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W.-C. Chen · F.-J. Tsai · J.-Y. Wu · H.-C. Wu
H.-F. Lu · C.-W. Li

Distributions of p53 codon 72 polymorphism in bladder cancer – proline form is prominent in invasive tumor

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Abstract Abnormal function of p53 is commonly associated with various cancer formations. High-grade and late-stage bladder cancers have been reported to have mutated or become inactive p53 when using immunohistochemical stains. Recently, p53 codon 72 polymorphism was extensively studied to determine the risk factors responsible for cancer formation. There was a general population of codon 72 sequence polymorphism of the wild type p53. A single base change from G to C caused the alteration of amino acid residue 72 from arginine to proline. The arginine form is considered to be a significant risk factor in the development of cancer. However, various reports had indicated discrepancies with regard to this polymorphism; some showed no significant difference between the control and cancer groups, while other series were associated with high risks in the proline form homozygotes. To resolve the undefined distribution of this polymorphism in bladder cancers, 58 patients with bladder cancer were enrolled onto this study. When checked using the Chi-squared test ($P = 0.952$) there were no differences between the control subjects and bladder cancer patients in the distribution of polymorphism. However, proline form homozygotes were more frequently found in the invasive group than the non-invasive group by Fisher's exact test (25% and 2.9%, respectively, $P < 0.001$). More than 70% of the non-invasive bladder cancers were the arginine form homozygotes. This result is consistent with those reported for hepatocellular carcinoma that showed a history of chronic liver disease and proline form homozygotes in a report by Yu et al. Our data suggest that proline form homozygotes are associated with invasive bladder cancer.

Key words p53 codon 72 polymorphism · Bladder cancer · Proline form · Arginine form

H.-C. Wu (✉)
2, Yu-Der Road, Taichung, 404, Taiwan
e-mail: wuhc@hpd.cmch.org.tw
Tel.: +886-4-205-2121, ext 4325

W.-C. Chen · H.-F. Lu
Department of Urology,
China Medical College Hospital,
School of Medicine, China Medical College,
Taichung, Taiwan

F.-J. Tsai · J.-Y. Wu
Department of Medical Genetics,
China Medical College Hospital,
School of Medicine, China Medical College,
Taichung, Taiwan

C.-W. Li
Institute of Life Science,
National Tsing Hua University,
Hsinchu, Taiwan

Introduction

Most bladder cancers are transitional cell carcinoma (TCC). Risk factors of TCC include cigarette smoking, chemical exposure, viral infection, and artificial sweetener ingestion [10]. In an area of endemic "blackfoot disease" in southern Taiwan a high incidence of TCC in the urinary tract has been reported [3]. Furthermore, late-stage and high-grade bladder tumors have been reported to have mutated or become inactive p53 which when using immunohistochemical stains can result in positive and false–positive mutations of p53 [1, 5]. The p53 gene and its encoded protein have been intensively studied due to its regulation of cell cycle, cellular growth, apoptosis, and mediation of the integrity of the genome. It is called a cellular gatekeeper or guardian of the genome [7, 8]. Because abnormal p53 is commonly found in bladder and other cancers, an understanding of the role of p53 and its related molecular pathway has led to many studies in the pathogenesis of such tumor formation.

Five repeats of the sequence pxxp between amino acids 61 and 94 of the p53 are considered to be a motif that play a role in signal transduction via its SH3

domain binding activity, which might mediate tumorigenesis [13]. Impairment of p53 to suppress tumor cell growth in culture was related to the deleted proline-rich domain but did not disrupt the upstream regulation of p53 by DNA damage. There was a general population of codon 72-sequence polymorphism of the wild-type p53. The single-base change (from CGC to CCC) caused the alteration of amino acid residue 72 from arginine (arg) to proline (pro) [2]. The effect of this polymorphism on the susceptibility of p53 to E6-mediated degradation has been investigated and the arginine form of p53 was found to be significantly more susceptible to tumor growth than the proline form. Therefore, p53 arg is considered to be a significant risk factor in the development of HPV-associated cancers [12]. Patients were seven times more likely to develop HPV-associated cervical cancer in p53 arg, which was also found in renal transplantation patients who had p53 arg homozygotes. There may be different structural functions from the codon 72 between arg and pro form. There has been no study that focused on bladder cancer. This study was performed to try and understand this polymorphism in bladder cancers.

Materials and methods

Fifty-nine healthy male volunteers each more than 40 years old were enrolled in this study as the control group. They were non smokers and had no history of familial cancer. Patients with bladder cancer from the department of urology who had been treated from 1998 through 1999 were included in this study. Informed consent was obtained from both groups of participants in this study. All the pathological cell types in the cancer group were TCC. The genomic DNA was prepared from peripheral blood by using a DNA Extractor WB kit (Wako, Japan).

Polymerase chain reactions (PCRs) were carried out in a total volume of 25 Pol, containing genomic DNA; 2–6 pmol of each primer; 1X Taq polymerase buffer (1.5 mM MgCl); and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer). The primer Pro72 was designed for p53 codon 72 in proline form and Arg72 for arginine form, according to the procedure described by Storey et al. [12]. PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer). Cycling condition for Pro72 was set as follows: one cycle at 94 °C for 5 min, 35 cycles of 94 °C for 15 s, 52 °C for 20 s, and 72 °C for 30 s, and one final cycle of extension at 72 °C for 7 min.; conditions for Arg72 were the same as Pro72 except temperature of 50 °C used for annealing.

The PCR products from Arg72 and Pro72 from the same individual were mixed together and 10 mml of this solution was loaded into 3% agarose gel containing ethidium bromide for electrophoresis. Patients were further subdivided into invasive and non-invasive cancer groups according to their pathological grading and clinical course. The patients in the noninvasive group were classified with pathological superficial tumors in surgical specimens (Ta and T1 by AJCC staging) and patients in the invasive group were defined as having pathological invasive tumors (T2a and T2b by AJCC staging) in surgical specimens. Statistical analysis for determining the relative risk of this polymorphism was done using the Chi-squared test to compare the pathological grading of control and bladder cancer groups. The Fisher's exact test (2 tail) was used in the analysis of distribution in invasive and non-invasive bladder cancer groups.

Results

Fifty-eight bladder cancer patients were enrolled in this study with ages ranging from 44 to 86 years (mean, 66.7 years). The mean age of the control group was 63.2 years (range, 42 to 80 years). The proline form of PCR results were in a product of 177 bp and the arginine form in one of 141 bp. Those bands revealing proline or arginine homozygotes and heterozygotes showed on the gel (Fig. 1). The frequencies of the genotype in the bladder cancer group and control group are shown in the Table 1. Using the Chi-squared test, we

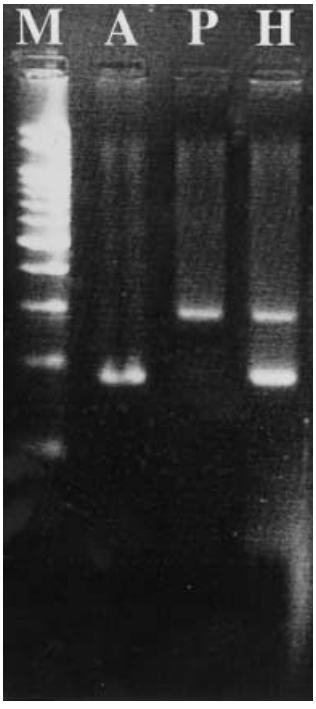

Fig. 1 PCR-base analysis of p53 codon 72 polymorphism. The polymorphic region was amplified by PCR which resulted an 141 bp fragment in arginine form (*lane 2*) and 177 bp fragment in proline-form (*lane 3*), or heterozygous form (*lane 4*). M, marker (*lane 1*), 100 bp ladder

Table 1 Distribution of p53 codon 72 polymorphism in healthy control subjects and bladder cancer patients

	Pro/Pro		Pro/Arg		Arg/Arg		Total		*P*-value
	n	%	*n*	%	*n*	%	*n*	%	
Control	8	13.5	26	44	25	42.4	59	100	0.952
Bladder cancer	7	12.0	25	43.1	26	44.8	58	100	

df = 2, X^2 = 0.097

compared the distribution of p53 codon 72 polymorphism. There were no significant differences between the healthy individuals and the cancer group patients. The distribution in the control group showed 42.4% in arginine homozygotes, 44% in heterozygotes and 13.6% in proline homozygotes. The cancer group showed a very similar distribution (df = 2, $X^2 = 0.097$, $P = 0.952$). For further classification into groups according to invasiveness, we used Fisher's exact test to analyze the data. There were significant differences between the invasive and non-invasive groups in the distribution of polymorphism ($P < 0.001$). Arginine was dominant in the noninvasive group ($n = 24$, 70.59%). Compared with the noninvasive group, there were an increasing number of proline homozygotes in the invasive group of 2.9% and 25% respectively (Table 2). The difference between controls and noninvasive bladder cancers revealed no statistical significance in the distribution of polymorphism (Table 3).

Discussion

This study was designed to examine the distribution of p53 codon 72 polymorphism in bladder cancer patients within different pathological groups. Invasive and noninvasive were the groups to be defined for this polymorphism. The finding showed that the arginine form of p53 codon 72 did not increase the risk of bladder cancer compared with the healthy individuals. The differences were found in the group with invasive bladder cancer that showed the number of the proline form was largely increased. A high percentage of proline form in the invasive group was compatible with hepatocellular carcinoma with a history of chronic liver disease as reported by Yu et al. [15]. In this distribution, patients with proline form of p53 could be susceptible to developing invasive bladder cancer. However, there was no chronic disease associated with bladder cancer like hepatocellular carcinoma in the patient group. Thus, these data cannot help us to predict those diseases related to the formation of bladder cancer. Bladder cancer patients who lived in the area of endemic "blackfoot disease" in southern Taiwan [3] should be further investigated by this method.

It is noteworthy that p53-deficient mice develop normally but have been found to be susceptible to spontaneous tumor formation [4]. According to the results of Walker and Levine, mutations of a novel p53 functional domain in 61–94 codon altered the growth rate but did not influence the binding of damaged DNA. Losses of this domain function resulted in the later development of cancer or cells, which did not undergo apoptosis. The proline-rich domain of p53 function as a discrete signaling molecule is well documented and pxxp-rich domains may play a role in the formation of signaling complexes [13]. These pxxp residues form a left-handed polyproline type II helix, which creates a binding site for SH3 (*src* homology 3) domains. These motifs have been found in all proteins known to bind directly to SH3 domains, despite the fact that specificity of such interactions appears to be determined by variable adjacent residues. These structural features of p53 (residues 61–94) have been well preserved throughout evolution except residue 72, which has been recognized as a polymorphism where arginine residue is substituted for the proline form [2]. It was accepted until Storey et al. reported that the proline form was seven times longer than the arginine form when p53 was degraded by the infection of HPV [12]. This explains the hypothesis

Table 2 Fisher's exact test for the distribution of polymorphism in bladder cancer patients according to the invasiveness

Bladder cancer	Pro/Pro		Pro/Arg		Arg/Arg		Total		*P*-value
	n	%	*n*	%	*n*	%	*n*	%	
Non-invasive	1	0.94	9	26.47	24	70.59	34	100	0.00000185***
Invasive	6	25.00	16	66.67	2	8.33	24	100	

[a] Fisher's exact test
***$P < 0.001$

Table 3 Chi-square test for the distribution of polymorphism between control and non-invasive bladder cancer, and, control and invasive bladder cancer

	Pro/Pro		Pro/Arg		Arg/Arg		Total		X^2	*P* value
	n	%	*n*	%	*n*	%	*n*	%		
Control	8	13.5	26	44	25	42.4	59	100		
Bladder cancer										
Non-invasive	1	0.94	9	26.47	24	70.59	34	100	7.547	0.023
Invasive	6	25.0	16	66.67	2	8.33	24	100	9.122	0.010*

df = 2
*$P < 0.05$

that the arginine form of p53 in residue 72 may be responsible for the less potent effect when the cell is destined to replicate. This mechanism may be independent of transmission of nonproliferative signals through transcriptional activation.

Rosenthal and coworkers first investigated the possible risk factor of cervical cancer through PCR amplification of p53 codon 72 polymorphism allele [11]. However, there were changes identified in the proportions of codon72 after comparing control with cervical cancer groups. The distribution of the proline homozygotes was all below 8% in the cancer and control groups. A large sample of cancer patients and control subjects were analyzed for this polymorphism in Costa Rica, Portland and the Eastern United States. The studies showed that p53 arg was not associated with any increase in the risk of development of cancer [6]. However, in Swedish and Italian women with HPV-16 positive cervical disease, the distribution of p53 codon 72 polymorphism showed arg homozygotes were enriched in cancer patients compared with control subjects and precursor lesions [16]. More than 70% of arg homozygotes were found in the patients with invasive cervical carcinomas. This discrepancy may be due to the strict classification of this form of cancer. However, there is evidence that pro allele homozygotes are a risk factor for lung cancer and hepatocellular carcinoma as reported by Yu and Wang et al. [14,15]. Different cancer cells showing different risk factors should also be classified.

We found that the proline form of codon 72 in p53 showed more aggressiveness in the tumor progression. Although the role has not yet been clarified, this polymorphism should receive more attention in the study of progression of cancer and in the development of gene therapy. Recently, single nucleotide polymorphism has been used as a tool for the searching of genetic variations for a disease gene [9]: by understanding this associated polymorphism it is expected that understanding of disease progression will increase.

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