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Progenitor Cells and Hypoxia in Angiogenesis

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ENGLISH SUMMARY

Despite the medical advantages made in the last decade, the aging population and increased occurrence of obesity and diabetes boosted the occurrence of chronic ischemic cardiovascular pathologies. Apart from pharmacological and surgical interventions, stimulation of the body's own ability to improve neovascularization would be an alternative approach to alleviate ischemia. A better understanding of the mechanism of vascularization may identify and overcome bottle-necks in the body's own revascularization system.

This thesis starts with three surveys on adult neovascularization (**Chapter 1-3**), which set out a framework for the experimental chapters described afterwards on vascularization-supporting endothelial progenitor cells (**Chapter 4 and 5**), pro-angiogenic mesenchymal stromal cells (**Chapter 6**), and the key regulator hypoxia (**Chapter 7**). In **Chapter 1** adult neovascularization is highlighted, which encompasses three distinct mechanisms whereby blood supply and perfusion can be restored or adapted to novel circumstances. First, sprouting of the existing vascular network i.e. angiogenesis. Second, arteriogenesis facilitates the adaptations of the proximal vessels to efficiently support the new vascular bed. Third, adult vasculogenesis has been proposed as an additional pathway whereby progenitor cells migrate and differentiate into vascular cells not only supporting resident endothelial cells, but also form new blood vessels *de novo*.

Chapter 2 reviews the process of sprouting angiogenesis, and one of its key regulators, being the group of membrane-bound proteases. This chapter surveys the different families of extracellular proteases and describes their 'classical' role in matrix degradation, guidance of cell invasion and lumen formation. However, the major role of proteases is not to simply degrade extracellular matrix. This chapter emphasizes on the role of proteases in homeostatic regulation of the extracellular environment, modification of matrix and circulating proteins with new biological activity, control of innate immunity, and recruitment of bone marrow-derived progenitor cells which enforce angiogenesis. Finally, this chapter describes the potential of some proteases as candidate biomarkers for a specific disease, and the perspective and complexity of protease inhibition as a target for therapy in diseases with neovascularization.

Chapter 3 extends on the work described in **Chapter 2** and surveys the mobilization, homing, and participation of progenitor cells in the endothelial lining of newly formed blood vessels. Specific emphasis has been given on two different pheno-

types of EPC, which currently have been recognized. The first population provides early-outgrowth cells, or colony-forming unit endothelial cells (CFU-EC), which represent myeloid cells with some endothelial properties but no ability to form endothelial colonies. They can stimulate neovascularization by paracrine means, but are not incorporated in the endothelial lining itself. The second population generates the late-outgrowth endothelial colony-forming cells (ECFC). ECFC have a very high proliferative potential, can insert into the endothelial lining of new blood vessels, and can also form endothelial tubes by themselves after stimulation with the proper angiogenic. Subsequently, **Chapter 3** discusses current insights into the molecular mechanisms that are involved in mobilization and recruitment of progenitor cells to areas of neovascularization, with specific emphasis on the role of proteases and receptors.

The contribution of CD34⁺ cells, as a suggested main source of EPC, to *in vitro* vascularisation is described in detail in **Chapter 4**. We demonstrate that human CD34⁺-cells selectively home to sites of angiogenic endothelium, proliferate, integrate (in low numbers) in the endothelial monolayer, are able to differentiate into endothelial cells *in situ* and have a mild but significant stimulatory effect on new capillary formation. In addition, this chapter provides evidence for the important supportive role of CD34⁻ on CD34⁺ differentiation into endothelial cells and the marked enhancement of neovascularization by co-cultures of CD34⁺ and CD34⁻.

In **Chapter 4** the contribution of CD34⁺ (-derived) cells to *in vitro* neovascularization is reported. More recent studies demonstrated that these cells predominantly give rise to so-called early EPC or colony-forming unit endothelial cells (CFU-EC). These cells of myeloid origin support angiogenesis mainly by paracrine mechanisms. In **Chapter 5** we discuss the second and later identified population of ECFC (identical to blood-outgrowth endothelial cells (BOEC)) and their contribution to *in vitro* tube formation. These highly proliferative BOEC express most known endothelial markers, are able to perform de-novo tube formation, and are pro-angiogenic *in vitro* and *in vivo*. A comparative analysis of cord blood- (CB) and peripheral blood (PB)-derived BOEC revealed that the endothelial phenotype and characteristics for homing, adhesion, migration, inflammation and angiogenic tube formation are almost equal for BOEC from CB and PB. This clearly demonstrates that both types of BOEC have suitable properties to be regarded as candidates for neovascularization therapy.

Another progenitor cell type which has gained a lot of interest in the scientific community is the mesenchymal stromal cells, described in **Chapter 6**. This progenitor

cell with a high degree of plasticity is capable of giving rise to a number of unique, differentiated mesenchymal and non-mesenchymal cell types. Recent reports show that the observed and potential therapeutic effects of MSC are (partly) mediated by paracrine factors secreted by these cells. The factors released by MSC could well play a role in enhancing angiogenesis. In **Chapter 6** we investigate whether human fetal MSC secrete products that can activate endothelial cells toward a pro-angiogenic state, and to identify the factors that mediate these effects. We demonstrate that human fetal MSC produce and secrete considerable amounts of pro-angiogenic growth factors. VEGF and HGF were identified as the major contributing factors to endothelial cell proliferation and outgrowth of capillary-like tubes.

In ischemia, when blood supply is impaired, hypoxia occurs rapidly. This state of inadequacy of oxygen delivery to meet demands of a tissue initiates adaptive processes to restore vascularization and oxygen transfer. The transcription factor family of hypoxia inducible factors (HIF) controls a large panel of genes which facilitate these effects. Because of the central role of endothelial cells in angiogenesis, numerous groups have studied the genomic responses of endothelial cells to hypoxic exposure. However, these studies have largely ignored the influence of prolonged exposure to hypoxia on hypoxia-regulated gene expression. This is rather contradictory since ischemic diseases are usually accompanied by prolonged hypoxia. In **Chapter 7** we show that endothelial cells exposed to prolonged hypoxia reprogram their transcription of genes considerably. Surprisingly, comparison with five previously published microarray studies on short-term hypoxia-exposed endothelial cells showed substantial overlap in identity of the genes. We demonstrate that the duration of hypoxia evokes a quantitative, rather than a qualitative difference in hypoxia-regulated gene expression. In addition to the undisputable role of HIF in hypoxia-induced gene transcription, additional complexity of the hypoxia response may also involve the transcription factor KLF2, which was not earlier recognized as a hypoxia-regulated factor.

Chapter 8 summarizes the results of this thesis and supplies a comprehensive model in which hypoxia and progenitor cells of different lineages collaborate in order to restore, or enhance, neovascularization. Both in the field of tissue engineering as well as in the development of new cardiovascular disease therapies, a better understanding of the contribution of (endothelial) progenitor cells and the specific adaptations of endothelial cells during chronic hypoxia is urgently required to make (pre)clinical studies on restoring efficient perfusion more successful.