Application of Antibodies to Intermediate Filament Proteins in Simple and Complex Tumors of the Female Genital Tract

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Summary: Polyclonal antibodies to cytokeratins, vimentin, and desmin and monoclonal antibodies to vimentin and to individual cytokeratin polypeptides, specific for glandular epithelia (RGE 53) or keratinizing stratified squamous epithelia (RKSE 60), have been applied in gynecological tumors with simple or complex composition. In general, tumors with simple composition showed reaction patterns fitting their known epithelial or mesenchymal nature, i.e., cytokeratin positivity in epithelial tumors only, vimentin positivity in mesenchymal tumors, and expression of desmin and vimentin in muscle cell tumors. Rather frequently, coexpression of cytokeratins and vimentin was noted in endometrial adenocarcinomas. Tumors with complex composition, such as müllerian mesodermal mixed tumors (MMMTs), that may pose considerable problems in conventional histopathology revealed various reaction patterns, with either expression of only cytokeratins or coexpression of cytokeratins and vimentin in carcinomatous areas and expression of only vimentin in sarcomatous areas. However, in addition, some MMMTs contained cells that were also positive for desmin. Intermediate filament protein immunohistochemistry appeared to be helpful in establishing a diagnosis of MMMT and in characterizing the different tumor components, which may prove to be useful in the evaluation of gynecological treatment protocols. Key Words: Gynecological tumors—Müllerian mesodermal mixed tumors—Intermediate filament proteins—Immunohistochemistry.

Biochemical and immunochemical studies have demonstrated that five types of intermediate filament proteins (IFPs) can be distinguished in vertebrate cells (1). These include the cytokeratins, vimentin, desmin, glial fibrillary acidic protein (GFAP), and the neurofilament protein triplet.

These different types of IFPs show a tissue-specific distribution (1–4). In general, cytokeratins are specific for epithelial cells, vimentin for mesenchymal cells, desmin for muscle cells, GFAP for glial cells, and neurofilaments for neuronal cells. Certain tissue types in embryos may contain IFPs different from those

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present in their adult counterparts, mostly concerning a transient expression of vimentin (5–7).

Mesothelial cells have been shown to coexpress cytokeratins and vimentin (8), a phenomenon also seen in ovarian surface epithelium, granulosa cells, and rete ovarii cells (9). Cells brought into tissue culture often express vimentin next to their cell type-specific IFP (10).

Generally, the IFP specific for a given cell type is retained on neoplastic transformation (for reviews see refs. 2–4). As a result, most epithelial and mesenchymal tumors express only one type of IFP. A restricted number of tumors have been reported to contain more than one type of IFP. For example, coexpression of cytokeratins and vimentin has been found in pleomorphic adenomas of the parotid gland (11), in renal cell carcinomas (12), in carcinomas of the thyroid gland (13), and in malignant mesotheliomas (8,14). Furthermore, metastatic carcinoma cells present in body cavity effusions may obtain an additional IFP of the vimentin type (15).

The cytokeratins represent a family of biochemically and immunochemically distinct polypeptides. So far, 19 cytokeratin polypeptides have been recognized in human epithelial tissues. These cytokeratins are not distributed randomly throughout different epithelia but occur in tissue-specific combinations (16). Also, the diverse types of carcinomas differ in their cytokeratin polypeptide content. The cytokeratin polypeptide patterns of epithelial tumors are either identical to the cytokeratin pattern present in the tissue of origin or at least closely related to it. As a result, identification of the cytokeratin pattern can be helpful in classifying a given type of epithelium or epithelial tumor (see, for example, refs. 16,17).

In recent years several reports have described the application of antibodies to cytokeratins and other types of IFPs in studies of normal and neoplastic tissues of the female genital tract (9,17–27). These studies have pointed to the potential usefulness of IFP typing in the recognition of different normal and neoplastic tissues.

In the present study we have applied a number of well-characterized and well-documented monoclonal and polyclonal antibodies to cytokeratins, vimentin, and desmin (4,26–32) in a series of gynecological tumors with simple or complex composition. Tumors with simple composition showed only one epithelial or mesenchymal component whereas complex tumors comprised several epithelial components or consisted of a combination of epithelial and mesenchymal tissues.

First, it was our aim to determine whether the reactivity patterns of the antibodies in gynecological tumors with simple composition were comparable with those obtained in tumors of other organs. The second goal of this study was to see if these antibodies were able to discriminate between the component parts in gynecological tumors with a more complex composition.

MATERIALS AND METHODS

Tissues

The tissue specimens used in this study comprised both samples from fresh surgical specimens, snap frozen in liquid nitrogen, and routinely formalin-fixed, paraffin-embedded tissues. A survey of the tissue specimens investigated is given
in Tables 1–3. In several cases both fresh material and formalin-fixed, paraffin-embedded tissue from the same surgical specimen were examined. Tumors with a more complex composition were almost exclusively available as routinely formalin-fixed, paraffin-embedded tissue blocs.

All tumors listed in Tables 1–3 were primaries, with the exception of the secondary tumors from breast in the ovary, two cases of leiomyosarcoma (metastases of uterine leiomyosarcoma in brain and vertebral column), and one case of granulosa cell tumor (metastasis in the abdomen). A primary diagnosis in all cases was made by routine light microscopy, using H&E-stained tissue sections or, when appropriate, supplemented with additional histochemical staining procedures. In selected cases electron microscopical examination was performed. Typing and classification of the tumors were analogous to the proposals of the World Health Organization (33,34).

Antibodies

The following primary antibody preparations were used in this study:

1. An affinity purified polyclonal rabbit antiserum directed against human skin keratins (pKer). For preparation and specificity testing see refs. 4,26–30.

2. An affinity purified polyclonal rabbit antiserum raised against vimentin, isolated from calf lens by preparative gel electrophoresis (pVim). Preparation and specificity testing have been described before (4,28–30.).

3. A mouse monoclonal antibody to calf lens vimentin (MVI). The specificity of this antibody has been shown in earlier reports (27,29).

4. A polyclonal rabbit antiserum directed against chicken gizzard muscle desmin (pDes). For preparation and specificity testing see ref. 4,30.

5. The mouse monoclonal antibody RGE 53 directed against cytokeratin 18. Preparation and specificity testing of this antibody have been described previously (31). The RGE 53 specifically recognizes glandular epithelial cells from breast; digestive, respiratory, and urogenital tracts; endocrine and exocrine tissues; and mesothelial cells. No reaction is found in stratified squamous epithelial or nonepithelial tissues.

6. The mouse monoclonal antibody RKSE 60 directed against cytokeratin 10. Preparation and specificity testing of this antibody were described elsewhere (4,32). The RKSE 60 recognizes only keratinizing stratified squamous epithelial tissues. No reaction is found in nonkeratinizing stratified squamous and glandular epithelia.

All antibodies mentioned above are available from Euro-Diagnostics B.V., Apeldoorn, The Netherlands. The polyclonal rabbit antisera can be applied to both frozen tissue sections and routinely formalin-fixed, paraffin-embedded tissue sections, whereas the monoclonal antibodies are only reactive in frozen tissue sections.

Indirect immunofluorescence technique

Air-dried cryostat sections (4–7 μm) from samples of fresh surgical specimens were fixed in methanol (−20°C, 5–10 min) and acetone (room temperature, 10–30 s). After air drying for at least 30 min at room temperature, they were rehydrated in phosphate-buffered saline (PBS), pH 7.4 (two steps of 5 min each), and incubated for the indirect immunofluorescence technique. Incubation proce-
<table>
<thead>
<tr>
<th>Tissue and diagnosis</th>
<th>Indirect immunofluorescence technique</th>
<th>Immunoperoxidase (PAP) technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nt</td>
<td>Age (years)</td>
</tr>
<tr>
<td>Vulva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>3</td>
<td>71–80</td>
</tr>
<tr>
<td>Uterine cervix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>3</td>
<td>31–46</td>
</tr>
<tr>
<td>Adenocarcinoma (endocervical type)</td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td>Clear cell (mesonephroid) adenosarcoma</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>13</td>
<td>29–70</td>
</tr>
<tr>
<td>Undifferentiated sarcoma</td>
<td>1</td>
<td>89</td>
</tr>
<tr>
<td>Uterine corpus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>6</td>
<td>56–87</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>3</td>
<td>43–74</td>
</tr>
<tr>
<td>Adeno component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>3</td>
<td>49–68</td>
</tr>
<tr>
<td>Leiomyoma</td>
<td>9</td>
<td>25–50</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>5</td>
<td>45–67</td>
</tr>
<tr>
<td>Tumor Type</td>
<td>Nf</td>
<td>Np</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Endometrial stromal sarcoma</td>
<td>8</td>
<td>36-81</td>
</tr>
<tr>
<td>Adenomatoid tumor</td>
<td>2</td>
<td>44, 50</td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous adenofibroma</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td>Adeno component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroma component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous papillary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cystadenocarcinoma</td>
<td>9</td>
<td>53-75</td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>6</td>
<td>47-63</td>
</tr>
<tr>
<td>Clear cell (mesonephroid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>1</td>
<td>57</td>
</tr>
<tr>
<td>Secondary (metastatic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tumor from breast</td>
<td>3</td>
<td>31-53</td>
</tr>
<tr>
<td>Granulosa cell tumor^</td>
<td>2</td>
<td>37, 63</td>
</tr>
<tr>
<td>Thecoma</td>
<td>3</td>
<td>52-75</td>
</tr>
</tbody>
</table>

Nf, number of patients from which frozen tissue material was examined; Np, number of patients from which paraffin-embedded tissue was examined; Nt, total number of patients examined. See text for description of antibodies.

^ In these types of tumors, differences in the number of immunoreactive cells were noted between individual cases, including several nonimmunoreactive tumors. RK56 60 was only detected in keratinizing cells.

^ Coexpression of cytokeratins and vimentin was seen in some of these cases in a varying number of tumor cells. For details see text.

^ In these types of tumors some metastatic lesions are included. For details see Materials and Methods section.
TABLE 2. Expression of intermediate filament proteins in müllerian mesodermal mixed tumors (MMMTs) [immunoperoxidase (PAP) technique]

<table>
<thead>
<tr>
<th>Tissue and diagnosis</th>
<th>Nt</th>
<th>Age (years)</th>
<th>Epithelial component</th>
<th>Mesenchymal component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pKer</td>
<td>pVim</td>
</tr>
<tr>
<td>Uterine cervix</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMMT—homologous</td>
<td>2</td>
<td>29, 69</td>
<td>+/−</td>
<td>−/+/bc</td>
</tr>
<tr>
<td>Uterine corpus</td>
<td></td>
<td>45−73</td>
<td>+/−</td>
<td>−/−sp+/*−c</td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homologous</td>
<td>2</td>
<td>71, 72</td>
<td>+/−</td>
<td>−/−sp+/*c</td>
</tr>
<tr>
<td>Heterologous</td>
<td>1</td>
<td>39</td>
<td>+/−</td>
<td>−/−sp+/*c</td>
</tr>
</tbody>
</table>

For further explanation of symbols see key to Table 1.

a In these types of tumors, differences in the number of immunoreactive cells were noted between individual cases, including several nonimmunoreactive tumors.
b Coexpression of cytokeratins and vimentin was seen in some of these cases in a varying number of tumor cells. For details see text.
c Vimentin positivity in epithelial cells was mainly confined to spindle cell areas.

dures for single- and double-labeling assays have been described before (4,15,28). For double-labeling studies, the following combinations of monoclonal and polyclonal antibodies were used: RGE 53 or RKSE 60 and pKer; RGE 53 or RKSE 60 and pVim; MVI and pKer.

As second antibodies, fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit, goat anti-mouse, or rabbit anti-mouse IgGs (Nordic Immunology, Tilburg, The Netherlands) and Texas Red conjugated sheep F(ab')2 anti-mouse Ig (New England Nuclear, Boston, MA, U.S.A.) were used. Overlap between the FITC and Texas Red fluorescence in the respective channels was checked using tissue sections labeled with a single second antibody. Incubations with the second antibodies alone were used as negative controls.

Immunoperoxidase technique

Parallel sections from the routinely formalin-fixed, paraffin-embedded tissues were deparaffinized using xylene, rinsed in ethanol 100%, treated with 1% hydrogen peroxide in methanol for 30 min to block the endogenous peroxidase activity, and subsequently brought to PBS using a descending ethanol series. Tissue sections to be used for incubation with pKer were predigested with 0.1% type

TABLE 3. Expression of intermediate filament proteins in ovarian mucinous cystadenocarcinomas with mural nodules [immunoperoxidase (PAP) technique]

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Nt</th>
<th>Age (years)</th>
<th>pKer</th>
<th>pVim</th>
<th>pDes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucinous cystadenocarcinoma with sarcoma-like mural nodule</td>
<td>1</td>
<td>28</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td></td>
<td></td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mural nodule</td>
<td></td>
<td></td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma with mural nodules, showing characteristics of müllerian mesodermal mixed tumors</td>
<td>2</td>
<td>29, 41</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td></td>
<td></td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mural nodules</td>
<td></td>
<td></td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

For explanation of symbols see key to Table 1.

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IFPs IN GYNECOLOGICAL TUMORS

XIV protease from Streptomyces griseus (Sigma, St. Louis, MO, U.S.A) in 0.05 M Tris-buffer, pH 7.6 (37°C, 10–15 min). To reduce background staining, the sections were preincubated with normal swine serum for 30 min. All antisera used in the immunoperoxidase technique were diluted in normal swine serum.

The sections were incubated with the primary rabbit antiserum overnight at 4°C. After washing in PBS (three steps of 10 min each), the sections were incubated with swine anti-rabbit Ig (DAKO, Glostrup, Denmark; diluted 1:30) for 30 min. After washing (PBS, 3 × 10 min), the sections were incubated for 30 min with rabbit peroxidase-antiperoxidase (PAP) complex (DAKO; diluted 1:100). After washing (PBS, 3 × 10 min), peroxidase activity was visualized using a freshly prepared 3,3′-diaminobenzidine tetrahydrochloride solution (Sigma) for 5–10 min. Harris hematoxylin was used as a counterstain.

RESULTS

The immunoreactivity patterns observed in gynecological tumors with the antibodies described above are summarized in Tables 1–3. The diagnoses mentioned therein represent final conclusions based on histopathologic as well as immunohistochemical criteria.

In general, gynecological tumors composed of only one epithelial or mesenchymal component (a simple tumor composition) showed reaction patterns that could be expected from their known epithelial or mesenchymal nature (Table 1 and Figs. 1 and 2). Strong cytokeratin positivity was observed in epithelial tumors only, vimentin positivity was mainly restricted to mesenchymal/stromal tumors, and variable coexpression of desmin and vimentin occurred in muscle cell tumors. In all uterine leiomyomas most tumor cells were desmin positive and, to a less extent, also vimentin positive. In four of five leiomyosarcomas most tumor cells were desmin positive, and in one uterine leiomyosarcoma a relatively small number of desmin-positive cells was present. Strong vimentin positivity was seen only in a restricted number of tumor cells in all cases of leiomyosarcoma. In normal endometrium as well as in some muscle cell tumors we have observed a (weak) focal or patchy cytokeratin positivity. Coexpression of cytokeratins and vimentin was found in cases of undifferentiated carcinoma of the uterine cervix (4 of 13) (Fig. 3a and b); adenocarcinoma (6 of 6) (Fig. 3c and d), as well as an undifferentiated carcinoma (1 of 3) and the two adenomatoid tumors (Fig. 3e and f) of the uterine corpus; and in serous papillary cystadenocarcinoma of the ovary (2 of 9). Expression of both cytokeratins and vimentin was also found in the primary ovarian granulosa cell tumor (Fig. 3g and h). Dual expression of different types of IFPs was concluded from double label immunofluorescence assays or presumed from IFP reactivity patterns in parallel sections in the PAP technique. The proportion of cells immunoreactive for cytokeratins as well as vimentin differed from <10% in, for example, undifferentiated carcinomas of the uterine cervix and serous papillary cystadenocarcinomas of the ovary to a substantial percentage (10–40%) in, for example, adenocarcinomas of the uterine corpus. The monoclonal cytokeratin 18 antibody, RGE 53, was reactive with different types of adenocarcinoma. No reaction was found in squamous cell carcinomas. It is emphasized that RGE 53 positivity was also found in part of the tumor cells in the primary ovarian granulosa cell tumor, whereas in the metastatic granulosa cell
FIG. 1. Photomicrographs show indirect immunofluorescence (a–d) and immunoperoxidase (e and f) staining patterns in (a and b): keratinizing squamous cell carcinoma (a: pKer; b: RKSE 60), (c and d): adenocarcinoma (c: pKer; d: pVim), and (e and f): undifferentiated carcinoma (e: pKer; f: pVim) of the uterine cervix. a and b, ×155; c, ×250; d, ×260; e and f, ×190.

tumor only vimentin but no cytokeratins could be demonstrated. The monoclonal cytokeratin 10 antibody, RKSE 60, gave a positive reaction in squamous cell carcinomas. Staining was restricted to keratinizing cells within such neoplasms. No reaction with RKSE 60 was found in adenocarcinomas. Interesting in this respect
is the staining pattern in adenosquamous carcinomas of the uterine corpus. The adenocarcinomatous part of these tumors reacted similarly to pure adenocarcinomas of the uterine corpus, although coexpression of cytokeratins and vimentin was less pronounced in two of three cases. In its immunoreactivity the squamous part resembled pure squamous cell carcinoma.

The single case of ovarian serous adenofibroma showed a clear differential IFP staining pattern, with cytokeratin positivity exclusively in the epithelial part and vimentin positivity only in the mesenchymal part of the tumor.

In addition, other tumors with combinations of epithelial and mesenchymal components (a complex tumor composition) were examined. These comprised MMMTs and several ovarian mucinous cystadenocarcinomas containing so-called mural nodules (see Tables 2 and 3 and Fig. 4). Most cases of MMMT have been designated as homologous, and only one MMMT of the ovary could be classified as heterologous. These tumors revealed various reaction patterns with either expression of only cytokeratins or apparent coexpression of cytokeratins and vimentin in the carcinomatous portions and expression of vimentin but no...
FIG. 3. Photomicrographs show immunoperoxidase (a, b, e, and f) and indirect immunofluorescence (c, d, g, and h) staining patterns in (a and b): undifferentiated carcinoma of the uterine cervix (a: pKer; b: pVim); (c and d): adenocarcinoma (c: RGE 53; d: pVim; double label immunofluorescence), and (e and f): adenomatoid tumor (e: pKer; f: pVim) of the uterine corpus, and (g and h): primary ovarian granulosa cell tumor (g: pKer; h: pVim). Note expression of both cytokeratins and vimentin in these different types of tumors. a and b, ×200; c and d, ×150; e and f, ×320; g, ×185; h, ×155.
FIG. 4. Photomicrographs show immunoperoxidase staining patterns in a and b: müllerian mesodermal mixed tumor (MMMT) of the ovary (a: pKer; b: pVim); c–f: MMMT of the uterine cervix (c and e: pKer; d and f: pVim); and g and h: mural nodule (left side of the micrographs) in ovarian mucinous cystadenocarcinoma (g: pKer; h: pVim). Note expression of both cytokeratins and vimentin in spindle cell areas of the MMMT in c–f (e and f: higher magnifications of spindle cell areas in c and d, respectively), as well as in the area of the mural nodule shown in g and h. In the ovarian MMMT depicted in a and b, the reactivity patterns of pKer and pVim are mutually exclusive. a and b, ×190; c, d, g, and h, ×185; e and f, ×290.
cytokeratins in the sarcomatous portions of the tumors. It is stressed here that an accurate distinction between carcinomaous and sarcomatous components could be made only on the basis of the immunoreactivity patterns, especially in poorly differentiated (sarcomatoid/spindle cell) areas. Well-differentiated carcinomaous areas were mainly composed of cells positive for cytokeratins only (Fig. 4a and b), whereas in areas with spindle cell morphology a varying number of cells appeared to contain cytokeratins as well as vimentin (Fig. 4c–f). Because of their cytokeratin positivity, the latter cells were identified as epithelial in nature and regarded as a part of the carcinomaous component of the tumor. Finally, groups of cells positive for vimentin only were invariably found in areas with spindle cell morphology, representing in our opinion the true sarcomatous elements of the MMMTs. In some of the MMMTs malignant-appearing desmin-positive cells were found. In one ovarian MMMT several of the desmin-positive cells unequivocally showed cross striations, which led us to classify them as heterologous MMMT. In two other cases of ovarian MMMT, cross striations could not be found in the mostly spindle-shaped desmin-positive cells. Although the smooth muscle character of these cells has not been further substantiated, these latter two examples of ovarian MMMT were regarded as homologous.

A distinct group of gynecological tumors with complex composition is represented by the ovarian mucinous cystadenocarcinomas containing so-called mural nodules (Table 3 and Fig. 4g and h). This entity has been recognized only recently, but already various types of mural nodules have been described in the literature (35–39). In our material the mural nodule in one of the cases was composed mainly of malignant-appearing spindle-shaped cells, while multinucleated giant cells were also present. In the immunoperoxidase staining, cells positive for vimentin only were found. The morphology and IFP pattern of this mural nodule fitted in well with the characteristics of the type 2 sarcoma-like mural nodule reported by Prat and Scully (36). The morphology of the mural nodules in the other two cases did not match the characteristics of one of the three types of sarcoma-like mural nodules described by Prat and Scully (36) or one of the other types of mural nodules reported to date (35,37–39). Furthermore, in these nodules we found malignant-appearing cells positive for cytokeratins only, cells positive for vimentin only, as well as cells apparently positive for both cytokeratins and vimentin, but no desmin-positive cells. These findings prompted their classification as lesions with characteristics of MMMT.

**DISCUSSION**

Using polyclonal and monoclonal antibodies to IFP, the results described above show that, in general, the immunoreactivity patterns of gynecological tumors with simple composition correspond with their epithelial or mesenchymal nature. In this way it has been demonstrated that in simple gynecological tumours reactivity patterns comparable with those obtained in tumors of other organs can be found. It is remarkable that several tumors appeared to show coexpression of cytokeratins and vimentin. In adenocarcinomas of the uterine corpus this type of coexpression was found in a substantial proportion of the tumor cells. Coexpression of cytokeratins and vimentin was also seen in nonneoplastic endometrial glandular epithelium (unpublished data) and has been described before in endo-
cervical columnar epithelium (40). Vimentin positivity in endometrial adenocarcinomas has also been noted by Bonazzi del Pogetto et al. (19), albeit in a few tumor cells. The observation of coexpression of cytokeratins and vimentin in adenocarcinomas of the uterine corpus adds another example of coexpression of distinct IFP classes in human neoplasms to the list recently compiled by Gould (41). Likewise, many cells coexpressing cytokeratins and vimentin occurred in adenomatoid tumors of the uterine corpus. In the literature, general agreement exists on the mesothelial origin of adenomatoid tumors of the genital tract (42–44). Since coexpression of cytokeratins and vimentin in mesothelium has been described before (8,9), this type of coexpression in adenomatoid tumors is not surprising.

With respect to undifferentiated carcinoma of the uterine cervix, it is of paramount importance that cytokeratin positivity was invariably demonstrable in all 13 cases, even in routine paraffin sections. In some cases of undifferentiated carcinoma of the uterine cervix, coexpression of cytokeratins and vimentin was found in a very small number of tumor cells. This phenomenon requires further study.

With regard to ovarian tumors similar to those mentioned in Table 1, Ganjei et al. (20) found that strong cytokeratin expression was limited to Brenner tumors and the squamous component of endometrioid carcinomas, which is in contrast with our previous results (26,30,31) and the results described in this paper, as well as those of others (17–19,24). Methodological aspects, such as formalin fixation, paraffin embedding, the use of nonpredigested paraffin sections, and a limited cross-reactivity of the cytokeratin antibody applied, may be responsible for this discrepancy (see also 23).

Since ovarian surface epithelial cells have been shown to coexpress cytokeratins and vimentin (9), it is remarkable that we, in accordance with findings reported by Miettinen et al. (18), could demonstrate this dual IFP expression only in a very small number of tumor cells from serous papillary cystadenocarcinomas, since these tumors belong to the group of so-called common epithelial tumors (34,45), also designated as tumors of surface epithelial (45) or müllerian epithelial origin (46). Similarly, it is striking that also in a case of ovarian serous adenofibroma, which is considered to be of surface epithelial-cortical stromal origin (47), vimentin expression could not be detected in the epithelial component of this tumor.

In the literature, there is no general agreement on the IFP content of granulosa cells. Miettinen et al. (18) found only vimentin and no cytokeratins in granulosa cells. Accordingly, Miettinen et al. (18,25) could show only vimentin but no cytokeratins in formalin- or ethanol-fixed tissue specimens or in frozen sections from granulosa cell tumors. Also Ganjei et al. (20) could not detect cytokeratins in granulosa cell tumors. Czernobilsky et al. (9), however, were able to demonstrate cytokeratins 8 and 18 in addition to vimentin in human granulosa cells. Our results in the primary ovarian granulosa cell tumor, i.e., a (weakly) positive reaction with the polyclonal cytokeratin antiserum and the monoclonal cytokeratin 18 antibody, as well as a positive reaction with the vimentin antibodies, are in line with the results obtained by the latter authors. The detection of only vimentin in the metastatic granulosa cell tumor corresponds with the findings of Miettinen et al. (18,25).
With respect to uterine leiomyosarcomas and endometrial stromal sarcomas, Bonazzi del Pogetto et al. (19) have noticed that in these two types of uterine sarcoma most tumor cells appeared to be positive for desmin, whereas in most of these tumors vimentin was present only in some neoplastic cells and in the vascular endothelial cells. Apparently, desmin was more frequently expressed in leiomyosarcomas than in stromal sarcomas, a phenomenon that these authors supposed to be potentially helpful in the differential diagnosis in some doubtful cases. Our results differ from those of Bonazzi del Pogetto et al. (19), in that expression of desmin appeared to be completely absent from the tumor cells in endometrial stromal sarcomas; vimentin positivity was noted in many tumor cells in these neoplasms. Desmin positivity was found in all muscle cell tumors examined, mostly in a major portion of the tumor cells, whereas a variable number of tumor cells showed vimentin positivity. The apparent absence of desmin from tumor cells in endometrial stromal sarcomas may turn out to be of diagnostic significance in distinguishing these neoplasms from muscle cell tumors.

It is much more common to encounter MMTTs in the uterine corpus than in the ovary, and primary MMTTs of the uterine cervix are rarely reported (45,47,48, and references therein). According to Norris and Zaloudek (49), the histogenesis of MMTTs has been a source of controversy since they were first described. With respect to MMTTs of the uterine corpus, these authors state that the presence of both epithelium and connective tissue differentiation within these neoplasms is a result of the embryologic heritage of the epithelium and stroma of the endometrium. Although rare examples of ovarian MMTTs have been demonstrated to originate in endometriosis, most ovarian MMTTs are considered as arising directly from the surface (celomic) epithelium with participation of the underlying stroma (45,47). In the uterine cervix, MMTTs are supposed to arise from cervical epithelium and stromal cells (50,51, and references therein). These theories point to the derivation of MMTTs, whether they occur in the ovary or in the uterus, from tissues with a common embryologic origin, i.e., the embryonic mesoderm. This idea is deduced from the fact that within the embryonic mesoderm the celomic cavity is formed, which is then lined by the celomic epithelium. On one hand, this epithelium gives rise to the surface epithelium of the ovaries, and on the other hand it gives rise to the müllerian duct, from which the fallopian tubes, the uterine body, the cervix, and probably part of the vagina are derived (47,52). This common mesodermal background of the ovarian surface epithelium and the müllerian epithelium and mesenchyme (49), in our opinion, accounts very well for the presence of both cytokeratins and vimentin in the epithelial tumor cells of cases of MMT. As a matter of fact, this may also apply to the coexpression of cytokeratins and vimentin in adenocarcinomas of the uterine corpus.

To the best of our knowledge, only a few detailed reports concerning the demonstration of IFPs in MMTTs of the female genital tract have appeared in the literature (19,22,26). Bonazzi del Pogetto et al. (19) mentioned a clear-cut differential expression of cytokeratins in the epithelial component and vimentin and/or desmin in the mesenchymal component of ovarian and uterine MMTTs. This IFP pattern was suggested to be an acquired property accompanying the particular pathway of differentiation and perhaps to be related to the mode of growth of the cell types. Our results point to the presence of vimentin in addition to cytokeratins in a varying number of epithelial cells, mainly in spindle cell areas of

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MMMTs. This phenomenon may indicate a relation between the expression of vimentin and spindle cell morphology.

The typing of IFPs has played an important role in the identification of carcinomatous and sarcomatous components in MMMTs. Two cases of MMT, one in the uterine cervix and one in the uterine corpus, in which initial histopathological diagnoses of undifferentiated carcinoma and poorly differentiated adenocarcinoma were made, respectively, were only recognized as MMT after IFP typing. Furthermore, IFP-typing has contributed substantially to a final classification in several other tumors. The usefulness of IFP typing is exemplified by the variable demonstrability of cytokeratins in undifferentiated carcinomas of the uterine cervix and by the ultimate classification of a uterine tumor as adenosquamous carcinoma in a case initially diagnosed as MMT. Mainly the demonstration of cytokeratin-positive cells in supposed chondroid areas led to the reclassification of this tumor (see also ref. 26). Another example concerns a uterine tumor, in which conventional light microscopic examination was not conclusive with respect to the distinction between poorly differentiated carcinoma and sarcoma. A preferential diagnosis of sarcoma was made because of the presence of vimentin-positive tumor cells and a complete lack of cytokeratin reactivity.

A conspicuous finding in our cases of MMT, pointing to a possible limitation of IFP typing in tumor diagnosis, was the indemonstrability of cytokeratins in a number of cells clearly recognizable as epithelial in nature. As far as we could verify, loss of expression of cytokeratins in tumors of epithelial origin has not yet been extensively studied in in vivo human tumor pathology. On the contrary, reversible loss of cytokeratins has been described in human mesothelial cells during rapid growth in culture (53), and Venetianer et al. (54) have noticed the cessation of cytokeratin expression in a rat hepatoma cell line. In view of these observations, it is suggested that cytokeratins may no longer be detectable in some epithelial tumor cells.

Much of what has been said about MMMTs also applies to the cases of ovarian mucinous cystadenocarcinoma with mural nodules, in which characteristics reminiscent of MMMT were found. Similar cases have so far not been described in the literature. Whether these tumors represent a special type of complex tumor has to be assessed in future studies.

It seems justified to conclude that IFP typing can be of practical significance in gynecological tumor pathology, by refining the differential diagnosis. In this way it may decrease the need for the application of time-consuming additional histopathologic techniques or at least limit their extent. It is stressed, however, together with Blobel et al. (14), Gould (41), and Erlandson (55), that well-defined antibodies should be used in combination with proper tissue handling and standardized laboratory techniques to avoid contradictory or noninterpretable results. When these prerequisites are guaranteed, IFP typing contributes to a differentiated descriptive diagnosis, which in turn may prove to be useful in the evaluation of gynecological treatment protocols.

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