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miR-144 negatively regulates the effect of CCNB1 protein on the biological behavior of hepatoma cells

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ABSTRACT

Liver cancer poses a great threat to the life safety of patients, which is a common malignant tumor worldwide. This study aims to explore the effect of miR-144 negatively regulating CCNB1 on the biological behavior of liver cancer cells, including the proliferation, apoptosis and migration of liver cancer cells, so as to provide a sufficient biological basis for the treatment of liver cancer. A 3 armour hospital at the records of 100 patients with liver cancer in 2015-2019 as the research object, and resection of the liver cancer cells and tissue adjacent to carcinoma as the research samples, using polymerase chain reaction (PCR) for the organization of miR-144 gene and detect CCNB1 protein expression level, and by using a technique called RNA interference to silence the CCNB1 gene, and try to transfer by transfection CCNB1 protein, thus all kinds of biological behaviour of hepatocellular carcinoma cells. The liver tissue of miR-144 is low, the level of gene expression CCNB1 protein expression level is higher, the expression level in liver cancer cells directly influences the curative effect of hepatocellular carcinoma patients, the miR-144 gene can negative regulation CCNB1 protein, through this kind of negative adjustment to the biological behavior of liver cancer cells have a profound impact.

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Introduction

At present, hepatocellular carcinoma ranks fifth in the global incidence of malignant tumors and third in the mortality rate of malignant tumors (1). The occurrence of liver cancer involves many factors. It is a complex and changeable progressive process, which will result in the transformation of multiple signal factors, thus forming a complex molecular spectrum (2). At present, the medical community has not reached a unified conclusion on the pathogenesis of liver cancer (3). For a long time, the preferred treatment for liver cancer is radical surgery, but the clinical treatment results of liver cancer show that more than half of the patients with liver cancer will have metastasis and disease recurrence within 5 years after treatment (4). In recent years, due to the emergence and development of targeted therapy for liver cancer, the research on liver cancer in the medical field has gradually deepened into the field of gene and protein levels (5). Many foreign researchers believe that there is a close relationship between the molecular level of many types of genes and proteins and the biological behavior of liver cancer cells, which affects the staging,and the precise treatment of gene targets (6). Existing studies have shown that miR-144 is involved in the biological behavior of tumor cells, and CCNB1 protein, as a prototypal product of some cancer genes, has been studied by some researchers, and the results indicate that CCNB1 protein can be a potentially effective target for tumor therapy (7).

miR-144 belongs to a type of microRNAs, or miRNAs for short. It is an endogenous non-coding RNA with a length of about 22 bases. It can degrade target genes by using non-coding regions, thus inhibiting normal gene translation(8-9). Therefore, abnormal expression of miRNAs can effectively explore the pathogenesis of liver cancer and explore new targets for the treatment of liver cancer (10). MiRNAs are small pieces of single-stranded RNA of a non-coding type with a certain evolutionary conservatism that exist in eukaryotic cells, with a length of about 18-25 nucleotides (11). It can complement the bases of target genes, thus affecting the stability of mRNA and the translation process, and on this basis, regulate protein expression (12). miRNAs affect cell proliferation and apoptosis and play an important role in the development of organisms (13). Existing studies have shown that the regulated gene types account for about 30% of the total number of human genes. All kinds of miRNAs are able to regulate the translation of target mRNA according to different types of pairing, with the highest regulated type up to 200 (14). Up-regulation and down-regulation of multi-type miRNA expression can be observed in all types of tumor tissues, thus influencing the gene expression of multi-type tumors and finally achieving the effect of inhibiting oncogenes (15). Therefore, miRNAs play an important role in the de-

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tection and treatment of early-stage tumors (16). miR-144 is an important component of miRNA target genes. Current research results indicate that the expression of miR-144 is down-regulated in multiple tumor tissues, which may inhibit the proliferation of cancer cell genes to a large extent and have an important impact on the biological behavior of cancer cells (17).

Materials and Methods

Experimental materials

In this study, a total of 100 patients with liver cancer admitted to a third-grade a hospital from 2015 to 2019 were selected to study the liver cancer tissues and adjacent tissue samples of these 100 patients. There were 60 male patients and 40 female patients, with an average age of 50.34. These patients in the treatment of liver cancer radical surgery, in this paper, the liver cancer patients after surgical treatment of liver cancer and tissue samples collected, the specimen within five minutes after leaving the body will not damage parts of the central region of the cancer cells to shear, to be in a 5 mlep tube disease quickly placed in a liquid nitrogen tank, and then put it into the refrigerator of temperature to - 80 ℃ cold storage processing, after the surgery for patients with follow-up, the follow-up period of 12 months.

Inclusion criteria

There was no targeted therapy or targeted therapy for liver cancer patients before the study; After surgery, pathological examination confirmed liver cancer; Based on the principles of the Declaration of Helsinki, the consent of the patients and their families was obtained and written consent was signed. The research in this paper conforms to the national standards of health ethics research and is carried out with the approval of the hospital ethics committee.

Research methods

The cell lines needed for this study are normal hepatocyte HL.7702 and human hepatoma cell line QGY-7703, respectively. These two cell lines are provided by Shanghai Living Cell Bank, and the cell lines are stored in a special laboratory. (2) experimental apparatus and reagents, this study used a variety of types of experimental apparatus and reagents, including RPMI1640, fetal bovine serum, CCK8 Tongren chemical company (Japan), miR-144 model as well as the negative control simulation Ying Jun Biotechnology Company (Shanghai), miR-144 and QRT-PCR kit (Japan Toyobo company), transfection reagent kit, flow cytometry, cell cycle. These experimental instruments have been professionally tested before use, and the test results all meet the experimental requirements of this paper. (3) the experimental process: first, cultivate transfection cell, will human liver cell line QGY-7703 and HepG2 in incubator temperature of 37 ℃ for culture, the HepG2 is RPMI1640 with a concentration of 10% fetal bovine serum, cultivation of cells in the night, miR-144 and the negative contrast simulation objects using transfection reagent to the mentioned above in the hepatocellular carcinoma cell line, keep the concentration of the simulation in l00txmol/L; Then, the reverse transcription of liver cancer cells, QRT-PCR and liver cancer tissues were detected, the total RNA was extracted with the reagent TRIzol, and the QRT-PCR reaction was quantitatively measured with the help of the kit. The specific operation was based on the kit instructions. Different primer sequences were detected respectively, and a detection solution curve was formed after 40 cycles. After all, reactions were completed, the detection results were analyzed by the detection system. 7721 (4) cultivating HepG2 cells and cells, the use of contains a concentration of 10% fetal bovine serum and 4 ml of glutamine and 1% of penicillin-streptomycin solution DMEM as cell culture medium, in carbon dioxide concentrations for 5% of the incubator for cell culture, the temperature of the incubator set at 37 degrees Celsius, the culture supernatant of acquisition, the loading capacity of 1.5 mLEP pipe respectively. Cell biological behavior experiment. MTF method is used to test the liver cancer cell proliferation condition, using cell scratch experiment to test the condition of liver cancer cell migration, on liver cancer cell invasion detection using the cell of experimental condition, the use of flow cytometry technique for detecting apoptosis situation, using cloning experiments to test the liver cancer cells form tumors ability.

Data analysis

After the completion of the experiment, data analysis software was used to analyze and process the experimental data, which were expressed in the form of % and x±s. Based on the obtained experimental data, the data chart is drawn with the help of computer software, and the data chart is deeply analyzed.

Results

Expression levels of miR-144 gene and CCNB1 protein in liver cancer cells

According to the median expression level of miR-144 and CCNB1, among 100 liver cancer patients, there were 25 patients with a high expression level of miR-144 and 75 patients. There were 68 patients with high CCNB1 expression levels and 32 patients with low CCNB1 expression levels. Table 1 shows the expression levels of miR-144 and CCNB1, respectively. From the data in Table 1, we can see that the postoperative survival time of patients with a high expression level of miR-144 is significantly higher than

Table 1. Influence of miR-144 gene and CCNB1 protein expression level on postoperative survival time of patients

Classification	Number of cases	Postoperative survival time (months)	Median expression level	
			Tissue adjacent to carcinoma	Cancer cell tissue
miR-144 high expression	25	12.51±2.35	-14	-16
miR-144 lower expression	75	8.12±1.26		
High expression of CCNB1 protein	68	8.51±1.05	0.6	0.9
Low expression of CCNB1 protein	32	11.65±1.53		

*Data came from the experimental results.

that of patients with a high expression level.

Effects of CCNB1 protein knockdown on apoptosis, proliferation and migration of hepatocellular carcinoma cells

Figure 1, 2, and 3 respectively CCNB1 protein on reduction of cell apoptosis, proliferation and migration ability, the influence of the data from the chart we can see that compared with the untreated and negative control simulation content, CCNB1 protein transfection after liver cancer cells apoptosis of HepG2 cells significantly increased, and the protein transfection CCNB1 ability after the type of cell proliferation and migration were significantly reduced.

The tumorigenesis ability of cells was detected by a clone formation experiment

Figure 4 for the negative control simulation and untreated cell clone formation test data, as a result, of the data from the picture we can safely draw the conclusion that CCNBI protein with QGY-77 transfection after treatment, obvious decline in the number of liver cancer cell cloning, meanwhile its tumor ability also significantly reduced, eventually making the probability of tumor patients is reduced greatly.

Discussion

Modern medical research results have proved that miRNA genes can participate in the normal physiological process of the body. Studies have shown that miRNA is an important regulatory factor in the process of tumor production, and abnormal expression of this factor will have an important impact on the formation and development of tumors (18-19). Therefore, the medical community has been paying increasing attention to miRNAs in recent years. At the beginning of the study, many scholars believed that miR-144 showed a down-regulated trend in multiple tumor types, and played a good inhibitory effect on the biological behavior of cancer cells (20). Foreign scholars in the study of different types of cancerous tissue and tissue adjacent to carcinoma analyzed gene expression levels of miRNAs detection, the analysis results show that miR-144 in cancerous tissue types have a similar expression trend, and expression levels were significantly higher than in the tissue adjacent to carcinoma, the expression level in cancerous tissue (21). The results of other researchers proved that miR-144 has a high expression level in thyroid cancer tissues and can target and regulate ZEBI and other proteins, thus playing a good inhibitory effect. From this, we were able to infer that the miR-144 gene is effective in inhibiting cancer (22). CCNB1 protein is formed under the action of progenitor cancer cell genes. At present, many studies have shown that CCNB1 protein is abnormally expressed in many tumor tissues, but few studies have involved the negative regulation of CCNB1 protein by miR-144 at this stage (23).

In this paper, the research results show that compared with the tissue adjacent to carcinoma, cancer of the liver tissue of miR-144 expression level dramatically cut, CCNB1 protein expression level is, by contrast, raised significantly, miR-144 is high expression levels of the survival of patients after surgery time is longer than the low expression level of patients, CCNB1 low protein expression

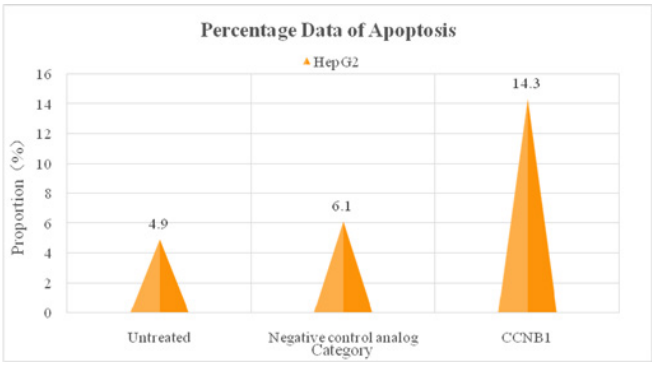

Figure 1. Percentage of apoptosis.

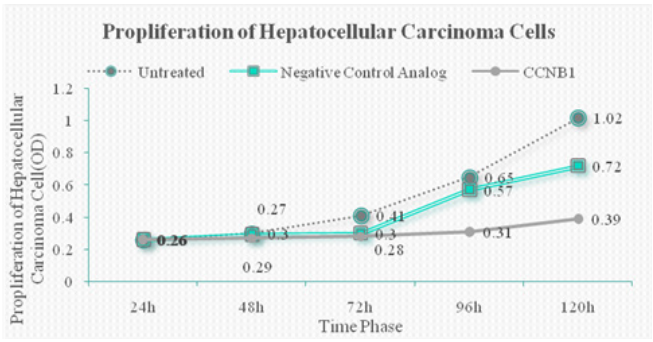

Figure 2. Proliferation ability of HCC cells.

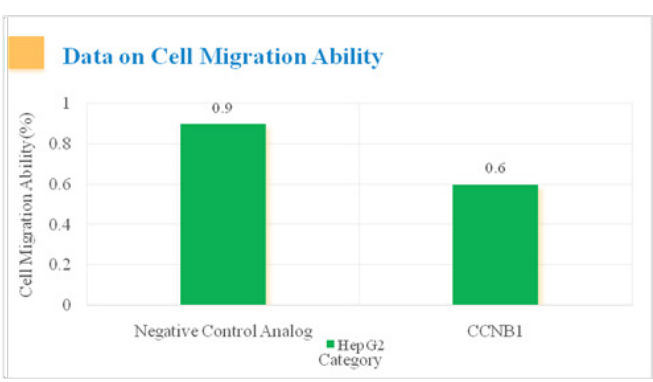

Figure 3. Cell migration ability data.

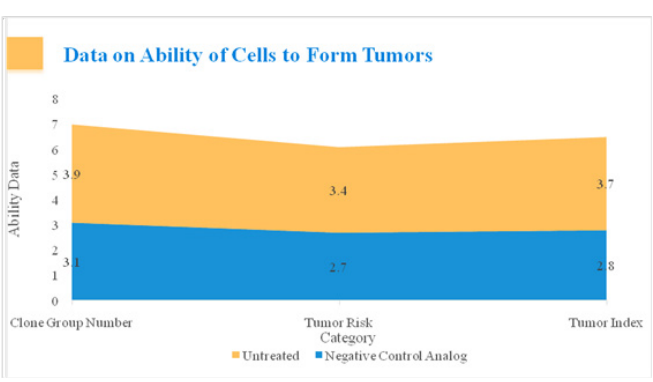

Figure 4. Data on the ability of cells to form tumors.

level in patients with postoperative survival time is longer than high expression level; Compared with untreated patients and negative control simulators, CCNB1 protein expression level of transfected HCC cells was significantly reduced. Compared with the negative control analog and untreated cells, QGY-7703 cells transfected with CCNB1-SIR had a continuously reduced ability to form tumors. The preliminary experimental results show that miR-144

gene expression in liver cancer cells was significantly reduced, and a marked increase in the CCNB1 appearance phenomenon, the miR-144 gene of CCNB1 protein negative regulation deeply influenced the final effect of treating liver cancer patients, and the various biological behavior of liver cancer cells, including cell proliferation, migration and apoptosis and miR-144 gene negative regulation CCNB1 there is a certain relationship between protein. This conclusion is similar to that of Guo S, et.al (24). The key factor that promotes the abnormal expression of cancer cell genes is the imbalance of cell cycle regulation, which further promotes the abnormal regulation of cancer cell proliferation and apoptosis. In this paper, the experimental study has found that miR-144 genes can effectively restrain CCNB1 protein expression level in cell lines HepG2, miR-144 gene expression in liver cells, significantly reduce the proliferation of liver cancer cells, to eliminate the effects on the liver cancer cells after CCNB1 protein has the similarity, this suggests that miR-144 gene can negative regulation CCNB1 protein, miR-144 as a kind of can inhibit cancer genes, its in liver cancer tissues often present a low level of expression, Wang J, et al. believed that the restoration of miR-144 gene expression level could promote the effective inhibition of liver cancer cells (25-26), and impede the normal promotion of various cell biological behaviors, such as migration and proliferation of liver cancer cells, proving that Mir-144 gene had a significant inhibitory effect on the biological behavior of liver cancer cells. In this paper, experimental studies also found that after the elimination of CCNB1 protein, the overall proliferation, migration and tumor formation ability of HCC cells was significantly reduced, but the rate of cancer cell decay was increasing, indicating that the negative regulation of miR-144 on CCNB1 protein could profoundly affect the biological behavior of HCC cells. CCNB1 protein as an important product of proto-oncogenes, its abnormal expression in the cancer cells will make factor adjustment in a state of imbalance. Using a lower CCNB1 expression level can make the active factor of the mitotic effectively suppressed, thus preventing the process of cell proliferation and division, inhibition of CCNB1 to cytoplasmic transfer leads to apoptosis, it is proved that CCNB1 protein abnormal expression is related to the formation and development of cancer cells.

Above all, cancer of the liver tissue of miR-144 is low, the level of gene expression CCNB1 protein expression level is higher, the expression level in liver cancer cells directly influences the curative effect of hepatocellular carcinoma patients, a miR-144 gene can negative regulation CCNB1 protein, through this kind of negative adjustment to the biological behavior of liver cancer cells have a profound impact.

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