A role for Fbxw7 in the vascular damage caused during phase I Retinopathy of Prematurity (ROP):

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Background:

Retinopathy of prematurity (ROP) is a vaso-proliferative disease affecting premature infants that proceeds from an early hyperoxic phase associated with stunting of normal endothelial cell (EC) proliferation to a later hypoxic phase with excessive EC growth. Transcriptomic screening for novel effectors of ROP and hyperoxia-associated oxidative stress suggested a potential new role for Fbxw7 in pathology. Fbxw7 is an F-box protein and the substrate recognition component of the E3 ubiquitin ligase SCF complex which triggers the proteasomal degradation of many target proteins crucial for cell growth, differentiation, and survival. Therefore, our aim was to determine how Fbxw7 is regulated in ROP.

Methods:

Freshly isolated primary retinal microvascular EC (RMECs) were cultured and their phenotypic purity confirmed by immunocytochemistry using EC-specific markers. Cells were then exposed to varying oxygen conditions and analysed by gene expression and protein analysis for the presence of Fbxw7 and its substrates. The impact of changes in Fbxw7 activity on RMEC function was investigated by siRNA knockdown using alpha-Fbxw7 specific siRNAs and a non-target (NT) control siRNA. To confirm knockdown, protein and RNA samples were analysed by western blot and qRT-PCR post-transfection. To determine the effect of Fbxw7 KD on RMEC function, wound healing was measured in scratch assay, tube formation in a 3D matrix scaffold and proliferation by 5-ethynyl-2'-deoxyuridine (EdU).

Results:

qPCR and western blotting confirmed that alpha-Fbxw7 is the predominant isoform expressed in EC. qPCR analysis confirmed increased Fbxw7 expression in response to altered oxygen levels with a 1.7 $\pm$0.1 fold-change increase in Fbxw7 expression. However, interestingly Fbxw7 protein levels were found to be decreased by high oxygen: a decrease that corresponded to elevated Notch 1 Intracellular Domain (NICD) and cyclin E substrate bioavailability indicative of a loss of Fbxw7 function. In agreement with this, Fbxw7 KD of the alpha isoform in RMEC produced a similar effect which was confirmed by decreased Fbxw7 protein expression and a reciprocal increase in NICD and cyclin E. RMEC transduced with the same Fbxw7 siRNAs demonstrated a significant increase in the rate of wound healing which was similar to Dll4 transfected cells which also showed increased Notch 1 function. Fbxw7 siRNA-treated RMEC also showed impaired tubulogenesis further corroborating a role for enhanced Dll4/Notch 1 signalling as a result of impaired Fbxw7 function, similar to hyperoxia.

Conclusion:

Our results demonstrate that Fbxw7 function is responsive to changes in oxygen and that KD or hyperoxia-induced reduction of Fbxw7 function alters EC proliferative and functional capacity concomitant with increased NICD activation and enhanced Notch signalling. Future work aims to investigate the effect of varying oxygen levels on Fbxw7 to understand its role in ROP.