Exosomes in Toxicology: Relevance to Chemical Exposure and Pathogenesis of Environmentally Linked Diseases

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ABSTRACT

Chronic exposure to environmental toxins has been known to initiate or aggravate various neurological disorders, carcinomas and other adverse health effects. Uptake by naïve cells of pathogenic factors such as danger-associated molecules, mRNAs, miRNAs or aggregated proteins leads to disruption in cellular homeostasis further resulting in inflammation and disease propagation. Although early research tended to focus solely on exosomal removal of unwanted cellular contents, more recent reports indicate that these nano-vesicles play an active role in intercellular signaling. Not only is the exosomal cargo cell type-specific, but it also differs between healthy and dying cells. Moreover, following exosome uptake by naïve cells, the contents from these vesicles can alter the fate of recipient cells. Since exosomes can traverse long distances, they can influence distantly located cells and tissues. This review briefly explores the role played by environmental toxins in stimulating exosome release in the context of progressive neurodegenerative diseases such as Alzheimer’s, Parkinson’s, and Huntington’s, as well as certain cancers such as lung, liver, ovarian, and tracheal carcinomas.

Key words: exosome; cell-to-cell transmission; neurodegeneration; environmental toxins; cancer; protein aggregation.

Intracellular communication is an essential biological phenomenon for the regulation of normal tissue function, which in turn, is important for the development and adaptation of multicellular organisms. Cells also communicate with one another following genetic or environmental stimuli and can help pass information in the form of signaling molecules and transcription modulators. Until recently, researchers have had only a limited understanding of these signaling pathways, which are often thought to be mediated through hormones, cytokines, growth factors and neurotransmitters and their specific recognition by cell-surface receptors. However, in the last 2 decades, a novel mechanism for intercellular communication has emerged that involves the transfer of extracellular vesicles (EVs). These vesicles play a key role in cell-to-cell communication, exerting their effects locally as well as across multiple organ systems. EVs are often classified based on their approximate size, origin and cargo. As determined by their biogenesis, the 3 major subclasses of EVs are apoptotic bodies, microvesicles, and exosomes (Figure 1). Apoptotic bodies, the largest EVs in this group (50–5000 nm), are released when plasma membrane blebbing occurs during programmed cell death. In contrast, microvesicles and exosomes are much
smaller in size and are generated by budding from the plasma membrane or are derived from the endo-lysosomal pathway, respectively. Furthermore, exosome biogenesis (Figure 2) differs from that of other EVs as the former is facilitated by the fusion of a multivesicular body (MVB) with the plasma membrane. Various published reviews (Colombo et al., 2013, 2014; Thery et al., 2002) have provided in-depth information on exosome biogenesis and hence will not be covered in this review.

Interest in exosome research has increased in the past few years mainly due to mounting evidence that implicates their dynamic role in immune activation, oncogenesis as well as cell death. In this review, we summarize recent progress in the study of exosomes as biomarkers, how exposure to environmental toxins alters the exosomal cargo and its relevance to various human disorders.

EXOSOME-MEDIATED INTERCELLULAR COMMUNICATION

Exosome involvement in cell-to-cell communication was first established by Dr Graça Raposo, who in 1996 demonstrated that B lymphoblastoid cells released exosomes containing MHC class II molecules, inducing antigen-specific, MHC class II restricted T cell responses (Raposo et al., 1996). This novel form of antigen presentation provided insight into the exosome’s role in immune cell activation and cell-to-cell transmission of biologically active components. The study was the first to implicate exosomes in antigen presentation in vivo. Since then, exosomes were primarily studied in immune cells in the context of antigen presentation, and were soon found to play an important role in the pathogenesis and progression of various diseases, including carcinomas, cardiovascular, neurodegenerative, and infectious diseases (Azmi et al., 2013; Graner et al., 2013; Hoshino et al., 2015; Rajendran et al., 2006).

Today, exosomes have been identified in all biological fluids, including blood, urine, saliva and cerebrospinal fluid (CSF), and their number and cargo are known to vary depending upon cell type and health status (eg, tumorigenic vs normal). Nonetheless, various in vitro and in vivo studies have demonstrated that exosomes contain a variety of biomolecules (eg, miRNAs, small RNAs, DNA as well as signaling peptides, and lipids), some of which have been implicated in creating a favorable microenvironment for neoplasia. Neoplastic cells can secrete factors, such as vascular endothelial growth factor and transforming growth factor β (TGF-β), that form blood vessels...
and produce mitogen, thus supporting the growth of newly transformed tumor cells (Challagundla et al., 2014; Massague, 2008). Importantly, the miRNA cargo can also modulate the gene expression of various biomolecules. For example, breast cancer cells secreting miR-200 are able to transform normal cells into neoplastic cells (Le et al., 2014). Similarly, miR-105 decreased the expression of tight junction protein-1, thus compromising the vascular-endothelial barrier and metastasizing neoplastic cells (Zhou et al., 2014). Metastasis is guided by the expression of specific integrins. The exosomal integrins α6β4 and α6β1 are associated with lung metastasis while αvβ5 is linked with liver metastasis (Hoshino et al., 2015). Integrins are activated by tetraspanins, such as Tspan8, CD9, CD81, and CD63, which are abundant in exosomes. In the case of prostate cancer cells, insulin-like growth factor-1, c-Src, and focal adhesion kinase are packaged in exosomes along with integrins and aid the proliferation and migration of the cells (DeRita et al., 2017). Exosomes can also contain Fasl and PD-L1, both of which promote the apoptosis of immune cells or aberrant surveillance, thus allowing tumors to grow undetected (Feng et al., 2011). The immunomodulatory effects of exosomes are best described in the context of parasitic infections. For example, when exosomes isolated from Leishmania donovani are added to leukocytes, inflammatory cytokine production is suppressed, which reprograms the leukocytes to a Th2 profile (Silverman et al., 2010). In fact, mice challenged with these exosomes prior to infection showed higher parasite titers compared with mice that had not been pre-exposed to the exosomes. In addition to disease-specific exosome cargo, exosomes contain certain membrane and cytosolic proteins commonly used as exosome-specific markers. These exosomal proteins belong to various functional groups, such as tetraspanins (CD9, CD63, and CD81), flotillins, integrins, heat shock proteins (HSC70 and HSC90), membrane transporters (GTPases), and lipid-bound proteins (Figure 3).

**ROLE OF EXOSOMES IN DISEASE PATHOGENESIS**

**Role of Environmental Toxicants in Exosome-mediated Carcinogenesis**

Cancer biology fuels most discoveries in exosome signaling. Simply put, “cancer” is a disease of abnormal and unregulated cell growth with the potential to spread to other parts of the body. Cancer is one of the leading causes of global mortality with an estimated 1.6 million newly diagnosed cases in 2016. One of the major questions in cancer biology is, “how do cancerous cells grow, metastasize and evade immune detection?” Cancerous cells release many signaling molecules that dampen the immune response to the unregulated cell growth as well as “recruit” naïve neighboring cells. This intercellular communication can occur via the release of exosomes that can carry a variety of biomolecules capable of binding to and activating or silencing downstream signaling pathways.
Exposure to tobacco smoke and air pollutants, such as asbestos, arsenic (As), radon, and soot, increases the risk of lung or bronchiolar carcinogenesis. Xu et al. (2015) induced human bronchial epithelial (HBE) cells to release miR-21, which in turn stimulated normal HBE cells to proliferate, simply by exposing the cells to 1 μM arsenite. Furthermore, these exosomes also increased the expression of phosphatase and tensin homolog, cementing the fact that exogenous miRNAs, when taken up by naïve cells via endocytosis of circulating exosomes, can function as endogenous miRNAs. Nicotine-derived nitrosamine ketone (NNK, or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) is a potent carcinogen present in tobacco smoke. Chronically exposing male rats to NNK down-regulated the tumor suppressor miR-206 (Wu et al., 2013), which is an effect also seen in metastatic lung cancer. Although not conclusively demonstrated, Wu et al. speculated that following exposure to NNK, exosomes derived from the tumor cells release biomolecules and other miRNAs that potentially down-regulate tumor suppressive factors including miR-206 and miR-133b. Other reports have shown that exosomes derived from highly metastatic lung cancer cells induced carcinogenesis in HBE cells by increasing vimentin expression and thus inducing the epithelial to mesenchymal transition (EMT) (Rahman et al., 2016) that serves as the basis for cancer propagation.

Exposure to ionizing radiation, principally seen in radiotherapy, can lead to genomic instability in distinct cell populations. Radiation of the tracheal mucosa can trigger invasive tracheal carcinomas, activating TGF-β, integrins and CXCL12 (Barcellos-Hoff et al., 1994; Mishra et al., 2008). This increase in growth factors and adhesion proteins leads to cellular remodeling and a microenvironment conducive to tumor metastasis. Similarly, exosomes derived from highly cancerous melanomas potentially re-program bone marrow progenitors by signaling through tyrosine kinase MET (Peinado et al., 2012). These reprogrammed cells now develop neoplastic behavior, thus propagating the cancer. In short, exposure to environmental toxins or carcinogens triggers genotoxic or mutagenic changes in susceptible cells, which in turn release exosomes containing miRNAs, integrins, cytokines or chemokines that modulate the microenvironment and potentiate tumorigenesis. Besides creating a favorable environment for cell metastasis, exosomes also play an important role in fostering chemo- and radiotherapy-resistant tumors. Exosomes produced by the irradiated head and neck cancer cell lines BHY and FaDu promoted the survival of irradiated recipient cells and increased the proliferation of nonirradiated recipient cells (Mutschelknaus et al., 2016).

The role of exosomes as important mediators of tumorigenesis can be further validated in other chemical-induced carcinogenesis. For example, media from arsenite-transformed human hepatic epithelial cells L-02, when added to normal L-02 or THLE-3 (liver cell lines) cells, induces the proinflammatory cytokines IL-6 and IL-8 and constitutively activates the STAT3 pathway.

Figure 3. Typical structure and content of exosomes. Depending on their cellular origin, exosomes can contain a diverse cargo. Thus exosomes isolated from immune cells, enterocytes and even melanocytes have the antigen-presenting receptors MHC Class I/II and scaffolding proteins such as tetraspanins. Since exosomes are a part of vesicular trafficking, various proteins involved in this pathway, such as Rab proteins, Annexins, and cytoskeletal proteins, constitute the exosomal payload. In addition, the cargo can also contain metabolic components such as enzymes, lipids, small carbohydrates as well as small RNAs and miRNAs.

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pathway, resulting in tumor progression (Chen et al., 2017). The authors found that this proinflammatory activation was mediated by exosomes. Media from arsenite-exposed L-02 cells, when depleted of exosomes, did not provoke inflammation in normal L-02 or THLE-3 cells. Moreover, among the various other biomolecules ferried by these nano-sized vesicles, miR-155 was also transferred to the normal liver cells where it specifically targeted and down-regulated the tumor suppressor gene QKI, activating NF-κB and promoting IL-6 and IL-8 production thus supporting oncogenesis.

The persistent use of platinum-containing drugs such as cis-diamminedichloroplatinum(II) (cisdiammine), cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin), and paclitaxel to treat ovarian cancer results in recurrences in 80% of cases due to acquired drug resistance. Resistant cancer cells have greater drug efflux potential, increased DNA damage repair and antioxidant properties, thus making them immune to chemotherapeutics (Stewart, 2007). To understand how these platinum-resistant cancer cells influence cancer progression, Crow et al. (2017) showed that a platinum-resistant epithelial ovarian cancer cell line released exosomes containing miR-21, which is postulated to promote EMT, an important step in cancer progression. Notably, these platinum-resistant cells had somatic mutations in SMAD4—a transcription factor that is involved in intracellular communication and EMT (Pohl et al., 2010). The authors hypothesized that exosomal miR-21 could increase the chances of or even induce somatic mutations in the SMAD4 gene in the presence of platinum-containing drugs, thus increasing cell survival and promoting cancer progression. Early life exposure to endocrine-disrupting compounds such as diethylstilbestrol and genistein, especially during reproductive tract development, could lead to the development of uterine fibroids (tumors arising from the myometrium and tumor stem cells) in later life. Yang et al. (2016) hypothesized that tumor stem cells release exosomes encapsulating important effectors of Wnt, Hedgehog and β-catenin, which are signaling factors vital for maintaining cell proliferation.

Advancements in nanotechnology are increasingly being applied in consumer product manufacturing, drug delivery, prosthetics, efficient fuel batteries, etc. (Brenza et al., 2016; Yang et al., 2017; Zhang et al., 2012). However, studies assessing the environmental impact of nanomaterials, their possible bioaccumulation and associated toxicities are not keeping pace. For instance, lead sulfide quantum dots (PbS-QD) have been proposed for biomedical imaging. By transferring energy, these nanoparticles can fluoresce in the far-red range and hence are valuable bio-imagers. However, the same energy transfer can also generate reactive oxygen species leading to oxidative stress and cell death. Human embryonic kidney cells responded to PbS-QD exposure by releasing altered exosomal cargo containing markers of DNA damage as well as factors promoting inflammation, including p53 as well as IL-8 and CXCL5, which are potent activators of neutrophil chemotaxis, and thus inflammation at the site of QD accumulation (Kim et al., 2015).

The link between chronic inflammation and cancer has been fairly well established (Chai et al., 2015), and the potential proinflammatory role of exosomes is also emerging. For instance, Levanen et al. (2013) showed that the exosomal cargo isolated from bronchoalveolar lavage from healthy controls and patients suffering from intermittent asthma, a chronic inflammatory condition of the lungs, was strikingly different in composition. The exosomal miRNAs isolated from intermittent asthma patients transcriptionally regulate the production of factors such as IL-13, IL-10, IL-6, and IL-8 that are known mediators of airway obstruction and immune cell infiltration. Thus, asthmatic lung epithelial cells are primed for immune cell infiltration and inflammation following exposure to particulate matter. IL-13 plays a critical role in the initiation and progression of asthma attacks. IL-13 and IL-12 in turn are transcriptionally regulated in part by miR-21 (Liu et al., 2009). Down-regulation of this miRNA can result in an exaggerated production of these interleukins when the lung epithelia encounter even small amounts of particulate pollutants. Interestingly, it has been observed that miR-21 expression positively correlates with increased resistance of non-small cell lung cancer against epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor treatment (Li et al., 2014). Cigarette smoke is a well-known carcinogen. Continued exposure to cigarette smoke can induce broncho-alveolar macrophages to shed exosomes and other microvesicles that in turn induce the epithelia to produce proinflammatory cytokines (Cordazzo et al., 2014). Exposure to cigarette smoke is also positively correlated to the overexpression of miRNAs that affect EGFR function, thus driving proliferation of epithelial cells and initiating lung carcinomas (Goldkorn and Filosto, 2010).

Thus, different components of the exosomal cargo have specific functions that aid in deregulating immune surveillance and promoting the tumor growth niche leading to malignant and resistant tumors.

### Exosomes in Neurological Disorders

Exosomes play an important role in brain development and physiology of the central nervous system, which requires precise orchestration of cellular events through the coordinated information exchange between distally located cells. Nevertheless, exosomes are often identified as the “Trojan horse of neurodegeneration” (Chidoni et al., 2008) because their cargo could contain potentially pathogenic proteins. Importantly, a variety of genetic and environmental factors can stimulate the release of exosomes and their composition. Given that environmental toxicants such as metals and pesticides, as well as traumatic brain injury (TBI) play a particularly significant role in the onset of neurodegenerative diseases (Berry et al., 2010; Gardner et al., 2015; Harischandra et al., 2015a; Rokad et al., 2016), here we focus on recent literature on the role of environmental factors in exosome biogenesis and disease progression.

Neurodegenerative disorders, including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), and prion diseases, share common cellular pathological features such as aggregation of disease-specific proteins (Rosa and Poirier, 2004). These diseases are also described as protein misfolding diseases where, in every instance, the native form of β-amyloid (Aβ), tau, α-synuclein (αSyn), Huntingtin, or prion protein (PrP) acquires an abnormal β-sheet structure that is absent under normal conditions. Recent experimental evidence indicates that these proteins also share prion-like behavior as abnormally folded proteins interact with each other to form cross-β species that assemble into a “nucleus”—an ordered aggregate possessing the ability of selfpropagation (Nelson et al., 2005). However, unlike classical prion diseases, these “prionoids” (Aguzzi and Lakkaraju, 2016) proteins have yet to be documented as transmissible between individuals. A wealth of evidence supports the theory that the exosomal pathway may be exploited for the cell-to-cell transmission of prionoids. Furthermore, pathogenic forms of Aβ, αSyn, and PrPSc have been identified in exosomes, highlighting the prospect of a potential intracellular spread of...
these misfolded proteins (Danzer et al., 2012; Emmanouilidou et al., 2010; Guo et al., 2016; Rajendran et al., 2006).

The idea that exosomes are involved in the intercellular dissemination of aggregated αSyn gained considerable attention when pathogenic αSyn species were discovered in CSF exosomes from patients with PD and dementia with Lewy bodies, both of which are complex and multifaceted disorders whose etiology is not fully understood. Evidence for gene-environment interactions triggering neurodegenerative disorders is provided by the juvenile-onset Parkinsonism-related gene ATP13A2/PARK9 (Gillier et al., 2009; Santoro et al., 2011). Although ATP13A2’s exact function is unknown, it belongs to a large group of lysosomal transport proteins in the 5-P type ATPase family involved in transporting multiple cations (eg, manganese [Mn], zinc, cadmium, selenium) from the cytosol to the lysosomal lumen (Kong et al., 2014; Schmidt et al., 2009). Importantly, overexpression of ATP13A2/PARK9 increases αSyn secretion through exosomes in h4 cells and mouse primary cortical cells (Tsunemi et al., 2014) Furthermore, Rentschler et al. (2012) showed that ATP13A2/PARK9 polymorphisms influence the neurotoxic effects of Mn in humans. However, given the possibility that neurons can pick up exosomes carrying toxic protein cargo capable of triggering their degeneration, whether exosomal release of αSyn is a cellular protective adaptation or solely pathological has yet to be defined.

Given the multifactorial etiology of PD and other neurodegenerative diseases, considerable effort has been devoted to studying chronic exposure to neurotoxic metals, such as copper (Cu), lead, mercury, Mn, cadmium, and As, and their potential for promoting protein aggregation (Goldman, 2014). For instance, transition metals such as Mn are known to increase αSyn expression (Cai et al., 2010; Peres et al., 2016) and the exosomal release of αSyn (Harischandra et al., 2015b). Since Mn exposure triggers transient or sustained increases of intracellular calcium levels (Xu et al., 2009), which has been linked to the rapid secretion of exosomes in neuronal cells (Emmanouilidou et al., 2010), this may provide mechanistic insight into how environmental neurotoxicants regulate exosome biogenesis and exosomal αSyn release. Importantly, αSyn-containing exosomes have been shown to provide a catalytic environment for the structural conversion of PrP protein, leading to its aggregation into PrPSc, implying an environmental source for the infectious agent. The existence of sporadic forms of prion diseases, such as scrapie, implies an environmental source for the infectious agent. Indeed, several transition metals are known to bind PrP, and metal-ion occupancy of PrP plays a pivotal role in the pathogenesis of prion diseases (Choi et al., 2001). The structural conversion of PrP protein, leading to its aggregation and toxicity. Importantly, PrP is one of the most commonly identified cargo proteins in exosomes (Fevrier et al., 2004), which can aid in the horizontal transmission of pathologic prion protein (PrPSc) (Liu et al., 2016). A recent study shows that stimulating the release of exosomes increases intercellular transfer of prions (Guo et al., 2016), highlighting an integral role for exosomes in facilitating the unique transmissible nature of prions.
Table 1. Environmental Toxicant-induced Exosomal Release and Associated Disease Pathogenesis

<table>
<thead>
<tr>
<th>Environmental Toxicants</th>
<th>Disease Pathogenesis</th>
<th>Exosome Cargo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenite</td>
<td>Lung carcinogenesis, liver carcinogenesis</td>
<td>miR-21, miR-155</td>
<td>Xu et al. (2015), Chen et al. (2017)</td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>Tracheal carcinoma</td>
<td>miRNAs, integrins and chemokines</td>
<td>Mutschelknaus et al. (2016)</td>
</tr>
<tr>
<td>Diethylstilbestrol, genistein</td>
<td>Uterine fibroids, ovarian cancer</td>
<td>Effectors of Wnt, Hedgehog and β-catenin</td>
<td>Yang et al. (2016)</td>
</tr>
<tr>
<td>Cigarette smoke</td>
<td>Lung carcinoma, AD</td>
<td>miR-21, IL-13, mediators of Wnt/β-catenin pathway, CNN1</td>
<td>Wang et al. (2015), Moon et al. (2014)</td>
</tr>
<tr>
<td>Mn</td>
<td>PD, Synucleopathies, other neurodegenerative disease</td>
<td>&gt; Syn</td>
<td>Harischandra et al. (2015b)</td>
</tr>
<tr>
<td>Monensin</td>
<td>Prion disease</td>
<td>PrP, &gt; Syn</td>
<td>Guo, Bellingham and Hill (2016)</td>
</tr>
<tr>
<td>Rotenone</td>
<td>PD, Synucleopathies, other neurodegenerative disease</td>
<td>&gt; Syn</td>
<td>Pan-Montojo et al. (2012)</td>
</tr>
<tr>
<td>Parauquat</td>
<td>Amyotrophic lateral sclerosis</td>
<td>TDP-43, Cu/Zn SOD</td>
<td>Grad et al. (2014)</td>
</tr>
<tr>
<td>Pyridostigmine bromide,</td>
<td>GWI</td>
<td>rno-piR-007899, rno-piR-019162</td>
<td>Pierce et al. (2016)</td>
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<tr>
<td>Permethrin</td>
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Furthermore, exosomes are known to shuttle many miRNAs between donor and recipient cells and regulate prion expression. The small RNA miR-146a increases during metal-induced neurotoxicity and oxidative stress conditions and has been identified in rare human prion diseases, including sporadic Creutzfeldt-Jakob disease and Gerstmann-Straussler-Scheinker syndrome (Lukiew et al., 2011).

ALS is another fatal, progressive neurodegenerative disease affecting predominantly motor neurons in the spinal cord and motor cortex. Previous studies have shown that environmental toxicants can alter superoxide dismutase and TAR-DNA-binding protein (TDP)-43 expression, both of which are associated with a subset of ALS cases. In addition, TDP-43 has been linked to sporadic and familial frontotemporal lobar degeneration (FTLD), resulting in a proteinopathy phenotype in the frontal and anterior temporal lobes of the brain. Interestingly, paraquat exposure induces caspase-dependent accumulation of TDP-43 fragments (Meyerowitz et al., 2011), which causes inclusion formation and cellular toxicity (Zhang et al., 2009). Given the previous reports on TDP-43 enrichment in exosomes isolated from TDP-43-expressing neuroblastoma cells, exosomes extracted from the CSF of patients with ALS and/or FTLD provide evidence of their possible role in propagating TDP-43 between cells (Nonaka et al., 2013; Thompson et al., 2016). Furthermore, the transmission of misfolded wild-type Cu/Zn SOD between cells seems to be mediated through exosomal release and uptake of protein aggregates (Grad et al., 2014), providing further evidence of exosome-mediated, prion-like intercellular transmission of misfolded proteins.

TBI is another example of how environmental factors and lifestyle can influence the development of neurodegenerative diseases later in life. Head injury is a signature injury in boxing, football and military combat (Bahrami et al., 2011; Mac Donald et al., 2011). Researchers have made a strong case linking TBI and neurological ailments such as AD, PD, and general dementia. One such study conducted with 7130 participants identified 117 cases of PD and 865 individuals with a history of TBI (Crane et al., 2016), and another study found that middle-to-older aged patients diagnosed with trauma have a 44% increased risk of being diagnosed with PD (Gardner et al., 2015). Furthermore, an increase in circulating exosomes in the blood of TBI patients following injury has been reported (Graner et al., 2013; Taylor and Gercel-Taylor, 2014). Similarly, increased exosomes and altered cargo have been identified in cultured cortical neurons in a stretch injury model of mild TBI (Ko et al., 2016). Collectively, such findings suggest that exosomes represent a potential biomarker and diagnostic tool for TBI. A study performed by Zhang et al. (2015) demonstrated that exosomes isolated from mesenchymal stem cells significantly increased the number of endothelial cells at the site of lesion thus restoring the blood-brain barrier in a rodent model of TBI. Although the specific exosomal content that improved endothelial cell function and immature neuron proliferation was not identified, it is posited that the mesenchymal stem cells released growth factors via EVs that in turn stimulated neurogenesis and improved memory in the rats following TBI.

Gulf War Illness (GWI) is a chronic multi-symptom neurological disorder comprising cognitive dysfunction, tremors and psychological disturbance in approximately 25% of veterans who were deployed to the Persian Gulf War, 1990–1991 (Parihar et al., 2013). Although the precise cause of GWI is unknown, combined exposure to the nerve gas drug pyridostigmine bromide and the pesticides DEET and permethrin has been proposed as one of the foremost causes (Parihar et al., 2013). The role of pesticide exposure in epigenetic alterations and gene expression changes is well established (Jin et al., 2014; Song et al., 2011), and GWI toxicants were recently shown to induce epigenetic changes in a rat model of GWI (Pierce et al., 2016). Here, exosome RNA-seq analysis of circulating rat exosomes identified GWI-induced changes in 2 rat exosomal piRNAs (an 8.2-fold upregulation of rno-piR-007899 and a 7.2-fold downregulation of rno-piR-019162), demonstrating a role for environmental chemical exposure in exosomal small RNA cargo changes (Pierce et al., 2016). This particularly interesting effect could be exploited for reliable quantitative biomarkers in diagnosing these neurological diseases.

FUTURE DIRECTIONS

In summary, there is a small but significant pool of research that elucidates the role of toxins and exosomes in carcinogenesis or neurodegeneration (Table 1). With the capability of
transferring various biomolecules, including miRNAs, lipids, and signaling peptides, over long distances, exosomes have become an attractive research focus for understanding disease progression, for biomarker discovery, and even as possible cell-based, therapeutic delivery platforms. Three reasons why exosomes show tremendous potential in the field of nanomedicines are (1) exosomes can cross the blood-brain barrier to deliver therapeutic drugs or antioxidants, (2) exosome membranes are rich in sphingomyelin, cholesterol and glycerophospholipids with long, saturated fatty acyl chains and phosphatidylyserine, which increases their stability and potentially favors their uptake, thus making them effective drug delivery vehicles, and (3) exosomes derived from the same species typically should have only minimal immunogenicity, thus reducing the potential risk of an immune response. However, the challenge here is to develop target-specific exosomes. Also, despite the abundant literature citing the potential diagnostic or therapeutic importance of these nanovesicles, it is premature to correlate changes in exosomal cargo with a disease state given the paucity of human studies validating results seen in preclinical experiments. Thus, it is imperative to understand the functional differences between cell type-specific and disease-related exosomal contents. As summarized in this review of recent reports, chronic exposure to environmental toxins can stimulate exosome biogenesis and subsequent disease pathology. In many cases, the exosomes isolated from targeted patient populations contain altered cargo. However, the links between toxin exposure, altered exosomal content and disease progression are not clearly understood. Hence, future studies need to focus on understanding how environmental toxins tweak the biochemical pathways involved in selecting cellular material as exosome cargo and on how released cargo reprograms the cellular microenvironment leading to disease progression.

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