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RNA in extracellular vesicles

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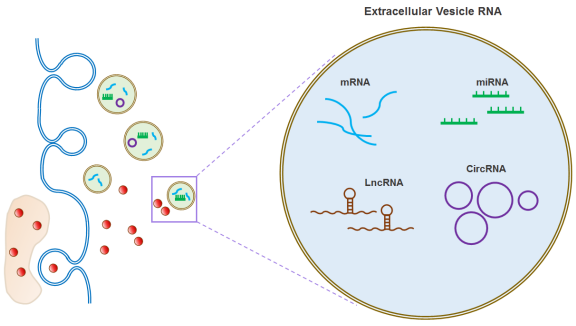
Abstract

Cells release a range of membrane-enclosed extracellular vesicles (EVs) into the environment. Among them, exosomes and microvesicles (collectively measuring 30-1000 nm in diameter) carry proteins, signaling lipids, and nucleic acids from donor cells to recipient cells, and thus have been proposed to serve as intercellular mediators of communication. EVs transport cellular materials in many physiologic processes, including differentiation, stem cell homeostasis, immune responses, and neuronal signaling. EVs are also increasingly recognized as having a direct role in pathological processes, notably cancer and neurodegeneration. Accordingly, EVs have been the focus of intense investigation as biomarkers of disease and prognostic indicators, and even therapeutic tools. Here, we review the classes of RNAs present in EVs, both coding RNAs (mRNAs) and noncoding RNAs (long noncoding RNAs, microRNAs, and circular RNAs). The rising attention to EV-resident RNAs as biomarkers stems from the fact that RNAs can be detected at extremely low quantities using a number of methods. To illustrate the interest in EV biology, we discuss EV RNAs in cancer and neurodegeneration, two major age-associated disease processes.

Graphical abstract



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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

Keywords

Extracellular vesicle; exosome; microvesicle; microRNA; long noncoding RNA; circular RNA; biomarker; gene therapy; drug delivery

INTRODUCTION

Extracellular Vesicles

Cells produce extracellular vesicles (EVs) of three main types, according to their size and biogenesis: exosomes, microvesicles, and apoptotic bodies.[1-4] Microvesicles, measuring 0.2-1 μm in diameter, arise through budding of the plasma membrane and are therefore enclosed by a fraction of plasma membrane.[5, 6] Apoptotic bodies are released from blebbing of the plasma membrane of apoptotic cells and have diameters of 0.5-2 μm.[7, 8] Exosomes, by contrast, arise from the endosomal pathway and form intracellular multivesicular bodies (MVBs) which fuse to the plasma membrane and are secreted as vesicles measuring 0.04-0.1 μm. EVs were first described by Pan *et al.* and Johnstone *et al.* in 1983 in sheep reticulocytes and were subsequently visualized using electron microscopy (EM) [9, 10, 11] EVs are secreted from numerous cell types and have been isolated from a wide variety of human body fluids such as blood, urine, saliva, and breast milk.[12-15]

A variety of molecules have been identified in EVs, including DNA, RNA, bioactive lipids, and proteins.[16] These molecules are protected by EV membranes from nucleases, proteases, fluctuations in pH and osmolarity, and other environmental factors.[17-20] EV components can be delivered from an originating cell to a recipient cell, whether the recipient cell is in the vicinity (horizontal transfer) or in a distant tissue, and the transferred molecules are capable of eliciting changes in function and gene expression in the recipient cell.[20-22] Since EVs are found in easily accessible body fluids, particularly blood, they are attractive sources of diagnostic and prognostic biomarkers.[23, 24] In addition, since EVs are derived from intracellular material, they are being explored as packaging tools for the therapeutic delivery of genetic material and drugs.[25-29] As we gain more information about EVs directed to specific tissues and organs, such delivery of therapeutic molecules could be targeted with high precision.[30] Given the rising interest in exploiting EVs in disease diagnosis and treatment, there is urgency to determine comprehensively the tissues of origin of EVs, their target tissues, and their molecular constituents. Towards the latter goal, the relatively low abundance of EVs presents some challenges, but highly sensitive methods of RNA detection developed in recent years [particularly RNA-sequencing (RNA-Seq) and reverse transcription and quantitative PCR analysis (RT-qPCR)] afford accurate and quantitative identification of RNAs, even if present in very low amounts. In this review, we discuss the progress made by the RNA and EV communities to identify the pools of transcripts present in exosomes and microvesicles.

DIVERSE RNAs IN EVs

The identification of RNAs in EVs has progressed immensely in recent years thanks to technical advances in the detection of low-abundance, complex RNA samples. RNA pools in

EVs have been identified comprehensively using high-throughput RNA-Seq and many have been validated using RT-qPCR analysis. These RNA populations include various protein-coding transcripts (mRNAs) and many types of non-coding RNAs, including microRNAs (miRNA), long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), small nucleolar RNA (snoRNAs), small nuclear RNAs (snRNAs), transfer RNA (tRNAs), ribosomal RNAs (rRNAs), and piwi-interacting RNAs (piRNAs).[31] These RNAs can be transferred from parent cells to recipient cells, where they can regulate or serve as templates for protein production,[32, 33] although the relative abundance of full-length and fragmented transcripts in EVs is not known at present because the analysis methods available (RNA-Seq and microarray) identify relatively small RNA segments. In this review, we focus on mRNAs, microRNAs, lncRNAs, and circRNAs identified in EVs.

Protein-coding RNA (mRNA)

Protein-coding RNAs are synthesized in the nucleus as pre-mRNAs and then typically undergo splicing, modification of the ends, and export to the cytosol, where they function as templates for protein translation. mRNAs have three basic segments, the 5′-untranslated region (5′UTR), the coding region (CR, which encodes protein), and the 3′UTR. A number of reports have identified full-length and sometimes fragmented mRNAs in EVs. For example, early microarray analysis revealed that EVs (primarily exosomes) derived from glioblastoma cells contained 27,000 mRNAs.[20] Interestingly, ~4,700 of these mRNAs were only detected in EVs, not in cells, and >3,000 mRNAs were preferentially included (2,238 mRNAs) or excluded (1,188 mRNAs) from EVs compared with mRNAs found in cells. To test whether mRNAs in glioblastoma-produced EVs were translated in recipient cells, the authors expressed *Gaussia* luciferase[4] (*Gluc*) mRNA in glioblastoma cells and purified EVs from the medium. *Gluc* mRNA encodes a luciferase protein that is secreted and emits intense fluorescence. They then added these EVs to recipient human brain microvascular endothelial cells (HBMVECs) and found that Gluc activity released by HBMVECs increased continuously over the ensuing 24 h. These findings supported the view that the cargo *Gluc* mRNA from the parent cell was translated in the recipient cell to generate a functional protein. These results contribute to a substantial body of evidence that EV-resident mRNAs can be translated in recipient cells.

Another study used microarrays to identify 13,000 mRNAs in EVs derived from MC/9 cells (a mouse mast cell line).[34] Interestingly, 270 mRNAs were only detected in EVs and not in cells. To test if the EV mRNAs could serve as a templates for the synthesis of functional proteins, they used rabbit reticulocyte lysate as an *in vitro* translation system. Following the completion of translation, two-dimensional polyacrylamide gel electrophoresis was employed to identify mouse proteins (COX5B, HSPA8, SHMT1, LDH1, ZFP125, GPI1, and RAD23B) from rabbit proteins by mass spectrometry analysis. The authors concluded that many EV-resident mRNAs were translated efficiently.

In another model system, human central nervous system (CNS)-patrolling macrophages, stimulation by β-amyloid peptide (Aβ) resulted in the secretion of exosomes containing a number of cytokine mRNAs relevant to Alzheimer's disease (AD) pathogenesis. Interestingly, macrophages derived from older subjects generated higher levels of exosomal

IL6, *TNF*, and *IL12* mRNAs, but did not exhibit differences in the levels of *IL8*, *IL1*, or *IL23* mRNAs. The authors proposed that cytokine mRNAs in exosomes may be a mechanism for spreading neuroinflammation induced by Aβ peptide.[35] In sum, many mRNAs residing in EVs can be translated and contribute to the protein expression programs of recipient cells.

microRNA (miRNA)

MicroRNAs are small (~22-nt) non-coding, highly conserved, single-stranded RNAs found both inside and outside of cells.[36, 37] The biosynthesis of microRNAs begins with the transcription of a primary (pri-) microRNA that is processed by the RNase III DROSHA into a ~70 nucleotide stem-loop transcript, the precursor (pre-)microRNA. XPO5 (Exportin-5) then exports pre-microRNAs to the cytoplasm where they are processed by RNase III DICER1 into mature ~22-nt microRNA duplexes;[37, 38] after unwinding the duplex, one strand associates with AGO (Argonaute) proteins to form RNA-induced silencing complex (RISC).[36, 38, 39] Alternative pathways for microRNA biogenesis have also been described.[40] Although microRNAs can affect gene transcription by influencing chromatin structure and transcription, they are best known for eliciting gene silencing by lowering the stability and/or translation of mRNAs with which they share partial complementarity, generally at the mRNA 3′UTR.[36, 41, 42] By modulating gene expression programs in the cells in which they are generated, microRNAs play a role in a wide range of biological processes, including development, cell proliferation and differentiation, apoptosis, and immune regulation.[41]

MicroRNAs can also participate in intercellular signaling. Many body fluids harbor abundant, stable microRNAs which avoid nucleolytic degradation by associating with RNA-binding proteins (RBPs) and high- and low-density lipoproteins, and by being encapsulated in EVs.[43-45] With the discovery of microRNAs in EVs, many new functions and applications have emerged – from new ways of cell-cell communication to potentially easy-access biomarkers and, given the non-immunogenicity of EVs, possibly novel therapeutics.[19, 46-51] MicroRNAs uptaken via EVs might function as gene expression regulators in recipient cells, but growing evidence from EVs in cancer and other processes has expanded the subset of EV functions.[47, 52-55] Particularly surprising and exciting was the discovery that EV microRNAs can act as ligands for Toll-like receptors (TLR) and induce immune responses or inhibit macrophage activation by suppressing TLR signaling.[47, 56-59] It is still unclear whether specific microRNAs are actively sorted into EVs, although some microRNAs appear to be selectively targeted to EVs and several mechanisms for selective inclusion of microRNAs have been proposed, as discussed in the section below.

Different microRNA profiles in patients and controls have been reported for many diseases, implicating them in disease pathogenesis.[43, 46, 50, 60-63] The easy access and stability of EV-microRNAs in biological fluids have created a niche for them as potential diagnostic biomarkers. Even though most of such studies to-date come from cancer research, the brain has the highest expression of tissue-specific microRNAs (70% of all reported microRNAs) and thus microRNAs in brain EVs may also be particularly informative in neurological disorders.[64, 65]

The presence in EVs of misfolded proteins associated with neurodegenerative disorders [Aβ, tau (MAPT), α-synuclein (SNCA), and prion proteins] prompted studies to test a role for EVs in disease propagation.[66-69] The discovery of microRNAs in EVs expanded the search for mechanisms of disease pathogenesis implicating dysregulation of endogenous microRNAs.[66, 68, 70, 71] For example, the EV-resident microRNA miR-193b was implicated in regulating the production of amyloid precursor protein (APP, the precursor of the neurotoxic peptide Aβ), perhaps by repressing APP expression.[72] Interestingly, the levels of soluble miR-193b in the blood of patients with MCI (mild cognitive impairment) and AD were similar to those in controls, but EV miR-193b levels in the blood and cerebrospinal fluid (CSF) of persons with MCI and AD was lower,[72] suggesting that miR-193b was selectively excluded from these disease-associated populations. Sixteen microRNAs implicated in AD were recovered from patients' EVs, reaching a sensitivity and specificity of 87% and 77% for predicting AD. Another set of seven EV microRNAs showed 83-89% accuracy in predicting AD status and recent reviews showcase various microRNA signatures for AD in different body fluids.[73] Six EV microRNAs have been associated with AD by multiple groups; among them, miR191-5b in plasma and serum was particularly informative in the diagnosis of AD.[74] Likewise, differential microRNA profiles were reported when comparing CSF EVs from Parkinson's disease (PD) and AD patients.[62]

Age-related microRNA and TLR signaling dysregulation may contribute to other diseases of aging, such as muscular and cardiovascular diseases.[75-78] Notably, sarcopenia in older individuals could be associated with microRNA dysregulation, and changes in microRNA signatures in muscle tissues have been observed with aging.[79, 80]

The potential of EV microRNAs to change gene expression locally and distantly, together with their non-immunogenic nature, further suggests that they hold great potential for therapeutic applications.[46, 81, 82] An important caveat is the variable stoichiometry in microRNAs contained in EVs.[83, 84] It is currently unknown whether all EVs in a population contain the same amount of microRNAs or whether some vesicles are selectively loaded with given microRNAs; this information is critical for therapeutic applications, since the successful suppression of a given mRNA depends on the types and concentrations of available microRNAs. Even though therapeutic applications of EV microRNAs are still in their infancy, several successful studies *in vitro* and *in vivo* have been reported. In renal fibrosis, EVs from mesenchymal stem cells were engineered to overexpress the microRNA let-7c and were selectively targeted to damaged kidneys, where they successfully attenuated kidney injury.[85] Similar approaches based on the EV-mediated delivery of microRNAs have been undertaken for the treatment of cancer and liver disease.[86, 87] This field is expected to boom with increasing knowledge of the targeted sorting, enrichment and packaging of microRNAs into EVs.

Long noncoding RNA (lncRNA)

This class of noncoding RNAs, defined as being >200 nt, was identified almost three decades ago,[88] but the advent of high-throughput RNA-Seq has begun to reveal the rich and diverse functions of mammalian lncRNAs.[89-91] LncRNAs are involved in controlling a

variety of cellular processes, such as chromatin organization, gene transcription, mRNA turnover, protein translation, and assembly of macromolecular complexes.[92-94]

Numerous lncRNAs have been found in EVs. In 2014, Gezer *et al.* identified lncRNAs including *MALAT1*, *HOTAIR*, *lincRNAp21*, *GAS5*, *TUG1*, and *ncRNA-CCND1* in EVs derived from human cervical and breast carcinomas (HeLa and MCF-7 cells, respectively) by RT-qPCR analysis.[95] Each lncRNA had different expression patterns in exosomes compared with cells: *lincRNAp21*, *HOTAIR*, and *ncRNA-CCND1* were highly enriched in EVs even if their expression was very low in cells, while expression of *MALAT1* was very high in both. Following exposure to DNA-damaging agents like bleomycin, *lincRNAp21* and *ncRNA-CCND1* were selectively found in EVs, while other lncRNAs such as *MALAT1*, *HOTAIR*, *GAS5* and *TUG1* were not. These results suggest that some lncRNA may be selectively sorted by as-yet unknown mechanisms that regulate the abundance of lncRNAs in EVs under specific cell conditions.

Other studies using prostate cancer cell lines (PC3, VCaP, LNCaP, DU145) identified lncRNAs enriched in EVs relative to EVs from a normal prostate epithelial line.[96] These lncRNAs have specific motifs which perfectly complement the seed regions of certain microRNAs, such as let-7 family members (let-7a, let-7b, let-7c, let-7d, let-7e and let-7i), miR-17, miR-18a, miR-20a, miR-93, and miR-106b. The most frequent sequence in this subset of lncRNAs is CCUCCC, which matched perfectly miR-7106-5p, miR-6883-5p, miR-6799-5p, miR-6785-5p, miR-4728-5p, miR-6887-5p, miR-6885-5p, miR-6799-5p, miR-328-5p and, miR-149*. Further analysis revealed that miR-149* was also highly enriched in EVs, but not in cells, leading the authors to suggest that the EV lncRNAs might function as a sponge for EV microRNAs, although it is also possible that lncRNAs may provide a mechanism for loading microRNAs into EVs. In addition, among the lncRNAs in EVs, they found enrichment in transcripts bearing RNA motifs recognized by RBPs ELAVL1 (HuR) and RBMX, prompting the additional hypothesis that specific lncRNA-RBP complexes may capture specific microRNA subsets and target them into exosomes.

Circular RNA (circRNA)

CircRNAs are a highly abundant, heterogeneous class of noncoding regulatory RNAs.[97-102] Although their biogenesis is not well understood, the splicing machinery is believed to generate most circRNAs via head-to-tail backsplicing.[103] RBPs like muscleblind (MBL), quaking I 5 (QKI5), and the RNA-editing enzyme ADAR were found to be involved in circRNA biogenesis.[104, 105] High-throughput RNA sequencing together with computational analysis have identified large numbers of circRNAs in mammalian cells.[97-102] Unlike linear RNAs, circRNAs have a long half-life because they lack free ends and thus cannot be degraded by exonucleases.[106, 107] This feature may enable critical transcriptional and posttranscriptional functions of circRNAs. CircRNAs have been shown to sponge some microRNAs and thereby control their function as regulators of mRNA stability and/or translation. For instance, *ciRS-7* sponges and regulates the function of miR-7 and *EIciEIF3J* interacts with the snRNP *U1* and the promoter EIF3J to enhance *EIF3J* transcription.[108] *CircMbl* regulates the splicing activity of MBL, controlling *MBL* pre-mRNA processing to mature *MBL* mRNA by competing with the splicing machinery.[109] *CircFoxo3* forms a

complex with CDK2 and p21 that limits the cell cycle-dependent interactions of CDK2 with cyclins A and E.[110]

Although these functions illustrate the emerging impact by EV-harbored circRNAs on cellular processes and cell fates, the extracellular functions of circRNAs via EVs are not known. Recently, circRNAs were found to be both enriched and stable in cancer EVs, suggesting that they could also potentially be used as cancer biomarkers.[111] In addition, it has been suggested that EVs could represent a mechanism for circRNA clearance[112] and that circRNAs in EVs could constitute potential biomarkers in disease processes as well as therapeutic targets.[113]

MECHANISMS FOR SPECIFIC SORTING OF RNAs INTO EVs

Specific subsets of RNAs are selectively mobilized to EVs and in some cases excluded from cells[114-117]. Although the mechanisms responsible for this packaging are not clear, some mechanisms responsible for sorting microRNAs to EVs have been suggested,[118, 119] as discussed below.

microRNA EXOmotifs

A recent survey using microarray analysis to identify microRNAs in primary T lymphoblasts and derived EVs[118] revealed specific subsets of microRNAs and mRNAs differentially present in cells and in EVs. Some microRNAs, including miR-575, miR-451, miR-125a-3p, miR-198, miR-601, and miR-877 were highly abundant in EVs (specifically in exosomes) compared to cells and were named EXOmiRNAs, while other microRNAs, including miR-17, miR-29a, let-7a, miR-142-3p, miR-181a, and miR-18a, were highly represented in cells. Comparing the two subsets, the authors found two conserved sequences in the subsets of microRNAs that accumulated in exosomes and not in cells, and termed them EXOmotifs. The EXOmotif GGAG is located in the 3′ half of the microRNA, while the EXOmotif C/UCCU/G can exist anywhere on the microRNA. In support of the authors' hypothesis, microRNAs with mutated EXOmotifs could not be exported into exosomes and were stored in cells. The RBP HNRNP (heterogeneous nuclear ribonucleoprotein) A2B1 was found to interact with EXOmiRNAs and helped transfer them into exosomes through EXOmotifs. Exosomal HNRNP A2B1 had a larger molecular weight than the cellular HNRNP A2B1 due to the attachment of a ubiquitin-related residue (SUMO) in exosomes, and this modification appeared to be crucial for the sorting of EXOmiRNAs to exosomes by HNRNP A2B1. While it has been shown that some specific lncRNAs are highly enriched in EVs compared with cells,[95] the mechanisms responsible for this preferential mobilization are unknown. Much remains to be elucidated about ribonucleoprotein (RNP) complexes, how they are transported to EVs, and whether RNP complexes dissociate in recipient cells or not.

Relative levels of microRNAs and target mRNAs

Another mechanism to sort microRNAs into EVs was identified while performing microRNA transcriptome analysis of BMDMs (bone marrow-derived macrophages) using TaqMan low-density quantitative PCR (qPCR) arrays.[119] A specific pool of microRNAs (including miR-150-5p, miR-146a-5p, miR-320-3p, miR-467-3p, and miR-467f) was

enriched in BMDM-derived exosomes but not in BMDMs, and another pool of microRNAs accumulated in BMDMs but not in exosomes. The different microRNA expression profiles in macrophages and macrophage-derived exosomes were investigated using wild-type cells and cells deficient in DICER1, the RNase that cleaves precursor (pre-)microRNAs to form mature microRNAs. Surprisingly, *Dicer1* knockout BMDMs produced EVs with marked deficiencies in microRNA levels, even though the BMDMs themselves showed only modest declines in microRNA levels.[123] Interestingly, ectopically changing the levels of microRNAs or the target mRNAs influences the sorting of microRNAs to EVs. This observation was made after artificially increasing the levels of miR-511-3p (which is expressed at low levels in BMDMs) and finding that it disproportionately elevated miR-511-3p levels in EVs and not in BMDMs, while raising the levels of a miR-511-3p sponge bearing miR-511-3p binding sites lowered miR-511-3p levels in EVs. These and other lines of evidence suggest that EVs can function as tools to maintain appropriate levels of microRNAs in cells and thereby preserve homeostasis.

EV RNA IN AGING-RELATED DISEASE

There is growing interest in studying EVs in several disease conditions, including those showing higher incidence in the elderly, such as neurodegenerative diseases and cancer.

Neurodegenerative disease (Parkinson's and Alzheimer's diseases)

Advancing age causes loss in the structure and function of neurons that can lead to neurodegenerative conditions such as Parkinson's disease (PD) and Alzheimer's disease (AD). Efforts are underway to find biomarkers for early detection of these neurodegenerative diseases. Gui and colleagues isolated exosomes in CSF from PD and AD patients and healthy controls by flow cytometry based on the presence of the exosomal surface protein CD63, and used microarray analysis to identify microRNAs with differential abundance among the AD, PD, and normal groups.[62] Compared with healthy control exosomes, PD exosomes showed that 16 microRNAs were more abundant (including miR-153, miR-409-3p, miR-10a-5p, and let-7g-3p) and 11 were less abundant (including miR-1, miR-19b-3p). AD exosomes showed fewer differences with healthy controls, with only miR-16-2, miR-29c, miR-132-5p, miR-136-3p, miR-331-5p, and miR-485-5p showing significantly altered levels. Further assessment of mRNAs and lncRNAs associated with PD and AD using RT-qPCR analysis revealed several mRNAs differentially expressed in CSF exosomes from PD and AD subjects. For example, compared with healthy CSF exosomes, the levels of *APP* (amyloid precursor protein) mRNA, *SNCA* (α-synuclein) mRNA, *DJ-1*/*PARK7* mRNA, and *CX3CL1* (Fractalkine) mRNA were lower in AD and PD exosomes, while the levels of *NEFL* (neurofilament L) mRNA was higher in AD and PD CSF exosomes. Interestingly, *MAPT* (Tau) mRNA was unchanged among the three exosome groups, and lncRNAs *RP11-462G22.1* and *PCA3* were enriched in CSF exosomes from PD and AD. Taken together, the authors propose that some of these RNAs could have diagnostic values, although additional analysis is necessary.

Cancer

There is also a great deal of interest in EVs in cancer diagnosis and therapy. In an early study in glioblastoma patients, numerous mRNAs and microRNAs were found to be highly enriched in EVs present in the serum.[20] In prostate cancer patients, EVs purified from the urine were found to contain prostate cancer biomarkers (*PCA3* and *TMPRSS2:ERG* mRNAs) by using nested PCR.[120] More recent studies showed RNAs specifically enriched in EVs from cultured prostate cancer lines PCA3, VCaP, LNCaP, and DU145[96] relative to exosomes from normal cells, as mentioned above. In ovarian cancer, exosomal RNAs were also identified by several groups. Several of these reports have found exosomal microRNAs, including miR-21, miR-141, miR-182, miR-200a, miR-200b, miR-200c, miR-203, miR-205, and miR-214 as being highly enriched in EVs from ovarian cancer patients.[121-124] In summary, there are intense efforts to exploit EVs in cancer diagnosis. In addition, new studies have begun to investigate the use of EVs in cancer therapy via the delivery of RNAs and chemotherapeutic drugs.[125-127]

PERSPECTIVES

The presence of various RNA molecules in EVs is well established,[31] although the mechanisms that control EV production, release, and uptake by recipient cells are poorly understood. Such mechanisms could be altered under pathological conditions and may provide therapeutic opportunities. Detection of EV RNAs by high-throughput (e.g., RNA-Seq and microarray analyses) or by transcript-specific RT-qPCR analysis has shown that some transcripts are selectively included into EVs and some are specifically excluded from EVs. How the selective localization of EV RNAs is regulated is largely unknown, but it likely involves their recognition by RBPs and the formation of RNP complexes that can regulate RNA uptake and/or retention. Although efforts to identify specific RNA signature sequences that target them for inclusion into EVs or exclusion from EVs have been initiated, additional investigation is needed. Similarly, the RBPs implicated in sorting RNAs to EVs, the signaling pathways that regulate this process, and possible post-translational modifications of the RNPs responsible for sorting EV RNAs are almost entirely unknown.

Another important consideration is whether the various RNA molecules in EVs are intact or partially degraded. This question is particularly important because intact mRNAs could serve as templates for functional proteins in the recipient cells, and full-length noncoding RNAs (including lncRNAs and microRNAs) might potentially retain their original function. Partially degraded mRNAs and ncRNAs, on the other hand, could assume new functions, as sponges or competitors for certain particular factors (e.g., RBPs, microRNAs), in turn affecting cellular processes in different ways. Advances in technical and analytical tools for investigating EV RNAs are needed in order to help elucidate these questions.

Body fluids contain EVs originating from different tissues, but each tissue is expected to release EVs containing distinct subsets of RNAs; for instance, neuronal EVs may have different RNA contents than EVs from other tissues like liver or muscle. Accordingly, identifying the source of EVs is particularly important. In this regard, robust efforts are also underway to identify markers unique to EVs from neurons, liver, pancreas, prostate, breast, muscle, as well as other organs and tissues. Markers can also be informative as to whether

EVs are microvesicles or exosomes, particularly when analyzed in conjunction with EV size assessments.

Finally, EVs can be retooled as delivery vectors for therapeutic intervention.[125, 126, 128] While an individual's EVs are recognized by the body as 'self' and thus allowed to circulate without rejection by the body's immune system, caution should be to avoid unwanted immune responses triggered by EVs.[129] With better knowledge of the EV determinants that target uptake by other tissues, EVs could be engineered to deliver therapeutic factors (nucleic acids, proteins, or drugs) to a target tissue. Therefore, it will be especially important to identify the factors that target EVs to a given cell type or tissue, whereupon it can be internalized via mechanisms such as membrane fusion, caveolin-mediated endocytosis, and phagocytosis.[130] The actual molecular mediators of EV uptake are not known, but ongoing efforts from our laboratory include screening of RNAi libraries to identify proteins implicated in this process. Progressively better understanding of the cargos (particularly RNA molecules) that EVs carry, the factors that govern EV release and uptake, and the tissues that originate and deliver EVs, this important class of intercellular transport vehicles will become increasingly valuable tools in many areas of physiology and pathology.

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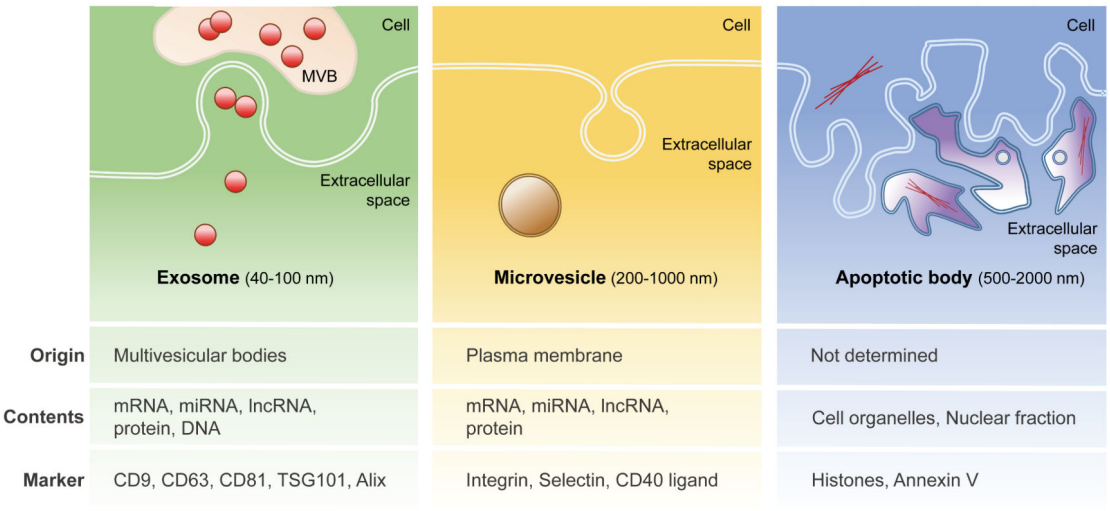

Figure 1. Different Types of Extracellular Vesicles

Schematic depiction of the distinct sizes, contents, and detection markers for exosomes (*left*), microvesicles (*middle*), and apoptotic bodies (*right*).

Table 1

EV RNAs

Types of RNAs identified in EVs, including coding RNAs (mRNA) and noncoding RNAs (microRNAs, lncRNAs, circRNAs). Disease processes in which EV RNAs are differentially enriched or excluded, forms of EV purification, and methods of EV RNA detection are indicated.

EV RNA			Altered levels in EVs from aging-related disease	EV Purification	Detection	Refs.
Protein-coding RNA	**mRNA**	*NEFL*	enriched in PD and AD EVs	ultracentrifugation	qPCR validation	[62]
		APP	lower in PD and AD EVs			
		SNCA				
		DJ-1/PARK7				
		Fractalkine/CX3CL1				
		PCA3	enriched in prostate cancer EVs		nested PCR	[120]
		TMPRSS2: ERG				
		Tau/MAPT	lower in AD EVs		qPCR validation	[62]
Noncoding RNA	**microRNA**	miR-153	enriched in PD	ultracentrifugation	TaqMan microRNA array qPCR validation	[62]
		miR-409-3p				
		miR-10a-5p				
		let-7g-3p				
		miR-1	lower in PD EVs			
		miR-19b-3p				
		miR-132-5p	enriched in AD			
		miR-485-5p				
		miR-29c	lower in AD EVs			
		miR-136-3p				
		miR-16-2				
		miR-331-5p				
		miR-21	enriched in ovarian cancer EVs	magnetic activated cell sorting (MACS), anti-epithelial cell adhesion molecule (EpCAM)	microRNA microarray	[121]
		miR-141				[121] [124]
		miR-200a				
		miR-200b				
		miR-200c				
		miR-203				[121]
		miR-205				
		miR-214				
		miR-182				
	lncRNA	*RP11-462G22.1*	enriched in PD and AD EVs	ultracentrifugation	qPCR validation	[62]
		PCA3				
		ENST00000501280	enriched in prostate cancer EVs		lncRNA array	[96]
		uc010bys.1				

EV RNA			Altered levels in EVs from aging-related disease	EV Purification	Detection	Refs.
		uc001qgn.1				
		ENST00000499690				
		ENST00000453968				
		G36642				
		AK055500				
		HIT000070262				
		ENST00000452932				
	circRNA	*Circ-CDYL*	MHCC-LM3 liver cancer cell EVs	ultracentrifugation	RNA-seq, qPCR validation	[111]
		Circ-CAMSAP1				
		Circ-EZH2				
		Circ-FOXK2				
		Circ-XPO1				
		Circ-KLDHC10	colorectal cancer EVs			