Journal of Clinical Neuroscience 35 (2017) 13–23

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Review article

The emergent role of exosomes in glioma

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ARTICLE INFO

Article history:
Received 14 July 2016
Accepted 26 September 2016

Keywords:
Glioma
Glioblastoma multiforme
Extracellular vesicles
Exosomes

ABSTRACT

Extracellular vesicles (EVs) are known mediators of intercellular communication for both normal and tumour cells. With the capability to transfer nucleic acids, proteins and lipids, EVs are able to influence numerous functional and pathological aspects of both donor and recipient cells. The tumour microenvironment possesses a high level of complex heterogeneity, particularly within the most prominent brain malignancy, glioblastoma multiforme (GBM). This complexity relies on a network-based communication between many different components of the local niche, including the various cell types, stroma, blood vessels, secreted factors and surrounding matrix. Exosomes are one type of EV which facilitates this intercellular communication and cross-talk within the tumour microenvironment. Exosomes secreted by tumour cells are increasingly recognized in a number of processes underlying tumour progression including facilitating the transport of receptors, signalling molecules, oncogenic genes and miRNA. They are emerging as a key component in the biogenesis of glioma, in addition to contributing to the modification of the surrounding microenvironment to support tumour progression. In this review we describe advancements in the understanding of the biology of exosomes, as well as their roles in tumour progression, as a tumour biomarker for tracking cancer progression, and as a potential therapeutic target/delivery system, with a contextual emphasis on GBM.

1. Introduction

Glioma tumours that arise from glia or glial precursor cells are the most prevalent brain tumour, with an estimated 23,000 new cases and 16,000 deaths in the USA in 2016 [1]. According to the CBTRUS Statistical report (2008–2012), gliomas account for over 32% of all central nervous system (CNS) tumours and approximately 80% of malignant primary CNS tumours [2]. The most prevalent form of glioma with the most dismal prognosis is the grade IV glioblastoma multiforme with an incidence rate of 3.2 per 100,000 population [2] and evidence suggesting that it is increasing every year [3].

With a median survival rate of only 14.6 months [4], GBM is the most intractable and lethal primary brain malignancy [5]. The customary treatment protocol usually involves surgery followed by post-operative fractionated radiation therapy and concomitant chemotherapy [4,5]. However, with near universal recurrence, this approach provides only a degree of palliation [6,7]. The invasive nature of GBM prevents total resection, making them surgically incurable. In 2005, a pivotal study was published showing that concurrent radiotherapy and temozolomide-based chemotherapy led to a modest 2.5 month increase in the dismal median survival of GBM patients and improvement in their health-related quality of life. This combinatorial treatment also led to a significant increase from 10% to 26% of patients who survived beyond two years [8]. The treatment, now referred to as the Stupp protocol, has been universally adopted as the standard of care for them [9]. Even alternative adjuvant therapies such as photodynamic therapy have only achieved similar median survival figures (14.8 months) as the Stupp treatment protocol for GBM patients [10–12].

GBM tumours are both morphologically and molecularly complex. Not only do they display properties indicative of diverse cellular phenotypes [13–17], they are also significantly heterogeneous at the inter-tumour and intra-tumour levels [18]. As a result, the classification and subsequent treatment of GBM are made considerably more difficult as a result of this heterogeneity [19]. Regional diversity observed in molecular pathways, cellular communication and tumour stem cell signalling may also contribute to the varied therapeutic response observed in GBM patients. Such diversity has driven the efforts in refining existing classification systems focus on the sub-typing of GBM by utilizing genomic and expression data [20,21]. Categorization of GBM based on distinct gene sets and particular signalling pathways yields

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http://dx.doi.org/10.1016/j.jocn.2016.09.021
0967-5868/

potential for more specific and targeted therapeutics [22,23]. Further complicating the aspect involving the heterogeneous nature of GBM, single cell sequencing has recently identified that different regions within an individual GBM lesion may exhibit several subtype-specific signatures [24,25]. With this taken into consideration, selection of particular sub-populations of GBM patients in the future could enable the design of personalized molecularly–targeted therapies for them [26–28].

Tumour cells possess the ability to communicate between the different compartments of the tumour microenvironment and ultimately influence neighboring cells. This effect can be varied with respect to the different subtypes of GBM, depending on the composition of the molecules they secrete. Mesenchymal cells have been shown to promote an invasive microenvironment by manipulating surrounding cells via the involvement of miRNA [29,30]. The molecules which are secreted by tumour cells and are subsequently utilized for communicative purposes are often encapsulated in structures known as extracellular vesicles (EVs). EVs are small portions of organelle-free cytosol enclosed by a spherical lipid bilayer. EVs can be categorized further depending upon their site of origin and their size, which can range from 30 to 2000 nm. Vesicles that are derived from multi-vesicular bodies (MVBs) are referred to as exosomes, whilst those produced directly from the plasma membrane are known as microvesicles [31]. Cells can also shed contents into the microenvironment in moments of stress or cell death and this involves the process of blebbing by apoptotic bodies [31]. It has now become evident that exosomes are particularly important structures as they are involved in a variety of physiological processed including the intercellular exchange of proteins and RNA [32,33], induction of angiogenesis [34] and immune regulation [35–37]. However, given the expanding and complex research field on EVs, this review will only focus on a brief overview of the current literature that has investigated the potential roles of exosomes in brain tumours.

2. Exosomes

2.1. Exosomal composition

Exosomes can be defined via a number of main morphological and physical characteristics. Firstly, they range in size between 40 and 120 nm in diameter, are of endocytic origin and sediment at approximately 100,000 g (sucrose density gradient of 1.13–1.19 g/ml) [38]. Morphologically they appear as spherical structures with a well-defined lipid bilayer when viewed with an electron microscope [37]. Contained within their aqueous core or in the lipid membrane, are various proteins, nucleic acids and receptors that are reflective of the parental donor cell (2,3). The variety of proteins and receptors that are present in exosomes is largely dependent on their cell of origin. Whilst the biogenesis of exosomes and cargo regulation is complex, we know that exosomes are generated first as intraluminal vesicles (ILVs) within multivesicular bodies (MVBs), via mechanisms that are either dependent or independent of the 'Endosomal Sorting Complex for Transport' (ESCRT).

Proteins can be sorted into ILVs independent of ESCRT machinery, through raft-based micro-domains of endosomes which are rich in sphingolipids. Sphingolipids are formed into ceramides by sphingomyelinases which induce the union of their micro-domains and trigger ILV formation [39]. ESCRT dependent mechanisms act on MVBs, allowing for selection of particular proteins and receptors into ILVs. The ESCRT family includes a variety of complexes, the first being ESCRT0 which identifies ubiquitinylated proteins protruding into the cytosolic side of the endosomal or MVB membrane. ESCRT0 has the ability to separate the ubiquitinylated proteins into specific micro-domains [31]. ESCRT0 then binds to the ESCRTI complex [35] which in turn recruits ESCRTII subunits, initiating the reverse budding of ILVs into MVBs [40]. Cytosolic RNAs and proteins have direct access into the forming vesicles during this internalisation stage. Next, the ESCRTII complex recruits ESCRTIII subunits inside the neck of the nascent ILVs, which results in their cleavage into free vesicles [32]. The MVB (or late endosome) can then fuse with the peripheral membrane and release the exosomes into the extracellular space [41]. A schematic representation of exosome biogenesis and release is presented in Fig. 1. Several functional RNA species including miRNA, mRNA, rRNA and tRNA have been identified within exosomes [42,43]. The protection from enzymatic RNAse degradation afforded by their inclusion inside exosomes, enables safe passage through the extracellular environment and vasculature system [44–47]. Subsequent, release of cargo mRNA and microRNA into the recipient cell can modulate gene expression through translational and post-translational regulation of target mRNAs [40]. Exosomes are able to alter the transcriptome and signalling activity within recipient cells, allowing them to induce specific phenotypic changes [48–50].

Many exosome associated proteins have been identified but the core composition of exosomes remains the focus of continued research. In addition, whilst contributing to the fusion and budding processes of vesicles, lipids can play a vital role in membrane rigidity and stability [51]. The lipid composition of exosomes has not been entirely elucidated as various lipids have been reported to exist in the lipid bilayer of exosomes. These include cholesterol, sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, ganglioside GM3, prostaglandins and lysobisphosphatidic acid [52,53]. Exosomes contain many membrane-associated proteins that contribute to a variety of functions, including adhesion via tetraspanins (CD63, CD81, CD9, CD37, CD53 and CD82), ICAM-1 and integrins; intracellular transport and membrane fusion (Rab proteins and Annexins); MVB formation (Alix, TSG101); and antigen presentation (MHC-I, MHC-II, HLA-G) [54]. Other proteins may be present depending on the cell type and method of isolation as outlined in the databases – ExoCarta [55,56], Vesiclepedia [57] and EVpedia [58].

2.2. Exosomes in glioma progression

Exosomes represent an important extension of the complex array of metabolites, growth factors, cytokines and ions that are secreted by tumour cells [59]. It has been established that exosomes mediate the transfer of histones [60], oncogenic species (EGFRvIII) [61], non-coding RNA (miRNA) [62] and tumour suppressors (PTEN) in glioma cells [63]. However, the ramifications of intercellular communication between cells in the GBM tumour microenvironment, facilitated by the exchange of exosomes, have not been fully elucidated.

Notably, this includes the possibility of different active intracellular signalling pathways within the various GBM subtypes leading to the activation of different vesicle biogenesis pathways. The four molecular subtypes of GBM (known as mesenchymal, classical, neural and pro-neural) have been observed to differ substantially in the mRNA levels of known markers of exosome biogenesis [21]. Exosomes released from glioma cells have been implicated in the shaping of the tumour microenvironment in an array of processes including the transfer of functional RNA transcripts [64], angiogenesis, clonogenicity and heightened proliferation arising from paracrine induction [61,64], spread of pro-migratory factors [65] and influencing immune-tolerance or inducing malignancy in normal cells [66–68]. In addition, exosomes of GBM origin may also modify cell surface protein expression and cytokine secretion, as well as influence the immunity functions of the