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 process of 'developmental pre-programming' in the thymus, after which a large fraction of γδ T cells migrate to peripheral sites already committed to producing either the IL-17 or IFN-γ. We have previously found that one microRNA, miR-146a, maintains peripheral γδ T cell identity by inhibiting IFN-γ production by the IL-17-committed $CD27^{-}$ γδ T cell subset. To further and more globally address the role of microRNAs in effector γδ T cell differentiation, we established a double reporter IL-17-GFP:IFN-γ-YFP mouse strain and isolated pure $IL\text{-}17^{+}$ and $IFN\text{-}\gamma^{+}$ γδ T cell populations from the peripheral lymphoid organs to perform small RNA-sequencing. This allowed us to identify clearly distinct microRNA signatures associated with cytokine expression in γδ T cells, from which we selected ten candidate microRNAs differentially expressed between $IL\text{-}17^{+}$ and $IFN\text{-}\gamma^{+}$ γδ T cells to study further. We characterized the detailed expression pattern of each candidate microRNA in γδ T cell subsets throughout mouse ontogeny and upon gain-of-function studies in *in vitro* cultures of γδ T cells. Our results indicate that while some microRNAs, such as miR-128-3p and miR181a-5p, regulate γδ T cell development in the thymus, other candidates, including miR-7a-5p, miR-139-5p, miR-322-5p and miR-450b-3p, modulate peripheral γδ T cell 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 *in vivo* 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 $IFN\text{-}\gamma^{+}$ γδ T cells, regulate peripheral γδ T cell effector functions, either acting as an IFN-γ auto-repressor (miR-139-5p) or promoting functional plasticity (miR-7a-5p). Finally, miR-322-5p and miR-450b-3p, overexpressed in $IL\text{-}17^{+}$ γδ T cells, may have therapeutic potential by modulating the production of IFN-γ, whose levels are critical in anti-tumoral and anti-viral responses. These data demonstrate the impact of microRNAs on the differentiation and functional identity of effector γδ T cell subsets, which may open new avenues for their manipulation in disease settings.