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Conditional expression of Foxo1 variants in mice

The transcription factor (TF) Foxo1 (forkheadbox protein o1) regulates a multitude of cellular processes and belongs to the class of forkhead proteins. Foxo1 binds via its forkhead domain to DNA recognition sequences and, dependent on the individual gene, can act as a transcriptional activator or repressor. The activity of Foxo1 is controlled via different mechanisms, such as subcellular distribution, degradation, association with co-regulators and modulation of DNA-binding. Upon stimulation with insulin or other growth factors, the main regulator of Foxo1, the phosphoinositide-3-kinase (PI3K), effects the translocation of the TF from the nucleus to the cytoplasm. Since both, *in vitro* as well as *in vivo* investigations, show the involvement of Foxo1 in hepatic glucose metabolism, proliferation of the pancreas and differentiation of adipocytes, a role of Foxo1 and diabetis has been discussed. In order to investigate the biological function of the TF, two different mutants have been constructed: a dominant negative (Foxo1DN) and a constitutively active (Foxo1ADA) form. The Foxo1DN has been discribed as a truncated protein, which has no transcriptional domain. It binds to the DNA site without being functionally active as a TF. In contrast, Foxo1ADA binds continuously to the DNA and operates as a TF, independent of PI3K and on the basis of its three point mutations T24A, S253D and S319A.
The main part of this thesis included the generation of transgenic mice, carrying conditional Foxo1DN and Foxo1ADA constructs, that can be activated through cre recombination in the ROSA26-*locus*. Both Foxo1 constructs were generated and subsequently cloned into the ROSA26 targeting vector. Transfection of murine ES-cells and verification of homologously recombinant clones were performed. I*n vitro* analyses indicated that Foxo1DN is functionally active. Additionally, a luciferase assay demonstrated that Foxo1DN is capable to reduce the transcriptional activity of the endogenous Foxo1 protein significantly. In addition, FACS analyses showed that Foxo1DN ES-cells proliferate less and/or undergo apoptosis. The injection of Foxo1DN and Foxo1ADA ES-cells into blastocysts led to a 90% chimeric mouse in each cases. Both of them are currently investigated for germline transmission. Since western blot analyses of Foxo1DN ES-cells led to unexpected results, two other Foxo1DN ROS26 targeting vectors were cloned. Both contain a nuclear localisation signal at different positions to enhance nuclear import.
Furthermore, a conditional Foxo1 reportergene mouse should be generated for visualisation of the activated PI3K signalling pathway *in vivo*. The reportergene encode a fusionprotein of Foxo1 and a fluorescent protein. In total four different reportergenes were constructed and integrated in the ROSA26-*locus*: Foxo1GFP, Foxo1DsRed and their variants destabilised by a PEST domain. Transfection of murine ES-cells and verification of homologously recombinant clones were performed. Fluorescence microscopy analyse Foxo1GFP and Foxo1GFPd2 ES-cells upon cre mediated recombination. Foxo1GFP transgenic ES-cells were injected in order to generate chimeric mice.