Loss of endothelial ZEB1 results in increased choroidal neovascularisation and leakage without an increase in inflammation.

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During physiological conditions the endothelium is maintained in a quiescent state which is rapidly lost as endothelial cells (EC) become activated during ischaemia, diabetes or inflammation. Regulated phenotypic switching between quiescence and activation is transcriptionally mediated, and is required for ECs to contribute to processes such as angiogenesis and inflammation.

In wet age-related macular degeneration (wAMD), vessel dysfunction occurs resulting in a destabilised endothelium and excessive EC growth and leakage resulting in vision loss. Here we uncover a potential role for ZEB1 in maintaining endothelial quiescence using a mouse model of wAMD.

Endothelial specific ZEB1 knockout (iECKO) or was induced in adult $Zeb1^{fl/fl}$:VECad-Cre-ER^{T2} and $Zeb1^{wt/wt}$:VECad-Cre-ER^{T2} (control) using tamoxifen. Laser induced choroidal neovascularisation was performed and the effect of $ZEB1^{iECKO}$ on lesion leakage post-lasering was measured using fundus fluorescein angiography on days 3, 7 and 14. Choroid flat mounts were dissected and stained for CD31 and CD45, to observe and quantify neovascularisation and inflammation respectively. In addition, to elucidate the role of ZEB1 within endothelial cells, RNAseq analysis was performed in Human Umbilical Vein Endothelial Cells treated with siRNA to knockdown ZEB1 expression.

Fluorescein leakage was observed to be significantly greater in $ZEB1^{iECKO}$ mice on days 3 (164±13% of control), 7 (174±16% of control) and 14 (207±13% of control) when compared to littermate controls. CD31 staining of choroidal flat mounts also indicated increased vascular lesion size in $ZEB1^{iECKO}$ mice (289±12% of control). However, quantification of inflammation at laser induced lesions indicated there was no significant difference in inflammation between ZEB^{iECKO} and control mice. Further to this, in vitro RNAseq analysis revealed a reduction in inflammation associated gene expression when ZEB1 expression was knocked down. Taken together, our results indicate knockdown of ZEB1 in vivo results in enhanced vascular growth and leakage, but with no increase in inflammation.