Cardiovascular disease is the leading cause of death worldwide. Aging is among the greatest risk factors for cardiovascular disease. Cardiovascular disease comprises several diseases, for example myocardial infarction, elevated blood pressure and stroke. Many processes are known to promote or worsen cardiovascular disease and in the present study, cellular senescence and inflammatory activation were of special interest, as they have a strong association to aging and can be seen as hallmarks of cellular aging.

Long noncoding RNAs (lncRNAs) are noncoding RNAs with a length of more than 200 nucleotides. In recent years, numerous regulatory functions were shown for these transcripts and lncRNAs were shown to directly interact with DNA, RNA and proteins. The long noncoding RNA H19 was among the first described noncoding RNAs and was initially shown to act as a tumor suppressor. More recently, several studies showed oncogenic roles for H19. In regards to the cardiovascular system, H19 was not analyzed before.

We show that H19 is the most profoundly downregulated lncRNA in endothelial cells of aged mice compared to young littermates. Microarray analysis of human primary endothelial cells upon pharmacological H19 depletion revealed an involvement of H19 in cell cycle regulation. Loss of H19 in human endothelial cells *in vitro* led to reduced proliferation and to increased senescence. H19 depletion was shown to counteract proliferation before, but none of the described mechanisms applied to endothelial cells. We show that the reduction in proliferative capacity and the pro-senescent function of H19 is most probably mediated by an upregulation of $p16^{ink4A}$ and p21 upon H19 depletion.

When we compared the angiogenic capacity of aortic endothelial cells from young and aged mice in an aortic ring assay, rings from aged mice showed a reduced cumulative sprout length. Interestingly, pharmacological inhibition of H19 in aortic rings of young animals, where H19 is highly expressed, was sufficient to reduce the cumulative sprout length to levels we observed from aged animals. Furthermore, overexpression of human H19 in aortic rings of aged mice, where H19 is poorly expressed, rescued the impaired angiogenic capacity of aged endothelial cells.

We generated inducible endothelial-specific H19 knockout mice ($H19^{iEC\text{-}KO}$) and subjected these animals to hind limb ischemia surgery followed by perfusion analysis in the hind limbs by laser-doppler velocimetry and histological analysis. Perfusion in the operated hind limb was increased in $H19^{iEC\text{-}KO}$ compared to Ctrl littermates, which was in contrast to a reduction in capillary density in the operated hind limbs of $H19^{iEC\text{-}KO}$ animals compared to Ctrl littermates and to our previous results. Analysis of arteriogenesis revealed an increase in collateral growth upon EC-specific H19 depletion in the ischemic hind limbs, which explains the increase in perfusion despite the reduction in capillary density. Further characterization of the animals revealed an increase in leukocyte infiltration into the

tissue in the ischemic hind limbs upon endothelial-specific H19 depletion, indicating a potential role of H19 in inflammatory tissue activation.

Reanalysis of the microarray data from human primary endothelial cells upon H19 depletion revealed an association of H19 with inflammatory signaling and more specifically with IL-6/JAK2/STAT3 signaling. Analysis of cell surface adhesion molecule expression revealed an upregulation of ICAM-1 and VCAM-1 on mRNA level and an increase of the abundance of the two proteins on the cell surface of human primary endothelial cells. Consequently, adhesion of isolated human monocytes to human primary endothelial cells was increased upon H19 depletion *in vitro*. Interestingly, TNF-α mediated inflammatory activation of primary human endothelial cells repressed H19 expression. H19 did not function *via* previously described mechanisms. We excluded a competitive endogenous RNA (ceRNA) function for H19 in endothelial cells and showed that miR-675, which is processed from H19, does not play a role in the endothelium. Furthermore, H19 did not regulate previously described genes or pathways.

Analysis of transcription factor activity upon H19 depletion and overexpression revealed a differential activity of STAT3. STAT3 phosphorylation at TYR705 and thus activation was increased upon H19 depletion. Inhibition of STAT3 activation using a small compound inhibitor abolished the effects of H19 depletion on mRNA expression of p21, ICAM-1 and VCAM-1 and on proliferation, indicating that the effects of H19 are at least partially mediated *via* STAT3. STAT3 was shown to have positive effects on the cardiovascular system before, most likely due to upregulation of VEGF in a STAT3-dependent manner. We were not able to confirm previously described mechanisms for STAT3 in the present study and propose a new mechanism of action for the H19-dependent regulation of STAT3. Taken together, these results identify the long noncoding RNA H19 as a pivotal regulator of endothelial cell function. Figure 1 summarizes the described functions of H19 in endothelial cells.

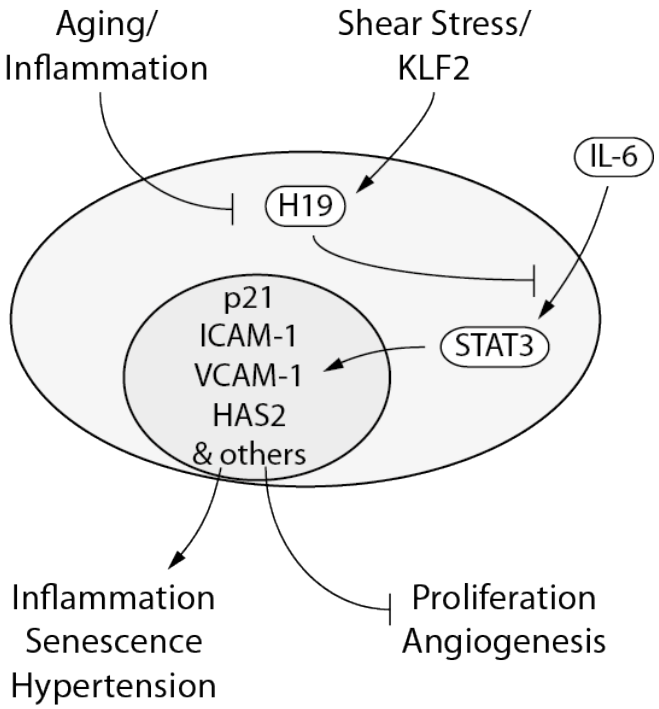

Figure 1: Summary of the described functions of H19 in endothelial cells. H19 expression is repressed with aging and inflammation and induced with shear stress *via* KLF2. H19 modulates STAT3 activation and regulates several genes in a STAT3-dependent manner. Loss of H19 leads to increased inflammation and cellular senescence, and hypertension. Loss of H19 reduces proliferation and angiogenesis.