KAWASAKI MED. J. VOL. 13 : NO. 2, 91-94 (1987)

Brief Note

Epithelial Membrane Antigen in Normal Lung Tissue —A Tool for Identifying Alveolar Epithelial Lining

Accepted for Publication on July 11, 1987

Key words : immunohistochemistry — epithelial membrane antigen — lung tissue — type I pneumocyte — type II pneumocyte

Epithelial membrane antigen (EMA) was originally described as a human milk fat globule related antigen, considered to be present specifically in breast tissue.[1] Through extensive studies with antisera against this antigen, however, it has now been shown that the antigen is rather non-specific and that it constitutes a surface membrane component in a variety of epithelial cells.[2] Many tumor tissues have also been extensively examined for the presence of this antigen, and of course lung tumors have not been excluded.[3] In normal lung tissue, especially, in alveolar lining epithelial cells, the presence of such antigen has not been clearly demonstrated to date. Damage of alveolar epithelium seems to play an important role in promoting the interstitial and/or intra-alveolar fibrosing process of fibrosing lung diseases. Therefore, identification of alveolar epithelial cells is essential to elucidate its disease mechanism. Several antibody preparations such as those for surfactant apoproteins have been reported to identify type II epithelial cells(pneumocytes) while none has identified type I pneumocytes.[4—6]

In order to test whether EMA may be a good tool for identifying either type I or type II pneumocytes in the normal lung tissue, we immunohistochemically stained human lung tissue sections. Herein described are our preliminary results.

Two surgically resected human lungs from lung cancer cases were utilized for this study. Resected lungs were fixed by infusion through airways with a 10% buffered formalin. Non-neoplastic, normal-looking areas were sectioned, processed routinely and embedded in paraffin. Deparaffinized sections were treated with 0.1% trypsin to unmask the immunoreactive sites of antigens.[7] Then, they were stained immunohistochemically with mouse anti-human EMA (DAKO, Santa Barbara, California). The principles of our peroxidase-antiperoxidase method have been described elsewhere in detail.[8] For some sections, imidazole was used to intensify diaminobenzidine staining.[9]

Light-microscopic observation of these lung sections showed positive stainings along the luminal surface of bronchiolar and alveolar epithelial cells. In the alveolar linings, there were two types of positive cells, each differing in their morphology (Fig. 1). Epithelial cells of the first type had thin, slender and elongated cytoplasms which were partially and linearly stained in ordinary preparations but were strongly and diffusely stained in places in imidazole preparations. Alveolar walls cut tangentially in thick tissue sections made small alveolar pores (Kohn's pores) clearly discernible in the cells of this type (Fig. 2).

原　宏紀，山口昌江，真鍋俊明

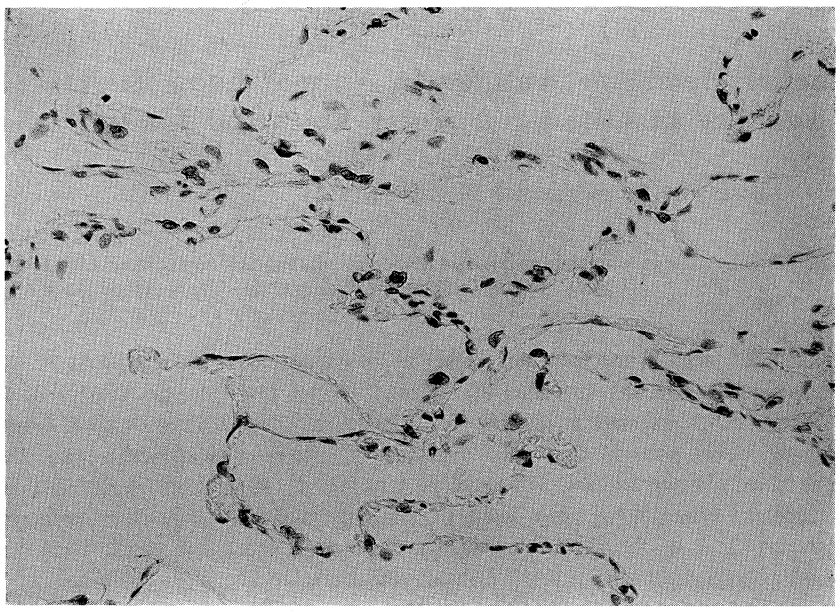

Fig. 1. Alveolar epithelia showing immunoreactivity for EMA. Note that two types of cells are discernible. Immunoreactive plump round cells represent type II pneumocytes and linear immunoreactivity represents the cytoplasm of type I pneumocytes. (Immunoperoxidase-hematoxylin for EMA, ×250)

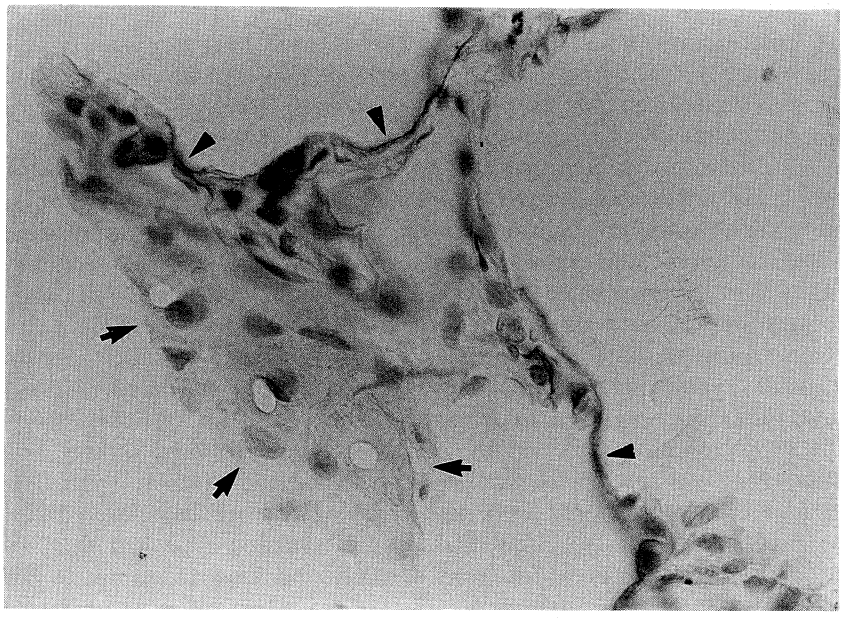

Fig. 2. A tangentially cut alveolar wall. The imidazole preparation intensifies the stainability of type I pneumocytes. Arrowheads indicate a cross section of type I pneumocytes and arrows indicate their en face view. Note the presence of Kohn's pores. (Immunoperoxidase-hematoxylin for EMA, ×500)

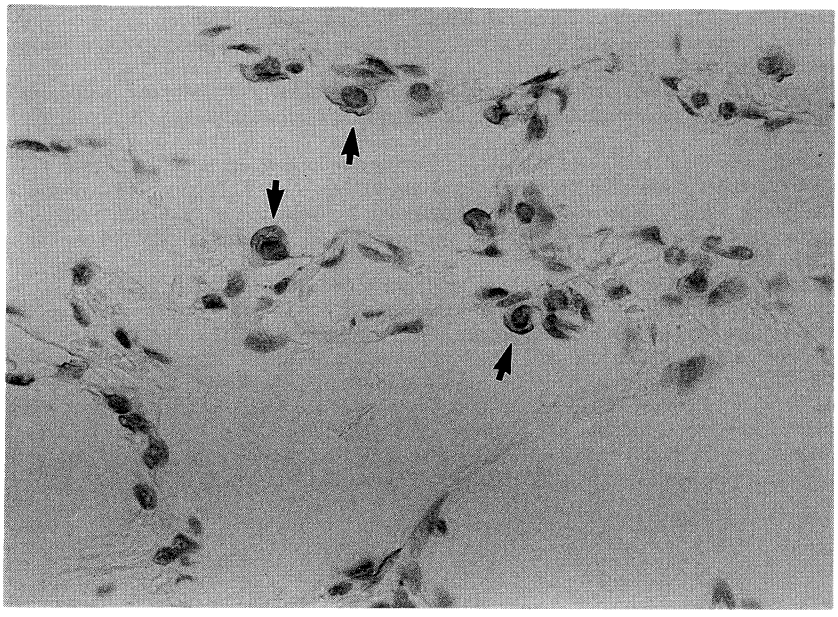

Fig. 3. Type II pneumocytes (arrows). Their luminal surfaces over the nuclei are strongly immunoreactive. (Immunoperoxidase-hemaoxylin for EMA, ×500)

On the other hand, epithelial cells of the second type had round cytoplasms with plump nuclei protruding toward the lumen. They were strongly immunoreactive with the antibody, especially along the luminal surface over the nuclei (Fig. 3). Morphologically, epithelial cells of the first type probably correspond to type I pneumocytes and those of the second to type II pneumocytes.

It is considered, therefore, that both type I and type II cells may express EMA normally. The amount contained in type II cells is much larger than that in type I cells. This might be the reason why only type II cells were stained by the ordinary immunoperoxidase method, and the stainability of type I cells was intensified by the imidazole preparation.

From this study, we have concluded that EMA may be a useful tool for detecting the presence or absence of alveolar epithelium in cases of fibrosing lung disease. When only type II cells are sought, the ordinary procedures of the immunoperoxidase method can be used. Intensification by imidazole may be applied when both type I and type II cells are sought.

Hiroki HARA, Masae YAMAGUCHI and Toshiaki MANABE

Department of Human Pathology II,
Kawasaki Medical School,
Kurashiki 701-01, Japan

REFERENCES

1) Ceriani, R.L., Thompson, K., Peterson, J.A. and Abraham, S.: Surface differentiation antigens of human mammary epithelial cells carried on the human milk fat globule. Proc. Natl. Acad. Sci. USA 74 : 582–586, 1977

2) Heyderman, E., Steele, K. and Ormerod, M.G.: A new antigen on the epithelial membrane: its immunoperoxidase localization in normal and neoplastic tissue. J. Clin. Pathol. **32** : 35-39, 1979
3) Sloane, J.P. and Ormerod, M.G.: Distribution of epithelial membrane antigen in normal and neoplastic tissues and its value in diagnostic tumor pathology. Cancer **47** : 1786-1795, 1981
4) Kuroki, Y., Dempo, K. and Akino, T.: Immunohistochemical study of human pulmonary surfactant apoproteins with monoclonal antibodies. Am. J. Pathol. **124** : 25-33, 1986
5) Nathrath, W.B.J., Rowlatt, C. and Ketheridge, F.: Localization of organ-specific antigens in mouse lung by light and electron microscopy. J. Histochem. Cytochem. **29** : 1365-1371, 1981
6) Funkhouser, J.D., Cheshire, L.B., Ferrara, T.B. and Peterson, R.D.A.: Monoclonal antibody identification of a type II alveolar epithelial cell antigen and expression of the antigen during lung development. Dev. Biol. **119** : 190-198, 1987
7) Huang, S., Minassiam, H. and More, S.D.: Application of immunofluorescent staining on paraffin sections improved by trypsin digestion. Lab. Invest. **35** : 383-390, 1976
8) Manabe, T., Ikeda, H., Moriya, T. and Yamashita, K.: Immunohistochemical localization of the secretory products of rat Clara cells. Anat. Rec. **217** : 164-171, 1987
9) Straus, W.: Imidazole increases the sensitivity of the cytochemical reaction for peroxidase with diaminobenzidine at a neutral pH. J. Histochem. Cytochem. **30** : 491-493, 1982