

Immunohistochemical Investigation of Nasopharyngeal Carcinoma Using Keratin, EMA, Laminin, Fibronectin, Collagen Type IV, Laminin Receptor, and Laminin/Collagen Receptor Antibodies

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Immunohistochemical investigation was carried out to examine the characteristics of nasopharyngeal carcinoma (NPC) using 12 biopsy cases obtained from southern China. These cases were divided into three groups according to their predominant pattern associated with the cell and the tissue differentiation using the World Health Organization (WHO) classification as follows: (i) 4 cases with squamous cell carcinoma, (ii) 4 cases with differentiated nonkeratinizing carcinoma, (iii) 4 cases with undifferentiated carcinoma. These three types of NPC cases were reacted with keratin and epithelial membrane antigen (EMA). All neoplastic tissues were stained with keratin stronger than EMA. Squamous cell carcinoma and differentiated non-keratinizing carcinoma cases were diffusely stained strongly positive by immunoperoxidase for keratin. On the other hand, most of undifferentiated carcinoma cases were weakly or negatively reacted with keratin immunohistochemistry, and a part of tumor cytoplasm was stained strongly positive by keratin immunoreaction. For EMA immunoperoxidase method, squamous cell carcinoma cases were diffusely positive and most of differentiated non-keratinizing carcinoma and undifferentiated carcinoma cases were partly positive. However, none of NPC cases reacted with immunoreactivity for laminin 200, laminin 400, fibronectin, collagen, laminin receptor, and laminin/collagen receptor. Therefore, immunohistochemical characteristics of NPC showed epithelial cell origin, arising from squamous cells in nasopharynx.

Introduction

Nasopharyngeal carcinoma (NPC) arises in the surface epithelium of the posterior nasopharynx, characterized by anaplastic cells surrounded by prominent infiltration of lymphoid cells, known as lymphoepithelioma. The nuclear features of the carcinoma cells are distinctive with large nucleus and prominent nucleolus. NPC have been confirmed as a tumor occurring from squamous epithelium by electron microscopical and immunohistochemical examination. According to the World Health Organization (WHO) classification, NPC was divided into three types; squamous cell carcinoma, differentiated non-keratinizing carcinoma, and undifferentiated carcinoma.¹²⁾ Squamous

cell carcinoma type of NPC showed squamous differentiation with the presence of intracellular bridges and/or keratinization over most of its extent. Differentiated non-keratinizing carcinoma type of NPC showed stratified of paved and non-syncytial appearance. Undifferentiated carcinoma of NPC exhibited syncytial rather than paved appearance. Undifferentiated NPC may resemble large cell or immunoblastic types of malignant lymphoma and may be distinguished from them by the cohesiveness of the tumor cells and by their positive immunoreactivity for keratin and negative reaction for leukocyte common antigen (LCA).¹²⁾

In this study, immunohistochemical characteristics of NPC were reported using biopsy cases obtained from southern China. This investigation was undertaken as an extension of our previous work.¹⁷⁾ The authors also described keratin and EMA staining patterns in NPC.

Materials and Methods

The NPC specimens from 12 biopsy cases at the Jinan University Hospital in southern China were used. Shanmugaratnam and Sobin criteria were applied in assigning the diagnosis of NPC to the tissue. These NPC cases were divided into three groups as follows: (1) squamous cell carcinoma type, (2) differentiated non-keratinizing carcinoma type, (3) undifferentiated carcinoma type.

The specimens were fixed in 10% formalin, and embedded in paraffin for histochemical and immunohistochemical studies. Sections were cut at 4 micron and stained with hematoxylin and eosin stain for histological examination. These specimens were stained by indirect peroxidase-antiperoxidase (PAP) method for keratin (polyclonal antibody; Dako corp., U. S. A.; No. A575; Lot. 020-f), EMA (monoclonal antibody; Dako corp., U. S. A.; No. M613; Lot. 010B), laminin 200 (monoclonal antibody; Chemicon, U. S. A.; Lot. 175TCP2), laminin 400 (monoclonal antibody; Chemicon, U. S. A.; Lot. 310TCP5), fibronectin (monoclonal antibody; Chemicon, U.S.A.; Lot. 254CCP3), collagen type IV (monoclonal antibody; Chemicon, U. S. A.; Lot. 259SCL11), laminin receptor (mono-

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clonal antibody; Chemicon, U. S. A.; Lot. 167CCL), laminin/collagen receptor (monoclonal antibody; Chemicon, U. S. A.; Lot. 129CCB2). These antibodies were diluted 1:50 with PBS pH 7.4. The indirect PAP method was performed for the staining of keratin using Dako universal kit polyclonal antibody (Dako corp., PAP kit K548, Lot. 112) and for the staining of EMA, laminin 200, laminin 400, fibronectin, collagen type IV, laminin receptor, and laminin/collagen receptor using Dako universal kit for monoclonal antibody (Dako corp., PAP kit K550, Lot. 072-6).

The steps involved in the immunoperoxidase procedure are as follows: (1) Deparaffinize and hydrate in distilled water. (2) Treat with hydrogen peroxidase for 5 minutes. (3) Wash in Tris buffer pH 7.4 using three cycle changes of 3 minutes each. (4) Treat with normal serum for 20 minutes. (5) Treat with blot excess normal serum from sections. (6) Treat with primary antibody for 3 hours in room temperature. (7) Wash in Tris buffer pH 7.4 using three cycle changes of 5 minutes. (8) Treat with second antibody for 40 minutes in room temperature. (9) Wash in Tris buffer pH 7.4 using three cycle changes of 5 minutes. (10) Treat with PAP for 40 minutes in room temperature. (11) Wash in Tris buffer pH 7.4 using three cycle changes of 5 minutes. (12) Treat with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) solution with hydrogen peroxidase for 5 minutes. (13) Wash in running water. (14) Nuclei stain in Mayer's hematoxylin for 2 minutes. (15) Wash in running water. (16) Dehydrate, clear, and mount.

Results

The results of immunohistochemistry for keratin, EMA, laminin 200, laminin 400, fibronectin, collagen type IV, laminin receptor, laminin/collagen receptor were summarized in Table 1. All cases of squamous cell carcinoma type, differentiated non-keratinizing carcinoma type, and undifferentiated carcinoma type reacted with keratin and EMA by immunoperoxidase method. All cases of tumor cells were stained with keratin stronger than EMA. Most of squamous cell carcinoma cases and differentiated non-keratinizing carcinoma cases were diffusely stained strong-

ly positive for keratin by immunoreactivity. For keratin immunoperoxidase method, most of undifferentiated carcinoma cases were stained weakly positive and partly strongly positive. Most of squamous cell carcinoma cases were diffusely reacted moderately positive for EMA by immunohistochemical method. By immunoreactivity for EMA, most of the differentiated non-keratinizing carcinoma cases and undifferentiated carcinoma cases were strongly positive. Of the 4 cases, one (25%) of differentiated non-keratinizing carcinoma type showed diffusely strongly positive. On the other hand, squamous cell carcinoma cases, differentiated non-keratinizing carcinoma cases, and undifferentiated carcinoma cases did not react with immunoreactivity for laminin 200, laminin 400, fibronectin, collagen IV, laminin receptor, and laminin/collagen receptor.

Discussion

NPC cases obtained from southern China were analyzed by immunoperoxidase method employing various antibodies. All cases of NPC reacted with keratin, and EMA. The immunohistochemistry of keratin antibodies of various molecular weights have proven useful in the diagnosis of all normal epithelia, and can also be demonstrated with various epithelia lineage of neoplasms, including NPC.^{8, 10, 13, 14, 15} Monoclonal antibodies to EMA have been reported as being of diagnostic value in the recognition of epithelia lineage in tumor cells. In our study, NPC tissues were shown to be of epithelial lineage, because their cytoplasm reacted with keratin and EMA by immunohistochemical procedure. NPC is a definite malignancy of epithelial origin and can be distinguished from malignant lymphoma by immunohistochemical method for keratin, EMA, and leucocyte common antigen (LCA). LCA is present in lymphocytes, thymocytes, granulocytes, and monocytes. However, LCA is not present in other tissues. Malignant lymphoma and leukemia can be distinguished from non-hematopoietic neoplasms by using immunohistochemistry of LCA. Keratin and LCA antibodies are well known useful immunohistochemistry in the differential diagnosis of anaplastic lymphomas.¹⁰ Usually, LCA anti-

Table 1. Immunoreactivity for keratine, EMA, laminin 200, laminin 400, fibronectin, collagen, laminin receptor, laminin/collagen receptor

Histological type	Case	Immunohistochemical reactivity							
		keratin	EMA	laminin 200	laminin 400	fibronectin	collagen	laminin receptor	laminin/collagen receptor
Squamous cell carcinoma	4	+	+	-	-	-	-	-	-
Nonkeratinizing carcinoma	4	+	+	-	-	-	-	-	-
Undifferentiated carcinoma	4	+	+	-	-	-	-	-	-

EMA: Epithelial membrane antigen, +: positive, -: negative

body for lymphomas and keratin antibody for carcinomas are first used. However, a case of undifferentiated malignant lymphoma of stomach was reported as LCA negative and keratin positive, and also LCA negative cases were described in Ki-1 positive large cell lymphoma which were often expressed neither MB-1 nor MT-1 antibodies.^{2,3)} Initially, EMA was expressed only in epithelial cells, and EMA antibodies were used as the diagnostic tool in differentiating anaplastic carcinoma from malignant lymphoma. Later, EMA was also expressed in some non-Hodgkin's lymphomas, particularly T-cell lymphomas, and anaplastic large cell lymphomas associated with Ki-1 antigen.^{2,3)} Although histochemical diagnosis of NPC is often difficult to differentiate from malignant lymphoma up to the present, immunohistochemical method may distinguished NPC from malignant lymphoma.

To understand the role of integrins in tumor growth and metastasis, thus, laminin, fibronectin, collagen type IV, laminin receptor, laminin/collagen receptor studies are important and these substances are distributed in a variety of human malignancies.^{4-9,11)} Extracellular matrices are composed of macromolecules that include fibronectin, laminin, collagen, and proteoglycans. It was found that extracellular

matrix has focused on the interaction of tumor proliferation, invasion, and metastasis.^{5,6,16)} The metastasizing tumor cells must interact with the matrix at many stages of tumor proliferation, invasion, and metastasis. The interaction of cells with the extracellular matrix is mediated by the surface receptors. These receptors for fibronectin, collagen type I, and laminin have been identified. The basement membranes are the important extracellular matrix structures that play key roles in neoplastic proliferation, invasion, and metastasis.^{5,6,16)} The authors investigated integrins in NPC cells, because NPC is often metastasis in the lymph nodes. However, in this study, these integrins did not react with NPC cells. Therefore, it is suggested that these findings are somewhat cell-type specific.

Acknowledgements

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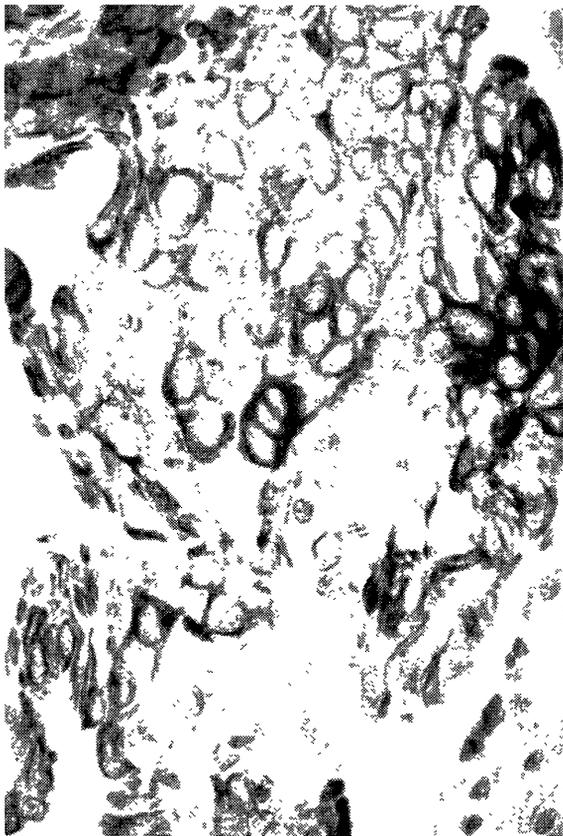


Fig. 1. Immunoreaction for keratin in differentiated non-keratinizing carcinoma type of nasopharyngeal carcinoma. The cytoplasm strongly reacted with keratin antibody. Keratin, X 200.

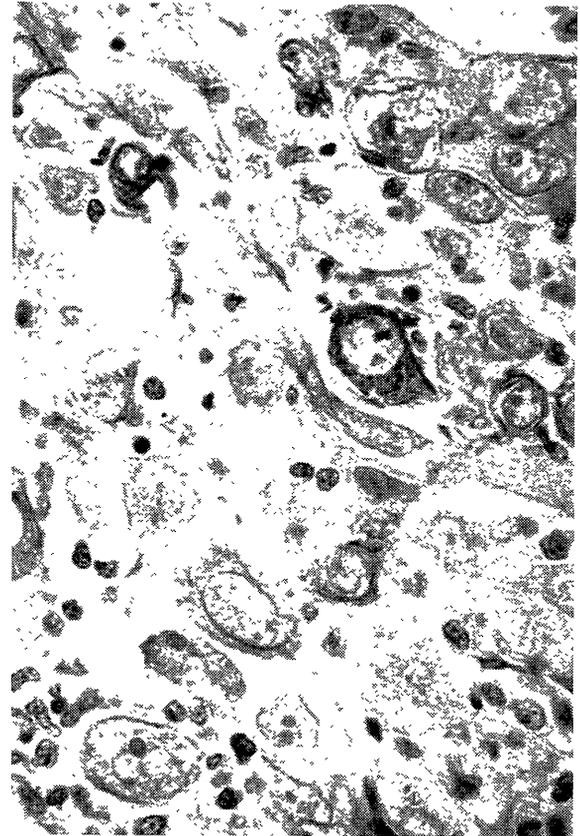


Fig. 2. Immunoreaction for keratin in undifferentiated carcinoma type of nasopharyngeal carcinoma. Several cytoplasmic areas were strongly stained with keratin antibody. Keratin, X 200.

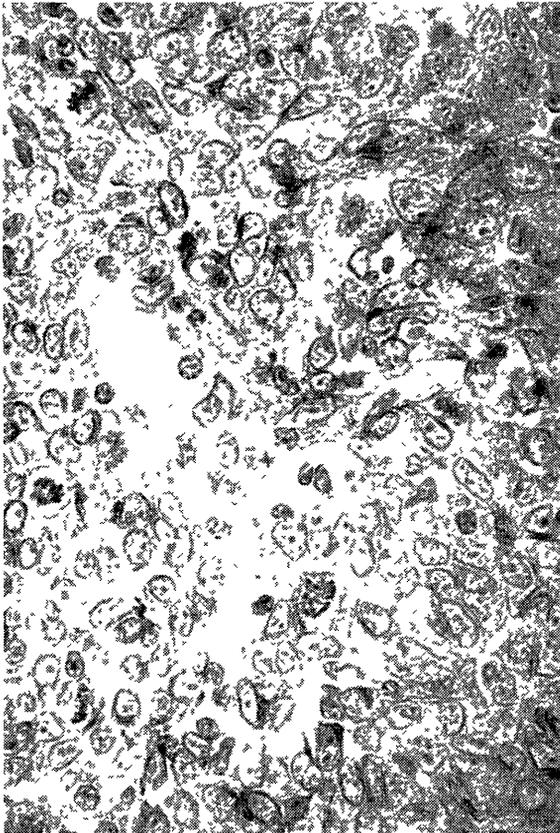


Fig. 3. Immunoreaction for EMA in differentiated non-keratinizing carcinoma type of nasopharyngeal carcinoma. The cytoplasm of the cells was moderately stained with EMA antibody. EMA, X 200

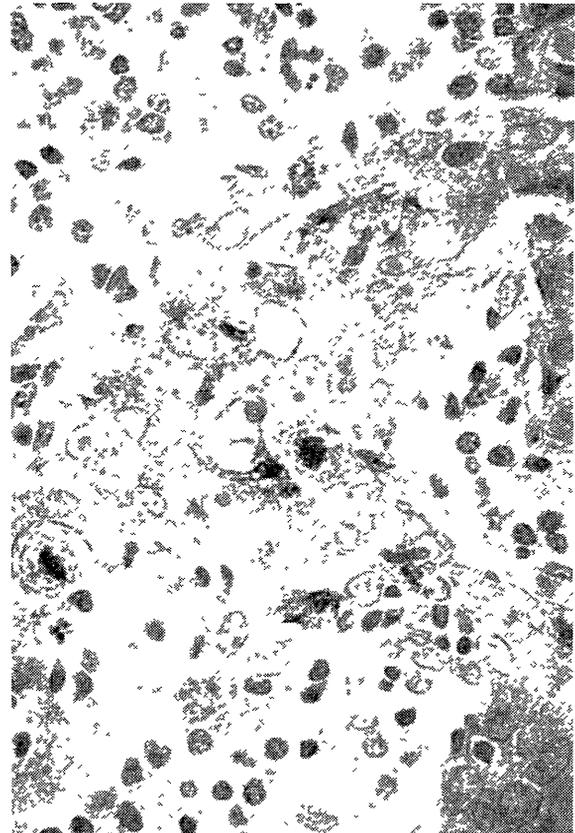


Fig. 4. Immunoreaction for EMA in undifferentiated carcinoma type of nasopharyngeal carcinoma. Some cytoplasmic areas were strongly stained with EMA antibody. EMA, X 200

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