Expression of miR-145 and miR-449 in U87 Glioblastoma Cells

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Abstract

MicroRNAs are known to play a critical oncogenic role in glioblastoma. Several miRNAs have been shown to play roles in growth and cell cycle control in glioblastoma, and have potential as diagnostic markers or as therapeutic targets. miRNA-145 is overexpressed in glioblastoma and is thought to downregulate srGAP1 (SLIT-ROBO Rho GTPase-activating protein1), which promotes an invasive phenotype. This is in contrast to miRNA-145's proposed tumor-supressive role in many other cancers (1). miRNA-449 is thought to be a tumor suppressor that interrupts the cell cycle and induces apoptosis via suppression of E2F1, CCND1, and GPR158. Therefore it is highly downregulated in many tumor cells (2,3). Both miRNAs-145 and -449 are the topic of continued inquiry in understanding their expression and their molecular targets in glioma cells.

Here we attempt to establish the baseline expression of miRNA-145 and miRNA-449 in the U87 cell line using RT-PCR. Weekly passaging of cells was carried out, followed by RNA isolation and RT-PCR. Baseline average concentrations were established for miRNA-145 and miRNA-449 using a set of serial dilutions and a standard curve. Further experimentation will be required to establish a baseline concentration for these miRNAs in healthy glial cells.

Objective: Quantify miRNA-145 and miRNA-449 expression in U87 glioblastoma cells using RT-PCR and establish baseline concentrations using a standard curve.

Results

Table 1. Average Ct and Concentration Values of U6 control, miRNA-145, and miRNA-449 Samples. Conc. values were calculated using the equation from the standard curve (Figure 1). Conc. (nM) appears to decrease as the Ct values of the miRNA samples increase.

	Avg. Ct	Avg. Conc. (nM)
U6 (Control)	19.53	4.97×10^{-2}
miR-145	28.48	6.75×10^{-5}
miR-449	29.63	2.75×10^{-5}

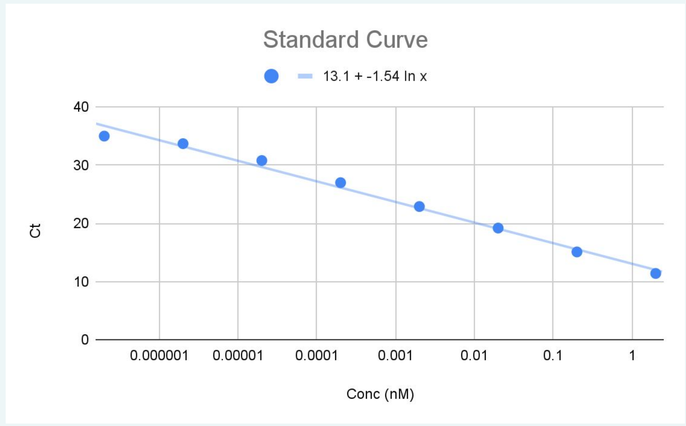

Figure 1. Standard Curve of the relationship between Ct value and Concentration (nM). Created using Conc. from serial dilutions and average Ct from several trials. We are able to determine the conc., or expression, of different microRNAs in glioblastomas using the standard curve based on its Ct value.

Conclusions

- This experiment established the baseline expression of miRNA-145 and miRNA-449 in our sample of glioblastoma cells.
- Fewer number of cycles required to reach the threshold value relates to a higher concentration of miRNA.
- The next step would be to establish the baseline expression of healthy glial cells.
- Further research should be continued to establish a more accurate standard curve. Further research should also include live cells through testing on rats or other animals.

Methods

1. Passaging adherent Cells

In this study, we observed a U87 cell line. To prevent our cell line from overgrowing, we weekly diluted and split our cell culture. We accomplished this by following these steps:

1. Wash cells in 5 mL of PBS
2. Add 5 mL of warmed trypsin and then incubate for 5 minutes
3. Add 5 mL of MEM alpha media to neutralize the trypsin.
4. Centrifuge and resuspend cells in 5 mL of media
5. Take 1 mL from this mixture and add it into a flask containing 20 mL MEM alpha media

2. RNA Isolation

We isolated the miRNA samples in our cells by using the mirVana™ RNA isolation kit protocol.

3. microRNA RT-PCR

A master mix was made from 1.5 uL of 10X RT Buffer, 0.15uL of 25x dNTP mix, 3uL 10X RT microRNA primer, 1uL of Multiscribe RTase, 0.19 uL of RNase inhibitor and 4.16 uL of Nuclease-free water.

10 uL of RT master mix was then combined with 5 ul of diluted RNA (10ng/ul) in PCR tubes. The tubes were then put in a thermocycler and the microRNA RT protocol was run.

A PCR mix was then prepared in new PCR tubes. It contained 5 uL of master mix, 0.5 uL of target primer, 3.5 uL of water and 2.0 uL of cDNA from the RT.. The tubes were then run through a thermocycler set to run the PCR protocol. The thermocycler was then able to give the Ct value of each tube.

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