GLOBAL ANALYSIS OF RXR-REGULATED GENES IN MACROPHAGES REVEALS A GENE NETWORK POTENTIALLY INVOLVED IN PROMOTING ANGIOGENESIS AND METASTASIS

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Introduction: Retinoid X receptor (RXR), a member of the family of nuclear receptors, is activated by vitamin A metabolite 9-cis-retinoic acid and fatty acids and acts as the obligate heterodimeric partner of several nuclear receptors, like PPAR, LXR, or RAR. Thus, RXR can mediate diverse signaling pathways simultaneously as a component of various heterodimers. Nevertheless, the global pattern of RXR-regulated gene expression changes in macrophages has not yet been analyzed. Our aim was to examine the genome-wide effect of the absence of RXR (loss of function) as well as specific activation of the receptor (gain of function) in macrophages, and identify novel RXR-dependent gene networks potentially involved in the regulation of macrophage metabolism and immune function.
Methods: Bone-marrow-derived macrophages (BMDMs) from Lys-M-Cre $RXR\alpha^{+/+}\beta^{-/-}$ (control) and Lys-M-Cre $RXR\alpha^{flox/flox}\beta^{-/-}$ (macrophage RXR KO) animals were treated with pan-RXR agonist LG268. Changes in mRNA expression induced by specific RXR activation or the absence of RXR were detected by microarray analysis. The angiogenic potential of RXR-activated BMDMs was assessed by human umbilical vein endothelial cell (HUVEC)-based tube formation assay. Primary and metastatic tumor development in animals transplanted with macrophage RXR KO bone marrow was studied in B16-F10 mouse melanoma xenograft model.
Results: The absence of RXR in BMDMs resulted in the altered expression of 141 genes (fold change$\geq$1.3; $p\leq0.05$), which comprised a gene network linked to macrophage adhesion and tissue accumulation. In addition, several of these genes (e.g. S100a8, S100a9, Camp, Sell) have been previously shown to regulate the pro-metastatic action of macrophages. We found 103 genes, which were regulated by specific RXR activation in BMDMs. Among the upregulated genes we found Vegfa, one of the crucial mediators of tumor angiogenesis. Using Ingenuity Pathway Analysis, we identified several additional RXR-induced genes (e.g. Hbegf, Nus1, Serpine1) also implicated in angiogenesis. Accordingly, RXR-activated macrophages showed enhanced angiogenic potential in HUVEC tube formation assay *in vitro*. However, primary B16-F10 melanoma tumor growth was unimpaired in macrophage RXR KO animals, suggesting that RXR expressed by tumor-infiltrating macrophages is dispensable for tumor vascularization. In contrast, mice lacking macrophage RXR had a significantly higher number of B16-F10 lung metastases.
Conclusion: Our results suggest that *in vitro* RXR activation generates a macrophage phenotype possessing pro-angiogenic capacity. Based on our *in vivo* observations we hypothesize that this signaling pathway is presumably either inactive or silenced in the primary tumor microenvironment, but has a potential role altering pro-metastatic

activity of macrophages.

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