Immunohistochemical Expression of Wilms Tumor Gene Protein in Different Histologic Subtypes of Ovarian Carcinomas

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• ***Context.*—Immunohistochemical expression of Wilms tumor gene protein (WT1) has previously been described in primary ovarian carcinomas.**

***Objective.*—To evaluate differences in WT1 expression among different histologic subtypes of ovarian carcinomas and the correlation to the histologic grade.**

***Design.*—Ninety-one primary ovarian carcinomas were reviewed, and 1 representative formalin-fixed and paraffin-embedded tissue block was selected. One slide from each case included in the study was immunostained using the WT1 clone 6F-D2. The immunoreactivity was graded according to the percentage of stained tumor cells. Only nuclear staining was considered a positive reaction. A tumor was regarded as negative if less than 1% of the tumor cells was stained.**

***Results.*—All serous carcinomas (28/28) showed WT1 expression, whereas all mucinous (14/14) and all clear cell carcinomas (14/14) were negative. The lone malignant Brenner tumor and 3 (60%) of 5 undifferentiated carcinomas included in the study were also negative. The endometrioid carcinomas showed either no reaction for WT1 or were diffusely positive with more than 50% of the tumor cells stained. All the grade 1 tumors (10/10) were negative, whereas 5 (45%) of the 11 grade 2 tumors and 5 (63%) of the 8 grade 3 tumors showed a positive reaction.**

***Conclusion.*—The present study demonstrates differences in immunohistochemical expression of WT1 among different histologic subtypes of primary ovarian carcinomas. Regarding the endometrioid subtype, the expression seems to be correlated to the histologic grade.**

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Worldwide, ovarian cancer is the fifth most common cancer in women, and malignant surface epithelial-stromal ovarian neoplasms account for approximately 90% of primary ovarian cancers. Differences in biological behavior and molecular characteristics among different histologic subtypes of malignant epithelial-stromal ovarian tumors have in recent years been the subject of several studies. One of the issues of interest has been the tumor suppressor genes. Losses and alterations of tumor suppressor gene function and expression seem to play a role in the development of most ovarian cancers. Alterations in the *p53* gene are the most frequent events described.[1–3]

Wilms tumor gene (*WT1*) was originally identified as a tumor suppressor gene located on chromosome 11p13.[4,5] Unlike most other tumor suppressor genes, WT1 expression in normal human tissue seems to be particularly limited to the urogenital system and mesoderm-derived tissues.[6,7] In neoplasms, WT1 protein expression has been described in Wilms tumor,[8] malignant mesotheliomas,[9] leukemia,[10] and desmoplastic small round cell tumors.[11] Among carcinomas, nuclear WT1 protein expression seems to be limited. One of the few types of carcinomas reported to demonstrate nuclear WT1 expression is ovarian and fallopian tube carcinoma.[12–21] In the few studies performed on the subject, there seem to be differences in the expression among different histologic subtypes of ovarian carcinomas, with serous carcinomas expressing WT1 more frequently than the other subtypes.[12,13,16,20,21] The number of nonserous carcinomas included in the former studies has been relatively limited, and the subject needs to be further investigated.

The aim of the present study was to evaluate the immunohistochemical expression of WT1 protein among different histologic subtypes of primary ovarian carcinomas. Regarding the serous and endometrioid cell type, correlation between WT1 protein expression and histologic grade of the carcinoma was analyzed.

MATERIALS AND METHODS

Ninety-one primary ovarian carcinomas of pure type were drawn from the files from the Institute of Pathology, Aalborg Hospital, Denmark. The numbers of cases included in each histologic subtype were as follows: 29 endometrioid carcinomas, 28 serous carcinomas, 14 mucinous carcinomas, 14 clear cell carcinomas, 5 undifferentiated, and 1 malignant Brenner tumor. The endometrioid and serous carcinomas were graded according to the International Federation of Gynecology and Obstetrics grading system.

All stained slides from the cases included in the study were reviewed. One formalin-fixed and paraffin-embedded tissue block with representative tumor was selected from each case. Sections 4 μm thick were cut and placed on charged slides. The

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Table 1. WT1 Expression in Different Histologic Subtypes of Primary Ovarian Carcinomas

Histologic Subtype*	<1%	1%–10%	11%–50%	>50%
Serous (n = 28)	0	0	7	93
Endometrioid (n = 29)	66	0	0	34
Mucinous (n = 14)	100	0	0	0
Clear cell (n = 14)	100	0	0	0
Undifferentiated (n = 5)	60	0	0	40
Malignant Brenner (n = 1)	100	0	0	0

* n indicates number of carcinomas.

slides were air dried in an incubator at 37°C followed by 1 hour at 60°C to ensure maximum adherence. Before immunostaining, the slides were dewaxed and endogenous peroxidase activity was blocked with 0.45% hydrogen peroxide in 99% ethanol for 15 minutes. The sections were then hydrated and subjected to microwave antigen retrieval in 10mM Tris and 1mM EDTA (pH 9) for 15 minutes. The sections were left to cool in the hot buffer for an additional 15 minutes. After antigen retrieval, the slides were washed in 0.05M Tris-buffered saline, pH 7.4 (DakoCytomation, Norden, Denmark) added to 0.05% Tween-20 and placed in an Autostainer. The slides were incubated with anti-human WT1, clone 6F-D2, dilution 1:100 (DakoCytomation), which is a mouse monoclonal antibody, for 30 minutes. The immunoreaction was visualized with the Envision-plus system (DakoCytomation) for 30 minutes and developed with diaminobenzidine (DakoCytomation) for 10 minutes. The immunoreaction was intensified in 0.5% $CuSO_4$ in 0.05M Tris buffer (pH 7.4) for 5 minutes. Finally, the slides were washed in rinse water, counterstained with Mayer hematoxylin, dehydrated, and coverslipped. A positive control slide was included in each run.

The immunoreactivity was graded according to the percentage of stained tumor cells of the entire tumor tissue present on the slide. Only nuclear staining, but of any intensity, was regarded as a positive reaction. A tumor was considered negative if less than 1% of the tumor cells was stained. Positive tumors were graded as follows: 1+, 1% to 10%; 2+, 11% to 50%; and 3+, more than 50% positive cells.

RESULTS

All the serous carcinomas (28/28) included in the present study (Table 1; Figure 1, A and B) showed a positive reaction for WT1. Except for 2 cases, the reaction was diffuse, with more than 50% of the tumor cells stained, and for the serous carcinomas, as well as other positive tumors, the tumor cells generally stained strong. Tumors of histologic grades 1, 2, and 3 were equally represented in the material.

All the mucinous (14/14) and all the clear cell carcinomas (14/14) (Figure 2, A and B) were negative, with less

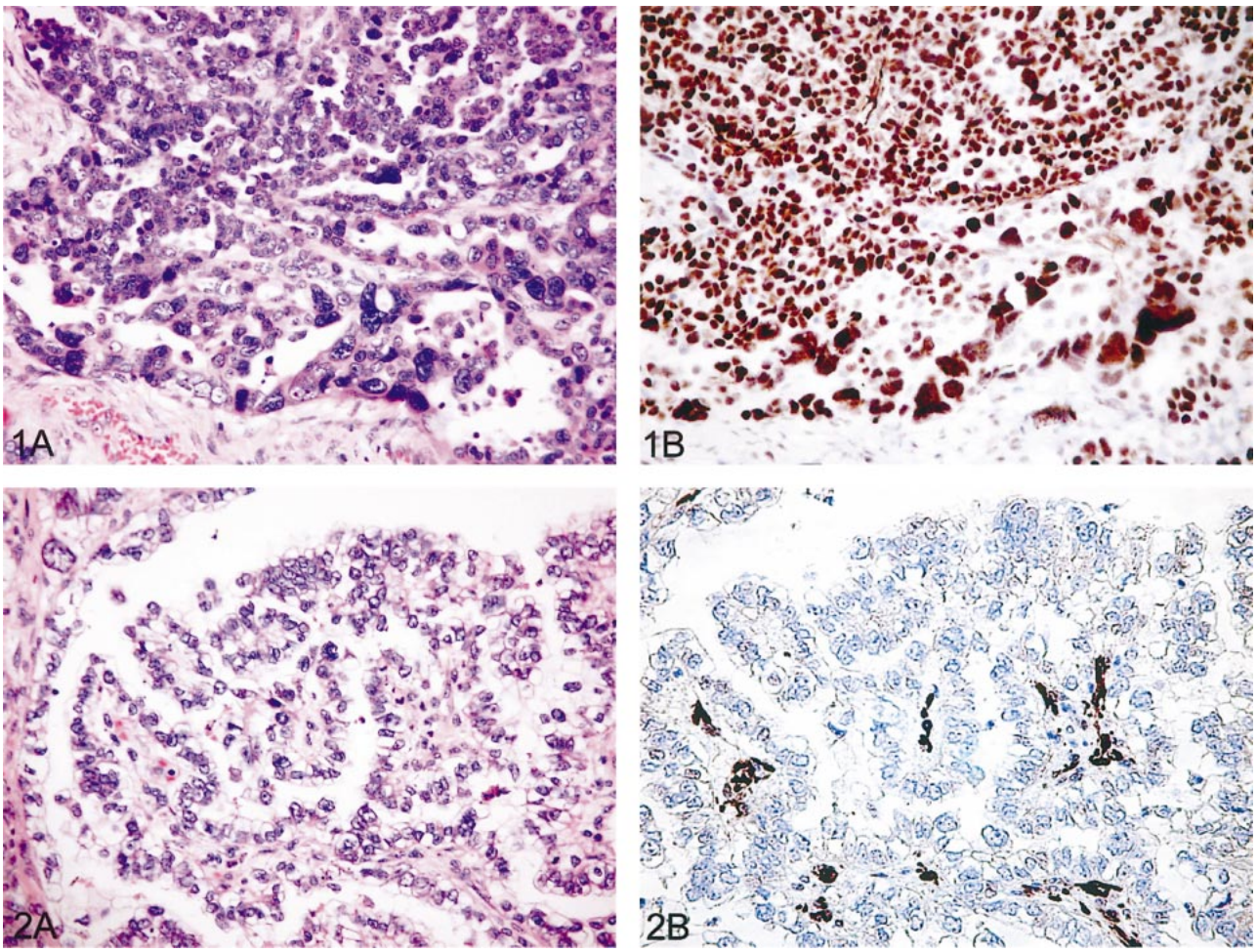

Figure 1. *Serous adenocarcinoma of the ovary (A and B) demonstrating diffusely immunohistochemical nuclear reaction for Wilms tumor gene (WT1) (B) (hematoxylin-eosin [A] and immunohistochemical stain for WT1 [B], original magnification ×400).*

Figure 2. *Clear cell adenocarcinoma of the ovary (A and B), with no nuclear staining for Wilms tumor gene (WT1) demonstrated (B) (hematoxylin-eosin [A] and immunohistochemical stain for WT1 [B], original magnification ×400).*

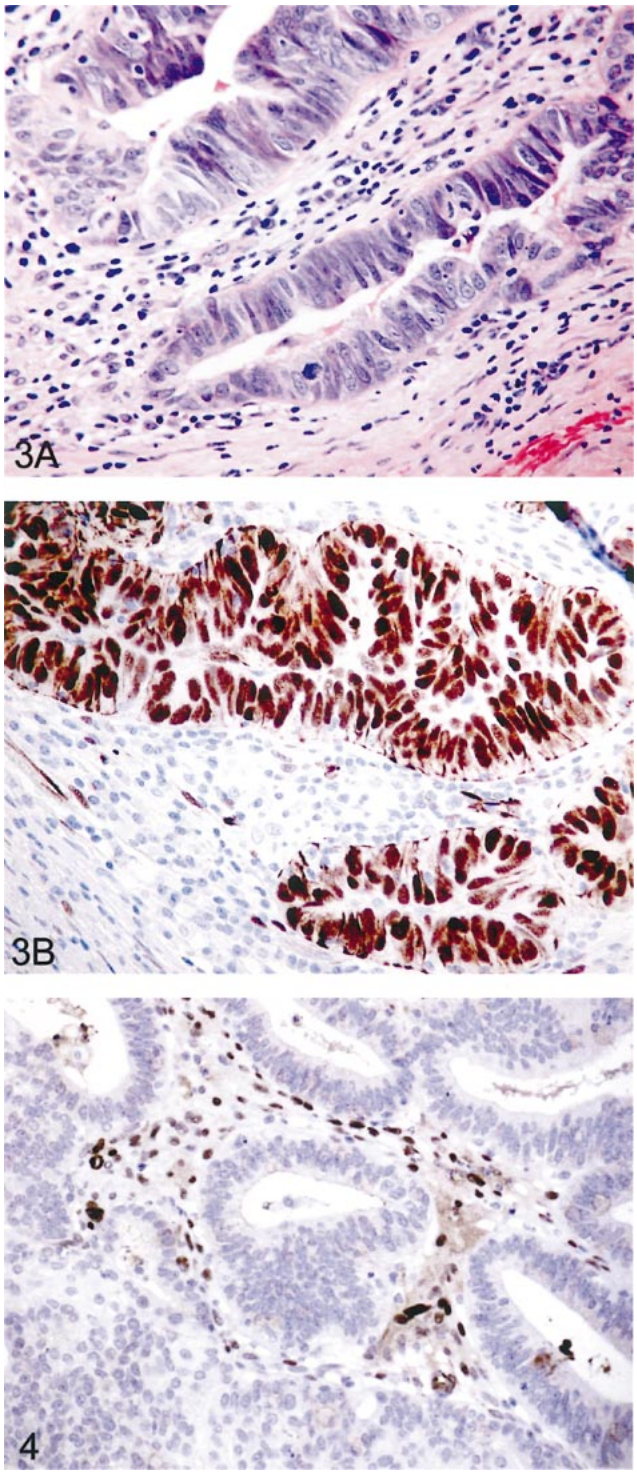

Figure 3. *Endometrioid adenocarcinoma of the ovary histologic grade 2 (A and B), with more than 50% of the tumor cells showing a positive reaction for Wilms tumor gene (WT1) (B) (hematoxylin-eosin [A] and immunohistochemical stain for WT1 [B], original magnification ×400).*

Figure 4. *Endometrioid adenocarcinoma of the ovary histologic grade 2 with no immunoreactivity for Wilms tumor gene (WT1) (immunohistochemical stain for WT1, original magnification ×400).*

Table 2. WT1 Expression and Histologic Grade in Endometrioid Carcinomas

Histologic Grade	WT1 Positive, %	WT1 Negative, %
1	0	100
2	45	55
3	63	37

than 1% of the tumor cells stained. The single malignant Brenner tumor was negative and so were 3 (60%) of 5 undifferentiated carcinomas.

Regarding endometrioid carcinomas, 10 (34%) of 29 were diffusely positive (Figure 3, A and B), whereas 19 (66%) of 29 were negative (Figure 4). Correlating to the histologic grade (Table 2), all the grade 1 endometrioid carcinomas (10/10) were negative, whereas 5 (45%) of 11 and 5 (63%) of 8 of the grades 2 and 3 endometrioid carcinomas, respectively, revealed a positive reaction.

COMMENT

In the present study, serous carcinomas showed the most frequent immunoreactivity for WT1 among ovarian carcinomas. All the serous carcinomas included in our study were positive. The result is comparable to that reported by others.[12,13,15–21] Since 93% of the serous carcinomas had a strong positive reaction for WT1 in more than 50% of the tumor cells, the sensitivity in the present study is higher than in the previous studies of Hashi et al[16] and Goldstein and Uzieblo,[19] which had a corresponding rate of diffuse reaction in 72% and 73% of their cases, respectively. Although 100% of the serous carcinomas in our study were positive, other studies[12,15,18–21] indicate that a WT1 negative reaction does not exclude the diagnosis of a serous ovarian carcinoma.

The mucinous and clear cell carcinomas in the present study were all negative. This finding correlates well with the results reported by Goldstein et al[12] and Hashi et al.[16] In contrast, Shimizu et al[13] found some immunohistochemical expression of WT1 in both mucinous and clear cell carcinomas. They found that serous carcinomas had a significantly higher expression than the clear cell carcinomas but not a significantly higher expression than the mucinous carcinomas. Differences in the results may be due to differences in the immunohistochemical protocols and the use of different primary antibody. Shimizu et al[13] used the C19 clone, whereas in the present study and in most other studies the 6F-H2 clone was the antibody chosen against WT1. Goldstein et al[19] compared the C19 and the 6F-H2 antibody in their study and found no differences in the percentage of stained tumor cells, but the 6F-H2 antibody produced a stronger and more homogeneous reactivity than did the C19 clone. Acs et al,[21] who used the same clone as in the present study, found 4 of 18 clear cell carcinomas with a positive reaction for WT1.

The endometrioid carcinomas included in the present study showed either no reaction or a diffuse reaction, with more than 50% of the tumor cells being positive for WT1. When correlating to the histologic grade (Table 2), 100% of the grade 1 tumors were negative, whereas 45% and 63% of the grade 2 and 3 endometrioid carcinomas, respectively, showed a positive reaction. The results indicate a significant difference in WT1 expression between highly differentiated endometrioid carcinomas and those of lower grade. Shimizu et al[13] found a low degree of WT1 expres-

sion among endometrioid carcinomas, and the expression was significantly less than among the serous carcinomas. Lee et al,[15] Hecht et al,[18] and Logani et al[17] found WT1 expression in some of the endometrioid subtypes, but only a very limited number of endometrioid carcinomas were included in their studies. Hashi et al,[16] Al-Hussaini et al,[20] and Acs et al[21] included 15, 13, and 11 endometrioid carcinomas, respectively, in their studies, and they were all negative. None of the previous studies have correlated WT1 expression to the histologic grade. It is well known that it can be difficult to distinguish poorly differentiated endometrioid carcinomas from the serous subtype,[22] but even after a second review of the positive cases, we did not find it to be a reasonable explanation of the results obtained.

The differences in WT1 expression of the serous subtypes compared with the clear cell and the mucinous cell type support others studies that showed that the immunohistochemical expression of WT1 seems to reflect biological cell type rather than mutations.[23] Regarding the endometrioid carcinomas, however, our results indicate that the expression may be related to the histologic grade of differentiation. Our results may reflect, as suggested by Gilks,[22] that low-grade endometrioid carcinomas differ from high-grade endometrioid carcinomas in biological behavior and gene expression profile. Low-grade endometrioid carcinomas are often associated with endometriosis, and this might suggest that they arise from ectopic endometrial tissue rather than ovarian surface epithelium. Normal endometrial epithelium is negative for WT1 staining, whereas normal ovarian surface epithelium shows a positive reaction.[13] Endometrial endometrioid carcinomas are also reported to be negative for WT1 staining.[20,21] The association between endometrioid carcinomas and endometriosis needs to be further investigated.

Sixty percent of the undifferentiated ovarian carcinomas included in the present study showed no WT1 expression, whereas the remaining 40% were diffusely positive. The result might reflect that some of the histologic undifferentiated carcinomas are very low differentiated serous (or endometrioid) carcinomas, whereas the WT1 negative carcinomas may be of other biological cell types. The single malignant Brenner tumor in our study was negative. Logani et al[17] found a positive reaction for WT1 in 14 of 17 transitional cell carcinomas of the ovary.

In summary, the present study demonstrates differences in immunohistochemical expression of WT1 among different histologic subtypes of ovarian carcinomas, supporting previous studies that identified serous carcinoma as the most frequent type expressing WT1. Additional studies on endometrioid carcinomas are needed.

To our knowledge, we present the largest number of endometrioid carcinomas so far examined for immunohistochemical expression of WT1 protein, with 19 (66%) of 29 being negative and the other 10 (34%) being diffusely positive. Correlating this to the histologic grade, our data suggest differences in WT1 expression among highly differentiated endometrioid carcinomas (100% were negative) and those of lower histologic grade (45% and 63% were positive). Our results emphasize the need for further investigations, including molecular analysis.

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