Molecular Therapy
Nucleic Acids
Commentary

Targeting microRNA-10 in glioma; a focus with potential therapeutic application in genome editing

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Glioblastoma (GBM) is the most common and aggressive mode of primary brain cancer.[1] Despite the recent development of highly effective medical treatments and well-tolerated therapeutic interventions in cancer, it is still challenging to apply effective treatments, which diminish the high mortality rates. As the number of patients and costs associated with GBM are continuing to increase in modern society, there is an urgent need for novel diagnostic and therapeutic options for GBM. In last years, the therapeutic potential of noncoding RNAs for the treatment of GBM has come to light.[2] Small noncoding RNAs (microRNAs [miRNAs or miRs]) are well-known regulators of gene expression, and changes in their levels can modify phenotypes and cellular behavior by regulating gene expression or transcription of their targets and may present an attractive treatment option for the disease. MiR-10b was discovered to possess pro-metastatic effects and plays an important role in the development of GBM.[3] In the elegant study by Zhang et al., a potential mechanism of miR-10b-mediated glioma cell death is proposed. The authors analyzed the effects of proteins secreted by glioma cells treated with lentivirus against miR-10b (donor cells) compared with non-treated glioma cells (recipient cells). They discovered that a potent bystander effect through miR-10b gene editing leads to the secretion of proteins that promote glioma cell death. By using proteome profiling via mass spectrometry analysis on the donor cells' secretome, two potential candidate proteins were found, namely, phosphoglycerate kinase 1 (PGK1) and insulin-like growth factor binding protein 2 (IGFBP2). These proteins are known regulators of cell viability and mediate the bystander effect by their controlled secretion into extracellular space, as the authors have elegantly demonstrated.

Cellular crosstalk is an important phenomenon that regulates cellular behavior in the normal physiological and pathological states. In co-culture experiments of gene-edited miR-10b glioma cells (donor cells) and non-edited glioma cells (recipient cells), reduced cell proliferation was observed, which was due to increased cell death in recipient glioma cells. The authors tested the potential bystander effect of miR-10b in the recipient cells by treating them with cell culture medium (CM) consisting of proteins secreted by the donor cells. As a consequence, reduced glioma cell growth and increased cell death were reported in recipient cells. Proteomic analysis and size range of secreted proteins were measured in the CM, and the effect of soluble proteins was determined in the recipient glioma cells. Recipient glioma cells were treated with different fractions of the donor cell proteins, and the glioma cell growth was determined. The analysis revealed that the death-promoting factors (proteins) were smaller than 50 kDa. These fractions were further analyzed in quantitative tandem mass tag mass spectrometry to identify potential protein candidates. In the analysis, 577 proteins were detected of which PGK1 and IGFBP2 were chosen for further characterization. Luciferase assays were performed to measure whether PGK1 and IGFBP2 could directly bind to miR-10b. It was shown that miR-10b triggered a reduction in the activity of PGK1 and IGFBP2 reporters, indicating that miR-10b directly regulates the expression of those proteins. Furthermore, the group analyzed whether the observed bystander effect of miR-10b on glioma cells was mediated by PGK1 and IGFBP2. Diluted proteins were added to glioma cell cultures, and cell growth and cell death were monitored. It was demonstrated that after treatment, glioma cells exhibited reduced cell growth and increased cell death. These effects were less pronounced after the treatment with IGFBP2. Rescue experiments, using specific inhibitors against PGK1 and IGFBP2, demonstrated that the effects were abrogated, and a reduction of the growth-inhibitory outcome was observed (Figure 1).

Intercellular communication is an umbrella term for the various and diverse ways of cell-cell crosstalk and transfer of messages through different forms of interaction. In the last few years, the role of extracellular vesicles (EVs) in intercellular communication has become a major research focus in various fields, and it has become clear that they play major roles in different pathophysiological states. EVs are small membranous vesicles released by healthy or diseased cells that circulate in various human bodily fluids, e.g., blood, urine, and saliva. They are involved in multiple physiological and pathological processes, including blood coagulation, inflammation, stem cell expansion, neuronal communication, tumorigenesis,[3] and cardiovascular biology,[4,5] acting as a vector for biological information. These EVs contain biological cargos (RNA, DNA, proteins, lipids) and regulate target cell function. The composition of EVs and their effects on target cells are extremely heterogeneous and dependent on the cell of origin and its functional state upon release of the EVs.

Multiple studies highlighted the importance of intercellular crosstalk via EVs for the development and homeostasis of GBM. The tumor microenvironment (TME) of GBM is highly complex and diverse, consisting of different cell types such as glioblastoma cells,

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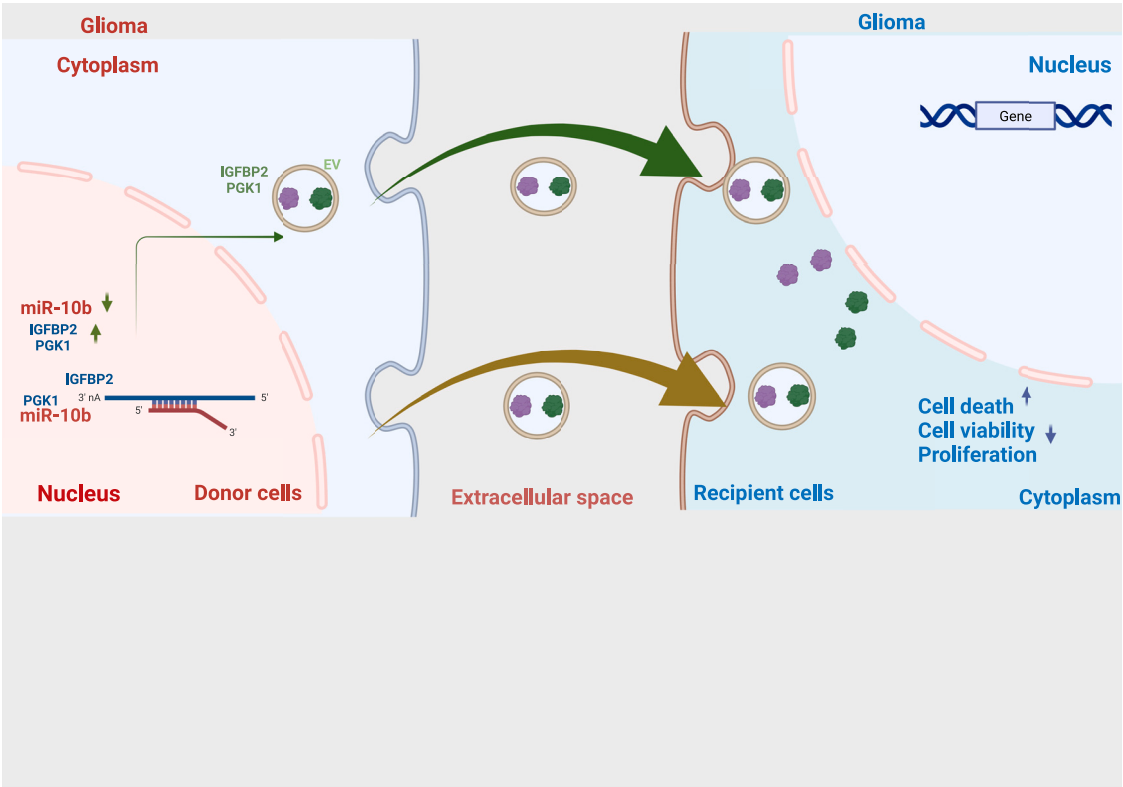

Figure 1. The schematic illustration of model proposed for miR-10b action

Gene editing of miR-10b contributes to release of PGK1 and IGFBP2 into extracellular space, which is shuttled to the recipient glioma cells to regulate cellular proliferation and death of target cells. The figure was created with BioRender.com. PGK1, phosphoglycerate kinase 1; IGFBP2, insulin-like growth factor binding protein 2; EV, extracellular vesicle; miRs, microRNAs.

normal stromal cells, astrocytes, and endothelial cells. Studies suggest that EVs play a critical role in mediating proliferation, migration, and invasion as well as therapeutic resistance of the GBM-TME.[6] Due to their implication in many different processes of GBM development and their easy accessibility in body fluids, EVs hold a great potential for the application as biomarkers of GBM. Indeed, studies propose that the miR and mRNA cargos of EVs could potentially be used as GBM biomarkers as they are unique to GBM patients. Interestingly, proteomic EV cargos were shown to be suitable biomarkers as they reflect the molecular signature of GBM. To this day, there is an urgent demand for treatment possibilities in GBM that can penetrate the blood-brain barrier (BBB) and are able to achieve high concentrations at the tumor site. EVs are suitable drug vehicles that could be very promising for GBM therapies as they are able to pass the BBB, are a rather non-invasive treatment option, and can tolerate harsh environmental factors.[7] The contents of EVs are highly variable including miRs, mRNAs, and proteins and can easily be modified. The study presented by Zhang et al. demonstrates the potential of miR-10b and its downstream targets for the treatment of GBM. MiR-10b is expressed in glioma cells and absent in normal neurological cells in the brain. Studies have shown that ablation of miR-10b is lethal in glioma cells. Therefore, gene editing, which is a novel potential modality of GBM treatment, seems to be more targeted and permanent, and its radio- and chemotherapy resistance could be very effective. However, currently, there are still limitations to gene editing therapies regarding their specificity and potential off-targets effects.[8,9]

Overall, the study by Zhang et al. strikingly highlights the potential of miR-10b gene editing and intercellular communication via EVs for the treatment of GBM. Further investigation of EV-cellular crosstalk in the setting of GBM-TME is warranted.[10] *In vitro* co-culture experiments could be useful to identify whether EVs would affect cell types like endothelial cells, normal stromal cells, astrocytes, or even immune cells, as it is suggested that EVs could even regulate the immune response to support immunosuppression. Extensive *in vivo* experiments in murine models of GBM would also be a feasible option to test for off-target effects by analyzing the effects of EV cargos on the GBM-TME, which will provide a better understanding of the observed effects, which is necessary before potential therapeutic application.

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DECLARATION OF INTERESTS

The authors declare no competing financial interest.

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