Characterizing Subpopulations of Neoplastic Cells in Serous Effusions

The Role of Immunocytochemistry

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OBJECTIVE: To analyze the role of immunochemistry in serous effusions.

STUDY DESIGN: We analyzed cell blocks of 18 pleural and 18 peritoneal effusions diagnosed as malignant (18), benign (14) and suspicious (4). They were immunostained by the avidin-biotin complex method with a panel of four monoclonal antibodies—CEA, Ber-EP4, LeuM1 (CD15) and p53—and, for lectins (Ulex europaeus) *UEA-l, ConA and ConBr.*

RESULTS: Seventeen of the 18 cases of adenocarcinoma were positive for CEA (95%), 12 (66.6%) for Ber-EP4, 11 (61%) for CD15 and 11 (61%) for p53. Twelve of the 18 (66.6%) were positive for UEA-1, CEA, Ber-EP4 and CD15. UEA-1 did not react with mesothelial cells. p53 Gave a positive reaction in only one case, reactive mesothelial cells. ConA and ConBr reacted indiscriminately with benign and malignant cells; thus, it was not useful in distinguishing between these cells.

CONCLUSION: In this context no antibody used alone is reliable for corroborating a diagnosis, but the selective use of a small panel of three markers (CEA, Ber-EP4 and LeuM1) can be very useful in solving diagnostic difficulties in the cytodiagnosis of serous effusions. (Acta Cytol 2001;45:18–22)

Immunocytochemistry with a small panel of monoclonal antibodies, such as CEA, Ber-EP4 and LeuM1, can give significant help in the interpretation of serous effusions in day-to-day work....

Keywords: cells, neoplasms, immunocytochemistry, serous effusions.

A major difficulty in the cytologic examination of serous effusions, and a challenge to the diagnostic skills of the cytopathologist, is distinguishing between reactive (hypertrophic and hyperplastic) mesothelial cells and adenocarcinoma cells.[4,41]

In most cases the diagnosis is based on routine cytologic techniques,[16] the sensitivity of which varies between 50% and 78%.[26,44,52] However, in about 15% of cases more sophisticated techniques, especially immunocytochemistry, may be necessary to improve diagnostic preci-

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sion,[6,10,12,14,15,19,20,22,23,27,38,40,44,50] and for this purpose various antibody panels have been proposed.[11,13,28,34,49,51]

In our study we made up a panel with some markers the sensitivity and specificity of which had already been tested, including antibodies against antigens CEA, Ber-EP4, LeuM1 and p53 and lectins *Ulex europaeus* (UEA-1) and Concanavalin A (ConA). CEA has been extensively evaluated for its usefulness in distinguishing mesothelial cells from adenocarcinoma cells.[13,38,40]

Ber-EP4 is a monoclonal antibody that identifies 34- and 39-kd cell surface glycoproteins present in epithelial cells but not in mesothelial cells.[33] Leu M1 (CD15) reactivity has been identified in a variety of carcinomas, particularly lung cancer, while reactive mesothelial cells are negative.[13,50] Mutated forms of p53 accumulate in the nucleus, where they are more readily detectable by immunohistochemistry.[7] It may be useful in distinguishing benign from malignant cells in pleural effusion.[52] UEA-1 may help in distinguishing reactive mesothelial cells from adenocarcinoma cells in effusions.[45,48] ConA is a protein isolated from the jack bean. The distribution of this lectin's binding sites on unfixed cells in effusions can help to distinguish cancer cells from mesothelial cells.[36] *Canavalia brasiliensis* (ConBr) had not been used before for this purpose.

Materials and Methods

Thirty-six specimens, 18 pleural and 18 ascitic fluids, were selected. The available material consisted of smears stained by the Papanicolaou method and cell blocks stained with hematoxylin and eosin, together with the clinical records, histopathology reports (if any) and follow-up information.

On cytology we identified 18 adenocarcinomas, 14 benign cases and 4 cases suspicious for adenocarcinoma. The 36 specimens were tested against the panel of monoclonal antibodies and lectins listed above. We used the avidin-biotin complex (ABC) immunocytochemical method.

Of the 18 cases positive for adenocarcinoma, 15 were from women and 3 from men; their average age was 63.6 years. Ten metastatic adenocarcinomas of known primary origin were diagnosed by examination of pleural fluid: 5 pulmonary, 3 mammary, 1 ovarian and 1 of unknown origin. The other eight neoplasms, all adenocarcinomas of known primary origin, were identified in peritoneal fluid: four ovarian, one mammary and one colonic. Of the four specimens reported as suspicious, 3 were from patients who had a history of neoplasms: adenocarcinomas of lung, stomach and skin.

Cell block sections were placed on slides covered with saline, deparaffinized in xylene and dehydrated in ethanol. Before incubation with the primary antibodies and lectins, the sections underwent treatment with trypsin or microwaves in citrate-buffered solution, 0.01 M, pH 6.0, with the aim of antigen recovery. After overnight incubation with primary antibodies/lectin (anti-CEA rabbit antiserum [Ortho Diagnostic Systems, Carpinteria, California, U.S.A.], 1:800; epithelial antigen, mouse Ber-EP4, M08D4 [Dako, Glostrup, Denmark], 1:100; mouse granulocyte-associated antigen MO733 [Dako], 1:100; p53 protein M701 [Dako], 1:50; UEA-1 agglutinin I, L1060 [Vector Laboratories, Burlingame, California, U.S.A.], 1:800; *Canavalia ensiformis* (CE), 1:100; ConBr, 1:100), the sections reacted with secondary antibodies for 60 minutes. The antigen-antibody complex and lectins were localized using the ABC-diaminobenzidine-peroxidase method. The smears were cross-stained with hematoxylin or methyl-green and subsequently analyzed by two observers (C.Q. and C.E.B.) according to the reactivity model for each marker and were classified as positive or negative. The statistical calculation of sensitivity and specificity used the cytologic diagnosis from cell blocks as the standard.

UEA-1 can be useful in distingushing adenocarcinoma cells from reactive mesothelial cells.

Results

The CEA reaction was positive in 17 of the 18 adenocarcinomas and in 1 cytologically suspicious specimen, with the positivity cytoplasmic. CEA was not detected in mesothelial cells, but there was a positive reaction in the accompanying polymorphonuclear leukocytes due to the nonspecific reaction of the cytoplasmic granules.

The Ber-EP4 reaction was positive in 12 cases of adenocarcinoma, with the positivity (usually intense) predominantly in the cell membranes. Mesothelial cells gave a negative reaction.

The CD15 (LeuM1) reaction was positive in 11 cases of adenocarcinoma and was not positive in any benign case. Immunostaining for CD15 was

predominantly in the cell membranes of carcinoma cells.

p53 Was positive in 15 of the 36 cases examined. It was positive in 11 cases of adenocarcinoma, 1 negative specimen and 3 cytologically suspicious specimens. The immunostaining was nuclear. Mesothelial cells gave a negative reaction.

The UEA-1 reaction was positive in 12 of the 18 cases of adenocarcinoma. The carcinoma cells that reacted positively showed a cytoplasmic staining reaction. The staining for UEA-1 was negative in all benign cases. The ConA reaction (cytoplasmic) was positive in all cases, in both benign and malignant cells, with 100% sensitivity and 0% specificity. The ConBr reaction (cytoplasmic) was positive in all cases, in both benign and malignant cells, with 100% sensitivity and 0% specificity.

Discussion

Distinguishing reactive mesothelial cells from adenocarcinoma cells in serous fluid is a common problem. Despite the absence of a specific, reliable marker for mesothelial cells, the detection of glycoprotein epitopes Ber-Ep4 and LeuM1 has been shown to offer valuable support to conventional diagnostic techniques.[11,13,28,33,51]

Detection of CEA in cells seems to definitively exclude a mesothelial origin.[1,8,10,25,26,34,37,43,46,47,51] In our specimens CEA was positive in 1 of 4 cases reported as suspicious and negative in 1 case of ovarian cystadenocarcinoma (the only case among the 18 cases of adenocarcinoma that was negative for this marker), which agrees with previous studies.[1,20] This case was also negative for Ber-EP4, CD15, p53 protein and UEA-1. In 10 cases of serous adenocarcinoma of the ovaries, Khoury et al, after finding that only three reacted with LeuM1 and B72.3 and 40% stained for CEA, concluded that immunonegative results must be interpreted with much caution, especially in serous tumors.[31]

Sheibani et al[47] analyzed > 500 diffuse malignant mesotheliomas of epithelial type that were well characterized clinically and morphologically with various monoclonal and polyclonal antibodies reactive against oncofetal antigens, intermediate filaments, lectins and mucoid material. They found that LeuM1, CEA and Ber-EP4 were the most useful reactors for distinguishing between mesotheliomas and adenocarcinomas.[35] Hartman concluded that expression of CEA and LeuM1 by tumor cells virtually excludes mesothelioma.[28] In the cases studied by Gaffey, where 10 of 49 pleural and peritoneal mesotheliomas were positive for Ber-EP4, it was not clear if they were peritoneal or pleural tumors.[17]

Of our four cytologically suspicious cases stained for p53, three were positive. Follow-up of these patients showed that three had histologic or clinical evidence of cancer. These results suggest that the reaction for p53 is a good pointer to malignancy, indicating, perhaps, that in these cases the reactive cells have undergone malignant change but that they have not yet acquired the phenotypic changes detectable by conventional cytologic techniques. The reaction for p53 was positive in one of 14 cases reported cytologically as benign, in a 6-year-old girl with a diagnosis of acute hepatitis. Reactivity with p53 in benign cells has been reported.[11]

According to the literature, ConA is not a useful marker for distinguishing between mesothelial and adenocarcinoma cells.[5,9,45,49] ConBr showed identical immunocytochemical behavior. Despite the fact that these lectins belong to the same genus and have a high level of homology (80%/90%) in relation to their primary structures, they have shown differences in biologic behavior.[3,21] These differences can be explained by the different sites of affinity between the lectins and receptors that trigger biologic effects, but they are not enough to show a distinct immunocytochemical reaction.[18,39]

In considering a combination of antibodies containing at least one positive marker sensitive for adenocarcinoma, both CEA/CD15 and Ber-EP4/CD15 had 100% sensitivity. A positive reaction for CEA and/or CD15 has been shown to be the best immunocytochemical combination for adenocarcinoma, and a negative reaction for both would be the best indicator of mesothelioma.[24]

A panel made up of CEA, Ber-EP4 and LeuM1 is highly sensitive and extremely specific in detecting adenocarcinoma cells in cell blocks from serous effusions, resulting in a more accurate diagnosis. The failure of cytologically positive cases to react with one or more of these markers (one case for CEA, six for Ber-EP4 and seven for LeuM1) was probably due more to the absence of expression of the detectable molecules by these antibodies rather than to genetic abnormalities occurring in clones of metastatic cells.[13] Perhaps some sort of masking of the epitopes during fixation and processing could have reduced the immunoreactivity. We cannot exclude, however, the true absence of these antigens in adenocarcimona cells that did not express these markers.

Immunoreactivity for p53 can aid in detecting neoplastic cells and is a strong indicator of malignancy even in cytologically benign cases.[7,29,30,32]

UEA-1 can be useful in distinguishing adenocarcinoma cells[2] from reactive mesothelial cells.

The lectins ConA and ConBr did not show any specificity and were not capable of distinguishing neoplastic populations from reactive mesothelial cells. However, the use of ConBr in immunocytochemistry opens up an avenue of research, in the sense of trying to find other Brazilian lectins that could be used in characterizing tumors.

Conclusion

Distinguishing reactive mesothelial cells from adenocarcinoma cells is a common problem in the cytodiagnosis of serous fluids. Despite the current absence of a specific and reliable marker for mesothelial cells, the detection of glycoprotein epitopes, including CEA and those recognized by Ber-EP4 and LeuM1 antibodies, has been shown to offer valuable support to conventional diagnostic techniques.

Immunocytochemistry with a small panel of monoclonal antibodies, such as CEA, Ber-EP4 and LeuM1, can give significant help in the interpretation of serous effusions in day-to-day work, increasing diagnostic accuracy and being easy and economical to perform.

Our results indicate that UEA-1 can be useful in distinguishing adenocarcinoma cells from reactive mesothelial cells. The lectins ConA and ConBr did not show any specificity and were not capable of distinguishing neoplastic populations from reactive mesothelial cells.

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