Exosomal circular RNAs in glioma: coexistence of opportunities and challenges for application

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Glioma is the most common primary tumor in the central nervous system. With the help of intercellular communication, glioma cells have the ability to survive in the tumor microenvironment (TME) and make it more suitable.[1]

Exosomes, a new type of intercellular communication, are nanoscale membrane-bound extracellular vesicles that are secreted by various types of cells. Circular RNAs (circRNAs) are enriched in exosomes and transported to neighboring or distant cells to exert their functions. The current progressive research of exosomal circRNAs in glioma emphasized their crucial roles in clinical responses and potential clinical applications with both opportunities and challenges.

Exosomal circRNAs could regulate various hallmarks of glioma and also provide a myriad of opportunities for application. In the following parts, we outline the current studies on the roles of exosomal circRNAs in the development and clinical responses of glioma, indicating their perspectives for clinical applications.

Rapid proliferation and aggressive invasion are the main hallmarks of high-grade glioma. Various types of exosomal circRNAs derived from glioma cells could exert important functions in promoting cell proliferation and invasion [Figure 1A], which are attributable to various types of mechanisms,[2] such as serving as microRNA (miRNA) sponges, encoding proteins, binding to RNA binding proteins, and involving in multiple signaling pathways.

In the TME, exosomal circRNAs could play a crucial role in the angiogenesis of glioma [Figure 1B]. Recently, He *et al*[3] found inhibition of glioma-exposed endothelial cells angiogenesis due to the downregulation of FUS or circ_002136, suggesting the regulatory function of FUS/circ_002136/miR-138-5p/SOX13 feedback loop in angiogenesis of glioma. In-depth understanding of detail mechanisms involved in glioma angiogenesis at cellular and molecular levels would be crucial to develop effective antiangiogenic therapies.

Immune cells play crucial roles in glioma progression and antitumor immune responses. The participation of circRNAs in regulating immune responses of hepatocellular carcinoma has been confirmed based on the close relationship between miRNAs and circRNAs.[4] The crucial role of miRNAs in glioma immunity is explicit,[5] while exosomal circRNAs may also be closely associated with the immune microenvironment [Figure 1C] by sponging their corresponding miRNA. The underlying mechanisms of circRNA/miRNA axis in regulating glioma immunity are still poorly understood and worthy of in-depth exploration.

CircRNAs delivered by exosomes also made significant contributions to therapeutic resistance in many types of cancers,[6] including glioma [Figure 1D].

CircRNAs could be packaged into exosomes to participate in the circulation with stability, revealing their potential as biomarkers [Figure 1E]. The capacity of crossing the

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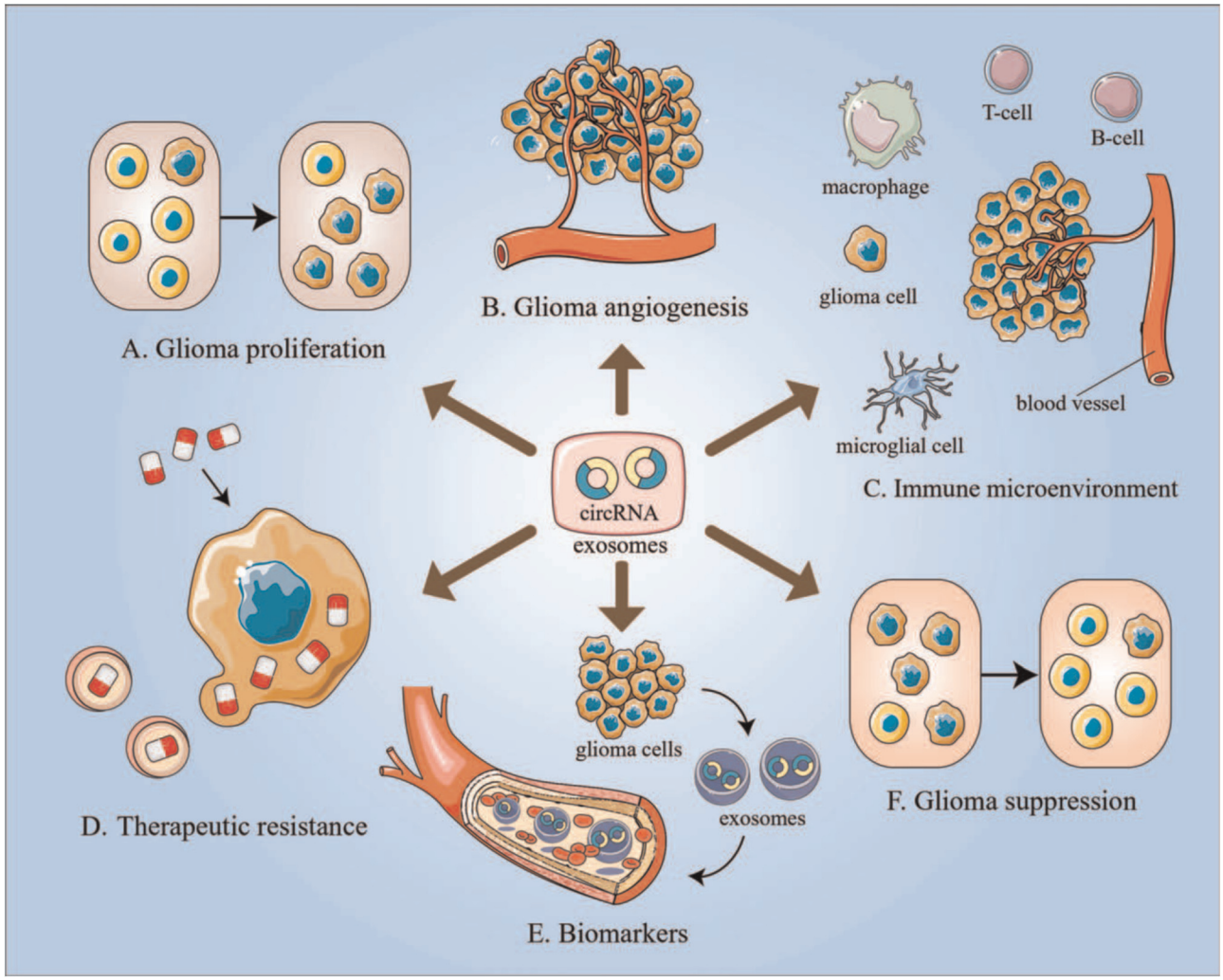

Figure 1: The roles of exosomal circRNAs in glioma development and clinical responses. (A) Exosomal circRNAs can promote glioma cell proliferation by exerting several important functions. (B) The potential role of exosomal circRNAs in regulating glioma angiogenesis. (C) The potential role of exosomal circRNAs in regulating glioma immunity by helping glioma cells to manipulate surrounding immune cells. (D) Exosomal circRNAs can contribute to therapeutic resistance in glioma. (E) CircRNAs could be packaged into exosomes to participate in the circulation serving as biomarkers. (F) Exosomal circRNAs can inhibit glioma cell proliferation. circRNA: Circular RNAs. The original elements used in the Figure were from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

blood–brain barrier (BBB) and preventing circRNAs from degradation via double-layer lipid membrane in exosomes also increase the reliability of circRNAs acting as biomarkers. Recently, Barbagallo *et al*[7] investigated that circSMARCA5 was significantly associated with glioma survival, revealing their potential in serving as prognostic biomarkers.

As the natural carriers of circRNAs, exosomes may deliver tumor-suppressive circRNAs [Figure 1F] based on their capacity of crossing the BBB. For instance, exosomal circ-FBXW7 was lowly expressed in glioblastoma samples and it could inhibit glioblastoma proliferation via upregulation of FBXW7-185aa, unveiling the potential of circ-FBXW7 in therapeutic applications.[8] With the advances of understanding, exosomal circRNAs could become attractive targets for glioma treatment.

Similar to exosomal miRNAs, there are still many challenges of application that coexisted with opportunities in exosomal circRNAs.[9]

The multiple steps involved in exosome isolation could affect the analytical outcomes. For example, during the sample collection procedure by blood drawing, physical forces could result in the presence of impurities. Therefore, larger needles and careful drawing are recommended to address this challenge. In addition, heparin could bind to nucleic acid, which is suggested to be avoided and replaced by citrate. Fresh sample is always recommended because inappropriate storage condition is also a technical challenge that could affect the analysis.

Although many methods have attempted to address these challenges, each of them has its own pros and cons. For example, although differential ultracentrifugation is considered as a gold standard, it is limited by time-consuming procedures and costly equipment. Immunoaffinity-based techniques are limited by high-cost antibodies and low yield though they are highly selective and specific. In addition, although polymer-based precipitation is relatively simple, it is limited by coprecipitation of non-exosomal contaminants.

The sample heterogeneity could affect exosome analysis and remains a significant biological challenge. For example, the amounts of disease-specific exosomes could be abnormal in healthy individuals due to their difference in age and sex. To address this challenge, a sample control bank containing as many variants as possible is recommended to establish.

To date, knockdown of circRNAs or their downstreaming miRNAs remains challenging. The protective layers of exosomes could also be a challenge when miRNAs and circRNAs are required to be released from exosomes for analysis. In addition, how circRNAs are selectively packaged into exosomes and targeted to specific cells is far from clear. Thus, the relationship between circRNAs, exosomes, and glioma requires further investigation.

Various techniques are utilized to detect and quantify exosomes and their cargos (i.e., miRNAs and circRNAs), such as enzyme-linked immunosorbent assay, flow cytometry, nanoparticle tracking analysis, electrochemistry-based techniques, and microfluidics.[9-14] The ongoing development of exosome detection techniques would assist the application of exosomes cargos.

Exosomal circRNAs with high stability and specificity have broad prospects in the application of glioma, including serving as a biomarker, alleviating treatment resistance, regulating immune response, and inhibiting malignant behaviors. After entirely elucidating the functional mechanisms of exosomal circRNAs and overcoming the challenges of isolation and detection, new avenues for the diagnosis and treatment of glioma will be opened.

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Conflicts of interest

None.

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