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Original Article

Polymorphisms in TP53 are associated with risk and survival of osteosarcoma in a Chinese population

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Abstract: Osteosarcoma (OS) is the most frequent histological form of primary bone cancer in adolescence. TP53 is a tumor suppressor gene which is essential for regulating cell division and preventing tumor formation. The purpose of this study is to examine whether genetic mutations in the TP53 gene are associated with OS risk and survival in a Chinese population. Five polymorphisms in the TP53 gene were selected in a case-control study, including 210 OS patients and 420 cancer-free controls. We found that subjects carrying rs12951053 CC genotype and rs1042522 GG genotype were significantly associated with risk of OS [odds ratio (OR) = 1.68, 95% confidence intervals (CI): 1.05-2.68; OR = 1.89, 95% CI: 1.16-3.07] compared with subjects carrying the common genotypes. Results of haplotype analysis also showed that A-G-G-A-C haplotype (rs12951053, rs1042522, rs8064946, rs9895829 and rs12602273) conferred significant decreased risk of OS (OR = 0.37, 95% CI: 0.19-0.72) compared with A-C-G-A-C haplotype. Besides, rs1042522 was an independent prognostic factor for OS with hazard radio (HR) = 1.94 (95% CI: 1.03-3.65) in GG genotype than in CC genotype. Our data suggest that genetic mutations in the TP53 gene are associated with risk and survival of OS in Chinese population.

Keywords: Osteosarcoma, TP53, polymorphism, haplotype, susceptibility, survival

Introduction

Osteosarcoma (OS) is the most common type of malignant bone tumors in children and adolescents, comprising 2.4% of all malignancies in pediatric patients. It commonly occurs in the femur (42%), the tibia (19%), and the humerus (10%) [1]. The incidence peaks of OS occurs at age 15-19 for males and age 10-12 for females, who are experiencing a growth spurt, with slightly higher incidence rate in males than in females [2, 3]. Despite significant advancements in the diagnosis and treatment of OS, the overall survival rate has improved only slightly over the past decades [4].

Studies to determine the etiology of OS involve epidemiologic, environmental and genetic factors. Although the exact etiology of OS remains unclear, factors related to patient characteristics include age, gender, ethnicity, growth and height, preexisting bone abnormalities (e.g., Paget's disease, fibrous dysplasia, enchondromatosis, and hereditary multiple exostoses), and genetic and familial factors [1, 5]. Genetic aberrations in OS have received increasing recognition as an important factor in its etiology. Several case-control studies have reported preliminary associations of common genetic variants in specific genes and pathways may play roles in the pathogenesis of OS [6, 7]. TP53 gene encodes a tumor suppressor protein p53, which is essential for regulating cell cycle and plays important roles in cancer prevention through regulating apoptosis, genomic stability, and inhibition of angiogenesis. Heritable mutations in TP53 are associated with the Li-Fraumeni syndrome, a Mendelian disorder, which can increase the incidence of multiple types of cancer [8]. Many studies also investigated the genetic link between TP53 variations and cancer susceptibility, including breast, lung, colorectal, ovarian, endometrial cancer, and etc. [9, 10]. However, little is known with regard to TP53 mutations and OS, and this association has not been studied in Chinese population.

The purpose of this study is to exam whether mutations in the TP53 gene (rs12951053, rs1042522, rs8064946, rs9895829 and rs-

Table 1. Characteristics of cases and controls

Characteristics	Cases, n (%)	Controls, n (%)	*P*-value
Age			
≤ 20	129 (61.4)	252 (60.0)	
> 20	81 (38.6)	168 (40.0)	
Mean age	28.0 ± 18.0	27.9 ± 17.3	0.95
Gender			
Male	130 (61.9)	240 (57.1)	
Female	80 (38.1)	180 (42.9)	0.25
Tumor location			
Long tubular bones	154 (73.3)		
Axial skeleton	56 (26.7)		
Metastasis			
Yes	57 (27.1)		
No	153 (72.9)		

12602273) are associated with risk and survival of OS in a Chinese population.

Materials and methods

Study subjects

In this study, a total of 210 newly diagnosed OS cases among Nanjing residents, aged 9-69 years, were recruited from Jinling Hospital between 2009 and 2014. Cases were all histologically confirmed with primary OS. 420 controls, without a history of cancer, were randomly selected from those who went to hospital during the same period and frequency matched to the cancer cases by age and gender. All cases and controls were provided with written, informed consent before participating in the study. Demographic information and clinical pathological data were obtained through in-person interviews conducted by trained interviewers. At the same time, 5 mL of peripheral blood was obtained. All patients were followed up regularly (every 2 months) until death or the end of follow-up. The study was approved by the Review Boards of the Jingling Hospital.

Genotyping

Tagging SNPs in the TP53 gene were selected by searching Han Chinese data from the HapMap project. Tagging SNPs were selected with criteria of a minor allele frequency (MAF) ≥ 0.1 and linkage disequilibrium of $r^2 \geq 0.90$. Finally, a total of 5 SNPs were selected. Genomic DNA was extracted from blood samples using Genomic DNA Extraction Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. DNA concentration was measured by spectrometry, and dilutions were made to a final concentration of 10 ng/μL. Genotyping for the selected SNPs was performed by ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the TaqMan SNP Genotyping Assay. The PCR results were analyzed by Sequence Detection System software. The genotyping completion rate was > 99%. Finally, 5% of DNA samples were randomly selected and re-genotyped to assess the quality and potential misclassification of the genotyping. The concordance rate for the duplicate samples was 100%.

Statistical analysis

The difference in distribution of demographic characteristics between cases and controls were assessed by chi-square test or t test. The Hardy-Weinberg equilibrium (HWE) for the genotypic distribution of the five SNPs in control group was evaluated by Chi-square test. The association between the five SNPs and OS risk was evaluated by odd ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression analysis adjusted by age. All statistical analyses were conducted using SAS 9.3. Linkage disequilibrium between SNPs was assessed by HaploView software (Daly Lab at the Broad Institute, Cambridge, MA, USA) [11]. Associations between haplotypes and OS risk were evaluated by SNPStats, using the most common haplotype as the referent category [12]. Effects of the different genotypes on osteosarcoma survival were evaluated by hazard ratios (HR) and 95% CIs, using univariate and multivariate Cox regression analysis. *P*-values of less than 0.05 (two-sided probability) were considered as statistically significant. The analyses were performed using SAS 9.3.

Results

The distributions of selected characteristics of the study subjects are shown in **Table 1**. Cases and controls were evenly matched by age and gender. Specifically, the mean age of the cases and controls were 28.0 ± 18.0 and 27.9 ± 17.3 years old, respectively. 61.9% of the cases and

Table 2. ORs and 95% CIs for OS in relation to polymorphisms of *TP53* gene

SNP	Genotypes	Cases, n (%)	Controls, n (%)	OR (95 CI)†	P trend
rs12951053	AA	54 (25.7)	140 (33.3)	1.00	
	AC	103 (49.1)	198 (47.2)	1.35 (0.91-2.00)	
	CC	53 (25.2)	82 (19.5)	1.68 (1.05-2.68)	0.03
	AC + CC	156 (74.3)	280 (66.7)	1.45 (1.00-2.09)	
rs1042522	CC	59 (28.2)	162 (38.6)	1.00	
	CG	106 (50.7)	194 (46.2)	1.50 (1.03-2.20)	
	GG	44 (21.1)	64 (15.2)	1.89 (1.16-3.07)	0.007
	CG + GG	150 (71.8)	258 (61.4)	1.60 (1.11-2.29)	
rs8064946	GG	68 (32.5)	135 (32.1)	1.00	
	GC	100 (47.9)	189 (45.0)	1.05 (0.72-1.53)	
	CC	41 (19.6)	96 (22.9)	0.85 (0.53-1.36)	0.56
	GC + CC	141 (67.5)	285 (67.9)	0.98 (0.69-1.40)	
rs9895829	AA	173 (82.8)	350 (83.3)	1.00	
	AG	36 (17.2)	70 (16.7)	1.04 (0.67-1.62)	
	GG	-	-	-	-
rs12602273	CC	89 (42.6)	155 (36.9)	1.00	
	CG	93 (44.5)	199 (47.4)	0.81 (0.57-1.16)	
	GG	27 (12.9)	66 (15.7)	0.71 (0.42-1.20)	0.15
	CG + GG	120 (57.4)	265 (63.1)	0.79 (0.56-1.11)	

†Adjusted for age.

Table 3. Association of haplotypes in the TP53 gene with risk of OS

Haplotype*	Cases	Controls	OR (95 CI)†
ACGAC	35.9	34.0	1.00
CGCAG	31.3	32.8	0.94 (0.70-1.25)
AGGAC	4.0	8.0	0.37 (0.19-0.72)
AGGGC	5.0	6.7	0.71 (0.39-1.29)
CGCAC	5.4	5.7	0.83 (0.49-1.38)

*In the order rs12951053, rs1042522, rs8064946, rs9895829 and rs12602273. †Adjusted for age.

57.1% of the cases were male. In addition, 73.3% of tumors located on the long tubular bones and others occur in the axial skeleton (26.7%). Distant metastases were found in 27.1% of cases.

The distributions of genotype and allele frequencies of the 5 tagging SNPs of TP53 (rs12951053, rs1042522, rs8064946, rs9895829 and rs12602273) in cases and controls are presented in **Table 2**. Hardy-Weinberg equilibrium test showed that no SNPs showed significant deviation ($P > 0.05$). Two SNPs in TP53 gene are significantly associated with risk of OS. Specifically, CC genotype of rs12951053 was associated with significant increased risk of OS (OR = 1.68, 95% CI: 1.05-2.68) compared with the AA genotype; subjects with the GG genotype of rs1042522 also had significant increased cancer risk (OR = 1.89, 95% CI: 1.16-3.07) compared with those who carrying the CC genotype.

Haplotype analysis showed that rs12951053, rs1042522, rs8064946, rs9895829 and rs12602273 were in linkage disequilibrium ($D' = 0.65\text{-}0.88$, $r^2 = 0.02\text{-}0.57$). The associations between TP53 haplotypes and OS risk were presented in **Table 3**. Carriers of the AGGAC haplotype had significant reduced risk of OS (OR = 0.37, 95% CI: 0.19-0.72) relative to the ACGAC haplotype carriers.

Based on the data from follow-up interview to October 2014, a total of 99 patients died of OS, with median survival time of 21.3 months. We found that patients with rs1042522 GG genotype had shorter survival time [hazard radio (HR) = 1.94, 95% CI: 1.03-3.65] compared with patients carrying the CC genotype. This association was still exist in multivariate Cox regression analysis after adjusting for age, gender, tumor location, and metastasis (HR = 1.95, 95% CI: 1.02-3.71). Other mutations in TP53

Table 4. Association between genotypes of *TP53* genes and OS patients' survival

Genotypes	Genotypes	Cases, n	Deaths, n	MST	HR (95% CI)†
rs12951053	AA	54	22	22.4	1.00
	AC	103	52	19.7	1.44 (0.87-2.40)
	CC	53	25	21.8	1.24 (0.69-2.23)
rs1042522	CC	59	14	28.0	1.00
	CG	106	51	21.8	1.61 (0.88-2.94)
	GG	44	34	19.4	1.94 (1.03-3.65)
rs8064946	GG	68	23	22.5	1.00
	GC	100	54	19.8	1.24 (0.76-2.03)
	CC	41	22	22.1	1.07 (0.59-1.95)
rs9895829	AA	173	81	21.8	1.00
	AG	36	18	19.4	1.39 (0.82-2.35)
	GG	-	-	-	-
rs12602273	CC	89	34	23.4	1.00
	CG	93	49	19.3	1.40 (0.89-2.22)
	GG	27	16	21.6	1.06 (0.58-1.92)

MST: median survival time. †Adjusted for age.

gene were not associated with survival of OS (**Table 4**).

Discussion

The p53 protein is crucial for normal cell growth, apoptosis, and DNA repair, and the TP53 gene has been observed to undergo somatic mutation in tumors. However, it has not been fully studied with regard to TP53 polymorphisms with risk and survival of OS in Chinese population. In this study, we found those two SNPs (rs12951053 and rs1042522) and one haplotypes (AGGGC) in TP53 gene significantly associated with OS risk, and one genetic variant (rs1042522) was associated with OS survival.

TP53 gene located on the short (p) arm of chromosome 17 at position 13.1, and is one of the most frequently mutated genes in human. It was reported that somatic TP53 gene alterations are frequent in most human cancers and germline TP53 mutations predispose to a wide spectrum of early-onset cancers [13, 14]. The majority of TP53 mutations are missense substitutions (75%), and other alterations include frame shift insertions and deletions, nonsense mutations, silent mutations, and etc. [15]. Different kind of TP53 mutation may produce p53 isomorphs that have different functional and biological effects.

Rs1042522, located in exon 4 of the TP53 gene, is a missense mutation that results in the substitution of arginine (Arg) for proline (Pro) at codon 72. Molecular epidemiological studies have shown that mutant allele of rs1042522 was associated with increased risk for developing various cancers [9]. Reports on rs1042522 and bone cancer risk are rare and lack consistency. Savage et al [16] genotyped common SNPs in TP53 in a case-control study of sporadic OS in a mixed population, and found that rs1042522 may be associated with increased risk for OS (OR = 7.5, 95% CI: 1.20-46.3). Another study did in Caucasian population also found that Pro/Pro genotype had a 2.90-fold increased risk of OS compared with Arg/Arg genotype [17]. However, a research on Ewing Sarcoma [18], the second most common bone tumor in children, in Brazil population failed to find any relation with rs1042522. Nevertheless, this study included a relatively small population (24 cases and 91 controls) which may result in low statistical power. So far, there was no report on rs1042522 mutation and bone cancer prognosis. In our study, we found rs1042522 may play roles both in susceptibility and prognosis of OS. The mutant alleles at this locus (rs1042522) can encode a protein isomorph that differs from the wild-type p53 protein in its capacities to induce target gene transcription and apoptosis [19, 20], its ability to bind p73 (a homologue of the tumor-suppressor protein p53) [21], and its targeting of the proteasome for degradation [22]. Thus, our finding is biologically plausible.

Although rs12951053 is an intronic mutation of TP53 gene, it is also found in positive association with many kinds of cancers (e.g. lung cancer, ovarian cancer, basal cell carcinoma) [23-25]. Here, we found rs12951053 was related to risk of OS, we failed to find its association with OS prognosis. The functional significance of this change is still unclear, and biological studies are needed to clarify the possible mechanism.

To the best of our knowledge, this is the first investigation on the association of TP53 polymorphisms and OS risk and survival in Chinese population. However, limitations of our study should also be noted that potential selection bias might occurred because of the hospital-based study design, and the relatively small sample size may hampered our ability to detect some relationships more reliably and to produce subgroup analyses.

In conclusion, TP53 is a key gene in tumor formation and progression, and variants in this gene may influence the susceptibility and prognosis of OS in our population. Future research should be performed in a larger population to confirm our findings.

Disclosure of conflict of interest

None.

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