Quantum Dots for the Targeting of HGF Binding Sites and Downstream Targets in Cancer Chemotaxis

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Abstract

Medulloblastoma is the most common form of malignant pediatric brain cancer. Medulloblastoma treatment has greatly improved in recent years, but still commonly results in disability and severely reduced life expectancy. The study seeks to utilize micro and nano-based technologies to examine chemotaxis of medulloblastoma cells *in vitro*. It has been shown that there is a resistance of medulloblastoma in some cases to radiation. It is proposed that higher Hepatocyte Growth Factor (HGF concentrations in the tumor microenvironment may play a role. The study primarily focuses on fluorescently tagging specific cellular receptors and down-stream targets utilizing liposome encapsulated Quantum Dots (QDs). The targeted application of the technology is to examine changes in growth factor receptor membrane localization, the HGF receptor, c-Met, and downstream molecule movement and activation in response to growth factor concentration gradients. The binding of HGF to c-Met corresponds to the activation of pathways critical for cell survival (PI3K/Akt/mTOR), and proliferation (RAS/RAF/MEK), as well as cell motility. Finding the relationship between cell movement and tumor self-renewal could provide a solution to the problem of treatment resistance.

Background

Brain Tumor Perivascular Niche

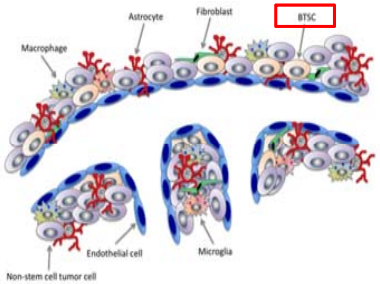

Figure 1: Brain tumor perivascular niche and related cell types[1]

The tumor vasculature forms an interface between healthy tissue, cancer tissue and the blood stream. It is responsible for nutrient and oxygen availability and plays a major role in cell signaling. The perivascular niche is comprised of cells which produce and are influenced by exogenous factors. A subset of vessel adjacent cells have been shown to exist in a stem-like state. It is proposed that this is a result of dedifferentiation of healthy cells. The stem-like properties result in increased resistance to treatment. It has yet to be determined the impact of elevated HGF in the vasculature (cell recruitment or plasticity).

C-Met Signaling Pathway

C-Met is upregulated in radioresistant medulloblastomas. There are two signaling pathways of interest for the study of c-Met activation as it relates to cell migration and survival. RAS/RAF/MEK is implicated in cell motility and tumor growth. PI3K/Akt/mTOR is implicated in cell resilience.

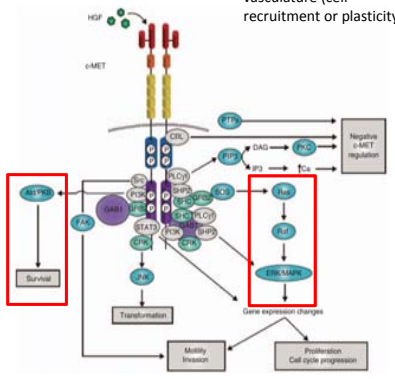

Figure 2: c-Met pathway activation by HGF binding[2]

Microtechnologies

µLane Device and Gradient Characterization

The µLane system is a platform for controlled observation of cell migration in a straight microscale channel molded from PDMS, and bonded to a glass substrate. Hydorstatic pressure is equalized by a bridge channel and 1-Dimensional convective flow can be assumed due to the small transverse cross-section of the channel.

$$\text{1-D Convective diffusion} \quad \frac{\partial C}{\partial t} + V\frac{\partial C}{\partial x} = D\frac{\partial^2 C}{\partial x^2}$$

Time to steady state is dependent on molecule size, solubility, and position within the channel. HGF has a molecular weight of 68 kDa, and would take >12 hours to reach steady state.

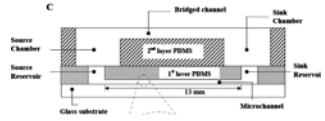

Figure 3: Longitudinal cross-section of µLane device showing diffusion axis, well structure, and bridge [3]

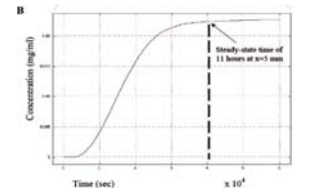

Figure 4: Concentration profile over time for 40 kDa Dextran used to model steady state development[3]

Early Cell Migration and Device Interfacing Experiments

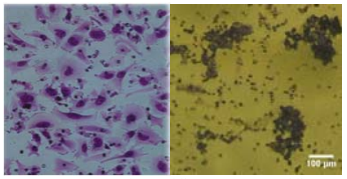

Figure 5: Typical Boyden Chamber assay for DAOY cells (left) and clustered CHLA-01-MED cells (left)

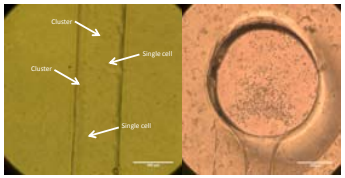

Figure 6: CHLA-01-MED cells in the channel ready for observation (left) and accumulated in well (right)

Boyden chamber assay was used to do preliminary tests on the affinity of CHLA-01-MED cells for varied HGF concentrations. It was found that due to cell clustering meaningful cells counts could not be determined.

The cells were placed in the microchannel as a check for cell compatibility with glass substrates and to examine their previously demonstrated colony-forming tendencies. It was found that though the cells clumped in the wells, they were both clumped and singular within the channel itself.

Nanotechnologies

Liposome Mediated Endocytosis

Liposome encapsulation of streptavidin conjugated QDs provides access to the intracellular space and selective molecule tagging. Conjugated QDs were complexed with anti-c-Met antibody to measure c-Met activation in the presence of varied HGF concentrations as a preliminary step to optimize receptor activity as a function of ligand gradient. The same was done with primary and secondary antibody labeling alone.

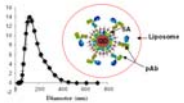

Figure 7: Illustration of QD conjugated with an antibody, and encapsulated in lipid[4]

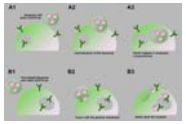

Figure 8: Schematic of liposome endocytosis with encapsulated QDs[5]

Liposome Preparations and Sizing

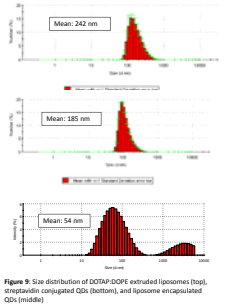

Figure 9: Size distribution of DOTAP:DOPE extruded liposomes (top), streptavidin conjugated QDs (bottom), and liposome encapsulated QDs (middle)

Sizing of QDs, extruded DOTAP:DOPE liposomes, and QDs encapsulated in liposomes was done to verify experimental parameters and optimize extrusion protocols.

A test of endocytosis via liposome-cell membrane fusion was done using non-specific endocytosis of liposome encapsulated QDs in DAOY cells.

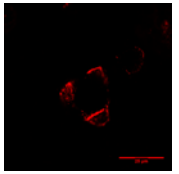

Figure 10: Non-specific QD endocytosis in DAOY cells

Immunocytochemistry

A primary anti-c-Met antibody was used to label activated c-Met under the stimulation of HGF. A secondary fluorescent antibody was used for imaging and expression quantification. 10 ng/mL of HGF showed the greatest receptor activation, and therefore greatest likelihood of creating down stream effects that impact cell behavior.

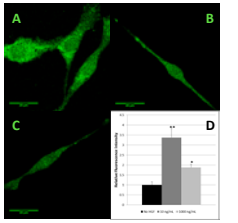

Figure 11: ICC results for CHLA-01-MED cells exposed to 10 ng/mL (A), 1000 ng/mL (B), and 0 ng/mL (C) HGF. Relative c-Met expression (D)

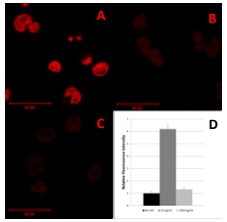

Figure 12: QD655 tagging results for DAOY cells exposed to 10 ng/mL (A), 1000 ng/mL (B), and 0 ng/mL (C) HGF. Relative c-Met expression (D).

QDs with emission wavelength of 655nm were conjugated to a primary c-Met antibody and used to tag c-Met activation under HGF stimulation. Similar to the results obtained by ICC, 10 ng/mL of HGF showed the greatest receptor activation.

Discussion and Future Work

Having established the relative optimal concentration of HGF for c-Met binding activity, characterized the labeling particles, developed protocols for molecule tagging, and verified a migratory platform device, it will be possible to tag intracellular proteins in the c-Met activation pathway, specifically those responsible for cell migration and cell survival. The next step is to study the response of intracellular proteins to HGF stimulation and to observe cell migratory behavior exposed to a concentration gradient within the microdevice.

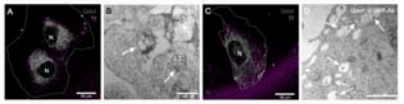

Figure 13: TEM of liposome encapsulated QD endocytosis and translocation to the cell nucleus[4]

References and Acknowledgements

References

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