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The Role of Exosome MicroRNA-103 in the Proliferation and Invasion of Hepatoma Cells and Its Mechanism

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ABSTRACT

Liver cancer (HCC) is a common malignant tumor whose incidence is increasing worldwide, but existing chemotherapeutic agents are not ideally effective drugs and have considerable resistance to chemotherapy. Exosome microRNA-103 plays an important role in the proliferation and invasion of liver cancer cells. The purpose of this article is to investigate the role and mechanism of exosome microRNA-103 in hepatocellular carcinoma cell proliferation and invasion. 84 patients with hepatocellular carcinoma diagnosed in a hospital from June 2017 to June 2020 were selected. The average age was 60.13±6.99 years. When the patient was fasting, 3 mL of peripheral venous blood was taken. And 50 healthy control groups were established, with an average age of 59.98±8.18 years old. 3 ml of peripheral venous blood was collected on an empty stomach to compare the cell proliferation rate and invasion rate. The results of the study showed that the number of stage III hepatocellular carcinoma cells invaded at 6h was 68.9, and it changed to 89.4 at 12h, and 106.4 at 24h; compared with that, the cell invasion rate in stage IV was higher. The number of stage IV hepatocellular carcinoma invasions at 6h was 68, which changed to 94.5 at 12h and 112.4 at 24h.

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer in the world and the third most common malignant tumor. According to the report, more than 50% of new liver cancers in the world occur in my country, and the incidence rate has been continuously rising, causing serious harm to people's health. Not only is the incidence of liver cancer high, but the treatment is not optimistic. At present, surgical treatment is still the best way to treat liver cancer, but the initial symptoms of liver cancer lack specificity, and only a few patients have the opportunity to undergo surgery. Therefore, new medical mechanisms are needed for treatment (1-3).

MicroRNA-103 plays an important role in the diagnosis and subsequent treatment of liver cancer. This is the consensus of experts in the medical field. Oze detected exosomes isolated from the serum of chronic hepatitis B (CHB), cirrhosis, HCC patients, and HCC patients, as compared with patients with chronic hepatitis B or cirrhosis, microRNA-103 levels were elevated, so exosome microRNA-103 can be used as a biomarker for the diagnosis of HCC (1). Tamkovich claimed that microRNA-103 was detected in the blood of HCC patients. MicroRNA-103 can bind and modify epigenetic factors at specific genomic sites, which ultimately leads to an imbalance in gene expression related to HCC development, thereby promoting the HCC process (2). Zaharie believes that microRNA-103 can also promote tumor cell migration by inhibiting the expression of liver cancer cell p120 catenin (3). Yao pre-treated monolayer endothelial cells with stable expression of hepatoma cell secretions, and found that it can promote the transendothelial infiltration of tumor cells, and the expression of this exosome can be reduced by inhibiting endothelial cells microRNA-103 (4). Lin's research showed that microRNA-103 secreted by liver cancer cells can be transduced into endothelial cells by exosomes by directly inhibiting

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the expression of vascular endothelial cadherin, p120 catenin and obturator region (5). In conclusion, exosome microRNA 103 secreted by liver cancer cells enhances vascular permeability and promotes tumor metastasis by targeting multiple endothelial connexins.

The development of liver cancer is a complicated process. In this complex process, hepatocytes undergo proliferation and abnormal differentiation and acquire a malignant phenotype that metastasizes in and beyond the liver. Currently, there are many studies in the medical field. In a human study involving 8 specimens of liver cancer and adjacent tissues. Soeda compared the differences in microRNA expression between cancer tissues and adjacent tissues and was highly correlated with the abnormal expression of 16 microRNAs (6). Wang used liquid chip technology combined with real-time RT-PCR to analyze 36 liver cancer patients to determine the grade of liver cancer, tumor differentiation, the presence of intrahepatic and extrahepatic metastases, and the presence of portal vein thrombosis. The degree of microRNA is closely related to other factors we found (7). Zhang analyzed 100 patients with HCC and pointed out that liver cancer is usually caused by viral or alcoholic hepatitis, and liver cancer cells have long been subjected to various harmful stimuli, including viral infections and inflammatory stimuli (8). Xuesong used microRNA chips to detect microRNA expression in the blood circulation of liver cancer patients and found that significantly up-regulated microRNA can be used as a marker for liver cancer (9). Nakashima revealed that comprehensive treatment for liver cancer includes surgical resection, hepatic artery chemoembolization, liver transplantation and radiofrequency ablation. Surgical treatment is the preferred method of treating liver cancer. Although liver transplantation can provide more satisfactory results, the development of liver transplantation is severely limited due to the lack of donor livers (10). Therefore, there is an urgent need to combine research on the causes of liver cancer to further develop new therapies aimed at improving the prognosis of liver cancer patients and improving the quality of life of liver cancer patients.

Cancer cell invasion is the invasion or possession of malignant tumors from primary or secondary tumors to adjacent host tissues (11). The main process of cell invasion is that the tumor cells are close to the organ surface → the endothelial cells with prosthetic feet have tumor cells attached to the surface → the tumor cells use the prosthetic feet to generate a natural cell gap to reach the basal layer → the tumor cells penetrate into the organ → form a cancer nest Factors involved in the process of cell invasion. It is secreted by tumor cells and can stimulate cell movement in many ways, such as chemotaxis and phagocytosis. Schizont is an important category (12). There are three types of mitogens, typical are an autocrine factor (AMF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and transforming growth factor (TGF). This is a type of cell surface structure that promotes cell-cell or cell-cell interaction. It consists of four superfamilies: integrin, cadherin, immunoglobulin and selectin. There are many studies on integrin, these studies can give cells the ability to interact with the basement membrane (13). Extracellular matrix and basement membrane are obstacles to tumor metastasis. They are composed of fibronectin, laminin, glass binding protein, type IV collagen and elastin (14). Once the decomposition is released, it acts as a chemokine, guiding it to the site of release. Proteolytic enzymes can degrade the substrate. Common ones include matrix metalloproteinase (MMP), cytosolic enzyme, urokinase plasminogen activator (UPA) and cathepsin. Angiogenesis is aggressive in nature, and tumor cells are eroded by collagen cracks that open along new blood vessels, specifically shown in Figure 1.

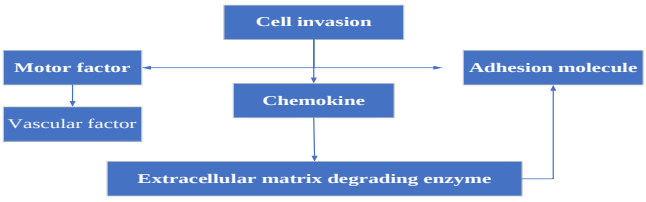

Figure 1. Factors involved in cell invasion

Micro RNA (mi RNA) is a group of natural non-coding small RNAs, approximately 21 to 25 nucleotides in length (15). MiRNA can specifically inhibit or degrade the translation of target mRNA and regulate the expression of target genes. MiR-103a-3p (also known as miR-103a) is a member of the mi RNA family and is the product of the 3'end of the precursor of mi R-103a (16). MiR-103a-3p is differentially expressed in different tissues and can be used as both a cancer promoter and tumor suppressor

to regulate tumor occurrence and development. Judging from the nature of such molecules, the molecule can regulate the entire life process of the cell (17). MiRNA and the 3'untranslated region of the target gene mRNA is complementary to each other, combining development and inhibiting the target gene according to the transcription mechanism, thereby controlling various biological processes (18).

This article mainly explores the role and mechanism of exosome microRNA-103 in the proliferation and invasion of liver cancer cells. 84 patients with hepatocellular carcinoma diagnosed in a hospital from June 2017 to June 2020 were selected, with an average age of 60.13±6.99 years old, when the patient is in a fasting state, then take 3 mL of peripheral venous blood. And 50 healthy control groups were established, with an average age of 59.98±8.18 years old. 3 ml of peripheral venous blood was collected on an empty stomach to compare the cell proliferation rate and invasion rate. The research results show that the OD value of the proliferation rate of liver cancer cells is only 0.26 at the beginning, and it has become 0.57 at 6h, and the value has become 1.23 at 24h; and after adding mi RNA-103 inhibitor, the OD value began to decline, from the initial 1.23 to 0.58. The innovation of this study is the first to link exosome microRNA-103 with the proliferation and invasion of liver cancer cells. At the same time, the microRNA-103 inhibitor was compared with the two groups without the inhibitor, and the mechanism of action of microRNA-103 was explored.

Materials and methods

Study Object Inclusion and Exclusion Criteria

We selected 84 patients with hepatocellular carcinoma diagnosed in a hospital from June 2017 to June 2020. The patients included 35 males and 49 females, with an average age of 60.13±6.99 years. When the patient was fasting, 3 mL of peripheral venous blood was taken. According to the latest TNM staging in 2015 (UICC / AJCC), there are 18 cases of stage IIIA, 22 cases of stage IIIB, 24 cases of stage IVA, and 20 cases of stage IVB. At the same time, peripheral blood is collected from healthy adults. There were 28 males, 22 females, and 50 healthy controls. The average age was 59.98±8.18 years old. 3 ml of peripheral venous blood was collected on an empty stomach. The details are shown in Table 1.

Table 1. Basic information about the subjects

Group	Age	Sex ratio	Type
Experimental group	60.13±6.99	35:49	IIIA:IIIB:IVA:IVB= 18:22:24:20
Control group	59.98±8.18	28:22	×

Inclusion criteria: The patient's pathological diagnosis was confirmed to be hepatocellular carcinoma, or he had a history of hepatitis, and imaging studies also suggested hepatocellular carcinoma. Exclusion criteria: Patients with liver cancer must exclude thyroid disease, systemic lupus erythematosus, rheumatoid arthritis, and other diseases that affect immune function and another tumor history.

Experimental Instrument Materials

Human liver cancer cell line Hep G2, PBS, isopropanol, dimethyl sulfoxide (DMSO), ultra-low temperature refrigerator, luciferase reporter gene kit, immunohistochemistry kit, mi RNA-103 mimics, RNA reverse transcription kit, Mi RNAs real-time quantification kit.

Experimental Testing Measures

All cell lines are routinely cultured in a PRMI-1640 medium containing 10% FBS and diabody (100 IU penicillin and 100 μg/ml streptomycin), 5% CO2 and 37°C (19). According to cell proliferation, subculture every 2-3 days.

After configuring the materials and reagents required for the experiment, perform a mixing operation to start the PCR reaction (20). The specific conditions of the reaction system are 16°C for 30 minutes, 42°C for 30 minutes and 85°C for 10 minutes (21). After the reaction was completed, the reaction product was stored in a refrigerator at 20°C. Centrifuge immediately, mix the above components and run 40 cycles at 95°C for 60 seconds. The PCR reaction was performed on an ABI7500 fluorometer (22). Lyse tissue and use lysate to achieve the goal. After adding the corresponding amount of lysate, gently shake the culture plate and place it on ice for 30 minutes to achieve complete cell lysis. Place the above materials in a centrifuge tube at 1500 rpm for 5 minutes at 4°C in a pre-cooled centrifuge (23). Process the material obtained by centrifugation, aspirate the supernatant, and then use the kit to

complete the protein concentration determination. After the measurement, please keep it at a low temperature below 70℃.

Inoculate 1 x 105 cells into the upper chamber, add 100 μl of medium and 600 μl of medium containing 10% FBS, and place the trans well chamber in a 37°C environment for 24 hours (24). After that, remove the remaining cells, fix the cells below, and use crystal violet to achieve the staining effect. After the cell staining was completed, the number of cells was measured with an optical microscope, and the data of the control group were compared and analyzed. For the scratch experiment, the cells were seeded in a 24-well plate until the cells covered the bottom of the plate (25). Then use a sterile pipette tip to draw a straight line in the center of the bottom of the well, and rinse off the floating cell debris with PBS. Add serum-free medium, incubate for 24 hours and calculate the cell penetration distance.

Observation Index

Collect sample protein supernatant for detection, and use dual-luciferase reporter gene detection system to detect luciferase activity. The ratio of firefly luciferase activity to nielli luciferase activity is used to eliminate errors in transfection efficiency. The relative activity of luciferase is expressed as a fold change.

In order to ensure the accuracy of the experimental results, it is necessary to statistically process the experimental operation flow. At the same time, the experimental data are expressed as mean ± standard deviation (x ± s). The test data should be analyzed by the T test. When the significance level reaches p <0.05, it indicates that the experimental results are statistically significant.

Results and discussion

Exosome Expression Correlation Comparison Results

The expression level of microRNA-103 in patients with liver cancer was positively correlated with clinicopathological parameters. With the development of liver cancer, the expression level of microRNA-103 in liver cancer tissues continues to increase. The indicators of the two groups of patients are shown in Table 2, the expression of microRNA-103 in hepatocellular carcinoma is only related to the TNM stage (P<0.05), but not related to age, smoking history, pathological type, tumor size, lymph node metastasis and survival (P>0.05).

Table 2. Comparison of the levels of peripheral blood regulatory T cells between the two groups

TNM staging	IIIA	IIIB	IVA	IVB
MiRNA expression	4.356	6.765	7.862	10.239

Analysis of the Relationship between MicroRNA-103 on Proliferation and Invasion of Liver Cancer Cells

The results show that the addition of a microRNA-103 inhibitor can significantly reduce the proliferation rate of liver cancer cells. Further analysis showed that the experimental group with hepatocellular carcinoma xenografts stably expressing microRNA-103 had higher tumor vascular permeability, higher exosome microRNA-103 levels and higher levels than the control group. If the internal tumor cells are large, it indicates an increased rate of liver and lung metastases. The OD value is an important expression that reflects cell proliferation. As shown in Figure 2, the initial OD value of hepatocellular carcinoma cell proliferation rate was only 0.26. By 6h, it had become 0.57, and by 24h, the value had become 1.23; and after adding mi RNA-103 inhibitor, the OD value. It began to decline, from the initial 1.23 to 0.58. This shows that microRNA-103 can promote the proliferation of liver cancer cells, and the addition of microRNA-103 inhibitors will reduce the expression of exosomes, thereby reducing the proliferation of liver cancer cells.

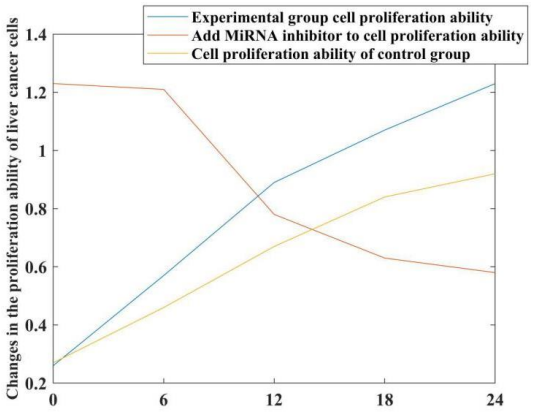

Figure 2. Comparison of proliferation ability of liver cancer cells

Studies have shown that the expression of microRNA-103 can significantly increase the invasion

rate of liver cancer cells. As shown in Figure 3, the number of stage III liver cancer cell invasion at 6h was 68.9, and it changed to 89.4 at 12h, and 106.4 at 24h; compared with this, the cell invasion rate in stage IV was higher. The number of hepatocellular carcinoma cells invaded at stage 6 at 6h was 68, and it changed to 94.5 at 12h, and 112.4 at 24h. After adding the microRNA-103 inhibitor, the number of cell invasions decreased from the initial 102.8 to 73.6, which reflects the expression of microRNA-103 is closely related to the invasion of liver cancer cells.

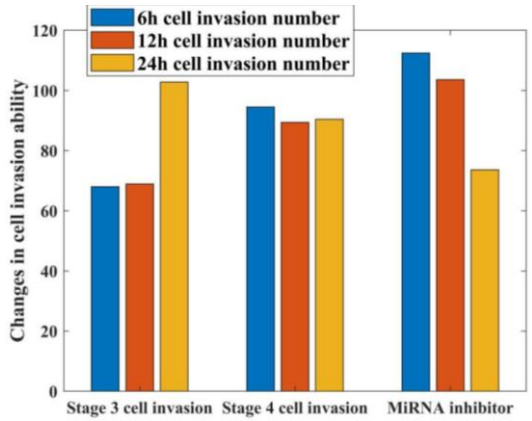

Figure 3. Change of invasion rate of liver cancer cells

Analysis of the Mechanism of Proliferation and Invasion of MicroRNA-103 on Liver Cancer Cells

Studies have shown that microRNA-103 secreted by liver cancer cells is transduced to endothelial cells through exosomes, thereby increasing the expression of vascular endothelial cadherin, p120 catenin and obturator zonules, improving the integrity of endothelial junctions. Proved that the reduction of microRNA-103 can promote vascular permeability and metastasis of liver cancer cells. As shown in Figure 4, after adding microRNA-103 inhibitor, the expression of P120 protein decreased and fluctuated in the range of 0.17-0.20; while the expression of P12 protein in normal liver cancer cells continued to increase. This shows that silencing microRNA-103 can reduce the content of p120-catenin, thereby reducing vascular permeability and inhibiting the proliferation and invasion of liver cancer cells.

The results of this study showed that the addition of microRNA-103 inhibitors reduced the proliferation and invasion ability of liver cancer cells, and no serious side effects occurred while the disease was under control. As shown in Figure 5, 17 cases of stage IIIA, 21 cases of stage IIIB, 17 cases of stage IVA, and 11 cases of stage IVB after treatment with microRNA-103 inhibitors were reduced by 1, 1, 7, and 9 respectively. For example, this illustrates the role of silencing microRNA-103. This article believes that after the use of microRNA-103 inhibitors, the patients with stage IVA and IVB stages are under control, and most of them are transferred to stages IIIA and IIIB. Therefore, the number of people with stage IIIA and IIIB has not decreased significantly compared with before.

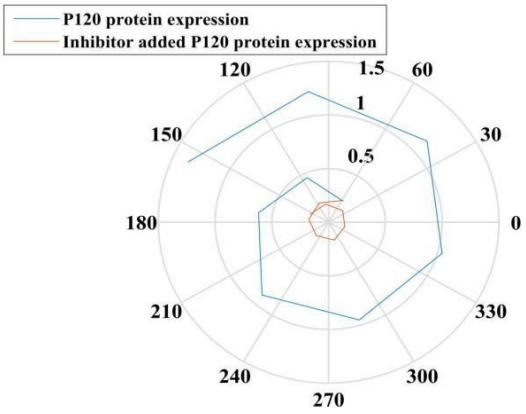

Figure 4. P120 protein expression

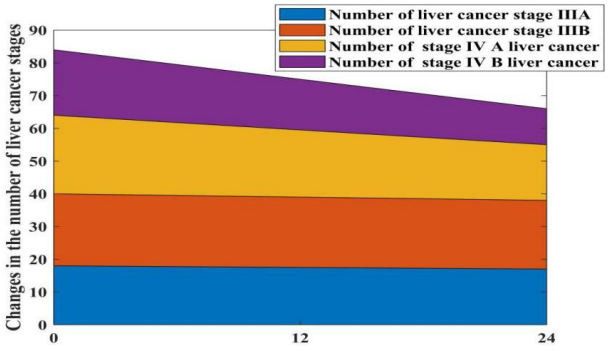

Figure 5. Changes in the number of patients with liver cancer staging

There are various strategies for growth inhibition or eradication of cancer cells (26, 27). Exosomes can participate in the exchange of materials and information between cells and play an important role in various physiological and pathological processes. During the formation of liver cancer, cancer cells must effectively communicate with neighboring cells and their local microenvironment. The complex tumor microenvironment and the interaction between cancer cells and non-cancer cells promote the occurrence of tumors. Cancer cells secrete exosomes and exchange

signals with surrounding fibroblasts, endothelial cells and immune cells, thereby promoting cancer invasion and metastasis, angiogenesis, matrix remodeling and drug resistance. The origin of tumors or tumor-related exosomes is an important mechanism regulating tumorigenesis and development. Tumor exosome analysis and detection are helpful for early tumor diagnosis, efficacy evaluation and prognostic analysis.

The high expression of microRNA-103 secreted by liver cancer cells enhances the permeability of monolayer endothelial cells. MicroRNA-103 delivered to endothelial cells through exosomes can not only improve low-metastatic tumor cells but also directly inhibit the expression of vascular endothelial cadherin, p120 catenin and obturator region, thereby enhancing endothelial junction. It directly reduces the integrity and can modify the metastasis to the microenvironment, thereby promoting the local spread of tumor cells and distant metastasis. In addition, microRNA-103 can also promote the migration of tumor cells by inhibiting the expression of p120 protein in liver cancer cells.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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