Int J Clin Exp Pathol 2015;8(9):11531-11536
www.ijcep.com /ISSN:1936-2625/IJCEP0013741

Original Article

Genetic polymorphisms in COL18A1 influence the development of osteosarcoma

Zhihao Guo, Tianji Zhang, Juntao Wu, Hongwei Wang, Xiaotan Liu, Linqiang Tian

Department of Orthopedics, The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, China

Received July 31, 2015; Accepted August 28, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: We conducted a case-control study to investigate the association of COL18A1 D104N polymorphism in the development of osteosarcoma in a Chinese population. Between May 2012 and May 2014, 141 patients with pathologically proven osteosarcoma and 341 were selected into this study. Genotyping of COL18A1 D104N was analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). By logistic regression analysis, we found that individuals with the NN genotype of COL18A1 D104N were significantly associated with an increased risk of osteosarcoma when compared with the DD genotype (OR=20.97, 95% CI=2.74-933.42). In dominant model, the NN+DN genotype of COL18A1 D104N had a 1.99 fold risk of osteosarcoma when compared with the DD genotype. Moreover, the NN genotype was correlated with a 20.45 fold risk of osteosarcoma when compared with the DN+DD genotype in recessive model. However, we did not find significant interaction between COL18A1 D104N polymorphism and Enneking stage, histological subtype, tumor metastasis and tumor location of patients with osteosarcoma. In conclusion, our study suggests that the homozygous DN and NN genotypes of COL18A1 D104N were associated with the risk of osteosarcoma.

Keywords: COL18A1, polymorphism, osteosarcoma

Introduction

Osteosarcoma is a highly malignant and aggressive bone tumor, which is derived from mesenchymal tissues and often occurs in the distal femur, proximal tibia and humeral metaphysis. Osteosarcoma is the most leading bone malignancy in adolescents [1]. It is estimated that the annual incidence of osteosarcoma is about $3/10^5$ worldwide [1]. The osteosarcoma is caused by complex, multistep and multifactorial processes, and many environmental and genetic factors are involved in its carcinogenesis [2, 3]. Previous experimental studies have conducted to investigate the cancer stem cells and their potential to cause tumors [4, 5]. The concept of genetic factors being involved in the susceptibility to osteosarcoma has gained many studies to investigate the genetic determinants for osteosarcoma in the last decade.

Angiogenesis, the formation of new blood vessels from preexisting endothelium, is a discrete event in carcinogenesis that is related to the aggressive potential of a tumor [6, 7]. Accumulating evidences suggest that the growth of tumors is associated with increased angiogenesis and that the formation of new blood vessels is a fundamental step in tumor development and expansion [8]. Previous study has reported that osteosarcoma is an angiogenesis dependent cancer [9]. A single nucleotide polymorphism, c.4309G>A (D104N), has been identified in the Endostatin domain of COL18A1; this polymorphism affects a site that is conserved in humans and mice [10, 11]. Previous studies have reported the COL18A1 D104N polymorphism could influence the development of several kinds of cancers, such as adrenocortical tumors and breast cancer [8, 11]. Until now, no study has been conducted to investigate the role of COL18A1 D104N polymorphism in the development of osteosarcoma. In our study, we conducted a case-control study to investigate the association of COL18A1 D104N polymorphism in the development of osteosarcoma in a Chinese population.

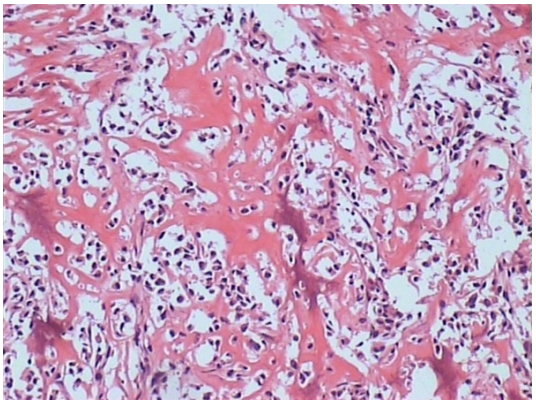

Figure 1. Chondroblastic osteosarcoma.

Materials and methods

Subjects

Between May 2012 and May 2014, 166 patients with pathologically proven osteosarcoma were selected from the Third Affiliated Hospital of Xinxiang Medical University. Osteosarcoma patients were newly diagnosed and histopathologically confirmed independently by two pathologists (**Figure 1**). Patients who had primary tumors other than osteosarcoma, tumors of an unknown origin or any histopathologicl diagnosis other than osteosarcoma were excluded. Finally, 141 patients with osteosarcoma were included into our study, and the participation rate was 89.24%.

A total of 352 adult healthy subjects without osteosarcoma were randomly collected from individuals who received regular health check-up in the Third Affiliated Hospital of Xinxiang Medical University during May 2012 and May 2014. The control subjects were confirmed to be without any primary tumors. Finally, 341 patients agreed to participate in this study, and the participation rate was 96.88%.

Patients with osteosarcoma and control subjects were investigated by doctors to obtain demographic parameters, including gender, age and family history of cancer. The clinical and pathological information of patients with osteosarcoma were collected from the medical records, including Enneking stage, histological subtype, tumor location and tumor metastasis. All individuals voluntarily participated in the study and gave their informed consent. The protocol of this study was approved by the ethics committee of the Third Affiliated Hospital of Xinxiang Medical University.

Genotyping

5 ml peripheral blood sample was drawn from each patient with osteosarcoma and control subject, and the peripheral blood sample was kept in -20°C until using. Genomic DNA was isolated from the blood samples using a Qiagen genomic DNA isolation kit according to manufacturer's instructions. Genotyping of COL18A1 D104N was analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primers and probes for COL18A1 D104N were designed using Primer premier v5.0 software (Applied Biosystems). The primers for COL18A1 D104N were 5'-ACTTTCACCCACAGGGATC-3' (forwards) and 5'-TTTCTCCTATCTGCAGGGC-3' (reverse), respectively. The PCR amplification cycle was one cycle of DNA denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, 55°C annealing step for 1 min with a 72°C extension step for 2 min, with a final extension step of 5 min at 72°C. The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme. Digestion products were separated by electrophoresis on ethidium bromide stained agarose gel and visualized under UV light.

Statically analysis

Frequencies were used to describe the distribution of categorical variables and median and interquartile range was used for continuous variables. The statistical significance of the differences between groups was calculated using a Chi-squared test or Fisher's exact test. Standard chi-square test was used to assess deviation from Hardy-Weinberg equilibrium (HWE). The odds ratios (ORs) and 95% confidence intervals (CIs) were evaluated for the association between COL18A1 D104N gene polymorphism and risk of osteosarcoma using logistic regression models adjusted for confounding factors. Multiple logistic regression models were established to estimate the interaction between COL18A1 D104N gene polymorphism and demographic characteristics in the risk of osteosarcoma. All tests were two-sided with a significant level of

Table 1. Clinicopathological characteristics of the patients with osteosarcoma and controls

Characteristics	Patients	%	Controls	%	χ^2 test	*P* value
Gender						
Female	51	36.17	133	39.00		
Male	90	63.83	208	61.00	0.34	0.56
Age, years						
<20	94	66.67	217	63.64		
≥20	47	33.33	124	36.36	0.40	0.53
Family history of cancer						
No	136	96.45	341	100.00		
Yes	5	3.55	0	0.00	21.22	0.002
Enneking stage						
I-II	78	55.32				
III	63	44.68				
Histological subtype						
Osteoblastic	86	60.99				
Chondroblastic	21	14.89				
Fibroblastic	19	13.48				
Other	15	10.64				
Tumor metastasis						
Negative	96	68.09				
Positive	45	31.91				
Tumor location						
Extremities	87	61.70				
Other	54	38.30				

P-value <0.05. Statistical analysis was conducted using the SPSS 16.0 package (SPSS Inc., Chicago, IL, USA).

Results

The distributions of the clinicopathological characteristics of the patients with osteosarcoma and controls were shown in **Table 1**. The mean ages of the included patients and controls at the time of enrolling into our study were 17.3±6.1 and 16.7±6.4 years old. By χ^2 test, patients with osteosarcoma were likely to have family history of cancer when compared with controls. Of the 141 patients with osteosarcoma, 78 (55.32%) patients were at I-II Enneking stage, 63 (44.68%) were at III stage, 86 (60.99%) were osteoblastic osteosarcoma, 21 (14.89%) were chondroblastic osteosarcoma, 19 (13.48%) were fibroblastic osteosarcoma, 45 (31.91%) had tumor metastasis and 87 (61.70%) showed extremities of osteosarcoma.

The genotype distributions of COL18A1 D104N were found to in line with Hardy-Weinberg equilibrium in the control group (**Table 2**). By χ^2-test, we found that genotype frequencies of the DD, DN and NN were significant difference between patients with osteosarcoma and controls (χ^2=16.37, *P*<0.001). By logistic regression analysis, we found that the NN genotype of COL18A1 D104N was significantly associated with an increased risk of osteosarcoma when compared with the DD genotype (OR=20.97, 95% CI=2.74-933.42). In dominant model, the NN+DN genotype of COL18A1 D104N had a 1.99 fold risk of osteosarcoma when compared with the DD genotype. Moreover, the NN genotype was correlated with a higher risk of osteosarcoma than the DN+DD genotype in recessive model (OR=20.45, 95% CI=2.68-909.89).

We further analyzed the association between COL18A1 D104N polymorphism and clinicopathological characteristics of osteosarcoma (**Table 3**). By χ^2-test, we did not find significant interaction between COL18A1 D104N polymorphism and Enneking stage, histological subtype, tumor metastasis and tumor location of patients with osteosarcoma (*P*<0.05).

Discussion

Osteosarcoma is generally believed to be a gene-environment interaction disease with high morbidity and mortality. However, the mechanism of osteosarcoma was unclear. A number studies has showed that gene polymorphism is closely correlated with the risk of osteosarcoma recently. In our study, we investigated whether the COL18A1 D104N polymorphism alters the susceptibility of osteosarcoma, and found that individuals with the homozygous DN and NN genotypes were associated with risk of osteosarcoma. This is the first report to analyze the association between the COL18A1 D104N polymorphism and development of osteosarcoma.

Table 2. Association between COL18A1 D104N polymorphism and the risk of osteosarcoma

Polymorphisms	Patients	%	Controls	%	HWE	χ^2-test	*P* value	Adjusted OR (95% CI)[1]	*P* value
COL18A1 D104N									
Codominant									
DD	119	84.40	312	91.50	0.66	16.37	<0.001	1.0 (Ref.)	-
DN	14	9.93	28	8.21				1.31 (0.62-2.68)	0.43
NN	8	5.67	1	0.29				20.97 (2.74-933.42)	<0.001
Dominant									
DD	119	84.40	312	91.50		5.31	0.02	1.0 (Ref.)	-
NN+DN	22	15.60	29	8.50				1.99 (1.04-3.74)	0.02
Recessive									
DN+DD	133	94.33	340	99.71		15.76	<0.001	1.0 (Ref.)	-
NN	8	5.67	1	0.29				20.45 (2.68-909.89)	<0.001

[1]Adjusted for sex, age and family history of cancer.

Table 3. Association between COL18A1 D104N polymorphism and clinicopathological characteristics of osteosarcoma

Patients	DD	%	DN+NN	%	χ^2-test	*P* value
Enneking stage						
I-II	64	53.78	14	63.64		
III	55	46.22	8	36.36	0.73	0.69
Histological subtype						
Osteoblastic	73	61.34	13	59.09		
Chondroblastic	17	14.29	4	18.18		
Fibroblastic	16	13.45	3	13.64		
Other	13	10.92	2	9.09	0.26	0.97
Tumor metastasis						
Negative	80	67.23	16	72.73		
Positive	39	32.77	6	27.27	0.26	0.61
Tumor location						
Extremities	72	60.50	15	68.18		
Other	47	39.50	7	31.82	0.46	0.5

The COL18A1 D104N polymorphism of Endostatin, a 183-amino-acid proteolytic fragment produced by the cleavage of the C-terminal non-collagenous domain (NC1) of human type XVIII collagen, is an efficient anti-angiogenic molecule [12]. As an angiogenic inhibitor, Endostatin could prevent tumor growth and expansion by controlling the formation of new blood vessels. *In vitro* and *in vivo* studies, they have shown that treatment with Endostatin can increase apoptotic activity and decrease microvessel density and metastasis in many tumors [13-15].

Previous studies have reported the role of the COL18A1 D104N polymorphism in the susceptibility of many benign and malignant tumors, such as hepatocellular carcinoma, prostate cancer, breast cancer, multiple myeloma and lung cancer as well as adrenocortical tumors [8, 11, 16-20]. Wu et al. conducted three independent case-control studies in a Chinese population, and they reported that COL18A1 rs7499 could contribute to the development of hepatocellular carcinoma [16]. Iughetti et al. have reported that the COL18A1 D104N polymorphism is associated with risk of prostate cancer [18]. Another three studies have reported that the COL18A1 D104N polymorphism contributes to the development of, prostate cancer, myeloma and lung cancer [17, 19, 20]. However, some studies reported inconsistent results. One study in Brazilian has reported that the COL18A1 D104N polymorphism has no associated with risk of adrenocortical tumors. Another study in a British population has reported that COL18A1 D104N polymorphism is not associated with susceptibility of breast cancer [11]. In our study, we have found that the COL18A1 D104N polymorphism is significantly associated with an increased risk of osteosarcoma, which is controversial with previous studies. These different results may be due to the heterogeneity of the different diseases, ethnicities, study design, source of patients and sample size.

In our study, we did not find that the COL18A1 D104N polymorphism has association with clinicopathological characteristics of osteosarcoma, including Enneking stage, histological

subtype, tumor metastasis and tumor location of patients with osteosarcoma. One previous study have also reported that the COL18A1 D104N polymorphism is no associated with tumour grade, nodal status, vascular invasion or overall survival of breast cancer [11]. Further studies are greatly needed to confirm our results.

There are three limitations in our study. First, patients with osteosarcoma and controls were selected from one hospital, and selection bias may exist and influence the results of our study. Second, we did not observe a significant association between the COL18A1 D104N polymorphism and clinicopathological characteristics of osteosarcoma. This result may be due to the small number of the NN genotype may influence their association. Third, the sample size of this study is relatively small, and small sample size may limit the statistical power to find differences between groups.

In conclusion, our study suggests that the homozygous DN and NN genotypes of COL18A1 D104N were associated with the risk of osteosarcoma. Therefore, our study has indicated that the COL18A1 D104N polymorphism may contribute to the development of osteosarcoma. Further large sample studies are greatly warranted to elucidate our finding.

Acknowledgements

This work was supported by the Scientific Research Fund of Xinxiang Medical University (2014QN104).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Linqiang Tian, Department of Orthopedics, The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, China. Tel: +86-373-3029595; Fax: +86-373-3029595; E-mail: xuwhued@163.com

References

[1] Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: Data from the Surveillance, Epidemiology, and End Results Program. Cancer 2009; 115: 1531-43.

[2] Powers M, Zhang W, Lopez-Terrada D, Czerniak BA, Lazar AJ. The molecular pathology of sarcomas. Cancer Biomark 2010; 9: 475-91.

[3] Bovée JV, Hogendoorn PC. Molecular pathology of sarcomas: concepts and clinical implications. Virchows Arch 2010; 456: 193-9.

[4] Osuna D, de Alava E. Molecular pathology of sarcomas. Rev Recent Clin Trials 2009;4: 12-26.

[5] Berger M, Muraro M, Fagioli F, Ferrari S. Osteosarcoma derived from donor stem cells carrying the Norrie's disease gene. N Engl J Med 2008; 359: 2502-4.

[6] Nakamura Y, Yasuoka H, Tsujimoto M, Imabun S, Nakahara M, Nakao K, Nakamura M, Mori I, Kakudo K. Lymph vessel density correlates with nodal status, VEGF-C expression, and prognosis in breast cancer. Breast Cancer Res Treat 2005; 91: 125-32.

[7] Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996; 86: 353-64.

[8] Mariani BM, Trarbach EB, Ribeiro TC, Pereira MA, Mendonca BB, Fragoso MC. Genotype analysis of the human endostatin variant p.D104N in benign and malignant adrenocortical tumors. Clinics (Sao Paulo) 2012; 67: 95-8.

[9] Kim HS, Lim SJ, Park YK. Anti-angiogenic factor endostatin in osteosarcoma. APMIS 2009; 117: 716-23.

[10] Visakorpi T. Molecular genetics of prostate cancer. Ann Chir Gynaecol 1999; 88: 11-6.

[11] Balasubramanian SP, Cross SS, Globe J, Cox A, Brown NJ, Reed MW. Endostatin gene variation and protein levels in breast cancer susceptibility and severity. BMC Cancer 2007; 7: 107.

[12] O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. Endostatin: An endogenous inhibitor of angiogenesis and tumor growth. Cell 1997; 88: 277-85.

[13] Xu M, Xu CX, Bi WZ, Song ZG, Jia JP, Chai W, Zhang LH, Wang Y. Effects of endostar combined multidrug chemotherapy in osteosarcoma. Bone 2013; 57: 111-5.

[14] Fu XH, Li J, Zou Y, Hong YR, Fu ZX, Huang JJ, Zhang SZ, Zheng S. Endostar enhances the antineoplastic effects of combretastatin A4 phosphate in an osteosarcoma xenograft. Cancer Lett 2011; 312: 109-16.

[15] Jiang WG, Lu XA, Shang BY, Fu Y, Zhang SH, Zhou D, Li L, Li Y, Luo Y, Zhen YS. Genetically engineered endostatin-lidamycin fusion proteins effectively inhibit tumor growth and metastasis. BMC Cancer 2013; 13: 479.

[16] Wu X, Wu J, Xin Z, Wang H, Zhu X, Pan L, Li Z, Li H, Liu Y. A 3' UTR SNP in COL18A1 is associated with susceptibility to HBV related hepatocellular carcinoma in Chinese: three independent case-control studies. PLoS One 2012; 7: e33855.

[17] Lourenço GJ, Cardoso-Filho C, Gonçales NS, Shinzato JY, Zeferino LC, Nascimento H, Costa

FF, Gurgel MS, Lima CS. A high risk of occurrence of sporadic breast cancer in individuals with the 104NN polymorphism of the COL18A1 gene. Breast Cancer Res Treat 2006; 100: 335-8.

[18] Iughetti P, Suzuki O, Godoi PH, Alves VA, Sertié AL, Zorick T, Soares F, Camargo A, Moreira ES, di Loreto C, Moreira-Filho CA, Simpson A, Oliva G, Passos-Bueno MR. A polymorphism in endostatin, an angiogenesis inhibitor, predisposes for the development of prostatic adenocarcinoma. Cancer Res 2001; 61: 7375-8.

[19] Ortega MM, Nascimento H, Costa FF, Teori MT, Lima CS. A polymorphism in the angiogenesis inhibitor, endostatin, in multiple myeloma. Leuk Res 2003; 27: 93-4.

[20] Zambon L, Honma HN, Lourenco GJ, Saad IA, Mussi RK, Lima CS. A polymorphism in the angiogenesis inhibitor, endostatin, in lung cancer susceptibility. Lung Cancer 2008; 59: 276-8.