A key role for microRNAs in the development and functional differentiation of γδ T cell subsets

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The ability of murine γδ T cells to rapidly produce the pro-inflammatory cytokines interleukin-17 (IL-17) or interferon-γ (IFN-γ) underlies their crucial roles in several (patho)physiological contexts. This capacity stems from a complex thymic process of 'developmental pre-programming', after which a large fraction of γδ T cells migrate to peripheral sites already committed to producing IL-17 or IFN-γ. We have previously found that miR-146a maintains the cell identity of peripheral IL-17-committed γδ T cells by inhibiting IFN-γ production. To further address the role of microRNAs in γδ T cell differentiation, we isolated pure IL-17$^+$ and IFN-γ$^+$ γδ T cell populations from the peripheral lymphoid organs of a double reporter IL-17-GFP:IFN-γ-YFP mouse strain to perform small RNA-sequencing. This allowed us to identify distinct microRNA signatures associated with cytokine expression in γδ T cells, from which we selected ten microRNAs differentially expressed between IL-17$^+$ and IFN-γ$^+$ subsets to further characterise. We first analyzed the expression pattern of each candidate microRNA in γδ T cell subsets throughout mouse ontogeny and upon gain-of-function studies in *in vitro* γδ T cell cultures. Our results indicate that while some microRNAs–regulate γδ T cell development in the thymus, other candidates modulate their peripheral effector functions. More specifically, using a miR-181a deficient mouse model, we have found that miR-181a, highly expressed in immature γδ T cell subsets in the thymus, shifts the IL-17/IFN-γ balance towards the IL-17-pathway in neonatal life, which is further maintained in the periphery during adult life. On the other hand, miR-7a-5p and miR-139-5p, overexpressed in peripheral IFN-γ$^+$ γδ T cells, regulate peripheral γδ T cell effector functions, either promoting functional plasticity or acting as an IFN-γ auto-repressor, respectively. Finally, miR-322-5p and miR-450b-3p, overexpressed in IL-17$^+$ γδ T cells, may have therapeutic potential by modulating IFN-γ levels, which are critical in anti-tumoral and anti-viral responses.