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1



predict whether inhibitor bound structure is the real active form of the enzyme. Therefore, the main aim of our project is to crystallize TG2 in presence of calcium.

TG2 besides crosslinking other proteins, also gets crosslinked it self in presence of calcium hence it has always been difficult to purify TG2 and crystallize it. We use wild type TG2 as well as active site mutant (C277S) for crystallization trials. The active site mutant lacks transamidation activity. Both the constructs were purified using cobalt affinity column and then gel filtrated to remove the aggregated and crosslinked proteins. Before loading the protein on the gel filtration column, it was incubated with 0.5 mM CaCl₂ for an hour. Gel filtration buffer also contained 0.5 mM CaCl₂. TG2 was concentrated to 20 mg/ml and crystallization screens were performed in 96-well-plates using PACT premier, JCSG+ and Structure1&2 screens available from Molecular Dimensions. There was good precipitation and phase separation but unfortunately we couldn't get any crystals. We designed another construct of TG2 with a short N terminal linker and a TEV cleavage site. The protein was purified as before and the N'terminal tag was removed by treating it with TEV protease overnight. The protein was subsequently purified again and gel filtered in presence of 0.5 mM CaCl₂ and concentrated to 18 mg/ml. PACT screen gave some positive hits with beautiful rod shaped crystals. The conditions have to be optimized further to get stable crystals, which could be used for X-ray diffraction in future.

Activation of retinoid X receptor induces a proangiogenic macrophage phenotype in vitro

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Tumors develop in a complex tissue microenvironment containing a wide spectrum of host cells such as fibroblasts, endothelial cells and various immune cells. Among immune cells, tumor-infiltrating macrophages significantly contribute to tumor progression by inhibiting anti-tumor immunity, increasing the invasive potential of tumor cells, and enhancing tumor angiogenesis. It is therefore of importance to identify signaling pathways, which are involved in the initiation of these effects. 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 γ , LXR, or RAR, thus mediating diverse signaling pathways simultaneously.

Our aim was to examine the effect of RXR activation on macrophage gene expression *in vitro*, and identify RXR-responsive gene networks with a potential role in generating an anti-/pro-tumoral macrophage phenotype. We also aimed to study tumor development in mice with RXR α/β -deficient macrophages.

Mouse bone-marrow-derived macrophages (BMDM) were treated with pan-RXR agonist LG268. Changes in mRNA expression induced by specific RXR activation 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. Tumor development in Lys-M-Cre RXR $\alpha^{\text{flox/flox}}\beta^{-/-}$ animals was studied in B16-F10 mouse

melanoma xenograft model.

Specific RXR activation in mouse BMDMs induced a pro-angiogenic gene network including VEGF α , one of the crucial mediators of tumor angiogenesis. Using a tube formation assay we found that RXR-activated BMDMs induce angiogenesis *in vitro*. However, primary tumor development in Lys-M-Cre RXR $\alpha^{\text{flox/flox}}$ $\beta^{-/-}$ mice was unimpaired in our xenograft tumor model.

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 macrophages residing in the tumor microenvironment.

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Ascorbic acid strongly enhances adipogenesis from mouse embryonic stem cells and primary embryonic fibroblasts

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Recently, ascorbic acid (AsA) has been identified as an epigenetic regulator that enhances various cellular differentiation processes; however the mechanism of action remained largely unknown. AsA is known to act as an antioxidant; and it also plays an essential role as a cofactor for many enzymes, including prolyl-4-hydroxylases (PHDs). Prolyl-4-hydroxylation is necessary for oxygen-dependent protein stability of hypoxia-inducible transcription factors (HIFs). Pharmacological inhibition of PHD activity during the initial stages of adipogenesis abrogates the formation of adipocytes.

We here demonstrate that addition of AsA to the adipogenic cocktail promote the differentiation of mouse embryonic stem cells (ESCs) and primary mouse embryonic fibroblasts (PMEFs) to adipocytes. Even in low concentration of AsA (40 μM) significantly improved adipocyte differentiation of PMEFs and resulted in an increased expression of fatty acid binding protein 4 (Fabp4). Addition of dimethyloxaloylglycine (DMOG), a PHD inhibitor, activated the hypoxic response pathway in PMEFs and could also induce the expression level of Fabp4. Moreover, we found evidence that addition of AsA also activates hypoxia inducible mechanisms of PMEFs.

These results suggest the existence of a link between AsA and low oxygen condition and implicate their regulatory function during formation of adipocytes.

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