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P53 EXPRESSION AS A PROGNOSTIC MARKER IN HEPATOCELLULAR CARCINOMA

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Abstract - Hepatocellular carcinoma (HCC) is one of the most frequent cancers worldwide with a high mortality. Immunohistochemical overexpression of the p53 protein was correlated with a poor prognosis in various human malignancies, including HCC. In our study, 45 resected HCCs were examined to evaluate the expression of p53 and its correlation with clinicopathological parameters. Immunohistochemical detection of p53 with monoclonal human antibody, revealed its overexpression in 20 tumors (44%), including diffuse positive in 7 cases (35%), heterogeneous in 5 (25%), and focal in 8 (40%). We considered a positive reaction only in the presence of immunostained nuclei in brown shades in more than 5% of the tumor nuclei. To elucidate the significance of p53 in HCC, we correlated its protein expression with major clinicopathological features. We did not observe significant correlation with sex, age, presence of cirrhosis, chronic hepatitis status, tumoral necrosis and tumor size. The density and intensity of p53 revealed significant correlation with histological grade (P=0.008 and P=0.014) and tumor stage (P=0.005 and P=0.007). In conclusion, our results suggest that overexpression of p53 is associated with HCC progression and contributes to disease progression. Moreover, p53 expression may be a valuable marker of HCC prognosis.

Key words: p53, hepatocellular carcinoma, prognosis, overexpression

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent cancers worldwide, with high risk in Asia and Africa and less common, but with increasing incidence, in Western developed countries (Stefan et al., 1992). The mortality in HCC is increased, with an estimated number of 1 million deaths annually and a survival rate less than 5% for 5 years (Ahmad et al. 2007; Blum et al. 2007).

In recent years, research has focused on the identification of prognosis markers in order to facilitate the early detection of HCC and to guide the development of targeted therapy. Strachan and Read (1999) described as p53 as "the guardian of the genome" due to its important role in preserving stability by preventing genome mutation. Mutation of the p53 suppressor gene is the most commonly detected anomaly in human cancers (Shuang-Jian et al., 1998; Yun et al., 1998). Immunohistochemical overexpression of p53 protein was correlated with a poor prognosis in various human malignancies including HCC (Qin et al., 2001; Hsia et al., 2000).

The purpose of our study was to evaluate the significance of p53 protein immunoreactivity in HCC and to determine whether p53 reactivity correlates with conventional parameters of prognosis.

MATERIALS AND METHODS

Forty-five patients with HCC who had undergone hepatic resection were selected for this retrospective study. The clinicopathological data including sex, age, tumor necrosis, histological grading, tumor stage, hepatitic status, the presence of cirrhosis and tumor size.

Specimens were fixed in 10% buffer formalin and paraffin-embedded. In order to assess the tumor grade and stage, histological sections were cut at 5-μm thickness and stained using the hematoxylin and eosin method.

For immunostaining, p53 was used a primary antibody. We performed heat-induced epitope retrieval with a citrate-based pH 6.0 solution (Novocastra, Newcastle uponTyne, UK) for 30 min. Endogenous peroxidase blocking was realized with 3% hydrogen peroxide for 5 min. This step was followed by incubation for 30 min with p53 (Bond ready to use primary antibody, clone DO-7, Leica Biosystems, Newcastle uponTyne, UK). The Bond Polymer Refine Detection System (Leica Biosystems, Newcastle uponTyne, UK) was used for visualization. 3,3 diaminobenzidine dihydrochloride was applied as chromogen, and hematoxylin was used for counterstaining. The entire immunohistochemical procedure was performed with Leica Bond-Max (Leica Biosystems, Newcastle upon Tyne, UK) autostainer.

Three independent microscopic fields (×400) were selected for each sample and all the tumor cells within each microscopic field were counted, and then the positive percent of p53 cells was calculated. We considered a positive reaction only in the presence of immunostained nuclei in brown shades, in more than 5% of the tumoral nuclei. Three staining patterns were recognized: diffuse nuclear staining that expressed uniform diffuse positivity on tumoral cells (>50%, Fig.1), heterogenous nuclear staining that expressed areas of strong positivity alternating with areas of weak positivity (10%-50%) and focal nuclear staining that expressed small nests or isolated tumoral cells (>5%, Fig.2).

The intensity of reaction was assessed as low (+), moderate (++) or intense (+++).

Examination was performed with an Eclipse E80i Nikon microscope and images were acquired with Lucia G software for microscopic image analysis.

Statistical analysis was performed using the commercially available SPSS version 17.0. We applied the Student's t-test and p-values of less than 0.05 values were considered significant.

RESULTS

Our study included samples from 45 patients (32 males and 13 females), aged between 29-77 years. In relation to tumor size, HCCs were divided into 2 categories: tumors smaller than 5 cm and tumors greater than 5 cm. From the 45 HCCs, 12 tumors were larger than 5 cm and 33 were smaller than 5 cm. According to the Edmondson and Steiner system (Edmondson et al., 1954), tumor grade was divided into three groups: well differentiated (grade I-4 cases), moderately differentiated (grade II-20 cases) and poorly differentiated (grades III and IV-21 cases). Histologically, most of the HCCs showed a trabecular pattern admixed with a pseudoglandular pattern (23 cases) or with a solid pattern (9 cases). Tumor stages were classified as I (12 cases), II (18 cases), III (14 cases), and IV (1 case). The nontumorous liver showed cirrhosis in 17 (38%) patients and chronic hepatitis in 18 (40%).

The main clinicopathological features of patients investigated and the correlations with p53 are presented in Table 1.

By immunohistochemical stain, the p53 protein was detected in the tumor cell nucleus in 20 HCCs (44%), including focal positive in 8 cases (40%) (Fig. 1A), diffuse positive in 7 (35%) cases (Fig. 1B), with heterogeneous pattern in 5 (25%) cases (Fig. 1C). Those without p53 expression or positive p53 expression in less than 5% of tumor cells were considered as negative (25 cases, 56%). From all of the 45 HCCs, analysis of p53 positivity revealed intense positivity

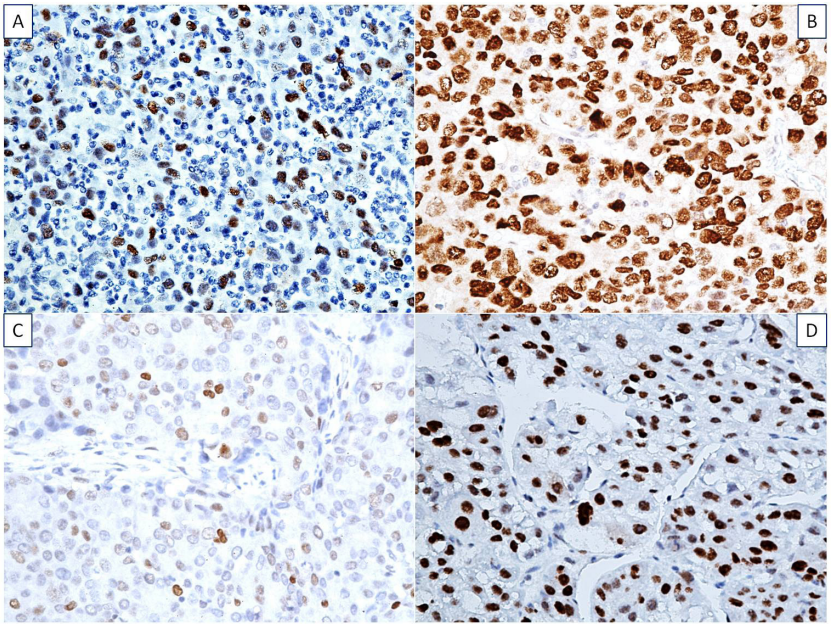

Fig. 1. Distribution patterns and intensity of p53 expression in HCC. Focal expression of p53 in HCC (A) compared with its diffuse expression (B). Heterogenous expression of p53 intensity in different tumor cells nuclei (C). Note the homogeneous expression in other cases with the same intensity in almost all nuclei (D).

Table 1.

Clinicopathological parameters		No. of cases	P53 positive cases (%)	P53 density p-value	P53 intensity p-value
Gender	Males	32	14 (43.7%)	>0.5	>0.5
	Females	13	6 (46.1%)	>0.5	>0.5
Age	<60 years	13	7 (58.8%)	>0.5	>0.5
	>60 years	32	13 (40.6%)	>0.5	>0.5
Grade	Well differentiated (gr. I)	4	0	0.008	0.014
	Moderately differentiated (gr. II)	20	7 (35%)		
	Poorly differentiated (gr. III, IV)	21	13 (62%)		
Stage	I	12	4 (33%)	0.05	0.07
	II	18	11 (61%)		
	III (A, B, C)	14	5 (36%)		
	IV	1	0 (0%)		
Tumor size	<5cm	33	14 (42%)	>0.5	
	>5 cm	12	6 (50%)		
Tumoral necrosis		17	7 (41%)	>0.5	
Cirrhosis		17	8 (47%)	>0.5	
Chronic hepatitis		18	8 (44%)	>0.5	

in 8 (40%) cases (Fig. 1D), moderate positivity in 7 (35%) cases and weak positivity in 5 (25%) cases.

Clinicopathological data including sex, age, presence of cirrhosis, chronic hepatitis, tumor stage, histological grading, tumor size and presence of tumoral necrosis. P53 expression showed positivity in 43.7% of males and 46.1% of females and appeared to be more frequently in young patients (53.8% in patients <60 years and 40.6% in patients >60 years).

Regarding tumor stage, the expression of p53 presented a maximum score in stage II (61%) and stage III (38%). A significant correlation was noted between overexpression of p53 and tumoral stage (P=0.005). As shown in Table 1, p53 protein expression tended to occur in HCCs with high grade (grade II-IV; P=0.008).

P53 showed positivity in 12 (50%) HCCs larger than 5 cm and in 33 (42%) HCCs smaller than 5 cm. We analyzed the relation of p53 with tumor size, which is known as an important prognostic factor in HCC, but the correlation was not significant (P>0.5).

P53 immunoreactions did not exhibit a relation with the other clinicopathological parameters.

DISCUSSION

The tumor-suppressor gene p53, which is located on the short arm of chromosome17, plays an essential role in preserving stability by preventing genome mutation. The major function of the gene is to block cell cycle progression in response to DNA damage (Cornelia et al., 2009). Wild-type p53 (wt) is at low levels in normal tissue and usually it cannot be detected with immunohistochemical stains. Unlike wt p53 protein, mutant forms are more stable and have a prolonged half-life, which favors intranuclear accumulation, becoming detectable immunohistochemically (Steele et al. 1998). In several studies, the overexpression of p53 in the serum or liver tissues of HCC patients was associated with poorer prognosis and a shorter survival time (Cornelia et al., 2009; Zhou et al., 2006; Liu el al., 2012).

In recent years, many studies explored the expression and the mutations of p53 in HCC and the results are various. Caruso et al. (1999), studied the immunohistochemical expression in a series of 193 HCC specimens, and overexpression of p53 was observed in only 29 (15%) HCCs, frequently in tumors with poor cellular differentiation, but without reaching statistical significance. In the study of Ng et al. (1995), overexpression of p53 was found in 31% HCCs and unlike the results of the study conducted by Caruso et al. (1999) here a significant correlation between p53 overexpression and the poor cellular differentiation was found. Zhao et al. (1994) analyzed p53 immunoreactivity using five anti-p53 protein antibodies and the results ranged from 67.5% to 10.8%, which strongly suggests that the immunoreactivity of p53 is influenced by the antibody used. Moreover, the results may vary depending on the geographical area, being lower in Europe where exposure to aflatoxin B1 does not occur (Boix-Ferrero et al. 1999). In our research, immunohistochemical analysis of p53 demonstrated accumulation of nuclear staining in 44% of cases, which is consistent with the results of Boix-Ferrero et al. (1999).

Several studies found that overexpression of p53 does not correlate with p53 mutation in HCC (Hsia et al., 2000; Boix-Ferrero et al., 1999; Anzola et al., 2004). An explanation could be the presence of other factors that might contribute to the inactivation of the p53 rather than mutations, such as viral proteins (Boix-Ferrero et al., 1999; Bourdon et al., 1995). Kang et al. (1998) compared overexpression and mutation of p53 in HCC and found that 50% of cases showed positivity for p53 but mutations were detected in only 25% cases. In the same study, p53 protein was positive in high-grade dysplastic nodules but none had mutations in the exons examined (Kang et al., 1998).

Our study showed that p53 overexpression was significantly associated with the poor histological differentiation of tumor cells (P=0.008), which is consistent with previous reports (Qin et al., 2001; Caruso et al., 1999; Ng el al., 1995; Sung et al., 2005; Hsu et al. 1993; Lee et al., 2002). Moreover, we found significant correlation with tumoral stage (P=0.005), which is known to be an independent prognosis factor.

CONCLUSIONS

The immunohistochemical expression of p53 in 45 patients with HCC showed a positive reaction in 44% of cases. We found a significant statistic correlation between p53 expression and tumoral stage and grade. Our results suggest that the overexpression of p53 is

associated with HCC progression. We conclude that the p53 protein has an important role in hepatocarcinogenesis and contributes to a poor prognostic indirectly by grade and tumor stage.

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