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# Belonging to a network—microRNAs, extracellular vesicles, and the glioblastoma microenvironment

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The complexity of glioblastoma multiforme (GBM) and its distinct pathophysiology belong to a unique brain microenvironment and its cellular interactions. Despite extensive evidence of a role for microRNAs in GBM cells, little is known about microRNA-dependent communication between different cellular compartments of the microenvironment that may contribute to the tumor phenotype. While the majority of microRNAs are found intracellularly, a significant number of microRNAs have been observed outside of cells, often encapsulated in secreted extracellular vesicles (EVs). The function of these circulating/secreted microRNAs has not been explored in the context of the brain tumor microenvironment. Establishing how microRNAs are involved in the regulation of oncogenic signaling networks between tumor cells and stroma is likely to add a needed additional layer of complexity to the tumor network, consisting of intercellular communication. More importantly, microRNA/EV signaling may provide an additional therapeutic target for this deadly disease.

**Keywords:** exosomes, extracellular vesicles, glioblastoma multiforme, microRNA, tumor microenvironment.

## Introduction: The Microenvironment of Glioblastoma Multiforme

Solid tumors do not exist autonomously, but rather as entities that are closely connected and dependent on their surrounding cellular and tissue environment. Tumor cells have the ability to both adapt to the local environment and change it to their own advantage. This requires complex multilevel communication and interaction both between themselves and with nonmalignant cells in their microenvironment. The microenvironment is defined as a localized niche composed of tumor cells, surrounding stroma, blood vessels, immune cells, and extracellular matrix (Fig. 1).[1]

Among all tumor glioblastoma is a highly heterogeneous structure containing not only tumor cells, including cells with stem cell–like capacity, but also intermingled nonneoplastic parenchymal cells. These tumor-associated parenchymal cells, including vascular cells, microglia, peripheral immune cells, and neural precursor cells, also play a vital role in controlling the course of pathology via multiple types of cell–cell communication. The tumor vasculature not only supports glioblastoma cells with nutrients and oxygen, but also provides a specialized niche for these stemlike cells. Microglial cells, which contribute significantly to the brain tumor mass,[2] play a role in glioblastoma cell invasion. In addition, nonneoplastic astrocytes can be converted into reactive cells by the glioma microenvironment and can secrete a number of factors that influence tumor biology.

All these factors add additional layers of complexity and underscore the importance of network-based communication in the brain microenvironment: yet, this organizational structure of communication remains poorly understood. Of relevance, the molecules and factors that play a role in this communication are not well studied. It is evident though that "secreted" factors must be important. In fact, secreted cytokines, growth factors, chemokines, and colony stimulating factors can all support tumor initiation, angiogenesis, proliferation, and invasion.[3] An important part of this "secretome" is composed of extracellular vesicles (EVs) with their multifunctional cargo, including microRNA. In this review, we first discuss the importance of microRNA in glioblastoma pathogenesis; we then review the current state of knowledge of EVs' role in glioblastoma multiforme (GBM) and their possible use as therapeutic delivery vehicles; and finally we review EVs' use as biomarkers of disease. For a recent systematic review of intracellular, rather than "secretome," microRNA function, the reader should consult the study by Floyd and Purow.[4]

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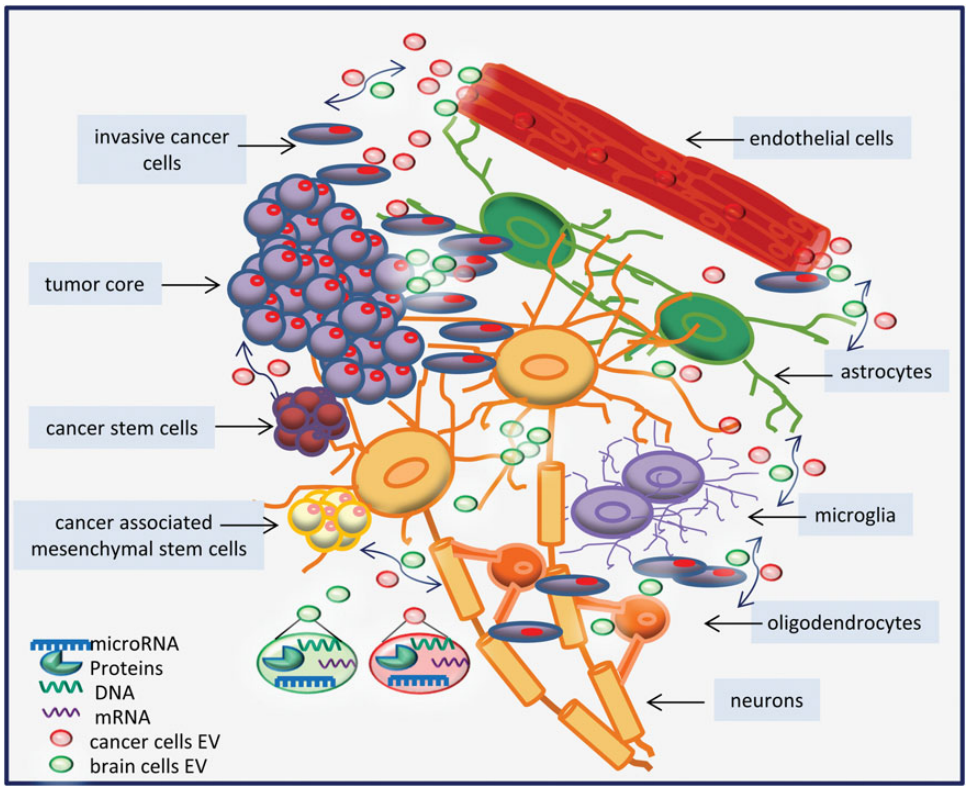


**Fig. 1.** The glioblastoma microenvironment. The microenvironment of glioblastoma is composed of several specialized cell types, which may contribute to tumor growth and invasion providing growth factors, neutrophic factors, and chemokines. Cells from the tumor microenvironment may also be recruited by cancer cells for their own benefit. Different cell types from the tumor microenvironment communicate via the release and uptake of EVs. Such communication can take place both locally and at distant ranges and contributes to tumor progression by transferring of bioactive molecules, including microRNAs.

## MicroRNAs in Glioblastoma

### *The Function of MicroRNAs in GBM Pathobiology*

The discovery of microRNAs changed our understanding of the regulation of gene expression. MicroRNAs are ~23-nucleotide-long noncoding RNAs that act as repressors of gene expression by reducing the stability and translation of target genes through regions of partial complementarity in their mRNA 3′ untranslated regions (UTRs). At present, ~1600 microRNAs are thought to exist in the human genome,[5] although the exact number is not yet clear. Each microRNA may have hundreds of target mRNAs, and many mRNAs are targeted by multiple microRNAs, thus leading to highly complex regulatory networks. Distinct patterns of microRNA expression have been observed in many cancers, including glioblastomas, and the functional significance of some of these microRNA alterations is beginning to emerge. MicroRNAs have been implicated as important regulators of glioblastoma stem cell maintenance,[6–8] epigenetic pathways,[6,9] pathogenesis,[10,11] signaling pathways,[12] and invasiveness.[13,14] Genetically distinct subclasses of glioblastoma are characterized by microRNA and mRNA expression signatures.[15] Moreover, numerous reports have linked microRNAs with alterations in radio- and chemotherapy resistance/sensitivity[16] and have postulated that microRNAs may be important biomarkers of diagnostic and prognostic relevance.[17,18] Only recently, researchers have focused on the possible role of microRNAs in the microenvironmental communication of glioblastoma, primarily through the release and uptake of EVs.[12,19,20]

### *Networks of MicroRNA "Targetomes"*

An emerging theme in microRNA cancer research relates to the ability of a single microRNA to simultaneously target multiple effectors, both directly (by binding to target multiple mRNA 3′-UTRs, resulting in blockade of translation) and indirectly (by targeting upstream regulators of gene expression such as master kinases or transcription factors) (Fig. 2). Work from many laboratories has established that indirect targeting may be of equal, if not greater, importance to direct targeting. When we analyzed the complete proteome of glioblastoma stem cells transfected with miR-128 or scrambled control, we found that out of 1321 proteins significantly downregulated in miR-128–expressing cells, only 454 (34%) of their corresponding mRNAs contained putative miR-128 target sites (unpublished data). This implied either that there were some possible "off-target" effects of miR-128 on other mRNAs that did not have target binding sites or, more likely, that the panoply of downregulation of the possible 454 multiple direct targets of miR-128 resulted indirectly in additional downstream

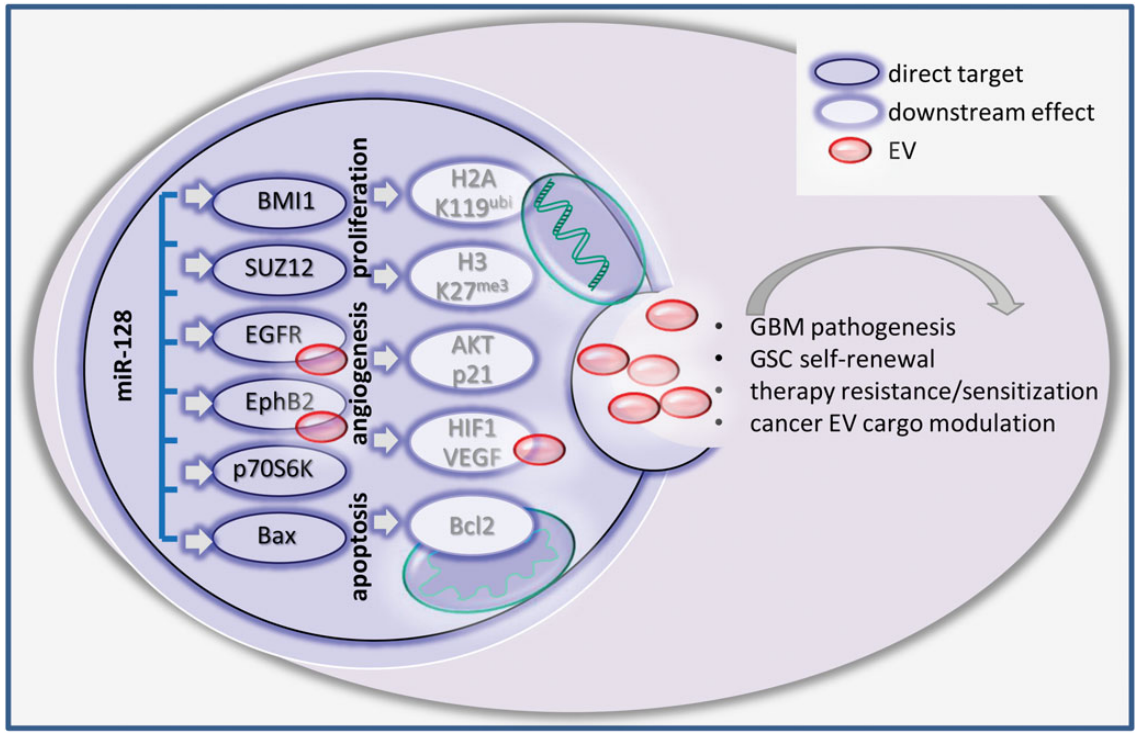


**Fig. 2.** Schematic representation of microRNA network action (example: microRNA-128). MiR-128 is one of the most downregulated microRNAs in glioblastoma cells. When reintroduced, either transiently by oligonucleotide precursors or permanently by lentiviral vector, it directly targets a broad array of oncogenes. The "blocked" function of many oncogenic pathways results in numerous indirect changes in level/activity of downstream effectors. Both direct and indirect effects of miR-128 result in antiproliferative, anti-angiogenic, and pro-apoptotic phenotypic rearrangements. Such phenotypes may be transferred to other cells (including glioblastoma stem cells, GSC) in the tumor microenvironment by EVs, either by direct transfer of miR-128 or by modulation of vesicular cargo, potentiating the effect of miR-128 replacement.

changes in expression of additional mRNAs and their proteins. Examples of coordinated microRNA targeting in glioblastoma, both in a tumor suppressor and in a protumorigenic context, are also increasingly being discovered.

### *The Pro-Oncogenic MicroRNA Network*

MiR-21 and its targets provide an example of an upregulated pro-oncogenic microRNA network.[21] In glioblastoma, it suppresses both the pro-apoptotic programmed cell death protein 4[22] and the anti-invasive matrix metalloproteinase regulators RECK (reversion-inducing cysteine-rich protein with kazal motifs) and tissue inhibitor of metalloproteinase 3.[23] Another one, miR-10b, inhibits pro-apoptotic signaling by direct targeting of Bim, activation protein 2γ, and the cell-cycle inhibitors p16 and p21[24] but is also capable of inducing migration and invasiveness of glioblastoma cells through multiple other mechanisms.[25] In another example of protumorigenic microRNA network targeting, it was demonstrated that miR-26a alone can transform cells and promotes glioblastoma cell growth in vitro and in mouse brain by decreasing phosphatase and tensin homolog (PTEN), retinoblastoma 1, and mitogen-activated protein 3 kinase 2 (MAP3K2)/MAP extracellular signal-regulated kinase kinase 2 (MEKK2) protein expression, thereby increasing Akt activation, promoting proliferation, and decreasing c-Jun N-terminal kinase (JNK)–dependent apoptosis.[26]

### *The Tumor-Suppressive MicroRNA Network*

In general, a majority of microRNAs deregulated in cancers are expressed at significantly lower levels in tumors compared with normal tissues.[27] This likely reflects a role for microRNAs in terminal differentiation and the relatively incomplete differentiation status of cancer cells, as microRNAs are thought to be master guardians of cell differentiation and tissue specificity. With this in mind, it may seem surprising that reexpression of nonexpressed microRNAs into tumor cells results in the coordinated targeting of pro-oncogenic pathways. The mechanism behind this observation likely lies in the existence of major tumor suppressor hubs such as p53[28] and PTEN,[29] which orchestrate multiple tumor-suppressive microRNAs and are lost or malfunctioning in malignant cells. MiR-34 targets multiple oncogenes, such as Notch-1, Notch-2, c-Met, and platelet derived growth factor receptor alpha (PDGFRA) in glioblastoma and medulloblastoma cells.[30,31] Expression of the miR-302–367 cluster is sufficient to suppress the "stemness" signature, self-renewal, and cell infiltration through inhibition of the C-X-C chemokine receptor type 4 pathway, which leads to disruption of the sonic hedgehog–Gli–Nanog network.[32] In our laboratory, scientific efforts have focused on the tumor suppressor microRNAs miR-128 and miR-1, and on the stress-regulated miR-451. Reintroduction of miR-128 into glioblastoma stem cells blocked their self-renewal and propagation. We demonstrated that miR-128 coordinately targets both

components of the polycomb repressor complex (PRC): PRC1 (by targeting Bmi1) and PRC2 (by targeting Suz12). The importance of such a mechanism that is so specific for microRNAs is that it prevents the partially redundant function of both complexes. This is not possible with small interfering (si)RNA, since specific siRNA-mediated silencing of one complex leads to an increase in redundant level/activity of the other.[11] Stable expression of miR-128 in glioblastoma cells diminished PRC-dependent chromatin modifications, reduced levels of phospho-Akt, and de-repressed expression of p21. Other research groups have demonstrated that miR-128 is capable of targeting the mRNAs for epidermal growth factor receptor (EGFR), PDGFRA, EphB2, p70S6K, Bax, and other proteins.[33] Additionally, using a genetically engineered mouse model of glioblastoma, we demonstrated that loss of miR-128 expression in brain cells precedes onset of symptoms (and likely gliomas) in mice and thus may be an early event in the pathogenesis of glioblastoma.[11,34] Coupled with recent findings related to the importance of epigenetic modifications in glioma pathogenesis,[35] these findings imply that early miR-128 loss in brain cells may be a critical step that alters the epigenetics of the pretumorigenic brain cells of origin for glioma. Complex functions and targeting of miR-124, one of the most enriched miRNAs in the brain, which is lost in GBM, have been previously reviewed.[36] Finally, miR-7 coordinately targets the activation of the Akt pathway via 2 independent mechanisms: direct inhibition of EGFR mRNA and concurrent EGFR-independent targeting of insulin receptor substrate 1 and 2.[37]

MiR-1 is profoundly downregulated in glioblastoma. Its reintroduction causes significant inhibition of growth, angiogenesis, and invasiveness in vitro and in vivo. We discovered that annexin A2 (ANXA2) and Met were among the direct targets of miR-1, and this led to diminished levels of EGFR, PRC1/2, and phospho-JNK.[12] The importance of miR-1 in EV intercellular communication will be discussed later.

### *The MicroRNA Network and Its Effects on Tumor Metabolism*

Expression of miR-451 is not significantly deregulated in glioblastoma. However, its expression is regulated by glucose availability, where it is high in a glucose-rich environment. MiR-451 is a potent inhibitor of the signaling pathway of adenosine monophosphate–activated protein kinase (AMPK), by operating at multiple levels. It directly targets calcium binding protein 39—a coactivator of the AMPK upstream master kinase, liver kinase B1, and 14-3-3ζ—a functional inhibitor of AMPK-phosphorylated Raptor.[38] Additionally, forced expression of miR-451 in glioblastoma cells strongly inhibits their migration in vitro[13,14] and invasiveness in vivo (unpublished data). The anti-AMPK and anti-invasive functions of miR-451 have also been independently confirmed by other groups.[39] Interestingly, miR-451 sensitizes cells from multiple cancers, including glioblastoma, to chemotherapy.[40,41] MiR-451 thus provides an almost unique example of a molecule that is not deregulated in GBM but is instead finely regulated, based on the biologic behavior that GBM cells require. In fact, cellular stress leads the GBM cell to downregulate miR-451 in order to allow for AMPK activation to occur and turn on energy-saving cell signaling and behavior. Forced expression of miR-451 during stress leads to cytotoxicity. The converse is true: cellular "happiness" due to a state of nutrient abundance leads to miR-451 upregulation with shut-off of AMPK activity and institution of a program of cell proliferation.

### *The MicroRNA Network in the Context of the Tumor Microenvironment*

It is noteworthy that microRNA alterations that take place in the course of glioblastoma progression are not mutually exclusive; that is, loss of expression of tumor suppressor microRNA happens simultaneously with induction of oncogenic microRNA expression, multiplying the aggressive phenotype. All these examples provide significant evidence for an essential role of microRNAs in glioblastoma cell pathophysiology, as modulators of either pro-oncogenic or tumor suppressor pathways.

How microRNAs and the secretome cooperate to regulate glioblastoma tumor progression is still unclear. Growing evidence suggests that direct targeting of several key secretome factors is responsible for molding the tumor microenvironment. Recently, it was shown that a miR-142-3p–driven autocrine and paracrine positive loop epigenetically regulates the progression and cancer stemlike property of glioblastoma by targeting the secretion of the pro-inflammatory cytokine interleukin-6.[42] MiR-124 was identified as an important inhibitor of signal transducer and activator of transcription 3 signaling in glioblastoma, a key pathway mediating immunosuppression in the tumor microenvironment.[43] In fact, the therapeutic effect of miR-124 depends on the presence of a T-cell–mediated antitumor immune response.[43]

Combinational approaches utilizing complex regulatory microRNA networks may provide a promising strategy for silencing important mediators of cancer-promoting pathways. Top deregulated networks of microRNAs targeting protein coding mRNAs are shown in Supplementary Table S1. Thus, targeting a single miRNA may in fact represent a combined intervention that affects feedback and compensatory pathways that the tumor cell utilizes to evade therapy.

## MicroRNA in the Glioblastoma Microenvironment—the Role of Extracellular Vesicles

### *The Biogenesis and Heterogeneity of Extracellular Vesicles*

EVs (including exosomes and microvesicles) are nanometer size and released by numerous cell types. They either can originate as endosomes released from the cell when multivesicular bodies containing them fuse with the cell membrane or are released directly from the cell membrane through a process known as "blebbing." EVs can carry a diverse spectrum of biomolecules. Their cargo can contain genomic DNA, cDNA, various RNAs (mRNA, microRNA, long noncoding RNA), and proteins (both cytoplasmic and membrane-bound). By transferring bioactive molecules between cells and tissues, they play an important role in the intercellular communication within multicellular organisms (Fig. 3). EVs have emerged as important mediators of intercellular communication in cancer too,

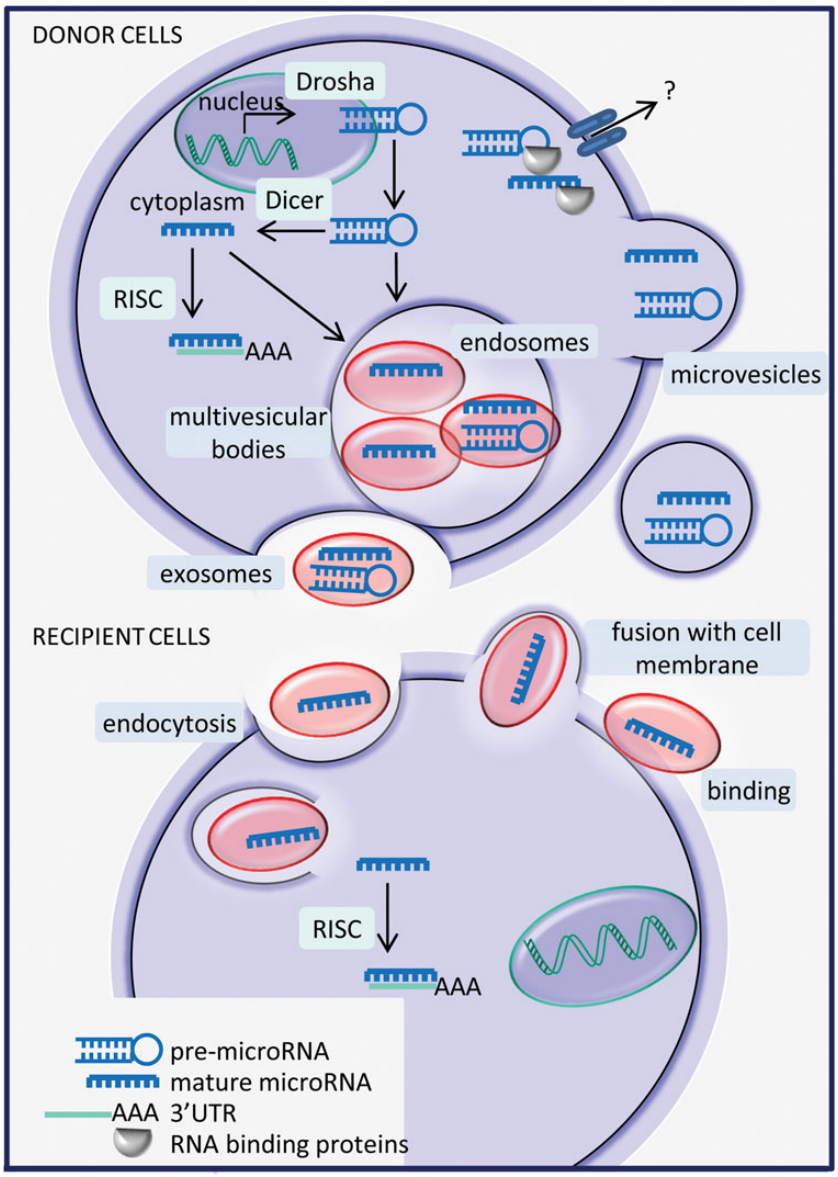


**Fig. 3.** Mechanisms of microRNA cellular release and extracellular transfer. In the nucleus, microRNAs are transcribed from DNA by pol II. A precursor hairpin microRNA (pre-microRNA) is formed after cleavage of primary transcript by the RNase III enzyme Drosha. Then, pre-microRNAs are transported into the cytoplasm and are further cleaved into 19- to 23-nucleotide mature microRNA duplexes by the enzyme Dicer. One strand of the microRNA duplex is loaded into the RNA-induced silencing complex (RISC), where it guides the RISC to specific mRNA targets, preventing the translation of mRNA into protein. In the cytoplasm, mature and pre-microRNAs can be incorporated into endosomes and are released from cells as exosomes when multivesicular bodies fuse with the cell membrane. Cytoplasmic microRNAs can also be secreted by microvesicles, which are released from the cell through blebbing of the cell membrane. MicroRNAs are also secreted in a microvesicle-free form associated with high-density lipoproteins or RNA-binding proteins such as Argonaute 2. Extracellular vesicles release microRNAs into recipient cells by either endocytosis or fusion with cell membrane.

and this may include the transport of tumor-promoting microRNAs between cells.[12,44,45] It is important to note that although individual EV/microRNA-mediated interactions may be relatively "weak," their combinatory regulatory effect through the negative regulation of multiple mRNA targets,[46] miR-mRNA "nanosponge" interactions,[47] and regulation of targets in neighboring or even distant cells[12,48] may generate substantial and significant modifications of the tumor microenvironment.[20,29,49] However, the mechanisms through which microRNAs in EVs modify the molecular phenotype of target cells are far from being fully understood. The complexity of the EV bioactive cargo suggests multipronged modes of action through which EVs alter the extracellular milieu to facilitate disease progression and therapy resistance.[19]

### *The Mechanism of Action of Extracellular Vesicles*

The analysis of EV/microRNA networks suggests that they can affect the tumor microenvironment in different ways: (i) direct reprogramming of cells in the tumor microenvironment (Fig. 4A), (ii) indirect reprogramming of cells in the tumor microenvironment (Fig. 4B), or (iii) modification of the extracellular microenvironment (Fig. 4C). These mechanisms, separately or in combination, may be utilized for sensitization to therapy (Fig. 4D). EVs are avidly taken up by cells in culture where they can change such target cells' translational, transcriptional, and proteome profile. In fact, it has been shown that the delivery of this tumor-derived EV cargo[50–52] becomes functional in recipient cells.[45,50] EVs carrying oncogenic and tumor-suppressive proteins such as EGFR, EGFR variant III, PDGFRA, Met, and PTEN have been discovered in several models of high-grade glioma.[12,53,54] The contents of glioma-derived EVs also were found to be deregulated in hypoxia[55] and after radiation.[56] In these studies, hypoxia-inducible factor 1 or PDGFRA was found to be overexpressed. Likewise, pro-oncogenic and tumor-suppressive microRNAs (miR-34a, miR-128, miR-1, miR-26) directly targeting these and many other factors have been documented as deregulated in GBM cells.[12,30,37,57] Therefore, EVs appear to provide a significant mode of communication between tumor cells.

### *The Extracellular Vesicle/MicroRNA Network in the Tumor Microenvironment*

Despite the fact that cellular material is actively and selectively packaged into the EVs within tumor cells,[58] it is reasonable to speculate that the EV content mimics at least in part that of its parent cell. Indeed, a number of microRNAs detected in GBM cell–derived EVs were also found to be upregulated in the GBM cells from which they were derived. For example, oncogenic microRNAs such as miR-21 and miR-26a were detected in glioblastoma EVs,[52,59,60] along with novel microRNAs such as

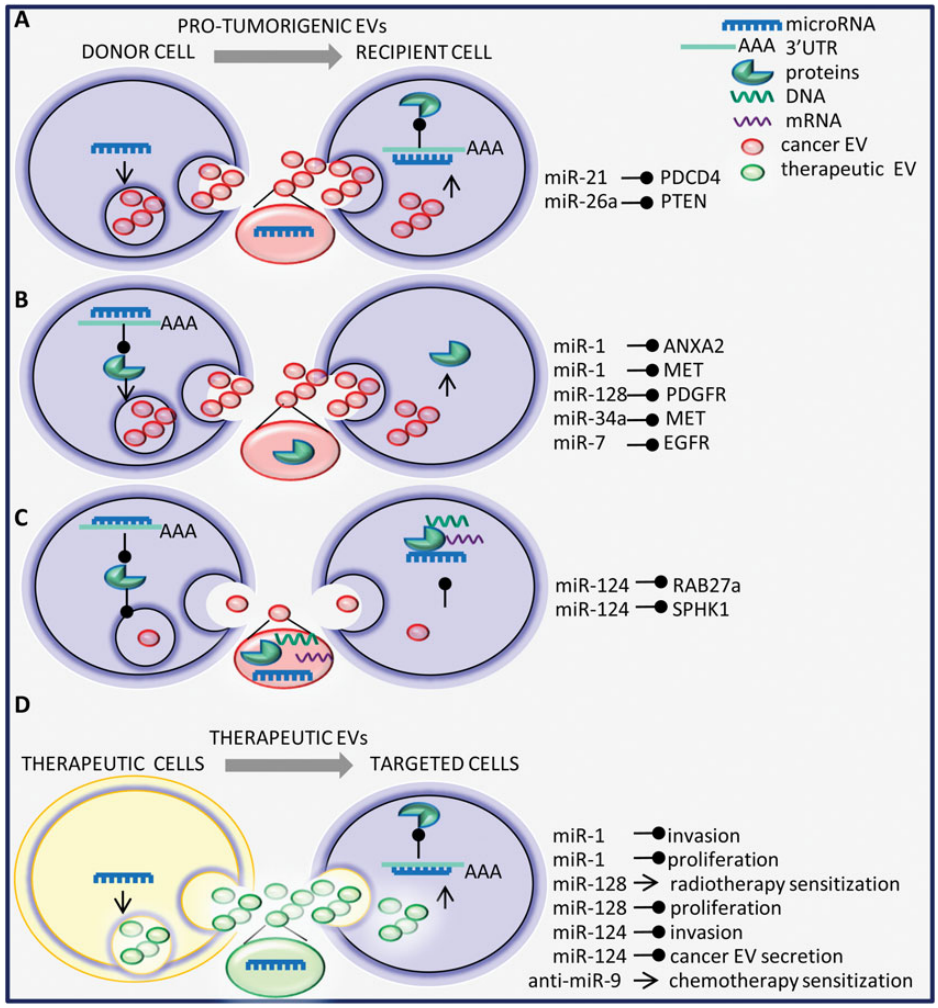


**Fig. 4.** The modes of action of EV/microRNAs in the microenvironment. (A) Direct reprogramming of cells in the tumor microenvironment by microRNA transfer, (B) indirect reprogramming of cells in the tumor microenvironment by miR-dependent targeting of EV cargo, (C) modification of extracellular microenvironment by miR-dependent alteration of EV release, (D) therapy sensitization by delivering therapeutic microRNA/anti-microRNA.

miR-4301 and miR-4454. When the cell of EV origin was queried, these miRs were also upregulated. Interestingly, though, there also appears to be a mechanism for selective enrichment of specific microRNAs (eg, miR-451) in EVs.[48] This included the generation of microRNA from cells transfected with the corresponding pre-microRNA (miR-520g).[12,61]

Our recently published study showed that ectopic expression of the novel tumor suppressor miR-1 in GBM cells blocked growth, neovascularization, and invasiveness of intracranial xenografts, partially by alteration of EV molecular cargo, including secretion of mature miR-1. The results of the study revealed a dramatic effect of a single microRNA on tumor cells and their microenvironment. In fact, we found that miR-1 rearranged a broad set of oncogenic signaling molecules in GBM, including reduction of JNK activity[62] and repression of members of PRCs,[11,34] Met,[63] and ANXA2.[64,65] In silico analysis suggested that the observed changes in the molecular milieu of miR-1–expressing GBM cells were likely consequences of both direct and indirect effects. The observed miR-1–dependent inhibition of GBM-derived EV stimulation of angiogenesis, invasion, and neurosphere formation in recipient cells suggested a multimodal mechanism. These included: molecular rearrangement of donor cells, miR-1–dependent alteration of EV cargo, and direct EV-mediated transfer of functional miR-1 to the microenvironment. We also provided proof of principle that after being transferred by EV, miR-1 can repress mRNAs in recipient cells. Analysis of the proteomic cargo of EVs demonstrated a global effect of miR-1 on factors linked to cancer signaling networks, including downregulation of ANXA2, fatty acid synthase, and 14-3-3ζ. Together, our results showed 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 glioblastoma management.

### *Extracellular Vesicles/MicroRNAs for Therapy*

Because both microRNAs and EVs are part of the natural homeostatic milieu of the cell and its microenvironment, evolution has been instrumental in the development of their regulatory signaling networks and secretory pathways. Therefore, 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. Indeed, it was recently demonstrated that systemically injected EVs can be delivered to tumors within an hour following injection.[66] Thus EVs could turn into a potent microRNA delivery system.[67] MicroRNAs have also been shown to play a direct role in EV secretion. The molecular mechanisms of exosome biogenesis and secretion are poorly understood, but the Rab27 family of small GTPases that regulate exocytosis of distinct vesicles was found to be an important player in EV secretion.[68] Interestingly, one of the tumor suppressor microRNAs lost in glioblastoma, miR-124,[69] was recently shown to target Rab27a in GBM cells,[70] suggesting an additional role for microRNAs in microenvironmental communication. The continuous activation of sphingosine 1-phosphate (S1P) receptors on multivesicular endosomes was 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.[71] The pro-proliferative, pro-invasive, pro-angiogenic, and anti-apoptotic role of SPHK1 in GBM is now well documented.[72–76] Thus, direct targeting of SPHK1 by miR-124[77] may multiply the effect of this microRNA on EV secretion and global EV cargo loading and ultimately lead to a decrease of SPHK1 secretion by EVs.[78]

This miR-dependent EV secretion may also be relevant in combination therapy. It was shown that EVs derived from irradiated GBM cells enhanced the migration of recipient cells, and their molecular profiling revealed an abundance of molecules related to signaling pathways important for cell migration.[56] Taking into account the prosensitization and antimigratory effects of miR-124 in GBM,[79,80] these data suggest that delivery of miR-124 could provide a multiplier of therapeutic effects through direct targeting of pro-invasive mRNA molecules, direct inhibition of promigratory EVs, and reduction of bystander effects.[81,82] Taking this one step further, engineered EVs with specific microRNA contents might even reverse tumor EV–induced host cell modulation.

The application of any microRNA or anti-microRNA approach to inhibit protumorigenic communication between cells within tumors will require the development of new methods of gene delivery to target cells. Whereas the EV-based approach is feasible in preclinical models, applying such an approach in the therapy of human disease is likely to become more difficult, as the effective delivery and uptake by relevant cell types in vivo may be limited. To test whether marrow stromal cell–derived EVs could be used as a vehicle for the delivery of antitumor microRNAs, Katakowski and colleagues[83] used EVs preloaded with miR-146b, by overexpression of the primary miR-146b transcript in donor cells. Intratumor injection of therapeutic EVs carrying this mature miR-146 significantly reduced glioma xenograft growth in a rat model of primary brain tumor.[83] In another study, EV-based delivery of miR-9 inhibitor decreased the expression of the multidrug transporter, responsible for chemotherapy resistance of GBM cells, and sensitized them to temozolomide.[84] These data showed that mesenchymal stem cells and their EVs could functionally deliver synthetic anti-microRNA to reverse the chemoresistance of GBM.

For efficient delivery of EVs to target cells, the surface of EVs can also be modified by the genetic engineering of donor cells. Successful targeted delivery of EVs was reported to occur by 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 tissue.[85] These experiments thus suggest that 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 summary, EV-based delivery of microRNAs with known anti-oncogenic properties, such as miR-128, miR-1, miR-34a, and miR-124 or inhibitors of protumorigenic miR-10b, may represent an important strategy for the treatment of the most aggressive cancers, like GBM.

## MicroRNA in Extracellular Vesicles as Biomarkers

### *The Cargo of Extracellular Vesicles as a Cancer Biomarker*

The clinical management of cancer patients could be improved through the development of noninvasive approaches for the detection of incipient, residual, and recurrent tumors. Recent advances in the understanding of microRNAs as circulating biomarkers provide the biologic rationale to explore whether they could become biomarkers for early detection of many diseases and in response assessment for patients subjected to the therapy.[86,87] Cancer cells secrete EVs containing genetic materials that partially reflect the microRNA and/or the intracellular signature of the tumor milieu. These EVs are released into the local environment and transgress anatomic compartments into CSF and the systemic blood circulation.[88,89] Indeed, cellular tumor-specific mutations and specific RNA expression were detected in EVs,[52,90] and EV-specific enrichment or downregulation of molecules was also reported to occur.[48,91] This suggests that profiling the EV content from biofluids may be useful for the detection of cancer cell signatures.

### *Extracellular Vesicles/MicroRNA as Glioblastoma Biomarkers*

The RNA profiles of EVs are highly enriched for small RNAs, including the size range of microRNAs.[12,90] Given the differences in the microRNA signature for healthy subjects and glioblastoma patients,[34,92] microRNAs may be very useful as biomarkers for glioblastoma. Recent studies suggest that circulating microRNA can exist not only within EV but also as complexes with Argonaute 2, the catalytic component of the RNA-induced silencing complex, or high-density lipoproteins.[93] Circulating, tumor-specific microRNAs have indeed been detected in the blood of GBM patients.[94,95] In these studies, miR-21[94] and miR-128[95] were found to be upregulated, whereas miR-342-3p[95] was downregulated in glioblastoma patients. Interestingly, miR-128 is one of the most downregulated microRNAs in glioblastoma and glioblastoma stem cells,[11,34] and there are no reports of this microRNA present in EVs.[48,52] MicroRNAs detected in the biofluids of brain tumor patients may originate from brain tumor cells but also from surrounding brain tissue or from extracranial tissues, due to the blood–brain barrier disruption associated with tumor progression and therapy. Thus it would be significant to verify the source of miR-128 in patients' blood. One explanation may be that neurons, where miR-128 is highly and specifically expressed, secrete miR-128 as a response in glioblastoma initiation.

An important study was performed to distinguish between primary glioblastoma tumor and metastatic (breast and lung) brain cancer through microRNA profiling in subjects' CSF.[96] This analysis revealed elevation of miR-10b and miR-21 in all brain neoplasms, compared with tumors in remission and with a variety of nonneoplastic samples. These 2 microRNAs have already established pleiotropic roles in glioblastoma pathobiology.[24,97] In contrast, members of the miR-200 family that suppress metastasis[98] were found to be highly elevated exclusively in the CSF of patients with brain metastases, allowing discrimination between primary and metastatic brain tumors. In a recent study, Akers and colleagues[59] showed that miR-21 levels in EVs isolated from CSF differentiated glioblastoma patients from non-oncologic patients. This study provides a basis for a prospective, multicenter study to validate CSF EV miR-21 as a biomarker for the presence of glioblastoma. The isolation and analysis of microRNA EVs within CSF or blood may enhance the sensitivity of microRNA-based biomarker assays compared with the analysis of free circulating nucleic acids, since genetic material within EVs is more stable than that in whole biofluids.[59]

## Conclusions

Recent data underline a role for microRNA in the regulation of intercellular communication in the glioblastoma microenvironment. This may be critical for the survival of tumor cells in the dynamically changing microenvironmental landscape. Extracellular vesicles seem to play a critical role in such communication. The role of these vesicles in other tissues and cancer cell types is being intensively examined. Exploiting this mechanism for the development of a novel, safe, and effective method of delivery of therapeutic molecules and therapy-sensitizing agents will have broad implications in cancer, but also in other diseases. The promising effects of microRNA/EV-based therapeutics in vivo make it a potentially important modulator of future glioma therapeutic strategies. It is likely that disease-specific microRNA signatures within EVs will be uncovered, validated, and then used for the development of sensitive and minimally invasive diagnostic alternatives that are urgently needed for heterogeneous brain tumors.[57,99,100] Such knowledge can also be utilized for understanding the communication networks between tumor cells, the tumor microenvironment, and host responses.

## Supplementary Material

Supplementary material is available at *Neuro-Oncology Journal* online (http://neuro-oncology.oxfordjournals.org/).

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