Original Article

Clinical significance of RAD51C and its contribution to ovarian carcinogenesis

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Abstract: Aim: The underlying mechanisms of chemoresistance-induced recurrence of ovarian carcinoma are largely unknown. The purpose of this study was to investigate the clinical significance of RAD51C and its role in ovarian tumorigenesis and progression. Methods: 60 cases of ovarian epithelial tumors (30 benign and 30 malignant tumors, respectively) were enrolled from 2014 to 2016. Immunohistochemistry was used to evaluate RAD51C expression in tumor tissues, and RT-PCR was employed to test RAD51C mRNA levels in SKOV3, A2780, and CAOV3 cell lines. Targeted knockdown of RAD51C was achieved with siRNA to explore the changes of cell proliferation, migration, and apoptosis. Results: RAD51C protein level in carcinoma tissues, especially in the high-grade group (P<0.001), was significantly higher than that of benign tumors and associated with pathological type, stage, and overall survival (P<0.05). Downregulation of RAD51C promoted apoptosis and decreased cell survival rate and migration. Conclusions: Our results supported that RAD51C contributes to the progression of ovarian carcinoma, suggesting its promising application as an independent prognostic marker for diagnosis and treatment.

Keywords: RAD51C, ovary, carcinoma, progression, immunohistochemistry

Introduction

Ovarian cancer, one of the most common malignant tumors in women, is the greatest contributor to death from gynecologic neoplasms [1, 2]. It is characterized by invasion, metastasis, and chemoresistance, resulting in short relapse-free and overall-survival [3]. However, the underlying molecular mechanisms of ovarian tumorigenesis and progression are largely unknown. Epithelial ovarian carcinoma (EOC) accounts for approximately 85-90% of ovarian malignant neoplasms. Thus, a biologic marker for diagnosis, treatment, and prognosis of EOC is urgently needed.

The RAD51C gene is a susceptibility gene of EOC and localizes to a region of chromosome 17q23 [4]. The overexpression or mutation of RAD51C can ruin the ability of homologous recombination repair, leading to the instability of genome [5, 6]. RAD51C protein, one of the elements of homologous recombination pathway, is involved in Fanconi anemia syndrome and several cancers, such as breast, ovary, pancreas, and prostate [7-9]. This study used immunohistochemistry (IHC) and siRNA to gain more insights into the role of RAD51C in EOC progression, malignant cell proliferation, invasion, metastasis, and apoptosis.

Materials and methods

Patients and clinical data

From January 2014 to December 2016, 60 cases of epithelial ovarian tumors were obtained, including 30 cases of EOC (15 serous and 15 mucinous carcinoma, respectively) and 30 cases of benign ovarian tumor (15 serous and 15 mucinous cystadenoma, respectively). The final diagnosis was established by two pathologists. Patient’s age ranges from 14-year to 70-year and the average age was 44.58±14.47-years. Inclusion criteria include: 1. Complete clinical data; 2. Definite pathologic diagnosis; 3. Signed informed consent; 4. No prior radio-, chemo-, or immunotherapy; 5. Other than the ovarian tumor, the patient’s past medical history was not significant. The study was
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approved by the Research Ethics Committee of the First Affiliated Hospital of Nanchang University (No. 2019KJJ024).

Immunohistochemistry

All tissues were fixed in 4% buffered formalin and paraffin-embedded. Tissue sections were cut at 2-5 μm thickness for immunohistochemical staining. Sections were dewaxed and underwent antigen retrieval process in citrate buffer for 15 min. These pretreated slides were incubated at 4°C overnight with antibody for RAD51C (Novusbio, 1:100). Evaluation of immunohistochemical results were performed by two independent pathologists, who were blinded regarding patient details. Immunoreactive score (IRS) was used to analyze the stained slides and gave a range of 0-12 as a product of multiplication between the percentage of positive cells: 0: ≤5%; 1: 6-25%; 2: 26-50%; 3: 51-75%, 4: >75%, and intensity: 0: none; 1: weak; 2: medium; 3: strong. A score ≤2 was considered as low expression, and >2 was high expression.

Cell culture and transfection

Cell lines SKOV3, A2780, and CAOV3 were plated in 1640 medium supplemented with 10% FBS. Targeted cells were transfected with siRNA using Lipofectamine 3000 according to manufacturer’s instruction.

Quantitative RT-PCR

RNA was extracted from cultured cells using Trizol. cDNA was synthesized with a mixture of random and Oligo dT primers using reverse transcriptase kit (Takara). Primers for measuring cDNA expression include RAD51C-F: CGC-TGTCTGACTACACAGA, R: GTTCCAACCTTTGCTTTCG, GAPDH-F: CAATGACCCCTTGCAGAC, R: GAGAAGCTTCCGTTCATCTCAG. Quantitative PCR was performed with the Applied Biosystems Prism 9700 PCR machine. Relative gene expression was normalized to GAPDH.

Cell proliferation

5×10^3 cells were digested, resuspended, counted, and plated. After 48 hours, 10 μl CCK8 was added into medium for 2 hours, followed by absorbance detection using ELISAs. Cell vitality% = Absorbance (experimental-blank)/Absorbance (control-blank) ×100%.

Cell migration

Scratch wound healing assay was employed to evaluate cell migration. When the plating density was up to 90%, a 200 μl pipette tip was used to scratch a wound through the center of the well, which was washed 3 times with PBS and cultured in 1640 medium without FBS. The wound width was measured at 0, 24, and 48 h, and \( R_m = \frac{(W_i-W_f)}{t} \) (\( R_m \): rate of cell migration; \( W_i \): initial wound width; \( W_f \): final wound width).

Flow cytometry analysis

Cultured cells were washed in PBS and centrifuged twice. The plaque was resuspended in 300 μl 1× pre-cooled binding buffer supplemented with 5 μl Annexin V-FITC and 5 μl PI-PE and cultured for 10 min. 200 μl 1× pre-cooled binding buffer was added into the mixture which was analyzed by flow cytometry (BD Accuri C6, Baijia, China).

Statistical analysis

All data are shown as the mean ± SD. Statistical analysis was performed using SPSS 19.0 and Prism 5.02 (Graphpad). Significance levels were set at \(* P<0.05; ** P<0.01; *** P<0.001.

Results

RAD51C expression in ovarian epithelial tissues

Positivity of RAD51C was observed in the cytoplasm, rather than nucleus, of ovarian epithelium (Figure 1). The score of malignant ovarian tissues was 4.43±2.43, and that of benign tumor was 1.50±1.47 (Figure 2).

Differential expression of RAD51C in tumor tissues

The high expression rate of RAD51C in ovarian cancer (73.3%, 22/30) was significantly higher than that of benign tumors (10.0%, 3/30) (P<0.05) (Table 1). The majority of serous ovarian cancer (93.3%, 14/15) and 2 (13.3%) cases of serous cystadenoma exhibited a high level of RAD51C (Table 2). The percentage of mucinous cancer (7/15, 46.7%) expressing RAD51C was higher than its benign counterpart (1/15, 6.6%) (Table 3).
The relation ship between RAD51C and clinical findings

There was no highly significant correlation between RAD51C and age of patients (P<0.05). The expression of RAD51C in high grade serous carcinoma was significantly upregulated compared with mucinous tumors (P<0.05). It seems that RAD51C promoted the progression of ovarian carcinoma because of the higher level of RAD51C at late stages (III-IV) than early stages (I-II), leading to a poor prognosis (P<0.05) (Table 4).

RNAi downregulated the expression of RAD51C in cell lines

The mRNA levels of RAD51C were evaluated using qPCR and the results indicated that the A2780 cell line exhibited the highest RAD51C (P<0.05) (Figure 3). Three constructs of RAD51C interference were transfected into A2780 followed by qPCR test. siRNA-3 showed the lowest level of RAD51C mRNA compared to normal controls (P<0.05) (Figure 4A and 4B).

RAD51C was involved in cell proliferation and apoptosis

To explore the role of RAD51C in the proliferation and apoptosis of ovarian cancer cells, RAD51C siRNA-3 was transfected into A2780 cells. After 24 h, CCK-8 was used to test and evaluate the proliferation of malignant cells. Compared with the control group, knockdown of RAD51C significantly decreased cell proliferation and increased cellular apoptosis (P<0.05) (Figure 4C and 4D).

RAD51C affected the migration of cancer cells

The results of scratch wound healing assay indicated that knock-down of RAD51C impaired the ability of cellular migration in the siRAD51C group compared with control cells at 24 h (P<0.05) (Figure 5A and 5B).

Table 1. Expression of RAD51C in ovarian tumors

<table>
<thead>
<tr>
<th>Type</th>
<th>Low n (%)</th>
<th>High n (%)</th>
<th>X^2 value</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Benign tumor</td>
<td>27 (90%)</td>
<td>3 (10.0%)</td>
<td>25.733</td>
<td>0.000</td>
</tr>
<tr>
<td>Malignant tumor</td>
<td>8 (26.7%)</td>
<td>22 (73.3%)</td>
<td></td>
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Figure 1. Immunohistochemical scores of RAD51C. A. 0 point: none of the tumor cells showed positivity for RAD51C antibody (original magnification ×200). B. 1 point: the cytoplasm of tumor cells exhibited light brown staining intensity (original magnification ×200). C. 2 points: brownish yellow intensity was seen in cytoplasm (original magnification ×200). D. 3 points: strong positivity for RAD51C (original magnification ×200).

Figure 2. IHC scores of benign and malignant ovarian tumors. The expression level of RAD51C in malignant carcinoma was higher than that of adenocarcinoma.
Discussion

The most concerned issues in ovarian cancer studies are molecular mechanisms, chemoresistance, and targeted therapy [10-12]. DNA homologous recombination repair is associated with EOC chemoresistance and PARP inhibitor. It is conceived that an increase in DNA homologous recombination repair contributes to chemoresistance. PARP inhibitor can promote cell apoptosis through the inhibition of DNA homologous recombination (HR) repair [13, 14].

Radio- and chemotherapy induce tumor cell death mainly through DNA double strand break (DSB), which can be repaired by HR in eukaryotic cells. RAD51C belongs to the HR-related RAD51 family. The homologous protein system of RAD51C forms two complexes: XRCC3-RAD51C heterodimeric protein structure (CX3) and RAD51B-RAD51C-RAD51D-XRC-C2 heterotetrameric protein structure (BCDX2), which are central to the early and late stages of HR [15-17]. Accumulating evidence supports the important role of RAD51C in several cancers, including breast cancer, prostate cancer, gastric cancer, and endocrine tumors [15, 16, 18-22]. In contrast, the level of RAD51C in gastric cancer tissues was significantly lower compared with para-cancer tissue [23].

Consistent with previous studies, our IHC results showed that ovarian cancer, especially high-grade serous cancer, expressed a higher level of RAD51C protein compared with benign tumors. RAD51C protein level increased in direct proportion to clinical stage, indicating poor prognosis. There was no substantial evidence to demonstrate a relationship between RAD51C and age. Transfection of siRNA-3 into A2780 significantly down-regulated RAD51C expression, which inhibited cellular proliferation and migration both in early and terminal stages of apoptosis, suggesting an important role of RAD51C during the carcinogenesis and progression of ovarian cancer.

This study provides preliminary evidence to support a role of RAD51C in OC. The findings provide more insights into the molecular mecha-
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**Figure 4.** RAD51C is involved in cellular proliferation and apoptosis. A. siRNA3 inhibited RAD51C expression in the cell line. B. Downregulation of RAD51C decreased cell viability. C and D. The inhibition of RAD51C increased cellular apoptosis.

**Figure 5.** RAD51C and cell migration. A and B. siRNA impaired migration ability of infected cells.

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**Disclosure of conflict of interest**

None.

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