MicroRNA-212 and -375 as Putative Regulators of Stem-like State in Glioblastoma

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Introduction

Only a small number of factors have been shown to regulate the cancer stem-like cell (CSLC) state in GBM. We sought to identify miRs affecting GBM CSLC that might confer sensitivity to radiation. We hypothesize that miRs driving proliferation and differentiation might serve to induce radiosensitivity in GBM CSLC.

Methods

Microarrays, containing 754 human miRs based on Sanger miRBase v14, were used to create a comprehensive expression profile, we pre-determined statistical significance to be p = 0.001. GBM neurosphere lines 20913 and 060909 were maintained in Neurocult culture medium. Lentiviral vectors were created for miR-212 and miR-375 plasmid insertion into cell lines. Resazurin and MTS assays were performed to assess cellular proliferation in each condition. Clonogenicity was assessed using soft agar assays in the 20913 cell line. Fluorescence immunocytochemistry was performed using primary antibodies including ki-67, Cleaved caspase 3, CD133, and CD15.

Results

A miR microarray was performed, comparing 3 differentiated GBM tumor samples to 3 GBM CSLC lines. The 20913 and 060909 GBM neurosphere lines were used to test selected miRs in vitro.

Proliferation was increased with induced miR-212 or miR-375 overexpression, by growth assays and ki-67 expression. After one-time radiation treatment of 4 Gy, cells overexpressing miR-212 or miR-375 exhibited significantly diminished proliferation.

With miR-212 and miR-375 overexpression, neurosphere lines witnessed a marked decrease in putative GBM CLSC markers CD133 and CD15. Conversely, radiation treatment resulted in increased percentages of CD133 and CD15 positivity among cells overexpressing miR-212 and miR-375.

Figure 1: Functional effect of induced miR-212 & 375 overexpression

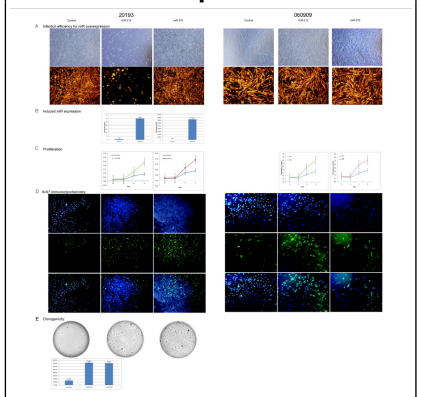
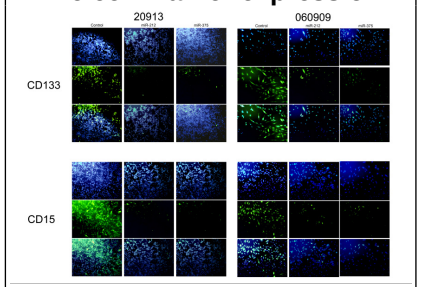
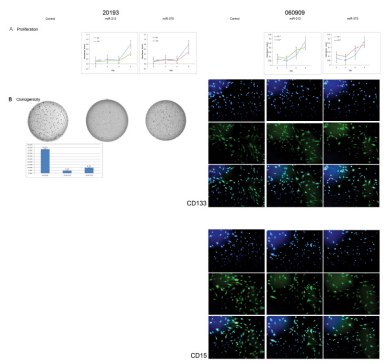


Figure 2: Putative cancer stem-like cell marker expression



Putative cancer stem-like cell markers CD133 and CD15 demonstrated significantly higher expression in control neurosphere cells relative to cells overexpressing miR-212 and miR-375, prior to radiation treatment.

Figure 3: Post-radiation functional studies



After a single 4Gy treatment, cellular proliferation and clonogenicity in miR-212 and miR-375 overexpressing cells were significantly diminished relative to control, where these cells had exhibited increased proliferation and clonogenicity without radiation treatment. In addition, putative cancer stem-like cell markers CD133 and CD15 were highly expressed after radiation treatment in all conditions.

Conclusions

MiR-212 and miR-375 are thought to induce differentiation among GMB CLSC, thereby increasing radiosensitivity. MiR-212 and miR-375 might offer avenues by which to improve care of GBM patients.

Learning Objectives

By the conclusion of this session, participants should be able to:

1) Describe the potential importance of microRNA function in GBM CLSC biology.

2) Discuss, in small groups, the potential risks and benefits of microRNA-based treatment regimens.

3) Identify a potential mechanism by which microRNA's drive GBM CSLC biologic function.

References

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