Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: miRNA:mRNA regulatory networks in the differentiation of pro-inflammatory γδ T cell subsets

Synopsis:
microRNAs (miRNAs) are a class of small conserved non-coding RNAs that repress mRNA expression, regulating gene expression networks and therefore cell differentiation. While many miRNAs have been identified as regulators of αβ T cell differentiation and effector function, much less is known about the function of these post-transcription regulators in γδ T cells, key providers of the proinflammatory cytokines interleukin-17 (IL-17) and interferon-γ (IFN-γ) in various contexts of (patho)physiology. We have previously found that miR-146a was selectively enriched in these cells and restricted their IFN-γ production by targeting Nod1 mRNA. In this project, we aim at further dissecting miRNA-mediated regulation of effector γδ T cell differentiation. To do so, we isolated pure IL-17$^+$ or IFN-γ$^+$ γδ T cell populations from the peripheral lymphoid organs of a double reporter IL-17-GFP:IFN□-YFP mouse strain and subjected them to next generation sequencing analysis of both miRNA and mRNA repertoires, which allowed us to identify miRNA and mRNA signatures directly associated with cytokine expression. Furthermore, differentially expressed miRNAs and mRNAs were bioinformatically integrated into networks that allowed the prediction of 6 and 3 miRNAs targeting key determinants of the IL-17 and IFN-γ programs of γ T cells, respectively. Further molecular assays are being performed on peripheral γδ T cells to provide a broader functional characterization of the impact of miRNAs on the identity and differentiation of effector γδ cell subsets. Building on this data that is currently being generated on the lab, the aims of this Masters project are the following:

1. Determine the functional impact of the miRNA candidates on γδ T cell differentiation by overexpression and downregulation strategies in *in vitro* γδ T cell development, differentiation and expansion models. These will be evaluated by measuring IFN-γ and IL-17 protein levels by flow cytometry, as well as parameters such as proliferation and apoptosis.

2. Analyse the regulation of candidate miRNA expression in IFN-γ$^+$ and IL-17$^+$ γδ T cells. The influence of external cues (such as T cell receptor (TCR) or cytokine receptor signalling) and that of intracellular transcriptional mechanisms (such as transcription factor regulation) will be tested in the *in vitro* differentiation of γδ T cells, followed by the assessment of candidate miRNA expression by RT-qPCR.

3. Identify specific mRNA targets that constitute the molecular basis of miRNA-dependent regulation of γδ T cell differentiation and effector function**,** using a combination of bioinformatics and biochemical assays, such as qCLASH.

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