The Applicability of a Keratin 7 Monoclonal Antibody in Routinely Papanicolaou-stained Cytologic Specimens for the Differential Diagnosis of Carcinomas

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The monoclonal antibody OV-TL 12/30, which detects keratin 7, was tested for its usefulness in cytologic diagnosis by reinculating previously Papanicolaou-stained slides. For this purpose malignant effusions of 73 patients with histologically confirmed cancers of the colon, ovary, mesothelium, breast, lung, esophagus, pancreas, urinary bladder, stomach, kidney, and prostate were used. All malignant cells from ovarian adenocarcinomas were positive, whereas malignant cells from colonic adenocarcinomas and malignant mesotheliomas were negative. Adenocarcinomas of gastric, renal, pancreatic, esophageal, and mammary origin demonstrated variable staining. Transitional cell carcinomas were positive, whereas squamous and small cell lung carcinomas were negative. Because OV-TL 12/30 does not react with normal and atypical mesothelial cells in these preparations, this reagent is a valuable tool in distinguishing benign mesothelial cells and adenocarcinoma cells.

The authors' results demonstrate that this antibody is an excellent tool in the differential diagnosis of malignant cells in effusions and can be used in routinely stained cytologic specimens to determine primary tumor localization. In addition to its ability to distinguish between ovarian and colonic adenocarcinomas, its negativity in mesotheliomas may prove helpful in several diagnostic considerations. (Key words: Colon cancer; Effusions; Immunochemistry; Lung cancer; Mammary cancer; Mesothelioma; Ovarian cancer; Transitional cell carcinoma)


Keratins comprise a group of at least 20 intermediate filament proteins that show a tissue-specific distribution in both normal tissues and in malignancies. Monoclonal antibodies developed against these individual keratins have become extremely useful in the classification of the major types of epithelial tumors, both in cytology and in histology.2,3 One drawback of these keratin monoclonal antibodies is that they frequently do not react with formalin-fixed material.4,5 OV-TL 12/30 is a recently developed monoclonal antibody that reacts solely with keratin 7. An important advantage over other keratin 7 monoclonal antibodies is that it reacts with formalin-fixed, paraffin-embedded tissues.6

In tissue studies,7 OV-TL 12/30 was invaluable, because positivity or negativity of the monoclonal antibody in carcinomas, particularly adenocarcinomas, may indicate the primary origin of the carcinoma or limit differential diagnostic possibilities. In studies performed on routinely formalin-fixed and paraffin-embedded tissues,8 OV-TL 12/30 intensely stained all ovarian adenocarcinomas, whereas adenocarcinomas of the colon and metastases of colonic adenocarcinomas were negative. All hepatocellular carcinomas tested were negative, whereas cholangiocarcinomas were positive. Prostatic carcinomas were usually negative, and transitional cell carcinomas of the urinary bladder were mostly positive. Surprisingly, mesotheliomas were negative with OV-TL 12/30 in formalin-fixed tissue.

In this study, we investigated whether the promising results observed with the keratin 7 monoclonal antibody OV-TL 12/30 in tissues could be reproduced in cytology, in particular for Papanicolaou (PAP)-stained slides. We examined whether OV-TL 12/30 could provide diagnostically helpful information by reusing PAP-stained slides from pleural and abdominal effusions in which the morphologic diagnosis had been established. Moreover, we investigated whether application of the antibody could provide information concerning the localization of the primary tumor.

MATERIALS AND METHODS

Cytologic Specimens

The cytologic material used in this study was retrieved from the files of the Department of Clinical Pathology of the SSDZ.
Delft, The Netherlands, and comprised 73 malignant and 15 benign pleural and abdominal effusions from patients with histologically confirmed cancers, diagnosed from 1987 to 1991. Most of the preparations studied were made by cyt centrifugation, whereas the remaining cases were direct smears. In all cases, both cytologic and histologic slides were reviewed.

**Antibodies Used**

OV-TL 12/30. OV-TL 12/30 (Dakopatts, Glostrup, Denmark) is a mouse monoclonal antibody specifically reactive with keratin 7. In immunoblotting studies, it reacted with cytoskeletal proteins of epithelial tissues, but not with nonepithelial cells. In tissue studies, it was reactive in formalin-, ethanol-, and methanol-fixed epithelial cells and tumors. It stained carcinomas of the thyroid gland, some adenocarcinomas of the stomach, and all carcinomas of the ovary and endometrium. Renal cell carcinomas and colonic carcinomas were usually not stained, and the majority of prostate cancers were negative.

RCK 105. RCK 105, also a keratin 7 antibody, was used for comparative purposes. Its reactivity in tissue sections and cytologic specimens has been extensively described.

**Staining Procedure**

All specimens used in this study had been previously examined by May Grünwald-Giemsa (MGG) staining, PAP staining (hematoxylin, Orange G, E50), or both. For the immunocytochemical assays, the PAP prestrained slides were reincubated with the monoclonal antibody after removing the coverslip by placing the slide in xylol and rehydrating the cells in a descending alcohol (95%, 70%, and 50%) series. The slides were then rinsed in distilled water for 10 minutes and in PBS, pH 7.4 for 10 minutes at room temperature. The primary monoclonal antibody as culture supernatant was tested at several dilutions, with OV-TL 12/30 showing optimal results for an antibody dilution of 1:200. The slides were incubated for 30 minutes, after which they were rinsed for 10 minutes in PBS and then incubated for 30 minutes with a biotin conjugated rabbit-antimouse IgG antiserum (Dakopatts, Glostrup, Denmark) diluted 1:600. The slides were subsequently rinsed with PBS and incubated with avidin-biotin-horseradish peroxidase complex (ABC-HRP, Dakopatts) for 30 minutes and again rinsed for 10 minutes in PBS. They were then developed in AEC (3-amino-9-ethylcarbazol) (0.5 mg/mL, Sigma, St Louis, MO) for 10 minutes as the final step to visualize the signal. After the slides were rinsed with distilled water, counterstained with hematoxylin.
for 2 minutes, and rinsed in tap water, they were mounted with gelatin/glycerin. Ten cases of unstained, acetone fixed (fixed 2 minutes at room temperature) smears were used as control specimens and immunocytochemically treated, as described above.

A similar immunostaining procedure was applied to MGG-prestained slides. As no immunoreactivity was observed, these slides were predigested with protease or treated with an antigen retrieval kit (BioGenex, San Ramon, CA). Because no immunoreactivity was observed with these MGG-stained slides, the procedures are not described further.

RESULTS

Papanicolaou-stained slides were destained in alcohol, hydrated, and incubated with OV-TL 12/30 or RCK 105. OV-TL 12/30 exhibited positivity in specific cases, whereas RCK 105 displayed a positive reaction in a more restricted number of cells, as compared with OV-TL 12/30.

The staining results with OV-TL 12/30 are schematically represented in Table 1 and depicted in Figures 1 and 2. All 11 adenocarcinomas of the ovary were positive, usually with intense expression (Figs. 1A and 2a); however, moderately and weakly staining cells were also observed (Fig. 1B). No significant differences in reactivity were observed between the different types of ovarian carcinoma. Adenocarcinoma cells from the breast displayed a variable reaction pattern. Staining was usually intense (Fig. 1C), but in two cases only a few cells stained. The staining intensity was not related to the degree of differentiation of the tumor. Effusions containing malignant cells from lung carcinomas did not stain in the case of undifferentiated small cell or squamous cell carcinoma. In a bronchialveolar cell carcinoma (Fig. 1D) or pulmonary adenocarcinoma, however, most cells stained. Surprisingly, all the PAP-prestained slides in which a mesothelioma was diagnosed were negative for OV-TL 12/30 (Fig. 1E). Of the tumors of the gastrointestinal tract, gastric adenocarcinomas were all positive, showing strong to moderate reactivity and variable numbers of nonreacting tumor cells (Figs. 1F and 2C). Malignant
cells from colonic adenocarcinomas (Figs. 1G and 2B) or esophageal adenocarcinomas were also not decorated by OV-TL 12/30. Of four cases of pancreatic adenocarcinoma, two were negative for OV-TL 12/30, whereas the other two showed a variable reactivity pattern (Fig. 1H). When effusions of urinary tract tumors were examined, malignant cells from transitional cell carcinomas (Fig. 1I) also showed a variable staining pattern that could not be related to the grade of differentiation. In cases in which malignant cells from a renal cell carcinoma were present, only a few of the malignant cells stained with antibody OV-TL 12/30. In the effusion from a prostatic adenocarcinoma, the majority of cells stained with moderate intensity.

Acetone fixed cells in nonpreserved slides were tested with RCK 105 and OV-TL 12/30 to examine the influence of the PAP staining procedure on the reactivity of both antibodies. Because it is well established that mesothelial cells, as well as ovarian carcinoma cells, contain relatively high concentrations of keratin 7, these two different cells are expected to react with both antibodies when their epitopes are preserved.

Unstained slides of benign and malignant effusions (10 cases) fixed in acetone were incubated with both OV-TL 12/30 or RCK 105. Compared with the results described above, these staining patterns were more difficult to interpret because of background reactivity (e.g., in erythrocytes). RCK 105 stained the majority of the benign mesothelial cells, whereas OV-TL 12/30 generally did not show any positivity. Malignant cells behaved as expected.

**DISCUSSION**

In routine cytology, as in histology, the value of immunohistochemistry in the differential diagnosis of the main types of malignancies is well known and has been proven to be a valuable supplement to conventional staining techniques. A drawback in the application of many immunohistochemical staining techniques is that these are often only reactive in mildly fixed and unstained tissues or cells. Fixation and conventional staining procedures often destroy or mask antigen epitopes. Recent reports indicate that with selected antibodies and modified fixation procedures, it should be possible to ascertain immunoreactivity in PAP-stained smears and maintain nuclear and cytoplasmic morphologic detail. OV-TL 12/30, directed against keratin 7, seemed promising in this respect, because it has the advantage of being reactive in formalin-fixed, paraffin-embedded tissues. In addition, its staining pattern in these tissues is generally equal to that observed in fresh-frozen tissue. However, OV-TL 12/30 showed an unexpected negative reaction in paraffin-embedded mesotheliomas. This must be ascribed to the effects of fixation on the keratin 7 epitope recognized by OV-TL 12/30 in this type of tumor. The phenomenon of epitope masking is discussed below.

The impact of the application of this keratin 7 antibody in cytology should be considerable. The antibody discriminates benign and malignant mesothelial cells from the keratin 7-containing adenocarcinomas. In addition, after identification of malignant cells in PAP-stained smears, the origin of the tumor cells is often not evident. OV-TL 12/30 can then be applied to these unclassified cells in the same preparation that was used for routine screening, which will often supply valuable information concerning primary tumor origin. Finally, retrospective studies on PAP-stained archival material can now easily be performed.

The differential diagnostic considerations in pleural effusions are much the same as described above for ascitic fluids.
The fact that OV-TL 12/30 was negative in small cell carcinomas is not surprising, as this carcinoma contained keratin 8, 18, and sometimes 19 in tissue cultures. Squamous cell carcinomas of the lung do not react with OV-TL 12/30 when tested in paraffin sections, whereas adenocarcinomas contained keratin 7. As shown in this study, OV-TL 12/30 can also distinguish between an adenocarcinoma of the lung (positive) and a squamous cell carcinoma (negative) in PAP-prestained slides. Our series is too small, however, to draw definitive conclusions regarding the discriminatory capacity of OV-TL 12/30 in distinguishing between these two tumor types.

Of particular interest was the negativity of OV-TL 12/30 in malignant mesothelial cells and its sporadic positivity in benign mesothelial cells. This is surprising, as both cell types contain keratin 7. RCK 105 reacted positively in mesothelial cells present in the cytologic specimens. The antibody determinant on keratin 7 recognized by OV-TL 12/30 in fresh tissue or after fixation is probably masked in mesothelial cells. This specific configuration of the epitope or pair formation with another keratin subtype may be specifically present in mesothelial cells. This staining characteristic may, however, be used in diagnostic situations when a mesothelioma is being considered. Application of the antibody on benign effusions from patients with known malignancies did not show that malignant cells had been missed during initial screening of the PAP- and MGG-stained slides. This means that the value of the antibody is mainly in the field of differentiation of malignant cells, rather than an aid for screening smears or cytospin preparations from effusions.

In summary, OV-TL 12/30 and the use of keratin 7 antibodies may be extremely useful in determining the origin of malignant cells in effusions. The added value of the OV-TL 12/30 antibody is its reactivity with routinely fixed and PAP-stained slides. However, protocols to ascertain immunoreactivity in MGG-stained slides would render the antibody even more useful.

REFERENCES


