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Massively parallel sequencing analysis of mucinous ovarian carcinomas: genomic profiling and differential diagnoses

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HIGHLIGHTS

- MOCs are heterogeneous at the mutational level.
- Mucinous ovarian cancers (MOCs) frequently harbor *TP53* and *KRAS* mutations.
- The current pathologic criteria to diagnose MOCs may result in misclassifications.
- Mutation analysis of a small gene panel may help improve the accuracy of MOC diagnosis.

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ABSTRACT

Objective. Mucinous ovarian cancer (MOC) is a rare type of epithelial ovarian cancer resistant to standard chemotherapy regimens. We sought to characterize the repertoire of somatic mutations in MOCs and to define the contribution of massively parallel sequencing to the classification of tumors diagnosed as primary MOCs.

Methods. Following gynecologic pathology and chart review, DNA samples obtained from primary MOCs and matched normal tissues/blood were subjected to whole-exome ($n = 9$) or massively parallel sequencing targeting 341 cancer genes ($n = 15$). Immunohistochemical analysis of estrogen receptor, progesterone receptor, PTEN, ARID1A/BAF250a, and the DNA mismatch (MMR) proteins MSH6 and PMS2 was performed for all cases. Mutational frequencies of MOCs were compared to those of high-grade serous ovarian cancers (HGSOCs) and mucinous tumors from other sites.

Results. MOCs were heterogeneous at the genetic level, frequently harboring *TP53* (75%) mutations, *KRAS* (71%) mutations and/or *CDKN2A/B* homozygous deletions/mutations (33%). Although established criteria for diagnosis were employed, four cases harbored mutational and immunohistochemical profiles similar to those of endometrioid carcinomas, and one case for colorectal or endometrioid carcinoma. Significant differences in the frequencies of *KRAS*, *TP53*, *CDKN2A*, *FBXW7*, *PIK3CA* and/or *APC* mutations between the confirmed primary MOCs ($n = 19$) and HGSOCs, mucinous gastric and/or mucinous colorectal carcinomas were found, whereas no differences in the 341 genes studied between MOCs and mucinous pancreatic carcinomas were identified.

Conclusions. Our findings suggest that the assessment of mutations affecting *TP53*, *KRAS*, *PIK3CA*, *ARID1A* and *POLE*, and DNA MMR protein expression may be used to further aid the diagnosis and treatment decision-making of primary MOC.

1. Introduction

Mucinous adenocarcinoma of the ovary is a rare type of epithelial ovarian cancer, representing approximately 3–4% of all epithelial ovarian malignancies [1]. These tumors are distinct at the biological, clinical and genetic levels from the common high-grade serous ovarian cancers (HGSOCs). Contrary to HGSOCs, mucinous ovarian cancers (MOCs)

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frequently present with early stage ovarian-confined disease associated with an overall favorable prognosis (stage I, 5 year survival rate 91%) [2,3]. In advanced stage disease, however, MOC is associated with poor outcome and overall survival rates lower than those reported for advanced stage HGSOC (5-year survival 11% MOC vs 26% HGSOC) [2,4–6]. MOCs are less sensitive to standard platinum/taxane chemotherapy regimens than HGSOCs, which may contribute to the observed poor outcome when diagnosed in advanced stages [4,7]. Previous studies employing targeted and whole-exome massively parallel sequencing have revealed that primary MOCs are heterogeneous at the genomic level, with *TP53* (52–57%), *KRAS* (45–65%), *BRAF* (23%), *ERBB2* (23%) and *CDKN2A* (60%) being the genes most commonly altered [8,9], and that these tumors may be distinct from other ovarian cancer subtypes at the genomic level.

The diagnosis of primary MOC and the differentiation from metastatic mucinous tumors originating in extraovarian primary sites, most commonly from the colorectum, especially appendiceal, is challenging [10,11]. Pathology review of 44 presumed MOCs as part of a prospective Gynecological Oncology Group phase 3 trial led to the reclassification of 61% of cases as ovarian metastases from tumors originating in other primary sites [6]. Therefore, the integration of clinical history and pathologic features has been shown to be essential for the diagnosis of primary MOC and to discriminate these tumors from their metastatic mimics [6,10]. Laterality and size provide important information as primary MOCs typically present as unilateral tumors measuring > 10 cm as compared to metastatic lesions [10]. In addition, immunohistochemistry has been used as an ancillary diagnostic test for the differentiation between MOCs and adenocarcinomas from other anatomical sites, in particular those demonstrating lower intestinal differentiation [11–16]. In advanced stage mucinous ovarian disease, upper and lower endoscopy, CT or PET imaging and/or serum tumor markers are warranted to rule out the presence of an extra-ovarian primary cancer (NCCN guidelines, https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf).

In this study, we sought to characterize the repertoire of somatic genetic alterations focusing on key cancer genes in MOCs and to define the contribution of massively parallel sequencing to the classification of tumors diagnosed as MOCs based on current clinicopathologic criteria. To achieve these aims, we subjected a series of 24 MOCs to whole-exome sequencing ($n = 9$) or massively parallel sequencing targeting all exons of 341 key cancer genes (MSK-IMPACT; $n = 15$).

2. Material and methods

2.1. Case selection

All primary MOCs diagnosed between July 2001–July 2013 with available tissue slides and blocks were retrieved from the files of the Department of Pathology at Memorial Sloan Kettering Cancer Center (MSKCC). This study was approved by the Institutional Review Board (IRB) of MSKCC, and patient consent was obtained where appropriate. Representative sections of each case diagnosed as primary MOC were re-reviewed by gynecologic pathologists (DD, YRH, RAS), and clinical information, including age, stage, laterality, therapy, endoscopy and follow-up, was obtained from the medical records. Invasive MOC was defined as the presence of confluent tumor cells with intracytoplasmic mucin, measuring > 10 mm^2 and at least 5 mm in one linear dimension [3]. Unilateral tumor size ≥ 10 cm and expression of CK7 with or without CK20 expression were used to confirm the diagnosis of primary MOC along with normal upper and lower endoscopy and prolonged clinical follow-up without evidence of gastrointestinal involvement in patients with advanced stage disease, and the absence of the development of a second primary tumor during follow-up; in addition, cases with a unilateral tumor size < 10 cm or bilateral disease with a tumor size ≥ 10 cm were acceptable if PAX8 was expressed [10,12,13,15,17]. The ovarian origin of the stage I carcinomas as opposed to a gastrointestinal carcinoma metastatic to the ovary was further confirmed by the prolonged survival, as 71% (12/17) of the stage I tumors had no evidence of disease at a median follow-up of 82 months (range 41–185 months), 12% (2/17) were alive with disease (40 and 326 months follow-up), and 18% (3/17) died of unknown cause (62, 98 and 102 months follow-up; Table 1). Following this review, 24 primary MOCs (15 formalin-fixed paraffin-embedded (FFPE), 9 fresh frozen) were included in this study.

2.2. Immunohistochemistry

Immunohistochemistry (IHC) for CK7, CK20 and PAX8, was performed in the diagnostic work-up of the tumors, following previously validated protocols [15,18]. For CK7 and CK20 only cytoplasmic staining was considered positive, and for PAX8 only nuclear staining was considered positive [15,18]. After sequencing, additional immunohistochemical analysis was performed for estrogen receptor (ER), progesterone receptor (PR), PTEN, ARID1A/BAF250a and the DNA mismatch repair proteins MSH6 and PMS2 using previously described protocol [19–22]. ER and PR expression was defined as positive when >1% of the tumor nuclei showed immunoreactivity, following the ASCO/CAP guidelines for breast cancer [23]. Loss of MSH6, PMS2 and ARID1A expression was defined as complete absence of protein expression in unequivocal tumor cell nuclei [24]. Normal epithelium and stroma were used as internal controls for PTEN, MSH6, PMS2 and ARID1A expression; PTEN expression was defined as lost if tumor cells displayed no immunoreactivity or less than the internal control.

2.3. DNA extraction

Tumor sections were reviewed by two gynecologic pathologists (YRH, RAS) to ensure >20% neoplastic cells. Matched normal DNA was extracted from peripheral blood lymphocytes or normal tissue sections (benign lymph node), confirmed to be devoid of any neoplastic cells. Genomic DNA from tumor- and matched normal samples was extracted using the DNeasy Blood & Tissue kit (Qiagen).

2.4. Whole-exome and targeted massively parallel sequencing

Tumor and matched normal DNA samples were subjected to whole-exome sequencing ($n = 9$) or massively parallel sequencing ($n = 15$) using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay targeting all exons, selected intronic and regulatory regions of 341 key cancer genes, as previously described [25,26]. Sequencing data were analyzed as previously described (Supplementary Methods) [24,26]. Cancer cell fractions of each mutation were inferred using ABSOLUTE (v1.0.6) [27], as previously described [24,26]. Mutational signatures were defined for cases with at least 20 somatic mutations using deconstructSigs [28].

2.5. Comparison with high-grade serous ovarian, colorectal, gastric and pancreatic carcinomas

The mutational frequencies of the 341 genes in our targeted sequencing panel of MOCs were compared to those of HGSOCs from The Cancer Genome Atlas (TCGA; $n = 316$) [29], mucinous colorectal carcinomas (TCGA; $n = 32$) [30], mucinous gastric adenocarcinomas (TCGA; $n = 18$) [31], pancreatic adenocarcinomas from the International Cancer Genome Consortium (ICGC; mucinous cystadenocarcinoma/intraductal papillary mucinous neoplasm with invasion, $n = 11$; pancreatic ductal adenocarcinoma, $n = 177$) [32] and to MOCs described by Ryland et al. ($n = 12$) [9]. The whole-exome sequencing-derived mutational data of the mucinous colorectal carcinomas and mucinous gastric adenocarcinomas were retrieved from GDAC Firehose (https://gdac.broadinstitute.org/; Mutation Annotation File) and of the HGSOCs and pancreatic adenocarcinomas from cBioPortal (http://www.cbioportal.org/) [33]. We restricted the comparison to the 341 genes

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