IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Role Of Extracellular Vesicles In Intercellular Communication

Arpita Prajapati[1], Avinash[2] Gond, DR. Rajneesh Kumar Gupta[1*]

[1]Department of Pharmaceutics, Goel Institute of Pharmacy & Science, Lucknow, Uttar Pradesh, India, Azamgarh, U.P INDIA

[2]Department of Pharmaceutics, Goel Institute of Pharmacy & Science, Lucknow, Uttar Pradesh, India

***Corresponding author**

Dr. Rajneesh Kumar Gupta

Designation: Professor and head of department,

Goel Institute of Pharmacy & Science,

Lucknow, Uttar Pradesh, India

Abstract

Extracellular vesicles are believed to play a vital role in various diseases. The study of extracellular vesicles (EVs) and nanoparticles (NPs) is rapidly expanding because recent discoveries have revealed a much greater complexity and diversity than was appreciated only a few years ago. New types of EVs and NPs have recently been described. Proteins and nucleic acids previously thought to be packaged in exosomes appear to be more enriched in different types of EVs and in two recently identified membranous NPs, exomeres and super meres. Thus, our understanding of cell biology and intercellular communication facilitated by the release of EVs and NPs is in flux. In this review, we describe the different types of EVs and NPs, highlight recent advances, and present major outstanding questions. Despite their clinical potential, the lack of sensitive preparatory and analytical technologies for EVs poses a barrier to clinical translation. New analytical platforms, including molecular ones, are thus actively being developed to address these challenges. Recent advances in the field are expected to have a far-reaching impact in both basic and translational studies.

Keywords: Extracellular vesicles, Nanoparticles, Exosomes, Exomeres, Supermeres, Intercellular communication, Cell biology, Clinical translation, Molecular analysis, Diagnostic biomarkers.

Introduction

Extracellular vesicles (EVs) have emerged as highly promising carriers of active pharmaceutical ingredients in the rapidly evolving field of precision medicine (1). Over the past decade, the study of EVs and extracellular nanoparticles has expanded significantly due to major scientific discoveries that revealed a much greater level of complexity, heterogeneity, and functional diversity than was previously appreciated (2). These naturally occurring nanosized particles are secreted by almost all cell types and play essential roles in both normal physiological functions and disease-related processes (3). Their unique biological properties, including biocompatibility, low immunogenicity, intrinsic targeting ability, and capacity to transport a wide range of biomolecules, have generated enormous interest in their potential applications in diagnostics, therapeutics, and drug delivery systems (4).

Extracellular vesicles are membrane-bound nanoparticles that mediate intercellular communication by transferring biologically active cargo molecules between cells (5). These cargoes include proteins, lipids, messenger RNAs (mRNAs), microRNAs (miRNAs), long non-coding RNAs, carbohydrates, metabolites, and DNA fragments (6). By transferring these molecules, EVs regulate numerous cellular and molecular pathways involved in immune modulation, inflammation, angiogenesis, tissue regeneration, cellular differentiation, and maintenance of tissue homeostasis (7). EVs are generally classified into several major subtypes, including exosomes, microvesicles, and apoptotic bodies, based on their size, composition, and mechanisms of biogenesis (8). Exosomes originate from the endosomal pathway through the formation of multivesicular bodies, whereas microvesicles are generated through direct outward budding of the plasma membrane. Apoptotic bodies are released during programmed cell death (9).

Recent advances in the field have demonstrated that extracellular particles are far more diverse than these traditional classifications suggest. Newly identified extracellular nanoparticles such as exomeres and supermeres have significantly transformed the understanding of extracellular communication (10). Exomeres are small non-membranous nanoparticles enriched with specific proteins, lipids, nucleic acids, and metabolites, while supermeres represent another recently identified bioactive nanoparticle population containing disease-associated proteins and RNAs (11). Importantly, several biomolecules previously believed to be predominantly packaged within exosomes are now known to be more enriched in exomeres and supermeres (12). These findings have challenged conventional concepts regarding EV composition and function, highlighting the remarkable heterogeneity and specialization of extracellular particles (13).

One of the most important features of EVs is their role in long-range intercellular communication. EVs can circulate through various biological fluids, including blood, urine, saliva, cerebrospinal fluid, lymph, and breast milk, enabling communication between distant tissues and organs (14). Because EVs carry molecular signatures that reflect the physiological or pathological state of their parent cells, they are increasingly being recognized as highly promising circulating biomarkers, often referred to as “liquid biopsies” (15). Unlike traditional tissue biopsies, liquid biopsies are minimally invasive and can provide real-time information about disease progression, treatment response, and molecular changes occurring within tissues (16). EV-derived biomarkers have shown significant potential in the diagnosis and monitoring of various diseases, including cancer, cardiovascular diseases, neurodegenerative disorders, autoimmune diseases, infectious diseases, and metabolic syndromes (17).

In cancer biology, tumor-derived EVs are known to influence multiple aspects of tumor progression, including angiogenesis, metastasis, immune suppression, and remodeling of the tumor microenvironment (18). They can transfer oncogenic proteins, nucleic acids, and signaling molecules to surrounding or distant cells, thereby promoting tumor growth and metastasis (19). Similarly, in neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease, EVs may facilitate the spread of pathological proteins between neurons, contributing to disease progression (20). In cardiovascular diseases, EVs participate in endothelial dysfunction, inflammation, thrombosis, and vascular remodeling (21). Furthermore, EVs play

significant roles in immune regulation and host–pathogen interactions during infectious and inflammatory diseases (22).

The natural biological properties of EVs make them particularly attractive candidates for therapeutic drug delivery. Unlike synthetic nanoparticles, EVs possess excellent biocompatibility and reduced toxicity because they are naturally derived from cells (23). They also exhibit low immunogenicity, enabling them to circulate within the body with minimal immune clearance (24). Another important advantage is their ability to cross biological barriers, including the blood–brain barrier, which is a major limitation for many conventional drug delivery systems (25). Additionally, EVs display tissue tropism mediated by specific surface molecules such as integrins, glycans, tetraspanins, and adhesion proteins, allowing selective interaction with target tissues and cells (26).

Various methods have been developed to load therapeutic agents into EVs for clinical use (27). Therapeutic cargoes may include small-molecule drugs, nucleic acids, proteins, peptides, and gene-editing tools (28). Loading strategies are generally classified into endogenous and exogenous approaches. In endogenous loading, donor cells are engineered or treated so that therapeutic molecules are naturally incorporated into EVs during their formation. Exogenous loading methods involve introducing therapeutic agents into isolated EVs using techniques such as electroporation, sonication, freeze–thaw cycles, extrusion, incubation, or chemical transfection (29). These methods aim to maximize drug loading efficiency while preserving EV integrity and biological activity (30).

In addition to cargo loading, several engineering and surface modification strategies have been explored to improve EV stability, circulation time, and targeting efficiency (31). Surface functionalization techniques involve modifying EV membranes with targeting ligands, peptides, antibodies, aptamers, or polymers to enhance selective delivery to specific tissues or disease sites (32). PEGylation and other chemical modifications have also been used to prolong systemic circulation and reduce rapid clearance by the mononuclear phagocyte system (33). Such advances are expected to improve the therapeutic efficacy and precision of EV-based drug delivery systems.

Despite the enormous promise of EVs, several technical and translational challenges remain. One of the primary limitations is the lack of standardized and highly sensitive methods for EV isolation, purification, characterization, and quantification (34). Conventional techniques such as ultracentrifugation, precipitation, filtration, and size-exclusion chromatography often result in low purity or inconsistent recovery of EV populations (35). Furthermore, the heterogeneity and nanoscale size of EVs complicate their accurate characterization and analysis (36).

To address these limitations, researchers are developing advanced analytical technologies and molecular characterization platforms (37). Techniques such as nanoparticle tracking analysis, tunable resistive pulse sensing, cryo-electron microscopy, atomic force microscopy, high-resolution flow cytometry, Raman spectroscopy, and surface plasmon resonance are improving EV detection and characterization (38). Molecular profiling methods including proteomics, transcriptomics, lipidomics, and metabolomics are enabling detailed analysis of EV cargo composition and biological functions (39). In addition, microfluidic platforms and single-vesicle analysis technologies are providing more rapid, sensitive, and high-throughput approaches for EV isolation and analysis (40).

Overall, extracellular vesicles and extracellular nanoparticles represent one of the most rapidly advancing areas in modern biomedical science. Their ability to mediate intercellular communication, carry therapeutic cargoes, and function as minimally invasive biomarkers highlights their immense potential in precision medicine (41). Continued advances in EV isolation, molecular characterization, and engineering strategies are expected to pave the way for innovative diagnostic tools, targeted drug delivery systems, and personalized therapeutic approaches for a wide range of human diseases (42).

EV BIOGENESIS AND CONTENTS

Several routes to EV generation exist, although the exact mechanisms remain largely unknown. Based on their biogenesis, EVs are currently classified into three broad groups: exosomes, microvesicles, and apoptotic bodies.

Major types of extracellular particles

Vesicle	Size (nm)	Density (g/mL)	Origin	Markers
Exosomes	40–200	1.13–1.18	Endosomes	Tetraspanins, Alix, TSG101
Microvesicles	200–2000	1.16–1.19	Plasma membrane	Integrins, selectins, and CD40
Apoptotic bodies	500–2000	1.16–1.28	Plasma membrane, endoplasmic reticulum	Phosphatidylserine, genomic DNA
High-density lipoprotein particles	7–13	>1.06	Hepatocyte	Apolipoproteins, phospholipids, cholesterol, and triglycerides
Low-density lipoprotein particles	21–27	1.02–1.06	Hepatocyte	Apolipoproteins, phospholipids, cholesterol, and triglycerides

Route of administration of EVs

Administration Route	Experimental Model	Source of EVs	Therapeutic Cargo	Target Tissues
Intravenous	Mice	Plasma		Liver
		Serum	miR-124	CNS
		Serum		Lung
		Serum		Systemic effects
		Mice serum, supernatant of cultured myotubes	miR-21	Kidney
		Blood	Dopamine	CNS
		Urine	Klotho	Kidney
		Milk		Liver, spleen, heart, lungs
		MSC	Paclitaxel	Subcutaneous tumors and distant metastases
		MSC		Heart
		MSC		Liver
		MSC	miR-210	Brain
		MSC	miR-let7	Atherosclerotic plaque
		MSC	miR-125b	Heart
		MSC		Bone marrow

		MSC		CNS
		MSC, liver stem cells		Subcutaneous tumor
		AdSC	miR-199[a]	Orthotopic tumor
		AdSC		CNS
		AdSC	miR-17	Liver
		AdSC		Skin
		Bone marrow stromal cells		Liver, lungs, bone
		HEK293T cells	Anti-miR-214	Subcutaneous tumor
		HEK293T cells	Curcumin, miR-143a	Tumor cells
		HEK293T cells	miR-199a-3p	Subcutaneous tumor
		HEK293 cells		Mammary tumor
		Dendritic cells		Spleen
		Dendritic cells	siRNA	Brain
		Immature dendritic cells	Doxorubicin	Mammary tumor
		Mouse brain endothelial cells	miR-126	CNS
		Endothelial colony-forming cells	miR-486-5p	Kidney
		Gastric epithelial cells		Aorta

		Neural primary stem cells		CNS
		BMD2a cells		Lungs, liver, spleen, brain
		Liver	miR-130a-3p	Systemic effects
		Schwann cells		Peripheral nerves
		Astrocytes		CNS
		Microglial cells	miR-124-3p	CNS
		Breast cancer cells	miR-126	Lung cancer cells
		Tumour-cell exocytosed-exosome biomimetic porous silicon nanoparticle	Doxorubicin	Tumor cells
		Gastric cancer cells		Blood myeloid-derived suppressor cells
		Pancreas carcinoma cells		Liver, spleen, lungs
		Macrophages	Brain-derived neurotrophic factor	CNS
		L929 cells	Methotrexate	Glioblastoma tissue
		Ginger roots	siRNA	Subcutaneous tumor
	Rats	Serum		CNS
		MSC	miR-544	CNS
		MSC	CC chemokine	CNS

			receptor type 2	
		MSC		CNS
		MSC		CNS
		MSC	miR-17-92 cluster	CNS
		MSC		Pulmonary vasculature
		MSC		Colon
		MSC		Vein graft
		MSC		Heart
		MSC		Lungs
		MSC	miR-29b	Spinal cord
		MSC		Pancreas
		MSC		Carotid artery
		MSC		Bone
		AdSC		Penile tissue
		AdSC	miR-126	CNS
		AdSC		Brain, spleen
		AdSC		Systemic effects
		AdSC		CNS
		Human urinary stem	miR-26a	CNS

		cells		
		Human fetal amniotic fluid stem cells		Heart
		Cardiac progenitor cells	miR-146a	Heart
		Cardiac progenitor cells		Cardiomyocytes
		Cardiac stem cells		Heart
		Human iPSCs		Bone
		Urinary stem cells		Kidney
		Aortic adventitial fibroblasts	miR-155-5p	Aorta and mesenteric artery
		HEK293T cells	miR-21 antisense	CNS
		HEK293T cells	siRNA	CNS
		Human renal tubular cells		Kidney
		Renal cells		Kidney
	Patients with COVID-19	MSC		Systemic effects
	Monkeys	MSC		CNS
Intraperitoneal	Mice	Liver stem cells, MSC		Subcutaneous tumor
Intramyocardial	Rats	MSC		Heart
Subcutaneous	Mice	AdSC		Skin
		Echinostoma caproni		Systemic immune

				response
Intramuscular	Mice	Primary mouse satellite cells	miR-29	Kidney, muscle
		Cardiac stem cells		Muscle
		Human iPSCs		Muscle
	Chicken	Serum		Systemic immune response
Intrathecal	Rats	MSC		Peripheral nerves
Oral	Mice	Bovine milk		Liver, spleen, heart, lungs, kidney
Intraocular	Rabbit	MSC		Retina
Subconjunctival	Rabbit	MSC		Retina

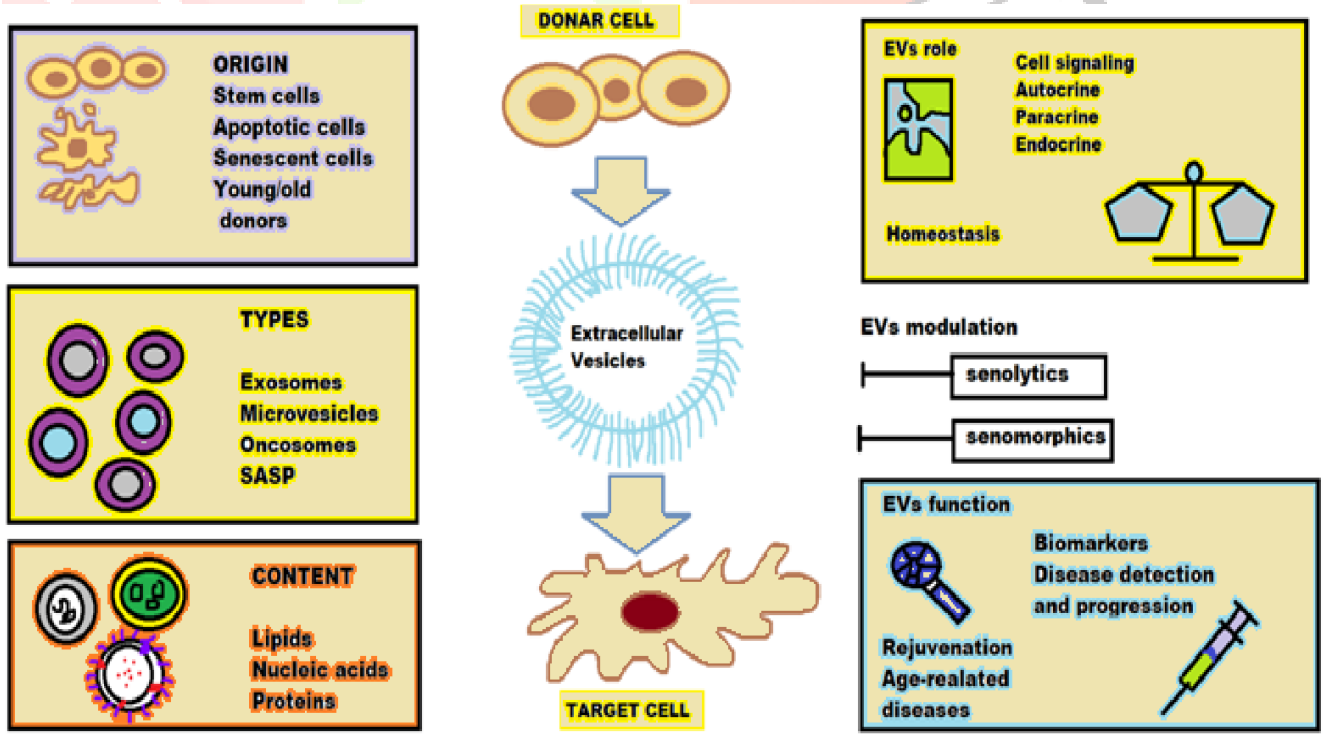

Fig1 schematic representative of extracellular vehicles (EVs) illustrating their origin, types, content, biological roles, modulation, and therapeutic application in cellular communication and age related disease.

Exosomes

Exosomes are among the most extensively studied subtypes of extracellular vesicles (EVs) and play major roles in intercellular communication, disease progression, biomarker discovery, and therapeutic drug delivery (43). Exosomes are produced through the inward invagination of the endosomal membrane pathway. Initially, inward budding of the plasma membrane forms early endosomes. Subsequently, further inward budding of the limiting membrane within the endosome generates small intraluminal vesicles (ILVs), leading to the formation of multivesicular bodies (MVBs), which are characterised by the presence of multiple vesicles within their lumen (44). During this process, cytosolic contents, transmembrane proteins, peripheral membrane proteins, lipids, and nucleic acids become incorporated into the invaginating membrane (45).

Multivesicular bodies may follow two distinct pathways. They can fuse with lysosomes, leading to degradation of their contents, or fuse with the plasma membrane and release their intraluminal vesicles into the extracellular environment via exocytosis (46). Once released, these vesicles are called exosomes. Exosomes are small membrane-bound lipid vesicles generally ranging from 30–200 nm in diameter (47). Due to the double invagination process involved in their biogenesis, the membrane orientation and protein topology of exosomes closely resemble those of the parent cell plasma membrane (48).

Exosomes contain a wide variety of biologically active molecules, including proteins, messenger RNA (mRNA), microRNA (miRNA), long non-coding RNA, lipids, enzymes, metabolites, and DNA fragments, enabling them to act as highly efficient mediators of cell-to-cell communication (49). They participate in numerous physiological and pathological processes, including immune regulation, inflammation, angiogenesis, tissue regeneration, tumour progression, and metastasis (50). Because exosomes are present in several body fluids, including blood, urine, saliva, breast milk, and cerebrospinal fluid, they are also being explored as minimally invasive biomarkers, or “liquid biopsies,” for disease diagnosis and prognosis (51).

In recent years, exosomes have attracted significant attention as promising nanocarriers for therapeutic drug delivery because of their low immunogenicity, excellent biocompatibility, ability to cross biological barriers, and natural targeting capabilities (52). Exosomes can transport a broad range of therapeutic cargos, including chemotherapeutic drugs, proteins, peptides, nucleic acids, and gene-editing molecules. Furthermore, the exosomal surface can be engineered or functionalized with antibodies, ligands, peptides, or other targeting molecules to improve selective delivery to tumour tissues and diseased cells (53).

In 2023, Ali Akbari, Fereshteh Nazari-Khanamiri, Mahdi Ahmadi, Maryam Shoaran, and Jafar Rezaie published a review titled *Engineered Exosomes for Tumour-Targeted Drug Delivery* (54). Their work highlighted the increasing interest in extracellular vesicle-mediated drug delivery systems as alternatives to synthetic nanocarriers for cancer therapy. The authors explained that exosomes can be loaded with various therapeutic cargos while maintaining high biocompatibility and low toxicity. They also discussed different methods for surface engineering and functionalization to improve tumour-targeting efficiency. Despite promising outcomes, they emphasised that the clinical translation of exosome-based therapies remains challenging due to challenges in large-scale production, purification, cargo-loading efficiency, and standardisation (55).

Similarly, Somaye Sadeghi, Fahimeh Ramezani Tehrani, Safa Tahmasebi, Abbas Shafiee, and Seyed Mahmoud Hashemi investigated exosome engineering in cell therapy and drug delivery (56). Their study demonstrated that cell-derived exosomes possess several advantageous features, including low immunogenicity, high physicochemical stability, efficient tissue penetration, and innate long-distance communication. The authors also highlighted the use of exosomes for delivering therapeutic agents, including immunomodulators, antisense oligonucleotides, therapeutic drugs, and vaccine molecules.

However, despite extensive investigation, routine clinical application of exosome-based therapeutics still requires substantial technological development and clinical validation (57).

Microvesicles

Microvesicles are another important subtype of extracellular vesicles, formed by outward blebbing or budding of the plasma membrane rather than via the endosomal pathway (58). They are generally larger than exosomes, typically ranging from 100 nm to 1 µm in diameter, although size variations may occur depending on the parent cell type and physiological conditions (59).

The plasma membrane maintains lipid asymmetry through the uneven distribution of phospholipids. The outer leaflet of the membrane is enriched with phosphatidylcholine and sphingomyelin, whereas the inner leaflet predominantly contains phosphatidylserine and phosphatidylethanolamine (60). During microvesicle formation, an increase in intracellular calcium (Ca^{2+}) concentration disrupts this membrane asymmetry by activating enzymes such as scramblases and floppases, leading to transbilayer phospholipid redistribution (61). This redistribution causes membrane instability and promotes outward membrane blebbing.

Simultaneously, Ca^{2+}-dependent proteolytic enzymes degrade membrane-associated cytoskeletal proteins, thereby facilitating vesicle budding and release from the plasma membrane (62). One proposed mechanism of microvesicle release involves actomyosin-mediated membrane abscission, regulated by activation of ADP-ribosylation factor 6 (ARF6) signalling pathways (63). Interestingly, certain aspects of microvesicle biogenesis resemble viral budding mechanisms observed in infected cells.

Microvesicles contain proteins, lipids, nucleic acids, enzymes, cytokines, and signalling molecules that reflect the molecular composition of their parent cells (64). They are involved in several biological and pathological processes, including coagulation, inflammation, angiogenesis, immune modulation, endothelial dysfunction, and tumour progression (65). In cancer, tumour-derived microvesicles can facilitate metastasis and modify the tumour microenvironment by transferring oncogenic molecules to recipient cells (66). Because of their biological significance and accessibility in body fluids, microvesicles are also being explored as potential biomarkers and therapeutic delivery systems.

Oncosomes and Large Oncosomes

The terms “oncosomes” and “large oncosomes” are distinct and should not be used interchangeably because they differ in origin, conceptual context, extracellular vesicle size, and molecular composition (67). These terms were introduced independently by different research groups to describe specific consequences of malignant transformation associated with the production of extracellular vesicles. Importantly, the term “oncosomes” does not simply refer to EVs released from cancer cells; rather, it highlights extracellular vesicles associated with the transfer of oncogenic macromolecules and abnormalities in vesicle biogenesis during tumour progression (68).

Large oncosomes are atypically large extracellular vesicles released predominantly from highly migratory and invasive tumour cells exhibiting amoeboid phenotypes (69). These vesicles contain oncogenic proteins, metabolites, lipids, nucleic acids, signalling molecules, and membrane receptors that can influence surrounding stromal and immune cells within the tumour microenvironment. Large oncosomes are strongly associated with tumour invasion, metastasis, angiogenesis, and aggressive cancer behaviour (70).

On 18 July 2019, Chiara Ciardiello, Alessandra Leone, Paola Lanuti, Maria S. Roca, Tania Moccia, Valentina R. Minciacchi, Michele Minopoli, and Vincenzo Gigantino published a study on large oncosomes overexpressing integrin alpha-V and their role in prostate cancer invasion via AKT activation (71). Their research identified large oncosomes in highly migratory and invasive prostate cancer cells and

demonstrated that these vesicles contribute to tumour adhesion, invasion, and aggressive phenotypic behaviour. The study also showed altered cytoskeletal dynamics and distinct proteomic profiles in resistant prostate cancer cell lines associated with large oncosome production.

In 2019, Maria Conchetta Cufaro and Damiana Pieragostino described large oncosomes as emerging mediators of intercellular communication during tumour progression and metastasis (72). Their work explained how tumour-derived extracellular vesicles interact with fibroblasts, endothelial cells, immune cells, and extracellular matrix (ECM) components within the tumour microenvironment. Large oncosomes released from amoeboid tumour cells facilitate tumour migration, angiogenesis, immune modulation, extracellular matrix remodelling, and metastatic dissemination by transferring oncogenic signalling molecules. The study also highlighted the involvement of molecules such as transforming growth factor-beta (TGF-β) and fibronectin-1 (FN1) in extracellular vesicle-mediated tumour progression.

Overall, exosomes, microvesicles, and oncosomes represent highly specialised extracellular vesicle populations with distinct mechanisms of biogenesis, molecular compositions, and biological functions. Their growing importance in disease progression, biomarker discovery, targeted drug delivery, and precision medicine continues to drive extensive research in modern biomedical science.

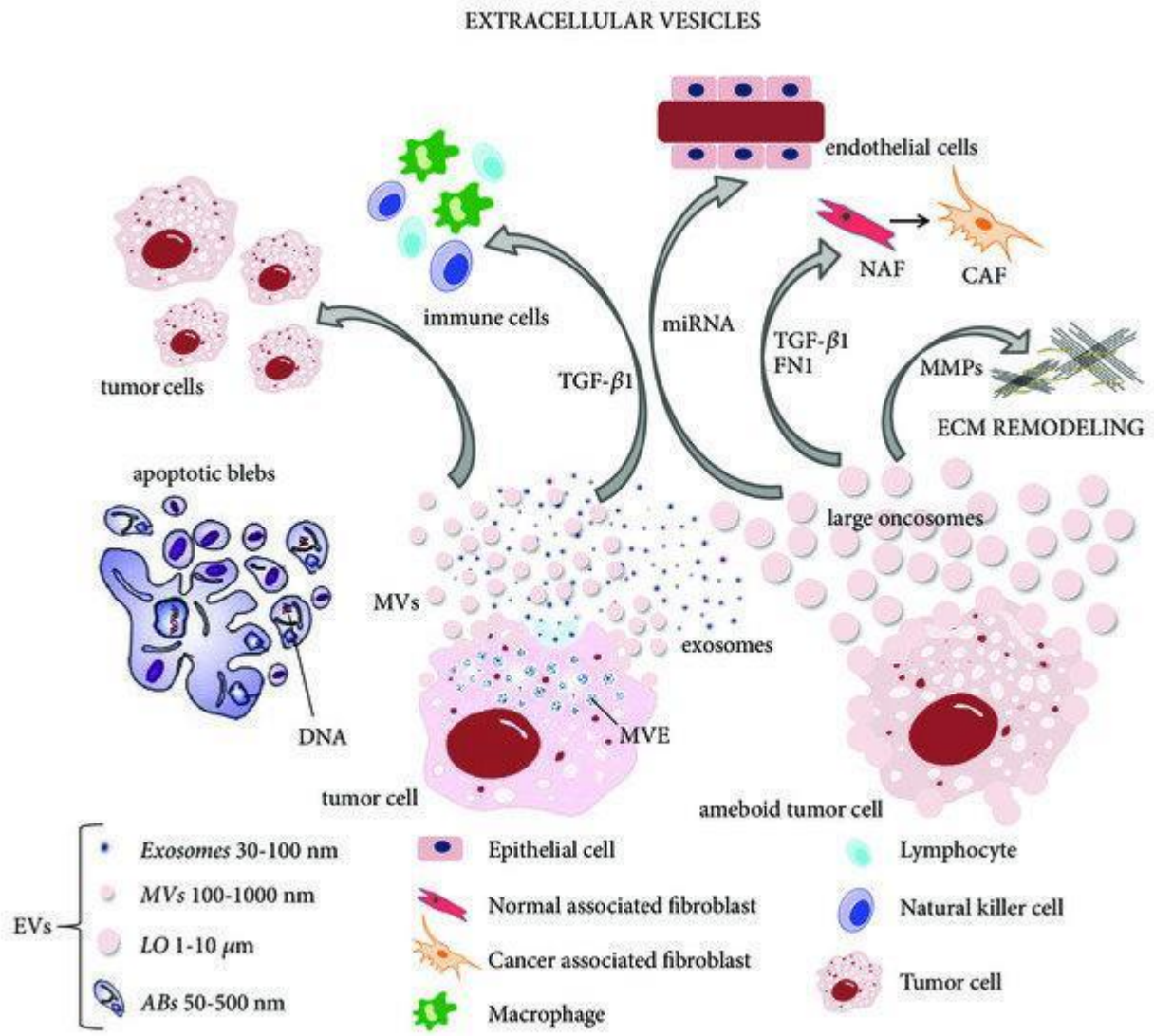

Fig 2 illustration of extracellular vesicle mediated communication in the tumour microenvironment , showing the formation and release of exosomes, macrovesicles , apoptin bodies , and large oncomes from tumour cell and their role in immune modulation extracellular matrix remoulding, and cancer progression.

Conclusion

The clinical applications of extracellular vesicles (EVs) in diagnostics, prognostics, therapeutics, and drug delivery have been widely demonstrated and continue to attract significant scientific interest (73). EVs are nanosized membrane-bound particles released by almost all cell types and are increasingly recognised as important mediators of intercellular communication. They carry a variety of biologically active molecules, including proteins, lipids, messenger RNA (mRNA), microRNA (miRNA), DNA fragments, and metabolites, which reflect the physiological or pathological condition of their parent cells (74). Because EVs are present in biological fluids such as blood, urine, saliva, cerebrospinal fluid, and breast milk, they are considered promising non-invasive biomarkers or “liquid biopsies” for disease diagnosis and monitoring (75).

EVs have shown considerable potential in cancer diagnosis and prognosis, where tumour-derived EVs carry oncogenic molecules associated with tumour progression, metastasis, and therapeutic resistance (76). Similarly, EV-associated biomarkers are being explored in cardiovascular, neurodegenerative, autoimmune, and infectious diseases. In addition to their diagnostic value, EVs also possess significant

therapeutic potential due to their natural biocompatibility, low immunogenicity, and ability to cross biological barriers such as the blood–brain barrier (77). Their intrinsic targeting capabilities make them promising carriers for delivering therapeutic agents, including drugs, proteins, peptides, small interfering RNA (siRNA), and gene-editing molecules (78).

Despite these advantages, one of the major challenges in EV research is the lack of standardised methods for EV isolation, purification, characterisation, and analysis (79). Several techniques are currently used to isolate EVs, including ultracentrifugation, precipitation-based methods, size-exclusion chromatography, ultrafiltration, and immunoaffinity capture. However, each method has its own advantages and limitations regarding purity, yield, sensitivity, scalability, and reproducibility (80). Importantly, studies have demonstrated that different isolation methods used to isolate exosomes from the same cell type can yield EV populations with distinct proteomic and molecular profiles, complicating comparisons between studies and affecting reproducibility.

This variability suggests that instead of attempting to establish a universal set of exosomal and non-exosomal marker proteins applicable to all cell types and isolation methods, it may be more beneficial to identify marker proteins specific to particular cell types or isolation techniques (81). Such an approach may improve consistency and reliability in EV characterisation studies.

To improve rigour and reproducibility in EV research, the International Society for Extracellular Vesicles (ISEV) introduced the Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018) guidelines (82). These guidelines provide recommendations regarding EV nomenclature, isolation procedures, characterisation methods, and reporting standards. MISEV2018 emphasises the importance of detailed methodological reporting, the use of multiple characterisation techniques, and the inclusion of appropriate controls to ensure reliability and comparability across studies. Incorporating these recommendations is essential for improving the standardisation and clinical translation of EV-based diagnostics and therapeutics in future biomedical research.

References

1. Yáñez-Mó M, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles.* 2015.

2. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science.* 2020.

3. Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J Cell Biol.* 2013.

4. EL Andaloussi S, et al. Extracellular vesicles: Biology and emerging therapeutic opportunities. *Nat Rev Drug Discov.* 2013.

5. Théry C, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018). *J Extracell Vesicles.* 2018.

6. Valadi H, et al. Exosome-mediated transfer of mRNAs and microRNAs. *Nat Cell Biol.* 2007.

7. Tkach M, Théry C. Communication by extracellular vesicles. *Cell.* 2016.

8. Doyle LM, Wang MZ. Overview of extracellular vesicles. *Methods Mol Biol.* 2019.

9. van Niel G, et al. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol.* 2018.

10. Zhang H, et al. Identification of distinct nanoparticles and subsets of extracellular vesicles. *Nat Cell Biol.* 2018.

11. Zhang Q, et al. Supermeres are functional extracellular nanoparticles. *Cell.* 2021.

12. Kalluri R. The biology and function of exosomes in cancer. *J Clin Invest.* 2016.

13. Mathieu M, et al. Specificities of secretion and uptake of exosomes and other extracellular vesicles. *Nat Cell Biol.* 2019.

14. Lässer C, et al. Human saliva, plasma and breast milk exosomes contain RNA. *BMC Genomics.* 2011.

15. Machado de Menezes M, et al. Extracellular vesicles as biomarkers in cancer. *Cancer Lett.* 2021.

16. Pantel K, Alix-Panabières C. Liquid biopsy in cancer patients. *Cancer Cell.* 2019.

17. Zhou B, et al. Extracellular vesicles in diagnosis and therapy. *Signal Transduct Target Ther.* 2020.

18. Becker A, et al. Extracellular vesicles in cancer. *Cell.* 2016.

19. Peinado H, et al. Melanoma exosomes educate bone marrow progenitor cells. *Nat Med.* 2012.

20. Guo M, et al. Role of extracellular vesicles in neurodegenerative diseases. *Neural Regen Res.* 2020.

21. Jansen F, et al. Endothelial microparticles in cardiovascular diseases. *Circ Res.* 2017.

22. Schorey JS, Harding CV. Extracellular vesicles and infectious diseases. *Nat Rev Microbiol.* 2016.

23. Vader P, et al. Extracellular vesicles for drug delivery. *Adv Drug Deliv Rev.* 2016.

24. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as drug carriers. *Nat Nanotechnol.* 2021.

25. Banks WA, et al. Extracellular vesicles and the blood-brain barrier. *Pharmacol Ther.* 2020.

26. Hoshino A, et al. Tumour exosome integrins determine organotropic metastasis. *Nature.* 2015.

27. Fuhrmann G, et al. Engineering extracellular vesicles for drug delivery. *Nat Rev Drug Discov.* 2023.

28. Kamerkar S, et al. Exosomes facilitate therapeutic targeting. *Nature.* 2017.

29. Johnsen KB, et al. A comprehensive overview of exosome loading techniques. *Drug Deliv Transl Res.* 2014.

30. Wiklander OPB, et al. Extracellular vesicle in vivo biodistribution. *J Extracell Vesicles.* 2015.

31. Armstrong JPK, Stevens MM. Strategic design of extracellular vesicle drug delivery systems. *Adv Drug Deliv Rev.* 2018.

32. Liang Y, et al. Engineered extracellular vesicles for targeted drug delivery. *Signal Transduct Target Ther.* 2021.

33. Kooijmans SAA, et al. PEGylated and targeted extracellular vesicles. *J Control Release.* 2016.

34. Konoshenko MY, et al. Isolation of extracellular vesicles. *Biomed Res Int.* 2018.

35. Li P, Kaslan M, et al. Progress in exosome isolation techniques. *Theranostics.* 2017.

36. Erdbrügger U, Lannigan J. Analytical challenges of extracellular vesicles. *Cytometry A.* 2016.

37. Shao H, et al. New technologies for analysis of extracellular vesicles. *Chem Rev.* 2018.

38. Wang Y, et al. Advanced technologies for extracellular vesicle analysis. *Biosens Bioelectron.* 2021.

39. Simpson RJ, et al. Extracellular vesicle proteomics and transcriptomics. *Proteomics.* 2009.

40. Contreras-Naranjo JC, et al. Microfluidics for exosome isolation and analysis. *Lab Chip.* 2017.

41. Kalluri R, LeBleu VS. Discovery and functions of extracellular vesicles. *Science.* 2020.

42. Murphy DE, et al. Extracellular vesicle-based therapeutics. *Nat Rev Drug Discov.* 2019.

43. Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. *J Cell Biol.* 2013.

44. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol.* 2018.

45. Théry C, Zitvogel L, Amigorena S. Exosomes: Composition, biogenesis and function. *Nat Rev Immunol.* 2002.

46. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles. *Nat Cell Biol.* 2019.

47. Doyle LM, Wang MZ. Overview of extracellular vesicles and methods of exosome isolation and analysis. *Methods Mol Biol.* 2019.

48. Valadi H, Ekström K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs between cells. *Nat Cell Biol.* 2007.

49. Tkach M, Théry C. Communication by extracellular vesicles. *Cell.* 2016.

50. Becker A, Thakur BK, Weiss JM, et al. Extracellular vesicles in cancer. *Cell.* 2016.

51. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease. *Cancer Cell.* 2019.

52. Vader P, Mol EA, Pasterkamp G, Schiffelers RM. Extracellular vesicles for drug delivery. *Adv Drug Deliv Rev.* 2016.

53. Liang Y, Duan L, Lu J, Xia J. Engineering exosomes for targeted drug delivery. *Signal Transduct Target Ther.* 2021.

54. Akbari A, Nazari-Khanamiri F, Ahmadi M, Shoaran M, Rezaie J. Engineered exosomes for tumour-targeted drug delivery. *Molecules.* 2023.

55. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as next-generation drug delivery systems. *Nat Nanotechnol.* 2021.

56. Sadeghi S, Tehrani FR, Tahmasebi S, Shafiee A, Hashemi SM. Exosome engineering in cell therapy and drug delivery. *J Control Release.* 2022.

57. EL Andaloussi S, Mäger I, Breakefield XO, Wood MJA. Extracellular vesicles: Biology and therapeutic opportunities. *Nat Rev Drug Discov.* 2013.

58. Cocucci E, Meldolesi J. Ectosomes and microvesicles: Shedding the confusion. *Trends Cell Biol.* 2015.

59. Tricarico C, Clancy J, D'Souza-Schorey C. Biology and biogenesis of microvesicles. *Front Immunol.* 2017.

60. van Meer G, Voelker DR, Feigenson GW. Membrane lipids and membrane asymmetry. *Nat Rev Mol Cell Biol.* 2008.

61. Hugel B, Martínez MC, Kunzelmann C, Freyssinet JM. Membrane microparticles and phospholipid redistribution. *Physiology.* 2005.

62. MacKenzie A, Wilson HL, Kiss-Toth E, et al. Microvesicle shedding mechanisms. *Proc Natl Acad Sci USA.* 2001.

63. Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C. Microvesicles in cancer progression. *Cancer Res.* 2009.

64. György B, Szabó TG, Pásztói M, et al. Membrane vesicles and extracellular communication. *Cell Mol Life Sci.* 2011.

65. Jansen F, Nickenig G, Werner N. Extracellular vesicles in cardiovascular disease. *Circ Res.* 2017.

66. Minciacchi VR, Freeman MR, Di Vizio D. Extracellular vesicles in cancer progression. *Semin Cell Dev Biol.* 2015.

67. Di Vizio D, Kim J, Hager MH, et al. Oncosome formation in prostate cancer. *Cancer Res.* 2012.

68. Minciacchi VR, You S, Spinelli C, et al. Large oncosomes represent a distinct class of tumour-derived EVs. *Oncotarget.* 2017.

69. Ciardiello C, Leone A, Lanuti P, et al. Large oncosomes and aggressive prostate cancer. *Oncogene.* 2019.

70. Becker A, Thakur BK, Weiss JM, et al. Tumour-derived extracellular vesicles and metastasis. *Cell.* 2016.

71. Ciardiello C, Leone A, Lanuti P, Roca MS, Moccia T, Minciacchi VR, Minopoli M, Gigantino V. Large oncosomes overexpressing integrin alpha-V promote prostate cancer adhesion and invasion via AKT activation. *Oncogene.* 2019.

72. Cufaro MC, Pieragostino D. Large oncosomes: New players in tumour progression and metastasis. *Biochim Biophys Acta Rev Cancer.* 2019.