**Hetero-cellular Tumor Spheroid Model of Hypoxia, Stem Cell, and Inflammatory Signaling in Pancreatic Cancer and Evaluation of Nano-particle Mediated MicroRNA-34a Delivery**

**Megha Suresh and Mansoor Amjii - Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston, MA 02115** (Email: suresh.m@husky.neu.edu)

**Abstract**

The first aim of the project was to establish an in vitro model of a three-dimensional spheroid composed of tumor cells, macrophages and fibroblasts, that can recapitulate certain components of the microenvironment of a pancreatic tumor more closely than two dimensional co-culture systems. Here, the hypothesis was that inflammatory and cancer stem cell signaling pathways are largely affected by the hypoxic conditions and by the arrangement and interaction of the different cell types in the 3D structure of the spheroid. There are three theories that are being tested in the multi-cellular spheroid model: (1) the effect of the cross-talk between inflammatory cytokines and the resultant development or suppression of an aggressive, metastatic phenotype (2) the effect of hypoxia on the increase or decrease of cancer stem cell-like phenotype and (3) the interplay of the inflammatory and cancer stem cell signaling pathways. Post confirmation of these two signaling pathways in the 3D spheroid model, a nano-scale hyaluronic acid-based, microRNA-34a delivery system was established as a therapeutic platform to evaluate the modulation of these pathways in the spheroid model. This nano formulation will be surface modified through attachment of CD44 and/or EGFR targeting ligands for selective uptake by the tumor cells with the aim of changing the tumor supportive phenotype to that of a tumor suppressive one.

**Results**

Morphology of homo-and hetero-cellular spheroids
Both homo-cellular and hetero-cellular spheroids were characterized using confocal microscopy and were found to be intact and spherical with a diameter of 93µm and 396µm respectively.

**Analysis of surface CD4+ receptor expression in homo- and hetero-cellular spheroids through FITC-conjugated CD4 antibody staining**

**Analysis of surface SCF receptor expression in homo- and hetero-cellular spheroids through DyLight594-conjugated SCF antibody staining**

**Analysis of expression of inflammatory markers: IL8, TNF-α and TGF-β in homo- and hetero-cellular spheroids through RT-PCR**

**Size and charge characterization of blank and therapeutic HA-PEI nanoparticles using the zetasizer**
Blank HA-PEI nanoparticles of a concentration of 2mg/ml were loaded with miR34a-25 in a HA-PEI: miRNA w/w ratio of 9:1 and characterized for size and zeta potential using the Malvern zetasizer.

**Conclusions**

The spheroid models were developed and characterized morphologically. Future studies include evaluation of hypoxic markers such as HIF1α, HIF2α, HIF1β and SCF receptors through western blot before and after treatment with the miRNA, followed by cytotoxicity evaluations.

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**Methods**

Both homo-cellular ( Panc-1 cells only) and hetero-cellular (Panc-1, NIH/3T3 fibroblasts, and J747.A1 macrophages) spheroids were developed using the hanging drop technique and harvested on day 5 of growth. The arrangement of different cells within the hetero-cellular spheroid was evaluated through cytoplasmic membrane labeling with fluorescent dyes for confocal imaging. Cell surface expression of CD29 and stem cell factor (SCF) was carried out through immunofluorescence using confocal microscopy. RT-PCR of inflammatory markers, IL6, TNF-α and TGF-β was carried out in the hetero-cellular spheroid model and compared to expression in homo-cellular spheroids. Blank and miRNA-34a expressing plasmid-loaded hyaluronic acid poly(ethylene imine) (HA-PEI) nanoparticles were characterized for size and charge using dynamic light scattering. Encapsulation of miRNA-34a expressing plasmid was also assessed through gel electrophoresis.

**References**