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Mismatch repair deficiency is associated with MSI phenotype, increased tumor-infiltrating lymphocytes and PD-L1 expression in immune cells in ovarian cancer

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HIGHLIGHTS

- MMR deficiency is generally presented at early-stage OCs with better prognosis.
- Complete loss of MLH1 and MSH2 is strongly associated with MSI-H phenotype.
- MMR deficient OCs show an increase of TILs and PD-L1+ intratumoral immune cells.

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ABSTRACT

Objective. The role of mismatch repair (MMR) deficiency in ovarian cancer (OC) pathogenesis and its association with other clinicopathologic features, such as microsatellite instability (MSI) and expression of checkpoint proteins, remain largely elusive.

Methods. We performed Immunohistochemistry (IHC) for MLH1, MSH2, MSH6 and PMS2 on full-section slides from 419 OCs to assess the MMR status. The clinical relevance of MMR deficiency was analyzed in combination with clinical data. The MSI status (by MSI assay) and expression of CD3, CD8, PD-1 and PD-L1 (by IHC) were compared in OCs with different MMR status.

Results. We found that 2.6% OCs were MMR-negative, 4.3% OCs were MMR-low, and 63.6% of MMR-negative OCs were of endometrioid subtype. A significantly higher proportion of MMR-negative OCs were diagnosed at stage I or II compared to MMR-proficient OCs ($p = 0.0041$). MSI was observed in all tested MMR-negative OCs, 14.3% of tested MMR-low OCs and 3.2% of tested MMR-proficient OCs. In addition, MMR-negative OCs had better progression free survival compared to MMR-proficient and MMR-low OCs ($p = 0.0046$). Furthermore, the majority of OCs were PD-1-positive in intratumoral lymphocytes regardless of MMR status; while MMR-negative OCs exhibited significantly increased CD3+ and CD8+ tumor-infiltrating lymphocytes, and PD-L1+ intratumoral immune cells compared to MMR-proficient OCs.

Conclusion. Our data suggests that MMR deficient OC is a unique molecular subgroup, characterized by early stage of diagnosis, MSI phenotype, and increased tumor-infiltrating lymphocytes. These patients may be good candidates for anti-PD-1/PD-L1 therapy.

1. Introduction

Ovarian cancer (OC) is a major cause of death among gynaecological cancers. There were an estimated 238,700 new cases of OC worldwide, leading to 151,900 deaths each year [1]. Although the risk of developing OC in women with germline mutations in *BRCA* genes is widely recognized, OC also occurs within the hereditary non-polyposis colorectal

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cancer (HNPCC, Lynch syndrome) due to germline mutations in the mismatch repair (MMR) genes [2–4]. MMR genes play an important role in maintaining genetic fidelity [5,6]. The deficiency of MMR genes leads to accumulation of thousands of mutations in the genome and causes widespread microsatellite instability (MSI) in tumors [5,6]. The most commonly affected MMR genes are *MLH1*, *MSH2*, *MSH6* and *PMS2* [7,8]. Besides germline mutations, MMR deficiency can also occur sporadically due to somatic mutations of MMR genes in tumor cells [3,9].

MMR deficient colorectal cancer is a well-defined subgroup, which has distinct clinicopathologic characteristics, exhibits increased tumor-infiltrating lymphocytes (TIL) and PD-1/PD-L1 expression, and responds well to immune checkpoint blockade [10–12]. However, the role of MMR deficiency in OC is poorly understood. Further, the clinicopathologic features of MMR deficient OCs remain largely undefined with conflicting findings on the concordance of MMR deficiency with MSI phenotype [13–19]. In addition, it is unclear whether OCs with MMR deficiency may also respond well to PD-1/PD-L1 blockade, as this kind of therapy showed only modest responses in OCs [20,21]. To address these knowledge gaps, we applied immunohistochemistry (IHC) for MLH1, MSH2, PMS2 and MSH6 to tumor sections from 419 OCs to assess the pathological features of MMR deficiency and its clinical implications. We also characterized the MSI status, the number of TILs and the expression of PD-1 and PD-L1 in a series of OCs with different MMR status, and evaluate their association with MMR deficiency. We present evidence showing that compared to MMR-proficient OCs, MMR-negative OCs were strongly associated with MSI phenotype, early stage of diagnosis, improved progression free survival, and increased number of TILs and PD-L1+ intratumoral immune cells. These findings suggest that the MMR status together with checkpoint proteins may serve as guides to design therapeutic strategies efficiently targeting OCs.

2. Material and methods

2.1. Collection of materials

All cases were retrieved from the pathology department of the Sichuan provincial people's hospital (Chengdu, China) after approval by the hospital's ethics committee. Cases were selected from the medical record database from 2008 to 2016 based on diagnosis of epithelial ovarian cancer. 493 formalin-fixed, paraffin-embedded (FFPE) ovarian tumor blocks were collected and hematoxylin and eosin (H&E) staining was performed on fresh 4-μm section from each block. H&E stained slides and original pathology reports were then reviewed by two senior pathologists (LS and DD). Tumors were classified histologically according to the criteria of the World Health Organization and staged according to the Federation of International Gynaecological Oncologists (FIGO) system.

2.2. Clinical data

Clinical data were retrieved from the medical record database and patient follow-up record which contain patients' information such as medication, medical examination and healthy status. After surgery, tumor progress or relapse was detected by physical examination, imaging and serum carcinoembryonic antigen assay. The duration of follow-up was defined as the time between surgery and disease progress/relapse, death, or last hospital visit/phone contact. Progression free survival (PFS) was defined as the time between the date of surgery and the first event (disease progress, relapse or death whichever occurs first). The cut-off time for survival analysis was July 2017.

2.3. Immunohistochemistry and scoring

Immunohistochemistry (IHC) was performed on 4 μm full-section slides from FFPE tumor blocks. Slides were incubated at 60 °C for 1 h. Then samples were deparaffinized by Xylol and rehydrated through a series of descending concentrations of alcohol. After heat induced antigen retrieval, slides were blocked in 3% H_2O_2 and then incubated at 4 °C overnight with primary antibodies, including MLH1, MSH2, MSH6, PMS2, CD3, CD8, PD-1 and PD-L1 (Supplementary data 1). Afterwards sections were incubated with secondary antibody for 30 min, and then developed with DAB. Slides were brief counterstained with hematoxylin and coverslipped. Interpretation of IHC results was by consensus of 2 senior pathologists (LS and DD). For evaluation of MMR protein expression, sections were scored by percentages of tumor cell nuclei staining: complete loss of staining (0%), reduced expression (1%–25%), and moderate to strong expression (26%–100%). Nuclear staining of normal lymphocytes and/or stromal cells in each slide was used as positive internal control. Tumors with moderate to strong expression of all four MMR proteins were defined as MMR-proficiency. Tumors lost staining or showed reduced expression of any MMR protein were defined as MMR-negative and MMR-low respectively. Counts of TILs, intratumoral lymphocytes (ITLs) and intratumoral immune cells (ITIs) were performed manually with blinding to MMR status, and the average was determined from counts of five high power fields (HPF). For statistical analysis, an average of one or greater PD-1-positive ITL per HPF was considered positive. Positive tumor expression of PD-L1 was defined as equal to or >5% tumor cells with PD-L1 positivity. Positive for PD-L1 in ITIs was defined as an average of one or greater ITIs with PD-L1 positivity per HPF.

2.4. Microsatellite instability (MSI) assay

DNA from both tumor and normal tissues was extracted from FFPE blocks for each patient. MSI was performed by multiplex polymerase chain reaction (PCR) amplification of the five microsatellite loci (BAT25, BAT26, D5S346, D2S123 and D17S250) recommended by the National Cancer Institute (NCI) [22]. The fluorescent labelled products were analyzed by capillary electrophoresis. A difference in the length of a microsatellite marker in tumor tissue compared with normal tissue was interpreted as microsatellite unstable. MSI-high (MSI-H) was defined if two or more markers were affected, and involvement of one marker was interpreted as MSI-low (MSI-L). Microsatellite stable (MSS) was reported if all 5 microsatellites showed stability.

2.5. Statistical analysis

Statistical analysis was performed by using GraphPad Prism 6. Fisher's exact test was used to analyze the association between MMR status and clinicopathological parameters. Mann-Whitney *U* test and Kruskal-Wallis test were used to compare two and three groups of unpaired variables respectively. Pearson Correlation analysis was used to measure of the linear correlation between two groups of variables. For PFS analysis, patients who were alive and relapse free on the last follow-up date were censored. Survival curves were estimated with the Kaplan-Meier method. Log-rank test was used to compare PFS. Probability value $p < 0.05$ was considered statistically significant.

3. Results

3.1. Clinicopathological features of MMR deficiency in ovarian cancer

After review of 493 ovarian cancer cases, 74 cases were excluded for the following reasons: borderline tumors (n = 56), reclassification of tumor type (n = 9), or chemotherapy prior to surgery (n = 9). The remaining 419 cases were subjected to IHC staining for MMR status and the characteristics of patients were summarized in Table 1. Since seromucinous OC does not exhibit a distinct immunophenotype or genotype and most of them have endometrioid genotype [23], the 30 seromucinous cases were combined with endometrioid OCs for further analysis.

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