RESEARCH ARTICLE

Predictive Impact of Common Variations in DNA Repair Genes on Clinical Outcome of Osteosarcoma

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Abstract

We aimed to assess the role of XPG, XPC and MMS19L polymorphisms on response to chemotherapy in osteosarcomas, and the clinical outcomes. One hundred and eighty five osteosarcoma patients who were histologically confirmed were enrolled in our study between January 2007 and December 2009. Genotyping of XPG, XPC and MMS19L was performed in a 384-well plate format on the MassARRAY® platform. Individuals with XPG TT genotype and T allele were more likely to be better response to chemotherapy than CC genotype, with the OR (95% CI) of 4.17 (1.64-11.54) and 2.66 (1.39-5.11), respectively. Those carrying MMS19L TT genotype and T allele showed better response to chemotherapy, with ORs (95% CI) of 4.8 (1.56-17.7) and 2.3 (1.22-4.36), respectively. Patients carrying TT genotype of XPG and MMS19L showed a significantly longer overall survival than CC genotype, with a 0.47 and 0.30-fold risk of death when compared with the wild-type of the gene. XPG and MMS19L are correlated with response to chemotherapy and prognosis of osteosarcoma, so that they could be used as predictive markers for prognosis.

Keywords: XPG - MMS19L - osteosarcoma - clinical outcome

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Introduction

Osteosarcoma is the most frequent primary malignant bone tumors, mainly occurring in children and adolescents and accounting for 20% of all primary sarcomas in bone (Marina et al., 2004). Although the advanced treatment for osteosarcoma consists of aggressive adjuvant chemotherapy and wide tumor excision, the survival of osteosarcoma is still poor (Longhi et al., 2006). Such a poor general outcome is related to both advanced stage at diagnosis and relatively low treatment response rate due to a limited radiosensitivity and chemosensitivity of the tumor (Longhi et al., 2006). Individuals may present differences in response and toxicity of each anticancer drug. Therefore, the genetic factors may participate in the process of influencing the drug absorption, metabolism and excretion as well as distribution, which may influence the individual susceptibility to anticancer therapy.

Efficiency of DNA damage repair systems is considered to be one of the most important mechanisms affecting interindividuals' differences in clinical outcome of patients treated with chemotherapy. Previous studies have indicated that the SNPs of NER genes are related with the response to chemotherapy in osteosarcoma (Caronia et al., 2009; Biason et al., 2012; Dogan et al., 2012). However, the response to chemotherapy of osteosarcoma by XPG, XPC, and MMS19L has not been studies. Therefore, in our study, we aimed to assess the role of XPG, XPC, and MMS19L polymorphisms on the response to chemotherapy in osteosarcoma, and the clinical outcome of osteosarcoma.

Materials and Methods

Subjects

One hundred and eighty five osteosarcoma patients were enrolled between January 2007 and December 2009 in our hospitals. All hospital patients with newly diagnosed osteosarcoma were asked to participate within one month after diagnosis, and all cases were histopathologically confirmed. All patients were followed up every month by telephone or clinic visiting until death or the end of follow-up, and written informed consents were obtained from all patients.

Patients received six chemotherapy cycles, two before operation and four after operation. Before operation, patients received doxorubicin at75 mg/m^2, 120 mg/m^2 cisplatin, and 14 g/m^2 methotrexate. After operation, patients received doxorubicin 75 mg/m^2 and 12 g/m^2 methotrexate. If patients showed non-hematology toxicity which was higher than grade three, the dosage of chemotherapy drug would be reduced by 25%.

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Chemotherapy response

The response to chemotherapy was classified into good and poor response to chemotherapy. Complete response or partial response was considered as good response, and stable disease or progressive disease was considered as poor response. Overall survival (OS) was used to assess the clinical outcome of osteosarcoma, and the OS was assessed from the date of entry to the date of death or last clinical follow-up. All patients were followed up for three years.

DNA extraction and quantification

Genomic DNA of the six genes polymorphisms was extracted from venous blood by using a Qiagen Blood Kit (Qiagen, Chastworth, CA). Genotyping of XPG, XPC and MMS19L was performed by Sequenom MassARRAY platform with a 384-well plate format (Sequenom, San Diego, USA).. PCR and single base extension (SBE) primers were designed using Sequenom® Assay Design 3.1 software (Sequenom®), according to the manufacturer's instructions.

Statistical analysis

The difference between the categorical variables was analyzed by Pearson χ^2 test, and the continuous variables were assessed by student's t-test. The patients were divided into responders who presented complete response (CR) or partial response (PR) and non-responders who showed stable disease (SD) or progressive disease. Odds ratios (OR) and 95% confidence interval (CI) were used for assessing the ssociation between response to chemotherapy and XPG, XPC and MMS19L genotypes. Homozygotes of XPG, XPC and MMS19L were regarded as the reference group. Association between genotypes of XPG, XPC and MMS19L and survival of osteosarcoma was assessed by Cox Hazard regression model with hazard ratios (HR) and their confidence intervals (CI). Survival distributions were estimated by using the Kaplan-Meier method. All analyses were conducted by the Statistical Package for the Social Sciences (SPSS) software 16.0 for windows. Statistical significance of all the tests was defined as two-sided with $p<0.05$.

Table 1. Clinical and Pathological Characteristics of Patients

	Patients	
	No	%
Median age, yr (range)		16.8(6-39)
Sex		
Female	77	41.7
Male	108	58.3
Location		
Femur	101	54.5
Tibia/flbula	67	36.3
Arm	11	5.9
Central	6	3.3
Metastasis		
No	108	58.3
At diagnosis	33	17.9
At follow-up	44	23.8
Histological response		
Good	106	57.3
Poor	79	42.7
Death during follow-up		
Alive	106	57.3
Dead	79	42.7

Results

Patients' characteristics were presented in Table 1. The median age at diagnosis was 16.8(range 6 to 37 years) The mean age of included patients was 16.8±8.6 years, and 97 were males. Among the subjects, 53.2% of the tumors were located in femur and 37.3% in the tibia. At the time of diagnosis, 33 patients showed metastasis, and 44 presented metastasis during the follow-up period., 39 patients died during the follow-up period.

During the treatment, 106 patients showed good response to chemotherapy, while 79 patients showed poor response. Our study found that polymorphisms of XPG and MMS19L influence the efficacy of chemotherapy (Table 2). Individuals with XPG TT genotype and T allele were more likely to be better response to chemotherapy than CC genotype, with the OR (95% CI) of 4.17 (1.64-11.54) and 2.66 (1.39-5.11), respectively. Similarly, individuals carrying MMS19L TT genotype and T allele showed better response to chemotherapy, with ORs (95% CI) of 4.8 (1.56-17.7) and 2.3 (1.22-4.36), respectively.

We found the individuals carrying XPG TT and MMS19L TT genotypes could increase the survival of patients. For XPG polymorphism, patients carrying TT genotype showed a significantly longer overall survival than CC genotype, they had 0.47-fold risk of death when compared with wide-type of this gene (HR=0.47, 95% CI=0.17-0.94). For MMS19L polymorphism, we found a

Table 2. Association Between XPG, XPC and MMS19L Polymorphisms and Prognosis of Osteosarcoma

Genotype		Cases	%	N=106	%	Odds ratio(95% CI)	*P* value	N=79	%	Hazard ratio(95% CI)	*P* value
XPG rs2296147	CC	99	53.6	46	43.8	-	-	43	54.2	-	
	CT	49	26.5	31	29.1	1.98(0.93-4.28)	0.14	25	31.3	1.36(0.64-2.85)	0.18
	TT	37	19.9	29	27.1	4.17(1.64-11.54)	<0.05	11	14.5	0.47(0.17-0.94)	<0.05
	CT/TT	86	46.4	60	56.2	2.66(1.39-5.11)	<0.05	36	45.8	0.84(0.58-1.55)	0.33
XPC rs2228001	AA	128	69.1	70	66.2	-	-	57	72.5	-	
	AC	29	15.7	18	16.9	1.36(0.55-3.45)	0.59	12	15.3	0.88(0.35-2.14)	0.44
	CC	28	15.2	18	16.9	1.49(0.60-3.91)	0.31	10	12.2	0.69(0.26-1.73)	0.71
	AC/CC	57	30.9	36	33.8	1.42(0.72-2.86)	0.38	22	27.5	0.78(0.39-1.55)	0.45
MMS19L rs29001322	CC	86	46.5	40	37.5	-	-	43	54.3	-	
	CT	73	39.6	45	42.5	1.85(0.94-3.67)	0.08	30	38.2	0.70(0.35-1.37)	0.26
	TT	26	13.9	21	20	4.8(1.56-17.7)	<0.05	6	7.5	0.30(0.09-0.88)	<0.05
	CT/TT	99	53.5	66	62.5	2.3(1.22-4.36)	<0.05	36	45.7	0.57(0.30-1.07)	0.06

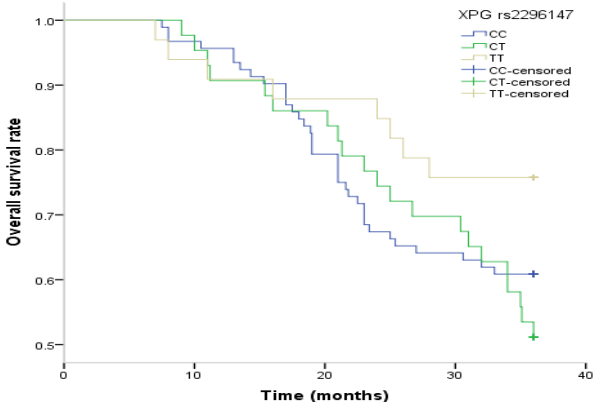

Figure 1. Overall Survival of XPG rs2296147 Polymorphisms

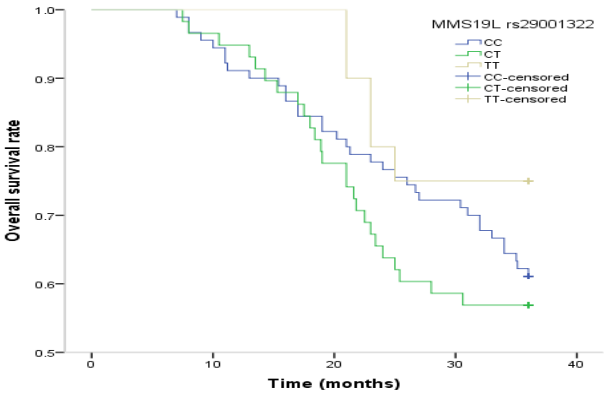

Figure 2. Overall Survival of MMS19L rs29001322 Polymorphisms

significantly decreased risk of death from osteosarcoma among patients carrying TT genotype also showed reduced risk of death from osteosarcoma when compared with CC genotype, and the HR (95% CI) was 0.30 (0.09-0.88) (Figure 2).

Discussion

This study assessed the association between XPG, XPC and MMS19L polymorphisms and prognosis of patients' osteosarcoma receiving chemotherapy, and is the most comprehensive pahrmacogenetic study in osteosarcoma patients to date. Our study identify molecular prognostic and predictive markers of osteosarcoma patients, and provide important information for customized chemotherapy to improve treatment efficacy. Previous clinical studies have indicated the XPD, XPG, XPA, CXCR4, survivin and MMP9 were associated with the clinical outcome of osteosarcoma in western countries (Giachino et al., 2007; Graf et al., 2011; Biason et al., 2012). Cisplatin is one of the effective chemotherapy for osteosarcoma, and polymorphisms of genes involved in the NER pathway plays an important role on changing the efficacy of chemotherapy (Rabik et al., 2007). Our study found that polymorphisms in XPG and MMS19L could influence the response to chemotherapy and clinical outcome of osteosarcoma with among patients with osteosarcoma, and XPG TT genotype and MMS19L TT genotype had an estimated 4.17 and 4.8-fold risk of good response to chemotherapy, and they could reduce 53% and 70% risk of death from cancer.

The XPG gene is laocated on the chromosome 13q33 and consisted of 15 exons spanning ~30kb of genomic DNA, and it participates in two incision steps to correct the excision repair deficiency (Mudgett and MacInnes, 1990; Takahashi et al., 1992). During the NER pathway, the XPG has a role of making one of the incisions required to excise a damaged oligonucleotide through cleaving 3' to DNA damaged site, and it also stabilizes the DNA repair complex to damaged DNA (Friedberg, 2003). Previous studies showed enhanced NER activity to the resistance of platinum agents and diminished NER activity to the sensitivity of platinum agents. The association between the XPG polymorphism and response to chemotherapy has been described in various cancers previously (Sakano et al., 2010; Italiano et al., 2011; Liu et al., 2012; He et al., 2013). Only three previous studies explored the association between polymorphism in XPG and osteosarcoma risk (Caronia et al., 2009; Graf et al., 2011; Biason et al., 2012), and no one was conducted in China. Biason et al., reported no association was found between XPG polymorphisms and osteosarcoma patients treatment with neoadjuvant chemotherapy (Biason et al., 2012), while Caronia reported a shorter event-free survival in osteosarcoma patients (Caronia et al., 2009). Our finding indicated the polymorphism of XPG was related with good response to cisplatin in osteosarcoma and longer survival time. The inconsistence of our findings was induced by different ethnicities, sample size and case selection, et al. Further studies are greatly warranted to confirm their association.

Variants of MMS19 have specific distinct functional domains, and it exerts its function in repairing and transcription. Specific MMS19 domains a specific role in NER pathway and transcription and contributes to regulating the switch between transcription and NER (Hatfield et al., 2006). Previous two studies reported that the association between MMS19L and risk of cancer or its prognosis (McWilliams et al., 2009; Zhang et al., 2012). Our study has showed polymorphism in MMS19 is associated with good response of cisplatin chemotherapy in osteosarcoma.

In conclusion, XPG and MMS19L are correlated with response to chemotherapy and prognosis of osteosarcoma, which could be used as predictive markers for the prognosis of osteosarcoma. Our study provides significant information on prognostic value of XPG and MMS19, and detecting of polymorphisms of these two genes could be used as predictive markers toward individualizing osteosarcoma treatment strategies.

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