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Research Paper

Variants of FasL and ABCC5 are predictive of outcome after chemotherapy-based treatment in osteosarcoma

Xu Leilei[a], Xia Chao[a], Sun Qi[b], Sheng Fei[a], Xiong Jin[a], Wang Shoufeng[a,*]

[a] Department of Orthopedic Surgery, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Zhongshan Road 321, Nanjing 210008, China
[b] Department of Pathology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, China

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ABSTRACT

Objectives: Previous pharmacogenetics studies showed that genetic variants could be indicative of the response to chemotherapy. We aimed to investigate whether variants of FasL, MSH2, ABCC5, CASP3 and CYP3A4 are associated with the outcome after chemotherapy-based treatment in osteosarcoma.

Methods: 132 osteosarcoma patients who had completed the neoadjuvant chemotherapy in our center were included. 5-year progression-free survival (PFS) was assessed from the initial treatment to the earliest sign of disease progression or death from any cause. 5 SNPs were genotyped using TaqMan SNP Genotyping Assay, including rs763110 of FasL, rs4638843 of MSH2, rs939338 of ABCC5, rs2720376 of CASP3 and rs4646437 of CYP3A4. Patients were classified into two groups according to the 5-year PFS (event/no event). The chi-square test was used to analyze difference of genotype frequency. Logistic regression analysis was used to determine the independent predictors of the PFS rate.

Results: The overall 5-year PFS was 61.4% (81/132). Genotype TT/CT of rs763110 and genotype GG/AG of rs939338 were significantly associated with the event of 5-year PFS ($p = 0.028$ for rs763110; $p = 0.039$ for rs939338). Patients with no risk allele showed a 5-year PFS of 73.7% (42/57), which was significantly higher than a PFS of 54.2% (26/48) for patients with one risk allele and 48.1% (13/27) for patients with two different risk alleles ($p = 0.03$). Logistic regression analysis showed that allele T of FasL rs763110 and allele G of ABCC5 rs939338 were independent risk factors of the 5-year PFS. The ORs were 2.14 (95%CI = 1.13–3.35, $p = 0.01$) for rs763110 and 1.73 (95%CI = 1.05–2.52, $p = 0.03$) for rs939338, respectively.

Conclusions: The association of variants of FASL and ABCC5 with survival outcome after chemotherapy was validated in patients with osteosarcoma. Our findings may provide a new insight into a more personalized treatment for patients with osteosarcoma.

1. Introduction

Osteosarcoma is a common primary malignant bone tumor predominantly located in the metaphyses of the distal femur, proximal tibia and proximal humerus [1]. Since its advent in 1970s, neoadjuvant chemotherapy has greatly promoted the survival rate of osteosarcoma patients [2,3]. The backbone drugs currently used in neoadjuvant chemotherapy of osteosarcoma included doxorubicin, cisplatin, methotrexate and ifosfamide [4]. Despite the reported effectiveness of these chemotherapeutics, patients were found to have highly varied sensitivity to these agents regarding the antitumor effect and the toxic side-effect [5]. To date, few predictors have been reported to be indicative of the overall survival after the completion of neoadjuvant chemotherapy [6–8]. Reliable predictive factors await to be uncovered to identify patients who may benefit less from the chemotherapy.

Pharmacogenetics of cancer treatment mainly refers to the inherited variability of drug response [9]. In previous studies, candidate gene analysis and pathways-based gene analysis were employed to explore pharmacogenetic markers [5]. To date, a few genetic polymorphisms have been found predictive of sensitivity and toxicity of chemotherapy in osteosarcoma through candidate gene analysis. Polymorphisms of ERCC1 were reported to play important roles in the response to cisplatin mediated by the DNA repair pathway [10]. MTHFR C677T polymorphism was identified as a predictor of MTX toxicity in osteosarcoma patients [11]. The SNPs of ABCB1 and ABCC3 were found significantly associated with pharmacokinetic parameters and the occurrence of MTX toxicity [12,13]. To facilitate risk stratification and personalized treatment, establishment of a prediction model based on these genetic variants are warranted.

In a recent study, Hagleitner et al. [14] investigated genes variations

* Corresponding author.
E-mail addresses: wangshoufeng@nju.edu.cn, wsf0135_drumtower@126.com (S. Wang).

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involved in the metabolism of cisplatin and doxorubicin in 126 patients with osteosarcoma. Five variants in FasL, MSH2, ABCC5, CASP3 and CYP3A4 were combined in a risk prediction model to differentiate patients with different progression-free survival (PFS) [14]. The authors reported a PFS of 100% in patients with none or only one risk allele [14]. Since there exists remarkable genomic heterogeneity in osteosarcoma, replication of these five SNPs in additional cohorts of patients is warranted to confirm the efficacy of the predictive model. In the current study, we investigated the association of five variants of FasL, MSH2, ABCC5, CASP3 and CYP3A4 with the 5-year PFS in a cohort of osteosarcoma patients who had completed the neoadjuvant chemotherapy. Moreover, we analyzed the functional role of these variants in the gene expression.

2. Methods

2.1. Subjects

This study was approved by the ethics committee of our University Medical Center. 132 osteosarcoma patients who had completed the neoadjuvant chemotherapy in our center were included. Informed consent was obtained from all patients or their guardians. All the patients underwent the same chemotherapy regimens consisted of cisplatin (300 mg/m^2), doxorubicin (80 mg/m^2) and methotrexate (10 g/m^2) with a minimum of 5-year follow-up. Clinical data were collected from the medical record retrospectively. PFS was assessed from the initial treatment to the earliest sign of disease progression or death from any cause. Good response to the treatment was defined as with $<10\%$ vital cells after two cycles of preoperative chemotherapy therapy [15].

2.2. Genotyping of target variants

The peripheral blood was collected from each patient at the initial visit. DNA was then extracted using the DNA extraction kit (QIAGEN Inc., Tokyo, Japan) according to the protocol of the manufacturers. 5 SNPs were genotyped using TaqMan SNP Genotyping Assay, including rs763110 of FasL, rs4638843 of MSH2, rs939338 of ABCC5, rs2720376 of CASP3 and rs4646437 of CYP3A4 [14]. The genotyping assay was performed with ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Ten percent of the samples were randomly selected to validate the genotyping results. The reproducible rate was 100%.

2.3. Expression analysis in tumor samples

The tumor samples were collected from 56 patients during the resection surgery and stored at −80 °C. The total RNA was extracted from the tumor tissue using the TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa, Tokyo, Japan). Real-time PCR was carried out to quantify the mRNA expression level for above-mentioned 5 genes on the ABI 7900HT Detection System (Applied Biosystems, Foster City, CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control gene. The specific primers of the target genes were listed in Table 1. All amplification procedures were repeated in triplicate, with a mean value of threshold cycle (Ct) scores used to calculate the expression level with ΔΔCt method.

2.4. Statistical analyses

The Hardy–Weinberg equilibrium (HWE) test was performed to exclude selection bias. Patients were classified into two groups according to the 5-year PFS (event/no event). For the inter-group comparison, the Chi-square test was used to analyze difference of genotype frequency and the Student's *t* test was used to analyze the difference of gene expression level. Specifically, a dominant model was used to compare the association between genotype and PFS. The odds ratios (OR) and 95% confidence interval (95% CI) were calculated with the risk allele as reference. In addition, the student *t* test was used to compare the gene expression level between patients with risk allele and those without risk-allele. Logistic regression analysis was used to determine the independent predictors of the PFS rate. Genotype was coded as 0 for homozygotes of non-risk allele and 1 for heterozygotes and homozygotes of risk allele. Response to preoperative chemotherapy treatment was coded as 0 for good response and 1 for bad response. According to different number of the risk alleles, the 5-year PFS curves were analyzed using the Kaplan–Meier method. All the statistical analyses were performed with the SPSS software (version 17.0, Chicago, IL). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Demographic data of the patients

The mean age of patients was 35.2 ± 19.8 years old. 74 patients (56.1%) had tumor metastasis at the initial visit. 102 (77.3%) patients received limb salvage surgery. 49 (37.1%) patients showed good response to chemotherapy. The overall 5-year PFS was 61.4% (81/132). By the end of the 5-year follow-up, 42 (31.8%) patients had died for tumor recurrence or metastasis.

3.2. Genetic association with 5-year PFS

The genotyping results of the 5 SNPs were listed in Table 2. Genotype TT/CT of rs763110 and genotype GG/AG of rs939338 were significantly associated with the event of 5-year PFS ($p = 0.028$ for rs763110; $p = 0.039$ for rs939338). The frequency of genotype CC of rs4638843 was 1. As for rs2720376 and rs4646437, no significant association with 5-year PFS was found. Patients with no risk allele showed a 5-year PFS of 73.7% (42/57), which was significantly higher than a PFS of 54.2% (26/48) for patients with one risk allele and 48.1% (13/27) for patients with two different risk alleles ($p = 0.03$, Fig. 1).

3.3. Logistic regression analysis of risk factors

Logistic regression analysis showed that allele T of FasL rs763110 and allele G of ABCC5 rs939338 were independent risk factors of the 5-year PFS. The ORs were 2.14 (95%CI = 1.13–3.35, $p = 0.01$) for rs763110 and 1.73 (95%CI = 1.05–2.52, $p = 0.03$) for rs939338, respectively. With the cut-off value set as 0.5, the sensitivity and

Table 1
Primers for the qPCR assay.

Gene	Forward primer	Reverse primer
FasL	TGCCTTGGTAGGATTGGGC	GCTGGTAGACTCTCGGAGTTC
ABCC5	TGTTTTGCTGCAGGGCTCA	AGTGCTGGTTCTCTCCCTCA
MSH2	**AGGCATCCAAGGAGAATGATTG**	**GGAATCCACATACCCAACTCCAA**
CASP3	**CATGGAAGCGAATCAATGGACT**	**CTGTACCAGACCGAGATGTCA**
CYP3A4	**AGATGCCTTTAGGTCCAATGGG**	**GCTGGAGATAGCAATGTTCGT**
GAPDH	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG