# Modulation of microRNAs for Treatment of Glioblastoma Multiforme

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**Statement of Purpose:** Glioblastoma multiforme (GBM) is the most common type of primary brain tumor found in adults. This type of malignant glioma accounts for approximately 60% of the 17,000 annually diagnosed cases in the United States [1]. The percentage of patients exhibiting re-occurrence of the tumor is relatively high, despite the use of aggressive multi-modal treatment approaches, including surgery, radiation therapy, and chemotherapy. GBM still represents the worst prognosis of all central nervous system (CNS) cancers [2]. It is believed that resistance to treatments is due to the population of cancer stem cells that have the capabilities of self-renewal and can repopulate the tumor [3]. Therefore, it is imperative to develop a therapeutic strategy that permanently eradicates the tumor.

The therapeutic advantages of microRNAs (miR), short 20-22 nucleotide sequences of non-coding RNA, have shown to be effective in treating various cancers. Levels of specific miRs have been found to be significantly altered in numerous cancers [4]. Specifically, in GBM, it has been discovered that an array of miRs including, miR-21 and miR-34a become highly deregulated in the oncological cells [5]. MiR-21 is anti-apoptotic, whereas miR-34a is pro-apoptotic. Therefore, we hypothesize that by modulating the two, we will increase the ability of the glioblastoma cells to undergo programmed cell death. This will be accomplished by using a targeted cationic liposomal based system to modulate apoptosis related miRs in glioblastoma cells, including the cancer stem cell population, as an effective therapeutic strategy.

**Methods:** *Liposomal Formulation* – Cationic liposomes were created at 3:1 molar concentrations of cationic lipid and neutral lipid respectively. Lipids were dissolved in methanol and chloroform and rotor-evaporated. Lipids were then hydrated in HEPES buffered saline and sequentially extruded using a LIPEX™ Extruder (Northern Lipids). An epidermal growth factor receptor targeting peptide was cross-linked to the surface of the liposome utilizing a post insertion technique. Liposomes were then characterized for size distribution and zeta potential using a digital light scattering system.

*miR Transfection* – Glioblastoma cell lines U87MG and A172, transformed glioblastoma cell lines U87NS and A172NS were plated at 10,000 cells per well in a 24-well plate and transfected with 12pmols of miR-34a and 30pmols of the antisense oligonucleotide (ASO-21) sequence to miR-21 individually and in combination. All conditions utilized 11nmols of cationic liposome for transfection and were compared to commercially available Lipofectamine (Invitrogen), the gold standard for RNA transfection. Cellular viability was assessed daily using the Cell Counting Kit-8 Assay (Dojindo).

*Stem Cell Isolation* – Primary glioblastoma cells obtained from University of Massachusetts Tissue Bank will be cultured in cancer stem cell defined media. For isolation of cancer stem cell population, CD133 (MACs) staining and fluorescence automated cell sorting will be used to isolate cells of interest.

*qRT-PCR* – After isolating mRNA and miR (RNAeasy Mini Kit, Qiagen), qRT-PCR will be performed to quantify levels of apoptosis related genes, such as p53, BCL-2, Caspases 3 and 8, and miR-34a and -21.

**Results:** *Liposomal fabrication* – Cationic liposome characterization yielded an average diameter of 155.2nm and a zeta potential of 28.0 ± 4.83mV. Stability was verified at 3 months after fabrication. Cytotoxicity studies were performed and did not demonstrate statistically significant toxic effects in confluent cellular monolayers compared to non-treated media only condition.

*miR Transfection* – In transfected A172 glioblastoma cell line, combination treatment of miR-34a and ASO-21 was statistically significant compared to media, liposome only controls, as well as the individual miRNA 34 and ASO21 treatments in reducing cellular viability over 7 days (Fig 1). Also, transfected with non-targeted liposomes, no statistically relevant changes in viability were detected up to day 3. Transformed neurospheres, U87NS, also showed decreased cellular viability with the combination miR treatment. Current studies are being performed using primary glioblastoma cells.

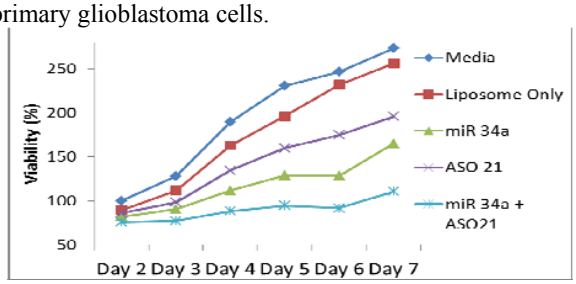


Figure 1. Cellular viability after miR transfection of A172 cells, normalized to day 2 media controls.

*qRT-PCR* - PCR results should suggest that after transfection of miR34a and ASO-21 there is an increase in miR-34a and decrease in miR-21 levels relative to untreated cellular controls. Additionally, apoptosis pathway modulation should yield increased levels of BCL-2, p53, and caspases 3 and 8, indicative of cells undergoing apoptosis.

**Conclusions:** Cationic liposomal delivery of miR effectively targets cells of interest with minimal cytotoxicity due to liposomes alone. Delivering miRs in combination to reduce the cells anti-apoptotic properties and in the meantime increase the miRNA that promotes apoptosis is an effective treatment regime to induce apoptosis and retard the growth progression of the tumor. In our future studies we plan to deliver these miRs in combination in an *in vivo* rodent model.

**References:**

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