Engineering extracellular matrix constructs to modulate endothelial cell secretion and its ability to control cancer.

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Statement of Purpose: The emerging angiocrine regulation of tumors adds further dimension to the vascular control of cancer. In addition, to the perfusing aspects that vessels provide as conduits of nutrients and blood, tumor vessels are lined by endothelial cells (ECs) and as they penetrate into the depths of tumors they carry EC to every part of a tumor. From these sites ECs provide paracrine biochemical regulation over tumor biology (Franses, 2011). ECs embedded within 3D matrices can be implanted adjacent to tumors to provide exogenous control in a manner dependent on secretion of perlecan, a heparan sulfate proteoglycan from extracellular matrix. We now ask whether we could modulate the tumor control potential of matrix embedded ECs (MEECs).

Methods/Results: As proteoglycans are essential for cell regulatory control (Dreyfuss, 2009) we added heparin to compressed denatured native type I 3D collagen gels (Gelfoam) and in doing so increased the secretion of heparan sulfate by MEECs and the anti-proliferative effect on lung

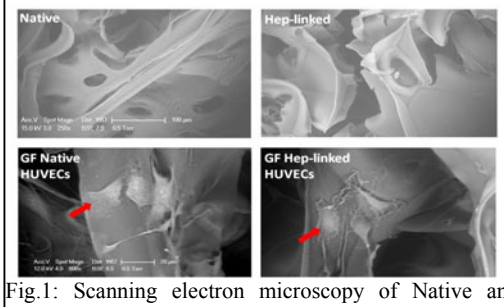

Fig.1: Scanning electron microscopy of Native and Heparin-linked collagen matrices. The upper panel shows the gelfoam without cells and the lower panel shows human umbilical vein ECs cultured in 3 D matrices. Arrows show ECs.

adenocarcinoma A549 cells and human breast carcinoma MDA-MB-231 cells. ECs within in heparin bound matrices proliferated identically to proliferation in native Gelfoam and under scanning electron microscopy appeared similar from ECs on native Gelfoam (Fig. 1). Heparan sulfate biosynthesis was however significantly different in EC in the heparin-Gelfoam (Fig. 2).

Immunofluorescent assays defined a reduction in heparan sulfate proteoglycan syndecan 4 and an increase in heparan sulfate present at extracellular matrix. qPCR documented selective upregulation of endothelial biological genes for EC in heparin matrices.

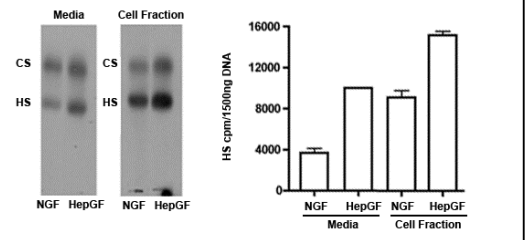

Fig.2: Heparan sulfate biosynthesis from ECs cultured in different 3D matrices. 35S-metabolic radiolabeling followed by agarose gel electrophoresis show the heparan sulfate synthesis in HUVECs cultured in Native Gelfoam (NGF) and Heparin-linked GF (HepGF).

These transcriptional and post-transcriptional changes correlated with increased tumor suppressive therapy. Experiments using co-culture systems of ECs cultured in heparin-linked 3D matrices and cancer cells showed the potential of these MEECs to suppress the cancer cell proliferation. ECs embedded in heparin-linked collagen matrices showed to be 50% more effective than ECs cultured in native collagen matrices regarding the anti-proliferative effect of cancer cells (Fig. 3).

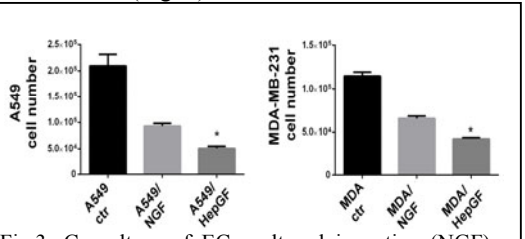

Fig.3: Co-culture of ECs cultured in native (NGF) or heparin-linked (HepGF) with cancer cells (A549 or MDA-MB-231) for 3 days. The co-culture was able to suppress cancer cell proliferation.

Conclusions: Novel EC constructs of 3D collagen type I matrices with linked heparin allowed for controlled engineering of EC regulation of tumors adding further insight into the potential of MEECs in cancer and added power to MEECs as therapeutic modalities.

References: Dreyfuss JL. An Acad Bras Cienc. 2009, 3:409-29; Franses JW. Sci Transl Med, 2011, 3:66ra5