Masters Project 2022-2023

Starts in Sept 2022

(duration: 9 - 12 months)

Project title: LAMA2-CMD: establishment of a new gene therapy strategy using an *in vitro* model

Names of the supervisors and contacts: Rita Carlos (arcarlos@fc.ul.pt) and Jose Escandell (jose.escandell@ibet.pt)

Laboratory/Institution, location: Development and Evolutionary Morphogenesis (DEM) laboratory, Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa and Health & Pharma Division, Animal Cell Technology Unit, Instituto de Biologia Experimental e Tecnológica (IBET).

Abstract

LAMA2-congenital muscular dystrophy (LAMA2-CMD), the most common congenital muscular dystrophy, is a life threatening and currently incurable disease. Several studies have tried to establish new therapeutic strategies, however none of these strategies has shown to be fully successful. Gene therapy is a strategy where genetic material is transferred into cells, providing new genetic instructions to help fighting diseases. One of the most commonly used viral vectors in gene therapy, and with the greatest potential recombinant, are adeno-associated virus (rAAV). In this project we aim at testing the efficiency of an all-in-one rAAV vector encoding a CRISPR-Cas9 system to revert a specific mutation in the *Lama2* gene. For that we will first establish a C2C12 myoblast cell line that carries a mutation in the *Lama2* gene (Cys79 to Arg) using CRISPR-Cas9 system. This mutation has been previously identified in mice (nmf417 mice) that carry a phenotype characteristic of LAMA2-CMD. This mutant cell line will be then characterized *in vitro* for proliferation (resazurin assay), changes in extracellular matrix components (immunofluorescence and western blot) and ability to differentiate into myotubes (immunofluorescence). After the characterization of the mutant cell line, we will use it to test the success of the infection with an all-in-one rAAV vector encoding a CRISPR-Cas9 system that includes the gRNA and the *Campylobacter jejuni* Cas9. In order to successfully revert the mutation, the efficiency of different gRNAs, which define the region where the Cas9 endonuclease cut the DNA, will be compared. Reversion of the mutation will be tested by PCR and deep sequencing technology. The best gRNA sequence with higher mutation reversion score will be selected for future studies. This project will be a proof-of-concept that precedes the *in vivo* studies using nmf417 mice and will contribute to establishing a new possible therapeutic strategy targeting LAMA2-CMD.

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3. Martins, S. G., Zilhão, R., Thorsteinsdóttir, S. & Carlos, A. R. Linking Oxidative Stress and DNA Damage to Changes in the Expression of Extracellular Matrix Components.

Front. Genet. **12**, 1279 (2021).

Masters Project 2022-2023

Starts in Sept 2022

(duration: 9 - 12 months)

Project title: Understanding the alterations in the expression of extracellular matrix genes in melanoma – a tissue transcriptomics approach

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Abstract

Cancer is a complex multivariable disease with an enormous impact worldwide. In order to rationalize the complexity of this disease, Hanahan and Weinberg initially defined six hallmarks that are necessary for the establishment of cancer, being vital for cancer cell proliferation and maintenance. In addition to these hallmarks, several other factors are now described to play an important role in tumorigenesis including DNA damage and DNA replication stress, oxidative stress and extracellular matrix (ECM) remodeling. Even though several studies have shed light on each individual cancer hallmark, the intricate network of events that leads to cancer onset and progression has not been fully uncovered. In particular, studies regarding ECM remodeling in the context of cancer have focused primarily on how different signaling pathways promote or are affected by changes in the biomechanical or biochemical properties of the ECM, and how this impacts metastatic spread. Whether changes in gene expression or mutations in ECM or ECM-related components are key steps during cancer progression, and if this relates to stress phenotypes of cancers, remains largely unexplored. Using state-of-the-art technology, laser capture microdissection (LCM) followed by RNA sequencing (RNAseq), this project aims at unravelling the transcriptional network of specific regions of a tumor, where we have observed different expression levels of two major ECM components, LAMA2 and collagen IV (COLIV). Validation of this analysis will be performed using A375 melanoma cell line and human primary melanocytes using RT-qPCR, western blot and immunofluorescence. A375 wildtype and *LAMA2*-deficient cell lines (available at the host laboratory), will be used to compare the expression of target genes in the presence or absence of *LAMA2*. This analysis will provide unique information, interconnecting ECM with different cancer hallmarks, opening the possibility to develop novel therapies.

1. Martins, S. G., Zilhão, R., Thorsteinsdóttir, S. & Carlos, A. R. Linking Oxidative Stress and DNA Damage to Changes in the Expression of Extracellular Matrix Components. *Front. Genet.* **12**, 1279 (2021).

2. Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov.* **12**, 31–46 (2022).

Masters Project 2022-2023

Start in Sept 2022

(duration: 9 - 12 months)

Project title: Understanding the mechanisms at the onset of LAMA2-congenital muscular dystrophy.

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Abstract

LAMA2 congenital muscular dystrophy (LAMA2-CMD) is a crippling, and often lethal, muscle disease which manifests itself from birth and is characterized by severe muscle weakness. LAMA2-CMD is caused by mutations in the *LAMA2* gene, which encodes the laminin α2 chain of laminin 211 (LN211), the most important laminin isoform in mature skeletal muscle. There is currently no treatment or cure for LAMA2-CMD so there is an urgent medical need to develop new therapies.

Using a *Lama2*-null mouse model for LAMA2-CMD we are studying the processes underlying disease onset. Our results so far showed that the disease starts as a defect in muscle growth during fetal stages without any signs of muscular dystrophy (Nunes et al., 2017). Moreover, our data demonstrated that LN211 is an important component of the niche of fetal muscle stem cells (MuSCs) and myoblasts and that it is required for these cells to reach their full developmental potential and therefore ensure muscle growth. To dissect out the mechanisms involved in the disease onset we have established a *Lama2*-deficient C2C12 myoblast cell line. Using this model, we found that *Lama2*-deficient cells present a reduction in proliferation, a defect in differentiation and an increase in DNA damage and oxidative stress markers in comparison wildtype C2C12.

This Masters project aims to address if treatment with different compounds targeting proliferation, differentiation and oxidative stress are able to revert the phenotype. For that we will treat *Lama2*-deficient and wildtype C2C12 cells with these compounds and analyse proliferation (resazurin assay), differentiation (immunofluorescence), oxidative stress (flow cytometry and western blot) and DNA damage (immunofluorescence). After identifying the compounds, and respective pathways, that are able to revert the phenotype, we will isolate MuSCs/myoblasts from muscles of both control and *Lama2*-null fetuses and test if the same compounds are able to revert the phenotype *ex-vivo*. With this project we expect to move closer to understanding the molecular or cellular mechanism(s) underlying the first steps of disease in *Lama2*-null muscles. Identifying these mechanisms is essential for the development of therapies that target disease onset directly.

Masters Project 2022-2023

Start in Sept/Oct 2022

(duration: 9 - 12 months)

Project title:
Effect of the absence of extracellular matrix laminin α2 chain in *in vitro* muscular dystrophy models using decellularized fetal muscle matrices.

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Abstract

Congenital muscular dystrophies have different causes, but the most frequent is due to a mutation in the *Lama2* gene, which encodes the α2 chain of laminin, part of laminin 211 ("LAMA2-congenital muscular dystrophy - LAMA2-CMD"). Laminin 211 is a glycoprotein present in the extracellular matrix that surrounds muscle fibers and serves as a niche for myoblast differentiation. Studies in our laboratory[1], using a mouse model of the disease, have shown that there is a defect in fetal myogenesis that could explain the muscle weakness present at birth. Our data indicate that in the absence of LAMA2, there is a defect in the proliferation and differentiation of fetal myoblasts, preventing normal muscle growth.

In this project we intend to use a myoblast cell line, the C2C12 cells ("WT C2C12"), as well as the same cell line where the expression of the *Lama2* gene was suppressed using CRISPR/Cas9 technology ("KO C2C12"). WT C2C12 and KO C2C12 cells will be compared in their ability to proliferate and differentiate in traditional cell culture systems and in cultures in decellularized fetal muscle matrices[2], obtained from normal and mutant fetal muscles. In this project, we hope to be able to evaluate the effect of the extracellular *versus* cellular environment on the onset of the first symptoms of the disease. The normal and edited C2C12 cells will be seeded in wildtype and mutant decellularized muscle matrices in crossed experiments. The capacity for proliferation, differentiation, and extracellular matrix production, secretion and remodelling by the C2C12 cells will be evaluated. Additionally, co-culture with neuronal cells will be tested to verify the mutual influence of myoblasts-neurons in the developmental progression of the observed defects or in their eventual mitigation[3]. This project will have a strong component of experimentation based on *in vitro* models for the study of cellular behavior and physiology using techniques such as immunocytochemistry, for example.

With this approach, we expect to dissect the contribution of both the cell behaviour and the established niche for the development of the early symptoms of the disease, which are yet largely unknown.

References:

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Masters Project 2022-2023

Starts in Sept 2022

(duration: 9 - 12 months)

Project title: Impact of environmental pollutants in the skin: cell physiology and genetic changes

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Abstract

Environmental pollutants have an enormous impact for the global ecosystem, being extremely harmful to all living organisms and leading to several human diseases. Sulphur dioxide (SO_2) is a frequent pollutant, which is mainly generated by the combustion of fossil fuels (for example by power plants or other industries), but it is also released by fireplaces, natural fires, or volcanic eruptions. Therefore, SO_2 can impact human health directly or through the contamination of crops and water (due to acid rains), entering our body through breathing, ingesting or by contacting the skin. This contamination may damage different cellular components leading to disease. The aim of this project is to analyse the impact of chronic exposure to SO_2 derivatives in the physiology of skin cells and their potential effect at the genetic level. For that the student will take advantage of human melanoma cell line (A375) and human primary melanocytes that are available at the DEM laboratory. These cell lines will be exposed to low doses of SO_2 derivatives for an extended period. Control cell lines will be grown under the same conditions, but without the exposure to SO_2 derivatives. During this time, cells will be monitored for proliferation (resazurin assay), oxidative stress (flow cytometry), DNA damage (immunofluorescence) and changes in extracellular matrix components (immunofluorescence and western blot). Changes in these parameters are suggestive of cell dysfunction and are often associated with skin ageing or tumorigenesis. To investigate whether the chronic exposure to SO_2 derivatives can by itself produce genomic alterations, the student will analyse genetic variation through time to identify mutations that may arise during exposure to the pollutant (Illumina sequencing). This data will then be compared with data already available for human populations living in areas with high SO_2 emission levels. For the top genes that present genetic alterations, a follow up analysis will be performed by RT-qPCR to evaluate transcriptional changes. Overall, this study aims to understand how environmental pollution can impact cells, from genes to physiological responses.

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