Immunohistochemical Analysis of Human Pulmonary Carcinomas Using Monoclonal Antibody 44-3A6¹

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ABSTRACT

A monoclonal antibody, 44-3A6, was raised against the human pulmonary adenocarcinoma cell line A549. This antibody recognizes a protein antigen at the cell surface, which is preserved after formalin fixation and paraffin embedding. Immunohistochemical staining of lung tissue with this antibody revealed diffuse immunoreactivity of type II pneumocytes. Bronchial epithelial cells were also focally immunoreactive. Immunostaining of various bronchopulmonary carcinomas demonstrated characteristic patterns of reactivity. All of the 42 adenocarcinomas and 18 carcinoids were strongly immunoreactive either diffusely or focally. The immunoreaction occurred at the cell membrane and/or in the cytoplasm. None of the 39 squamous cell carcinomas, 12 bronchioalveolar carcinomas, and 30 small cell neuroendocrine carcinomas was immunostained. Ten intermediate cell neuroendocrine carcinomas and 8 well-differentiated neuroendocrine carcinomas were focally immunoreactive, while 7 and 2 of them were negative. Six adenosquamous carcinomas were focally positive in glandular and “basaloid” areas, whereas squamous areas were negative. Twenty-one large cell carcinomas were focally immunoreactive, while 6 were negative. It appears that MCA 44-3A6 is an effective marker for certain features of “glandular” differentiation, which may be present even in tumors lacking obvious glands, and that it may be useful for the differential diagnosis of various bronchopulmonary carcinomas.

INTRODUCTION

The increasing clinical and epidemiological significance of bronchopulmonary carcinomas is a matter of serious concern (1). Pathologically, bronchopulmonary neoplasms encompass several types: SCC; AC; ASC; LCC; BAC; SCNC; ICNC; WDNC; carcinoid; and other rare entities such as mucoepidermoid carcinoma, adenoid cystic carcinoma, and carcinosarcoma (2-5).

The classification of bronchopulmonary neoplasms on the basis of conventional morphology is difficult, because the pattern of differentiation of individual neoplasms may vary markedly, and the diagnostic criteria may vary considerably among pathologists. Moreover, one may see carcinomas with variable admixtures of SCC, AC, and NE carcinoma differentiation, etc. There are also “poorly differentiated carcinomas,” which by conventional light microscopy do not express a definite pattern of differentiation; these are usually classified as LCCs. Recent studies have suggested that “LCCs” may include poorly differentiated ACs and various NE carcinomas (6, 7).

There have been a number of attempts to establish simple and objective differential diagnostic methods of bronchopulmonary neoplasms using phenotypic markers. It was demonstrated that a significant proportion of the LCCs are in fact NE carcinomas, mostly ICNCs, which express one or more NE markers including NSE, serotonin, and various neuropeptides (4, 5, 7). It was also demonstrated that SCCs and ACs express different types of cytokeratin polypeptides (6, 8, 9). Preliminary studies revealed that LCCs include at least two different populations of neoplasms which demonstrate either an SCC-type cytokeratin pattern or an AC-type cytokeratin pattern (6). Also, MCAs were raised against several SCNC cell lines (10). They immunoreact with a specific sugar sequence in lacto-N-fucopentaose, which is present in various human tissues and neoplasms (11). Preliminary results revealed that those antibodies also immunoreact with certain bronchopulmonary carcinomas (10).

Recently, a MCA, 44-3A6, was developed against a human AC cell line, A549 (12). In this article, we describe the characteristic patterns of immunoreactivity of various human bronchopulmonary neoplasms to MCA 44-3A6. Our observations suggest that this MCA may be a useful marker for certain aspects of “glandular” differentiation of bronchopulmonary neoplasms and could thus be helpful in differential diagnosis.

MATERIALS AND METHODS

MCA 44-3A6 was produced using the well-defined hybridoma technology (13); details of the procedure have been described elsewhere (12).

For immunohistochemical staining, 7-μm sections of tissue were prepared. Following routine deparaffinization of tissue sections, immunohistochemical staining was performed with the avidin:biotin complex method (Vector Laboratories, Burlingame, CA) as previously described (14). MCA 44-3A6 was used with a concentration of about 1 μg/ml. The slides were then counterstained with hematoxylin for 1.5 min, dehydrated, and conventionally mounted. Negative controls were performed by omitting the primary antibody and substituting nonimmune serum.

Samples of primary bronchopulmonary neoplasms were formalin fixed and paraffin embedded; they were obtained from the surgical pathology files of the Rush-Presbyterian-St. Luke’s Medical Center and VA Lakeside Medical Center, Chicago, from 1979 to 1984. They included 42 ACs, 39 SCCs, 6 ASCs, 12 BACs, 27 LCCs, 18 carcinoids, 10 WDNCs, 17 ICNCs, and 30 SCNCs. Also cases of organizing pneumonia were included to study the pattern of immuno-
staining of the reactive pneumocytes. No metastatic neoplasms were studied.

The non-NE bronchopulmonary neoplasms were assessed according to the classification of the WHO and Fascicle 17 of the Armed Forces Institute of Pathology (2, 3). The ACs were defined as malignant neoplasms with definite glandular differentiation, at least focally, which might produce mucin. The BACs were defined as primary bronchopulmonary carcinomas with relatively bland cytological features which tend to grow along the alveolar septa, preserve the alveolar architecture, and often produce mucin abundantly. The SCCs were defined as primary bronchopulmonary carcinomas with individual cell keratinization, pearl formation, or extensive intercellular bridges. The LCCs were poorly differentiated carcinomas without any clear glandular, squamous, or NE differentiation by conventional microscopy. The ASCs consist of variable admixtures of malignantpolygonal cells with focal but definite glandular and squamous differentiation. They may express NE markers by immunohistochemistry. They tend to have prominent peripheral nuclear palisading, either focal or diffuse. Clusters of solidly arranged small and intermediate cells could be seen.

The NE neoplasms were assessed by the classification of Gould et al. (4, 5); their NE differentiation was demonstrated by various NE marker immunohistochemistry and/or electron microscopy. The NE markers included NSE, serotonin, bombesin, leu-enkephalin, calcitonin, somatostatin, VIP, substance P, ACTH, and MSH. All of the NE neoplasms revealed one or more of these NE markers. Carcinoids were defined as NE neoplasms with classic "solid nests and ribbon" pattern of cells without significant cellular pleomorphism or mitotic activity.

**RESULTS**

The MCA 44-3A6-associated antigen was well preserved after routine formalin fixation and paraffin embedding; contrast between immunoreactive and negative cells by immunohistochemistry was readily apparent.

Type II pneumocytes were immunoreactive, while type I pneumocytes were negative (Fig. 1). The immunoreactivity was evidently stronger in areas adjacent to inflammatory lesions or at the periphery of tumors. Bronchial epithelial cells, distributed irregularly from the large bronchi to respiratory bronchioles, were also immunoreactive.

All of the 42 ACs examined were strongly immunoreactive either diffusely or focally (Fig. 2). The connective tissue stroma and the nuclei of the neoplastic cells were not immunostained. The immunoreaction occurred at the cell membrane and/or in the cytoplasm. The cells at the periphery of the tumor often showed stronger immunoreactivity. Some of the ACs had focal mucin production; in these areas, immunostaining was weak or altogether absent.

None of the SCCs examined was immunoreactive (Fig. 3); the BACs and the adenoid cystic carcinoma were also negative (Fig. 4). Twenty-one of 27 LCCs showed focal but convincing immunoreactivity. The latter was largely confined to the large cells with abundant clear cytoplasm and poor cohesion to adjacent cells (Fig. 5); the intensity of the immunoreaction varied widely. Six cases of LCC were not at all immunoreactive. All of the ASCs showed focal but strong immunoreactivity. The immunoreactivity was confined to the glandular and/or "basaloid" areas. The squamoid areas were not immunoreactive (Fig. 6).

The NE neoplasms showed a wide spectrum of staining patterns. None of the 30 SCNCs examined was immunoreactive (Fig. 7). Ten of 17 ICNCs and 8 of 10 WDNCs showed areas of weak immunoreactivity (Figs. 8 and 9). All of the carcinoids were immunoreactive; the immunoreaction was often focal but strong (Fig. 10).

**DISCUSSION**

Using immunohistochemistry with MCA 44-3A6, we have studied the patterns of immunoreaction of various bronchopulmonary carcinomas. MCA 44-3A6 was raised against human AC cell line A549. The MCA 44-3A6 antigen appears to be related to cell membranes. Although the precise role of the MCA 44-3A6 antigen is not clear, it may be related to certain "glandular" or "secretory activity related" features of differentiation.

Various types of lung carcinomas demonstrated distinct and characteristic patterns of immunoreactivity. All of the ACs were strongly immunoreactive either diffusely or focally, whereas all of the SCCs were negative. The 6 ASCs revealed focal immunoreactivity at the glandular and/or basaloid areas, while the squamous areas were not immunoreactive.

It is of interest that the majority of the LCCs showed focal but obvious immunoreactivity; this may indicate that the LCCs comprise heterogeneous tumor cell populations, and that a subset of them may in fact represent poorly differentiated ACs. This notion parallels the results of previous studies using other differentiation markers such as cytokeratins and/or NE markers. It was demonstrated that diverse types of bronchopulmonary carcinoma express characteristic cytokeratin polypeptides as their intermediate filament cytoskeletal component (6, 8, 9). ACs and NE carcinomas express "simple epithelium-type cytokeratins" such as polypeptides 7, 8, 18, and 19, whereas SCCs express "stratified epithelium-type cytokeratins" such as polypeptides 4, 5, 6, 13, 14, 15, and 17. It was suggested that the LCCs are composed of at least 2 different subsets of neoplasms which express either an SCC-type cytokeratin pattern or an AC-type cytokeratin pattern (6).

It has also been demonstrated that about 40% of the LCCs express NE markers including NSE, serotonin, and one or more neuropeptides such as bombesin, calcitonin, leu-enkephalin, VIP, somatostatin, substance P, ACTH, and MSH (7). It was indeed suggested that bronchopulmonary carcinomas with basaloid nuclear palisading should be considered as possible NE carcinomas, and systematic immunohistochemical study of these neoplasms for NE markers was recommended.

The family of bronchopulmonary NE neoplasms encompasses a wide spectrum of pathologically and clinically distinct entities (4, 5). It is of interest that the immunoreactivity of NE neoplasms to 44-3A6 showed a spectrum of intensity which roughly correlates inversely with their clinical behavior and pathological grade of differentiation. All of the SCNCs were negative, while all of the carcinoids were strongly immunoreactive either focally or diffusely; and, not surprisingly, the immunoreactivity of ICNC and WDNC fell between the extremes of the spectrum. This indicates that the NE neoplasms tend to lose their glandular features of differentiation as they approach the least differentiated morphologically and clinically most aggressive end of the spectrum. In this context, it should be stressed that exocrine and endocrine differentiation may readily coexist in pulmonary and other neoplasms, and this coexistence may have important implications for our understanding of the histogenesis of these neoplasms (15).

It was also interesting that all of the BACs did not show any
immunoreactivity. Moreover, even ACs with focal mucin production did not express any immunoreactivity in the mucin-producing areas. It appears, therefore, that the 44-3A6 antigen is either not expressed or becomes "masked" in foci of marked mucosubstance production. BAC is one of the controversial entities within bronchopulmonary carcinomas. The morphological diagnostic criteria of BAC vary especially widely among different pathologists. MCA 44-3A6 might thus be used as a differential diagnostic tool for BAC.

MCA 44-3A6 appears to be a promising adjunct tool for the differential diagnosis of pulmonary carcinomas, particularly when used in conjunction with other phenotypic markers such as cytokeratin-polypeptides, NE markers, and/or other possible new MCAs against different types of carcinomas in the future. Areas in which application of 44-3A6 might prove useful include the identification of subsets of apparently undifferentiated LCCs, discrimination between ACs and BACs, discrimination between pulmonary ACs and pleural mesotheliomas, and the monitoring of emerging AC features of differentiation as the phenotypic characteristics of non-ACs may be altered by longevity or therapy. The encouraging results obtained when MCA 44-3A6 is applied to conventional cytological specimens may further facilitate and broaden its usefulness (16).

REFERENCES

Fig. 1. Immunohistochemical staining of lung tissue near the tumor with MCA 44-3A6; notice strongly immunoreactive type II pneumocytes and clear fibrotic alveolar septa. × 150.

Fig. 2. AC with diffuse and strong immunoreaction; notice the immunoreactivity is at the cell membrane and/or in the cytoplasm. × 400.

Fig. 3. SCC is not immunoreactive. × 400.

Fig. 4. BAC is not immunoreactive. × 400.

Fig. 5. LCC with focal, convincing immunoreactivity. × 400.

Fig. 6. ASC with focal immunoreactivity; the immunoreactivity is confined to the glandular and "basaloid" areas, while the squamous area is negative. × 150.
Fig. 7. SCNC is not immunoreactive; notice small cells with hyperchromatic nuclei, scanty cytoplasm, and numerous mitoses. × 400.

Fig. 8. ICNC with focal immunoreactive cells; at identical magnification, the neoplastic cells are evidently larger than those in Fig. 7. × 400.

Fig. 9. WDNC with relatively weak immunoreactivity; the neoplastic cells are in "organoid" clusters, but they are evidently pleomorphic. × 400.

Fig. 10. Carcinoid with diffuse and strong immunoreactivity. × 400.
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