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Study on multidrug resistance mechanism and reversal strategy of hepatocellular carcinoma based on exosome

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Abstract: *MDR (multidrug resistance) refers to the phenomenon that after tumor cells develop resistance to a chemotherapeutic drug, they also develop cross-resistance to other chemotherapeutic drugs with different chemical structures and mechanisms. HCC (Hepatocellular carcinoma) is the fourth most common cause of cancer-related deaths in the world. Exosomes, produced by polyhedra, are extracellular lipid nano-vesicles with various bioactive substances, which contain lipid RNA, DNA and protein. Exosomes produced by tumor cells carry important information about tumor cells. Studies have found that non-coding RNAs, as an important part of exosomes, are involved in the regulation of the tumor microenvironment of HCC, including tumorigenesis, tumor metastasis, angiogenesis, immune regulation and other processes, and play an important role in the diagnosis, treatment and prognosis of tumors. Therefore, studying the molecular mechanism of MDR and exploring the reversal strategy is a hot topic in tumor research at present. This paper reviews the research progress in this field.*

Keywords: *Exosomes, Hepatocellular carcinoma, multidrug resistance, Reversal strategy*

1. Introduction

The mechanism of multi-drug resistance (MDR) is complex. Many studies have found that MDR is composed of and participated in by multiple resistance proteins, and there are multiple resistance-related proteins participating together [1]. With the deepening of research on the mechanism of MDR, the generation of multi-resistance mechanism has entered the gene level, but the mechanism of MDR is still not fully understood, indicating that there are other ways to generate multi-resistance mechanism [2]. MDR is the main reason for the failure of tumor chemotherapy, and it is also an urgent problem to be solved in tumor chemotherapy. Chemotherapy drugs that easily cause MDR such as vincristine, doxorubicin, and mitomycin are called MDR drugs. Overexpression of p-glycoprotein, an expression product of MDR gene 1(MDR1), is an important cause of MDR in tumor cells.

The immune cell population is closely related to local inflammation occurring in tumor interstitium, cancer cells, and tumor microenvironment. Studies have found that exosomes can cause the expression of pro-inflammatory cytokines by activating the TLR2 and TLR4 signaling pathways, and have similar effects on cell signaling as TLR agonists [3]. At present, a variety of biomarkers have been found in exosomes, such as the detection of EGFR, MET, ALK and BRAF in exosomes to be markers of lung cancer, which has provided a new idea for the study of biomarkers of liver cancer. In addition, exosomes are easy to be obtained clinically, causing less trauma to patients and showing high sensitivity. Therefore, exosomes have great research value in the diagnosis and treatment of Hepatocellular carcinoma (HCC).

2. Exosomes and their biological effects as molecular markers

2.1 Biological properties of exosomes

Exosome is a vesicle with a diameter of 30–100 nm and a double-layer membrane structure, which can be secreted into the microenvironment by a variety of cells through exocytosis. The secretion of exosomes and their composition are shown in Fig. 1.

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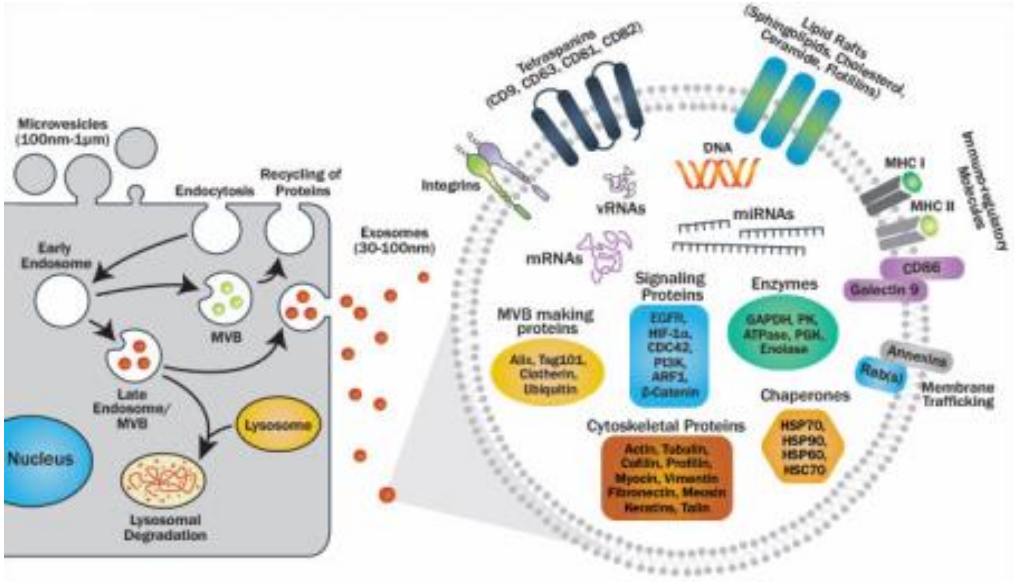

Figure 1: Exosome secretion and exosome composition

Exosomes were first identified in reticulocytes by JOHNSTONE in 1987. Subsequent studies have revealed that exosomes can be secreted by lymphocytes, platelets, dendritic cells, endothelial cells, mast cells, fibroblasts, tumor cells, mesenchymal stem cells and other cells, and exist in blood, urine, amniotic fluid, intraperitoneal effusion, bronchoalveolar lavage fluid, synovial fluid, milk and other various body fluids. It has been proved that exosomes can participate in cell communication, immune response, cell migration, angiogenesis, and tumor cell growth [4].

2.2 Proteins in exosomes

Nearly 10,000 different proteins exist in exosomes of different origins, and many of them have been widely used as biomarkers due to their frequent expression. Exosomes from different sources have similar biosynthetic pathways, so most of the proteins contained in exosomes are related to the MVB formation process. The proteins most commonly identified in exosomes are fusion proteins, membrane transporters, heat shock proteins, and the superfamily of four-transmembrane proteins. Some specific proteins, such as CD81 and CD63, are considered to be marker proteins of exosome and can be used for the identification of exosome [5].

2.3 Biological effects of exosomes as molecular markers

More and more evidences show that the contents in exosome have potential biological marker function and can be used to reflect some diseases. In particular, the marker proteins contained in exosome have the function of reflecting the physiological traits of cells. Such as CD63 and CD81 of the tetraspanins family of exosome-labeled proteins. It has been found in the literature [6] that the expression level of CD81 in exosomes is significantly increased in serum of patients with chronic hepatitis C disease, indicating that CD81 can be used as a potential diagnostic marker of hepatitis C. In the previous study [7], the protein spectra of urine-derived exosomes from healthy people and patients with bladder cancer were compared, and significant differences were found in eight proteins, of which five proteins were related to the epidermal growth factor receptor pathway. With the deepening of research on exosome-related proteins, more early diagnosis methods for diseases are provided for clinic.

In addition to exosome-related proteins, many studies have shown that miRNA in exosome can also be used as ideal biomarkers for clinical diagnosis. Literature [8] has revealed that there are four statistically different miRNAs in the bronchoalveolar lavage fluid-derived exosomes of patients with COPD and non-COPD, and the common action target of these four miRNAs is s6 kinase, which is a part of mTOR signaling pathway that can mediate autophagy of skeletal muscle cells, suggesting that the amount of miRNAs in the exosomes of patients can be compared with that of non-patients as a new method for clinical diagnosis.

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3. Mechanism of drug resistance in HCC

3.1 Protein related drug resistance

Drug transporters are the general names of the protein that take drugs as the matrix and exist on the cell membrane surface of tissues or organs, serving as the transmembrane transport function of drugs. They are divided into two superfamilies: ATP Binding Cassette Super Family (ABC) and solute carrierfamily (SLC) (Fig. 2). Glycoprotein (P-gp) is a transmembrane glycoprotein encoded by tumor MDR gene MDR-1 gene and belongs to the transporter ABC [9].

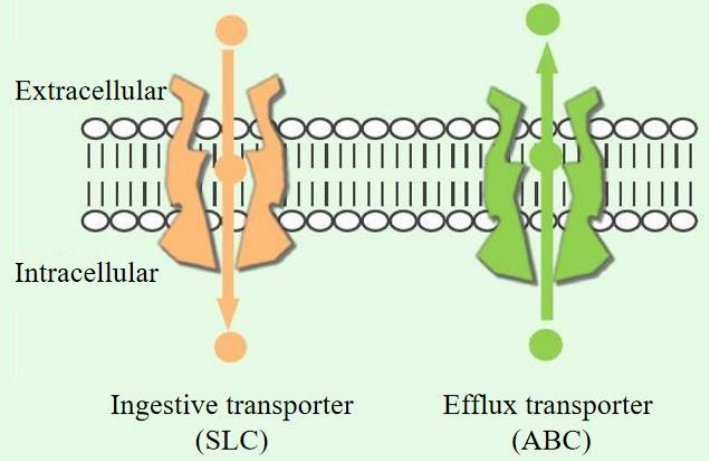

Figure 2: Drug swivel and its classification

Many lipophilic anticancer drugs need a long time to cross the cell membrane, which is enough for them to combine with P-gp between lipid bilayer and cause the drugs to be discharged before entering the cells, thus playing the role of the so-called "hydrophobic vacuum pump". Similar to P-gp, Multidrug Resistance Associated Protein (MRP) uses ATP to transport drugs to the outside of the cells, belonging to the ABC superfamily [10]. However, MRP is not inhibited by the regulators of MDR-1.

3.2 Abnormal intracellular enzyme system

DNA topoi-somerase (Top) is the main ribozyme for cellular DNA replication and transcription. There are two main types of topoisomerases in eukaryotic cells, one is topoisomerase I (TOPO I), and the other is topoisomerase II (TOPO II). Compared with Topo I, Topo II plays a more critical role in the process of cellular DNA replication, transcription and mitosis. Topo II, as a target of anti-cancer drugs such as doxorubicin, etoposide (VP-16) and mitoxantrone [11], its expression is positively correlated with VP-16 resistance and negatively correlated with amoxicillin resistance [12].

Glutathione S-transferase (GST) is a family consisting of a series of isozymes and serves as an important intracellular detoxification system. GST-π expression was positively correlated with cisplatin resistance. The endogenous expressions of GST-π and TopoII were different in four human lung cancer cell lines with different tissue types, which might be related to the change of cisplatin chemotherapy sensitivity. Among the related proteins, GST-π might be very useful for predicting cisplatin resistance [13].

3.3 Apoptosis regulatory gene mediated

Bcl-2 family is a family of gene related to apoptosis and also a family of homologous protein. These homologous proteins are mainly located on the inner membrane of cells and act to prevent or promote the escape of various death activators from the mitochondria. The most important two members are bcl-2 and bax. The enhanced expression of this gene significantly inhibits apoptosis, and bax is an apoptotic gene [14]. At present, more and more studies have shown that bcl-2 not only promotes chemotherapy tolerance, but also improves tumor recurrence rate, radiotherapy tolerance and malignant transformation potential of tumors [25].

The p53 gene, located in the short arm of chromosome 17, encodes a 53kd nuclear phosphoprotein p53 protein consisting of 393 amino acids. There are two kinds of p53 gene:

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Wild-type. wt-p53gene is a tumor suppressor gene that induces apoptosis of tumor cells, thereby inhibiting the growth and proliferation of cancer cells;

The mutant p53 gene not only lose that function of a tumor suppressor gene, but also leads to the los of apoptosis signals of cancer cells, thereby increase the tolerance of the cancer cells to apoptosis induced by anticancer drugs, and reducing or losing the efficacy of the anticancer drugs. C-myc, c-fos and c-jun as well as Rb and NF-κB genes are also involved in apoptosis regulation related to drug resistance.

3.4 Exosomes and drug resistance of HCC

HCC is not sensitive to traditional chemotherapeutic drugs, which is related to its specific microenvironment and tissue specificity. Exosomes, as an important component of its microenvironment, regulate the sensitivity of chemotherapy through multiple ways.

Exosomes produce resistance to HCC in vitro and in vivo by activating the HGF/c-Met/AKT signaling pathway and inhibiting sorafenib-induced apoptosis. In addition, a study in the literature [17] has revealed that exosomes produced by macrophages can significantly reduce the sensitivity of pancreatic ductal adenocarcinoma cells to gemcitabine in vitro and in vivo. This effect is mediated by the transfer of miR-365 in exosomes produced by macrophages. MiR-365 inhibits the activation of gemcitabine by overexpressing a nucleotide triphosphate library and inducing cytidine deaminase in cancer cells, resulting in drug resistance.

In addition, it has been found in the literature [18] that the treatment of liver tumor cells with the chemotherapeutic drug sorafenib resulted in the proliferation of exosomes rich in lincRNA-ROR, which activated the transforming growth factor-β pathway and led to drug resistance of HCC. In summary, exosomes can directly or indirectly regulate the sensitivity of chemotherapeutic drugs through a variety of pathways, leading to the development of drug resistance.

4. Reversal strategy of mdr in tumor cells

4.1 P-gp reversal strategy

P-gp reversal strategies mainly include: (1) the use of P-gp reversal agents, which mainly achieve the purpose of reversal by specifically or competitively binding to P-gp; (2) Immunotherapy, namely, P-gp function is blocked by specific antibodies; (3) Gene therapy, including single-chain antibody technology, ribozyme technology and small molecule interfering RNA technology, can block the action of MDR-1 at the level of DNA or RNA [19].

4.2 Reversal agent for apoptosis regulatory gene

The addition of pro-apoptotic substances such as all-trans retinoic acid could reduce the expression of genes such as BCL-2 and BCL-XL in drug-resistant cells. Implantation of pro-apoptotic genes, such as wild-type p-53 gene, etc. The results showed that wild-type p-53 transfection could play a synergistic or additive role with some drugs such as 5-FU, cisplatin and bleomycin. In clinical trials, literature [20] showed that among 24 patients with advanced NSCLC and abnormal p53, intratumoral injection of adenovirus-mediated p53(Adp53) combined with CDPP was effective in 17 cases.

4.3 Immunotherapy reversal

Exosomes secreted by tumor cells can induce apoptosis of natural killer cells and cytotoxic T cells by delivering TRAIL, FasL, and others, which is beneficial for tumor cells to escape from immune surveillance. Macrophages transfer specific endogenous miRNA into HCC in a cell-contact-dependent manner and inhibit tumor cell proliferation by affecting protein expression.

Literature [21] introduced the role of exosomes in the process from chronic hepatitis B to hepatocellular carcinoma. The relationship between exosomes and hepatocellular carcinoma: Application of exosomes in diagnosis and prognosis of liver cancer: Application of exosomes in the treatment of liver cancer. We summarized the latest related research contents to reveal the importance of exosome in the occurrence, development, diagnosis and treatment of liver cancer, so as to provide reference for researchers to further clarify the mechanism of exosome in liver cancer, and promote the application of exosome in clinical diagnosis and treatment of liver cancer (Fig. 3).

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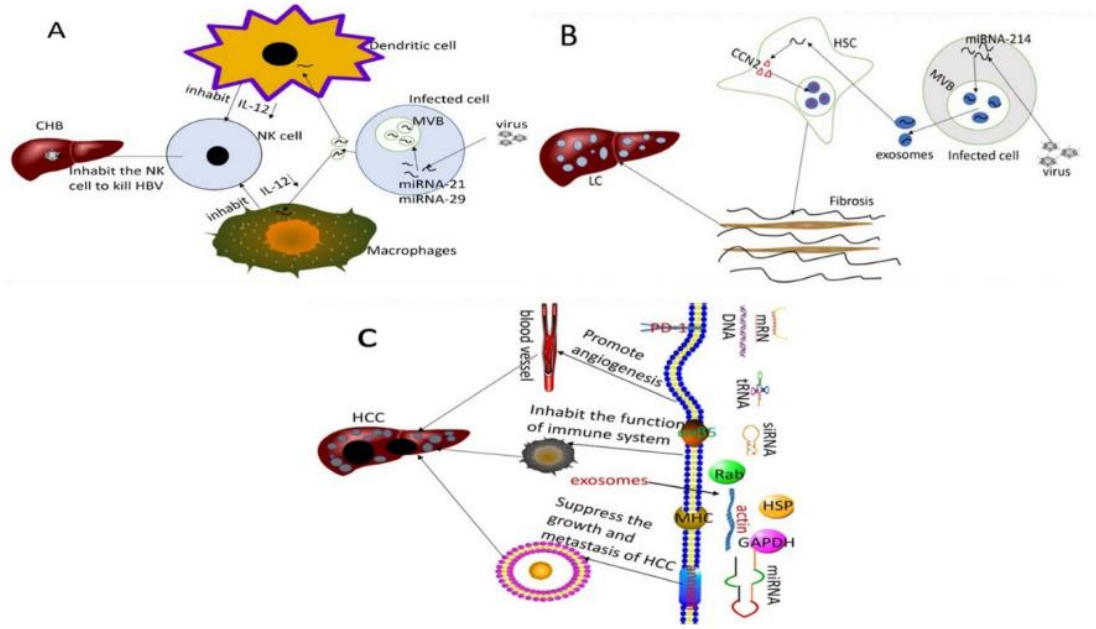

Figure 3: Mechanism of exosome in the transformation of chronic hepatitis b into HCC

HCC metastasis is an important part of the development process of HCC. The exosome ncRNAs can promote the invasion and metastasis of HCC by mediating biological processes such as epithelial-mesenchymal transition, extracellular matrix degradation and vascular leakage. The exosome ncRNAs can mediate epithelial-mesenchymal transformation of HCC cells, even if tumor cells lose their epithelial phenotype to acquire the mesenchymal phenotype, resulting in decreased intercellular adhesion, enhanced mobility, and eventually tumor invasion and metastasis. In vitro and in vivo studies have shown that miR-320a can up-regulate cyclin-dependent kinase 2 by directly binding to the downstream target PBX3, thereby inhibiting the proliferation and growth of HCC cells [22]. Literature [23] has revealed that exosome and ubiquitination-related cyclic RNAcirc-DB is up-regulated in patients with HCC with high body fat. CIRC-DB promotes the growth of liver cancer cells and reduces DNA damage by inhibiting miR-34a and activating USP7/CY-Clina2 signaling pathway related to deubiquitination.

The development of cancer is closely related to the tumor immune microenvironment. By regulating the immune response, tumors can escape from immune destruction. The high malignancy and poor overall survival result of liver cancer are closely related to the inhibition of host immune response. The exosomes released by liver cancer cells can inhibit the immune system of patients and prepare for metastatic spread. The exosome miR-23a-3p can be transferred from endoplasmic reticulum-stressed HCC cells to macrophages, and induce tumor immune escape by inhibiting PTEN to activate PI3K-Akt pathway, increase PD-L1 expression and inhibit T cell activation [24].

It has been reported in the literature [25] that exosome-derived HMGBl activates B cells, promotes the expansion of TIM-L+Breg cells through Toll-like receptor 2/4 and mitogen-activated protein kinase signaling pathway, expresses high-level immunosuppressive cytokine IL-10, and displays strong inhibitory activity on CD8+T cells, thereby promoting immune escape of HCC. In another study, the exosome lncRNA TUC339 derived from HCC regulated macrophages, resulting in decreased production of pro-inflammatory cytokines such as IL-1β and TNF-α, impaired phagocytosis, and M2 polarization, thereby promoting the progression of HCC.

5. Summary

Although domestic and foreign scholars have carried out extensive and in-depth research on the drug resistance mechanism of tumors, especially MDR, the understanding of the drug resistance mechanism is still incomplete. Therefore, it is of great significance to find high-sensitivity and high-specificity biomarkers for early diagnosis and treatment of HCC. With the continuous discovery of high-efficiency and low-toxicity reversal agents, the continuous development of gene therapy technology and the application of evidence-based medicine to guide clinical practice, the strategy for reversing tumor MDR will be continuously improved. More and more studies have found that exosome ncRNAs play an

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important role in the occurrence, development, diagnosis and prognosis of HCC. Stable exosome can be used as good biomarkers and is of great significance for the early and specific diagnosis and treatment of tumors. However, the understanding of exosome is still in the initial stage and many problems still need to be clarified. Further studies are needed on how to develop exosomes for the early diagnosis and treatment of tumors.

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