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Exploring the Role of Endothelial-to-Mesenchymal Transition Related Transcription Factors in Sprouting Angiogenesis

Angiogenesis is the process of new blood vessel formation from existing vessels. It occurs either during the development of new tissues or during tissue repair. Endothelial cells (ECs), which line the lumen side of the blood vessel, are a key player in angiogenesis. ECs are normally quiescent but upon stimulation by growth factors like vascular endothelial growth factor (VEGF), they start to proliferate and migrate to form new vascular sprouts. However, after a mature blood vessel is formed, activated ECs completely regain their original identities to stabilize the blood vessel. Our lab hypothesizes that this involves partial endothelial-to-mesenchymal transition (pEndoMT), where ECs partially and reversibly acquire mesenchymal-like characteristics, like increased proliferation and migration. Interestingly, this pEndoMT process may be dysregulated in tumor angiogenesis during which we see leaky and uncontrolled vascular outgrowth.

SNAI1, SNAI2, and TWIST1 are transcription factors that play key roles in EndoMT progression. Additionally, OVOL1 and OVOL2 are regulators of SNAI1, SNAI2, and TWIST in the related process of epithelial-to-mesenchymal transition, and may similarly regulate EndoMT in EC. Previous studies have shown that both SNAI1 and SNAI2 are required in EC for tumor neovascularization in mice. Thus, there may exist a delicate balance between the expression level of pro- and anti-EndoMT factors to drive pEndoMT during angiogenesis. To determine the precise role of those factors in sprouting angiogenesis (potentially through pEndoMT), we generated SNAI1, SNAI2, TWIST, OVOL1 and OVOL2 overexpression lentiviral vectors, which will drive constitutive expression of each transcription factor in virus-transduced cells. Next, we determined the titer of the virus by puromycin selection, and we further validated transcription factor overexpression in transduced EC by quantitative PCR (qPCR).

Our future experiments include additional further validation of our overexpression vectors at the protein level by immunofluorescence. After that, we will perform the Fibrin Gel Bead Sprouting Assay with wild-type or lentivirus-transduced Human Umbilical Vein Endothelial Cells (HUVECS). The Bead Assay is a classic *in vitro* angiogenesis assay which involves coating plastic beads with HUVECs and embedding into a fibrin hydrogel matrix alongside primary fibroblasts. After a few days of culture in suitable growth conditions, coated ECs undergo sprouting angiogenesis. Different morphometric features including sprout number, branching, and length can be quantified for each bead. This assay will help us understand what effect (e.g., hyper-sprouting or loss of sprouting) the overexpression of EndoMT transcription factors will have on angiogenesis. Additionally, it will provide valuable insights into how pEndoMT may be dysregulated by the tumor microenvironment, and how this may contribute to tumor neovascularization in cancer.