

## **EphrinA 1-Targeted Nanocarriers for Intracellular Co-Delivery of FdUMP[10] and Phospholipid Gemcitabine to Glioblastoma Cells via the EphA2 Receptor**

Simultaneous delivery of multiple chemotherapeutic agents that target alternative pathways is potentially advantageous in achieving greater toxicity in tumor cells. Nanoscale carriers such as liposomes provide a method to achieve this simultaneous delivery by incorporation of drugs into a single carrier. Further, targeting ligands directed at uniquely expressed or highly over-expressed surface receptors on glioma cells can achieve intracellular delivery for increased selectivity of the drug carriers and their payloads.

Therefore, the proposed studies are driven by two hypotheses. First, because phospholipid gemcitabine analogs and 5-fluorouracil analogs such as FdUMP[10] operate on alternative pathways, we hypothesize that co-delivery of these two agents with a nanocarrier system will achieve greater levels of toxicity than either agent delivered alone. We further hypothesize that the use of a nanocarrier system targeted toward the EphA2 receptor with an EphrinA1 ligand will achieve selective intracellular delivery of these drugs into cells expressing the receptor.

The objective of the proposed studies is to incorporate EphrinA1 ligands into a liposomal nanocarrier and to incorporate phospholipid gemcitabine and FdUMP[10] into these formulations to examine the intracellular uptake and toxicity in cell lines positive or negative for the EphA2 receptor. The proposed system offers selectivity for tumor cells via the targeting of GBM-upregulated receptors and through the use of drugs selective for tumor cells. In order to achieve these objectives and test the hypotheses, we propose the following two specific aims:

Specific Aim 1. Formulate a liposomal delivery system targeted to the EphA2 receptor for co-delivery of phospholipid gemcitabine and FdUMP[10].

A ligand to the EphA2 receptor, EphrinA1, will be conjugated to phosphoethanolamine (DSPE) via a polyethylene glycol2000 (PEG2000) spacer. The resulting conjugate, DSPE-PEG2000-EphrinA1, will be incorporated into a liposomal system containing the phospholipid gemcitabine

analog in the formulation. FdUMP[10] will be incorporated into the liposomes by a remote loading procedure. Liposomes will be characterized for diameter, charge, the number of incorporated targeting ligands, the amount of each drug incorporated, and the rate of drug leakage.

Specific Aim 2. Assess cellular uptake and cytotoxicity of the delivery system in a glioma cell line over-expressing the EphA2 receptor.

The formulations developed in Specific Aim 1 will be applied to U-251 cells, a glioblastoma cell line that expresses the EphA2 receptor. Cellular uptake will be assessed by fluorescently labeling the liposomes and assessing cell-associated fluorescence. Cytotoxicity will be assessed by colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(e-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS) assay. Initial experiments to assess whether cytotoxicity occurs by apoptosis will be conducted by examining caspase-3 activity and through the DNA ladder assay. Cells that do not express the EphA2 receptor (U-87MG and H4 cell lines) will be utilized as negative controls. Figure 3 shows a schematic of the system.

When complete, these studies will allow assessment of the utility of simultaneous delivery of phospholipid gemcitabine and FdUMP[10] through targeting the EphA2 receptor with a liposomal nanocarrier system. In addition to the scientific contribution, the proposed studies also bring together scientists from the School of Biomedical Engineering and Sciences (Dr. Lee at Virginia Tech and Dr. Saul at WFU) and the WFU Comprehensive Cancer Center (Drs. Kucera, Gmeiner, Debinski) interested in the design and development of novel technologies to more effectively treat GBM. Thus, while the primary objective of the studies is to achieve the specific aims described, the collaborations built will provide the opportunity to explore alternative treatment methods beyond those proposed herein.