

Meeting abstract

Grainyhead like 3 – a newly identified TNFalpha regulated transcription factor – is regulated by the Src kinases & NO

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One major independent risk factor of cardiovascular diseases (CAD) is aging. Therefore, it is important to understand vascular aging on a cellular level. It is known that TNFalpha is increased in serum of CAD patients, induces senescence and apoptosis of endothelial cells (EC) and decreases their migratory capacity. Recently, we discovered the transcription factor Grainyhead like 3 (GRHL3) as a TNFalpha regulated gene. GRHL3 $-/-$ mice demonstrated defects in cell migration. Therefore, the aim of our study was to investigate the role of GRHL3 in EC migration and the underlying mechanisms.

After verifying expression of GRHL3 in EC, GRHL3 was cloned and overexpressed in EC. GRHL3 induced migration to a similar degree as vascular endothelial growth factor (VEGF). To get insights in the underlying mechanisms, we analyzed a known VEGF-induced migration activator, endothelial nitric oxide synthase (eNOS). eNOS phosphorylation on S1179 was increased upon overexpression of GRHL3, suggesting that GRHL3 increased EC cell migration through a nitric oxide (NO) dependent pathway. To determine whether a potential positive feedback loop of nitric oxide (NO) and GRHL3 exists, we next tested the effect of NO on endogenous GRHL3 expression by real time PCR. Incubation with the NO donor papanonoate dramatically increased GRHL3 mRNA expression (papanonoate: 3.75-fold \pm 1.34 of control). Furthermore, decreased migratory capacity of senescent EC concomitant with an increase in Src kinase activity has been shown. To analyze whether Src has an influence on GRHL3, we determined the nuclear levels of this promigratory transcription factor in mouse embryonic fibro-

lasts (MEF), deficient for Src, Fyn and Yes (SFY $-/-$). A higher concentration of GRHL3 protein was observed in nuclear extracts of SFY $-/-$ MEF. To demonstrate that the Src kinase family is indeed a negative regulator of GRHL3 expression in EC, we treated the cells with the Src kinase family inhibitor PP2 and analyzed expression of GRHL3. Indeed, expression of GRHL3 was dramatically increased by PP2 (PP2: 4.50-fold \pm 1.72 of control).

In conclusion, GRHL3 is an important inducer of EC migration mediated through activation of eNOS/NO. NO in turn induces GRHL3 expression, suggesting a positive feedback loop. In addition, the Src kinase family negatively regulates endogenous GRHL3.