

Original Article

Effect of variation of FGF2 genotypes on the risk of osteosarcoma susceptibility: a case control study

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Abstract: Objective: Genetic factors play an important role in osteosarcoma (OS) etiology and fibroblast growth factor 2 (FGF2) gene single polymorphisms may be involved. The aim of this study was to test whether FGF2 variants are associated with susceptibility to OS in a Chinese population. Methods: A total of 151 subjects who were diagnosed as OS and 225 healthy age-matched controls were enrolled in the present study. The rs11737764 C/T SNP in FGF2 gene was genotyped in all the subjects. The SPSS software was used to investigate the association between the rs11737764 genotypes and OS susceptibility or severity. Results: The genotype frequencies of the FGF2 rs11737764 C/T polymorphism were 44.4% (CC), 50.3% (CT) and 5.3% (TT) in OS patients, and 55.6% (CC), 43.1% (CT) and 1.3% (TT) in controls. Rs11737764 C/T was found to be significantly associated with increased risk of OS no matter what genetic model was used. Conclusion: In conclusion, our data demonstrated the FGF2 SNP rs11737764 was significantly associated with increased osteosarcoma susceptibility in Chinese Han Population.

Keywords: FGF2, SNP, osteosarcoma

Introduction

Osteosarcoma (OS) is a life-threatening malignancy characterized by high local aggressiveness and a tendency to metastasize to the lungs and distant bones. Patients with localized OS at presentation have a 60-80% rate of long-term survival, while metastatic disease carries a poorer prognosis [1, 2]. Although many studies have been conducted on the etiology of OS, the exact mechanism remains poorly understood. Cumulative evidences suggest that OS is a complex disease resulting from the interaction between environmental factors and genetics [3-7]. Recently, more and more attention was paid to common genetic variants with osteosarcoma risk in biologically plausible pathways [8-12].

OS occurs mostly in teenagers, correlates with the puberty and bone growth, comprising 2.4% of all malignancies in pediatric patients [13-15]. This incidence peak in adolescence is followed by a rapid decline and a plateau when bone growth is complete (after age 24 years). Also, OS mostly occurs at sites of long bones,

such as the femur and the tibia, which always experience a rapid bone growth [16]. At the same time, several studies have suggested that being taller than average at diagnosis is associated with increased OS risk [17, 18], which may be an explanation of the sex difference of OS susceptibility. Given above, these data suggest that growth and development during puberty, take an important role in OS etiology. It is possible that variation in genes important in bone development and growth are modifiers of OS risk. Moreover, the variants in DNA repair genes may be associated with OS risk, especially during the rapid bone growth period.

The fibroblast growth factor 2 gene (FGF2) is a member of the fibroblast growth factor (FGF) family, which is implicated in diverse biological processes, such as limb and Musculoskeletal system development, wound healing, and tumor growth. FGF2 was focused on as the factors highly expressed in the tumor environment rather than in osteosarcoma cells in previous studies. Further investigation revealed that the hyperphosphorylation of extracellular signal-regulated kinase (Erk)1/2 in AX cells induced by

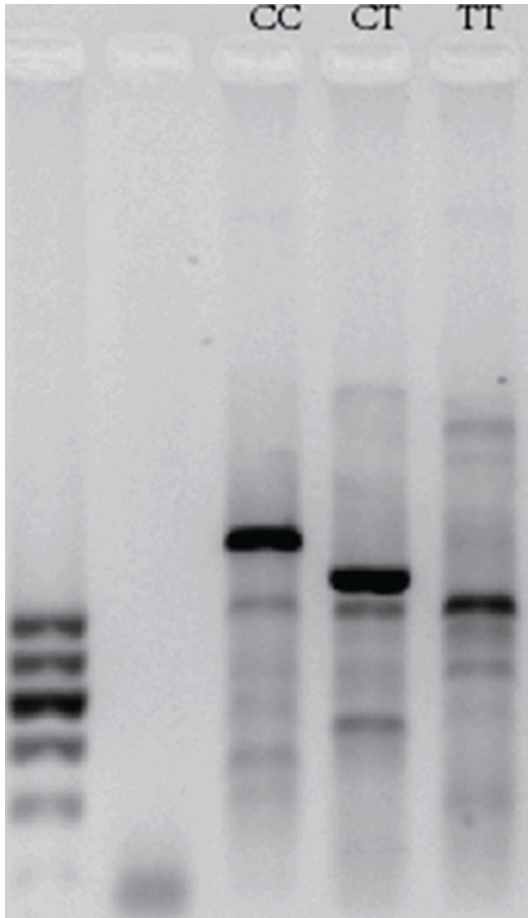


Figure 1. The gel pictures for genotype distribution assessment.

FGF2 potently suppressed osteogenic differentiation. Erk1/2 hyperactivation was detected at the immature areas of tumors, suggesting that it plays key roles in maintaining immaturity in osteosarcoma cells *in vivo*. In addition, FGF2 afforded a growth advantage and the enhancement of cellular motility, as well as resistance to Adriamycin, to AX cells, indicating the contribution of FGF2 to tumor progression and refractoriness to treatment. Given above, FGF2 may take an important role in the etiology and pathology of osteosarcoma.

Accordingly, the aim of this study is to explore the association of FGF2 gene polymorphism (rs11737764) and the susceptibility of OS. We performed genotyping analyses with a case-control study in a Chinese Han population.

Method

The study was approved by the ethics committee of the Yantai Yuhuangding Hospital, and

informed consent was obtained from all patients and control participants. A total of 151 patients diagnosed with OS and 225 age and sex matched healthy controls were enrolled in this study. All subjects included in this study were Chinese Han Population. Patients or the controls with familial cancer syndromes were excluded.

Genotyping

DNA samples were obtained from all the participants from peripheral blood with the Chelex-100 method [19]. The SNP was then genotyped using Taqman assay (Applied Biosystems 7500, ABI, Foster City, CA) and dual-labeled probes in real-time PCR (**Figure 1**). The primer and probe were designed and synthesized by Sigma (Sigma-Proligo, The Woodlands, TX). Genotyping was performed by independent laboratory personnel who were blinded to the study, and three authors independently reviewed the genotyping results, data entry, and statistical analyses. In addition, we randomly selected 5% samples of case and control subjects for reproducibility tests at least twice in different days and yielded a 100% concordant.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL, USA), version 16.0 for Windows. The demographic and clinical data were presented as Mean \pm SD and compared between groups by the Student's t-tests. The genotype and allelic frequencies were evaluated by Hardy-Weinberg equilibrium and compared by the Chi-square test. The association between the FGF2 gene SNP rs-11737764 and OS susceptibility was assessed under the following genetic models, which were treated as a dichotomous variable: (i) T-allele versus C-allele for allele level comparison; (ii) CT+TT versus CC for a dominant model of the T allele; (iii) TT versus CT+CC for a recessive model of the T-allele; and (iv) TT versus CC for the extreme genotype. The $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics

Demographic data of the population studied and the number of individuals in each group

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Table 1. The summary of the basic characteristics of the groups

Clinical Characteristics	AS Patients n=151	Controls n=225	P-Value
Age (years)	16.7 ± 8.5	17.3 ± 7.9	n.s
Female/Male	54/97	77/148	n.s
Smoking Status (n)	12	21	n.s
Tumor Location			
Long Tubular bones	117		
Axial skeleton	34		

Table 2. The genotype and allele distributions of the FGF2 SNP rs11737764 for the cases and controls

Genotype	Group		OR	p value
	control	case		
CC	125	67	1	/
CT	97	76	1.46 (0.96 to 2.23)	<0.001
TT	3	8	4.98 (1.28 to 19.38)	0.0203
CT+TT	100	84	1.57 (1.04 to 2.37)	0.0358
CT+CC	222	143	1	/
TT	3	8	4.14 (1.08 to 15.9)	0.0313
Allele				
C	77.1	69.5	1	/
T	22.9	30.5	1.48 (1.21 to 1.80)	0.0001

were shown in **Table 1**. There were no significant differences between groups in terms of age and gender.

Association of FGF2 SNP rs11737764 with OS

As expected, the distribution of the genotype of SNP rs11737764 conformed to the Hardy-Weinberg equilibrium and the genotyping success rate was 100%. **Table 2** listed the genotyped and allele distributions of the SNP rs11737764 for the cases and controls. The genotype frequencies of the FGF2 rs11737764 C/T polymorphism were 44.4% (CC), 50.3% (CT) and 5.3% (TT) in OS patients, and 55.6% (CC), 43.1% (CT) and 1.3% (TT) in controls. For allele level comparison, the FGF2 rs11737764 T allele was associated with an increased risk of OS in terms of the frequency of allele comparison (T vs. C: OR = 1.48; 95% CI = 1.21 to 1.80), P = 0.0001). For a dominant model of the T allele, the CT + TT genotypes were associated with the risk for OS (CT + TT vs. CC, OR = 1.57, 95% CI = 1.04 - 2.37, P = 0.0358). For a recessive model of the T allele, the TT homozygote

genotype was associated with susceptibility to OS (TT vs. CT + CC. OR = 4.14, 95% CI = 1.08-15.9, P = 0.0313). For the extreme genotype, the TT genotypes were associated with the risk for OS (TT vs. CC, OR = 4.98, 95% CI = 1.28-19.38, P = 0.0203).

Discussion

OS involves interactions among genetic and environmental factors, among which the genetic background is important determinants of OS. The most important finding of this study was that the FGF2 SNP rs-11737764 variant was significantly associated with increased osteosarcoma susceptibility in Chinese Han Population.

Osteosarcoma is the most common primary malignant bone tumor in children and adolescents. In young adults it usually occurs where there is rapid bone growth, such as in the distal femur, proximal tibia, and proximal humerus. Osteosarcoma is a primary malignant tumor of the skeleton characterized by the direct formation of immature bone or osteoid tissue by the tumor cells. Most osteosarcoma tumors are of high grade and tend to produce pulmonary metastases. Despite clinical improvements, patients with metastasis or recurrent diseases still have a poor prognosis. The biology of the more common sporadic osteosarcoma pathogenesis is complex and remains unknown. Epidemiologic studies suggest that growth and development play an important role in the etiology as it occurs primarily in adolescents during puberty when bone growth is rapid [15, 20]. The rapidly growing bone is highly susceptible to carcinogenesis, possibly due to rapidly proliferating osteogenic cells being more vulnerable to DNA repair errors [21].

FGF2 mediates various cellular events and promotes tumor progression [22, 23]. Osteosarcoma is a highly vascular tumor characterized by a malignant and metastatic potential [24]. Angiogenesis is a critical step in tumor growth and metastasis [25]. Previous studies suggested FGF2 introduced tumor progression by angiogenesis [26, 27]. FGF2 can stimulate proliferation and migration of endothelial cells, alter their patterns of gene expression, increase microvascular permeability, cause extravasation of plasma proteins into the extravascular

space, and also induce plasma-derived matrix. It is also suggested to involve in differentiation and/or cell death in some tumors such as Ewing tumor and osteosarcoma [28, 29]. As mentioned above, OS appears mostly during rapidly bone growing, variants of FGF2 may influence the bone growth and finally OS formation. The roles of FGF2 on osteogenic differentiation is complicated and has not been fully clarified, but it seems to have a potential of promoting proliferation and osteogenesis [30-33]. However, sustained exposure to a high concentration of FGF2 inhibited osteogenesis by suppressing the production of bone materials [34, 35].

The most important limitation of the present study is the relatively small sample size. A single center case-control study is not sufficient to fully interpret the relationship between FGF2 polymorphism and susceptibility to osteosarcoma. Further study with multiple population and larger sample size is needed.

Conclusion

In conclusion, our data demonstrated the FGF2 SNP rs11737764 was significantly associated with increased osteosarcoma susceptibility in Chinese Han Population.

Disclosure of conflict of interest

None.

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