Non-coding RNAs as epigenetic regulator of glioma stem-like cell differentiation

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Glioblastomas show heterogeneous histological features. These distinct phenotypic states are thought to be associated with the presence of glioma stem cells (GSCs), which are highly tumorigenic and self-renewing sub-population of tumor cells that have different functional characteristics. Differentiation of GSCs may be regulated by multi-tiered epigenetic mechanisms that orchestrate the expression of thousands of genes. One such regulatory mechanism involves functional non-coding RNAs (ncRNAs), such as microRNAs (miRNAs); a large number of ncRNAs have been identified and shown to regulate the expression of genes associated with cell differentiation programs. Given the roles of miRNAs in cell differentiation, it is possible they are involved in the regulation of gene expression networks in GSCs that are important for the maintenance of the pluripotent state and for directing differentiation. Here, we review recent findings on ncRNAs associated with GSC differentiation and discuss how these ncRNAs contribute to the establishment of tissue heterogeneity during glioblastoma tumor formation.

Keywords: epigenetics, glioma, cancer stem cells, long non-coding RNA, micro RNA
or oncogenic functions in many types of cancer (Esteller, 2011; Wapinski and Chang, 2011; Hu et al., 2012; Zhang et al., 2013).

In this review, we provide a summary of the current understanding of miRNAs and IncRNAs in gliomas with a focus on their roles in GSCs.

**miRNAs IN GSC DIFFERENTIATION**

miRNAs are short sequences of 17–25 nucleotides that are not transcribed but have a regulatory function. An RNase III enzyme converts pri-miRNA into pre-miRNA hairpin transcripts that are processed into mature miRNAs and incorporated into a ribonucleoprotein complex called the RNA-induced silencing complex (RISC). The RISC and associated mature miRNA then binds to mRNA and causes a physical block to translation (Ambros and Lee, 2004; Bartel, 2004). Many miRNAs form imperfectly complementary stem-loop structures on the sense strand of the target mRNA. Thus, each miRNA can target multiple mRNA species through recognition of complementary sequences. Upregulation of mature miRNAs may occur as a consequence of transcriptional activation or amplification of the corresponding pre-miRNA locus, whereas downregulation of miRNAs may result from epigenetic silencing or deletion of the corresponding region (Schickel et al., 2008). Although dysregulation of the miRNA-mRNA network has been reported in glioblastoma, little attention has so far been paid to its role in GSCs (Godlewski et al., 2010a). In this section, we describe the information available on the significance of miRNAs in GSCs (Table 1).

**miR-17-92 CLUSTER**

The miR-17-92 cluster is thought to be involved in the regulation of GSC differentiation, apoptosis, and proliferation (Ernst et al., 2010). The level of transcripts from miR-17-92 clusters are significantly higher in primary astrocytic tumors than in normal brain tissues and increase significantly with tumor grade progression. A High-level amplification of the miR-17-92 locus has also been found in glioblastoma specimens. Inhibition of miR-17-92 induces apoptosis and decreases cell proliferation in GSCs. mir-17-92 inhibition is also associated with induction of cyclin-dependent kinase inhibitor 1A (CDKN1A), E2F transcription factor 1 (E2F1), PTEN, and connective tissue growth factor (CTGF). Of these, the CTGF gene was shown to be a direct target of miR-17-92 in GSCs.

When GSCs are exposed to the differentiation-promoting conditions, downregulation of the oncogenic miR-17-92 cluster is directly related to the concomitant upregulation of CTGF (Ernst et al., 2010).

**miR-124 AND miR-137**

The initial analysis of miR-124 showed that it promotes neuronal differentiation by targeting the polyipyrimidine tract-binding protein 1 (PTBP1) that encodes a global repressor of alternative pre-mRNA splicing; miR reduces the level of PTBP1, which results in an increase in the production of nervous system-specific alternative RNA splicing and promotes the differentiation of progenitor cells to mature neurons (Makeyev et al., 2007). Subsequent analysis showed that both miR-124 and miR-137 are downregulated in high-grade gliomas and up-regulated during adult neural stem cell differentiation (Silber et al., 2008). Transfection of miR-124 or miR-137 inhibits proliferation of GSCs, via suppression of cyclin-dependent protein kinase 6 (CDK6), and induces morphological changes in human GSCs and expression of neuronal differentiation markers. Overexpression of miR-124 has consistently been found to inhibit the CD133+ cell subpopulation of the neurosphere and to downregulate stem cell markers, such as BMI1, Nanog, and Nestin. These effects could be rescued by re-expression of SNAI2, another direct target of miR-124 (Xia et al., 2012).

**miR-451**

Analysis of the miRNA profiles of GSC (CD133+ cells) and non-GSC (CD133− cells) populations showed that several miRNAs, including miR-451, miR-486, and miR-425, are upregulated in CD133− cells. Transfection of cells with miR-451 has been shown to induce disruption of glioblastoma neurospheres (Gal et al., 2008). Interestingly, this study also showed that SMAD

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**Table 1 | List of miRNAs dysregulated in GSCs.**

| MicroRNAs | Direct targets | Roles in GSC | Reference |
|-----------|---------------|--------------|-----------|
| mir-17-92 cluster | CTGF | Differentiation (−), proliferation (+), apoptosis (−) | Ernst et al. (2010) |
| mir-451 | CAB39 | Differentiation (−), proliferation (+), apoptosis (−) | Godlewski et al. (2010b) |
| mir-1275 | CLDN1 | Differentiation (−), proliferation (+) | Katsushima et al. (2012) |
| mir-138 | CASP3, BLCAP, MXD1 | Differentiation (−), proliferation (+), apoptosis (−) | Chan et al. (2012) |
| mir-137 | CDK6 | Differentiation (−), proliferation (−) | Silber et al. (2008) |
| mir-34a | MET, NOTCH1, NOTCH2, CDK6 | Differentiation (−), proliferation (−), apoptosis (−) | Li et al. (2009b), Guessous et al. (2010) |
| mir-302-367 cluster | CXCR4 | Differentiation (−), proliferation (−), invasion (−) | Fareh et al. (2012) |
| mir-124 | SNAI2 | Differentiation (−), proliferation (−), invasion (−) | Xia et al. (2012) |
| mir-204 | SOX4, EPHB2 | Differentiation (−), proliferation (−), invasion (−) | Ying et al. (2013) |
| mir-128 | BMI1, SUZ12 | Differentiation (−), proliferation (−), radiosensitivity (−) | Godlewski et al. (2008), Peruzzi et al. (2013) |

(+): increased, (−): decreased.
miR-34a

miR-34a is tumor-suppressive and is downregulated in human glioma tissues; miR-34a directly inhibits the expression of c-Met, Notch-1, and Notch-2 in GSCs (Li et al., 2009b). Notch is a critical regulator of cell-fate during development and also of normal stem cell maintenance (Fan et al., 2006; Shih and Holland, 2006; Fan et al., 2010). Activation of the Notch pathway enhances the stemness, proliferation, and radioreistance of GSCs (Wang et al., 2010). Ectopic expression of miR-34a in glioma cells inhibits cell proliferation, survival, and migration. In addition, miR-34a induces GSC differentiation as evidenced by the decreased expression of stem cell markers and increased expression of differentiation markers (Guessous et al., 2010).

miR-128

Two studies have described a link between miR-128 and the polycomb repressor complex (PRC). Two major complexes, PRC1 and PRC2, are recognized as key epigenetic regulators during development (Lund and van Lohuizen, 2004) and are required for maintaining self-renewal and multi-potential capability (Richly et al., 2011). The first study demonstrated that miR-128 has a tumor-suppressive function and that this is downregulated in glioblastoma tissue. miR-128 expression significantly reduces glioma cell proliferation both in vitro and in vivo via downregulation of the oncogene Bmi-1 that is a component of PRC1. In addition, miR-128 inhibits GSC self-renewal (Godlewski et al., 2008). The second study showed that miR-128 directly targets SUZ12, a key component of PRC2. Ectopic expression of miR-128 in GSCs significantly increases their radiosensitivity (Peruzzi et al., 2010). The PRC has been shown to promote normal and cancer stem cell self-renewal and is also implicated in GSC regulation (Abdouh et al., 2009; Suva et al., 2009; Natsume et al., 2013). The findings of these various studies therefore indicate that miR-128 mediates an important epigenetic regulatory pathway in GSCs.

OTHER miRNAs

Several other miRNAs have been implicated in glioma malignancy. Ectopic expression of the miR-302-367 cluster in GSCs inhibits the CXCR4 pathway resulting in the suppression of stemness signatures, self-renewal, and cell infiltration. Inhibition of the CXCR4 pathway leads to the disruption of the SHH-GLI-NANOG network, which is important for cell self-renewal and tumorigenic properties (Fareh et al., 2012). In both GSCs and non-GSCs, miR-1275 is controlled by a polycomb-mediated silencing mechanism and regulates expression of the oligodendroglial-lineage gene claudin 11 (CLDN11). These data illustrate that miR-1275 is regulated by an epigenetic pathway and that it contributes to the phenotypic diversity of glioblastoma tissues. The increased insight into the roles of these miRs may provide a better understanding of basis for the heterogeneity of glioblastomas in the context of human neurodevelopment (Katsushima et al., 2012). Recently, miR-204 was shown to suppress self-renewal, a stem cell characteristic, and the migration of GSCs by targeting the stemness-governing transcriptional factor SOX4 and the migration-promoting receptor EphB2 (Ying et al., 2013).

LncRNAs IN CANCER

Genome-wide studies showed that there are a large number of ncRNAs, including a group termed IncRNAs (Birney et al., 2007). LncRNAs are generally greater than 200 nucleotides and up to 100 kb in length (Mercer et al., 2009). It is known that IncRNAs are mainly transcribed by RNA polymerase II, are polyadenylated and spliced (Wu et al., 2008; Mercer et al., 2009; Ponting et al., 2009). Approximately 15,000 IncRNAs are estimated to occur in human cells and these are frequently expressed in tissue-specific patterns (Derrien et al., 2012). IncRNAs appear to play important roles in a wide range of biological cellular processes including maintenance of stemness, development, and cell survival (Koziol and Rinn, 2010; Zhang et al., 2013). Currently studies detected a set of IncRNAs in each disease using RNA immunoprecipitation with RNA binding proteins coupled with computational approaches.

Long non-coding RNAs are believed to regulate gene expression through four different pathways (Koziol and Rinn, 2010; Hu et al., 2012). First, IncRNAs can bind to chromatin modifying proteins (which have a scaffold function) and recruit these proteins to target loci. These IncRNA complexes can target genes that are closely situated in the genome (cis-regulation) or genes that are genomically distant (trans-regulation) (Nagano et al., 2009; Prensner et al., 2011; Wang et al., 2011). Second, IncRNAs can act as an RNA decoy, that is, they can interact directly with a DNA binding domain to prevent transcription factors interacting with their DNA targets (Kino et al., 2010; Ng et al., 2012). Third, IncRNAs can act as an mRNA sponge, that is, they prevent specific miRNAs from binding to their target mRNAs by competitive binding (Poliseno et al., 2010; Cesana et al., 2011; Karreth et al., 2011). Fourth, IncRNAs can bind to specific combinations of regulatory proteins, such as RNA splicing proteins within ribonucleoprotein complexes (Tripathi et al., 2010; Ng et al., 2012; Schor et al., 2012).

There is increasing evidence to show that a set of IncRNAs is associated with cancer pathogenesis and that these IncRNAs function as regulators in cancer development (Prensner and Chin-nayani, 2011). IncRNAs that are dysregulated in cancers are listed in Table 2. Below, we provide a brief description of some IncRNAs that are associated with glioma tumorigenesis.
## Table 2 | List of IncRNAs dysregulated in cancers.

| Name | Cancer type | Biological function | Molecular function | References |
|------|-------------|---------------------|--------------------|------------|
| **Oncogenic** | | | | |
| HOTAIR | Breast, hepatocellular, colorectal, pancreatic, GIST | Promotes invasion and metastasis, modulates cancer epigenome | Scaffold (PRC2, LSD1), guide (trans-regulation) | Gupta et al. (2010), Kogo et al. (2011), Yang et al. (2011), Niinuma et al. (2012), Kim et al. (2013) |
| ANRIL | Prostate, leukemia, melanoma | Suppresses senescence via INK4A | Scaffold (PRC1, PRC2), guide (cis-regulation) | Pasmant et al. (2007), Yu et al. (2008), Popov and Gil (2010), Pasmant et al. (2011) |
| MALAT1 | Lung, prostate, breast, colon, hepatocellular | Regulates alternative splicing of pre-mRNA | Splicing (nuclear paraspeckle) | Ji et al. (2003), Muller-Tidow et al. (2004), Lin et al. (2007), Tano et al. (2010), Tripathi et al. (2010) |
| PCAT-1 | Prostate | Promotes cell proliferation, inhibits BRCA2 | Scaffold (PRC2), guide (trans-regulation) | Prensner et al. (2011) |
| CTBP1-AS | Prostate | Promotes cell proliferation | Scaffold (PSF), guide (trans-regulation) | Takayama et al. (2013) |
| PCGEM1 | Prostate | Inhibits apoptosis, promotes cell proliferation | Unknown | Srikanth et al. (2000), Petrovics et al. (2004) |
| TUC338 | Hepatocellular | Promotes cell proliferation | Unknown | Braconi et al. (2011) |
| uc. 73a | Leukemia, colorectal | Promotes cell proliferation, inhibits apoptosis | Unknown | Calin et al. (2007) |
| SPRY4-IT1 | Melanoma | Promotes cell proliferation and invasion, inhibits apoptosis | Unknown | Khaitan et al. (2011) |
| ncRAN | Neuroblastoma, bladder | Promotes cell proliferation and invasion | Unknown | Yu et al. (2009), Zhu et al. (2011) |
| PRNCR1 | Prostate | Promotes cell proliferation | Unknown | Chung et al. (2011) |
| H19 | Breast, hepatocellular | Promotes cell proliferation, both oncogenic and tumor suppressive functions reported | Unknown | Gabory et al. (2006), Matouk et al. (2007) |
| **Tumor suppressive** | | | | |
| GAS5 | Breast | Induces growth arrest and apoptosis | Decoy (glucocorticoid receptor) | Mourtada-Maarabouni et al. (2008), Kino et al. (2010) |
| MEG3 | Meningioma, hepatocellular, leukemia, pituitary, gliomas | Mediates p53 signaling, inhibits cell proliferation | Unknown | Zhou et al. (2007, 2012), Wang et al. (2012) |
| PTENP1 | Prostate, colon | Inhibits cell proliferation | Sponge (PTEN) | Poliseno et al. (2010) |
| LincRNA-p21 | Mouse models of lung, sarcoma, lymphoma | Induces apoptosis by repressing p53 targets | Scaffold (hnRNP-k), guide (trans-regulation) | Huarte et al. (2010) |
MEG3

Maternally expressed gene 3 (MEG3) is a maternally expressed imprinted gene that can also act as an IncRNA. MEG3 is generally expressed in normal tissues, and its downregulation by aberrant DNA methylation has been found in many types of human cancer (Zhou et al., 2012; Shi et al., 2013). For example, MEG3 expression in glioma tissues is decreased compared to adjacent normal tissues (Wang et al., 2012). The tumor-suppressive role of MEG3 is supported by the fact that it can associate with p53 and that this association is required for p53 activation (Lu et al., 2013). Ectopic expression of MEG3 inhibits cell proliferation and induced cell apoptosis in glioma cell lines (Wang et al., 2012).

CRNDE

Colorectal neoplasia differentially expressed (CRNDE) transcripts are categorized as IncRNAs and have the potential to interact with chromatin-modifying proteins to regulate gene expression through epigenetic changes (Ellis et al., 2012). CRNDE is expressed in the fetal brain and in induced pluripotent stem cells; levels of expression increases during neuronal differentiation but no transcripts can be detected in the adult brain (Lin et al., 2011). Intriguingly, CRNDE is highly expressed in gliomas. The recent study of Ellis et al. demonstrated a direct interaction between CRNDE transcripts and components of PRC2 and the CoREST chromatin-modifying complex. CRNDE provides specific functional scaffolds for regulatory complexes, such as PRC2 and CoREST, and may contribute the maintenance of pluripotent state as well as neuronal differentiation (Ellis et al., 2012).

CONCLUDING REMARKS

Following the discovery of cancer stem cells, it became important to elucidate the mechanisms and the environmental cues that control the differentiation of these cells into the diverse array of cell types that form during tumorigenesis. Epigenetic dysregulation has recently been shown to change the balance between differentiation and self-renewal of cortical progenitor cells and, thereby, to alter the rate and developmental timing of neurogenesis (Pereira et al., 2010). Given that cancer is a disease of faulty cellular differentiation, it is likely that aberrant epigenetic mechanisms involving ncRNAs are involved in glioma tumorigenesis. IncRNAs are increasingly important because of their potential for use in clinical diagnosis and treatment. To date, however, the functions of only a few IncRNAs have been elucidated with respect to tumor biology and there are still many aspects that remain to be resolved. Further investigations are required to clarify the functional roles of IncRNAs in order to elucidate the gene regulatory mechanisms in glioma-genesis. Understanding of the interplays between IncRNAs and genomes, which are reversible alterations, may offer a novel opportunity for the development of molecularly targeted therapies. Nevertheless, a better understanding of the glioblastoma core signaling pathways regulated by ncRNAs and other epigenetic mechanisms will undoubtedly provide novel therapeutic targets and strategies with applications in diagnosis and therapy in glioblastoma.

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