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# Exosomal RNAs in diagnosis and therapies

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ABSTRACT

The field of extracellular vesicles has been rapidly developing after it became evident that a defined subset of vesicles, called exosomes, can modulate several biological functions in distant cells and tissues. Exosomes range in a size from 40 to 160 nm in diameter, are released by majority of cells in our body, and carry molecules which reflect the cell of origin. The types of biomolecules packed, their respective purpose, and their impact on the physiological state of distinct cells and tissues should be understood to advance the using of exosomes as biomarkers of health and disease. Many of such physiological effects can be linked to exosomal RNA molecules which include both coding and non-coding RNAs. The biological role(s) of various exosomal RNAs have started being recognized after RNA sequencing methods became widely available which led to discovery of a variety of RNA molecules in exosomes and their roles in regulating of many biological processes are beginning to be unraveled. In present review, we outline and discuss recent progress in the elucidation of the various biological processes driven by exosomal RNA and their relevance for several major conditions including disorders of central nervous system, cardiovascular system, metabolism, cancer, and immune system. Furthermore, we also discuss potential use of exosomes as valuable therapeutics for tissue regeneration and for conditions resulting from excessive inflammation. While exosome research is still in its infancy, in-depth understanding of exosome formation, their biological effects, and specific cell-targeting will uncover how they can be used as disease biomarkers and therapeutics.

## 1. Introduction

Extracellular vesicles (EVs) are a diverse group of cell-derived membranous structures secreted by both prokaryotic and eukaryotic cells as part of their normal physiology. They are formed during invagination of cellular plasma membrane resulting in the formation of multivesicular bodies [1]. The latter can subsequently intersect with other intracellular vesicles and organelles, yielding great diversity in their composition [2]. EVs can be broadly categorized into ectosomes and exosomes. Ectosomes are EVs that pinch off the surface of the plasma membrane via outward budding and include microvesicles, microparticles, and large vesicles with sizes ranging from 50 nm to 1 mm [1]. Exosomes are EVs with a size ranging from 40 to 160 nm in diameter with an endosomal origin [3]. Secreted exosomes are primarily made of DNA, RNA, lipids, metabolites, and cytosolic and cell-surface proteins which are all indicative of cells they originate from [4]. Subsequently, exosomes are released into the extracellular space where they are taken up by other cells [5]. Therefore, exosomes serve as an additional mediator of intercellular communication, facilitating both short and long-distance communication between cells and tissues (Figs. 1 and 2). By delineating the RNA, DNA, and protein composition of exosomes which are reflective of the cell of origin, it is possible to both identify biomarkers of pathological conditions and to design therapeutic interventions.

Because exosomes are secreted by all cells, they can be found in many biological fluids such as blood, urine, cerebrospinal fluid, breast milk, ascites fluid, amniotic fluid, bile, semen, saliva, and sputum [6]. Because they originate from various cells/tissues, there is an abundance of diversity in both exosome composition and in their biological functions. Multiple roles have been studied and attributed to exosomes, including the elimination of obsolete molecules, facilitation of the immune response, antigen presentation, apoptosis, inflammation,

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angiogenesis, coagulation, dissemination of oncogene from tumor cells, and the spread of pathogens such as viruses from one cell to another (reviewed in Ref. [7]). Because exosomes can deliver various RNAs and proteins to adjacent cells, they facilitate cell-cell communication and signaling.

Exosomes were first discovered in sheep reticulocytes, and subsequently found to be secreted by other mammalian cell types. Initially, exosomes were considered vehicles for abandoned membrane parcels and molecular fragments [8]. However, exosomes were subsequently found to have a role in B lymphocyte antigen presentation which indicated their specific role in the immune response [9]. Following a series of investigations on the immune response, a landmark finding in the 2010s demonstrated that miRNA and mRNA can be transported in exosomes. This discovery evolved into a newfound function of exosomes: the vesicles serve as communication mediators between cells by transducing signals between cells [10]. With an especially important role in cellular communication, re-encoding genes of target cells and contributing to various disease pathologies, it is now understood that exosomes share a critical function in physiological and pathological processes, including immune surveillance, inflammation, tumorigenesis, and drug resistance [10].

Due to their role(s) in information exchange between cells resulting in modulation of cellular functions, exosomes possess capacity to serve as both diagnostic biomarkers and therapeutic interventions [11]. While an abundance of information exists for the biological roles of exosomal proteins, less is known about the use of exosomal RNAs for either diagnostic or therapeutic purposes. The purpose of this manuscript is to explore functions of RNA molecules delivered by exosomes and the potential clinical applications of such RNA molecules as either biomarkers or therapeutics.

As noted above, exosomes contain a mixture of genetic material (DNA and RNA), proteins, and lipids. Their composition is determined by the physiological state and stimuli of the cells at the tissue of origin at the time of exosome production. Exosomal RNA is a mixture of coding and noncoding RNA and is also dependent on the microenvironment of the secreting cells, allowing the specific material to be indicative of the cell or cellular system of origin [5]. Though the underlying mechanisms as to how RNA is loaded into exosomes is still unknown, a few RNA-binding proteins have been shown to possess a capacity to selectively bind RNA molecules through specific motifs, and to deliver them into intracellular vesicles.

The intent of this manuscript is to review several extensively studied roles through which exosomes modulate biological processes, laying clear the fundamental deficiencies in understanding and areas for future investigation, and emphasizing the critical importance of continued investigation into exosomal research. This article has been framed to direct readers to several recently published comprehensive reviews in various neoplastic disease.

We review here some key roles of exosomes in modulating biological processes with a particular emphasis on roles of RNA molecules that are delivered via exosomes.

## 2. The profile and sorting of RNA into exosomes

To evaluate the potential of exosomal RNA as diagnostic tools and therapeutics, it is necessary to understand the roles of such RNA molecules in the context of infectious diseases, transplant medicine, fetal monitoring, cancer, and a range of other areas (Table 1). As a dynamic and diverse biomolecule with numerous essential roles in biological processes, RNA-based measurements have tremendous clinical potential to provide insight on the onset and progression of diseases. Specifically, it should be noted that modern RNA sequencing methods allow for the detection of a great variety of RNA species and provide a historically unmatched opportunity to quantify known, pre-defined RNA species and rare RNA transcript variants within a sample. These sensitive methods allow RNA to be leveraged as a biomarker, providing an insight into the biochemical reactions and protein syntheses taking place within defined biological compartments. Moreover, circulating RNAs and small regulatory RNAs, such as microRNAs, are stable and currently undergoing rigorous investigation as biomarkers [12]. As an integral component of exosomes, it is likely that investigation into exosomes generally, as well as exosomal RNA, more specifically, will provide critical insight into the mechanism by which a diseased state progresses (Table 1). Byron et al. describe in thorough detail the various RNA species, their general

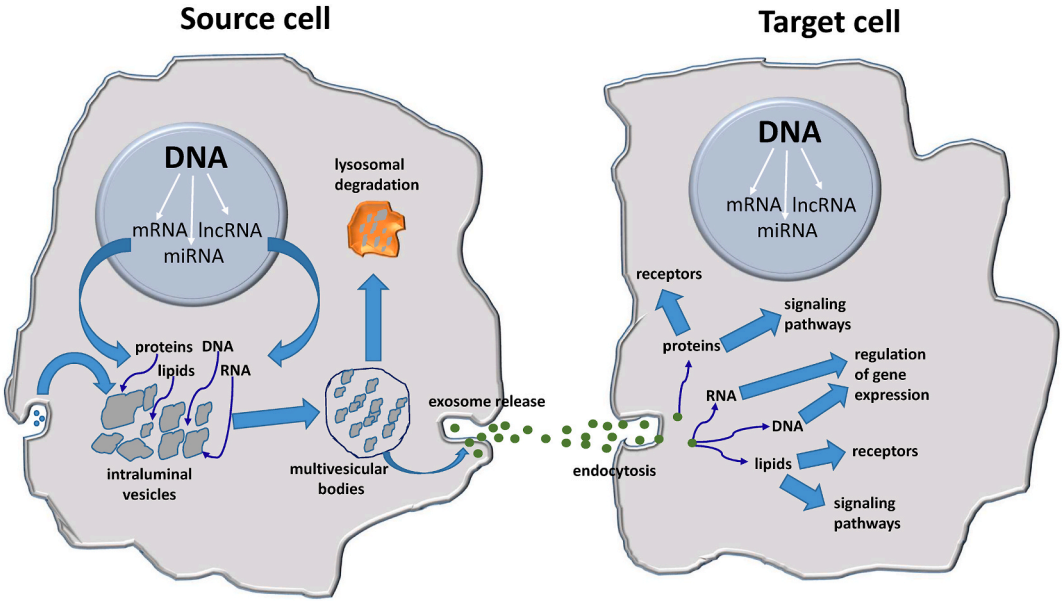


**Fig. 1.** Schematic representation of the biogenesis of exosomes and their effects on target cells: Exosomes are formed during the invagination of the endocytic membrane resulting in formation of cytoplasmic intraluminal vesicles. Nucleic acids, proteins and lipids are subsequently incorporated into such vesicles and their maturation gives rise to multivesicular bodies (MBs). MBs can be recycled, delivered to lysosomes for degradation, or they can fuse with the plasma membrane and release exosomes into the extracellular space. Exosomal cargoes from the source cell can be further delivered to target cells resulting in the modulation of target cell signaling, gene expression and/or immune response.

characteristics, and their potential clinical applications – while the RNA species described in this manuscript are more specifically tailored to certain conditions, they remain rooted in similar clinical applications respective to each species [13].

Valadi et al. first reported abundance of both messenger (mRNA) and micro RNA (miRNA) in exosomes, with little or no 18S and 28S ribosomal RNA present [14]. Subsequent studies have also identified long non-coding RNA (lncRNA) and circular RNA (cirRNA) in exosomes [15]. According to ExoCarta, an internet exosome database (http://www.exocarta.org/), 286 studies identified 2838 miRNAs and 3408 mRNAs, respectively (June 2021). In addition to miRNAs and mRNAs, exosomes have also been documented to contain rRNAs, tRNAs, small Cajal body-specific RNAs (scaRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), and piwi-interacting RNA (piRNA) from a previous investigation that assessed the exosomal content of exosomes derived from serum, plasma, urine, and Cerebrospinal Fluid [16]. Recently, exoRBase, a database developed by Fudan University Shanghai Cancer Center collected and characterized RNA species in human blood exosomes, and through RNA-sequencing data analyses and experimental findings from other literature, they detected 58,330 cirRNAs, 15,501 lncRNAs, and 18,333 mRNAs [17]. In a different study, exosomes were collected from patients diagnosed with 17 different diseases and, using RNA sequencing, over 1000 miRNAs specific for each subject and disease were detected [17,18]. A database they developed, EVmiRNA, provides information on miRNA expression profile of EVs including exosomes from different cells from lymphoma exosomes, and while total of 394 miRNAs were detected, only 23 miRNAs were specific for such exosomes [17]. It should be noted that lncRNA and cirRNA are yet to be thoroughly investigated in lymphoma derived exosomes.

The total levels of mRNA, miRNA, and lncRNA differ between exosomes and their cells of origin, indicating that the cytoplasmic RNA incorporation into secreted vesicles involves a sorting mechanism [14]. While the latter is still under investigation, the discovery of EXOmotifs within RNA has provided insight on this process [19]. EXOmotifs are short, specific sequences in exosomal RNA that can bind to RNA-binding proteins (RBPs), such as hnRNPA2B1, hnRNPA1, YBX1, or SYNCRIP; they control the sorting of mRNA, lncRNA, and small interfering RNA (siRNA) into exosomes via binding to the specific sequence motifs present in exosomal shuttle RNA, a long non-coding and circular type of RNA shown to transfer ribonucleic acids [20]. Therefore, EXOmotifs and RNA-binding proteins share a critical function in the loading of exosomal RNA.

Because exosomal RNA is involved in regulating a variety of functions, it is not surprising that the RNA content and protein content largely differ among exosomes from different sources.

## 3. Exosome uptake

As mentioned above, exosomes carry diverse cargo molecules including lipids, proteins, and nucleic acids, each of which mediate intercellular communication by catalyzing a range of biological responses by acceptor cells. Once secreted, exosomes are released into the extracellular space and incorporated into bodily fluids [1]. Recipient cells then uptake exosomes through various processes: they may enter such cells through phagocytosis/endocytosis, or fuse with the plasma membrane while simultaneously releasing molecular cargo. This process is facilitated by surface proteins displayed on exosomes and recipient cells, including integrins, lectins/proteoglycans, and T Cell Immunoglobulins [21]. These proteins ensure that recognition of the recipient cell takes place, allowing protein-protein interactions to occur through the ligands on the cell surface [21]. This general process is subject to different forms of internalization and intracellular signal cascades depending on the exosome released and its respective function. Specific protein-protein interactions also facilitate receptor-mediated endocytosis (RME) of exosomes, offering additional targets for therapeutic manipulation.

Identification of receptors responsible for uptake of exosomes is work in progress although some of the well-described endocytosis receptors such as low-density lipoprotein receptor and transferrin receptor also mediate uptake of exosomes [22]. Receptors that selectively facilitate exosomal internalization include C-type lectin receptor, siglec-1, siglec-3, cadherin 11, lymphocyte function-associated antigen 1, vascular cell adhesion molecule 1, integrin α6β4, integrin αvβ5, p-selectin, tetraspanin-29, tetraspanin-28, tetraspanin-8, heparan sulfate proteoglycan 2, and T cell immunoglobulin and mucin domain containing 1 and 4, each of which facilitate direct uptake, and epidermal growth factor receptor, which facilitates indirect uptake of exosomes [5,23–25]. The receptors that mediate protein-protein interactions involved in exosomal uptake include lectins such as C-type/selectin, (sialic-acid-binding immunoglobulin-like lectins) siglecs, galectins, adhesion molecules such as cadherins, selectins, mucins, integrins, and immunoglobulins, heparan sulfate proteoglycans, T cell

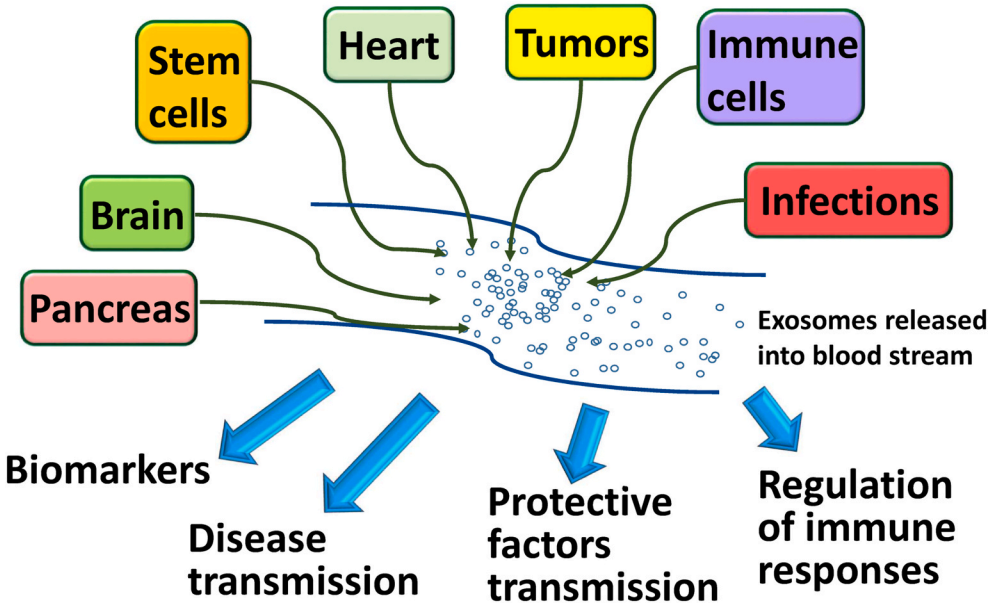


**Fig. 2.** Pathologic function and therapeutic potential of exosomes secreted form various tissues. Multiple cell types can yield exosomes and release them into surrounding tissues and ultimately into blood stream. These exosomes can be taken up by various distant cells leading to changes in signaling of the later. Importantly, biochemical composition of secreted exosomes depends on types of cells secreting them, their stress levels, apoptosis/necrosis, inflammatory response and other physiological states that can be used for both diagnostic and therapeutic purposes.

**Table 1**
Summary of the characteristics and clinical applications of various RNA species found in exosomes.

| RNA Species | Description | Potential Clinical Application |
|---|---|---|
| miRNA | miRNAs are ~18–24 nucleotides in length and represent the most extensively characterized group of small ncRNAs having activity in gene repression. | miRNAs are being pursued as potential biomarkers in a broad spectrum of diseases, from cancer to Alzheimer disease to cardiovascular disease. A microarray-based miRNA test is currently available for use in characterizing cancer origin [103]. |
| piRNA | piRNAs are ~26–32 nucleotides in length, with functions in transposon repression and maintenance of germline genome integrity. | piRNAs have been implicated in cancer, with an initial study demonstrating an association between increased expression of piRNA and poor prognosis in soft-tissue sarcomas [104]. |
| snRNA | snRNAs are ~100–300 nucleotides in length, localized to the nucleus, with functions in RNA processing and splicing. | Circulating levels of U2 snRNA fragments (RNU2-1f) have been proposed as potential diagnostic biomarkers in various tumour types, including pancreatic cancer and colorectal cancer [105]. |
| tRNA | tRNAs help with translation of mRNA to protein. tRNAs are highly structured and have many modifications to bases, making them difficult to sequence through. | Recent evidence suggests that tRNA fragments are cleaved in the presence of hypoxic or other stressful conditions. They can, in some cases, act as decoys for RNA binding proteins, causing destabilization of other transcripts [106]. |
| circRNA | circRNAs are lncRNAs that contain a covalent bond between the 5 and 3 end, resulting in a continuous circular loop. circRNAs can act as miRNA sponges and regulators of splicing and transcription. | Although little is known about the association of circRNAs with disease, initial studies are exploring circRNA levels as potential biomarkers in cancer; a recent study showed an association between reduced levels of a specific circRNA (hsa_circ_002059) in gastric tumors compared to adjacent non-tumour tissue [107]. |
| lncRNA | lncRNAs represent the category of ncRNAs that are greater than 200 nucleotides in length and function to regulate gene expression. | lncRNAs have been associated with cancer prognosis, with potential utility as biomarkers in cancer. Tests such as ExoIntelliScore Prostate include lncRNA as a biomarker [108]. |
| snoRNA | snoRNAs have two main classes, box C/D snoRNAs, ~60–90 nucleotides in length, and box H/ACA snoRNAs, ~120–140 nucleotides. snoRNAs play a key role in ribosome biogenesis and rRNA modifications. | Levels of snoRNA and/or their functional fragments have been proposed as potential clinical diagnostic measures, with applications being pursued in fields such as cancer and neurodegenerative disorders. Two snoRNAs were recently identified in sputum samples and shown to have potential use as diagnostic biomarkers in lung cancer [109]. |

immunoglobulin mucin domain-containing molecules, and epidermal growth factor receptor [21]. Though these proteins have been linked to exosome internalization, it is presently unclear which additional mechanisms may contribute to internalization and whether different cell types preferentially utilize different internalization mechanisms. Moreover, it has been suggested that exosomes may utilize several mechanisms in the same cell at varying times, though additional research is needed to determine the link between uptake mechanisms and phenotypic changes in the recipient cell. It is also yet to be elucidated whether these mechanisms are cell-cycle-dependent, cell type, or simple opportunistic processes of the extracellular vesicles [26].

## 4. Exosomes as diagnostic tools

### 4.1. The central nervous system disorders

Cellular communication via exosomes plays a major role in progression of central nervous system diseases such as Alzheimer's disease (AD), Parkinson's Disease (PD), and Huntington's Disease (HD) [27]. Therefore, exosomes are currently being evaluated as prognostic indicators for traumatic brain injury, stroke, and other neurodegenerative diseases [28,29]. Exosomes are found both in cerebrospinal fluid and in peripheral fluids, and their protein and RNA contents may undergo alterations in the years or even months leading up to the official prognosis of these diseases, allowing them to serve as potential diagnostic tools. Specifically, these exosomes leave the blood brain barrier to join peripheral circulation, thus can be detected to monitor disease progression and/or to enable early diagnosis [27].

In the context of AD, promising results were obtained when levels of exosomal RNAs were examined in individuals diagnosed with the disease. For diagnostic purposes, comparison of miRNA-135a, miRNA-193b, and miRNA-384 levels in serum-derived exosomes from individuals with AD dementia, PD dementia, and vascular dementia, resulted in conclusion that the levels of miRNA-384 can be used to differentiate between the three dementias. Moreover, miRNA-384, miRNA-193b, and miRNA-135a appeared to be effective indicators for early diagnosis of AD [30]. These findings indicate that exosomal miRNAs can serve as potential biomarkers for the diagnosis of AD, providing a new tool for disease identification and prevention.

Parkinson's Disease (PD) is the second most common neurodegenerative disease and exosomes have been shown to both contribute to its pathology and to be neuroprotective by contributing to elimination of misfolded proteins. For example, results from several studies indicated that exosomes transmit toxic (misfolded) α-synuclein between cells, contributing to the pathological stages of PD [31]. The results from a different study which examined exosomes from PD patients indicated that exosomes can weaken the neuronal stress response by causing cellular apoptosis [32]. Exosomes were also shown to potentially contribute to neuroprotection due to their ability to eliminate misfolded proteins [33]. In terms of potential biomarkers for PD, 13 miRNAs were identified in Cerebrospinal Fluid derived exosomes of PD patients, each of which target genes included in the KEGG pathway "Dopaminergic synapse"; another 9 miRNAs targeting 41 genes in the Dopaminergic synapse pathway and the KEGG pathway "Cholinergic synapse" which was significantly enhanced among PD patients via 11 miRNAs targeting 40 genes in the Cholinergic synapse pathway [34–37]. Comparing these with miRNA concentrations in AD patients, the reliability of exosomal RNA molecules as biomarkers to distinguish PD and AD was supported, proving that exosomes can be utilized in diagnosis of these three diseases.

Huntington's Disease (HD) is characterized by abnormal amplification of CAG repeats in the Huntington gene, leading to accumulation of mutant Huntingtin protein (mHTT) [38]. While it has been documented that mHTT can be transmitted across the nervous system via tunneling nanotubes or vesicle mechanisms, there is no evidence suggesting that mHTT can be transported via exosomes during *trans*-nervous transmission [39,40]. However, when HTT-exon 1 polyQ-GFP construct was overexpressed in 293T cells, the secreted exosomes contained both polyQ-GFP protein and amplified RNA. Furthermore, these exosomes were taken up by striatal mouse neurons, leading to an increase in polyQ-GFP RNA in cells [41]. This demonstrated that exosomes also have a high potential to deliver toxic amplified trinucleotide repetitive RNA and it also indicated that they can eliminate polyQ proteins from cells to reduce their accumulation in the brain. Moreover, these amplified RNAs in HD patients' body fluids may be a reliable biomarker for monitoring disease progression.

### 4.2. The cardiovascular system disorders

As with the central nervous system, the cardiovascular system is highly dependent on exosomes for homeostasis and these vesicles are released by cardiomyocytes (CMs) constitutively [42]. In a hypoxic environment, such as the one seen in arterial blockage, the release of these vesicles is tripled, with a concurrent change in exosomal content [42]. For example, the pro-inflammatory cytokine TNF-α is not normally produced within the heart tissue. However, during hypoxia, TNFα is found in exosomes produced by cardiomyocytes indicating these cells are capable of producing it under stress to induce apoptosis in neighboring healthy cells [43]. This indicates that the release of exosomes is a mechanism in which stressed cells share a capacity to respond to a pro-inflammatory event. Additionally, DNA and RNA can be transferred between different types of cardiac cells to alter gene expression within recipient cells, including cardiomyocytes and fibroblasts, suggesting that exosomes serve in communication between different types of cells in heart tissue [44].

MiRNAs are the most investigated RNA species contained in exosomes for their function as new biomarkers in cardiovascular diseases [45]. For example, two cardiac-specific miRNAs, hsa-miRNA-1 and hsa-miRNA-133a, were found to be upregulated in exosomes isolated from serum of patients with acute coronary syndrome (ACS) [46]. In addition to ACS, rapid diagnosis of acute myocardial infarction (AMI) may also utilize exosomal miRNAs. Troponin and creatinine kinase levels in serum are routinely used as biomarkers for AMI; however, troponin levels peak after 12 h following cardiac damage, a time duration that can be improved upon if another biomarker is discovered [47]. Recently, several miRNAs were found upregulated in the plasma exosomes from AMI patients, reaching their peak level faster than troponin. Specifically, miRNA-208a was undetectable in healthy patients, but was present only in 10% of AMI patients after 4 h following the initiation of chest pain. In addition, miRNA-208b, miRNA-1, miRNA-133a, and miRNA-499 were all found to be significantly increased in AMI patients within 12 h, serving as a reliable biomarkers though not superior to troponin [48]. In the context of heart failure, increased levels of miRNA-208b and miRNA-499–5p corresponded to increased risk of death or heart failure, establishing it as a potential prognostic indicator [49]. Furthermore, three miRNAs found within exosomes isolated from the serum of AMI patients, miRNA-192, miRNA-194, and miRNA-34a, were upregulated in subjects who experienced worsening heart failure symptoms within 12 months, also establishing these miRNAs as potential prognostic biomarkers [50].

### 4.3. Metabolic disorders

Noncoding RNA-containing exosomes have been shown to be involved in a wide range of processes underlying diabetic progression for both Type 1 and Type 2 diabetes [51]. These noncoding RNA have been found to exert their effects on remote tissues through mechanisms including the recruitment of epigenetic modifier proteins, control of mRNA decay and translation, and DNA sequestration of transcription factors.

Type 1 Diabetes is an autoimmune disorder caused by an irreversible destruction of insulin producing pancreatic β cells by innate and adaptive immune cells and a lack of pancreatic β cells serves as a pathological means to type 1 diabetes [51]. There is a noteworthy relationship between exosomes and autoimmunity observed in type 1 diabetes. Studies of type 1 diabetes have also found that both rat and human pancreatic islets release intracellular β cell autoantigens and proinsulin in exosomes, which are taken up by dendritic cells (DCs) [52]. Thus, exosomal proteins contribute to activation of B and T cells leading to pancreatic β cells apoptosis and development of type 1 diabetes [51]. Interestingly, exosomes derived from benign tumors of pancreas, insulinoma, were also found to stimulate the innate immune response via pro-inflammatory signaling downstream of toll-like receptor (TLR) and interleukin-1 (IL-1) signaling pathways. Moreover, exosomes derived from pancreatic mesenchymal stromal cells directly induce the T cell response and stimulate the release of IFNγ to induce inflammation, contributing to the autoimmune responses in Type 1 Diabetes.

Type 2 Diabetes accounts for ~90% of the diabetic cases and is characterized by high plasma glucose levels due to insulin resistance which is driven by excess accumulation of fat in adipocytes [53]. Studies have demonstrated that adipocytes can produce and release exosomes containing molecules which contribute to the development of such resistance [54]. For example, exosomes isolated from type 2 diabetic patients contain increased levels of miRNA-20b-5p; this miRNA downregulates AKT signaling leading to reduction of glycogen accumulation in primary human skeletal muscle and potential increase in insulin resistance [51]. Additionally, miRNA-155 found in exosomes derived from adipose tissue macrophages of diabetic mice was found to cause glucose intolerance and insulin resistance by targeting peroxisome proliferator-activated receptor γ, a transcription factor that regulates lipid metabolism [55]. Similarly, exosomes from obese mice that have increased levels of miRNA-192, miRNA-122, miRNA-27a-3p, and miRNA-27b-3p could induce glucose intolerance in lean mice [56]. Each of these miRNAs was increased in obese mice, and, when injected into lean mice, the mice developed glucose intolerance and insulin resistance. These data also demonstrate that the exosomal miRNAs can be used to diagnose and potentially treat diabetic patients.

### 4.4. The immune system

It is well established that exosomes are involved in communication within the immune system, mediating immunomodulation for both immune and non-immune cells [57]. For example, the activation of T helper cells and the initiation of adaptive immune response is highly regulated by DCs and other antigen presenting cells. Upon infection, mature DCs release exosomes with MHC molecules that can bind to T cell receptors and induce T helper cell activation resulting in adaptive immune response [58]. These T helper cells both activate B cells and further increase release of MHC complex-containing exosomes. The B cell derived exosomes can increase stimulation of T helper cells, indicating that B cell exosomes play a role in modulating the immune response. Additionally, the exosomes released by DCs can transfer antigens to other DCs [42,59].

Immature and suppressive DCs can release exosomes responsible for reducing adaptive immune responses by inducing cytotoxic T cell apoptosis and ensuring a tolerogenic immune response. These exosomes contribute to balancing the pro-inflammatory and anti-inflammatory effector T cells by their interaction with T helper cells resulting in T cell differentiation into regulatory T cells [42,59]. On the other hand, viral nucleic acids found in exosomes of infected cells can contribute to triggering of immune responses. For example, latent Epstein-Barr virus (EBV) infected cells trigger antiviral immunity through the exosome transfer of 5′ppp-RNA in vitro. This indicates that 5′ppp-recognizing sensors such as RIG-I can also recognize exosomal RNA. T cells can also release exosomes that contain genetic and mitochondrial DNA through the interactions with DCs from T cells [60]. This leads to the enhancement of antiviral responses via intracellular sensors such as cGAS/STING pathway and induction of IRF-3-stimulated genes. These findings indicate existence of a feedback mechanism that enhances T cell activity via DCs, resulting in more efficient response to infections by the same or similar pathogen. The changes in DCs induced by T cell-derived exosomes occur in a specific antigen-dependent manner indicating that DCs respond to certain stimuli. Furthermore, both oxidized mitochondrial DNA and genomic DNA are also present in T cell exosomes suggesting another role for these exosomes as contributors to T cell homeostasis by eliminating harmful or damaged components [61].

The molecular basis for autoimmune diseases is complex and is currently being extensively studied; existing data indicate that noncoding RNAs (ncRNAs) encapsulated in exosomes play a critical role in

autoimmune diseases. Rheumatoid Arthritis (RA) is one type of a chronic autoimmune disease characterized by infiltration of leukocytes into joints, causing a proliferation of inflammatory mediators and destruction of bone and cartilage tissue [62]. MiR-150–5p is associated with T cell maturation and can regulate angiogenesis; it has been demonstrated that exosomal micRNA-150 alleviates RA symptoms by downregulating MMP14 and VEGF as well as inhibiting angiogenesis [63]. It has also been shown that miR-548a-3p is significantly reduced in serum exosomes of RA patients, negatively correlated with serum levels of C-Reactive Protein (CRP), rheumatoid factor (RF), and erythrocyte sedimentation rate in RA patients [64]. It has also been demonstrated that exosomal miR-548-a-3p is involved in the regulation of macrophage mediated inflammation through the TLR4/NF-kB signaling pathway in RA, laying clear the influence that exosomal RNA has on RA [65].

Systemic Lupus Erythematous (SLE) is multisystem autoimmune disease characterized by persistent inflammation and autoantibody production, and miRNAs carried by exosomes have been demonstrated to also be involved in the pathogenesis of SLE, specifically in the context of regulating inflammation and immune imbalance [66].

### 4.5. Cancer

Exosomes are also released from tumor cells (tumor-derived exosomes) into their surrounding and growing evidence indicates that these vesicles regulate multiple processes, including tumor initiation and progression, epithelial-to-mesenchymal transition (EMT), suppression of anti-tumor response(s), angiogenesis, invasiveness, and drug resistance (reviewed in Ref. [67]). As an illustration, we review several key roles for cancer-related exosomes below; more comprehensive reviews of exosomes in various neoplastic diseases have been published recently [68–72].

Exosomal miRNAs isolated from MDA-MB-231 breast metastatic cell line was used to treat non-metastatic MCF-7 cells in vitro resulting in an increased ability of MCF-7 cells to both grow in an anchorage-independent manner, and in acquisition of metastatic behavior [73]. Furthermore, both miR-9 and miR-155 were shown to be present in MDA-MB-231-derived exosomes, and RT-PCR results showed that the mRNA levels of two miR-9 and miR-155 targets, PTEN and DUSP14, were decreased in exosome-treated MCF-7 cells [73]. Additional study evaluated a role for miR-155 in Transforming Growth Factor beta (TGF-β)-induced EMT; exosomes secreted from breast cancer stem cells were found to be enriched with miR-155, and the transfer of this miRNA via exosomal uptake upregulated EMT process in breast cancer recipient cells [74].

Exosomes from tumor cells can also transfer oncogenic proteins and nucleic acids from tumor cell to normal recipient target cells resulting in cell signaling pathway modulation in both transformed and non-transformed cells [75]. This transfer of various biomolecules is important in tumor cell-mediated angiogenesis, an important step in preparing a site for future colonization by cancer cells. To this end, cancer cell-secreted exosomes can stimulate the formation of pre-metastatic niche, and facilitate cancer cell migration [76]. It has been demonstrated that many tumor-released exosomal miRNAs which stimulate angiogenesis and promote metastasis can be utilized as biomarkers for the diagnosis and prognosis of human cancers using liquid biopsies because their circulating levels are deregulated [77–80]. Mechanistically, such microRNAs can target multiple pathways essential for tumor metastasis. For example, miR-9 activates the JAK/SAT pathway by reducing the suppressor of cytokine signaling-5 (SOCS-5) levels to promote tumor angiogenesis while miR-105 induces vascular leakiness and promotes metastasis [81].

## 5. Therapeutic properties of exosomes

Due to their versatile roles in both local and distance cell communication, both cell-secreted and engineered exosomes have been explored in pre-clinical and recently, smaller clinical trials [82]. The most direct method of exosome production involves the use of cells to take advantage of exosomes' "natural ability" to modulate biological processes. The most widely studied therapeutic exosomes thus far are secreted by mesenchymal stromal cells (MSCs) [83]. Exosomes secreted from MSCs appear to have similar impact on the phenotype of cells that also respond to MSCs. For example, both MSCs and MSC-derived exosomes have shown both vascular and cardiac benefits such as suppressing pulmonary hypertension (PH) and vascular remodeling in murine models of PH [84]. Similarly, exosomes derived from rat bone marrow MSCs protect cardiomyocytes from ischemic injury in a rat model of myocardial infarction [85]. Importantly, exosome-secreting cells can also be modified to enhance exosomal therapeutic properties; this can be achieved through cellular exposure to cytokines or gene transfection of the exosome-producing cells. For example, the treatment of human platelets with medications such as aspirin have shown evidence of decreased cargo proteins in platelet-derived exosomes. Also, murine bone marrow derived dendritic cells treated with recombinant murine IL-10 protein release exosomes that have an enhanced immunosuppressive effect. Similarly, pulsing murine DCs with tumor peptides led to an increase in ability of DC-derived exosomes to prime cytotoxic T cell immune responses against murine tumors. Therefore, this approach can be utilized to enhance therapeutic effects of exosomes.

## 6. The use of MSC-derived exosomal RNA in tissue regeneration and immunomodulation

Multiple reports indicated that stem cells engrafted into heart tissue after myocardial infraction survive only for a few days but this engraftment still led to improved heart function. These seemingly controversial data were subsequently explained by the presence of paracrine factors secreted by stem cells, in addition to direct benefits of the engrafted donor stem cells [86]. This idea has been corroborated by investigations that conditioned media from stem cells can improve cardiomyocyte survival following hypoxic impairment [87], inducing angiogenesis in infarcted myocardium [88], and reducing infarct size in mice [89] and porcine [90]. These therapeutic molecules include both nucleic acids and proteins packed inside exosomes that contribute to both tissue regeneration and in immunomodulation.

Mesenchymal stromal/stem cells (MSCs) are adult stromal cells with high regenerative capacity and with an established safety record in humans [91]. Specifically, MSCs facilitate regeneration of various tissues, including bone as demonstrated by the formation of new bone with supporting vasculature, improved morphological, biomechanical, and histological outcomes after fractures [89]. While the mechanisms by which MSC exosomes exert their therapeutic effects for bone regeneration are yet to be fully understood, it is expected that through a multifaceted mechanism, MSC exosomes promote survival, proliferation, migration, and ultimately support osteogenesis and angiogenesis with their protein and nucleic acid contents [89]. The cargo of MSC exosomes includes over 850 proteins including growth factors, angiogenesis-stimulating factors, and factors that promote bone formation in situ [92]. In terms of the role of exosomal RNA in this process, several miRNAs, including miRNA-126 [92] and miRNA-224–3p [93], have been associated with angiogenic effects of MSC exosomes [89]. Specifically, miRNA-126 was upregulated in exosomes isolated form MSCs maintained under hypoxic conditions, resulting in an enhanced angiogenic effect, increased $CD31^{+}$ endothelial cell proliferation and enhanced callus formation in a mouse femoral fracture model [94]. In another study, downregulation of miRNA-224–3p in bone marrow MSC exosomes was found to enhance proliferation, rearrangement, and tube formation of human umbilical vein endothelial cells through upregulation of a focal adhesion kinase family interacting protein [93].

MiRNAs can also contribute to tissue regeneration by stimulating cell proliferation and reducing apoptosis. For example, exosome-delivered miR-144, miR-21–5p, and miR-19a decrease apoptosis in damaged

cells. One common pathway that miRNAs target is the downregulation of PTEN which upregulates the pro-survival Akt signaling pathway resulting in decreased levels of pro-apoptotic capases-3, -8, and -9 [95–97]. Additionally, MSC exosome-delivered miR-100–5p targets mTOR mRNA which promotes autophagy in osteoarthrosis leading to bone growth [98].

Exosomal RNA also contributes to immunomodulatory properties of exosomes, and thus their therapeutic properties. For example, miRNA-223, an essential component in the regulation of inflammatory response, protected cardiac tissue through lessening the inflammatory response in a polymicrobial murine cecal ligation puncture sepsis model [99]. miRNA-223 has been identified in many tissues [100] and its low levels have been associated with increased inflammation and sepsis; in a clinical study, serum exosomal miR-223 levels in subject that died of sepsis were drastically lower compared to survivors [101]. Delivery of miR-223 via MSC-derived exosomes to macrophages and cardiomyocytes led to a downregulation of Sema3A and Stat 3 (miR-223 targets and inflammation related genes) which caused an inhibition of inflammatory response in macrophages and lessened death of cardiomyocytes during sepsis. However, when exosomes were released from MSCs that did not contain miR-223, no effects on Sema3A and Stat3 levels were observed [102]. Further research needs to be conducted to understand the potential of using of MSC-derived exosomes as a therapy for sepsis.

## 7. Concluding remarks

Investigation into the therapeutic and biomarker potential of exosomes is still in its infancy and we stress here the importance of continued investigation in this field, given the role that these vesicles play in human biology. Transporting a diverse information via RNA and proteins, exosomes facilitate the communication processes that may contribute to disorders of the central nervous system, metabolism, the immune system, the cardiovascular system, and many other conditions. While the mechanisms by which exosomes modulate biological processes are yet to be elucidated, it is apparent that exosomal RNA not only provides early diagnostic information but also contributes to therapeutic properties of exosomes isolated from stem cells. With the numerous roles of these vesicles, the field of exosomal therapy has a versatile potential because such vesicles can also be engineered to carry and transmit genetic material that can direct the progression of a disease. Specifically, by reorganizing the genetic information carried in such vesicles, certain organ systems and cells can be targeted to alter the processes that lead to physiological abnormalities. Moreover, to take a full advantage of this approach, it is necessary to understand the complex cellular interactions that exosomes contribute to. By investigating these interactions both in breadth and depth, the mechanisms by which information is transmitted can be exploited both for diagnostic and therapeutic purposes.

## Declaration of competing interest

No potential conflict of interest was reported by the authors.

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## List of abbreviations

| | |
|---|---|
| EVs | extracellular vesicles |
| DNA | deoxyribonucleic acid |
| RNA | ribonucleic acid |
| miRNA | microRNA |
| mRNA | messenger RNA |
| lncRNA | long non-coding RNA |
| cirRNA | circular RNA |
| rRNA | ribosomal RNA |
| tRNA | transfer RNA |
| scaRAN | small Cajal body-specific RNA |
| snoRNA | small nucleolar RNA |
| snRNA | small nuclear RNA |
| piRNA | piwi-interacting RNA |
| siRNA | small interfering RNA |
| RBPs | RNA-binding proteins |
| RME | receptor-mediated endocytosis |
| AD | Alzheimer's disease |
| PD | Parkinson's Disease |
| HD | Huntington's Disease |
| mHTT | mutant Huntingtin protein |
| TNF-α | tumor necrosis factor alpha |
| ACS | acute coronary syndrome |
| AMI | acute myocardial infarction |
| DCs | dendritic cells |
| MHC | major histocompatibility complex |
| MBs | multivesicular bodies |

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