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REVIEW ARTICLE

Exosomal communication in glioma – a review

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Summary

Cancer research has revealed the existence of cancer stem cells (CSCs). However, the influence of the surrounding stromal cells present in the microenvironment on CSCs is still poorly understood. The latest studies on gliomas suggested that the microenvironment of human gliomas contains both glioma stem cells (GSCs) and glioma associated (GA)-mesenchymal stem cells (MSCs; (GA-MSCs). Also, studies have suggested that nano-sized vesicles, termed exosomes, have been recently observed to contribute towards intercellular communication within the tumor niche. The present review article will highlight the current view of exosomal communication in gliomas.

Key words: *gliomas, microenvironment, exosomal communication*

Introduction

The mode of intercellular communication in the form of ions, proteins etc, has been well studied and understood in various recent studies [1-4]. Moreover, stress is also being given to alternative forms of communication like exosomes. Exosomes belong to a class of extracellular vesicles, and are the smallest in the group. An exosome is a 40-100 nm diameter particle lipid bi-layer membrane from the originating cell [5]. The formation of exosomes takes place intracellularly within the endosomal/lysosomal system, after which they are excreted from the cell via exocytosis (Figure 1). This process also distinguishes exosomes from larger micro-vesicles and apoptotic bodies, which are formed by external membrane budding or blebbing, and fragmentation of the plasma membrane, respectively [6].

Exosomes were first described as a smaller subset of a group of larger micro-vesicles derived from the C-6 rat glioma cell line with the help of electron microscopy [7]. The process basically involved recycling of unutilized receptors within the cell, which then were excreted into the extracellular space in exosomes [8]. So, exosomes are part of cell biology, and since that time the literature on exosomes has increased exponentially. Exosomes are formed within the endosomal system. This system is responsible for intracellular protein trafficking between various organelles and the cell membrane. The formation of exosomes initially involves the recycling of plasma membrane receptors and proteins, via clathrin-associated endocytosis, resulting in the extracellular domain of membrane proteins now within the intra-endosomal compartment. This process signifies the creation of the early endosomes in the cytosol [9]. Early endosomes then follow one of two pathways, depending on their content and

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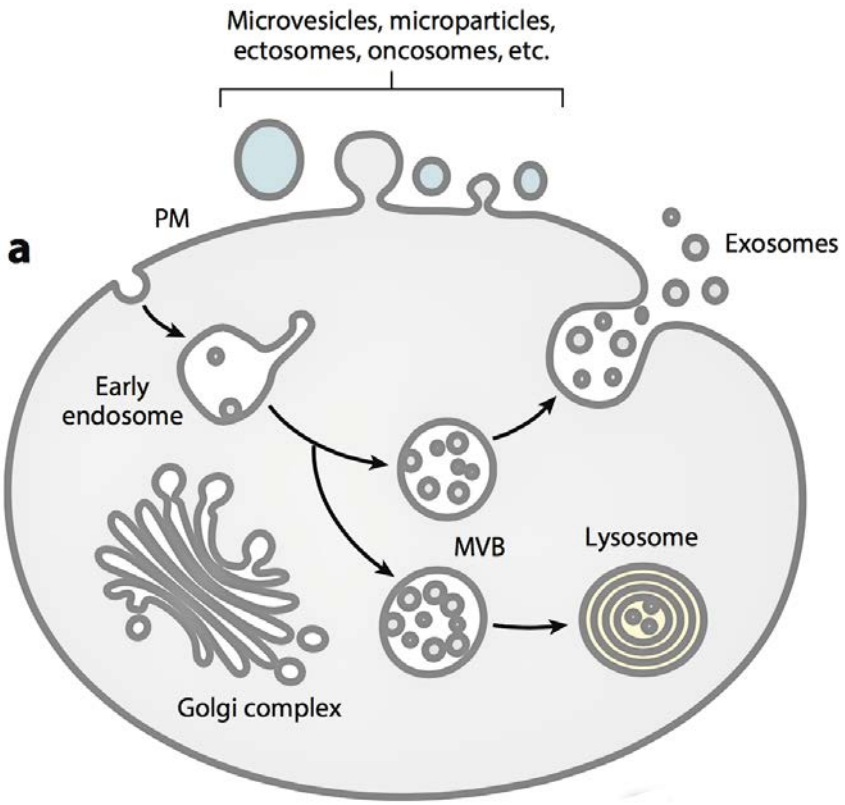

Figure 1. Intracellular process of exosome formation.

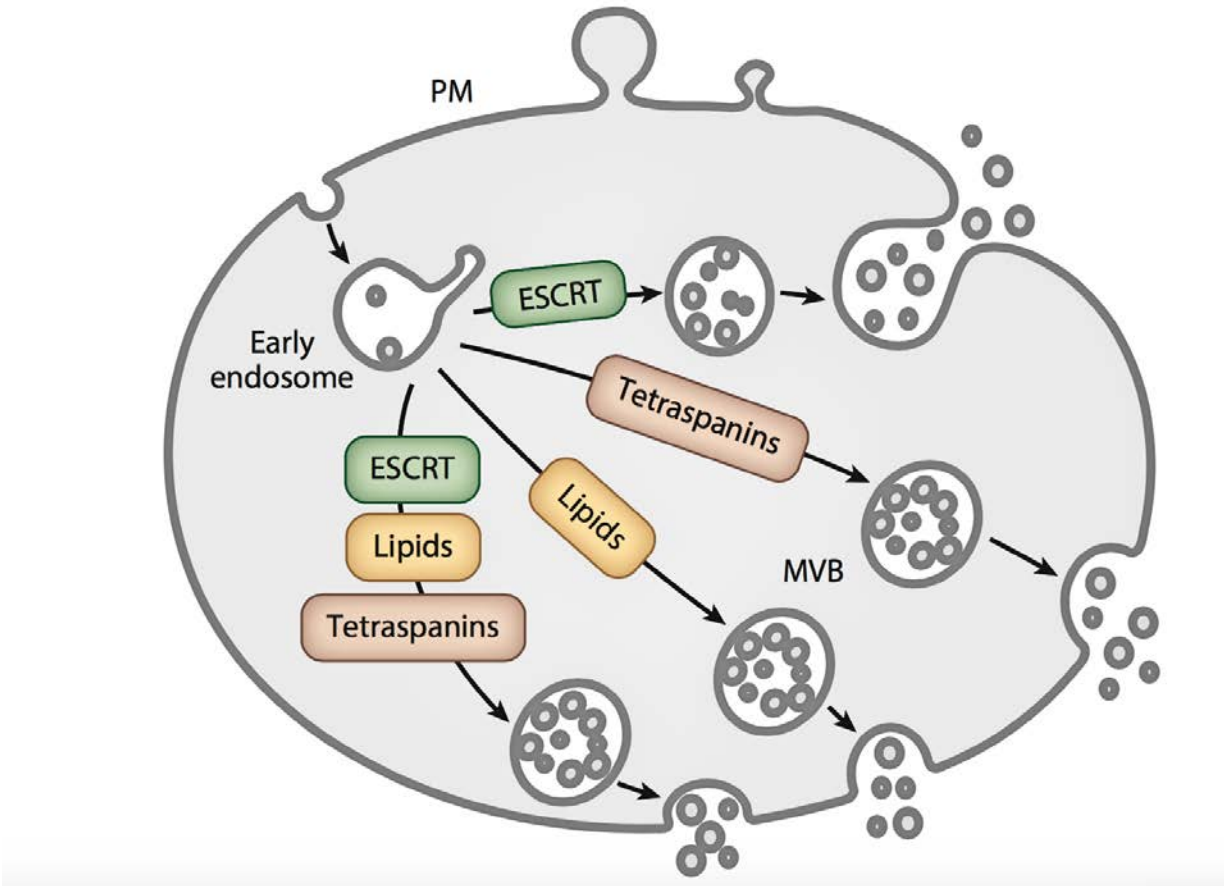

Figure 2. Initialization of endocytosis for internalization as well as fusion of exosomes with the plasma membrane of the recipient cell for the release of their contents into the cytoplasm.

function. The first pathway involves fusion with a lysosome, resulting in degradation and recycling of intra-endosomal contents (Figure 2) [9]. The second pathway involves lysosomal escape, which is signaled by specific endosomal membrane components. Lysosomal escape results in the eventual maturation of early endosomes into late endosomes. The membrane of late endosomes then undergoes the process of reverse endosomal budding, which is essentially endocytosis of the endosomal membrane. This process forms smaller vesicles within the endosome, which now have the extracellular domain of recycled plasma membrane proteins in the extra-vesicular compartment. The formation of these intra-endosomal vesicles, which are future exosomes, signifies the creation of the multi-vesicular body (MVB). MVBs then traffic their cargo of exosomes through the cytoplasm to the plasma membrane.

Fusion of MVBs with the cell membrane occurs in an energy-dependent, calcium-mediated process [10]. This process is similar to the mechanism used by neurons to release pre-packaged neurotransmitter vesicles from the axon terminal into the synaptic space. After the MVB fuses with the plasma membrane, the exosomes are released with similar, but not identical, membrane receptors and proteins characteristic of the originating cell. Once in the extracellular milieu, exosomes are able to bind to target cells and complete the intercellular interaction by transferring their internal cargo.

One key aspect of exosome formation, which remains unclear, is the molecular mechanism of cellular elements systematic packaging in the exosomal membrane. The composition of the exosomal membrane is not identical to that of the cell membrane from which it was derived. Although the two membranes are similar to each other, exosomal membranes lack many of the common clusters of differentiation (CD) and fragment crystallizable (Fc) surface proteins, as well as various integrins, that are present on the plasma membrane [11]. Thus, the components of the membrane are altered during protein recycling and exosome formation, with certain lipids and proteins being removed or concentrated. For instance, compared to the cell of origin, exosomes have an increased concentration of ceramide, an important lipid membrane constituent capable of cellular signaling [12]. This indicates that the composition of the exosome membrane is distinctive and purposeful, and is not indiscriminately compiled. Similarly, the intra-exosomal content is comparable, but not identical, to that of the parental cell [13]. The packaging of cellular elements into exosomes has been linked to the ESCRT (endosomal sorting complex required for transport) machinery, which is part of the endosomal system and responsible for trafficking of proteins and remodeling of cellular membranes during exosome formation [14]. Variations in this process can lead to either enrichment or depletion of exosomal content depending on the needs of the cell. For instance, specific nucleotide motifs on RNA molecules can be recognized by ribonucleoproteins (RNPs), which lead to the cell preferentially packaging messenger-RNA (mRNA) or microRNA (miRNA) into exosomes [15]. The unique differences between the composition of exosomes and the cell of origin indicate that exosome formation is not a random process, and that both the membrane constituents and intra-exosomal content are assembled for a distinct purpose in cellular biology.

Cellular elements in exosomes

Exosomes are known to contain widely variable cellular elements in the exosomal membrane (Figure 3) [16]. Likewise, the function of these membrane constituents is broad. Evidence shows that exosome membrane can contain integrins, which can mediate binding in the extracellular space and may play a role in internalization by a recipient cell [17]. Additionally, studies show that the membranes of exosomes can contain major histocompatibility complexes (MHC) which could participate in antigen presentation and immune responses to infection and cancer [18]. Many other membrane proteins have been described in exosomes, some of which have no known or accepted function at this time. Specifically, proteins in the tetraspanin family, which are thought to be membrane scaffold proteins, have been ubiquitously described in exosomes. However, the role of these proteins in exosomes is still under investigation. Included in the tetraspanin family are various cluster of differentiation proteins, including CD9, CD37, CD63, and CD81, which are all used as surface markers for exosomes [19]. CD63 is one of the most utilized tetraspanin surface markers in exosomes from a variety of cell types, and has been linked to antigen presentation via association with MHC molecules [20]. This assortment of proteins represents only a fraction of the exosomal membrane constituents, and new functional components are still being discovered. Similar to the exosomal membrane components, the intra-exo-

somal compartment is home to a wide variety of cellular elements. However, unlike the membrane components, the roles of nearly all cellular elements within exosomes are well known. The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is known to play a major role in intracellular vesicular trafficking, and may also indicate a metabolic role for exosomes [21]. The intra-exosomal compartment can also contain functional genetic elements, not present in exosomal membranes. The transfer of mRNA via exosomes has been described in a variety of disease processes, including cancer.

Role of MSCs in intracellular communication

The findings from studies investigating the role of MSCs in gliomas, and thus the intercellular communication between MSCs and GSCs, is limited to the description of soluble tumor-promoting proteins by MSCs. However, one key component of paracrine intercellular interactions that has been overlooked is that of exosome communication. Although the physiologic and pathologic release of exosomes has recently been investigated in a variety of disease processes, including cancer, the function of exosomes secreted from non-cancer cells within the tumor niche has never been explored. This research disparity, combined with the extensive evidence describing the various contents of exosomes, highlights a gap in knowledge as to the function of MSC-derived exosomes. Thus, further examination is necessary to evaluate this novel intercellular exosomal communication pathway between MSCs and GSCs.

Exosomes and microenvironment

Currently, there are only two reports investigating the role of tumor stroma- derived exosomes within the microenvironment. In 2013, Luga et al.

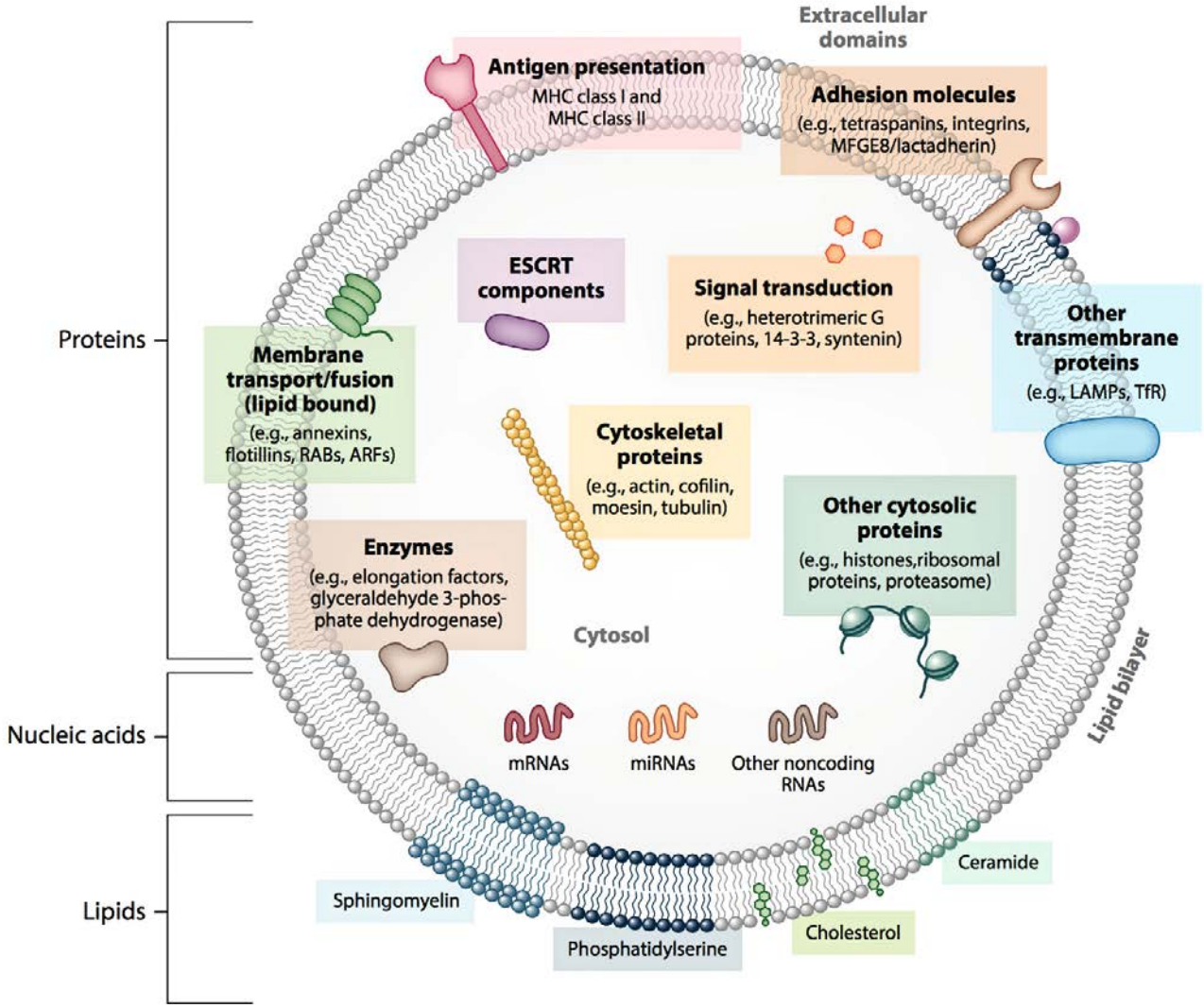

Figure 3. Variety of biomolecules in the exosomal membrane.

found that exosomes released from cancer-associated fibroblasts (CAFs) in breast cancer increased the invasion and motility of breast cancer cells [22]. This effect was linked to the activation of the Wnt-planar cell polarity (Wnt-PCP) signaling pathway, via Wnt11 molecules packaged in CAF-derived exosomes. Luga and colleagues concluded that this intercellular communication system led to enhanced metastatic capabilities of breast cancer [23]. This is the first study demonstrating the tumor-promoting capabilities of exosomes derived from stromal cells isolated from the tumor microenvironment. Recently, in 2014, Wang and colleagues, demonstrated that exosomes from gastric carcinoma-mesenchymal stem cells (GC-MSCs) were capable of increasing the growth and migration of human gastric carcinoma (HGC) cells [24]. These effects were found mediated by the delivery of miR-221 to HGC cells by GC-MSC-derived exosomes. The onco-miRNA miR-221 is linked to the down-regulation of certain cyclin dependent kinase inhibitors and anti-apoptotic factors. Together, these two studies demonstrate the tumor-promoting role of stroma-derived exosomes, and define a mechanism by which the exosomal contents can mediate the tumor-enhancing effects.

Another study published by Roccaro et al. showed the tumor-promoting properties of BM-MSC-derived exosomes in multiple myeloma (MM), albeit using cells that were not part of the microenvironment in the primary tumor [25]. Here the researchers found that MSCs harvested from the bone marrow (BM-MSCs) of patients with MM had different genetic and proteomic profiles than BM-MSCs harvested from normal subjects. These differences carried over to differences in the genetic and proteomic profiles of exosomes derived from both BM-MSC lines. Roccaro and colleagues found that BM-MSC-derived exosomes from MM patients had higher levels of levels of oncogenic proteins, cytokines and adhesion molecules, when compared to BM-MSC-derived exosomes from normal subjects. These tumor promoting proteins were shown to transfer to plasma cells in both *in vitro* and *in vivo* mouse models [25]. Importantly, the BM-MSCs of MM patients were harvested from abnormal bone marrow, not primary tumor sites, and therefore were not associated with the microenvironment. However, MM is thought to arise from abnormal bone marrow, and these abnormal BM-MSCs may play a role in the initial malignant transformation of plasma cells in MM. Nevertheless, this study demonstrates the tumor-promoting capabilities of exosomes derived from BM-MSCs, which can be recruited to, and engrafted in gliomas.

In 2012 Zhu et al. investigated the tumor promoting effects of BM-MSC- derived exosomes on human gastric carcinoma and colon cancer cell lines [26]. They found that tumor stem cells treated with BM-MSC-derived exosomes had increased proliferation *in vitro,* and promoted tumorigenicity in *in vivo* mouse xenografts. Additionally, they showed that these results were due to the increased expression of vascular endothelial growth factor (VEGF) in the cancer stem cells, via exosomal activation of extracellular signal-regulated kinase 1/2 (ERK1/2). Their findings suggested a novel mechanism by which intercellular communication could take place within a tumor.

Although the Roccaro and Zhu articles demonstrate the tumor promoting properties of BM-MSC-derived exosomes, these cells are not the most accurate representation of MSCs in the tumor microenvironment. Once recruited and entrenched in the tumor niche, BM-MSCs are influenced by neighboring CSCs and undergo specific changes that alter their biology [27]. Although they still exhibit the classical MSC morphology, surface markers, and tri-differentiation capabilities, TA- MSCs differ from normal BM-MSCs in proliferation rate and genetic signature [27]. Therefore, the efficacy of these studies could be improved by investigating the effects of TA-MSC-derived exosomes on the progression of malignancy.

Conclusions

From the above citations it can be concluded that exosomal mode of communication plays an important role during gliomas. However, more detailed studies are still required in order to develop better therapeutic strategies targeting exosomes.

Conflict of interests

The authors declare no confict of interests.

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