Deciphering the transcriptional regulation underlying miR-34c-5p TCR-dependent induction in CD4+ T cells

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microRNAs (miRs) are small non-coding RNAs of 21-23 nucleotides in length that regulate gene expression post-transcriptionally that play a key role in cell fate determination processes including the regulation of T cell activation, proliferation, and differentiation, a central process for mounting an effective immune response. miR-155 is a well characterized regulator of the innate and adaptive immune responses, being induced in response to CD4+ T cell activation and required for the polarization of different Th subtypes. In addition to miR-155, miR-34c-5p has been identified by the host lab as a novel regulator of T cell activation, displaying an activation dynamic significantly distinct from miR-155. The transcriptional mechanisms, as well as key regulators involved in the TCR-dependent induction of miR-34c-5p remain poorly characterized, thus defining the core aims of this project. Prior work from the lab has led to the identification of two genomic regions with the potential to enhance or inhibit miR-34c-5p expression, and some putative regulatory transcription factors (TFs) acting on them. In this project, we aim to expand this work to generate a highly detailed mapping of the transcriptional control of miR-34c expression.
Luciferase reporter platforms will be used to investigate additional genomic segments and address the combinatorial effects of key TFs that act on these regions. Validation of the sequence-dependent nature of the observed effects will be demonstrated by analyzing the direct binding of TFs to the genomic regions and introducing point mutations to abrogate the observed interactions.
The identified transcriptional regulatory elements will be further validated in primary CD4+ T cells by a combination of approaches: 1) transfection of the luciferase vectors coupled to TCR stimulation to assess their ability to simulate the miR-34c transcriptional response; 2) time-course analysis of the expression level, subcellular localization, and post-transcriptional modification of the candidate regulatory factors in response to TCR stimulation; and (if time permits) 3) chromatin-immunoprecipitation (ChIP) in unstimulated and stimulated cells to validate direct association to the target genomic region in a physiological context.
This project will allow the student to acquire a deep understanding of the transcriptional regulation in T cell activation, develop significant expertise in genetic engineering methods, as well as in the growth, manipulation and analysis of human cells.