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• LIVER CANCER •

Expression of p53 and C-myc genes and its clinical relevance in the hepatocellular carcinomatous and pericarcinomatous tissues

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Abstract

AIM: To investigate the possible roles of p53 and C-myc genes in the primary hepatocellular carcinogenesis and the relationship between the liver hyperplastic nodule(LHN) and hepatocellular carcinoma(HCC).

METHODS: The expression of p53 and C-myc genes was detected immunohist-ochemically in 73 and 60 cases of HCC and pericarcinomatous tissues, respectively.

RESULTS: The positive expression of p53 in HCC was significantly higher than that in pericarcinomatous tissues ($P<0.05$). In pericarcinomatous tissues, the p53 expression was observed only in LHN, but not in liver cirrhosis (LC) and normal liver tissues. The positive expression rate of C-myc in HCC or LHN was significantly higher than that in LC or normal liver tissues ($P<0.05$ and $P<0.01$), however, no significant difference was found between HCC and LHN ($P>0.05$). The positive expression rate of p53 and C-myc in HCC was correlated with the histological differentiation, that in the poorly differentiated was significantly higher than that in well differentiated samples ($P<0.05$).

CONCLUSION: The overexpression of p53 and C-myc genes might play a role in the carcinogenesis of HCC; And LHN seems a preneoplastic lesion related to hepatocarcinogenesis; No evidence supports that LC contribute directly to the hepatocarcinogenesis.

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INTRODUCTION

Primary hepatocellular carcinoma (HCC) is one of the most common malignant tumors in China[1-11], and the incidence of HCC reported has apparently increased in recent years. Despite a variety of therapeutic strategies, HCC remains a significant cause of cancer death. Therefore, to study the HCC pathogenesis is of the utmost importance to the prevention and treatment of this disease. With the advancement of HCC study, it becomes clear that the biologic behavior of HCC is closely related with the overactivation of the oncogenes and the inactivation of the tumor suppressor genes[12,13].

In the present study, the immunohistochemical LSAB (labelled streptavidin biotin) method was used to detect the expression of p53 and C-myc genes in HCC and pericarcinomatous tissues, in order to investigate the possible roles of these genes played in the HCC carcinogenesis, and to find out the relationship between the liver hyperplastic nodule and HCC. In addition, the relationship between the expression of p53 and C-myc genes and clinicopathological parameters of HCC was preliminarily investigated.

MATERIALS AND METHODS

Materials

HCC specimens of 100 cases obtained from surgical resections or biopsies performed at the Affiliated Hospital of Medical College of Qingdao University, China. Of these patients, 76 were male and 24 female with an average of 50.4 years. None of the patients had received chemo- or radio-therapy before resection. We randomly selected 73 and 60 cases of HCC to detect the expression of p53 and C-myc genes respectively owing to the limitation of antibodies. The specimens for detecting p53 were classified into 4 grades according to Edmondson's grading criteria, 4 specimens were in grade Ⅰ, 24 in grade Ⅱ, 39 in grade Ⅲ, and 6 in grade Ⅳ. All 73 specimens contained pericarcinomatous tissues, in which including 39 liver hyperplastic nodules (LHN), 35 liver cirrhosis(LC) and 10 normal liver tissues. Among the specimens for detecting C-myc gene, 4 were in grade Ⅰ, 17 in grade Ⅱ, 30 in grade Ⅲ, and 9 in grade Ⅳ. All 60 specimens contained pericarcinomatous tissues, in which including 37 LHN, 30 LC and 12 normal liver tissues.

Methods

All specimens were routinely processed, alcohol-fixed and paraffin-embedded. Serial paraffin sections of 4 um thickness were cut and used for hematoxylin and eosin(HE) and immunohistochemical stains. Immunohistochemical LSAB method was used to detect p53 and C-myc genes. Anti-p53 monoclonal antibody DO-7, anti-C-myc monoclonal antibody and LSAB kits were purchased from Dako Co. Before staining, the sections were heated with microwave in 0.05 mol • L^{-1} citric acid solution for antigen retrieval. In each staining , a known p53 or C-myc positive section was added as the positive control, and PBS was used as the substitute of the first antibody for the negative control.

Analysis of immunohistochemical staining

Cells with brown granules under microscope were regarded as positive. The criteria for the evaluation of the p53 expression in the present study were as follows: the positive nuclei number was semiquantitatively evaluated by counting that in 8-10 randomly-chosen medium power (×100 magnification), and the four degrees of the p53 expression were considered as: negative (-), no positive cells; weak positive (+), the positive cells <10 %; moderately positive (+ +), the positive cells between 10-50 %; strong positive (+ + +), the positive cells >50 %. For the evaluation of C-myc expression, the percentage of positively stained cells was employed as an index, which was obtained from counting 500 cells at more than 5 high power fields for each section, and classified into 4 grades: grade Ⅰ, the positive cells between 1-25 %; grade Ⅱ, the positive cells 26-50%; grade Ⅲ, the positive cells 51-75 %; grade Ⅳ, the positive cells 76-100 %.No positive cells were scored negative (-).

Statistical analysis

Results were analysed by χ^2 test. Differences at $P<0.05$ were considered to be statistically significant.

RESULTS

Expression of p53 gene in HCC and its pericarcinomatous tissue

The positive staining for p53 gene expressed as brown granules, which was mainly located in the cell nuclei of tumor cells (Figure 1). The staining intensity and extent varied among tumors, different tumor regions and individual tumor cells. Immunostaining of p53 protein was negative in all tumor stroma, bile duct epithelia, LC and normal liver tissues. A few p53 weakly positive cells were found in LHN (Figure 2). The significant difference existed between HCC and LHN (χ^2 value 10.57, $P<0.01$), and so did between LHN and LC (χ^2 value 6.94, $P<0.01$) (Table 1).

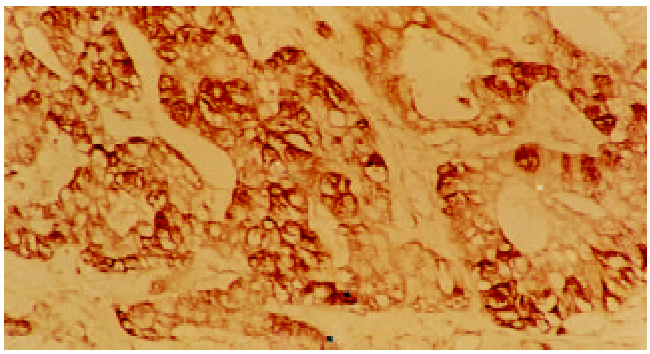

Figure 1 The positive expression of p53 gene in HCC. LSAB×200

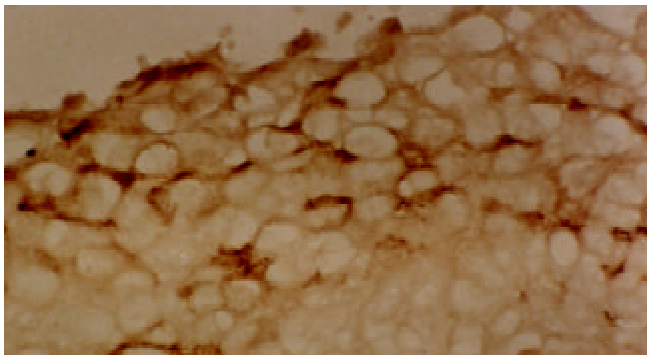

Figure 2 The positive expression of p53 gene in LHN. LSAB×200

Expression of C-myc gene in HCC and its pericarcinomatous tissue

The positive staining for C-myc gene was also expressed as brown granules, which was distributed mainly in cell nuclei, partly in cytoplasms. Although the expression rate of C-myc was higher in LHN than that in HCC, the statistical significance did not reached (χ^2 value 0.05, $P>0.05$). The expression of C-myc gene in HCC and LHN was significantly higher than that in LC (χ^2 values 4.38, 4.51, $P<0.05$). In HCC (Figure 3) and LHN (Figure 4) showing strong expression of C-myc, the positive-staining cells were distributed dominantly in a diffused pattern; whereas in LC (Figure 5) showing weak expression of C-myc, they preferred in a focalized pattern. The expression of C-myc was negative in normal liver tissues (Table 2).

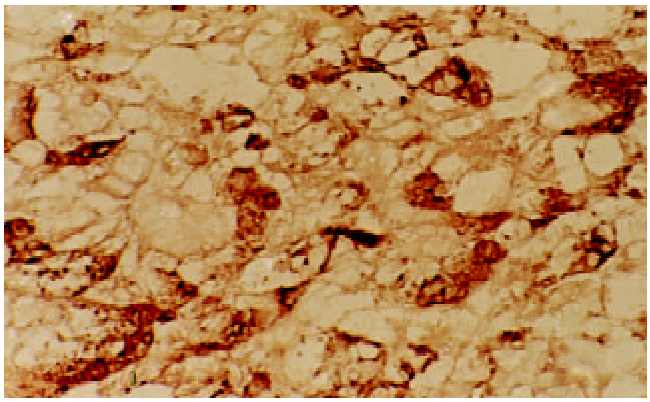

Figure 3 The positive expression of C-myc gene in HCC. LSAB×200

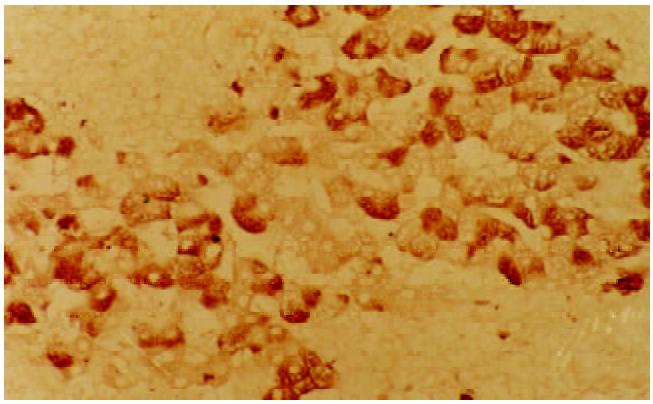

Figure 4 The positive expression of C-myc gene in LHN.LSAB×200

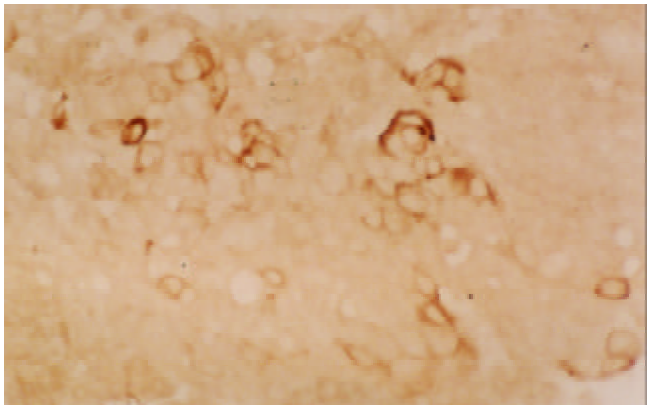

Figure 5 The positive expression of C-myc gene in LC. LSAB×200

Table 1 Expression of p53 gene in HCC and its pericarcinomatous tissue

Histological type	*n*	Expression of p53 gene				Positive rate
		-	+	++	+++	(%)
HCC	73	37	12	19	5	49.3
LHN	39	32	7	0	0	17.9
LC	35	35	0	0	0	0
Normal liver tissues	17	17	0	0	0	0

Table 2 Expression of C-myc gene in HCC and its pericarcinomatous tissue

Histological type	*n*	Expression of C-myc gene				Positive rate (%)
	-	grade Ⅰ	grade Ⅱ	grade Ⅲ	grade Ⅳ	
HCC	60	37	0	2	9	12	38.3
LHN	37	22	1	3	3	8	40.5
LC	30	25	4	1	0	0	16.7
Normal liver tissues	12	12	0	0	0	0	0

The relationship between the expression of p53 and C-myc genes and histological grade of HCC

There were varieties of positive rates of p53 and C-myc genes in different HCC histological grades of HCC, with a close relationship between the genes expression and the tumor differentiation. The positive rates of p53 expression in Edmondson' s grading Ⅲ and Ⅳ and Edmondson' s grading Ⅰ and Ⅱ were 60 %(27/45), 32.1 %(9/28), respectively, which manifested a significant difference (χ^2 value 5.36, P<0.05); The positive rates of C-myc expression in Edmondson' s grading Ⅲ and Ⅳ and Edmondson' s grading Ⅰ and Ⅱ were 48.7 %(19/39), 19 %(4/21), respectively, their differences were also significant(χ^2 value 5.08, P<0.05) (Table 3).

Table 3 The relationship between the expression of p53 and C-myc genes and histological grade of HCC

Edmondson's grading	Expression of p53 gene		Expression of C-myc gene	
	Negative	Positive	Negative	Positive
Ⅰ	4	0	3	1
Ⅱ	15	9	14	3
Ⅲ	18	21	18	12
Ⅳ	0	6	2	7

Correlation between p53 and C-myc protein expressions and HCC clinicopathological parameters

No significant relation was found between p53 or C-myc gene expression and patient age, sex and tumor size (P>0.05).

DISCUSSION

The p53 gene is one of the most important tumor suppressor genes determined so far[14-19]. In recent years, it has been found that the p53 protein seems not express in the benign and preneoplastic lesions, and that there was no obvious relation between the p53 protein and carcinogenesis[20]. However, in the present study, the positive rate of p53 protein staining in LHN was 17.9 %, which coincided basically with some results in literature[21-25]. The facts that the p53 protein expressed low in LHN and high in HCC, indicate that the high expression of p53 protein is probably associated with the cancerous transformation of hepatocytes, the early event in the carcinogenesis of HCC. It has been proved that wild type p53 protein can induce cell apoptosis whereas the mutant p53 protein can inhibit cell apoptosis and promote cell transformation and proliferation, resulting in carcinogenesis[26-30]; The p53 protein confirmed by immunohistochemical staining was considered as the mutation type[31]; Therefore, we speculate that p53 protein may exert its carcinogenic effect in the early stage of carcinogenesis on hepatocytes by two ways: **(1)** As described above, the mutant p53 protein might be associated with cell rapid proliferation and cell transformation, which ultimately results in hepatocellular carcinogenesis; **(2)** with the increase of the mutant p53 expression, and its inhibiting effect on apoptosis there will be an abnormal in cell numbers, which may eventually initiate the hepatocellular carcinogenesis. In addition, our results indicated that there existed a close relationship between the p53 gene expression and tumor cell differentiation in HCC, which suggests that the expression of p53 gene might serve as an index for the judgement of HCC malignant degree and its clinical prognosis.

It has been reported that the inept expression of C-myc gene correlated with carcinogenesis[23,32,33]. However, some authors held that the overexpression of C-myc gene could not be observed until the hepatocytes had thoroughly transformed into malignancy in the late stage of HCC, which reflected the continuous proliferation of tumor cells[34-36]. On the contrary, most experiments demonstrated the expression of C-myc in HCC and its pericarcinomatous tissue[24,37]. In the present study, the expression of C-myc gene was observed both in LHN and LC with varied degree and LHN was similar to HCC in the expression of C-myc, which were coincident with the results of others and suggests that the overexpression of C-myc gene occurs in the early phase of HCC formation, and correlates with preneoplastic transformation and proliferation. Our results also indicated that the expression of C-myc gene in HCC was related to the cell differentiation, which suggests that C-myc gene expression may exist in the sequential process of hepatocarcinogenesis, and be related to the phase of hepatocarcinogenesis.

In the present study, the expression of p53 gene in LHN was similar to that in HCC to some extent and the overexpression of C-myc gene was seen in both samples. The mutation of p53 gene that may lead to cell malignization, thus may reflect the alterations in different biologic state of LHN, and suggest that LHN is probably in the process of malignant transformation and in relation to hepatocarcinogenesis. In the present study the expression of C-myc gene in LHN had no significant difference from that in HCC, which reveals that parts of LHN were actually in the preneoplastic state or might be cancerous though they seemed normal in histology. Some study indicated that the increased oncogene expression brought cells into a state of active proliferation that resulted in an increased frequency of mutation[38]. This suggests that the overexpression of C-myc gene may make LHN be transformed

malignantly. Another study showed that not all altered hepatocyte foci manifested abnormal expression of C-myc in the early stage of experimental HCC and the high expression of C-myc was only seen in the poorly differentiated foci[39]. It shows that the overexpression of C-myc gene may relate to the tumor' s differentiation. Therefore, the overexpression of C-myc gene may be responsible for the low differentiation of LHN. It has been postulated that C-myc products might serve as a valid index for identifying preneoplastic lesion of HCC, the foci overexpressed C-myc were in danger of carcinogenesis[24,33]. However, few research reports till now have been found on the relationship between LHN and HCC. The present study thus provides a new possible way to diagnose HCC at earliest possible stage, which is of great importance in improving the prognosis because early diagnosis usually means high curability.

For many years, it has been generally considered that LC was closely associated with HCC and hepatocarcinogenesis. According to carcinogenic hypothesis on oncogene, at least two activated oncogenes are required-namely, the ras gene which was representative of transforming gene and the C-myc representative of immortalizing gene. Only when the two sorts of oncogenes function coordinately can stock-cultured cells be transformed malignantly. In the present study, we found that the expression rate of C-myc protein in LC was 26.7 %, but, the expression intensity of C-myc in LC was significantly less than that in LHN. Our previous studies have indicated that there was no mutation of the ras gene in LC, and that the expression of c-erbB-2 oncogene was negative in LC, indicating that there are no mutation and activation of c-erbB-2 in LC, that is, it is impossible in this situation for malignant transformation. In addition, according to the present study, there was no mutation of p53 in LC, suggesting that LC does not necessarily link with hepatocarcinogenesis. Alcohol is the major cause of cirrhosis in European countries and the United States, responsible for 60 to 70 percent of all cases of cirrhosis, but it only infrequently leads to HCC; carbon tetrachloride can lead to LC rather than HCC. However, the reason why the ratio of HCC accompanied by LC in China is obviously higher than that in European countries and the United States perhaps lies in HBV infections, there is not a cause and effect but a accompanying relationship between LC and HCC. The significance of C-myc expression in LC remains to be investigated.

What requires a special explanation is that the LHN used in the present study is totally different from the nodules in LC. We noticed that though the majority of the LHN developed from LC, it is a cell population that differs from LC in properties and proliferative patterns. Although the liver cell cords in LC are in disarray, the hepatocytes are primarily arranged in a single line, most of which are normal in morphology; However, the hepatocytes in LHN grow by expansion, one nodule primarily contains a sort of cells, and in mixed cell nodules there exists a clear margin between the cellgroups, suggesting the nodules are of clone origin.

As to the relationship between the expression of p53 and C-myc genes and clinicopathological parameters of HCC, we found that there was no link of p53 or C-myc gene expression with patient age, sex and tumor size, which was in accordance with the previous reports[40]. Some studies indicated that the overexpression of p53 or C-myc was closely related to the prognosis of HCC[18,33, 41-50], which was not our results have not yet confirmed that p53 or C-myc gene expression is directly associated with the prognosis of HCC. However, in the present study, the low expression of p53 gene and the overexpression of C-myc gene were found in LHN. It can be deduced that although normal in histology, LHN is surely abnormal in gene expression. Whether the phenomenon has a tie with the recurrence of HCC after resection needs to be further studied.

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