less than 12 months (Legler et al., 1999). Composed of a heterogeneous mixture of poorly differentiated neoplastic astrocytes, malignant gliomas primarily affect adults, and preferentially occur in the cerebral hemispheres. Treatment of malignant gliomas is palliative and includes surgery, radiotherapy, and chemotherapy and frequently recur after radiation as focal masses (Garden et al., 1991).

Despite the recent advances in malignant glioma treatment, patients relapse after an initially favorable response to therapies. This is potentially due to a sub-population of cells with tumorigenic potential that are intrinsically resistant to therapy. These cells, referred to as tumor–initiating "stem cells" or cancer stem cells, were identified in a variety of solid tumors including brain tumors (Galli et al., 2004; Hemmati et al., 2003; Singh et al., 2004). This cellular fraction of the tumors is capable of initiating tumors similar to the parental tumor when transplanted into a secondary site (Reya et al., 2001).

It has been found that a malignant glioma is a heterogeneous tumor composed of a small portion of tumor-initiating cells, i.e., glioma stem cells (GSCs). GSCs are phenotypically similar to normal neural stem cells (NSCs). They express CD133 and Nestin, which are characteristic marks for NSCs. Like NSCs, GSCs also possess the self-renewal potential. On the other hand, GSCs have the potential to recapitulate original polyclonal tumors when xeno-grafted to nude mice. They are chemo-resistant and radiation-resistant and therefore responsible for tumor progression and recurrence after conventional therapy.

Altered expression (e.g., reduced/loss-of expression) of a miRNA may lead to aberrant expression of its target proteins, resulting in altered phenotype. For example, over-expression of miR-34a inhibits proliferation, disrupts tumorsphere formation, or induces differentiation of GSCs, but fails to eliminate GSCs (Guessous et al., 2010; Li et al., 2009).

SUMMARY

The present disclosure is based on unexpected discoveries that (i) the levels of microRNA-138 (miR-138) increase in glioma stem cells (GSCs) isolated from MG patients and in GBM patient tumor samples (primary and secondary) as compared to those in normal neural stem cells (NSCs) and in healthy controls, and (ii) knockdown of miR-138 results in suppression of cell proliferation, induction of apoptosis, and reduction of tumorigenicity.

These discoveries demonstrate that miR-138 is a reliable biomarker for monitoring brain tumor progress, assessing efficacy of MG treatment, and identifying GSCs, the presence of which is indicative of an increased risk in MG development, progression, and metastasis. They also demonstrate that miR-138 is a target in treating malignant glioma, such as grade- III anaplastic astrocytoma and grade-IV glioblastoma multiforme.

In one aspect, there is provided use of an oligonucleotide targeting miR-138 in the manufacture of a medicament for treating malignant glioma in a subject in need thereof.

Disclosed is a method for treating malignant gliomas, including administering to a subject in need thereof an effective amount of an oligonucleotide (an antigomir) specifically targeting miR-138, which can be a hairpin oligonucleotide. The oligonucleotide can comprise a core sequence having the nucleotide sequence of 5'-CGGCCTGATTCACAACACCAGCT-3' (SEQ ID NO.1), which targets the sequence of human mature miR-138. In one example, the oligonucleotide has the nucleotide sequence of 5'-GAGCTGGTATTGTGAATCAAGCAGCTTCCTGTCAGCGGCCTGATTC ACAACACCAGCTTTTTT-3' (SEQ ID NO:2). In another example, one or more of the nucleotides in the oligonucleotide are modified by, e.g., a 2'-O-methoxyethyl group, a 2'-O-methoxy group, or a phosphorothioate group.

In another aspect, there is provided use of an oligonucleotide targeting miR-138 in the manufacture of a medicament for inhibiting glioma stem cell growth, wherein the medicament is to be contacted with the glioma stem cell.

Disclosed is a method for inhibiting GSC cell growth, including contacting GSCs with an effective amount of any of the oligonucleotides described above. In this method, the contacting step can be performed by administering the oligonucleotide to a subject in need of the treatment.

In another aspect, there is provided use of an oligonucleotide targeting miR-138 in the manufacture of a medicament for prolonging survival of a subject suffering from malignant glioma.

Disclosed is a method for prolonging survival in malignant glioma patients (e.g., human patients) with an effective amount of any of the above-described oligonucleotides. Optionally, the patients subjected to this method have undergone or are in the course of conventional therapy, including surgery, radiotherapy, and/or chemotherapy.

Any of the methods described above can be performed, when applicable, on a human subject having, suspected of having, or at risk for malignant glioma. The oligonucleotide administered to such a subject can be in an amount sufficient to suppress GSC proliferation or in an amount sufficient to induce GSC apoptosis in the subject.

Also within the scope of this disclosure are (a) a pharmaceutical composition for use in treating MG, prolonging survival of a MG patient, or inhibiting GSC growth, the

pharmaceutical composition containing any one or more of the oligonucleotides described herein and a pharmaceutically acceptable carrier; and (b) the use of the just-described pharmaceutical composition in manufacturing a medicament for any of these purposes.

In addition, the present disclosure provides methods for monitoring MG progress, methods for assessing efficacy of a MG treatment, and methods for identifying GSCs, all relying on the level of miR-138 as a biomarker.

In another aspect, there is provided a method for monitoring tumor progress in a malignant glioma patient, comprising: quantifying levels of miR-138 in samples containing glioma stem cells that have been obtained from the patient at multiple time points, and assessing whether malignant glioma progresses in the subject, wherein an increase in miR-138 levels over time indicates progress of malignant glioma.

A method for monitoring MG progress includes at least the following steps: obtaining samples containing tumor cells from a patient (e.g., a human patient or a laboratory animal) suffering from MG at multiple time points, quantifying the levels of miR-138 in the samples, and assessing whether MG progresses in the subject. An increase in miR-138 levels over time indicates progress of MG. In this method, the quantifying step can be performed by any conventional method, including, e.g., quantitative real-time PCR or in-situ hybridization.

In another aspect, there is provided a method for assessing efficacy of a malignant glioma treatment in a malignant glioma patient, the method comprising: quantifying levels of miR-138 in samples containing tumor cells that have been obtained from the patient before, during, and/or after the treatment, and assessing efficacy of the treatment based on the levels of miR-138, wherein no change or a decrease in the levels of miR-138 after the treatment or along the course of the treatment indicates that the treatment is effective.

A method for assessing efficacy of a MG treatment in a patient (e.g., a human patient or a laboratory animal) includes obtaining samples containing tumor cells from the patient before, during, and/or after the treatment, quantifying levels of miR-138 in the samples via a conventional method (e.g., those listed above), and assessing efficacy of the treatment based on the level of miR-138. No change or a decrease in the levels of miR-138 after the treatment or along the course of the treatment indicates that the treatment is effective. In some embodiments, the samples are obtained before and after the treatment and no change or a decrease in the level of miR-138 after the treatment indicates that the treatment is effective. In other embodiments, the samples are obtained at multiple intervals during the treatment and no change or a decrease in the levels of miR-138 along the course of the treatment indicates that the treatment is effective.

In another aspect, there is provided a method for identifying glioma stem cells, comprising: quantifying the level of miR-138 in a sample suspected of containing glioma stem cells, and determining whether the sample contains glioma stem cells based on the level of miR- 138, wherein an elevated miR-138 level and/or a decreased level in the sample as compared to a predetermined standard indicates presence of glioma stem cells in the sample.

A method for identifying GSCs includes quantifying the level of miR-138 in a sample suspected of containing GSCs via a conventional method as those described herein, and determining whether the sample contains glioma stem cells based on the level of miR-138. An elevated miR-138 level in the sample as compared to a predetermined standard (e.g., the level of miR-138 in non-tumor cells such as normal neural stem cells) indicates presence of glioma stem cells in the sample. The sample suspected of containing GSCs can be obtained from a human subject. In some embodiments, this method further comprises a step of

assessing whether the human subject is suspected of having or at risk for malignant glioma. Presence of GSCs in the sample indicates that the human subject is suspected of having or at risk for malignant glioma. In other embodiments, the method further comprises assessing the risk of tumor progression or tumor metastasis in a malignant glioma patient. Presence of GSCs in the sample is indicative of an increased risk for malignant glioma progression or metastasis.

The details of one or more embodiments of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings are first described.

Figure 1 is a diagram showing that GSCs are multipotent and tumorigenic. A: a photo of representative images showing that, like NSCs, GSCs also form spheres. Scale bar: 100 μm. B: a photo showing immune-blot analysis of cellular lysates from GSCs. NSCs, and GSCs on differentiation. Like NSCs, GSCs express neural stem cell markers CD133 and Nestin. C: a bar graph showing the percentage of tumorspheres formed by GSCs, demonstrating the GSCs' ability to self-renew (left panel) and a photo showing formation of a tumorsphere. Sub-sphere forming assay was performed up to 3 generations. The bar graph represents the mean and standard deviation from triplicate experiments. D: a photo of representative images showing differentiation of GSCs to neurons, astrocytes, and oligodendrocytes, which were determined by specific markers Tuj1, GFAP, and O4, respectively. E: a photo showing characteristic glioblastoma phenotype observed in H&E stained tumor sections via light micrograph.

Figure 2 is a diagram showing identification and validation of differentially expressed miRNAs in GSCs. A: a graph showing differential miRNA expression in GSCs. The scatter plot represents the transcript abundance of miRNAs in GSCs (vertical axis) and NSCs (horizontal axis). B : a bar graph showing levels of miR-138 in NSCs and GSCs as determined by quantitative real-time PCR (qRT-PCR). C: a photo showing the expression