Laboratory rotations

2015-16

- Master Program in Health Sciences, ECS
- PhD Programs
  - Health Sciences, ECS, University of Minho
  - PhD-iHES (Health Sciences in an Industrial Setting), ECS, University of Minho
  - PhDOC – PhD program in Aging and Chronic Diseases
    (ECS – University of Minho; FM – University of Coimbra; FM – Nova University of Lisbon)
- MD/PhD – Summer rotations

- Internships with Foreign Universities

School of Health Sciences, Life and Health Sciences Research Institute
1 Surgical Sciences Research Domain
1.1 Role of cancer cell metabolism in the modulation of RTKs targeted therapy response

Summary
The discovery of alterations on receptor tyrosine kinases (RTKs) signaling paved the way for the development of such successful molecular targeted therapies. However, similarity to chemotherapy, the main challenge of targeted therapy in cancer treatment is drug resistance. These targeted agents have largely achieved only modest clinical results. Resistance commonly occurs through the acquisition of compensatory mechanisms that bypass the “addiction” of the mutated gene (i.e., oncogene) that is targeted pharmacologically.

The differences in glucose metabolism that distinguish most malignant and normal tissues have called attention to the importance of understanding the molecular mechanisms by which tumor energy metabolism is regulated. RTKs pathways that are implicated in proliferation and transformation have been also linked to several aspects of tumor glucose metabolism. Previous results from our group, using glioblastoma cells, showed that imatinib (an anti-RTK) treatment induced an increase in glucose uptake and lactate release into the extracellular medium. Additionally, it was very recently described that metabolic rewiring can potentially drive resistance to targeted cancer therapy. However little is known about this topic and the mechanisms through which cellular metabolism modulates targeted therapies are unknown.

Aims
To shed light on the role of cancer cells metabolism modulation of RTK targeted therapies in solid tumors. Firstly, to screen for metabolic alterations before and after cells treatment with anti-RTKs agents; in second, to study the influence of pharmacology modulation of glucose metabolism in the re-sensitization of cancer cells to anti-RTKs therapy.

References

Skills to be achieved in this project
As specified in excel file.

Supervisors
Olga Martinho and Fátima Baltazar
1.2 Molecular insights into the response of cervical cancer cells to molecular targeted therapies

**Summary**

Cervical cancer (CC) is a common malignancy which kills 288,000 women annually. However, despite technological advances, up to 35% of all CC patients will develop a metastatic disease. Infection of human keratinocytes by oncogenic HPV subtypes is critical for cervical carcinogenesis, however, it is not sufficient for cancer development. Among other molecular cofactors investigated by our group, receptor tyrosine kinases (RTKs), mainly the Erbb family, and MAPK pathway modulators (such as RKIP) are frequently altered in CC, constituting in some cases predictors of poor prognosis. In the past two decades we have seen the successful development of RTKs signaling targeted drugs, expanding treatment options for cancer patients. However, actually there is no FDA approved molecular targeted therapies in CC mainly because the preclinical studies are scarce.

**Aims**

The aim of this study is firstly to screen for effective molecular targeted therapies in cervical cancer cell lines and secondly to find out potential predictive factors of therapy response.

**References**


**Skills to be achieved in this project**

As specified in excel file.

**Supervisors**

Olga Martinho and Rui M. Reis
1.3 Raf kinase inhibitor protein (RKIP): A modulator of RTKs molecular targeted therapies response in solid tumors?

Summary

Cancer treatment is being revolutionized by the translation of knowledge achieved in cancer biology studies in the development of new drugs that act by molecular recognition. However, the understanding of cancer molecular genetics suggests that the complex heterogeneity of human malignancies is a major limitation for the application of these novel cancer treatment approaches, and the prognosis of the majority of solid tumors remains largely dismal. Similarity to chemotherapy, the main challenge of targeted therapy in cancer treatment is drug resistance. In particular, deregulation of one signaling pathway can sometimes alleviate or bypass the “addiction” to another pathway. For example, disruption of the intracellular signaling pathways, such as the mitogen-activated protein kinase (MAPK) cascade can modulate the response to anti-RTKs inhibitors. Raf kinase inhibitor protein (RKIP), firstly described as an inhibitor of MAPK pathway, is involved in other intracellular signaling pathways. Thus, RKIP downregulation is associated with prognosis and malignant progression in several tumor types. Moreover, downregulation of RKIP led to tumor cells resistance to chemotherapy. Due to the important role of RKIP in the regulation of important intracellular signaling pathways, and its involvement in some tumors malignancy, we believe that RKIP can also modulate tumor cells response to RTKs molecular targeted therapies. Surprisingly, besides our preliminary and exciting results, there are no studies screening for RKIP expression as a possible predictor or modulator of cancer patients response to targeted therapies.

Aims

Thus, the major aim of this project is to shed light on the mechanisms of cancer cells response to RTK targeted therapies, by studying the role of RKIP expression in the modulation of tumors response to both established and emergent anti-RTKs therapies in solid tumors.

References


Skills to be achieved in this project

As specified in excel file.

Supervisors

Olga Martinho and Rui M. Reis
1.4 Exploitation of Monocarboxylate transporters (MCTs) as therapeutic targets in solid tumours

Summary
Most solid tumours rely on glycolysis for energy, which results in an increased production of acids, namely lactic acid. To reduce the intracellular level of acids and maintain the physiological pH, tumour cells upregulate several membrane transporters, of which MonoCarboxylate Transporters (MCTs). Therefore, acid efflux through MCTs constitutes an important mechanism involved in the maintenance of tumour intracellular pH. Recent evidence demonstrates that MCTs are upregulated in some in solid tumours. We are currently evaluating the role of MCT expression and activity in several types of solid tumours and, so far in human tissues, we found MCT overexpression in colorectal (1), cervix (2), prostate, breast (3) and brain tumours. However, the dependence of tumour cells on MCT activity is poorly understood. Besides studying the expression of the MCT isoforms MCT1 and MCT4 in human tumors, we are also characterizing the effect of MCT inhibition on several tumour cell lines, by exposing the cells to known MCT inhibitors or with specific inhibition of MCT expression. MCTs are attractive targets for cancer therapy, which are now starting to be explored in the clinical context (first clinical started in 2011, AstraZeneca).

Aims
Evaluation of MCT expression in samples of human solid tumours. Growth and maintenance of tumour cell cultures; assessment of tumour cell viability upon MCT activity/expression inhibition.

Skills to be achieved in this project
As specified in excel file.

Supervisor(s)
Fátima Baltazar and Marta Costa
1.5 Lung development orchestration by tissue macrophages

Summary
Lung dysfunction represents a great challenge, since current therapies simply provide symptomatic relief, without reversing organ damage. Strong evidences support that developmental genes and pathways are commonly associated with disease pathogenesis. Thus, it is essential to deeply understand lung developmental processes. Importantly, macrophages are one of the cell populations present throughout foetal and postnatal lung development (1,2). They are a plastic and heterogeneous cell population that shape their cellular neighbourhood, in different pathological situations (3,4). Interestingly, growing evidences demonstrate developmental morphogenesis regulation by macrophages, namely in vasculature and branching, key processes in lung organogenesis (6). Although, these data highlights a promising role for macrophages in lung development, nothing is known about it. Our unpublished data show that macrophage deficient fetus present impairment in lung morphology, vasculature and alveolar differentiation.

Aims
- Understand the consequences of tissue macrophage ablation in lung morphogenesis (e.g. microvasculature, proliferation and apoptosis, differentiation) and in lung developmental molecular pathways;
- Identification of molecules induced by tissue macrophages regulating lung development;
- Understand the contribution of macrophages-derived molecules to lung epithelial differentiation and repair.

References

Skills to be achieved in this project
As specified in excel file.

Supervisor
Sandra Costa
1.6  Characterization of intercellular signaling pathways involved in chick lung development

Summary
Lung development is directed by intrinsic epithelial-mesenchymal interactions that regulate cell proliferation, fate, migration and differentiation, leading to branching morphogenesis and ultimately to proper lung formation. Pulmonary development is controlled by genetically conserved intercellular signaling pathways also known to be involved in body pattern formation, and that include bone morphogenetic proteins, fibroblast growth factors, sonic hedgehog and Wnt signaling pathways, insoluble extracellular matrix proteins and their receptors, as well as various transcription factors. The intricate mechanisms that determine a correct lung development depend on the involvement of several paths. Aiming to untangle an association between these signaling pathways in chick lung morphogenesis, inhibitors of these signaling pathways will be added to chick lung explants culture to evaluate their effect in lung morphology. Moreover, the expression pattern of the genes belonging to these pathways will be characterized as well as the intracellular mechanisms involved in these processes.

With this project, we expect to confirm the relationship between regulators of lung development (FGF, Notch, SHH members), disentangling its potential role in the overall process of fetal lung morphogenesis.

Aims
To characterize the expression pattern of the members of known molecular players involved branching, in the chick model
To dissect the links between different signaling pathways

References

Skills to be achieved in this project
As specified in excel file.

Supervisor
Rute Moura
1.7 Study the role of the tumour suppressor gene SPINT2 in Melanoma

Summary
Serine protease inhibitor Kunitz type 2 (SPINT2) has been identified as a tumour suppressor gene in various solid tumours. SPINT2 inhibits serine proteases involved in the extracellular matrix degradation and HGFA, regulating the HGF/MET signalling. Still, few mechanistic studies have explored SPINT2 deregulation and an extensive characterization of its clinical relevance is needed. We have screened SPINT2 promoter methylation on 77 cancer cell lines, from 13 different solid tumours and found that it was a frequent event in several tumour types. Further, we have extensively characterized series of colorectal, brain and prostate cancer, regarding SPINT2 methylation and protein expression and found correlations between promoter methylation, and absence or dislocation of protein expression, and tumour aggressiveness, particularly in brain and colorectal cancers. Recent findings from our group suggest that SPINT2 is highly frequently down-regulated in advanced stages of melanoma and promoter methylation is the epigenetic mechanism responsible for silencing the gene.

Aims
Determine the clinical relevance of the tumour suppressor SPINT2 in melanoma and its functional role in the genesis and progression of this tumor type. Growth and maintenance of melanoma cell cultures for assessment of cell viability and tumorigenic behaviour after SPINT2 expression modulation.

References

Skills to be achieved in this project
As specified in excel file.

Supervisors
Marta Viana-Pereira and Rui Manuel Reis
2  Microbiology and Infection Research Domain
2.1 Molecular mechanisms underlying inflammasome activation in antifungal immunity

Summary
Invasive aspergillosis (IA) is an infectious disease caused mainly by the fungus Aspergillus fumigatus. This infection typically affects immunocompromised patients such as hematological patients or recipients of hematopoietic stem cell transplants. Owing to a constantly increasing incidence, IA is a leading cause of death among transplant recipients with a 1-year mortality reaching 75%, despite the availability of several antifungal drugs.

Inflammasomes are multimeric protein complexes consisting of a sensor molecule, the adaptor ASC, and procaspase-1. During infection, tissue damage or metabolic imbalances, a range of substances able to trigger inflammasome formation emerge. Once the protein complexes form, inflammasomes activate caspase-1, which proteolytically activates and induces the release of the proinflammatory cytokines IL-1beta and IL-18. Although this process contributes to normal host defense to several infectious diseases and to immune homeostasis, gain-of-function mutations in the gene encoding the NLRP3 inflammasome leading to excessive IL-1beta production result in severe autoinflammatory diseases.

We now know that genetic variants affecting immune responses can compromise specific modules of antifungal immunity and predispose to infection. Preliminary data from our laboratory has disclosed an association between a single nucleotide polymorphism (SNP) in NLRP3 underlying an amino acid substitution (Q705K) and susceptibility to IA in a large Portuguese multicenter cohort of recipients of hematopoietic stem cell transplantation. Moreover, primary macrophages carrying the mutation and stimulated in vitro with live conidia of A. fumigatus were found to be high producers of IL-1beta, suggesting that a hyperactivation of the NLRP3 inflammasome might be detrimental to antifungal immunity and underlie increased susceptibility to infection.

Aims
Characterize the consequences of the Q705K mutation in NLRP3 to antifungal effector mechanisms of macrophages and epithelial cells;
Elucidate the molecular aspects underlying the mutation-driven hyperactivation of the NLRP3 inflammasome.

Skills to be achieved in this project
As specified in excel file.

Supervisors
Cristina Cunha and Agostinho Carvalho
2.2 Dissecting immunometabolism traits of antifungal immunity activation

Summary

Invasive aspergillosis (IA) is an infectious disease caused mainly by the fungus Aspergillus fumigatus. This infection typically affects immunocompromised patients such as hematological patients or recipients of hematopoietic stem cell transplants. Owing to a constantly increasing incidence, IA is a leading cause of death among transplant recipients with a 1-year mortality reaching 75%, despite the availability of several antifungal drugs.

Alterations in cellular metabolism represent a fundamental mechanism by which immune cells maintain homeostasis. In response to pathogens or tissue damage, immune cells must rapidly adapt their metabolic programs to meet specialized host defense needs. This adaptation is bioenergetically expensive, requiring precise control of cellular metabolic pathways relying on the consumption of substrates such as glucose, fatty acids or amino acids. By fueling the cell fate decisions and effector functions of immune cells, metabolic pathways of oxidative metabolism, glycolysis and glutaminolysis are critical during immunity and inflammation processes.

Although several evidence has implicated immunometabolic dynamics in shaping immunity to several pathogens, nothing is know about these processes in defining antifungal immune responses. Our laboratory is now focussing on investigating how immunometabolism dynamics converge to influence the function of macrophages and ultimately the immune response against A. fumigatus. To do so, we are using in vitro models of infection and clinical specimens of human patients suffering from IA.

Aims

Characterize the immunometabolism dynamics and its modulation in primary human macrophages infected with live conidia of A. fumigatus;
Define the peripheral and lung metabolome of stem-cell transplanted patients suffering from IA.

Skills to be achieved in this project

As specified in excel file.

Supervisor(s)

Cristina Cunha and Ricardo Silvestre
2.3 Impact of IL-10 on the plasticity of the spleen

Summary

The spleen is a lymphoid organ extremely well organized to answer to blood born infections. The spleen organization is mediated by cytokines and chemokines. We have an experimental model were mice, over expressing IL-10 for 30 days show a disorganization of the spleen. Interestingly, as IL-10 levels are set normal the spleen acquires what seems to be a normal organization. These observations raise important questions.

Aims

What are the molecular mediators, regulated by IL-10, that are involved in spleen disorganization?
Does the, apparently normal, organization of the spleen upon exposition to high levels of IL-10 is really normal?

Skills to be achieved in this project

As specified in excel file.

Supervisor(s)

Gil Castro and Bruno Silva
2.4 Dissecting the role of IL-10 in monocyte/macrophage differentiation and function during anti-tumor immunity

Summary

Tumor-induced immune suppression is a significant impediment to immunosurveillance and immunotherapy in cancer. Clinical studies and experimental mouse models suggest that tumor-associated macrophages are key for tumor progression as they are generally associated with aggressive tumor growth with a high metastatic potential.

IL-10 is an anti-inflammatory cytokine with a broad impact in the immune system but with a still controversial role in anti-tumor immunity. Interestingly, therapeutic doses of IL-10 used to treat several inflammatory diseases were shown that IL-10 can: i) inhibit angiogenesis, by repressing IL-1α/b, TNF and IL-6; ii) reduce the secretion of matrix metalloproteinases (MMP)-2 and MMP-9; iii) promote a retention of CD14 monocytes in the blood; iv) induce high levels of IFNγ form peripheral blood mononuclear cells; and v) promote NK and CD8 T cell cytotoxicity. These mechanisms have been associated with anti-tumor immunity and suggest that this cytokine may be of therapeutic value as part of combination therapy in cancer treatment.

The aim of this project is to study the role of IL-10 and the mechanisms whereby this cytokine modulates the anti-tumor immune response. To this end, we have developed a genetically modified mouse strain wherein IL-10 is under the control of a zinc-inducible metallothionein promoter (pMT-10), thus allowing the temporal control of IL-10 expression.

Specific aims

1- To determine the temporal role of IL-10 in tumor progression and metastasis.
2- To determine the extent to which IL-10 modulates the anti-tumoral immune response, including the accumulation, differentiation and polarization of monocytes/macrophages and activation of T cells in the tumor microenvironment.
2- To define the pathways modulated by IL-10 that impact tumor progression and metastasis.

Skills to be achieved in this project

As specified in excel file.

Supervisors

Egídio Torrado and Ana Margarida Barbosa
2.5 Immunogenetics in Buruli Ulcer: the role of TNF-α (Tumour Necrosis Factor Alpha) polymorphisms

Summary

Susceptibility to an infectious agent and variability in disease outcomes are dependent on the interaction between environmental and host genetic factors. Nevertheless, little is known about the role of host genomics in susceptibility to Buruli Ulcer (BU), a neglected necrotizing skin disease caused by Mycobacterium ulcerans infection. Data from a population-based genetic association study performed by our group using samples from a BU endemic population in Benin report that a single nucleotide polymorphism (SNP) rs1800629 located in the promoter region of the TNFA (Tumour Necrosis Factor Alpha) gene is associated with the development of BU.

TNF-α is a proinflammatory cytokine produced by a wide range of cell types, including macrophages, and induce different cellular responses. The relevance of this cytokine in the control low and intermediate virulence M. ulcerans infection was previously shown by our lab, since TNF-α absence led to an increased bacterial proliferation.

Therefore, we propose to uncover if this polymorphism impacts TNFA gene transcription during M. ulcerans infection, leading to a differential production of TNF-α depending on the genotype. To accomplish our goal, macrophages will be differentiated from human Peripheral Blood Mononuclear Cells (PBMCs). Cells with different genotypes for the SNP will be challenged with a mycolactone-negative M. ulcerans strain in the presence/absence of non-cytotoxic doses of mycolactone. At different time-points post-infection, TNF-α expression and protein production will be measured by Quantitative Real-Time PCR (qRT-PCR) and Enzyme-Linked Immunosorbent Assay (ELISA), respectively.

Aim

Determine the impact of TNFA rs1800629 polymorphism in TNF-α production during M. ulcerans infection.

Skills to be achieved in this project

As specified in excel file.

Supervisors

Alexandra Fraga and Jorge Pedrosa
2.6 The interplay between autophagy and DNA replication stress during aging: the glutaminolysis contribution

Summary

Aging is characterized by the progressive accumulation of damaged macromolecules/organelles associated with the decline of housekeeping processes. The inefficient removal of nonfunctional cellular components due to autophagy impairment has been proposed to be the main cause of the biological “waste” accumulation that appears to be critical in the progression of aging. How autophagy regulation is coordinated with other cellular processes during aging is still matter of intensive research. DNA replication stress, caused by DNA replication forks that delay the repair of damages, is also an important factor in aging progression. Our previous work demonstrated that under a proteotoxic context, elicited by the heterologous expression of the human alpha-synuclein, aged yeast cells presented increased autophagy activity linked to the promotions of DNA replication stress. This connection is reflected by lower ATP intracellular levels associated with the autophagic-dependent Rnr1 (ribonucleotide reductase 1, responsible for the dNTPs synthesis) degradation and concomitantly decreased dNTPs pool. Nucleotides are essential for a wide variety of biological processes and are constantly synthesized in cells, either de novo or via the nucleoside salvage pathways. Glutaminolysis is a mitochondrial pathway that comprises a series of biochemical reactions by which glutamine is degraded to glutamate that is further metabolized by conversion to alpha-ketoglutarate and then into malate. During this reactions, it is produced NADPH that among other processes is used in the nucleotide metabolism. On the other hand, a crosstalk between glutaminolysis and autophagy was already established. Therefore, the present project aims to elucidate mechanisms underlying the crosstalk among DNA replication stress, glutaminolysis and autophagy in yeast aged cells. The understanding of the molecular mechanisms coordinating DNA replicative stress and autophagy is essential to understand aging and age-related diseases.

Aims

- To monitorize autophagy activity by the GFP-Atg8 processing assay and cell cycle during chronological lifespan (CLS) of wild-type cells submitted or not to conditions of DNA replication stress, induced by proteotoxic stress;
- To determine glutamine, NADH and glutamate concentrations during the cells CLS;
- To determine intracellular amino-acids, particularly serine and glycine, by HPLC.

Skills to be achieved in this project

As specified in excel file.

Supervisors

Paula Ludovico and Belém Sampaio-Marques
2.7 Regulation of mitophagy: the Parkin contribution

Summary
Mitophagy is a process that mediates selective removal of damage mitochondria. In yeast, the mitophagy pathway is very well characterized. When mitophagy is induced, the mitochondrial outer membrane protein Atg32, a specific mitochondrial receptor, is phosphorylated by an unidentified kinase. This phosphorylation mediates the Atg32-Atg11 interaction that recruits mitochondria to be degraded. It is assumed that mitophagy is conserved in eukaryotes, but the mammalian homologues of Atg32 and Atg11 are not yet identified. In mammalian cells, two mitophagy mechanisms have been identified: PINK/PARKIN- and NIX-dependent, being NIX the mammalian protein that shares some functional homology with Atg32. These recognized mechanisms of mitochondrial degradation are triggered by different stimulus and operate with different efficiencies, from partial to complete elimination of mitochondria. Parkin is a cytosolic E3 ubiquitin ligase, and mutations in this protein are associated with autosomal recessive forms of Parkinson disease. Parkin ubiquitylates several mitochondrial proteins, which in turn recruit other proteins to mitochondria to initiate mitophagy.

To disclose the relevance and the complex role of mammalian mitophagy in cell physiology, it is critical to understand the distinct regulatory mechanisms involved in the regulation of mitochondrial degradation. Thus, the present project aims to further elucidate the Parkin role and its contribution to the mitophagic process.

Aims
- To evaluate chronological lifespan and mitophagy activity, by the alkaline phosphatase assay and by the GFP-Om45 processing, in yeast wild type cells heterologous expressing the human Parkin or harboring the vector control or co-expressing alpha-synuclein;
- To study the involvement of Parkin in the mitophagy process in conditions in which mitophagy is inhibited (using Dats32 cells) or induced (co-expression with α-synuclein).

Skills to be achieved in this project
As specified in excel file.

Supervisors
Paula Ludovico and Belém Sampaio-Marques
2.8 Diabetes/obesity and acute myeloid leukemia: molecular analysis of risk factors and bio prognostic markers

**Summary**

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults, yet has the lowest survival rate. Such dismal prospect has not changed over the past decades as well as the therapeutic strategy that has also not been significantly modified over the last 30 years. Although the therapeutic regimen results in a positive response in young patients, intensified therapy is highly toxic, has limited application and poor outcomes among old individuals, the most affected population, resulting in high mortality. Advancing treatment regimens for this malignancy is therefore critical from a societal perspective, representing a strong interest in public health. The presence of several metabolic alterations/disorders, including diabetes mellitus (DM), obesity and high body mass index (BMI), has been recently associated with the occurrence (or poor prognosis) of different hematological malignancies, including AML. Epidemiological evidence indicates that DM is associated with increased incidence of AML and worst chemotherapy results, while the risk of developing leukemia increases in individuals with increased BMI. However, the biological mechanisms underlying these observations remain to be elucidated. Therefore, the main objective of this project is to dissect the correlation between these altered/abnormal metabolic conditions and the prognosis and/or survival of AML patients, as well as the molecular basis behind this association.

**Aims**

- To characterize the genetic and epigenetic changes induced by exposure to potentially high glucose concentrations in genes related to the proteolytic systems and epigenetic known regulators involved in AML;
- To determine metabolic biomarkers and signatures with prognostic value in AML.

**Skills to be achieved in this project**

As specified in excel file

**Supervisors**

Paula Ludovico
2.9 Molecular mechanisms underlying IL-10 and IL-17F modulation of tumor microenvironment and macrophage function

Summary
The dynamic nature of signaling networks and their interactions between cancer cells and macrophages allows tumor cells to survive and progress. Within the tumor microenvironment IL-10 is a double-edged sword with the potential to display pro- or anti-tumoral activities. On the one hand, IL-10 can favor tumor growth by stimulating cell proliferation and inhibiting both cell apoptosis and T cell activation. In contrast, IL-10 may favor anti-tumor immunity by inhibiting tumor-induced angiogenesis, enhancing the production of tumor-toxic molecules or inducing the anti-tumor activity of T and NK cells. IL-17F is a proinflammatory cytokine involved in tumor-specific immune response and thereby decrease tumorigenicity. Nevertheless, the true implication of IL-10 and IL-17F in tumorigenesis and macrophage function is far from being clarified. With this proposal, we want to dissect the true role of IL10 and/or IL17F in both tumor cells and macrophages.

Aims
Characterize the proliferative and bioenergetics profile of Lewis lung carcinoma (LLC1) mouse cell line exposed to recombinant IL-10 and/or IL-17F
Define the impact of macrophages on tumor cells response to IL-10 and/or IL-17F

Skills to be achieved in this project
As specified in excel file.

Supervisors
Inês Mesquita and Ricardo Silvestre
2.10 Role of IL-10 in the metabolic profile of gut mucosa: influence on monocyte differentiation pattern

Summary

Mononuclear phagocytes are one of the most abundant leucocytes in the intestinal mucosa being involved in gut homeostasis and the pathogenesis of intestinal inflammation. Intestinal macrophages do not fit the current paradigm that tissue-resident macrophages are derived from embryonic precursors that self-renew in situ, but are instead dependent on the continuous recruitment of Ly6Chi blood monocytes. In the steady-state intestinal mucosa, the recruited Ly6Chi monocytes experience a differentiation process through a number of intermediate subsets. During this process, each intermediate monocyte display specific cell-surface markers evidencing a gradual functional specialization in relation to antigen presentation, toll-like receptor (TLR) signaling, phagocytosis and inflammatory/anti-inflammatory profile. Despite of these findings, the mechanisms driving monocyte differentiation in the intestinal mucosa remain largely unknown. This is particularly relevant since the disruption of the differentiation pattern of monocytes in this tissue plays a critical role in the pathogenesis of several chronic diseases namely inflammatory bowel diseases (IBD). Multiple factors have been accounted for the phenotypical and functional signature of the monocytes subsets during differentiation. Among those, much attention has been given to the IL-10/IL-10 receptor (IL-10R) axis. Moreover, recent studies have revealed an intricate relationship between immune cell biology and extracellular or intracellular metabolite profiles. In this context, we hypothesize that the interplay between IL-10 and the metabolic environment play a chief role in the phenotypic changes observed during monocyte differentiation in the gut mucosa, which appears to be unique to the intestine. Within this laboratory rotation we aim to elucidate the interplay between IL-10 and the modulation of environmental metabolic factors and pathways accountable for monocyte training in the mucosa.

Aims

Optimization of a monocyte enrichment protocol from gut samples of IL-10-deficient and wild-type mice, both colitic or healthy.

Development of a flow cytometry strategy, using specific surface markers, to identify the different monocytic populations and their activation status in gut samples of IL-10-deficient and wild-type mice, both colitic or healthy.

Skills to be achieved in this project

As specified in excel file.

Supervisors

Joana Gaifem and Ricardo Silvestre
2.11 The increased susceptibility of diabetic patients to tuberculosis: characterization of parameters of the host immune system

**Summary**

Tuberculosis (TB) remains one of the world’s deadliest communicable diseases. Despite recent investments in TB research and a global effort to implement control programs, the mechanisms underlying the disease remain elusive and the disease incidence is declining at an unsustainably slow rate. High-risk groups are an important factor undermining the success of TB control programs. Current control strategies must, therefore, target interventions to such groups, redirecting efforts towards those who are most likely to develop and to transmit the disease. Of relevance is the evidence that individuals with diabetes mellitus (DM) are one of such high-risk groups. Not only are persons with DM three times more likely to develop active TB compared to those without DM, but TB patients with DM are at higher risk of failure and death than are other non-HIV-infected TB patients. Moreover, the adjusted hazard ratio for contracting TB increases with the severity of DM. This project aims to unveil host factors that are involved in the DM-patients increased susceptibility to TB, characterizing a series of immune features in DM patients with or without TB, as well as in a control group of TB patients that do not have DM.

**Aims**

- Measurement and correlation of a series of immune parameters among the different groups included in the study, including production of cytokines and generation of polyfunctional T cells.

**Skills to be achieved in this project**

As specified in excel file.

**Supervisors**

Teresa Rito and Margarida Correia-Neves
2.12 The increased susceptibility of diabetic patients to tuberculosis: the role played by the invading bacteria

Summary
Tuberculosis (TB) remains one of the world’s deadliest communicable diseases. Despite recent investments in TB research and a global effort to implement control programs, the mechanisms underlying the disease remain elusive and the disease incidence is declining at an unsustainably slow rate. High-risk groups are an important factor undermining the success of TB control programs.

Current control strategies must, therefore, target interventions to such groups, redirecting efforts towards those who are most likely to develop and to transmit the disease. Of relevance is the recent evidence that individuals with diabetes mellitus (DM) are one of such high-risk groups. Not only are persons with DM three times more likely to develop active TB compared to those without DM, but TB patients with DM are at higher risk of failure and death than are other non-HIV-infected TB patients. Moreover, the adjusted hazard ratio for contracting TB increases with the severity of DM. Understanding the molecular and clinical basis of the DM-TB interaction is a crucial step to develop preventive and therapeutic measures to control TB in this group. Whereas a growing body of evidence suggests that DM patients’ immune system may be impaired, making it particularly susceptible to Mycobacterium tuberculosis, the role of the microbe itself and the possibility of specific adaptations have so far been overlooked.

Here, we propose to address the DM-TB problematic from the M. tuberculosis side, examining specific genotypes and/or phenotypes that may be more prone to cause infection in DM patients.

Aims
Analysing the distribution and clustering patterns of M. tuberculosis strains infecting DM and non-DM patients.

Skills to be achieved in this project
As specified in excel file.

Supervisors
Teresa Rito and Margarida Correia-Neves
2.13 Implication of amino acid metabolism in monocyte differentiation

Summary
Nutritional immunology is a research area that has grown in the past years, aiming to define the role of nutrients in the metabolism and function of immune cells, at a cellular and molecular level. Protein malnutrition reduces the amino acid pool leading to the impairment of immune function and the increase of susceptibility to inflammatory and infectious diseases. Recent studies have shown that amino acids have an important role in immune responses through regulation of the activation of immune cells, the balance of cellular redox state, cell proliferation and the production of immune effector molecules (e.g., antibodies, cytokines, acute phase proteins). Arginine, glutamine and cysteine are the main studied players in this hot topic with expanded applications to human nutrition. Glutamine has shown to be essential for nucleotide synthesis, modulation of intermediary amino acid metabolism and glutathione (GSH)-mediated antioxidant defense, being considered a key substrate for the cell survival and maintenance. The metabolism of arginine is crucial for the production of nitric oxide (NO) and the polyamine precursor L-ornithine, which are involved in host protection against infections and cell growth and proliferation, respectively. Dietary cysteine has been shown to play a key role in the effectiveness of antioxidant defense which influences immune function by modulating the actions of oxidant stress at the transcription factor activation level. Thus, we propose to assess the role of non-essential amino acids in the differentiation and effector pattern of monocytes. For this, we will perform an in vitro study of healthy monocyte differentiation in conditional media containing physiological levels of amino acids.

Aims
Address the implication of non-essential amino acid metabolism in the differentiation and effector pattern of monocytes

Skills to be achieved in this project
As specified in excel file.

Supervisors
Ana Belinha and Ricardo Silvestre
2.14 Ammonium and amino acids as key players on dietary restriction and its consequences on yeast longevity in culture medium

Summary

The aging process is conserved from yeasts to mammals, with several studies showing that reducing growth factors/nutrients intake has profound positive effects in extension of life span and also improves overall health by delaying or reducing aged-related diseases in mammals. Further studies have revealed that the major 3 nutrient-signaling pathways TOR, SCH9 and RAS/AC/PKA are involved in longevity regulation by glucose, promoting cell division and growth in response to nutrients. New evidences have recently emerged from studies in yeast and in higher eukaryotes showing the importance of nutrient balance in dietary regimes and its effects on longevity regulation. In this context, we have focused on the role of nitrogen sources, (ammonium and amino acids) as new key players in the modulation of longevity, showing that i) manipulation of ammonium concentration in the culture and/or aging medium can drastically affect chronological lifespan (CLS) of the yeast Saccharomyces cerevisiae, especially in amino acid restricted cells; ii) the CLS shortening under amino acid restriction can be completely reverted by removing ammonium from the culture medium and iii) the absence of ammonium, and of any rich nitrogen source like glutamine, was so effective in extending CLS that no beneficial effect could be observed by further imposing calorie restriction conditions. Our data also show that Tor1p, Ras2p and Sch9p seem to be involved in the mediation of ammonium toxicity under amino acid restriction, highlighting ammonium as a key effector in the nutritional equilibrium between rich and essential nitrogen sources and glucose required for longevity promotion. The present project aims to further elucidate the involvement of these key proteins in ammonium and amino acids interplay on dietary restriction and its consequences on longevity.

Aims

- Evaluation of the chronological lifespan of S. cerevisiae mutants cells deleted on Tor1p, Ras2p and Sch9p (tor1Δ, ras2Δ and sch9Δ), in the presence or absence of ammonium, under caloric restriction by decreasing glucose concentration in culture medium;
- Assessment of other rich nitrogen sources like glutamine and glutamate as potential modulators of nutrient signaling pathways, by evaluating tor1Δ, ras2Δ and sch9Δ cell’s longevity phenotype, under non-caloric and caloric restriction conditions.

Skills to be achieved in this project

As specified in excel file.

Supervisors

Júlia Santos and Cecília Leão
2.15 Gene editing: the cross-kingdom system for genohacking

Summary

Herein, we aim to use the trans-kingdom RNA-guided clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 [nuclease]/[nickase; Cas9n] technology to edit genomes from human cell lines to fungal cells. We will develop and apply the programmable guide sequences to direct cleavage by the Cas9/Cas9n and produce either double (DSB)- or single (SSB)-strand breaks at target sites triggering the formation of small insertions/deletions (INDELs) or allele-specific substitution via exogenous donor template. It is currently known that the introduction of DSB at specific loci allows modification of genomes with high precision and efficiency, where HDR increases in several orders of magnitude. Our main goal is to use the CRISPR/Cas9 system to perform genetic manipulation by 1) the insertion of mutations and 2) targeted homologous recombination, which represent a step towards the comprehension of cell and molecular biology aspects revealed by gene function in its own genetic context. This system offers a promising way of circumvent the resilience of Paracoccidioides spp. to target homologous molecular manipulation, allowing the generation of knock-out strains and also fine-genomic-editing.

Aims

Taking advantage of the resolution offered by this newly developed technology, we expect to successfully manipulate the genome of Paracoccidioides spp., thus moving the field beyond the state of art. So, the generation of targeted loss/gain of function mutants in Paracoccidioides spp. is still both a huge gap and a challenge in the field. In addition, we will apply this system to human primary cell lines targeting pre-known Single Nucleotide Polymorphisms associated to increased susceptibility to immune response.

References


Skills to be achieved in this project

As specified in excel file.

Supervisors

Cristina Cunha and Fernando Rodrigues
2.16 Impact of strain variability on the response to Paracoccidioides spp

Summary
The mammalian immune system has innate and adaptive components, which cooperate to protect the host against infection. Pathogenic fungi can subvert the immune response to prevent elimination by the host. Genetic variability of the Paracoccidioides spp clade have also been recently acknowledge. In the context of this project we aim at defining how different clinical isolates of Paracoccidioides spp modulate the inflammatory response and how does that impact the ability of the host to control infection. To this end, we will use a model of bone-marrow derived macrophages to probe the inflammatory response of these cells to different clinical isolates of Paracoccidioides spp and correlate this information with the ability of mice to control infection.

Aims
Define immune response variability associated with Paracoccidioides spp strain variability

References

Skills to be achieved in this project
As specified in excel file.

Supervisors
Egidio Torrado and Fernando Rodrigues
2.17 The role of Pentraxin 3 in the establishment of paracoccidioidomycosis

Summary

Pentraxin (PTX)3 is a member of a family of multimeric pattern-recognition proteins with known proinflammatory activity. PTX3 acts as a non-redundant component of the humoral branch of innate immunity being also involved in fine-tuning inflammation. It has been shown that peritoneal macrophages derived, from mice overexpressing PTX3, have an improved opsonin-independent phagocytosis of yeast form of Paracoccidioides brasiliensis. Human genetic variants that conditioning the expression levels of PTX3 were shown to be associated with increased susceptibility to aspergillosis. The manipulation the host response with a protective effect of PTX3 by recombinant-PTX3-treating may reveal PTX3 as a potential therapeutic tool in paracoccidioidomycosis, either alone or in combination with antifungals therapy.

Aims

Evaluate the effects of the absence of PTX3 on the profile of resistance/susceptibility to Paracoccidioides spp.

References


Skills to be achieved in this project

As specified in excel file.

Supervisors

Agostinho Carvalho and Fernando Rodrigues
3 Neurosciences Research Domain
3.1 Searching for therapies for Machado-Joseph disease

**Summary**

Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by the expansion of a polyglutamine (polyQ) tract within the C-terminal of the ataxin-3 protein. Ataxin-3 is known to interact with polyubiquitin chains and to have a deubiquitylase (DUB) activity in vitro, however the cellular and physiological role(s) of this protein remain unknown. The leading hypothesis concerning the pathogenesis of polyQ diseases is that the expanded polyQ tract confers a toxic gain of function to the mutant proteins. These disease proteins acquire the ability to self associate and form aggregates. The lack of therapeutic strategies that effectively prevent neurodegeneration in MJD patients prompted us to search for compounds that modulate mutant ataxin-3 aggregation and neurological dysfunction. Recent data from our lab reveal that many aspects of MJD can be properly modeled in the round worm Caenorhabditis elegans, and others have shown that this animal provides a suitable platform for both the discovery of new bioactive compounds and therapeutic target identification. This project is based on the idea that the finding of effective drugs can be accomplished by looking simultaneously at protein aggregation (conformational disorder) in the live neuronal cells, and on its impact on neuronal-regulated behavior of the whole-animal (neurodegenerative disorder). With this in mind, the student will test some small molecules for their ability to prevent or delay the formation of mutant ataxin-3 aggregates by feeding them to our MJD C. elegans model. Additionally, we will address their effect on motor neuron dysfunction by performing motility analysis. The drugs thus identified will in the future be tested in a mouse model of the disease.

**Aims**

-To evaluate the effect of pharmacological compound(s) on the aggregation of mutant ATXN3, using imaging-based criteria;
-To evaluate the effect of the same compound(s) on mutant ATXN3-mediated motor neuron dysfunction (motility assay).

**References**


**Skills to be achieved in this project**

As specified in excel file.

**Supervisors**

Andrea Teixeira-Castro and Patrícia Maciel
3.2 Temporal modulation of the subventricular zone neural stem cell niche by choroid plexus-cerebrospinal fluid derived factors

Summary

With this project we aim at determining the contribution of molecules secreted from the choroid plexus (CP) towards the cerebrospinal fluid (CSF) in the modulation of the subventricular zone (SVZ) neural stem cell population during brain maturation. Lining the lateral walls of the CSF-filled brain ventricles and in the proximity of the CP, the subventricular zone (SVZ) is one of the main neural stem cell niches in the postnatal brain. Several studies suggest that the SVZ functions as a reservoir of progenitor cells for brain repair. Adult neural progenitor cells in the SVZ are derived from radial glia, the embryonic neural stem cells; during development, radial glia generates neurons and glial cells for the assembly of the mature brain. Interestingly, at birth, the lateral wall of the brain ventricles is still comprised of radial glial cells similarly to what is observed in the ventricular zone in early embryonic stages. Then, in the initial postnatal days, radial glia cells are converted into adult neural stem cells; the latter are the neural progenitors at juvenile and adult stages. The mechanism through which this cellular transition occurs and is regulated in such a short time window period is largely unknown. We propose that factors secreted by the CP towards the CSF play a role in this timely transition of brain morphology.

Aims

We currently aim at exploring the specific contribution of CP-born CSF molecules in the homeostasis of the SVZ neural stem cell niche by:

i) Determining the temporal changes in the CP transcriptome in specific milestones of SVZ development (pre-natal, early post-natal and adult);

ii) Study in vitro and in vivo the impact of identified CP-derived proteins/molecules in the SVZ; in vitro by using a transwell co-culture system of CP epithelial cells and SVZ neurospheres; in vivo with viral vectors that specifically target CP epithelial cells and thus abrogate or overexpression of CP proteins secretion towards the CSF; the impact of this changes in the SVZ is analyzed by using cell specific markers; cell fate of SVZ born cells is analyzed in the olfactory bulb and in the corpus callosum.

References


Skills to be achieved in this project

As specified in excel file.

Supervisors

João Carlos Sousa and Diana Afonso
3.3 The role of N-glycans in cell-cell communication in neural cells

Summary
This project aims at gaining insight in the role of cell surface and extracellular matrix glycans in the modulation of neural cells communication. Glycans are complex carbohydrate molecules composed of variable length chains of simple sugars that are associated with intracellular and cell surface proteins and lipids. Glycosylation is an important post-translational modification with a strong impact in organism development and homeostasis. In the brain glycans are relevant for neural cells differentiation and function, and were recently implicated in synapse formation and synaptic transmission. Nevertheless how different glycosylation patterns affect nervous system wiring and function is still largely unknown. We are currently using animal models (C.elegans and rodents) to address how N-glycans modulate neuronal function. Specifically, we are exploring the effects of disturbing specific enzymatic pathways of glycosylation on the nervous system.

Aims
We are using specific C.elegans loss-of-function mutants for key enzymes in the synthesis of N-glycans to evaluate:
1) neuronal dependent animal behaviour;
2) structure of the nervous system;
3) the effects on glycosylated proteins of the synapse; and
4) variations in the type and levels of glycans.
Furthermore we explore the effects of the stress response in the glycosylation process in the neural cells. Knowledge obtained will be of value to understand pathophysiological processes such as the stress response and neuronal regeneration.

References

Skills to be achieved in this project
As specified in excel file.

Supervisor
João Carlos Sousa and Ana Veloso
3.4 Exploring the Therapeutic Potential of the Secretome of Mesenchymal Stem Cells and Bioreactors in Parkinson’s Disease Animal Models

Summary

Human Mesenchymal stem cells (hMSCs) have been proposed as possible therapeutic agents for central nervous system (CNS) disorders. Nowadays it is suggested that their effects are mostly mediated through their secretome, which contains several neuroregulatory molecules capable of increasing cell proliferation, differentiation and survival (1). In light of the actual knowledge of the MSCs therapeutic potential is extremely relevant to establish the best culture parameters of MSC populations because little is known about the secretome of MSCs and their applications in the CNS (2). Additionally, as MSCs are highly responsive to dynamic culturing environments, one could expect to modulate and possibly increase the level of the above referred neuroregulatory factors in the secretome through the use of bioreactors. Thus, it is logical to hypothesize that when subjected to different dynamic culturing conditions, the secretome of these cells might change. Moreover, as high yields of cells will be obtained, the possibility of having higher concentrations of neuroregulatory factors in their conditioned media (CM) will also increase. We have previously shown that the secretome of MSCs cultured in bioreactors is able to induce higher differentiation rates in populations of human neural stem cells. Following this initial results the goal is to test its therapeutic properties in a Parkinson’s Disease rat model. For this purpose the 6-OHDA unilateral model (4) will be used, and the secretome of MSCs locally injected in the striatum and substantia nigra and compared with MSCs and NSCs transplanted groups. Follow up will be assessed through behavioral and histological analysis.

Aims

Unveil how the dynamic cultured obtained secretome of MSCs modulates: 1) the motor symptoms of PD in the 6-OHDA model; 2) the non-motor symptoms in the 6-OHDA model; 3) Neuronal cell survival and differentiation in the affected areas. Experimental Techniques: Cell and Tissue Culture, Behavioral Analysis, Immunohistochemistry, Histology, Neurostructural Analysis, Fluorescence/Confocal Microscopy.

References

- Teixeira FG et al., Stem Cells Reviews and Reports, 2015, 11(2): 288
- Teixeira FG et al., Stem Cell Research and Therapy, 2015, 6(1): 133
- Salgado AJ et al., Frontiers in Cellular Neuroscience, 2015, 9: 249

Skills to be achieved in this project

As specified in excel file.

Supervisors

António Salgado and Fábio Teixeira
Pharmacotherapies and Extracellular Matrix like Hydrogels as tools for Spinal Cord Injury Regeneration: A combinatorial Approach

Summary

Spinal cord injury (SCI) is a major medical problem world-wide that affects 11,000 people/year in EU only and usually results in devastating and permanent loss of function (paraplegia and quadriplegia). Therefore it is urgent to find novel strategies that can lead to the regeneration of SCI affect sites and individuals, as the present ones (mainly pharmacological agents) do not elicit regeneration. Due to the complexity of SCI, only regenerative strategies based on multidisciplinary and integrative approaches such as those presented by tissue engineering concepts, will adequately tackle the problem. Tissue engineering, a field of science that has been developed through the last 15 years, stands on the interface between materials science, biology and medical sciences, and aims at developing tissue hybrids that induce tissue regeneration [1]. By following these concepts we have previously developed extracellular matrix like hydrogels with enhanced cell adhesion and proliferation properties (mimicking the natural extracellular matrix) [2-4]. The role of the hydrogel is to promote axonal migration, in order to restore the cord’s functional properties. Therefore we have modifier it with peptides that are involved in this process, namely GRGRDS (fibronectin) and YIGSR (laminin). On the other hand, while the regenerative step can be fostered by these structures (with or without the combination with stem cells), it is also need to render the injured spinal cord more amenable characteristics for reparative processes to happen, namely by partially inhibiting processes such as inflammation and glial scar formation. Such processes can be achieved by using drugs that target them. Therefore the objectives of this rotation will be to test different combination of drugs and ECM-like hydrogels (with or without cells) and their impact on in vitro and in vivo models of axonal degeneration/regeneration.

Aims and Techniques

Test different combination of drugs and ECM-like hydrogels (with or without cells) and their impact on in vitro and in vivo models of axonal degeneration/regeneration.

Experimental techniques: cell and explant culture; biomaterials modification; immunohistochemistry; neurostructural analysis; fluorescence/confocal microscopy; in vivo models of SCI; behaviour analysis

References

- Assunção-Silva RC et al., Biomedical Materials, 2015, in press

Skills to be achieved in this project

As specified in excel file.

Supervisors

António Salgado and Nuno Silva
3.6 Modulation of Mesenchymal Stem Cells Secretome through Peptide Grafted 3D Culture Environments: A Focus on Spinal Cord Injury Repair

Summary

Spinal Cord Injury (SCI) is a chronic condition for which there is still no clinical treatment. In the last decade, the transplantation of Mesenchymal Stem Cells (MSCs) has been suggested as a possible therapy for SCI. This pro-regenerative capacity of MSCs has been linked to the secretion of bioactive molecules that provide trophic support to the damaged tissues, known as the secretome (1,2).

In spite of the aforementioned beneficial roles of MSC transplantation, very low numbers of cells survive within the lesion site, which represents a drawback for their clinical application. A possible alternative is to combine them with biodegradable biomaterials. They can protect encapsulated MSCs, while stimulating the production of growth factors by them, which will enhance the regeneration of SCI (3). Moreover by using peptides from proteins present in MSCs ECM (e.g. fibronectin, laminin and collagen) involved in processes such as cell survival, adhesion and proliferation, to modify these hydrogels, it will be possible to further modulate MSCs secretome, and thus increase the regenerative potential of these cells (4). Thus, it is possible to envision the development of tissue responsive/inductive GG based hydrogels that modulate the action of encapsulated MSCs in the injured spinal cord [11]. The aims of this rotation will be focused on testing a number of different peptides, grafted into hydrogel matrix based on gellan gum, a naturally occurring hydrogel, and assesse their effects on the regenerative potential of MSCs

Aims and Experimental techniques

- Test a number of different peptides, grafted into hydrogel matrix based on gellan gum, a naturally occurring hydrogel, and assess their effects on the regenerative potential of MSCs
- Experimental techniques: cell and explant culture; biomaterials modification; immunohistrochemistry; neurostructural analysis; fluorescence/confocal microscopy;

References

1- Teixeira FG et al., Cellular and Molucar Life Sciences, 2013, 70(20): 3871
2- Silva NA et al., Progress in Neurobiology, 2014, 114: 25
3- Assunção-Silva RC et al., Stem Cells International, 2015, 948040
4- Silva NA et al., Biochimie, 2013, 95(12): 2314

Skills to be achieved in this project

As specified in excel file.

Supervisors

António Salgado and Nuno Silva
3.7 Sorting Nexin 3 role in neuronal development and behavior

**Summary**

Protein assembly and turnover abnormalities are hallmarks of several neurodegenerative disorders [1]. The Sorting Nexins family of proteins (SNXs) plays pleiotropic functions in protein trafficking and intracellular signal in neuronal and non-neuronal cells, and has been associated with several human diseases that result from abnormal endosomal function, namely, Alzheimer’s disease [2]. Despite the reported roles of SNXs in protein homeostasis in neurodegeneration, not much is known about SNXs function in the nervous system. The aim of this project was to use the nematode Caenorhabditis elegans that encodes in its genome eight SNXs orthologs, and is a reference model organism to study the function and malfunction of the nervous system, to functionally characterize SNXs. We found that SNX3 gene mutation led to an array of developmental defects, namely, reduced brood size, embryonic lethality, delayed hatching, and to a decreased life span. Additionally, SNX3 mutant worms presented distinct behavioral deficits, such as, increased motor uncoordination, impaired chemotaxis and susceptibility to osmotic, thermo and oxidative stresses, which implies perturbed neuronal functions. Altogether, our data supports a prominent role of SNX3 in nervous system development. In this manner, we would like to pursue with C. elegans SNX3 characterization, by (i) quantifying in the wild-type (WT) worm SNX3 expression during the developmental cycle (egg to adulthood); (ii) unraveling the neuronal architectural defects of SNX3 mutants by backcrossing with strains that express GFP in all (pan-neuronal), or specific types of neurons, namely in GABAergic and cholinergic neurons; and (iii) quantifying, in the SNX3 mutant, the expression level of several receptors and neurotransmitters by RT-PCR and Western-blot analysis. We will finally assess susceptibility to seizures, memory, learning, and social behavior of this SNX3 mutant.

**Aims**

- Characterization of C. elegans SNX3 mutant expression during development, and after stress exposure.
- Evaluate the neurological phenotype and nervous system morphology of SNX3 mutant.
- Evaluate behavioral deficits of the SNX3 mutant.

**References**


**Skills to be achieved in this project**

As specified in excel file.

**Supervisor**

Neide Vieira
3.8 Exploring Sorting Nexin 27 role in Stress

Summary
Sorting nexins (SNXs) are a family of proteins that play pleiotropic roles in protein trafficking and that have been associated with endocytic events underlying neurodegeneration, synaptic plasticity and cognition [1-4]. Despite this, not much is known about SNXs role in the nervous system and how their function is modulated by aging, age-related pathologies and stress; conditions that are inextricably linked to endocytic dysregulation, cognitive decline and synaptic malfunction. Previous data from the host lab demonstrated that SNX27 expression is significantly reduced in the PFC (a brain region severely affected by stress exposure [5]) of rats exposed to a mild stress protocol. Interestingly, a C. elegans snx-27 deletion mutant strain displayed an increased susceptibility to heat shock, osmotic or oxidative stresses, which implies a role for SNX-27 in stress tolerance. Taking into consideration the recent findings that SNX27 is tightly associated with Down’s syndrome [3] and Alzheimer's disease [6], were its expression is markedly reduced; that stress is a major trigger in neurodegenerative disorders; and that stress-related pathologies display, mirroring neurodegenerative diseases, cognitive impairments, synaptic malfunction/atrophy, and decreased proteostasis [7-8], we aim to dissect SNX27 role in stress. For that, SNX27 heterozygous mice (KO mice are not viable) and littermate controls will be exposed to a 6 week Chronic Mild Stress (CMS) protocol. Animals will then be analysed for several behavioral dimensions: locomotion, emotion and cognition. Behavioral assessment will be performed in the following order: elevated plus maze (EPM; anxiety-like behavior), open field (OF; locomotor and exploratory behavior), forced-swim test (FST; depressive-like behavior), working memory water maze (WM; spatial short-term memory), Morris water maze (MWM; spatial reference memory) and spatial reversal (behavioral flexibility). Following behavioral characterization, animals will be anesthetized, CSF samples will be collected, as well as blood serum samples (for posterior analysis of metabolites, cytokines, among others). Tissue samples will also be collected (liver, adrenal glands, thymus, spleen and brain). Half of the brain will then be macrodissected for further RNA or protein analysis, and the remaining brain processed for Golgi staining (for posterior stereological analysis and 3D neuron reconstructions).

Aims
- Characterize SNX27 expression by RT-PCR and western-blot analysis in control and stress exposed wild-type and Snx27+/− mice.
- Evaluate the subcellular localization and membrane-association of SNX27 in control and stress exposed wild-type and Snx27+/− mice.
- Evaluate distinct behavioral dimensions in control and stress exposed wild-type and Snx27+/− mice.
- Analyze neuronal volumes, cell numbers and neuronal morphology.

References

Supervisor
Neide Vieira and Susana Roque
3.9 Exploring Sorting Nexin 27 role in Pain

Summary

Sorting nexins (SNXs) are a large family of phosphoinositide-binding proteins that play fundamental roles in orchestrating cargo sorting through the endosomal network. SNX27 is one of the most studied members of this family and has a well-established role in glutamate receptors trafficking regulation [1, 2]. Glutamatergic transmission is critical for nociception (acute pain) and underlies the onset and maintenance of hypersensitivity in chronic pain conditions [3] but surprisingly, no previous study assessed SNX27 potential as a therapeutical target for pain. Based on this rationale we propose to study the manifestation of acute and chronic pain in a mice model with reduced expression of SNX27. Male SNX27 -/+ mice and littermate controls will be used to assess pain-related behaviors in models of acute noxious thermal (tail- and paw-flick), tonic inflammatory (formalin) and chronic neuropathic pain (spared nerve injury, SNI). In the later, emotional and cognitive behaviors will also be characterized as these are frequently affected in chronic pain conditions [4], using the following paradigms: elevated plus maze (EPM; anxiety-like behavior), open field (OF; locomotor and exploratory behavior), forced-swim test (FST; depressive-like behavior), working memory water maze (WM; spatial short-term memory), Morris water maze (MWM; spatial reference memory) and spatial reversal (behavioral flexibility). Animals will then be anesthetized, spinal cord will be removed for c-fos immunohistochemistry and density estimation. Sciatic nerve ultrastructure will also be studied for g-ratio calculation [axon diameter/ (axon diameter+myelin thickness)]. A- and C-fiber conduction velocity and compound action potential will also be followed.

Aims

- Evaluate the impact of decreased SNX27 expression in distinct behavioral dimensions.
- Analyze nociception of wild-type and Snx27+/- mice.
- Assess Snx27+/- mice tolerance to peripheral neuropathic pain.
- Evaluate neuronal volumes, cell numbers and neuronal morphology of wild-type and Snx27+/- mice exposed to SNI.
- Quantify spinal cord neuronal activation by c-fos immunostaning in wild-type and Snx27+/- mice exposed to SNI.

References


Skills to be achieved in this project

As specified in excel file.

Supervisor

Neide Vieira and Hugo Almeida
3.10 A Poly-pharmacological Therapy to Restore the Injured Spinal Cord

Summary

Spinal cord injury leads to devastating neurological deficits that have a strong impact in the physiological, psychological and social behavior of patients. For these reasons, it is urgent to develop therapeutic strategies that can specifically target this problem. When the spinal cord suffers a mechanical trauma it begins a cascade of cellular and biochemical reactions that leads to further damage. This cascade of reactions, also known as "secondary injury", it is characterized by a strong inflammatory response, glutamate excitotoxicity, release of myelin-derived inhibitors and the formation of a glial scar. These events are known to have a crucial contribution for axon regeneration failure after a SCI. The modulation of the secondary events will most likely play a central role in future clinical therapy. Several authors already demonstrated that the neutralization of a single secondary event leads to some motor recovery and higher neurite extensions in SCI animals. For instance, the modulation of inflammation using demonstrated to promote behavioral and histological improvements in injury rats. Moreover, the administration of Mg or Riluzole revealed to reduce the glutamate excitotoxicity and to promote motor improvements. In addition, the glial scar degradation with has shown to enhance axon regeneration and improve motor function in SCI rat. It was also demonstrated that blocking myelin inhibitory proteins, such as Nogo, MAG or OMgp, facilitates the regeneration of the injured spinal cord. Finally, it was previously shown that the prevention of cAMP hydrolysis stimulates axonal regeneration and motor improvements. These are promising results, however it is missing an integrative approach that combines the activation of growth promoting programs; while at the same time attenuate growth inhibitory pathways and promotes neuroprotection. For this reason, we are studying a poly-pharmacotherapy that can tackle most of the molecular issues responsible for the failure of axon regeneration upon SCI.

Aims

- Determination of the bioactivity of the selected drugs when combined into a single pharmacotherapy approach;
- Establish a contusion model of SCI in rats.

Skills to be achieved in this project

As specified in the excel file.

Supervisor

Nuno Silva
3.11 Neural circuits of reward and motivation

Summary
The mesolimbic circuit, comprising dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is involved in processing emotionally salient stimuli of both positive and negative valence, being crucial for reward and aversion modulation.

The NAc is mainly composed of dopamine receptor D1 and D2-type medium spiny neurons (MSNs) that have been proposed to have an opposing role in behavior; whereas D1 signaling promotes reinforcement and is associated with positive valence, D2 activation mediates aversion and encodes negative valence. However, recent evidence from our team suggests that this canonical perspective is oversimplistic and that both types of MSNs are important for reward and reinforcement.

In this work, we will use optogenetic and electrophysiological tools in order to dissect what is the role of the NAc MSNs in reward and motivation.

Aims
- Optogenetic manipulation/inhibition of D1 and/or D2 neurons in reward/motivation-related tasks
- Electrophysiological measurements

References

Skills to be achieved in this project
As specified in excel file.

Supervisor
Ana João Rodrigues
3.12 Dendrimers Nanoparticles as Novel Carriers for the Delivery of Temozolomide Chemotherapy into Malignant Brain Tumors

Summary

Glioblastoma (GBM) is the most common and most malignant tumor of the central nervous system, for which curative therapies are not available partly due to suboptimal access of the different anti-cancer chemotherapeutic drugs to the tumor. Recently, the use of nanoparticles as drug delivery systems (DDS) for cancer treatment has gained particular interest. We recently showed that carboxymethylchitosan/poly(amidoamine) (CMCh/PAMAM) dendrimer nanoparticles may be an attractive DDS for these tumors, as GBM cell models can efficiently and rapidly uptake these nanoparticles. Since temozolomide (TMZ) is the standard chemotherapeutic agent used to treat GBM patients, it is herein proposed the study of the therapeutic value of TMZ-loaded CMCh/PAMAM nanoparticles, both in vitro and in vivo. Overall, this work will develop a novel dendrimer-based nanocarrier for TMZ delivery into cancer cells, and evaluate its therapeutic potential for glioblastoma. Importantly, it may also constitute a proof-of-principle for future studies evaluating other solid tumors that may benefit of a TMZ-based chemotherapy (e.g., melanoma). Our findings may reveal novel therapeutic strategies for GBM, which can also serve as a proof-of-principle applicable to other human cancers treated with TMZ (e.g., melanoma).

Aims

- In vitro evaluation of TMZ-loaded dendrimers in Glioblastoma cell lines: Internalization kinetics and Cytotoxicity
- In vivo evaluation of the therapeutic potential of TMZ-loaded dendrimers in Glioblastoma mice models: Biodistribution and effects on tumor kinetics

Skills to be achieved in this project

As specified in excel file.

Supervisor

Bruno M. Costa
3.13 Relevance of HOTTIP in Glioblastoma: Molecular, Functional and Prognostic Insights

Summary

Glioblastoma (GBM) is a highly malignant and the most common primary brain tumor, for which curative therapies are not available. We previously showed that HOXA9 overexpression is a negative prognostic factor in GBM, and functionally impacts cells behavior. Recent data from our group indicates that HOTTIP, a long non-coding RNA (lncRNA) that regulates the expression of HOXA genes in normal tissues, is also prognostically valuable in GBM patients. Interestingly, the relevance of HOTTIP in the context of cancer has not been addressed to date. We intend to: i) evaluate the molecular status of HOTTIP in GBM, by assessing its gene expression levels, DNA methylation, and copy number and mutational alterations in databases; ii) study its functional roles in critical GBM hallmark features, by modulating HOTTIP levels with shRNA approaches; and iii) validate the prognostic value of HOTTIP in prospectively-collected GBM cohorts. This project will provide novel insights that may translate into a better clinical management of this dramatic disease.

Aims

- Evaluate HOTTIP expression in a panel of glioblastoma tumor samples from patients and in cell lines;
- Silence HOTTIP expression in glioblastoma cell lines with shRNAs, and evaluate its functional roles on cell viability, invasion, and response to chemotherapy, which are critical hallmarks of glioblastoma;

Skills to be achieved in this project

As specified in excel file.

Supervisor

Bruno M. Costa
Summary

Glioblastoma (GBM) is a highly malignant and the most common primary brain tumor, for which curative therapies are not available. We previously showed that HOXA9, a critical transcription factor during development, is overexpressed in a subset of glioblastoma samples, and is associated with increased tumor resistance to therapy and shorter patient survival. A recent study demonstrated that HOXA9 affects the aggressiveness of ovarian cancer cells by affecting the expression of P-Cadherin, which encodes a transmembrane protein with roles in cell adhesion. Since this HOXA9/P-Cadherin link has not been described in other cancer types, and nothing is currently known on the importance of P-Cadherin in the context of malignant brain tumors, we will study: i) how HOXA9 may affect P-Cadherin levels in glioblastoma; ii) the functional roles of P-Cadherin in glioblastoma, particularly at the levels of cell migration, invasion, viability, and response to chemotherapy; iii) the roles of P-Cadherin in in vivo intracranial models of glioblastoma; iv) the clinical value of P-Cadherin as a prognostic marker in glioblastoma. These approaches will clarify for the first time the relevance of a critical Cadherin in the context of malignant brain tumors.

Aims

- Evaluate P-Cadherin expression in a panel of glioblastoma tumor samples from patients and in cell lines, and correlate them with HOXA9 levels;
- Evaluate how the levels of P-Cadherin influence the aggressiveness of glioblastoma using orthotopic in vivo mice models.

Skills to be achieved in this project

As specified in excel file.

Supervisor

Bruno M. Costa
3.15 The role of IL10 in depression and antidepressant treatment

**Summary**

Previous studies from our laboratory showed that female mice lacking IL10 expression show a depressive-like behavior, a phenotype that is rescued by IL10 administration. In accordance, mice overexpressing IL10 present decreased learned helplessness. Interestingly antidepressant treatment, both in patients with depression and in animal models, increases IL10 production. In fact, the reversion of the inflammatory milieu has been shown to play a role for the efficacy of antidepressant treatment. Indeed, treatment-resistant patients have been shown to present a persistent inflammatory profile after antidepressant treatment. In accordance, the concomitant treatment of those patients with antidepressants and anti-inflammatory drugs improved the efficacy of antidepressant therapy. Thus, the goal of this work is to unravel the mechanisms underlying the role of the anti-inflammatory cytokine IL10 in the etiology of depression and in response to antidepressant therapy. Since two of the most widely discussed mechanisms underlying depression are the altered HPA axis and the imbalance production of cytokines, based respectively, on the frequently observed increased levels of glucocorticoids and pro-inflammatory cytokines in depressed patients, respectively, we will investigate these two mechanisms in mice lacking IL-10 expression. Moreover, preliminary data suggest that IL10KO mice seem to be resistant to antidepressant therapy (both with fluoxetine and imipramine), suggesting that IL10 expression is crucial for the reversion of the depressive-like phenotype. Thus, further studies will be performed to clarify if this animal model could help to better understand the causes of treatment-resistant depression.

**Aims**

- Assess whether colon inflammation and corticosterone levels could be associated with depressive-like behavior;
- Clarify the role of IL10 in antidepressant treatment

**Skills to be achieved in this project**

As specified in excel file.

**Supervisors**

Susana Roque and Margarida Correia-Neves
Impact of chronic pain in decision-making - the role of dopamine

Summary
Chronic pain impacts brain structure and function. Indeed, besides sensory abnormalities, chronic pain is frequently accompanied by alterations in emotional and cognitive behavior. The neurotransmitter dopamine emerges here as an interesting link between chronic pain, depression and decision-making as it is individually related with each of these dimensions.

Recently we demonstrated in a rat model of chronic pain that i) chronic pain impacts in a lateralized way decision-making and ii) there are side-specific alteration in the expression of dopamine receptors in the prefrontal cortex and nucleus accumbens, areas involved in decision-making.

In this project we intend to further explore these observations, unraveling the lateralized impact of chronic pain in the dopaminergic system and its posterior involvement in the observed impairments in decision-making.

Aims
- Study the impact in decision-making of nucleus accumbens unilateral dopamine depletion;
- Manipulate the dopaminergic system unilaterally and study side-specific effects on behavioural readouts in a chronic neuropathic pain model;

References

Skills to be achieved in this project
As specified in the excel file.

Supervisors
Hugo Leite-Almeida and Margarida Cunha
3.17 Asymmetries in rodents' brain function

**Summary**

Brain functional asymmetry in a nearly symmetrical body remains paradoxical. Nevertheless, since the 1861’s Paul Broca description of a left dominance for language, many side-biased brain processes and behaviors have been described. Such functional asymmetry is not exclusively human; it has also been described in various vertebrate and invertebrate classes, suggesting that laterality is highly conserved across species. The relevance of brain functional asymmetry for cognition and executive function is not understood. For instance, brain imaging studies in older individuals report bilateral activity during the execution of cognitive tasks that in younger subjects were associated with lateralized activity. It remains an open question if the lack of asymmetry is a compensatory mechanism operating from the non-dominant hemisphere and therefore rendering individuals with lower asymmetry functional advantage or if it simply is a correlate of poorer executive function.

The present project proposal is designed to study these questions. We aim to characterize corticostriatal activity in a behavioral paradigm of intertemporal decision-making (impulsivity) and to correlate task-solving efficiency with activity side-biases particularly, its magnitude and directionality (left>right or left<right).

**Aims**

- Characterize trait impulsivity in rats;
- Obtain bilateral local-field potentials recordings in relevant areas (e.g. prefrontal cortex) during the execution of the paradigm;

**References**


**Skills to be achieved in this project**

As specified in the excel file.

**Supervisors**

Hugo Leite-Almeida and Madalena C. Esteves
3.18 Asymmetries in human brain function

**Summary**

Brain functional asymmetry in a nearly symmetrical body remains paradoxical. Nevertheless, since the 1861’s Paul Broca description of a left dominance for language, many side-biased brain processes and behaviors have been described. Such functional asymmetry is not exclusively human; it has also been described in various vertebrate and invertebrate classes, suggesting that laterality is highly conserved across species. The relevance of brain functional asymmetry for cognition and executive function is not understood. For instance, brain imaging studies in older individuals report bilateral activity during the execution of cognitive tasks that in younger subjects were associated with lateralized activity. It remains an open question if the lack of asymmetry is a compensatory mechanism operating from the non-dominant hemisphere and therefore rendering individuals with lower asymmetry functional advantage or if it simply is a correlate of poorer executive function.

The present project proposal is designed to study these questions. We aim to characterize both structural and functional hemispheric asymmetries in humans. In order to achieve this we are currently taking advantage of ICVS’ large database of MRI and fMRI data from several different cohorts.

**Aims**

- Characterize structural hemispheric asymmetries and find influencing factors;
- Determine the importance of functional and structural asymmetries for cognitive function;

**References**


**Skills to be achieved in this project**

As specified in the excel file.

**Supervisors**

Hugo Leite-Almeida and Madalena C. Esteves
3.19 Social or anti-social? The disruptive power of a peripheral neuropathic lesion

**Summary**

Mood disorders are frequently accompanied by alterations in the amount and quality of social interactions. While it is well-established that in experimental models of chronic pain animals develop anxiety- and depressive-like phenotypes, no study has been carried to understand how the social environment contributes to pain manifestation and to the emergence of associated comorbid emotional disorders. Recently, we have observed that in chronic neuropathic pain conditions the rodent’s natural ability to interact with an unfamiliar animal conspecific (with or without pain) is disrupted. This proof-of-concept prompted us to further explore these findings. In a series of experiments we will study mutual influences in pain- and mood-related behaviors between cage-mate pairs pain/no-pain, pain/pain and control no-pain/no-pain. Also, social interactions will be studied between familiar and unfamiliar conspecifics in the conditions above. The spared nerve injury (SNI) model of neuropathic pain will be used and putative side-specific effects in left- (SNI-L) or right (SNI-R) –lesions will be analysed as previous studies demonstrated that SNI-L is more anxiogenic than SNI-R. Finally, analgesic pharmacotherapy will be employed attempting to rescue the phenotypes.

**Aims**

- Study mutual influences between cage mates on different conditions of pain
- Characterize social behaviour between familiar and (un)familiar animals with and without pain
- Study possible lateralized effects of pain (SNI-L vs SNI-R) on social behaviour.

**References**


**Skills to be achieved in this project**

As specified in the excel file.

**Supervisors**

Hugo Leite-Almeida
3.20 Is memory formation dependent solely on neuronal function? Dissecting the role of astrocytes in cognitive processes.

**Summary**

The classical paradigm that brain information processing is exclusively neuronal has been challenged in the past ten years by an exciting body of evidence. Indeed, the importance of glial cells is rising due to emerging data supporting dynamic neuron-glia interactions. These inter-cellular interactions are nowadays widely accepted. Nevertheless, little is known about effects of glial cells, namely astrocytes, in complex behaviour outputs. Astrocytes are able to sense, integrate and respond to neurons. In the lab we have currently mice strains that incorporate genetic modifications to impair specifically each of these 3 functions. The goal of this rotation is to test their performance in cognitive tasks and correlate this performance with electrophysiological or molecular/morphological alterations. This way, the applicant may choose between different approaches to solve the same problem.

By disclosing the net consequence of astrocytic modulation of the neuronal networks we expect to better understand behaviour computation and therefore understand the possible therapeutic approaches in diseases characterized by cognitive dysfunction (e.g. Alzheimer’s, Major Depression...).

**Aims**

- To test the performance of mice with astrocytic dysfunction in working memory and long term memory paradigms (hole board and Morris Water Maze).
- To correlate the performances obtained in the tests with the observed expression of the transgenes in the specific areas by microscopic observation of brain slices containing the targeted areas (prefrontal cortex and hippocampus, respectively), as well as quantification of altered protein levels.
- To analyse the morphological changes in both neurons and astrocytes by means of immunohistochemistry and unbiased stereological techniques.

**References**


**Skills to be achieved in this project**

As specified in excel file.

**Supervisor**

João Oliveira
3.21 Adult neuroplasticity as a pathological trigger to recurrence in depression

**Summary**
Depression is a highly prevalent neuropsychiatric disorder, frequently associated with the occurrence of relapses and recurrences. Although the vast majority of studies focus the onset and treatment of depression, knowledge regarding recurrence into a new depressive episode remains scarce. Giving the high rate of recurrence in depressive patients, it is of great relevance to understand the molecular mechanisms behind this phenomenon. According to recent studies, the impact of depression on neuronal morphology and adult cytogenic processes namely, hippocampal neurogenesis and gliogenesis may determine the progression to a further depressive episode.

Modeling recurrent depression using an animal model of depression, we intend to unravel the behavioral and molecular response to a repeated depressive episode, determine impact of treatment with typical antidepressants and characterize detectable molecular alterations in biological fluids. Unveiling these mechanisms may pave the way to the development of efficient treatments to prevent recurrent depression.

**Aims**
- Determine the behavioral and neuroplastic alterations associated to the recurrence into a new depression episode;
- Characterize the therapeutic relevance of initial antidepressants treatment to promote resilience to recurrent depression;
- Identify on peripheral fluids, physiological and neuromolecular correlates of recurrence in depression

**References**

**Skills to be achieved in this project**
As specified in excel file

**Supervisor**
Luísa Pinto
3.22 Exploring the role of the transcription factor AP2gamma in the control of post-natal glutamatergic neurogenesis

**Summary**

The view of the central nervous system (CNS) as an immutable system in now outdated, and several lines of evidence demonstrate that the CNS is endowed with significant regenerative and neuroplastic potential. In fact, it is now well established that new cells are continuously generated during adulthood in specific brain regions, a process called neurogenesis. The current challenge relies on dissecting the regulatory mechanisms of the adult neurogenic process. Recently, we have identified AP2gamma (AP2\(\gamma\)) as an important transcription factor involved in developmental corticogenesis. We now seek to explore whether AP2\(\gamma\) has a role on the regulation of adult neurogenesis and its importance for different emotional and cognitive modalities.

**Aims**

- Analyze different behavioral parameters in WT and AP2gamma conditional knockout mice.
- Evaluate the presence of AP2gamma protein by western-blot in different brain regions from WT and AP2gamma conditional knockout mice.

**References**


**Skills to be achieved in this project**

As specified in excel file.

**Supervisor**

Luísa Pinto
3.23 Drug screening for chronic pain disorders

Summary
Chronic pain and depression are enormous health and financial burdens on our society, with depression also being the major comorbidity of chronic pain disorders. Chronic pain induces a profound change in the expression of peptides and their receptors that leads to changes in brain wiring (neuronal plasticity) of central pathways mediating pain [1]. In this work, chronic pain will be induced in Wistar han adult rats for a period of 4 weeks followed by the administration of drugs for an additional 3 weeks. At the end of this period, behavioural analysis to evaluate anxiety- (acoustic startle, open field and elevated plus maze), depressive-like (forced swimming test and sucrose preference test) behaviour as well as nociception [pain behaviour - tail and paw-flick tests (heat noxious stimulation), cold allodynia (acetone test), the Randall-Selitto and the pressure application measurement tests (mechanical noxious stimulation)] will be performed.
At the end of the behavioural task the animals will be sacrificed and brains will be removed for the evaluation of changes in neurotransmitters pathways, either through RT-PCR or immunohistochemistry. Emotional and nociceptive behavioural data will be correlated to the expression of neurotransmitters and its receptors in several areas of the brain involved in pain modulation.

Aim
- To evaluate the ability of different types of drugs to reverse the impact of chronic inflammatory pain upon mood disorders in rodents;
- To correlate drug efficiency with changes in the neurochemistry of brain areas involved in pain modulation.

References

Skills to be achieved in this project:
As specified in excel file.

Supervisors
Diana Amorim and Filipa Pinto-Ribeiro
Summary

DNA methylation, or 5-methylcytosine (5mC), is an epigenetic mark that plays a pivotal role in development and cellular differentiation. Genome-wide erasure of SmC occurs during epigenetic reprogramming of preimplantation embryos and primordial germ cells (PGCs), by mechanisms that may also drive locus-specific DNA methylation dynamics in differentiated tissues. Recent advances have suggested that, amongst other potential pathways, oxidation of 5mC to 5hmC by TET enzymes (of which there are three in mammals) might initiate DNA demethylation.

In postmitotic cells DNA methylation has been traditionally regarded as a highly stable epigenetic mark; however, there is evidence that active DNA demethylation occurs in terminally differentiated neurons following synchronous neuronal activation. Interestingly, 5hmC is highly abundant in several brain regions and neuronal cell types, suggesting that it may play a role in post-mitotic DNA demethylation. In particular, it has been shown that high 5hmC content is a feature of both neuronal progenitors and post-mitotic neurons. Tet transcripts have also been shown to be highly expressed in the brain with Tet3 being the most abundant. Overexpression of Tet1 in the mouse adult dentate gyrus resulted in reduced levels of methylation at Bdnf gene and increased expression, suggesting that Tet1 could be involved in the active demethylation process in post-mitotic neurons. However, little is known about the function of TET enzymes in neuronal differentiation and function, apart from a recent study showing that overexpression of TET2 and TET3 enhance neuronal differentiation resulting in a higher number of cells migrating from the ventricular/subventricular and intermediate zones to the cortical plate in vivo.

We propose to functionally test the role of TET enzymes during neurogenesis using a well established in vitro model and shRNA-mediated depletion of Tet transcripts. The impact on the transcriptome and epigenome will be studied using genome-wide approaches.

Aims

Test the role of TET enzymes in neuronal differentiation
Test the role of TET enzymes in maintenance of the neural lineage
Establish a functional link between 5hmC, 5mC and the regulation of neurogenesis

References


Skills to be achieved in this project

As specified in excel file.

Supervisor

Joana Marques
3.25 Stress-driven changes in synaptic interactome: a link between depression and Alzheimer’s disease

**Summary**
World Health Organization estimates that the leading cause of mental disability in the coming years will be depression and Alzheimer’s disease (AD), raising these two diseases as significant public health problem. Focusing on risks factors of these diseases, previous clinical and experimental studies suggest a causal role of environmental parameters e.g. chronic stress and the subsequent elevation of stress hormones, glucocorticoids (GC), in pathogenesis of depression while recent findings involve stress in the onset/progression of AD. Neuronal atrophy and synaptic failure have been suggested to play an essential role in stress-related pathologies such as depression as well as in AD. Furthermore, important clues of synaptic disruption mechanism(s) previously implicated in pathophysiology of AD have been recently suggested to also contribute in stress-driven brain pathology involving, for the first time, Tau missorting in mechanism(s) of synaptic damage beyond Alzheimer’s disease (Pinheiro et al., 2015; Sotiropoulos et al., 2011). Based on the recently suggested role of Tau in synaptic structure and function interacting with NMDA receptors, PSD-95 and Fyn proteins, this project examines the alterations of synaptic interactome underlying stress-induced neuronal atrophy and synaptic loss searching for molecular targets with neuro- and synapto-protective properties.

**Aims**
- Molecular characterization of stress/GC impact on postsynaptic vs extra-synaptic/presynaptic interactome
- In vitro and/or in vivo monitoring of intracellular and synaptic trafficking of receptors and related cell signalling under stressful conditions.

**References**

**Skills to be achieved in this project**
As specified in excel file.

**Supervisors**
Ioannis Sotiropoulos and Nuno Sousa
3.26 Synaptic Tau protein: an unknown target for anesthetics malfunction?

Summary
General anesthetics (GA) are widely used drugs with various clinical applications but many studies report various cognitive problems (some persistent) after GA exposure. Previous studies have shown that different anesthetics induced Tau hyperphosphorylation whereas Tau hyperphosphorylation is related to memory deficits through brain synaptic loss. Based on the recently suggested role of Tau in synaptic structure and function interacting with various proteins such as NMDA receptors (Ittner and Gotz, 2011), we hypothesize that synaptic Tau may have a unique involvement in anesthetics action. First results of this project have shown that NMDA-related anesthetics modifies neuronal structure altering dendritic arborization and synapses in hippocampus accompanied by cognitive impairment in WTs while this effect was attenuated in absence of Tau. Thus, this project aims to clarify the role of Tau and its interaction with NMDA receptors on synaptic mechanisms underlying anesthetic-induced neuroremodeling and cognitive deficits.

Aim
Perform detailed molecular and structural analysis of synaptic cell signalling and synaptic interactome of Tau

Skills to be achieved in this project
As specified in excel file.

Supervisors
Ioannis Sotiropoulos and Hugo Almeida
3.27 Pain-triggered synaptic plasticity: identifying the mechanistic involvement of Tau protein

Summary
Tau protein hyperphosphorylation and consequent malfunction is a hallmark of Alzheimer’s disease pathology; importantly, pain perception is diminished in these patients. In physiological conditions, Tau contributes to cytoskeletal dynamics and in this way, influences a number of cellular mechanisms including axonal trafficking, myelination and synaptic plasticity. While it is known that these cellular processes are also implicated to pain perception, it was just recently that we demonstrated the direct in vivo role of Tau in nociception (Sotiropoulos et al., 2014) showing that Tau-deficient mice display altered pain perception but the underlying mechanisms remain unknown. Interestingly, Tau has an important role in the postsynaptic density, where, via interaction with Fyn, contributes to NMDA receptor/PSD95 complex formation the latter has also been implicated in nociception (Ittner and Gotz, 2011; D’Mello et al., 2011). Thus, this project will analyze the molecular alterations of pain-stimulated synapses with particular focus on the suggested interaction of Tau with synaptic receptors responsible for synaptic plasticity in both spinal cord and brain.

Aims
- Monitor the effect of pain stimuli on synaptic TAU phosphorylation;
- Identify the influence of Tau on synaptic plasticity mechanisms (e.g. trafficking of synaptic receptors, interaction with scaffold proteins)

References

Skills to be achieved in this project
As specified in excel file.

Supervisors
Ioannis Sotiropoulos and Hugo Leite-Almeida
3.28 “Stressed” proteostasis: the chronic stress impact on the orquestration of proteasomal and autophagic pathways in Alzheimer’s disease pathology

Summary

Tau aggregation is a common feature in Alzheimer’s disease (AD), frontotemporal dementia (FTD) and other tauopathies. Consistent with suggestions that lifetime stress may be a clinically-relevant precipitant of AD pathology, we previously showed that chronic stress and the main stress hormones, Glucocorticoids (GC), trigger Tau aggregation affecting molecular chaperones such as Hsp90 which is known to be involved in both GC signaling and Tau degradation (Sotiropoulos et al., 2015); still, the underlying mechanisms of the above stress/GC effects remain unclear. This project focuses on how stressful conditions converge on cellular mechanisms underlying Tau aggregation in AD brain monitoring tress/GC-triggered downstream degradation mechanisms (proteasome- and lysosome-related ones) mechanisms using both animal and cell culture experimental approaches.

Aims

- Molecular and cellular characterization of ubiquin-proteasome and autophagic pathways under chronic stress and/or GC treatment.
- Clarification of stress/GC-driven molecular mechanisms relating molecular chaperones such as Hsp90 to Tau degradation pathways.

References


Skills to be achieved in this project

As specified in excel file

Supervisors

Joana Silva and Ioannis Sotiropoulos
3.29 Coaching strategies to prevent stress anxiety in test performance

**Summary**

Modern lifestyle exerts an enormous burden on individuals by pushing towards increasing productivity and longer working hours. This feeling of persistent underachievement, that modern working conditions exert, leads to burnout and stress-related mental disorders such as anxiety. Additionally, workplace environment is often inappropriate to provide a stress-free environment due to poor lighting, temperature or noise conditions.

Higher education is a transition period before students reach the working environment. Students are subjected to increasing periods of work with a progressive focus on autonomy and continuous assessment as mandated by current educational policies. The increasing workload is perceived as stressful and commonly leads to mental disorders and perception that their cognitive performance is bellow their expected standards. This is corroborated by the high prevalence of anxiety disorders among higher education students.

Stress is known to affect an individual’s cognitive performance in a biphasic mode. Too little stress impairs adequate performance, increasing with physiological levels of acute stress and followed by a decrease in performance again with prolonged or disproportioned levels of stress. When an individual is exposed to stressful stimuli (physical or psychological), the organism perceives it as a threatening event and mounts an adequate biological and behavioral responses. The main biological agents are the release of cathecolamines, like epinephrine (adrenaline), and corticosteroids (cortisol). The positive effects of acute stress are attributed mainly to the effect of catecholamines while exposure to chronic stress results in repeated activation of the hypothalamic-pituitary-adrenal axis, leading to cortisol secretion, and elevated circulation of pro-inflammatory cytokines, affecting glucocorticoid sensitivity, brain function and behavior.

Assessment is a fundamental phase in the training and certification process that a higher education student is submitted to. It is also one of the strongest stress factors due to the high-stake implications in the academic progress and self-perceived image. Stress is a risk factor for anxiety and may lead to worsening of performance in assessment tasks.

The present proposal will use cohorts of higher education students as a model in order to study the effect of stress/anxiety in the performance of high demand tasks. We intend to correlate stress/anxiety markers with the response pattern in high-stakes exams and develop coaching strategies in order to improve the students’ performance.

**Aims**

Specifically we aim to:
- characterize how anxiety affects medical student’s performance in their assessment, correlating basal levels of self-perceived stress with anxiety levels and test performance;
- address potential biological mechanisms underlying the adverse effects of stress/anxiety on cognitive overload and overall performance of high demand tasks. We hypothesize that these effects are mediated by elevations in stress hormones and pro-inflammatory signals during challenging cognitive tasks;
- evaluate if a directed coaching strategy will improve performance in exams by reducing the level of anxiety and/or stress.

**References**

Davide Carneiro, Paulo Novais, José Miguel Pêgo, Nuno Sousa, and José Neves. Using Mouse Dynamics to Assess Stress During Online Exams. E. Onieva et al. (Eds.): HAIS 2015, LNAI 9121, pp. 1–12, 2015.

**Skills to be achieved in this project**

As specified in excel file.

**Supervisor**

José Miguel Pêgo
Inflammatory response in AD: the role of interferons

Abstract
Alzheimer's disease (AD) is the most prevalent form of dementia. Cognitive decline and the incapacity to form new memories are the major constrains of AD patients. Herein we intend to target cognitive decline by addressing the impact of the levels of interferons (IFNs) on cognitive performance in AD. Here we propose to explore how peripheral and central interferons (IFNs) impact on Alzheimer's disease (AD). Our motivation is triggered by our recent observation of a shift in the expression levels from the IFN type II to type I response during aging, both in the liver and in the choroid plexus of an AD mouse model. In face of publish data and our preliminary results, the present project is biologically relevant in proposing to (i) explore the novel concept that the modulation of peripheral and central IFNs in vivo may hamper cognitive decline in AD, and (ii) unravel how IFNs influence pathology, namely in amyloid precursor protein processing/Aβ levels, neurotrophins expression, inflammation and synapse functioning.

Aims
1-Investigate the impact of IFNs, on AD-related cognitive decline and pathology.
2-Determine to what level cognitive impairment is preventable and/or (fully or partially) reversible by targeting central and/or peripheral IFNs.

References

Skills to be achieved in this project
As specified in excel file.

Supervisor
Fernanda Marques and Sandro Mesquita
The impact of lipocalin 2 in the central nervous system homeostasis: friend or foe?

Abstract
Lipocalin 2 (LCN2), an acute-phase protein that, by binding to iron-loaded siderophores, is a potent bacteriostatic agent since it participates in the iron-depletion strategy of the immune system to control pathogens. The recent identification of a mammalian siderophore also suggests a physiological role for LCN2 in iron-homeostasis, specifically through iron delivery to cells via a transferrin-independent mechanism. LCN2 participates, as well, in a wide variety of cellular processes, including apoptosis and proliferation. In the central nervous system, less is known about the processes involving LCN2, namely which cells are producing this molecule or its impact on cell proliferation and death or on emotional behaviours. Importantly, LCN2 has recently emerged as a relevant clinical biomarker, namely in multiple sclerosis; again, there are conflicting views on the role of LCN2 in pathophysiological processes, with some studies pointing to its neurodeleterious effects, while others revealing it as neuroprotective.

Aims
Determine the role of LCN2 in cognition and cell proliferation

References

Skills to be achieved in this project
As specified in excel file.

Supervisor
Fernanda Marques and Catarina Ferreira
3.32 Behavioral and brain histological characterization of phospholipase D knock-out mice

Summary
Since lipids are the major constituent of the brain, the modulation of its levels can potentially have an impact in its functioning. One the enzymes that can modulate the levels of signaling lipids is phospholipase D (PLD). Specifically, PLD is responsible for the generation of phosphatidic acid (PA) from phosphatidylcholine. PA is a central signalling lipid with membranar fusogenic properties. Consequently, the modulation of its levels can potentially alter cellular/neuronal functioning.

In mammals there are two main PLD isozymes: PLD1 and PLD2. They both catalyse the same reaction, but they differ in their regulatory properties and cellular location. Thus the genetic ablation of either PLD1 or PLD2 has a differential impact. To date there are only four published studies with PLD knock-out mice, which show a role for PLD1 in platelet functioning and autophagy and the ablation of PLD2 was shown to be protective in a Alzheimer’s disease mouse model. However the precise role of PLD1 and PLD2 in brain functioning is still elusive. We propose to study the impact of both PLD1 and PLD2 ablation in mice cognitive-associated behavior and brain hippocampal organization.

Aims
- To characterize the impact of PLD1 and PLD2 ablation in mice behavior.
- To characterize the impact of PLD1 and PLD2 ablation in mice brain histological organization.

Skills to be achieved in this project
As specified in excel file.

Reference

Skills to be achieved in this project
As specified in excel file.

Supervisor
Tiago Gil Oliveira