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ORIGINAL ARTICLE

Correlation between expression levels of PTEN and p53 genes and the clinical features of HBsAg-positive liver cancer

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Summary

Purpose: *To determine the expression levels of PTEN and p53 genes in HBsAg-positive liver tumors and analyze the data for correlations of the expression levels of each gene with pathological features of primary liver cancer (PLC).*

Methods: *Blood and postoperative tissues were collected from 43 cases diagnosed with PLC treated in our hospital. The cases included 29 HBsAg-positive and 14 HBsAg-negative PLC. The mRNA expression levels of PTEN and p53 in normal liver, tumor-adjacent and liver tumor samples were detected via RT-PCR. Additionally, protein expression levels of PTEN and p53 in different liver tissues were detected via immunohistochemistry (IHC).*

Results: *RT-PCR results showed that the relative mRNA expression levels of PTEN and mutant p53 in PLC and tumor-adjacent tissues were significantly different ($p<0.05$). IHC showed that the positive rate of protein expression of PTEN was only 34.88% in PLC and 86.05% in tumor-adjacent tissues. The protein expression levels of PTEN were further related to tumor characteristics such as the pathologic grade, and metastasis and invasion capabilities of the tumor cells ($p<0.05$). However, the levels of PTEN were not associated to the presence of the hepatitis B virus (HBV) antigen, the tumor diameter or the AFP levels ($p>0.05$). The protein expression levels of p53 were highest in cancer tissues, but the levels revealed no correlation with the presence of the HBV antigen, the tumor diameter, the AFP levels, the pathologic grade, or the invasion and metastasis capabilities of the tumor tissues ($p>0.05$). Finally, Spearman correlation analysis showed that the levels of PTEN exhibited no correlation with the levels of mutant p53 ($rs=-0.021$, $p>0.05$).*

Conclusion: *This study showed that the expression of PTEN was significantly reduced in PLC when compared to its expression in normal liver; and the expression levels were associated with the pathologic grade, invasion and metastasis capabilities of the tumor. On the other hand, p53 expression was high in PLC tissues but no correlations to the cancer's characteristics were found.*

Key words: *HbsAg, p53, primary liver cancer, PTEN gene*

Introduction

PLC is one of the most common malignancies. The therapeutic approaches used to treat this condition have outcomes that are far behind those applied to treat other solid tumors [1]. Molecular biology advances have guided clinical research to focus on studying the effects of single genes as well as developing and testing specific therapeutic approaches. So far, PTEN is the only tumor-suppressor gene known to have dual (lipid and protein) phosphatase activities [2,3]. Studies have shown associations between the expression levels of the PTEN gene and the presence of the liver cancer [4]. In addition, wild-type p53 gene is a well-known tumor-suppressor gene, but its function can be significantly affected during mutations and may lead to the development of more than 50 kinds of tumors, including liver cancer [5].

This study was designed to determine the expression levels of both PTEN and p53 genes in different samples from patients with PLC in order

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to find any associations between these levels and the clinicopathological features present in the patients.

Methods

Patients

Registered in this study were 43 patients with PLC admitted to our hospital from February 2013 to February 2015 and included 31 males and 12 females, with an average age of 46.1±12.8 years. For the experimental study, preoperative venous blood, postoperative liver cancer, tumor-adjacent (about 2 cm from the tumor's edge) and normal liver (>5 cm from the tumor's edge) tissue samples were obtained from all subjects. Pathology confirmed PLC diagnosis in all of the patients [6]. Nineteen stage I-II and 24 stage III-IV cases were determined after Edmondson grading [7]. Additionally, sample tissues for IHC and RT-PCR were also collected from all subjects. All patients signed informed consent forms. Relevant clinicopathological characteristics of the 43 patients with liver tumors were recorded and are shown in Table 1. Furthermore, the risk of liver cancer metastasis for each patient was estimated according to the EL-Assal scoring system [8]. According to this system, a score ≥3 indicates a high risk and a score < 3 indicates a low risk of metastasis.

Table 1. The clinical pathological characteristics of the 43 liver tumor patients

	Group	*Number of cases*
HBV	+	29
	-	14
Tumor diameter (cm)	<5	16
	≥5	27
AFP (ng/ml)	<8.1	22
	8.1-400	6
	≥400	15
Pathological grade	I-II	19
	III-IV	24
Invasion and metastasis	high risk	30
	low risk	13

HBV(+): HBsAg positive, HBV(-): HBsAg negative

Real-time PCR

Total RNA extraction from tissue samples was performed according to the Trizol kit (Invitrogen, Carlsbad, CA, USA) instructions. Primers were designed according to conventional criteria and were synthetized by TaKaRa (Table 2). The experiments were performed in triplicate to reduce errors and bias, and averages were shown. The β-actin gene was used as a housekeeping gene. Standard RT-PCR protocols were followed to obtain relative mRNA levels. The integrated optical density (IOD) defined the absolute integral of amplification band, and the relative amount of mRNA was obtained by the formula: mRNA amount = IOD of target gene/ IOD of reference gene.

Table 2. Primer sequence

Molecule	*Amplified fragment length (bp)*	*Primer (5 →3)*
PTEN	466	Upstream: AGTTCGTGGTCTGCCAGCTA
		Downstream: TCAGAGTCAGTGGGTTCAGA
Mutant p53	482	Upstream: CGTATGGAAACTACTTCCTGAAAACTA
		Downstream: ACAGCATCAATTCATCCATTGC
β-actin	365	Upstream: GACGATGGAGGGGCCGGACTCATG
		Downstream: CAAAGACCTCTATGCCAACACAGC

Streptavidin-peroxidase immunohistochemical analysis

The SP immunohistochemical method was utilized for the tissue sample experiments. After conventional sample preparations, 5 high-power field areas were chosen from each section. Senior pathologists studied the results under light microscope. The PTEN protein was considered to be present if there was granular brown staining only in the cytoplasm of cells. Positivity for p53 protein meant a granular brown stain in both nucleus and the cytoplasm of p53–probed samples. Samples were classified according to the percentage of positive cells present in them: a percentage from 0-10% was marked as negative, from 10-25% as (+), from 25-50% as (++), and >50% as (+++). All samples with at least (+) were considered positive .

Statistics

The SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data were presented as mean ± standard deviation. Correlation analyses were performed according to the Spearman's method. A $p<0.05$ meant that a given difference was statistically significant.

Results

RT-PCR

mRNA expression levels of PTEN and p53 in different tissues

RT-PCR showed 14 PTEN positive bands in tumor tissues and 33 in tumor-adjacent tissues (Figure 1). The relative expression of mRNA levels of PTEN and mutant p53 in PLC and tumor-adjacent tissues are shown in Figure 2. PTEN was

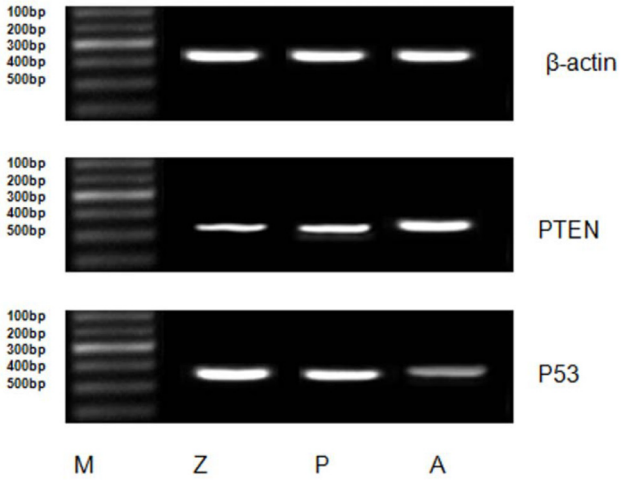

Figure 1. Expression levels of PTEN and mutant p53 mRNAs were detected by RT-PCR, with β-actin as the housekeeping gene. PTEN was downregulated and p53 was upregulated in tumor tissues compared with normal or adjacent tissues. Z: normal liver tissues; P: tumor-adjacent tissues; A: tumor tissues; M: Marker.

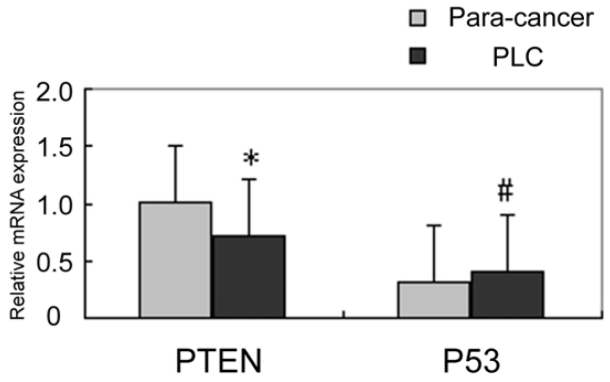

Figure 2. Relative expression levels of PTEN and mutant p53 mRNAs in PLC and tumor-adjacent tissues: *Compared with PTEN in tumor-adjacent tissues, p<0.05; #Compared with p53 in tumor-adjacent tissues, p<0.05.

downregulated and p53 was upregulated in tumor tissues compared with normal and adjacent tissues.

IHC results

Relative protein expression levels of PTEN in different liver tissues and their correlation with clinicopathological features of PLC

Positive staining of PTEN protein was most striking in normal liver tissues (100%), and the expression levels declined progressively from tumor-adjacent (86.05%) to tumor tissues (34.88%, 15 of 43 samples). The differences between the groups were significant (p<0.05; Figure 3).

In liver tumor tissues, the expression levels of PTEN were related to the grade of tumor differentiation. Compared with III-IV pathological stage samples, the expression levels of stage I-II samples were significantly higher (p<0.05). The expression levels of PTEN were also associated to the probability of invasion and metastasis of PLC. Furthermore, the expression levels in the low risk metastasis group were significantly higher than the levels in the high risk metastasis group (p<0.05; Table 3).

Relative p53 protein expression levels in different liver tissues and their correlation to clinicopathological features of PLC

Positive staining of p53 was mostly seen in the nucleus of cells. However, in contrast to the case of the PTEN protein, there was no positive staining of p53 in normal liver tissues. The protein expression levels of the p53 protein showed a

Table 3. Correlation between the expression of PTEN gene protein and liver cancer

Clinicopathological factors	*Group*	*Cases*	*Expression of PTEN gene*				*Positive rate (%)*	*p value*
			-	+	++	+++		
HBV	+	29	18	7	2	2	37.93	>0.05
	-	14	9	3	2	0	35.71	
Tumor diameter (cm)	<5	16	9	5	1	1	43.75	>0.05
	≥5	27	18	6	2	1	33.33	
AFP (ng/ml)	Negative	22	16	5	1	0	27.27	>0.05
	Positive	21	12	5	3	1	42.86	
Pathological grade	I-II	19	9	5	5	1	57.89	<0.05
	III-IV	24	19	5	0	0	20.83	
Risk	High risk	30	24	4	2	0	20.00	<0.05
	Low risk	13	5	5	2	1	61.54	

Table 4. Correlation between the expression of p53 gene protein and liver cancer

Clinicopathological factors	*Group*	*Cases*	*Expression of p53 gene*				*Positive rate (%)*	*p value*
			-	+	++	+++		
HBV	+	29	19	5	3	2	34.48	>0.05
	-	14	8	4	1	1	42.86	
Tumor diameter (cm)	<5	16	9	3	3	1	43.75	>0.05
	≥5	27	17	6	2	2	37.04	
AFP (ng/ml)	Negative	22	13	6	2	1	40.91	>0.05
	Positive	21	13	4	3	1	38.10	
Pathological grade	I-II	19	12	5	1	1	36.84	>0.05
	III-IV	24	13	5	3	3	45.83	
Risk	High risk	30	16	8	3	3	46.67	>0.05
	Low risk	13	9	1	2	1	30.78	

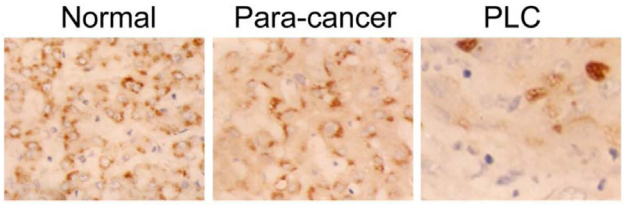

Figure 3. Protein expression levels of PTEN in different tissues. PTEN was low-expressed in tumor tissues (x400).

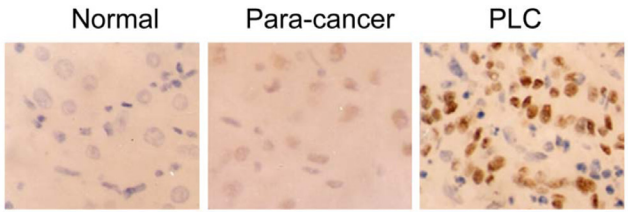

Figure 4. Protein expression levels of p53 in different tissues. p53 protein was highly expressed in tumor tissues (x400).

rising trend from tumor-adjacent to tumor tissues (Figure 4). However, the expression of mutant p53 protein was not related to the levels of HBsAg in serum, and the tumor tissues, AFP level, grade of differentiation, invasion or metastasis (p>0.05; Table 4) .

Correlation between PTEN and p53 in liver tumor tissues

The expression levels of PTEN and p53 in liver tumor tissues are shown in Table 5. According to the analysis obtained by Spearman's correlation test, there was no correlation between the mRNA or protein levels of PTEN and those of p53.

Discussion

The PTEN gene is a tumor suppressor located on chromosome 10q23.3 with total length of 200 KB [7]. It is a well-known fact that PTEN exerts its antitumor effects by the dephosphorylation of PIP3, blocking its recruitment for the AKT signaling pathway. The protein also acts by antagonizing PI3K/AKT pathway, inducing cell apoptosis and inhibiting cell growth . Additionally, there are reports revealing that PTEN inhibits the adhesion, invasion and metastasis of tumor cells by the dephosphorylation of FAK [8]. Furthermore, PTEN seems to inhibit cell differentiation by blocking the cell signal transduction pathway of MAPK [9,10]. Earlier studies have confirmed that the deletion of PTEN gene leads to development of malignant tumors of the prostate, breast and sporadic melanomas . There is also a report on PTEN heterozygosity in liver cancer [11]. The present study showed that the mRNA and protein expressions levels of PTEN were clearly low in cancer tissues relative to the levels in adjacent or normal tissues (p>0.05). Nevertheless, the protein expression levels of PTEN were not related to the levels of HBsAg or AFP in serum, or to the diameter of the tumors. On the other hand, the levels were associated to the pathologic differentiation features of the tumor tissues, and these results are consistent with earlier reports [12,13].

Table 5. Correlation between PTEN and p53 in liver tumor tissue

p53	*PTEN*		*Total, n*
	+	-	
+	6	12	18
-	9	16	25
Total	15	28	43

p>0.05, showing no correlation between PTEN and p53

p53 is an important tumor suppressor gene, which participates in the expression /regulation of cell cycle, apoptosis, cell proliferation and DNA damage and repair [14-17]. The mutant p53 gene can result in cell transformation and promote tumor formation [18,19]. There are mainly two kinds of mutations: recessive (where the mutant p53 gene loses its anticancer activity), and dominant

(where an allele mutates into a tumor cell inducer) [20]. Of the 43 cases in this study, there was no positive p53 staining in normal liver tissues. The expression levels of PTEN protein showed a rising trend from tumor-adjacent to tumor tissues. Our results confirmed that the mutant p53 gene participating in the occurrence of liver tumor is frequent in PLC. However, the expression of mutant p53 protein was not associated to serum HBsAg levels, the size of tumors, the AFP levels, the grade of differentiation, invasion or metastasis ($p>0.05$). This result is not surprising as earlier reports also failed to confirm any associations .

In conclusion, based on our findings PTEN has a low expression in liver tumor cells and its expression levels decrease as the cells become malignant and are able to invade and metastasize. The expression levels of wild type p53, on the other hand, are high in liver tumor cells but they cannot be reliably used as biological markers for PLC.

Conflict of interests

The authors declare no conflict of interests.

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