Analysis of the regulation of the mdm 2-p53 pathway by MiRNA-370-3P in hepatocellular carcinoma

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Abstract: *Liver cancer tumor is one of the most common liver malignant tumors in the world, among which hepatocellular carcinoma accounts for about 90% of primary liver cancer. In recent years, its incidence has been increasing rapidly in many major regions of the world, and it is also the second leading cause of tumor death in the world.In recent years, although our country liver cancer clinical early diagnosis and early treatment of patients, surgery and perioperative patient care technology improved, and part of the late primary liver cancer targeted therapy and the use of biological immunotherapy drugs, improve the majority of patients with clinical survival time, but still because of the current high incidence of liver cancer disease, high surgical recurrence rate and relatively limited drug treatment strategy, its future overall survival rate is still relatively not idealTherefore, it is very important to find new methods or new ways to treat liver cancer. In this paper explores one of the mechanisms of liver cancer occurrence, and understand and analyze liver cancer through credit analysis.analyze the regulation of miRNA-370-3p on mdm 2-p53 pathway in hepatocellular carcinoma. Methods Through QuickGo database, David database, String, Cytoscape, BGI, the mechanism of mdm 2 regulating the mdm 2, then cRNA, and the ceRNA regulation mechanism of abnormal mdm 2 gene expression by bioinformatic analysis.*

Keywords: miRNA-370-3P; hepatocellular carcinoma; mdm 2-p53 regulation

1. Introduction

Liver cancer tumor is one of the most common liver malignant tumors in the world, among which hepatocellular carcinoma accounts for about 90% of primary liver cancer. In recent years, its incidence has been increasing rapidly in many major regions of the world, and it is also the second leading cause of tumor death in the world [1,2]. The early occurrence of liver cancer lesions can be inherited by the internal environment many factors such as basic and body epigenetic genetic conditions interact directly, and the development of chronic toxic hepatitis, alcohol consumption, long-term exposure to aflatoxin and genetic metabolic diseases are the risk factors that promote the occurrence of liver cancer [3]. In recent years, although our country liver cancer clinical early diagnosis and early treatment of patients, surgery and perioperative patient care technology improved, and part of the late primary liver cancer targeted therapy and the use of biological immunotherapy drugs, improve the majority of patients with clinical survival time, but still because of the current high incidence of liver cancer disease, high surgical recurrence rate and relatively limited drug treatment strategy, its future overall survival rate is still relatively not ideal [4]. At present, the treatment methods for liver cancer in the world are still limited in the world, especially the treatment of advanced liver cancer, which is also true in China, so the overall 5-year survival rate of liver cancer in China is only 10.1% [5].

P53, widely defined as a tumor suppressor gene, has a mutant of p53 in nearly 50% of malignancies[6]. One of the most important regulatory protein mechanisms in the many regulatory proteins of p53 is realized by mdm 2 and its homoheteromeric complex protein mdmX[7]; The Mdm 2 gene can also effectively and directly inhibit the transcription of the p53 gene and the expression of the immune activity level and the cytogenetic functional stability, at the same time, The Mdm 2 gene itself is yet another major target gene directly encoding for p53 protein expression, The amount of expression and the level of activity are directly controlled by the p53 gene, And that mdm 2 has the ability to inhibit p53 induction of apoptosis, So mdm 2 is considered the functional target most closely associated with p53[8].

Mdm 2 is an oncogene that can repress p53, The Mdm 2 gene is currently a novel oncogenene that

is known to be effectively used in vivo to inhibit p53 expression in mouse sarcomas in vitro, It was discovered in 1982 that it was a DNA amplification sequence of a double microbody in a cell sequence of 3T3DM, The amplified sequence was amplified and amplified nearly 50-fold in an additional cellular sequence of 3T3DM, In 1991, studies found that the amplified sequence could also contain another Mdm 2 gene, Is considered to be overexpressed in multiple tumor cell lines, Scholars agree that it may be another new expression-amplified oncogene[9,10].

MiRNA is a class of mature non-coding small molecule of miRNA, which is a class of mature genes with endogenous structure, regulation and transcription detected by human scientists in the early system structure of eukaryotic organisms. Mature non-coding RNA is generally about 20 to 25 nucleotides in size, which is processed and transformed from a class of mature small molecules with functions such as gene transcription or regulating gene transcription. Research and data comparison show that miRNA can participate in complex biological processes including the growth, proliferation, differentiation, apoptosis and other response regulation processes, and the immune regulation of various tumor cell cycles. Therefore, miRNA is directly involved in controlling the occurrence, development, invasion and cancer metastasis of other cancer cells. In the study of the mechanism of ceRNA transcription regulation model, lncRNA in the upstream of miRNA, miRNA gene expression bidirectional selective inhibition and regulation of transcription, through direct activation of miRNA targeted mRNA gene transcription and translation inhibition or interference and bidirectional block transcription regulation, or directly by prompting target mRNA gene bidirectional selective inhibition of degradation, involved in the target cell normal differentiation, growth, regulation of apoptosis process, and the migration of target genes, etc important process[11]. At present, it has been widely recognized and proved to clinical researchers that it can regulate the abnormal differentiation and occurrence mechanism of various tumor genes. Therefore, it will be of special academic significance to analyze the mechanisms of the occurrence of tumor targeted gene variation in hepatocellular carcinoma and the clinical prevention and treatment of patients with advanced HCC and actively improve the survival prognosis of patients with cirrhosis. In this paper, the network bioinformatic analysis of mdm 2-p53 pathway regulation by miRNA-370-3P in hepatocellular carcinoma[12].

2. Method

2.1. Search for the Bp-related genes of interest-p53

The p53 genes were identified by entering in the QuickGo database and then finding p53 and 82 related genes. These 82 genes were downloaded for breakdown and then put into the David database for analysis to obtain the KEGG plots of p53 signal pathway.

2.2. Significant genes were selected

The 82 genes from QuickGo database were put into the BGI Multi-omics system to search for expression, and the genes with expression levels were downloaded for screening: Qvalue (p1_C / p1_H) <0.05, log2 (p1_C / p1_H) <-1 or log2 (p1_C / p1_H)> 1, p1_H Expression or p1_C Expression was more than 1. A total of 22 meaningful differentially expressed genes meeting the above conditions were finally selected. When the gene log2 (p1_C / p1_H) is negative and the value is less than or equal to-1, the gene can achieve high expression in cancer tissue cells. When the gene log2 (p1_C / p1_H) is also a positive value and a value greater

2.3. Determine the core gene to be analyzed mdm2

Combining the KEGG map and the selected 22 meaningful differentially expressed genes, we can get the core genes that you want to study - -Mdm 2, or you can put these 22 genes into a PPI network map by String, and then send the map to Cytoscape for a computational drawing of top10. After obtaining the top10 map, the most critical gene- -mdm 2, which can then be selected for study. Because the core gene- -mdm 2 was upregulated in cancer tissues in the above analysis, it can be analyzed that miRNA regulating mdm 2 is downregulated, because miRNA has the effect of inhibiting the corresponding target genes.

2.4. Look for the miRNA for mdm 2

Input mdm 2 on the Gene Symbol through the BGI multi-omics system, find the miRNA of mdm 2,

then download it, find the expression level of the corresponding miRNA in the BGI multi-omics system, and then download it for screening. Screening method: Qvalue (p2_C / p2_H) <0.05, log2 (p2_C / p2_H) <-1 or log2 (p2_C / p2_H)> 1, and then because mdm 2 is highly expressed in cancer tissue, miRNA with downregulated expression in cancer tissue, namely miRNA with screening log2 (p2_C / p2_H) value was positive and> 1. We obtained 14 miRNA with low expression.

2.5. For looking for a ceRNA with low miRNA expression

Find 14 lncRNA corresponding to low expression miRNA through the BGI multiomics system, download them, and then go to the BGI multiomics system to find the expression level of lncRNA and download it. It is usually Qvalue (p1_C / p1_H) <0.05, log2 (p1_C / p1_H) <-1 or log2 (p1_C / p1_H)> 1, because mdm 2 gene has high expression in cancer cell tissue cells and miRNA is relatively low expression, so lnRNA concentration is highly expressed in cancer cell tissue proteins, so log2 (p1_C / p1_H) should be negative and less than-1. That is, 24 lncRNA.

2.6. Find the targeting relationship of miRNA and the corresponding lncRNA

14 miRNA with low expression were put into the BGI multi-omics system to find corresponding targeting relationships and downloaded. Then the 24 ceRNA are used to screen miRNA and the corresponding targeting relationship, and then the two tables are put into Sytoscape to draw the ceRNA network diag

3. Analysis

3.1. Analysis of the KEGG pathway map made through the David database.

Figure 1: mdm 2-p53 signaling pathway in Fig

From the the *figure 1* to find out their genes of interest-mdm 2, we can see that mdm 2 to the downstream of p53, resulting in p53 downstream of a series of target genes for the next step of regulation, and the development of hepatocellular carcinoma belongs to mdm 2-p53 Apoptosis (apoptosis) the result of the pathway process, when apoptosis reduced or suppressed, will cause the number of cells, finally evolved into cancer cells. As seen from this mdm 2-p53 pathway. High expression of Mdm 2 will inhibit the expression of p53, which causes reduced apoptosis and increases the risk of cell carcinogenesis. So we can screen out the corresponding miRNA with low expression based on the high mdm 2 expression.

3.2. Top10 Analysis of the PPI network map and PPI of the differential genes made by String and Sytoscape, and the network map top10

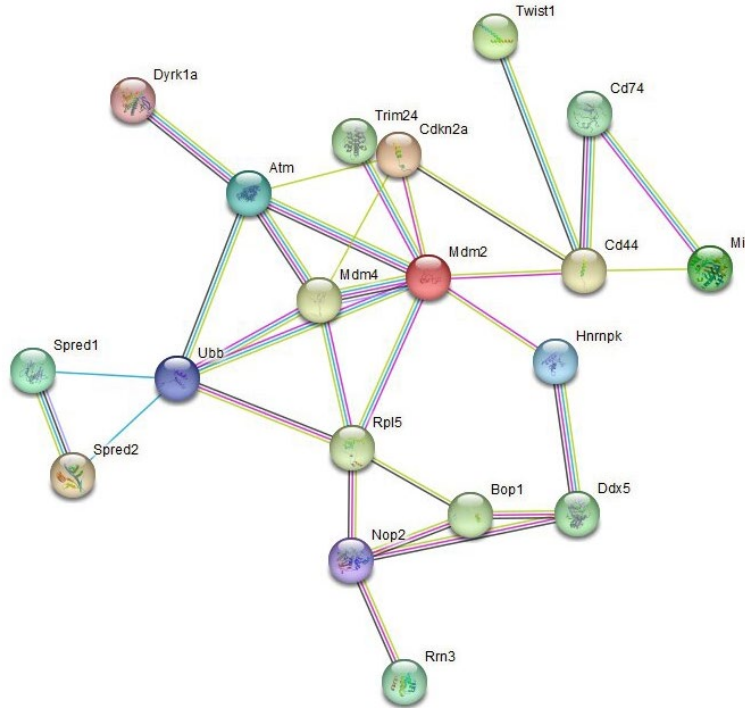### 3.2.1. Analysis of PPI network plots of differential genes made from String.

Figure 2: PPI of differential genes, network Fig

Can be made according to the String differentially expressed gene diagram from *Figure2*, so as to get meaningful genes, from the figure can intuitively see the 19 genes correlation and interaction, and then put the 19 genes PPI network graph into Stoscape for a calculation and top10 a composition, namely select the 10 most important genes for an analysis.

3.2.2. Analysis from the String combined SytoscapePPI network graph top10
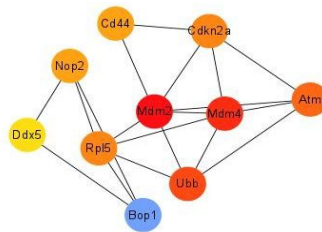

Figure 3: PPI network map top10

As can be seen from the top10 diagram from *Figure 3*, the 10 genes or expressed proteins, the darker the color, the more critical the gene, so the key gene to be studied- -mdm 2. Analysis of the expression level cluster heat map
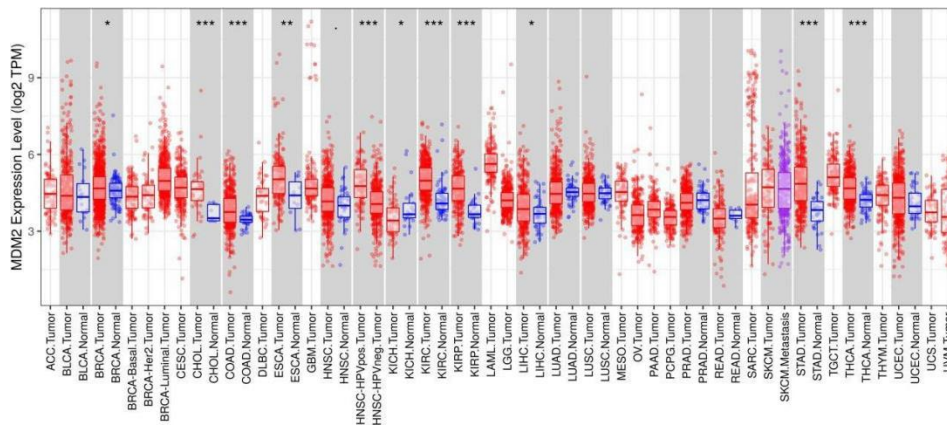
3.3. Cluster heat maps of expression levels were analyzed

Figure 4: Heatmap of differential gene expression magnitude clustering

The 82 differentially expressed genes s elected from the Quick GO database were constructed through the BGI multi-omics system. From this cluster heat map from Figure 4, we can know tha t mdm 2 gene show ed high expression in cancer ours tissue, that is, up-regulated expression in cancer ours tissue and relatively low expression in adjacent tissues, that is, down expression in adjacent tissues. Moreover, the expression in three cancer tissue samples showed similar strong correlation in three cancer tissue samples, and similar expression in three adjacent tissues, indicating a strong correlation between three adjacent tissue samples.

3.4. Analysis of the ceRNA network plot of mdm 2

Figure 5: mdm2 ceRNA network in Fig

By selecting the 14 downregulated miRNA of mdm 2 and the corresponding targeting relationship, then identify the corresponding lncRNA and lncRNA, and select the corresponding lncRNA in the 14 downregulated miRNA with targeting relationship, and then the targeted miRNA of lncRNA can be found. The 14 downregulated miRNA selected again correspond to the key gene, mdm 2. By establishing a correspondence table, then put the table into Sytoscape to draw the ceRNA network diagram. It can be seen from the figure that miRNA-370-3p is regulated by three lncRNA, Gm8883,0610005C13RiK, Gm30262, the three lncRNA upregulated and caused the downregulation of miRNA-370-3p like *Figure5* , thus causing the upregulation of mdm 2 gene.

3.5. Analysis of the mdm 2 expression levels in Fig

P-value Significant Codes: $0 \leq *** < 0.001 \leq ** < 0.01 \leq * < 0.05 \leq . < 0.1$

Figure 6: Expression level plots of mdm 2

According to the analysis results in the figure above, for P-value in LIHC Tumor (hepatocellular carcinoma tissue) and in LIHC Normal (hepatocellular carcinoma adjacent tissue) from *Figure 6*, the factor is only * At 0.01 and * 0.05, the difference has certain clinical statistical reference significance. From the analysis results in the above figure, it can be seen that mdm 2 has high specificity and high expression in hepatocellular carcinoma tissue cells, and low expression in adjacent tissue cells of liver parenchymal cell carcinoma.

3.6. Analysis of the survival plots of mdm 2
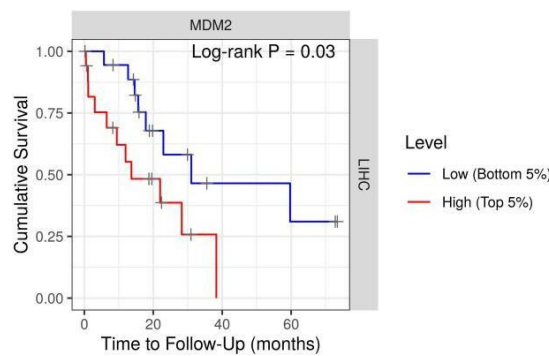
Figure 7: The survival plots of mdm 2

From *Figure 7* we can see that *r*ed lines represent high mdm 2 expression in LIHC samples, and blue lines represent low mdm 2 expression in LIHC samples; at month 0, both samples survived 100%, and survival rates in both groups decreased over time. However, it can be seen from the figure that the sample curve of the mdm 2 high expression group decreased faster than that of the mdm 2 low expression group; at month 20, the survival rate of the samples of the mdm 2 high expression group was about 49%, while the survival rate of the mdm 2 low expression group was about 71%. And at month 40, the survival rate of the mdm 2 expression group was 0, while the survival rate of the mdm 2 expression group was about 47%. All the above indicated that the survival rate of the high mdm 2 expression group was significantly lower than the low mdm 2 expression group. Because log-rank P=0.03, less than 0.05, it is known that the survival of mdm 2 in LIHC and mdm 2 in LIHC samples is significantly different, indicating that mdm 2 is related with the survival of LIHC samples, which can promote the development of hepatocellular carcinoma, thus reducing the survival rate.

4. Analyze and discuss

The mdm 2-p53 pathway, in which mdm 2 is involved, is not only a pathway contributing to the formation of hepatocellular carcinoma (by inhibiting the apoptosis pathway), but also has a certain effect on the development and development of hepatocellular carcinoma. However, miRNA-370-3p, as a miRNA regulating mdm 2, can upregulate the expression of mdm 2 and promote the progression of mdm 2-p53 pathway, because mdm 2 suppresses p53 expression in mdm 2-p53 pathway, it will inhibit the progression of apoptosis, which will promote normal cell carcinogenesis.

5. Outlook and summary

miRNA as a therapeutic target is one of the most promising directions in cancer research [13]. Studies have shown that miR-370-3P can directly inhibit the normal development and proliferation of human articular chondrocytes (HC-a) cells by directly or releasing signaling molecules such as targeted JAK 2 and STAT5B, and then directly regulate the process of normal cartilage growth and development of normal joints in human bones [14]. This paper is only a bioinformatic analysis of the regulation of mdm 2-p53 pathway by miRNA-370-3p in hepatocellular carcinoma, and the regulatory mechanism needs to be confirmed by numerous design experiments. The mdm 2-p53 pathway plays an indispensable role in the development of most tumors. From the bioinformatic analysis, we show that miRNA-370-3P is the miRNA that regulates mdm 2[15]. The study of the mechanism of mdm 2-p53 pathway by miRNA-370-3p is not only for us to better understand the formation and development process of hepatocellular carcinoma, but also to extract the mechanism of this pathway to provide new ideas for the clinical treatment of tumors.miRNA Is regulated by lncRNA in the ceRNA mechanism, So lncRNA can be used as an entry point for research, From the bioinformatic analysis of miRNA-370-3p regulation of mdm 2-p53 pathway, the ceRNA network map found that miRNA-370-3p.Non-coding gene RNA (including micRNA and long no-coding RNA) plays a fundamental role in the regulation of major vital chemical activities such as cellular response and material metabolism balance [16]. The LncRNA receptors and miRNA receptors jointly affect the development of some neoplastic diseases through mutual cross-regulating mechanisms of immune action. Because now found that most of the lncRNA gene molecule expression of cell structure essentially also with mRNA gene expression structure has a certain cell number of molecular similarity, that miRNA is likely through its similar to the mRNA gene structure through the negative mechanism of the regulation of certain lncRNA gene expression molecules, and then can effectively play the role of this series of biological [17]. Some lncRNA may be expressed to specific target gene mRNA, and 3′-URT on the surface, which indirectly inhibit the regulation of some negative effects on the function of some specific target genes, and inhibit the repeated expression of in other miRNA gene segments by exerting the transcriptional function of the endogenous miRNA sponge. Therefore, by integrating and analyzing the functional relationship between lncRNA, miRNA and target genes, we can have a clearer understanding of the causes and development of human disease mechanism, so as to provide a new research and therapeutic entry point for treatment in clinical practice[18].

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