Extracellular Vesicles in Glioblastoma

Subjects: Oncology

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Glioblastoma (GB) is the most aggressive form of brain cancer in adults, characterized by poor survival rates and lack of effective therapies. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression post-transcriptionally through specific pairing with target messenger RNAs (mRNAs). Extracellular vesicles (EVs) are cell-derived vesicles which transport miRNAs, mRNAs and intracellular proteins, and have been shown to promote horizontal malignancy into adjacent tissue, as well as resistance to conventional therapies. Furthermore, GB-derived EVs have distinct miRNA contents and are able to penetrate the blood–brain barrier and could be used as biomarkers, while EVs carrying specific miRNAs or miRNA inhibitors have great potential as therapeutic nanotools in GB.

Keywords: extracellular vesicles ; glioblastoma ; microRNA ; biomarkers ; nanocarriers ; therapy

1. Overview

Glioblastoma (GB) is the most aggressive form of brain cancer in adults, characterized by poor survival rates and lack of effective therapies. MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression post-transcriptionally through specific pairing with target messenger RNAs (mRNAs). Extracellular vesicles (EVs), a heterogeneous group of cell-derived vesicles, transport miRNAs, mRNAs and intracellular proteins, and have been shown to promote horizontal malignancy into adjacent tissue, as well as resistance to conventional therapies. Furthermore, GB-derived EVs have distinct miRNA contents and are able to penetrate the blood–brain barrier. Numerous studies have attempted to identify EV-associated miRNA biomarkers in serum/plasma and cerebrospinal fluid, but their collective findings fail to identify reliable biomarkers that can be applied in clinical settings. However, EVs carrying specific miRNAs or miRNA inhibitors have great potential as therapeutic nanotools in GB, and several studies have investigated this possibility on *in vitro* and *in vivo* models. In this review, we discuss the role of EVs and their miRNA content in GB progression and resistance to therapy, with emphasis on their potential as diagnostic, prognostic and disease monitoring biomarkers and as nanocarriers for gene therapy.

2. Glioblastoma

Glioblastoma (GB) is the most aggressive form of brain cancer in adults, characterized by fast growth and invasiveness, high tumor heterogeneity, poor survival and lack of effective therapies [1][2][3]. The diagnosis and classification of brain tumors have undergone several modifications over the last two decades. Thus, the latest classification of central nervous system (CNS) tumors released by the World Health Organization (WHO) [4] takes into account molecular markers along with histological assessment and clinical presentation into the diagnosis and classification of GB. In this regard, it has been demonstrated that GBs with identical histopathological classification, but of a different molecular subtype, have distinctive clinical outcomes and treatment responses: the Proneural subtype is associated with longer survival and low treatment response compared to other subtypes, while Classical and Mesenchymal subtypes respond significantly better to aggressive treatment [5][6]. Following diagnosis, the current standard of treatment for GB includes maximum safe surgical resection (often aided by 5-aminolevulinic acid (5-ALA)-induced tumor fluorescence), radiotherapy and chemotherapy using temozolomide (TMZ) or other agents [7][8][9][10][11].

Despite recent therapeutic advances and improved imaging techniques, *de novo* GB diagnosis is frequently done in advanced stages of the disease, when the impact on patients' quality of life is severe. Furthermore, recurring GB tumors are still difficult to manage, and magnetic resonance imaging (MRI) follow-ups are expensive and sometimes misleading, as it is difficult to distinguish between recurrence and pseudo progression. Despite ongoing efforts to develop new diagnostic and therapeutic tools, minimal advances have been made, and no reliable biomarkers are being used in clinical practice [12]. Therefore, there is a need for minimally invasive, easy to measure and cost-effective biomarkers for early diagnosis of GB and therapeutic response monitoring. The advancements in molecular biology in the last decades have

led to the discovery of new potential biomarkers, among which microRNAs (miRNAs) seem to be the most promising ones.

MiRNAs are small, single-stranded, non-coding RNAs that regulate gene expression post-transcriptionally by inhibiting translation and/or promoting messenger RNA (mRNA) degradation through specific pairing with target mRNAs [13]. MiRNAs are stress response molecules, have modified expression levels during disease progression and are known to be involved in the initiation and development of various types of cancer [14][15]. Furthermore, miRNAs have been shown to circulate in the blood stream and cerebrospinal fluid (CSF), associated with extracellular vesicles (EVs), lipoproteins or protein complexes, and their circulating profiles reflect their modified tissue expression or an increased intercellular communication [16][17]. This, combined with the fact that miRNAs are relatively easy to measure in biological fluids, supports their potential use as biomarkers for diagnosis, prognosis and therapeutic response monitoring of CNS malignancies. However, many studies have attempted to identify specific serum or CSF miRNAs as biomarkers for brain tumors, including GB [18], but their collective findings fail to identify reliable biomarkers that can be applied in clinical settings. Serum biomarkers are easy to measure and can be useful in clinical practice, but EVs have a more disease- and tissue-specific cargo and could differentiate between pathologies more accurately.

EVs represent a heterogenous group of lipid vesicles that are secreted by numerous cell types, under physiological or pathological conditions, exhibit specific markers and transport particular molecules from their cells of origin, including miRNAs [19][20][21][22][23][24]. Furthermore, EVs bind and fuse with their target cells, delivering their cargo and promoting horizontal malignancy into adjacent tissues [23][25], as well as resistance to therapeutic interventions [26][27][28][29][30][31]. On the other hand, EVs derived from healthy cells have been shown to improve pathological conditions in recipient cells [32][33][34]. Considering the ability of miRNAs to target multiple transcripts, EV-mediated transfer of miRNAs to recipient cells could have an extensive impact.

EVs can be isolated from biological fluids [35][36][37][38][39][40][41][42] or cell culture medium [43], providing an extensive platform for studying pathological processes. Moreover, EVs have been shown to contain a significantly distinct miRNA signature compared to their cells of origin, suggesting a selective miRNA packaging into EVs [24], and their number and miRNA content change under pathological conditions [23][44][45]. These aspects could be exploited in a clinical setting as EVs have been shown to have diagnostic potential in various pathologies, including GB [26][46], as well as biomarker potential for treatment response monitoring and disease recurrence [47][48][49][50][51][52].

Due to the ability of EVs to cross the blood–brain barrier [53][54] and to transfer their cargo to a wide array of cells [23][25][32][33][34], they could be used as therapeutic tools in GB. This possibility opens up many new avenues in cancer treatment, aided by the fact that EVs can be enriched in endogenous [55] or synthetic miRNAs [56], or miRNA inhibitors [57][58]. The production and clinical use of EV-based therapeutics depend on numerous safety, biological and manufacturing aspects and are still not clearly regulated [59]. Despite current limitations and drawbacks, EV-based miRNA nanocarriers could represent an important adjuvant in GB therapy, combined with the current standard of treatment.

3. Conclusions

Despite recent advancements in diagnostic techniques, GB diagnosis is frequently done in advanced stages of the disease. There is a need for minimally invasive, easy to measure and cost-effective biomarkers for early diagnosis and therapeutic response monitoring of GB. The research data obtained in the last decades has led to the discovery of new potential biomarkers, among which miRNAs seem to be the most promising ones. Moreover, there is increasing evidence that EV-associated miRNAs may provide a more specific discrimination between studied cohorts and, therefore, could have great diagnostic and prognostic value. However, these biomarkers have not been yet introduced in clinical practice due to great differences between studies. In order to identify reliable biomarkers, larger studies with well characterized cohorts of patients need to be undertaken.

Furthermore, EV-based miRNA nanocarriers can be taken into consideration as adjuvants in GB therapy, combined with the current standard of treatment.

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