Transfection studies into the role of Endoglin in regulation of monolayer permeability of Human Umbilical Vein Endothelial Cells (HUVECs)

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Objectives:

The transmembrane glycoprotein, Endoglin (ENG) (CD-105), is expressed on endothelial cells and mesenchymal stem cells and is thought to function as a co-receptor for several ligands of the transforming growth factor beta (TGF-β) family. Moreover, endothelial ENG is associated with several proteins critically involved in endothelial cell adhesion, proliferation, migration, angiogenesis (VE-cadherin), and vascular permeability which includes integrins, VEGF receptor type 2 and vascular endothelial-cadherin. In this study the role of ENG, present on peri-vascular mesenchymal stem cells in umbilical Wharton's Jelly (WJ-MSCs), on endothelial permeability and the effect of silencing this was investigated, using a co-culture permeability study model (WJ-MSCs +HUVECs).

Methods:

WJ-MSCs were transfected with the ENG-siRNAs (s4677 and s4679) or Silencer® Select Negative Control #1 siRNA and Control # 2 siRNA and lipofectamine transfection reagent duplex when cells reached 80% confluence in reduced serum medium. Specific silencing was confirmed after 72hrs post transfection by flow cytometry. Culture supernatants were collected and VEGF-A levels were measured by ELISA. WJ-MSCs (ENG$^{+/+}$ and ENG$^{-/-}$; 10,000 cells) were placed on top of confluent HUVEC monolayers on trans-well inserts. After 24 hours, FITC-albumin (1mg/ml) was added to the apical chamber, and albumin without FITC was added to the basal chamber. Samples (50 µL) were collected from the lower compartment at different time intervals (1, 2, 4h) and measured using a fluorescence plate reader (SpectraMax M2e). The data was expressed as the amount of leaked protein to the basal compartment in the two co-culture groups (HUVEC+ ENG$^{-/-}$WJ-MSCs and HUVEC+ ENG $^{+/+}$WJ-MSCs). Six replicates per groups and three independent experimental repeats were performed.

Results:

The knockdown in WJMSC was 96.87%± 1.5 at 24hrs. Serial transfection for passages 1 and 2 had the same efficiency. ENG-siRNA transfection had no significant effect on the cell viability (87%±2.1, *P* value =0.47) compared to the siRNA non-silencing control (90.6%±9.5). The cells survived the sub-culturing with no significant statistical difference in the calculated doubling time (1.6 d ±0.16) in ENG$^{-/-}$WJ-MSCs compared to its control (1.6 d ±0.5). The FITC-albumin transfer was significantly higher (30.97%) when HUVEC was co-cultured with silenced ENG$^{-/-}$ WJ-MSCs (13.11 µg/mL ±1.5; P value ≤ 0.005) compared to HUVEC+ ENG$^{+/+}$ WJ-MSCs (10.01 µg/mL ±1.3). The ENG deficient cell cultures secreted more VEGF-A (184.6 pg/mL ± 4.048) compared to the ENG$^{+/+}$ group (87.93 pg/mL ± 8.508) P value ≤0.0001. GraphPad Prism software 9.2.0 was used to analyse the data; unpaired *t* tests were used to compare the experimental groups (mean ± SD).

Conclusion:

Our findings suggest that loss of endoglin in perivascular WJMSCs resulted in increased permeability of albumin-FITC across HUVEC monolayers. This may be linked to the observed increase of VEGF-A secretion in ENG$^{-/-}$WJMSCs. Whilst ENG depletion in endothelial cells per se have been linked to junctional instability and increased permeability, the loss of ENG in peri-vascular cells such as WJMSC, and the impaired cross-talk between perivascular and endothelial cells may also lead to endothelial junctional disassembly and reduced barrier function.