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Defining the cut-point between low- and high-grade ovarian serous carcinomas: A clinicopathologic and molecular genetic analysis

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Abstract

A two-tier grading system based on nuclear grade divides ovarian serous carcinomas into low- (nuclear grade 1) and high-grade (nuclear grade 3). In most instances the separation is straightforward but at times, the morphologic distinction between them can be difficult. We studied eleven ovarian serous carcinomas with features that were “intermediate” (nuclear grade 2) between low and high grade. All the cases were high stage and had a poor clinical outcome. None of the tumors showed mutations in *KRAS*, *BRAF* and *ERBB2* genes which characterize most low-grade serous carcinomas. In contrast, 10 (90.9%) of 11 cases contained non-synonymous *TP53* mutations characteristic of high-grade serous carcinomas. In summary, the molecular genetic profile and behavior of serous carcinomas with grade 2 nuclei are virtually the same as those of serous carcinomas with grade 3 nuclei, supporting the use of the two-tier grading system for classifying ovarian serous carcinomas.

Keywords

Ovarian cancer; serous carcinoma; grade; TP53; two-tier grading system

Introduction

Recently, it has been proposed that the three-tier (well, moderately and poorly differentiated) grading system for ovarian serous carcinomas be replaced by a two-tier (low-grade and high-grade) system based on clinical, morphological and molecular studies.^{6, 10, 12, 16} The two-tier system was initially proposed based on morphologic and molecular genetic features¹² and subsequently was shown in a multivariate analysis to be useful in predicting outcome.⁶ In the two-tier system, high-grade serous carcinomas exhibit a papillary or solid growth pattern and the tumor cells contain markedly atypical, large pleomorphic nuclei and frequent mitotic figures, many of them abnormal.^{5, 6} These nuclei are classified as grade 3. In contrast, the majority of low-grade carcinomas display a micropapillary pattern. The tumor cells contain small, uniform nuclei with minimal atypia and low mitotic activity corresponding to grade 1.

Clinically, high-grade serous carcinomas are aggressive neoplasms frequently affecting women in the peri-menopausal or postmenopausal age group while low-grade serous carcinomas are relatively indolent affecting younger women.⁴ Low-grade serous carcinomas are more refractory to platinum-based chemotherapy as compared to high-grade serous carcinomas⁹ probably because of their low proliferative rate. They develop slowly in a stepwise fashion from non-invasive micropapillary serous carcinomas which in turn arise from atypical proliferative serous (borderline) tumors. Molecular genetic studies have demonstrated that low-grade micropapillary serous carcinomas contain either *KRAS*, *BRAF* or *ERBB2* mutations in approximately two thirds of cases but only rarely harbor *TP53* mutations.^{3, 7, 13} In contrast, high-grade serous carcinomas are believed to develop from intraepithelial carcinoma in ovarian surface inclusion cysts or the fallopian tube. High-grade serous carcinomas frequently contain *TP53* mutations (>80%)⁸ but on rare occasion harbor mutations in *KRAS*, *BRAF* and *ERBB2* genes.^{3, 7, 13}

Although high-grade and low-grade serous carcinomas are usually easily distinguished, it may be difficult for carcinomas with nuclear features that are intermediate between grade 1 and grade 3. These intermediate grade tumors have relatively uniform nuclei that are midway in size between grade 1 and grade 3 tumors and therefore qualify as grade 2. Compared to typical high-grade nuclei (Fig. 1), grade 2 nuclei are more uniform in size but are larger and more atypical than grade 1 nuclei. Moreover, the mitotic index in these intermediate grade tumors is increased compared to low-grade serous tumors but lower than high-grade serous carcinomas. In this study, we analyzed a small group of serous carcinomas displaying these intermediate features corresponding to what would be classified as moderately differentiated (nuclear grade 2) carcinomas for mutations of *TP53*, *KRAS*, *BRAF* and *ERBB2* genes in order to determine whether these tumors displayed a molecular genetic profile that paralleled the genotype of low or high-grade serous carcinomas. In addition, we evaluated their clinicopathological features to determine if their behavior was it similar to low or high grade tumors.

MATERIALS AND METHODS

Case Review and Selection of Cases

A total of eleven cases of ovarian serous carcinomas with grade 2 nuclei were studied. Four cases were identified from the archival files of the Department of Pathology at the Johns Hopkins Hospital; two cases were collected from the Department of Pathology at the Emory University School of Medicine, and five cases from Washington Health Center. Tumor sampling was approximately one section/cm of the greatest tumor dimension. Hematoxylin and eosin stained sections were reviewed and the corresponding paraffin blocks retrieved. Collection and analysis of the anonymized specimens were approved by the Institutional Review Boards from the four institutions. The morphologic criteria used for nuclear grading are shown in Fig. 1. Grade 1 nuclei were relatively small and uniform in size whereas grade 3 nuclei were large, pleomorphic with coarse clumping of chromatin. Grade 2 nuclei in this study were relatively uniform in size; they were larger than grade 1 nuclei and smaller than grade 3 nuclei. Nucleoli were more prominent than in grade 1 tumors (Fig. 1). Mitotic activity was determined by counting 50 high power field (HPF; 40x) using an Olympus BH2 microscope. Ten high power fields were counted in the most active areas.

Mutational analysis

Genomic DNA was isolated from fresh tumor tissue in three cases and from paraffin sections in the remaining 8 cases. Purification of genomic DNA was performed by proteinase K digestion followed by a Qiagen DNA purification kit for fresh tissues, and the Forma Pure kit (Agencourt, MA) for sections from formalin fixed paraffin embedded samples. Genomic

regions containing the exon 1 of *KRAS*, exon 15 of *BRAF*, exon 20 of *ERBB2*, exons 4 to 9 of *TP53*; and exons 1-3, 9 and 20 of *PIK3CA* were amplified by the polymerase chain reaction (PCR). Purification of PCR products was performed using a Qiagen PCR purification kit. Sequencing was performed at the Johns Hopkins DNA sequencing facility. The sequences of primers for PCR and nucleotide sequencing have been previously reported.^{7, 8}

RESULTS

The clinical features of the eleven serous ovarian carcinomas with grade 2 nuclei are summarized in Table 1. The ages of the patients ranged from 43 to 81 years with an average of 65.4 years. The frequency of serous carcinomas with grade 2 nuclei was estimated to be approximately 4% of all serous carcinomas based on reviewing archival specimens at the Johns Hopkins Hospital and the Emory University Hospital. Ten cases were primary ovarian serous carcinomas, and one was a primary peritoneal serous carcinoma. Those cases were originally reported as papillary serous cystadenocarcinoma without specifying either low- or high-grade. All patients presented with FIGO stage IIIC disease. Eight patients died of disease and two patients died from surgical complications one a month and the other six months after surgery. Follow up information was not available on one case. The survival time for the eight patients varied from 6 to 30 months. Histopathologic features of the tumors are presented in Table 2. Six tumors (55%) had no necrosis; five tumors (45%) had multiple small foci of tumor necrosis. Four tumors (36%) displayed a solid and slit-like pattern and six displayed a micropapillary pattern involving at least 25% of the tumor. Mitotic counts ranged between 4.4 and 18 per 10 HPFs with a medium of 10.2, and a mean of 10.7 per 10 HPFs. Representative hematoxylin-and-eosin stained sections from two tumors are shown in Fig. 2.

To determine the molecular genetic features of the serous ovarian carcinomas with grade 2 nuclei, *KRAS*, *BRAF*, *ERBB2*, *PIK3CA* and *TP53* genes were sequenced because somatic mutations of *KRAS*, *BRAF* and *ERBB2* are the most common molecular genetic changes in low-grade micropapillary serous carcinomas while *TP53* mutations are the most frequent mutation in high-grade serous carcinomas. *PIK3CA* kinase gene was also sequenced in this study because the gene has been analyzed in ovarian cancer tissues in several reports.^{2, 7, 17, 18} We found no *KRAS*, *BRAF*, *ERBB2*, mutations in any of the grade 2 tumors while 10 of the 11 tumors carried non-synonymous *TP53* mutations (Table 3 and 4). Wild-type *PIK3CA* sequences were present in all the specimens examined.

DISCUSSION

Grading systems for ovarian serous carcinomas include the FIGO system which is based solely on architecture¹, the WHO grading system based on cytological and architectural features¹⁵ and the Silverberg-Shimizu grading which takes into account architectural and nuclear features and mitotic activity.¹¹ More recently, a two-tier grading system that divides serous carcinomas into low and high-grade has been proposed.^{6, 12} This grading system is primarily based on nuclear grade with mitotic activity used as a secondary feature. In contrast to the three tier system, the two-tier grading system has a molecular genetic underpinning. This is important because it provides biologic support rather than being based entirely on morphologic criteria.^{3, 10} Using the two-tier system, the majority of serous carcinomas can be classified into either low or high-grade, but there are occasional tumors with nuclear features that are intermediate between conventional low-grade and high-grade. The current study provides clinical and molecular genetic evidence that tumors with grade 2 nuclei are more closely related to high-grade rather than low-grade serous carcinomas. Specifically, the aggressive behavior of the tumors, the high proportion of *TP53* mutations (90%) and the absence of *KRAS*, *BRAF*, *ERBB2* mutations are similar to tumors with grade 3 nuclei as compared to tumors with grade 1 nuclei.

The results of this study have important clinical implications because the architectural features such as solid growth pattern (poorly differentiated), papillary (moderately differentiated) or micropapillary/macropapillary (well-differentiated) may overlap. The findings in the present study support those in our previous study in which we showed that serous carcinomas with micropapillary features and grade 3 nuclei were similar to conventional high-grade carcinomas based on absence of *KRAS* mutations.¹⁴ Thus, nuclear grade 2/3 versus grade 1 is the major microscopic feature that distinguishes low from high-grade serous carcinomas. As there were only eleven cases in this study, more cases are needed to corroborate our conclusion.

We found that the mitotic activity ranged widely among the 11 carcinomas with grade 2 nuclei (Table 2). There were seven tumors with mitotic counts below 12 mitoses/10 HPFs, a cutoff proposed to distinguish low-grade versus high-grade serous carcinomas in a previous study.⁶ Thus, mitotic activity should be used as an ancillary feature in the two-tier grading system. In fact, we believe the proposed mitotic count cut point of 12 mitotic figures/10 HPFs should be revised. In our experience low grade serous carcinomas almost never have mitotic counts greater than 5 mitotic figures/10 HPFs. A larger study will be necessary to come up with firm guidelines.

In conclusion, we analyzed a series of ovarian serous carcinomas with grade 2 nuclei to delineate their clinicopathologic and molecular genetic relationship to low- and high-grade serous carcinomas. All the tumors were high stage and clinically aggressive. Mutations of *KRAS*, *BRAF* and *ERBB2* which characterize most low-grade serous carcinomas were not identified in any of them. In contrast, 10 (90%) of 11 tumors contained non-synonymous *TP53* mutations characteristic of high-grade serous carcinoma. The clinical and morphologic data in this study showing close similarity between ovarian serous carcinomas with grade 2 nuclei and those with grade 3 nuclei together with our recent report showing that subclassification of high-grade serous carcinoma into moderately and poorly differentiated serous carcinomas is not relevant¹⁶ provide strong support for the use of a two-tier grading system for ovarian serous carcinomas.

Acknowledgments

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Fig. 1.

Grade 1 nuclei (Gr 1) are small and uniform in size and there are few mitotic figures. Grade 2 nuclei (Gr 2) are larger compared to grade 1 but are still relatively uniform. Nuclear chromatin is coarser than in grade 1 and mitotic figures are more frequent. Grade 3 nuclei (Gr 3) are highly pleomorphic and large with coarsely clumped chromatin. Numerous mitotic figures including many abnormal ones are present.

Fig. 2.

Case 5 shows a micropapillary pattern (A) and tumor cells contain grade 2 nuclei (B,C). Case 3 demonstrates a slit-like growth pattern (D) and tumor cells contain grade 2 nuclei (E,F).

TABLE 1

Clinical features of serous carcinomas with grade 2 nuclei

Case	Age	Laterality	Stage	Survival
1	71	left ovary	IIIC	dead of disease at 13 months
2	58	right ovary	IIIC	dead of disease at 22 months
3	43	bilateral	IIIC	dead of sepsis at 6 months
4	57	peritoneal *	IIIC	no evidence of disease at 6 months
5	77	bilateral	IIIC	dead of post-operation complication
6	58	bilateral	IIIC	dead of disease at 20 months
7	73	bilateral	IIIC	dead of disease at 6 months
8	81	bilateral	IIIC	dead of disease at 13 months
9	46	bilateral	unstaged	dead of disease at 30 months
10	75	bilateral	IIIC	lost to follow up
11	80	bilateral	IIIC	dead of disease at 15 months

* Primary peritoneal carcinoma with very minimal ovarian surface involvement.

TABLE 2

Histopathologic features of serous carcinomas with grade 2 nuclei

Case	Micropapillary pattern	Necrosis	Mitosis (10HPFs)
1	Yes	None	9.6
2	Yes	None	10.2
3	No	Focal	12
4	Yes	Minute	4.4
5	Yes	Focal	18
6	Focal	Focal	7.5
7	No	None	10.4
8	No	None	13.6
9	Yes	None	14.2
10	No	None	9.6
11	Yes	Focal	8.5

TABLE 3
Mutational analysis of *TP53*, *KRAS*, *BRAF*, *ERBB2* and *PIK3CA* in serous carcinomas with grade 2 nuclei

Case	<i>TP53</i>	<i>KRAS</i>	<i>BRAF</i>	<i>ERBB2</i>	<i>PIK3CA</i>
1	mutation	wild type	wild type	wild type	silent at codon 20
2	mutation	wild type	wild type	wild type	wild type
3	mutation	wild type	wild type	wild type	wild type
4	mutation	wild type	wild type	wild type	wild type
5	mutation	wild type	wild type	wild type	wild type
6	mutation	wild type	wild type	wild type	wild type
7	mutation	wild type	wild type	wild type	not examined
8	wild type	wild type	wild type	wild type	not examined
9	mutation	wild type	wild type	wild type	not examined
10	mutation	wild type	wild type	wild type	not examined
11	mutation	wild type	wild type	wild type	not examined

TABLE 4Mutational status of the *TP53* in serous carcinomas with grade 2 nuclei

Case	Location	Nucleotide change	Presumed protein change
1	Exon 5	Thymine deletion	frameshift
2	Exon 8	Thymine to Guanine	Phenylalanine to Cysteine
3	Exon 4	Cytosine deletion	frameshift
4	Exon 4	Cytosine deletion	frameshift
5	Exon 8	Guanine to Thymine	Lysine to Asparagine
6	Exon 6	Guanine to Adenine	Valine to Methionine
7	Exon 4	Cytosine to Adenine	Proline to Threonine
8	wild type	wild type	wild type
9	Exon 5	Thymine deletion	frameshift
10	Exon 5	Guanine to Adenine	Arginine to Histidine
11	Exon 5	Cytosine to Adenine/Cytosine hetero	Serine to Serine/Stop