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Research

Polymorphisms in SULF1 Associated with Platinum Resistance and Survival in Chinese EOC Patients

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ABSTRACT

Background: Ovarian cancer is the leading cause of death from gynecologic cancers and platinum resistance remains a major obstacle in the treatment of ovarian cancer. This study aims to examine the role of polymorphisms in sulfatase 1 (SULF1) in platinum resistance and survival in advanced epithelial ovarian cancer (EOC) patients.

Methods: We genotyped 12 SNPs of SULF1 in 195 EOC patients treated with platinum usingMassarray method and test the association between the SNPs and platinum response.

Results: We found that rs3802278 was marginal significantly associated with platinumresistance in recessive model with p value of 0.055, the patients with rs3802278 AA were moreresistant to platinum-based chemotherapy comparing to those with AG/GG genotype (OR:2.317, 95 CI: 0.982~5.465). In survival analysis, we found that rs3802278 was significantlyassociated with both of PFS and OS after adjusted by FIGO stage and age. Patients with AAgenotypes showed a shorter PFS and OS than with AG/GG genotypes (median PFS: 15 months 58vs. 21 months, p=0.010, HR=1.876, 95 CI: 1.165-3.022; median OS: 42 months vs. 73 months, 59p=0.031, HR=1.928, 95 CI: 1.061-3.504).

Conclusion:SULF1 rs3802278 may serve as a potential candidate biomarker for theprediction of platinum resistance and prognosis in Chinese EOC patients.

Keywords:SULF1; polymorphism; platinum resistance; epithelial ovarian cancer.

INTRODUCTION

Ovarian cancer is the leading cause of death from gynecologic cancers. It consists of severalhistopathologic entities and epithelial ovarian cancer (EOC) constitutes comprises the majorityof malignant ovarian neoplasms (~ 90). The standard treatment for epithelial ovarian cancer(EOC) is primary debulking surgery followed by platinum+taxane-based chemotherapy. Themajority of patients respond well to initial platinum-based chemotherapy with 60–80 ofpatients achieving clinical remission. However, approximately 15 of patients will be primaryresistance, and an additional 30 will recur within 6 months of completing initial platinum-based chemotherapy, eventually, the majority of patients becomes resistant or refractory toplatinum compounds[1,2]. Hence, identifying

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patients prior to treatment who are more orless likely to benefit from chemotherapeutic agents is essential/crucial. Pharmacogenetics isthe study of how the genetic variations between individuals affect their response to drugs andother xenobiotics. Great efforts had been made to discover SNP biomarkers applicated foridentifying patients who are most likely to benefit from platinum, but no definite SNP withhigh consistency and repeatability are found so far so good[3-7]. SULF1 is an extracellularheparan sulfate endosulfatase, it can selectively remove 6-O-sulfate groups from heparansulfate chains of heparan sulfate proteoglycans (HSPGs) that act as coreceptors for diverseprotein ligands, including growth factors, growth factor receptors, cytokines and resulted inthe alteration of HSPGs biological functions [8-10]. So SULF1 can regulate many signalpathway through the substrate HSPG and played a key role in the development of cancer[8,11-13]. On the other hand, many studies also had reported that SULF1 expression wasassociated with platinum resistance and our previous study using the genome-scale CRISPR-Cas9 knockout (GeCKO) library to screen gene modulating response to cisplatin in ovariancancer cell lines also found that loss of SULF1 was involved in resistance to cisplatin[14], so we are interested whether some SNPs of SULF1 are associated with platinum response inovarian cancer.

Materials and Methods

Patients and Clinical Data

The study population and data collection were described previously[15]. A total of 195 ovarian cancer patients from Hunan Cancer Hospital were recruited for retrospective analysis. Clinical data were obtained from 2006 to 2018. The study protocol was approved by the Committee for Medical Ethics, Institute of Clinical Pharmacology, Central South University (registration no.CTXY-140002-10), and was registered in the Chinese Clinical Trial Registry (ChiCTR-TNC-15007604). Patients had signed their written informed consent prior to participation. All included patients were serious or mucinous EOCs with stage of II, III, or IV, according to the FIGO (International Federation of Gynecology and Obstetrics) criteria, they all received standard (at least 3 cycles of) platinum-based chemotherapies within 7 days after maximalcy to-reductive surgery. Patients with other cancer or underlying disease that may affect the treatment were excluded.

Primary recurrence was used to determine platinum sensitivity and resistance. Platinum resistance was defined if the patient had persistent or progressive disease during treatment or recurrence within 6 months after the last cycle of chemotherapy. Platinum sensitivity was defined if the progression or recurrence time is six months or greater after completing a platinum-based regimen. Progression free survival (PFS) was calculated as the duration, in months, from the date of initial surgical resection to the date of progression, or the date of recurrence which was defined by physical examination, CA125 or radiographic studies. Overall survival was defined as the duration from diagnosis to death from any cause. Informationbout PFS was available for 181(92.8) patients and overall survival information was availablefor 168 (86) patients.

DNA Extraction

5 mL venous blood from each patient was collected in EDTA (ethylenediaminetetraaceticacid)-containing tubes. Genomic DNA was isolated using a DNA easy Blood & Tissue Kit(QIAGEN, Germantown, MD, USA) according to standard protocols.

SNP Selection and Genotyping

SULF1 gene is located on chromosome 8 with 26 exons. All single-nucleotide polymorphisms(SNPs) were chosen/selected by Haplo view 4.2 (Broad Institute, Cambridge, MA, USA) using pair-wise tagging with default settings (pair wise r2 threshold ≥ 0.8). The following criteria were used to select SNPs: 1) minor allele frequency (MAF) ≥ 5 in the south Chinese population according to the 1000 Genomes database; 2) these SNPs were located in the promoter region , the 5'untranslated region (5'-UTR), coding regions, or 3'-UTRs; 3) that were reported to be clinically relevant according to the previous literature. If SNPs are in high link age disequilibrium (LD) (r 2 ≥ 0.8), only one SNP were genotyped. As a result, 12 SNPs were selected for genotyping and analysis. Sequenom Mass ARRAY system was used to genotype.

Statistical Analysis

The SPSS 20.0 (SPSS Inc, Chicago, IL) and Plink 1.07 were used for general statistics analyses. The Hardy-Weinberg equilibrium (HWE) of SNPs among all subjects was examined via a goodness-of-fit χ 2-test to compare the expected genotype frequencies with those that were observed. The logistic regression was performed for categorical variables, and the Coxproportional hazard models was used for multivariate survival analysis. Full models (allelic,dominant, recessive and genotypic) association analysis were conducted. Before association analysis, the forward stepwise method was used to screen the covariates. The candidate covariates include age, body mass index (BMI), FIGO stage, tumor differentiation and histological type. The receiver operating characteristic (ROC) curve analysis was used to test the predictive ability of the factors that association with chemotherapy response. To draw the survival curve, Kaplan-Meier method and the log-rank test were utilized.

RESULTS

The basic characteristics of these EOC patients were summarized in Table 1. Among these patients, 149 (76.4) were platinum-sensitive and 46 (23.6) were platinum-resistant. No difference was found in age, histologic subtype and tumor differentiation type between sensitive and resistant group, except for FIGO stage. The basic information of all tested SNP were shown in Table 2. Among the 195 samples, 11 SNPs were successfully determined inwhole samples, only 1 SNP didn't be determined in 4, samples. All tested SNPs were consistent with Hardy-Weinberg equilibrium.

Table 1:-. Basic clinical characteristic of EOC patients treated with platinum- based chemotherapy.

Charateristics	No.of patients	Sensitive	Resistant	p value#
Total	195	149	46	
Age at treatment start				
Mean ± SD;years		50.60 ± 8.43	53.20 ± 7.61	0.066
BMI Mean ± SD;m2/kg		23.42 ± 3.01	23.03 ± 2.50	0.678
FIGO stage				
II-III	172(87.69)	126	45	0.042
IV	24(12.31)	23	1	
Histologic subtype				
Serous	98(50.26)	72	26	0.332
Non-serous	97(49.74)	77	20	
Tumor differentiation				
Low	142(72.82)	104	38	0.082
Middle and High	28(14.36)	25	3	
Unknown	25(12.82)	20	5	

Table 2:- Twelve selected SNPs in SULF1.

SNP (rs number)	Allele	Region	Call rate	MAF	HWE
rs2623047	A>G	promoter	100	0.423	0.394
rs59465016	G>C	promoter	100	0.474	0.972
rs10957496	A>C	intron	100	0.451	0.126
rs1372150	G>A	intron	97.9	0.289	0.165
rs1441199	C>T	intron	100	0.097	0.488
rs16936018	C>T	intron	100	0.441	0.372
rs16936195	G>A	intron	100	0.187	0.308
rs1899274	T>A	intron	100	0.228	0.950
rs2028442	A>G	intron	100	0.210	0.059
rs2583092	G>A	intron	100	0.100	0.968
rs4737999	A>G	intron	100	0.285	0.067
rs3802278	G>A	3'UTR	100	0.362	0.436

Table 3:- Association between SULF1 SNP and platinum response in 195 EOC patients.

SNPrsnumber)	Additive model	Recesscive model		Dominant model	Allelic model		
	p	OR(95%CI)	p	OR(95%CI)	p	OR(95%CI)	p
rs3802278	0.132	2.317(0.982-5.465)	0.055	1.045(0.529-2.064)	0.900	1.294(0.808-2.073)	0.283
rs10957496	0.367	0.524(0.214-1.282)	0.157	0.825(0.409-1.663)	0.590	0.764(0.483-1.210)	0.251
rs1372150	0.534	1.203(0.399-3.627)	0.743	1.471(0.748-2.894)	0.264	1.286(0.781-2.116)	0.323
rs1441199	0.885	NA	0.999	1.183(0.518-2.701)	0.690	1.117(0.505-2.470)	0.784
rs16936018	0.717	1.057(0.467-2.393)	0.894	0.777(0.385-1.569)	0.482	0.914(0.574-1.455)	0.703
rs16936195	0.289	1.429(0.342-5.968)	0.625	0.616(0.292-1.303)	0.205	0.770(0.419-1.413)	0.398
rs1899274	0.498	0.371(0.044-3.074)	0.358	0.708(0.352-1.422)	0.331	0.699(0.381-1.282)	0.247
rs2028442	0.572	1.844(0.570-5.961)	0.307	1.051(0.524-2.110)	0.889	1.155(0.684-1.950)	0.591
rs2028444	0.654	1.599(0.555-4.606)	0.385	1.020(0.508-2.045)	0.957	1.118(0.675-1.853)	0.664
rs2583092	0.968	NA	0.999	1.028(0.440-2.402)	0.948	0.941(0.427-2.074)	0.881
rs2623047	0.152	1.555(0.667-3.628)	0.307	0.635(0.315-1.281)	0.205	0.926(0.564-1.519)	0.760

rs4737999	0.614	1.043(0.354-3.079)	0.939	1.382(0.705-2.709)	0.346	1.199(0.737-1.952)	0.465
rs59465016	0.135	1.621(0.749-3.509)	0.221	0.6684(0.3253-1.374)	0.273	1.004(0.6243-1.616)	0.986
rs2244817	0.121	1.758(0.768-4.023)	0.182	0.6841(0.3403-1.375)	0.287	1.003(0.6174-1.63)	0.990

Association between SULF1 SNPs and platinum sensitivity

To tested the association between these selected SNP and platinum sensitivity in EOC patients. A total of 195 ovarian cancer patients with platinum chemotherapy were recruited forenotyping. Table 3 summarizes the association analysis results between 12 SULF1 SNPs and platinum response. Only rs3802278 was found to be marginal significantly associated with platinum response in recessive model with p value of 0.055. Comparing to the patients withrs3802278 AG/GG genotype, those with AA were more resistant to platinum-based chemotherapy (OR: 2.317, 95 CI: 0.982~5.465). ROC analysis for platinum response found that the area under the curve of FIGO stage and rs3802278 were 0.566 and 0.563, respectively. When combining these two factors, the AUC reached to 0.655 which was improved by about 8.9 (Figure 1).

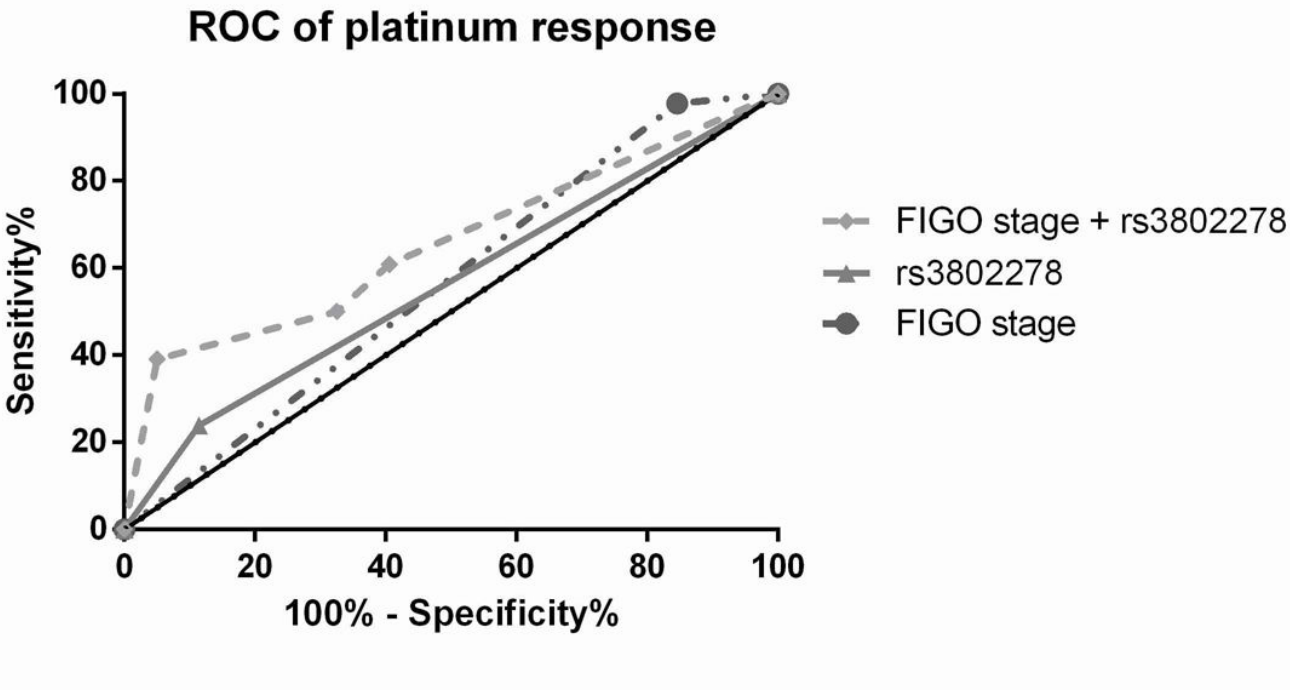

Figure 1: ROC of platinum response

Association between SULF1 SNPs and prognosis

In survival analysis, we evaluated the effect of SNPs on OS and PFS of patients. We found thatrs3802278 was significantly associated with both of PFS and OS after adjusted by FIGO stage and age. Patients with AA genotypes showed a shorter PFS and OS than patients with the AG/GG genotype (median PFS: 15 months vs. 21 months, p=0.010, HR=1.876, 95 CI: 1.165-1663.022; median OS: 42 months vs. 73 months, p=0.031, HR=1.928, 95 CI: 1.061-3.504) (Figure 2), whereas this association with PFS or OS was not observed for other SNPs of SULF1.

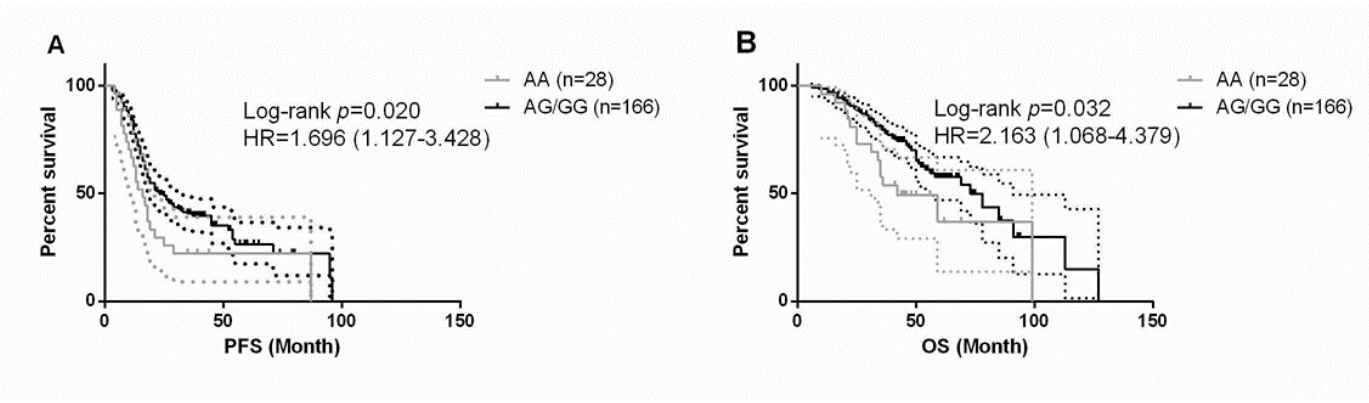

Figure 2:- Kaplan-Meier estimates of survival according to SULF1 rs3802278 genotype. (A) progression free survival; rs3802278 AA vs.rs3802278 AG/GG (B) overall survival; rs3802278 AA vs.rs3802278 AG/GG. In the group of patients with rs3802278 AA genotype, there was 1.696/2.163 higher risk, as compared with the group with rs3802278 AG/GG genotype.

DISCUSSION

Several researches about polymorphisms of SULF1 focused on the influence on risk of disease, such as carriers of SULF1 rs4737999 AA genotype were almost 3-fold less likely of having low grade lesions compared to GA/GG genotypes, and SULF1 rs3802278 and rs2623047 may serve as biomarker for predicting the susceptibility to breast cancer[16-18]. Up to now, no research about the association between the polymorphism of SULF1 and chemo resistance were reported. In our study, we selected 12 important SNPs of SULF1 based on their localization, function prediction and published paper, then investigated the associations between these SNPs and platinum chemotherapy response in ovarian cancer patients. We found that SULF1 rs3802278 G>A polymorphism was only marginal significantly associated with platinum resistance, but was significantly associated with both of PFS and OS. Patients with rs3802278 AA genotype had poor prognosis than those with AG/GG genotype. However, the mechanism is still unknown.

MRNAs can recognize the specific sites in the 3'-UTR of target mRNAs and induce target gene translational repression or mRNA cleavage, and rs3802278 is located in the 3'-UTR of SULF1. Therefore, the polymorphism rs3802278 may influence the interaction between iRNAs and SULF1, and ultimately change the expression of SULF1 genes. On the other hand, several studies had reported that the expression of SULF1 was down-regulated in many ancers, including hepato cellular (HCC), ovarian and breast cancers. And many evidences had indicated that hsulf1 expression were associated with chemotherapy. HCCs cells with high expression of hSulf1 were sensitive to staurosporine- or cisplatin-induced apoptosis, whereas low expressing cells were resistant. Over expression of hSulf1 in hSulf1-negative cells restored staurosporine and cisplatin sensitivity. Similarly, down regulation of HSulf-1 expression in ovarian cancer OV167 and OV202 cells transfected with HSulf-1 siRNA lead to an attenuation of cisplatin-induced cytotoxicity. Moreover, patients with ovarian tumors expressing higher levels of hSulf-1 showed better response rate to chemotherapy than those with weak or moderate levels[19]. Based on the previous research, we speculated that rs3802278 affected the prognosis of platinum chemotherapy in EOC patients by influencing the expression of SULF1 mediated by miRNA. However, this specific mechanism needs further study to confirm. our study also has several limitations. First, the number of the subjects are limited, from which we only observed the marginal significance. Second, our study was single-center study, multicenter study will provide more reliable results. Thirdly, a serial of studies are need to be carried out for exploring the mechanisms. In conclusion, we analyzed the polymorphism of SULF1, and found that rs3802278 AA genotype was associated with worse PFS and OS, and reached the marginal significantly associated with platinum resistance.

CONCLUSION

This suggested that the polymorphism may serve as a biomarker for predicting platinum response in EOC patients . Nevertheless, future multicenter studies with larger samples will be needed to validate our study result.

Statements

Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. subjects had given their written informed consent and that the study protocol was approved by the Committee for Medical Ethics,

Institute of Clinical Pharmacology, Central South University (registration no. CTXY-140002-10).

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