THE ANGIOGENIC RESPONSE IN HYPOXIC HEART
Experimental studies in mice

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ABSTRACT

Coronary artery disease is the leading cause of death in the western world today. Although induction of angiogenesis would appear to be an ideal therapeutic strategy, clinical trials of pro-angiogenic factors have proved disappointing. Angiogenesis is a complex process involving many signaling pathways and mediators, and further insights into the underlying cellular and molecular mechanisms are urgently needed. Here, we used two mouse models, systemic hypoxia and myocardial infarction (MI), to study the effects of hypoxia on angiogenesis in the myocardium, and the cellular and molecular mechanisms involved.

Hypoxia-inducible factor-1α (HIF-1α) is an important transcriptional regulator of angiogenesis. Small ubiquitin-related modifier-1 (SUMO-1) has been shown to stabilize transcription factors and modulate their activity. In our mouse model of systemic hypoxia, we showed that SUMO-1 expression is enhanced by hypoxia in brain and heart. Furthermore, SUMO-1 co-localizes and directly interacts with HIF-1α under hypoxic conditions, indicating that hypoxia-mediated increases in SUMO-1 expression could modulate HIF-1α function.

We combined our mouse model of systemic hypoxia with a model of MI and showed that chronic hypoxia protects the heart from infarct injury and promotes angiogenesis. A proteomics analysis demonstrated that protein disulfide isomerase (PDI) is upregulated in the myocardial capillary endothelial cells of mice exposed to chronic hypoxia. Furthermore, PDI knockdown in endothelial cells *in vitro* increases apoptosis and inhibits migration and adhesion, indicating that PDI may play an integral role in angiogenesis.

Endoglin is a co-receptor for transforming growth factor-β. In our mouse model of MI, we showed increases in endoglin expression in endothelial cells in the heart one week after surgery. Similarly, endoglin expression is increased in endothelial cells *in vitro* after exposure to hypoxia. Furthermore, we showed that hypoxia promotes activation of the endoglin/ALK-1/SMAD1/5 but not the endoglin/ALK-5/SMAD3 signaling pathway in endothelial cells. The induction of this pathway represents another potential mechanism for regulation of angiogenic responses in endothelial cells after MI.

The results presented advance our understanding of the complex pathways involved in hypoxia-mediated angiogenesis in the heart. Our findings could play a role in identifying new strategies for the treatment of ischemic heart disease.

Key words: angiogenesis, endoglin, hypoxia, mice, PDI, SUMO

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Avhandlingen baseras på följande delarbeten:

Paper I **Increase of SUMO-1 expression in response to hypoxia: direct interaction with HIF-1α in adult mouse brain and heart *in vivo***

Ruijin Shao, Fu-Ping Zhang, Fei Tian, Anders Friberg, Xiaoyang Wang, Helen Sjöland, Håkan Billig

FEBS Letters 2004, 569:293-300

Paper II **Expression of protein disulfide isomerase is increased in vascular endothelial cells during myocardial infarction in mice exposed to chronic hypoxia: role in angiogenesis?**

Fei Tian, Johannes Wikström, Helen Karlsson, Xianghua Zhou, Helén Sjöland, Li-Ming Gan, Levent M. Akyürek, Jan Borén

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Paper III **Endothelial cells are activated during hypoxia via endoglin/ ALK-1/SMAD1/5 signaling *in vivo* and *in vitro***

Fei Tian, Xianghua Zhou, Erik Larsson, Carl-Henrik Heldin, Jan Borén, Levent M. Akyürek

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