

Application of exosomes as markers and drug carriers in tumors

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Abstract: Exosomes are extracellular vesicles with a diameter of about 40-100nm and a double-layer lipid structure. Exosomes secreted by a variety of cells contain a variety of RNA and proteins like microRNA, LncRNA, Circ RNA, exosome synthesis-related proteins and transfilm protein, etc. Exosomes play an important role in the clinical application of tumor treatment. It can be used as a marker for early diagnosis of tumors, diagnostic typing, recurrence monitoring, treatment monitoring, etc. In addition, exosomes can also be used as protein drug carriers, RNA drug carriers, and chemical drug carriers for tumor treatment. This article introduces the different applications of exosomes as markers in tumors and their therapeutic effects as different kinds of drug carriers in tumors.

1. Introduction

The accurate prediction of power load is of great significance for the electric power production and the safe operation of the power grid and the national economy [1]. Short term load forecasting is an important part of energy management system. The prediction error directly affects the analysis results of subsequent safety check of power grid, which is of great significance for dynamic state estimation, load scheduling and cost reduction [2-4]. Traditional prediction methods are based on linear regression, such as time series method, analysis method and pattern recognition method has defects of respectively [5].

2. Introduction

Cancer, widely regarded as a serious global disease, has been widely watched and studied. With the rise of tumor immunotherapy, the interaction between exosome and immune system in tumor has also attracted much attention. However, due to the difficulty of external urinary purification, there is still a deviation in the study of exosomes. This article focuses on microRNAs, LncRNAs, CircRNAs, and protein that make up exosomes. The clinical effects of colon cancer, non-small cell lung cancer, pancreatic cancer, bladder cancer, glioblastoma, diffuse large B-cell lymphoma, multiple bone marrow cancer, Hodgkin's lymphoma, eight common cancers and exosomes in these eight types of cancer are summarized. Finally, through data analysis and literature reference, compared with the defects of ordinary drugs, analysis of the natural presence of exosomes can be used as a protein delivery carrier, can be naturally carried into the target cells such as the advantages of drug carriers. The research on the future of external urinary bodies finds and uses the prospect.

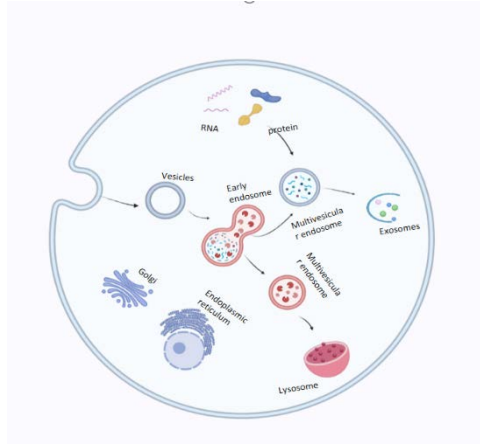


Figure 1. Formation of exosomes

3. Overview of exosomes

In 1985, Johnstone et al. discovered a kind of small vesicles in the multivesicular bodies of reticulocytes that could be released to extracellular surroundings, and then termed this small vesicle as “exosome”. Thereafter, these unique vesicles have been found in various body fluids, such as blood, saliva, semen, amniotic fluid, ascites, urine, cerebrospinal fluid, breast milk, and bile [1].

EVs are a nanoscale membrane bubble secreted by cells, whose origin, characteristics and properties are different, and are mainly divided into exosomes, microcystic bubbles and apoptosis miniasses according to their size.

Exosome is a type of extracellular vesicle with biphospholipid membrane and protein, lipids, nucleic acids, and other components inside. Exosomes are small and 30 to 200 nm in diameter, and almost all cells secrete exosomes, which play an important role in intercellular connections and can be detected in a variety of body fluids. The components contained in exosomes are obviously specific and can be used as biomarkers for diagnosis of diseases such as cancer and viral infections, polycystic ovary syndrome, etc.). Exosomes are involved in signaling from cancer cells to other cancer cells, as well as transmitting cell growth, transformation, and survival signals. Rains are also tried as a carrier for delivering drugs, such as anti-tumor drugs. 10 times obtained from most body fluids (blood, saliva, urine, ascites, cerebrospinal fluids).

Non-coding RNA (no coding RNA, ncRNA) in exosomes is a class of RNA with no protein coding capability, such as tiny RNA (microRNA, miRNA), long-chain non-coding RNA (long no coding RNA, lncRNA), ring RNA (circular RNA, circRNA). With the further study of external urinary function, proteins and tiny RNA (microRNA) from the source of tumor extracellular urinary bodies have been found to be diagnostic markers for many types of cancer. In addition to diagnosing cancer as a biomarker, exosomes can also be used as tools for disease treatment and drug transport.

3.1 MicroRNA

MiRNA is a small, non-coding RNA with a length of about 22 bases, the main biological function of which is to silence the target gene by binding the target mRNA. Most miRNAs are first transcribed in the nucleus by RNA polymerase II, forming a primary transcript with a card-like structure, pri-miRNA, which contains the miRNA sequence. Pri-miRNA is nucleated by Exportin-5 (EXP-5), then called pre-miRNA, and contains a stem ring structure of about 70 bases. This is then done by RNase endoenzyme III-Dicer, resulting in a double chain of miRNA. MiRNA double-stranded and AGO proteins bind into the RISC complex, one of which is selected as a mature miRNA. The biological function of can be in the cytoplasmic silence target mRNA, inhibit the expression of the target gene, or act in the nucleus of the cell by binding to the promoter region of the target gene, resulting in the target gene silence or overexpression, and even combined with the extraocrine secretion secreted to other cells to play a biological role.

For example, through the extraction, separation, sequencing and experimental verification of blood internal and external urinary bodies in NSCLC patients, it is found that exosome-mediated intercellular signaling plays an important role in tumor metastasis, and microRNA has judgmental value for early NSCLC metastasis. Exosome miRNAs may become new indicators of hepatocellular carcinoma (Hepatocellular carcinoma, HCC) diagnosis and prognosis EVs miRNAs are promising indicators of PCRC diagnosis and prognosis miR-21-5p、 miR-1246、 miR-1229-5p and miR-96-5p.

It may promote the chemical sensitivity of Osari platinum and 5-fluorouracil, and may be a promising strategy to improve CRC resistance to colorectal cancer. LncRNA is a class of non-coding RNA with a length of more than 200 nucleotides. They participate in a range of biological processes, such as tumor development, by interacting with DNA, RNA, or proteins. Studies have shown that certain lncRNA disorders and mutations are associated with human diseases such as lung cancer, cervical cancer, osteosarcoma, intracranial aneurysms, etc. SCIENCE have reported that LncRNA has a high prospect of malignancy diagnosis. Example: Exosome ncRNAs can effectively inhibit the growth of HCC invasion, enhance the sensitivity of cancer cells to chemotherapy drugs, and have the advantages of low biotoxicity, low immunogenicity, low pathogenicity, better biocompatibility, etc.

3.2 Circ RNA

Unlike linear RNA, circ RNA is a special convalescence closed continuous ring without a 5' cap and 3' tail polydenotides, a structure that makes it more resistant to RNA enzymes than linear mRNA, acts as a sponge body for miRNA to regulate gene expression, and is a key factor in tumor development.

3.3 Protein

Based on the biological pathways of the nucleosome, exosome-specific protein markers include small Rab family GTPases, annexins and flotillin, exosome synthesis-related proteins (Alix, Tsg101, and ESCRT complexes), and transmembrane protein families (CD9, CD37, CD53, CD63, CD81, CD82), and epithelial cell adhesion molecules, etc. The specific surface proteins carried by exosomes can reflect their source cells

4. Clinical application of exosomal markers in tumors

The research of exosomes in the field of tumors is the most reported. It is involved in many processes of tumorigenesis and development, including promoting angiogenesis, regulating immunity, mediating treatment resistance, and many other aspects. Exosomes are also very important in the clinical application of tumors. Among them, exosomal markers play an important role in the clinical application of tumors, including early detection, diagnostic typing, recurrence monitoring, diagnosis, chemotherapy resistance markers, and treatment monitoring.

Colorectal cancer is the third largest tumor in the world, 70% of which are located in the colon and are called colon cancer [2]. More and more studies have proved that the contents of exosomes can be used as markers in the early detection of many diseases. Among them, plasma exosomal miRNAs can play a diagnostic role in colon cancer [3], and the researchers verified four miRNAs (Let-7b-3p, miR-150-3p, miR-145-3p, miR-139-3p). After comparing the diagnostic performance of four miRNAs in plasma exosomal miRNA and plasma total miRNA, the results showed that the diagnostic performance of plasma exosomal miRNA is significantly better than that of plasma total miRNA [3].

Lung cancer is the main cause of cancer death in China [4], and advanced diagnosis is the main obstacle to improving the prognosis of lung cancer. The average 5-year survival rate of non-small cell carcinoma is about 15% [5]. Recent studies have shown that exosomal miRNA can be used as a diagnostic marker for early non-small cell lung cancer. The results show that compared with the control group, lung adenocarcinoma has 11 high-expressed and 13 low-expressed miRNAs, and lung squamous cell carcinoma has 6 high-expressed and 8 low-expressed miRNAs [6]. Among them, four miRNAs, let-7, miR-21, miR-24, and miR-486, are different in lung adenocarcinoma and lung

squamous cell carcinoma [6] miR-181-5p, miR-30a-3p, miR-30e-3p and miR-361-5p are four adenocarcinoma-specific miRNAs[6]. miR-10b-5p, miR-15b-5p and miR-320b are three distinct miRNAs that are associated with lung squamous cell carcinoma [6]. The diagnostic performance of these miRNAs was further evaluated in the test group, and the results showed that the diagnostic performance of these three cancers [6].

Bladder cancer is the most common urinary system malignant tumor worldwide [7]. About 75% of patients are classified as non-invasive bladder cancer (NMIBC), which is characterized by a particularly high recurrence rate [8]. In the study, it was found that urinary exosomes Ln RNA have a high potential as a marker for the diagnosis and recurrence prediction of bladder cancer [9]. And three LncRNAs including MALAT1, PCAT-1 and SPRY4-IT1 are significantly up-regulated in urine exosomes of patients with bladder cancer [9]. Experimental results show that the diagnostic performance of these three groups of LncRNA is significantly higher than the diagnostic results of urine cytology [9]. Later, when observing the patient's recurrence time, it was found that for NMIBC patients, patients with high urinary exosomes MALAT1 and PCAT-1 had lower recurrence-free survival, it shows that MALAT1 and PCAT-1 can be used as markers for monitoring the recurrence of non-invasive bladder cancer [9].

Table 1. Application of exosomes as markers

type of tumor	markers	marker function
Colon cancer [3]	Let-7b-3p, miR-150-3p, miR-145-3p, miR-139-3p	Early detection
Non-small cell lung cancer [6]	let-7, miR-21, miR-24, miR-486 , miR-181-5p, miR-30a-3p, miR-30e-3p , miR-361-5p, miR-10b-5p, miR-15b-5p , miR-320b	Diagnostic typing
Bladder Cancer [9]	MALAT1, PCAT-1	Recurrence monitoring
Glioma [10]	miR-301a	Diagnosis
Diffuse large B-cell lymphoma [11]	miR-99a-5p, miR-125b-5p	Chemotherapy resistance markers
Multiple myeloma [12]	let-7b, miR-18a	Diagnosis
Hodgkin's Lymphoma [13]	miR127-3p, miR155-5p, miR21-5p, let7a-5p	Treatment monitoring

5. Clinical application of exosomes as drug carriers in tumors

5.1 Advantages of Exosomes Delivery:

Traditional drug delivery systems like viruses or synthetic nanoparticles, although these systems have the natural ability to invade the host cell and bring the genetic material from viruses to the genome in the cell but combined with the high risks of induction of genetic variation, which tumor might occur [14]. Other drugs delivery systems, such as liposome and cation complex. These complexes might be the successful nano vector treatment with low immunogenicity and mutagenicity but have the disadvantage of short blood circulation, low drug delivery efficiency in the cell, relative high cell toxicology, and low transfection efficiency in vivo [15]. Compared with traditional drug delivery systems, exosomal drug delivery systems have the following advantages: firstly, the auto-derived exosome has the properties of limited or no adverse immunogenicity when deposits in different places [16]. Secondly, the exosome expresses Gpi-anchored complement regulators CD55 and CD59, which promise the exosome stability in the blood and delivery of the drug to the cytoplasm of the target cell [17]. Compared to most Artificial carriers, exosomes can avoid macrophage phagocytosis, which extends the half-life of the drug [18]. At the same time, exosomes can enter the cell through the interaction between their own membrane proteins and the recipient cell, which escape the half-life of the fatal drug that is eliminated by metabolism. This sure the transported drugs are not decomposed

and prolongs the time of systemic circulation. In addition, the lipid bilayer of exosomes can protect the content in vivo [19]. Thirdly, exosomes act as the drug delivery system to some extent improve the accuracy of targeting. Due to same-sex characteristics, especially the integrins of tumor-derived exosomes extra, the exosome has natural targeting that tends to go back to maternal tumors [20]. Fourthly, exosomes have self-contained transmembrane and membrane-anchored protein. This enhances the endocytosis, which facilitates the delivery of payload [21]. Fifthly, with the advantage of being small in diameter, exosome with the cell surface substances to enhanced permeability and retention effect which provide the strong permeability [22].

5.2 Exosomes to deliver drug carrier

5.2.1 Examples of exosomes as protein drug carriers

Exosomes are an efficient biomolecule delivery system in nature. They can act as a delivery system mainly because they transfer the content from the mother cell to the neighboring cell, particularly act as a protein delivery vector [23]. Naturally occurring exosomes effectively present bioactive ligands, and these membrane-associated protein ligands gathered and formed the micro-domains on exosomes during biogenesis. Therefore, exosomes can provide a natural membrane environment for a membrane protein, which helps stabilize the stability and bioactivity, thus contributing a great help in enhancing the efficiency of membrane protein Therapy [24]. As a result, exosomes can act as protein delivery vectors. For example, when comparing an exosome and a ferritin nano protein cage together delivering signal-controlled protein (antigen of CD47), it seemed that although exosome is a complex vector with a variety of protein and lipid, compared to other delivery systems itself has an extinct advantage. This is because macrophages enhance the effect of phagocytes, which contribute to stronger inhibition using exosomes to induce tumor to inhibit effect than nano protein cage [24]. At the surface of the exosome, some signal-relevant membrane proteins present strong immunogenicity. This protein activates the immune cell to inhibit tumors. For example, the research uses the Dentric cell-derived exosome to carry MHC-I combined with the tumor-derived peptide. This complex can activate the immune cell to inhibit tumors, which can become the new anticancer vaccine [25].

5.2.2 Exosomes as examples of RNA drug carriers

Research shows exosomes innately carry the genetic material (e.g., miRNA, siRNA, mRNA) to the target cell [26], which induces genetic modification in biology and pathogenesis. Exosome carried genes can fuse with the target cell membrane directly and release the nucleic acid drug into the cytoplasm [27], so exosomes can act as the nucleic acid drug carrier vector, which suggests exosome is capable of delivering the miRNA to the target cell. For example, the high expression of let-7 in cancer cell lines will change the cell cycle progress and reduce cell division. This shows that let-7 has the function of tumor suppressor [28]. In addition, the GE11 positive exosomes is a promising carrier for delivering the drug to tumor which expresses EGFR [27]. The research uses the exosomes from embryonic kidney cells, delivering let-7 by intravenous injection. The results show that let-7 effectively inhibits the proliferation of EGFR-expressing xenografts in mouse breast cancer tissues.[27] Another research found out that exosomes derived from co-cultured stellate cells of liver cancer cells promote the growth and invasion of liver cancer cells. The expression of miR-335-5p in both derived exosome and the growing liver cancer cell are down-regulated. When injecting Stellate cell-derived exosomes enriched with up-regulated miR-335-5p to the tumor in the HCC xenograft mouse model, the size of the tumor will decrease [29]. Furthermore, another research finds that high-level expression of miR-145-5p is associated with the progression of pancreatic ductal adenocarcinoma. Exosomes which derived from human umbilical cord mesenchymal stromal cells to deliver exogenous miR-145-5p, the Smad3 pathway expression decreases, inhibiting the proliferation and invasion of the PDAC cell and increasing the effect of apoptosis and cell cycle arrest. The research uses the mouse model to modify the overexpression of miR-145-5p will reduce the growth of xenograft tumors in the body. [30]

5.2.3 Examples of exosomes as chemical drug carriers

Most of the targets of anti-tumor chemicals are located inside the cell. This anti-tumor chemical drug needs to travel through the cell membrane to get to the target to play its role. Due to poor water solubility or premature degradation, the transportation of chemical drugs currently has low efficiency and severe side effects. The presence of the exosome to some extent helps to solve this problem. Moreover, anti-cancer drugs carried by exosomes have an enhanced effect. One research estimates the exosome that carries Paclitaxel origin from Prostate cancer (Pca) cell line PC-3 and LNCaP. Paclitaxel (PTX) can inhibit tumor cell growth. However, due to the low solubility, PTX can lead to exosome to increase its solubility. [31] Another research shows that exosome is continually absorbed by prostate cancer cells. The PTX carried by the exosome goes into the cancer cell by endocytosis, then released to the cell cytoplasm, which successfully leads to apoptosis. The result shows that the extent of exosomes will enhance the cytotoxic effects of drugs. Therefore, the self-derived exosome might have the potential to deliver chemotherapeutic drugs to cancer cells effectively.[32] In addition, another research use macrophage-derived exosomes loaded with paclitaxel which gives better stability and loading efficiency than other drug loading methods. The research results show macrophage-derived exosomes loaded with paclitaxel can effectively inhibit the proliferation of Lewis lung cancer cells and showed an anti-tumor effect in mouse Lewis's lung cancer model. [33]. Doxorubicin is an anti-tumor anthracycline antibiotic, but long-term doxorubicin chemotherapy can cause cardiotoxicities, such as arrhythmia, bone marrow suppression, cardiomyopathy, and congestive heart failure (CHF).[34] By using exosomes to load Doxorubicin, the resulting cytotoxicity will be correspondingly reduced. Yang et al. used a zebrafish model to examine the effect of brain endothelium-derived exosomes to deliver the anti-tumor drug doxorubicin through the blood-brain barrier. The results show that exosomes successfully carried the drug through the blood-brain barrier and inhibited the growth of the tumor. [35] Another research uses combined DC-derived exosome with specific IRGD peptides to load Doxorubicin, thus inhibiting the proliferation and invasion of breast tumor cells. The research result shows that exosomes carrying chemical drugs can target breast cancer more effectively than chemical drugs alone. [36]

6. Conclusion

Exosomes have opened a new chapter as exosomal markers in clinical research for a variety of tumor treatment processes. It is also regarded as a promising delivery system that transfers proteins, nucleic acids, and chemical drugs. Despite the advantages of using exosomes to treat cancer, detailed understanding of the secretion and fusion mechanisms is still unsure. The yield and purity of exosomes during manufacturing still needs to improve. Although several studies have shown techniques for mass production of exosomes and improvements in biocompatibility [37], more preclinical and clinical research is needed to confirm these findings.

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