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SIRT3 expression in hepatocellular carcinoma and its impact on proliferation and invasion of hepatoma cells

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ABSTRACT

Objective: To observe expression of SIRT3 in normal liver tissue, cirrhotic tissue and hepatocellular carcinoma (HCC) tissues, and to explore the significance of SIRT3 in primary HCC .**Methods:** SIRT3 expression was detected in 10 normal cases, 30 cases with, 30 HCC cases by immunohistochemical and Western-blotting method. **Results:** Immunohistochemical assay showed that the SIRT3 positive expression rates were 100.0% (10/10), 96.7% (29/30) and 60.0% (18/30), respectively in normal group, paracancer group and HCC group. And the SIRT3 expression in HCC group was significantly lower than in normal group and paracancer group (P<0.05). Western-blotting showed the SIRT3 expression in cancer tissue was 0.29±0.07, significantly lower than that in paracancer group and normal group (P<0.05). SIRT3 expression was related to the differentiation degree and portal vein tumor thrombus (P<0.05). **Conclusions:** Abnormal expression of SIRT3 is closely related to the biological behavior of primary HCC.

1. Introduction

Primary hepatocellular carcinoma (HCC) is one of the most common malignancy in the world, its incidence and mortality rank the second of all cancers, which is a serious threat to people's health. The first options of treatment for HCC is still surgery, but the surgical resection rate is low, and the recurrence rate postoperative is high, so the death rate is always high[1,2]. So it is extremely important to explore the pathogenesis of HCC and look for new drugs. This article detected the silent information regulator protein 3 (SIRT3) expression of primary HCC by immunohistochemistry and Western-blotting, in order to understand the possible role of SIRT3 in the process and development of liver cancer.

2. Materials and methods

2.1. Materials

In this study, all samples were taken from tissues of patients who underwent surgical removal from January 2011 to December 2012, and diagnosed with primary HCC by pathological analysis. All patients with HCC did not receive preoperative treatment programs such as chemotherapy, radiation or radio frequency. Cancer adjacent tissues without necrosis (the distance was beyond 2 cm from

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tumor specimen edge) were taken as the paracancer group. Specimens of 10 normal liver tissues of patients suffering from surgical resection at the same period in our hospital were selected as the normal group. Part of them were fixed in 10% formalin, dehydrated and embedded in paraffin, 3-4 μm sections were prepared for immunohistochemical assay; Part of them were collected from the body within half an hour after surgical resection, immediately quick-frozen in liquid nitrogen, stored at -80 ℃ and prepared for Western-blotting.

2.2. Reagents

Mouse anti-human SIRT3 monoclonal antibody was purchased from Thermo, fluorescence-labeled goat anti-mouse secondary antibody were purchased from Genomics Company. Immunohistochemistry kit was purchased from Sigma, USA. Total protein extraction kits were purchased from Invitrogen Corporation.

2.3. Immunohistochemistry method

Immunohistochemistry was adopted as follows: The antigen of all specimens were repaired by high temperature and high pressure, and then stained in accordance with the instructions. The SIRT3 protein staining was mainly located in cytoplasm, which was yellow or brown. SIRT3-positive cells cytoplasm showed brown or brown-yellow particles, otherwise they were considered to be SIRT3-negative cells. Double-blind method was adopted by two experienced pathologist, and then the results were determined by semi-quantitative method. Five randomly visions (400×) of each tissue section were observed, at least 1 000 cells were counted, and the percentage of positive cells was calculated. Positive degree of tumor cells from 10% to 25% were (+), from 26% to 50% were (++), more than 51% were (+++); positive cells rate of section ≥10% were regarded as positive, <10% as negative (-).

2.4. Western-blotting

Liver tissue was removed from the liquid nitrogen, and cut approximately to 50 mg. It was added to homogenate protein by the ratio of 1:9, the total protein was extracted for protein quantification. It was denatured at 95-100 ℃ for 5 min, 30 μg total protein were taken from each group for electrophoretic, transfer and BSA blocking. Mouse anti-human SIRT3 monoclonal antibody was added, and diluted at 1:1 000. It was gently shaked at 4 ℃, incubated overnight, and added with second antibody after washing. They were diluted at 1:5 000. Avoid light, it was incubated for 1 h at room temperature, and then was washed. With β-actin as an internal control, the gray integral value of each stripe were automatically read and recorded by the computer, statistical analysis was conducted based on sample integral value/internal reference ratio.

2.5. Statistical analysis

All of the data were analyzed by SPSS 11.5 statistics software. *t*-test, χ^2 examination, exact probabilities and the Spearman rank correlation analysis were applied. $P<0.05$ was considered as statistical significance.

3. Results

3.1. SIRT3 protein expression

Immunohistochemical results showed that SIRT3 expressions were all positive in normal group, the positive expression rate was 100.0%. A total of 29 cases were positive in paracancer group (96.7%), and 18 cases were positive in HCC group (60.0%). SIRT3 positive rate and the mean score of tissues in HCC group was significant higher than those of other two groups ($P<0.05$) (Table 1).

Western-blotting experimental results showed that SIRT3 protein relative expression in HCC group (SIRT3/β-actin) was 0.29±0.07, which was significantly lower than in paracancer group (0.57±0.16) and normal group (0.59±0.15) ($P<0.05$).

Table 1
SIRT3protein expression (*n*).

Groups	SIRT3			
	-	+	++	+++
HCC group	12	15	3	0
Paracancer group	1	2	11	16
Normal group	0	1	1	8

3.2. Relationship between SIRT3 protein expression and clinical pathological features of primary HCC

Immunohistochemistry and Westem-boltting showed that SIRT3 protein relative expression gradually decreased with the degree of the differentiation of hepatoma cell. In hepatoma cells with various differentiation degree, the differences of the SIRT3 protein relative expressions had statistically significant ($P<0$ 05). The statistical analysis showed patients with portal vein tumor thrombus had significantly lower positive expression rate and relative expression ($P<0.05$). There was no significant difference between SIRT3 protein expression and age, sex, liver size and AFP values ($P>0.05$) (Table 2).

Table 2

Relationship between SIRT3 protein expression and clinical pathological features of primary HCC.

Groups	Cases	SIRT3 positive expression by immunohistochemical assay	SIRT3 protein levels by Western-blotting	*t* value	χ^2
Age					
≥60	12	5	0.28±0.08	0.18	2.80
<60	18	13	0.29±0.07		
Gender					
Male	21	14	0.27±0.07	1.49	1.30
Female	9	4	0.31±0.12		
Tumor diameter					
≥5 cm	13	7	0.28±0.06	1.45	0.36
<5 cm	17	11	0.30±0.09		
Differentiation degree					
Well-differentiated	8	7	0.35±0.09	2.24	3.86
moderately differentiated	15	7	0.24±0.08		
Poorly differentiated	7	4	0.17±0.09		
AFP value					
≥20 ng / L	20	14	0.27±0.08	1.36	2.50
<20 ng / L	10	4	0.30±0.08		
Portal vein thrombosis					
with	9	2	0.16±0.09	2.43	7.65
without	21	16	0.34±0.12		

4. Discussion

Silent information regulator factor family (Sirtuins) is a highly conserved protein family, which contain a conserved catalytic core domain, and according to its subcellular localization, it is divided into seven subtypes[3]. The studies suggest that Sirtuins (SirT) involved in the regulation of life, SIRT3 as a potential tumor inhibitors attracting more and more attention in recent years. SIRT3 is the main histone deactylase of the mammalian mitochondrial, it can participate in amino acid metabolism by adjusting the enzyme activity, and also participate in the protein metabolism adjust of entire body by the regulation of cell autophagy. The substrates mainly include glutamate dehydrogenase and succinate deoxy enzymes. They can activate the transferase activity of the deacetylation enzymes and ribosomes to achieve post-translational regulation of the target protein[4-6].

Mice with the SIRT3 knockouted was more susceptible to cancer if they received many stress.SIRT3 in tumor cells decreased significantly compared with in the normal tissue cells, which showed that the SIRT3 may have tumor suppressor function in the body. Once the body lose the SIRT3-mediated regulation of mitochondrial protein, it may lead to oncogene infection, and that lead to immortalization and transformation. Therefore members of histone deactylase family plays an important role in development of tumor[7-10].

Immunohistochemical results showed that SIRT3 SIRT3 positive rate and the mean score of tissues in HCC group was significant higher than those of other two groups (P<0.05). Western-blotting experimental results showed that SIRT3 protein relative expression of 30 cases in the hcc group (SIRT3/ β -actin) was also significant higher than those of other two groups (P<0.05). The SIRT3 protein expression of the cirrhotic tissue was slightly lower than the normal group, but the difference had no statistically significant (P>0.05).

The immunohistochemistry and Westem-bloting results showed there was no significantly different of SIRT3 expression between the normal liver tissues and the cirrhotic tissue, and there was significantly low expression in HCC tissues. Immunohistochemical assay showed the positive expressions were mainly weakly positive, and there was no significant expression, which is consistent with other studies. There was no significant change of the SIRT3 protein expression levels during the development process from normal liver tissue to liver cirrhosis, but the expression in HCC tissues was decreased or lost, which showed the SIRT3 protein did not play a biological role in this process from the normal liver tissue to cirrhosis. Once the expression was decreased or lost, the nature of the cirrhotic liver tissues may become carcinogenesis[11].

In this study, we analyzed the effect of SIRT3 protein expression levels on the clinical and pathological features of the primary hepatocellular carcinoma. We presume that the SIRT3 protein expression in HCC is related with the pathological features of liver cancer cell, the differentiation degree and complication with portal vein tumor thrombus. SIRT3 protein relative expression gradually decreased

with the degree of the differentiation of hepatoma cell. SIRT3 protein expression level showed the downtrend in the deterioration and progression of HCC cells. Therefore, we believe that SIRT3 protein also plays a role in the progression of liver cancer. Its mechanism may be related to reactive oxygen species (ROS) in mitochondrial. The occurrence and development of liver cancer are the same as other tumors, which is a complex process involved in polygenic and multifactorial. But liver cancer still has its own characteristics, we summarizes the carcinogenesis of various animal models and found that ROS increased almost in all of the animal models. Thus we can infer ROS played a very important role in the occurrence and development of liver cancer[12-14].

Mitochondria are the main source of ROS in the body. Studies have shown that SIRT3 can reduce the level of ROS. It is easy to cause mitochondrial metabolic abnormalities when cells lack of SIRT3. Cells suffered ROS damage. With the increase of ROS, cell damage becomes more severe, and there will be mutations of the gene stability in particular intracellular environment, and ultimately there will be de-differentiation and carcinogenesis. This conjecture has been confirmed in SIRT3-deficient rats[15-18].

Therefore, the lack of SIRT3 expression can easily cause the occurrence and development of HCC. By revealing SIRT3′s anti-tumor mechanism, we try to provide an effective method for early diagnosis and treatment of tumor in clinic.

Conflict of interest statement

We declare that we have no conflict of interest.

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