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Advances in exosomes for cancer therapy

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Abstract: *Over the past few decades, cancer research has advanced substantially reaveling illumination on previously enigmatic aspects of cancer biology and paving the way for revolutionary therapeutic approachces. However, the resources and methods of treating cancers are still less in number which people still felt threatened by it passively. There is a nanosized extracellular vesicle (EV) called exosome have been widely used for treating cancer as well as research of it in nowadays, which contains and transports proteins, lipids, and nucleic acids. We discuss how exosome is preserved currently which uses different methods and techiniques. Next, we compared what kind of technology could be the most efficient in purifying exosome. We also point out that exosomes can either suppress or promote the cancer cells, depending on what they carrying and what kinds of enviorment they in. In this comprehensive review, we will focus on the mechanism of cargo sorting and how exosome is created and characterised from endosomes, which contains how they distribute specific cargo and how exosome carries those. Eventually, we provide a brief view of clinical courses and application of exosomes in canaer therapy in recent years and prospects for exosomes being used in further medical research.*

Keywords: *Exosomes, Extracellular vesicle, Cancer cells, Purifying exosome, Cargo sorting*

1. Introduction

The word "exosome "was initially coined in late 1980s by Johnstone [1], exosomes are small membrane vesicles of 30-150nm [2,3] that houses some of the protein [4], lipids [5], DNA, and RNA [4,6,7] of the cell. There are initially made from endosome by carrying out endocytosis and by some other important processes, which is going to be introduced in the first part of this comprehensive review. The exosome possesses the ability to transport the cargo to the target cells [8], some might be stimulated by the proteins covered outside such as some antigens (MHC class I and MHC class II) or other signalling receptors on the membrane. Therefore, this the one of reasons why researchers recommend taking advantage of exosome. The cargo inside of exosome is sorted by some mechanisms, they will have different cargo as exosomes are in different Multivesicular bodies (MVBs). Nowadays, researchers are still discovering the best methods to isolate and purify exosomes, they try to make up for shortcomings and keep enhancing advantages. We highlighted three techiniques for purifying exosomes and analysed the advantages and disadvantages of them, ultracentrifugation, ultrafiltration, and polymer precipitation, these three methods are popular and recommended from researchers. However, as far as we are concerned, we tend to recommond ultracentrifugation and polymer percipitation, the reasons will be explained in detail. Moreover, we keep the topic continue to the preservation of exosome, comparing with different temperature of environment and, as similar before, we chose three symbolic methods and analysed benefits and drawbacks. In the end of this comprehensive review, we endeavor to provide a brief view to the recent application of exosome in medical development. The progress of application in exosome is outstanding as the time passes, researchers have invented different ways to take precautions againt tumor development, such as vaccination. In the end, this review is mainly concentrating on the mechanism and biogenesis of exosome.

2. Exosome formation and exosome cargo

2.1. Exosome Formation

Nucleic acids, proteins, lipids and matabolites are the cargo carried by exosome.There is four stages before forming the exosome, Early, sorting endosome,Late sorting exosome,Intraluminal vesicles and

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Multivesicular bodies(MVBs)[9].To begin with, early sorting exosome by carrying out endocytosis in plasma membrane[10], in this moment, the cargo is proteins, lipids, nucleic acids, ions, proteins from cell surface and extracellular milieu.Those early sorting endosomes possess ability to fuse with endoplasmic reticulum and Trans-Golgi network[9] which means some cargo from theirs can be transported into early sorting endosome.Therefore,the luminal membrane is formed when early sorting endosome rise to late sorting endsome.In the late sorting endosome,the cargo has secreted out to the Trans-golgi network and cargo is sent back which has been modified by Trans-golgi network. Furthermore, Intraluminal vesicles start to have the size of exosome insides the MVB, and the cells surface protein is separated on each Intraluminal vesicle. In addition, MVBs fuse with lysosome to degrade. In the end the MVBs, that contain a lot of exosomes that carry cargo, has carried out exocytosis to release exosome to extracellular environment. [9,11].

2.2. Exosome cargo

Accoding to the details of exosome formation,most of the cargo in exosome is already been packed in the stage called Intraluminal vesicles.Before that the membrane of it still depends on the host cell [11].There is also some environmental factors might stimulate the change in cargo such as hypoxia [11].To be specific,the main machnism of sorting cargo is ESCRT-dependent sorting mechanism[11-13].One of t0he regulatory mechanisms for controlling the loctaion,stability,and function of proteins is represented by ubiquitination and ubiquitin-like modifiers [15].Apart from that, the nucleic acids that carried by exosome might either promote or suppress the cancer cell by upregulate and downregulate particular such as miRNA-21-5p in exosomes will be upregulated more when it is under hypoxic conditions which is going to promote the thyroid cancer progression by inhibiting TGFBI and COL4A1 [15],by contrast, some exosomes that contain hepaCAM are able to downregulate VEGF to suppress renal cancer [16]. Furthermore, there is another mechanism called ESCRT-independent sorting mechanism [11], which distributes different protein in terms of enrinching it into the exosome by the post transitional modification (PTMs).

3. Methods for purifying exosomes

3.1. Ultracentrifugation

This section will start with advantages of using ultracentrifugatian. It is widely used in nowadays research [17]. Ultracentrifugation rotates samples in ultracentrifugation tube at high speeds for high gravity force separations.The separation occured because of difference in density and scale of exosome and other material.Meanwhile,it is always recommended keep refrigerated about 4°C .To beigin with,the scalability is the second advantage of this technology,this procedure may be modified to isolate exosomes in large quantities for possible therapeutic uses or from tiny volumes of material for fundamental study. Consequently, cost-effectiveness,compared to some other techniques, ultracentrifugation is relatively cost-effective and does not require the use of expensive consumables,it only require the ultracentrifugation device and the tube.Nevertheless,There is some potential damage to exosomes as they experiencing a high-speed centrifugation, consequently,leading to loss of function or altered cargo content [18].Overall,even though there is potential damage to exosome,ultracentrifugation is still the most commmon method to purify exosome.

3.2. Ultrafiltration

This method is mainly concentrating on the size of the exosome and other impurities, this method required semipermeable which means there is molecular weight cut off (MWCO) controls the material that is passing. Generally, exosome cannot pass through it and other impurities (smaller than exosome) will pass through the membrane. Compared to other methods, ultrafiltration is relatively straightforward, the specialised equipment is not necessary for this method. However, the centrifugation is still needed for ultrafiltration, and it is much slower than ultracentrifugation [19]. Furthermore, there is possibility that lossing some small exosomes that can pass through MWCO which can lead to some potential error because of only larger exosomes are being purified. In the end, there is a limited in the purity so that ultrafiltration is somehow not recommended compared to other techniques.

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3.3. Polymer Precipitation

The process of polymer precipitation is frequently used to separate exosomes from biological materials. To cause exosome precipitation, polymers like polyvinyl alcohol (PVA) or polyethylene glycol (PEG) are used [19-20]. Ease of use is the first advantage of this method; polymer precipitation is a simple and cost-effective method for exosome purification which means it is accessible for lots of condition. Moreover, using this technology can purify larger volume of exosome simultaneously which means high yield. Furthermore, strong and evenly distributed products can be purified after using PEG polymer precipitation which other methods, such as ultracentrifugation, cannot reach that quality. Nonetheless, the biological sample that with high viscosity or high lipid content is hard to process out because it does not suitable for polymer precipitation.

4. Preservation methods of exosomes

Exosomes are a promising therapeutic option, so we feel it is necessary to summarize some preservation options for them.

4.1. Cryopreservation

It usually refers to temperatures of 4°C, -80°C, and -196°C. However, this storage method is prone to osmotic imbalances during freezing and the formation of ice crystals inside the biological particles. To overcome this defect, one or more antifreeze agents of appropriate concentration are usually selectively added [21-23].

Table 1: Exosomes at different temperatures

4°C	-80°C	-196°C
·The highest concentration. ·not suitable for long-term preservation (more than one month), If kept them for longer than a month,the proteins will be almost completely degraded with no activity.	·Suitable for long-term preservation ·Relatively intact	·Suitable for long-term preservation ·Relatively intact

In Table 1, the effects of different temperatures on exosomes during cryopreservation were introduced. Select the desired temperature for different needs.

4.2. Freeze drying

This method allows the material containing water to be cooled in advance. Then freeze it below freezing point to form a solid. It then sublimates into ice under a vacuum. Finally, the ice is removed with steam. To meet the storage requirements. The storage effect is like that of freezing at -80 ° C.

4.3. Spray drying

This method requires the material to be atomized in the drying chamber. Exposure to hot air vaporizes the water quickly, producing a dry final product. The method can directly dry the solution and emulsion into powder or granular products. As a continuous process, this can eliminate evaporation, crushing and other processes. More affordable and economical. The three methods for preserving exosomes are reviewed above. So, compared with these three methods, which is more suitable? To this end, we summarize their advantages and disadvantages in Table 2.

Table 2: Advantages and disadvantages of different storage technology

	cryopreservation	Freeze drying	Spray drying
advantages	·Practical ·Long storage time(-80°C,-196°C)	·High stability	·Rapid drying process ·Certain health protection
disadvantages	High cost	Particle number reduction [24]	High cost

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5. Application of exosomes in therapy

5.1. Delivery through exosomes allows the drug to work more efficiently.

We have been concerned in the previous literature that exosomes can promote drug resistance in cancer [25]. However, we can adjust the properties of exosomes to make the drug work more effectively [26]. We are aware of a paper published on March 19, 2019 [27]. It documented the negative regulation of MRP5 and Bmi1 delivered through the exosome miR-128-3p in a stable oxaliplatin resistant CRC system, effectively improving the chemical sensitivity of CRC [27].

5.2. As a vaccine

We observe that exosomes generated from dendritic cells are one of the most researched tumor vaccines now. They practically all include antigen-presenting molecules, which allows them to activate innate immunity by inducing or amplifying acquired immunity in natural killer cells.

5.3. The latest effect and application progress

The number of studies on the usefulness of exosomes in the treatment of various pathologies has increased substantially. We learned that exosomes include different and have a lot of cargo. These allow the introduction of multiple diagnostic groupings in the process of detecting and monitoring the disease. The nature of exosomes to deliver goods to target cells also facilitates their use as therapeutic vectors at the basic and applied levels to maximize their usefulness [28]. At present, it is speculated that exosome technology can be used in the future in the fields of metabolic processes, cardiovascular diseases, cancer, immunity, and nerve repair. It can be said that this technology is a promising new star. Or will be another possibility for later treatment.

6. Conclusion

In this review, we summarized the formation of exosome and how cargo is exchanged and disrtibuted in each of the exosome. Additionally, we classified different purification methods by comparing their superior part and shortcoming part. Meanwhile, the preservation of the exosome has been highlighted with the different environment (temperature) by taking advantage of tables. Consequently, some examples of application of exosomes in therapy recently. Compared with previous years, we find that quite a lot of researchers have paid attention to clinical and technical uses of exosome during some research and treatments. The unknowned exosomes or other medical treatment of cancer are about to be revealed in the coming years. So far, we are looking forward to and sincerely hope to see some more convenient and economical storage methods for various types of exosomes, as well as long-distance transportation methods for it, to support more researchers' research. For the treatment of cancer, we also believe that exosomes will become a key factor worth considering because of its distinguished function on marking targeted cells, for instant. As far as we are concerned, in the future, there are going to be other technology that replace exosomes, which will show an out-standing performance in treating cancers.

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