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IL-4-regulated microRNA expression changes during human and mouse alternative macrophage activation

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Introduction: Tissue macrophages are polarized into different functional states depending on the microenvironmental stimuli. Interferon gamma (IFN γ) induces classical macrophage activation resulting in M1-type inflammatory macrophages. In contrast, interleukin-4 (IL-4) stimulus leads to alternative activation, generating M2-type macrophages with anti-inflammatory properties favouring tissue regeneration. This functional plasticity requires stringent regulation both at transcriptional and at translational level. MicroRNAs inhibit target gene expression by inhibiting translation or by inducing degradation of mRNAs, thereby play a role in fine-tuning several aspects of the immune response. However, microRNAs participating in the regulation of alternative macrophage activation remain largely unknown.

Aim: Our goal was to examine the expression of IL-4-regulated microRNAs during alternative macrophage activation and explore their potential roles in regulating the M2 phenotype.

Methods: Macrophages were differentiated from human peripheral blood-derived monocytes and mouse bone marrow-derived progenitors. IL-4-induced changes in microRNA expression were detected by microRNA microarray analysis and RT-qPCR measurements.

Results: Based on the microarray analysis, miR-99b, miR-125a-5p and miR-342 were selected to be validated via RT-qPCR from the 25 microRNAs showing a significant ($p=0.05$) change larger than 1.3x upon IL-4 treatment of human macrophages. The expression of miR-99b and miR-125a-5p decreased, whereas the expression of miR-342 increased in response IL-4 stimulus. These changes were found to be conserved in mouse macrophages, allowing us to identify the key elements of IL-4-induced regulatory network in gene knock-out mouse macrophages. In IL-4 receptor KO macrophages, IL-4-induced decrease of miR-99b and miR-125a-5p as well as increase of miR-342 were not detected. Similarly, the IL-4-dependent expression pattern was not observed in the absence of STAT6, a transcription factor downstream to IL-4 receptor. *In silico* target prediction analyses showed that a mutual target of miR-99b and miR-125a-5p is IRF-4, a key transcription factor controlling M2 polarization.

Conclusion: Our results show that IL-4 regulates the expression of miR-99b, miR125a-5p and miR-342 microRNAs in human and mouse macrophages by activating STAT6 via IL-4 receptor. This signaling pathway could potentially serve as a fine-tuner of the regulation of macrophage polarization.