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Diagnostic and therapeutic potentials of exosomes in CNS diseases

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Abstract

A newly discovered cell-to-cell communication system involves small, membrane-enveloped nanovesicles, called exosomes. We describe here how these extracellular nanoparticles were discovered and how it became gradually apparent that they play fundamental roles in regulation of physiological functions and pathological processes. Exosomes enable intercellular communication by transporting genetic material, proteins and lipids to cells in their vicinity or at distant sites, and subsequently regulating functions of targeted cells. Relatively recent experiments indicate that exosomes are released also by CNS cells, including cortical and hippocampal neurons, glial cells, astrocytes and oligodendrocytes, and that exosomes have significant impact on pathophysiology of the brain. How it is decided what individual exosomes will carry to their targets is not understood, but it appears that the contents may represent “signature cargos” that are characteristic for various conditions. Exploration of such characteristics could result in discovery of novel diagnostic biomarkers. Exosomes are also promising as a vehicle for therapeutic delivery of micro RNA or other compounds. How to deliver exosomes to selected sites has been a tantalizing question. Recent experiments revealed that at least some exosomes carry antibodies on their surface, suggesting that it may be feasible to deliver exosomes to unique sites based on the recognition of antigens by those antibodies. This discovery implies that rather precise targeting of both natural and engineered exosomes may be feasible. This would reduce distribution volume of therapeutics, and consequently minimize their side effects.

Introduction

Exosomes are key players in intercellular communication that can fundamentally affect various physiological functions at targeted cells. Exosomes are generated inside cells from multivesicular bodies (MVB) and released into extracellular space via exocytosis. These membrane enveloped nanovesicles carry genetic material, proteins and lipids that are employed in regulation of targeted cells and travel in extracellular body fluids that allow them to act on nearby cells, as well as on distant targets. Here we highlight some of critical

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discoveries in exosomal research, describe ongoing efforts and challenges in this rapidly developing field with the emphasis on CNS, and finally, propose how immunological principles could be employed in the design of exosome-based treatment.

MVB and the discovery of exosomes

MVB are endosomal organelles that are characterized by internal membrane-enveloped vesicles, which were described for the first time in neurons by Palay and Palade in 1955 (Palay *et al.*, 1955). Originally, these vesicles were regarded to be pre-lysosomal structures involved in protein degradation (Piper *et al.*, 2007), but more recent evidence indicates that MVB mediate diverse intra- and intercellular trafficking of molecules (Von Bartheld *et al.*, 2011).

Nearly three decades after the discovery of intracellular MVB, Trams and co-workers analyzed cell-free supernatants collected from human neuronal neoplastic cell lines. The supernatants were spun at the speed of 100,000 g for 90 minute, and the pellets of these supernatants were then studied by electron microscope (Trams *et al.*, 1981), which resulted in the discovery of extracellular membrane-enveloped vesicles ranging in size from 40 to 1,000 nm that were visualized by electron microscopy (for comparison, obtaining a pellet of cells from peripheral blood requires a centrifugation force of about 400g). The supernatant pellets possessed enzymatic activities, including 5-nucleotidase and ATPase, suggesting that the vesicles could play some physiological roles rather than just be cellular waste products (Trams *et al.*, 1981).

The extracellular vesicles found by these researchers included subsets that are today known as nanovesicles or exosomes (<100 nm), as well as larger ectosomes (also referred to as microvesicles or microparticles) that typically range in the size 0.1–1.2 μ m. While exosomes have been shown to be derived from MVB, ectosomes shed from cellular membranes (Fleury *et al.*, 2014; Harding *et al.*, 1984; Heijnen *et al.*, 1998; Heijnen *et al.*, 1999; Peters *et al.*, 1991; Raposo *et al.*, 1996; Thery *et al.*, 2009) and differ in their content, implying that exosomes and ectosomes may have distinct purposes (Lazaro-Ibanez *et al.*, 2014; Revenfeld *et al.*, 2014). In this review we focus primarily on exosomes.

Exosomes are functional

The early work of Rose Johnstone and colleagues supported the idea that the extracellular vesicles obtained after ultracentrifugation of cell culture supernatants may be functional. They found that the vesicles from *in vitro* cultured sheep red blood cell precursors, reticulocytes, possess unique enzymatic activities resembling those of reticulocyte cell membrane, and a lipid composition reflecting sphingomyelin content of red blood cell membrane (Johnstone *et al.*, 1987). For a long period, however, it remained unclear what is the purpose of these vesicles, and the general view of these early studies was that they serve as “garbage cans” removing unwanted molecules.

After a decade without a follow up, experiments by Raposo and co-workers turned around this concept, as their results indicated that exosomes seem to play an active role in intercellular communication within the immune system (Raposo *et al.*, 1996). The authors

reported that cultured B cell lines secrete 60–80 nm vesicles containing complexes of antigens and major histocompatibility complex (MHC) II, which are known to be essential for presentation of antigens to T lymphocyte to induce their activation (Raposo *et al.*, 1996). In follow up studies, Zitvogel and co-workers demonstrated that dendritic cells (professional antigen-presenting cells) also release nanovesicles expressing MHC class I, MHC class II, as well as co-stimulatory molecules that are critical for T cell activation and proliferation (Zitvogel *et al.*, 1998). The investigators then pulsed vesicles from the dendritic cells with tumor derived peptides and administered them to mice bearing tumors. This resulted in stimulation of antitumor T lymphocyte responses *in vivo* and improved tumor eradication (Zitvogel *et al.*, 1998), clearly indicating a critical contribution of exosomes to important immune responses *in vivo*.

Exosomes transport RNA

A seminal breakthrough that identified exosomes as an entirely new form of intercellular communication came from the discovery of Jan Lotvall's group who demonstrated the presence of RNA in exosomes (Valadi *et al.*, 2007). Exosomes derived from human and mouse mast cells contained messenger RNA (mRNA), as well as micro RNA, which encoded approximately 1,300 genes. The transfer of exosomal RNA led to the protein translation in the target cell (Valadi *et al.*, 2007), establishing, that exosomes transfer genetic information, at least *in vitro*. These data placed exosomes among fundamental, physiological regulators and opened a wholly new world of controlling mechanisms that were till then completely unknown.

The presence of RNA in extracellular fluids has been known for a long time, but it had been largely assumed that it comes from dying cells and it is quickly degraded to small fragments by omnipresent ribonucleases. While the majority of RNA is indeed located within cells, numerous studies over the last decade made it abundantly clear that all body fluids contain cell-free micro RNA that is functional (Chen *et al.*, 2007; Chevillet *et al.*, 2014). The circulating microRNA travels mostly protected by cell membrane as an exosomal cargo (Gallo *et al.*, 2012), or to a lesser degree in association with secreted proteins, such as Ago 2 (Wang *et al.*, 2010), or with high density lipoproteins (Vickers *et al.*, 2011). The size of micro RNA is approximately 20–25 nucleotides (Lee *et al.*, 1993). It is encoded by introns or by their own genes within exons (Rodriguez *et al.*, 2004). Precursors of mature micro RNA form hairpin structures that are then cleaved by Dicer, a ribonuclease of Family III (Ambros *et al.*, 2003). The removal of hairpin's loops yields double stranded RNA fragments of about 22 nucleotides. Dicer then helps to recruit the micro-RNA induced silencing complex (RISC)(Ji, 2008) that guides the base-pairing of one strand of microRNA with complementary sequences at the 3' end of mRNA in target cells, and thereby inhibits translation of specific mRNA, while the other strand of micro RNA is degraded (Ambros *et al.*, 2003; van den Berg *et al.*, 2008).

Exosomal cargos in their complexity may turn to be relevant diagnostic biomarkers

What determines the content of exosomes and how is the cargo uploaded? The answers to these fundamental questions are far from clear. It is known that exosomes transport a variety of molecules. Together with micro RNA and mRNA (Valadi *et al.*, 2007), exosomes carry also genomic DNA (Lazaro-Ibanez *et al.*, 2014), mitochondrial DNA (Guescini *et al.*, 2010b) (Zhang *et al.*, 2012), proteins (Kalra *et al.*, 2013) and lipids (Llorente *et al.*, 2013; Subra *et al.*, 2007).

Current evidence appears to indicate that exosomal cargos have “signature patterns” that corresponds to individual cell-types and conditions, and as such, these “patterns” are of considerable interest as biomarkers for various pathological conditions. Given that exosomes may regulate a high number of functions both in health and disease, it will not be trivial to distinguish individual “signatures” and relate them to distinct physiological or pathological conditions. For example, all cell-cell interactions in neuronal and immune synapses may need to be reassessed with consideration to regulatory roles of exosomes and combination of regulatory molecules that they carry (Chivet *et al.*, 2013; Gutierrez-Vazquez *et al.*, 2013; Smythies *et al.*, 2012). Nevertheless, a remarkable experimental study of Peinado and co-workers showed the feasibility of elucidating complex interactions in melanoma where exosomes mediate multi-organ communications among tumor cells, tissue stroma at sites of future metastases and cells of the immune system (Peinado *et al.*, 2012).

In this investigation, patients with metastatic melanoma were found to have exosomes in their plasma that contained a set of proteins representing their “signature” (TYRP2, VLA4, HSP70, MET and Rab27a). Particularly MET and TYRP2 correlated with metastatic invasiveness of patient’s melanomas (Peinado *et al.*, 2012). Setting up an experimental model of mouse of melanoma, they administered intravenously exosomes derived from supernatant of a highly metastatic mouse melanoma cell line (Peinado *et al.*, 2012). These exosomes homed to sites where melanoma commonly metastasizes, such as lungs and lymph nodes. At these sites, the exosomes altered the microenvironment by facilitating plasma leakage and inflammatory changes that promoted cell migration into these localities, and in such a way promoted metastases. These exosomes also increased the levels of the oncoprotein MET in the bone marrow progenitors. When the expression of MET in the exosomes, was reduced, the metastatic invasiveness of the melanoma significantly decreased. In parallel, the investigators analyzed bone marrow derived cells of patients with highly metastatic melanoma and observed high level of MET, suggesting that it may serve as a diagnostic and prognostic biomarker of metastatic melanoma, as well as a novel therapeutic target for this highly aggressive cancer that belongs to one of tumors with rising worldwide incidence and great difficulty to manage when it reaches the metastatic stage (Peinado *et al.*, 2012).

This elegant study highlighted how characterization of disease-associated exosomes, understanding their “signature patterns” and following their functional consequences in experimental disease models and patients may bring new level of clarity of the intricate

interplay among cell populations in different organs. Undoubtedly such approaches should result in significant diagnostic and therapeutic advances.

What makes the evaluation of exosomes challenging?

Intense efforts in the field have led to the development of new methodologies that make it possible to detect and isolate these nanoparticles in small amounts of various body fluids (Lasser *et al.*, 2012), including plasma/serum (Kalra *et al.*, 2013; Lasser, 2013; Muller *et al.*, 2014), urine (Gonzales *et al.*, 2010) or cerebrospinal fluid (Chiasserini *et al.*, 2014; Street *et al.*, 2013). This makes it possible to investigate exosomal content by rapidly evolving nucleic acid sequencing, proteomic and lipidomic techniques, and to evaluate exosomal surface molecules that may be involved in directing exosomes to their targets (Momen-Heravi *et al.*, 2013; Schageman *et al.*, 2013; Witwer *et al.*, 2013).

A major challenge in the field is reliable quantification of minuscule amounts of micro RNA in and out of exosomes (Zampetaki *et al.*, 2012). There are three platforms available for analysis of the micro RNAs: new generation deep sequencing, microarrays and quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). New generation sequencing permits unbiased analysis of minute amounts of microRNA, but unfortunately, does not allow quantifying the amounts of RNA. The weakness of microarrays analyses and quantitative RT-PCR assays is in the requirement of pre-designed primers and thus may lead to omission of previously not yet identified molecules of micro RNAs.

Quantitative proteomic methodologies have revealed that exosomes contain both conserved sets of proteins that are found in cells of diverse origins, as well as unique proteins that depend on conditions and cell types from which exosomes were secreted. The latter proteins are being now explored as possible biomarkers for various diseases (de Jong *et al.*, 2012).

With regards to lipid composition of exosomes, high levels of sphingomyelin were detected already at the time of their discovery. However, results of comprehensive lipidomic analyses of exosomes were reported only recently (Del Boccio *et al.*, 2012; Llorente *et al.*, 2013; Subra *et al.*, 2007) and lipid portion of exosomal cargos remain poorly characterized.

How are exosomes uptaken by their target cells?

This is yet another fundamental question that is of strong relevance to both understanding basic physiology of exosomes, as well as application of the discovered principles in designing new exosome-based therapies.

It appears that the uptake of exosomes in their final destination involves several routes, including attachment to tetraspanins, integrins, immunoglobulins or proteoglycans, and then internalization via clathrin and caveolin mediated uptakes, macropinocytosis, phagocytosis and lipid rafts mediated internalization. These mechanisms are comprehensively discussed by Mulcahy and co-worker in their recent review (Mulcahy *et al.*, 2014).

Recently we discovered that exosomes can deliver micro RNA in antigen-specific manner due to antigen-specific antibodies on their surface (Bryniarski *et al.*, 2013). This new finding

opens the possibility of engineering exosomes that can be delivered to various sites with specificities that are uniquely recognized by specific antibodies, and thus help resolve the problem of specific targeting of therapeutic exosomes.

Exosomes can act in antigen-specific manner

Our observations were made in the classical, experimental model for investigation of effector T cell-driven immunity *in vivo* that employs hapten-induced delayed type hypersensitivity (DTH) in the mouse skin. In this model, mice are immunized with a reactive hapten antigen, like picryl chloride (PCI, TNP-Cl) by painting it on their shaved abdomen. Four days later, they are exposed to second, low-dose, antigen challenge, by painting the TNP hapten on skin of their ears. Twenty-four hours later, ears respond by tissue swelling and local inflammation driven by antibody-mediated recruitment of a antigen-specific T cells that orchestrate infiltration of neutrophils and mononuclear cells (Askenase, 2001).

In contrast, the immunization by intravenous administration of high doses of the antigen, results in tolerance to subsequent exposure to the antigen (non reactivity in the skin). Understanding how is tolerance mediated is essential for designing therapies for autoimmune and allergic diseases. The mechanism causing the tolerance in the DTH model involves antigen-specific CD8⁺ suppressor T cells, that we knew for long time to produce a soluble suppressor factor into serum (Herzog *et al.*, 1990). However, the full nature of the suppression factor remained obscure until recently, when we discovered that the tolerance is mediated by the micro RNA, MIR-150, delivered by exosomes (Bryniarski *et al.*, 2013). The exosomes not only transferred the tolerance, but they did so in an antigen-specific manner. Thus, the mice tolerized to TNP-Cl could transfer the tolerance only to mice that were immunized to picryl chloride antigen, and not to another distinct hapten, oxazolone, and vice versa. Flow cytometry of the nanovesicles revealed the presence of antibody kappa light chains on their surface, suggesting that B-cell derived antibody were responsible for the antigen specificity of the T cell exosomes delivering inhibitory MIR-150. In fact, tolerized mice that are pan-immunoglobulin deficient produced exosomes that did not have immunosuppressive properties. Importantly, when these non-suppressive exosomes were coated with hapten-specific light chain antibodies, they became immunosuppressive *in vivo* (Bryniarski *et al.*, 2013), clearly demonstrating the key role of antigen-specific antibodies in tissue targeting.

Thus, in principle, it should be feasible to engineer exosomes that can carry selected micro RNAs to specific sites, provided that light chains of antibodies are raised against molecular structure(s) that are specific for the site. There already are vast libraries of antibodies against a huge number of specific antigens, and raising additional antibodies against specific antigens involves methodologies that are commonly used in immunology. Precision in delivering exosomes might be of particular importance in the brain where functionally distinct regulatory areas are in close vicinity from each other. Future experiments will show whether this vision is feasible.

Inter-cellular communication and exosomes in CNS

The communication of cells within the brain involves two integrated systems - wiring transmission and volume transmission (Agnati *et al.*, 2014; Agnati *et al.*, 2010). The wiring transmission engages point-to-point transmission, such as synapses or gap junctions, and more recently discovered tunneling nano-tubes that can transmit proteins, RNA and mitochondrial DNA (Agnati *et al.*, 2010). The volume transmission involves communication via extracellular and cerebrospinal fluid where exosomes were believed to play primary roles (Agnati *et al.*, 2010). Recently, however, exosome release was found also in synapses (Lachenal *et al.*, 2011), suggesting that exosomes may have previously unanticipated role also in synaptic cleft physiology.

The first direct evidence of exosomal release from neural cells came from Remy Sadoul's laboratory (Faure *et al.*, 2006). The investigators employed isolated primary, embryonic cortical neurons from rats and mice, and found that the *in vitro* stimulated neurons released exosomes into the supernatants (Faure *et al.*, 2006). Later the same group followed with another report where exosomal release from differentiated cortical and hippocampal neurons was dependent on calcium influx and glutamatergic synaptic activity (Lachenal *et al.*, 2011). The release of exosomes was demonstrated also by other cell types in CNS, including astrocytes (Guescini *et al.*, 2010a), microglial cells (Poticchio *et al.*, 2005) and oligodendrocytes (Kramer-Albers *et al.*, 2007). CNS cell type communicate via exosomes as is suggested by the release of nanovesicles from oligodendrocyte that are uptaken by glial cells and support neuro-glial interactions (Fruhbeis *et al.*, 2012). Another example of critical role of exosomes in the intercellular interactions in the brain is glioblastoma cells. They release exosomes containing mRNA, micro RNA and angiogenic factors, and upon exposure to hypoxia, these exosomes induce angiogenesis, a critical component of cancer survival (Kucharzewska *et al.*, 2013; Skog *et al.*, 2008).

With regards to the role of exosomes in human neurological and psychiatric diseases, it will be essential to develop a workable protocol for isolation and analysis of exosomes from cerebrospinal fluid (CSF). The first isolation and proteomic analysis of human CSF exosomes was performed on patients who underwent thoraco-abdominal aortic repair and large volume of CSF (200–500ml) was obtained as a therapeutic part of the procedure (Street *et al.*, 2012). This work broke the grounds by demonstrating the existence of exosomes in CSF, but the methodology using such large volumes is not easily applicable to other clinical scenarios. The volume of CSF that is commonly collected for diagnostic purposes is about 10–20 ml. In more recent report, investigators evaluated by proteomics samples of 6 ml that represent a pool of individual 0.5 ml CSF samples, making such analyses clinically attainable (Chiasserini *et al.*, 2014).

Recent reviews addressed the roles of exosomes in several neuropsychiatric conditions, including Alzheimer disease, Parkinson's disorder, glioblastoma multiforme, prion disease, multiple sclerosis or schizophrenia, where exosomes seem to play important roles in causing pathological changes that are typical for the diseases, clearly implying the need for learning how to interfere with detrimental exosomal pathways (Kalani *et al.*, 2014b; Lai *et al.*, 2012; Pegtel *et al.*, 2014; Tsilioni *et al.*, 2014). Several studies have already shown promising

effects that can be applied in therapy of the CNS diseases (Alvarez-Erviti *et al.*, 2011; Cooper *et al.*, 2014; Kalani *et al.*, 2014a; Katakowski *et al.*, 2013; Munoz *et al.*, 2013; Pusic *et al.*, 2014; Zhuang *et al.*, 2011).

The field of autism spectrum disorders (ASD) is currently one of most active research areas. Surprisingly, there is no publication on the role of exosomes in ASD. It can be speculated that exosomes may play a role since several reports indicated genetic alterations in micro RNA genes (Mellios *et al.*, 2012), and a recent comparison of serum of children with healthy control subjects revealed differences in levels of (Mundalil Vasu *et al.*, 2014). There is no information available with regards to micro RNA in CSF of patients with ASD. Performing studies that address the role of micro RNAs and exosomes further is highly relevant in such complex disorder that is associated with numerous psychiatric and non-psychiatric comorbidities and profiles of sera and CSF samples may help identifying subsets of patients with different pathogenesis and facilitate administering relevant treatment corresponding to affected pathways (Angelidou *et al.*, 2011; Kohane *et al.*, 2012).

Exosomes as a promising therapeutic vehicle

Since the discovery that exosomes contain micro RNA, they became of considerable interest to pharmaceutical industry as a possible vehicle for therapeutic delivery of designed short interfering RNA molecules (Johnsen *et al.*, 2014). Significant attempts have been done in relation to loading micro RNA into liposomes, which are also membrane enveloped vesicles. Exosomes, however, appear to be more favorable vehicle. The differences in biochemical and pharmacodynamic properties between these two kinds of corpuscles were recently summarized, by van der Meel (van der Meel *et al.*, 2014).

From the existing evidence it appears that future design of therapeutic vehicles may include more regulatory molecules delivered at the same time. Numerous details will need to be considered during exosomal engineering and some lessons may be learned from the efforts that lead to the development of liposome-based therapies, e.g. structural composition, viscosity and size of particles that affects number of functions, such as plasticity of the particles, response to osmotic shock, entrapment efficiency and drug release (Ravouru *et al.*, 2013; Salama *et al.*, 2012).

Interestingly, exosomes may at least partially replace stem cell based-cell therapies, since it appears that a number of desired effects of mesenchymal stem cells were mediated via exosomes (Braccioli *et al.*, 2013)

Considerations for exosomal therapy in CNS

Unique anatomy, restrictive space of the cranium and the complexity of CNS requires special attention when examining possibilities of directing therapeutics into brain tissue.

Compounds that are administered intravenously need to negotiate two barriers: blood brain barrier and blood–CSF barrier. To increase the amounts of therapeutic vesicles that reaches the brain tissue may be achieved by increasing permeability of these barriers, by exploiting

receptor-mediated endocytosis or by employing cell penetrating exosomes (Larsen *et al.*, 2014; Yang *et al.*, 2013).

Therapeutics can also be delivered also directly into cerebrospinal fluid via intrathecal administration, or may be given intranasally. The simplicity and rapid onset of effects achieved by intranasal delivery makes this route a promising candidate. When a compound is applied into nasal cavity, it can travel along olfactory to the brain tissue (Kozlovskaya *et al.*, 2014). The effectiveness of intranasal therapy was shown for example in a rat model of Parkinson disease where the intranasal administration of liposomes containing glial derived neurotrophic factor turned to be protective (Migliore *et al.*, 2014).

For clinical purposes, it will be of utmost importance to quantify the amounts of administered material that reaches and then remains in the brain. Kozlovskaya and co-workers devoted detailed attention to the existing literature on intranasal drug delivery and performed quantitative analysis of data from 73 studies using 82 compounds that were administered intranasally (Kozlovskaya *et al.*, 2014). They found that there is a large variability in brain targeting efficiency by different methodologies, but that absorption enhancers, mucoadhesive compounds or targeting residues do result in increased drug delivery via the intranasal route (Kozlovskaya *et al.*, 2014). Thus individual approaches to intranasal drug administration will need to be adequately characterized prior their clinical applications.

With regards to intranasal treatment with exosomes, the research group of Lawrence Steinman reported that exosomes that were loaded with an anti-inflammatory compound execute their potent anti-inflammatory effects in the mouse model of experimental autoimmune encephalitis (EAE) (Zhuang *et al.*, 2011). The anti-inflammatory effects were achieved only by curcumin-containing exosomes, whereas there was no effect when empty exosomes or curcumin alone were administered. This was tested in three distinct CNS inflammatory conditions: lipopolysaccharide-induced activation of microglia, T-cell driven, chronic inflammation in a well-established mouse model of multiple sclerosis and microglia activation associated with tumor growth. The exosomal treatment inhibited microglia activation and cytokine production and resulted in a profound improvement of clinical scores in the mice with EAE (Zhuang *et al.*, 2011).

Of note, the exosomes used in these studies did not have any surface molecules that would target them specifically to certain cells or sites in the CNS. Localization into the brains was achieved only through choosing the intranasal route of delivery. Our recent discovery that exosomes may be equipped on their surface with antibodies recognizing specific antigens (Bryniarski *et al.*, 2013) implies, that it may be feasible to further improve the targeting by focusing the delivery of the exosomes to distinct areas of brain by employing the recognition of specific antigens at such sites, provided that such distinct antigens for individual brain areas will be identified.

For example, we could speculate that exosomes carrying relevant micro RNA (determined by analyses of microRNA and exosomal profiles in serum and CSF of ASD patients) and other regulatory molecules (determined by analyses of exosomes in serum and CSF of ASD

patients) could be administered intranasally, then travel along olfactory nerve into the brain tissue and then through “volume transmission system” (Agnati *et al.*, 2010) reach different brain areas. Initially, enrichment of such therapeutic exosomes in cerebellum could be considered since this is a site where numbers of Purkinje cells in post-mortem brains of ASD patients were consistently found to be decreased (Fatemi *et al.*, 2012), perhaps due to chronic inflammation that was demonstrated at this site (Vargas *et al.*, 2005). Theoretically, the enrichment of exosomes in the cerebellum could be achieved by identifying cerebellum-specific antigens and then placing antibodies against these antigens on the surface of engineered therapeutic chromosomes. Such hypothesis would have to be first established experimentally, for example in the mouse model where ASD is induced in off-spring of dams that were exposed to immune activation during their pregnancy and have all key symptoms of ASD (Malkova *et al.*, 2012). If pre-clinical studies would warrant further clinical testing, then administration of precisely targeted exosomes carrying a therapeutic cargo could turn to be an effective intervention with minimized side effects. That may be particularly critical for diseases with immune pathogenesis, since systemic administration of immunosuppressive agents is associated with the dreaded increase in susceptibility to infections, and as recently suggested by the work of Kipnis’ lab, also alterations of cognitive functioning (Filiano *et al.*, 2014).

Concluding remarks

The vision of completely new forms of diagnostic and therapeutic approaches for numerous medical conditions has been driving the rapid development of the research on exosomes and their functions. Significant challenges in the field include the elucidation of processes that control selecting and uploading specific exosomal cargos, as well as mechanisms of recognition and uptake of exosomes by targeted cells at distinct locations. The development of new analytical technologies that are sensitive enough to detect tiny amounts of material within exosomes represent essential tools enabling these developments. On the way towards finding the answers about fundamental physiology and pathology of exosomes, new meaningful diagnostic markers will likely be identified, and understanding the basic principles of exosomal roles in health and disease should revolutionize designs of new therapeutics. With regards to the CNS disorders, specific biomarkers are practically non-existent, making it very difficult to distinguish subsets of patients who present with same clinical symptoms but ineffectiveness of medications in large numbers of the patients implies the involvement of different biological pathways in these subpopulations. Recent discovery of antigen-specific exosomes raises high hopes for developing engineered exosomes that could enable precise targeting of relevant regulatory molecules to areas of pathology, while minimizing side effects in other areas of the brain or body. This may be in particular useful for the conditions where autoimmune mechanisms were implicated such as Tourette syndrome, autism or depression (Lotrich, 2014; Martino *et al.*, 2014). Immune therapies should be tested at least in some patients with these disorders, but thus far this has been done only minimally. The reason is in the absence of relevant biomarkers that makes it very difficult to clearly identify the patients who could benefit from immune therapies and concerns that they could rather be uselessly exposed to the risks of systemic immune therapy, such as serious infections complications. The field of exosomes brings a hope of

developing adequate diagnostic tools that could distinguish patient subpopulations and enable personalized treatment of patients with neuro-psychiatric disorders.

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HIGHLIGHTS

- Exosomes are membrane-enveloped nanovesicles of endosomal origin
- They carry nucleic acids, proteins and lipids to nearby or distant cells
- Exosomal cargo regulates functions at target cells
- At least some exosomes recognize their targets in antigen specific manner
- Exosomes revolutionize the search for biomarkers and new therapies