The Frequency and Clinical Implication of Mismatch Repair Protein Deficiency in Chinese Patients with Ovarian Clear Cell Carcinoma

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Research

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Abstract

Background: To assess the prevalence of deficient mismatch repair (MMR) in Chinese ovarian clear cell carcinoma (CCC) patients and its association with clinicopathologic features.

Methods: Immunohistochemistry with four antibodies against MLH1, PMS2, MSH2 and MSH6 were performed on whole section slides. Results were correlated with clinicopathologic variables.

Results: A total of 108 cases were included in the study, with a median age of 52 years at first diagnosis. Early-stage disease and platinum-sensitive recurrence accounted for 62.3% and 69.6%, respectively. Overall, the estimated 5-year overall survival was 70.3% and 20.7% in patients with early and late stage tumor, respectively. Deficient MMR were identified in 5.6% (6/108) of the cohort and included MSH2/MSH6 (n=4) and MLH1/PMS2 (n=2). The average age of the six patients was 45.6 years. The rate of MMR-deficient tumors in women ≤ 50 years was relatively higher than that those over 50 years (10.0% Vs. 2.9%; \( P=0.266 \)). A half of the patients with deficient MMR were diagnosed with a synchronous (endometrial or colorectal) and metachronous (endometrial) cancer, significantly more than those intact counterparts (\( P=0.002 \)). All the six patients had early-stage tumor and the majority (83.3%) were platinum-sensitive. The median progression-free survival was slightly higher in patients with defective MMR expression than those intact counterparts (30 months Vs. 27 months), although significance was not achieved (\( P=0.471 \)).

Conclusions: Ovarian CCC patients with young age and concurrent diagnosis of endometrial and colorectal cancer are more likely to have MMR-deficient tumors. It merits further evaluation whether patients harboring MMR abnormality has favorable prognosis.

Background

The histologic subtypes of ovarian cancer are distinct diseases, each with different clinical and molecular characteristics [1]. Ovarian clear cell carcinoma (CCC) has unique epidemiologic correlations with ethnicity and endometriosis, genetic/epigenetic alterations, and specific immune-related molecular profile [2]. Besides, it is considered as a great challenge due to its disease aggressiveness and chemo-resistance [3]. The objective response rate to conventional chemotherapy is 9% in platinum sensitive and 1% in platinum resistant recurrence [4]. In recent clinical trials, ovarian CCC patients showed surprising sensitivity to immune checkpoint inhibitors, despite that ovarian cancer patients (mainly high-grade serous carcinoma) showed modest responses in all [5, 6]. However, given disease rarity, only small cases were included in the trials and further verification is need.

Mismatch repair (MMR) deficiency is proved to be biomarker of sensitivity to immune checkpoint blockade with antibodies to programmed death receptor-1 across various kinds of tumors [7]. Defective MMR leads to accumulation of mutations in the genome and microsatellite instability in tumors [8]. Lynch syndrome is characterized by loss of expression of MMR genes [9], and the mostly clinically significant genes are MLH1, MSH2, MSH6 and PMS2 [8, 10]. Women with Lynch syndrome are at increased risk of ovarian carcinoma, mostly clear cell and endometrioid histology [9]. In the past five years, there have been some publications focusing on the MMR deficiency in ovarian CCC [11–18]. The frequency of deficient MMR varies from study to study and most studies included small cases of clear cell carcinoma patients [13–16, 18].

In the present study, we assessed the status of MMR protein in a well-annotated unselected cohort of Chinese ovarian CCC patients. The frequency of MMR deficiency by immunohistochemistry and the associations with clinicopathologic variables were evaluated.

Results

Clinical features and follow-up of the study patients

There were a total of 108 patients after excluding eight cases with immunohistochemistry technical failure. For the entire cohort, median age was 52 years (mean: 51.8 years; range: 26–79 years). Of them, 37.0% (40/108 of the patients were 50
years or younger. Nine patients had a personal history of cancer and/or a synchronous cancer. Of them four were diagnosed synchronously with a malignancy of the endometrium (n = 3) or colon (n = 1). The patient with synchronous colon and ovarian cancer developed endometrial cancer two years later. A previous history of breast cancer and thyroid cancer was noted in two and one patients, respectively. The rest two patients had metachronous urothelial cell cancer and lung cancer after the diagnosis of ovarian CCC. In addition, 23 patients (21.3%) reported a family history of cancer, mostly colorectal cancer (n = 4), pancreatic cancer (n = 3) and urothelial cell cancer (n = 3).

As clearly shown from Table 1, 62.3% (66/106) of the patients presented with early-stage disease (FIGO I + II), and most of them (48 patients, 45.3%) were of FIGO stage I. Totally, 91.2% patients had residual disease ≤ 1 cm. For the patients with advanced disease, the debulking results were 41.7% (15/36) with no gross residual disease, and 66.7% (24/36) with residual disease ≤ 1 cm. Concerning chemotherapy response, platinum-sensitive recurrence accounted for 69.6%.

Table 1
Clinical features of the study population (n = 108)

<table>
<thead>
<tr>
<th>Age at diagnosis</th>
<th>52 (26–79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal history of cancer</td>
<td>8.3% (9/108)</td>
</tr>
<tr>
<td>Family history of cancer</td>
<td>21.3% (23/108)</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
</tr>
<tr>
<td>FIGO stage I</td>
<td>45.3% (48/106)</td>
</tr>
<tr>
<td>FIGO stage II</td>
<td>17.0% (18/106)</td>
</tr>
<tr>
<td>FIGO stage III</td>
<td>29.2% (31/106)</td>
</tr>
<tr>
<td>FIGO stage IV</td>
<td>8.5% (9/106)</td>
</tr>
<tr>
<td>Extent of debulking</td>
<td></td>
</tr>
<tr>
<td>Residual disease = 0 cm (%)</td>
<td>79.4% (81/102)</td>
</tr>
<tr>
<td>Residual disease ≤ 1 cm (%)</td>
<td>88.2% (90/102)</td>
</tr>
<tr>
<td>Platinum response</td>
<td></td>
</tr>
<tr>
<td>Platinum-sensitive</td>
<td>69.6% (71/102)</td>
</tr>
<tr>
<td>Platinum-resistant</td>
<td>30.4% (31/102)</td>
</tr>
<tr>
<td>Follow-up time (mean, range)</td>
<td>46 (1-178)</td>
</tr>
<tr>
<td>Disease status at last follow up</td>
<td></td>
</tr>
<tr>
<td>Dead (%)</td>
<td>46.1% (48/104)</td>
</tr>
<tr>
<td>Alive with disease (%)</td>
<td>20.2% (21/104)</td>
</tr>
<tr>
<td>No evidence of disease</td>
<td>33.7% (35/104)</td>
</tr>
</tbody>
</table>

Follow-up information was available in the majority of the patients (96.3%, 104/108). After a mean follow-up time of 46 months (range, 1-178 months), 46.1% (48/104) died of disease, 20.2% (21/104) were still alive with disease and 33.7% (35/104) had no evidence of disease. Of note, 26 patients had follow-up time less than 24 months: six were alive without disease and the remaining 20 patients all died. Figure 1 demonstrates the survival curves for the entire cohort stratified by stage. The median PFS of patients with early and late disease was 35 and 12 months (P = 0.002), respectively. Similarly, the median OS of patients with early stage tumor was significantly better than that of patients with advanced disease (109 Vs. 31
months, \( P < 0.001 \). The estimated 5-year overall survival was 70.3% in patients with early stage tumor, and 20.7% in those with advanced disease.

**Clinicopathologic Features Of Patients With Defective MMR**

A total six (5.6%) patients harbored abnormal MMR expressions, including MSH2/MSH6 \((n = 4)\) and MLH1/PMS2 \((n = 2)\) (Fig. 2). Table 2 presents the clinical and pathological characteristics of the ovarian CCC patients with deficient MMR. The average age of the six patients was 45.6 years, compared to 52.1 years for MMR-intact tumors, although statistic significance was not achieved \((P = 0.153)\). In addition, the rate of MMR-deficient tumors in women \(\leq 50\) years was relatively higher than that those over 50 years \((10.0\% \text{ Vs. } 2.9\%; P = 0.266)\). In terms of personal history of cancer, a half of the patients with deficient MMR were diagnosed with a synchronous or metachronous cancer, significantly more than those counterparts with intact expression \((P = 0.002)\). Two patients had a synchronous endometrioid endometrial cancer (No. 3 and No. 5). The patient (No. 2) had an accidental diagnosis of ovarian CCC during the scheduled colorectal cancer surgery and was diagnosed with metachronous endometrioid endometrial cancer two years later. Family history of cancer was reported in two patients (No. 3, No. 6). All the six patients had early-stage (FIGO I + II) tumor at first diagnosis. Concerning platinum response, the majority \((5/6, 83.3\%)\) of the patients were platinum-sensitive. During study period, three patients relapsed. At last follow-up, two patients were still alive with disease and four had no evidence of disease. The median PFS was higher in patients with abnormal MMR expression than those with intact expression \((54 \text{ months Vs. } 27 \text{ months})\), although statistic significance was not achieved \((P = 0.471)\) (Fig. 1). Overall survival comparison was not made give that all the patients with MMR deficient tumors were still alive (data censored) at last contact.

<table>
<thead>
<tr>
<th>No</th>
<th>Age (years)</th>
<th>Personal history</th>
<th>Family history</th>
<th>Stage</th>
<th>Platinum response</th>
<th>Status</th>
<th>PFS (months)</th>
<th>OS (months)</th>
<th>dMMR pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td></td>
<td></td>
<td>IC</td>
<td>Sensitive</td>
<td>NOD</td>
<td>71</td>
<td>71</td>
<td>MSH2/MSH6</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>Colon, endometrial</td>
<td></td>
<td>IA</td>
<td>Sensitive</td>
<td>NOD</td>
<td>30</td>
<td>30</td>
<td>MSH2/MSH6</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>Endometrial</td>
<td>Endometrial, colon, pancreatic</td>
<td>IIB</td>
<td>Sensitive</td>
<td>NOD</td>
<td>30</td>
<td>36</td>
<td>MSH2/MSH6</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td></td>
<td></td>
<td>IIB</td>
<td>Sensitive</td>
<td>AWD</td>
<td>19</td>
<td>126</td>
<td>MSH2/MSH6</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Endometrial</td>
<td></td>
<td>IIB</td>
<td>Sensitive</td>
<td>NOD</td>
<td>57</td>
<td>57</td>
<td>MLH1/PMS2</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td></td>
<td>Colon</td>
<td>IIA</td>
<td>Resistant</td>
<td>AWD</td>
<td>4</td>
<td>33</td>
<td>MLH1/PMS2</td>
</tr>
</tbody>
</table>

Abbreviations: PFS = Progression-Free Survival; OS = Overall Survival; dMMR = Deficient Mismatch Repair; AWD = Alive With Disease; NOD = No evidence Of Disease

The two patients with family history underwent subsequent genetic testing. Patient No.6 was proved to carry a MHL1 germline mutation: c.1756G > C (p.Ala586Pro) (Class 5). Patient No.3 had synchronous endometrial cancer and ovarian CCC at diagnosis. Besides, she had a significant family history: mother had endometrial cancer, and two brothers of her mother had colorectal and pancreatic cancer, respectively. She was found to have a complex gene rearrangement in MSH2 which has never been reported (MSH2 variant of uncertain significance). Further multiplex ligation-dependent probe amplification test turned out to be negative.
**Discussion**

Several recent publications focusing on MMR evaluation in ovarian CCC have been summarized in Table 3. Five of the eight studies include relatively small sample size due to disease rarity [13–16, 18]. The prevalence of MMR deficiency ranged from 0 to 13% [11–18]. Bennett et al conducted the largest series on whole section slides and correlated the MMR expression to histologic features [11]. They highlighted that diffuse intratumoral stromal inflammation and the presence of peritumoral lymphocytes might be associated with MMR loss in ovarian CCC [11]. Colleagues from Canada performed another study with a large sample size, which assessed the PROMISE algorithm related markers, including MMR in ovarian CCC by tissue microarray [17]. They reported a low frequency of abnormal MMR (2%) and no pathogenic DNA polymerase ε (POLE) mutation [17].

**Table 3**

A review of the recent studies focusing on MMR immunohistochemistry in ovarian clear cell carcinoma in chronological order.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>dMMR Rate</th>
<th>Sample</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bennett et al</td>
<td>USA</td>
<td>6% (6/109)</td>
<td>Whole section slides</td>
<td>MSH2/MSH6 (3), MLH1/PMS2 (1), MSH6 (1), PMS2 (1)</td>
</tr>
<tr>
<td>Rambau et al</td>
<td>Canada</td>
<td>2.4% (4/164)</td>
<td>Tissue microarray</td>
<td>MSH2/ MSH6 (3), MSH6 (1)</td>
</tr>
<tr>
<td>Willis et al.</td>
<td>USA</td>
<td>13% (3/23)</td>
<td>Whole section slides</td>
<td>MSH2/ MSH6 (3)</td>
</tr>
<tr>
<td>Stewart et al</td>
<td>Australia</td>
<td>6% (2/32)</td>
<td>Whole section slides</td>
<td>MSH2/ MSH6 (2)</td>
</tr>
<tr>
<td>Howitt et al</td>
<td>USA</td>
<td>10% (3/30)</td>
<td>Whole section slides</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Xiao et al</td>
<td>China</td>
<td>4% (2/50)</td>
<td>Tissue microarray</td>
<td>MLH1/PMS2 (1), PMS2 low (1)</td>
</tr>
<tr>
<td>Parra-Herran et al</td>
<td>Canada</td>
<td>2% (2/90)</td>
<td>Tissue microarray</td>
<td>MSH2/ MSH6 (1), MSH6 (1)</td>
</tr>
<tr>
<td>Fraune et al</td>
<td>Germany</td>
<td>0/23</td>
<td>Tissue microarray</td>
<td>/</td>
</tr>
<tr>
<td>Our study</td>
<td>China</td>
<td>5.6% (6/108)</td>
<td>Whole section slides</td>
<td>MSH2/MSH6 (4), MLH1/PMS2 (2)</td>
</tr>
</tbody>
</table>

Abbreviations: dMMR = Deficient Mismatch Repair

Universal testing of MMR in ovarian cancer is not routine in most hospitals and National Comprehensive Cancer Network (NCCN) guideline recommends it as clinically indicated. Not surprisingly, higher frequencies of defective MMR were reported in younger patients in several studies [9, 11, 12, 19]. Rambau and colleagues tested MMR protein in 612 ovarian cancer patients by tissue microarray and found that deficient MMR was related to age < 50 years, synchronous endometrial endometrioid cancer and absence of ARID1A [12]. In a relatively large sample size study focusing on ovarian CCC alone (n = 109), the patients with abnormal MMR expression were significantly younger, with a mean age of 40 years in contrast to 53.2 years for the overall cohort [11]. In our series, the mean age of the six patients with loss of MMR expression was 45.6 years, compared to 52.1 years for MMR-intact tumors, which was not statistical significant. Nevertheless, we noticed a rate of 10.0% MMR deficiency in patients of 50 years and below.

MMR-deficient tumors have peculiar clinical behaviors, including early-onset metastatic potential but generally favorable prognosis, and remarkable responses to immune therapy [20]. The possible clinical implications of MMR deficiency in ovarian CCC have been evaluated in literature [11, 12, 17]. However, no consensus has ever been reached mainly due to disease rarity and low frequency of MMR abnormality. In the present work, we noticed that all the six patients had early-stage disease, which was quite consistent with the finding that MMR-deficient colorectal cancers were strongly enriched in the early stages of diagnosis [21]. What's more, patients with loss of MMR expression tended to have longer progression-free survival than those
patients with preserved expression though without significance. Albeit based on a small number of cases, it raises the possibility that MMR-deficient tumors might confer a good prognosis in ovarian CCC. Similarly, the prognostic implication of MMR deficiency in ovarian CCC has been evaluated in two studies [11, 17]. However, no conclusion has ever been achieved. Stewart et al. reported two patients with advanced tumor harboring MMR abnormality were alive 160 months and 124 months following surgery [14].

To the best of our knowledge, the present study represents one of the largest series measuring MMR protein in ovarian clear cell carcinoma patients. Several limitations should be pointed out. Firstly, considering disease rarity, we collected the cases over a long period of time, which leads to some missing data. Secondly, the cohort might be limited by the selection and surveillance biases often associated with studies from a single institution.

Conclusions

We showed MMR loss in 5.6% of unselected tumors of ovarian clear cell carcinoma, but this rate increased to 10% when selecting for age (50 years and below). All patients presented with early-stage disease and half of the patients had synchronous/metachronous endometrial/colorectal cancer. Patients with MMR deficiency seemed to have better progression-free survival.

Materials And Methods

Study population

After obtaining the institutional review board approval (050432-4-1212B), we identified all the patients by searching the surgical pathology archives for “ovarian clear cell carcinoma” from 2008 to 2018. In our institution, one surgical specimen is usually reviewed by two pathologists (one young and one senior doctor) as a routine. A third experienced pathologist will review the slides in some difficult cases or to resolve discrepancy. In the current study, we included all the patients with archived tissue blocks and all the available hematoxylin & eosin-stained slides were reviewed to confirm the diagnosis. The cases were excluded if it was focal carcinoma or the clear cell component was less than 50% in mixed tumor. The requirement for written informed consent was waived considering its retrospective design.

Clinicopathologic information and survival outcomes were abstracted from medical records. The following data was extracted: the age at diagnosis of ovarian CCC, personal and family history of cancer, date and type of primary surgery, International Federation of Gynecology and Obstetrics (FIGO) stage at initial diagnosis [22], residual disease, platinum-free interval (the time interval from completion of the last platinum-based chemotherapy to disease recurrence), time of disease progression or recurrence, and tumor status at last contact. Patients were considered as platinum-sensitive if the platinum-free interval was more than six months. Progression-free survival (PFS) and overall survival (OS) was defined as the time interval from the date of the primary surgery to the date of first recurrence and death or last contact, respectively. Due to the retrospective design, some clinicopathologic information was missing.

Immunohistochemistry

Four-µm-thick, formalin-fixed, paraffin-embedded whole-block sections were used for immunohistochemistry. All stains were performed on an automated stainer (Ventana BenchMark ULTRA). MMR protein immunohistochemistry has been routinely conducted in some circumstances in our institution [23, 24]. Primary antibodies included anti-MLH1 (Clone G168-728), anti-MSH2 (Clone G219-1129), anti-MSH6 (Clone 44), and anti-PMS2 (Clone EPR3947), which were purchased from Roche (Basel, Switzerland).

All the slides were reviewed independently by two pathologists who were blind to the clinical information. MMR stains were interpreted as abnormal (loss of nuclear staining in all tumor cells) and normal (retained nuclear staining) [23, 24].
Lymphocytes and stromal cells served as positive internal controls.

**Statistical Analyses**

Continuous data were presented as median/mean (range) and categorical data as proportions. Parametric Student’s *t*-tests were employed in evaluating continuous variables, while chi-square tests were used for the categorical variables. Survival time was evaluated using the Kaplan-Meier model.

All of the *P* values reported were two-sided, and a value of *P* < 0.05 was considered statistically significant. Statistical Package for Social Science (SPSS) (Version 17.0, SPSS, Inc., Chicago, IL, USA) was used for the analyses and GraphPad Prism (Version 5.0, GraphPad Software, Inc., La Jolla, CA, USA) was used for figure illustration.

**List Of Abbreviations**

MMR, mismatch repair; CCC, clear cell carcinoma

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the institutional review board at Fudan University Shanghai Cancer Center. The written informed consent was waived due to retrospective design.

**Consent for publication**

Not applicable.

**Availability of data and material**

The dataset supporting the conclusions of this article is available upon request. Please contact Prof. Huijuan Yang (huijuanyang@hotmail.com).

**Competing interests**

All the authors have nothing to declare.

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**Authors’ contributions**

Shuang Ye: Conceptualization, methodology, software, analysis, investigation, writing-original draft, funding acquisition; Shuling Zhou: Conceptualization, methodology, analysis, investigation, writing-original draft; Siyuan Zhong: Conceptualization, methodology, analysis, investigation, writing-original draft; Boer Shan: Conceptualization, methodology, investigation, writing-original draft; Wenhua Jiang: Conceptualization, methodology, investigation, writing-original draft; Xiaohua Wu: Conceptualization, writing-review and editing, supervision, project administration; Wentao Yang: Conceptualization, writing-review and editing, supervision, project administration; Xu Cai: Conceptualization, methodology, investigation, writing-review and editing, supervision, project administration; Huijuan Yang: Conceptualization, methodology, investigation, writing-review and editing, supervision, project administration.
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None

References


**Figures**

![Figure 1](image-url)

**Figure 1**

Kaplan-Meier curves of survival based on stage and mismatch repair status. Abbreviations: dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.
Figure 2

Immunohistochemistry of patients with proficient mismatch repair (1st row, A-D), loss of MSH2/MSH6 protein expression (2nd row, E-H) and loss of MLH1/PMS2 protein expression (3rd row, I-L). Abbreviations: pMMR, proficient mismatch repair.