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Usefulness of E-Cadherin Expression in Malignant Effusion

Sung-Jig Lim, M.D., Gou Young Kim, M.D., Youn Wha Kim, M.D.,
Yong-Koo Park, M.D., Juhie Lee, M.D., Moon Ho Yang, M.D., and Nam Hee Won, M.D.*

Department of Pathology, College of Medicine, Kyung Hee University,
College of Medicine, Korea University*, Seoul, Korea

The usefulness of E-cadherin immunostaining as a marker of malignancy in the body fluids was investigated in the present study. Thirty-three histologically proven cases of cell blocks from the pleural, peritoneal, and pericardial fluids were studied by immunocytochemistry for E-cadherin antibody using LSAB method. These cases were cytologically diagnosed as adenocarcinoma (25 cases) and atypical cells (8 cases). Tumor cells showed strong positive membranous staining for E-cadherin antibody in 21 out of 25 cases (84%) of adenocarcinoma. E-cadherin staining was not found in 6 of 8 cases of suspicious maligancy. The sensitivity and specificity were 84% and 75%, respectively. Reactive mesothelial cells and inflammatory cells scattered were all negative. In conclusion, E-cadherin is an useful adjunctive marker to distinguish reactive mesothelial cells from the carcinoma cells in the body fluids.

Key words: E-cadherin, Body fluids, Carcinoma, Reactive mesothelial cells, Immunocytochemistry

Introduction

Routine cytologic examination of body fluids is the preferred method for the detection of metastatic malignancy. But this method has a limitation in distinguishing adenocarcinoma from reactive mesothelial cells. Various morphometric features do not greatly assist in this matter since there is a considerable overlap between benign and malignant cells.[1,2] Histochemical stains may be helpful, however mucin production cannot be demonstrated in many adenocarcinomas.[3,4] Application of immunocytochemical techniques to the effusions using a number of different monoclonal and polyclonal antibodies provides significant improvement in this cytologic distinction. In general, immunocytochemical stain is more sensitive and specific than other methods such as histochemistry, electron microscopy

책임저자 : 이주희
주 소 : (130-702) 서울특별시 동대문구 회기동 1번지, 경희의료원 해부병리과.
전 화 : 02-958-8741
팩 스 : 02-957-0489
E-mail adress : Leejuhie@chollian.net

and flow cytometry.[5] It offers greater availability of commercial reagents for use. Keratin, CD15, and carcinoembryonic antigen(CEA) are frequently used markers for the epithelial malignancy.[6] However a single universal marker distinguishing between mesothelial cells and cancer cells has not yet to be identified.

E-cadherin, an epithelial specific homotypic adhesion protein, can be a marker with high sensitivity and specificity for the detection of carcinoma cells. It is a 120-KD transmembrane glycoprotein whose calcium sensitive homotypic adhesion is the primary stabilizing interaction in cell-cell adhesion and a signal for polarization and cell differentiation.[7,8] Cells of mesenchymal origin, like mesothelial cells, express not E-cadherin but a related cadherin called N-cadherin.[9] In our study, we evaluated the efficacy of E-cadherin expression to differentiate reactive mesothelial cells and malignant cells in the body fluids.

Materials and Methods

One hundred sixty six cases of the body fluids were selected for two years from 1989 to 1990 at the Kyung Hee Medical Center. The cytologic slides stained by Papanicolaou method were reviewed, and then classified as malignant, atypical, and reactive. The cases whether malignant or not cannot be determined are grouped into atypical. Thirty three cases of body fluid cytology were chosen by the presence of cell blocks and the tissue diagnoses. They included 13 peritoneal fluids, 18 pleural fluids, and 2 pericardial fluids. Cytologic diagnosis were 25 cases of adenocarcinomas and 8 cases of atypical cells.

Paraffin-embedded cell blocks were serially sectioned at 5 μm in thickness. Sections were deparaffinized in two changes of xylene (7 minutes each) and placed in a series of graded alcohols(from 100 to 70 %). To block endogenous peroxidase activity, the sections were placed in 5 mL of 3% hydrogen peroxide for 15 minutes. They were immersed with primary antibody, E-cadherin (Monoclonal mouse antibody, Transduction, USA) diluted at 1:300, for 1 hour at 37℃. Ordinary avidin-biotin complex(ABC) method was processed using labelled streptavidin-biotin(LSAB) kit(DAKO, USA). The sections were conuterstained with Mayer's hematoxylin.

Slides were interpreted as positive if any areas of the slide showed definite cytoplasmic or membranous staining. A weak cytoplasmic blush, just over the staining level of the background, was interpreted as negative. Many reactive mesothelial cells scattered in the cell block were used for negative internal controls.

Results

Table 1 shows the cytologic and histologic diagnoses with results of E-cadherin immunostaining in 33 cases. Twenty five cases were diagnosed as malignant, and 8 as atypical, suspicious for malignancy by conventional cytology. The E-cadherin immunohistochemical staining pattern was predominantly membranous, but some cases showed dotted pattern in the cytoplasm of malignant cells (Fig. 1 & 2). Many reactive mesothelial cells scattered were negative for E-cadherin as shown in Fig. 3. E-cadherin immunoreactivity was identified in 21 of 25 (84%) malignant cells, while 3 cases of metastatic adenocarcinoma from the stomach, pancreas, and prostate and a case of malignant lymphoma were negative on E-cadherin staining. As the primary sites of the metastatic adenocarcinoma, E-cadherin posi-

Table 1. Results of immunocytochemistry for E-cadherin in effusion with comparision to histologic diagnosis*

Cytologic diagnosis \ Histologic diagnosis	Adenocarcinoma		No malignant cells	
	E-cadherin		E-cadherin	
	+	−	+	−
Adenocarcinoma	21	3	0	0
Atypical cells	2	0	0	6

*A case of malignant lymphoma was excluded.
$p<0.05$, sensitivity; 84%, specificity; 75%

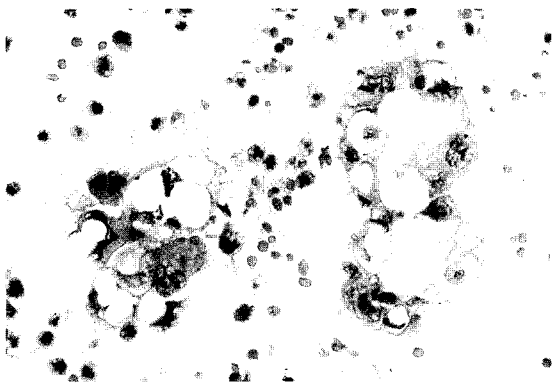

Fig. 1. Cytologic finding of adenocarcinoma from a pleural effusion of a patient with known lung tumor: A few cohesive clusters of malignant cells(H-E, ×400).

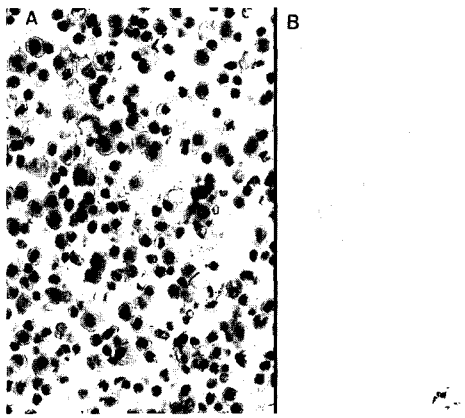

Fig. 3. Cytologic finding of reactive mesothelial hyperplasia: Many reactive mesothelial cells(A: H-E, x400) are negative for E-cadherin(B: Immunohistochemistry, ×400).

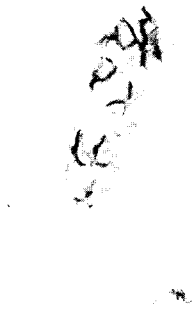

Fig. 2. Representative immunohistochemical staining of E-cadherin in lung adenocarcinoma from a pleural effusion cell block: Membranous and dotted cytoplasmic staining (Immunohistochemistry, ×400)

tive cells were derived from the lung (11/11), ovary (3/3), stomach (6/7), pancreas (2/3), and adenocarcinoma of unknown primary origin (1/1). One case of prostatic adenocarcinoma was negative. Among 8 cytologically atypical cases, 6 cases which were later proven to be benign lesions on the histologic examinations, were negative for E-cadherin. The other two atypical cases showed positive immunostaining for E-cadherin and were found to have gastric and pancreatic adenocarcinoma after the histologic confirmation.

E-cadherin immunopositivity was found both in 84% and in 25% of cytologically malignant and atypical cells, respectively. The sensitivity and specificity were 84% and 75%, respectively ($p<0.05$).

Discussion

It is known that the suppression of cadherin activity triggers tumor invasion and metastasis.[10] Well-differentiated tumors generally maintain homogenous strong expression of E-cadherin. Poorly differentiated tumors show altered expression; E-cadherin-positive and -negative cells can be mixed or scattered expression because they cannot form close contacts with each other.[10] E-cadherin is highly specific for epithelial cells. Although E-cadherin is lost in some tumors such as lobular carcinoma of the breast[11] and gastric signet ring cell carcinoma,[12] most well differentiated epithelial tumors maintain the expression of E-cadherin.

Many monoclonal and polyclonal antibodies are introduced for differential diagnosis of reactive mesothelial cells and cancer cells, which consist of keratin, CD15(Leu-M1), and CEA. Nearly all carcinomas are positive for keratin, but so are most mesothelial cells. CEA is more helpful to differentiate carcinoma cells from mesothelial cells because nearly all mesothelial cells are negative for CEA. However, only 50~67% of adenocarcinomas expressed this marker.[5] CD15 is also helpful because many carcinomas are stained with this marker but mesothelial cells are not.[6] But a single marker for the differentiation between carcinoma cells and mesothelial cells has not yet been defined.

Kevin et al.[6] reported E-cadherin expression of carcinoma cells in body fluids from the peritoneal, pleural, and pericardial cavity, with 86.5%(32/37) of sensitivity and 87.5% of specificity. Simsir et al.[7] also reported E-cadherin expression in pleural effusions in adenocarcinoma and reactive mesothelial cells. Adenocarcinoma and reactive mesothelial cells showed E-cadherin expression at 97% and 14%, respectively. The sensitivity and specificity in this present study were 84% and 75%, which were similar to the previous study.[6,7]

In this study, there were four cases of malignancies that did not stain with E-cadherin antibody. One case of those was malignant lymphoma, which was not expected to express E-cadherin. The other three cases were metastatic adenocarcinomas originated from the prostate, pancreas, and stomach, respectively. All three cases were poorly differentiated adenocarcinomas on histologic sections. The negative staining of E-cadherin expression in these latter cases can be explained by loss or alteration of E-cadherin gene.[6] We also compare the staining results of E-cadherin according to the primary sites of metastatic adenocarcinoma. Adenocarcinoma cases from the lung, ovary, stomach, and pancreas carcinomas showed positive staining for E-cadherin, but that from the prostate negativity. Our cases are too small in number to determine the organ predilection for E-cadherin expression.

There were two cytologically atypical cases showing positive E-cadherin staining. One was metastatic adenocarcinoma from the stomach, and the other from the pancreas. On retrospective review, the cytologic smears of two cases were less cellular and only a few malignant cells were scattered with minimal pleomorphism. It was very difficult to distinguish reactive mesothelial cells from malignant cells in the cytologic smear of the ascitic fluids. Six atypical cases, which were confirmed as benign after the histologic examinations, were all negative for E-cadherin staining. Therefore, E-cadherin staining might be helpful for the cytologic discri-

mination of atypical cells on hypocellular smear. Although a few poorly differentiated tumors lost the expression of E-cadherin, this study indicates that E-cadherin is a reliable adjunctive marker to distinguish reactive mesothelial cells from the carcinoma cells in the body fluids.

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= 국문초록 =

악성 삼출액에서 E-Cadherin 발현의 유용성

임 성 직 · 김 교 영 · 김 윤 화 · 박 용 구 · 이 주 희 · 양 문 호 · 원 남 희*

경희대학교 의과대학 병리학교실, 고려대학교 의과대학 병리학교실*

체강액 내의 악성 종양세포와 중피세포의 구분이 종종 어려우나 환자의 치료나 종양의 임상기 결정에 큰 영향을 미치기 때문에 정확히 감별하는 것이 매우 중요하다. E-cadherin은 상피세포에서 표현되는 유착단백질이다. 본 연구에서는 체강액의 세포학적 검사에서 악성세포의 표지자로서 E-cadherin의 유용성을 알아보기 위하여 세포검사후 조직검사로 진단을 확인한 33예를 대상으로 체강액으로부터 만든 세포 블록에 대하여 E-cadherin에 대한 면역세포화학염색을 시행하였다. 33 예의 세포학적 진단은 선암종 25예, 비정형세포 8예였다. 선암종으로 진단하였던 25예중 21예(84%)에서 E-cadherin 에 양성이었다. 비정형세포라고 진단하였던 8예중 6예에서 음성이었으며 양성으로 염색된 2예는 조직학적 검사로 전이성 암종임을 확인하였다. 반응성 중피세포나 염증세포는 모두 음성이었다. 민감도와 특이도는 각각 84%와 75%였다. 결론적으로 E-cadherin은 체강액에서 악성 종양세포와 반응성 중피세포와의 구분에 유용한 보조적인 표지자이다.