Applications of Exosomes in Cancer Therapy

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Abstract

This systematic review explores the field of utilizing exosomes for cancer therapy, providing a comprehensive analysis of their mechanisms, applications, advantages, and limitations. Exosomes, specialized extracellular vesicles, demonstrate promising attributes as potential vehicles for drug delivery due to their biocompatibility, immunotolerance, and ability to traverse biological barriers. The review categorizes exosomes based on their in vivo sources, including milk, dendritic cells, mesenchymal stem cells, erythrocytes, and tumor cells, elucidating the unique advantages and challenges associated with each type. Additionally, the study delves into various exosome-loading techniques such as transfection, incubation, and electroporation. The clinical implications of exosomes as cancer biomarkers are detailed, including their role in early detection and diagnosis through exosomal RNA and protein analysis. Despite the promising potential, the review also highlights existing challenges in industrial-scale production, standardization, and long-term biosafety. Finally, the paper outlines future research directions aimed at refining exosome-based therapies, addressing existing limitations, and realizing their full therapeutic potential against cancer.

Keywords: Exosomes, Cancer Therapy, Drug Delivery, Biomarkers, Loading Techniques, Biocompatibility, Clinical Implications.

Introduction

1.1 Cancer

Cancer, or uncontrollable cell growth, is a major public health problem across the globe. Across the world, an estimated 10 million die annually from cancer. Cancer is the second leading cause of death globally, surpassed only by cardiovascular disease[1]. Traditional treatments such as chemotherapy or radiotherapy have been shown to be incredibly effective, but they all have downsides, such as nonspecific treatment and major side effects. Recently, immunotherapy (cancer treatment by stimulation of the immune system) has emerged as a way to combat cancer that addresses the shortcomings of traditional therapy. However, this method has disadvantages as well, including potential toxicity to the patient and the inability to clear certain barriers within the body [5]. The emergence of exosome based therapy provides a clinically viable method that addresses the shortcomings of traditional cancer therapy using natural mechanisms.

1.2 Exosomes

Exosomes are derivatives of extracellular vesicles (EVs). Extracellular vesicles are portions of the cell membrane that bud out from a cell. All cells synthesize EVs as a normal part of their functioning; and exosomes are a subset of EVs that are created in endosomal

compartments. They contain various biomolecules such as proteins, lipids, and nucleic acids. They are typically 40-160 nm in diameter[3].

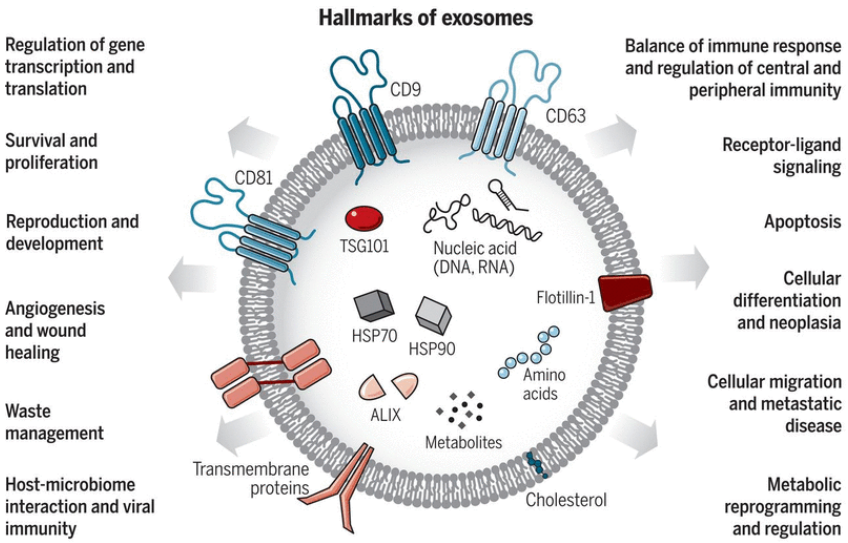

Figure 1 (above). Adapted from [3]. This details all the functions of exosomes.

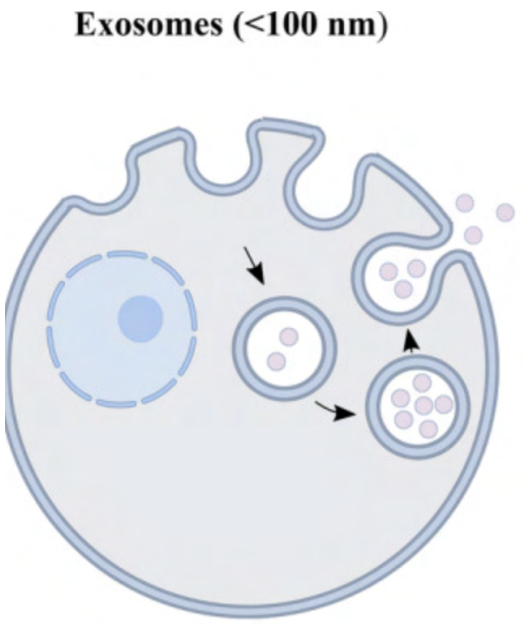

Figure 2 (above). Adapted from [4]. This visualizes the formation of exosomes.

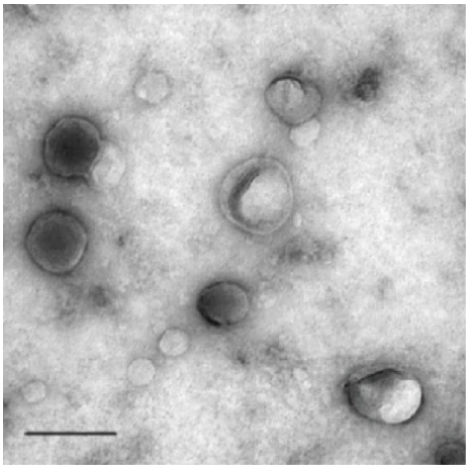

Figure 3 (above). Adapted from [26]. Exosomes under a microscope.

1.3 Exosomes' Mechanism of Action in the body

Exosomes are present in a variety of bodily fluids, such as blood, saliva, and urine, and travel in these fluids toward their target cells. Exosomes are synthesized in complexes known as multivesicular bodies (MVBs). They typically interact with the target cells via endocytosis into the plasma membrane, but can also do it via ligand-receptor binding. Due to exosomes' small size, they are able to easily move through capillaries, and can penetrate the blood-brain barrier. Within the body, exosomes perform a variety of functions, with their most notable being detection of chemicals released by cells as they age and subsequent promotion of apoptosis in those cells [2].

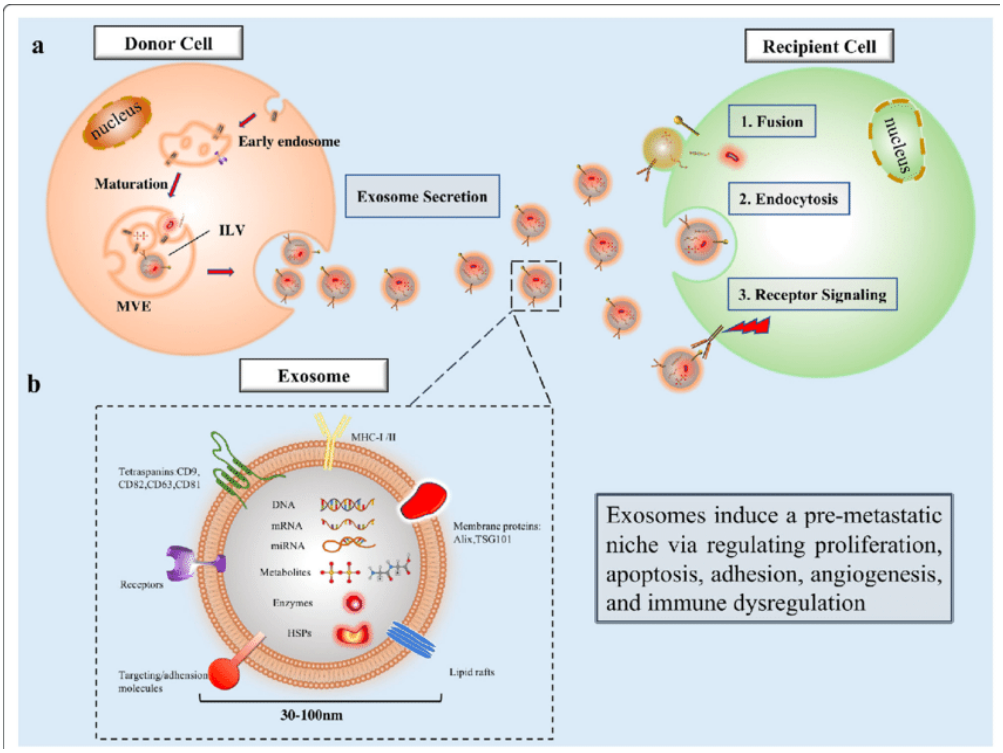

Figure 4. Adapted from [51]. How exosomes work.

Background

2. Utilizing Exosomes for Drug Delivery

The primary use of exosomes in cancer therapy is for drug delivery. They are used extensively because of many features that are not present in other treatments, including specific targeting abilities and biocompatibility[3]. In addition, exosomes are secreted naturally by cells to send messages or transfer compounds to a recipient cell [5], and they are nontoxic to the host body [26]. In drug delivery, exosomes act as vessels to carry various compounds to the site of cancer [8]; enhancing the effectiveness of drugs as well. The lipid bilayer of exosomes protects internal compounds from degradation, and also avoids destruction from the immune system [8]. This is confirmed by a study by Vashisht et al., where the compound circumin was coated with an exosome, and it was able to pass through the digestive tract undigested [10].

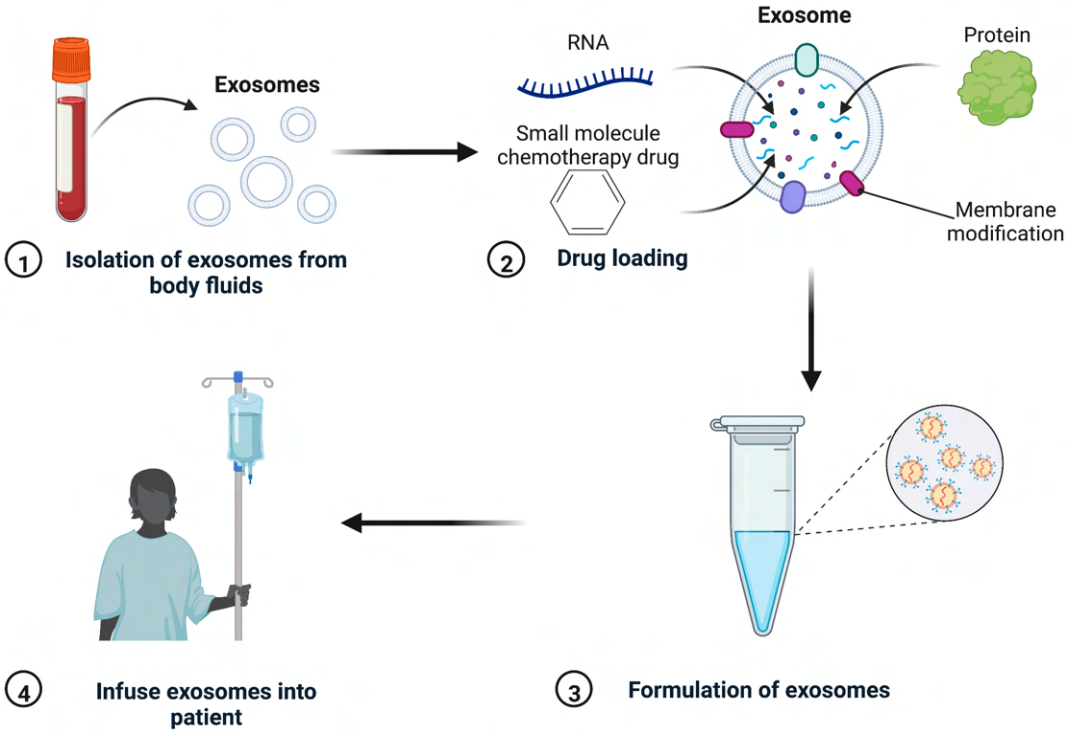

Figure 5. Adapted from [52].

In this approach, exosomes are first isolated from various *in vivo* tissues in the body, and then loaded *ex vivo* with compounds that kill cancer [19]. They are then reintroduced into the body [6]. Exosomes can merge with the cell membrane of target cells and transfer their contents [26]. This review will detail 5 *in vivo* sources of exosomes, and 2 *ex vivo* methods of loading exosomes.

Note that isolation techniques such as ultracentrifugation are also important, but this review does not cover them. Figure 5, above, details the general workflow of drug delivery using exosomes (adapted from [52]).

2.1 *In vivo* Sources of Exosomes

2.1.1 Milk Derived Exosomes

There are biologically active exosomes found in human breast milk. A study done by Reif et al. incubated these milk exosomes with normal and tumor cells, and found that the compounds within the exosomes were able to affect the levels of Collagen *I* within normal cells only [7]. It also induced proliferation in normal cells [7]. In contrast, the exosomes ability to change the mRNA profile of tumor cells was largely dependent on miRNA-148a expression [7]. A similar study backed up this result; milk-derived exosomes loaded with the drug paclitaxel (marketed in the US as Taxol) inhibited tumor growth by 60%, whereas the plain drug inhibited growth by 31% [9].

A study by Vashisht et al. has also proved that milk derived exosomes can pass through the digestive tract unscathed, which opens up the option of oral delivery of the drug [5,10].

2.1.2 Dendritic Cell (DC) Derived Exosomes

Dendritic cells, a specific type of monocyte in the immune system, is another potential source of exosomes for cancer treatment. It is known that DCs have capabilities to present antigens to T cells of the immune system to activate them [11], but the exosomes of DCs also have capabilities to form MHC complexes and present antigens [12].

In the context of drug delivery, DCs have been shown to elicit a strong antitumor response. When exosomes were injected with peptides eluted from MHC class I molecules and put in contact with a tumor cell line derived from a mouse, there was greatly reduced tumor growth [13]. One important thing to note was that this response was only observed in mice with T cells, implying that this method is only effective in a viable immune system with helper T cells [14].

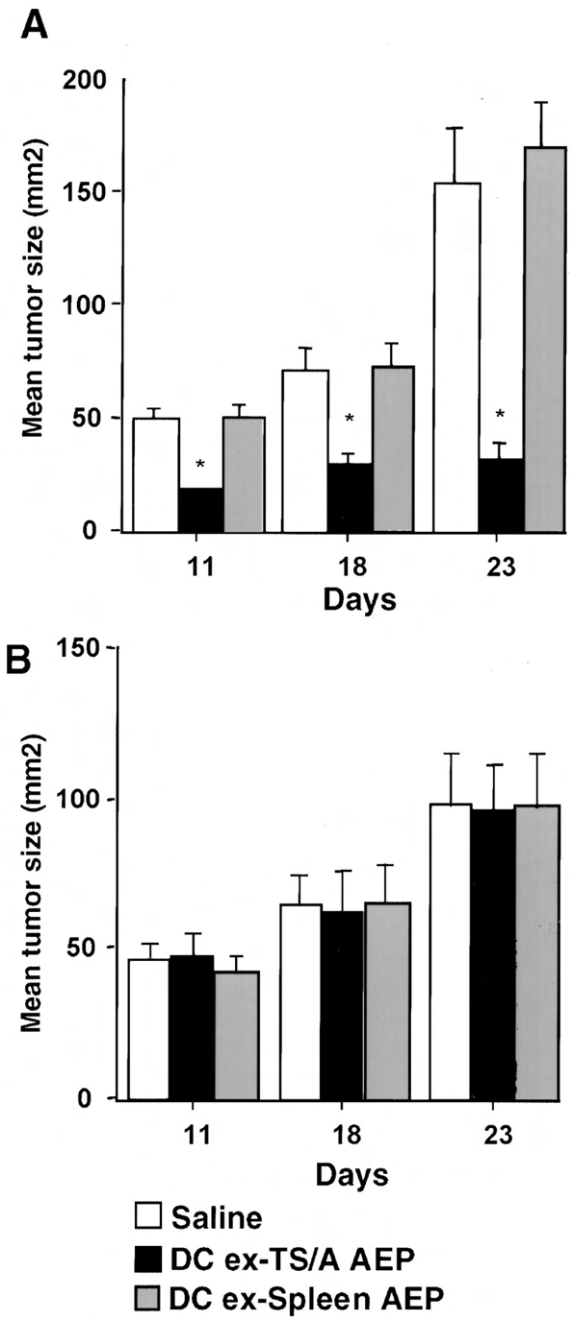

Figure 6. Adapted from [13]. This chart shows the effects of various DC derived exosomes on tumor size over time.

2.1.3 Mesenchymal Stem Cell Exosomes

Mesenchymal stem cells (MSCs) are a subset of adult stem cells which can be isolated from a variety of tissues, including bone and blood, and have the ability to differentiate into multiple types of cells. They display a variety of unique surface markers, including (CD)29, CD44, CD73, CD90, CD105, which allow it to differentiate into tissues such as adipocytes, chondrocytes, and myocytes [15,16].

Mesenchymal derived exosomes (MDEs) show similar physiological functions as their parent cells, including tissue repair functions, and unlike MSCs, they don't get rejected by the host immune system [17]. This, coupled by MSC's high rate of exosome production, make MDEs an attractive option for therapeutic drug delivery [18].

One specific application of MDEs is its use in the brain; a study has confirmed that MDEs in certain diseases including Alzheimer's accumulate in the brain; bypassing the blood-brain barrier [25].

Multiple studies have confirmed the viability of MDEs. A study by Lee et al. has shown that MDEs are able to downregulate the expression of growth factors promoting the expansion of blood vessels near breast cancer tumors, slowing tumor growth significantly [18]. This is further backed up by a study done by Naseri et al. Increased expression of miRNAs 142-3p and 150 is correlated to increased tumorigenesis of breast cancer cells [20]. Naseri at al. loaded MDEs with nucleic acids that increased upregulation of genes that counter the cacogenic miRNAs [20]. However, some studies refute the results of these studies, by showing that MDEs can in fact increase stem cell proliferation and promote the growth of tumors [21]. However, these effects can usually be offset by changes in experimental design [21].

2.1.4 Erythrocyte Derived Exosomes

During hematopoiesis, or the differentiation of blood cells into erythrocytes, leukocytes, and thrombocytes, the final stage of erythrocyte differentiation involves ejection of all organelles, including the nucleus [22]. The only way that RBC derived exosomes are formed is during this differentiation process [23].

The main appeal of erythrocyte derived exosomes is that they automatically target the liver (due to the nature of erythrocyte function and the liver's job of detoxifying the blood [24]]), and because exosomes can travel through the blood brain barrier, as outlined in Section 1.2 [26]. In addition to the normal drug-carrying capabilities of exosomes, RBC derived exosomes have been shown to not induce death of RBCs when loaded with chemicals [26].

2.1.5 Cancer Exosomes

Although it may seem paradoxical, exosomes derived from tumor cells can induce proliferation in tumor cells [5]. One study done by Xu et al. loaded exosomes derived from HT29 colon cancer cells with arsenite, a drug that kills cancer, and decreased tumor growth was observed once exosomes were reinjected to the body [27].

Another interesting phenomenon known as the Trojan Horse effect can potentially be utilized in cancer derived exosomes. Qiao et al. conducted a study where they isolated exosomes from the HT1080 cell line, loaded it with drug, and reinjected it back into the body [28]. The major conclusion of this study was that because cancer-derived exosomes have certain proteins that they share with their parent cells, they automatically "home" towards them [28]. In other words, if cancer-derived exosomes are isolated from the body, loaded with drugs, and reintroduced to the body, they automatically return to and fuse with tumorous cells, transferring the compounds they were loaded with [28]. This makes them a Trojan Horse in a sense, and although there is not much research in this field currently, it can be explored in the future to further utilize this property [28].

2.2 Methods of Loading

Once exosomes are isolated from various sources within the body, they are loaded with compounds outside the body [8]. There are a plethora of methods that are used to load exosomes, and this section will go into detail on 3 of those methods.

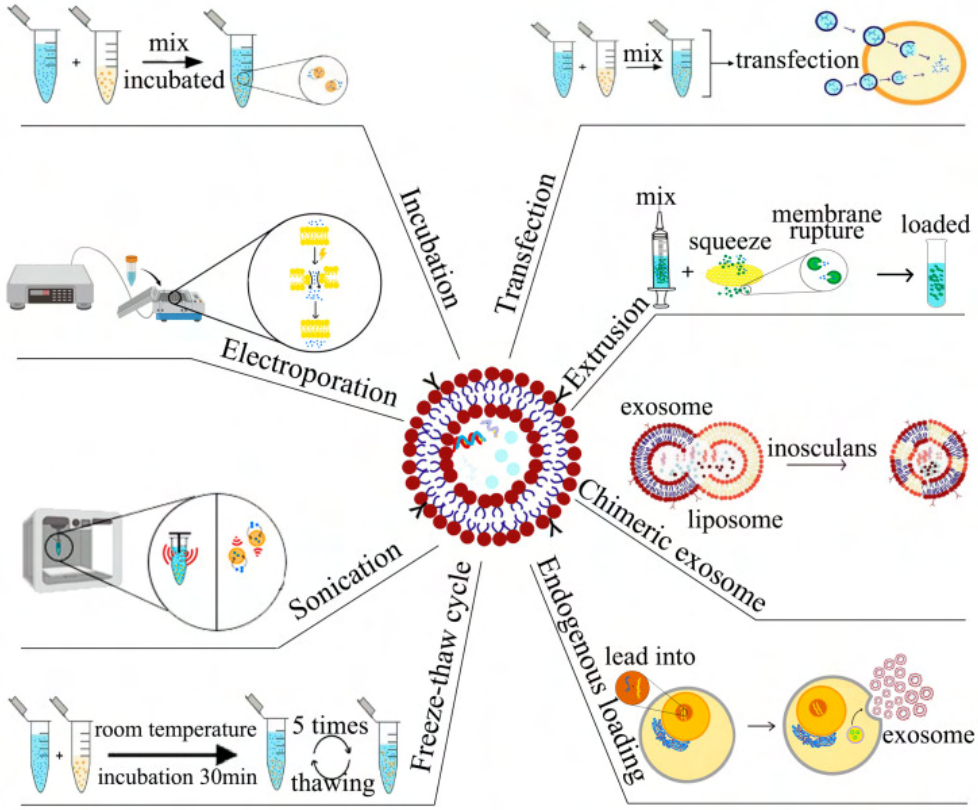

Figure 7. Adapted from [8]. This image details all the methods of loading exosomes.

2.2.1 Transfection

This approach involves using mRNA to generate exosomes in cells. Plasmids which contain specific RNA instructions to make the compounds are injected into cells which make exosomes; therefore the compound can be produced and put into exosomes by the parent cells [29].

2.2.2 Incubation

This method involves incubating exosomes with the drug for a period of time. It is a relatively simple method, and it works best with small molecules or bioactive enzymes. One advantage of this method is that it is simple, and can be effectively used with many small molecules. However, a disadvantage is its low efficiency, which limits its practical use [8].

2.2.3 Electroporation

Electroporation is a method where an electrical charge temporarily breaks the lipid bilayer of the exosome, which allows small compounds to migrate into the cell. The exosomes are then incubated in order to restore a normal lipid bilayer [8].

Electroporation is one of the more efficient loading methods; as a study by Lennaárd et al.found [30].

3. Exosomes as Cancer Biomarkers

The presence of exosomes in heightened levels typically means the presence of a late stage of cancer [42]. This is the case because tumor cells produce exosomes in order to facilitate cell to cell communication, growth, and exchange of molecules, and an increased number of tumor cells means an increased number of tumor cell derived exosomes [47]. Researchers can utilize this property by drawing tissue samples and analyzing exosome levels and levels of compounds in exosomes to diagnose cancer.

3.1 Exosomal RNA as Biomarkers

Certain tumor cells produce miRNAs and encapsulate them in exosomes. The miRNA produced is typically unique to a type of cancer, and by analyzing the levels and types of miRNA, a cancer diagnosis can be made [43].

In a study done by Hannafon et al., exosomal miRNA levels were analyzed, and it was found that increased levels of miR-1246 was found in two breast cancer cell lines: MCF-7 and MDA-MB-231 [44].

A similar study done in lung cancer patients by Hydbring et al. has shown that miR-200b, miR-200c, miR-141 and miR-375 all are upregulated in the presence of tumor cells [45].

Finally, Xue et al. conducted a study that found that 8 exosomal miRNAs; miR-17-5p, miR-29a, miR-106a, miR-122, miR-125b, miR-145, miR-192, and miR-194, are present in elevated amounts in the existence of gastrointestinal (colon) cancer [46].

3.2 Exosomal Proteins as Biomarkers

When exosomes pinch off their parent cell's membrane, they incorporate some of their proteins into their membrane. Techniques are present that detect proteins which are only present on tumorous cells' membranes, which can then be used to detect and diagnose cancer [48].

3.3 Exosomal Biomarkers Applications in Cancer Therapy

One effective and clinically viable way to detect various biomarkers is drawing blood samples and analyzing them for certain markers such as miRNAs and proteins [42]. This can be used as a method to detect cancer in its early stages, where other treatments can then be administered. However, other methods of drawing samples, such as pleural samples (which is specific to lung cancer), can be more invasive to the patient and unrealistic [43]. Tests that detect specific miRNAs can be used to diagnose specific subsets of cancer, due to the high prevalence of miRNA in various types of cancer.

Discussion

4. Analyzing the Effectiveness of Exosomes

Overall, exosomes are a viable and effective means of combating cancer. Their properties of being immunotolerant, nontoxic to the host body, being able to easily travel throughout the host, and being of natural origin make them highly appealing [26]. Additionally, a plethora of *in vivo* sources give a lot of flexibility on the specific type of exosome used for treatment. Table 1 compares the different sources and their advantages and disadvantages

(Note: The general characteristics of all exosomes outlined in section 1.2 are not included; only advantages and disadvantages unique to specific types of exosomes are included).

Origin	Advantages	Disadvantages	References
Breast Milk	• Oral biocompatibility • Ability for large scale production • Easy to access source • Pass through the gastrointestinal barrier	• Low loading efficiency • Lack of specificity ro target cells	31, 32
Dendritic Cells	• Activate adaptive and innate immunity • Facilitate immune cell–dependent tumor rejection • Ability to present antigens • Upregulate immune response	• Low circulation time • Activation of an immune response can cause complications in patients with autoimmune complications	33, 34, 35
Mesenchymal Stem Cells	• Ability to be loaded with a variety of compounds • Nanoengineering	• Accumulation within body • Potentially toxic to target cells	17, 36, 37
Erythrocytes	• Biological barrier permeability	• Targeted by immune system as	24, 26, 38, 39

Table 1
(above).

Table 2
outlines
the

advantages and disadvantages of the 3 methods of loading outlined in Section 2.2 (Note: The general characteristics of all methods outlined in section 2.2 are not included; only advantages and disadvantages unique to specific types of loading methods are included).

Method	**Advantages**	**Disadvantages**	**References**
Transfection	• High purity and stability • Able to load bioactive substances	• Low efficiency	40, 41
Incubation	• Simple • Does not affect membrane integrity	• Very low efficiency by itself, so it has to be combined with other methods to be viable	8
Electroporation	• Good	• Efficiency	8

	efficiency • Maintains original drug properties • Not time consuming	varies with conditions	

Table 2.

However, despite the array of advances made in exosome therapy and the advantages exosomes have over other treatment methods, there are still major disadvantages that must be overcome. One of the major downsides to using tumor cell derived exosomes is their ability to upregulate tumor cell division, instead of stopping it as intended [5]. A study done by Fang et al. found that pancreatic tumor cell derived exosomes carry compounds that promote metastasis of the cells [47]. If manipulated incorrectly, researchers can increase metastasis instead of promoting proliferation of tumor cells.

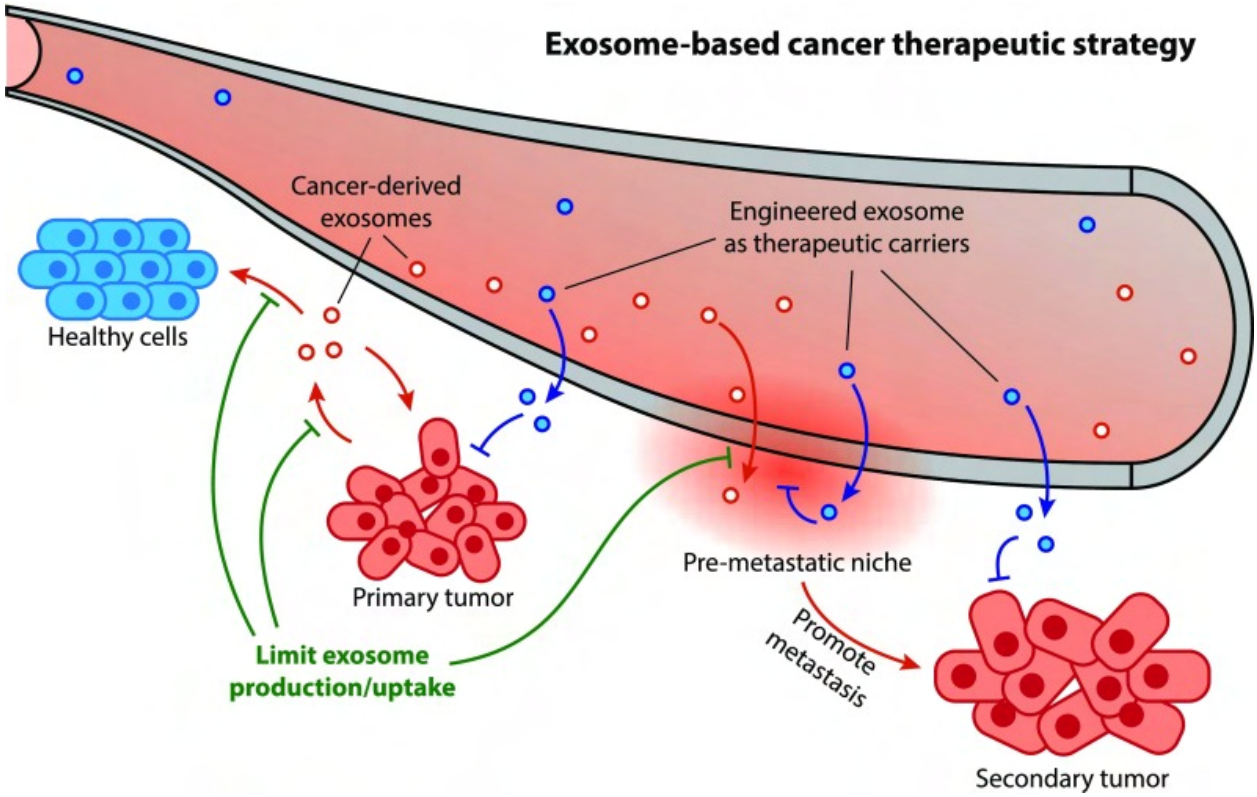

Figure 8. Adapted from [41]. This shows how tumor-derived exosomes can promote metastasis.

Additionally, the long term effects of exosomes to the body have not been studied widely to date [48].

The major disadvantage to exosome based therapy comes in an industrial lens. At a small level, exosomes are easily able to be derived and loaded with compounds, but for a drug to be FDA approved and put into commercial use, the technology and quality control required to

mass produce are not developed yet [48]. Additionally, there are no widely-agreed upon standards for production and isolation of exosomes [41,48]. There is also no common source for cells from which exosomes should be derived; no studies have been done on major differences on exosomes between cell types [40].

Although biomarkers are an excellent way to detect cancer, they do not offer an explicit way to combat it. Additionally, other molecules may contaminate biomarker samples, leading to false diagnosis of cancer or missing a diagnosis entirely [49].

5. Future Steps

In essence, the biological mechanism of exosomes is highly sophisticated and trumps many other methods. There are few drawbacks with exosomes themselves, but fine distinctions must be made between the *in vivo* source of exosomes, isolation techniques, and loading methods in order to maximize efficiency of exosomes. While tumor cell derived exosomes can promote cancer, new methods can be researched to work around this ability or silence it entirely. Additionally, because the long term biosafety of exosomes has not been thoroughly researched, that should be a priority [50].

However, the industrial mechanism of exosomes needs to be improved. Safe and efficient techniques for isolating and loading exosomes need to be developed. Additionally, the specifics about exosomes *in vivo* must be figured out, such as stability, drug dosage, and concentration. Finally, development of cost-efficient and mass producible methods for exosome extraction, isolation, and loading must be developed [50]. Overall, exosomes are a great cancer treatment, but some hurdles must be overcome for them to become viable on a large scale.

6. Conclusion

The review on the applications of exosomes in cancer therapy presents a comprehensive overview of the potential of exosomes as therapeutic agents. Exosomes offer promising avenues in cancer treatment due to their inherent properties such as biocompatibility, ability for specific targeting, and natural origin. The review elucidates various in vivo sources of exosomes, each with distinct advantages and limitations, underscoring the versatility of exosome-based therapies.

Furthermore, the review sheds light on methods for loading exosomes with therapeutic compounds, emphasizing techniques like transfection and electroporation. These methods provide insights into optimizing the effectiveness of exosome-based drug delivery systems.

Importantly, the role of exosomes as potential biomarkers for cancer detection has been highlighted, showcasing their diagnostic value. Exosomal RNA and proteins serve as promising indicators for the presence and type of cancer, thereby aiding in early diagnosis and personalized treatment approaches.

However, it is crucial to acknowledge the existing challenges and limitations associated with exosome-based therapies. Concerns such as potential upregulation of tumor growth by certain exosomes and the lack of standardized production methods need to be addressed to ensure safe and effective clinical applications.

In conclusion, while exosomes present a groundbreaking approach in cancer therapy with remarkable advantages over traditional treatments, further research is imperative. Addressing existing challenges, refining production methods, and conducting comprehensive studies on long-term safety will pave the way for exosomes to realize their full potential as a transformative cancer therapy on a broader scale.

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