Cytoskeletal Proteins as Tissue-Specific Markers in Cytopathology

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Key Words. Cytokeratin · Vimentin · Cytopathology · Immunocytochemistry

Abstract. Antibodies to intermediate filament proteins react in a tissue-specific manner and can be used to characterize tumor cells present in thin-needle aspirates from solid tumors, from palpable lymph nodes and cells present in samples from peritoneal pleural effusions. From our studies so far the following conclusions can be drawn: (a) Polyclonal antisera to cytokeratins can identify carcinoma metastases in thin-needle aspirates from palpable lymph nodes and distinguish them from malignant lymphomas and nonmalignant lesions such as chronic lymphadenitis, which show only vimentin-positive cells. (b) Monoclonal antibodies to specific cytokeratin polypeptides are able to distinguish between different types of epithelial tumor metastases, i.e., metastases from adenocarcinomas and metastases from squamous cell carcinomas. (c) Cells present in peritoneal pleural effusions can be partly characterized using intermediate filament antisera. We have found that metastatic adenocarcinoma cells from breast, ovary, endometrium, cervix, colon and stomach, as well as squamous cell carcinomas and malignant mesothelioma stain specifically with antibodies to cytokeratin while mesenchymally derived tumors such as malignant lymphomas, malignant melanomas, and fibrosarcomas, are positive for vimentin only. (d) Metastatic tumor cells of epithelial origin present in aspirates from human serous body cavity fluids may coexpress vimentin next to their original cytokeratin intermediate filaments. Benign mesothelial cells present in body cavity fluids frequently coexpress cytokeratins and vimentin. (e) Tumor cells present in thin-needle aspirates from solid tumors such as pleomorphic adenomas of the parotid gland can be identified as such because of their typical patterns of intermediate filament (co-)expression. Antibodies to the different components of the intermediate filament cytoskeleton can thus be helpful in cytopathologic diagnosis where a definitive diagnosis cannot be made on the basis of conventional cytologic features.
Introduction

All human cells contain an intracellular matrix of proteinaceous fibrous elements recognized as microfilaments, microtubules and intermediate-sized filaments (IF). These IF often constitute a considerable part of the cytoskeleton and are composed of different types of proteins which are specific for the various cell types as summarized in Table I (for reviews see 1 and 2).

In general cytokeratins are specific for epithelial cells, whereas vimentin is specific for mesenchymal cells, and desmin is present in cells of myogenic nature.

Malignant tumors of these cell types, the carcinomas and malignant lymphomas or sarcomas, respectively, retain the intermediate filament protein type specific for their cell of origin. Most epithelial and mesenchymal types of tumor cells do normally not express new types of intermediate filament proteins as a result of malignant transformation. However, some exceptions to this rule have been reported [1, 2].

In contrast to their normal (adult) human tissue counterparts, certain neoplastic tissues may coexpress different types of intermediate filament proteins. Vimentin IF have been found next to cytokeratin IF in pleomorphic adenomas of the parotid gland, in adenoid cystic carcinomas of the salivary gland and of the lung, in thyroid carcinomas, in malignant mesotheliomas of the spindle cell type, in renal cell carcinomas (Gravitz tumor) and nephroblastomas. Furthermore, metastatic carcinoma cells growing in body cavity fluids (ascites or pleural fluid) may obtain an additional vimentin cytoskeleton (see below).

Polyclonal antiserum directed against cytokeratin (mainly skin keratins) recognize (nearly) all epithelial tissues including squamous epithelia, glandular epithelia and mesothelial cells. Therefore, immunohistochemical localization of cytokeratin proteins has been useful in characterizing, classifying and determining the derivation of primary epithelial neoplasms and metastases.

Recent studies have detected 19 different cytokeratin polypeptides in human epithelial tissues. These polypeptides are not randomly distributed throughout epithelial tissues. Different epithelia express distinctive profiles of cytokeratins, generally maintained in primary tumours and metastases. These findings offer a new approach to tumor characterization, especially since monoclonal antibodies specific for different cytokeratin proteins are already proving to be useful as immunologic probes in the study of epithelial neoplasms [3].

Furthermore, antisera to the other types of intermediate filament proteins (vimentin, desmin, GFAP and neurofilaments) can also be used as immunohistochemical markers in tumor diagnosis (Table I).

Until now IF antisera have mainly been used for help with the differential diagnosis of solid tumors and tumor metastases in routine paraffin sections or frozen sections. However, cytokeratin antibodies have recently been described as a useful tool to distinguish between mesothelial cells and metastatic adenocarcinoma cells present in smears from pleural and peritoneal effusions [4]. Furthermore, we have recently described that cytokeratin and vimentin may be used as immunocytochemical markers in cytologic aspirates [5]. Here we briefly summarize the potential usefulness of antibodies to cytokeratins and vimentin in the differential diagnosis of epithelial and nonepithelial tumor cells in cytologic material.
Intermediate Filaments in Cytopathology

Table I. Tissue and tumor specificity of intermediate filament proteins

<table>
<thead>
<tr>
<th>Type of IF protein</th>
<th>Tissue type</th>
<th>Tumor type</th>
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</thead>
<tbody>
<tr>
<td>(Cytokeratins)</td>
<td>epithelial tissues</td>
<td>carcinomas</td>
</tr>
<tr>
<td>Vimentin</td>
<td>mesenchymal tissues</td>
<td>lymphomas, sarcomas</td>
</tr>
<tr>
<td>Desmin</td>
<td>muscle tissues</td>
<td>myosarcomas</td>
</tr>
<tr>
<td>GFAP</td>
<td>astroglial cells</td>
<td>astrocytomas</td>
</tr>
<tr>
<td>Neurofilament proteins</td>
<td>nerve tissues</td>
<td>some neural tumors</td>
</tr>
</tbody>
</table>

Methodology

Cell Preparations

The cytologic samples used in our studies consisted of material from body cavity effusions and thin-needle aspirates from lymph nodes and from solid tumors. In the body cavity effusions routine diagnosis was made on Papanicolaou-stained smears and the preparations were shown to contain either epithelial tumor cells (metastatic adenocarcinomas from breast, ovary, endometrium, cervix, large intestine and stomach as well as squamous cell carcinomas and a malignant mesothelioma) or nonepithelial tumor cells (malignant lymphoma, leukemia, malignant melanoma, fibrosarcoma) in addition to mesothelial cells and blood cells. For the immunocytochemical examination of cells from peritoneal and pleural effusions ethanol/carbonic acid was used, unstained parallel smears were used. Some of the preparations used in our experiments had been stored at −50 °C for up to 4 years. Thin-needle aspirates from palpable lymph nodes were smeared onto glass slides using conventional techniques or brought onto glass slides using a pressure-fixation technique ("Cytopress"). These preparations could be shown to contain cells from squamous cell carcinomas, adenocarcinomas or malignant lymphomas in addition to normal lymphocytes and other blood cells. Also tumor cells in thin-needle aspirates from pleomorphic adenomas and an adenoid cystic carcinoma were examined.

Antisera and Indirect Immunofluorescence Technique

We have used the following antibody preparations: (1) an antiserum directed against human foot callus keratins raised in rabbits; (2) a rabbit antiserum directed against bovine lens vimentin; (3) a mouse monoclonal antibody (RGE 53), directed against cytokeratin 18 isolated from HeLa-cells and shown to react specifically with glandular epithelial cells from various organs but not with stratified squamous epithelia or nonepithelial tissues. This antibody reacts with adenocarcinomas but not with squamous cell carcinomas, sarcomas or malignant lymphomas [3]; (4) a monoclonal antibody to bovine lens vimentin (obtained from Euro-Diagnostics BV, Apeldoorn, The Netherlands).

Mostly these antibodies were applied to the cytologic smears in a double-label procedure combining one monoclonal antibody and one polyclonal antiserum on the same smear. This allows the detection of coexpression of intermediate filament proteins and has proven to be an efficient procedure for the recognition of epithelial and nonepithelial (tumor) cells.

The indirect immunofluorescence technique with these antisera was performed as follows. Cell smears were fixed in cold methanol and acetone and incubated with the first antibodies. After incubation with the first antiserum or antiserum (a combination of a polyclonal antiserum to cytokeratin and a monoclonal antibody to vimentin, or vice versa) for 30 min in a humidified box at room temperature, the slides were washed with phosphate-buffered saline (PBS) in three subsequent washing steps of 10 min each. The cell smears were then incubated for another 30 min with the second antibodies (fluorescein-conjugated goat-anti-rabbit or rabbit-antimouse IgG, diluted 1:20), obtained from Nordic Immunology, Tilburg, The Netherlands and sheep F(ab')2 antiserum Ig conjugated with Texas Red, diluted 1:50, obtained from New England Nuclear, Boston, Mass.). After a second series of washes, the slides were stained with hematoxylin for 60 s and mounted with 50% glycerol in PBS, pH 7.4.
Cells were viewed as soon as possible with a Leitz Dialux 20 EB microscope equipped with epifluorescent illumination (HBO 100 bulb), using the appropriate filter systems. Pictures are preferably taken with a Leitz Fluotar 40x objective using an automated Leitz camera (ASA setting 400 or 800) and Kodak Tri-X film.

Results

Applications of IF Antisera to Lymph Node Aspirates

Table II summarizes some significant pathological and immunocytochemical data obtained with lymph node aspirates. In all cases lymphocytes were negative for cytokeratin antibodies, but (weakly) positive for vimentin. When examining frozen sections of total lymph nodes, we have found no cytokeratin-positive cells. As a result, the detection of cytokeratin-positive cells in aspirates from lymph nodes is a very strong indication for the presence of metastatic carcinoma cells. Table II summarizes typical examples of staining patterns of cell preparations from lymph nodes containing malignant or benign cells. It is obvious that epithelial cells present in these preparations all show strong positivity with the polyclonal cytokeratin antisera, but are negative for vimentin. Normally no cytokeratin-positive cells can be observed in cases of chronic lymphadenitis or malignant lymphomas. Instead the cells are vimentin positive.

When using the monoclonal cytokeratin antibody RGE 53 no reaction with cells from metastatic squamous cell carcinomas or with lymphocytes is seen. A rather strong reaction, however, is seen in cells from metastases of adenocarcinomas. These reaction patterns are consistent with our results in frozen sections of several types of epithelial tumors [3].

Application of the immunocytochemical techniques described above thus seems promising in those cases where lymph node aspirates have to be evaluated. Epithelial tumor cells present in these preparations mostly contain exclusively cytokeratin IF and do not coexpress vimentin (as in the case of ascites and pleural fluids; see below). Furthermore, a keratin-positive reaction in cells is detected quickly in the immunofluorescence microscope, also in cases where only few tumor cells are present in the preparation. This positive reaction then indicates very strongly the presence of metastatic carcinoma cells in the lymph nodes examined. Moreover, since the monoclonal antibody directed against cytokeratin from glandular epithelial tissues (RGE 53) can distinguish between cells derived from adenocarcinomas and squamous cell carcinomas it is obvious that application of this antibody in cytopathology can help to make a more precise differential diagnosis and can thus give valuable information with respect to the nature of a primary tumor.

Application of IF Antisera to Aspirates from Pleomorphic Adenomas and Adenoid Cystic Carcinoma

IF typing in aspirates from pleomorphic adenomas of the parotid gland, which normally coexpress cytokeratin and vimentin has been shown to be most helpful in confirming or establishing the cytologic diagnosis of such neoplasms.

In frozen sections of these tumors several cell types have been detected, which differ in their IF pattern. Amongst these are cells coexpressing cytokeratin and vimentin, but which are negative for RGE 53. Another cell type is positive for the polyclonal cytokeratin antiserum as well as for RGE 53, but is nega-
Table II. Patterns of cytokeratin and vimentin expression in cells from lymph node aspirates

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Keratin</th>
<th>RGE 53</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinomas</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenocarcinomas</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Malignant lymphomas</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Chronic lymphadenitis</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Table III. Patterns of cytokeratin and vimentin expression in benign and malignant cells in body cavity effusions

<table>
<thead>
<tr>
<th>Cytologic diagnosis</th>
<th>Keratin</th>
<th>RGE 53</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinomas</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenocarcinomas</td>
<td>++</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Malignant lymphomas</td>
<td>-</td>
<td>-</td>
<td>++/++</td>
</tr>
<tr>
<td>Leukemias</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Malignant melanomas</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fibrosarcomas</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Benign mesothelial cells</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Macrophages and lymphocytes</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

tive for vimentin. These two combinations are typical for solid pleomorphic adenomas. When examining thin-needle aspirates from such neoplasms, these two cell types can be detected on the basis of their IF pattern.

All cells from an adenoid cystic carcinoma of the trachea showed coexpression of cytokeratin and vimentin, which was helpful in its recognition.

Application of IF Antisera to Body Cavity Effusions

Metastatic tumor cells of epithelial and nonepithelial origin, present in body cavity effusions can be examined for their intermediate filament protein type(s) using the double-label technique as described in the methodology part.

Within aspirates from these effusions only epithelial tumor cells and mesothelial cells react positively with the cytokeratin antisera in the indirect immunofluorescence assay, while on the contrary all nonepithelial tumor cells and nonmalignant nucleated blood cells are negative for cytokeratin, but positive for the vimentin antisera (table III). Erythrocytes are negative for both the cytokeratin and vimentin antisera. This implies that antibodies to cytokeratin can be of help in cytopathologic diagnosis when a differential diagnosis between epithelial and nonepithelial cells cannot be made on the basis of routine
cytologic techniques. In the interpretation of these preparations it is of help that cell nuclei can be counterstained with hematoxylin. This staining procedure may however influence the fluorescence pattern insofar that erythrocytes show nonspecific fluorescence. Furthermore, for optimal results preparations should be viewed within a few hours after incubation.

We have occasionally observed that clusters of epithelial-like tumor cells in effusions were negative for cytokeratin, while single tumor cells showed a positive reaction for these antibodies. This result may be partially explained by the compact nature of these three-dimensional tumor cell groups.

As in lymph node aspirates, also in preparations from body cavity fluids the monoclonal cytokeratin antibody RGE 53 can distinguish between cells derived from adenocarcinomas and cells derived from squamous cell carcinomas. Unfortunately, this antibody does not distinguish between benign or malignant mesothelial cells and adenocarcinoma cells.

When interpreting intermediate filament patterns in cells from ascites or pleural fluid the following should be kept in mind. We have noticed that cytokeratin and vimentin filaments may be expressed simultaneously in metastatic epithelial tumor cells present in body cavity fluids [6]. This is in contrast to what has been found with most carcinoma cells in solid tumors.

Apparently epithelial tumor cells which are shed into body cavity fluids adapt to their new environment or growth conditions by the development of a vimentin cytoskeleton. Expression of vimentin as adaptation to changing growth conditions has also been observed when epithelial cells are brought into tissue culture [2]. It is noteworthy in this respect that cells from squamous cell carcinomas did not express vimentin when growing in body cavity fluids. This phenomenon may be explained by the highly differentiated state of these cells, although keratinocytes in culture can coexpress vimentin next to cytokeratin. Also the onset of vimentin expression may be less rapid in cells from squamous cell carcinomas than in cells from adenocarcinomas or in mesothelial cells.

All nonepithelial tumor cells present in effusions are negative for the cytokeratin antisera, but positive for the antisera directed against vimentin. Macrophages and lymphocytes, as well as tumor cells of mesenchymal origin could be shown to express exclusively vimentin-type intermediate-sized filaments. These nonepithelial neoplastic cells included malignant lymphomas, leukemias, fibrosarcomas, and cells from malignant melanomas. These findings are in complete agreement with data obtained in tissue sections from malignant lymphomas, malignant melanomas or fibrosarcomas.

In conclusion we can state that antibodies to different types of intermediate filament proteins (especially those directed against cytokeratins and vimentin) can be of help in cytopathologic diagnosis. The most important field of application seems to be their use in lymph node aspirates.

In cytologic preparations from body cavity fluids such antibodies are of limited value since, so far, no IF markers specific for mesothelial cells have been prepared.

References


Furthermore, we refer to the special issue of Acta Cytologica (vol. 28, No. 4, July–August 1984) which is partly devoted to application of intermediate filament antibodies in cytopathology.

Received: June 13, 1985
Accepted: September 30, 1985

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