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Extracellular Vesicles and MicroRNAs: Their Role in Tumorigenicity and Therapy for Brain Tumors

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Abstract

MicroRNAs are small non-coding RNAs which mediate post-transcriptional gene regulation. Recently, microRNAs have also been found to be localized to the extracellular space, often encapsulated in secreted extracellular vesicles (EVs). This tandem of EVs and tissue-specific expressed/secreted microRNAs that can be taken up by neighboring or distant recipient cells, leading to changes in gene expression—suggests a cell-specialized role in physiological and pathological conditions. The complexity of solid tumors and their distinct pathophysiology relies on interactive communications between the various cell types in the neoplasm (tumor, endothelial, or macrophages, for instance). Understanding how such EV/microRNA-mediated communication occurs may actually lead to avenues for therapeutic exploitation and/or intervention, particularly for the most formidable cancers, such as those in the brain. In this review, the role of microRNAs/EVs in brain tumors will be discussed with emphasis on how these molecules could be utilized for tumor therapy.

Keywords

Glioblastoma multiforme; GBM; Cancer stem cells; microRNA; Exosomes; Extracellular vesicles

Introduction

Glioblastoma multiforme (GBM) is an incurable brain cancer with a very poor prognosis and a mean survival rate of only 3.3 % at 2 years and 1.2 % at 3 years (Furnari et al. 2007; Ohgaki et al. 2004), which makes it one of the most lethal human malignancies (Al-Nedawi et al. 2008). GBM accounts for 12–15 % of all brain tumors and approximately 70 % of all diagnosed astrocytomas (Ohgaki and Kleihues 2005; Ohgaki and Kleihues 2007). It is highly heterogeneous morphologically and, even at the single-cell level, recent data show significant genetic heterogeneity within tumor per se (Friedmann-Morvinski 2014; Meyer et al. 2015; Patel et al. 2014). GBM tumors are also highly invasive, infiltrating surrounding brain tissue (Al-Nedawi et al. 2009; Skog et al. 2008). These features make it impossible to

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cure GBM by local therapies or single-target therapy. This failure of complete eradication leads to inevitable tumor relapses.

Additional insights into the mechanisms underpinning GBM pathobiology are therefore urgently needed. GBM is recognized as a complex “ecosystem” composed of cells with distinct phenotypes, genotypes, and epigenetic landscapes. To date, analyses on molecular diversity have focused mostly on protein-coding genes (Patel et al. 2014), but the engagement and contribution of non-coding RNAs remains insufficiently studied or characterized.

The conventional view of the mammalian genome was that ~20,000 protein-coding genes were dispersed across mostly repetitive and largely non-transcribed sequences. Over the past decade, this view has been challenged by increasingly thorough examinations of the RNA species in mammalian cells. These studies have revealed the fascinating complexity of the transcriptome, in which protein-coding genes produce many alternative products. On the other hand, genomic regions previously thought to be transcriptionally silent give rise to a range of processed and regulated transcripts that do not code for functional proteins. The vast majority of these non-coding RNAs have no known function. In fact, only a small fraction of these transcripts are precursors for small regulatory RNAs, such as microRNAs, with documented pro-tumorigenic and tumor suppressor functions in many cancers including GBM (Floyd and Purow 2014; Godlewski et al. 2015; Sergeeva et al. 1987).

GBMs contain certain cellular niches enriched for distinct phenotypic properties (Patel et al. 2014), including transient quiescence and self-renewal (Chen et al. 2012a; Meacham and Morrison 2013; Singh et al. 2004), adaptation to hypoxia (Li et al. 2009c), and resistance to radiation-induced DNA damage (Bao et al. 2006; Bhat et al. 2013). This diversity in phenotypes could be rendered further complex by the intercellular transfer of functional EV-encapsulated RNA molecules, including microRNAs. EVs include exosomes, microvesicles, apoptotic blebs, and oncosomes, and thus the full comprehension of functional consequences of such EV diversity is still incomplete (Bobrie et al. 2012). MicroRNAs exist in bodily fluids, including blood and CSF (Holdhoff et al. 2013; Rao et al. 2013; Roth et al. 2011), as a component of EVs but can also be found inside high-density lipoproteins (HDLs) (Tabet et al. 2014; Vickers et al. 2011), or bound by AGO2 outside of vesicles (Arroyo et al. 2011). Insofar as extracellular vesicles, increasing evidence is showing that they contain microRNAs that are selectively packaged and then transferred to recipient cells, where they regulate gene expression (Fitzner et al. 2011; Godlewski et al. 2015; Villarroya-Beltri et al. 2013). In the context of a GBM “ecosystem” composed of cells from distinct genetic subclasses and functional role of EVs and their cargo in maintaining such diverse landscapes, the EV/microRNA complex may be responsible for the molecular/phenotype “switch.” Indeed, although protein-coding gene-based signatures predict molecular subtype classification in GBM (Verhaak et al. 2010), there is no strong correlation between global signature of microRNA expression/detection and molecular subtypes. Therefore, uncovering EVs/microRNA rearrangements adds an additional layer to the complexity of tumor heterogeneity (Fig. 1). EVs with their microRNA components may also represent a novel target for therapeutic intervention or a vehicle for the transfer of anticancer therapeutic molecules. Unlike other cellular nanoparticles (e.g., lipoproteins), EVs consist of a plasma

membrane surrounding the inner space (lumen), containing complex mixtures of cellular proteins, bioactive lipids, and nucleic acids (mRNA, microRNA, mtRNA, and DNA) whose roles in GBM pathology have been somewhat described (Garnier et al. 2013; Godlewski et al. 2015; van der Vos et al. 2011). Correspondingly diverse are their alleged functions, ranging from immunomodulation, bone formation, removal of degraded cellular material, glandular secretion, erythropoiesis, blood coagulation, angiogenesis, tumor, metastasis, and several other processes. This review aims to discuss the mechanisms, function, and potential therapeutic properties of cellular and vesicle-transferred microRNAs in GBM. It is hoped that the review will help frame the scientific context for additional research in this highly efficient intercellular mechanism of transfer of regulatory genetic information in brain tumors.

MicroRNAs—Mode of Action

Less than two percent of the human genome encodes genes that are translated into proteins: the rest of transcriptome gives rise to non-protein-coding RNAs (Djebali et al. 2012). It is now well appreciated that these molecules play essential roles in a variety of biological processes (Iorio and Croce 2012; Tay et al. 2014; Wilusz et al. 2009). Among these non-protein-coding genes are small non-coding RNAs known as microRNAs that now appear to have a significant role in cancer biology. Twenty years after the first microRNA gene (*lin-4*) was discovered in *Caenorhabditis elegans* (Lee et al. 1993), vast numbers of other microRNAs have been identified. So far over 2500 have been described in the human genome (miRBase (www.mirbase.org)) (Kozomara and Griffiths-Jones 2014).

MicroRNAs are a family of small (17–24 nucleotides long), non-coding RNAs that deregulate gene expression by various means. The established mechanism of action is that they regulate levels of target mRNA/proteins through a combination of rapidly occurring mRNA destabilization (dominant function) and translational repression (Eichhorn et al. 2014). MicroRNAs initiate the formation of inhibitory complexes by binding to the 3′ untranslated region (3′ UTR) of specific mRNAs (Carthew and Sontheimer 2009). Other targets, though, include the 5′ untranslated region (5′ UTR) or coding sequence (CDS) of the targeted messenger RNA (mRNA) (Miranda et al. 2006; Zhou and Rigoutsos 2014), simultaneous 5′ UTR and 3′ UTR bridging interaction (Lee et al. 2009), acting as a ligand for receptor (Fabbri et al. 2012), or interaction with other non-coding RNAs (Guil and Esteller 2015) (Fig. 2). Repression of gene expression can also occur by other means as evidence is emerging that microRNAs can also alter global protein synthesis (Eiring et al. 2010; Lee et al. 2009; Orom et al. 2008; Vasudevan et al. 2007). Therefore, the capability to deregulate protein-coding and non-protein-coding genes allows non-coding RNAs to modulate significant epigenetic rearrangements.

MicroRNAs in Brain Tumors

Typically in cancer, including GBM, microRNAs can mediate oncogenic or tumor suppressor functions (Floyd and Purow 2014; Godlewski et al. 2015; Sergeeva et al. 1987). In the first instance, microRNAs are oncogenic by post-transcriptionally lowering mRNA and subsequently tumor suppressor proteins. In the second instance, they act as tumor

suppressors by reducing oncogenic mRNAs and their protein product levels. Altered expression of microRNAs in tumor cells coupled with the function of the targeted molecules drives the oncogenic phenotype.

Recent reviews have comprehensively analyzed microRNAs deregulated in GBM stem cells (GSCs) (Floyd and Purow 2014; Godlewski et al. 2015; Yang et al. 2015). Here we will focus on the influence of selected microRNAs over GBM tumor cell growth, invasion, stem cell regulation, as well as chemo-resistance in the context of therapy (Fig. 3).

Proliferation—Pro or Anti-microRNA

One of the most investigated microRNAs upregulated in cancer is miR-21, reproducibly shown to be overexpressed in GBM (Chan et al. 2005; Ciafre et al. 2005; Conti et al. 2009; Gabriely et al. 2008; Gaur et al. 2011; Kwak et al. 2011; Lakomy et al. 2011; Malzkorn et al. 2010; Papagiannakopoulos et al. 2008; Zhou et al. 2010). Experimentally, the downregulation of miR-21 was found to reduce the oncogenic potential of GBM cell lines through the inhibition of several cellular signaling pathways involved in malignancy maintenance (Krichevsky and Gabriely 2009). A variety of targets for miR-21, such as PTEN, SPRY2, LRRFIP1, HNRPK, and TAP63 (Kwak et al. 2011; Li et al. 2009b; Papagiannakopoulos et al. 2008; Zhou et al. 2010), are known to regulate GBM proliferation. Additionally, indirect targeting of key components of proliferation-signaling pathways, e.g., NF- κ B and Ras, by miR-21 has also been reported (Kwak et al. 2011). It was also shown that downregulation of miR-21 diminished tumor growth in immunodeficient mice (Corsten et al. 2007; Gaur et al. 2011; Zhou et al. 2010). Transcriptional profiling of cells where miR-21 was knocked down revealed changes in the expression of genes associated with DNA damage response, regulators of cell cycle arrest, and positive regulators of apoptosis (Gabriely et al. 2008). These data, together with miR-21-dependent sensitization of GBM cell to both radio- and chemotherapy (Li et al. 2011; Ren et al. 2010a; Ren et al. 2010b; Shi et al. 2010a), make miR-21 a particularly interesting focus for therapeutic exploitation.

The other strongly upregulated microRNA in GBM is miR-10b (Gabriely et al. 2011; Lang et al. 2012; Sasayama et al. 2009). MiR-10b has been shown to promote cell cycle progression in both S-phase and mitotic transitions (Gabriely et al. 2011; Guessous et al. 2013; Sasayama et al. 2009), and miR-10b inhibition suppresses the growth of subcutaneous and intracranial GBM xenografts (Gabriely et al. 2011; Lin et al. 2012). Direct anti-proliferative targets of this microRNA were shown to be CDKN1A/p21 and CDKN2A/p16 (Gabriely et al. 2011). Recently, miR-10b-dependent targeting of E2F1 in a CDKN1A/p21-dependent fashion was also shown to regulate cell death and proliferation signaling (Teplyuk et al. 2015).

Other microRNAs upregulated in GBM that drive the pro-proliferative phenotype are miR-125 (Shi et al. 2010b), the miR-17-19 cluster (Ernst et al. 2010), and miR-381 (Tang et al. 2011).

The loss of expression of tumor-suppressive microRNAs (Godlewski et al. 2008; Qiu et al. 2013; Rani et al. 2013; Zhao et al. 2013) is also an important cause of aggressive growth of

GBM cells. MiR-7 is a well-documented example of an inhibitor of mitogenic signaling via the simultaneous repression of multiple mRNAs and their proteins within the EGFR pathway (Kefas et al. 2008). In addition, transfection of miR-7 in GBM promoted the generation of a primary GSC as well as the inhibition of proliferation of GBM cells in vitro (Kefas et al. 2008). These results implicate miR-7 as a key molecule and possibly a therapeutic molecule for GBM. Other microRNAs consistently reported as downregulated in GBM are miR-137 that have often been investigated in combination with neuronal-specific miR-124 (Chen et al. 2012b; Lavon et al. 2010; Setty et al. 2012; Silber et al. 2008; Vo et al. 2011; Wuchty et al. 2011). Their anti-proliferative effect mediated through targeting of CDK6 (Silber et al. 2008), Msi1 (Vo et al. 2011), and Cox-2 (Chen et al. 2012b) with concurrent induction of differentiation of GSCs and cell cycle arrest (Silber et al. 2008) suggest that targeted delivery of miR-137 and/or miR-124 may be therapeutically efficacious for GBM.

Invasion—Go or Grow—MicroRNAs

As the complete surgical resection of GBM tumors is not possible due to their highly invasive infiltration of surrounding brain tissue (Rao 2003; Zhong et al. 2010), the development of therapies targeting the invasive properties of GBM cells is critically needed. Experiments with cultured GBM cells (Giese et al. 2003) suggest an almost exclusive relationship between migratory and proliferative performance. This phenomenon has been known as the migration/proliferation dichotomy (or ‘Go or Grow’ mechanism) (Giese et al. 1996). Using a GBM cell migration assay, we initially identified miR-451 as the most downregulated microRNA during cell migration (Godlewski et al. 2010b). Its targeting of the major energy biosensor pathway of cells by downregulation of the LKB1 cofactor CAB39 and subsequent modulation of AMPK led to GBM cell inability to survive glucose deprivation (Godlewski et al. 2010b; Nan et al. 2010). The other element of the phenotype—deceleration of migration rate—was also found to be due to miR-451 targeting of YWHAZ (also known as 14-3-3 ζ) or TSC1 (Godlewski et al. 2010a). The expression of this miR-451 is regulated by microenvironmental changes (e.g., glucose availability) (Ansari et al. 2015); this knowledge could also be exploited therapeutically by targeting this miR-mediated mechanism of rapid adaptation of cancer cells to dynamic changes in energy availability.

Biologic evidence indicates that migratory and proliferative processes share common signaling pathways, suggesting a unique intracellular mechanism that regulates both behaviors (Giese et al. 2003). The observed dual pro-proliferative/anti-invasive phenotype driven by a single microRNA (i.e., miR-451) may thus reflect an effect of network targeting of such pathways and adaptation of GBM cells to cellular and microenvironmental changes. Indeed, a number of microRNAs deregulated in GBM have been reported to have such a dual phenotype. Functional assessment of miR-21 suppression in GBM cells was shown to lead to decreased cell growth and tumorigenicity (discussed in the previous section) but also to reduced invasiveness by targeting RECK and TIMP3 (Gabriely et al. 2008). Similarly, microRNAs like miR-137, miR-10b, miR-128, or miR-124 shown to deregulate proliferation and neurosphere formation were found to target also pro-migratory/invasive targets (SFRP1 (Delic et al. 2014), HOXD10 (Sun et al. 2011), SP1 (Dong et al. 2014), and Rab27a (Wu et al. 2013)).

GBM is characterized by cellular heterogeneity with a subpopulation of self-renewing and highly resistant GSCs. GSCs are thought to be crucial in the initiation and hierarchical maintenance of the GBM tumor niche (Charles et al. 2012; Gilbertson and Rich 2007; Infanger et al. 2013; Pietras et al. 2014; Yan et al. 2013). The genetic alterations of GBM are often driven by Notch, Wnt, or Polycomb signaling—documented targets for microRNAs in GBM. Notch is a critical regulator of cell-fate during development and also of normal stem cell maintenance (Fan et al. 2010; Fan et al. 2006; Shih and Holland 2006). Activation of the Notch pathway enhances the stemness, proliferation, and radio-resistance of GSCs (Wang et al. 2010), and miR-34a, downregulated in human GBM, was shown to directly inhibit the expression of c-Met, Notch-1, and Notch-2 in GSCs (Li et al. 2009a). Ectopic expression of miR-34a in glioblastoma 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). Several other microRNAs such as miR-326 or miR-524-5p have been reported to target Notch family members and ligands. MiR-326 has also been shown to suppress the Hedgehog pathway (Chen et al. 2012d; Ferretti et al. 2008; Kefas et al. 2009; Li et al. 2009a). The Wnt pathway in GSC was shown to be promoted by miR-92b, which targets the tumor suppressor Wnt-inhibiting NLK gene, and by miR-328 targeting the tumor suppressor SFRP1 (Delic et al. 2014; Wang et al. 2013). The indirect targeting of molecular markers of stem cells (Nanog and Nestin) by miR-124 resulted in downregulation of the CD133+ (GSC marker) cell subpopulation in neurospheres and could be rescued by re-expression of SNAI2, also a direct target of miR-124 (Xia et al. 2012a). Several other microRNAs including miR-451, miR-486, and miR-425 have also shown to be inversely correlated with the frequency of the CD133+ cell population, thought to represent GSCs (Gal et al. 2008).

The key epigenetic regulators required for maintenance of the self-renewal and multi-potential capability of GSCs are Polycomb Repressor Complexes 1 and 2 (PRC1, PRC2) (Richly et al. 2011). Data from our group clearly show that one of the significantly downregulated microRNAs in GBM—miR-128—is responsible for targeting of both these complexes (Peruzzi et al. 2013). In fact, re-expression of miR-128 significantly reduces GBM cell proliferation via downregulation of the oncogene BMI-1, a component of PRC1, and leading to inhibition of GSC self-renewal (Godlewski et al. 2008). MiR-128 also directly targets SUZ12, a key component of PRC2. Therefore, miR-128 leads to a reduction in PRC1 and PRC2 activities (ubiquitination of histone H2A and methylation of histone H3) (Peruzzi et al. 2013). This simultaneous action of a single microRNA on PRC1 and PRC2 is an important epigenetic process, because it leads to a global inhibition of PRC in neural stem cells.

In addition to microRNA-dependent inhibition of stemness in glioblastoma cells, pro-differentiation signaling was shown to increase the expression of the tumor-suppressive miR-302–367 cluster and miR-137 (Bier et al. 2013; Fareh et al. 2012; Silber et al. 2008). Functions in neuronal differentiation consist of both direct (Fareh et al. 2012) and indirect (Bier et al. 2013) targeting of CXCR4, which in turn modulates the expression of the stem cell regulators SHH, GLI1, and NANOG (Fareh et al. 2012). GSCs display resistance to radiation and chemotherapy and contribute to tumor growth through the stimulation of

angiogenesis (Cheng et al. 2010); these microRNAs thus could be used in combinatorial modes to sensitize GSCs to radio- and chemotherapy.

Overcoming Therapy Resistance—MicroRNA Targeting and Re-expression

Since its approval for use in newly diagnosed GBM patients, temozolomide (TMZ), an oral alkylating agent, has proven to be a somewhat effective chemotherapy drug with tolerable side effects. TMZ is most effective when administered concomitantly with radiotherapy (Stupp et al. 2005). Its active metabolite methylates DNA and such therapeutic benefit is inhibited by the DNA repair enzyme, methylguanine methyltransferase (MGMT) (Stupp et al. 2007; Zhang et al. 2012b). Reports have shown that certain microRNAs are implicated in acquired TMZ resistance. For instance, Ujifuku et al. demonstrated that miR-195, miR-455-3p, and miR-10a were upregulated in TMZ-resistant cells (Ujifuku et al. 2010). Slaby et al. also showed that GBM patients who responded to radiotherapy and TMZ also had downregulated miR-181b and miR-181c and upregulated miR-21 (Slaby et al. 2010). Additional analyses from The Cancer Genome Atlas (TCGA) of microRNAs associated with overall survival in TMZ-treated patients indicated that miR-130a could be a positive predictive marker for TMZ response in patients with GBM (Chen et al. 2015). Remarkably, in all these studies, the methylation status of MGMT was an independent predictor of response to TMZ suggesting that MGMT expression is unlikely to be the only pre-requisite for acquired TMZ resistance in all GBM phenotypes.

The exact mechanisms underlying MGMT modulation of TMZ and radiation responses remain uncertain, but a number of studies indicate MGMT's influence on various GBM signaling pathways. With this knowledge, microRNAs reported to influence TMZ resistance in GBM (miR-195, miR-455, miR-10 (Ujifuku et al. 2010), miR-181b (Li et al. 2010; Slaby et al. 2010), miR-21 (Shi et al. 2010a; Wong et al. 2012), miR-125b (Shi et al. 2012), miR-145 (Yang et al. 2012), miR-211 (Asuthkar et al. 2012), miR-17 (Comincini et al. 2013), miR-9 (Munoz et al. 2013), the miR-183/96/182 cluster (Tang et al. 2013), and miR-221/222 (Chen et al. 2012c)) were shown to target pathways involved in proliferation, apoptosis, and stem cell maintenance. Some studies suggested that targeting GSCs may effectively reduce tumor recurrence and significantly improve GBM sensitization to radiation (Huang et al. 2010). Because ectopic expression of miR-128 in GSCs significantly increases their radio-sensitivity, miR-128 may be a key microRNA in addressing the therapeutic challenges of therapy resistance (Peruzzi et al. 2013).

EVs and MicroRNAs—Targeting of EV Secretion, Composition of EV Cargo, and Delivery of MicroRNAs

In diseases such as cancer, EVs can facilitate tumor progression by altering their vesicular content and by supplying the tumor niche with molecules that favor the progression of oncogenic processes such as proliferation, invasion, cancer stem cell propagation, or even drug resistance. EVs derived from GBM cells were reported to modify recipient cells (of tumor, endothelial, or monocytic origin) via the transfer of cell-transforming proteins and messenger RNAs and ncRNAs (Al-Nedawi et al. 2009, 2008; Balaj et al. 2011; Skog et al. 2008).

Our recently published data suggest that the mechanism for the transfer of certain proteins via EV may depend on microRNA targeting of the EV cargo in GBM cells (Bronisz et al. 2014). Many experimental strategies are based on re-expression of downregulated microRNAs or inhibition of overexpressed microRNAs in tumor cells. However, only recently we have started to elucidate exactly how these forced expression approaches affect neighboring cells in the tumor microenvironment. We found that ectopic expression of the novel tumor suppressor miR-1 in GBM cells blocked growth of tumor cells but also inhibited the neovascularization of intracranial xenografts. Interestingly, we also found that cells forced to re-express miR-1 were less tumorigenic and that purified EVs from such cells were less pro-tumorigenic when added onto tumor and endothelial cells than EVs from control cells. This was due to an altered EV molecular cargo that also included mature miR-1. In fact, we showed that EV miR-1 led to direct repression of its mRNA targets (MET, AnnexinA2) and indirectly reduced levels of EGFR and inhibited JNK activity in recipient GBM cells. There was a global effect of miR-1 on the EV proteome linked to cancer signaling networks, such as AnnexinA2, fatty acid synthase, and 14-3-3 ζ . Together, our results indicated that EV signaling promotes the oncogenic properties of GBM within the tumor and in its microenvironment and that ectopic expression of miR-1 can mitigate these effects, with possible implications for the development of a unique miR-based therapy for GBM.

Additional input related to the role of microRNA in GBM microenvironment came from a very recent study by van der Vos et al. (van der Vos et al. 2015). Using one of the most enriched microRNAs in EVs—miR-451 and one of the most upregulated microRNA in GBM (Gabriely et al. 2008; Li et al. 2013)—miR-21, the authors demonstrate that GBM-derived EVs are internalized by microglia and that these two microRNAs are taken up by recipient microglial cells. This study also includes elegant visualization of the process in vivo. However, the functional modification of microglial phenotype by these microRNAs is still not clear. The proposed downregulation of c-MYC by miR-451 was already shown before (Chen et al. 2014; Wang et al. 2014). However, the 3'UTR of c-MYC mRNA is lacking a canonical miR-451 target site and mutation analysis of c-MYC mRNA needs to be performed to exclude indirect targeting by miR-451. Additionally, miR-451 was shown to be enriched in EVs from other cells including normal cells (Collino et al. 2010; Guduric-Fuchs et al. 2012; Pigati et al. 2010) in contrast to miR-21, calling into question the specificity of action that this microRNA may exert on microglia. Finally, the analysis of transcriptional regulation (primary transcript) of microRNA-451 in recipient cells may shed new light on a described mode of action, as this particular microRNA was found to be dramatically deregulated upon exposure to stress (Ansari et al. 2015; Godlewski et al. 2010b) and stress also is an additional mechanism of EV secretion/loading (de Jong et al. 2012; Garcia et al. 2015; Yu et al. 2006). The finding that microRNAs naturally enriched in EVs are post-transcriptionally regulated (Koppers-Lalic et al. 2014) questions whether such modification (uridylation of 3' end of microRNA) has an impact on the affinity of target recognition. This can represent an additional regulatory mechanism of cell-specific targeting by microRNA.

Although several EV-secreted microRNAs were shown to produce functional effects in different types of cancer (Ismail et al. 2013; Kogure et al. 2011; Kosaka et al. 2013; Ostensfeld et al. 2014; Singh et al. 2014; Valadi et al. 2007; Zhang et al. 2010), knowledge of

the role of secreted endogenous microRNAs by GBM cells as it relates to tumor progression is limited. This may be because the cellular heterogeneity of GBM cells and GSCs (Mao et al. 2013; Patel et al. 2014) complicates the analysis of the microRNA and proteome component in EVs. Until recently, several approaches were initiated to uncover the role of microRNAs secreted by GBM cells into the microenvironment or the circulation. Despite different models used in these studies, it became clear that miR-21 was the only one identified in all of them regardless of whether EVs were derived from GBM cells, CSF or serum (Table 1) (Akers et al. 2015; Li et al. 2013; Manterola et al. 2014; Roth et al. 2011; Shi et al. 2015; Skog et al. 2008; Sun et al. 2015; Teplyuk et al. 2012; Wei et al. 2014; Zhang et al. 2015). Interestingly, one of the established tumor-suppressive microRNAs in GBM—miR-128 (Godlewski et al. 2008; Peruzzi et al. 2013)—was found in blood (Roth et al. 2011) and serum (Sun et al. 2015) showing elevated level in these fluids of GBM patients (Sun et al. 2015). Additionally, the level of miR-128 in preoperative serum of GBM patients was significantly decreased in comparison to normal controls, and its levels were significantly elevated after surgery. Finally, low serum levels of miR-128 correlated with high pathological grade and low Karnofsky Performance Status score. These findings indicate that serum miR-128 could be a sensitive and specific biomarker of GBM. In the context of different modes of sorting of microRNAs into EVs, these additional comprehensive studies combining the analysis of microRNA signature with bioinformatic analysis of the mechanism of secretion are necessary. In summary, EV microRNA profiles may become useful biomarkers for GBM patients (Akers et al. 2013; Godlewski et al. 2015). Before this can be clinically applicable, we will need to resolve the significance of differences in the microRNA signature of healthy subjects compared to those of GBM patients (Godlewski et al. 2008; Piwecka et al. 2015), the significance of extracellular cancer EVs containing genetic materials that partially reflect the intracellular microRNA signature of the tumor milieu (Camussi et al. 2011; Noerholm et al. 2012; Skog et al. 2008), and the relevance of transgression of anatomic locations (blood to CSF and vice versa) by EVs (Holdhoff et al. 2013; Roth et al. 2011; Teplyuk et al. 2012).

Mechanisms of EV MicroRNA Processing and Loading

The processing of microRNAs is carried out by the RNA-induced silencing complex (RISC) (Bartel 2009; Maniataki and Mourelatos 2005) and RISC-loading complex (RLC) (Chendrimada et al. 2005; Gregory et al. 2005; MacRae et al. 2008; Maniataki and Mourelatos 2005; Melo et al. 2009), and the components of RISC were found in EVs, suggesting that processing of microRNA precursors in EVs is in fact possible. Recently, Monia et al. (Melo et al. 2014) showed in an in vitro cell-free model that precursor processing could occur in EVs. The authors argued that processing in recipient cells was unlikely due to the rate-limiting availability of processing enzymes. This however would not explain why delivered synthetic microRNA precursors are processed and become functional in recipient cells.

Recent evidence suggests that the microRNA repertoire in EVs only partially mirrors that of cellular microRNA (Skog et al. 2008) and, in fact, its specific pattern may be surprisingly different from that of secreting cells (Guduric-Fuchs et al. 2012; Li et al. 2013; Mittelbrunn et al. 2011; Nolte-Hoehn et al. 2012; Valadi et al. 2007). Yet, the active mechanisms that

control the selective sorting of RNA into EVs are poorly understood. An initial insight into this mechanism comes from the identification by Ostrowski and colleagues of the regulatory molecules involved in the release of EVs. They observed that Rab27a and Rab27b were associated with EV secretion. Knockdown of Rab27 or their effectors, SYTL4 and EXPH5, could inhibit secretion of EVs in HeLa cells (Ostrowski et al. 2010). GBM cells are characterized by massive secretion of EVs (Li et al. 2013) and interestingly one of the tumor suppressor microRNAs lost in GBM—miR-124 (Fowler et al. 2011)—was recently shown to target Rab27a in GBM cells. This suggests that miR-124 loss in GBM may be needed to increase EV secretion in GBM (Wu et al. 2013).

One additional factor/metabolic pathway may also be important: sphingosine 1-phosphate (S1P) receptors in multivesicular endosomes have been shown to be essential for the sorting of intracellular cargo into intraluminal vesicles destined for extracellular vesicle release. This process is regulated by sphingosine kinase-1 (SPHK1), which catalyzes the biosynthesis of S1P (Kajimoto et al. 2013). The pro-proliferative, pro-invasive, pro-angiogenic, and anti-apoptotic role of SPHK1 in GBM is well documented (Abuhusain et al. 2013; Estrada-Bernal et al. 2011; Guan et al. 2011; Kapitonov et al. 2009; Zhang et al. 2012a). It turns out that miR-124 also targets SPHK1 (Xia et al. 2012b); thus, lack of miR-124 in GBM results in elevated expression of SPHK1 (Xia et al. 2012b). Forced re-expression of miR-124 leads to a decrease of SPHK1, multiplying the effect on EV secretion and global EV cargo loading (Rigogliuso et al. 2010). Therefore, miR-124 may also be a useful target for a possible therapeutic strategy.

As always, there is also evidence that cannot be explained by the models. For example, the tumor repressor protein p53 and its downstream effector TSAP6 are enhancers of EV production (Yu et al. 2006). Therefore, one would expect that p53 loss of function, as often seen in cancers, would lead to decrease rather than the observed increase in EV production. This dissonance remains unresolved, but it is possible that the p53 cell signaling pathway is cell or stress dependent rather than a global mechanism relevant to all cells.

A novel study by Villarroya-Beltri adds to the knowledge of how microRNAs are sorted into EVs (Villarroya-Beltri et al. 2013). The authors identified short sequence motifs over-represented in microRNAs (EXOmotifs) that guide their loading into EVs and, by directed mutagenesis, were able to modulate loading of pre-selected microRNAs into these vesicles (Villarroya-Beltri et al. 2013). The heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) is a ubiquitously expressed RNA-binding protein that controls the transport and subcellular localization of specific mRNAs in neurons (Munro et al. 1999). It was identified as an interactive partner that binds a specific subset of microRNAs through their EXOmotifs and controls their loading into EVs. Moreover, hnRNPA2B1 in EVs was found to be sumoylated and this post-translational modification was responsible for controlling hnRNPA2B1–microRNA binding (Villarroya-Beltri et al. 2013). In contrast, partial regulation of microRNA sorting to EVs by cell activation-dependent changes of targeted transcript was proposed by Squadrito et al. (2014). In this report, the authors demonstrated profiling of RNA in macrophages and their EVs as well as subsequent genetic perturbation of the expression of individual microRNAs or their targets. This promoted bidirectional microRNA relocation from the cell cytoplasm/P bodies (sites of microRNA activity) to

MVB (sites of exosome biogenesis) and controlled sorting of microRNAs to exosomes (Squadrito et al. 2014). Interestingly, the functional microRNAs enriched in EV identified in this study (e.g., miR-188-5p, miR-146a-5p) (Squadrito et al. 2014) carry EXOmotifs (Villarroya-Beltri et al. 2013). This may suggest that passive mechanism to dispose excessive microRNAs may be the first step for selective microRNA incorporation into EVs (Villarroya-Beltri et al. 2013). Significantly, in both studies mature microRNAs, but not pre-microRNAs (Melo et al. 2014), were identified in secreted EVs, suggesting different mechanisms for sorting of microRNA that are at different stages of maturation. Another mode of action responsible for the sorting of microRNAs was proposed by Koppers-Lalic et al. (Koppers-Lalic et al. 2014). RNA sequencing analysis of small RNA profiles from a panel of human B cells and their secreted EVs not only revealed non-random distribution of microRNA species subsets between cells and EVs, but also discovered post-transcriptional modifications that may contribute, at least partially, to ncRNA sorting into EVs. The analysis of 3'-ends of microRNAs demonstrated that adenylated microRNA isoforms are relatively enriched in cells, and microRNA isoforms uridylated at 3' ends are in fact over-represented in EVs. Although this study was not validated in the brain cancer model, this type of mechanism may indeed exist independently of the cellular context, as microRNAs known to be generally EV enriched (e.g., miR-451) were indeed detected in EVs derived from GBM cells (Table 1). It is also possible that there are several mechanisms of ncRNA sorting explaining different mechanisms of microRNA enrichment in EVs: (1) secretion of the most abundantly expressed microRNAs; (2) secretion of microRNAs with low cellular levels; and (3) secretion of microRNAs dependent on microenvironmental context/stimuli. In fact, it was shown (Squadrito et al. 2014) that sorting of microRNAs into EVs is modulated by cell activation-dependent changes of microRNA targets in the donor cells. These studies (Koppers-Lalic et al. 2014; Squadrito et al. 2014; Villarroya-Beltri et al. 2013) show the functional transfer of EV-enriched microRNA; however, the alternative explanation that secretion of microRNAs occurs because they are not functional and thus disposable molecule may also be entertained.

Therapeutic Applications of EVs and EV MicroRNAs

Since EVs naturally carry microRNAs with established function in GBM (Godlewski et al. 2015), it seems logical to explore this as an approach for the delivery of therapeutic microRNAs (Fig. 4).

The half-life of EVs in the circulation should be greater than that of liposomes due to their endogenous origin and unique surface composition. Only a few studies have investigated this question, and while some reported that injected EVs accumulated in the liver only to a small degree, others have provided pharmacokinetic evidence of a circulatory EV half-life comparable to that of synthetic liposomes, partially due to the accumulation in the liver (Alvarez-Erviti et al. 2011; Takahashi et al. 2013). The well-designed approach by Lai and colleagues combined *Gaussia* luciferase and metabolic biotinylation to create a sensitive EV reporter system (EV-GlucB) for multimodal imaging in vivo as well as for monitoring EV levels in the organs and biofluids ex vivo. After administration of EVs, they showed that EVs first underwent a rapid distribution phase followed by a longer elimination phase via hepatic and renal routes within six hours (Lai et al. 2014). Moreover, they demonstrated that

EVs injected systemically can be delivered to tumor sites within an hour following injection. To date, liposomes/nanoparticles have been used to deliver genetic material, drugs, cytokines, adjuvants, or antigens into the body. However, therapeutic EVs could also be introduced exogenously and, as they are natural endogenous vesicles, they may have advantageous biosafety features related to inflammatory response (Dai et al. 2008; Escudier et al. 2005; Mignot et al. 2006; Morse et al. 2005) and to rapid delivery (Zhuang et al. 2011). As microRNAs and EVs are part of the natural homeostatic milieu of the cells, under normal homeostatic circumstances, microRNAs and EVs should have few, if any, sequence-specific “off-target” effects: this may render them ideal therapeutic agents for personalized medicine with efficient and site-specific delivery.

To validate the efficacy of EV-loaded tumor-suppressive microRNA (Katakowski et al. 2010a), Katakowski and colleagues (Katakowski et al. 2013) used mesenchymal stem cell (MSC)-derived EVs preloaded with miR-146b by overexpression of the primary miR-146b transcript in donor cells. Intra-tumoral injection of therapeutic EVs carrying this mature miR-146 significantly reduced the growth of a glioblastoma xenograft in a rat model. This underscores the relevance of both EV-based drug delivery and microRNA-based therapy (Katakowski et al. 2013, 2010b). In another study, MSC EV-delivered inhibitor of miR-9 decreased the expression of the multidrug transporter, responsible for chemotherapy resistance of GBM cells, and sensitized them to TMZ (Munoz et al. 2013). This study also suggests that EVs were responsible for most of the communication between MSCs and GBM cells, as the transfer of fluorescently labeled anti-miRs still occurred after blocking the formation of gap junctions. However, another study showed that the delivery of miR-124 and miR-145 into GBM has functional consequences inhibiting GBM cell migration and GSC self-renewal and that such a mechanism was mediated by both gap junctions and EVs (Lee et al. 2013). Additionally, the functional transfer of microRNAs naturally expressed in donor cells via gap junctions was also reported (Lim et al. 2011; Xin et al. 2012) including the functional transfer of microRNA between GBM cells and astrocytes (Hong et al. 2015). These data suggest that the extracellular transfer of microRNAs has functional consequences that depend on the gap junction, protein complex loading (Tabet et al. 2014), or EV encapsulation which should be considered in the targeted delivery of microRNAs.

EVs are very similar to nanoparticles because they could express/present certain antigens on their surface; however, their toxicity is minimal or none (Dai et al. 2008; Escudier et al. 2005; Mignot et al. 2006; Morse et al. 2005). The surface of EVs can also be modified by the genetic engineering of donor cells for efficient delivery of EVs to target cells. The successful targeted delivery of EVs was reported using cells engineered to express a peptide that specifically bound to tumor cells with EGFR on their surfaces as the source of the delivered EVs. These modified EVs efficiently and specifically delivered let-7a microRNA to EGFR-expressing cancer cells (Ohno et al. 2013). This strategy may be most relevant to specific targeting of GBM cells that express EGFR on their surface. The above-mentioned studies provide an illustration of how EVs can be used for microRNA replacement therapy: by restoring the expression of microRNA downregulated in target cells or by delivering antagonists of tumor-specific, pro-oncogenic microRNA in combination with standard therapy.

Perspective

There is little doubt that microRNAs are important modulators of GBM pathobiology (Godlewski et al. 2015). There is also evidence that other types of RNAs (including lncRNA) may be relevant in GBM biology (Du et al. 2013; Han et al. 2012; Yan et al. 2015; Zhang et al. 2013). GBM-derived EVs as well as the functional co-interaction between different classes of ncRNA are also not well characterized. The modification of gene expression in recipient cells by the transfer of genetic material could account for several EV functions. This EV-mediated transfer of regulatory ncRNAs is potentially a powerful means of orchestrating gene expression which increases the complexity of communication between cells.

The role of EVs as a novel drug delivery system appears to be advantageous over existing ones because of their small size, lack of toxicity, and target specificity. However, therapeutic loading of EVs without compromising their biological properties remains a challenge. Several important questions remain to be answered: what are the mechanisms through which EV ncRNAs are selected to be secreted; are the ncRNAs conveyed into EVs a mere reflection of the cellular ncRNA composition or are they loaded selectively; what are the mechanisms by which the ncRNAs sequestered in EVs influence the phenotype of cancer cells?

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References

- Abuhusain HJ, et al. A metabolic shift favoring sphingosine 1-phosphate at the expense of ceramide controls glioblastoma angiogenesis. *J Biol Chem.* 2013; 288:37355–37364. DOI: 10.1074/jbc.M113.494740 [PubMed: 24265321]
- Akers JC, et al. MiR-21 in the extracellular vesicles (EVs) of cerebrospinal fluid (CSF): a platform for glioblastoma biomarker development. *PLoS One.* 2013; 8:e78115.doi: 10.1371/journal.pone.0078115 [PubMed: 24205116]
- Akers JC, et al. miRNA contents of cerebrospinal fluid extracellular vesicles in glioblastoma patients. *J Neurooncol.* 2015; 123:205–216. DOI: 10.1007/s11060-015-1784-3 [PubMed: 25903655]
- Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat Cell Biol.* 2008; 10:619–624. DOI: 10.1038/ncb1725 [PubMed: 18425114]
- Al-Nedawi K, Meehan B, Kerbel RS, Allison AC, Rak J. Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. *Proc Natl Acad Sci USA.* 2009; 106:3794–3799. DOI: 10.1073/pnas.0804543106 [PubMed: 19234131]
- Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol.* 2011; 29:341–345. DOI: 10.1038/nbt.1807 [PubMed: 21423189]
- Ansari KI, et al. Glucose-based regulation of miR-451/AMPK signaling depends on the OCT1 transcription factor. *Cell Rep.* 2015; 11:902–909. DOI: 10.1016/j.celrep.2015.04.016 [PubMed: 25937278]
- Arroyo JD, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci USA.* 2011; 108:5003–5008. DOI: 10.1073/pnas.1019055108 [PubMed: 21383194]

- Asuthkar S, Velpula KK, Chetty C, Gorantla B, Rao JS. Epigenetic regulation of miRNA-211 by MMP-9 governs glioma cell apoptosis, chemosensitivity and radiosensitivity. *Oncotarget*. 2012; 3:1439–1454. [PubMed: 23183822]
- Balaj L, Lessard R, Dai L, Cho YJ, Pomeroy SL, Breakefield XO, Skog J. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nature Communications*. 2011; 2:180.doi: 10.1038/ncomms1180
- Bao S, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006; 444:756–760. DOI: 10.1038/nature05236 [PubMed: 17051156]
- Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136:215–233. DOI: 10.1016/j.cell.2009.01.002 [PubMed: 19167326]
- Bhat KP, et al. Mesenchymal differentiation mediated by NF-kappaB promotes radiation resistance in glioblastoma. *Cancer Cell*. 2013; 24:331–346. DOI: 10.1016/j.ccr.2013.08.001 [PubMed: 23993863]
- Bier A, et al. MicroRNA-137 is downregulated in glioblastoma and inhibits the stemness of glioma stem cells by targeting RTVP-1. *Oncotarget*. 2013; 4:665–676. [PubMed: 23714687]
- Bobrie A, Colombo M, Krumeich S, Raposo G, Thery C. Diverse subpopulations of vesicles secreted by different intracellular mechanisms are present in exosome preparations obtained by differential ultracentrifugation. *J Extracell Vesicles*. 2012; doi: 10.3402/jev.v1i0.18397
- Bronisz A, et al. Extracellular vesicles modulate the glioblastoma microenvironment via a tumor suppression signaling network directed by miR-1. *Cancer Res*. 2014; 74:738–750. DOI: 10.1158/0008-5472.CAN-13-2650 [PubMed: 24310399]
- Camussi G, Deregibus MC, Bruno S, Grange C, Fonsato V, Tetta C. Exosome/microvesicle-mediated epigenetic reprogramming of cells *American J Cancer Res*. 2011; 1:98–110.
- Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. *Cell*. 2009; 136:642–655. DOI: 10.1016/j.cell.2009.01.035 [PubMed: 19239886]
- Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*. 2005; 65:6029–6033. DOI: 10.1158/0008-5472.CAN-05-0137 [PubMed: 16024602]
- Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H. The brain tumor microenvironment. *Glia*. 2012; 60:502–514. [PubMed: 22379614]
- Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF. A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature*. 2012a; 488:522–526. DOI: 10.1038/nature11287 [PubMed: 22854781]
- Chen L, et al. miR-137 is frequently down-regulated in glioblastoma and is a negative regulator of Cox-2. *Eur J Cancer*. 2012b; 48:3104–3111. DOI: 10.1016/j.ejca.2012.02.007 [PubMed: 22406049]
- Chen L, et al. Downregulation of miR-221/222 sensitizes glioma cells to temozolomide by regulating apoptosis independently of p53 status. *Oncol Rep*. 2012c; 27:854–860. DOI: 10.3892/or.2011.1535 [PubMed: 22075712]
- Chen L, et al. The putative tumor suppressor miR-524-5p directly targets Jagged-1 and Hes-1 in glioma. *Carcinogenesis*. 2012d; 33:2276–2282. DOI: 10.1093/carcin/bgs261 [PubMed: 22871495]
- Chen D, et al. MicroRNA-451 induces epithelial-mesenchymal transition in docetaxel-resistant lung adenocarcinoma cells by targeting proto-oncogene c-Myc. *Eur J Cancer*. 2014; 50:3050–3067. DOI: 10.1016/j.ejca.2014.09.008 [PubMed: 25310895]
- Chen H, Li X, Li W, Zheng H. miR-130a can predict response to temozolomide in patients with glioblastoma multiforme, independently of O6-methylguanine-DNA methyltransferase. *J Transl Med*. 2015; 13:69.doi: 10.1186/s12967-015-0435-y [PubMed: 25890369]
- Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*. 2005; 436:740–744. DOI: 10.1038/nature03868 [PubMed: 15973356]
- Cheng L, Bao S, Rich JN. Potential therapeutic implications of cancer stem cells in glioblastoma. *Biochem Pharmacol*. 2010; 80:654–665. DOI: 10.1016/j.bcp.2010.04.035 [PubMed: 20457135]

- Ciafre SA, et al. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun.* 2005; 334:1351–1358. DOI: 10.1016/j.bbrc.2005.07.030 [PubMed: 16039986]
- Collino F, et al. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. *PloS one.* 2010; 5:e11803.doi: 10.1371/journal.pone.0011803 [PubMed: 20668554]
- Comincini S, et al. microRNA-17 regulates the expression of ATG7 and modulates the autophagy process, improving the sensitivity to temozolomide and low-dose ionizing radiation treatments in human glioblastoma cells. *Cancer Biol Ther.* 2013; 14:574–586. DOI: 10.4161/cbt.24597 [PubMed: 23792642]
- Conti A, et al. miR-21 and 221 upregulation and miR-181b downregulation in human grade II-IV astrocytic tumors. *J Neurooncol.* 2009; 93:325–332. DOI: 10.1007/s11060-009-9797-4 [PubMed: 19159078]
- Corsten MF, Miranda R, Kasmieh R, Krichevsky AM, Weissleder R, Shah K. MicroRNA-21 knockdown disrupts glioma growth in vivo and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas. *Cancer Res.* 2007; 67:8994–9000. DOI: 10.1158/0008-5472.CAN-07-1045 [PubMed: 17908999]
- Dai S, Wei D, Wu Z, Zhou X, Wei X, Huang H, Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. *Mol Ther.* 2008; 16:782–790. DOI: 10.1038/mt.2008.1 [PubMed: 18362931]
- de Jong OG, et al. Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. *J Extracell Vesicles.* 2012; doi: 10.3402/jev.v1i0.18396
- Delic S, et al. MiR-328 promotes glioma cell invasion via SFRP1-dependent Wnt-signaling activation. *Neuro-oncology.* 2014; 16:179–190. DOI: 10.1093/neuonc/not164 [PubMed: 24305703]
- Djebali S, et al. Landscape of transcription in human cells. *Nature.* 2012; 489:101–108. DOI: 10.1038/nature11233 [PubMed: 22955620]
- Dong Q, et al. An axis involving SNAIL, microRNA-128 and SP1 modulates glioma progression. *PLoS One.* 2014; 9:e98651.doi: 10.1371/journal.pone.0098651 [PubMed: 24959930]
- Du Z, et al. Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. *Nat Struct Mol Biol.* 2013; 20:908–913. DOI: 10.1038/nsmb.2591 [PubMed: 23728290]
- Eichhorn SW, et al. mRNA destabilization is the dominant effect of mammalian microRNAs by the time substantial repression ensues. *Mol Cell.* 2014; 56:104–115. DOI: 10.1016/j.molcel.2014.08.028 [PubMed: 25263593]
- Eiring AM, et al. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. *Cell.* 2010; 140:652–665. DOI: 10.1016/j.cell.2010.01.007 [PubMed: 20211135]
- Ernst A, et al. De-repression of CTGF via the miR-17-92 cluster upon differentiation of human glioblastoma spheroid cultures. *Oncogene.* 2010; 29:3411–3422. DOI: 10.1038/onc.2010.83 [PubMed: 20305691]
- Escudier B, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial. *J Transl Med.* 2005; 3:10.doi: 10.1186/1479-5876-3-10 [PubMed: 15740633]
- Estrada-Bernal A, Lawler SE, Nowicki MO, Ray Chaudhury A, Van Brocklyn JR. The role of sphingosine kinase-1 in EGFRvIII-regulated growth and survival of glioblastoma cells. *J Neurooncol.* 2011; 102:353–366. DOI: 10.1007/s11060-010-0345-z [PubMed: 20938717]
- Fabbri M, et al. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proc Natl Acad Sci USA.* 2012; 109:E2110–2116. DOI: 10.1073/pnas.1209414109 [PubMed: 22753494]
- Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res.* 2006; 66:7445–7452. DOI: 10.1158/0008-5472.CAN-06-0858 [PubMed: 16885340]
- Fan X, et al. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells.* 2010; 28:5–16. DOI: 10.1002/stem.254 [PubMed: 19904829]

- Fareh M, et al. The miR 302–367 cluster drastically affects self-renewal and infiltration properties of glioma-initiating cells through CXCR4 repression and consequent disruption of the SHH-GLI-NANOG network. *Cell Death Differ.* 2012; 19:232–244. DOI: 10.1038/cdd.2011.89 [PubMed: 21720384]
- Ferretti E, et al. Concerted microRNA control of Hedgehog signalling in cerebellar neuronal progenitor and tumour cells. *EMBO J.* 2008; 27:2616–2627. DOI: 10.1038/emboj.2008.172 [PubMed: 18756266]
- Fitzner D, et al. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J Cell Sci.* 2011; 124:447–458. DOI: 10.1242/jcs.074088 [PubMed: 21242314]
- Floyd D, Purow B. Micro-masters of glioblastoma biology and therapy: increasingly recognized roles for microRNAs. *Neuro-oncology.* 2014; 16:622–627. DOI: 10.1093/neuonc/nou049 [PubMed: 24723563]
- Fowler A, et al. miR-124a is frequently down-regulated in glioblastoma and is involved in migration and invasion. *Eur J Cancer.* 2011; 47:953–963. DOI: 10.1016/j.ejca.2010.11.026 [PubMed: 21196113]
- Friedmann-Morvinski D. Glioblastoma heterogeneity and cancer cell plasticity. *Crit Rev Oncog.* 2014; 19:327–336. [PubMed: 25404148]
- Furnari FB, et al. Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev.* 2007; 21:2683–2710. DOI: 10.1101/gad.1596707 [PubMed: 17974913]
- Gabriely G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS, Krichevsky AM. MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. *Mol Cell Biol.* 2008; 28:5369–5380. DOI: 10.1128/MCB.00479-08 [PubMed: 18591254]
- Gabriely G, et al. Human glioma growth is controlled by microRNA-10b. *Cancer Res.* 2011; 71:3563–3572. DOI: 10.1158/0008-5472.CAN-10-3568 [PubMed: 21471404]
- Gal H, et al. MIR-451 and imatinib mesylate inhibit tumor growth of glioblastoma stem cells. *Biochem Biophys Res Commun.* 2008; 376:86–90. DOI: 10.1016/j.bbrc.2008.08.107 [PubMed: 18765229]
- Garcia NA, Ontoria-Oviedo I, Gonzalez-King H, Diez-Juan A, Sepulveda P. Glucose starvation in cardiomyocytes enhances exosome secretion and promotes angiogenesis in endothelial cells. *PloS one.* 2015; 10:e0138849.doi: 10.1371/journal.pone.0138849 [PubMed: 26393803]
- Garnier D, Jabado N, Rak J. Extracellular vesicles as prospective carriers of oncogenic protein signatures in adult and paediatric brain tumours. *Proteomics.* 2013; 13:1595–1607. DOI: 10.1002/pmic.201200360 [PubMed: 23505048]
- Gaur AB, Holbeck SL, Colburn NH, Israel MA. Downregulation of Pcd4 by mir-21 facilitates glioblastoma proliferation in vivo. *Neuro-oncology.* 2011; 13:580–590. DOI: 10.1093/neuonc/nor033 [PubMed: 21636706]
- Giese A, Loo MA, Tran N, Haskett D, Coons SW, Berens ME. Dichotomy of astrocytoma migration and proliferation. *Int J Cancer.* 1996; 67:275–282. DOI: 10.1002/(SICI)1097-0215(19960717)67:2<275:AID-IJC20>3.0.CO;2-9 [PubMed: 8760599]
- Giese A, Bjerkvig R, Berens ME, Westphal M. Cost of migration: invasion of malignant gliomas and implications for treatment. *J Clin Oncol.* 2003; 21:1624–1636. DOI: 10.1200/JCO.2003.05.063 [PubMed: 12697889]
- Gilbertson RJ, Rich JN. Making a tumour's bed: glioblastoma stem cells and the vascular niche. *Nat Rev Cancer.* 2007; 7:733–736. DOI: 10.1038/nrc2246 [PubMed: 17882276]
- Godlewski J, et al. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res.* 2008; 68:9125–9130. DOI: 10.1158/0008-5472.CAN-08-2629 [PubMed: 19010882]
- Godlewski J, Bronisz A, Nowicki MO, Chiocca EA, Lawler S. microRNA-451: a conditional switch controlling glioma cell proliferation and migration. *Cell Cycle.* 2010a; 9:2742–2748. [PubMed: 20647762]
- Godlewski J, et al. MicroRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells. *Mol Cell.* 2010b; 37:620–632. DOI: 10.1016/j.molcel.2010.02.018 [PubMed: 20227367]

- Godlewski J, Krichevsky AM, Johnson MD, Chiocca EA, Bronisz A. Belonging to a network—microRNAs, extracellular vesicles, and the glioblastoma microenvironment. *Neuro-oncology*. 2015; 17:652–662. DOI: 10.1093/neuonc/nou292 [PubMed: 25301812]
- Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. *Cell*. 2005; 123:631–640. DOI: 10.1016/j.cell.2005.10.022 [PubMed: 16271387]
- Guan H, et al. Sphingosine kinase 1 regulates the Akt/FOXO3a/Bim pathway and contributes to apoptosis resistance in glioma cells. *PLoS One*. 2011; 6:e19946.doi: 10.1371/journal.pone.0019946 [PubMed: 21625639]
- Guduric-Fuchs J, O'Connor A, Camp B, O'Neill CL, Medina RJ, Simpson DA. Selective extracellular vesicle-mediated export of an overlapping set of microRNAs from multiple cell types. *BMC Genomics*. 2012; 13:357.doi: 10.1186/1471-2164-13-357 [PubMed: 22849433]
- Guessous F, et al. microRNA-34a is tumor suppressive in brain tumors and glioma stem cells. *Cell Cycle*. 2010; 9:1031–1036. [PubMed: 20190569]
- Guessous F, et al. Oncogenic effects of miR-10b in glioblastoma stem cells. *J Neurooncol*. 2013; 112:153–163. DOI: 10.1007/s11060-013-1047-0 [PubMed: 23307328]
- Guil S, Esteller M. RNA-RNA interactions in gene regulation: the coding and noncoding players. *Trends Biochem Sci*. 2015; 40:248–256. DOI: 10.1016/j.tibs.2015.03.001 [PubMed: 25818326]
- Han L, et al. LncRNA pro file of glioblastoma reveals the potential role of lncRNAs in contributing to glioblastoma pathogenesis. *Int J Oncol*. 2012; 40:2004–2012. DOI: 10.3892/ijo.2012.1413 [PubMed: 22446686]
- Holdhoff M, Yovino SG, Boadu O, Grossman SA. Blood-based biomarkers for malignant gliomas. *J Neurooncol*. 2013; 113:345–352. DOI: 10.1007/s11060-013-1144-0 [PubMed: 23670054]
- Hong X, Sin WC, Harris AL, Naus CC. Gap junctions modulate glioma invasion by direct transfer of microRNA. *Oncotarget*. 2015; 6:15566–15577. [PubMed: 25978028]
- Huang Z, Cheng L, Guryanova OA, Wu Q, Bao S. Cancer stem cells in glioblastoma—molecular signaling and therapeutic targeting. *Protein Cell*. 2010; 1:638–655. DOI: 10.1007/s13238-010-0078-y [PubMed: 21203936]
- Infanger DW, et al. Glioblastoma stem cells are regulated by interleukin-8 signaling in a tumoral perivascular niche. *Cancer Res*. 2013; 73:7079–7089. DOI: 10.1158/0008-5472.CAN-13-1355 [PubMed: 24121485]
- Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med*. 2012; 4:143–159. DOI: 10.1002/emmm.201100209 [PubMed: 22351564]
- Ismail N, et al. Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. *Blood*. 2013; 121:984–995. DOI: 10.1182/blood-2011-08-374793 [PubMed: 23144169]
- Kajimoto T, Okada T, Miya S, Zhang L, Nakamura S. Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. *Nat Commun*. 2013; 4:2712.doi: 10.1038/ncomms3712 [PubMed: 24231649]
- Kapitonov D, et al. Targeting sphingosine kinase 1 inhibits Akt signaling, induces apoptosis, and suppresses growth of human glioblastoma cells and xenografts. *Cancer Res*. 2009; 69:6915–6923. DOI: 10.1158/0008-5472.CAN-09-0664 [PubMed: 19723667]
- Katakowski M, Buller B, Wang X, Rogers T, Chopp M. Functional microRNA is transferred between glioma cells. *Cancer Res*. 2010a; 70:8259–8263. DOI: 10.1158/0008-5472.CAN-10-0604 [PubMed: 20841486]
- Katakowski M, Zheng X, Jiang F, Rogers T, Szalad A, Chopp M. MiR-146b-5p suppresses EGFR expression and reduces in vitro migration and invasion of glioma. *Cancer Invest*. 2010b; 28:1024–1030. DOI: 10.3109/07357907.2010.512596 [PubMed: 20874002]
- Katakowski M, et al. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. *Cancer Lett*. 2013; 335:201–204. DOI: 10.1016/j.canlet.2013.02.019 [PubMed: 23419525]
- Kefas B, et al. microRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. *Cancer Res*. 2008; 68:3566–3572. DOI: 10.1158/0008-5472.CAN-07-6639 [PubMed: 18483236]

- Kefas B, et al. The neuronal microRNA miR-326 acts in a feedback loop with notch and has therapeutic potential against brain tumors. *J Neurosci*. 2009; 29:15161–15168. DOI: 10.1523/JNEUROSCI.4966-09.2009 [PubMed: 19955368]
- Kogure T, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. *Hepatology*. 2011; 54:1237–1248. DOI: 10.1002/hep.24504 [PubMed: 21721029]
- Koppers-Lalic D, et al. Nontemplated nucleotide additions distinguish the small RNA composition in cells from exosomes. *Cell Rep*. 2014; 8:1649–1658. DOI: 10.1016/j.celrep.2014.08.027 [PubMed: 25242326]
- Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. Neutral sphingomyelinase 2 (nSMase2)-dependent exosomal transfer of angiogenic microRNAs regulate cancer cell metastasis. *J Biol Chem*. 2013; 288:10849–10859. DOI: 10.1074/jbc.M112.446831 [PubMed: 23439645]
- Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. 2014; 42:D68–73. DOI: 10.1093/nar/gkt1181 [PubMed: 24275495]
- Krichevsky AM, Gabrieli G. miR-21: a small multi-faceted RNA. *J Cell Mol Med*. 2009; 13:39–53. DOI: 10.1111/j.1582-4934.2008.00556.x [PubMed: 19175699]
- Kwak HJ, et al. Downregulation of Spry2 by miR-21 triggers malignancy in human gliomas. *Oncogene*. 2011; 30:2433–2442. DOI: 10.1038/onc.2010.620 [PubMed: 21278789]
- Lai CP, et al. Dynamic biodistribution of extracellular vesicles in vivo using a multimodal imaging reporter. *ACS nano*. 2014; 8:483–494. DOI: 10.1021/nn404945r [PubMed: 24383518]
- Lakomy R, et al. MiR-195, miR-196b, miR-181c, miR-21 expression levels and O-6-methylguanine-DNA methyltransferase methylation status are associated with clinical outcome in glioblastoma patients. *Cancer Sci*. 2011; 102:2186–2190. DOI: 10.1111/j.1349-7006.2011.02092.x [PubMed: 21895872]
- Lang MF, et al. Genome-wide profiling identified a set of miRNAs that are differentially expressed in glioblastoma stem cells and normal neural stem cells. *PloS one*. 2012; 7:e36248.doi: 10.1371/journal.pone.0036248 [PubMed: 22558405]
- Lavon I, et al. Gliomas display a microRNA expression profile reminiscent of neural precursor cells. *Neuro-oncology*. 2010; 12:422–433. DOI: 10.1093/neuonc/nop061 [PubMed: 20406893]
- Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*. 1993; 75:843–854. [PubMed: 8252621]
- Lee I, et al. New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res*. 2009; 19:1175–1183. DOI: 10.1101/gr.089367.108 [PubMed: 19336450]
- Lee HK, et al. Mesenchymal stem cells deliver synthetic microRNA mimics to glioma cells and glioma stem cells and inhibit their cell migration and self-renewal. *Oncotarget*. 2013; 4:346–361. [PubMed: 23548312]
- Li Y, et al. MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res*. 2009a; 69:7569–7576. DOI: 10.1158/0008-5472.CAN-09-0529 [PubMed: 19773441]
- Li Y, et al. MicroRNA-21 targets LRRFIP1 and contributes to VM-26 resistance in glioblastoma multiforme. *Brain Res*. 2009b; 1286:13–18. DOI: 10.1016/j.brainres.2009.06.053 [PubMed: 19559015]
- Li Z, et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell*. 2009c; 15:501–513. DOI: 10.1016/j.ccr.2009.03.018 [PubMed: 19477429]
- Li P, et al. MiR-181b suppresses proliferation of and reduces chemoresistance to temozolomide in U87 glioma stem cells. *J Biomed Res*. 2010; 24:436–443. DOI: 10.1016/S1674-8301(10)60058-9 [PubMed: 23554660]
- Li Y, Zhao S, Zhen Y, Li Q, Teng L, Asai A, Kawamoto K. A miR-21 inhibitor enhances apoptosis and reduces G(2)-M accumulation induced by ionizing radiation in human glioblastoma U251 cells. *Brain Tumor Pathol*. 2011; 28:209–214. DOI: 10.1007/s10014-011-0037-1 [PubMed: 21618027]
- Li CC, et al. Glioma microvesicles carry selectively packaged coding and non-coding RNAs which alter gene expression in recipient cells. *RNA Biol*. 2013; 10:1333–1344. DOI: 10.4161/rna.25281 [PubMed: 23807490]

- Lim PK, et al. Gap junction-mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. *Cancer Res.* 2011; 71:1550–1560. DOI: 10.1158/0008-5472.CAN-10-2372 [PubMed: 21343399]
- Lin J, Teo S, Lam DH, Jeyaseelan K, Wang S. MicroRNA-10b pleiotropically regulates invasion, angiogenicity and apoptosis of tumor cells resembling mesenchymal subtype of glioblastoma multiforme. *Cell Death Dis.* 2012; 3:e398.doi: 10.1038/cddis.2012.134 [PubMed: 23034333]
- MacRae IJ, Ma E, Zhou M, Robinson CV, Doudna JA. In vitro reconstitution of the human RISC-loading complex. *Proc Natl Acad Sci USA.* 2008; 105:512–517. DOI: 10.1073/pnas.0710869105 [PubMed: 18178619]
- Malzkorn B, Wolter M, Liesenberg F, Grzendowski M, Stuhler K, Meyer HE, Reifenberger G. Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. *Brain Pathol.* 2010; 20:539–550. DOI: 10.1111/j.1750-3639.2009.00328.x [PubMed: 19775293]
- Maniatakis E, Mourelatos Z. A human, ATP-independent, RISC assembly machine fueled by pre-miRNA. *Genes Dev.* 2005; 19:2979–2990. DOI: 10.1101/gad.1384005 [PubMed: 16357216]
- Manterola L, et al. A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool. *Neuro-oncology.* 2014; 16:520–527. DOI: 10.1093/neuonc/not218 [PubMed: 24435880]
- Mao P, et al. Mesenchymal glioma stem cells are maintained by activated glycolytic metabolism involving aldehyde dehydrogenase 1A3. *Proc Natl Acad Sci USA.* 2013; 110:8644–8649. DOI: 10.1073/pnas.1221478110 [PubMed: 23650391]
- Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. *Nature.* 2013; 501:328–337. DOI: 10.1038/nature12624 [PubMed: 24048065]
- Melo SA, et al. A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet.* 2009; 41:365–370. DOI: 10.1038/ng.317 [PubMed: 19219043]
- Melo SA, et al. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. *Cancer Cell.* 2014; 26:707–721. DOI: 10.1016/j.ccell.2014.09.005 [PubMed: 25446899]
- Meyer M, et al. Single cell-derived clonal analysis of human glioblastoma links functional and genomic heterogeneity. *Proc Natl Acad Sci USA.* 2015; 112:851–856. DOI: 10.1073/pnas.1320611111 [PubMed: 25561528]
- Mignot G, Roux S, Thery C, Segura E, Zitvogel L. Prospects for exosomes in immunotherapy of cancer. *J Cell Mol Med.* 2006; 10:376–388. [PubMed: 16796806]
- Miranda KC, et al. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell.* 2006; 126:1203–1217. DOI: 10.1016/j.cell.2006.07.031 [PubMed: 16990141]
- Mittelbrunn M, et al. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat Commun.* 2011; 2:282.doi: 10.1038/ncomms1285 [PubMed: 21505438]
- Morse MA, et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *J Transl Med.* 2005; 3:9.doi: 10.1186/1479-5876-3-9 [PubMed: 15723705]
- Munoz JL, Bliss SA, Greco SJ, Ramkissoon SH, Ligon KL, Rameshwar P. Delivery of functional anti-miR-9 by mesenchymal stem cell-derived exosomes to glioblastoma multiforme cells conferred chemosensitivity molecular therapy. *Nucleic Acids.* 2013; 2:e126.doi: 10.1038/mtna.2013.60 [PubMed: 24084846]
- Munro TP, Magee RJ, Kidd GJ, Carson JH, Barbarese E, Smith LM, Smith R. Mutational analysis of a heterogeneous nuclear ribonucleoprotein A2 response element for RNA trafficking. *J Biol Chem.* 1999; 274:34389–34395. [PubMed: 10567417]
- Nan Y, et al. MiRNA-451 plays a role as tumor suppressor in human glioma cells. *Brain Res.* 2010; 1359:14–21. DOI: 10.1016/j.brainres.2010.08.074 [PubMed: 20816946]
- Noerholm M, et al. RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls. *BMC Cancer.* 2012; 12:22.doi: 10.1186/1471-2407-12-22 [PubMed: 22251860]
- Nolte-t Hoen EN, Buermans HP, Waasdorp M, Stoorvogel W, Wauben MH, Hoen PA. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of

- small non-coding RNA biotypes with potential regulatory functions. *Nucleic Acids Res.* 2012; 40:9272–9285. DOI: 10.1093/nar/gks658 [PubMed: 22821563]
- Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligo-dendroglial gliomas. *J Neuropathol Exp Neurol.* 2005; 64:479–489. [PubMed: 15977639]
- Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. *Am J Pathol.* 2007; 170:1445–1453. DOI: 10.2353/ajpath.2007.070011 [PubMed: 17456751]
- Ohgaki H, et al. Genetic pathways to glioblastoma: a population-based study. *Cancer Res.* 2004; 64:6892–6899. DOI: 10.1158/0008-5472.CAN-04-1337 [PubMed: 15466178]
- Ohno S, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol Ther.* 2013; 21:185–191. DOI: 10.1038/mt.2012.180
- Orom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. *Mol Cell.* 2008; 30:460–471. DOI: 10.1016/j.molcel.2008.05.001 [PubMed: 18498749]
- Ostenfeld MS, et al. Cellular disposal of miR23b by RAB27-dependent exosome release is linked to acquisition of metastatic properties. *Cancer Res.* 2014; 74:5758–5771. DOI: 10.1158/0008-5472.CAN-13-3512 [PubMed: 25261234]
- Ostrowski M, et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nature Cell Biol.* 2010; 12:19–30. 11–13. DOI: 10.1038/ncb2000 [PubMed: 19966785]
- Papagiannakopoulos T, Shapiro A, Kosik KS. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. *Cancer Res.* 2008; 68:8164–8172. DOI: 10.1158/0008-5472.CAN-08-1305 [PubMed: 18829576]
- Patel AP, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science.* 2014; 344:1396–1401. DOI: 10.1126/science.1254257 [PubMed: 24925914]
- Peruzzi P, et al. MicroRNA-128 coordinately targets polycomb repressor complexes in glioma stem cells. *Neuro-oncology.* 2013; 15:1212–1224. DOI: 10.1093/neuonc/not055 [PubMed: 23733246]
- Pietras A, et al. Osteopontin-CD44 signaling in the glioma perivascular niche enhances cancer stem cell phenotypes and promotes aggressive tumor growth. *Cell Stem Cell.* 2014; 14:357–369. DOI: 10.1016/j.stem.2014.01.005 [PubMed: 24607407]
- Pigati L, et al. Selective release of microRNA species from normal and malignant mammary epithelial cells. *PloS One.* 2010; 5:e13515.doi: 10.1371/journal.pone.0013515 [PubMed: 20976003]
- Piwecka M, et al. Comprehensive analysis of microRNA expression profile in malignant glioma tissues. *Mol Oncol.* 2015; doi: 10.1016/j.molonc.2015.03.007
- Qiu S, Huang D, Yin D, Li F, Li X, Kung HF, Peng Y. Suppression of tumorigenicity by microRNA-138 through inhibition of EZH2-CDK4/6-pRb-E2F1 signal loop in glioblastoma multiforme. *Biochim Biophys Acta.* 2013; 1832:1697–1707. DOI: 10.1016/j.bbadis.2013.05.015 [PubMed: 23707559]
- Rani SB, Rathod SS, Karthik S, Kaur N, Muzumdar D, Shiras AS. MiR-145 functions as a tumor-suppressive RNA by targeting Sox9 and adducin 3 in human glioma cells. *Neuro-oncology.* 2013; 15:1302–1316. DOI: 10.1093/neuonc/not090 [PubMed: 23814265]
- Rao JS. Molecular mechanisms of glioma invasiveness: the role of proteases. *Nat Rev Cancer.* 2003; 3:489–501. DOI: 10.1038/nrc1121 [PubMed: 12835669]
- Rao P, Benito E, Fischer A. MicroRNAs as biomarkers for CNS disease. *Front Mol Neurosci.* 2013; 6:39.doi: 10.3389/fnmol.2013.00039 [PubMed: 24324397]
- Ren Y, et al. Co-delivery of as-miR-21 and 5-FU by poly(amidoamine) dendrimer attenuates human glioma cell growth in vitro. *J Biomater Sci Polym Ed.* 2010a; 21:303–314. DOI: 10.1163/156856209X415828 [PubMed: 20178687]
- Ren Y, et al. MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol. *BMC Cancer.* 2010b; 10:27.doi: 10.1186/1471-2407-10-27 [PubMed: 20113523]
- Richly H, Aloia L, Di Croce L. Roles of the Polycomb group proteins in stem cells and cancer. *Cell Death Dis.* 2011; 2:e204.doi: 10.1038/cddis.2011.84 [PubMed: 21881606]
- Rigogliuso S, Donati C, Cassara D, Taverna S, Salamone M, Bruni P, Vittorelli ML. An active form of sphingosine kinase-1 is released in the extracellular medium as component of membrane vesicles

shed by two human tumor cell lines. *J Oncol.* 2010; 2010:509329.doi: 10.1155/2010/509329 [PubMed: 20508814]

- Roth P, et al. A specific miRNA signature in the peripheral blood of glioblastoma patients. *J Neurochem.* 2011; 118:449–457. DOI: 10.1111/j.1471-4159.2011.07307.x [PubMed: 21561454]
- Sasayama T, Nishihara M, Kondoh T, Hosoda K, Kohmura E. MicroRNA-10b is overexpressed in malignant glioma and associated with tumor invasive factors, uPAR and RhoC. *Int J Cancer.* 2009; 125:1407–1413. DOI: 10.1002/ijc.24522 [PubMed: 19536818]
- Sergeeva TI, Raushenbakh TI, Shevchenko MO, Rybal'chenko VG. Excretion of 5-hydroxyindole-3-acetic and 5-methoxyindole-3-acetic acids in cancer patients. *Vopr Onkol.* 1987; 33:20–25.
- Setty M, et al. Inferring transcriptional and microRNA-mediated regulatory programs in glioblastoma. *Mol Syst Biol.* 2012; 8:605.doi: 10.1038/msb.2012.37 [PubMed: 22929615]
- Shi L, Chen J, Yang J, Pan T, Zhang S, Wang Z. MiR-21 protected human glioblastoma U87MG cells from chemotherapeutic drug temozolomide induced apoptosis by decreasing Bax/Bcl-2 ratio and caspase-3 activity. *Brain Res.* 2010a; 1352:255–264. DOI: 10.1016/j.brainres.2010.07.009 [PubMed: 20633539]
- Shi L, et al. MiR-125b is critical for the suppression of human U251 glioma stem cell proliferation. *Brain Res.* 2010b; 1312:120–126. DOI: 10.1016/j.brainres.2009.11.056 [PubMed: 19948152]
- Shi L, et al. MicroRNA-125b-2 confers human glioblastoma stem cells resistance to temozolomide through the mitochondrial pathway of apoptosis. *Int J Oncol.* 2012; 40:119–129. DOI: 10.3892/ijo.2011.1179 [PubMed: 21879257]
- Shi R, et al. Exosomal levels of miRNA-21 from cerebrospinal fluids associated with poor prognosis and tumor recurrence of glioma patients. *Oncotarget.* 2015; 6:26971–26981. [PubMed: 26284486]
- Shih AH, Holland EC. Notch signaling enhances nestin expression in gliomas. *Neoplasia.* 2006; 8:1072–1082. DOI: 10.1593/neo.06526 [PubMed: 17217625]
- Silber J, et al. miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. *BMC Med.* 2008; 6:14.doi: 10.1186/1741-7015-6-14 [PubMed: 18577219]
- Singh SK, et al. Identification of human brain tumour initiating cells. *Nature.* 2004; 432:396–401. DOI: 10.1038/nature03128 [PubMed: 15549107]
- Singh R, Pochampally R, Watabe K, Lu Z, Mo YY. Exosome-mediated transfer of miR-10b promotes cell invasion in breast cancer. *Mol Cancer.* 2014; 13:256.doi: 10.1186/1476-4598-13-256 [PubMed: 25428807]
- Skog J, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol.* 2008; 10:1470–1476. DOI: 10.1038/ncb1800 [PubMed: 19011622]
- Slaby O, et al. MicroRNA-181 family predicts response to concomitant chemoradiotherapy with temozolomide in glioblastoma patients. *Neoplasia.* 2010; 57:264–269. [PubMed: 20353279]
- Squadrito ML, et al. Endogenous RNAs modulate microRNA sorting to exosomes and transfer to acceptor cells. *Cell Rep.* 2014; 8:1432–1446. DOI: 10.1016/j.celrep.2014.07.035 [PubMed: 25159140]
- Stupp R, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New Engl J Med.* 2005; 352:987–996. DOI: 10.1056/NEJMoa043330 [PubMed: 15758009]
- Stupp R, Hegi ME, Gilbert MR, Chakravarti A. Chemoradiotherapy in malignant glioma: standard of care and future directions. *J Clin Oncol.* 2007; 25:4127–4136. DOI: 10.1200/JCO.2007.11.8554 [PubMed: 17827463]
- Sun L, et al. MicroRNA-10b induces glioma cell invasion by modulating MMP-14 and uPAR expression via HOXD10. *Brain Res.* 2011; 1389:9–18. DOI: 10.1016/j.brainres.2011.03.013 [PubMed: 21419107]
- Sun J, Liao K, Wu X, Huang J, Zhang S, Lu X. Serum microRNA-128 as a biomarker for diagnosis of glioma. *Int J Clin Exp Med.* 2015; 8:456–463. [PubMed: 25785017]
- Tabet F, et al. HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. *Nat Commun.* 2014; 5:3292.doi: 10.1038/ncomms4292 [PubMed: 24576947]

- Takahashi Y, Nishikawa M, Shinotsuka H, Matsui Y, Ohara S, Imai T, Takakura Y. Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *J Biotechnol.* 2013; 165:77–84. DOI: 10.1016/j.jbiotec.2013.03.013 [PubMed: 23562828]
- Tang H, et al. Interaction of hsa-miR-381 and glioma suppressor LRRC4 is involved in glioma growth. *Brain Res.* 2011; 1390:21–32. DOI: 10.1016/j.brainres.2011.03.034 [PubMed: 21435336]
- Tang H, et al. The miR-183/96/182 cluster regulates oxidative apoptosis and sensitizes cells to chemotherapy in gliomas. *Curr Cancer Drug Targets.* 2013; 13:221–231. [PubMed: 23252827]
- Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature.* 2014; 505:344–352. DOI: 10.1038/nature12986 [PubMed: 24429633]
- Tepluyuk NM, et al. MicroRNAs in cerebrospinal fluid identify glioblastoma and metastatic brain cancers and reflect disease activity. *Neuro-oncology.* 2012; 14:689–700. DOI: 10.1093/neuonc/nos074 [PubMed: 22492962]
- Tepluyuk NM, et al. MicroRNA-10b inhibition reduces E2F1-mediated transcription and miR-15/16 activity in glioblastoma. *Oncotarget.* 2015; 6:3770–3783. [PubMed: 25738367]
- Ujifuku K, et al. miR-195, miR-455-3p and miR-10a(*) are implicated in acquired temozolomide resistance in glioblastoma multiforme cells. *Cancer Lett.* 2010; 296:241–248. DOI: 10.1016/j.canlet.2010.04.013 [PubMed: 20444541]
- Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol.* 2007; 9:654–659. DOI: 10.1038/ncb1596 [PubMed: 17486113]
- van der Vos KE, Balaj L, Skog J, Breakefield XO. Brain tumor microvesicles: insights into intercellular communication in the nervous system. *Cell Mol Neurobiol.* 2011; 31:949–959. DOI: 10.1007/s10571-011-9697-y [PubMed: 21553248]
- van der Vos KE, et al. Directly visualized glioblastoma-derived extracellular vesicles transfer RNA to microglia/macrophages in the brain. *Neuro-oncology.* 2015; doi: 10.1093/neuonc/nov244
- Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science.* 2007; 318:1931–1934. DOI: 10.1126/science.1149460 [PubMed: 18048652]
- Verhaak RG, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell.* 2010; 17:98–110. DOI: 10.1016/j.ccr.2009.12.020 [PubMed: 20129251]
- Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol.* 2011; 13:423–433. DOI: 10.1038/ncb2210 [PubMed: 21423178]
- Villarroya-Beltri C, et al. Sumoylated hnRNP A2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat Commun.* 2013; 4:2980. doi: 10.1038/ncomms3980 [PubMed: 24356509]
- Vo DT, Qiao M, Smith AD, Burns SC, Brenner AJ, Penalva LO. The oncogenic RNA-binding protein Musashi1 is regulated by tumor suppressor miRNAs. *RNA Biol.* 2011; 8:817–828. DOI: 10.4161/rna.8.5.16041 [PubMed: 21881409]
- Wang J, et al. Notch promotes radioresistance of glioma stem cells. *Stem cells.* 2010; 28:17–28. DOI: 10.1002/stem.261 [PubMed: 19921751]
- Wang K, et al. miR-92b controls glioma proliferation and invasion through regulating Wnt/beta-catenin signaling via Nemo-like kinase. *Neuro-oncology.* 2013; 15:578–588. DOI: 10.1093/neuonc/not004 [PubMed: 23416699]
- Wang R, et al. Acquisition of radioresistance in docetaxel-resistant human lung adenocarcinoma cells is linked with dysregulation of miR-451/c-Myc-survivin/rad-51 signaling. *Oncotarget.* 2014; 5:6113–6129. [PubMed: 25026294]
- Wei X, Chen D, Lv T, Li G, Qu S. Serum microRNA-125b as a potential biomarker for glioma diagnosis. *Mol Neurobiol.* 2014; doi: 10.1007/s12035-014-8993-1
- Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev.* 2009; 23:1494–1504. DOI: 10.1101/gad.1800909 [PubMed: 19571179]

- Wong ST, Zhang XQ, Zhuang JT, Chan HL, Li CH, Leung GK. MicroRNA-21 inhibition enhances in vitro chemosensitivity of temozolomide-resistant glioblastoma cells. *Anticancer Res.* 2012; 32:2835–2841. [PubMed: 22753745]
- Wu X, Hu A, Zhang M, Chen Z. Effects of Rab27a on proliferation, invasion, and anti-apoptosis in human glioma cell. *Tumour Biol.* 2013; 34:2195–2203. DOI: 10.1007/s13277-013-0756-5 [PubMed: 23553027]
- Wuchty S, et al. Prediction of associations between microRNAs and gene expression in glioma biology. *PloS One.* 2011; 6:e14681.doi: 10.1371/journal.pone.0014681 [PubMed: 21358821]
- Xia H, et al. Loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells. *J Biol Chem.* 2012a; 287:9962–9971. DOI: 10.1074/jbc.M111.332627 [PubMed: 22253443]
- Xia J, et al. miR-124 inhibits cell proliferation in gastric cancer through down-regulation of SPHK1. *J Pathol.* 2012b; 227:470–480. DOI: 10.1002/path.4030 [PubMed: 22450659]
- Xin H, et al. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells.* 2012; 30:1556–1564. DOI: 10.1002/stem.1129 [PubMed: 22605481]
- Yan K, Yang K, Rich JN. The evolving landscape of glioblastoma stem cells. *Curr Opin Neurol.* 2013; 26:701–707. DOI: 10.1097/WCO.0000000000000032 [PubMed: 24152818]
- Yan Y, et al. LncRNA and mRNA interaction study based on transcriptome profiles reveals potential core genes in the pathogenesis of human glioblastoma multiforme. *J Cancer Res Clin Oncol.* 2015; 141:827–838. DOI: 10.1007/s00432-014-1861-6 [PubMed: 25378224]
- Yang YP, et al. Inhibition of cancer stem cell-like properties and reduced chemoradioresistance of glioblastoma using microRNA145 with cationic polyurethane-short branch PEI. *Biomaterials.* 2012; 33:1462–1476. DOI: 10.1016/j.biomaterials.2011.10.071 [PubMed: 22098779]
- Yang HW, Xing H, Johnson MD. A major role for microRNAs in glioblastoma cancer stem-like cells. *Arch Pharmacol Res.* 2015; 38:423–434. DOI: 10.1007/s12272-015-0574-y
- Yu X, Harris SL, Levine AJ. The regulation of exosome secretion: a novel function of the p53 protein. *Cancer Res.* 2006; 66:4795–4801. DOI: 10.1158/0008-5472.CAN-05-4579 [PubMed: 16651434]
- Zhang Y, et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell.* 2010; 39:133–144. DOI: 10.1016/j.molcel.2010.06.010 [PubMed: 20603081]
- Zhang H, Li W, Sun S, Yu S, Zhang M, Zou F. Inhibition of sphingosine kinase 1 suppresses proliferation of glioma cells under hypoxia by attenuating activity of extracellular signal-regulated kinase. *Cell Prolif.* 2012a; 45:167–175. DOI: 10.1111/j.1365-2184.2012.00807.x [PubMed: 22288844]
- Zhang J, Stevens MF, Bradshaw TD. Temozolomide: mechanisms of action, repair and resistance. *Curr Mol Pharmacol.* 2012b; 5:102–114. [PubMed: 22122467]
- Zhang XQ, et al. A long non-coding RNA signature in glioblastoma multiforme predicts survival. *Neurobiol Dis.* 2013; 58:123–131. DOI: 10.1016/j.nbd.2013.05.011 [PubMed: 23726844]
- Zhang R, et al. Plasma miR-221/222 family as novel descriptive and prognostic biomarkers for glioma. *Mol Neurobiol.* 2015; doi: 10.1007/s12035-014-9079-9
- Zhao S, et al. MicroRNA-153 is tumor suppressive in glioblastoma stem cells. *Mol Biol Rep.* 2013; 40:2789–2798. DOI: 10.1007/s11033-012-2278-4 [PubMed: 23397238]
- Zhong J, Paul A, Kellie SJ, O'Neill GM. Mesenchymal migration as a therapeutic target in glioblastoma. *J Oncol.* 2010; 2010:430142.doi: 10.1155/2010/430142 [PubMed: 20652056]
- Zhou H, Rigoutsos I. MiR-103a-3p targets the 5' UTR of GPRC5A in pancreatic cells. *Rna.* 2014; 20:1431–1439. DOI: 10.1261/rna.045757.114 [PubMed: 24984703]
- Zhou X, et al. Downregulation of miR-21 inhibits EGFR pathway and suppresses the growth of human glioblastoma cells independent of PTEN status. *Lab Invest.* 2010; 90:144–155. DOI: 10.1038/labinvest.2009.126 [PubMed: 20048743]
- Zhuang X, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Mol Ther.* 2011; 19:1769–1779. DOI: 10.1038/mt.2011.164 [PubMed: 21915101]

Fig. 1.
Transfer of microRNAs encapsulated in EVs between the tumor cells and between tumor and non-malignant cells and circulation underscores additional layer of complexity of tumor heterogeneity

Fig. 2. Diverse mechanisms of microRNA-targeting strategies. The microRNAs recruited to the RNA-induced silencing complex (RISC) regulate the output of protein-coding genes, non-protein-coding genes, and proteins (including RNA-binding proteins—RBPs) through diverse mechanisms

Fig. 3.

MicroRNAs can affect GBM tumorigenesis by deregulation of gene expression. Such signals can be transferred between cells by the exchange of EVs, leading to complex pro-oncogenic phenotypes, including enhanced resistance to therapy. These microRNAs were selected as examples of pro-oncogenic (miR-10b) and tumor suppressor (miR-1) signaling in GBM and their potential engagement in EV-mediated communication

Fig. 4. EV/microRNAs' tandems can be used for the delivery of therapeutic microRNA (TmicroRNA). Whether EV/TmicroRNA-shedding cells or purified EV/TmicroRNA would be more efficacious as a vehicle for the delivery of microRNAs needs to be determined

Table 1

MicroRNAs deregulated in GBM and identified in EV derived from GBM patients' serum, CSF, or GBM cells

ID	Expression in GBM	Targets	Extracellular detection	References
hsa-miR-125b	Deregulated	BAX, BMF, PDPN, BMF	EV GBM cells, GBM CSF, GBM serum	Li et al. (2013); Teplyuk et al. (2012); Wei et al. (2014)
hsa-miR-130a	Deregulated	–	EV GBM CSF	Akers et al. (2015)
hsa-miR-328	Deregulated	SFRP1,	EV GBM CSF	Akers et al. (2015)
hsa-miR-451	Deregulated	CAB39, YWHAZ, MYC	EV GBM cells, EV GBM CSF	Li et al. (2013); Akers et al. (2015)
has-miR-29b	Downregulated	PDPN	EV GBM cells	Li et al. (2013)
hsa-miR-128	Downregulated	BMI1, SUZ12, EGFR, E2F3A	GBM blood, GBM serum	Roth et al. (2011); Sun et al. (2015)
hsa-miR-146b	Downregulated	TRAF6, EGFR, MMPs	EV GBM serum, EV GBM CSF	Manterola et al. (2014); Akers et al. (2015)
hsa-miR-15b	Downregulated	CCND1, CDKN1A, NRP2	EV GBM cells, GBM CSF	Skog et al. (2008); Teplyuk et al. (2012)
hsa-miR-16	Downregulated	CCND1	EV GBM cells, EV GBM CSF	Skog et al. (2008); Akers et al. (2015)
hsa-miR-10b	Upregulated	CDKN1A, CDKN2A, E2F1	GBM CSF	Teplyuk et al. (2012)
hsa-miR-19b	Upregulated	PTEN	EV GBM cells, EV GBM CSF	Skog et al. (2008); Akers et al. (2015)
hsa-miR-20a	Upregulated	TRP53INP1, CCND1	EV GBM cells, EV GBM CSF	Skog et al. (2008); Akers et al. (2015)
hsa-miR-21	Upregulated	PTEN, SPRY2, LRRFIP1,	EV GBM cells, EV GBM serum, EV GBM CSF,	Skog et al. (2008); Shi et al. (2015); Teplyuk et al. (2012); Akers et al. (2015)
hsa-miR-221/222	Upregulated	MGMT, SEMA3B, TIMP2, PUMA	EV GBM cells, GBM plasma	Li et al. (2013); Zhang et al. (2015)
hsa-miR-26ab	Upregulated	PTEN, MAP3K2, IFNB, EphA2, Rb1	EV GBM cells	Skog et al. (2008); Akers et al. (2015)
hsa-mir-27	Upregulated	SFRP1, BTG2, SPRY2	EV GBM cells	Skog et al. (2008); Li et al. (2013)
hsa-mir-339	Upregulated	ICAM1	EV GBM cells	Li et al. (2013) 23807490
hsa-miR-92b	Upregulated	ND	EV GBM cells	Skog et al. (2008); Li et al. (2013)
hsa-miR-93	Upregulated	ITGB8	EV GBM cells, GBM CSF	Skog et al. (2008); Teplyuk et al. (2012)
hsa-miR-17	Upregulated	CDKN1A, E2F1, PTEN,	EV GBM cells, GBM CSF, EV GBM CSF	Skog et al. (2008); Teplyuk et al. (2012); Akers et al. (2015)