the investigation of neurodegenerative disorders, including AD and FTD was set up. The phylogenetic resolution was improved and 10 new haplogroups are now available for analysis. The frequency of 27 mtDNA haplogroups in 358 patients (mean age 71.2 years) and 230 age matched controls (mean age 64.3 years), was evaluated. So far, no relevant differences were detected, considering individual haplogroups. The grouping analyses will be performed in order to increase the statistical probability of finding significant differences in the frequency distribution.

A collaboration project with the Centre for Hereditary Eye Diseases, Department of Ophthalmology, University Hospital of Coimbra, and Visual Neuroscience Laboratory, Faculty of Medicine of Coimbra, has been initiated, in order to set up the genome screening and evaluate the involvement of mtDNA sequence variations and MRC activity in eye diseases.
RNA analysis for the investigation of genetic expression variations is being performed.

During the last year we have continued to focus on the nuclear genetic factors known to underlie the process of neurodegeneration, mainly in Alzheimer's (AD) and Parkinson's (PD) diseases. Given the socio-economic impact of neurodegenerative disorders such as Parkinson's and Alzheimer's disease, it is important to design a viable strategy for the delineation of genetic predisposition in complex traits. We are tackling this problem from different perspectives: first, by studying rare familial forms of disease and then extrapolating the function of genes involved to related conditions – for this purpose, we are continuing to collect samples from individuals with positive familial history who do not present a clear genetic cause to their condition; second, identifying common genetic variability that confers risk for disease – in order to accomplish this, we are collecting as many samples from idiopathic forms of these diseases as possible, in collaboration with the Movement Disorders and Dementia Clinics nation-wide. Additionally, we have collected control samples for a series representative of the elderly Portuguese population – 400 samples from healthy individuals (mean age 67,9 years) were collected, which will allow to obtain a clear view of the genetic variability present in the Portuguese population.

PUBLICATIONS

Grazina M, Pratas J, Silva F, Oliveira S, Santana I, Oliveira C (2006). Genetic basis of Alzheimer's dementia. *Genes, Brain and Behaviour* 5(2), 92–107. Review.

Guerreiro RJ, Bras JM, Santana I, Januario C, Santiago B, Morgadinho AS, Ribeiro MH, Hardy J, Singleton A, Oliveira C. (2006) Association of HFE common mutations with Parkinson's disease, Alzheimer's disease and mild cognitive impairment in a Portuguese cohort. *BMC Neurol.* 6:24.

1.1.5. Cardiovascular Research - Basic Research Unit in Cardiology

(Lino Gonçalves (HUC/IBILI), Pedro Monteiro (HUC/CNC))

The basic research unit in Cardiology has focused its research in diabetes and ischemic cardiomyopathy. We used an animal model of type 2 diabetes, the Goto-Kakisaki rat, fed with an atherogenic diet or submitted to a hypocaloric regime; this animal model was then used to study several anti-diabetic and lipid-lowering drugs (both isolated and in association). This model was used in two different experimental settings: an acute one, with the drugs used only during ischemia-reperfusion, which was induced in the whole heart, later submitted to differential centrifugations to isolate the mitochondrial and citosolic fractions. Several parameters were studied, including mitochondrial swelling, calcium buffering capacity, oxidative stress and caspase cascade activation.

In the other setting, animals were treated with several drugs during four weeks (insulin, metformin, atorvastatin, gliclazida and combinations of these drugs) and then submitted to global ischemia-reperfusion or preconditioning. After this protocol, the same parameters as in the previous setting were evaluated. Before being treated with a drug and again before being sacrificed, blood and urine were taken to measure inflammatory and oxidative stress markers (as well as adipokines), in order to assess their modification by the drugs studied.

We have also used another study protocol, designed to study the cell and molecular biology of the cardiac tissue from hearts explanted from subjects with advanced heart failure and treated with cardiac transplantation.
Besides that, we are continuing our studies with nicorandil, trough cooperation with the University of São Paulo.

PUBLICATIONS

Carreira RS, Monteiro P, Goncalves LM, Providencia LA. 2006, Carvedilol: just another Beta-blocker or a powerful cardioprotector? *Cardiovasc Hematol Disord Drug Targets.* 6:257-266.

Facundo HT, Carreira RS, de Paula JG, Santos CC, Ferranti R, Laurindo FR, Kowaltowski AJ. 2006, Ischemic preconditioning requires increases in reactive oxygen release independent of mitochondrial K+ channel activity. *Free Radic Biol Med.* Feb 1;40(3):469-79.

Campos CB, Degasperi GR, Pacifico DS, Alberici LC, Carreira RS, Guimaraes F, Castilho RF, Vercesi AE. 2004, Ibuprofen-induced Walker 256 tumor cell death: cytochrome c release from functional mitochondria and enhancement by calcineurin inhibition. *Biochem Pharmacol.* Dec 1;68(11):2197-206.

1.1.6. Dermatology research: contact dermatitis

(Margarida Gonçalo (HUC), Américo Figueiredo (HUC), Teresa Cruz (CNC), Celeste Lopes (CNC))

As part of the collaboration with the Department of Dermatology of the University Hospital, we have two main objectives: the identification of new therapeutic targets for allergic contact dermatitis and the identification of cellular markers that allow the in vitro recognition of the skin sensitization potential of environmental chemicals. Our results show that among all the chemicals studied, namely the contact sensitizers (2,4-dinitrofluorbenzene (DNFB), 1,4-phenylenediamine (PPD), nickel sulphate (NiSO4)), the inactive analogue of DNFB, 2,4-dichloronitrobenzene (DCNB) and two irritants (sodium dodecyl sulphate and benzalkonium chloride), only NiSO4 increased the expression of inducible nitric oxide synthase (iNOS) in skin dendritic cells. We compared the effects of three different immunosuppressors on the iNOS expression and NO production elicited by the contact sensitizer NiSO4. The results show that dexamethasone, unlike cyclosporin A and sirolimus, inhibited iNOS expression and NO production stimulated by the contact sensitizer nickel sulphate. This can explain, at least in part, why topical and systemic steroids are more effective in the treatment of allergic contact dermatitis than the new immunosuppressors. More recently, we found that the skin sensitizers, DNFB, PPD and NiSO4, modulate the expression of dendritic cell surface molecules (chemokine and cytokine receptors), the cytokines production and transcription factors activation differently from skin irritants.

PUBLICATIONS

Cruz M.T., Gonçalo M., Figueiredo A., Duarte C.B. and Lopes M.C. Effect of skin sensitizers on inducible nitric oxide synthase expression and nitric oxide production in skin dendritic cells: role of different immunosuppressive drugs. *Immunopharmacology and Immunotoxicology* (*in press*).

Gonçalo M., Cruz M.T., Duarte C.B., Figueiredo A., Lopes M.C. (2006). Immunossupressive Drugs in Ni-induced iNOS expression in skin dendritic cells. *Contact Dermatitis*, 55 S1: 43

1.1.7. Arthritis research: inflammation

(Fernando Judas (HUC), Alexandrina Mendes (CNC), Celeste Lopes (CNC))

In collaboration with the Orthopaedic and Bone Bank Departments of the University Hospital, we are currently developing the projects entitled "Regulation of the response of normal and arthritic chondrocytes to pro- and anti-inflammatory cytokines" and "Evaluation of chondrocyte viability and metabolic activity in different conditions of cryopreservation of osteochondral allografts" using normal and osteoarthritic human articular chondrocytes. The first project aims at elucidating the role of the NOS isoforms in the regulation of the chondrocyte response to pro- and anti-inflammatory cytokines and at identifying molecular targets that can lead to new therapeutic strategies to arthritic diseases. The major objective of the second project is to develop a method for the cryopreservation of osteochondral allografts that maintains chondrocyte viability and metabolic activity and that is suitable for use at the Bone Bank Department of the University Hospital. The results of these two projects are expected, respectively, to identify molecular mechanisms that can be translated into new therapeutic strategies for arthritic diseases and to improve the survival rate of implanted osteochondral allografts, thus direct and positively affecting the clinical outcome.

PUBLICATIONS

Rosa S.C., Teixeira L., Lopes C., Mendes A.F. (2006). Chondrocyte viability in fresh and frozen large human osteochondral allografts: effect of cryoprotective agents. *Transp. Proc.* (*in press*)

Mendes A.F., Rosa S.C., Judas F., Lopes M.C. (2006). Chondrocyte Viability in Human Tibial Plateaus Cryopreserved with the Natural Glycosylated Hydroquinone, Arbutin. *Osteoarthritis Cartilage*. 14 (supp.B): P385.

Rosa S.C., Mobasheri A., Lopes M.C., Mendes A.F. (2006). Glucose transport in immortalized human chondrocytes (C-28/I2) in normoxia and hypoxia. *Osteoarthritis Cartilage*. 14 (supp.B): P179.

1.1.8. Research in brain cancer: genetic heterogeneity of gliomas

(Alberto Orfao (Centro de Investigación del Cancer, Salamanca), Fernando Gomes (HUC), Celeste Lopes (CNC))

The project 'Assessment of intratumoral genetic heterogeneity in gliomas: impact on the clinical and biological behaviour of the disease' is being developed in collaboration with the Neuropathology Laboratory and Neurosurgery Service of University Hospital of Coimbra and with the Research Unit of the University Hospital and Center for Cancer Research of Salamanca. In this project, allelic imbalances in chromosome regions of human gliomas are evaluated using interphase fluorescence *in situ* hybridization (iFISH) and the gene expression profiling is now starting to be performed by cDNA micro-arrays and SNP-array analysis. Tissue samples are obtained from patients diagnosed with gliomas, undergoing surgery at the Neurosurgical Unit of the University Hospital of Coimbra. The iFISH evaluation of allelic imbalances detected numerical abnormalities in the following chromosome regions: 1p36, 19q13, 7q11, 9p21, 9q34, 10q23, 13q14, 17p13 and 22q11, confirming the existence of complex cytogenetic abnormalities in these tumours. The analysis of larger groups of patients will help to establish the potential clinical relevance of these findings.

PUBLICATIONS

Sayagues JM, Tabernero MD, Maillo A, Trelles O, Espinosa AB, Sarasquete ME, Merino M, Rasillo A, Vera JF, Santos-Briz A, de Alava E, Garcia-Macias MC, Orfao A. (2006). Microarray-based analysis of spinal versus intracranial meningiomas: different clinical, biological, and genetic characteristics associated with distinct patterns of gene expression. *J Neuropathol Exp Neurol*, 65:445-54.

Espinosa AB, Tabernero MD, Maillo A, Sayagues JM, Ciudad J, Merino M, Alguero MC, Lubombo AM, Sousa P, Santos-Briz A, Orfao A. (2006). The cytogenetic relationship between primary and recurrent meningiomas points to the need for new treatment strategies in cases at high risk of relapse. *Clin Cancer Res.*;12:772-80.

2-INTERACTION WITH INDUSTRY AND LOCAL AUTHORITIES

2.1 BIOCANT – Center for Innovation in Biotechnology

Biocant is a non-profit association aiming of the transfer of the technology and human resources in the Biotechnology Area. The founding partners of this association are CNC (Center for Neuroscience and Cell Biology), CMC (Cantanhede Municipal Council), ABAP (an association that congregates seven Municipal Councils of the center region of Portugal).

Biocant started to operate in September 2005, under the scientific supervision of Prof. Carlos Faro (U. Coimbra) with four technological units, each headed by a senior scientist: Molecular Biotechnology (Prof. Euclides Pires, U. Coimbra), Genómica (Prof. M. Santos, U. Aveiro), Microbiologia (Prof. M. Costa, U. Coimbra), Biologia Celular (Prof. C. Duarte, U. Coimbra).

Each unit proceeded during 2006 the development of projects in collaboration with industry (pharmaceutics, wine, cork). Investigators from CNC were invited to integrate the research teams responsible for those projects.

Several companies from the Biomedical and Biotechnological areas showed interest in settling at Biocant Park to benefit from the capacity of Biocant to transfer technology and human resources. Criostaminal, the first company to settle at Biocant, started to fully operate in Biocant Park in 2006. At present the following companies have settled in the Park: Biognosis; Biocant Ventures; Crioestaminal – Saúde e Tecnologia, SA; geneBOX; GeneLab; Gene PreDiT, Lda; Haloris; Novexem Portugal Lda.; Vectorpharma *(http://www.biocantpark.com/default.asp)*.

3-SOCIETY EDUCATION PROGRAMME

CNC as promoter of Science Divulgation

Interaction with high school students: students came to CNC

Outline of the 2006 "Ciência Viva" programme at CNC

In July, nine students from different schools participated in the "Ciencia Viva" programme at CNC which was run for 10 solid days. Students were first introduced to the organization and research activity of CNC and visited the CNC animal facility and several laboratories located at IBILI and Faculty of Medicine. Students attending the programme were organized in groups in order to follow ongoing projects and the experiments that actually were going to be performed. Each group had also the opportunity to plan and to run themselves several molecular and cell biology techniques as part of a brief project intitled *"Neurons in stress"* and *"Neurotoxic effects of drugs of abuse",* specially prepared for this event. At the end each group had to prepare a Power Point document to be presented and discussed with the researchers involved in the scientific activity.

http://www.cienciaviva.pt/estagios/jovens/ocjf2006/inscricao.asp?accao=showentidadedetail&id_entidade=13

European Science Week programme activities at CNC

During the European Science Week CNC received 23 high school students (Escola Secundária José Falcão). During one afternoon the students visited the laboratories and observed and/or performed some techniques currently used in neuroscience as in other areas of research: cell viability test, behavioural tests using laboratory animals, cell culture of neurons.

http://www.cienciaviva.pt/semanact/edicao2006/index.asp?accao=listeventosentidade&id_entidade=1002

Outline of the Brain Awareness Week 2006 activities

Neuroscience Activities at schools

Several activities were organized, during the Brain Awareness Week 2006, for 5-17 years old students in 12 schools of the Center region of Portugal (Table 1) The major goal of the proposed activities (designed accordingly to the grade of education) was to promote contact between students and neuroscientists at school and to show the brain structure and function, what's the role of the brain in connection to the world (five senses), how brain is affected by several conditions (e.g. by drugs of abuse consumption), how can we contribute for a healthy brain. Activities included short PowerPoint presentation about brain structure and function, structure of a neuron and neuronal communication, five senses, a healthy brain (role of nutrients, sleep, physical and mental exercise, neuronal damage by drugs of abuse), making a neuron model by using playdough, puzzles and painting. More then 600 students participated in these activities.

Table 1 – Schools visited by CNC researchers during Brain Awareness Week 2006

School	City	Researchers	Date
Colégio de São Teotónio (1º ciclo do ensino básico)	Coimbra	Teresa Oliveira; Rosa Resende; Sílvia Neves; Ana Isabel Duarte	March 16
Colégio João XXIII (jardim de infância e pré-escola)	Coimbra	Manuela Grazina; Marta Simões; Cândida Mendes; João Pratas	March 17
2º Jardim Escola João Deus (1º ciclo do ensino básico)	Coimbra	Manuela Grazina; Marta Simões; Cândida Mendes; João Pratas	March 16
1º Jardim Escola João Deus (pré-escola, 1º ciclo do ensino básico)	Figueira da Foz	Cláudia Pereira; Rosa Resende; Elisabete Ferreiro; Sandra Cardoso	March 17
Escola EB1 de Águeda (1º ciclo do ensino básico)	Águeda	Ildete Luísa Ferreira; Ana Raquel Esteves; Ana Filipa Domingues	March 16
Jardim de Infância da Solum (pré-escola)	Coimbra	Joana Barbosa de Melo; Rosa Resende	March 28,
Infantário da Universidade (pré-escola	Coimbra	Paula Canas; Ana Raquel; Esteves; Ana Filipa Domingues; Márcio Baptista; Rui Costa	March 15
Eugénio de Castro (2º ciclo- 5º ano)	Coimbra	Cláudia Cavadas; Ana Rita Álvaro	March 15
Instituto Educativo de Souselas (3º ciclo- 9º ano)	Coimbra	Liliana Montezinho	March 17
Colégio S. Miguel (3º ciclo- 9º ano e secundário)	Fátima	Rodrigo Cunha	March 28,
EB1 Santa Cruz de Trapa (3º ciclo- 9º ano)	São Pedro do Sul	Ildete Luísa Ferreira	March 14
Escola Secundária da Mealhada (ensino secundário- 12º ano)	Mealhada	Paulo Santos	March 17

General public information about neuroscience

A public session untitled *"Brain and Drugs: beyond will"* was carried out at Casa Municipal da Cultura, Coimbra.
http://www.cienciaviva.pt/divulgacao/semanacerebro2006/home/drogasecerebrocoimbra.pdf

Several CNC researchers participated and organized part of an interactive exposition at the "Pavilhão do Conhecimento", Lisbon, untitled *"Drugs of abuse and Brain"*. More than 500 persons of different ages visited the exposition and learned how acute and chronic consumption of drugs of abuse changes brain function.
http://www.cienciaviva.pt/divulgacao/semanacerebro2006/home/lisboa.asp

General public session about science during the European Science Week

A public session untitled *"Scientific culture for all: in memorium of Romulo de Carvalho"* took place at Livraria Almedina, Coimbra.
http://www.contanatura.net/arquivo/2006/11/cultura_cientifica_para_todos.html

Services

8

CNC SERVICES

INTRODUCTION

Under the present contract, CNC Laboratório Associado (CNC/AIBILI), has the obligation to provide community services and to interact with clinical departments

Biochemical and Molecular Biology Analysis:

Coordinators: *Catarina Oliveira, Carlos Faro*.

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Maria Helena Garruncho
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Rita Guerreiro
Sónia Cristina Costa Oliveira

1- Biochemical and Molecular Biology analysis

Mitochondrial Respiratory Chain (MRC)

Oxygen consumption and double wavelength spectrophotometry evaluation
During 2006, 73 patients suspected of Mitochondrial Cytopathy were studied, corresponding to the analysis of 88 samples, including 55 lymphocytes isolated of peripheral blood, 23 muscular biopsies, 7 liver biopsies and 3 heart biopsies. MRC deficiences were detected in 42 patients.

Mitochondrial DNA (mtDNA) studies

The molecular differential analysis of mitochondrial cytopathies, as a highthrouput screening, by sequencing analysis, of 11 mtDNA regions, covering a total of 424 mtDNA point mutations/polymorphisms was developed. We have continued to screen deletions by flanking PCR of 6 hot-spot regions.

Several tissues in a total of 173 samples from 150 patients suspected of Mitochondrial Cytopathy were screened, for point mutations and deletions analysis. Deletions were detected in 15 samples (27 confirmed by sequencing analysis) and point mutations/polymorphisms in 88 samples. PCR-RFLP analysis was performed to reconfirm point mutations in 32 samples.

Amino Acid Analysis

Amino acids analysis is a very important approach in early metabolic disorder diagnosis, and frequently helps to prevent mental retardation or even death. Plasma amino acid evaluation is also very important for the follow-up of patients submitted to therapeutic protein restrictive diets. In fact, patients with chronic metabolic disorders are periodically screened in order to adjust the diet. On the other hand, alterations in amino acid profile can have a secondary cause, and may point to diagnosis of other types of metabolic disorders.

Our laboratory received 417 samples (354 - plasma, 47 - urine and 16 - cerebrospinal fluid) for amino acid analysis.
The evaluation of homocysteine in plasma samples, was performed for the diagnosis and follow-up of metabolic disorders and for the diagnosis / risk factors evaluation in vascular and neurodegenerative disorders. Accordingly, 81 plasma samples were analysed in patients suffering from stroke, dementia and mild cognitive impairment.

Genetic testing in neurodegenerative disorders

As of 2006, the Neurogenetics Laboratory at CNC offers genetic testing for AD and PD patients.

A total of 610 samples have been processed in the Neurogenetics Laboratory in 2006, which include: 400 from controls, 97 from dementia patients, 36 from patients displaying clinical features of parkinsonism, 10 from Frontotemporal dementia patients and 59 from Mild Cognitive Impairment patients. Genes associated with these diseases were screened in most samples, and mutations were found in *LRRK2*, *PS-2* and *PARK2*. We have also found that common variability in a region of chromosome 6 may be associated with PD in our series of patients, and we are currently following up on these results. Apolipoprotein-E genotype, the common risk factor for Alzheimer's disease, was determined in 567 individuals and the COMT functional SNP in exon 4 (rs165688) was assessed in 415 subjects.

APO E genotyping in patients with stroke was carried out according with the research project "Evaluation of the involvement of *apolipoprotein-E* gene in ischaemic cerebral vascular accident", Portuguese Health Ministry– Project nº 101/01.

PORTUGUESE FOUNDATION
FOR SCIENCE AND TECHNOLOGY (FCT)

AIBILI

REPORT 2006

CONTENTS

1. Introduction

AIBILI - Association for Innovation and Biomedical Research on Light and Image is a private non-profit organisation, founded in 1989, established to support technology transfer to industry.

AIBILI is a Research Technology Organisation (RTO) in the Health Market dedicated to help the development of new products for pharmaceutical and biotechnology companies.

AIBILI is certified by ISO 9001:2000 since 2004. Clinical Trials are performed in accordance with ICH Good Clinical Practice Guidelines and the Bioavailability and Pharmacokinetic Studies are also developed in compliance with the OCDE Principles of Good Laboratory Practice.

AIBILI has the following Units:

- Centre for Clinical Trials (CEC)
- Centre for Bioavailability Studies (CEB)
- Centre of New Technologies for Medicine (CNTM)
- Administrative Services (SA)
- Quality Management Unit (UGQ)
- Technology Transfer Unit (UTT)

AIBILI is located at the Health Campus of Coimbra University since 1994 and has 15.296 sq. feet with state-of-the-art equipment. Regarding human resources we have 7 investigators, 12 technicians, 5 study coordinators and 4 administrative staff. Also collaborate regularly with AIBILI 36 investigators, 4 technicians for diagnostic procedures and 8 nurses.

2. Areas of Expertise / Research / Staff

2.1. Centre for Clinical Trials

The Centre for Clinical Trials (CEC) performs randomized clinical trials with special emphasis on Ophthalmology and, more specifically, in diabetic retinopathy, age-related macular degeneration, glaucoma, cataract surgery and ocular inflammation.

It is the purpose of the Centre for Clinical Trials to work with the Industry and to function as liaison between the Drug and Medical Device Industry and the Health Services.

CEC has dedicated facilities and the most modern ophthalmological equipment. Its permanent staff includes two Ophthalmologists full time, one Pharmacist, five experienced Study Coordinators, three Technicians for Diagnostic Procedures, two Nurses, one Laboratory Technician and two Administrative Secretaries. Fifteen Ophthalmological Consultants and two Medical Doctors, Internal Medicine and Endocrinology, collaborate regularly with the Centre.

The professional organisation of the Centre for Clinical Trials with a Manual of SOP (Standardized Operating Procedures) and its convenient location, next to the University Hospital of Coimbra and its Department of Ophthalmology are a guarantee that the deadlines are successfully met and in compliance with the ICH Good Clinical Practice Guidelines. The Centre for Clinical Trials is also certified by ISO 9001:2000 to perform clinical trials, thus guaranteeing the continual improvement of its work in Ophthalmological Research.

Areas of Expertise

- Characterisation and evaluation of the most recent methods to study the initial stages of diabetic retinopathy. Evaluation of new methodologies for multimodal mapping of the macula.
- Studies of the diseases of the choroid and retina and especially of their blood circulation, particularly in age-related macular degeneration.
- Testing new methods of early diagnosis and characterisation of macular edema and retinal vascular pathology.
- Evaluation of new drugs to treat glaucoma. Development of methods to correlate clinical indicators of disease progression, particularly regarding optic nerve degeneration and the mechanisms of the actions of drugs being tested.
- Evaluation of the quality of cataract microsurgery, establishing references and evaluating different biomaterials.

Research

Ongoing Clinical Trials

Clinical Trials in Ophthalmology

Macular Edema after CRVO

- A Six- Month, Phase 3, Multicenter, Masked, Randomized, Sham-Controlled Trial (with Six-Month Open-Label Extension) to Assess the safety and Efficacy of 700µg and 350µg Dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS) Applicator System in the Treatment of Patients with Macular Edema Following Central Retinal Vein Occlusion or Branch Retinal Vein Occlusion.

Diabetic Macular Edema

- Characterization of the mechanisms of resolution of Diabetic Macular Edema after photocoagulation treatment with laser.

- Protocol B7A-MC-MBDL. Reduction in the Occurence of center-threatening Diabetic Macular Edema.

- The effect of Ruboxistaurin on clinically significant Macular Edema in patients with diabetes Mellitus, as assessed by optical coherence tomography.

- An extension of the double-blind, randomized, placebo-controlled, parrallel-group, multicenter study evaluating safety, tolerability and effect on MRI lesion parameters of FTY720 vs placebo in patients with relapsing multiple sclerosis.

- A randomized, double-masked, multicenter, phase II study assessing the safety and efficacy of two concentrations of ranibizumab (intravitreal injections) compared with non-treatment control for the treatment of diabetic macular edema with center involvement.

- A randomized, double-masked, parallel group, multi-center, dose-finding comparison of the safety and efficacy of ASI-001A 0.5 µg/day and ASI-001B 0.2 µg/day fluocinolone acetonide intravitreal inserts to sham injection in subjects with diabetic macular edema.

Glaucoma

- A five-year, multicenter, open-label study to evaluate the safety of once-daily evening instillation of travoprost 0,004% eyedrops (Travatan®) in subjects with open-angle glaucoma or ocular hypertension.

Age-Related Macular Degeneration

- A phase II/III randomized, double-masked, controlled, dose-ranging, multi-center comparative trial, in parallel groups, to establish the safety and efficacy of intravitreal injections of Eye001 (Anti-VEGF Pegylated Aptamer) given every 6 weeks for 54 weeks, in patients with exudative age-related macular degeneration (AMD).

- An evaluation of efficacy and safety of posterior juxtascleral injections of anecortave acetate 15mg (0.5ml of 30 mg/ml anecortave acetate sterile suspension) versus vehicle in patients with subfoveal choroidal neovascularization (CNV) due to exudative age-related macular degeneration (AMD).

- An evaluation of efficacy of posterior juxtascleral administration of Anecortave Acetate for depot suspension (15mg or 30mg) versus sham administrations in patients (enrolled in study "A" or study "B") at risk for developing sight-threatening choroidal neovascularization (CNV) due to exudative age-related macular degeneration (AMD).

- A phase 3B, randomized, active controlled, double-masked, single dummy, multi-center comparative trial, in parallel groups, to compare the safety and efficacy of intravitreous injections of Macugen™ given every 6 weeks for 54 weeks (to be extended to 102 weeks as indicated), to Macugen™ plus PDT with Visudyne®, in subjects with predominantly classic subfoveal chorodial neovascularization (CMV) secondary to age-related macular degeneration (AMD).

- An open label evaluation of long term efficacy and safety of posterior juxtascleral injections of anecortave acetate 15mg in patients with subfoveal exudative age-related macular degeneration (AMD).

- A randomized, double-masked, active-controlled, multicenter study comparing the efficacy and safety of ranibizumab (0.3mg and 0.5mg) administered as two dosing regimens in patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration.

- An open label, non-comparative protocol for the use of pegaptanib sodium injection every 6 weeks in patients with exudative age-related macular degeneration (AMD).

- A 102-week, open label, multicenter trial to investigate the efficacy of macugen for the preservation of visual function in subjects with neovascular age-related macular degeneration (AMD) and to assess the benefit of treating early choroidal neovascularization (CNV).

- A phase IIIb, open-label, multi-center 12 month study to evaluate the safety, tolerability and efficacy and of ranibizumab (0.3mg) in patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration.

Uveitis

- An open label trial of Anti-TNF a chimeric Monoclonal Antibody (Infliximab, Remicade) in the Treatment of endogenous uveitis or vasculitis unresponsive to standard therapy.

- An 8-week, multicenter, masked, randomized trial (with an 18-week masked extension) to assess the safety and efficacy of 700 µg and 350 µg dexamethasone posterior segment drug delivery system (DEX PS DDS)

applicator system compared with sham DEX PS DDS applicator system in the treatment of non-infectious ocular inflammation of the posterior segment in patients with intermediate uveitis.

Cataract Surgery

- Randomised Multicentre European Clinical Trial of Antibotic Prophylaxis for Endophthalmitis Following Cataract Surgery.

- Nepafenac 0,1% Eye Drops, suspension compared to ketorolac Trometamol 0,5% Eye Drops, solution and placebo (nepafenac vehicle) for the prevention and treatment of ocular inflammation and ocular pain associated with cataract surgery: european study.

Observational Studies

- Observational Study on Diabetes type II

  To evaluate the natural story of Diabetic retinopathy on initial stages, 116 type 2 diabetic patients are being followed up in an observational study. Diabetic retinopathy was diagnosed as mild to moderate at at least 7 years ago. The evolution of retinopathy is monitorized yearly with color fundus photography, fluorescein angiography, retinal leakage analysis and optical coherence tomography. The metabolic control is performed by the family doctor according to the standard.

- Functional Genomics of the Retina in Health and Disease (EVI-GENORET)

  The phenotyping component of EVI-GENORET Project is collecting data on well-defined phenotypes of patients with Age-Related Macular Degeneration and Monogenic Retinal Dystrophies. A case report form (e-CRF) has been developed and is fully implemented as an internet based Database.

  Medical doctors from AIBILI are also including data according to this specific protocol to the consortium database.

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2.2. Centre for Bioavailability Studies

The Centre for Bioavailability Studies (CEB) responds to different needs of the Pharmaceutical Industry when introducing new or generic drugs or a modified dosage or type of pharmaceutical formulation.
One of the actual activities is the control of raw material and pharmaceutical formulations during the manufacturing process or in the finished product (identifying and quantifying active ingredients or interfering substances).
CEB also performs Bioavailability and Bioequivalence Studies in human healthy volunteers, assessing efficacy and security of the drugs, namely evaluating the velocity and extension in which a drug reaches the local of action and the "closeness" of a test formulation versus a reference one. These types of studies can be requested by the Regulatory Authorities to the Pharmaceutical Industry, when a market authorisation process is submitted for evaluation. This Centre also performs clinical trials.
Another area of interest is the development, optimisation and validation of analytical methods, according to the most recent ICH and FDA Guidelines. The dosage of drugs (in different matrixes) can be done through standard or internal analytical methods and CEB has the ability to ensure the existence of feasible and reliable methods according to specific requirements of each study.

CEB is certified by INFARMED (Portuguese Regulatory Authority) for the performance of Physical-Chemical Testing: Bioavailability/Bioequivalence Studies and Pharmacokinetic Studies, in compliance with the OECD Principles of Good Laboratory Practice.
CEB is also certified to perform Clinical Trials, Bioavailability/Bioequivalence Studies and Drug Dosages according to NP EN ISO 9001: 2000.
In case a study involves a clinical phase it is performed in compliance with Good Clinical Practice (ICH GCP Guidelines).

Areas of Expertise

- Studies of absolute bioavailability of a drug.
- Bioequivalence studies of pharmaceutical products having the same drug in the same formulation or different formulations.
- Elaboration of documents associated with clinical trials performance.
- Clinical trials performance.
- Development and validation of analytical methods.
- Dosage of drugs in the finished product or during the manufacturing process and in biological matrixes.
- Clinical studies on the variability of different batches of preparation from a single manufacturer.
- Chemical control of raw materials and manufactured products.
- Organisation and scientific coordination of reviews or reports for the introduction of drugs in Portugal and the European Union.

Research

Ongoing Studies

- **Bioavailability/Bioequivalence Studies**
  - Execution of an open, randomized and crossover study on the bioequivalence between suspensions containing 400 mg/5 ml of acyclovir from two different pharmaceutical laboratories
  - Execution of an open, randomized and crossed-over study on the bioequivalence between coated tablets containing 30 mg of deflazacort from two different pharmaceutical laboratories
  - Execution of an open, randomized and crossed-over study on the bioequivalence between coated tablets containing 6 mg of deflazacort from two different pharmaceutical laboratories

- **Clinical Trials**
  - Real-Life Effectiveness and Care Patterns (in Portugal) of Diabetes Management – The RECAPP-DM study
  - A 54-week, double-blind, randomized, placebo-controlled, parallel-group study to investigate the effects of rosiglitazone as adjunctive therapy to donepezil on cognition and overall clinical response in APOE e-stratified subjects with mild to moderate Alzeheimer's disease (REFLECT-2)
  - Food-effect and dosage form proportionality study of esclicarbazepine acetate market formulation in healthy subjects
  - A randomized, comparative, double-blind, parallel-Group, Multicenter, Monotherapy, study of Pregabalin (Lyrica) and Lamotrigine (Lamictal) in patients with newly diagnosed partial seizures
  - Multicentre, double-blind, randomized, active and placebo controleed trial to investigate the efficacy and tolerability of nebicapone in parkinson's disease patients with "wearing-off" phenomenon treated with levodopa/carbidopa or levodopa/benserazide
  - Safety and efficacy of lidocaine 5% medicated plaster in comparison with pregabalin in postherpetic neuralgia and diabetic polyneuropathic pain

- *Drug Dosages Studies*
  - Identification and quantification of azithromycin and related known and unknown substances in different batches of raw material.
  - Dosage of S- and R-warfarin in plasma samples from a clinical trial.

- **Marketing authorisation**
  - Elaboration, presentation and format of the applicant dossier for a medicine marketing authorisation

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3. Publications

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4. Financial Expenses

Following is a chart showing the financial expenses of AIBILI with respect to the Associate Laboratory:

(Euro)

Name	Total Annual Expenses
Carla Alexandra Fernandes Neta	31.374,62
Maria Luisa Soares Silva Reis Ribeiro	36.268,52
Rui Manuel Dias Cortesão Santos Bernardes	43.651,89
	111.295,02

Funding

9

ONGOING PROJECTS

Title	Financing Agency	Duration	Total Financing (CNC)	Financing 2006
National Projects:				
Real-time measurement of nitric oxide in hippocampal brain slices: modulation by tissue redox state with implications for neuronal degeneration Coordinator: João António Nave Laranjinha	FCT Refª: POCTI/BCI/42365/2001	15/03/2002 to 14/01/2006	81.065,00	0,00
Dynamic Interactions of Phenolic Compounds from Wine and Olive Oil in Plasma and Lipoproteins with Relevance for Atherosclerosis Prevention Coordinator: João António Nave Laranjinha	FCT Refª: POCTI/AGR/42418/2001	01/06/2002 to 15/06/2006	93.618,00	11.702,25
Liver cholestasis and steatosis: bile acid therapy. Cellular mechanisms with relevance to mitochondrial functionCoordinator: Carlos Manuel Marques Palmeira	FCT Refª: POCTI/CBO/42486/2001	01/07/2002 to 30/06/2006	82.500,00	0,00
K-ATP Channel dependent and independent pancreatic beta cells from normal and non obese type 2 diabetic rodents Coordinator: Luís Manuel de Oliveira Martinho do Rosário	FCT Refª: POCTI/NSE/42123/2001	01/07/2002 to 30/06/2006	100.000,00	0,00
Regulation of the expression of ionotropic glutamate receptors by BDNF in hippocampal neurons Coordinator: Carlos Jorge Alves Miranda Bandeira Duarte	FCT Refª: POCTI/BCI/46466/2002	01/03/2003 to 28/02/2007	97.964,00	4.944,33
Neuroprotective changes in the proteome induced by brain-derived neurotrophic factor (BDNF): proteomic studies in cultured hippocampal neurons Coordinator: Carlos Jorge Alves Miranda Bandeira Duarte	FCT Refª: POCTI/NSE/46441/2002	01/03/2003 to 28/02/2007	116.000,00	6.444,33
Crystal structure determination of normal and expanded ataxin 3: Analysis of the structural determinants implicated in Machado-Joseph disease Coordinator: Sandra de Macedo Ribeiro	FCT Refª: POCTI/MGI/47550/2001	01/03/2003 to 31/12/2006	75.000,00	4.166,67

Development of new polyurea/polyurethane-based membranes for blood oxygenators Coordinator: António Jorge Rebelo Ferreira Guiomar Participants: Instituto Superior Técnico (IST)	FCT Refª: POCTI/FCB/47661/2002	01/06/2003 to 31/10/2006	10.320,00	1.433,33
Immunological and functional identification of ionotropic presynaptic glutamate receptores Coordinator: João José Oliveira Malva	FCT Refª: POCTI/FCB/46804/2002	01/09/2003 to 31/12/2006	93.000,00	24.000,00
Neuroprotective effect of neuropeptide y in excitotoxicity and in temporal lobe epilepsy Coordinator: Catarina Isabel Neno Resende de Oliveira Participants: Faculdade de Medicina da Universidade de Coimbra (FMUC)	FCT Refª: POCTI/NSE/46848/2002	01/09/2003 to 31/08/2006	45.000,00	12.083,33
Targeted antisense therapy: a novel approach for the treatment of human small cell lung cancer Coordinator: João Nuno Sereno de Almeida Moreira Participants: Instituto Nacional de Engenharia, Tecnologia e Inovação (INETI)	FCT Refª: POCTI/FCB/48487/2002	01/09/2003 to 31/08/2007	33.700,00	11.250,00
Targeted Contrast Agents for MRI and Nuclear Scintigraphy (TARCAS) Coordinator: Carlos Frederico de Gusmão Campos Geraldes Departamento de Bioquímica, FCT, Universidade de Coimbra Participants: IBILI-Universidade de Coimbra; Universidade de Aveiro; Universidade de Minho	FCT Refª: POCTI/QUI/47005/2002	01/10/2003 to 30/09/2007	19.230,00	5.516,25
Biochemical and molecular analysis of hepsin, protease implicated in prostate cancer Coordinator: Carlos José Fialho da Costa Faro Participants: Instituto Português Oncologia (IPO)	FCT Refª: POCTI/CBO/49334/2002	01/10/2003 to 31/01/2007	68.400,00	15.898,50
Amyloid Fibril Formation by Transthyretin: searching for the amyloidogenic intermediates Coordinator: Rui Manuel Pontes Meireles Ferreira de Brito	FCT Refª: POCTI/BME/49583/2002	02/01/2004 to 01/01/2007	123.528,00	41. 176,00
Aspartic proteinase content of Arabidopsis genome: identification, expression and characterization Coordinator: Carlos José Fialho da Costa Faro	FCT Refª: POCTI/BCI/48400/2002	02/01/2004 to 01/01/2007	60.000,00	20.200,00

Development of novel synthetic viral vectors for gene transfer: expression of angiogenic growth factors vascular diseases therapy Coordinator: Sérgio Paulo de Magalhães Simões	FCT Refª: POCTI/BIO/48735/2002	01/03/2004 to 28/02/2008	115.000,00	21.355,67
Mechanisms of nucleo-cytoplasmic trafficking of ASFV Egenome and design of anti-NLS/NS backbone cyclic peptides to block viral infection Coordinator: Mª da Conceição Monteiro Pedroso de Lima	FCT Refª: POCTI/CVT/44854/2002	01/12/2004 to 30/11/2007	80.000,00	26.666,67
Relação entre alterações metabólicas no hipocampo e défices de memória induzidos por diabetes. Coordinator: Rui Albuquerque Carvalho	FCT Ref.ª : POCI/SAU-NEU/56098/2004	01/01/2005 to 31/12/2007	89.985,00	32.338.28
Neuroprotecção por receptores de adenosina: acoplar o aumento da formação de adenosina com o bloqueio de receptores A2A Coordinator: Catarina Resende de Oliveira	POCI/SAU-FCF/59215/2004	01/01/2005 to 31/12/2006	85.000,00	42.309,00
Caracterização dos receptores purinérgicos nas fibras musgosas do hipocampo – papel na epilépsia Coordinator: Rodrigo Pinto dos Santos Antunes da Cunha	FCT Refª: POCI/SAU-NEU/59135/2004	01/01/2005 to 28/02/2007	86.500,00	37.400,00
Controlo pela adenosina da neuro-inflamação Coordinator: Rodrigo Pinto dos Santos Antunes da Cunha	FCT Refª: POCI/BIA-BCM/59980/2004	01/01/2005 to 28/02/2007	85.000,00	46.600,00
Endereçamento dos receptores AMPA para a sinapse: papel dos parceiros proteicos no endereçamento sináptico das subunidades GluR4 e GluR2L Coordinator: Ana Luísa Monteiro de Carvalho	FCT Refª: POCI/SAU-NEU/58955/2004	01/01/2005 to 31/12/2007	93.000,00	35.928,00
Ecologia e diversidade microbiana em ambientes abissais hipersalinos do Mar Vermelho Coordinator: Milton Simões da Costa	FCT Refª: POCI/BIA-BDE/56014/2004	01/01/2005 to 31/03/2008	90.000,00	30.860,00
Defesas contra agressão ambiental. Biossíntese de solutos compatíveis em bactérias extremamente resistentes a radiaçõesdo género Rubrobacter" Coordinator: Milton Simões da Costa Participants: Instituto Tecnologia Quimica e Biológica (ITQB),	FCT Refª: POCI/BIA-MIC/56511/2004	01/01/2005 to 31/12/2007	71.500,00	24.528,00
Targetin of genes (…). Entidade Proponente INEB Porto Coordinator CNC: Sérgio Magalhães Simões	FCT Ref.º POCI/SAU-BMA/58170/2004	01/01/2005 to 31/12/2007	8.700,00	0,00

New nanocrystalline ductile coatings for biomedical applications Entidade Proponente FCT Coordinator CNC: Carlos Duarte	FCT Ref.ª POCI/CTM/55967/2004	01/01/2005 To 31/12/2006	6.000,00	0,00
Compatible solutes from extremophiles: screening, biosynthesis and heterologous production Coordinator: Milton Simões da Costa	FCT Ref.ª : 010.6/A005/2005	01/03/2005 to 29/02/2008	99.168,00	48.577,00
O ATP e a retinopatia diabética: papel na regulação da microglia e na morte neuronal Coordinator: Paulo Fernando Martins dos Santos	FCT Refª: POCI/SAU-NEU/59003/2004	01/03/2005 to 29/02/2008	87.016,00	34.008,00
Caracterização funcional de espermatozóides equinos. Avaliação molecular da fertilidade de garanhões. Coordinator: João Ramalho de Sousa Santos	FCT Refª: POCTI/CVT/49102/2002	01/04/2005 to 31/03/2008	65.000,00	20.550,00
Interacção entre catecolaminas e neuropeptídeo Y nas células cromafins humanas Coordinator: Claúdia Margarida Gonçalves Cavadas	FCT Refª: POCI/SAU-FCF/60399/2004	01/04/2005 to 31/03/2008	55.000,00	19.500,00
Determinantes moleculares de neurotoxicidade e agregação da ataxina-3 na doença Machado-Joseph Coordinator: Sandra de Macedo Ribeiro	FCT Refª: POCI/SAU-MMO/60156/2004	01/06/2005 to 31/05/2008	94.996,00	34.128,00
Acção protectora de polifenóis do vinho tinto na inflamação e disfunção do endotélio vascular: Implicações na prevenção da aterosclerose Coordinator: Leonor Martins de Almeida	FCT Refª: POCI/AGR/59919/2004	02/05/2005 to 30/06/2009	64.280,00	24.704,00
Influência das Soluções de Preservação para Transplante nos Perfis Metabólico e Imunológico Cardíacos Coordinator: Rui de Albuquerque Carvalho	FCT Refª: POCI/SAU-OBS/55802/2004	01/07/2005 to 30/06/2008	89.985,00	27.528,00
Formulações de inibidores de microtubulinas no tratamento de leishmaniose visceral zoonótica Coordinator: Sérgio Paulo de Magalhães Simões Participants: Instituto Nacional de Engenharia, Tecnologia e Inovação (INETI); Instituto de Higiene e Medicina Tropical	FCT Refª: POCI/CVT/56995/2004	01/07/2005 to 30/06/2008	5.644,00	5.644,00
Manipulação de DNA em solução e interfaces Coordinator: Sérgio Paulo de Magalhães Simões Participants: Faculdade de Ciências e Tecnologia	FCT Refª: POCI/QUI/58689/2004	01/07/2005 to 30/06/2008	4.440,00	2.400,00

Depressão Pós-parto e Sono Coordinator: Sandra Maria Rodrigues de Carvalho Bos Participants: Instituto de Psicologia Médica	FCT Refª: POCI/SAU-ESP/57068/2004	01/08/2005 to 31/07/2008	38.250,00	11.750,00
Estratégias de melhoramento do efeito terapêutico de fármacos antimicobacterianos Coordinator: Sérgio Paulo de Magalhães Simões Participants: Instituto Nacional de Engenharia, Tecnologia e Inovação (INETI), Universidade do Minho	FCT Refª: POCI/SAU-FCF/58355/2004	01/08/2005 to 31/07/2008	4.330,00	4.330,00
Algumas gotas de água; a diversidade microbiana na água de estalactites e estalagmites Coordinator: António Manuel Veríssimo Pires	FCT Refª: POCI/BIA-BDE/60704/2004	15/08/2005 to 14/08/2008	84.000,00	35.328,00
Controlo da morte celular dependente da mitocôndria por regulação da transcrição na presença de FK506 e BDNF - papel protector na doença de Huntington. Coordinator: Ana Cristina Rego	FCT POCTI/SAU-NEU/57310/2004	01/09/2005 To 31/08/2008	98.103,00	41.934,00
Vectores lentivirais e silenciamento de genes em doenças de poliglutaminas: Expressão de RNAs de cadeia dupla mediada por vectores lentivirais para silenciamento do gene da ataxina-3. Coordinator: Luis de Almeida	POCI/SAU-MMO/56055/2004	01/09/2005 To 31/08/2008	30.000,00	19.388,00
Vectorização de fármacos para os vasos sanguíneos tumorais: uma nova terapia para o cancro da mama humano. Coordinator: João Nuno Moreira	FCT POCI/SAU-OBS/57831/2004	01/09/2005 To 31/08/2008	45.000,00	21.534,25
A Microglia na Neo-neurogénese Associada à Epilepsia do Lobo Temporal: Papel das Citocinas Pró-inflamatórias e do Neuropeptídeo Y na Neuroprotecção dos Novos Neurónios Coordinator: João José Oliveira Malva	FCT Refª: POCI/SAU-NEU/58492/2004	01/09/2005 to 31/08/2008	76.000,00	25.333,00
Metabolismo hepático intermediário da glucose em crianças com e sem actividade da glucose-6 fosfatase hepática Coordinator: John Jones	FCT POCI/QUI/55603/2004	01/10/2005 To 30/10/2007	65.500,00	52.762,00
Efeito do consumo crónico de cafeína da neuromodulação exercida pela adenosina - possível relevância em processos de aprendizagem e memória. Coordinator: Rodrigo Cunha	FCT Refª: POCI/SAU-FCF/59601/2004	01/10/2005 a 30/09/2008o	85.000,00	32.400,00

Desenvolvimento de novos compostos de Vanádio. Sua aplicação como agentes antidiabéticos e anticancerígenos. Coordinator: Maria Margarida Catalão Almiro e Castro Participants: Instituto Superior Técnico; Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA)	FCT Refª: POCI/QUI/56949/2004	01/10/2005 to 30/09/2008	29.500,00	7.440,00
Stress oxidativo na Doença Cardíaca: acção das catecolaminas Coordinator: Rui Albuquerque Carvalho Participants: Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA); Universidade de Aveiro;	FCT Refª: POCI/SAU-OBS/55849/2004	01/10/2005 to 30/09/2008	11.900,00	0,00
Neurociências e Doença Coordinator: Catarina Isabel Resende de Oliveira	FCT Refª: REEQ/651/SAU/2005	15/02/2005 to 30/07/2007	1.600.000,00	604.000,00
Estrutura e Função de Proteínas Coordinator: Euclides Manuel Vieira Pires	FCT Refª: REEQ/1028/BIO/2005	01/04/2005 to 30/07/2007	200.000,00	100.000,00
Rede Espectrometria de Massa Coordinator: Euclides Vieira Pires	FCT Ref. REEQ/1506/REM/2005ª	01/01/2006 to 31/12/2006	290.103,00	290.103,00
O Mundo dos Micróbios – Colecção Portuguesa de Bactérias (CPB) e Laboratório Acreditado de Controlo Microbiológico Coordinator: Milton Simões da Costa	FCT Refª: REEQ/851/BIO/2005	01/04/2005 to 30/07/2007	129.936,00	77.647,00
Estudos populacionais e clínicos para a quantificação da sobrevivênvia e identificação de factores de prognóstico – "Estudo da heterogeneidade genética intratumoral de gliomas: impacto no seu comportamento clínico e biológico Coordinator: Maria Celeste Fernandes Lopes	FCG Refª: 68708	01/02/2005 to 31/01/2008	30.000,00	10.000,00
Estudos estruturais da biogénese do proteassoma: determinação da estrutura do Ump1 e do seu complexo com precursores do proteassoma Coordinator: Sandra de Macedo Ribeiro	FCT Refª: POCI/BIA-PRO/58638/2004	01/03/2006 to 28/02/2009	26.094,00	17.963,00
Proteases aspárticas secretadas em Candida albicans: potencial uso como antigénicos alvo para vacinação contra candidíases sistémicas Participants: Universidade do Minho Coordinator: Carlos José Fialho da Costa Faro	FCT Refª: POCI/SAU-IMI/58014/2004	01/06/2005 to 31/05/2008	9.600,00	4.800,00
Estudo dos possíveis factores ambientais e moleculares que levam ao desenvolvimento de diabetes tipo 2 e obesidade em Portugal Coordinator: Eugénia Maria Lourenço de Carvalho	FCT Refª: POCI/SAU-MO/57598/2004	15/10/2005 to 30/06/2008	90.250,00	3.840,00

Luso Coordinator: Milton Costa	Luso	01/07/2005 to 30/06/2006	13.000,00	4.590,61
Sub - Total			**5.432.105,00**	**2.038.982,46**
International Projects:				
New Applications for Compatible Solutes from Extremophiles (Hotsolutes) Coordinator: Milton Simões da Costa Participants: Stab Vida	União Europeia COOP-CT-2003-508644	15/02/2004 to 14/05/2006	137.650,00	4.074,37
Noninvasive measurement of hepatic glycogen kinetics in Type 1 diabetics. Coordinator: John Griffth Jones	JDRF Refª: 1-2006-74	01/05/2006 to 31/04/2007	49.269,00	16.722,85
EMIL Coordinator: Carlos Geraldes	EMIL Ref.ª EMIL: LSHC-CT-2004-503569	01/07/2005 to 30/06/2006	13.594,68	11.206,27
Sub – Total			**200.513,68**	**32.003,49**
Other:				
Alzheimer`s disease, nicotine exposure and nicotinic acetylcholine receptors. A study in rat cortical neurons and humam lymphocyte Coordinator: Paula Agostinho	Janssen Cilag Farmacêutica, lda	21/10/2004 to 20/10/2006	10.000,00	2.024,45
Characterisation of FKS gene(s) in Alternaria infectoria Coordinator: Teresa Gonçalves	Merck Sharp Dohme MSG – P 1599	01/01/2006 to 31/03/2007	30.640,00	26.615,00
Sub - Total			**40.640,00**	**28.639,00**
TOTAL			**5.673.258,68**	**2.099.624,95**

Staff Lists

10

LIST OF STAFF AND RESEARCH STUDENTS | GENERAL LIST

Members holding Ph.D.		Time % at CNC
Adriana Teixeira	(MD, HUC)	Collaborator
Alexandrina M. Ferreira S. Pinto Mendes	(Assistant Prof., FFUC)	60
Américo Costa Figueiredo	(M.D., Assistant Prof., FMUC)	Collaborator
Amílcar Falcão	(Full Professor, FFUC)	50
Ana Bela Sarmento A. Cruz Ribeiro	(M.D., Assistant Prof., FMUC)	80
Ana Cristina Carvalho Rego	(Assistant Prof., FMUC)	80
Ana Fagulha	(M.D. HUC)	Collaborator
Ana Margarida Malaquias P. Urbano	(Assistant. Prof., FCTUC)	Collaborator
Ana Margarida Vieira da Silva	(Assistant Prof., UVG)	50
Ana Luísa Monteiro de Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Kuan Yon Chung	(Investigator, I.A.V.)	Collaborator
Ana Paula Pereira da Silva	(Aux. Investigator, FMUC)	Collaborator
António Francisco Gomes Ambrósio	(Principal Investigator, FMUC)	Collaborator
António Freire Gonçalves	(M.D., Associate Prof., FMUC)	15
António João F. Macedo Santos	(M.D., Assistant Prof. FMUC)	Collaborator
António Joaquim Matos Moreno	(Assistant Prof., FCTUC)	Collaborator
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Armanda Emanuela Castro e Santos	(Assistant Prof., FFUC)	80
Armando Jorge Amaral Matias Cristóvão	(Assistant Prof., FCTUC)	80
Armindo Salvador	(Auxiliar Investigator, CNC)	100
Arsélio Pato de Carvalho	(Full Professor, FCTUC)	80
Caetana Angélica E. Monteiro de Carvalho	(Full Professor, FCTUC)	80
Carla Baptista	(M.D. HUC)	Collaborator
Carlos Eduardo Paz Ferreira	(M.D., HDE, Açores)	Collaborator
Carlos Frederico G. Campos Geraldes	(Full Professor, FCTUC)	Collaborator
Carlos Jorge Alves M. Bandeira Duarte	(Associate Prof., FCTUC)	80
Carlos José Fialho da Costa Faro	(Associate Prof., FCTUC)	80
Carlos Manuel Marques Palmeira	(Assistant Prof., FCTUC)	20
Carlos M. Neves Pato	(M.D., Associate Prof.,USA)	Collaborator
Catarina Isabel N. Resende de Oliveira	(M.D., Full Prof., FMUC)	80
Cláudia Margarida Gonçalves Cavadas	(Assistant Prof., FFUC)	80
Cláudia Maria Fragão Pereira	(Auxiliar Investigator, FMUC)	100
Cristina Maria Tristão Sena	(Assistant Prof., FMUC)	80
Emília Cortesão	(MD, MSc Student, FMUC)	Collaborator
Emília da Conceição Pedrosa Duarte	(Assistant Prof., FCTUC)	80
Euclides Manuel Vieira Pires	(Associate Prof., FCTUC)	80
Eugénia Carvalho	(Aux. Investigator, CNC)	100
Fernando Gomes	(M.D., HUC)	Collaborator
Fernando Judas	(M.D., Ph.D.)	Collaborator
Fernando Nogueira	(FCTUC)	Collaborator
Hermínio José Tão Espírito Santo	(M.D.)	Collaborator
Inês Baldeiras	(Investigator, HUC)	Collaborator
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
Isabel Sousa	(MD, HUC)	Collaborator
Joana Margarida M. Gago da Câmara	(M.D., HDE, Açores)	Collaborator
João António Nave Laranjinha	(Associate Prof., FFUC)	80
João José Oliveira Malva	(Principal Investigator, FMUC)	100
João Nuno Sereno Almeida Moreira	(Assistant Prof., FFUC)	80
João Ramalho Santos	(Assistant Prof., FCTUC)	80
Joaquim Adelino Ferreira Vicente	(Associate Prof., FCTUC)	Collaborator
John Griffith Jones	(Principal Investigator, CNC)	100
José António Pereira da Silva	(M.D., Associate Prof., FMUC)	20
José Antunes Barata Custódio	(Associate Prof., FFUC)	80
Leonor Martins de Almeida	(Full Professor, FFUC)	80
Lino Gonçalves	(M.D., Assistant Prof., FMUC)	Collaborator
Luís Almeida	(Assistant Prof., FFUC)	80
Luís Augusto Salgueiro Cunha	(M.D., Full Prof., FMUC)	15

Luis Pedro B. Sousa Inês	(M.D., HUC)	Collaborator
Luísa Barros	(M.D. HUC)	Collaborator
Luísa Maria Abreu Freire Diogo Matos	(M.D., CHC)	20
Madalena Caldeira	(Assistant Prof., FCTUC)	Collaborator
Manuela Carvalheiro	(M.D. HUC)	Collaborator
Manuela Lacerda	(M.D., Ph.D., IPO**)	Collaborator
Margarida Bastos	(M.D. HUC)	Collaborator
Maria Augusta de S. Fernandes dos Santos	(Investigator, FCTUC)	Collaborator
Maria Amália Silva Jurado	(Assistant Prof., FCTUC)	80
Maria Carmen M. de Carvalho Alpoim	(Associate Prof., FCTUC)	Collaborator
Maria Celeste Fernandes Lopes	(Full Professor, FFUC)	80
Maria Conceição Egas	(Auxiliar Investigator, CNC)	100
Maria da Conceição M. Pedroso de Lima	(Full Professor, FCTUC)	80
Maria Fernanda P. N. Gomes Nobre	(Principal Investigator, FCTUC)	100
Maria Graça Santos Pratas Vale	(Full Professor, FCTUC)	80
Maria Helena Pinto de Azevedo	(M.D., Full Prof., FMUC)	Collaborator
Maria Isabel Jacinto Santana	(M.D., Assistant Prof., FMUC)	80
Maria Manuela Monteiro Grazina	(Assistant Prof., FMUC)	80
Maria Margarida Catalão Almiro e Castro	(Assistant Prof., FCTUC)	Collaborator
Maria Margarida Gonçalo	(M.D., HUC)	Collaborator
Maria Sancha Santos	(Investigator, FCTUC)	100
Maria Teresa de Teixeira Cruz Rosete	(Assistant Prof. FFUC)	80
Marília Dourado	(M.D., Ph.D., FMUC CIMAGO*****)	Collaborator
Michelle Pato	(M.D., Associate Prof., USA)	Collaborator
Miguel Castelo-Branco	(Investigator, IBILI)	Collaborator
Miguel Marques	(FCTUC)	Collaborator
Milton Simões da Costa	(Full Professor, FCTUC)	80
Olinda Rebelo	(M.D., HUC)	Collaborator
Otilia Vieira	(Aux. Investigator, CNC)	100
Paula Cristina Veríssimo Pires	(Assistant Prof., FCTUC)	80
Paula Garcia	(M.D. HPC)	Collaborator
Paula Maria Garcia Agostinho	(Auxiliar Investigator, FMUC)	100
Paulo Fernando Martins dos Santos	(Assistant Prof., FCTUC)	80
Paulo Figueiredo	(M.D., IPO**)	Collaborator
Paulo J. Oliveira	(Auxiliar Inv., CNC)	100
Pedro Monteiro	(M.D., HUC)	Collaborator
Raquel Seiça	(M.D., Associate Prof. FMUC)	80
Rodrigo Pinto dos Santos A. Da Cunha	(Associate Prof., FMUC)	80
Romeu António Videira	(Assistant, I.P.V.)	80
Rui de Albuquerque Carvalho	(Assistant Prof., FCTUC)	Collaborator
Rui M. Pontes Meireles F. de Brito	(Assistant Prof., FCTUC)	35
Rui Manuel Silva Gomes Barbosa	(Assistant Prof., FFUC)	80
Sandra de Macedo Ribeiro	(Auxiliar Investigator, CNC)	30
Sandra Morais Cardoso	(Assistant Prof., FMUC)	100
Sérgio Paulo Magalhães Simões	(Assistant Prof., FFUC)	80
Teresa Carmo Pimenta Dinis	(Associate Prof., FFUC)	80
Teresa Maria Caldeira Martins	(Associate Prof.)	20
Teresa Maria Fonseca de Oliveira Gonçalves	(Assistant Prof., FMUC)	80
Tiago Alfaro	(M.D.)	Collaborator
Vítor Manuel Calado Madeira	(Full Professor, FCTUC)	80

Post-Doc Menbers

Ana Cristina Saavedra Martins	100
Ana Sofia Fraga de Almeida	100
Ana Sofia Bregieiro Eulálio	100
Anália do Carmo	100
Anabela Pinto Rolo	100
André Antunes	100
Attila Kofälvi	100
Carla Cardoso	100

Carla Margarida Pereira Cardoso	100
Catarina Isabel Ribeiro Pimentel	100
Cristina Isabel dos Santos Fonseca	100
Fabienne Agasse	100
Fernanda Maria Lopes Ferreira	100
Geanne Matos de Andrade Cunha	100
Henrique Faneca	100
Ildete Luísa Ferreira	100
Inês Maria Pombinho de Araújo	100
Isaura Simões	100
Jorge Fernandes dos Anjos	100
Lisiane de Oliveira Porciúncula	100
Miguel Luís Cunha Mano	100
Nuno Miguel Empadinhas	100
Paulo César da Silva Pinheiro	100
Pedro Castanheira	100
Teresa Almeida	100
Teresa Girão	100

Ph.D. Students

Adriana Oliveira dos Santos	100
Alexandra Amaral	100
Alexandra Sofia Beirão Mendes	100
Ana Catarina Oliveira	100
Ana Cristina da Silva Filipe	100
Ana Francisca Soares	100
Ana Luísa Cardoso	100
Ana Luisa Vital Carvalho	100
Ana Margarida Cruz Ledo	100
Ana Paula Sousa	100
Ana Raquel Esteves	100
Ana Rita Álvaro	100
Ana Rita Araújo Santos	100
Ana Simões Pinto Oliveira	100
Ana Isabel Marques Duarte	100
André Rodrigues de Abreu Gomes	100
Andrea Lobo	100
Artur Augusto Paiva	100
Áurea Castilho	50
Bruno Carreira	100
Bruno Gago	100
Bruno José Fernandes Oliveira Manadas	100
Bruno Miguel das Neves	100
Bruno Silva	50
Cândida Susana Gonçalves da Silva	100
Carla Nunes	100
Carlos Melo	100
Célia Aveleira	Collaborator
Chantal Fernandes	100
Claudia Silva	100
Daniela Arduíno	100
Daniela Maria Barroso de Moura Cipreste Vaz	100
Duarte A. Marques	100
Elisabete Batista Ferreiro	100
Ermelindo Leal	100
Filomena Silva	100
Francisco José Baptista Melo Simões de Deus	100
Gabriel Costa	100
Giovannia Araujo de Lima Pereira	100
Hugo Prazeres	100
Igor Clemente Tiago	100
Inês Crespo	100

Inês Vasconcelos Miranda Santos	100
Isabel Maria Nunes Correia	100
Jean P. Oses	100
Joana Cardoso da Costa	100
Joana Gaspar	100
Joana Gil	100
Joana Lourenço	100
Joana Rosmaninho Salgado	100
João Carlos Gomes	100
João Duarte	100
João Gonçalo Leal Frade	100
João Pedro Oliveira da Silva Paulino Lopes	100
José Mário Tenera Morgado	100
José Miguel Brás	100
Jorge Oliveira	100
Lígia Ferreira	100
Liliana Inácio Bernardino	100
Liliana Mendonça	100
Luísa Cortes Bastos	100
Marco Alves	100
Manuel Joaquim M. Gonçalves Matos	50
Margarida Alexandra Vaz Caldeira	100
Maria Joana Lima Barbosa de Melo	80
Maria José Simões	100
Maria Fátima Pereira	100
Maria Teresa de Jesus Matos	100
Marta Viegas da Silva	100
Mário Laço	100
Nelson Rebola	100
Nuno Ricardo Ferreira	100
Paula Isabel Moreira	100
Paula Canas	100
Paula Matos de Brito	100
Paula Mota	100
Pedro Manuel Venâncio Garção	100
Pedro Miguel Brás Macedo Coelho	100
Raquel Ferreira	100
Raquel Santiago	100
Ricardo J. Rodrigues	100
Ricardo Santos	100
Ricardo Tomé	100
Rita Guerreiro	100
Rita Perfeito	100
Rita Rocha	100
Rita Susana Rosa Branco	100
Rita Videira	100
Romeu Miranda Francisco	100
Rosa Maria Matos Branco Resende	100
Rui Costa	100
Rui Nobre	100
Sandra Amaral	100
Sandra Cristina Vicente e Almeida	100
Sandra Domingues Santos	100
Sandra Gamboa	100
Sandra Varum	100
Sandro José Paiva Fernandes Alves	100
Sara Gonçalves	100
Sara Alves Xapelli	100
Sílvia Sousa Neves	100
Sofia Domingues	100
Sofia Grade	100
Susana Carvalho Rosa	100

Susana Isabel Elias Alarico	100
Tatiana Catarino	100
Teresa Jesus Delgado	100
Teresa Oliveira	100
Tiago Alves	100
Tiago Brandão Rodrigues	100
Vera Moura	100
Vilma Marisa Arrojado Soares Sardão	100

MSc Students

Ana Catarina Fonseca	100
Ana Filipa Domingues	100
Ana Rita Costa	100
Ana Cristina Silva	100
João Martins	100
Marco Matos	100
Márcio Ribeiro	100
Maria Viegas Nascimento	100
Rui Sanches	100
Tiago Pereira	100
Ana Cristina Gomes	100
Luís Bimbo	100
Rui Abreu	100
Ana Carina Fernandes Pais	100
Cátia Marques	100
Dália Isabel Reis Gonçalves	100
João Demétrio Gonçalves Boto Martins	100
João Pedro Santos Prata Monteiro	100
Nuno Ricardo Ferreira	100
Ricardo Marques	100
Sandra Marina de Almeida Santos	100
Teresa Laura Serafim	100
Ana Luísa Nabais Gomes Nobre	100
Cristina da Silva Oliveira Paulo	100
Joana Medeiros Marques	100
Sofia Ramos	100
Ester Escribano Aranda	100
Ana Isabel Rafael	100
Maria João Silva	100
Ana Sofia Rodrigues	100
Ana Cristina Gonçalves	Collaborator
Elsa Nunes	100
Maria Inês Morte	100
Marta Alexandra Fernandes Neto	100
Paulo Nuno Centeio Matafome	100
Raquel Brito	100
Sara Diniz	100
Teresa Margarida Monteiro Garcia Louro	100

SERVICE STAFF

		Time % at CNC
Ana Cristina Franco dos Santos	(Graduate Technician, CNC)	100
Ana Margarida Ferreira	(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
Luciana C. Albuquerque Pinto	(Graduate Technician, CNC)	100
Luís Miguel M. Vidal Oliveira	(Graduate Technician, CNC)	100
Maria Helena Garruncho	(Graduate Technician, CNC)	Collaborator
Marta Sofia Marques Simões	(Graduate Technician, CNC)	100
Sónia Cristina Costa Oliveira	(Graduate Technician, CNC)	100
Teresa Proença	(Graduate Technician, CNC)	Collaborator

TECHNICAL STAFF

		Time % at CNC
Alda Maria Oliveira Rodrigues	(Technician, CNC)	100
Alexandre Simão Vieira Pires	(Graduate Technician, CNC)	100
Andrea Madeira	(Graduate Technician, CNC)	100
Cármen Lídia Graça Semeão	(Graduate Technician, CNC)	100
Cristina Barosa	(Graduate Technician, CNC)	100
Diana dos Santos Simões Graça	(Technician, CNC)	100
Elisabete Conceição R. Carvalho Lopes	(Technician, FCTUC)	Collaborator
Fátima Cristina dos S. Carvalho Graça	(Technician, CNC)	100
Isabel Nunes Correia	(Graduate Technician, CNC)	100
Maria do Céu Mendes Gomes	(Technician, CNC)	100
Maria Isabel Gonçalves	(Technician, CNC)	100
Núria Filipa Simões	(Graduate Technician, CNC)	100
Odete Pereira Cabanelas	(Graduate Technician, CNC)	100
Patrícia Nunes	(Graduate Technician, CNC)	100
Preciosa Carregado Galhote Esteves	(Technician, CNC)	100
Sandro Pereira	(Graduate Technician, CNC)	100
Teresa Serafim	(Graduate Technician, CNC)	100

ADMINISTRATIVE STAFF

		Time % at CNC
Arménio Pereira Ferreira	(Administrative Assistant, FCTUC)	Collaborator
Benilde Maria Jesus Correia	(Administrative Assistant, FCTUC)	Collaborator
Emilia Viola	(Administrative Assistant, CNC)	100
Elisabete Machado	(Graduate Administrative, CNC)	100
Heidi Maria da Silva Lopes Gonçalves	(Graduate Administrative, CNC)	100
Jasmim António de Jesus	(Graduate Administrative, FCTUC)	Collaborator
José Alberto Correia	(Administrative Assistant, FCTUC)	Collaborator
Lia da Costa Jordão Aparício Lopes	(Graduate Administrative, CNC)	100
Paulo Jorge Machado Barata	(Administrative Assistant, CNC)	30
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

RESEARCH STAFF AND STUDENTS | RESEARCH AREA

Neuroscience and Disease

Catarina Resende de Oliveira, M.D., Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
Alda Maria de Abreu Cardoso Ambrósio	(Investigator, FMUC)	Collaborator
António Francisco Gomes Ambrósio	(Principal Investigator, FMUC)	Collaborator
António Freire Gonçalves	(M.D., Associate Prof., FMUC)	15
António João F. Macedo Santos	(M.D., Assistant Prof. FMUC)	Collaborator
Ana Cristina Carvalho Rego	(Assistant Prof., FMUC)	80
*Ana Luísa Monteiro de Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Pereira da Silva	(Aux. Investigator, FMUC)	Collaborator
*Armanda Emanuela Castro e Santos	(Assistant Prof., FFUC)	80
Armando Jorge Amaral Matias Cristóvão	(Assistant Prof., FCTUC)	80
Arsélio Pato de Carvalho	(Full Prof., FCTUC)	80
Caetana Angélica E. Monteiro de Carvalho	(Full Prof., FCTUC)	80
*Carlos Jorge Alves M. Bandeira Duarte	(Associate Prof., FCTUC)	80
Carlos M. Neves Pato	(M.D., Associate Prof.,USA)	Collaborator
*Catarina Isabel N. Resende de Oliveira	(M.D., Full Prof., FMUC)	80
Cláudia Margarida Gonçalves Cavadas	(Assistant Prof., FFUC)	80
Cláudia Maria Fragão Pereira	(Auxiliar Investigator, FMUC)	100
Emília da Conceição Pedrosa Duarte	(Assistant Prof., FCTUC)	80
Inês Baldeiras	(Investigator, FMUC)	Collaborator
João José Oliveira Malva	(Principal Investigator, FMUC)	100
Luís Augusto Salgueiro Cunha	(M.D., Full Prof., FMUC)	15
Maria Helena Pinto de Azevedo	(M.D., Full Prof., FMUC)	Collaborator
Maria Isabel Jacinto Santana	(M.D., Assistant Prof., FMUC)	80
Maria Manuela Monteiro Grazina	(Assistant Prof., FMUC)	80
*Maria Sancha Vieira Santos	(Investigator, FCTUC)	100
Michelle Pato	(M.D., Associate Prof., USA)	Collaborator
Miguel Castelo-Branco	(Investigator, IBILI)	Collaborator
Paula Maria Garcia Agostinho	(Auxiliar Investigator, FMUC)	100
Paulo Fernando Martins dos Santos	(Assistant Prof., FCTUC)	80
Rodrigo Pinto dos Santos A. da Cunha	(Associate Prof., FMUC)	80
Sandra Morais Cardoso	(Assistant Prof., FMUC)	100

Post-Doc Members

Attila Kofâlvi	100
Ana Cristina Saavedra Martins	100
Fabienne Agasse	100
Geanne Matos de Andrade Cunha	100
Ildete Luísa Ferreira	100
Lisiane de Oliveira Porciúncula	100
Inês Maria Pombinho de Araújo	100
Paulo César da Silva Pinheiro	100
Teresa Almeida	100

M.D. Members

Carlos Eduardo Paz Ferreira	(M.D., HDE, Açores)	Collaborator
Joana Margarida M. Gago da Câmara	(M.D., HDE, Açores)	Collaborator
Luísa Maria Abreu Freire Diogo Matos	(M.D., CHC)	20
Tiago Alfaro	(M.D.)	Collaborator

Ph.D. Students

Ana Catarina Oliveira	100
Ana Raquel Esteves	100
Ana Rita Álvaro	100

Ana Rita Araújo Santos	100
Ana Isabel Marques Duarte	100
André Rodrigues de Abreu Gomes	100
Andrea Lobo	100
Áurea Castilho	50
Bruno José Fernandes Oliveira Manadas	100
Bruno Carreira	100
Bruno Silva	50
Carlos Melo	100
Célia Aveleira	Collaborator
Daniela Arduíno	100
Elisabete Batista Ferreiro	100
Ermelindo Leal	100
Gabriel Costa	100
Joana Gaspar	100
Joana Gil	100
João Pedro Oliveira da Silva Paulino Lopes	100
Joana Lourenço	100
Joana Rosmaninho Salgado	100
*João Duarte	100
José Miguel Brás	100
Jean P. Oses	100
João Carlos Gomes	100
João Pedro Lopes	100
Jorge Oliveira	100
Lígia Ferreira	100
Liliana Inácio Bernardino	100
Margarida Alexandra Vaz Caldeira	100
Maria Joana Lima Barbosa de Melo	80
Maria Fátima Pereira	100
Mário Laço	100
Nelson Rebola	100
*Paula Isabel Moreira	100
Paula Canas	100
Pedro Manuel Venâncio Garção	100
Raquel Ferreira	100
Raquel Santiago	100
Ricardo J. Rodrigues	100
Rita Guerreiro	100
Rita Perfeito	100
Rosa Maria Matos Branco Resende	100
Rui Costa	100
Sandra Cristina Vicente e Almeida	100
Sandra Domingues Santos	100
Sara Alves Xapelli	100
Sofia Domingues	100
Sofia Grade	100
Tatiana Catarino	100
Teresa Oliveira	100

MSc Students

Ana Catarina Fonseca	100
Ana Filipa Domingues	100
Ana Rita Costa	100
Ana Cristina Silva	100
João Martins	100
Marco Matos	100
Márcio Ribeiro	100
Maria Viegas Nascimento	100
Rui Sanches	100
Tiago Pereira	100

Underegraduate Students

A. P. Simões	100
C. G. Silva	100
Susana Louros	100
Sueli Cristina Ferreira Marques,	100
Carla Jesus Cardoso Sousa	100
Cristina Carvalho	100
Sónia Correia	100

**Investigator doing collaborative research involving different groups at CNC*

Molecular Biotechnology and Health

Euclides Pires, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
*Ana Luisa Monteiro de Carvalho	(Assistant Prof., FCTUC)	80
Ana Margarida Vieira da Silva	(Assistant Prof., UVG)	50
Armindo Salvador	(Auxiliar Investigator, CNC)	100
Carlos José Fialho da Costa Faro	(Associate Prof., FCTUC)	80
Euclides Manuel Vieira Pires	(Associate Prof., FCTUC)	80
João Nuno Sereno Almeida Moreira	(Assistant Prof., FFUC)	80
Luís Almeida	(Assistant Prof., FFUC)	80
Maria Conceição Egas	(Auxiliar Investigator, CNC)	100
Maria da Conceição M. Pedroso de Lima	(Full Prof., FCTUC)	80
Paula Cristina Veríssimo Pires	(Assistant Prof., FCTUC)	80
Rui M. Pontes Meireles F. de Brito	(Assistant Prof., FCTUC)	35
Sandra de Macedo Ribeiro	(Auxiliar Investigator, CNC)	30
Sérgio Paulo Magalhães Simões	(Assistant Prof., FFUC)	80

Post-Doc Members	
Ana Sofia Fraga de Almeida	100
Ana Sofia Bregieiro Eulálio	100
Catarina Isabel Ribeiro Pimentel	100
Cristina Isabel dos Santos Fonseca	100
Henrique Faneca	100
Isaura Simões	100
Miguel Luís Cunha Mano	100
Pedro Castanheira	100
Teresa Girão	100

Ph.D. Students	
Adriana Oliveira dos Santos	100
Alexandra Sofia Beirão Mendes	100
Ana Luísa Cardoso	100
Ana Cristina da Silva Filipe	100
*Áurea Castilho	100
Cândida Susana Gonçalves da Silva	100
Daniela Maria Barroso de Moura Cipreste Vaz	100
Inês Vasconcelos Miranda Santos	100
*Isabel Maria Nunes Correia	100
Liliana Mendonça	100
Luísa Cortes Bastos	100
Maria José Simões	100
Nuno Ricardo Ferreira	100
Pedro Miguel Brás Macedo Coelho	100
Rita Rocha	100
Rita Videira	100
Sandro José Paiva Fernandes Alves	100
Sílvia Sousa Neves	100
Ricardo Tomé	100
Vera Moura	100

MSc Students	
Ana Cristina Gomes	100
Luís Bimbo	100
Rui Abreu	100

Undergraduate Students	
Alexandra Faustino	100
João Pedro Pereira	100
Pedro Manuel Batista Branco	100

Cell and Molecular Toxicology

Leonor Almeida, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
Amílcar Falcão	(Full Professor, FFUC)	50
*António Joaquim Matos Moreno	(Assistant Prof., FCTUC)	Collaborator
Carlos Manuel Marques Palmeira	(Assistant Prof., FCTUC)	20
João António Nave Laranjinha	(Associate Prof., FFUC)	80
Joaquim Adelino Ferreira Vicente	(Associate Prof., FCTUC)	Collaborator
José Antunes Barata Custódio	(Associate Prof., FFUC)	80
Leonor Martins de Almeida	(Full Prof., FFUC)	80
Lino Gonçalves	(M.D., Assistant Prof., FMUC)	Collaborator
Maria Amália Silva Jurado	(Assistant Prof., FCTUC)	80
Maria Augusta de S. Fernandes dos Santos	(Investigator, FCTUC)	Collaborator
*Maria Sancha Santos	(Investigator, FCTUC)	100
Paulo J. Oliveira	(Auxiliar Inv., CNC)	100
Romeu António Videira	(Assistant, I.P.V.)	80
*Rui Manuel Silva Gomes Barbosa	(Assistant Prof., FFUC)	80
Teresa Carmo Pimenta Dinis	(Associate Prof., FFUC)	80
Vítor Manuel Calado Madeira	(Full Prof., FCTUC)	80
Post-Doc		
Anabela Pinto Rolo		100
Carla Margarida Pereira Cardoso		100
Fernanda Maria Lopes Ferreira		100
M.D. Members		
Luis Pedro B. Sousa Inês	(M.D., HUC)	Collaborator
Pedro Monteiro	(M.D., HUC)	Collaborator
Ph.D. Students		
*Ana Margarida Cruz Ledo		100
Bruno Gago		100
Carla Nunes		100
Filomena Silva		100
João Gonçalo Leal Frade		100
Manuel Joaquim M. Gonçalves Matos		50
*Marco Alves		100
Vilma Marisa Arrojado Soares Sardão		100
Paula Matos de Brito		100
*Paula Moreira		100
Ricardo Santos		100
MSc Student		
Ana Carina Fernandes Pais		100
Cátia Marques		100
Dália Isabel Reis Gonçalves		100
João Demétrio Gonçalves Boto Martins		100
João Pedro Santos Prata Monteiro		100
Nuno Ricardo Ferreira		100
Ricardo Marques		100
Sandra Marina de Almeida Santos		100
Teresa Laura Serafim		100
Undergraduate Student		
Júlio Matos		100

Gonçalo Pereira	100
Ana Filipa Branco	100
Cláudia Pereira (undergraduated student)	100
*Cristina Carvalho (undergraduated student)	100
*Sónia Correia	100
*Cristina Carvalho	100
Cláudia Pereira	100
Nuno Alexandre Gonzalez	100
Tânia Laranjeiro	100

**Investigator doing collaborative research involving different groups at CNC*

Microbiology

Milton Costa, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
Ana Paula Kuan Yon Chung	(Investigator, I.A.V.)	Collaborator
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Maria Fernanda P. N. Gomes Nobre	(Principal Investigator, FCTUC)	100
Milton Simões da Costa	(Full Prof., FCTUC)	80
Teresa Maria Fonseca de Oliveira Gonçalves	(Assistant Prof., FMUC)	80
Post-Doc Members		
André Antunes		100
Nuno Miguel Empadinhas		100
Jorge Fernandes dos Anjos		100
Ph.D. Students		
Ana Simões Pinto Oliveira		100
Chantal Fernandes		100
Igor Clemente Tiago		100
Joana Cardoso da Costa		100
Susana Isabel Elias Alarico		100
MSc Students		
Ana Luísa Nabais Gomes Nobre		100
Cristina da Silva Oliveira Paulo		100
Joana Medeiros Marques		100
Sofia Ramos		100
Undergraduate Students		
João Paulo Carvalho		100
Alexandra Abrunheira		100

**Investigator doing collaborative research involving different groups at CNC*

Biophysics and Biomedical NMR

Carlos Geraldes, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
•Ana Margarida Malaquias P. Urbano	(Assistant. Prof., FCTUC)	Collaborator
•Carlos Frederico G. Campos Geraldes	(Full Prof., FCTUC)	Collaborator
John Griffith Jones	(Principal Investigator, CNC)	100
Madalena Caldeira	(Assistant Prof., FCTUC)	Collaborator
•Maria Carmen M. de Carvalho Alpoim	(Associate Prof., FCTUC)	Collaborator
•Maria Margarida Catalão Almiro e Castro	(Assistant Prof., FCTUC)	Collaborator
•Rui de Albuquerque Carvalho	(Assistant Prof., FCTUC)	Collaborator
Rui Manuel Silva Gomes Barbosa	(Assistant Prof., FFUC)	80
M.D. Members		
Ana Fagulha	(M.D. HUC)	Collaborator
Carla Baptista	(M.D. HUC)	Collaborator
Luísa Barros	(M.D. HUC)	Collaborator
Luísa Diogo	(M.D. HPC)	Collaborator
Manuela Carvalheiro	(M.D. HUC)	Collaborator
Margarida Bastos	(M.D. HUC)	Collaborator
Paula Garcia	(M.D. HPC)	Collaborator
Ph.D. Students		
• Ana Francisca Soares		100
*Ana Margarida Ledo		100
Francisco José Baptista Melo Simões de Deus		100
• Claudia Silva (Postgraduate research fellow)		100
Duarte A. Marques		100
•Giovannia Araujo de Lima Pereira		100
*João Duarte		100
• Marco Alves		100
Rita Susana Rosa Branco		100
Romeu Miranda Francisco		100
• Sara Gonçalves		100
•Teresa Jesus Delgado		100
• Tiago Alves		100
•Tiago Brandão Rodrigues		100
MSc Students		
Ester Escribano Aranda		100
Ana Isabel Rafael		100
Maria João Silva		100
Undergraduate Students		
• Daniela Ribeiro		100
Inês Violante		100
• Manuela Almeida		100
Sara Figueiredo		100
Tiago Rito		100

**Investigator doing collaborative research involving different groups at CNC*

• *Collaboration NMR Spectroscopy Unit (61/94)/CNC*

Cell and Development Biology

Celeste Lopes, Ph.D., João Ramalho Santos Ph.D., Coordinators

Members holding Ph.D.		**Time % at CNC**
Alexandrina M. Ferreira S. Pinto Mendes	(Assistant Prof., FFUC)	60
Américo Costa Figueiredo	(M.D., Assistant Prof., FMUC)	Collaborator
*António Joaquim Matos Moreno	(Assistant Prof., FCTUC)	Collaborator
*Maria Sancha Santos	(Investigator, FCTUC)	100
Ana Bela Sarmento A. Cruz Ribeiro	(M.D., Assistant Prof., FMUC)	80
*Carlos Jorge Alves M. Bandeira Duarte	(Associate Prof., FCTUC)	80
Cristina Maria Tristão Sena	(Assistant Prof., FMUC)	80
Eugénia Carvalho	(Aux. Investigator, CNC)	100
João Ramalho Santos	(Assistant Prof., FCTUC)	80
José António Pereira da Silva	(M.D., Associate Prof., FMUC)	20
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
Maria Celeste Fernandes Lopes	(Full Prof., FFUC)	80
Maria Graça Santos Pratas Vale	(Full Prof., FCTUC)	80
Maria Teresa de Teixeira Cruz Rosete	(Assistant Prof. FFUC)	80
Otilia Vieira	(Aux. Investigator, CNC)	100
Raquel Seiça	(M.D., Associate Prof. FMUC)	80
Teresa Maria Caldeira Martins	(Associate Prof.)	20

M.D. Members		
Adriana Teixeira	(M.D, HUC)	Collaborator
Américo Costa Figueiredo	(M.D., Ph.D.)	Collaborator
Emília Cortesão	(M.D, MSc Student, FMUC)	Collaborator
Fernando Judas	(M.D., Ph.D.)	Collaborator
Fernando Gomes	(M.D., HUC)	Collaborator
José António Pereira da Silva	(M.D., Ph.D.)	Collaborator
Hermínio José Tão Espírito Santo	(M.D.)	Collaborator
Isabel Sousa	(M.D, HUC)	Collaborator
Manuela Lacerda	(M.D., Ph.D., IPO**)	Collaborator
Marília Dourado	(M.D., Ph.D., FMUC**** and CIMAGO*****)	Collaborator
Maria Margarida Gonçalo	(M.D., HUC)	Collaborator
Olinda Rebelo	(M.D., HUC)	Collaborator
Paulo Figueiredo	(M.D., IPO**)	Collaborator

Post Doc	
Anália do Carmo	100
Carla Cardoso	100

Ph.D. Students	
Alexandra Amaral	100
Ana Paula Sousa	100
Ana Luisa Vital Carvalho	100
Artur Augusto Paiva	100
Bruno Miguel das Neves	100
Hugo Prazeres	100
Inês Crespo	100
José Mário Tenera Morgado	100
Maria Teresa de Jesus Matos	100
Marta Viegas da Silva	100
Paula Mota	100
*Paula Moreira	100
Rui Nobre	100
Sandra Amaral	100

Sandra Gamboa	100
Sandra Varum	100
Susana Carvalho Rosa	100

MSc Student

Ana Sofia Rodrigues	100
Ana Cristina Gonçalves	Collaborator
Elsa Nunes	100
Maria Inês Morte	100
Marta Alexandra Fernandes Neto	100
Paulo Nuno Centeio Matafome	100
Raquel Brito	100
Sara Diniz	100
Teresa Margarida Monteiro Garcia Louro	100

Undergraduate Student

Renata Tavares	100
Marta Baptista	100
Luís Martins	100

**Investigator doing collaborative research involving different groups at CNC*

***IPO – Portuguese Institute for Oncology, Coimbra*

*****FMUC - Faculty of Medicine, University of Coimbra*

******CIMAGO – Center of Investigation in Environment, Genetics and Oncobiology*