Use of intermediate filament antibodies in the differential diagnosis of gynecological neoplasia

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All eukaryotic cells contain an intracellular network of protein filaments with varying diameters, the cytoskeleton. Next to microfilaments and microtubules, 10-nanometer filaments can also be recognized. These so-called intermediate-sized filaments comprise a considerable part of the cytoskeleton and have been described to be tissue-specific (Franke et al., 1981). Recent investigations have shown that all types of epithelial cells contain cytokeratins, while mesenchymal cells contain vimentin as the protein content of their intermediate filaments (I.F.). Muscle cells contain desmin I.F., while neural tissues contain GFAP or neurofilaments (Osborn et al., 1981). Antibodies raised against these different types of I.F. proteins can be used in the immunohistochemical characterization of normal tissues as well as of benign and malignant tumors. For example, carcinomas contain only cytokeratins, while malignant lymphomas contain only vimentin (Osborn and Weber, 1983; Ramackers et al., 1983a).

Since cytokeratins consist of a number of different proteins which occur in tissue-specific combinations (Moll et al., 1982), monoclonal antibodies to these cytokeratin subsets allow distinction between different kinds of epithelia. For example, we have prepared the monoclonal antibody RGE 53 which is able to distinguish squamous from columnar epithelium (Ramackers et al., 1983b). When applied to frozen sections of the squamo-columnar junction of the uterine cervix, for example, a polyclonal cytokeratin antibody stains both ectocervical squamous and endocervical columnar epithelium, whereas the monoclonal antibody only stains the columnar cells (Fig. 3). This antibody can also discriminate between tumors originating from these different types of epithelial cells. It shows a positive reaction in adenocarcinomas but is negative in squamous cell carcinomas. We have applied polyclonal and monoclonal antibodies to different I.F. proteins in the diagnosis of gynecological tumors. The specificity of the antibodies (see Table IV) was first tested on gynecological tumors which normally do not present problems in the differential diagnosis.

In cases of papilliferous serous cystadenocarcinomas of the ovary (Fig. 4), a positive staining reaction with polyclonal cytokeratin antibodies is seen. The tumor cells are negative with the vimentin antibody, while, however, stromal elements are strongly positive for this antibody. Similar results are obtained with papilliferous mucinous cystadenocarcinomas of the ovary. Also adenocarcinomas of the uterine corpus and cervix (Fig. 5) show this positive reaction for cytokeratin only, both in

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Figs. 1 and 2. (see p. 342) Immunofluorescent staining of human tissue sections and ascitic tumor cells with monoclonal antibody (OV-TL3) to ovarian carcinoma. Frozen sections were non-fixed, while cytospin slides were briefly fixed in cold methanol. 1a. Primary ovarian adenocarcinoma, strongly positive. 1b. Omental metastasis of ovarian carcinoma, strongly positive. 2. Clusters of ascitic tumor cells derived from an ovarian carcinoma; Note the strong positive membrane-like fluorescence pattern.

Figs. 6-9. (opposite page, and see pp. 351, 353) Sarcomatous nodule in a papillary serous mucinous cystadenocarcinoma of the ovary (second case). Fig. 6. H & E stained section of the nodule. Fig. 7. Cytokeratin-positive reaction in tubular structures present in the nodule (as demonstrated by the immunoperoxidase technique; PAP). Fig. 8. Individual cytokeratin-positive tumor cells in the nodule (PAP technique). Fig. 9. Vimentin-positive tumor cells in the nodule, with no reaction in the tubules (PAP technique).

Figs. 10-13. (opposite page, and see p. 353) Tumor in the uterus, initially diagnosed as a carcinosarcoma (third case). Fig. 10. H & E stained section, showing supposed chondrosarcomatous areas next to carcinomatous differentiation. Fig. 11. Detail of Fig. 10 showing squamous and adeno- components. Fig. 12. Positive reaction with the polyclonal antibody to cytokeratin in both carcinomatous and supposed chondrosarcomatous areas. Fig. 13. Immunofluorescence micrograph of RKSE 60-positive cells, indicating squamoid differentiation.
Fig. 3. Immunofluorescence micrographs of the cervical squamocolumnar junction. Note that a polyclonal rabbit antiserum to cytokeratins stains both squamous and glandular epithelial cells (A), while the monoclonal antibody to cytokeratin 18 (RGE 53) only reacts with the glandular and columnar epithelial cells.

the indirect immunofluorescence and in immunoperoxidase techniques. Furthermore, the polyclonal cytokeratin antiserum reacts strongly with squamous cell carcinomas of the cervix and with keratinizing squamous cell carcinomas of the vulva.

Next to the monoclonal antibody already mentioned, which specifically recognizes columnar epithelium and adenocarcinomas (RGE 53), we have prepared a monoclonal antibody (RKSE 60) which is specific for keratinizing squamous epithelium and recognizes keratinizing cells in squamous cell carcinomas. In the vulvar tumor mentioned above extensive keratinization could be demonstrated using this monoclonal antibody.

Non-epithelial tumors do not stain with the cytokeratin antibodies, but may show a reaction with vimentin or desmin antisera, as is the case for leiomyomas of the uterus.

TABLE IV
Tissue-specific reactivity patterns of intermediate filament antibodies

All antibodies are available through Euro-Diagnostics B.V., Apeldoorn, The Netherlands.

<table>
<thead>
<tr>
<th>Type of antibody</th>
<th>Specificity in normal tissues</th>
<th>Specificity for tumors</th>
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<tbody>
<tr>
<td>Polyclonal anticytokeratin PKE</td>
<td>all epithelia</td>
<td>all carcinomas and mesotheliomas</td>
</tr>
<tr>
<td>Monoclonal antibody RGE 53</td>
<td>glandular and columnar epithelia, and mesothelial cells</td>
<td>adenocarcinomas and mesotheliomas</td>
</tr>
<tr>
<td>Monoclonal antibody RKSE 60</td>
<td>keratinizing stratified squamous epithelia</td>
<td>keratinizing squamous cell carcinomas</td>
</tr>
<tr>
<td>Polyclonal antivimentin PVI</td>
<td>mesenchymal tissues</td>
<td>sarcomas, lymphomas, melanomas</td>
</tr>
<tr>
<td>Polyclonal antidesmin PDE</td>
<td>muscle tissues</td>
<td>rhabdomyosarcomas, leiomyosarcomas</td>
</tr>
</tbody>
</table>
Fig. 4. Immunofluorescence staining of a cystadenocarcinoma papilliferum serosa with antibodies directed against cytokeratin (CK) and vimentin (V). Note a positive reaction in the tumor cells only for cytokeratin, while stromal cells are positive for vimentin only (polyclonal antisera were used).

After the specificity of the antibodies had been proven to hold true for gynecological tumors, their applicability and usefulness in the differential diagnosis of gynecological tumors with a heterogeneous composition was tested. These tumors represented considerable diagnostic problems after use of routine histological techniques with important implications for clinical management. Our results with some cases will be described and illustrated below.

The first case concerns a 58-yr-old woman, who presented with cystic tumors in both ovaries. These were interpreted histologically as poorly differentiated adenocarcinoma. In the course of her illness a pure pleomorphic rhabdomyosarcoma was found in the mesocolon as confirmed by antidesmin staining. Next to this tumor multiple masses of malignant tissue were found in the uterus, lymph nodes and omentum, which again were interpreted as poorly differentiated adenocarcinoma. Re-evaluation of the original tumor and tumor anti-desmin serum revealed scattered rhabdomyoblasts in the adenocarcinomatous areas, establishing the diagnosis of mesodermal mixed tumor for the original neoplasm (see Ramaekers et al., 1983c).

The second case (see Figs. 6–9) deals with a solid mural nodule in an otherwise typical well-to-moderately differentiated papilliferous mucinous cystadenocarcinoma of the ovary. From the histological point of view it was difficult to determine whether the nodule represented an area of poorly differentiated carcinoma with or
Fig. 5. Immunofluorescence photomicrograph of an adenocarcinoma of the uterine cervix, positive for cytokeratin (polyclonal antiserum).
without a concomitant sarcoma or a mixed type of tumor (Fig. 6). When using intermediate filament antibodies to cytokeratin and vimentin it became obvious that the nodule consisted of both carcinomatous and sarcomatous components which were strongly intermingled. A positive cytokeratin reaction was seen in tubular structures (Fig. 7) as well as in some individual cells (Fig. 8), while vimentin positivity was found in the sarcomatous element lying between the carcinomatous tubules and cells (Fig. 9). From these results we concluded that the nodule represented a carcinosarcomatous lesion.

The third case (Figs. 10–13) concerns a tumor of the uterus, initially diagnosed as a carcinosarcoma. This was deduced from the supposed presence of chondrosarcomatous areas next to apparent carcinomatous tissue (Fig. 10), showing both adenocarcinoma and squamous cell differentiation (Fig. 11). Since the chondrosarcomatous and carcinomatous structures seemed to be confluent, without clear demarcation, the diagnosis, however, was given with some hesitation. When applying the I.F. antisera it became clear that both the carcinomatous and the supposed chondrosarcomatous areas were positive for cytokeratin (Fig. 12), indicating the epithelial nature of both histologically distinct components. Furthermore, the monoclonal antibody to skin keratin, specific for keratinizing cells, showed a positive reaction in several locations (Fig. 13). This led us to the diagnosis of an adenosquamous carcinoma.

These examples of complex gynecological tumors illustrate that antibodies to I.F. can be valuable tools in surgical pathology and can help to obtain a proper classification of the different components within heterogeneous gynecological tumors, and thus facilitate proper staging and proper treatment.

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References