Infrequent mutations of the *PPP2R1A* and *PPP2R1B* genes in patients with ovarian cancer

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Abstract. Protein phosphatase 2, regulatory subunit A, α (*PPP2R1A*) and β (*PPP2R1B*) are paralogous subunits of the heterotrimeric protein phosphatase 2 (PP2A) holoenzyme that catalyzes the dephosphorylation of target substrate proteins. Subtype-specific *PPP2R1A* mutations have been frequently observed in ovarian and endometrial cancer. Mutations in the paralogous genes were frequently observed in human malignancies. Thus, the present study aimed to analyze the mutation frequencies of the paralogous *PPP2R1A* and *PPP2R1B* genes in patients with primary and secondary ovarian cancer. A total of 251 patients with primary (n=234) and secondary (n=17) ovarian cancer were analyzed for the presence of *PPP2R1A* and *PPP2R1B* mutations by direct sequencing. For *PPP2R1A*, a heterozygous, somatic mutation (c.771G>T, p.W257C) was identified in 1 out of 37 patients (2.7%) with primary ovarian endometrioid carcinoma. The mutant sample was that of a 46-year-old female, who was also diagnosed with ectopic endometriosis in the benign ovary. No *PPP2R1A* mutations were detected in the remaining 250 patients with ovarian cancer. For *PPP2R1B*, no mutations were detected in our samples. The results of this study suggested that *PPP2R1A* mutations are less common in Chinese patients with ovarian cancer when compared with European and American patients. Furthermore, our study also supported previous observations that *PPP2R1B* mutations were absent in ovarian cancer, suggesting that *PPP2R1B* mutations are not actively involved in the pathogenesis of ovarian cancer.

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Introduction

Protein phosphatase 2, regulatory subunit A, α (*PPP2R1A*) and β (*PPP2R1B*) are paralogous subunits of the heterotrimeric protein phosphatase 2 (PP2A) holoenzyme, which catalyzes the dephosphorylation of target substrate proteins (www.ensembl.org) (1). *PPP2R1A* and *PPP2R1B* belong to the Huntington-elongation-A subunit-TOR (HEAT) repeat protein family, and are required as scaffolds for the formation of the heterotrimeric PP2A complex (2). *PPP2R1A* and *PPP2R1B* each contain 15 tandemly repeated HEAT motifs. It has been previously demonstrated that the HEAT repeats 2-8 and 11-15 were involved in binding to the regulatory and catalytic subunits of PP2A, respectively (3). In addition, mutations located in the HEAT 2 and 11 motifs promoted tumorigenesis; the mutant *PPP2R1A* proteins were defective in binding to the regulatory and catalytic subunits of PP2A, resulting in a decrease in PP2A activity and thus facilitating tumor progression (4).

A previous whole-exome sequencing attempt identified recurrent *PPP2R1A* mutations in 3 out of 42 patients (7.1%) with ovarian clear cell carcinoma (OCCC) (5). Subsequent studies confirmed this finding and also revealed frequent *PPP2R1A* mutations in patients with ovarian endometrioid carcinoma (6-8). Additionally, a high frequency of *PPP2R1A* mutations was also identified in several subtypes of endometrial carcinoma, with the highest frequency demonstrated in the serous subtype (6-9). Furthermore, previous studies revealed that *PPP2R1A* mutations were present in patients with breast and lung malignancies, but absent in patients with

melanoma and Wilms' tumor (10,11), suggesting a general effect of *PPP2R1A* mutations on human cancer. Until presently, the majority of *PPP2R1A* mutations identified in human cancer were restricted to codons 179-183 and 256-258, which correspond to the HEAT 5 and HEAT 7 motifs, respectively (http://www.uniprot.org/) (5-9). Therefore, the *PPP2R1A* codons 179-183 and 256-258 were considered potential mutational hotspots in human malignancies, particularly in ovarian and endometrial carcinoma.

PPP2R1B, the paralogous gene of *PPP2R1A*, was demonstrated to be mutated in breast and colorectal cancer, but not in ovarian cancer and certain other types of cancer (10,12-21). Although point mutations in the *PPP2R1B* gene have not been detected in ovarian cancer, it should be noted that a relatively small sample size was analyzed in these studies (16,17), and loss of heterozygosity (LOH) of *PPP2R1B* has been frequently observed in different subtypes of ovarian cancer (16). Additionally, accumulating evidence suggested that mutations in the homologous residues of paralogous genes were frequently observed in human malignancies (22-24), which suggested that *PPP2R1B* mutations may also present in patients with ovarian cancer. We hypothesized that *PPP2R1A* and *PPP2R1B* mutations are also involved in the pathogenesis of non-primary ovarian cancer. Therefore, to examine whether *PPP2R1A* and *PPP2R1B* mutations may be involved in the development of primary and secondary ovarian cancer, 251 Chinese patients with diverse subtypes of ovarian cancer were recruited to test this hypothesis.

Materials and methods

Patients and clinical data. A total of 251 formalin-fixed, paraffin-embedded (FFPE) ovarian cancerous and paired normal tissues were recruited from the Jiangxi Provincial Maternal and Child Health Hospital (Jiangxi, China). The sections were reviewed in a blinded manner by two pathologists, and only those with >70% cancerous cells were included in this study. In total, there were 234 primary and 17 secondary ovarian cancer samples. The primary ovarian cancer subtypes included ovarian serous cancer (n=76), OCCC (n=43), ovarian endometrioid carcinoma (n=37), mucinous ovarian carcinoma (n=15), ovarian germ cell tumor (n=33), ovarian sex cord-stromal tumor (n=18), other rare subtypes (n=12) and secondary ovarian cancer (n=17) (Table I); while all secondary types of cancer were Krukenberg tumors. This study conformed to the principles of the Declaration of Helsinki, and written informed consent was obtained from each subject prior to the study. The institutional review board of the Jiangxi Provincial Maternal and Child Health Hospital approved this study.

PPP2R1A and PPP2R1B mutational analyses. Genomic DNA was isolated from archival FFPE tissues using FFPE DNA kits (Omega Bio-Tek Inc., Doraville, GA, USA). For *PPP2R1A*, two short PCR fragments spanning codons 179-183 and 256-257 were amplified with the following primer pairs, respectively: Forward 1: 5'-GTACTTCCGGAACCTGTGCT-3' and reverse 1: 5'-AGCAAAACTCACCTGCTCGT-3'; forward 2: 5'-CTCTCCTCTCCCTAGGACTCG-3' and reverse 2: 5'-TGTGAACTTGTCAGCCACCA-3'. For *PPP2R1B*, the following primer pairs were used to amplify the PCR fragments spanning codons 191-195 and 268-269, corresponding to the homologous residues of codons 179-183 and 256-257 in *PPP2R1A* (Fig. 1): Forward 1: 5'-ATTCCGTTCCTTGTGCTCAG-3' and reverse 1: 5'-GGAGCATATCTGTGTCCCTTAAA-3'; forward 2: 5'-TAGGATTCAGTGCGCCTCCT-3' and reverse 2: 5'-TGAAAATCTGTCAGCCACCA-3'. The PCR products were sequenced using an ABI Prism 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The identified mutation was confirmed as either somatic or germline by sequencing the paired normal tissue.

Evolutionary conservation and protein sequence homology analyses. Evolutionary conservation analysis of the *PPP2R1A* mutation was performed using data obtained from GenBank for 14 different species, including *Homo sapiens* (*PPP2R1A*, GenBank accession no. NM_014225), *Pan troglodytes* (XM_001174546), *Pongo abelii* (NM_001132813), *Macaca mulatta* (NM_001257922.1), *Nomascus leucogenys* (XM_003269709), *Callithrix jacchus* (XM_002762433), *Rattus norvegicus* (NM_057140), *Mus musculus* (NM_016891), *Cricetulus griseus* (XM_003509396), *Canis lupus familiaris* (XM_845900), *Bos taurus* (NM_001037477), *Sus scrofa* (NM_214024), *Xenopus laevis* (NM_001086666) and *Danio rerio* (NM_213376).

Protein sequence homology analysis of human PPP2R1A (GenBank accession no. NP_055040.2) and PPP2R1B (NP_859050.1) was performed to determine the relationship between the two paralogous genes, using DNASTAR software (DNASTAR, Inc., Madison, WI, USA).

Results

Patient characteristics. A total of 251 patients with primary and secondary ovarian cancer were recruited from the Department of Pathology, Jiangxi Provincial Maternal and Child Health Hospital. The median age of the patients was 47 years (range, 5-75), and 106 out of the 251 patients were affected in both ovaries, while the remaining 145 patients were affected in either the left or the right ovary (Table IA).

PPP2R1A mutations in ovarian cancer. We screened the 234 primary and 17 secondary ovarian cancer samples for the presence of *PPP2R1A* hotspot mutations. The mutation type and frequency distribution are shown in Table IB. A somatic, heterozygous *PPP2R1A* mutation (c.771G>T, p.W257C) was identified in 1 out of 37 primary ovarian cancer patients (2.7%) with ovarian endometrioid carcinoma. The mutant sample was that of a 46-year-old female, who was also diagnosed with ectopic endometriosis in the benign ovary (Fig. 2). In addition, no *PPP2R1A* mutations were detected in the remaining 250 patients with ovarian cancer (Table IB).

The evolutionary conservation analysis suggested that the PPP2R1A p.W257C mutation was highly conserved among the 14 species ranging from *Homo sapiens* to *Danio rerio* (Fig. 3).

PPP2R1B mutations in ovarian cancer. We sequenced the genomic region of *PPP2R1B* spanning p.P191-R195 and p.S268-W269, the homologous residues corresponding to the potential mutational hotspots in the *PPP2R1A* gene. However,

Table I. Patient characteristics and *PPP2R1A* mutation distribution in Chinese patients with primary and secondary ovarian cancer.

A. Patient characteristics.

Characteristics	Value
Age at diagnosis (years)	
Median	47
Minimum	5
Maximum	75
Affected ovary (no. of patients)	
Both	106
Left	63
Right	82

B. *PPP2R1A* mutation distribution.

Cancer type	Frequency no. (%)	Nucleotide change	Amino acid change
Primary			
Epithelial			
Serous	0/76 (0.0)	-	-
Clear cell	0/43 (0.0)	-	-
Endometrioid	1/37 (2.7)	c.771G>T	p.W257C
Mucinous	0/15 (0.0)	-	-
Undifferentiated	0/3 (0.0)	-	-
Unclassified	0/4 (0.0)	-	-
Transitional cell	0/3 (0.0)	-	-
Mixed	0/2 (0.0)	-	-
Non-epithelial			
Germ cell tumor			
Yolk sac	0/11 (0.0)	-	-
Dysgerminoma	0/7 (0.0)	-	-
Teratoma	0/9 (0.0)	-	-
Mixed	0/6 (0.0)	-	-
Sex cord-stromal tumor			
Granulosa cell	0/16 (0.0)	-	-
Sertoli-Leydig	0/2 (0.0)	-	-
Secondary			
Krukenberg tumors	0/17 (0.0)	-	-
Total	251 (100)	c.771G>T	p.W257C

PPP2R1A, protein phosphatase 2, regulatory subunit A, α.

no mutations were detected among the 234 primary and 17 secondary patients with ovarian cancer.

Discussion

Recurrent *PPP2R1A* mutations have been identified in OCCC patients in a whole-exome sequencing study (5). This observation was confirmed by four studies that followed, and frequent *PPP2R1A* mutations were also identified in several other subtypes of ovarian and endometrial carcinoma (6-9). In addition, previous studies detected *PPP2R1A* and *PPP2R1B* mutations in human malignancies with variable frequencies (10-21,25).

In the present study, 234 primary and 17 secondary Chinese patients with ovarian cancer were analyzed for the presence of potential *PPP2R1A* and *PPP2R1B* hotspot mutations. A previously reported PPP2R1A p.W257C (c.771G>T) somatic mutation was detected in the sample from one patient with ovarian endometrioid carcinoma out of our 251 samples. This particular patient was also diagnosed with ectopic endo-

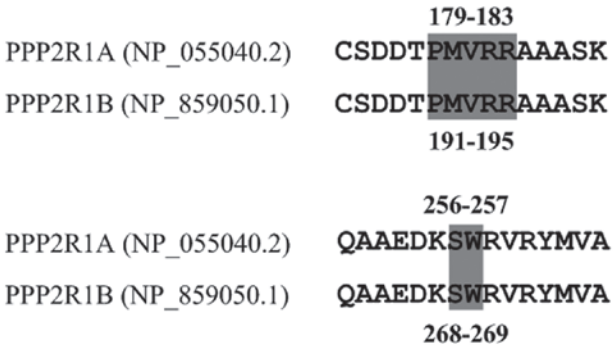

Figure 1. Protein sequence homology analysis of protein phosphatase 2, regulatory subunit A, α (PPP2R1A) and β (PPP2R1B). Underlined, affected codons.

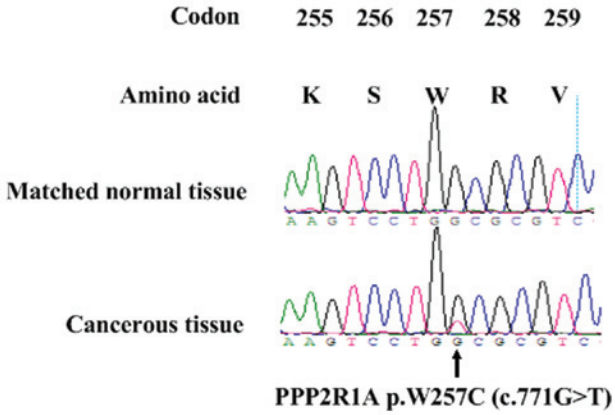

Figure 2. Sequencing electropherograms of the protein phosphatase 2, regulatory subunit A, α (*PPP2R1A*) p.W257C mutation. The arrow refers to the location of the mutation.

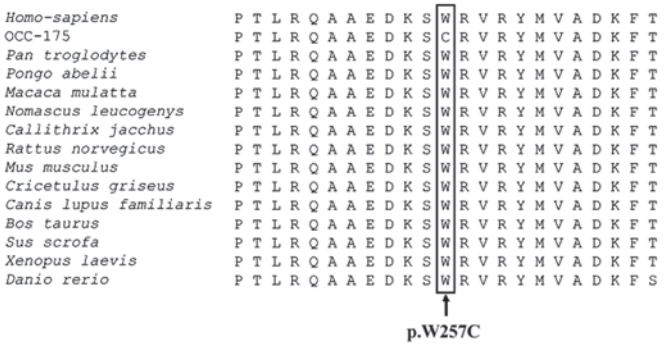

Figure 3. Evolutionary analysis of the protein phosphatase 2, regulatory subunit A, α (*PPP2R1A*) p.W257C mutation. Patient 'OCC-175' harbored the *PPP2R1A* p.W257C mutation, as indicated by the arrow.

metriosis in the benign ovary. We failed to detect the presence of any mutations in the remaining individuals. In addition, no *PPP2R1B* mutations were detected in our samples.

The combined results of previous studies demonstrated that *PPP2R1A* mutations frequently occurred in breast, lung and endometrial carcinoma, as well as in OCCC and ovarian endometrioid carcinoma (5-11). Furthermore, the mutation frequencies of *PPP2R1A* in OCCC and ovarian endometrioid carcinoma patients were 4.1-9.1% (mean, 7.4%) and 10.0-12.2% (mean, 11.1%), respectively (5-8). However, the mutation frequencies of *PPP2R1A* in our OCCC and ovarian endometrioid carcinoma samples were 0% (0/43) and 2.7% (1/37), respectively, which were lower than those observed in the European and American counterparts (5-8). One reason for these discrepancies may be population differences, suggesting that *PPP2R1A* mutations may increase the propensity for ovarian cancer when present in European and American individuals compared with Chinese individuals. An alternative explanation for these differences may be the relatively small sample size analyzed in our study, therefore, larger sample sizes would be required to test this theory. Moreover, we did not detect any *PPP2R1A* mutations in OSC or ovarian mucinous carcinoma, which was consistent with previous observations (6,8).

A previous study demonstrated that PPP2R1A p.E64D, p.E64G and p.R418W mutations located in the HEAT 2 and 11 motifs, respectively, contributed to carcinogenesis by impairing the activity of PP2A (http://www.uniprot.org/) (4). Mutations of the *PPP2R1A* codon 257 have been frequently observed in ovarian cancer (6-8), and are highly conserved across multiple species (Fig. 3). Additionally, the PPP2R1A p.W257C mutation was located in the HEAT 7 repeat motif (http://www.uniprot.org/), which has been proposed to be the domain interacting with the regulatory subunits of PP2A, and thus crucial for PP2A activity (3). Overall, we speculated that the PPP2R1A p.W257C mutation may contribute to the pathogenesis of Chinese patients with ovarian endometrioid carcinoma. Nevertheless, the pathogenicity of the PPP2R1A p.W257C mutation remains unclear, therefore, functional assays are required to decipher the potential role of this mutation in the development of ovarian cancer.

We also screened for potential mutations of p.P191-R195 and p.S268-W269, the homologous residues corresponding to potential mutational hotspots in the *PPP2R1A* gene. However, no mutations were evident in our samples. This was consistent with previous observations that *PPP2R1B* mutations were absent in patients with ovarian cancer (16,17). Combined with the results of previous studies (16,17), the absence of *PPP2R1B* mutations in the ovarian cancer samples analyzed in our study suggested that *PPP2R1B* mutations may not be actively involved in the development of primary and secondary ovarian cancer. However, this conclusion should be treated with caution, as the most frequent genetic aberrations of *PPP2R1B* observed in human cancers were heterozygous or homozygous LOH (10,19). Furthermore, we did not exclude the possibility that there may be mutations in other regions of this gene contributing to the development of ovarian cancer.

In conclusion, we analyzed 251 Chinese patients with primary and secondary ovarian cancer for the presence of *PPP2R1A* and *PPP2R1B* mutations. The mutation frequencies of *PPP2R1A* were 0.0 and 2.7% in OCCC and ovarian endometrioid carcinoma patients, respectively, which were lower than those of their corresponding European and American counterparts. In addition, we did not detect any *PPP2R1A* mutations in OSC and ovarian mucinous carcinoma, which was consistent with previous observations. Moreover, no *PPP2R1B* mutations were identified in our samples, and this observation was consistent with previous studies on ovarian

cancer. Our results suggested that mutations of *PPP2R1A*, but not *PPP2R1B*, may be involved in the pathogenesis of Chinese patients with ovarian cancer.

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