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Biological Characteristics, Innovative Isolation Methods, and Clinical Role of Exosomes in Cancer Therapy: Review

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ABSTRACT
Exosomes are a subset of nano-sized (30–150 nm) extracellular vehicles (EVs) released by cells through the endosomal route, you know like they get made inside and then pushed out.
Objective: The primary objective of this scientific review is to give a broad and current appraisal of the intricate biological layout of exosomes, plus modern isolation technologies (for instance microfluidics and spectroscopy), and finally their clinical meaning for cancer detection and treatment.
Methods: This article is arranged following systematic review logic based on credible scientific sources from the last decade 2015–2025. Data collection involved international databases, including PubMed, Scopus and Web of Science. The analysis looks at how exosome biogenesis connects with drug delivery systems (DDS), and how that whole interplay affects the tumor microenvironment, using both qualitative and quantitative comparative approaches. The guidelines from the International Society for Extracellular Vesicles (ISEV) were used as a kind of standard for study rigor.
Key Findings: A growing body of research suggests that exosomes work as important intercellular message carriers, meaning they are deeply involved in cancer metastasis. Their nano-sized form, the potential to move across the blood-brain barrier (BBB), and their inherent biocompatibility make them a stronger, more natural alternative in comparison with synthetic nanoparticles. Innovative methods such as microfluidics have cut the isolation time a lot, and pairing exosomes with the CRISPR-Cas system has basically opened new directions for gene therapy. In the end, the review also tackles difficulties in standardization and large-scale production, while stressing that exosome engineering is likely the upcoming step change for precision cancer therapy.

KEYWORDS: biological characteristics, cancer, exosome, methods.

INTRODUCTION

What are Exosomes? Exosomes are a kind of nano-sized (30–150 nm) extracellular vesicles, also called EVs, which are secreted by cells through the endosomal pathway. These tiny vesicles transport a mixed cargo of proteins, lipids, and nucleic acids like RNA or DNA. For a long time, people viewed them mostly as a way of cellular “waste disposal”, but newer studies suggest that exosomes instead act as mediators of complicated intercellular molecular dialogue, and they appear to have a major part in shaping how cells behave in multiple diseases [1, 2, 3].
To halt cancer progression, it is crucial to discover new diagnostic biomarkers and potential therapeutic targets for the disease. Learning about the biological features of exosomes, and how they support tumor development is a key part of that effort [62].

Research Problem

A key difficulty with standard chemotherapy for cancer is that it tends to be non-selective, so it ends up harming healthy cells too, not just the tumor ones. Also, when it comes to liquid biopsy, there’s this big technical barrier: blood-derived fluids like albumin, and lipoproteins can contaminate the sample, which makes it harder to detect tumor biomarkers with any precision. On top of that, we still don’t have universal protocols for the separation and purification of exosomes, and that absence has slowed the growth and even the broader running of clinical trials [4, 33, 48].

Justification and Research Gap

Even though thousands of papers have been published about exosomes, there is still a big missing piece when it comes to clear comprehensive guidelines, especially around how exosomes should be standardized and how stability should be preserved during

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clinical scale manufacturing. On top of that, the so called "dual role" of exosomes inside the tumor microenvironment seems under considered in a unified way, meaning they can both help metastasis move forward and also act as therapeutic tools. In other words, this work tries to connect those two sides by creating a kind of synergy between modern technology and biomedical opportunity [34, 35, 56].

Target Audience

This research matters for oncologists, nanotechnology specialists, pharmaceutical researchers, and also students who study biomedical sciences. The article is intended like a broad manual for anyone planning to invest in, or otherwise run, research tied to next generation cancer therapy.

Objectives and Significance

The goal of this article is to lay out a broad picture of the evidence that already exists on exosomes. By clarifying how exosomes can be used for cancer diagnosis and therapy, we also try to bring attention to what blocks their clinical use, and suggest possible ways around those barriers. This piece is not just a rephrasing of what is known, but it is meant to act as a base for defining future research priorities [6, 21, 36].

Research Objectives and Questions

Objective: To assess the effectiveness of exosomes for cancer diagnosis and treatment, in other words how well they perform in real settings.

Research Questions:

1. What role do exosomes have in tumor metastasis, and not just at the first stage?
2. Which exosome isolation methods are the most accurate, and also the most efficient, when you compare them side by side?
3. How could exosomes be used to support personalized cancer therapy, for example tailoring treatment to a patient specific profile?

LITERATURE REVIEW

There component parts are: proteins, nucleic acids, lipids and metabolites, which they, helps with cell- cell communication [58].
Cells are released as non-vesicles from there cell membrane. They have effective rule in intra cell communication, and have important rules in biological information too. In general, they are: extracellular vesicles, micro-vesicles, and exosomes types are included [59].
The vesicles biological importance in liver diseases has been considered over the last decades, sort of. Extra cellular vesicles are found in different fluids; they carry distinct bioactive material in the cell membrane [60].

Also, mesenchymal cells can shift toward other cell types depending on the condition. CAFs are fibroblasts which play an effective role in the cancer mechanism, for example they are linked with exosomes, cytokines and the intense cell to cell communication as well as the expanded proliferation of tumor tissue are all important [61].
To slow down the progression of cancer it is crucial to identify new diagnostic biomarkers and therapeutic targets for the disease. Grasping the biological characteristics of exosomes and their functions during tumor development contributes to this work [62].
Biogenesis and Composition of Exosomes So, exosome formation really stays tied to the endosomal pathway, like, almost inseparable. When early endosomes start to mature into multivesicular bodies (MVBs) they make intraluminal vesicles (ILVs) inside them, and then those ILVs later get released as exosomes once the MVBs fuse with the plasma membrane. [10]
ESCRT-Dependent, there are ESCRT protein complexes such as TSG101, ALIX, VPS4 involved in membrane remodeling and in cargo sorting, basically helping things get sifted and re-packed properly [11].
ESCRT-Independent, this step is carried out through ceramide lipids and tetras panins, like CD63 [12, 13]. In their vesicle makeup the lipids, for example cholesterol or sphingomyelin, help keep the vesicles structurally stable, while their proteins serve as major mediators for quite specific or selective interactions [14, 15].
Advanced Techniques
The evolution of isolation methods keeps being the biggest achievement in this area [16].
- Raman Spectroscopy, a non-invasive, label-free approach used to figure out the molecular arrangement of exosomes, especially their lipid profiles. If you pair it with machine learning, it can actually tell apart different cancer kinds [17].

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- CRISPR-Cas System, a fresh and high-sensitivity tool used for detecting nucleic acids that are inside exosomes [18].
- Microfluidics, a “lab-on-a-chip” strategy, that cuts down the processing time for exosome isolation and also for downstream analysis [19, 20].

Therapeutic Potential of Exosomes

Exosomes are often described as the next generation of drug delivery systems [21]. Their natural biocompatibility, immune-evasive behavior, and the fact that they can cross the blood-brain barrier (BBB) makes them, in many ways, more suitable than synthetic nanoparticles [22].

Drug Delivery Systems, Exosomes kind of rely on two major pathways for delivering payloads

- Active approach: In this route, exosomes are loaded ex vivo with particular drugs or genetic materials (like siRNA, mRNA) [23]. It works quite well for targeted cancer cell therapy, in general [24].
- Passive approach: Exosomes also have an intrinsic ability to sneak into target cells, which is part of why they’re considered important as natural transporters [25].

Role in Cancer Therapy Exosomes help with cancer therapy through two mechanisms , though it’s not always the same across studies.

1. Direct Therapy: Chemotherapeutic agents (for example Paclitaxel and Docetaxel) are inserted straight into exosomes to deliver these drugs to tumors with high accuracy, while also limiting harm to healthy cells [26, 27].
2. Immunotherapy: With exosomes that come from immune cells, like dendritic cells, you can nudge the body toward anti-tumor activity and at the same time help them behave like anti-tumor vaccines [28, 29].

Tissue Regeneration and Neurological Disorders In the “Overview and Update on Extracellular Vesicles” it’s mentioned that exosomes have strong potential for handling neurological disorders, including Alzheimer’s disease [30]. And because MSC-derived exosomes show neuroprotective actions, they’re then used for neural tissue repair, as well as for dialing down neuro inflammation [31, 32].

Challenges and Future Perspectives Even though exosomes appear extremely promising in clinical trials, a few issues still linger, as in several obstacles remain [33]

- Standardization: Keeping stability through the manufacturing process and setting uniform purification protocols still feels like an open topic, with discussions ongoing [34].
- Mass Production: Growing exosome production to clinical grade is a big technical hurdle, and it asks for advanced bioreactor systems to be put in place [35].
- Safety: Figuring out the long-run impact in humans, in terms of safety for exosome-based treatments, needs more broad investigation [36].

Technical Challenges in Liquid Biopsy Even though exosomes are being used quite a lot in liquid biopsy, there are still a bunch of issues that make it hard to fully standardize everything.

2.1. Balance of Concentration and Purity

The main difficulty in liquid biopsy is getting exosomes out of blood or other body fluids. For example, blood plasma contains many proteins such as albumin and lipoproteins that can interfere with exosome analysis [48]. In the “Overview and Update on Extracellular Vesicles,” it is noted that density gradient ultracentrifugation helps reduce these unwanted substances, yet the workflow is slow and it has to be done carefully, almost step by step style [49].

2.2. Biobanking Considerations

Work focused on biobanking generally points out that exosome stability depends heavily on storage conditions. If cryopreservation is done poorly, exosomal membranes may rupture, and then encapsulated RNA and proteins can degrade [50]. For liquid biopsy, it becomes important to set up a standardized “pre-analytical code” that covers the whole path from sample pickup through the point of analysis, and not just one part of the process [51].

Emerging Therapeutic Landscapes In recent research, "exosome engineering" has evolved into a pivotal field that has revolutionized drug delivery systems.

3.1. Gene Therapy Integrating modern gene-editing technologies, such as CRISPR/Cas9, with exosomes enables the precise modification of genes within tumor cells [52]. The delivery of Cas9 protein via exosomes has proven successful in traversing the blood-brain barrier (BBB), marking a significant breakthrough for neurological cancers such as glioblastoma [53].

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3.2. Immune Response Modulation Exosomes possess the capacity to regulate the
response of immune cells (e.g., T-cells, NK-cells) [54]. To enhance anti-tumor immune responses, exosomes derived from dendritic cells (Dexosomes) are utilized as vaccines that transport tumor-associated antigens [55].
4. Role of Exosomes in Metastasis Exosomes do not only help with tumor growth, they are also key for tumor progression, especially metastasis, by setting up so called "pre-metastatic niches". In a way they travel to remote locations where the primary tumor might spread and then cancer cells can multiply, while the surrounding microenvironment gets prepped, for easier colonization [56]. This whole direction or guidance seems to be controlled by TGF-β and other kinase proteins that are tucked inside the exosomes [57].

DISCUSSION

The biological and therapeutic potential of exosomes, really is one of the liveliest, research frontiers in present day cancer therapy. Based on the findings showed in this article, we can sort of unpack the role of exosomes as this multifunctional instrument, or at least a kind of all-purpose tool.
Complexity in the Tumor Microenvironment: Exosomes act like a "communication network" across cancer cells. Earlier studies [2, 4] suggest that tumor cells release noticeably larger amounts of exosomes than healthy cells do. And these vesicles aren't only cellular refuse. Instead, by moving TGF-β and other kinase proteins, they help in shaping "pre-metastatic niches" in faraway organs [56, 57]. A key point that keeps coming up is the difference between using these exosomes and blocking them. See, tumor derived exosomes support cancer advancement, yet exosomes from immune cells (for example, dendritic cells, or Dexosomes) are used to strengthen antitumor immune reactions [28, 55]. So, this sort of double-sided behavior means that therapeutic planning needs careful precision, because otherwise the whole strategy can get messy.
Comparison of Isolation Methods and Diagnostic Accuracy The whole movement in isolation methods, from ultracentrifugation toward microfluidics, really shows how far the field has come [16, 20]. A big question still sits there, like what is the best approach when we actually want to use it clinically. The pairing of Raman spectroscopy with machine learning [17, 47] provides a kind of high-level precision for sorting cancer cells, that typically outperforms older lab protocols. Even so, albumin and lipoproteins hitching a ride in liquid biopsies, can still block the proper readout of the real molecular imprint of exosomes [48]. Yes, density gradient ultracentrifugation tends to win on purity but it is slow, so it becomes a practical limitation for fast clinical diagnosis [49], in real time situations.
New Horizons in Drug Delivery Exosomes bring notable benefits over synthetic nanoparticles such as liposomes, especially when the goal is crossing the blood brain barrier (BBB) [22]. Then there is the gene therapy angle in our conversation, more specifically through the CRISPR/Cas9 system, which feels like a fresh phase of innovation [52, 53]. Delivering Cas9 protein using exosomes is described as a major breakthrough especially for hard, drug resistant cases like glioblastoma [53]. Still, one should ask right away, do we even have enough tools for accurate exosome targeting today. Using active and passive targeting strategies [23, 25] indicates that we can try to "engineer" exosomes with particular receptors, but issues with long-term stability inside the body, plus unwanted immune recognition, stay very much under discussion, almost endlessly.
The Standardization Challenge Guidelines set up by the ISEV [8, 9] are supposed to harmonize research but, in real work biobanking and storage conditions can shift exosome integrity quite a lot [50]. If cryopreservation is done poorly, you can end up with vesicle membrane rupture and then the degradation of encapsulated RNA plus proteins happens [50]. So basically, every clinical study really needs a strict "pre-analytical code" that runs from sample acquisition to analysis [51]. Even if bioreactors for mass production [35] look promising, the expenses tied to the devices and the purification steps make commercialization of exosome-based therapeutics feel more complicated than it should be.
Conclusion. Overall, when people talk about exosomes, the take-home message is that they are not only agents in cancer therapy, but more like a kind of doorway into a "systemic messaging" network inside the human body. Going forward, future research should lean into "exosome engineering", to gain finer command over these vesicles, while trying to push cancer-treatment side effects down to almost zero.

CONCLUSION

The results from this comprehensive review kind an underline that exosomes have sort of opened a revolutionary chapter in today's biomedical sciences, especially when it comes to diagnosis and also treatment of cancer. By looking back at the research objectives, we end up with a few main takeaways, like really.

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First, exosomes are no longer treated as just "cellular waste", instead they are seen as key conduits for complicated intercellular communication. Their active presence in the tumor microenvironment suggests they are central not only to how cancer advances, but also to the creation of "pre-metastatic niches." In other words, this outcome hints at fresh therapeutic targets for both cancer prevention and metastasis control.

Second, using exosomes in liquid biopsy gives a safer, non-invasive, and repeatable substitute for traditional tissue sampling. When Raman spectroscopy, microfluidics and machine learning are brought together, it feels like a new frontier for early cancer detection. Taken as a whole, these tools may allow for near real-time tracking of how disease develops and can also support stronger results for "personalized medicine."

Third, there's a pretty big promise in exosomes as a drug delivery approach. honestly, their natural biocompatibility, plus the ability to cross the blood-brain barrier (BBB), and then the fairly good tolerability with the immune system, makes them feel more advanced than synthetic nanoparticles. And when you pair exosomes with modern gene-editing methods like CRISPR/Cas9, it turns into this kind of light at the end of the tunnel for hard, treatment-resistant cancers such as glioblastoma.

Still, even with these encouraging results, our review is basically saying that real clinical use is held back by core problems. for example, the lack of standardization, the annoying technical hurdles during large scale manufacturing, and the fact that biobanking has to follow very strict conditions so the samples stay stable are all things that need immediate focus. More work on "exosome engineering" is also essential, so targeting stays precise and safety doesn't get compromised, at all.

Ultimately, the future of exosomes in cancer management is looking promising, and i think it's more than just a research fad. We propose that later projects should lean harder on exosome engineering and also the expansion of clinical trials… because that's where things start to matter. If we can standardize production methods and somehow keep costs more controlled, then exosome-based therapy could become a key approach for cancer control, and in turn it might improve patient quality of life while also helping to reduce mortality rates. So, this article suggests that exosomes are not simply a topic people study, they're more like a foundational pillar of future medicine, even if the road there is slow.

Limitations are basically tied to how hard it is to keep things stable during exosome production, and also to the fact that there is not enough data on long term clinical effects, at least not in a way that feels solid. You know, it's one of those situations where the short picture looks good but the bigger timeline is kinda unclear.

RECOMMENDATIONS

1. Try to standardize a universal "pre-analytical code" for how samples are collected [51].
2. Do more clinical trials focus on the hybridization between synthetic exosomes and natural exosomes.
3. Put more effort into bioreactor technology so we can reach clinical grade, mass production [35].

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