Influencing the homing and differentiation of MNCs in hereditary hemorrhagic telangiectasia

Dingenouts, C.K.E.

Summary
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Summary

Hereditary hemorrhagic telangiectasia (HHT) or Rendu-Osler-Weber disease, is a rare genetic disorder, known for its endothelial dysplasia causing vessel malformations, severe nose bleeds and internal bleedings. In the majority of patients mutations are found in genes belonging to the TGFβ superfamily, causing a disbalance in the TGFβ signaling pathway by haploinsufficiency of the remaining functional protein. HHT type 1 (HHT1) is the most prevalent HHT variant, and its mutation lies in the endoglin gene, encoding a protein which functions as co-receptor of TGFβ, and is crucial to neo-angiogenesis and vascular repair.

The TGFβ signaling pathway is tightly controlled and involves many activators and inhibitors. Although endothelial cells and pericytes have been the main research focus for HHT, it is now known that mononuclear cells (MNCs) also play an important role in vascular homeostasis, integrity and repair. The process by which MNCs are attracted to ischemic, damaged or inflamed tissue is tightly regulated via the stromal cell derived factor-1 (SDF1) – CXCR4 axis. SDF1 is produced in tissues shortly after an ischemic event, and mobilizes MNCs from the bone marrow to the circulation. Subsequent homing of $CXCR4^{+}$ cells from the blood to the site of injury is mediated by the SDF1 gradient and its receptor CXCR4. The enzyme DPP4 enzymatically inactivates SDF1, therefore playing a critical role in limiting MNC recruitment, limiting the inflammatory response.

There is a delicate balance between SDF1 and CXCR4, and skewing of either one of the proteins or regulators involved in their activation or inhibition will result in impaired homing, a defective inflammatory response and hamper wound healing and tissue repair.

HHT1 was long considered a disorder affecting angiogenesis only. In recent years it has become clear that endoglin heterozygosity disturbs the function of many more cell types and processes. In **Chapter 2**, we discussed HHT genetics, etiology and signaling, in particular focusing on the role of circulating mononuclear cells, both their impaired homing and contribution to tissue repair in HHT context.

We then showed that inhibiting DPP4 *in vivo* by treating $Eng^{+/-}$ mice after experimentally induced myocardial infarction (MI), restored homing of MNCs and benefits short term cardiac recovery by reducing fibrosis in **Chapter 3**. Here, the number of reparative M2 macrophages increased, suggesting that DPP4 inhibition reduced the pro-inflammatory immune response after MI.

By inhibiting BMP signaling using LDN, we aimed to stimulate TGFβ signaling in the $Eng^{+/-}$ animals in **Chapter 4**. *In vitro* analysis of macrophage differentiation revealed that LDN treatment increased the number of reparative macrophages. Treatment of $Eng^{+/-}$ mice with LDN restored cardiac function and reduced fibrosis after MI. In a second ischemia model, experimentally induced hind limb ischemia, and we showed that LDN improved blood flow recovery of $Eng^{+/-}$ mice. We found that macrophage signaling via canonical and non-canonical pathways is severely impaired by endoglin heterozygosity.

As macrophage differentiation and tissue repair is impaired in HHT1, in **Chapter 5** we studied the effect of DPP4 inhibition in a dermal wounding model in $Eng^{+/-}$ animals, assessing the healing of the lesion. Compared to untreated animals, dermal application of a DPP4 inhibitor increased wound closure speed and increased M2 macrophage numbers in the lesion area. Levels of fibrosis were decreased, signifying a reduction in scarring of the wound site. Furthermore, investigation of intracellular signaling in macrophages showed that in cultured

Eng$^{+/-}$ macrophages, non-canonical signaling was severely deregulated.

In **Chapter 6,** we described the abnormal epicardial response after myocardial damage in HHT1 mice. In this study, we analyzed the composition and the behavior of the epicardial layer at different timepoints post-MI and found that epicardial thickening is delayed. Furthermore, the epicardium was hyperactive in its response to cardiac ischemic injury. Treatment of *Eng*$^{+/-}$ mice with a DPP4 inhibitor reduced epicardial thickening and increased the percentage of macrophages present in the epicardial infarct border zone.

In conclusion, in this thesis we studied different aims and approaches to influence HHT1-MNC homing and differentiation to restore their contribution to tissue repair. In various experimental methods inducing ischemic and/or direct tissue damage, we aimed to improve tissue repair in the *Eng*$^{+/-}$ mice. Using DPP4 inhibition, we increased the SDF1-CXCR4 homing mechanism, to restore the impaired homing capacity of the HHT1-MNCs. Furthermore, we focused on correcting the M1/M2 differentiation in *Eng*$^{+/-}$ mice. Via use of the BMP receptor inhibitor LDN we aimed to restore the skewed BMP/TGFβ signaling; stimulating the TGFβ pathway signaling to induce M2 differentiation. We concluded that DPP4 inhibition can be used to improve the HHT1 immune system and tissue repair, and is best used in concert with other drugs or therapies that stimulate cardiac or tissue repair, like anti-coagulants or cell therapy.