HYALURONAN IS REQUIRED FOR SMOOTH MUSCLE CELL DIFFERENTIATION FROM STEM CELLSR Simpson, X Hong, Q Xu *King's College London*

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Background Emerging evidence indicates that the mobilization and recruitment of circulating or tissue-resident stem/progenitor cells give rise to smooth muscle cells (SMCs) which participate in numerous cardiovascular diseases. Understanding the regulatory mechanisms that control SMC differentiation and their recruitment from vascular progenitors is essential for stem cell therapy for vascular diseases and regenerative medicine. Hyaluronan (HA) is a linear glycosaminoglycan and ubiquitous component of the intercellular matrix; the identification of HA as a critical component of the stem cell niche led us to investigate its role in regulating stem cell differentiation.

Methods and Results Stem cells were seeded on collagen-IV-coated flasks and cultured in differentiation medium to induce SMC differentiation as demonstrated by the expression of SMC-specific genes smooth muscle α -actin, calponin, SM22- α and smooth muscle myosin heavy chain. These phenotypic changes coincided with dynamic changes in HA synthesis and organization. It was demonstrated that SMC differentiation was associated with increased HA synthase (HAS)2 expression, extracellular HA synthesis and loss of pericellular HA. Inhibition of HA synthesis by hyaluronidase, 4-methylumbelliferone (4MU), or specific gene silencing of HAS2 led to abrogation of SMC marker expression. Furthermore, It was found that SMC differentiation was coupled to phosphorylation of the EGF-receptor and ERK1/2, this was shown to be HA-dependent as pre-treatment with 4MU prevented downstream events. Paradoxically, when applied exogenously as a culture medium supplement, low molecular weight HA promotes, whereas high molecular weight HA attenuates SMC differentiation. Subsequently, using a bioreactor system, low molecular weight HA was circulated through a decellurised vessel scaffold ex vivo. In contrast to differentiation medium alone the addition of low molecular weight HA promoted migration of stem cells seeded around the outside of the vessel into the media and their differentiation into SMCs, which coincided with localisation of HA and its receptor CD44.

Conclusions We demonstrate for the first time that endogenously produced HA is critical for SMC differentiation from stem cells, via activation of EGF-R and ERK1/2. The prospect of maintaining stemness or inducing differentiation in stem cells by manipulation of HA concentration/molecular weight, or by targeting genes activated by HA, may provide new therapies for vascular disease.