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LINEAGE-DEPENDENT DIFFERENCES BETWEEN HUMAN SMOOTH MUSCLE CELLS IN ABILITY TO SUPPORT VASCULOGENESIS

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Lineage-tracking studies in avian and mouse embryos have revealed that smooth muscle cells (SMCs) in different regions of the vasculature are derived from different embryological origins. We have developed an *in vitro* protocol to differentiate human embryonic stem cells (hESCs) into SMCs through different embryonic lineages, namely neuroectoderm, lateral plate mesoderm (LM) and paraxial mesoderm. As mural cells are thought to augment the formation of functional blood vessels during revascularisation, this study aims to determine whether embryologically-distinct SMCs differ in their ability to support vasculogenesis. We assessed the hypothesis that LM-SMCs are superior in supporting endothelial network formation *in vitro*.

Using an mStrawberry-expressing hESC line, we derived the three origin-specific SMCs and co-cultured them with GFP-expressing HUVECs in a 3D *in vitro* matrigel assay. Endothelial network formation was assessed using real-time confocal imaging. Quantitative analysis revealed that LM-SMCs alone had a supportive effect on network formation and survival, with an increase in HUVEC network area and total cord length after 4 days (2.89-fold increase±0.41-fold, $p<0.01$ and 3.25-fold increase ±0.25-fold, $p<0.001$, respectively; n=3) and 8 days (6.30-fold increase ±1.14-fold, $p<0.01$ and 5.04-fold increase±0.56-fold, $p<0.001$, respectively; n=3), compared with HUVECs alone. In addition, LM-SMCs facilitated more complex endothelial networks, with narrower cords ($p<0.001$) and more branch points ($p<0.001$), compared with the other SMC types and HUVECs alone. To identify whether the LM-SMC-specific influence on network formation and survival was a paracrine effect, the three SMC types were tested in a paracrine 3D *in vitro* assay, where they shared media with HUVECs seeded in an adjacent well. Preliminary results reveal that the supportive effect of LM-SMCs is, at least partly, paracrine, with an increase in HUVEC network area after 4 days (7.08-fold increase; n=1) and 8 days (21.39-fold increase; n=1) compared with HUVECs alone. Currently, we are exploring possible soluble factors that may be responsible and investigating this effect in a murine *in vivo* matrigel assay. In conclusion, the embryological origin of SMCs influences their functional ability to support vasculogenesis. This research will provide insight into which SMC type will be most effective in future revascularisation therapy.