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Generation and validation of plasmid-based viral transduction systems for constitutive and inducible overexpression of murine Gata4 to study the role of Gata4 in endothelial differentiation in vitro

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Introduction: Liver sinusoidal endothelial cells (LSEC) are highly specialized microvascular EC which are involved in immune mechanisms and endocytosis. However, little is known about the differentiation process of these cells. The transcription factor Gata4 was found to be overexpressed in freshly isolated LSEC in comparison to other EC, and is downregulated when LSEC are isolated and cultivated. To further investigate the role of Gata4 in LSEC differentiation, Gata4-overexpressing plasmids were generated, established and scrutinized upon their biological functionality in the current study.
Materials and Methods: An artificial Gata4 cDNA sequence (synGata4), which encoded for a transgenic murine Gata4 protein, was cloned into the plasmids MIR and ADR3. Human embryonic kidney (HEK) cells and human umbilical venous endothelial cells (HUVEC) were infected with lentivirus harboring the resulting constitutively active plasmid ADR3-synGata4 or the inducible plasmid MIR-synGata4. Overexpression of synGata4 mRNA and transgenic murine Gata4 protein was confirmed by RT-PCR, qRT-PCR, immunocytochemistry and Western blot, respectively. The regulatory activity of transgenic murine Gata4 protein was assessed by analyzing the expression levels of genes known to be regulated in HUVEC by ectopically expressed human GATA4 via qRT-PCR.
Results: The functionality of both plasmids was verified based on the observed overexpression of synGata4 mRNA and transgenic murine Gata4 protein in HEK and HUVEC. Infection with the ADR3-synGata4 plasmid resulted in increased expression levels of the selected GATA4-regulated genes, including BMP2, CLEC2, IFITM1, IFIT3, IFI44L, RSPO3 and TSPAN7, in HUVEC. In contrast, the MIR-synGata4 plasmid was not able to induce changes in the expression level of GATA4-regulated genes in HEK cells or HUVEC.
Discussion: This thesis demonstrates the induction of selected GATA4-regulated genes by ectopically expressed transgenic murine Gata4 protein in HUVEC. Differences in the capacities of the MIR-synGata4 plasmid and the ADR3-synGata4 plasmid to alter gene expression levels may result from discrepant plasmid structures, such as the promoter type or a selection marker, differing time spans between the infection, induction and the analysis of the cells, and the potency of the ectopically expressed transgenic murine Gata4 protein in human cell lines. Optimizations of the experimental conditions will improve the applicability of the plasmids, e.g. in experiments regarding the differentiation of embryonic stem cells or induced pluripotent stem cells into (LS)EC.