TR3 nuclear orphan receptor in cardiovascular disease
Arkenbout, E.K.

Citation for published version (APA):
Chapter 9

Summary
Summary

Atherosclerosis is a major health problem in the Western world, and the effectiveness of interventional therapeutic modalities for symptomatic atherosclerotic lesions is limited by (in-stent) vessel restenosis. Proliferation and migration of smooth muscle cells (SMCs) are key events in various vasculo-proliferative diseases like atherosclerosis, post-interventional restenosis and vein-graft disease. Pharmaceutical interventions, such as targeting proteins that regulate SMC growth and movement, are promising new approaches to treat cardiovascular disease. For our goal to find new genes that are causative in atherosclerosis and specifically expression in SMCs, we used the differential display RT-PCR technique. We established that the expression of the family members of the NGFI-B family of nuclear hormone receptors is increased in cultured SMCs after atherogenic stimulation. The NGFI-B family constitutes a subfamily of the nuclear receptor superfamily, and comprises three mammalian members; TR3 orphan receptor (TR3), mitogen induced nuclear orphan receptor (MINOR) and nuclear orphan receptor of T-cells (NOT). In this thesis, several studies are described to reveal the function of this NGFI-B subfamily of transcription factors in different vasculo-proliferative diseases. Chapter 1 describes different techniques to identify novel genes involved in atherogenesis. Moreover, the process of atherosclerosis and a detailed description of the cell types involved are outlined. Features of the NGFI-B transcription factor subfamily are presented in Chapter 2. In Chapter 3 we report that TR3-, MINOR- and NOT mRNA is synthesized in human atherosclerosis, specifically in SMCs, ECs and macrophages. To investigate the role of TR3 in the vasculo-proliferative disease, transgenic mice were designed that overexpress TR3 or the dominant-negative variant (ΔTA), which specifically inhibits all family members in SMCs of the arterial vessel wall. Carotid artery ligations were performed to show that TR3 has a powerful effect in vivo. Overexpression of TR3 in these transgenic mice protects against lesion formation, while the dominant-negative variant augments SMC proliferation. Anti-proliferative effects of TR3 are mediated by modulating the expression of critical cell-cycle regulators, including p27Kip1 and cyclin A, that control progression of cell cycle from the G1 phase into the S phase. In Chapter 4, it was shown that TR3 inhibits proliferation through modulation of the expression of cell-cycle proteins in endothelial cells. Using adenoviral vehicles to accomplish overexpression of TR3 or the dominant-negative inhibitor in endothelial cells and subsequent fluorescence-activated cell sorting (FACS) analysis revealed that TR3 provokes G1 arrest of the endothelial cell-cycle. Vein grafts are employed in coronary artery bypass surgery. The role of TR3 in vein-graft disease is studied in Chapter 5. Vein grafts are subject to increased tensile stress due to exposure to arterial blood pressure, which has been hypothesized to induce endothelial cell-
and SMC injury, resulting in vein-graft disease. TR3 is induced in vein segments when they are subjected to perfusion at arterial pressure. In addition, cultured venous SMCs that are exposed to cyclic stretch start to proliferate and express TR3 mRNA, whereas arterial SMCs remain quiescent and exhibit no TR3 mRNA induction. Adenovirus-mediated overexpression of TR3 in venous SMCs results in decreased DNA synthesis in response to mechanical strain. Application of 6-MP, an agonist of TR3, during stretch also results in decreased DNA synthesis of SMCs, indicating that endogenous TR3 may prevent mechanical strain-induced excessive proliferation.

In Chapter 6, expression of TR3 and its family members in in-stent restenosis is described and several candidate genes that are regulated by TR3 were identified. In Chapter 7, the effects of (inhibition of ) TR3 on arteriogenesis as well as contraction and dilatation of aortic rings and small resistance mesenteric arteries are investigated in transgenic mice. No difference in responsiveness to vasoactive substances was observed between the vessels of TR3-overexpressing mice, ATA-transgenic mice or wild-type mice. Furthermore, collateral artery development is not influenced by overexpression or full inhibition of TR3.

Taken together, in this thesis we demonstrate a functional involvement of TR3-like factors in vasculo-proliferative disease. TR3 is expressed in atherosclerotic lesions, in-stent restenosis as well as in vein-graft arteriosclerosis and inhibits proliferation of SMCs and endothelial cells. Consequently, we propose that this transcription factor fulfills an athero-protective function. In view of the findings reported, this thesis may be the starting point for an ultimate clinical application of TR3 agonists in the treatment of SMC proliferation in (in-stent) restenosis, vein-graft disease and atherosclerosis.