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HNF-1 β in Ovarian Carcinomas With Serous and Clear Cell Change

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Summary

Many ovarian tumors, including high-grade serous carcinoma (HGSC), show clear cell change. Accurate diagnosis is important, however, as ovarian clear cell carcinoma (OCCC) is known to be less responsive to traditional types of ovarian cancer chemotherapies. In a previous study, the clinical, morphologic, and immunohistochemical features of 32 ovarian carcinomas, which had been previously diagnosed as pure OCCC (n = 11), pure HGSC (n = 11), and mixed serous and clear cell (MSC) (n = 10), were analyzed. The immunoreactivities of WT1, ER, and p53, as well as the mitotic indices and stages of presentation of the MSC, were similar to those of HGSC. It was consequently concluded that MSC represented HGSC with clear cell change. Hepatocyte nuclear factor-1 β (HNF-1 β) is a relatively new immunohistochemical marker that has been shown to be rather sensitive and specific for OCCC. We thus sought to evaluate this marker in this specific group of tumors. One block each of pure HGSC and pure OCCC were stained with HNF-1 β . In the cases of MSC, 2 blocks were stained when the serous and clear cell components were not present on the same slide. None (0/11) of the pure HGSC showed immunoreactivity for HNF-1 β , whereas all (11/11) of the pure OCCC were positive. In the cases of MSC, both the serous and clear cell components were negative for HNF-1 β . HNF-1 β seems to be a sensitive and specific marker for OCCC and is not expressed in HGSC with clear cell change. The pattern of immunoreactivity of HNF-1 β in tumors with both serous and clear cell change supports the conclusion that MSC are HGSC with clear cells.

Keywords

HNF-1 β ; Ovary; Clear cell carcinoma; High-grade serous carcinoma

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Many ovarian tumors, including high-grade serous carcinoma (HGSC), show clear cell changes. The presence of clear cytoplasm, in addition to other overlapping architectural features, has often led to misclassification. This is reflected in the reported incidence of ovarian clear cell carcinoma (OCCC), which has varied from 3% to 12% of all epithelial ovarian cancer in the western world and approximately 20% in Japan (1-5). There have also been discrepant reports regarding responsiveness of OCCC to chemotherapy. In one study, the response rate to platinum-based therapy was 70%; however, many other studies have shown that OCCC is not particularly responsive to traditional ovarian cancer chemotherapies and actually has a worse outcome compared with that of HGSC when at advanced stage (3,6-8). HGSC is the most common of the ovarian epithelial malignancies, usually presents at advanced stage, and is initially sensitive to chemotherapy; these data suggest that in some cases HGSC may have been misclassified as OCCC. As our knowledge of the molecular changes in these tumors increases, so does the potential for targeted therapies, and therefore accurate classification is crucial.

In a previous study, 32 ovarian carcinomas originally diagnosed as pure OCCC (n = 11), mixed serous and clear cell (MSC) (n = 10), or pure HGSC (n = 11) were evaluated in an attempt to determine accurate cell type (9). Parameters evaluated included clinical, morphologic, and immunohistochemical features. All pure HGSC and most MSC presented at Stages III or IV, whereas most pure OCCC presented at Stage I or II. The immunoreactivities for WT1, ER, and p53 in MSC were similar to those of HGSC but significantly different from those of pure OCCC. In addition, the mitotic indices of MSC and HGSC were similar and much higher compared with those seen in pure OCCC. On the basis of these results, it was concluded that MSC represents HGSC with clear cell change. Recently, immunohistochemical studies have shown that the antibody hepatocyte nuclear factor-1 β (HNF-1 β) is a sensitive and specific marker for OCCC and may be a useful diagnostic tool in ovarian tumors with clear change (10,11). We thus sought to analyze this marker in this specific group of tumors.

MATERIALS AND METHODS

The selection of cases has been described in a previous study (9). One block of each case of pure HGSC and OCCC was selected for immunohistochemical staining. In the cases of MSC, 2 blocks were evaluated when the serous and clear cell components were not present on the same slide. Four-micrometer sections of formalin-fixed, paraffin-embedded sections were stained with an antibody specific to HNF-1 β (Santa Cruz, Santa Cruz, CA, 1:80). Results of the immunohistochemical staining patterns, including the percentage of positive cells, in pure HGSC, pure OCCC, and each component of the MSC were recorded. The result was considered positive if nuclear staining was present. Appropriate positive and negative controls were also performed.

RESULTS

The results of the immunohistochemical studies are shown in Table 1. All (11/11) of the pure OCCC were positive for HNF-1 β . More than 75% of the cells were positive, with strong nuclear staining in all 11 cases. None (0/11) of the HGSC showed immunoreactivity

for HNF-1 β . In the cases of MSC, both the serous and clear cell components were negative for HNF-1 β (Figs. 1-3).

DISCUSSION

Many ovarian epithelial tumors show clear cell change, which often leads to difficulty in accurate diagnoses. In a previous study, the characteristics of tumors previously diagnosed as pure OCCC, pure HGSC, and mixed serous/clear cell (MSC) were analyzed. The reproducibility of pure OCCC was excellent ($\kappa = 0.82$) among gynecologic pathologists, whereas that of pure HGSC was moderate ($\kappa = 0.59$) (9). Tumors that had been previously diagnosed as MSC caused the most diagnostic difficulty as the reproducibility was only fair ($\kappa = 0.32$). This lack of reproducibility underscores the challenge of correctly diagnosing a tumor that shows clear cell change. The study also showed that the tumors could be divided into 2 separate groups on the basis of certain clinical and pathologic factors. Tumors diagnosed as pure OCCC showed early stages of presentation [International Federation of Gynecology and Obstetrics (FIGO) Stages I–II], low mitotic indices, and were usually negative for the immunohistochemical markers WT1, ER, and p53. The tumors diagnosed as pure HGSC usually showed the converse; presentation at advanced stage (FIGO Stages III–IV), high mitotic indices, and positivity for WT1, ER, and p53 using immunohistochemical study. The MSC, however, showed results similar to those of pure HGSC. They also presented at advanced stage and high mitotic indices. Both components were analyzed immunohistochemically and both demonstrated immunoreactivities similar to pure HGSC. On the basis of these results, it was concluded that tumors that appeared to have both serous and clear cell components were actually variants of HGSC, that is, HGSC with clear cells.

HNF-1 β , a transcription factor that is involved in glucose metabolism and is upregulated in most OCCC (12), has been shown to be a rather sensitive and specific immunohistochemical marker for OCCC. In a study of ovarian clear cell tumors, including 26 carcinomas, 3 borderline tumors, and 1 adenofibroma, all 30 tumors showed positive nuclear staining with the antibody. HNF-1 β was also positive in adjacent typical and atypical endometriosis in up to 75% of the cases (13). It was negative in non-clear cell tumors including 20 endometrioid, 15 serous, 11 mucinous, and 4 Brenner tumors. The only exception was focal (<5%) staining in 4/15 mucinous tumors. An additional study of HNF-1 β expression in ovarian tumors showed positive reactivity in 40/40 OCCC but was negative in 77/78 cases of serous carcinoma, 30/31 endometrioid carcinomas, and 37/38 mucinous carcinomas (14). HNF-1 β seems to be especially helpful when the differential diagnosis includes HGSC. In a series of 237 OCCC, HNF-1 β showed a sensitivity of 82.5% and a specificity of 95.2% for a diagnosis of OCCC versus HGSC (10). Although this antibody is useful in the appropriate context, one must keep in mind that it may be positive in carcinomas in other organ sites including clear cell carcinoma of the pancreas (15), renal cell carcinoma, and hepatocellular carcinoma (16). In addition, the sensitivity and specificity of HNF-1 β for endometrial clear cell carcinoma seem to be inferior to that of OCCC (17,18).

OCCC and HGSC often have overlapping morphologic features, including papillary architecture. The papillae in HGSC, however, usually show hierarchical branching with abundant tufting and budding of the cells; in comparison, the cells in OCCC usually show a

monolayer, that is, only one cell layer lining the papillae. HGSC also shows marked nuclear pleomorphism, whereas the cells in OCCC are relatively uniform. The mitotic index is also helpful in differentiating these tumors as that of OCCC is usually low, with a mean of approximately 4 per 10 high-power fields, whereas that of HGSC is almost always well above 12. Furthermore, the distinct tubulocystic architectural pattern is also very commonly present in OCCC. This typical clear cell morphology has been shown to correlate with HNF-1 β immunoreactivity. When only tumors with typical OCCC morphology were included, the sensitivity of HNF-1 β was 94% (11). If all of these described features of typical OCCC are present, immunohistochemistry may not be necessary for an accurate diagnosis. If the tumor shows atypical features, however, including abundant tufting and budding of the papillae, marked nuclear pleomorphism, elevated mitotic index, or presentation at advanced stage, immunohistochemistry may be a valuable diagnostic tool.

The use of HNF-1 β is an additional helpful tool in the diagnosis of OCCC. Until recently there has been a lack of positive immunohistochemical staining patterns in OCCC that help differentiate it from other ovarian epithelial malignancies. Instead, OCCC has been shown to be usually negative for many of the antibodies used in the differential diagnosis including WT1, ER, PR, and p53 (19-22). Although a negative result may support a morphologic impression of OCCC, positive staining provides useful additional information that may aid in a correct diagnosis, as other tumors in the differential diagnosis may be negative for these markers a certain percentage of the time. Kobel et al (10) found the combination of WT1, ER, and HNF-1 β to be the most sensitive and specific combination to differentiate OCCC from HGSC. It may also be useful to add this antibody to the immunohistochemical panel to aid the interpretation of positive cytology specimens from patients who are being considered for upfront chemotherapy. Accurate classification of HGSC and OCCC is important for several reasons and having a positive marker for clear cell differentiation is valuable. As mentioned previously, OCCC has a worse prognosis compared with HGSC when at advanced stage, which is likely because of its chemoresistance to platinum- and taxane-based therapies. Alternative treatment options are being explored to improve prognoses in these patients. As an alternative or adjunct to chemotherapy regimens, it appears that radiotherapy may provide an additional survival benefit. In patients with FIGO Stages Ic and II, irradiation improved disease-free survival by 20% at 5 years. Other promising therapies include targeting the PI3-kinase/mTOR pathway, as it seems to be involved in the pathogenesis of OCCC. The gene PIK3CA, which encodes the catalytic subunit of PI3-kinase, thus an activator of the pathway, has been found to be mutated in up to 56% of OCCCs (23-25). Downstream targets of this pathway, including genes important in angiogenesis, are specific potential targets of therapy. In a recent study, *in vivo* experiments showed that OCCC xenografts were quite sensitive to antiangiogenesis therapy (26). Clinical trials with inhibitors of the PI3-kinase/mTOR pathway are currently under way; thus, a correct diagnosis is essential if new treatment options are to be advanced. HNF-1 β may also be a potential target for therapy. As described previously, HNF-1 β is upregulated in OCCC, and this upregulation has been shown to be crucial for OCCC cells to survive (12). Consequently, it has been hypothesized that HNF-1 β may regulate genes that are important to cell survival and may thus be a molecular target for therapy.

Another important reason to ensure accurate classification of OCCC and HGSC is that they are associated with specific genetic syndromes. Approximately 15% of HGSC have been shown to occur in patients with germline *BRCA1* and/or *BRCA2* mutations (27-32). OCCC in young patients has been shown to be associated with Lynch syndrome, a disorder due to defects in DNA mismatch repair proteins. In a series of patients less than 50 years old with ovarian cancer, 60% (3/5) of the tumors with mismatch repair abnormalities were of clear cell histology (33). In a similar series of 70 patients less than 40 years old with endometrial cancer, 9 patients had mismatch repair abnormalities detected and 1 patient had a synchronous OCCC. In both of these syndromes, the affected patient and her family members are at risk for at least 1 additional cancer and the diagnosis may lead to additional genetic testing.

HNF-1 β seems to be a sensitive and specific marker for OCCC and is not expressed in HGSC with clear cell change. The pattern of immunoreactivity of HNF-1 β in tumors with both serous and clear cell change supports the conclusion that MSC are HGSC with clear cells. Typical OCCC morphology, as well as immunohistochemistry, should aid in correct and reproducible classification.

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FIG. 1.
(A) Pure ovarian clear cell carcinoma. (B) HNF-1 β in pure ovarian clear cell carcinoma.

FIG. 2.
(A) Serous component of mixed serous and clear cell carcinoma. (B) HNF-1 β in the serous component of mixed serous and clear cell carcinoma.

FIG. 3.
(A) Clear cell component of mixed serous and clear cell carcinoma. (B) HNF-1 β in the clear cell component of mixed serous and clear cell carcinoma.

TABLE 1
HNF-1 β in ovarian clear cell carcinoma, high-grade serous carcinoma, and serous and clear cell components of mixed carcinoma

Tumor type	No. HNF-1 β + cases
Clear cell carcinoma	11/11
Serous carcinoma	0/11
Clear cell component of mixed serous/clear cell carcinoma	0/10
Serous component of mixed serous/clear cell carcinoma	0/10

HNF-1 β indicates hepatocyte nuclear factor-1 β .