Review article

Unlocking the potential of extracellular vesicles: A new frontier in liquid biopsies towards precision cancer diagnostics

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Abstract

Extracellular vesicles (EVs) are nano-sized, membrane-bound particles secreted by cells under physiological conditions and are involved in multiple functions, especially cell signaling. They are found in body fluids including blood, saliva, semen, bile, cerebrospinal fluid, amniotic fluid, breast milk, and urine. The molecular cargoes encapsulated by the EVs provide information about the cells of their origin. More recent developments in liquid biopsies have enabled uncovering the potential diagnostic uses of these vesicles offering an alternative to traditional biopsy enquiries in pathology. In the field of cancer, cancer-associated exosomes have been explored for early diagnosis with biomarkers to determine prognosis and therapy responses. This review filters recent advancements in EV biology and focuses on EV biogenesis and how EVs play a pivotal role in mediating intercellular communication in cancer. We draw attention to EV molecular profile with cargo heterogeneity which warrants scientists to develop precision therapeutic strategies to enhance treatment efficacy. Integrating EV analysis into clinical practice holds significant potential to revolutionize cancer diagnostics and therapeutic approaches, paving the way for more effective patient-centered healthcare solutions.

Introduction

Extracellular vesicles are nano-sized, membrane-bound particles secreted by all cells and released into the extracellular environment through various mechanisms. These vesicles carry various biomolecules from the cell membrane and cytoplasm of their origin. Different varieties of lipids, proteins, and nucleic acids are encapsulated within the vesicles as cargo and play an important role in cell signaling. EVs were once considered biologically irrelevant cellular debris. Today, they have been recognized for their significant roles associated with interaction with surface membrane receptors of the recipient cell, resulting in intercellular communication.[1]

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The importance of cell-to-cell communication via EVs is evident by the evolutionary conserved process of vesicle formation. These vesicles are produced by prokaryotes to advanced eukaryotes including mammals. EVs are synthesized and released by numerous healthy cells such as epithelial cells, neurons, fibroblasts, and adipocytes. They can be isolated from bodily fluids including blood, serum, saliva, semen, breast milk, and urine.[2]

A key importance of EVs lies in the potential of their unique properties for cancer diagnostics and precision medicine. Early detection of cancer is possible because the cargo molecules reflect the characteristics of the tumor from which they originate. In addition, cancer patients synthesize high levels of EV proteins. Moreover, EVs can be isolated non-invasively from bodily fluids and integrated into liquid biopsy approaches, offering an alternative to traditional biopsies which cause patient discomfort.[3] Precision medicine is a recent approach that considers individual risk profiles for disease prevention and treatment. By analyzing a patient's molecular EV profile, personalized therapies are selected and are more likely to be effective than conventional treatment methods.

Methodology

A narrative literature search was conducted across multiple databases, including PubMed and Google Scholar. The search was performed using a combination of keywords and phrases: "extracellular vesicles," "exosomes," "cancer diagnostics," "biomarkers," and "liquid biopsies." The search was limited to literature published in English from 2008 to 2024, ensuring a focus on recent advancements in the field and developing an overview of the narrative.

Classification of EVs

EVs are classified based on their biogenesis mechanism, concept (manner of origin such as oncosome, matrix vesicle, stress EV, etc) and size. Exosomes, micro vesicles, apoptotic EVs, autophagic EVs, stressed EVs (stresses), and matrix vesicles are different forms of EVs found in body fluids.[4]

Biogenesis of extracellular vesicles

The biogenesis of extracellular vesicles (EVs) involves membrane remodeling processes facilitated by cytoplasmic proteins interacting with lipid bilayers, along with alterations in membrane composition that stabilize energetically unfavorable intermediates essential for vesicle budding (Figure 1).[5] Exosomes form as cell membrane invaginations leading to the creation of endocytic vesicles, which fuse with each other to become endocytic cisternae. Inward budding within these cisternae forms intraluminal vesicles (ILVs), which are

Table 1. Classification of extracellular vesicles

Category	*Size*	*Markers*	*Biogenesis*
Exosome	40-150 nm	CD63, CD9, CD81	Multivesicular endosome
Micro vesicle	40 nm -10 µm	Annexin A1, ARF6, ARRDC1, TSG101	Plasma membrane shedding
Apoptotic EV	100 nm-5 µm	Annexin V, PS	Apoptosis
Autophagic EV	40-1000 nm	LC3B-PE, p62, Nucleosomes (dsDNA/Histones)	Autophagosome-endosome fusion
Stressed EV	40-1000 nm	HSP90, HSPs	Plasma membrane shedding, autophagy
Matrix vesicles	40-1000 nm	Fibronectin, Proteoglycans	Matrix binding and release

A range of extracellular vesicles based on their sizes and cargo composition. ARF6, ADP-ribosylation factor 6; ARRDC1, Arrestin-Domain Containing Protein 1; TSG101, Tumor Susceptibility Gene 101; EV, extracellular vesicle; PS, phosphatidylserine; LC3B-PE, microtubule-associated protein light chain B-phosphatidylethanolamine; HSP, Heat Shock Protein.

identified as the EVs following their secretion. ILVs are formed within multivesicular bodies (MVBs) and the maturation and acidification of early MVBs alter protein content enhancing fusion potential with other vesicles and membranes.[6] Exocytosis of exosomes occurs when exocytic MVBs fuse with the plasma membrane, releasing ILVs as exosomes into the extracellular space through constitutive or regulated pathways[7].

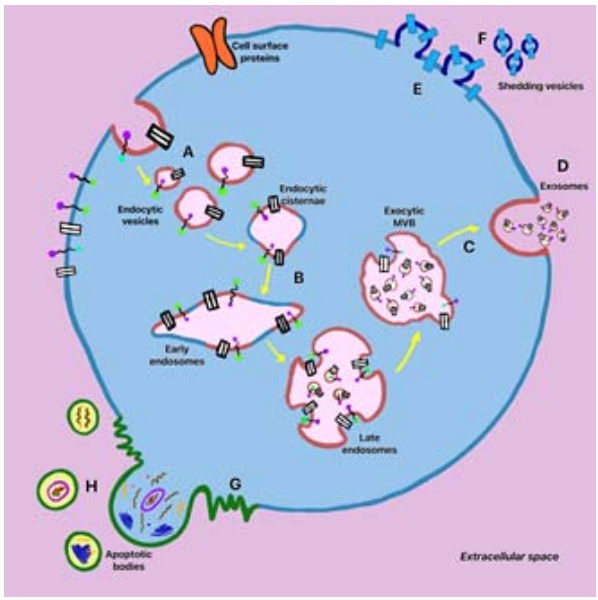

Figure 1. Routes of EV biogenesis.

Shedding vesicle biogenesis involves the budding of small cytoplasmic protrusions that eventually detach by fission, creating distinct membrane-bound structures. Although shedding vesicles share similar membrane components with their cells of origin, they selectively incorporate specific proteins through certain mechanisms that remain unclear.[7] Cholesterol-rich microdomains, known as lipid rafts, play a crucial role in the formation of shedding vesicles, as shown by reduced vesicle production following cholesterol depletion. Calcium (Ca^{2+}) influx is a significant factor that induces vesicle shedding, leading to different types (microvesicles and nanovesicles) based on their composition. Additionally, shedding vesicles differ among cell types and conditions, with some containing enzymes like metalloproteinases in tumor cells or integrins in platelets.[6]

Apoptotic bodies are heterogeneous vesicles that form during the final stages of apoptosis, a programmed cell death process essential for tissue homeostasis, development, and aging. Apoptosis begins with cellular shrinkage and chromatin condensation, facilitated by caspase activation, followed by membrane blebbing due to cytoskeletal breakdown. These blebs encapsulate fragmented DNA, proteins, and organelles, eventually detaching as apoptotic bodies, ranging from 50 to 5000 nm.[7] Entire cells can sometimes transform into a single large apoptotic body.[8]

The schematic shows several routes of EV biogenesis in a cell. (A-D) MVB-generated exosomes involve multiple sequential steps in the endocytic pathway: endocytosis occurs at the plasma membrane, which leads to the formation of early endosomes. Further membrane sorting forms MVBs which are found in intraluminal vesicles. Upon stimulation, exocytic MVBs fuse with the plasma membrane releasing exosomes into the extracellular matrix. (E-F) Vesicles may also be shed from the plasma membrane by the processes of blebbing. (G) By another route, apoptotic bodies may also form some vesicles during programmed cell death.

Molecular composition of EVs

EVs are complex structures with distinct molecular compositions, including lipids, RNA, DNA, and proteins, which contribute to their functional versatility. Their lipid bilayer is analogous to plasma membranes but differs significantly from single-layered lipoproteins such as HDL and LDL. The protein composition of EVs includes membrane proteins like integrins and tetra spanins (e.g., CD9, CD63, CD81), cytosolic proteins such as heat-shock proteins (HSP70, HSP90), and proteins involved in EV biogenesis like Alix and TSG101 in the ESCRT (endosomal sorting complexes required for transport) pathway. Exosomes, a subset of EVs, are enriched in sphingomyelin and de-saturated lipids, enhancing membrane rigidity, while cholesterol levels are elevated, promoting stability and potentially inducing apoptosis in recipient cells. Exosomes, a subset of EVs, which are secreted from MVBs have their membranes enriched with phosphatidylserine on the outer leaflet, facilitating internalization into recipient cells. They are known to be regulated specifically and show alteration of their cargoes under stress conditions9. Bioactive lipids like arachidonic acid and prostaglandin E2 are transported via EVs, along with lipid metabolic enzymes such as phospholipases D and A2, and fatty acid synthase under specific conditions. EVs primarily carry RNA molecules under 200 nucleotides, including microRNAs (miRNAs), transfer RNAs (tRNAs), and, in some cases, long noncoding RNAs (lncRNAs). Selective packaging mechanisms favor miRNAs through sequence motifs and modifications, and EV-RNAs can be translated after delivery. Additionally, certain EVs, particularly cancer-derived ones, contain circulating tumor DNAs and retrotransposon sequences, including Human Endogenous Retroviruses (HERVs) and Alu elements. Functionally, EVs can deliver bioactive proteins, including enzymes, transcription factors, and lipid metabolism modifiers, to recipient cells, influencing various cellular processes.[10]

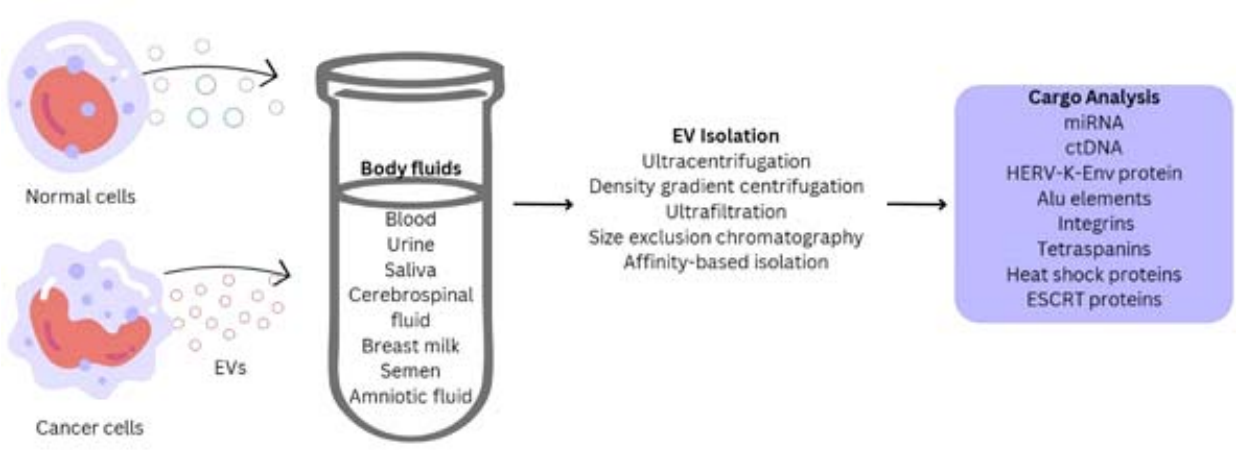

Figure 2. Schematic diagram of liquid biopsy-based cancer-derived EV analysis.

Body fluids and liquid biopsies

Biological fluids such as blood, urine, saliva, serum, cerebrospinal fluid, bile, and amniotic fluid are rich in EVs.[11] They reflect their cell of origin and are associated with the physiological and pathological status of the body. These fluids are easily accessible and are particularly useful in liquid biopsy applications. Blood contains a high density of EVs and is the most current source for liquid biopsies. Even though EV concentrations vary across different fluids, blood is the most commonly studied due to its high vesicle content and as it directly reflects systemic conditions as well as conditions like cancer and cardiovascular diseases.[11] EVs released from tumors to blood are crucial in cancer diagnosis as they reflect molecular alterations in tumors, enabling early detection and continuous monitoring.[12]

The EVs derived from both normal and cancer cells are released to the body fluids. By isolating and analyzing the EVs in body fluids, cancers can be diagnosed. EVs, Extracellular Vesicles; miRNA, microRNA; ctDNA, circulating tumor DNA; HERV, Human Endogenous Retrovirus; ESCRT, Endosomal Sorting Complexes Required for Transport.

One of the major advantages of using EVs in biological fluids for liquid biopsies is its non-invasive nature. This accessibility makes them ideal for repeated sampling without subjecting the patient to discomfort or a need for surgical procedures. Liquid biopsies allow for frequent and real-time monitoring of disease progression as well as therapeutic responses.[12] By tracking changes in EV biomarkers, shifts in the molecular profile of diseases like cancer can be detected, aiding in identifying recurrence or metastasis.[13] For example, a high amount of tumor material can be identified in circulating plasma EV DNA/RNA in metastatic prostate cancer patients. These can often be recognized before clinical symptoms become apparent. The ability to trail the molecular signature changes in response to medications allows for personalized treatment approaches such as optimizing drug routines based on the evolving disease profile of a patient. Therefore, liquid biopsies using EVs provide dynamic, real-time insights into healthcare compared to static traditional biopsies to make informed decisions regarding therapeutic options.

Relationship of EVs with cancers

EVs play a pivotal role in cancer biology by facilitating intercellular communication and influencing various aspects of tumor progression, including metastasis, immune evasion, and drug resistance. These vesicles carry a wide range of molecular cargoes, making them integral to cancer progression and therapy. In particular, cancer-associated fibroblast-derived exosomes (CDEs) serve as key signaling molecules between cancer cells and stromal cells, promoting oncogenic transformation. CDEs induce metabolic reprogramming in cancer cells, while also carrying miRNAs (e.g., miR-21, miR-378e, miR-143) that promote stemness and epithelial-mesenchymal transition (EMT), thereby increasing invasiveness. These exosomes further support metastasis by inducing neoplastic angiogenesis and activating key pathways like Wnt, contributing to drug resistance. Additionally, CDEs carry TGF-β1, which regulates long non-coding RNAs (lncRNAs) such as HOTAIR, further promoting metastasis. For example, in breast cancer, exosomes containing TGF-β1 stimulate EMT and metastasis, highlighting potential therapeutic targets to block these effects.[14]

Exosomes are emerging as powerful diagnostic and prognostic tools in oncology. Their stability in liquid biopsies makes them a non-invasive alternative to tissue biopsies, providing opportunities for early cancer detection and disease monitoring. For instance, in prostate cancer, exosomes exhibit unique protein profiles associated with the androgen receptor (AR) pathway, which can serve as biomarkers for castration-resistant prostate cancer (CRPC) and therapeutic resistance.[15]

Extracellular vesicle (EV)-derived nucleic acids, including microRNAs (miRNAs) and messenger RNAs (mRNAs), are increasingly recognized as valuable biomarkers in cancer diagnostics. These nucleic acids enable the detection of tumor presence, metastatic progression, and responses to treatment.[16] Mutational analyses of EV-derived DNA, such as KRAS and p53 mutations, have shown utility in identifying early-stage cancers like pancreatic cancer and in monitoring genetic changes associated with cancer progression and therapeutic resistance. Specific EV-associated mutations, such as the EGFR T790M mutation in non-small cell lung cancer (NSCLC), offer high sensitivity and specificity, improving diagnostic accuracy and aiding in treatment decisions.[17] Additionally, mRNA and miRNA profiles within EVs provide comprehensive tumor-specific information, revealing mutations and regulatory RNA expression patterns linked to cancer. These features make EV nucleic acids crucial tools for early cancer detection and the assessment of therapeutic outcomes across various cancer types.[16]

Future perspectives

EVs are emerging as precision tools, offering non-invasive means to diagnose and monitor diseases. EVs derived from tumors (tumor-derived extracellular vesicles or TDEs) present in the blood and other bodily fluids of cancer patients may allow early detection and may act as markers of prognosis and therapy response.

EVs may increase the efficiency of cancer therapeutics, by acting as natural nanocarriers for targeted drug delivery, potentially reducing systemic toxicity and enhancing treatment efficacy. To increase the targeting efficacy, EVs can be modified by surface engineering to enhance binding to specific cell types or loading with chemo-therapeutic agents to directly inhibit tumor growth.

Despite the promising application of EVs, several challenges and considerations remain in clinical translation. The yield of isolated EVs can be low, and their stability during storage and transportation poses additional challenges. Developing robust methods that ensure high yields while maintaining integrity is crucial for clinical applications. The regulatory landscape also has to be navigated carefully, establishing clear guidelines for the clinical use of EV-based therapies, to facilitate their integration into standard care practices.

Conclusion

EVs are unfolding a promising new area in precision medicine and cancer diagnostics. As reviewed, EVs are essential for facilitating intercellular communication using a wide range of biomolecules. In cancer, they are constantly being reprogrammed by their microenviron-ment and are highly equipped to re-educate sites for metastasis. Their capacity to be separated non-invasively from body fluids makes them perfect candidates for liquid biopsies, which have several benefits over conventional tissue biopsies. Moreover, the molecular cargoes in EVs offer critical information in oncology to evaluate drug resistance, and metastatic cues and to modify therapies accordingly. Therefore, the use of EVs in personalized medicine holds promise for improving treatment efficacy by customizing therapies based on individual molecular profiles. In conclusion, the integrated outlook of EV analysis in the cancer clinic has the potential to transform diagnosis and therapy, for developing more efficient and patient-centered medical solutions.

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