Clemens Scherer
Dr. med.

From osmotic to biomechanical stress - The role of nuclear factor of activated t-cells 5 in determining the vascular smooth muscle cell phenotype

Fach/Einrichtung: Physiologie

Doktorvater: apl. Prof. Dr. rer. physiol. Thomas Korff

Nuclear factor of activated T-cells 5 (NFAT5) is a well-known osmoprotective transcription factor the amount and nuclear distribution of which is increased in a hyperosmolaric environment. Recently, NFAT5 was shown to control the phenotype of vascular smooth muscle cells (VSMCs) in the context of arteriosclerosis and neointimal hyperplasia by regulating the expression of smooth muscle cell differentiation markers. As an increase in wall stress is a well-known determinant of the VSMC phenotype, it was hypothesized in this thesis, that wall stress or stretch may affect NFAT5 activity as well. Furthermore, this study tried to reveal potential signal transduction pathways leading to the activation of NFAT5 in stretch-stimulated VSMCs and explore the function of NFAT5 in the context of vascular remodeling.

Human arterial smooth muscle cells (HUSMCs) were exposed to cyclic stretch and examined by immunofluorescence, mRNA and protein analyses. NFAT5 translocation to the nucleus was increased after 24 hours in comparison to control VSMCs but declined thereafter. c-Jun N-terminal kinase (JNK) was found to be responsible for nuclear translocation of NFAT5 as well as to regulate its protein abundance. Furthermore, NFAT5 translocation depended on activation of palmitoyl transferases. Concordantly, the expression of carnitin palmitoyl transferase 1 was increased and NFAT5 was palmitoylated in stretch-stimulated VSMCs. Additionally, atorvastatin inhibited nuclear translocation of NFAT5 in stretch-stimulated VSMCs. DNA microarray analyses of stretch-stimulated VSMC with and without prior knockdown of NFAT5 revealed over 2,000 differentially regulated gene products. As one of the transcriptional targets of NFAT5, tenascin-C (TN-C) enhanced the 2D and 3D migratory capacity of VSMCs. Another transcriptional target of NFAT5, actin beta-like 2 (ACTBL2), the expression of which was up-regulated in stretch-stimulated VSMCs, likewise increased VSMC migration.

The data collected for this thesis suggests that biomechanical stretch can activate NFAT5 in cultured VSMCs. Nuclear translocation and activity of NFAT5 likewise depends on the activity of JNK and palmitoyl transferases. NFAT5 activity promotes expression of tenascin-C and ACTBL2. Both tenascin-C and ACTBL2 increase migration of VSMCs, thereby potentially enhancing maladaptive remodeling processes such as arterial stiffening in the context of hypertension. As statins inhibit nuclear translocation of NFAT5, the latter may be responsible for part of the pleiotropic effects of statin therapy. However, the significance of NFAT5 in the context of vascular remodeling *in vivo* is not known so far and has to be characterized in future experiments.