University of Minho
School of Medicine

Research project portfolio for Mobility Students

Braga | Portugal

Brain Circuits and Neuron-glia Adaptations

The other brain: Do neurons solely process learning and memory? Dissecting the role of astrocytes in cognitive function

Summary

An exciting body of evidence in the past years challenged the classical paradigm that braininformation processing is exclusively neuronal. Indeed, the importance of glial cells is risingdue to emerging data supporting dynamic neuron-glia interactions, and these inter-cellular interactions are nowadays widely accepted. Nevertheless, little is known about the involvement of glial cells, namely astrocytes, in the circuit computation of complex behavior outputs. We showed that blockade of neurotransmitter release from astrocytes (also calledgliotransmission) in vivo is enough to affect cortico-limbic circuits leading to severe cognitiveimpairments.

Aims

The main aim is to explore how further astrocyte genetic manipulations influences cognitivecomputation in cortico-limbic regions. The student will find available complementary techniques running in the lab to evaluate genetically modified mouse models of astrocyte dysfunction at different levels: functional (e.g., behavior and *in vivo* electrophysiology); structural (e.g., 3D cell reconstruction, estimation of cell density, etc.); and molecular (e.g., Transcriptomic analysis, WB, qRT-PCR, etc.).

This proposal is part of a project in the lab funded by the Bial Foundation. The budget includes support for the experiments required for this MSc thesis.

According to the student's expectations, the project is versatile regarding the technicalcombination. More than one student can be enrolled in the project. https://observador.pt/programas/mentes-brilhantes/as-celulas-que-ajudam-a-entender-a- depressao/

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Supervisors

João Filipe Oliveira and João Viana

Neuron-astrocyte signaling in depression: learning from astrocytes how to treat depression effectively

Summary

The prevalence of depressive disorders is dramatically increasing, affecting more than 280 million people worldwide. The COVID-19 pandemic has worsened these numbers as factors like bereavement, grief, isolation, loss of income, and stress trigger new mental health conditions or exacerbates existing ones. Still, the pathophysiology of depression remains poorly understood, which justifies the lack of effective treatments, leading to a failure of complete remission in 65% of the patients. Depressive behavior has been classically linked to neuronal impairments in cortico-limbic networks; however, recent data suggest unexpected roles for astrocytes - a ubiquitous glial cell type in the brain that has been overlooked – in the pathophysiology of depression.

Astrocytes maintain critical dialogues with neurons allowing normal synaptic function in these cortico-limbic regions. Our laboratory has preliminary data showing that silencing astrocytes protects the brain from the insults that cause depressive-like behavior in mouse models.

Aims

The main aim of this project is to pursue these revolutionary findings to IDENTIFY and TEST new astrocytic molecular targets to treat depression. For that, the student will be able to use a combination of cutting-edge methodologies to correlate behavioral, electrophysiological, and transcriptomic data to identify gene targets modulated by astrocytes responsible for the resilience to the depressive phenotype. We will generate tools to modulate these new molecular targets with translational potential and test their therapeutic potential in animal models.

This project is part of the recently funded La Caixa Foundation Grant to study the physiology of cortico-limbic circuits involved in cognition and depression. The overall budget is 0.5 million euros and includes support for the experiments required for this MSc thesis (info under https://fundacionlacaixa.org/en/caixaresearch-health-call-2021-project-depression-therapies).

According to the student's expectations, the project is versatile regarding the technical combination, and more than one student can be enrolled in the project. Together, we will explore the translational potential of the findings for new and more effective therapies to treat depression, reaching far beyond the current state-of-the-art. https://observador.pt/programas/mentes-brilhantes/as-celulas-que-ajudam-a-entender-a-depressao/

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Supervisor

João Filipe Oliveira and João Viana

On the role of adult neuro- and glio-plasticity in the healthy and depressedbrain

Summary

Depression is estimated to affect around 20% of the world population and is the leading cause of disability worldwide. Notably, Portugal has one of the highest rates of psychiatric disorders in Europe (22.9%), with anxiety (16.5%) and depressive disorder (7.9%) being themost relevant. Depressive disorder affects all communities across the world and is more prevalent in women than in men (2:1). Despite these striking numbers, the neuropathologicalbasis of depression remains obscure; moreover, about 65% of patients fail to respond to current first-line therapies, making this field of research a top priority.

Thus, the aim of this project is to unveil how hippocampal adult-born (ABAs) and pre-existingastrocytes (Pre-As) control neuroplasticity, neurophysiology and complex behaviors inhealth and depression. For this, we will use an original and innovative approach toselectively ablate or silence astrogliogenesis and Pre-As by developing an unprecedentedgenetic tool that will promote targeted cell-death or silencing of ABAs and Pre-As in the adultbrain, while not affecting the neuronal lineage. Complemented by other state-of-the-arttechniques (optogenetics, neuroimaging), this will allow fine dissection of the relevance ofthese cells in the remodeling and functioning of neuroglial networks for behavioral control. This project has the potential to open a new array of therapeutic targets for depression,promoting the re-investment of the pharmaceutical industry in neuropsychiatry and thedevelopment of novel therapeutic interventions to treat depression.

Aims

1) Determine how ablation/silencing of hippocampal ABAs or Pre-As in the healthy adult brain impacts on brain neurophysiology, neuronal connectivity, and behavior;
2) Assess the role of hippocampal ABAs and Pre-As for adult brain neurophysiology, neuronal connectivity, and behavior in the context of depression and AD treatment, using astress-induced rat model of depression.

References

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Supervisors

Luísa Pinto and Nuno Dinis Alves

Rewiring the brain: How rewards impact the functionality of thelaterodorsal tegmentum

Summary

The Laterodorsal Tegmentum (LDT) has attracted attention due to its involvement in reward information processing and reinforcement learning, and thus, in the development of addictive behaviors induced by drugs of abuse. Interestingly, evidence from our group revealed a key role for LDT excitatory projections to nucleus accumbens (NAc) (cholinergic and glutamatergic) to incentive salience – by shifting preference, enhancing motivation, anddriving positive reinforcement in natural reward-related behavioral tests. However, it is still unclear what is the contribution of these novel direct projections from the LDT to the NAc and their functional relevance when exposure to either natural rewards or drugs of abuse occurs is still unknown.

The main aim of this project is to understand if different LDT neuronal ensembles respond to positive stimuli differently, that can influence NAc neurons to drive rewarding behavior. This will significantly advance our understanding of LDT-NAc circuit dynamics and provide a necessary foundation for understanding aberrations in this circuitry which may contributeto reward-related disorders.

Aims

In order to achieve the main aim, we are employing a multitude of state-o-the-art techniques, ranging from electrophysiology, fiber photometry, calcium imaging using miniscopes, optogenetics and behavior, to understand and include the LDT as an important player not only in the perception and processing of natural rewards, but also for the understanding of the addictive process.

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Supervisors

Bárbara Coimbra & Ana João Rodrigues

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 ofAD. Neuronal atrophy and synaptic failure have been suggested to play an essential role instress-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 andFyn proteins, this project examines the alterations of synaptic interactome underlying stress-induced neuronal atrophy and synaptic loss searching for molecular targets with neuro- andsynapto-protective properties.

Aims

1) Molecular characterization of stress/GC impact on postsynaptic vs extra-synaptic/presynaptic interactome;

2) In vitro and/or in vivo monitoring of intracellular and synaptic trafficking of receptors andrelated cell signaling under stressful conditions.

References

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Supervisors

Ioannis Sotiropoulos and Nuno Sousa

Assessing the role of SNX3 and SNX12 in the nervous system: insightsfrom a "stressed" synapse

Summary

Sorting Nexins (SNXs) are a family of proteins that plays pleiotropic roles in intracellular trafficking, particularly mediating endocytic events underlying synaptic plasticity, cognition and neurodegeneration [1-4]. Importantly, cognitive and synaptic deficits have been rescuedby restoring SNXs hippocampal expression levels [3]. SNX3 and SNX12 are closely relatedbrain-enriched SNXsknown to regulate neurite formation [7], both of which single nucleotidepolymorphisms (SNPs) and decreased expression levels were also described in Alzheimer'sdisease (AD) patients [5, 6]. SNX12 has also been shown to regulate BACE-1 mediated amyloid precursor protein (APP) processing [6]. Recently, using the nematode *Caenorhabditis elegans* as a model, our group demonstrated for the first time *in vivo* that the SNX3/12 homolog gene is crucial for (neuro)behavioral performance in a wnt- and retromer-independent manner [8]. The *snx-3/12*worm deletion mutant displays marked neuronal and behavioral deficits that were rescued upon pan-neuronal expression of wormSNX3 cDNA in the mutant background [8]. Additionally, the *snx-3/12*mutant worms are short-lived and susceptible to environmental stressors. Here we propose to perform a characterization of SNX3 and SNX12 expression and subcellular localization in neurons, both *in vivo,* using rodent brain samples, and *in vitro*, using primary and established neuronalcultures. The distribution of SNX3 and SNX12 will be addressed by immunofluorescence using selected SNXs antibodies together with endocytic markers (*e.g* EEA1, LAMP1) and synaptic markers, using confocal microscopy. Their co-localization with endocytic cargoes of interest, such as glutamate receptors, glucocorticoid receptors, growth-receptors, amongothers, will also be inferred. Membrane fractionation studies will be additionally performed to study SNXs membrane-specific association, including synaptosomal membranes. Concomitantly, primary neuronal cultures will be treated acutely or chronically with corticosterone, the major stress hormone, and/or dexamethasone, and SNX3 and SNX12 expression and localization assessed, as well as of receptors of interest, to infer possible effect of stress-exposure on SNX-mediated functions. Down-regulation of selected SNXs will be achieved by RNAi approaches, in established neuronal cultures (e.g, SH-SY5Y) and/or primary neuronal cultures. These studies will allow to follow SNX3 and SNX12 expression levels, subcellular localization, membrane-association and to analyze the impactof their down-modulation in fundamental aspects of neuronal function, including cell survival,dendrite morphology, neurite outgrowth and receptor-mediated signaling. Overall, we aim tounravel the molecular mechanisms underlying SNX3 and SNX12 function in the nervous system, and pave the way to the identification of SNX3 and SNX12 interactomes and novelpathways of intervention in neurodegenerative disorders.

Aims

1) Characterize SNX3 and SNX12 expression by qRT-PCR and western-blot analysis *in vivo* using rodent brains;

2) Characterize SNX3 and SNX12 expression by qRT-PCR and western-blot *in vitro,* usingprimary neuronal cultures, and established neuronal cultures SH-SY5Y, in control conditionsor treated acutely- or chronically- with corticosterone or dexamethasone;

3) Characterize the subcellular localization and distribution of SNX3 and SNX12 by immunofluorescence of brain slices and cells in culture, including co-localization withendocytic and synaptic markers and endosomal cargo, and by performing a membrane fractionation protocol to study SNXs membrane-specific association, including synaptosomal membranes *in vivo* and subcellular compartments *in vitro*;

4) Evaluate the effect of down-modulating SNX3 and SNX12 levels in neuronal morphology,neurite outgrowth and cell survival.

References

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Supervisors

Neide Vieira

The role of Tau in the regulation of translational stress response and itsimportance for brain pathology

Summary

Clinical evidence suggests lifetime stress and high levels of glucocorticoid (GC) as risk factors for Alzheimer's Disease (AD) while previous experimental studies from our team highlight the essential interplay between Tau and chronic stress, via probably GC and theirreceptor signaling, in the precipitation of Tau-related neuronal malfunction and toxicity; however, the exact stress- driven molecular mechanisms are not understood. Because GC receptors are transcription factors and translational control contributes to cell adaptation to stressful conditions with a key role for RNA-binding proteins (RBPs) and stress granules (SGs) in the reprogramming of mRNA translation, we aim to understand the critical involvement of SGs and RNA dyshomeostasis in stress-induced Tau pathology. To further dissect the importance of Tau and SGs in translational stress response, we will use WT, Tau-KO, and P301L-Tg mice subjected to chronic stress, followed by behavioral characterization related to anxiety, depression, and cognition, followed by molecular analysis.

We will use integrated proteomic analysis of all proteome together with proteomic analysis targeted to the TIA-1 antibody (the initiator of SG formation), followed by WB analysis of cell fractionation protocols and immunofluorescence studies.

Aims

1) Monitor the trafficking and movement of RNA-binding proteins and Stress granuleassembly after exposure to chronic unpredictable stress protocol.

2) Decipher the role of TIA-1, the suggested initiating RBPs, in the stress/GC-driven SGsformation and Tau aggregation.

References

- Sotiropoulos et al., (2015) J Alz Dis;
- Silva JM et al., (2019) Cell Death and Diff.

Supervisors

Joana Silva and Ioannis Sotiropoulos

The effect of chronic stress and exosomes on Tau pathology propagation

Summary

Chronic stress and increased glucocorticoids (GCs) levels are suggested risk factors for Alzheimer's disease (AD). Indeed, previous data shows that chronic stress and GCs triggercytoplasmic accumulation of Tau towards synaptic malfunction and neurotoxicity, leading tocognitive deficits. However, it is unknown how stress triggers Tau pathology. Filling this essential gap, we recently found that the major degradative pathways (i.e., autophagy and endolysosomal pathway) are block upon chronic stress and high GCs. Concomitantly, exosomes, nano-sized extracellular vesicles (EVs) derived from the endolysosomal pathway, have recently been demonstrated to play key roles in brain physiology and pathology. However, no study to date has looked to the effect of stress on these vesicles and how this can impact tau pathology propagation. Thus, this project will use novel and advanced in vivo and ex vivo set-ups to evaluate the impact of chronic stress/GC on exosomes biogenesis and dissect the role of these vesicles on Tau pathology propagation.

Aims

1) Evaluate the impact of stress on brain Tau pathology
2) Study the impact of stress on exosome dynamics

References

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Supervisors

Patrícia Gomes and Ioannis Sotiropoulos

The physiological role of Tau in neurons – away from the axon

Summary

Tau protein is suggested to play important functions in neurons, however, the in vivo significance of these functions remains uncertain as conventional Tau-KO models exhibit nomajor deficits, possibly due to the presence of compensation mechanisms. While previous data by the group show that Tau absence is protective against stress-induced deficits in adult hippocampal neurogenesis, implicating Tau as an important regulator of the cellular cascades; the use of a novel conditional KO is opening our view on the essential role of Tauin the adult brain. The tau-cKO model presents behavior deficits and associated neuronal functional defects, with alterations in signaling cascades relevant to synaptic function as wellas neuronal differentiation and plasticity. Moreover, we revealed that Tau protein is essentialfor chromatin landscape maintenance of DNA/RNA homeostasis, altering the way genes areexpressed. Given these novel findings, this project aims to further dissect the role of Tau inthe nucleus and dendrites, understanding its impact on gene expression and consequent plasticity of the hippocampus.

Aims

1) Evaluate the impact of conditional Tau deletion on different neuronal cell types in thehippocampus and consequent behavior characterization.
2) Analyze nuclear structure in different cell types after Tau ablation.
3) Analyze the transcriptional repression of genes observed in Tau-cKO mice and theirrelationship with synaptic plasticity.

References

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- Silva JM et al (2019) Cell Death & Diff

Supervisors

Joana Silva

Sensory signals of associative learning

Summary

Pursuing rewards and avoiding punishment are two fundamental forces that drive animal's behavior. To survive and thrive in an ever-changing environment, individuals learn to assignvalence to environmental stimuli if they are emotionally relevant, such as those associated with the acquisition of food or other rewards, or the avoidance of predators and discomfort.This process of associative learning is key for successfully obtain rewards and avoid dangers and has shown to be dysfunctional in disorders such as depression and addiction.Strong evidence highlights the nucleus accumbens (NAc) as a critical hub in associative learning since it receives innervation from sensory cortical and limbic regions and sends information about those signals to output effector brain regions, being thus considered the limbic-motor interface of the mammalian brain[1,2]. In addition, our team has shown that NAcneurons encode both rewards and aversive events, as well as cues that predict those outcomes[3–6]. Considering this, we hypothesize that sensory signals are directly decoded bythe NAc, and significantly contribute for associative learning.

Aims

This project aims to determine if/how NAc neurons directly decode sensory signals to generate appropriate behaviors. To achieve that we will use genetically encoded calcium indicators together with fiber photometry or imaging through miniaturized microscopes (miniscopes) and optogenetics in freely behaving mice to determine if the NAc receives signals from the sensory cortex and if those signals are decoded by neurons within this brain region during the performance of associative learning tasks.

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5. Soares-Cunha, C. *et al.* Nucleus accumbens medium spiny neurons subtypes signalboth reward and aversion. *Mol. Psychiatry* **25**, 3241–3255 (2020).
6. Soares-Cunha, C. *et al.* Distinct role of nucleus accumbens D2-MSN projections to ventral pallidum in different phases of motivated behavior. *Cell Rep.* **38**, 110380 (2022).

Supervisors:

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Prefrontal Serotonin Input in Cognitive (In)Flexibility

Summary

Throughout our day-to-day lives, we are continuously exposed to different stressful experiences and stimuli. While in some situations a stress response is useful and manageable, permanent exposure to stressful events may severely impact and affect our mental health, often leading to the precipitation of neuropsychiatric disorders. Among one the hallmark symptoms observed in patients with neuropsychiatric disorders whose etiologyis highly associated with stressful experiences, is cognitive rigidity and inflexibility, characterized by an incapacity to make efficient decisions and a shift in attention. In humansand rodents, stress has a major impact on the Prefrontal Cortex (PFC), a brain region well-known to be involved in cognitive flexibility. Upon stress exposure, the PFC suffers anatomical and molecular changes that negatively impact its function, and ultimately lead tobehavioral impairments, including deficits in cognitive flexibility. Recently, we found that serotonin release in the Prelimbic subregion of the medial PFC plays necessary and sufficient roles in cognitive flexibility. In mice, by modulating the activity of serotonergic neurons projecting to the medial PFC, we observed either improvement or impairment in their performance in cognitive flexibility, in particular in the extradimensional set-shift task. Complementarily, others observed that serotonergic neurons specifically projecting to the orbitofrontal cortex (OFC) are involved in the intradimensional set-shift task. Recognizing the importance and urgency to develop novel and effective strategies to rescue or mitigate deficits promoted by stressful experiences, here we propose to study a treatment strategy to improve cognitive flexibility through the manipulation of PFC serotonergic pathways. Webelieve that the results of this study will provide critical insights on how to better treat cognitive flexibility impairments in stress-related disorders, and pave the way for upcomingprecision medicine-based treatments to alleviate the symptoms and burden caused by mental illness.

Aims

We aim to (i) reveal novel imaging correlates of performance in cognitive flexibility tasks (ii)understand the impact of stress and/or depression in specific serotonergic circuits, and ultimately (iii) rescue cognitive inflexibility. To achieve this goal, we will monitor and modulateneuronal activity while animals perform cognitive flexibility tasks. In this project, we will applystate-of-the-art techniques including fiber photometry, PET imaging, optogenetics, viral tracing approaches, electrophysiology, and behavior assessments, among others.

References

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Supervisors

Nuno Dinis Alves and Luísa Pinto

Raf kinase inhibitor protein (RKIP): A modulator of RTKs moleculartargeted therapies response in solid tumors?

Summary

Cancer treatment is being revolutionized by the translation of knowledge achieved in cancerbiology studies in the development of new drugs that act by molecular recognition. However,the understanding of cancer molecular genetics suggests that the complex heterogeneity ofhuman malignancies is a major limitation for the application of these novel cancer treatmentapproaches, 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 alleviateor 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 the 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 cell resistance to chemotherapy. Due to the important role of RKIP in the regulation of importantintracellular signaling pathways, and its involvement in some tumor's malignancy, we believethat RKIP can also modulate tumor cell response to RTKs molecular targeted therapies. Surprisingly, besides our preliminary and exciting results, there are no studies screening forRKIP 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. The work involves an in-silico analysis that will be done remotely.

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Supervisors

Olga Martinho

Therapeutic relevance of the tumor suppressor gene SPINT2 in Melanoma (skin cancer)

Summary

Serine protease inhibitor Kunitz type 2 (SPINT2) has been identified as a tumor suppressorgene in various solid tumors, including melanoma. The outcome of patients with metastaticmelanoma has greatly improved with targeted therapies, particularly BRAF and MEKinhibitors, nevertheless, tumors ultimately develop resistance to these therapies. Besides these targeted therapies, other biomarker-directed therapeutic strategies that allow the selection of personalized treatments are still lacking in melanoma.

It has been demonstrated that the secretion of HGF is a mechanism of resistance to BRAFinhibitors. SPINT2 has a role on regulating HGF activation and, therefore, might play a rolein this mechanism of resistance and consequently modulate the therapeutic response of melanoma cells. Additionally, HGF is the only ligand known for MET receptor activation and,therefore, MET inhibitors may play a role in overcoming therapeutic resistance in melanomawhen used in combination with BRAF inhibitors.

Aims

1) Growth and maintenance of melanoma cell lines to understand how SPINT2 expression modulation (downregulation of SPINT2 in cells previously expressing the proteinor *SPINT2* transfection and expression in cells without protein expression) can affect the therapeutic response of the cells;

2) Evaluate the efficiency of the targeted therapies and the mechanisms of resistance in melanoma cell lines genetically modified.

References

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Supervisors

Marta Viana-Pereira and Sónia Celeiro

Embryonic T-box transcription factor Brachyury: Dissecting its role inprostate cancer biology and clinical application.

Summary

Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men and therapy resistance is a major problem for PCa patients. Recently, we identified Brachyury as a new biomarker of PCa aggressiveness and poor prognosis, and demonstrated that thistranscription factor is a direct regulator of androgen receptor expression, contributing to tumor chemotherapy resistance and constituting an attractive target for advanced PCa.

Since the successful therapy of patients with PCa is highly dependent on reliable diagnosticand prognostic biomarkers, we aim to dissect the molecular mechanisms through which Brachyury is modulating therapeutic response, discover the network of genes and pathwaysregulated by Brachyury, and finally to develop a chip RNA-based platform to detectBrachyury, its targets and other major known prostate biomarkers, which could constitute atool for early diagnosis and potentially therapeutic prediction in PCa patients using non- invasive liquid biopsy.

Aims

Brachyury has been associated with increased in vitro and in vivo resistance to radio and chemotherapy in lung (eg. docetaxel) and in breast (tamoxifen)cancer models. Yet, lite is known about Brachyury role in PCa therapy response. Based on the previous data and combined with our own previous published studies, we are suggesting that Brachyury promotes PCa resistance. To test this hypothesis, we aim to use 5 human prostate cell lines representing in vitro models of PCa progression used worldwide, with modulated Brachyuryexpression, and evaluate the effect of Brachyury on the new generation of androgen-target therapies (abiraterone and enzalutamide). he work involves an in silico analysis that will bedone remotely.

References

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Supervisors

Olga Martinho and Rui M. Reis

Oncogenic interplay between RKIP and PD-L1 in lung cancer: implications to immunotherapy response

Summary

Immune checkpoint inhibitors, notably antibodies targeting programmed death-1 (PD-1) andprogrammed death ligand-1 (PD-L1), have modified the management of patients with locally advanced or metastatic cancers. However, because only a subset of patients responds, anurgent need exists to develop clinically practical tools to identify the subset of patients mostlikely to derive clinical benefit.

Recently, tumor-intrinsic functions of PD-L1 and their interplays with other oncogenic pathways have drawn much attention: PD-L1 expression influences cell growth and autophagy via mTOR signaling and can promote epithelial-to-mesenchymal transition (EMT)without a clear mechanism identified. Raf kinase inhibitor protein (RKIP), a signaling modulator, is a well-known regulator of metastization, through the regulation of EMT mechanisms, its downregulation contributing to chemotherapy resistance in several tumor types. Hence, in the present project we are hypothesizing whether RKIP can also be a predictor of tumor cell response to PD-1/PD-L1 targeted therapies in lung cancer.

Aims

Recently, high expression of PD-L1 was associated with the presence of NSCLC driver alterations such as EGFR and KRAS mutations, reinforcing the importance of studying PD-L1 tumor-intrinsic functions, to discover and validate new predictive biomarkers, beyond tumor PD-L1 expression. Thus, the major aim of this project is to shed light on the mechanisms of cancer cells' response to immunotherapy, by studying the role of RKIP expression in the modulation of NSCLC cells' response to PD-1/PD-L1 targeted therapies. The work involves an in-silico analysis that will be done remotely.

References

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Supervisors

Olga Martinho

Theranostic solutions for malignant brain tumors: bridging fundamentaland applied research through HOXA9

Summary

Glioblastoma (GBM) is the most common and lethal type of primary brain tumor in adults. Patients diagnosed with GBM present a short overall survival (OS) of roughly 15 months after standard treatment. Previously, our group demonstrated that the transcription factor HOXA9 is an important oncogene in GBM, modulating distinct cancer hallmarks and being associated with poor patient prognosis. More recent data suggest that HOXA9 influences the immune system in the context of GBM, while others have shown that HOXA9 regulatesimmune responses in ovarian cancer. Therefore, we now aim to: i) better determine the mechanisms by which HOXA9 impacts the aggressiveness of GBM, including immune responses; and ii) test therapies targeting HOXA9, alone or in combination with standard chemotherapeutic agent and/or immunotherapies. The completion of this project will shed light on novel mechanisms of aggressiveness in GBM and innovative therapeutic strategiesfor this deadly tumor.

Literature reading, writing of the report, and bioinformatic analyses supporting the laboratoryresults will be performed in a remote environment.

Aims

1) Characterize the molecular and cellular landscape signatures mediated by HOXA9 inGBM;
2) Investigate the therapeutic targeting of HOXA9 in GBM (alone and in combination withchemotherapy or immunotherapies), in a paradigm of precision medicine.

References

Costa BM, Smith JS, Chen Y, Chen J, Phillips HS, Aldape KD, et al. Reversing HOXA9 oncogene activation by PI3K inhibition: epigenetic mechanism and prognostic significance in human glioblastoma. Cancer Res. 2010;70: 453–62.

Pojo M, Gonçalves CS, Xavier-Magalhães A, Oliveira AI, Pinto L, Pinto AA, et al. A transcriptomic signature mediated by HOXA9 promotes human glioblastoma initiation, aggressiveness and resistance to temozolomide. Oncotarget. 2015;6: 7657–74.

Supervisors

Bruno M. Costa, Céline S. Gonçalves and Eduarda P. Martins

The influence of the tumor suppressor gene SPINT2 on melanoma cells'metabolism

Summary

The reprogramming of energy metabolism has emerged as an important hallmark of cancersince it supports continuous cell growth and proliferation. Similar to other cancer types, melanoma cells metabolize glucose into lactate, regardless of the oxygen levels, a processknown as Warburg Effect. Nevertheless, mitochondrial oxidative phosphorylation (OXPHOS) can also play a significant role in melanoma cancer.

The tumor suppressor gene Serine protease inhibitor Kunitz type 2 (SPINT2) has an inhibitory activity against serine proteases implied in the activation of several pathways deregulated in cancer, however there are no studies regarding SPINT2 influence in cancermetabolism. Preliminary results from our group suggest that SPINT2 influences glucose consumption/lactate efflux in melanoma cells and, therefore, we intend to understand SPINT2 influence in melanoma metabolism.

Aims

1) Growth and maintenance of melanoma cell lines genetically modified (SPINT2overexpression or SPINT2 absence);

2) Evaluate the expression of metabolic markers in the same melanoma cell lines.

References

- Pereira MS, Celeiro SP, Costa AM, et al. Loss of SPINT2 expression frequently occurs inglioma, leading to increased growth and invasion via MMP2. Cell Oncol (Dordr) 2020; 43(1): 107-21.

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Supervisors

Marta Viana-Pereira and Sónia Celeiro

Lactate as a key player in the metabolic symbiosis between tumo3eur cellsand tumour-associated macrophages

Summary

Tumour cells are characterized by high rates of glycolysis, with abundant expression of glucose transporters, glycolytic enzymes and monocarboxylate transporters (MCTs). However, little is known about how the expression/activity of MCTs mediate the metabolic symbiosis between tumour cells and tumour associated-macrophages (TAMs). TAMs are one of the most common stromal cell types, and their presence correlates with the outcome of several cancers. Macrophages can be differentiated in the classically activated type 1 macrophages (M1) and the alternatively activated type 2 macrophages (M2). There is evidence suggesting that M1 macrophages act as tumour suppressors, promoting an immune response that eliminates tumour cells, while M2 macrophages promote tumour growth, increasing tissue remodeling, angiogenesis and extracellular matrix degradation. Thus, we aim to understand the role of lactate on the metabolic symbiosis between the two cell populations.

Aims

As the crosstalk between tumour cells and TAMs are far from being clarified, the main aim of the present project is to characterize the metabolic symbiosis between tumour cells and macrophages. Firstly, M1 and M2 macrophages will be characterized by immunohistochemistry in a series of lung cancer, and secondly the metabolic profile of M1 and M2 will be evaluated in vitro and in vivo using a transgenic mouse model..

Skills to be achieved in this project

As specified in excel file.

Supervisors

Sara Granja and Fátima Baltazar

WNTrack – Tracking brain tumors through their exosomes: a WNT6-drivenapproach

Summary

Brain tumors have one of the highest mortality rates and rank 1st in average years of life lost among all tumor types. In Europe, they are the leading cause of cancer-related death inindividuals under 40 years old. Among primary brain tumors, glioblastoma (GBM) is the mostmalignant and common tumor type in adults, with a median overall survival of ~15 months and a 5-year survival rate of only 5%. We have recently shown that WNT6, a signaling molecule of the WNT pathway, is expressed in virtually all GBM, and that its increased expression is associated with patients' shorter overall survival and with increased GBM aggressiveness.

Besides the ineffectiveness of GBM therapy, patients' dismal prognosis is also linked to thefact that diagnosis and tumor monitoring is technically challenging, relying on risky brain biopsy and expensive magnetic resonance imaging (often with limited clinical value due to chemotherapy-induced pseudoprogression). In this context, several efforts have been madeto use liquid biopsies for non-invasive diagnosis and monitoring of GBM, namely using circulating tumor cells (CTCs) and cell-free nucleic acids (cfNAs) detected in blood or cerebrospinal fluid (CSF), but frustrating results have been obtained so far.

More recently, tumor-derived exosomes detected in patients' blood has emerged as an appealing alternative to CTCs and cfNAs. Exosomes are secreted by all viable tumor cells,have been found in higher levels in GBM patients, and their cargo includes nucleic acids and proteins, being globally better representations of the intratumoral heterogeneity.

Considering that WNT6 exerts its paracrine and autocrine role upon being secreted into exosomes, and that WNT6 potential as a serum-based biomarker tool was already demonstrated in osteosarcoma, in this project, we intend to identify a WNT6-driven exosomal molecular signature associated with GBM aggressiveness and poor prognosis, and explore its utility as a blood-based biomarker tool for GBM patients' management.

Aims

To achieve our goal, we intend to pursue two specific aims:

Aim 1: Identify the molecular landscape mediated by WNT6 in GBM, characterizing WNT6 full exosomal transcriptome and proteome and critically assessing their functional and clinical value in GBM.

Aim 2: Explore the potential of a WNT6 molecular signature for GBM liquid biopsies as a novel biomarker tool in GBM patients' blood.

References

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Supervisors

Céline S. Gonçalves and Bruno M. Costa

Exploiting the therapeutic value of WNT6 oncogene inhibition in malignantbrain tumors

Summary

In Europe, brain tumors are the leading cause of cancer-related death in individuals under 40 years old. Glioblastoma (GBM) is the most malignant and common primary brain tumor in adults, with a 5-year survival rate of 5%, highlighting an urgent unmet need for better therapies.
We recently described WNT6 as a new prognostic biomarker in GBM [1]. This protein is anactivator of the WNT pathway, with critical roles in embryogenesis and aberrantly activatedin cancer. We showed that WNT6 is significantly overexpressed in GBM and associated withpatients' poor prognosis in several independent cohorts [1]. WNT6 also increased GBM cellviability, proliferation, invasion, migration, stemness, and resistance to temozolomide-basedchemotherapy, implicating it as an important oncogene in glioma [1]. Using intracranial GBM mice models with both WNT6 overexpressing and silencing cell models, we showed that WNT6-high tumors present increased features of tumor aggressiveness, which ultimately associated with mice shorter overall survival [1]. Also, we identified the WNT, SFK and STAT3 signaling pathways as WNT6-mediated mechanisms in GBM [1]. Finally, we identified both DNA methylation and HOXA9, an oncogenic transcription factor in GBM, as WNT6 transcriptional regulators in glioma [2], which may shed new clues into therapeutic strategies targeting WNT6.
In this context, we now aim to: (i) understand the molecular mechanisms regulated by Wnt signaling/WNT6 that may contribute to glioma aggressiveness; (ii) evaluate the impact of WNT6 inhibition in GBM, alone or in combination with standard-of-care chemotherapeuticsand/or immunotherapies, which recently emerged as promising therapies for cancer; and
(iii) understand the role of WNT6 in anti-tumor immune responses.

Aims

1) Study new mechanisms underlying the observed WNT6-associated chemoresistance(e.g., status of MGMT, MMR and BER pathways) in GBM cell models and patients;
2) Assess the therapeutic potential of a new combinatorial treatment including an inhibitorof the Wnt pathway and temozolomide in glioma models (*in vitro* and *in vivo*);
3) Evaluate how WNT6 may modulate anti-tumor immune responses, both in basalconditions and upon the use of immunotherapies (e.g., immune checkpoint inhibitors).

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Supervisors

Bruno M. Costa and Céline S. Gonçalves

Unravelling the functional impact of P-Cadherin in aggressive braintumors

Summary

Glioblastoma (GBM) is a highly malignant and the most common primary brain tumor in adults, for which curative therapies are not available. We previously showed that HOXA9, acritical transcription factor during development, is overexpressed in a subset of GBM samples, and is associated with increased tumor resistance to therapy and shorter patient survival. A recent study also demonstrated that HOXA9 affects the aggressiveness of ovarian cancer cells by affecting the expression of P-Cadherin, which encodes a transmembrane protein with critical roles in cell adhesion. Consistently, we recently found that there is a correlation between HOXA9 and P-Cadherin in GBM, and that P-Cadherin might be a novel oncogenic molecule in GBM, by affecting cell viability, invasion ability, andstemness capacity *in vitro*. Critically, we also observed that P-Cadherin expression associates with shorter overall survival *in vivo* and in GBM patients of different cohorts.

In this context, we now aim to better understand the cellular and molecular mechanisms bywhich P-Cadherin mediates GBM aggressiveness. For example, genome-wide transcriptomic signatures of various GBM models will be profiled, and its effects on GBM cellular energetic metabolism, a critical hallmark of cancer, will be investigated.

Aims

1) Study the transcriptome of P-Cadherin (e.g., gene ontology characterization and pathwayenrichment analysis).
2) Study the impact of P-Cadherin expression in the energetic metabolic reprogramming ofGBM cell models.

References

Costa BM, Smith JS, Chen Y, Chen J, Phillips HS, Aldape KD, et al. Reversing HOXA9 oncogene activation by PI3K inhibition: epigenetic mechanism and prognostic significance in human glioblastoma. Cancer Res. 2010;70: 453–62.

Pojo M, Gonçalves CS, Xavier-Magalhães A, Oliveira AI, Pinto L, Pinto AA, et al. A transcriptomic signature mediated by HOXA9 promotes human glioblastoma initiation, aggressiveness and resistance to temozolomide. Oncotarget. 2015;6: 7657–74.

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Supervisors

Bruno M. Costa and Eduarda P. Martins

Unravelling the role of alveolar macrophages in the control of Mycobacterium tuberculosis by susceptible and resistant hosts

Summary:

Tuberculosis (TB) is the second worldwide leading cause of death from an infectious diseasebehind COVID-19. The causative agent, *Mycobacterium tuberculosis*, is an intracellular bacterium that spreads among people via infected aerosol droplets. Once inside the alveolarspace bacteria infect the tissue-resident alveolar macrophages (AMs). These cells are known to be the main host of *M. tuberculosis* and permissive to the bacterial growth. After replication in AMs, the bacteria disseminate to other lung-myeloid cells, an event that is required for the activation and recruitment of the acquired immunity. Our previous results showed that the mouse strain C3HeB/FeJ – susceptible to *M. tuberculosis* infection – is better able to control the bacterial growth during the early phase of infection. Interestingly,

M. tuberculosis remains in AMs longer than in the resistant strain C57BL/6. Accordingly, C57BL/6 displayed a faster dissemination of the bacteria and a more rapid activation of theacquired immune response mediated by $CD4^+$ T cells. Therefore, our hypothesis is that theharmful delay in the acquired immune response observed in the lungs of C3HeB/FeJ mice might be related to an improved ability of AMs from this strain to better control *M. tuberculosis*. Hence, the purpose of this project is to investigate the causes that predisposethe AMs to delay the bacterial dissemination, and consequently the response of activated $CD4^+$ T cells in C3HeB/FeJ mice.

Aims:

1. Collect alveolar macrophages through bronchioalveolar lavage from C57BL/6 andC3HeB/FeJ mice and infect them with *M. tuberculosis in vitro*;
2. Investigate the frequency of AMs' cell death *in vitro* and afterwards evaluate thelocalization of apoptotic cells *in vivo* using flow cytometry.
3. Evaluate *in vivo* the ability of AMs to migrate from the alveolar space to the lungparenchyma;
4. Determine the expression and production of cytokine or chemokines from these cellsusing RT-PCR and ELISA, respectively.

References:

Cohen SB et al. (2018). Cell Host Microbe. 24(3): 439-446.Huang L et al. (2018). J Exp Med. 215(4): 1135-1152.

Supervisors

Egídio Torrado and Consuelo Micheli

Lipid droplets and lipid mediators as early regulators of innate immunityduring tuberculosis

Summary:

Mycobacterium tuberculosis is an intracellular pathogen and the etiological agent of tuberculosis – an infectious respiratory disease that causes 1.4 million deaths worldwide every year. The first cells encountering *M. tuberculosis* are alveolar macrophages (AMs), which have been described as permissive to bacterial growth. Importantly, *M. tuberculosis* has been shown to induce a metabolic reprogramming of AMs, with impact in the initiation of the immune response. Furthermore, our recent data suggest that AMs play a crucial role in determining host resistance or susceptibility to tuberculosis. Therefore, we hypothesize that resistance/susceptibility may be dependent on metabolic reprogramming of AMs, specifically lipid accumulation, which in turn generate cytoplasmatic lipid droplets (LDs). Indeed, lipid droplets play a central role in the synthesis of lipid mediators, whose role in theimmune response against tuberculosis is not completely understood.

In this work, we aim to elucidate the impact of LDs and lipid mediators produced by foamy macrophages, specifically eicosanoids, in the early control of *M. tuberculosis* infection.

Aims:

1. Collect AMs through bronchioalveolar lavage from resistant and susceptible miceand infect them with *M. tuberculosis in vitro*;
2. Evaluate *in vitro* and *ex vivo* the presence of lipid droplets in infected and non-infected AMs using immunofluorescence methodologies;
3. Characterize the cytokine profile of the infected and non-infected AMs using ELISAand qPCR;
4. Modulate the generation of LDs *in vitro* in AMs of resistant and susceptible miceusing chemical activators/inhibitors;
5. Modulate the synthesis of lipid mediators *in vitro* using specific inhibitors.

References:

Cohen SB et al. (2018). Cell Host Microbe. 24(3): 439-446.Huang L et al. (2018). J Exp Med. 215(4): 1135-1152.
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Supervisors

Egídio Torrado and Consuelo Micheli

The role of branched-chain amino acids in shaping intestinal environment

Summary

Inflammatory Bowel Disease (IBD) is a complex set of inflammatory disorders comprising Crohn's disease and ulcerative colitis. Although the precise etiology of IBD is unknown, bothforms of IBD seem to result from an inappropriate immune response triggered by intestinal microbes and environmental cues in genetically susceptible individuals. Dietary nutrients have emerged as a potential therapeutic approach in IBD, due to the impact on intestinal homeostasis through the modulation of immune response, epithelial barrier and gut microbiota composition. Several nutrients have already been described as having a beneficial effect on IBD outcome. Previous data from our research group have described increased levels of branched-chain amino acids (BCAA; leucine, isoleucine and valine) in colonic samples of mice protected against colitis induction. Preliminary results showed thatBCAA supplementation worsens colitis outcome and increases recovery time. Nevertheless, the precise mechanisms by which these metabolites can influence the intestinal environmenttowards susceptibility are still unclear. By understanding the mode of action of these aminoacids, we aim to explore novel dietary supplementation strategies in order to promote intestinal protection and subsequently act as putative adjuvants for IBD.

Aims

1) Evaluation of the effect of BCAA in intestinal epithelial barrier integrity, in both homeostaticand colitic settings, using an in vitro gut-on-a-chip model;
2) Transcriptomic analysis of colonic epithelial cells from mice upon BCAA supplementation.
3) Metagenomic analysis of murine stool samples before and after colitis induction and BCAA supplementation.

Supervisors

Ana Frias and Ricardo Silvestre

Gene editing: the cross-kingdom system for genohacking

Summary

Herein, we aim to use the trans-kingdom RNA-guided clustered regularly interspaced shortpalindromic repeats (CRISPR)/Cas9 [nuclease]/[nickase; Cas9n] technology to edit genomes from human cell lines to fungal cells. We will develop and apply the programmableguide 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 ofcircumvent the resilience of *Sporothrix* 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 *Sporothrix* spp., thus moving the field beyond thestate of art. So, the generation of targeted loss/gain of function mutants in *Sporothrix* 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 associatedto increased susceptibility to immune response.

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Supervisors

Cristina Cunha and Fernando Rodrigues

Defining the mechanisms that prevent the rapid response of CD4 T cellsto Mycobacterium tuberculosis infection in the lung

Summary

One of the most intriguing aspects of the immune response to *Mycobacterium tuberculosis*(*M. tuberculosis*) infection is the delayed activation of the antigen-specific CD4 T cell response. This delayed activation is also seen during the secondary responses with memoryCD4 T cells colonizing the lungs, the organ where infection is established. Taking into account that *M. tuberculosis* is an intracellular pathogen that is rapidly phagocytosed by alveolar macrophages, we hypothesize that these macrophages do not provide the signalsnecessary for T cell activation. Therefore, in this project we will test this hypothesis using effector or memory *M. tuberculosis*-specific CD4 T cells and adoptive transfer experiments.

Aims

1) To generate effector or memory Th1 CD4 T cells specific to *M. tuberculosis in vitro*;
2) To perform adoptive transfers of effector or memory CD4 T cells.
3) To test the protective function of effector and memory CD4 T cells in the mouse model of *M. tuberculosis* infection.
4) To characterize the cytokine profile of the generated CD4 T cells by Flow cytometry,ELISA and qPCR;

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Supervisors

Egídio Torrado and Consuelo Micheli

Immunometabolic networks in the regulation of visceral Leishmaniasis

Summary

In response to pathogens, innate immune cells must rapidly adapt their metabolic programsto meet specialized host defense needs. This adaptation is bioenergetically expensive, requiring precise control of cellular metabolic pathways. Leishmania spp. parasites reside obligatorily in phagocytes (neutrophils and macrophages), use host cell metabolism and energy sources, intercept signaling pathways, and inflict the potentially fatal disease visceral leishmaniasis, which is fatal if left untreated. Despite the parasite's obligatory dependence on host cell metabolism and the lack of effective, non-toxic, orally bioavailable anti- leishmanial drugs, Leishmania-perturbed host cell metabolomes and their relation to anti- leishmanial immune responses remain unexplored. Thus, deciphering multilevel interactions between metabolic and innate immune cells during infection offers considerable therapeuticor prophylactic promise.

Aims

Elucidate and analyze the immunometabolic networks regulating the innate immune anti- leishmanial response that plays a crucial role in defining susceptibility to infection.

Supervisors

Maria João Peixoto and Ricardo Silvestre

Metabolic regulation of the immune response to fungal infection

Summary
Invasive pulmonary aspergillosis (IPA) is an infectious disease caused by the fungalpathogen *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, IPA is a leading cause of death amongtransplant recipients with a 1-year mortality reaching 75%, despite the availability of several antifungal drugs. Alterations in cellular metabolism represent a fundamental mechanism bywhich immune cells maintain homeostasis. In response to pathogens or tissue damage, immune cells must rapidly adapt their metabolic programs to meet specialized host defenseneeds. This adaptation is energetically expensive, requiring a 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 duringimmunity and inflammation processes. We have recently unveiled a novel mechanism whereby macrophages sense fungal melanin to direct metabolism toward the activation of glycolysis and protective immunity. Our laboratory is now focusing on investigating how additional metabolic pathways converge to influence the function of macrophages and ultimately the immune response against *A. fumigatus*.

Aims
Characterize the immunometabolic profile in primary human macrophages during infectionwith *A. fumigatus*;
Assess the functional impact of the manipulation of metabolic pathways on the effectorfunctions of macrophages;
Define the peripheral and lung metabolome of immunocompromised patients at-risk of IPA.

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Supervisors
Agostinho Carvalho and Cristina Cunha

The role of tryptophan metabolism in the regulation of granuloma formation in sarcoidosis

Summary

Sarcoidosis is a multisystemic disease of unknown etiology with lung involvement in most cases. The pathological hallmark is the formation of granulomas driven by the accumulationof activated macrophages and T-cells, and the production of proinflammatory cytokines. Despite their clinical significance, the molecular sequences of events that promote macrophage aggregation and transformation to initiate and maintain granulomas remain elusive. Indoleamine 2,3-dioxygenase 1 (IDO1) catalyzes the rate-limiting step of tryptophancatabolism along the kynurenine pathway to produce immunoregulatory metabolites. Our preliminary data show that the activity of IDO1 influences the alveolar immune profile of patients with sarcoidosis. In this project, we propose to further explore the functional activityof IDO1 during granuloma formation, resolve the mechanisms whereby IDO1 regulates granuloma formation, and identify and characterize genetic variation in IDO1 associated with susceptibility and the clinical course of sarcoidosis. These approaches will provide novel insights into the mechanisms linking IDO1 and susceptibility to sarcoidosis.

Aims

Evaluate the functional activity of IDO1 during granuloma formation;
Unravel the molecular and cellular mechanisms whereby IDO1 regulates granulomaformation;
Identify and characterize genetic variation in IDO1 associated with susceptibility and theclinical course of sarcoidosis.

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Supervisors

Agostinho Carvalho and Diana Ribeiro

Treatments for cognitive deficits in multiple sclerosis

Summary

Multiple Sclerosis (MS) is an autoimmune disorder of the central nervous system characterized by progressive demyelination, axonal damage and neuronal loss. Cognitive dysfunction is highly prevalent in MS and one of the main determinants of quality of life loss.Unfortunately, its underpinnings are still largely unknown making them poorly amenable to treatment.

Brain networks, created by the interaction of many different brain areas, are highly dynamic,changing at a fast pace, and essential for cognitive processes. As MS mostly targets whitematter fiber tracts, it might have a huge impact in the communication between different brainregions, thus disturbing the interactions between them and, as a result, cognitive function. This project uses advanced connectomics techniques to try to predict the efficacy of differentdrugs to improve cognitive dysfunction in Multiple sclerosis.

Aims

The aim of the project is to predict the efficacy of modafinil and other cognitive enhancer inimproving cognitive dysfunction in patients with multiple sclerosis by analyzing the connectivity patterns of MS patients upon the first challenge with each drug.

Supervisors

João Cerqueira

Dissecting pentraxin-3 role in the modulations of Multiple Sclerosis

Summary

Multiple Sclerosis (MS) is a demyelinating autoimmune disease, characterized by degradation of the myelin sheath and axonal loss. It generally affects young adults and causes disabilities at several levels, such as motor, visual and cognitive. In fact, an area frequently affected is the cerebellum, involved in fine motor skills and coordination. Due to the disabling features of this disease, there is an increasing need for the discovery of novel biomarkers and possible therapy targets. Natalizumab is one of the most effective drugs, since it inhibits leukocyte recruitment into the brain, however, also induces immunosuppression. In this context, pentraxin-3 (PTX3) seems to be a good alternative, playing a similar role to Natalizumab but without its side effects. Therefore, the goal of this work is to study the immunoregulatory role of PTX3 and its potential therapeutic effects in the experimental autoimmune encephalomyelitis (EAE) MS mouse model and through the analysis of human samples.

Aims

1) Compare the disease course and the cerebellar demyelination and inflammatory patternsof PTX3-/- mice and WT animals, induced with EAE;
2) Assess the potential effects of PTX3 exogenous administration in EAE mice, regardingdisease course and demyelination/inflammatory patterns in the cerebellum;
3) Evaluate the correlation between PTX3 genetic variants and PTX3 CSF levels, diseasesubtypes and disease progression in MS patients.

Supervisors

Fernanda Marques

Exploring the role of astrocytes in the pathophysiology of MultipleSclerosis

Summary

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS), in which there is a strong immune response against the myelin sheath of CNS axons. Besidesoligodendrocytes and neurons, which are directly affected in MS, astrocytes also play different roles throughout disease development. However, their involvement in MS pathogenesis is not fully elucidated. We intend to explore the role of astrocytes in MS pathogenesis, and identify astrocytic proteins which could be used as diagnostic or prognostic markers. We will use an MS mice model to isolate astrocytes, which will be usedfor RNAseq analysis to identify differentially expressed genes. Then, we will explore which genes influence neuronal degeneration and regeneration, using the Drosophila melanogaster model, and those found to play an important role will be further modulated ina mice model of disease. Furthermore, we will quantify the levels of proteins of interest in human samples.

Aims

1) Explore astrocytic RNA transcriptome during EAE development;
2) Quantification of candidate markers in the cerebrospinal fluid (CSF) and serum of MSpatients.

References

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Supervisors

Fernanda Marques

The choroid plexus as a shaper of (re)myelination

Summary

The choroid plexus (CP) is an often-overlooked tissue whose most described function is thecerebrospinal fluid (CSF) production. Despite that CSF components have access to the central nervous system (CNS) parenchyma, our understanding of the role of CP-derived molecules in brain cell dynamics is still scarce. Moreover, although it is known that CP is a source of biological active molecules, only recently breakthrough findings show that these molecules are modulating the quiescence and proliferation of neural stem cells (NSC) fromthe ventricular walls (1, 2). These discoveries open new doors and raise more questions. For instance, is CP providing instructing cues for cell type specification and differentiation?Likewise, considering the high rate of CSF renewal and that CP transcriptome and secretome is quickly altered in response to insults and in neurological disorders, can CP- borne molecules dynamically influence the course of diseases such as multiple sclerosis? We are interested in these questions and we hypothesize that CP is acting as a "signaling epicenter" monitoring and influencing CNS cells dynamics both in health and in response toneurological disorders such as multiple sclerosis. Thus, in this project we will tackle this specific question: what is the role of CP-borne molecules in myelination and remyelination?

Aims

The general goal of this project is to investigate the role of CP-borne factors in theoligodendrocyte lineage progression. Specifically, we aim to:

i) Determine the impact of CP secretome in the specification of a NSC into a committedoligodendrocyte precursor, in health;

ii) Determine the impact of CP secretome in the differentiation of an oligodendrocyteprecursor into a myelinating oligodendrocyte, in health;

iii) Objectives i) and ii) in disease.

To achieve these goals, the student will learn how to isolate and dissociate different cell types from the mouse brain and further maintain them in culture. Immunofluorescence techniques will be performed to analyze the cellular identities and behavior of these cells. The data analysis, scientific brainstorming and the report writing will be performed in a remote environment.

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Supervisors

Ana Mendanha Falcao

Exploring T cell differentiation defects underlying multiple sclerosis onset

Summary

Multiple sclerosis (MS) is a highly debilitating autoimmune disease of the central nervous system that affects more than 2.3 million people worldwide, and over 5000 in Portugal. Thisautoimmune disease is mediated to a great extent by autoreactive T cells. The initial courseof MS follows one of two paths: the relapse-remitting (RRMS) and the primary progressive (PPMS), the former representing 85-90% of total MS cases. The natural progression of MSincreases treatment-related costs and impacts highly on patient's wellbeing which highlightsthe urgency to find a cure or treatment to halt disease progression (*1*).

Autologous hematopoietic stem cell transplantation (aHSCT), devoted to the most severe forms of disease, leads to a relevant delay of disease progression and, in 3 out of 10 patients, to suppression of disease manifestation assessed 10 years following aHSCT (*2*). These trials' success suggests that newly differentiated T cells, generated by the patient thymus, do not directly drive MS. Two main exciting hypotheses need to be tested: are thecircumstances that lead to the differentiation and activation of autoreactive T cells transitory(ie. happening before disease onset), and/or rely on a slow and progressive process that takes several years to manifest. Addressing these hypotheses is the main goal of this proposal. To tackle these issues, the project is based on a cohort of newly-diagnosed RRMSand PPMS patients, and healthy controls (HC). Studying MS patients in such an initial phaseof the disease manifestation is essential to identify immunological alterations, specifically atthe T cells' level, closer to the initial events that drive MS.

In contrast to the reported prematurely aged immune system in MS patients (e.g. (*3*)) our results show that newly-diagnosed RRMS patients have lower overall percentages and numbers of memory CD4+ and CD8+ T cells, suggestive of general T cells alterations at disease onset (Annex1). To identify these modifications, we will first undertake an exploratory approach by evaluating the transcriptional profile of T cell subsets, at the single cell level. We will first focus on the most immature RTE, to the naïve and memory T cells, though we expect other T cell subsets to arise based on the transcriptome analysis. As bloodT cells' phenotype tips towards a less activated phenotype in newly-diagnosed MS patients,we will perform functional assays that will allow us to understand if, in these patients,: i) naïve T cells have impaired homeostatic proliferation [upon stimulation of CD31+ naïve T cells (RTE) with IL7]; ii) the T cell receptor activation threshold is altered thus impairing differentiation of naïve T cells into the memory phenotype; iii) memory T cells are less proliferative and/or more prone to die upon stimulation with specific (myelin peptides) or unspecific stimuli; iv) overall memory T cells are more prone to migrate to tissues. All theseanalyses will be performed in newly-diagnosed RRMS patients, and HC.

Aims

We aim to understand why the peripheral immune system from MS patients displays a lessmature phenotype in comparison to HC. The less mature phenotype could be related to: i) thymic function; ii) intrinsic features of MS patients' T cells; iii) impairment on naïve T cells to differentiate into memory T cells; iv) greater predisposition of memory T cells to die; v)enhanced homing capacity of memory T cells to home to tissues; and/or, vi) cyto/chemokineenvironment more prone to direct memory T cells to tissues.

Skills to be acquired:

- handle human PBMCs and work under aseptic conditions
- perform flow cytometry and data analysis
- Perform statistical analyses, literature search and report writing

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Supervisors

João Canto-Gomes

The role of the choroid plexus in aging and Alzheimer's disease

Summary

The brain is an unusual tissue since it is protected from the free exchange of substances that are travelling in the bloodstream. This is attained via the presence of blood-brain barriers. Three main barriers exist: the blood-brain barrier (BBB), the blood-cerebrospinal fluid (CSF) barrier (BCSFB) and the blood-meningeal barrier (BMB). All these barriers contribute to the brain homeostasis. We propose to study the BCSFB, which is located in the brain ventricles and that is constituted by the choroid plexus (CP) epithelial cells. In addition to be part of the BCSFB, the CP has an additional function that is the production ofCSF, the liquid that surrounds the entire central nervous system (CNS) – brain and spinal cord. The CSF exchanges substances with the brain interstitial fluid, participating in the delivery of nutrients and removal of waste products produced by the brain. Thus, the BCSFBis not only a barrier to the brain but also a clearance route. Importantly, dysfunction of brainbarriers has been observed during aging and numerous neurological conditions such as Alzheimer's disease. Despite these observations it is still unknown what exactly happens when the BCSFB is disrupted and how CSF production impacts other barrier/clearance systems. The aim of this proposal is to study the BCSFB at single cell level in order to understand how CP cells change during aging or Alzheimer's disease, and how those changes impact their functional role of producing and delivering CSF to the brain.

Aims

The general goal of this project is to investigate the role of CP in normal aging and in Alzheimer's disease using the J20 animal model. For that we will have samples from CP ofwild-type and J20 animals with 3, 12 and 20 months of age that will be used for single cell RNA sequencing.

References

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Supervisors

Fernanda Marques

Inflammation, Cell Signaling and Therapeutics (InflammaSignal)

Implications of the Autophagy Core Gene Variations in Acute Myeloid Leukemia

Summary

Acute myeloid leukemia (AML) comprises a heterogeneous group of neoplasms characterized by an impaired differentiation and/or proliferation of immature progenitor's cells (Dohner H et al., 2015). AML has still an aggressive clinical course and limited chemotherapy options (Dohner H et al., 2015). Considering the essential role of autophagyin AML cells response to chemotherapy, we hypothesized that genetic variants in autophagycore genes might contribute to outcomes of AML (Fernandes Â et al., 2015; Pereira O et al.,2018). Recently we have also uncovered the potential of screening for ATG10 genetic variants in AML prevention strategies, in particular for subjects carrying other AML risk factors such as elderly individuals with clonal hematopoiesis of indeterminate potential (Castro, I et al., 2021). Therefore, in this laboratory rotation, we will examine genetic polymorphisms in other autophagy core genes among 280 samples of a Spanish cohort of AML patients. The polymorphisms to study are: ATG5 rs688810, ATG5 rs510432, ATG7 rs8154, ATG12 rs26538, ATG16L1 rs2241880 and ATG16L2 rs11235604. Pluripotency- associated (PA) proteins such as OCT4 and SOX2 are transcriptional factors crucial to maintain pluripotency in cancer stem cells. It was also shown that autophagy acts together with the ubiquitin-proteasome system (UPS) to modulate the levels of PA proteins in humanembryonic stem cells (Cho YH et al., 2014). Therefore, we will determine whether autophagycore gene variations will impact on the levels of these PA proteins providing a new insight in the regulation of pluripotency in AML.

Aims

In the present project, we aim to investigate the role of autophagy core gene variations in acute myeloid leukemia (AML) pathophysiology. For that, the following specific objectives are proposed:

1) To examine the presence of functional genetic polymorphisms in 8 autophagy core genesamong 280 samples of AML patients;

2) To evaluate the impact of the functional genetic polymorphisms associated with AML onautophagy flux and protein levels of OCT4 and SOX2.

References

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Supervisors

Paula Ludovico and Belém Sampaio-Marques

Role of extracellular vesicles on the remodeling of bone marrow niche withclonal hematopoiesis of indeterminate potential (CHIP)

Summary

In recent years, the average human lifespan increased progressively, accompanied by the inevitably rising of age-associated diseases. Special attention is being paid to an age-relatedcondition known as Clonal Hematopoiesis of Indeterminate Potential (CHIP) which isconsidered a high-risk factor for developing Acute Myeloid Leukemia (AML) and cardiovascular diseases (CVDs). CHIP is characterized by the expansion of hematopoietic stem cells (HSCs) clones harboring specific somatic mutations. But why does sometimes CHIP progress to AML or CVD and others never express a symptom? Extracellular vesicles(EVs) might have a key role in this context considering their ability of both short- and long- distance cell communication. Given the instrumental role played by autophagy in HSCs self-renewal and differentiation, aging conditions, and leukemia, along with its impact in EVs secretion, we hypothesize that alterations in EVs and autophagy profile may determine thefate of CHIP-HSCs and be used as biomarkers to predict CHIP conditions.

Aims

- To characterize the profile of CHIP-related EVs released from HSCs;
- To investigate the impact of the metabolic, autophagic, and immunological profile ofCHIP-HSCs on EVs' cargo;
- To uncover the contribution of autophagy, particularly of the ATG5-ATG12-ATG16L1complex, on EVs generation.

References

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Supervisors

Paula Ludovico and Belém Sampaio-Marques

New insights on the role of autophagy in extracellular vesicle biogenesisand secretion during cellular aging

Summary

As the demographics of the modern world age, understanding and mitigating the effects of aging is of extreme importance within biomedical research. Recent studies in modelorganisms demonstrated that aging is frequently modified by the organism's ability to perceive and respond to changes in its environment. A large body of evidence supports thenotion that cellular intercommunication, particularly horizontal transmission mediated by extracellular vesicles (EVs), might have a deep impact on aging. It is described that EVs trafficking can enroll conventional, but also unconventional secretory pathways, like secretory autophagy. Nevertheless, the sorting for secretion, as well as the pathways and players associated with EVs secretion, biogenesis and uptake by receptor cells are still poorly understood. Given the advantages of the yeast Saccharomyces cerevisiae as a cellular aging model, we will explore the role of intercellular communication by EVs in agingmodulation, along with the mechanism underlaying EVs trafficking.

Aims

- To uncover EVs as a horizontal transmission process regulating chronological agingin *S. cerevisiae* cells;
- To use the yeast model for synucleinopathies for the study of horizontaltransmission;
- To disclose the involvement of the secretory autophagy in the biogenesis andsecretion of EVs.

References

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Supervisors

Belém Sampaio-Marques and Paula Ludovico

Sweeteners and their impact on the chronological aging of yeast

Summary

Sweeteners have become an elective choice to substitute sugar, particularly for those who may struggle with obesity or cannot tolerate sugar in their diets, such as diabetics. Currently,FDA have approved the consumption of eight natural and artificial sources sweeteners. These include steviol glycosides extracted from *Stevia rebaudiana* leaves as natural sweeteners and aspartame, sodium cyclamate, sucralose, and acesulfame potassium as chemically synthesized sweeteners (artificial sweeteners). Although in the last years there has been an increasing consumption of these sweeteners, their effects remain controversialand not completely understood (1,2). Yeast is a eukaryotic microorganism commonly used as a model in molecular biology research, biomedicine and related fields. Nevertheless, theimpact of the most common sweeteners on yeast chronological lifespan has never been exploited.

Aims

The global aim of our study is to evaluate the effects promoted by sweeteners on yeast chronological aging. To tackle this challenge, the following specific aims were defined:

- To determine how sweeteners (aspartame, sodium cyclamate, sucralose, and acesulfame potassium) supplementation impacts on yeast CLS;
- To evaluate the effects on cellular proliferation of acute exposure to sweeteners (aspartame, sodium cyclamate, sucralose, and acesulfame potassium) supplementation;
- to elucidate the acute and chronic effects of the sweeteners (aspartame, sodium cyclamate, sucralose, and acesulfame potassium) supplementation on cell cycle, reactive oxygen species accumulation and autophagy.

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Supervisors

Belém Sampaio-Marques and Paula Ludovico

Study of the impact of cell signaling on the regulation of premature agingpromoted by proteotoxic stress

Summary

Aging is an intricate physiological process that results in the progressive accumulation of molecular alterations and disruption of different cellular functions. Genome instability,deregulated nutrient sensing, mitochondria dysfunction and loss of proteostasis are some of the proposed aging hallmarks (1). To counteract these detrimental changes, cellular repairmechanisms and compensatory responses, known as cell autonomous mechanisms, are activated. Nevertheless, it is known that these autonomous mechanisms are not sufficient to explain all the aging associated alterations. A body of evidence supports the notion that emergent cell non-autonomous mechanisms involved in the intercellular communication through the transmission of various longevity factors, have a deep impact on aging (2). Thenetwork allowing to send and receive these messages is complex. It consists of an army ofmessenger molecules to spread the signal, which could circulate freely or as extracellular vesicles (EVs) cargo (3). However, the nature of the transmissible molecules is still unclear,as well as how the communication network is regulated. Studies using the yeast *Saccharomyces cerevisiae* as a model have resulted in pivotal findings regarding the cell autonomous mechanisms regulating aging (4, 5).

Aims

The global aim of the present project is to obtain new insights the role of the cell-cell communication under conditions of premature aging promoted by the heterologous expression of α-synuclein, a natural misfolded protein that promotes loss of proteostasis and premature aging.

References

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Supervisors

Belém Sampaio-Marques and Paula Ludovico

Using AI to build a virtual patient assessment item

Summary

Objective Structured Clinical Examinations are the hallmark of modern clinical assessmentin medical schools. While effective and reliable the costs associated limit its widespread availability. Moreover the complexity of its setup make its use by medical students limited. OSCE are based on carefully designed scripts and training of standardize patients. These scripts can potentially be used for creating standardized texts using ChatBots that serve asthe basis of virtual patients speeches that could be used for formative or summative assessment

Aims

In order to achieve the main aim, we are employing a multitude of AI base techniques that combine virtualization of image and speech and multiple iterations of text based onChatGPT.

References

Combine Chat GPT-3, DALL-E 2 & Synthesia To Create AI News Videos | by Techletters | Medium

Supervisors

José Miguel Pêgo

Behavioral and brain histological characterization of phospholipase Dknock-out mice

Summary

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

In mammals there are two main PLD isozymes: PLD1 and PLD2. They both catalyze 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 fourpublished 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 proposeto study the impact of both PLD1 and PLD2 ablation in mice cognitive-associated behavior and brain hippocampal organization.

Aims

1) To characterize the impact of PLD1 and PLD2 ablation in mice behavior;
2) To characterize the impact of PLD1 and PLD2 ablation in mice brain histologicalorganization.

References

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Supervisors

Tiago Gil Oliveira

The role of lipid signaling in Alzheimer's disease

Summary

Lipids are major constituents of the brain, and the modulation of its levels impacts brain functioning and dysfunction. One of the major diseases that affects the brain is Alzheimer'sdisease (AD). Importantly, lipid signaling associated genes are the major risk factors for ADand accordingly we have previously shown that the brains of AD patients and mouse modelspresent major lipidomic changes implicating membrane trafficking defects at the level of endosomal, lysosomal and autophagy dysfunction.

In this project we will focus on experimental approaches using cellular and mouse models of AD and test the impact of lipid signaling modulation in AD-dependent phenotypes.

Students will have the chance to learn a multitude of techniques, such as protein and lipid biochemistry methods - linked to lipidomic alterations we previously observed; cytological and histological imaging approaches with confocal microscopy - focusing on neuronal organelle mistrafficking; and mouse behaviors in the context of learning and memory deficitspresent in AD.

Aims

1) To test the impact of phospholipid modulation in AD mice behavior and associatedhippocampal biochemical and histological signatures;

2) To test the impact of phospholipid modulation in membrane trafficking deficits linked toAD.

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Supervisors

Tiago Gil Oliveira

Brain Magnetic Resonance Imaging in Alzheimer's disease

Summary

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder. AD is clinicallycharacterized by slowly progressive neurocognitive impairment predominantly characterizedby short-term memory loss (typical senile AD) and with disease progression, multiple neurocognitive domains can be involved, such as language impairment or mood disbalance.At the level of neuropathology, AD diagnosis consists of grading the "ABC score", which is achieved by grading amyloid plaques (A) by Thal phases, NFT by Braak staging (B) and the neuritic plaque score by CERAD assessment (C). While neuropathological diagnosis is performed post-mortem, strategies to diagnose AD and other neurodegenerative disorderswhile the patients are alive, can make all the difference for clinical trial planning. One of these strategies is using magnetic resonance imaging (MRI) to identify regions of the brainthat are most susceptible to neurodegeneration.

In collaboration with various institutions in the US, we have gathered cohorts with confirmedneuropathology diagnosis and pre-mortem MRI, ranging from AD, to a multitude of tauopathies and to trauma associated with concussion. In this project we propose to identifysignatures of disease susceptibility and resistance by using this multidimensional information. The final goals are to improve diagnosis, assess prognosis and select the right patients for the right clinical trials. Additional strategies will benefit from artificial intelligencemethodologies that we have been implementing in order to deal with the diversity of the datasets we have processing.

Aims

1) To identify MRI signatures of susceptibility and resistance in AD;
2) To identify predictors of conversion into AD.

References

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Supervisors

Tiago Gil Oliveira

Factors of susceptibility/resistance to chronic pain development

Summary

It is presently unknown why upon similar subacute painful episodes some individuals evolveto chronic pain conditions while others do not. While anxiety and depression are the most studied predictors of this evolution in humans, its neurobiological underpins remain poorly explored. A small number of neuroimaging studies demonstrate that the mesocorticolimbic network plays a critical role in pain trajectories. In addition, studies from the group show thatthere is a neuroimmune involvement in the process and that the resistance to pain can be experimentally promoted. Such is clinically relevant as the pain triggering event is in many cases well defined (e.g. surgeries, pharmacological treatments, etc.).

In this project we will characterize a subpopulation of rats that do not manifest pain-related behaviors after the installation of peripheral neuropathy. The core of the analysis will focus on the period prior to pain onset and will consist of behavioral, molecular and neurophysiological studies.

Aims

1) identify behavioral phenotypes that predict favorable pain trajectories;
2) obtain molecular endophenotypes of pain resistance;
3) understand at circuit level the neurophysiological underpins of pain resistance;
4) modulate pain resistance.

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Supervisor

Hugo Leite-Almeida

Social behavior, empathy and chronic pain – the role of the oxytocinergicsystem

Summary

Chronic pain is a debilitating disorder that is frequently accompanied by a multitude of behavioral manifestations including anxiety, depression and cognitive deficits. Sociability has been in this context poorly explored but both clinical and experimental evidence indicate reduced drive for social interplay in chronic pain conditions. Interesting, there is however evidence that social interaction has a positive impact in pain, particularly in experimental acute nociception, suggesting a bidirectional interplay. The oxytocinergic system has a well-known role in affiliative and sexual behaviors and has been therefore explored by our groupin this context. We observed in a neuropathic pain model (Spared Nerve Injury; SNI) that pain was associated with decreased circulating levels of oxytocin. More interestingly, in controls but not in SNI, oxytocin levels were associated with a preference toward animals inpain suggesting a manifestation of empathy-like behavior. Continued analgesic treatment partially improved pain-related behavior and fully recovered sociability. A singleadministration of systemic oxytocin transiently reduced pain and increased sociability.

In this project, we aim to decipher and manipulate the circuits underpinning pain and socialbehavior using targeted pharmacogenetic approaches. Also, we will study how pain-relatedinformation is conveyed across individuals (ultrasonic vocalizations, USVs) and how such influences empathetic-like manifestations.

Aims

1) use viral vectors to manipulate the activity of oxytocinergic neurons (pharmacogenetics)and
2) study its impact on pain and sociability;
3) register and playback USVs to study its impact on animals' behavior;
4) study the impact of pain in mesocorticolimbic networks activity during the socialencounters.

References

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Supervisor

Hugo Leite-Almeida

Screening of new drugs for the management of chronic pain disorders

Summary

Chronic pain and comorbid emotional disorders are enormous health and financial burdensat the individual and societal levels. 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] as well as activation of glial cells that furtherpotentiate this pathological condition [2]. Unfortunately, most chronic pain management therapies are inefficient or tend to become ineffective in the long term and often present sideeffects. Thus, due to the need for discovering/creating safer alternatives to current chronic pain therapies, we propose to screen a series of natural extracts displaying an anti- inflammatory and antioxidant profile.

In this work, chronic pain will be induced in Wistar han adult rats for a period of 4 weeks followed by the administration the extracts for an additional 3 weeks. At the end of this period, behavioral analysis to evaluate anxiety- (open field and elevated plus maze), depressive-like (forced swimming test and sucrose preference test) behavior as well as nociception [pain behavior - 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 occised, brains removed and processed for the evaluation of changes in neurotransmitters content. Additionally, samples from all major internal organs will be sampled to determine the extract's potential toxicity.

Aims

1) To evaluate the ability of different natural extracts to reverse chronic pain-inducednociceptive and emotional impairments;
2) To correlate behavioral data with changes in the neurochemistry of brain areas involvedin pain modulation;
3) To correlate extract efficacy and changes in the number of neurons and glial cells in painmodulatory areas.

References

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Supervisors

Diana Rodrigues, Inês Laranjeira and Filipa Pinto-Ribeiro

Assessing decision-making in rodents: task development and validation

Summary

Decision-making is a part of every-day life, varying from the simplest "what to wear today" to life-changing ones as whether to move to a different country or to choose this rotation. Itis a process that includes many dimensions, including attention to cues that may signal thenecessity for behavioral inhibition, outcome monitoring, updating of goals/beliefs and shiftingof strategies. A failure in this machinery may introduce errors in the decision-making process, which has been identified in pathologies such as drug addiction or obsessive- compulsive disorder.

In this project, we are mainly interested in two dimensions, which our group has shown to be altered in chronic pain conditions: cognitive flexibility and impulsivity. Cognitive flexibility is commonly assessed using the Attentional Set-Shifting Task (ASST), in which the animal learns that cue A, but not cue B, is associated with a reward. After establishment of this behavior, the reward becomes associated with cue B, and the animal's ability to shift strategies is assessed. While this task is well established, it is currently performed manuallyby the researcher, which implies high time consumption and larger probability of errors. Impulsivity assessment, on the other hand, is currently fully automatized in the Variable Delay-to-Signal (VDS) task. Here, the animal learns that nosepoking in a lighted hole delivers a reward, while nosepoking prematurely in the 3 s delay before the light turns on, ispunished with a time out in complete darkness. At the end of training, the number of premature responses is associated with action impulsivity, while in the test session, in whichdelays are variable, delay intolerance impulsivity is assessed. However, this task is currentlyperformed using commercial hardware and software, which limits adaptations of the task. Thus, the main goal of this project is to automate these two tasks using Arduino hardware and Bonsai software, and to validate them for usage in rats.

Aims

1) Create an automated version of VDS and ASST using Arduino and Bonsai;
2) Validate these automated tasks in rodents.

References

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Supervisors

Hugo Leite-Almeida and Madalena Esteves

Pathogen Evolutionary Genomics and Experimental Genetics (PEvoGen)

Phylogenetic analysis of HIV-1 genomic sequences

Summary

Globally, more than 30 million people are estimated to be infected with HIV-1. Phylogenetically, HIV-1 is divided into four genotype groups: M, O, N, and P. The vast majority of HIV-1 infections in the world are caused by M group viruses that can be further divided into at least nine genetic subtypes, A to D, F to H, J, and K, as well as different circulating and unique recombinant forms (CRFs and URFs, respectively). The geographic patterns of the M group subtypes are dynamic and can change in response to human population migrations and active transmission networks. Presently, HIV-1 B subtype is the most prevalent subtype in Western Europe, however in a part of the Iberian Peninsula (including the provinces of Minho, Portugal and Galicia, Spain) there is a higher HIV-1 subtype diversity. The in-detail study of this region is of great importance since it may be considered a "hotspot" of viral recombination and might also constitute a starting point for the geographic expansion in Western Europe of non-B HIV-1 genotypes. The introduction of non-B HIV-1 subtype in the Iberian Peninsula is likely a consequence of migratory movements with African and South American countries. However, it is unclear if the transmission of non-B subtypes is becoming established among local individuals and if it has the potential to influence the geographic pattern of HIV-1 subtype diversity in Western Europe. In order to address these questions, this project focus on the monitoring of the phylodynamics of HIV-1 viruses in the high subtype diversity region of the Iberian Peninsulaand its comparison to HIV-1 viral sequences isolated from other locations. Although currentantiretroviral regimens appear to have comparable efficacies across all HIV-1 subtypes, there is evidence suggesting that particular HIV-1 subtypes have a transmission advantage,higher replicative efficiency or a higher predisposition to drug resistance reinforcing the needfor continuous monitoring of HIV-1 subtype diversity.

Aims

1) Perform molecular epidemiologic characterization of HIV-1 sequences;
2) Identify viral transmission networks and possible relationships with other globallydescribed transmission clusters.

References

Carvalho A, Costa P, Triunfante V, Branca F, Rodrigues F, Santos CL, Correia-Neves M, Saraiva M, Lecour H, Castro AG, Pedrosa J, Osório NS. Analysis of a local HIV-1 epidemic in portugal highlights established transmission of non-B and non-G subtypes. J Clin Microbiol. 2015 May;53(5):1506-14. doi:10.1128/JCM.03611-14.

Supervisors

Nuno S. Osório

Defining drug resistance pathways in the malaria parasite

Summary

Artemisinin-based combination therapies (ACTs) have been highly effective over the past decade in treating Plasmodium falciparum infections and reducing the global burden of malaria, a disease that in 2015 alone caused near half million deaths. However, resistanceto the core artemisinin component has emerged. Since there are no new drugs commerciallyavailable to replace ACTs should they fail globally, there is a compelling need to define themolecular basis of P. falciparum multidrug resistance.
Efficiency of ACT drugs can be modulated by mutations in genes involved in different biological process including the ones involved on drug extrusion or in the detoxification pathways or even being the gene coding for the biological drug target. Molecular epidemiology studies, genome wide association studies supported by in vitro studies have linked mutations with altered parasite susceptibility to multiple ACTs. However, its role in resistance remained constrained by complexities intrinsic to field–based studies, including contributions of polyclonal infections and genomic variability.

Aims

To overcome these issues, leveraging recent advances in *P. falciparum* genome editing likethe zinc-finger nucleases (ZFNs) and the CRISPR/Cas9 system, we aim at validating the contribution of these mutations in drug resistance. Moreover, we will investigate the complexcrosstalk among resistant factors and tackle the molecular basis of multidrug resistance byunveiling how known resistance determinants impair the potency of ACTs. This knowledgewill provide necessary data to reformulate the already available drugs, find drugs with potential of reverting multidrug resistance phenotype and possibly unveil new targets. As ashort-term impact, data from this work will reveal the molecular weakness that compromisesthe efficacy of ACTs, the nowadays core antimalarial drug, vital for policy makers' decisions.

Specifically, this rotation will include:
- Molecular cloning: plasmid design in silico and wet lab
- Transfection based assays
- *P. falciparum* in vitro culture; Drug susceptibility assays

The project has an estimation of 60-70% activities at the ICVS facilities with the remainingtime for data analysis, bioinformatics, literature review and writing.

References

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Supervisors

Maria Isabel Veiga and Vitoria Baptista

Study of opto-acoustic properties of the malaria parasite for thedevelopment of non-invasive diagnostic device

Summary

An accurate malaria diagnosis is critical for the disease control and elimination. The latest 2018 malaria report from World Health Organization reveals that insufficient levels of accessand uptake of lifesaving malaria tools hinder early diagnosis and treatment on the patients,especially at the village level. This further exacerbates the transmissions of Malaria, a disease that puts today 1/2 world population at risk and half million deaths estimated per year, with around 80% occurring in children < 5 years old. The presence of malaria parasitesin human blood leads to biochemical and morphological changes in Red Blood Cells (RBC).One of those is related with the degradation of Hemoglobin (Hb) for parasites metabolism, producing Hemozoin (Hz). The Hb and Hz molar extinction coefficients differ significantly, especially at certain optical wavelengths, leading to different absorbance and reflectance spectra between infected and normal RBC. Additionally, as the infection proliferates, RBC become stickier and the flow velocity decreases, which can obstruct the microcirculation in blood vessels and capillaries. Taking advantage of these particular features in infected blood (RBC deformability and Hz formation), the main innovation herein to be studied in this project is the on-chip integration of acoustic measurement of RBC flow velocity and optical detection of parasitic Hz by reflectance spectrophotometry. Sensors, actuators, optical components and microelectronics will be integrated on the same chip, and a decision algorithm based onthe optical and acoustical signals will be implemented.

Development of such microdevice will meet the growing clinical demands for reliable, rapidand quantitative diagnosis suitable for field use at the patient's side. Because is a non- invasive device, besides being patient's favored (not painful), it will meet ecologic matters with no disposable parts and consequently no added costs.

Aims

The project aims to study the opto-acoustic properties of the malaria parasite for the development of an innovative, rapid and non-invasive device for malaria testing, based on the integration of optical and acoustic sensors for detecting, in few seconds, the presence of parasites directly in patient's skin.

The project has an estimation of 60-70% activities at the ICVS facilities with the remaining time for data analysis, bioinformatics, literature review and writing.

References

Catarino SO, Felix P, Sousa PJ, Pinto V, **Veiga MI**, Minas G. Portable Device for Optical Quantification of Hemozoin in Diluted Blood Samples. IEEE Trans Biomed Eng. 2020 Feb;67(2):365-371. doi: 10.1109/TBME.2019.2913454.

Supervisors

Maria Isabel Veiga and Susana Catarino (DEI- Department of Industrial Electronics, University of Minho, https://www.dei.uminho.pt/recursos-humanos/susana-catarino/)

The role of IL10 on cognitive performance

Summary

The crosstalk between the immune and the nervous systems is known to be essential for the maintenance of the body homeostasis in general and, in particular, for the correct functioning of the central nervous system. In fact, disruption in the brain/immune communication is associated with mood and cognitive alterations (Kipnis 2016; Miller and Raison 2016). Previous studies from our lab revealed that mice lacking the expression of the anti-inflammatory interleukin 10 (IL10KO mice) present depressive-like behavior, a phenotype that is rescued through IL10 administration (Mesquita *et al.* 2008). Recently we observed that the absence of IL10 is associated with cognitive impairment in female mice. Moreover, preliminary results also suggest that female IL10KO mice also present alterationson the immune cells profile. Accordingly, in humans $CD4^+$ effector memory T cells have been shown to be a significant predictor of cognitive performance (Serre-Miranda *et al.* 2015). Currently, we are unraveling the mechanisms that could be associated with the alterations observed in the cognitive performance of IL10 KO female mice.

Aims

1) Further evaluate the immune profile of IL10KO mice with cognitive impairment;
2) Explore the cytokine milieu of the brain meninges and CSF;
3) Analyze the neuronal plasticity and hippocampal cell proliferation.

References

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Supervisors

Susana Roque

Lipoarabinomannan as an important molecule for tuberculosis diagnosis

Summary

Tuberculosis (TB) is the world's deadliest infectious disease according to recent reports fromWHO. One third of the world's population is estimated to be infected with bacteria of the *Mycobacterium tuberculosis* (Mtb) complex and TB accounts for 1,5 million deaths annually,and one-fifth of adult' deaths in poor/low-income countries. WHO reports that, if leftuntreated, each person with active TB infects between 10 to 15 new individuals annually. Therefore, interrupting disease transmission is of major importance and requires early and accurate detection/diagnosis, paired with appropriate treatment. One of the most importantlimitations in what concerns interrupting tuberculosis transmission is the early and accuratediagnosis to tuberculosis, which is still a very difficult task. Lipoarabinomannan (LAM) is structurally unique glycolipid component of the outer cell wall of all mycobacterial species. Itis the main carbohydrate antigen and accounts for up to 15% of the bacterial weight. Severalreports have suggested that LAM has the potential to be used as a biomarker for tuberculosis.

Aims

Investigate how LAM might be used to improve tuberculosis diagnosis. Cell culture, Flow Cytometry and other techniques are being used in this project.

References

Källenius G, Correia-Neves M, Buteme H, Hamasur B, Svenson SB. Lipoarabinomannan, and its related glycolipids, induce divergent and opposing immune responses to Mycobacterium tuberculosis depending on structural diversity and experimental variations. Tuberculosis (Edinb). 2016;96:120-30

Supervisors

Margarida Correia-Neves

Diabetes and Infection – immunological determinants underlying the impact of glycemic dysregulation on the susceptibility of patients with diabetesto infection

Summary

Diabetes Mellitus (DM) is considered by the United Nations one of 4 prioritynoncommunicable diseases. Still on the rise, it is predicted by the World Health Organization(WHO) to affect 629 million people by 2045. In Portugal, it is estimated that 9,9% of the population have DM and 5% of the total deaths are attributable to DM. DM represents a great socioeconomic burden, not only because patients require expensive medical care, butalso because it is associated with premature death and disabling morbidities, leading to reduced individual's quality of life and working years.

In a very general way, DM can be categorized as type 1 (T1DM), type 2 (T2DM), and others.This project focuses on T2DM which is the most common type, affecting around 90% of all individuals with DM. T2DM is characterized by insulin resistance and several levels of b-celldysfunction, and is generally associated with overweight and obesity. Among other health complications, patients with T2DM present higher susceptibility and poor outcome toinfectious diseases, such as urinary tract, skin and soft tissue infections, pneumonia (the most recently demonstrated being pneumonia caused by SARS-CoV-2 infection) andtuberculosis. The process by which T2DM leads to increased susceptibility to infection is certainly multifactorial and far from being completely understood. It is known that increasedsusceptibility to infection is directly associated with uncontrolled glycemia, but the underlyingmechanism is still not known.

Several studies accessed immune mechanisms of susceptibility to infection in T2DM, suchas neutrophil or monocyte impairment, or decreased production of pro-inflammatory cytokines. Still, most studies use the mouse model or in vitro cultures of either cells from healthy individuals or from patients with T2DM. The few studies comparing DM and non-DMindividuals looked at a specific cell population or molecule and did not take into account different levels of glycemia.

Aims

This project aims to study the correlation between the blood immune characterization with infection events in patients with T2DM, taking into account the glycemia status (e.g. fastingglucose levels, Hb1Ac, glucose tolerance). This study will take advantage of techniques such as flow cytometry of blood cells, RNA expression analysis, multiplex analysis of cytokine/chemokine and other markers, and big data statistical analysis.

Supervisors

Palmira Barreira-Silva and Margarida Correia-Neves

Novel Approaches in Nervous System Regenerative Medicine

Exploring the Therapeutic Potential of the Secretome of MesenchymalStem Cells in Central Nervous System Regenerative Medicine

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 current knowledge of the MSCs therapeutic potential is extremely relevant to establish the best culture parameters of MSC populations because little is knownabout 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 andpossibly 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 horizontal stirred bioreactors is able to induce higher differentiation rates in populations of human neural stem cells (3). Moreover when applied in both Spinal Cord Injury and Parkinson's Disease animal modesl it was possible to observe both functional and histological improvements. Following these initial results we set to improve the conditions in which the secretome of MSCs can be obtained within bioreactors. Having this in mind the stirred based system were replaced byvertical oriented ones, due to the beneficial outcomes obtained with the latter.

Aims

Study the impact of the secretome of MSCs cultured in vertical wheel oriented bioreactors in: 1) Differentiation of neural progenitors and 2) functional outcomes of a C.Elegans modelof PD based on the aggregation of alpha-synuclein; 3) functional outcomes in a SCI mice model; 4) Neuronal cell survival and differentiation in the affected areas.

Skills (on site): Cell and Tissue Culture, Behavioral Analysis, Immunohistochemistry, Histology, Fluorescence/Confocal Microscopy.

Skills (remote environment): Neurostructural Analysis, Behaviou Analysis, Literature Search, Cell Counts, writing, literature search

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Supervisors

António Salgado and Ana Marote

Pharmacotherapies and Extracellular Matrix like Hydrogels as tools forSpinal Cord Injury Regeneration: a combinatory 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 tothe 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 thosepresented 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 onthe interface between materials science, biology and medical sciences, and aims at developing tissue hybrids that induce tissue regeneration. By following these concepts, we have previously developed extracellular matrix like hydrogels I with enhanced cell adhesionand proliferation properties (mimicking the natural extracellular matrix) [1-3]. The role of thehydrogel 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 stepcan 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 glialscar formation. Such processes can be achieved by using drugs that target them (4,5). 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 ofaxonal degeneration/regeneration.

Aims

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

Skills (on site): cell and explant culture; biomaterials modification; immunohistrochemistry; n fluorescence/confocal microscopy; in vivo models of SCI;

Skills (remote environment): Neurostructural Analysis, Behavioral Analysis, LiteratureSearch, Cell Counts, writing, literature search

References

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Supervisors

António Salgado and Nuno Silva

Inducing vascularization in SCI sites through the use of gellan gum basedhydrogels

Summary

Spinal cord injury (SCI) represents an extremely debilitating condition for which no efficacious treatment is available. One of the main contributors to the inhospitable environment found in SCI is the vascular disruption that happens at the moment of injury that compromises the blood-spinal cord barrier (BSCB) and triggers a cascade of events that includes infiltration of inflammatory cells, ischemia and intraparenchymal hemorrhage. Due to the unsatisfactory nature of revascularization following SCI, restoring vascular perfusion and the BSCB seems an interesting way of modulating the lesion environment intoa regenerative phenotype, with a potential increase in functional recovery. Having this in mind our lab is seeking strategies to pre-vascularize 3D hydrogels for SCI applications, whilesimultaneously combine them with populations related to this process particularly MSCs andendothelial cells. The aim of this rotation will be focused on testing the impact of such modification on the behavior of ECs and MSCs

Aims

Test a number of different peptides, grafted into hydrogel matrix based on gellan gum, a naturally occurring hydrogel, and assess their effects on the angiogenic potential of ECs andMSCs

Skills (on site): cell and explant culture; biomaterials modification; immunohistrochemistry;neurostructural analysis; fluorescence/confocal microscopy;

Skills (remote environment): Behavioral Analysis, Literature Search, Cell Counts, writing,literature search

References

- Rocha L et al., Frontiers in Pharmacology, 2018, 9: 164
- Gomes ED et al., Biomaterials, 2016, 105:38-51
- Oliveira E et al., Stem Cells International, 2017, 2017: 6319129
- Assunção-Silva RC, Biomedical Materials, 2015, 2015:948040

Supervisors

António Salgado

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 thephysiological, 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 reactionsthat 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 havea 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 authorsalready 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 waspreviously shown that the prevention of cAMP hydrolysis stimulates axonal regeneration andmotor 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

1) Determination of the *in vitro* bioactivity of the selected drugs when combined into a singlepharmacotherapy approach;
2) Test new therapies in a rat contusion model of SCI.

Supervisors

Nuno Silva

Immunomodulatory strategies for spinal cord injury

Summary

Spinal Cord Injury (SCI) triggers a strong inflammatory response locally, activating both endogenous (microglia) and infiltrating immune cells that progressively engage neurotoxic phenotypes. As such, is not surprising that inflammation has become a target for therapeuticapproaches aiming at protecting the spinal cord from further damage. Acute inflammation, however, is part of the immune response to injury and is by definition a protective responsetriggered by injured tissue from trauma, infection or any other harmful cause. But for the specific case of Spinal Cord Injury (as well as for other CNS injuries) the inflammation- associated infiltration of immune cells has mostly been associated with fibrosis, oxidative damage and further neurodegeneration contributing for the regeneration failure.

As more as we understand the functional role of the inflammatory response, the more evident becomes that this response can support both beneficial and detrimental effects to recovery This observation has shifted the research focus from developing anti-inflammatoryto immunomodulatory therapies aiming at boosting immune cells displaying repairing/ regenerative while abrogating pro-inflammatory cells with strong oxidative response.

In this project we will at first investigate the mechanisms underlying the disruption of balanced immune response to injury that happens after SCI. For this we will focus on understanding how a thoracic (T8) SCI impacts the plasticity of neurons innervating lymphoid organs and on how it consequently influences the immune response. Then, we willmodulate the immune response to SCI by stimulating or inhibiting the neuronal circuit controlling the splenic immune response, using chemogenetics tools (DREADDs (designerreceptors exclusively activated by designer drugs)).

The second aim of this project is to test different immunomodulatory therapies both targetinglocal inflammation (microglia) and systemic inflammation (neutrophils, monocytes, T and Bcells).

Aims

1) To understand how SCI impacts the neural control of the immune response by thoroughlycharacterize the spinal-splenic circuit in a mouse model of compression SCI;

2) To evaluate the therapeutic value of different immunomodulatory strategies either targeting local inflammatory response (microglia) and systemic inflammatory response (neutrophils, monocytes/macrophages, T and B cells).

References

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- Susana Monteiro, António Salgado, Nuno Silva *Immunomodulation as a neuroprotective strategy after spinal cord injury–Neural Regeneration Research* – 13(3):423-424. doi: 10.4103/1673-5374.228722

Supervisors

Susana Monteiro and Nuno Silva

Baclofen as a potential treatment to promote recovery from Spinal CordInjury.

Summary

Spinal cord injury (SCI) causes severe neurological impairments with no effective treatments. Most patients are young, so their long-term disability leads to a high psychological and financial burden. Therefore, the identification of pharmacological strategies to improve SCI patient's recovery is a high priority.
Baclofen, a GABA agonist that promotes the activation of $GABA_B$ receptors, is mainly usedfor spasticity control in SCI patients. However, emerging data in lampreys showed that Baclofen could promote neuronal survival through the suppression of apoptotic cell death and lead to axonal regrowth. In addition, preliminary data from our lab showed that Baclofenadministration after SCI in a mouse model leads to functional improvements. Nevertheless,the mechanism of action behind these observations is not known yet.
This project aims to understand at the cellular and molecular level the role of Baclofen in the functional improvements after SCI and lastly, introduce Baclofen as a viable option for SCI treatment.

Aims

Treat SCI mouse model with different Baclofen concentrations, assessing the neurological improvements (locomotor, sensory and functional) and processing samples (spinal cord, brain, bladder) for histological and molecular analysis.
Skills (on site): SCI mouse model surgeries; SCI mouse model post-operative care; Anatomical and Behavioral Characterization of SCI mouse model; Cellular and Molecular Biology (Immunohistochemistry, qPCR's, RNA extraction, Microscopy)
Skills (remote environment): Animal Behavioral Analysis, Images Analysis, Literature Search, Cell Counts, Writing

References

de Sousa, N. et al., 2021 (doi.org/10.1089/neu.2020.7591)
Romaus-Sansurjo, D. et al., 2018 (https://doi.org/10.1038/s41419-018-0704-9)

Supervisors

António Salgado and Nídia de Sousa

Near field sensing and imaging for neuronal science

Summary

Mesenchymal stem cells (MSCs), and within them adipose tissue derived stem cells (ASCs),have been shown to have therapeutic effects on central nervous system (CNS) cellpopulations and the Influence of passage number on the impact of the secretome of adiposetissue stem cells on neural survival, neurodifferentiation and axonal growth has been studiedrecently [1]. One powerful tool to assess the neuronal growth is fluorescence bioimaging ofimmunostained cell models.

Here, fluorescence immunostained neurofilaments in neural cells shall be prepared (ICVS)on top of optical grade coverslips or on top of INL pre-functionalized nanostructured substrates suited for near field sensing and super resolution imaging [2,3].

Details of the neurite outgrowth and differentiation shall be characterized by the topology and spatial organization of the neurofilaments, reaching into the nanoscale and therewith into unreached spatial resolution for these neurofilaments.

Aims

The candidate shall be acquainted with cell labeling and near field imaging techniques. Thissuper resolution imaging technique requires nanofabricated metal films on top of coverslips.In addition to the lab work the candidate will be able to be involved in bioimage data analysisusing custom developed Matlab toolboxes.

References

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Supervisors

INL – Jana Nieder/Ima GhaeliICVS –
Antonio Salgado

Development of malaria parasite with genetically encoded sensors for antimalarial screening and discovery

Summary

Development and establishment of biosensors for the monitoring of essential cellular physiological parameters, as calcium oscillation and/or oxidative stress, has been animportant recent contribution for the advance of basic science research. Moreover, detectionof cellular homeostasis disturbance by drugs is a crucial assessment for the understandingof their action and underlying mechanisms of action. As a consequence, genetically encoded sensors (GES) which are fluorescent protein based chimeric proteins with sensing capacity,are being applied for basic science research of physiologic cellular mechanisms, drug actions, as well as, for drug high-throughput screening (HTS) platforms stimulating a truly translational research technology. At present we are establishing and developing such sensors into *Plasmodium falciparum* in order to deliver novel antimalarial drugs. Our network is pioneer on this malaria research area establishing the first calcium yellow-chameleon nano-sensors for *Plasmodium falciparum* (Pandey K, et al. 2016) and recently redox sensors(unpublished).

Aims

Development of biosensors for the monitoring of different cellular physiological parameters,as calcium oscillation and/or oxidative stress in *Plasmodium falciparum*.

References

K. Pandey, P. E. Ferreira, T. Ishikawa, T. Nagai, O. Kaneko, and K. Yahata, “Ca(2+) monitoring in Plasmodium falciparum using the yellow cameleon-Nano biosensor.,” Sci. Rep., vol. 6, no. March, p. 23454, 2016.

Supervisors

Pedro Eduardo Ferreira

Leveraging artificial intelligence to assist minimally invasive inguinalhernia repair in children

Summary

Inguinal hernia is the cause of frequent surgery in children, with an estimated 20 million interventions per year worldwide. Although open surgical repair has been the routine, the laparoscopic approach has had an increasing number of adopters over the past decade. Supporting this trend are innumerous advantages, such as less invasiveness, no groin incision, minimal risk of vas deferens and testicular vessels injury, and the possibility to diagnose asymptomatic contralateral defect reducing the reoperation rate due to metachronous hernia from 10% to almost zero. Nonetheless, there are still many surgeonspreferring the open repair, mostly because they do not feel confident with the laparoscopicview, which results in a higher recurrence rate and in potential serious vascular (ileo- femoral) injuries.

Given this scenario and leveraging recent progresses in the field of artificial intelligence(AI) in the medical arena, this project aims to develop AI-based software tools to assist surgeons during minimally invasive inguinal hernia repair, which could increase the adoption rate of these surgical approaches but also pave the way towards the full automation of this intervention.

Aims

The student will be tasked with:

1) A systematic literature review on the topics of computer-assisted and automatic(robotic) surgery.
2) A survey of clinical requirements for the implementation of computerized assistancetools for inguinal hernia repair and its ultimate automation.

References

Barroso, C., Etlinger, P., Alves, A.L., Osório, A., Carvalho, J.L., Lamas-Pinheiro, R., andCorreia-Pinto, J. (2017). Learning curves for laparoscopic repair of inguinal hernia and communicating hydrocele in children. Frontiers in Pediatrics 5, 207.

Hashimoto, D.A., Rosman, G., Rus, D., and Meireles, O.R. (2018). Artificial intelligence insurgery: promises and perils. Annals of Surgery 268, 70.

Supervisors

Jorge Correia-Pinto and Sandro Queirós

Automating analysis and training in Focused Cardiac Ultrasound

Summary

Focused cardiac ultrasound (FoCUS), also named cardiac point-of-care ultrasound (POCUS), refers to the use of ultrasound imaging to evaluate cardiac structure and function at the bedside by a treating physician. In recent years, FoCUS has become an indispensable first-line diagnostic tool, complementing the traditional physical examinationand accelerating patients' evaluation in acute care settings. Nevertheless, proficiency in FoCUS requires dedicated (and continuous) training in both image acquisition and interpretation, with its clinical efficacy tightly dependent on the user skill. This dependency,together with its ever-growing usage in daily practice by a wide range of medical professionals with very diverse backgrounds and expertise, has led medical schools around the world to introduce bedside ultrasound, including FoCUS, in their curriculum.

Since POCUS/FoCUS training relies heavily on lengthy hands-on training sessions and daily practice on image interpretation skills, the schools' efforts raised one key challenge - the insufficient volume and availability of expert faculty members. In addition, practitioners need to maintain high skill levels, as misinterpretation of suboptimal images, incorrect diagnosis or overestimation of the diagnostic potential of FoCUS may all lead to critical complications under the emergency scenarios in which this tool is used. This highlights theneed to develop strategies for continuous medical education on this topic, as well as tools that facilitate interpretation of these images.

Leveraging recent advances in the field of artificial intelligence (AI), this project aims to address the abovementioned challenges by pursuing two intertwined research lines: (1) creation of AI-based medical training software for FoCUS/POCUS; and (2) development ofsoftware for automatic diagnosis in FoCUS.

Aims

The student will be tasked with:

1) A systematic literature review on learning approaches for FoCUS/POCUS.
2) A survey of clinical requirements for the implementation of an AI-powered medicaltraining platform for FoCUS/POCUS.

References

Neskovic, A.N., Skinner, H., Price, S., Via, G., De Hert, S., Stankovic, I., Galderisi, M., Donal, E., Muraru, D., and Sloth, E. (2018). Focus cardiac ultrasound core curriculum andcore syllabus of the European Association of Cardiovascular Imaging. European Heart Journal-Cardiovascular Imaging 19, 475-481.

Shokoohi, H., LeSaux, M. A., Roohani, Y. H., Liteplo, A., Huang, C., Blaivas, M. (2019). Enhanced point-of-care ultrasound application by integrating automated feature-learningsystems using deep learning. Journal of Ultrasound in Medicine 38, 1887-1897.

Supervisors

Sandro Queirós

Metabolic defects in SCA3: contribution of the hypothalamus malfunctionto disease progression and pathology

Summary

Besides the well-known protein aggregation, neuronal loss and neuroinflammation, metabolic changes are also well-described in neurodegenerative diseases. Spinocerebellarataxia type 3 (SCA3) is not an exception, this aspect of the disease being the main focus ofthis project proposal. SCA3 is a rare genetic disease caused by an expanded triplet CAG repeat within the ataxin-3 gene. This leads to the formation of a mutant form of ataxin-3 (ATXN3), that above a certain threshold (usually CAG repeats higher than 55) causes the disorder. Despite being ubiquitously expressed, when ATXN3 is mutated, only specific populations of neurons are affected in particular brain regions, mainly the cerebellum, brainstem, and spinal cord. This region-specificity makes motor-related symptoms the hallmark of SCA3. Nevertheless, other non-motor manifestations are also present in SCA3,from depression to metabolic alterations. SCA3 patients often show low body mass index (BMI), which is a predictor of poor outcome and disease progression. But what is contributingto the low BMI in SCA3 patients? The answer may be complex, and multifactorial, ranging from deficits associated with the fact that SCA3 patients have difficulties to intake food dueto dysphagia, or being intrinsic to the disease itself, as longer CAG repeats are known to associate with more severe pathology in the neocortex and striatum, and possibly related toother brain regions, including the hypothalamus, which is responsible to keep the brain andbody in a metabolic homeostasis status. Very recently, volumetric studies in SCA3 patientsshowed significant atrophy of the hypothalamus. Despite the clues about the probable relevance of the hypothalamus (dys)function in SCA3, hypothalamic pathology has receivedvery limited attention until now. Because we (and also others) did not characterize deeply this brain region in mouse models where a full-length mutant version of ATXN3 is present, we thought that this could be (i) a good candidate that may explain some of the SCA3 patients' symptoms, (ii) a good source of biomarkers (because it regulates many circulating hormones) and, importantly, (iii) a potential target for therapies.

Aims

Aim 1. Characterize the neuropathology of the hypothalamus in the Q135 and YAC84Q SCA3 mouse models during disease progression, by performing several standard techniques to quantify (i) neuronal death in the hypothalamic sub-regions (Nissl staining, NeuN immunohistochemistry), (ii) mutant ataxin-3 aggregates, and (iii) neuroinflammation (GFAP and IBA1 immunohistochemistry).

Aim 2. Measure the levels of circulating hormones produced by the hypothalamus (or whose secretion is regulated by the hypothalamus) in both mouse models and SCA3 patients' blood samples, to assess potential biomarkers and therapeutic targets. Those willinclude outputs from the hypothalamus: CRH, GHRH, AgRP, cortisol/corticosterone, estradiol, growth hormone, IGF-1, IGF1-binding proteins, NPY, orexin, prolactin, testosterone, TSH, vasopressin and somatostatin; and inputs to the hypothalamus: adiponectin, ghrelin, glucagon, glucose, insulin, and leptin.

Supervisors

Patrícia Maciel and Sara Duarte-Silva

Novel genetic modifiers of Machado-Joseph disease: the role of asps-1(ASPSCR1) gene

Summary

Machado-Joseph disease (MJD) is caused by a CAG repeat expansion in *ATXN3*. This genetic alteration is translated as a toxic polyglutamine tail in the corresponding protein ATXN3 followed by neuronal dysfunction and neuronal loss in specific brain regions. Despite major scientific advances in the last years, this inherited neurodegenerative disease still implies a costly and debilitating course, with a fatal outcome.

In MJD, the CAG segment in *ATXN3* is clinically relevant since longer repeats are associatedwith earlier onset and more severe progression of symptoms. However, the CAG length partially explains the variability of disease' clinical presentation, namely 50% of the age-at- onset (AO) variance. There is evidence for familial factors modulating AO, which suggests that the genetic background of the patients should contribute for the remaining phenotypic heterogeneity observed in MJD, even considering the possibility of shared environmental effects. The identification of these genetic modifiers of MJD severity, which remain insufficiently uncovered, is expected to clarify genotype-phenotype correlations, and therefore elucidate about disease-associated biological mechanisms and can precede the development of effective therapeutic interventions for patients.

Recently, whole exome sequencing of 16 MJD patients, grouped as pairs of age-at-onset (AO) concordant and discordant first-degree relatives revealed potential genetic modifiers of this disease, including a missense variant (rs807449) at the *ASPSCR1* gene.(1) This modifier effect was corroborated when its orthologue *asps-1* was modulated in a *C. elegans*model of MJD by RNAi.

We aim to validate and characterize in more detail the modifier impact of *asps-1* (or other modifier genes identified) in the *C. elegans* model of MJD(2), using a knockout strain for thisgene.

Students will learn how to:

1. Culture and handle *C.elegans*;
2. Perform genetic crossings in *C.elegans*;
4. Evaluate motor behavior, mutant ATXN3 aggregation in *C. elegans* neurons (confocalmicroscopy) and perform survival assays;
5. Perform data analyses and report writing.

References

1. Raposo M, Bettencourt C, Melo ARV, Ferreira AF, Alonso I, et al. 2022. Novel Machado-Joseph disease-modifying genes and pathways identified by whole-exome sequencing. *Neurobiology of Disease* 162:105578
2. Teixeira-Castro A, Ailion M, Jalles A, Brignull HR, Vilaça JL, et al. 2011. Neuron- specific proteotoxicity of mutant ataxin-3 in C. elegans: rescue by the DAF-16 and HSF-1 pathways. *Human Molecular Genetics* 20:2996-300

Supervisors:

Marta Costa and Patrícia Maciel

Potential therapeutic impact of multi-target drugs in neurodegenerativediseases

Summary

Neurodegenerative diseases are characterized by the progressive loss of nerve cells in thecentral nervous system which compromise specific brain functions such as movement, cognition and learning. The treatment of these diseases constitutes a greatest challenge given their devastating social and economic consequences. Although palliative managing ofthese diseases has improved significantly during the last years, there are no cure. It means that the design and development of new drugs able to treat neurodegenerative diseases arean urgent need.

Until now, approved drugs for distinct neurodegenerative diseases have targeted one pathological event at a time. More recently, the transition from this mono-therapeutic concept to the use of multi-functional/hybrid molecules has opened a new avenue of therapeutic possibilities for those disorders. These compounds combine in a single moleculeligands/pharmacophores targeting distinct disease mechanisms. (1; 2)

Motivated by the novelty and the potentiality brought by these new therapeutic concept, wepropose to explore the therapeutic potential of multi-target drugs in C. elegans models of neurodegenerative diseases, evaluating the impact that the drug treatment will have in specific disease hallmarks.

Students will learn how to:

1. Culture and handle C. elegans;
2. Perform drug assays in *C. elegans*; chronic treatments;
4. Evaluate behavior, neuronal survival (confocal/fluorescencemicroscopy)
5. Perform data analyses and report writing.

1. Blaikie L, Kay G, Kong Thoo Lin P. 2019. Current and emerging therapeutic targetsof alzheimer's disease for the design of multi-target directed ligands. *Medchemcomm* 10:2052-72
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Supervisors:

Marta Costa and Patrícia Maciel