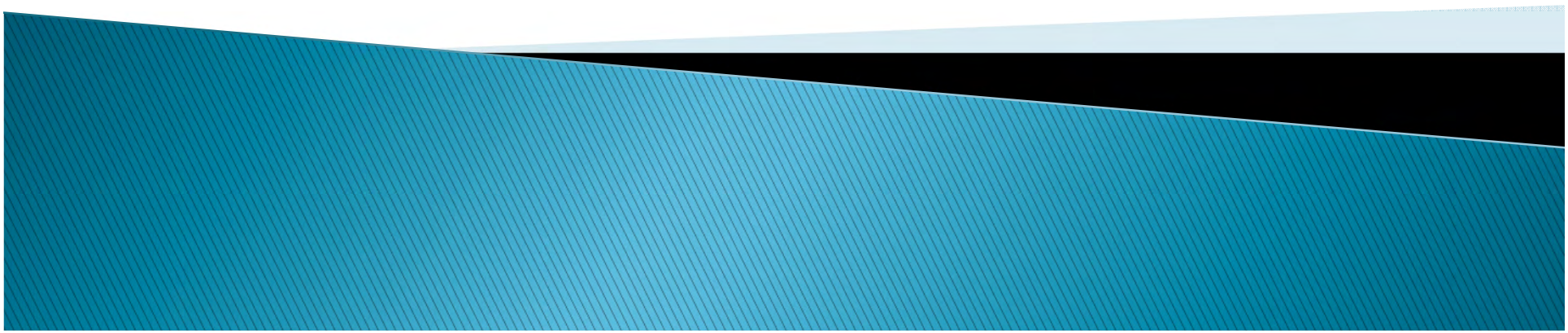
The Role of the MicroRNA-106b-25 Cluster in the Promotion of Gliomagenesis

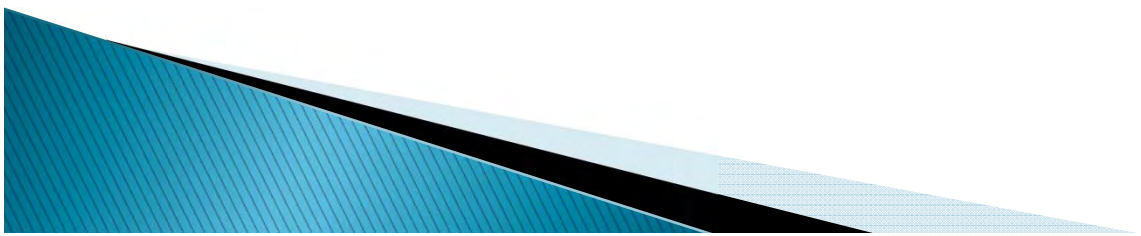
By Jovy Paily

School: Briarcliff High School



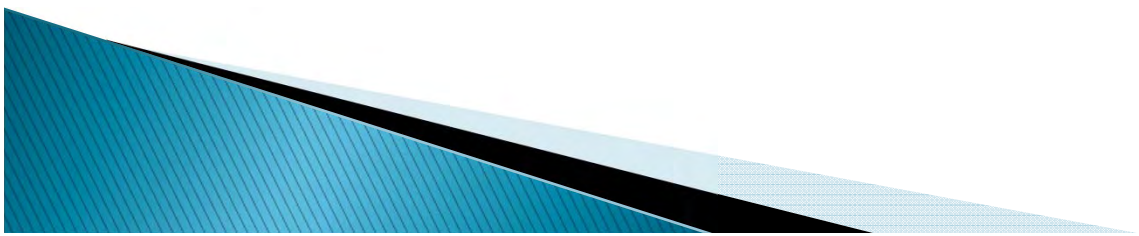
What's the Problem?

- Brain tumors are the second leading cause of cancer-related deaths in
  - 1. Children under age 20
  - 2. Males up to age 39
  - 3. Females under 20
- Additionally, 13,000 people die from brain tumors every year
- Brain tumors are presently incurable
- Scientists have tried chemotherapy, radiation, and surgery
- Difficulties like damaging healthy brain tissue, tumors growing back, and chemotherapy failing



Central Research Problem

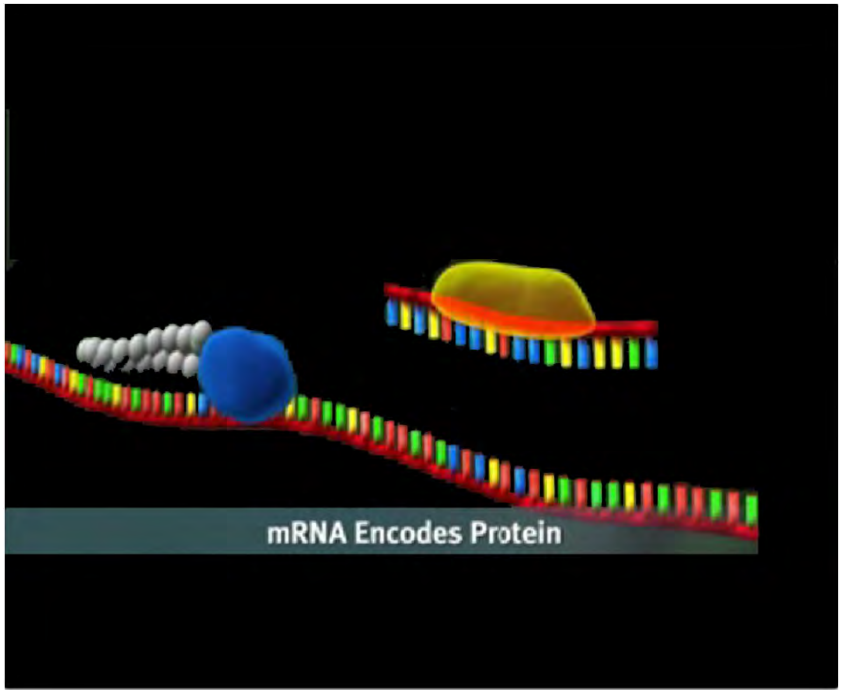
- How can we decrease the cell proliferation of brain tumors without the former popularized methods?

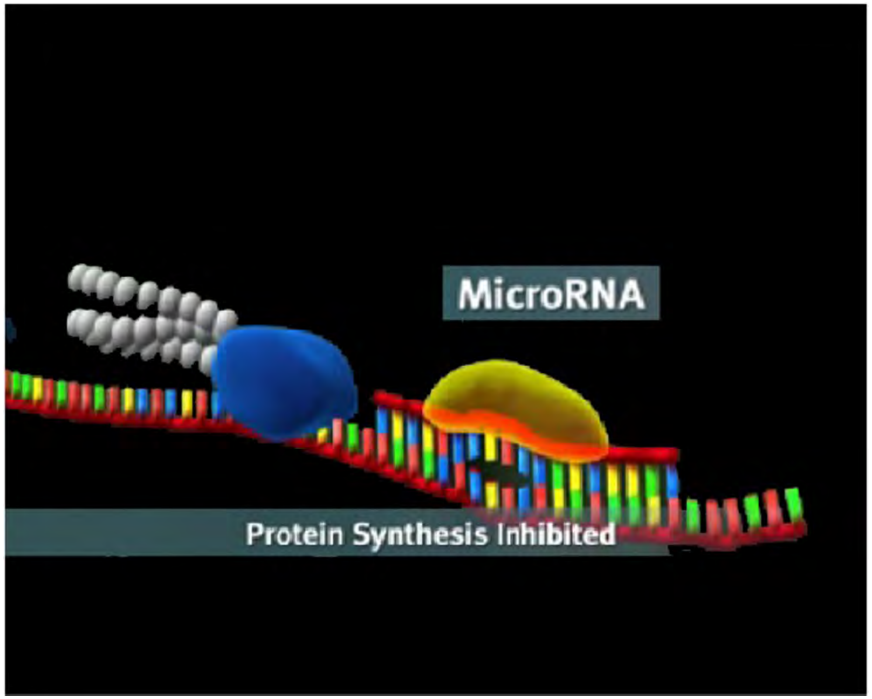


What are MicroRNAs?

- Small, non-coding RNAs that regulate gene expression
- Bind to the 3'UTR region of mRNAs
- They either block the translation of the mRNA or directly degrade it

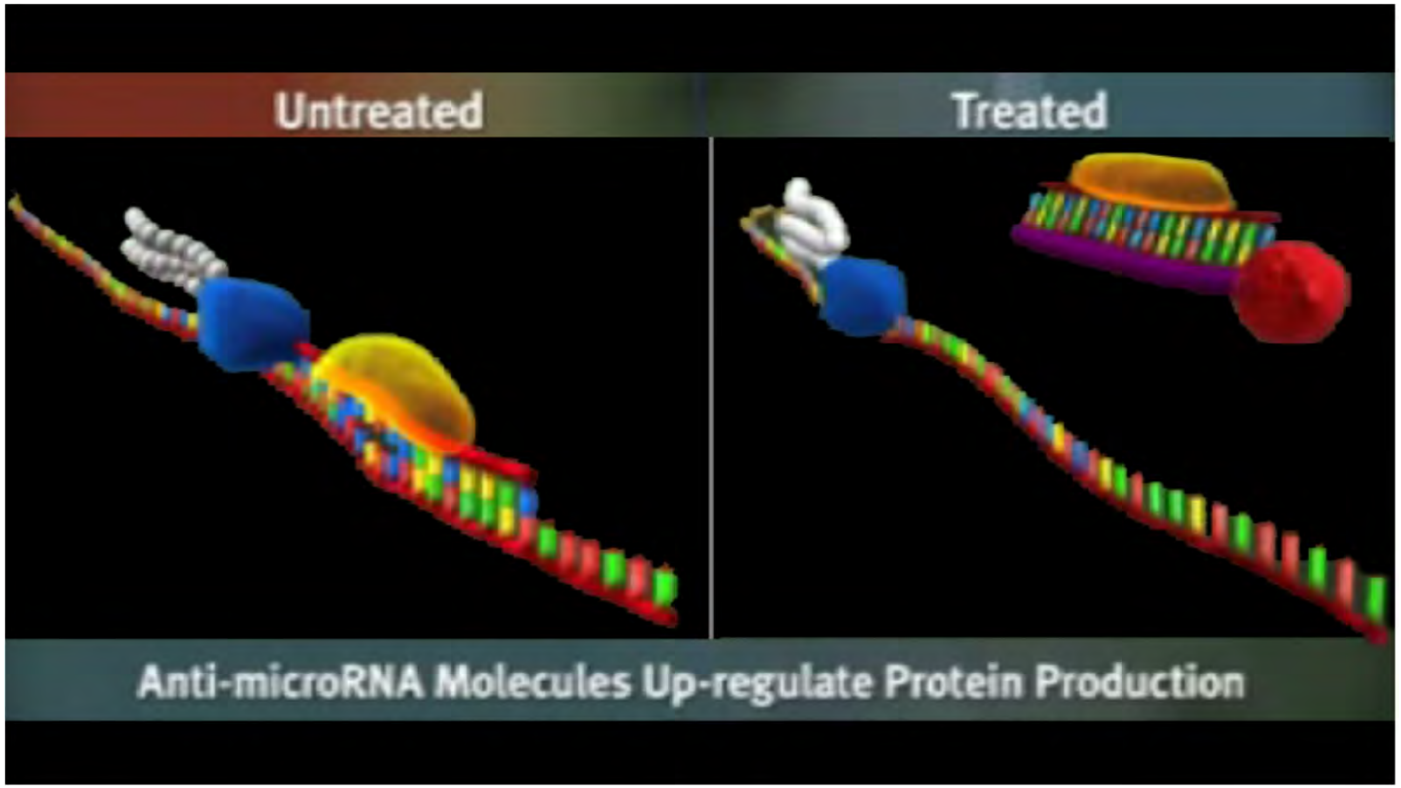
How MicroRNAs Work





Rosetta, 2009

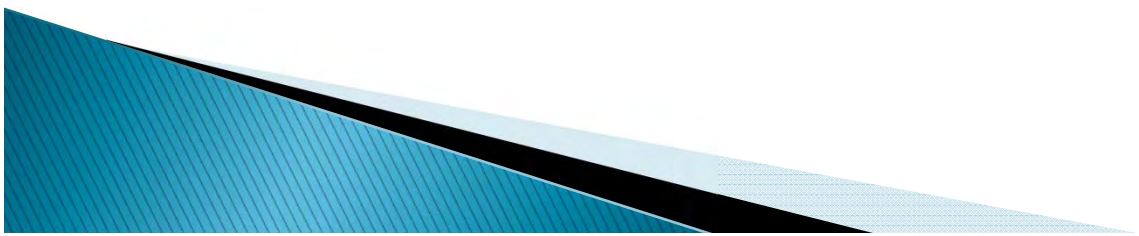
How Anti-mirs work



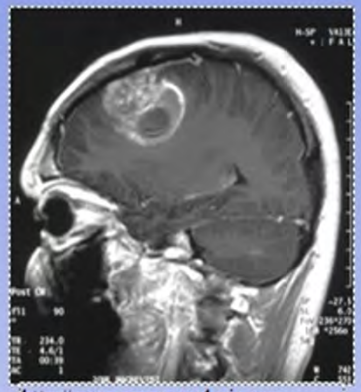
Rosetta, 2009

What is a Glioma?

- A type of cancer that starts in the brain
- Poor Prognosis
  - Of 10,000 Americans diagnosed each year with malignant gliomas, about half are alive 1 year after diagnosis, and 25% after two years (Kennedy's, 2009)
- Classified by grade of severity. Grade 1-4



Glioblastoma Multiforme



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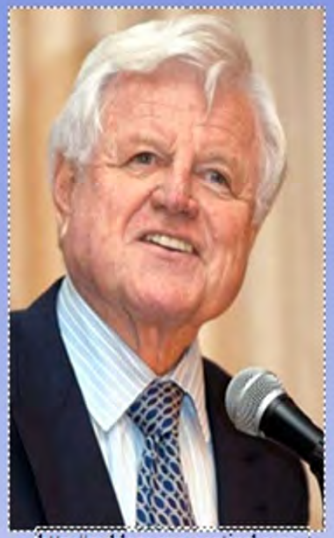

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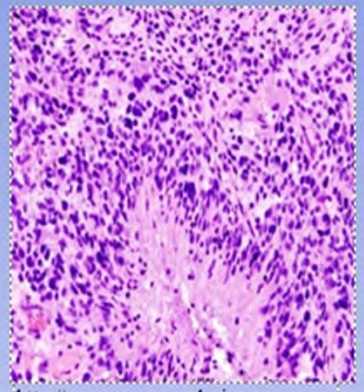

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. The glioma I studied is called Glioblastoma multiforme.

. This is the same brain tumor that former Massachusetts Senator, Ted Kennedy, died from

. It is the most common and malignant type of primary brain tumors.

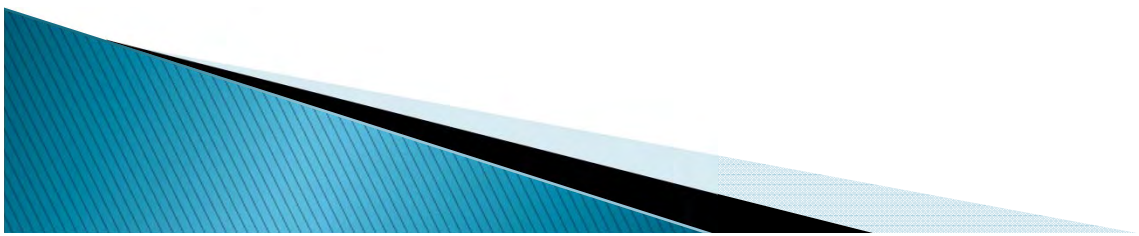
. Many genetic alterations in human GBMs have already been described but few have been shown to be directly linked with microRNAs. This is key because it shows the novelty of my project.

MiRNAs involved in GBMS

- When microRNAs are involved in cancers, they tend to be one of two things: oncomirs or tumor suppressors

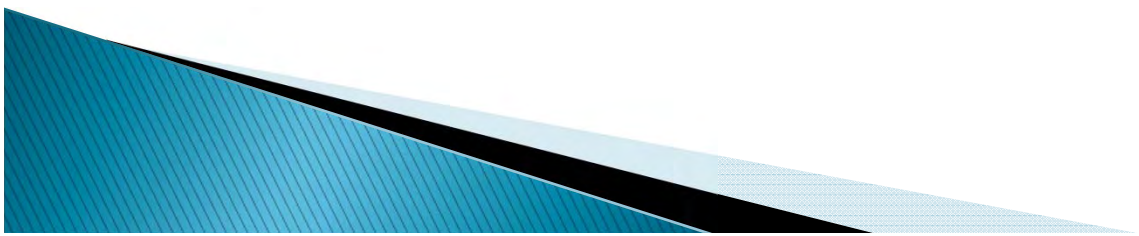
- **Oncomirs** are microRNAs that are often upregulated in cancers and display oncogenic properties. These oncomirs often suppress essential tumor suppressors, contributing to the growth of cancers

- **Tumor Suppressor** microRNAs are often downregulated in cancers and can often be the reason for the uninhibited growth of the cancer



Oncomirs

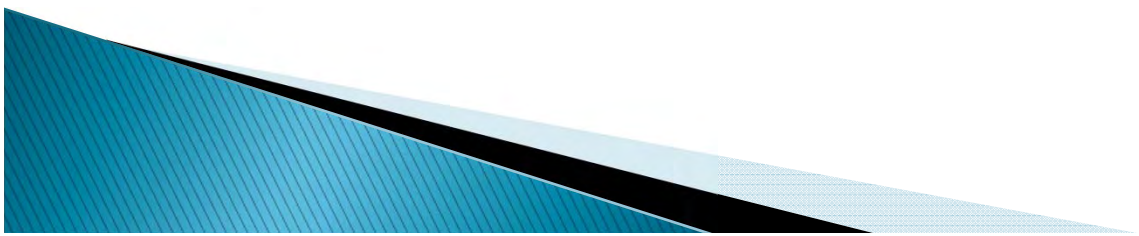
- MiR-221: increased in GBMs and targets p27 (Ciafre et al. 2005)
- MiR-21: increased in GBMs (among others); targets p53 (Corsten et al. 2007).
- MiR-26a: increased in GBMs and targets p21 (Huse, 2009)



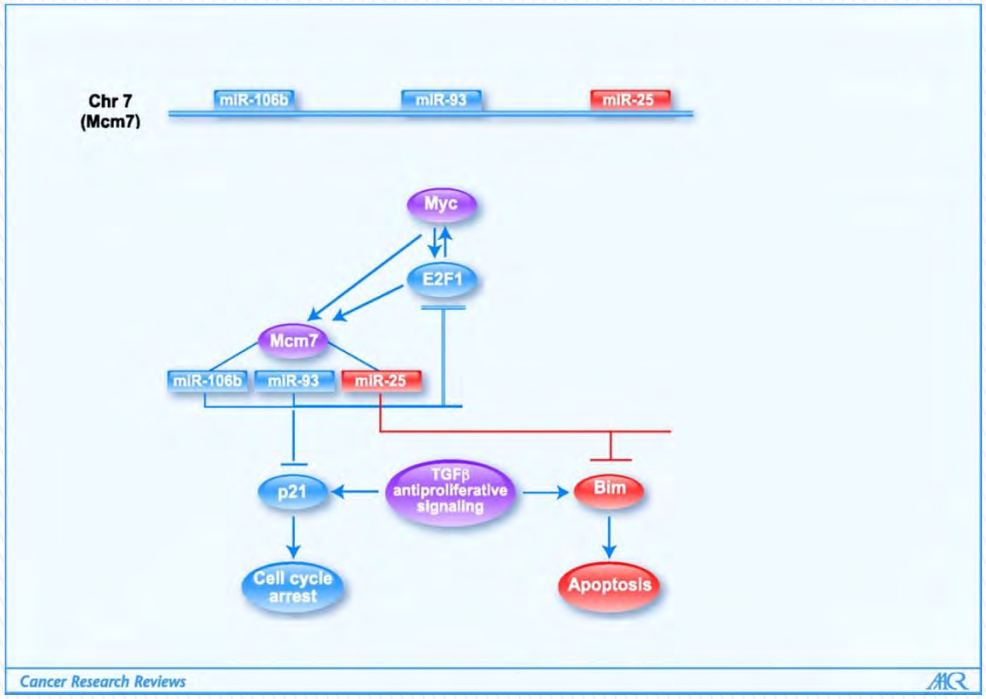
Tumor Suppressors

- MiR-124a and miR-137: decreased in GBM; reduce glioma cell proliferation and induce differentiation *in vitro* (Silber, 2008)

- MiR-7: decreased in GBM and targets EGFR; reduces proliferation, survival, and invasiveness in glioma cell lines (Kefas, 2008)



MicroRNA-106b-25 Cluster



A viable target for anti-mir treatment

- Microarray
- MicroRNA-106b, MicroRNA-25, and MicroRNA-93
- MiR-106-25 is strikingly similar to the miR-17-92 cluster
- Overexpressed in multiple tumor types
- Correlated with the expression of genes that regulate the cell cycle (Ivanovska, 2008)
- Knock-down studies show that it is essential for cell proliferation (Ivanovska, 2008)

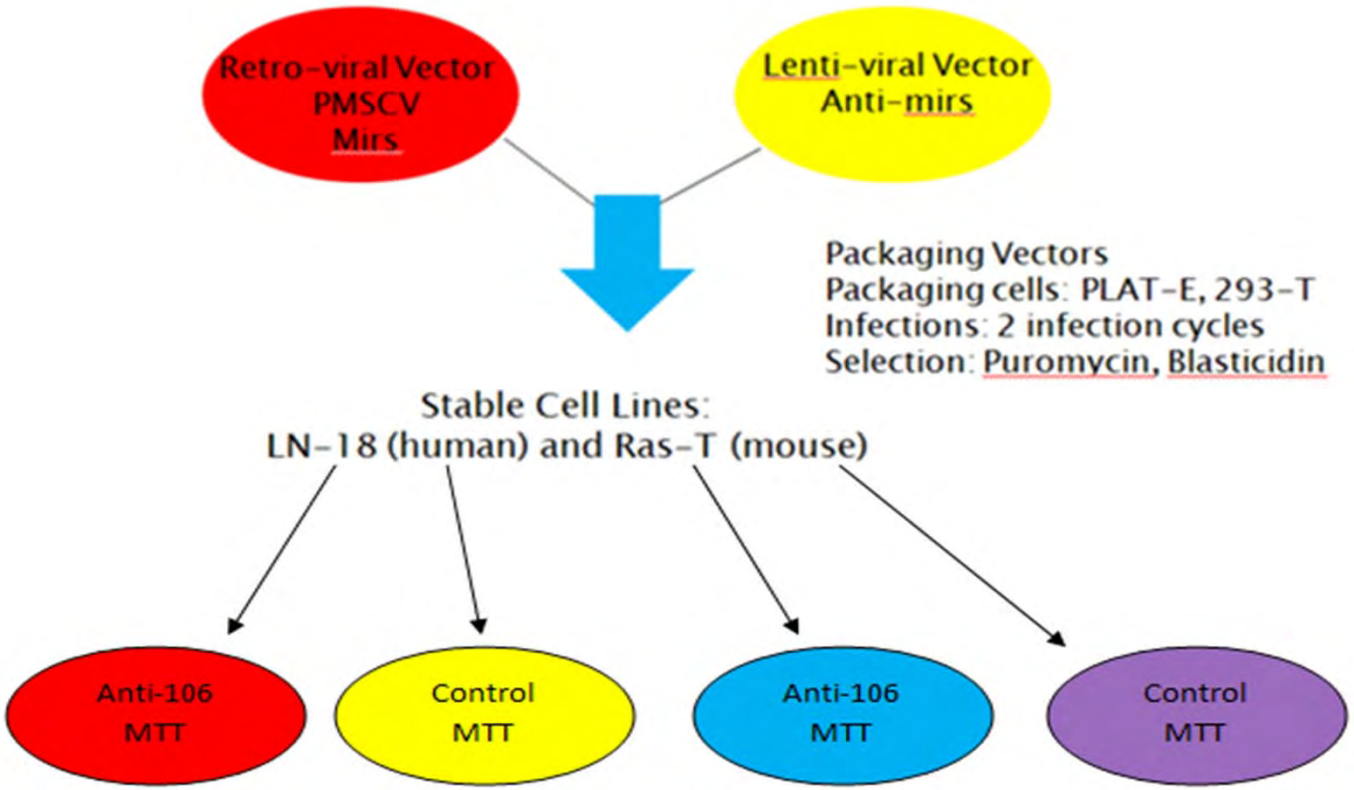
Hypothesis

- MiR-106b and miR-25 contribute to the pathogenesis of malignant gliomas by modulating cell proliferation and apoptosis.

- Questions addressed:
  - a. Does knockdown of miR-106b cause a decrease in cell proliferation of gliomas?

  - b. What molecular pathways do miR-106b and miR-25 regulate?

In Vitro Modeling



Transfection

- MiR-Vec constructs for miR-106 were purchased to perform this experiment
- The microRNA plasmids were inserted into PLATE-E packaging cells
- The anti-mir plasmids were inserted in 293-T cells

Infection

- Following two days of incubation at 37°C, the target cells, Ras-T and LN-18 cells were infected with the retrovirus produced by the plat-E cells and the lentivirus produced by the 293-T cells.
- The infection process consisted of a two cycle infection, with each cycle lasting 3 hours.
- Using a 0.45mM filter, the plat-E cells and the 293-T cells were removed, and Polybrene (5ug/ml final concentration) (Invitrogen) was added before adding to the target cells.
- After the final three hour incubation, the retrovirus and lentivirus were removed, and DMEM medium with serum was added.

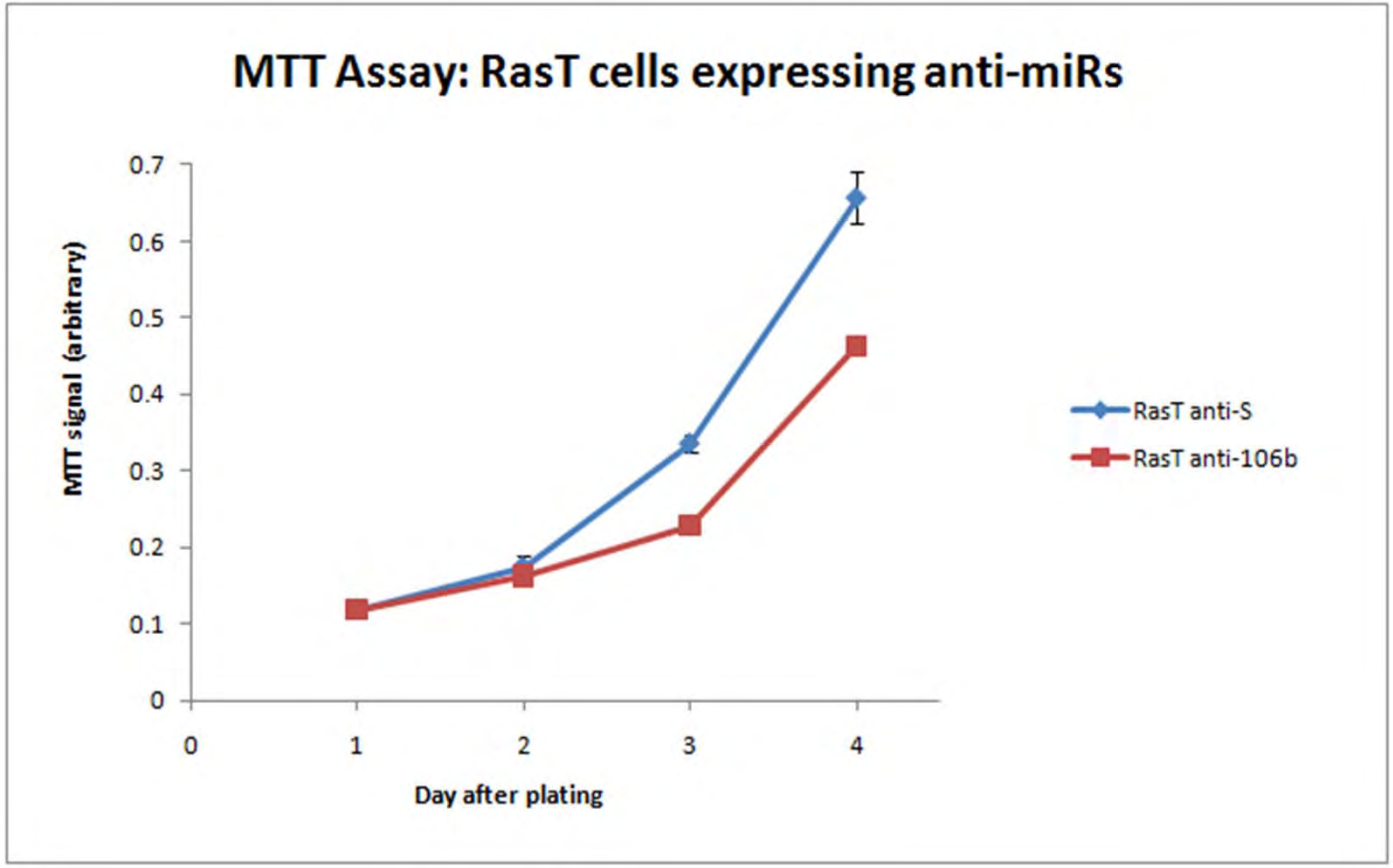
MTT Cell Proliferation Assay

- Colorimetric Assay
- Measures decrease in Methylthiazol Tetrazolium which is used in cell metabolism
- MTT measured every other day for 7 days
- MTTs performed twice
- The experiment was repeated twice
- The results were averaged and plotted using Microsoft Excel 2007

Western Blots

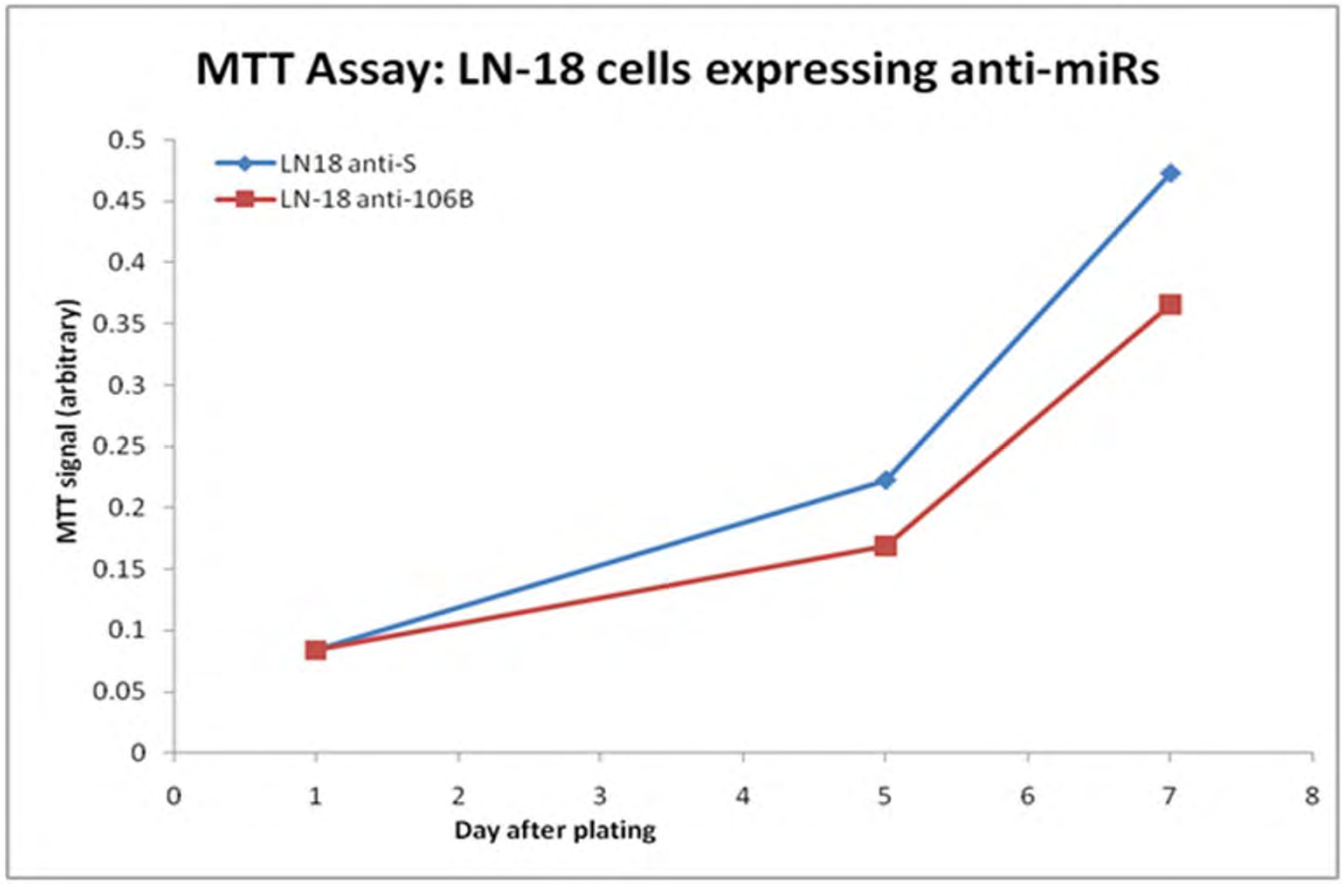
- The cells were lysed to extract the protein
- The proteins were transferred to PVDF membrane
- Primary antibodies (actin, p21, and Bim) were used. Then washed. Then Secondary Antibodies were used
- Membranes were then exposed to film

Mir-106b knockdown decreases cell proliferation in Ras-t cells



28%

Mir-106b knockdown decreases cell proliferation in LN-18 cells



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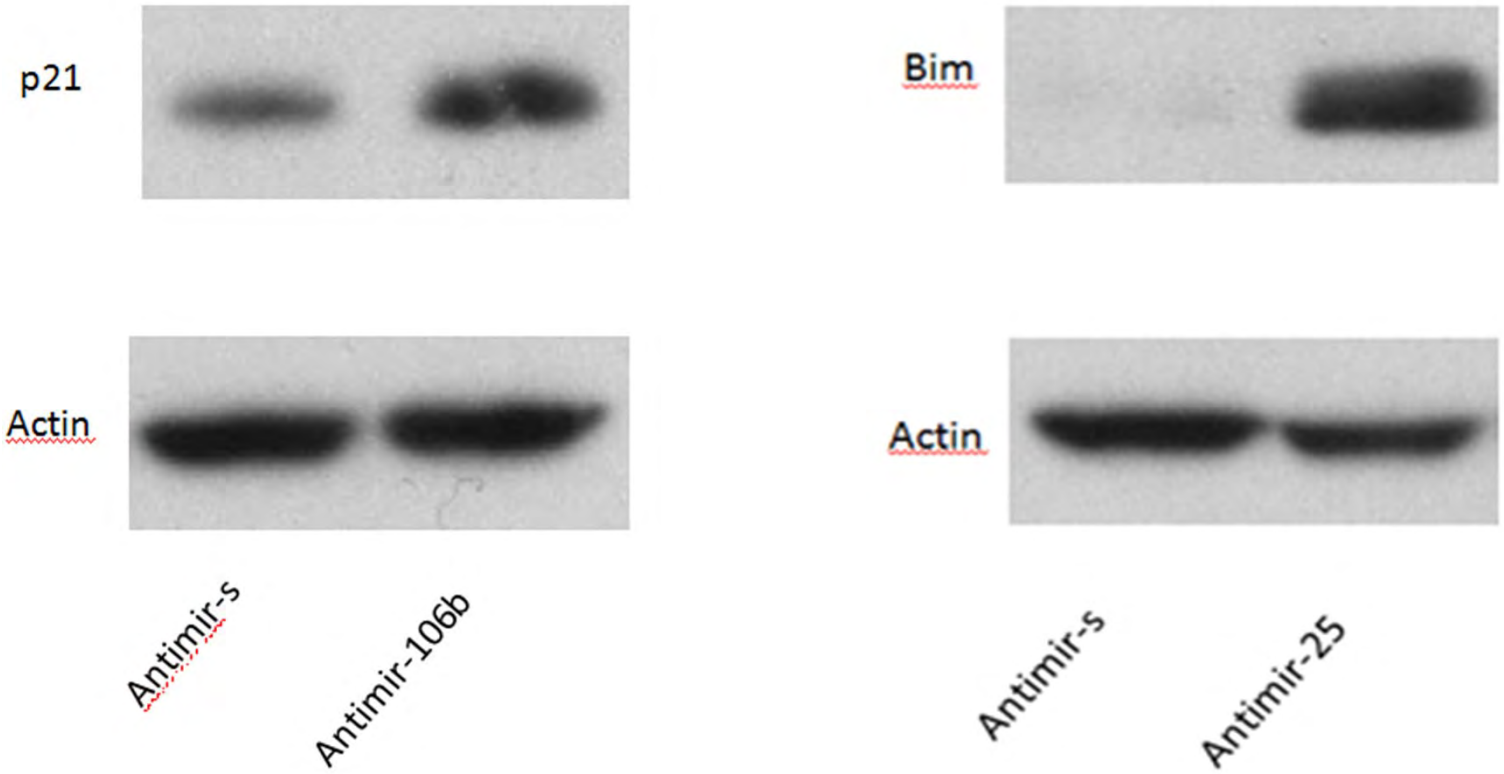
Identifying Targets of Mir-106 and Mir-25

RasT Cells



Discussion

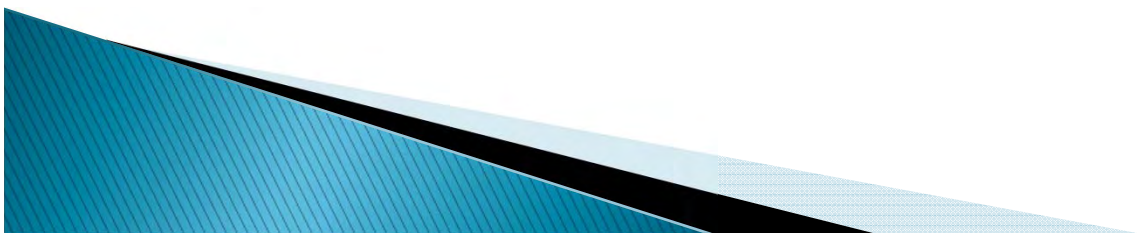
- MiR-106b has shown to have consistent roles in other cancer forms. As with studies conducted with hepatocellular carcinomas, miR-106b has been shown to be overexpressed by two-fold as compared to normal tissue (Ivanovska,2008)

- The identified role of miR-106b in this paper as a regulator of p21 is consistent with reports coming from esophageal adenocarcinomas, gastric cancers, prostate cancers, pancreatic cancers (Kan, 2009).

- These results are consistent with studies confirming the role of miR-106b in cell cycle progression, specifically in the G1-to-S transition (Ivanovska, 2008).

Future Research

- Running apoptosis assays on mir-25
- Cell Cycle analysis of MiR-106
- The use of mir-106b-25 as a biomarker
- In Vivo experiment



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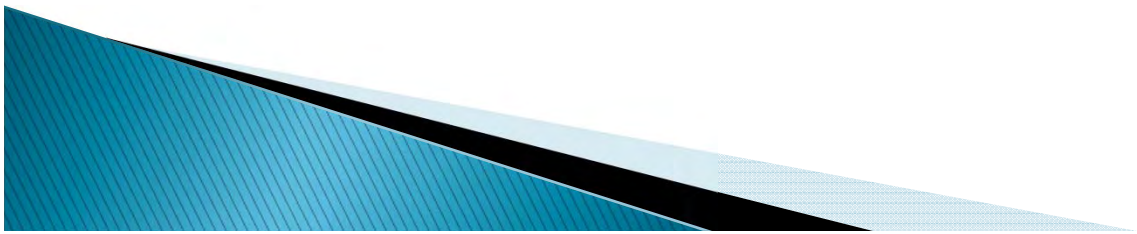

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Summary

- MiR-106b-25 is frequently upregulated in high-grade gliomas.
- MiR-106b knockdown in murine and human glioma cells leads to reduced cell proliferation and tumor development.
- MiR-106b targets the tumor suppressor p21 in glioma cell lines
- MiR-25 targets the tumor suppressor Bim in glioma cell lines