A potential role of ZEB1 in promoting ischaemic lymphangiogenesis

Zarah B. Tabrizi, Heunglam Mok, Joseph L. Horder, Kathryn R. Green, Sohni Ria Bhalla, Mussarat Wahid, Nicholas Beazley-Long, David O Bates, Andrew V. Benest
Endothelial Quiescence Group, Centre for Cancer Sciences, Biodiscovery Institute, School of Medicine, University of Nottingham, Nottingham, NG7 2RD, UK
Zarah.tabrizi@nottingham.ac.uk

Lymphangiogenesis is the growth of new lymphatic vessels from existing vasculature. Lymphatic endothelial cells (LECs) line all lymphatic vessels and are essential to vessel homeostasis. The switch between LEC quiescence and activation is highly regulated, controlled by multiple signalling pathways and transcription factors. In ischaemic injury, lymphatic vessels play a crucial role in transport of immune cells to the affected area and help decrease inflammation, which decreases the amount of permanent tissue damage. Chronic restriction of blood flow, such of that in peripheral arterial disease, can result in critical limb ischaemia (CLI), a third of cases of CLI ultimately end in amputation of the affected area. Resolution of inflammation has shown to improve reperfusion of tissue in several models of ischaemia. Here we demonstrate a role of endothelial ZEB1, a transcription factor primarily implicated in transcriptional activation and repression in epithelial to mesenchymal transition, regulates the shift to lymphatic endothelial quiescence in ischaemic conditions.

An endothelial cell specific and inducible ZEB1 knock out transgenic mouse model (ZEB1fl/fl*cdh5CRE-ERT2, ZEB1^{iECKO}) was generated, ischaemia was induced into the hindlimb by cauterisation at two points in the femoral artery. Mice were perfused fixed after 28 days and gastrocnemius muscle sectioned and stained for LYVE1 to observe and quantify lymphatic density. The density of LYVE1 positive structures was significantly increased in ZEB1^{iECKO} mouse muscle fibres in comparison to littermate controls (0.44% vs 0.88% LYVE1 positive area). In the non-surgery leg, ZEB1^{iECKO} displayed an increase in observed increase in LYVE1 positive structures (91.42mm^{-2} vs 158.7mm^{-2}).

To identify the potential signalling pathways ZEB1 regulates, Human Dermal LECs (HDLECs) were treated with siRNA to knockdown ZEB1 expression and RNAseq analysis performed. Key pathways linked to known lymphangiogenic and immunomodulatory genes were identified. ChIPSeq analysis of DNA binding motifs highlighted potential interaction of ZEB1 within gene sequences of other transcription factors and transcription factor interacting proteins such as the hypoxia regulated BRD2.

Taken together we identify a potential lymphangiogenic role for ZEB1 in ischaemic hindlimbs.