Short Communication

RNF43 mutations are recurrent in Chinese patients with mucinous ovarian carcinoma but absent in other subtypes of ovarian cancer

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1. Introduction

Ovarian cancer is a heterogeneous group of malignant neoplasms and a leading cause of gynecologic cancer deaths (Eitan et al., 2009; Visintin et al., 2008). The high mortality of this disease is attributed mainly to the fact that over 70% of patients were diagnosed at their advanced stages (Rossing et al., 2010). Approximately 90% of ovarian cancers are epithelial and 10% are nonepithelial (Munkarah et al., 2004). However, multiple lines of evidence showed that the RNF43 mutations identified in human cancers were distributed across the entire gene and the mutated samples usually harbored loss of heterozygosity (LOH) of the RNF43 gene (Ong et al., 2012; Wu et al., 2011), both of which were characteristics of tumor suppressor genes (Baker et al., 2009; Hagstrom and Dryja, 1999; Ong et al., 2012; Ryland et al., 2013).

RNF43 is an E3 ubiquitin-protein ligase that accepts ubiquitin from an E2 ubiquitin-conjugating enzyme and directly transfers the ubiquitin to targeted substrate proteins. Recently, large-scale sequencing efforts have identified prevalent RNF43 mutations in pancreatic and ovarian mucinous carcinomas. In the present study, we sequenced the entire coding sequences of RNF43 in 251 Chinese patients with distinct subtypes of ovarian cancers for the presence of RNF43 mutations. A total of 2 novel heterozygous nonsynonymous RNF43 mutations were identified in 2 out of 15 (13.3%) patients with mucinous ovarian carcinoma, these mutations were evolutionarily highly conserved; while no mutation was detected in other samples. In addition, none of the RNF43-mutated samples harbored DICER1 (dicer 1, ribonuclease type III), PPP2R1A (protein phosphatase 2, regulatory subunit A, alpha), TRRAP (transformation/transcription domain-associated protein) and DNMT3A (DNA (cytosine-5')-methyltransferase 3 alpha) hot-spot mutations. Recurrent RNF43 mutations existed in mucinous ovarian carcinomas implicated that these mutations might play crucial roles in the tumorigenesis of these patients, while the absence of DICER1, PPP2R1A, TRRAP and DNMT3A hot-spot mutations suggested that these genetic alterations might not play synergistic roles with RNF43 mutations in these individuals. Additionally, the absence of RNF43 mutations in other subtypes of ovarian carcinoma implicated that RNF43 mutations might not be actively involved in the pathogenesis of these disorders.

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Abbreviations: RNF43, ring finger protein 43; DICER1, dicer 1, ribonuclease type III; PPP2R1A, protein phosphatase 2, regulatory subunit A, alpha; TRRAP, transformation/transcription domain-associated protein; DNMT3A, DNA (cytosine-5')-methyltransferase 3 alpha; COSMIC, Catalogue of Somatic Mutations in Cancer; FFPE, formalin-embedded; PCR, polymerase chain reaction; SPSS, Statistical Product and Service Solutions; TCGA, The Cancer Genome Atlas; A, adenosine; G, guanosine; Arg, arginine; Asp, aspartic acid; Ile, isoleucine; Val, valine.

Keywords: RNF43 Mutation Ovarian cancer Chinese

ARTICLE INFO

Article history:
Accepted 14 August 2013
Available online 31 August 2013

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showed a general high frequency of DICER1 and PPP2R1A mutations as well as the presence of RNF43 mutations. Furthermore, prior studies have also examined RNF43 in 251 samples with distinct subtypes of ovarian cancers for assessing whether there is an internal link among different mutated genes, which might play synergistic roles in the pathogenesis of human malignancies. Almost all of these mutations in these four genes were limited to certain codons (Heravi-Moussavi et al., 2012; Shih et al., 2011). In addition, frequent TRRAP and DNMT3A mutations were initially identified in melanoma (Wei et al., 2011) and acute myeloid leukemia (Ley et al., 2010), subsequent studies also showed that infrequent TRRAP and DNMT3A mutations existed in ovarian serous carcinomas (The Cancer Genome Atlas Research Network, 2011) (COSMIC, http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). A critical concern in the molecular genetics of human carcinoma is to determine whether specific mutations found in a tumor type are also common in other cancers, and whether there is an internal link among different mutated genes, which might play synergistic roles in the pathogenesis of human malignancies. Almost all of these mutations in these four genes were limited to certain codons (Heravi-Moussavi et al., 2012; Shih et al., 2011). Therefore, we also sequenced the RNF43-mutated individuals for the presence of hot-spot mutations of these four genes in ovarian cancers, with the aim of identifying additional genetic alterations cooperating with the RNF43 mutations in the pathogenesis of these samples.

2. Materials and methods

2.1. Patients

Formalin-fixed, paraffin-embedded (FFPE) cancerous and their corresponding adjacent non-cancerous tissues were collected from a total of 251 patients with ovarian carcinoma in the Jiangxi Provincial Maternal and Child Health Hospital as well as the Jiangxi Provincial Cancer Hospital in China, all samples were reviewed by two pathologists on histopathological typing and purity of samples in a blinded fashion, only those with over 70% of cancerous cells were included in this study. The sample cohort was comprised of 76 ovarian serous carcinoma (high grade, n = 63; low grade, n = 13), 43 ovarian clear cell carcinoma, 37 ovarian endometrioid carcinoma, 33 ovarian germ cell tumor, 15 mucinous ovarian carcinoma, 18 ovarian sex cord–stromal tumor, 12 other rare subtypes and 17 Krukenberg tumor (Table 1). This study conformed to the tenets of the Declaration of Helsinki and signed written consent was obtained from each subject before collection of the samples. Institutional Review Board approval for this study was received from the Jiangxi Provincial Maternal and Child Health Hospital as well as the Jiangxi Provincial Cancer Hospital.

Table 1

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
<th>Median age</th>
<th>Minimal age</th>
<th>Maximal age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (years)</td>
<td>47</td>
<td>5</td>
<td>75</td>
</tr>
<tr>
<td>Affected ovary</td>
<td>Both ovaries</td>
<td>106</td>
<td>63</td>
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</table>

Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Target region</th>
<th>Amplicon length</th>
<th>Samples tested/total sample</th>
<th>Annealing</th>
<th>Forward primers (5'-3')</th>
<th>Reverse primers (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNF43</td>
<td>Exon 2-1</td>
<td>255 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>GAGAAGGAAAGCGCCAAAAC</td>
<td>GCTGGTGCCGATAGCTC</td>
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<td>RNF43</td>
<td>Exon 2-2</td>
<td>215 bp</td>
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<td>52 °C</td>
<td>CCTGCTCCGAGCGTGCTC</td>
<td>TACGCGTTAATTCTCTCT</td>
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<tr>
<td>RNF43</td>
<td>Exon 3</td>
<td>246 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>CTCTAGGCGATTCTCTTG</td>
<td>CCTCTCTTCAGCCAGCT</td>
</tr>
<tr>
<td>RNF43</td>
<td>Exon 4</td>
<td>208 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>CCTCTCTCTTGTTCTAGT</td>
<td>AACGCGTTGCTGCTCTCT</td>
</tr>
<tr>
<td>RNF43</td>
<td>Exon 5</td>
<td>224 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>ACATTGTCGTTGCTGCTC</td>
<td>TCAGTCGATTCTCATCCAG</td>
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<tr>
<td>RNF43</td>
<td>Exon 6</td>
<td>195 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>TGACCTCTTCTCCCTCTTG</td>
<td>TCAACACACACACACACAC</td>
</tr>
<tr>
<td>RNF43</td>
<td>Exon 7-1</td>
<td>246 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>ACTGGGAGCGTACGCACAAG</td>
<td>CTGCGCTCAAGACATCTCC</td>
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<tr>
<td>RNF43</td>
<td>Exon 7-2</td>
<td>236 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>ATCTAGGCGATTCTCTTG</td>
<td>CTAACACACACACACACAC</td>
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<td>RNF43</td>
<td>Exon 8</td>
<td>310 bp</td>
<td>251/251</td>
<td>52 °C</td>
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<td>GTGCGCTGCTGCTCTCTCT</td>
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<td>RNF43</td>
<td>Exon 9-1</td>
<td>152 bp</td>
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<td>52 °C</td>
<td>CACGACATTCTCTTTGGAAG</td>
<td>GGCTACAGGTGCTGCTC</td>
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<td>RNF43</td>
<td>Exon 9-2</td>
<td>205 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>ACTAGGACATTCTCTTG</td>
<td>CATTGCGTGCTGCTGCTC</td>
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<td>RNF43</td>
<td>Exon 9-3</td>
<td>332 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>TCCAGGCTCAGGGCGCTC</td>
<td>GAAGTGGCTGCTGCTGCTC</td>
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<tr>
<td>RNF43</td>
<td>Exon 9-4</td>
<td>374 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>AAAGGTGCTGCTGCTGCTC</td>
<td>TATGCGGAGGCCAGTTGCTC</td>
</tr>
<tr>
<td>RNF43</td>
<td>Exon 9-5</td>
<td>367 bp</td>
<td>251/251</td>
<td>52 °C</td>
<td>GCCTGTTCCGCTGCTGCTC</td>
<td>TATGCGGAGGCCAGTTGCTC</td>
</tr>
<tr>
<td>Dicer1</td>
<td>p.E1705D1709</td>
<td>159 bp</td>
<td>251/251</td>
<td>55 °C</td>
<td>CGAGCTGAGGATCATATTG</td>
<td>CTAGAAGAATGTGATGTG</td>
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<tr>
<td>Dicer1</td>
<td>p.D1810E1813</td>
<td>171 bp</td>
<td>251/251</td>
<td>55 °C</td>
<td>TGCGCTGTGCTGCTGCTG</td>
<td>TATGCGGAGGCCAGTTGCTC</td>
</tr>
<tr>
<td>PPP2R1A</td>
<td>p.P179R183</td>
<td>160 bp</td>
<td>251/251</td>
<td>55 °C</td>
<td>GTACGTGCGTGCTGCTGCTC</td>
<td>GAAGGACATCTGTGCTGCTC</td>
</tr>
<tr>
<td>PPP2R1A</td>
<td>p.S256W257</td>
<td>168 bp</td>
<td>251/251</td>
<td>55 °C</td>
<td>CTCTGCTCTGCTGCTGCTG</td>
<td>GTACGTGCGTGCTGCTGCTC</td>
</tr>
<tr>
<td>TRRAP</td>
<td>p.2722</td>
<td>183 bp</td>
<td>251/251</td>
<td>55 °C</td>
<td>TGACGTGCTGCTGCTGCTG</td>
<td>GTACGTGCGTGCTGCTGCTC</td>
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<tr>
<td>DNMT3A</td>
<td>p.R8882</td>
<td>177 bp</td>
<td>251/251</td>
<td>55 °C</td>
<td>TGCGCTGTGCTGCTGCTG</td>
<td>TATGCGGAGGCCAGTTGCTC</td>
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</tbody>
</table>

RNF43 mutations were distributed throughout the entire gene (Furukawa et al., 2011; Ong et al., 2012; Ryland et al., 2013; Wu et al., 2011). Simultaneous functional studies implicated that the potential cancer-suppressing roles of RNF43 might be mediated via negatively regulating Wnt/β-catenin signaling pathway (Hao et al., 2012; Koo et al., 2012). In the present study, we sequenced the entire coding sequence of RNF43 in 251 samples with distinct subtypes of ovarian cancers for the presence of RNF43 mutations. Furthermore, prior studies have also showed a general high frequency of DICER1 and PPP2R1A mutations in certain subtypes of ovarian carcinomas: frequent DICER1 mutations in Sertoli–Leydig cell tumors (Heravi-Moussavi et al., 2012) and recurrent PPP2R1A mutations in clear cell and endometrioid ovarian cancer (Nagendra et al., 2012; Shih et al., 2011). In addition, frequent TRRAP and DNMT3A mutations were initially identified in melanoma (Wei et al., 2011) and acute myeloid leukemia (Ley et al., 2010), subsequent studies also showed that infrequent TRRAP and DNMT3A mutations existed in ovarian serous carcinomas (The Cancer Genome Atlas Research Network, 2011) (COSMIC, http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). A critical concern in the molecular genetics of human carcinoma is to determine whether specific mutations found in a tumor type are also common in other cancers, and whether there is an internal link among different mutated genes, which might play synergistic roles in the pathogenesis of human malignancies. Almost all of these mutations in these four genes were limited in certain codons (Heravi-Moussavi et al., 2012; Ley et al., 2010; Nagendra et al., 2012; Shih et al., 2011; Wei et al., 2011). Therefore, we also sequenced the RNF43-mutated individuals for the presence of hot-spot mutations of these four genes in ovarian cancers, with the aim of identifying additional genetic alterations cooperating with the RNF43 mutations in the pathogenesis of these samples.

Table 2

The primers for the mutational analyses of the RNF43, DICER1, PPP2R1A, TRRAP and DNMT3A genes.
2.2. Mutational analysis of the RNF43, DICER1, PPP2R1A, TRRAP and DNMT3A genes

Genomic DNAs were extracted from archival FFPE tissues by using FFPE DNA kits (OMEGA Bio-tek Inc., Doraville, GA, USA). The entire coding region of the RNF43 gene and genomic region spanning the potential hot-spot mutations in DICER1, PPP2R1A, TRRAP and DNMT3A were amplified by polymerase chain reaction with specific primers (Table 2), the PCR reactions were cycled in a Thermal Cycler 2720 (Applied Biosystems, CA, USA) using the following conditions: an initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s, 52–55 °C (Table 2) for 30 s and 72 °C for 30 s; and a final extension of 72 °C for 10 min. The PCR products were then sequenced on an ABI Prism 3730 DNA sequencer (Applied Biosystems, CA, USA). The identified mutations were confirmed by bidirectional sequencing and somatic mutations were confirmed by sequencing their corresponding adjacent non-cancerous tissues.

2.3. Evolutionary conservation analysis of missense mutation in the RNF43 gene

Evolutionary conservation analysis of RNF43 mutation was performed using thirteen species, including Homo sapiens (RNF43, GenBank accession No. NM_017763.4), Pan troglodytes (XM_001172611.3), Macaca mulatta (XM_001106574.2), Gorilla gorilla gorilla (XM_004041273.1), Saimiri boliviensis boliviensis (XM_003929103.1), Rattus norvegicus (NM_001135921.1), Mus musculus (NM_172448.3), Bos taurus (NM_001191194.1), Canis lupus familiaris (XM_548234.3), Ovis aries (XM_004012397.1), Felis catus (XM_003996633.1), Oreotolagus cuniculus (XM_002719265.1) and Gallus gallus (XM_003642379.1) from GenBank.

2.4. Statistical analysis

Fisher exact test was used to compare the mutation frequency in the present and prior studies. Statistical analysis was performed using the SPSS 11.5 system software, a p value of less than 0.05 was considered statistically significant.

3. Results and discussion

Among the 251 patients with distinct subtypes of ovarian carcinoma, the median age was 47 years (age range, 5–75 years), 106 of 251 patients affected both of the ovaries, while the remaining 145 patients affected either the left or right ovary (Table 1).

We sequenced the whole protein-encoding exons of the RNF43 gene for the presence of RNF43 mutations in the cancerous tissues of 251 patients with distinct subtypes of ovarian cancer. Only two of the 251 tumors had somatic mutations in RNF43 and both were detected in mucinous tumors, that is, 2 novel heterozygous nonsynonymous RNF43 mutations were identified in 2 out of 15 (2/15, 13.3%) patients with mucinous ovarian carcinoma, including a truncating mutation (c.118delA, p.Arg40AspfsX11) and a missense mutation (c.142A>G, p.Ile48Val) (Fig. 1). The somatic origin of these mutations was confirmed by sequencing of the corresponding adjacent non-cancerous DNAs. Evolutionary conservation analysis suggested that the RNF43 missense mutation (c.142A>G, p.Ile48Val) was highly conserved among the thirteen species ranging from H. sapiens to G. gallus (Fig. 3). The sample with c.118delA truncating mutation (p.Arg40AspfsX11) was a 42-year woman affecting both ovaries; while RNF43 c.142A>G (p.Ile48Val) mutated sample was 50-year old and affected in the right ovary. The mutation frequency of the RNF43 gene in our mucinous ovarian...
Homo sapiens (NF_060233.3)  
OCC-4  
Pan troglodytes (XP_001172611.1)  
Macaca mulatta (XP_001106574.1)  
Gorilla gorilla gorilla (XP_004041321.1)  
Saimiri boliviensis boliviensis (XP_003929152.1)  
Rattus norvegicus (NP_001123993)  
Mus musculus (NP_766036.2)  
Bos taurus (NP_01178123.1)  
Canis lupus familiaris (XP_548224.3)  
Ovis aries (XP_004012446.1)  
Felis catus (XP_003966682.1)  
Oryctolagus cuniculus (XP_002719311.1)  
Gallus gallus (XP_003642457.1)

**Fig. 3.** Evolutionary analysis of RNF43 missense mutation, patient “OCC-4” harbored RNF43 p.Ile48Val (c.142A>G) mutation.

carcinomas (13.3%, 2/15) was slightly lower than that of the previous observation (15.7%, 8/51) (Ryland et al., 2013), and there was no statistical difference between them (Fisher’s exact test, p > 0.05). Of note, this comparison should be treated with caution due to that the sample size evaluated was small in this study and relatively underpowered compared to that in the previous study (Ryland et al., 2013). These results showed that RNF43 mutations might play crucial roles in the tumorigenesis of mucinous ovarian carcinoma. Further mutational analysis of the two RNF43-mutated samples showed that none of them harbored DICER1, PPP2R1A, TRRAP and DNMT3A hot-spot mutations (Fig. 2). In addition, no mutations in these genes were detected in the remaining 13 mucinous ovarian carcinomas without RNF43 mutations.

These results implicated that it is likely that some other genetic alterations, but not these hot-spot mutations in these genes, played synergistic roles with RNF43 mutations in the carcinogenesis of these patients.

No RNF43 mutation was detected in our 63 high-grade or 13 low-grade specimens with ovarian serous carcinoma (Table 1). It should be noted that a strikingly low frequency (0.6%, 2/316) of RNF43 mutation was detected in 316 high-grade ovarian serous carcinomas in a large-scale sequencing study performed by The Cancer Genome Atlas (TCGA) (The Cancer Genome Atlas Research Network, 2011). Taken together, we speculated that RNF43 mutations might not be actively participated in the progression of ovarian serous carcinoma.

Additionally, we also did not detect any RNF43 mutations in other subtypes of ovarian carcinomas, including 43 ovarian clear cell carcinoma, 37 ovarian endometrioid carcinoma, 33 ovarian germ cell tumor, 18 ovarian sex cord–stromal tumor, 12 other rare subtypes and 17 Krukenberg tumor (Table 1). These results implicated that RNF43 mutations might not be actively involved in the carcinogenesis of these tumors. Nevertheless, larger sample size for several subtypes of ovarian carcinomas should be recruited to test this conclusion, such as ovarian sex cord–stromal tumor, Krukenberg tumor and the remaining rare subtypes.

As RNF43 is a critical enzyme in the process of ubiquitin-dependent protein degradation (Hao et al., 2012; Koo et al., 2012; Sugiuira et al., 2008), it is quite natural to speculate that RNF43 mutations might affect RNF43 enzyme activity, and then intracellular protein degradation, ultimately contribute to the development of human neoplasms. Functional assays are performing in our laboratory to determine whether the RNF43 mutations could promote ovarian cancer progression.

In summary, we analyzed 251 Chinese patients with distinct subtypes of ovarian cancers for the presence of RNF43 mutations. Recurrent RNF43 mutations were identified in Chinese patients with mucinous ovarian carcinoma and this was consistent with the previous observation (Ryland et al., 2013); and DICER1, PPP2R1A, TRRAP and DNMT3A hot-spot mutations were not detected in the RNF43-mutated patients. In addition, not any RNF43 mutation was found in other subtypes of ovarian cancer. Our study implicated that RNF43 mutations might play crucial roles in the pathogenesis of Chinese patients with mucinous ovarian carcinoma, while might not be actively involved in other subtypes of ovarian carcinomas in China.

**Conflict of interest**

The authors declare that they have no competing interests.

**Acknowledgments**

We thank the sample donors involved in this study. This study was supported by the National Natural Science Foundation of China (No. 81260384, No. 81060052) and the Natural Science Foundation of Jiangxi Province (No. 20114BAB215033).

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