Original Article

TP53 variants in p53 signatures and the clonality of STICs in RRSO samples

Tomoko Akahane 1,2,4 Kenta Masuda 1,3 Akira Hirasawa 1,4 Yusuke Kobayashi 1,2,3 Arisa Ueki 2,5,1 Miho Kawaida 3,1 Kumiko Misu 2, Kohei Nakamura 3,2 Shimepi Nagai 1, Tatsuyuki Chiyoda 1, Wataru Yamagami 3, Shiminenori Hayashi 1, Fumio Kataoka 6, Kouji Banno 1, Kokichi Sugano 2,7 Hajime Okita 2, Kenjiro Kosaki 3, Hiroshi Nishihara 3, Daisuke Aoki 1

1Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan
2Department of Medical Genetics, Keio University School of Medicine, Tokyo, Japan
3Department of Clinical Genomic Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
4Department of Diagnostic Pathology, Keio University School of Medicine, Tokyo, Japan
5Department of Obstetrics and Gynecology, International University of Health and Welfare School of Medicine, Chiba, Japan
6Department of Genetic Medicine, Sasaki Foundation, Kyoundo Hospital, Tokyo, Japan
7Department of Genetic Medicine, Keio University School of Medicine, Tokyo, Japan

ABSTRACT

Objective: Precursor lesions may be identified in fallopian tube tissue after risk-reducing salpingo-oophorectomy (RRSO) in patients with pathogenic variants of BRCA1/2. Serous tubal intraepithelial carcinoma (STIC) is considered a precursor of high-grade serous carcinoma, whereas the significance of the p53 signature remains unclear. In this study, we investigated the relationship between the p53 signature and the risk of ovarian cancer.

Methods: We analyzed the clinicopathological findings and conducted DNA sequencing for TP53 variants of p53 signatures and STIC lesions isolated using laser capture microdissection in 13 patients with pathogenic variants of BRCA1/2 who underwent RRSO and 17 control patients with the benign gynecologic disease.

Results: TP53 pathogenic variants were detected significantly higher in RRSO group than control (p<0.001). No difference in the frequency of p53 signatures were observed between groups (53.8% vs 29.4%; p=0.17). TP53 sequencing and next-generation sequencing analysis in a patient with STIC and occult cancer revealed 2 TP53 mutations causing different p53 staining for STICs and another TP53 mutation shared between STIC and occult cancer.

Conclusion: The sequence analysis for TP53 revealed 2 types of p53 signatures, one with a risk of progression to STIC and ovarian cancer with pathological variants in TP53 and the other with a low risk of progression without pathological variants in TP53 as seen in control.

Keywords: Prophylactic Surgical Procedures; Salpingo-Oophorectomy; Genes, p53; Genes, BRCA1; Genes, BRCA2; Carcinoma in Situ; Cystadenocarcinoma, Serous

Synopsis
We conducted a DNA sequencing for TP53 variants in p53 signatures from patients with pathogenic variants of BRCA1/2 who underwent risk-reducing salpingo-oophorectomy (RRSO) and control patients with the benign gynecologic disease. TP53 pathogenic variants were detected significantly higher in RRSO group. The characteristics of p53 signatures might be different depending on BRCA status.
INTRODUCTION

TP53 mutation is one of the most common genetic alterations in cancer. Ovarian cancer, particularly high-grade serous carcinoma (HGSC), has the highest frequency of TP53 mutations compared to all cancer types, with more than 90% of cases reported to have TP53 mutations [1].

Serous tubal intraepithelial carcinoma (STIC), characterized by non-ciliated tubal epithelial cells that show marked nuclear atypia, mitotic figures, apoptotic bodies, loss of cellular polarization, abnormal p53 staining (a pattern compatible with either missense or deletion mutations), and an increased Ki-67 labeling index, has been recognized as a precancerous lesion of HGSC [2]. It is often found in fallopian tube tissue after risk-reducing salpingo-oophorectomy (RRSO) in hereditary breast and ovarian cancer (HBOC) patients with pathogenic variants of BRCA1 and BRCA2 and also found in fallopian tube tissue from patients with HGSC [3-6]. The coincidental finding of the TP53 mutation in both HGSC and STIC has led to the recognition of STIC as an early lesion and the TP53 mutation as an early driver mutation of HGSC [1,7-11]. The similarity in morphology, immunophenotype, and gene expression patterns between fallopian tube epithelium and HGSC provides additional evidence that HGSC develops from the fallopian tube [12].

The p53 signature is another lesion that can be seen in epithelial cells in the fallopian tube and is characterized by 12 or more consecutive cells with abnormal p53 immunostaining in morphologically normal tubal epithelium. Often found in RRSO specimens, the p53 signature is also considered to be a precursor lesion for STIC. However, the p53 signature can also be detected in benign specimens without pathogenic BRCA1/2 variants. Therefore, the association of the p53 signature and the risk of cancer development is unknown [13-15].

In this study, to investigate the risk of ovarian cancer in p53 signatures, we selectively analyzed TP53 variants in p53 signatures on specimens from RRSO group and benign control group. We also analyzed the clonality of cancer and STIC by combining TP53 variant analysis and panel sequencing for a case with different p53 staining STICs. This study clarified the significance of TP53 variant analysis in p53 signatures and found new insights into the carcinogenesis of HGSC.

MATERIALS AND METHODS

1. Study groups

We investigated 13 HBOC patients with pathogenic germline BRCA1 or BRCA2 variants in whom RRSO was performed at Keio University Hospital between February 2013 and September 2019 (Table S1). All patients underwent genetic counseling, and their family history of cancer was obtained as previously described [16]. Seventeen patients with benign gynecologic disease in whom bilateral salpingo-oophorectomy was performed between February 2013 and September 2020 were also included as controls (Table S2). Clinical information was collected from the medical records. The study was approved by the ethics committee of Keio University School of Medicine (approval numbers: 20070081, 20130477, and 20160443). Informed consent was obtained from all patients prior to enrolment in the study.
2. Pathologic analysis
All RRSO samples were analyzed using the Sectioning and Extensively Examining the FIMbriated end protocol [17]. In total, 313 embedded blocks were obtained from 13 patients; the mean number of blocks for each fallopian tube was 12 (range=6–22). The mean number of blocks per one fimbria was 3 (SD, ±1.5) in the RRSO group, no significant difference compared to the control group (2.5±1.0). The p53 signature was diagnosed as morphologically normal in tubal epithelial cells if there were at least 12 consecutive p53-positive secretory cells showing a low proliferative index (i.e., Ki67 <10%). STIC was defined as consecutive non-ciliated tubal epithelial cells showing marked nuclear atypia, mitotic figures, apoptotic bodies, loss of cellular polarization, an abnormal p53 staining pattern (compatible with either missense or deletion mutations), and an increased Ki-67 labeling index (Ki67 >10%). The samples were also evaluated for the presence or absence of occult cancer [13,18-21].

3. Immunohistochemistry
Expression levels of p53, Ki67/MIB1, c-Myc, Pax8, and WT-1 were analyzed in formalin-fixed, paraffin-embedded (FFPE) samples. Antigen retrieval was performed by autoclaving the samples in 10 mM sodium citrate buffer (pH 6.0) at 121°C for 1 minute. The following primary antibodies were used: anti-human p53 protein mouse monoclonal antibody (DO-7; Dako, Glostrup, Denmark), anti-human Ki67/MIB1 protein rabbit monoclonal antibody (SP6; Nichirei Bioscience, Tokyo, Japan), anti-human c-Myc protein rabbit monoclonal antibody (EP121; Nichirei Bioscience), anti-human Pax8 protein mouse monoclonal antibody (BC12; Nichirei Bioscience), and anti-human Wilms’ tumor (WT-1) protein mouse monoclonal antibody (WT49; Leica Biosystems, South San Francisco, CA, USA). The secondary antibody was mouse and rabbit Histofine Simple Stain MAX-PO (Nichirei Bioscience). Protein expression was visualized by 3, 3′-diaminobenzidine and 3-amino-9-ethylcarbazole (codes 425011 and 415131, Nichirei Bioscience). The nuclei were counterstained with Mayer’s hematoxylin. The stained slides were imaged using a Nano Zoomer-XR C12000 virtual slide scanner (Hamamatsu Photonics, Shizuoka, Japan).

4. Laser capture microdissection (LCM)
The FFPE tissue was cut into 3-µm sections and placed on a slide for LCM (Carl Zeiss Microscopy GmbH, Jena, Germany). The sections were deparaffinized and stained with hematoxylin. Approximately 20–50 epithelial cells from fallopian tube tissue were collected into 0.5-mL AdhesiveCap 200 under the microscope using the selective laser in the LCM system (PALM MB-IV; Carl Zeiss Microscopy). Genetic engineering research grade distilled water (Wako Pure Chemical Industries, Osaka, Japan) was then added to the micro tube cap and incubated overnight at room temperature.

5. I-PEP-PCR and direct sequencing of TP53
Exons 4–10 of the TP53 gene were amplified from genomic DNA using the primer extension and I-PEP-PCR (improved primer extension and preamplification-polymerase chain reaction) method [22-24]. First-round PCR was performed using a multiplex method. The final volume of the reaction mixture was 30 µL and contained Ex Taq Hot Start Version and PCR reaction mix (Takara Bio Inc., Shiga, Japan). The second-round PCR was performed in a hemi-nested condition using AccuPrime Pfx DNA polymerase and Accu-Prime Pfx Reaction mix (Invitrogen, Carlsbad, CA, USA). The primer sequences are shown in Table S3. The PCR cycling conditions were as follows: preheating for 10 minutes at 95°C, 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, 72°C for 10 minutes, and 4°C
thereafter. Direct sequencing was then performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA). The pathogenicity of the TP53 mutations was assessed using the Catalog of Somatic Mutations in Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cosmic) and the IARC TP53 database (https://p53.iarc.fr/TP53GeneVariations.aspx).

6. DNA analysis using next-generation sequencing
DNA was isolated from whole blood and HGSC tissue samples using the QIAamp Tissue and Blood Kit and the GeneRead DNA FFPE kit (Qiagen, Hilden, Germany). The quality of DNA was examined using the 4200 TapeStation (Agilent Technologies, Santa Clara, CA, USA) and Qubit 3.0 Fluorometer dsDNA BR assay kit (Thermo Fisher Scientific). A next-generation sequencing (NGS) library for amplicon sequencing was constructed from 40 ng (blood) and 250 ng (FFPE) of DNA using the QIAsseq Targeted DNA Human Comprehensive Cancer Panel (Qiagen), which targets 275 cancer-related genes (qiaseq targeted dna panels - GeneGlobe (qiagen.com). The constructed library was quantified by quantitative PCR using the QIAseq Library Quant Assay kit (Qiagen). The library was denatured with 0.2 N NaOH (cat#72068; Sigma-Aldrich, St. Louis, MO, USA), diluted with hybridization buffer to a concentration of 20 pM pooled for multiplexed sequencing. High-throughput sequencing was then performed using an Illumina MiSeq instrument in 2×150 bp paired-end reads (Illumina, San Diego, CA, USA).

7. Bioinformatics analysis
Bioinformatics analysis of the QIAseq Targeted DNA Human Comprehensive Cancer Panel was performed using the GeneGlobe Data Analysis Center (https://geneglobe.qiagen.com/jp/analyze).

The read processing, including read trimming, read alignment, post-alignment read pair filtering, marking reads putatively from the same input molecule, and gene-specific primer alignment masking prior to variant calling, was performed, and variant calling was performed using the UMI-aware variant caller smCounter2. SnpEff was used to annotate variants (http://snpeff.sourceforge.net/index.html). Somatic variants were selected by the criteria that germline and somatic pair variants have allelic frequencies greater than 0.4% or somatic-only variants, that are registered as “pathogenic/likely pathogenic” in ClinVar or Cosmic database or the annotation impact of SnpEff was “HIGH”. Copy number variant was analyzed by the published algorithm (https://github.com/reineckef/quandico).

8. Statistical analysis
Data were analyzed using a 2-tailed student’s t-test under the assumption of normal distribution for biological parameters and the χ² test for the contingency table analysis by GraphPad Prism 8. The p-value of <0.05 was considered statistically significant.

RESULTS

1. p53 signatures in the RRSO and control specimens
Seven (53.8%) of the 13 patients with BRCA1/2 pathogenic variants (BRCA-positive) harbored at least one p53 signature in their fallopian tube tissue (Table S1). One patient harbored both STIC lesions in the fallopian tube and occult cancer in the ovary. Seventeen lesions in 7 patients were diagnosed to contain p53 signatures. Twelve (70.5%) of these 17 lesions were identified in the fimbria, 2 (11.7%) in the infundibulum and ampulla, and 1 (5.8%) in the isthmus (Table 1, Fig. 1). There was no significant difference in age, body mass index,
parity, frequency of hormonal therapy for breast cancer, or frequency of oral contraceptive use according to p53 signature status in the RRSO group, but there was a trend towards differences by pathogenic variants of \textit{BRCA1} or \textit{BRCA2} (p=0.053) (Table 2). Next, the frequency of the p53 signature was compared in the fimbria, where p53 signatures are usually found, between the 13 BRCA-positive women and the 17 control women with no family history of cancer (Fig. 1). There was no significant difference in the frequency of the p53 signature in the fimbria between the 13 BRCA-positive women (n=7, 53.8%) and the 17 control women (n=5, 29.4%, p=0.17, Table 3). Furthermore, there was no difference in background characteristics between the BRCA-positive group and the control group, except in parity (Table S4).

2. TP53 variants in lesions with a p53 signature

Next, we isolated the p53 signature lesions in fallopian tube epithelial cells by LCM and analyzed the TP53 variants. In the RRSO group, 10 types of TP53 variant were identified in 5 (71.4%) of 7 patients with the p53 signature. All 5 patients had germline \textit{BRCA1} pathogenic variants. Nine (90%) of the 10 types of TP53 variant were pathogenic and one was of unknown significance (Tables S1 and S5). In the control group, 5 types of TP53 variant were identified in 3 (50%) of 6 patients with the p53 signature. None of these 5 variants were pathogenic. More pathogenic variants were identified in the RRSO samples than in the control samples (p<0.001) (Table S5). We also sequenced normal fallopian tube epithelial cells (without the
TP53 variants in p53 signatures

Table 2. Characteristics of patients with and without p53 signatures

| Characteristics                      | RRSO patients (n=13) | Control patients (n=17) | p-value | RRSO patients (n=13) | Control patients (n=17) | p-value |
|--------------------------------------|----------------------|-------------------------|---------|----------------------|-------------------------|---------|
| Mean age at operation (yr)           | 49.4                 | 47.3                    | 0.57*   | 49                   | 44.9                    | 0.11*   |
| BMI (mean)                           | 20.95                | 21.03                   | 0.95*   | 22.97                | 21.83                   | 0.48*   |
| Parity                               |                      |                         |         |                      |                         |         |
| Nulliparous                          | 1                    | 1                       | 0.9†    | 4                    | 7                       | 0.85†   |
| Parous                               | 6                    | 5                       |         | 2                    | 4                       |         |
| Hormone therapy for breast cancer    |                      |                         |         |                      |                         |         |
| No                                   | 5                    | 4                       |         |                      |                         |         |
| Yes                                  | 2                    | 2                       |         |                      |                         |         |
| GnRH analog                          |                      |                         | 0.11†   |                      |                         |         |
| No                                   | 2                    |                         |         |                      |                         |         |
| Yes                                  | 4                    |                         |         |                      |                         |         |
| OC use                               |                      |                         |         |                      |                         |         |
| Never                                | 7                    | 6                       |         | 6                    | 11                      |         |
| Ever                                 | 0                    | 0                       |         | 0                    | 0                       |         |
| Race                                 |                      |                         |         |                      |                         |         |
| Asian                                | 7                    | 6                       |         | 6                    | 11                      |         |
| BRCA1/2 pathogenic variant           |                      |                         | 0.053†  |                      |                         |         |
| BRCA1                                | 6                    | 2                       |         |                      |                         |         |
| BRCA2                                | 1                    | 4                       |         |                      |                         |         |

NA, not available.
*t-test; †χ² test.

Table 3. Frequency of p53 signature in fimbria of RRSO and control samples

| Group                  | RRSO (13) | Control (17) | Total | p-value |
|------------------------|-----------|--------------|-------|---------|
| p53 signature in fimbriae |           |              |       | p=0.17 (χ²) |
| Positive               | 7 (53.8)  | 5 (29.4)     | 12    |         |
| Negative               | 6 (46.2)  | 12 (70.6)    | 18    |         |

Values are presented as number (%).
RRSO, risk-reducing salpingo-oophorectomy.

p53 signature) as a negative control in 6 patients in which the p53 signature was identified, but no variant was identified in any of these cases (Table S2).

3. The TP53 variant analysis suggested a relationship between STIC and the origin of the cancer

One of the 13 patients in the RRSO group had occult HGSC and 2 STIC lesions with morphologic changes. Ki67 stained diffusely in both STICs. One STIC lesion showed positive p53 staining while the other did not; therefore, these lesions were respectively termed “p53-positive STIC” and “p53-null type STIC” (Fig. 2). WT1 and PAX8, both of which are markers of HGSC, were positive in both STICs, and the pattern was comparable to that of occult cancer. Next, TP53 variants were analyzed in both STICs using LCM (Fig. 3). In the p53-null type STIC, a nonsense mutation (c.617T>A) was detected, while in the p53-positive STIC, 2 mutations causing a frameshift of TP53 were identified. One mutation (c.617delT) occurred at the same codon as the mutation (c.617T>A) detected in the p53-null type STIC, and the other mutation (c.983delT) was identical to that in the occult HGSC. The latter mutation in HGSC was confirmed by DNA analysis using NGS, which also revealed amplification of MYC and CCND1, the gene alteration commonly found in HGSC (Fig. S1). Immunohistochemistry confirmed that c-Myc protein expression was higher in the STIC and occult cancer than in normal fallopian epithelial cells (Fig. 2).
To our knowledge, this is the first report to reveal that the frequency of pathogenicity of TP53 variants in p53 signatures was different between HBOC patients and control patients. Firstly, we found that the frequency of p53 signatures in the fimbria was similar between RRSO and control patients. HGSC, high-grade serous carcinoma; STIC, serous tubal intraepithelial carcinoma.

**DISCUSSION**

To our knowledge, this is the first report to reveal that the frequency of pathogenicity of TP53 variants in p53 signatures was different between HBOC patients and control patients. Firstly, we found that the frequency of p53 signatures in the fimbria was similar between RRSO and control patients. HGSC, high-grade serous carcinoma; STIC, serous tubal intraepithelial carcinoma.

**Fig. 2.** Immunohistochemistry staining for p53, Ki67, c-Myc, PAX8, and WT1 in the normal fallopian tube epithelial cells without p53 signature (A, E, I, M, Q) and p53-null type STIC (B, F, J, N, R) and p53-positive STIC (C, G, K, O, S) and HGSC (D, H, L, P, T) in a patient who underwent risk-reducing salpingo-oophorectomy. HGSC, high-grade serous carcinoma; STIC, serous tubal intraepithelial carcinoma.
samples and benign control samples, which indicates that the likelihood of a p53 signature in the fimbria does not depend on whether patients have germline BRCA1/2 pathogenic variants. The frequency of the p53 signature in RRSO samples was 53.8% in this study, which is within the range of 11%–71% in previous reports [13,15,21,25]. Secondly, in the sequence analysis, we identified 15 variants of TP53 in the RRSO and control specimens, of which 9 (60%) variants were categorized as pathogenic while the others were of unknown pathogenic significance. In the RRSO samples, 9 (90%) of 10 variants identified were pathogenic, whereas none of the 5 variants identified in the control group were pathogenic. The proportions of pathogenic variants were significantly different between RRSO samples and controls (p<0.001). These results suggest that even if p53 signatures are identified at a similar frequency in RRSO samples and controls, its characteristics and the risk of carcinogenesis might be different. Another finding was that in RRSO samples, patients with TP53 mutations in p53 signatures...
BRCAl pathogenic variants tended to have a higher frequency of p53 signature than those with BRCAl2 pathogenic variants. In addition, all 5 patients with TP53 pathogenic variants had BRCAl pathogenic variants, whereas none of the patients with BRCAl2 pathogenic variants had TP53 pathogenic variants. This may be due to the higher risk of developing ovarian cancer in HBOC patients with BRCAl pathogenic variant than in those with BRCAl2 pathogenic variant [26]. These results suggest that there might be 2 types of p53 signatures, one with a low risk of progression to STIC as seen in the control group and the other with a risk of progression to STIC with pathological variants in TP53. These 2 types of p53 signatures could be classified by analyzing TP53 variants.

Recent genomic analysis suggests that accumulation of TP53 mutations leads to aging and development of cancer in various organs throughout the body, most notably in sun-exposed skin cells [27]. However, the impact of the p53 signature on carcinogenesis in the fallopian tube is unknown. Studies of p53 signatures in fallopian tube tissue from patients with HGSC have found that some p53 signatures show loss of heterozygosity in addition to TP53 mutations, suggesting that some p53 signatures may already have a characteristics of cancer [28, 29]. A basic study found that mouse fallopian tube-derived organoids with double-knockout of Tp53 and Brca1 formed tumors when implanted subcutaneously [30], which indicates that TP53 mutations in cells with BRCAl mutations may increase the risk of carcinogenesis compared to cells without BRCAl mutations. In this study, however, the high prevalence of p53 signatures in normal controls, who are expected to have a low incidence of ovarian cancer, suggests that not all p53 signatures are associated with carcinogenesis. It has been reported that different types of TP53 mutations have different impacts on the prognosis of HGSC [31]; thus, the pathogenicity of mutations may be important for the characteristics of the p53 signature. Therefore, to assess the risk of carcinogenesis for a p53 signature, it would be necessary to evaluate its characteristics, such as loss of heterozygosity and BRCAl status in addition to TP53 mutation. The type and pathogenicity of TP53 mutations must also be considered. The concept of precursor escape has also been proposed [32], and how the p53 signature is involved in the development of peritoneal carcinoma will also require further exploration. The clinical significance of identifying p53 signature needs to be investigated in basic and clinical research in the future.

In this study, TP53 variants were detected in most p53 signatures, but not in all. One potential explanation for why not all p53 signatures had TP53 variants is that the section of the p53 signature from the tissue block was not the same as the section used for LCM, which could have prevented the p53 signature from being collected by LCM. In a previous study, the mutation detection rate for the p53 signature was found to be 57%, indicating the difficulty of analyzing the p53 signature in small lesions [13].

Two types of STIC, namely p53-positive STIC and p53-null type STIC, were identified in one patient who underwent RRSO. Both STICs were adjacent to each other, and both had mutations in the same codon of TP53, suggesting that they may have originated from the same cell. Furthermore, the p53-positive STIC had a mutation (TP53 c.983delT) in common with the occult cancer in this patient, suggesting that p53-positive STIC was likely the origin of HGSC. These findings suggest a relationship between the 2 STICs and HGSC in terms of clonal evolution. Although only the p53-positive STIC had the same mutation as that in cancer, the patterns of PAX8, WT-1, and c-Myc protein expression and the Ki-67 index in both STICs were similar to those of HGSC, suggesting that both STICs possessed the characteristics of cancer. It has been reported that STICs could have a pattern of strong
expression or loss of p53 protein [7,33]. Therefore, Ki-67 is important when screening for STIC in RRSO specimens, considering that p53-null type STIC might not be identified by p53 staining alone.

The main limitation of this research is that the analysis was based on a small number of cases from a single institution. To validate the results and obtain a more scientifically significant result, it would be needed to conduct a multicenter study with a large sample size. Further analysis of RRSO and control specimens will provide more detailed information on the characteristics of the p53 signature.

In summary, clinicopathologic analysis of the p53 signature and TP53 variant analysis in RRSO and control samples found no difference in the frequency of p53 signatures but did reveal a difference in the frequency of TP53 pathogenic variants in p53 signatures. Furthermore, TP53 variant analysis allowed us to evaluate the clonality of STIC and occult cancer in an actual patient who had undergone RRSO.

ACKNOWLEDGEMENTS

We thank K. Abe, T. Noda, S. Yamaguchi, and other laboratory members for technical assistance, as well as Collaborative Research Resources, Keio University School of Medicine, for technical support. This study was supported by Keio Gijuku Academic Development Funds (to KM); and by JSPS KAKENHI Grant Number 16K11155, 19K09833 (to TA), and 20K18174 (to KM).

SUPPLEMENTARY MATERIALS

Table S1
RRSO patients’ profile
Click here to view

Table S2
Normal control patients’ profile
Click here to view

Table S3
Primer sequence
Click here to view

Table S4
Characteristics of RRSO patients and control patients
Click here to view
Table S5
TP53 pathogenicity
Click here to view

Table S6
Gene list detected in high-grade serous carcinoma by next-generation sequencing
Click here to view

Fig. S1
Copy number variant analysis using DNA panel sequencing data of high-grade serous carcinoma lesion. Genes which copy number were elevated above 3.5 were MYC (3.87), FOXL2 (3.74), and CCND1/FGF4 (3.74).
Click here to view

REFERENCES

1. Ahmed AA, Etemadmoghadam D, Temple J, Lynch AG, Riad M, Sharma R, et al. Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. J Pathol 2010;221:49-56. PUBMED | CROSSREF

2. Shih IM, Wang Y, Wang TL. The origin of ovarian cancer species and precancerous landscape. Am J Pathol 2021;191:26-39. PUBMED | CROSSREF

3. Paley PJ, Swisher EM, Garcia RL, Agoff SN, Greer BE, Peters KL, et al. Occult cancer of the fallopian tube in BRCA1 germline mutation carriers at prophylactic oophorectomy: a case for recommending hysterectomy at surgical prophylaxis. Gynecol Oncol 2001;80:176-80. PUBMED | CROSSREF

4. Pick JM, van Diest PJ, Zweemer RP, Jansen JW , Poort-Keesom RJ, Menko FH, et al. Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. J Pathol 2001;195:451-6. PUBMED | CROSSREF

5. Leeper K, Garcia R, Swisher E, Goff B, Greer B, Paley P. Pathologic findings in prophylactic oophorectomy specimens in high-risk women. Gynecol Oncol 2002;87:52-6. PUBMED | CROSSREF

6. Kobayashi Y, Hirasawa A, Chiyoda T, Ueki A, Masuda K, Misu K, et al. Retrospective evaluation of risk-reducing salpingo-oophorectomy for BRCA1/2 pathogenic variant carriers among a cohort study in a single institution. Jpn J Clin Oncol 2021;51:213-7. PUBMED | CROSSREF

7. Kuhn E, Kurman RJ, Vang R, Sehdev AS, Han G, Soslow R, et al. TP53 mutations in serous tubal intraepithelial carcinoma and concurrent pelvic high-grade serous carcinoma—evidence supporting the clonal relationship of the two lesions. J Pathol 2012;226:421-6. PUBMED | CROSSREF

8. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. Am J Surg Pathol 2007;31:161-9. PUBMED | CROSSREF

9. Gilks CB, Irving J, Köbel M, Lee C, Singh N, Wilkinson N, et al. Incidental nonuterine high-grade serous carcinomas arise in the fallopian tube in most cases: further evidence for the tubal origin of high-grade serous carcinomas. Am J Surg Pathol 2015;39:357-64. PUBMED | CROSSREF

10. Morrison JC, Blanco LZ Jr, Vang R, Ronnett BM. Incidental serous tubal intraepithelial carcinoma and early invasive serous carcinoma in the nonprophylactic setting: analysis of a case series. Am J Surg Pathol 2015;39:442-53. PUBMED | CROSSREF
11. Koç N, Ayas S, Uygur L. The association of serous tubal intraepithelial carcinoma with gynecologic pathologies and its role in pelvic serous cancer. Gynecol Oncol 2014;134:486-91.

12. Karnezis AN, Cho KR, Gilks CB, Pearce CL, Huntsman DG. The disparate origins of ovarian cancers: pathogenesis and prevention strategies. Nat Rev Cancer 2017;17:65-74.

13. Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol 2007;211:26-35.

14. Visvanathan K, Shaw P, May BI, Bahadirlil-Talbott A, Kaushiva A, Risch H, et al. Fallopian tube lesions in women at high risk for ovarian cancer: a multicenter study. Cancer Prev Res (Phila) 2018;11:697-706.

15. Folkins AK, Jarboe EA, Saleemuddin A, Lee Y, Callahan MJ, Drapkin R, et al. A candidate precursor to pelvic serous cancer (p53 signature) and its prevalence in ovaries and fallopian tubes from women with BRCA mutations. Gynecol Oncol 2008;109:168-73.

16. Hirasawa A, Masuda K, Akahane T, Ueki A, Yokota M, Tsutara T, et al. Family history and BRCA1/BRCA2 status among Japanese ovarian cancer patients and occult cancer in a BRCA1 mutant case. Jpn J Clin Oncol 2014;44:49-56.

17. Medeiros F, Muto MG, Lee Y, Elvin JA, Callahan MJ, Feltmate C, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 2006;30:230-6.

18. Carcangi ML, Radice P, Manoukian S, Spatti G, Gobbo M, Pensotti V, et al. Atypical epithelial proliferation in fallopian tubes in prophylactic salpingo-oophorectomy specimens from BRCA1 and BRCA2 germline mutation carriers. Int J Gynecol Pathol 2004;23:35-40.

19. Jarboe E, Folkins A, Nucci MR, Kindelberger D, Drapkin R, Miron A, et al. Serous carcinogenesis in the fallopian tube: a descriptive classification. Int J Gynecol Pathol 2008;27:1-9.

20. Vang R, Visvanathan K, Gross A, Maambo E, Gupta M, Kuhn E, et al. Validation of an algorithm for the diagnosis of serous tubal intraepithelial carcinoma. J Clin Oncol 2012;31:243-53.

21. Visvanathan K, Vang R, Shaw P, Gross A, Soslow R, Parkash V, et al. Diagnosis of serous tubal intraepithelial carcinoma based on morphologic and immunohistochemical features: a reproducibility study. Am J Surg Pathol 2011;35:1766-75.

22. Shuber AP, Grondin VJ, Klinger KW. A simplified procedure for developing multiplex PCRs. Genome Res 1995;5:488-93.

23. Heinmoller E, Liu Q, Sun Y, Schlake G, Hill KA, Weiss LM, et al. Toward efficient analysis of mutations in single cells from ethanol-fixed, paraffin-embedded, and immunohistochemically stained tissues. Lab Invest 2002;82:443-53.

24. Akahane T, Sekizawa A, Purwosunu Y, Nagatsuka M, Okai T. The role of p53 mutation in the carcinomas arising from endometriosis. J Gynecol Pathol 2007;26:345-51.

25. Mehra KK, Chang MC, Folkins AK, Raho CJ, Lima JF, Yuan L, et al. The impact of tissue block sampling on the detection of p53 signatures in fallopian tubes from women with BRCA 1 or 2 mutations (BRCA+) and controls. Mod Pathol 2011;24:152-6.

26. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. JAMA 2017;317:2402-16.

27. Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. Science 2015;349:1483-9.

28. Labidi-Galy SI, Papp E, Hallberg D, Niknafs N, Adleff V, Noe M, et al. High grade serous ovarian carcinomas originate in the fallopian tube. Nat Commun 2017;8:1093.
29. Wu RC, Wang P, Lin SF, Zhang M, Song Q, Chu T, et al. Genomic landscape and evolutionary trajectories of ovarian cancer precursor lesions. J Pathol 2019;248:41-50.

30. Lõhmussaar K, Kopper O, Korving J, Begthel H, Vreuls CPH, van Es JH, et al. Assessing the origin of high-grade serous ovarian cancer using CRISPR-modification of mouse organoids. Nat Commun 2020;11:2660.

31. Tuna M, Ju Z, Yoshihara K, Amos CI, Tanyi JL, Mills GB. Clinical relevance of TP53 hotspot mutations in high-grade serous ovarian cancers. Br J Cancer 2020;122:405-12.

32. Meserve EEK, Brouwer J, Crum CP. Serous tubal intraepithelial neoplasia: the concept and its application. Mod Pathol 2017;30:710-21.

33. Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih IM, et al. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. Mod Pathol 2011;24:1248-53.