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ORIGINAL RESEARCH

Significant association of the *EXO1* rs851797 polymorphism with clinical outcome of ovarian cancer

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Background: Exonuclease 1 (*EXO1*), one of DNA mismatch repair pathway genes, functions in maintaining genomic stability and affects tumor progression. We hypothesized that genetic variations in *EXO1* may predict clinical outcomes in epithelial ovarian cancer (EOC).

Methods: In this cohort study with 1,030 consecutive EOC patients, we genotyped four potentially functional polymorphisms in *EXO1* by the Taqman assay and evaluated their associations with patients' survival.

Results: Using multivariate Cox proportional hazards regression models, we found that rs851797AG/GG genotypes were significantly associated with recurrence and cancer death (HR =1.30 and 1.38, 95% CI =1.11–1.52 and 1.02–1.88, respectively). Kaplan–Meier survival estimates showed that patients who carried rs851797AG/GG genotypes had poorer progression-free survival and poorer overall survival, compared with rs851797AA genotype carriers (log-rank test, P=0.002 and 0.025, respectively). Moreover, patients with older age at menophania, advanced stage tumor, or being received incomplete cytoreduction were more likely to be recurrent and dead.

Conclusion: *EXO1* rs851797 polymorphism can predict the clinical outcomes in EOC patients. In addition, age at menophania, FIGO stage, and complete cytoreduction might be independently prognostic factors of ovarian cancer. Large studies with functional experiments are warranted to validate these findings.

Keywords: *EXO1*, ovarian cancer, polymorphism, prognosis

Introduction

Ovarian cancer is the third most commonly diagnosed gynecologic cancer and the first leading cause of death from gynecologic malignancies, up to 238,700 new cases and 151,900 cancer deaths worldwide in 2012.[1] In China, there were 52,100 new ovarian cancer cases and 22,500 related deaths in 2015.[2] More than 90% of these cases are epithelial ovarian cancer (EOC), among which 70% are diagnosed with bulky intra-abdominal disease or distant metastases.[3] Despite improvements in surgical techniques and chemotherapeutic options, most of advanced-stage patients will relapse within 18 months, and 5-year overall survival still remains at ~46% in the United States.[4] Recently, genetic variations have been highly strengthened along with the development of molecular subtyping and targeting therapy in ovarian cancer and related research. Considerable efforts on prognostic genetic variations have been focused on germline or somatic mutations, such as *BRCA1/2* and other DNA repair pathway genes.[5] However, few reports were performed on the predictive value of single nucleotide polymorphisms (SNPs), especially in Chinese Han ethnics.

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Exonuclease 1 (*EXO1*) is a member of the RAD2 nuclease family with evolutionarily conserved domains,[6] and exhibits both 5′ to 3′ exonuclease activity and 5′ flap structure-specific endonuclease activity.[7] A large number of studies have demonstrated that *EXO1* can function in DNA replication, repair, and recombination by participating in various DNA repair pathways, such as mismatch repair (MMR), DNA double-strand break repair, and error-free DNA damage tolerance pathway, and thus may play a critical role in genome maintenance and tumor suppression.[8,9] Recently, three meta-analysis publications reported the significant associations of *EXO1* SNPs with cancer susceptibility.[10–12] Moreover, several investigations focused on the prognostic role of *EXO1* polymorphisms in human cancers. For example, *EXO1* N279S[13] and R354H[14] could predict the overall survival in pancreatic cancer patients. *EXO1* K589E (rs1047840) might be a prognostic biomarker for relapse-free survival in head and neck squamous cell carcinoma.[15] Recently, *EXO1* rs9350 was reported to be associated with poor survival of non-small cell lung cancer patients who were treated by platinum-based chemotherapy.[16] To date, only a pooled genome-wide association study showed the *EXO1* polymorphism region (1q43) to be associated with EOC susceptibility.[17] No investigations were reported on the association of *EXO1* polymorphisms with EOC survival, let alone the mechanism of *EXO1* polymorphisms in regulating gene and protein expression.

In this study, we hypothesized that potentially functional genetic variations in *EXO1* may affect the clinical outcome in EOC patients. We also conducted a relatively large-scale cohort study to identify four SNPs in the functional region of *EXO1* and their associations with EOC prognosis in Chinese Han women.

Materials and methods

Study subjects

The study population consisted of 1,165 consecutive EOC patients between March 2009 and August 2012 from Shanghai Ovarian Cancer Study as described previously in the Chinese EOC genome-wide association study,[18] mainly from Fudan University Shanghai Cancer Center (FUSCC). Among all the 1,165 patients, 135 cases were lost to follow-up. Thus, 1,030 EOC patients were involved in the final survival analysis. All cases were genetically unrelated ethnic Han Chinese, who were mainly from Eastern China where they lived, according to the records of in-patient registration and cancer registration system. The tumors were histopathologically confirmed independently as primary epithelial ovarian carcinoma based on World Health Organization Classification criteria, including serous, endometrioid, clear cell, and so on, by two gynecologic pathologists as routine diagnosis.[19] Patients with borderline ovarian tumors were not included. Age at menarche was defined as the age at the first menstruation. We defined post-menopause as the absence of menstrual periods for ≥12 months since the last period, or pre-menopausal hysterectomy. Cancer family history was defined when first-, second-, or more-degree relatives had cancer history. We defined female cancer family history as breast, ovarian, cervical, endometrial cancers in first-, second-, or more-degree relative women.

The detailed clinicopathological information was extracted from the patients' electronic database, including FIGO stage (International Federation of Gynecology and Obstetrics, 2013), histopathology, tumor grade, tumor type according to the dualistic model of carcinogenesis (categorized as type I tumor [low-grade serous carcinomas, low-grade endometrioid, clear cell, and mucinous carcinomas] and type II tumor [high-grade serous carcinoma, high-grade endometrioid carcinoma, malignant mixed mesodermal tumors and undifferentiated carcinomas]),[20] pelvic lymph node metastasis, the expression of estrogen receptor and progesterone receptor (dichotomized into positive [+] if >10% of cells stained positive and negative [–] if ≤10% stained positive),[21] neoadjuvant chemotherapy, residual disease after primary cytoreduction (categorized as 0 [no grossly visible tumor], 1 [0.1–0.5 cm], 2 [0.5–1.0 cm], and 3 [>1.0 cm]), tumor recurrence, and death. The residual disease was reviewed in the pelvis, middle abdomen, and upper abdomen. Complete cytoreduction was defined as no grossly visible tumor overall after surgical procedure. Optimal cytoreduction was defined as no more than 1 cm of residual tumor overall after surgical procedure. After surgery, all patients received adjuvant chemotherapy with platinum and paclitaxel for six to eight cycles. Unfortunately, in our data set, there were no enough information about patient's response to platinum.

SNP selection and genotyping

By searching the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and the International HapMap Project database (http://hapmap.ncbi.nlm.nih.gov/), we found that there were 1064 SNPs in *EXO1*, including 602, 22, and 43 SNPs located in the coding region, 5′-UTR, and 3′-UTR, respectively. Among them, four SNPs were finally selected, based on the following criteria: 1) minor allele

frequency of at least 5% in Chinese populations, 2) with low linkage disequilibrium by using an r^2 threshold of <0.8 for each other, 3) predicted to be a potentially functional SNP by the SNP function prediction platform (http://snpinfo.niehs.nih.gov/snpfunc.htm), 4) not included in the published genome-wide association studies, and 5) meet the Hardy–Weinberg equilibrium criteria. They are rs1047840G>A (NM_130398.3:c.1765G>A, Glu589Lys, exon 10); rs9350C>T (NM_130398.3:c.2270C>T, Pro757Leu, exon 12); rs851797A>G [NM_130398.3:c.*140A>G, 3′-untranslated region (UTR)]; and rs3754093A>G (NM_130398.3:c.-1959A>G, 5′-flanking). The RNAfold online tool (http://rna.tbi.univie.ac.at/) was used to estimate the RNA secondary structure based on minimum free energy (MFE) values for the potentially functional SNP.

DNA extraction and genotyping

Genomic DNA was obtained from the whole blood, and the Taqman method by 384-fomate was conducted for genotyping, as described previously.[22] As a result, the discrepancy rate in all positive controls (ie, duplicated samples, overlapping samples from previous studies, and samples randomly selected to be sequenced) was <0.1%.

Statistical analysis

Progression-free survival (PFS) and overall survival (OS) times were calculated from the date of first treatment to the date of disease recurrence and to the date of death, respectively. Patients without progression, lost to follow-up, or died from other causes were censored at their last date of record. Kaplan–Meier survival estimate and log-rank test were calculated to evaluate PFS and OS. We performed univariate and multivariate Cox proportional hazards regression analyses to evaluate the effects of *EXO1* genotypes on the cumulative probability of survival in EOC patients. Multivariate analyses were adjusted by those variables that were independently associated with survival in the univariate model. All statistical analyses were performed with SAS 9.1 software (SAS Institute, Cary, NC, USA), unless stated otherwise. All *P*-values were two-sided with a significance level of $P<0.05$.

Ethics approval and consent to participate

The research was approved by the Institutional Review Board of FUSCC. Each patient signed a written informed consent.

Table 1 Baseline characteristics of epithelial ovarian cancer patients

Characteristics	Patients	
	N=1,030	%
All subjects		
Age, years (median, range)	54.5 (18–85)	
≤50	358	34.76
>50	672	65.24
Age, years		
≤48	291	28.25
49–60	462	44.85
>60	277	26.90
Age at menophania, years		
≤15.5 (median)	615	59.71
>15.5 (median)	407	39.51
Missing	8	0.78
Menopausal status		
Pre-menopausal	311	30.19
Post-menopausal	697	67.67
Missing	22	2.14
BMI[a], kg/m^2		
<25	744	72.23
≥25	269	26.12
Missing	17	1.65
Cancer family		
No	767	74.47
Yes	251	24.37
Missing	12	1.17
Female cancer family		
No	965	93.69
Yes	53	5.15
Missing	12	1.17

Note: [a]According to the current WHO recommendations.
Abbreviation: BMI, body mass index.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Results

Population characteristics

Among the 1,165 consecutive EOC patients, 135 cases were lost to follow-up. Thus, 1,030 EOC patients were involved in the final analysis (Tables 1 and 2). The patients' median age at diagnosis was 54.5 years (range, 18–85 years). Totally, there were 32 (3.11%), 55 (5.34%), 492 (47.77%), and 74 (7.18%) patients diagnosed with stage I, II, III, and IV, respectively. The rates of complete and optimal cytoreduction were 33.40% and 70.68%, respectively. The median follow-up time was 37.7 months, and there were 752 (73.01%) recurrences and 207 (20.10%) cancer deaths during the follow-up period.

Table 2 Clinical characteristics of epithelial ovarian cancer patients

Characteristics	Patients	
	N=1,030	%
FIGO stage		
I	32	3.11
II	55	5.34
III	492	47.77
IV	74	7.18
Missing	377	36.60
Histopathology		
High-grade serous	725	70.39
Low-grade serous	105	10.19
Endometrioid	58	5.63
Clear cell	51	4.95
Mucinous	32	3.11
Others	57	5.53
Missing	2	0.19
Tumor grade		
Grade 1	14	1.36
Grade 2	146	14.17
Grade 3	750	72.82
Missing	120	11.65
Tumor type		
I	224	21.75
II	781	75.83
Unknown	25	2.43
Pelvic LN metastasis		
Negative	262	25.44
Positive	216	20.97
Missing	552	53.59
ER expression		
Negative	217	21.07
Positive	526	51.07
Missing	287	27.86
PR expression		
Negative	471	45.73
Positive	283	27.48
Missing	276	26.80
Neoadjuvant chemotherapy		
No	896	86.99
Yes	134	13.01
Residual disease after primary cytoreduction		
0 (no grossly visible tumor)	344	33.40
1 (0.1–0.5 cm)	169	16.41
2 (0.5–1.0 cm)	215	20.87
3 (>1.0 cm)	237	23.01
Missing	65	6.31
Recurrence		
No	278	26.99
Yes	752	73.01
Death		
No	823	79.90
Yes	207	20.10

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; LN, lymph node; ER, estrogen receptor; PR, progesterone receptor.

Table 3 Prognostic factors of epithelial ovarian cancer by Cox proportional hazards regression models

Prognostic factors	Recurrence	Univariate		Multivariate		Death	Univariate		Multivariate	
	N (%)[a]	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	N (%)[a]	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
All subjects	752 (73.0)					207 (20.1)				
Age, years			0.054[b]		0.493			**0.004**		0.394
≤50	262 (73.2)	1.00		1.00		56 (15.6)	1.00		1.00	
>50	490 (72.9)	1.16 (1.00–1.35)		1.18 (0.74–1.89)		151 (22.5)	1.58 (1.16–2.14)		1.28 (0.73–2.25)	
Age at menophania, years			0.393		**0.0003**			**0.047**		**0.017**
≤15.5 (median)	442 (71.9)	1.00		1.00		111 (18.1)	1.00		1.00	
>15.5 (median)	302 (74.2)	1.07 (0.92–1.24)		1.81 (1.31–2.50)		93 (22.9)	1.32 (1.00–1.74)		1.50 (1.07–2.08)	
Menopausal status			**0.023**		0.431			**0.005**		0.714
Pre-menopausal	222 (71.4)	1.00		1.00		48 (15.4)	1.00		1.00	
Post-menopausal	515 (73.9)	1.20 (1.03–1.41)		0.84 (0.54–1.30)		156 (22.4)	1.60 (1.15–2.20)		0.91 (0.53–1.54)	
BMI, kg/m^2			0.227		0.677			0.937		0.735
<25	526 (70.7)	1.00		1.00		147 (20.0)	1.00		1.00	
≥25	210 (78.1)	1.10 (0.94–1.30)		1.08 (0.75–1.57)		58 (21.6)	0.99 (0.73–1.34)		0.94 (0.65–1.36)	
Cancer family			0.690		0.626			0.152		0.093[b]
No	560 (73.0)	1.00		1.00		160 (20.9)	1.00		1.00	
Yes	181 (72.1)	0.97 (0.82–1.14)		0.92 (0.65–1.30)		43 (17.1)	0.78 (0.56–1.10)		0.70 (0.46–1.06)	

Association between clinicopathological characteristics and survival

As shown in Table 3, age at menophania, FIGO stage, and complete cytoreduction were independently associated with

Female cancer family			0.835		0.380			0.343		0.331
No	698 (72.3)	1.00		1.00			1.00		1.00	
Yes	43 (81.1)	1.03 (0.76–1.41)		0.69 (0.30–1.58)			0.72 (0.37–1.41)		0.61 (0.22–1.66)	
FIGO stage			**<0.0001**		**0.042**			**<0.0001**		**0.001**
I–II	48 (55.2)	1.00		1.00		3 (3.4)	1.00		1.00	
III–IV	419 (74.0)	2.48 (1.82–3.37)		1.67 (1.02–2.75)		149 (26.3)	11.34 (3.60–35.75)		6.94 (2.14–22.48)	
Histopathology			**<0.0001**		0.344			**0.016**		0.591
Others	207 (68.3)	1.00		1.00		55 (18.2)	1.00		1.00	
High-grade serous	543 (74.9)	1.49 (1.26–1.75)		1.31 (0.75–2.31)		152 (21.0)	1.47 (1.08–2.02)		1.12 (0.74–1.71)	
Tumor type			**<0.0001**		0.328			0.097[b]		0.971
I	149 (66.5)	1.00		1.00		42 (18.8)	1.00		1.00	
II	585 (74.9)	1.49 (1.24–1.79)		1.23 (0.82–1.84)		161 (20.6)	1.34 (0.95–1.88)		0.99 (0.62–1.59)	
Differentiation			**0.0001**		0.440			0.122		0.866
Morderate–low	110 (68.8)	1.00		1.00		32 (21.9)	1.00		1.00	
High grade	560 (74.7)	1.44 (1.17–1.77)		1.32 (0.65–2.70)		157 (20.9)	1.35 (0.92–1.99)		1.08 (0.47–2.49)	
Pelvic LN			**0.026**		0.089[b]			0.519		0.507
Negative	169 (64.5)	1.00		1.00		41 (15.7)	1.00		1.00	
Positive	164 (75.9)	1.28 (1.03–1.59)		0.75 (0.54–1.04)		41 (19.0)	1.15 (0.75–1.78)		0.83 (0.48–1.44)	
ER expression			0.100		0.267			0.359		0.625
Negative	151 (69.6)	1.00		1.00		51 (23.5)	1.00		1.00	
Positive	383 (72.8)	1.17 (0.97–1.42)		1.24 (0.85–1.83)		104 (19.8)	0.86 (0.61–1.20)		0.90 (0.60–1.36)	
PR expression			0.089[b]		0.848			0.354		0.757
Negative	351 (74.5)	1.00		1.00		104 (22.1)	1.00		1.00	
Positive	193 (68.2)	0.86 (0.72–1.02)		0.97 (0.68–1.37)		51 (18.0)	0.85 (0.61–1.19)		0.94 (0.63–1.40)	
Neoadjuvant chemotherapy			0.112		0.822			**0.001**		0.056[b]
No	656 (73.2)	1.00		1.00		170 (19.0)	1.00		1.00	
Yes	96 (71.6)	1.19 (0.96–1.48)		1.06 (0.64–1.77)		37 (27.6)	1.87 (1.31–2.68)		1.53 (0.99–2.36)	
Complete cytoreduction			**<0.0001**		**<0.0001**			**<0.0001**		**<0.0001**
No	498 (80.2)	1.00		1.00		161 (25.9)	1.00		1.00	
Yes	194 (56.4)	0.39 (0.33–0.47)		0.46 (0.31–0.68)		31 (9.0)	0.27 (0.18–0.40)		0.40 (0.25–0.63)	
Optimal cytoreduction			**<0.0001**		**0.005**			0.054[b]		0.148
No	213 (89.9)	1.00		1.00		59 (24.9)	1.00		1.00	
Yes	479 (65.8)	0.49 (0.41–0.57)		0.59 (0.41–0.85)		133 (18.3)	0.74 (0.54–1.01)		0.76 (0.53–1.10)	

Notes: [a]The percentage was defined as number of recurrent/dead patients divided by total number of patients in each subgroup. [b]Boardline significant association if $0.05 \leq P < 0.10$. The result is in bold, if $P < 0.05$.
Abbreviations: BMI, body mass index; FIGO, International Federation of Gynecology and Obstetrics; LN, lymph node; ER, estrogen receptor; PR, progesterone receptor.

tumor recurrence and death by multivariate Cox proportional hazards regression models. Specifically, patients with age at menophania above 15.5 years or with advanced stage tumor (III–IV) were more likely of poor survival (for recurrence: adjusted HR =1.81 and 1.67, 95% CI =1.31–2.50 and 1.02–2.75; for cancer death: adjusted HR =1.50 and 6.94, 95% CI =1.07–2.08 and 2.14–22.48; respectively). Complete cytoreduction was significantly associated with better survival (adjusted HR =0.46 and 0.40, 95% CI =0.31–0.68 and 0.25–0.63 for recurrence and death, respectively).

EXO1 genotypes predict clinical outcomes

Using multivariate Cox proportional hazards regression models, we found that rs851797AG/GG genotypes were significantly associated with recurrence and cancer death (Table 4, adjusted HR =1.30 and 1.38, 95% CI =1.11–1.52 and 1.02–1.88, respectively). Kaplan–Meier survival estimates showed that patients who carried rs851797AG/GG genotypes had poorer PFS and OS, compared with rs851797AA genotype carriers (log-rank test, P=0.002 and 0.025, respectively; Figure 1A and B). However, in the subgroup of type II tumor, the prognostic value of rs851797 was only observed in tumor recurrence (adjusted HR =1.44, 95% CI =1.01–2.07; Table S1). More interestingly, when combining all four *EXO1* SNPs, we found that patients who carried more than one risk genotype had a poor PFS than 0–1 risk genotype carriers (adjusted HR =1.30, 95% CI =1.02–1.65; Table 4).

The mRNA secondary structure is critical for mRNA–miRNA interactions. Thus, we explored whether the *EXO1* rs851797 SNP in the 3′-UTR of *EXO1* could alter the local secondary structure of the *EXO1* mRNA based on the MFE value. Using the RNAfold online tool and inputting 201-nt long DNA sequence of the *EXO1* 3′-UTR containing the rs851797 locus, we found that the MFE changed from −31.2 kcal/mol to −34.0 kcal/mol, when the rs851797 allele changed from A to G (Figure 2).

Discussion

To the best of our knowledge, this is the first study that investigates associations between potentially functional SNPs in *EXO1* and clinical outcomes in EOC patients. In the present study with a total of 1,030 EOC cases, we found that patients who carried rs851797AG/GG genotypes had poorer PFS and OS, compared with rs851797AA genotype carriers. Further in silico analysis indicated that rs851797 might be a functional SNP by affecting mRNA secondary structure of *EXO1*, thus contribute to tumor progression.

Table 4 *EXO1* genotypes predict prognosis in epithelial ovarian cancer patients

EXO1 genotypes	Recurrence	Univariate		Multivariate		Death	Univariate		Multivariate	
	N (%)[a]	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	N (%)[a]	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
All subjects	752 (73.0)					207 (20.1)				
rs1047840 (HWE =0.581)			0.263		0.170			0.458		0.306
GG	495 (72.2)	1.00		1.00		143 (20.9)	1.00		1.00	
AG/AA	257 (74.7)	1.09 (0.94–1.27)		1.25 (0.91–1.73)		64 (18.6)	0.89 (0.67–1.20)		1.20 (0.85–1.71)	
rs9350 (HWE =0.594)			0.400		0.693			0.716		0.501
CC	246 (72.6)	1.00		1.00		68 (20.1)	1.00		1.00	
CT/TT	503 (73.1)	0.94 (0.80–1.09)		0.94 (0.67–1.31)		138 (20.1)	0.95 (0.71–1.27)		0.89 (0.62–1.26)	
rs851797 (HWE =0.791)			**0.002**		**0.001**			**0.025**		**0.038**
AA	240 (69.8)	1.00		1.00		59 (17.2)	1.00		1.00	
AG/GG	512 (74.6)	1.28 (1.10–1.49)		1.30 (1.11–1.52)		148 (21.6)	1.41 (1.04–1.92)		1.38 (1.02–1.88)	
rs3754093 (HWE =0.904)			0.650		0.405			0.712		0.551
AA	300 (73.9)	1.00		1.00		83 (20.4)	1.00		1.00	
AG/GG	451 (72.4)	1.04 (0.89–1.20)		1.14 (0.84–1.56)		124 (19.9)	1.05 (0.80–1.40)		0.90 (0.65–1.26)	
Combination effect			**0.014**		**0.034**			0.253		0.247
0–1 risk genotype	130 (69.5)	1.00		1.00		34 (18.2)	1.00		1.00	
>1 risk genotypes	622 (73.8)	1.27 (1.05–1.53)		1.30 (1.02–1.65)		173 (20.5)	1.24 (0.86–1.80)		1.29 (0.84–1.98)	

Note: [a]The percentage was defined as number of recurrent/dead patients divided by total number of patients in each subgroup. The result is in bold, if $P<0.05$.
Abbreviation: HWE, Hardy–Weinberg equilibrium.

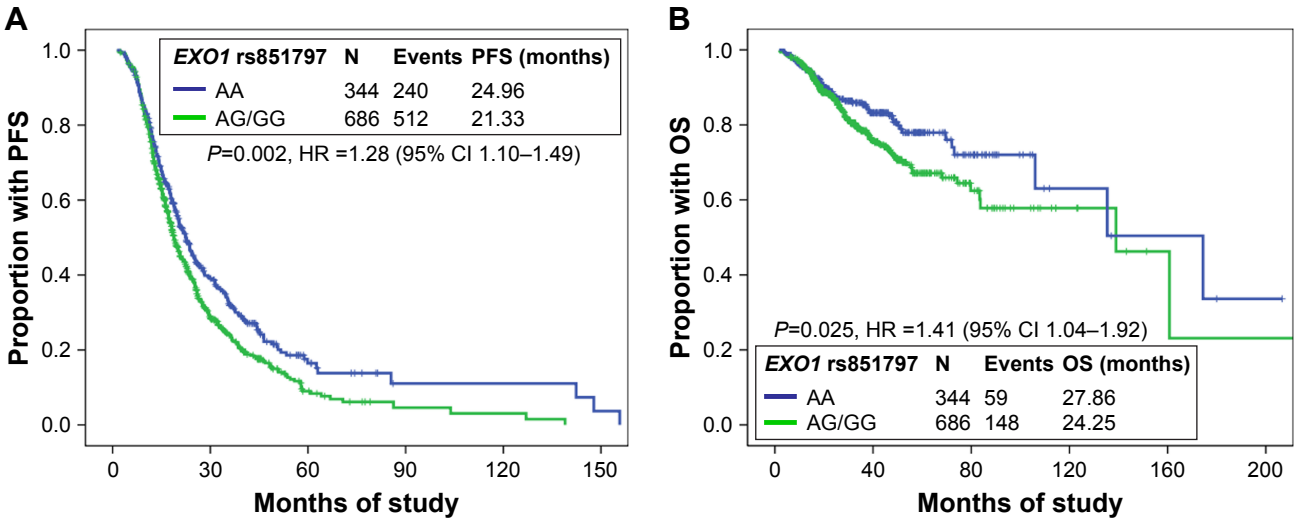

Figure 1 *EXO1* genotypes predict clinical outcomes in Chinese ovarian cancer patients. *EXO1* rs851797 AG/GG genotypes were significantly associated with poor (**A**) progression-free survival (PFS) and (**B**) overall survival (OS).

EXO1 polymorphisms have previously been reported to be associated with the development of many other types of human cancer. Meta-analysis showed that *EXO1* rs851797 was conferred an increased overall susceptibility to cancer in an allelic model.[11] Recently, a pooled genome-wide association study reported that the *EXO1* polymorphism region (1q43) was associated with the risk of EOC.[17] However, in our unpublished case–control study with a total of 1,320 EOC patients and 1,383 normal female controls, there were no significant associations between *EXO1* rs851797 genotype and EOC susceptibility in Chinese Han women (unpublished data). Based on the HapMap database, the frequency of rs851797 AG/GG genotype varies among ethnics, with 100%, 82.6%, and 70% in European, African–American, and Asian, respectively. On the other hand, the risk factor and mechanisms of genetic susceptibility might be different

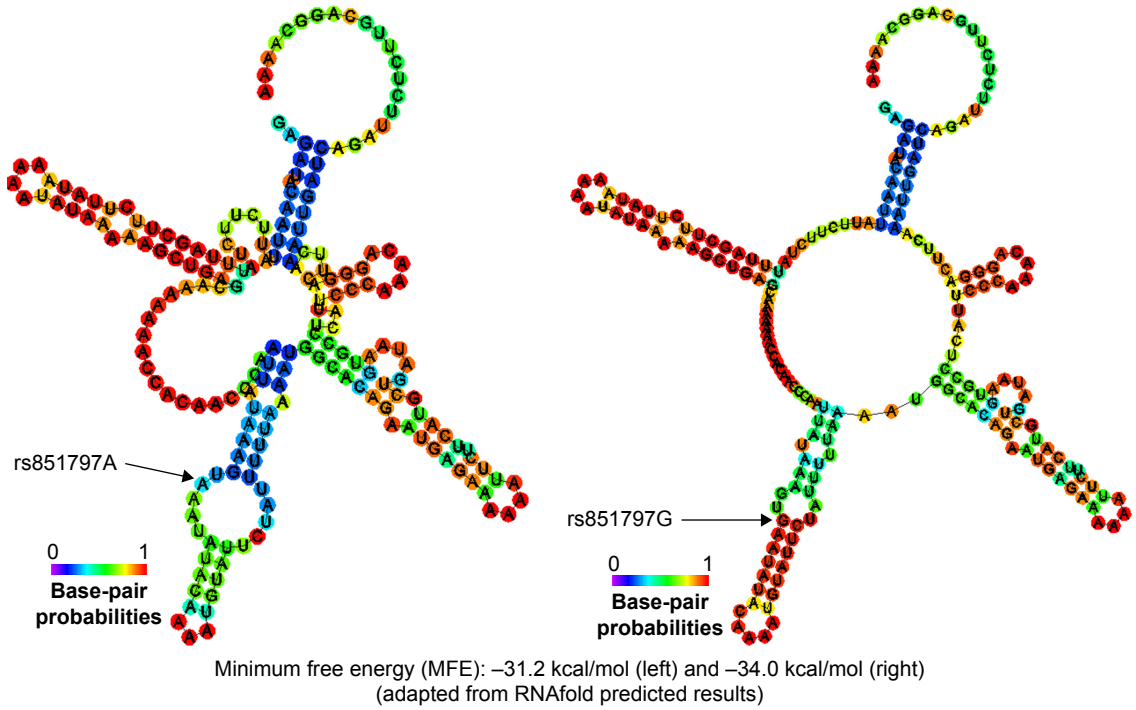

Figure 2 In silico analysis of potential functional rs851797 variant. The predicted secondary structure of the *EXO1* mRNA. The secondary structures of the *EXO1* 3′-UTR were predicted by inputting two 201-nt long DNA sequences centering rs851797 into RNAfold, with either the A (left) or G (right) allele. The figures and the values of minimum free energy were generated by RNAfold (http://rna.tbi.univie.ac.at).

from that of tumor progression. It could be necessary to further evaluate the prognostic role of *EOX1* polymorphisms. We here reported a potentially functional variant in *EXO1* (rs851797) that involved in the process of ovarian cancer progression and prognosis. Unlike the findings from head and neck squamous cell carcinoma[15] and non-small cell lung cancer,[16] we did not observe predictive values of rs1047840 and rs9350 polymorphisms in EOC survival. It might be caused by the heterogeneity among various types of human cancer.

EXO1, which is located at chromosome 1q42–1q43, contains one untranslated exon followed by 13 coding exons, encodes a protein with 846 amino acid, and acts as a double-stranded DNA exonuclease.[6,7] Accumulated data have demonstrated that *EXO1* participates in the process of DNA damage repair, replication, and the maintenance of genomic stability through its exonulease activity to correct overhanging flap structures.[6,7] *EXO1*-mutant cells showed increased microsatellite instability and incomplete MMR capability.[23] In addition, higher mutation rates were accompanied with higher susceptibility to lymphomas.[23] *EXO1* has also been implicated in hereditary nonpolyposis colorectal cancer due to its role in DNA MMR.[24]

SNPs are the most common type of genetic variations. At least 14,304 SNPs have been identified in the *EXO1* gene (http://www.ncbi.nlm.nih.gov/projects/SNP). The majority of SNPs are silent or have limited influences on the function and expression of genes. Only a small fraction of SNPs have been identified to be involved in the process of tumor progression as potentially functional variants.[25] It is well in accordance with the theory of the driver and passenger somatic mutations in human cancer genome.[26] Rs851797, located at 3′-UTR of *EXO1* gene, was found to be associated with risk of several human cancers.[11] In silico analysis by using the RNAfold online tool showed that rs851797 could alter the local secondary structure of the *EXO1* mRNA based on the MFE value, thus contribute to tumor progression and prognosis. Given that the mRNA secondary structure is critical for mRNA–miRNA interactions, it was reasonable to suspect the rs851797 variant as a functional SNP. Moreover, the intrinsic mechanism might be explained by that the 3′-UTR could contain sequence motifs crucial for the regulation of transcription, mRNA stability, and cellular location of the mRNA or the binding of microRNA.[27] Further functional studies are warranted to validate the association data.

Several limitations in the present study need to be addressed. First, there are selection bias and information bias by the study design, which may have been minimized by the adjustment for potential confounding factors in final multivariate analyses. Second, because of the retrospective nature of the study design and the recall bias, it is difficult to evaluate all prognostic factors exactly, especially, no enough information about patient's response to platinum. Third, further investigations of genotype–phenotype associations and functional analysis for this SNP are warranted.

In summary, in the current cohort study with 1,030 ovarian cancer patients, we found that the *EXO1* rs851797 polymorphism could predict clinical outcomes of EOC. In addition, age at menophania, FIGO stage, and complete cytoreduction might be independently prognostic factors for ovarian cancer. However, well-designed larger, prospective studies with functional analysis are warranted to validate our findings.

Abbreviations

EOC, epithelial ovarian cancer; SNP, single-nucleotide polymorphism; *EXO1*, Exonuclease 1; MMR, mismatch repair; FUSCC, Fudan University Shanghai Cancer Center; FIGO, International Federation of Gynecology and Obstetrics; UTR, untranslated region; MFE, minimum free energy; PFS, progression-free survival; OS, overall survival.

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Author contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. R Zang and T Shi were involved in study concept, design, and drafting of the manuscript. All the authors contributed to acquisition, analysis, or interpretation of data and critical revision of the manuscript for important intellectual content. T Shi, R Jiang, and P Wang contributed to statistical analysis.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 *EXO1* genotypes predict prognosis by the subgroup of histopathological tumor type

EXO1 genotypes	Recurrence N (%)[a]	Univariate		Multivariate		Death N (%)[a]	Univariate		Multivariate	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Tumor type I										
*EXO1*_rs1047840			0.253		0.925			0.459		**0.040**
GG	89 (63.6)	1.00		1.00		24 (17.1)	1.00		1.00	
AG/AA	60 (71.4)	1.21 (0.87–1.69)		1.05 (0.37–3.02)		18 (21.4)	1.26 (0.68–2.33)		2.74 (1.05–7.15)	
*EXO1*_rs9350			0.861		0.596			0.382		0.253
CC	49 (62.8)	1.00		1.00		17 (21.8)	1.00		1.00	
CT/TT	99 (68.3)	1.03 (0.73–1.46)		0.73 (0.23–2.34)		24 (16.6)	0.76 (0.40–1.42)		0.59 (0.24–1.46)	
*EXO1*_rs851797			0.381		0.866			0.100		0.167
AA	57 (64.0)	1.00		1.00		12 (13.5)	1.00		1.00	
AG/GG	92 (68.2)	1.16 (0.83–1.63)		1.09 (0.40–2.95)		30 (22.2)	1.76 (0.90–3.44)		2.14 (0.73–6.31)	
*EXO1*_rs3754093			0.674		0.578			0.591		0.738
AA	56 (71.8)	1.00		1.00		14 (18.0)	1.00		1.00	
AG/GG	93 (63.7)	0.93 (0.66–1.31)		0.76 (0.29–1.99)		28 (19.2)	1.20 (0.62–2.31)		0.85 (0.34–2.17)	
Tumor type II										
*EXO1*_rs1047840			0.449		0.055[b]			0.438		0.459
GG	398 (74.7)	1.00		1.00		115 (21.6)	1.00		1.00	
AG/AA	187 (75.4)	1.07 (0.90–1.27)		1.40 (0.99–1.98)		46 (18.6)	0.87 (0.62–1.23)		1.16 (0.78–1.73)	
*EXO1*_rs9350			0.193		0.379			0.953		
CC	189 (75.3)	1.00		1.00		49 (19.5)	1.00		1.00	
CT/TT	394 (74.6)	0.89 (0.75–1.06)		0.85 (0.60–1.22)		112 (21.2)	1.01 (0.72–1.41)		0.90 (0.61–1.33)	
*EXO1*_rs851797			**0.004**		**0.045**			0.130		0.595
AA	176 (71.8)	1.00		1.00		45 (18.4)	1.00		1.00	
AG/GG	409 (76.3)	1.30 (1.09–1.55)		1.44 (1.01–2.07)		116 (21.6)	1.31 (0.93–1.85)		1.11 (0.75–1.65)	
*EXO1*_rs3754093			0.325		0.159			0.958		0.726
AA	240 (75.0)	1.00		1.00		68 (21.3)	1.00		1.00	
AG/GG	344 (74.8)	1.09 (0.92–1.28)		1.28 (0.91–1.80)		93 (20.2)	1.01 (0.74–1.38)		0.94 (0.66–1.34)	

Notes: [a]The percentage was defined as number of recurrent/dead patients divided by total number of patients in each subgroup. [b]Boardline significant association if $0.05 \leq P < 0.10$; the result is in bold, if $P < 0.05$.