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Short Communication

Somatic Mutations of *PPP2R1A* in Ovarian and Uterine Carcinomas

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Exome sequencing of ovarian clear-cell carcinoma has identified somatic mutations in *PPP2R1A*, a subunit of protein phosphatase 2A. The present study was performed to determine the frequency of *PPP2R1A* mutations in exon 5, which harbors previously reported mutation hot spots, and adjacent exon 6, in 209 ovarian and 56 uterine tumors of various histologic subtypes. *PPP2R1A* mutations were demonstrated in 10 of 110 type I ovarian tumors (9.1%) including low-grade serous, low-grade endometrioid, clear-cell, and mucinous carcinomas. In contrast, none of 71 type II ovarian (high-grade serous) carcinomas exhibited *PPP2R1A* mutations. Moreover, *PPP2R1A* mutations were observed in 2 of 30 type I uterine (endometrioid) carcinomas (6.7%) and 5 of 26 type II uterine (serous) carcinomas (19.2%). Of the 18 mutations, 13 affected the R182 or 183, and there were 5 novel mutations including 3 involving S256, 1 involving W257, and 1 involving P179. All mutations were located in the α-helix repeats near the interface between the A subunit and the regulatory B subunit of the enzyme complex. These data provide new evidence that *PPP2R1A* somatic mutations occur in certain types of uterine and ovarian neoplastic lesions, especially uterine serous carcinomas, and suggest that mutation of *PPP2R1A* may participate in the pathogenesis of ovarian type I and uterine type II carcinomas. *(Am J Pathol 2011, 178:1442–1447; DOI: 10.1016/j.ajpath.2011.01.009)*

A recent genome-wide sequencing analysis of all exons from ovarian clear cell carcinomas led to the discovery of somatic missense mutations in *PPP2R1A* in approximately 7% of these tumors.[1] *PPP2R1A* encodes a constant regulatory subunit of the protein phosphatase 2A holoenzyme, which is one of four major serine-threonine phosphatases.[2] Protein phosphatase 2A is composed primarily of a catalytic C subunit and a scaffolding subunit A (PPP2R1A and PPP2R1B) and a regulatory subunit B. Protein phosphatase 2A can be regulated by binding of at least 18 different regulatory B subunits to the core enzyme. The regulatory B subunit has been implicated in controlling the substrate specificity, cellular localization and enzymatic activity of protein phosphatase 2A, which participates in negative regulation of cellular proliferation among several other cellular functions.[2,3] Moreover, the regulatory subunit competes with virus-producing proteins including the small T antigens of the papovaviruses SV40 and polyoma, and the middle T antigen of polyoma,[4] which suggests that protein phosphatase 2A has a role in the pathogenesis of virus-associated tumors.

Uterine carcinomas can be classified as type I or type II, and develop along unique molecular pathways. Type I uterine tumors are composed of endometrioid carcinomas, which frequently harbor mutations in *CTNNB1*, *PTEN*, *ARID1A (BAF250A)*, and *PIK3CA*, whereas type II tumors are composed of serous carcinomas, which contain *TP53* mutations in most cases.[5] Patients with type I tumors are typically younger, and carcinoma develops at an earlier stage and has a more indolent clinical course than in patients with type II tumors. Type I uterine carcinomas arise from endometrial hyperplasia, whereas type

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II uterine carcinomas develop from endometrial intraepithelial carcinoma. Compared with uterine carcinoma, ovarian carcinoma is far more complex and includes more histologic subtypes than uterine tumors do.[6] To provide a conceptual framework for the study of the pathogenesis of ovarian cancer, a dualistic model was proposed that organizes the clinical, pathologic, and molecular features of ovarian cancer by categorizing them into type I and type II groups, similar to the classification of uterine carcinoma.[7] Type I tumors include low-grade serous, low-grade endometrioid, clear-cell, and mucinous carcinomas, which develop in a stepwise fashion from well-established precursor lesions such as borderline tumors and endometriosis. Type I tumors typically are manifested as a large mass confined to one ovary, and are associated with a relatively good prognosis. They typically exhibit mutations in *KRAS*, *PIK3CA*, *PTEN,* and *CTNNB1*, and rarely in *TP53*.[6,8] In contrast, type II tumors include high-grade serous carcinomas, malignant mixed mesodermal tumors (carcinosarcomas), and undifferentiated carcinomas. Type II tumors constitute most ovarian cancers and account for most deaths. They are detected at an advanced stage (stages II to IV) in greater than 75% of cases, grow rapidly, and are highly aggressive. Type II tumors, of which high-grade serous carcinoma is the prototypic type, are chromosomally highly unstable,[9,10] and harbor *TP53* mutations in most cases but rarely demonstrate mutations in *KRAS*, *PIK3CA*, *PTEN*, and *CTNNB1*.[11,12]

The present study sought to extend the previous observation of somatic mutations of *PPP2R1A* in ovarian clear-cell carcinomas[1] by examining the mutational profiles of a variety of histologic types of ovarian and uterine carcinomas that have been broadly classified as type I and type II tumors.[5,7] Sequence analysis was performed in 256 ovarian and uterine tumors, focusing on exon 5, which harbors the codons that encode amino acids 182 and 183, which, according to the previous study, represent mutational "hot spots" in *PPP2R1A*.[1] Exon 6 was also analyzed in all tumors.

Materials and Methods

Tissue Specimens

Tissue specimens from 265 ovarian and uterine tumors were analyzed for mutations at exons 5 and 6 including the codons 182 and 183 of *PPP2R1A*.[1] Type I ovarian tumors included 44 clear-cell carcinomas, 6 mucinous tumors, 20 low-grade serous carcinomas, and 40 low-grade endometrioid carcinomas. Type I uterine carcinoma included 30 low-grade (grade 1) endometrioid carcinomas. Type II ovarian tumors included 71 high-grade serous carcinomas. Type II uterine carcinomas included 26 serous carcinomas, 3 of which contained clear-cell carcinoma components. Those uterine carcinomas with mixed serous and clear-cell histologic features were classified as serous carcinoma. The diagnosis of uterine serous carcinomas was supported at immunohistochemistry, which demonstrated diffuse p53 staining and a high (>50%) Ki-67 labeling index. One hundred sixty-seven specimens were affinity-purified using BerEP4 antibody-conjugated magnetic beads (Invitrogen Corp., Carlsbad, CA) following a previously described protocol[13]. The remaining samples were obtained from FFPE tissues after microdissection to enrich tumor cells. The ovarian clear-cell carcinomas analyzed represented new cases that had not been studied in the previous report.[1] In addition, 14 serous, 13 mucinous, and 1 seromucinous ovarian borderline tumors were analyzed. Specimens were obtained from The Johns Hopkins Hospital or the National Taiwan University Hospital. H&E-stained sections were reviewed to confirm the diagnosis before performing the experiments. All human tissue was collected anonymously using protocols approved by the institutional review boards of both hospitals.

DNA Extraction and Mutation Analysis

Genomic DNA was isolated using a DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA) for BerEP4 bead–purified tumor cells, and a QIAamp DNA Micro Kit (Qiagen, Inc.) for paraffin-embedded tissues. To enrich tumor cells from paraffin sections, pipette tips were used to individually scrape off the normal tissues, followed by tumor tissues, which were collected in separate microfuge tubes. For tumor tissues, only regions containing at least 60% of tumor cells were dissected. The selection of normal and tumor areas was made by two pathologists (I.M.S. and E.K.). PCR amplification was performed using the following primer pairs, which were designed to amplify the genomic DNA fragment of exon 5 flanking the arginine couplet at codons 182 and183: forward primer 5′-TACTTCCGGAACCTGTGCTC-3′ and reverse primer 5′-CCAGGAAGCAAAACTCACCT-3′. The PCR primers used to amplify exon 6 were: forward primer 5′-GTTCCTGCCCATGAAAGAGA-3′, reverse primer 5′-TTATTGCTCAAACGCCCAAT-3′, and sequencing primer 5′-AATGGTTCCATCGGCCTAAT-3′.

PCR conditions were as follows: 94°C for 2 minutes; three cycles at 94°C for 15 seconds, 64°C for 30 seconds, and 70°C for 30 seconds; three cycles at 94°C for 15 seconds, 61°C for 30 seconds, and 70°C for 30 seconds; three cycles at 94°C for 15 seconds, 58°C for 30 seconds, and 70°C for 30 seconds; and 30 cycles at 94°C for 15 seconds, 57°C for 30 seconds, and 70°C for 30 seconds, followed by 70°C for 5 minutes. Sanger DNA sequencing was performed by Beckman Coulter Genomics (Danvers, MA) using the sequencing primer 5′-CAAAACTCACCTGCTCGTCA-3′. Mutational analysis was performed using a software package (Mutation Surveyor; SoftGenetics LLC, State College, PA). To confirm somatic mutations, DNA from matched normal tissue was sequenced using the same method. All of the detected mutations were repeated at least twice to confirm the results and rule out potential PCR errors.

Table 1. Summary of *PPP2R1A* Mutation in Uterine and Ovarian Tumors

Tumor	Tumor type	No. of cases	Cases with mutation, no. (%)
Uterine cancer			
Endometrioid	I	30	2 (6.7)
Serous*	II	26	5 (19.2)
Ovarian cancer			
Low-grade serous	I	20	0 (0)
Clear cell	I	44	4 (9.1)
Low-grade endometriod	I	40	4 (10)
Mucinous	I	6	2 (33.3)
High-grade serous	II	71	0
Ovarian borderline tumor			
Serous		14	0
Mucinous		13	0
Seromucinous		1	1 (100)

*Three uterine carcinomas exhibited mixed features of clear-cell and serous carcinoma.

Results

Of 265 tumor tissues analyzed, *PPP2R1A* mutations at either exon 5 or 6 were detected in 18 tumors (Table 1). Their associated normal tissues contained wild-type sequences, confirming that the *PPP2R1A* mutations were somatic. The tumors with *PPP2R1A* mutations were of various subtypes of ovarian or uterine tumors except ovarian high-grade and low-grade serous carcinoma, in which no somatic mutations were found. Specifically, in ovarian carcinomas, *PPP2R1A* mutations were found in 4 of 44 ovarian clear-cell carcinomas (9.1%), 2 of 6 ovarian mucinous carcinomas (33.3%), and 4 of 40 ovarian low-grade endometrioid carcinomas (10%). In addition, *PPP2R1A* mutation was also recorded in 1 of 28 ovarian borderline tumors (3.6%). The mutation was observed in the seromucinous borderline tumor at codon 183 but not in the other borderline tumors. In uterine carcinomas, mutations were detected in 5 of 26 serous carcinomas (19.2%) (including those cases with mixed clear-cell and serous carcinoma), of which mutation frequency was greater than 6.7% in the uterine endometrioid carcinomas. Among the mutations, 1 uterine carcinoma demonstrated mixed clear-cell and serous components. Thus, 18 *PPP2R1A* mutations were identified. Of these, 13 mutations affected the R182 and R183 hot spots, which were identified in a previous report,[1] and there were 5 novel mutations including 3 involving S256, 1 involving W257, and 1 involving P179 (Table 2). All *PPP2R1A* mutations identified were missense mutations and were heterozygous. Representative chromatograms of somatic alterations from an ovarian low-grade endometrioid carcinoma (6026T) and a uterine serous carcinoma (319T) are shown in Figure 1.

In addition, mutation profiles of *PPP2R1A*, *KRAS*, and *PIK3CA* were determined. This analysis included cases with *PPP2R1A* mutations that had been previously reported (three clear-cell carcinoma tissue samples, three clear-cell carcinoma cell lines, and one pancreatic cancer tissue),[1,14] to increase the number of cases. The

Table 2. Mutation Profiles of *PPP2R1A*, *KRAS*, and *PIK3CA*

Sample	Histologic subtype	Gene		
		PPP2R1A	*K-RAS*	*PIK3CA*
TW-7	Ovarian clear cell	R183W (547 C>T)	No mutation	No mutation
TW-11	Ovarian clear cell	R183W (547 C>T)	No mutation	No mutation
TW-24	Ovarian clear cell	R183Q (548 G>A)	No mutation	No mutation
TW-28	Ovarian clear cell	R183W (547 C>T)	No mutation	No mutation
OV81†	Ovarian clear cell	R183W (547 C>T)	No mutation	No mutation
109T†	Ovarian clear cell	R182W (544 C>T)	No mutation	E545K (1633 G>A)
192TCS†	Ovarian clear cell	R183G (547 C>G)	G12D (35 G>A)	No mutation
KK†	Clear-cell carcinoma cell line	R183Q (548 G>A)	No mutation	E545A (1634 A>C)
OVISE†	Clear-cell carcinoma cell line	R183W (547 C>T)	No mutation	No mutation
OVTOKO†	Clear-cell carcinoma cell line	R183G (547 C>G)	No mutation	No mutation
KT-41	Mucinous	R183Q (548 G>A)	G12D (35 G>A homo)‡	No mutation
KT-44	Mucinous	R183Q (548 G>A)	G12D (35 G>A)	No mutation
KT-36	Ovarian endometrioid	R183W (547 C>T)	No mutation	E545A (1634 A>C)
TS	Ovarian endometrioid	R183W (547 C>T)	No mutation	M1043T (3128T>C)
OS	Ovarian endometrioid	R182W (544 C>T)	No mutation	N1044K (3132T>A)
EM-17T	Uterine endometrioid	R183W (547 C>T)	No mutation	No mutation
EM-21T	Uterine endometrioid	R182W (544 C>T)	No mutation	H1047R (3140 A>G)
314TCS	Ovarian seromucinous borderline	R183W (547 C>T)	G12D (35 G>A)	H1047R (3140 A>G)
6026TCS	Ovarian endometrioid	R183Q (548 G>A)	No mutation	No mutation
702TCS	Uterine serous	S256Y (767 C>A)	No mutation	No mutation
319TCS	Uterine serous	S256F (767 C>T)	No mutation	H1047R (3140 A>G)
UPSC_5§	Uterine serous	P179R (536 C>G)	NA	NA
UCC_1T§	Uterine serous/clear cell	W257G (769 T>G)	NA	NA
UCC_3T§	Uterine serous	S256F (767 C>T)	NA	NA
PA10X¶	Pancreatic carcinoma	R183W (547 C>T)	G12R (34 G>C)	No mutation

†*PPP2R1A* mutation identified in a previous report.[1]
‡Unless otherwise indicated, all mutations are heterozygous.
§Genomic DNA isolated from paraffin-embedded tissues.
¶*PPP2R1A* mutation identified in a previous report.[14]

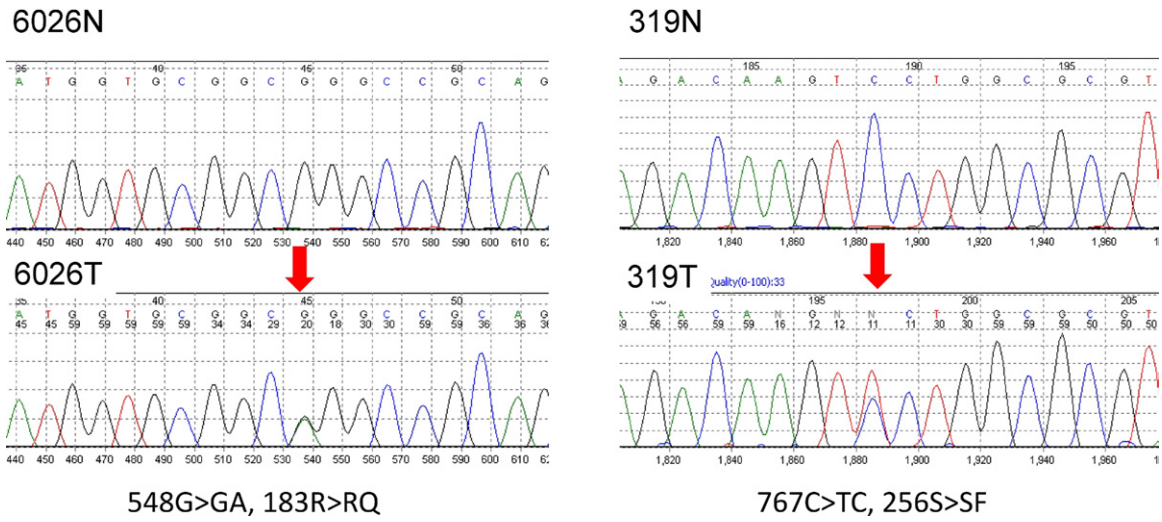

Figure 1. Examples of somatic mutations in *PPP2R1A*. Chromatogram of the sequences demonstrates a somatic mutation (548G>GA, 183R>RQ) in an ovarian endometrioid carcinoma, 6026T, and a novel mutation (767C>TC, 256S>SF) in a uterine serous carcinoma, 319T. The matched normal tissues (6026N and 319N) do not show the mutations. Both tumor tissues were affinity-purified. **Arrows** indicate the nucleotides with sequence mutation.

results for mutation status of *KRAS, PIK3CA,* and *PPP2R1A* are given in Table 2. In contrast to the mutually exclusive pattern of *KRAS* and *BRAF* mutations previously reported,[15,16] a mutually exclusive mutation pattern between *PPP2R1A* and *KRAS* or between *PPP2R1A* and *PIK3CA* was not observed. Of 25 tumors with *PPP2R1A* mutations, 5 tumors harbored concurrent *KRAS* mutations and 8 tumors harbored concurrent *PIK3CA* mutations (Table 2). Sequencing *PIK3CA* and *KRAS* in 54 ovarian and uterine carcinomas with wild-type *PPP2R1A* demonstrated a mutation frequency in *PIK3CA* and *KRAS* similar to that in tumors that had mutations in *PPP2R1A*. Of those *PPP2R1A* wild-type cases, 19 (35%) demonstrated *PIK3CA* mutations and 5 (9%) demonstrated *KRAS* mutations. There was no significant correlation of *PPP2R1A* mutation status with either *KRAS* or *PIK3CA* mutations ($P > 0.2$, Fisher exact test).

Discussion

The present study, by using an independent set of tumor samples, not only verified that somatic *PPP2R1A* mutations occur in nearly 10% of ovarian clear-cell carcinomas[1] but also demonstrated that *PPP2R1A* mutations were detected in other types of ovarian and uterine carcinomas except ovarian serous carcinoma, the conventional type of ovarian cancer. Also identified were novel somatic mutations located outside the previously reported hot-spot region of residues of R182 and R183. The results of this study should have several implications in understanding the molecular pathogenesis of both ovarian and uterine carcinomas. It also provides a biological foundation for studying the roles of *PPP2R1A* mutations in the development of uterine serous carcinoma.

The presence of *PPP2R1A* mutations in type I but not type II ovarian carcinomas is of great interest and provides further molecular genetic evidence to support the dualistic model of ovarian carcinogenesis. Although the overall frequency of *PPP2R1A* mutations in ovarian clear-cell, endometrioid, and mucinous carcinomas was only 9.1%, this observation suggests that *PPP2R1A* mutations most likely contribute a functional effect in development of some type I ovarian tumors, whereas type II ovarian carcinomas such as high-grade serous carcinomas may use *PPP2R1A*-indepent pathways for their development. Furthermore, the data suggest that *PPP2R1A* mutations may be related to those tumors arising either from endometrium or endometriosis. This is because uterine endometrioid and serous carcinomas develop from endometrium and ovarian clear-cell and endometrioid carcinomas, and seromucinous borderline tumors are closely associated with endometriosis.[17–20] Thus, *PPP2R1A* mutations were observed in15 of 140 tumors (10.7%) related to endometrium or endometriosis, and the frequency was significantly greater than 2.4% in tumors not related to endometrium and endometriosis ($P = 0.0072$, Fisher exact test). The present study evaluated only gynecologic malignant lesions; however, other tumor types may also harbor *PPP2R1A* mutations. For example, a previous genome-wide sequencing analysis of pancreatic cancer identified a point mutation at the R183 residue of *PPP2R1A* in 1 of 24 tumors.[14] Further studies analyzing *PPP2R1A* mutations in other types of cancer in humans are necessary to determine whether such mutations are generally enriched in tumors related to endometrium and endometriosis.

That *PPP2R1A* mutations occurred only in uterine serous carcinomas (uterine type II) but not in ovarian high-grade serous carcinomas (ovarian type II) suggests that although both tumor types share several clinicopathologic features, the pathogenesis is different in uterine serous carcinomas and ovarian high-grade serous carcinomas. The relatively high frequency of *PPP2R1A* mutations (19.2%) in uterine serous carcinoma warrants further investigation of its biological role in the development

of this highly aggressive uterine carcinoma. In addition to *TP53* mutations, *PPP2R1A* mutation is the most common sequence mutation in uterine serous carcinoma. All mutations in uterine serous carcinomas were located outside the R182 and R183, which raises the possibility that distinct mechanisms may be involved in generating the mutations at different locations.

Because the protein phosphatase 2A is implicated in regulation of signaling pathways including MAPK and AKT,[21,22] whether mutations of *PPP2R1A* coexisted with *KRAS or PIK3CA* mutations was analyzed because genes that regulate the MAPK and AKT pathways are frequently mutated in ovarian and uterine type I tumors.[5,6] From the perspective of cancer genetics, the lack of a mutually exclusive pattern between *PPP2R1A* and *KRAS* mutations or between *PPP2R1A* and *PIK3CA* mutations suggests that *PPP2R1A* mutations likely do not participate in signaling pathways involving *KRAS* and *PI3K*. However, future cell biology studies are needed to delineate whether there is cross-talk between protein phosphatase 2A pathway and the MAPK or AKT pathway.

A previous study[1] of ovarian clear cell carcinomas demonstrated that all of the *PPP2R1A* mutations were located in the pairs of arginine residues at R182 and R183.[1] However, analysis of the 18 *PPP2R1A* mutations in the present study identified new mutation positions at P179, S256, and W257. All mutations were located in the codons that are evolutionary conserved, and structural biology studies have demonstrated that the amino acids P179, R182, and R183 are located in the α-helix repeat 5, and amino acids S256 and W257 in the α-helix repeat 7. Both repeats are near the interface between the A subunit and the regulatory B subunit.[23] Mutations in several residues in the interface of A and B subunits such as P179, M180, and W257 have been reported to disrupt the interaction between these two subunits.[24] Because the mutations observed in this study were all at or close to those positions, it can be speculated that missense mutations at those residues could alter the binding affinity and/or specificity between A and B subunits and, thus, affect substrate recognition and/or phosphatase activity. All mutations identified in the previous and present studies were heterozygous missense mutations, which suggests a dominant attribute of *PPP2R1A* mutations in cancer pathogenesis. The mutated subunit A proteins encoded by mutant *PPP2R1A* may compete with their wild-type counterparts in binding to subunit B and forming a stable and functional enzyme complex. As a result, the enzymatic activity of protein phosphatase 2A, which may be important for tumor suppression, is reduced in the presence of mutant PPP2R1A proteins. This is the preferred interpretation, although there are other possibilities. For example, *PPP2R1A* may have a tumor-suppressor function, and the mutant proteins may function in a dominant negative manner. Additional studies should be undertaken to clarify the biological role of mutant PPP2R1A proteins in cancer development.

In summary, the present study provides new evidence of *PPP2R1A* mutations in several histologic subtypes of ovarian and uterine neoplasms in addition to ovarian clear-cell carcinoma. The relatively high frequency of *PPP2R1A* mutations in uterine (type II) serous carcinomas suggests that the alterations in the protein phosphatase 2A pathway may participate in its pathogenesis, which is different from ovarian (type II) high-grade serous carcinoma. The observation that all *PPP2R1A* mutations involve the α-helix repeats between the interface of subunits A and B strongly suggests that binding of both subunits is critical in cancer development. Considered together, the results indicate that *PPP2R1A* is a new cancer-associated gene that deserves further functional exploration to understand the roles of mutations in this gene in regulating protein phosphatase 2A functions and in tumor development.

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