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# Expression of the Antigenic Determinant Recognized by the Monoclonal Antibody 44-3A6 on Select Human Adenocarcinomas and Normal Human Tissues

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**Abstract.** The $IgG_1$ monoclonal antibody, 44-3A6, was raised against the human lung adenocarcinoma cell line, A549. It has been shown to react with a 40,000 MW protein found on the cell surface, which is preserved in formalin-fixed paraffin-embedded tissues. A recent study of pulmonary carcinomas utilizing immunohistochemical methods showed exclusive binding to lung adenocarcinomas, subsets of neuroendocrine tumors, some carcinoids and a subset of large cell carcinomas. Reactivity was not seen in squamous cell carcinomas and small cell neuroendocrine carcinomas. In addition, melanomas, sarcomas and hematologic malignancies do not express this antigen. We now report on the reactivity pattern of 44-3A6 in adenocarcinomas of nonpulmonary primary sites and in normal adult organs. Strong diffuse staining of neoplastic cells in adenocarcinomas of the stomach, colon, pancreas, gallbladder and breast was noted. Adenocarcinomas arising in the endometrium, ovary, kidney, prostate, thyroid and liver were either negative or showed weak and/or focal reactivity. Strong staining patterns were even noted in adenocarcinomas which had an 'undifferentiated' component; i.e., lacking well-defined glandular elements.

Immunoreactivity was noted in epithelial cells in several tissues from which these adenocarcinomas arose including the bronchial tract, stomach, small intestine, pancreas and colon, whereas epithelial cells from the endometrium, kidney, ovary, prostate and thyroid were negative or showed diffuse weak immunoreactivity. Our findings indicate that monoclonal antibody 44-3A6 recognizes an epithelial antigen on subsets of normal as well as transformed glandular epithelia. The differential pattern of expression of its target antigen probably reflects differences in tumor genesis and/or differentiation.

## Introduction

The $IgG_1$ mouse monoclonal antibody (MCA) 44-3A6 is a recently described antibody which is directed against the A549 human lung adenocarcinoma cell line. Fluorescent-activated cell-sorter analysis, immunofluorescence and live cell radioimmunoassays have shown that MCA 44–3A6 reacts with a cell surface antigen [1]. The antigen is preserved in formalin-fixed, paraffin-embedded tissue sections; a recent study described the immunostaining patterns of various bronchopulmonary neoplasms. That study revealed strong reactivity in primary lung adenocarcinomas, but not in squamous cell carcinomas, a subset of bronchioloalveolar carcinomas, or small cell neuroendocrine carcinomas [a]. Focal reactivitiy was seen in pulmonary carcinoids, and in subsets of large cell carcinomas and intermediate and well-differentiated neuroendocrine carcinomas. Immunoreactivity was noted both along the cell surface and in the cytoplasm. Based on those and related observations in cytologic specimens [3], the authors concluded that this antibody may be a useful tool in the differential diagnosis of pulmonary carcinomas especially those with glandular differentiation. The antigen is not expressed in melanomas, sarcomas and hematologic malignancies [4].

This study represents an extension of the aforementioned; it was undertaken to determine whether or not the antigen recognized by MCA 44-3A6 is expressed in adenocarcinomas of nonpulmonary primary origin. In addition, immunohistochemical analysis of normal adult organs from which these adenocarcinomas arose was undertaken to determine the target antigen's normal tissue biodistribution.

## Materials and Methods

The MCA 44-3A6 was produced by classic hybridoma methodology; a detailed description has been published elsewhere [5].

Hematoxylin and eosin-stained tissue sections were examined for the selection of tumors and normal tissue from the files of Northwestern Memorial Hospital, University of Illinois Hospital, MacNeal Memorial Hospital, and Rush-Presbyterian-St. Luke's Medical Center. Surgically resected neoplasms selected for this study (the number of cases used in parentheses) were as follows: adenocarcinomas of the breast (22); stomach (26); colon-rectum (50); gallbladder (2); endometrium (10); ovary (3); pancreas (1); prostate (15); islet cell neoplasms (6); renal cell carcinomas (8); adrenal cortical carcinoma (2); pheochromocytoma (2), and salivary gland tumors (9). Additional material was also submitted from the City of Hope National Medical Center and consisted of breast carcinomas (66) and adenocarcinomas from sites other than the lungs (10). The total number of tissues used are summarized in table I.

Autopsy tissues were studied (4 cases) to determine the normal adult distribution of the antigen; only cases less than 6 h postmortem were used in order to minimize the autolysis and to ensure antigen preservation. Tissues studied were trachea, lung, esophagus, stomach, duodenum, jejunum, ileum, colon, pancreas, liver, adrenal gland, kidney, prostate, bladder, ovary, uterus, cervix, testes, thyroid and skin.

All normal and neoplastic tissues were fixed in 10% buffered formalin and embedded in paraffin; tissue sections were prepared by routine surgical pathology procedures. Immunostaining was performed on 6-μm tissue sections. Following conventional deparaffinization, 6-μm thick tissue sections were stained by the avidin-biotin-complex (ABC) technique (Vector Laboratories, Burlingame, Calif.,) as previously described [6]. Tissue culture supernatnat from the clone which produces the MCA 44-3A6 was used throughout this study and was found to contain 1 mg/l of the antibody. Sections were subsequently counterstained in hematoxylin for 2 min. Negative controls were performed by omitting the primary antibody and substituting MCA 44-3A6 with another MCA, DWP. This MCA is of the same class as the MCA 44-3A6, but has been shown to detect a different epitope in formalin-fixed, paraffin-embedded tis-

**Table I.** Adenocarcinoma distribution of MCA 44-3A6

| | Positive case | Total cases |
|---|---|---|
| Breast adenocarcinoma | | |
| Intraductal | 6 | 8 |
| Infiltrating ductal | 41 | 62 |
| Lobular | 5 | 8 |
| Medullary | 3 | 8 |
| Colloid | 1 | 2 |
| Adenocarcinoma of stomach | 8 | 28 |
| Adenocarcinoma of gallbladder | 1 | 2 |
| Adenocarcinoma of pancreas | 3 | 3 |
| Adenocarcinoma of colon | 13 | 52 |
| Adenocarcinoma of liver | 0 | 5 |
| Adenocarcinoma of endometrium | 3 | 12 |
| Adenocarcinoma of ovary | | |
| Serous | 1 | 3 |
| Mucinous | 1 | 2 |
| Renal cell carcinoma | 0 | 8 |
| Adenocarcinoma of prostate | 0 | 15 |
| Adenocarcinoma of thyroid | 5 | 6 |
| Adrenal cortical carcinoma | 1 | 2 |
| Salivary gland tumors | 5 | 9 |

**Table II.** Normal tissue distribution of 44-3A6

| |
|---|
| Bronchial epithelial cells |
| Brunner's glands |
| Colonic enterocytes |
| Gastric chief and parietal cells |
| Myoepithelial cells and ductal cells of breast |
| Pancreatic islet cells |
| Peritracheal mucous glands |
| Sebaceous glands |
| Transitoneal cell epithelium of bladder and renal pelvis |
| Testicular interstitial cells |

sue. Sections submitted by the City of Hope were stained in a similar but slightly different manner; the method has been reported elsewhere in detail [7]. All immunostained tissue sections were scored for immunoreactivity on a scale of 0 to 4+.

## Results

Immunostaining of the neoplastic (table I) and normal tissues (table II) showed a heterogenous staining pattern. All cell types not expressly listed in the text, found in the tissues studied, did not show immunoreactivity with MCA 44-3A6.

Neoplasms of the gastrointestinal tract showed a varied staining pattern throughout the system. Strong immunoreactivity was seen in adenocarcinomas of the stomach, colon, pancreas and gallbladder (table I). Staining appeared to be predominantly cytoplasmic in nature, although membrane staining could not be excluded. Well-differentiated areas showing a glandular pattern (fig. 1, 3, 4) as well as poorly-differentiated areas (fig. 2) showed this strong immunoreactivity. Hepatocellular carcinomas were consistently negative as were islet cell tumors of the pancreas. Normal tissues within the gastrointestinal system showed a strong staining pattern in parietal cells and chief cells of the stomach, duodenal Brunner's glands, islet cells of the pancreas and enterocytes of the colon.

Renal cell neoplasms were consistently negative as were adenocarcinomas of the prostate. In the female genital tract, endometrial carcinomas were largely negative with the exception of focal cytoplasmic staining in 3 cases. In a limited number of ovarian neoplasms, both serous and mucinous cystadenocarcinomas showed focal staining which was especially prominent in the mucinous type. Prominent immunoperoxidase staining was seen in several transitional cell carcinomas of the bladder and renal pelvis.

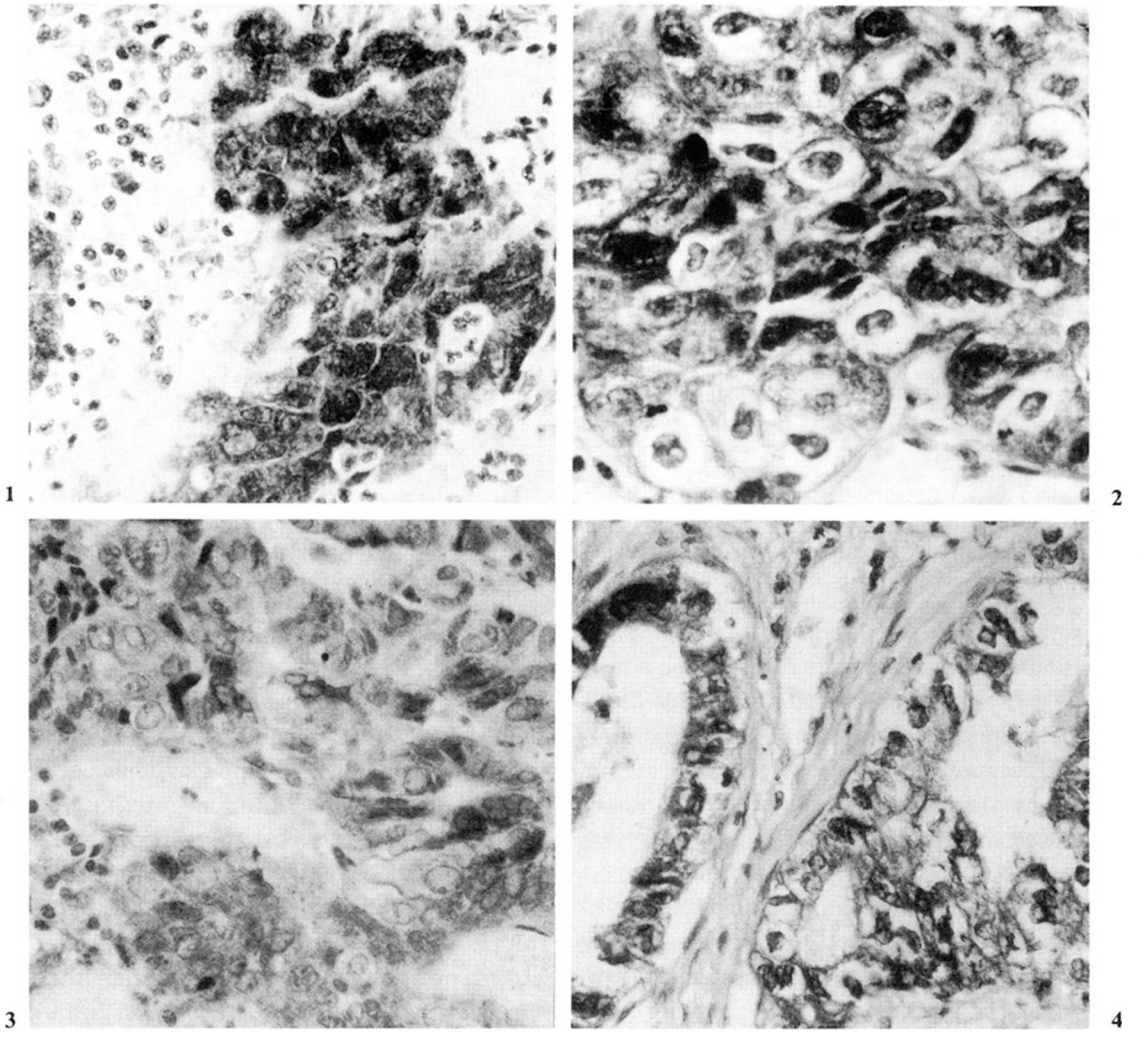

**Fig. 1.** Well-differentiated adenocarcinoma of the stomach. Strong immunoreactivity is noted in the glandular areas of the neoplasm. × 385.

**Fig. 2.** Poorly differentiated adenocarcinoma of the stomach. Strong immunoperoxidase staining is seen in the malignant cells. × 493.

**Fig. 3.** Adenocarcinoma of colon. The cells of the neoplastic glands show light diffuse immunoperoxidase staining. × 493.

**Fig. 4.** Adenocarcinoma of pancreas. Strong immunoperoxidase staining is noted in cells from well- differentiated areas. × 194.

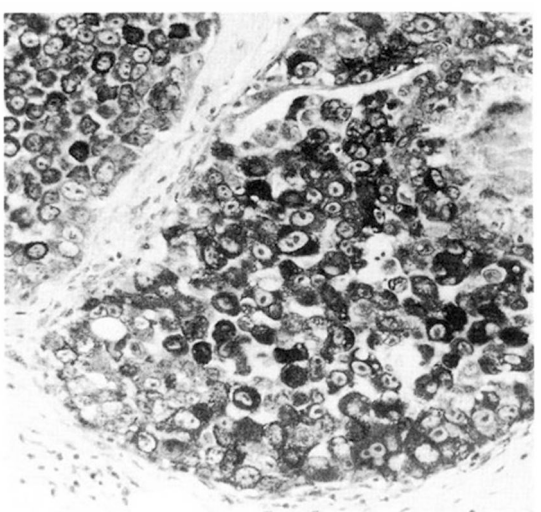

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**Fig. 5.** Intraductal carcinoma of breast. Prominent immunoreactivity is noted in the neoplastic cells. × 194.

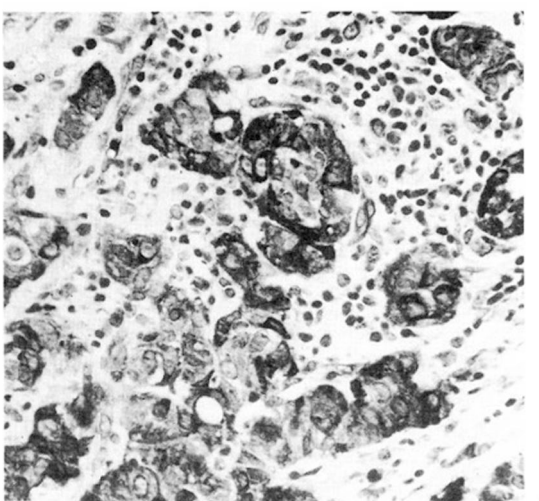

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**Fig. 6.** Infiltrating ductal carcinoma of the breast. Prominent immunoperoxidase staining is seen in the neoplastic cells. × 493.

The staining was predominantly cytoplasmic; however, in the cases from the renal pelvis the staining appeared prominent along the cell membrane and in the adjacent cytoplasm. An interstitial cell tumor of the testis showed strong focal cytoplasmic staining. Interstitial cells of the testes were also strongly positive. Normal tissues in the female genitourinary system were negative as were other tissues from the male genitourinary tract with the exception of light staining in transitional cell epithelium.

Thyroid and adrenal glands showed immunoreactivity in both normal and neoplastic tissues. Follicular carcinomas of the thyroid showed diffuse staining of the follicular cells while only focal staining was seen in the cells of the papillary carcinomas studied. Diffuse staining was also noted in the two thyroid medullary carcinomas. Spotty weak cytoplasmic staining was seen in adrenal cortical carcinomas; however, pheochromocytomas were negative. Thyroid follicular cells showed diffuse weak immunoreactivity as did cells of the fasciculata of the adrenal gland.

Breast malignancies showed intense immunostaining in the subtypes studied. Strong cytoplasmic and membrane staining was seen in cases of intraductal (fig. 5), infiltrating ductal (fig. 6), colloid, lobular and medullary carcinomas. This strong immunoreactivity was noted in both well-differentiated and poorly-differentiated cases. Focal staining was seen in normal ductal epithelium and rarely in myoepithelial cells.

Several salivary gland tumors were studied which showed positive staining in the epithelial component of Warthin's tumor and ductal elements in pleomorphic adenomas; however, an acinic cell carcinoma was negative. Normal salivary glands showed no

immunoreactivity. Sebaceous glands of the skin were found to express the antigen recognized by MCA 44-3A6.

## Discussion

MCA 44-3A6 has been shown to recognize a 40,000 MW protein antigen on lung adenocarcinomas as well as a subset of other pulmonary neoplasms. Immunohistochemical and immunocytologic analysis of a large panel of lung cancer specimens has demonstrated selective immunoreactivity. The present study examines the binding pattern of MCA 44-3A6 on adenocarcinomas outside the pulmonary tract. We have also evaluated normal adult tissues from which these tumors are derived. A heterogenous staining pattern was observed with an altered antigenic profile in adenocarcinomas from different sites. In addition, a variable antigen expression was noted within each adenocarcinoma category.

Positive immunoperoxidase staining was seen in 36% of tumors of the gastrointestinal tract. Both well- and poorly-differentiated neoplasms demonstrated strong diffuse or focal immunoreactivity in the stomach, colon, pancreas and gallbladder, with no reactivity seen in all five hepatocellular carcinomas. A similar staining pattern was seen with neoplasms from the genitourinary tract with 13% of tumors staining; breast with 63% of tumors staining and in the endocrine and salivary glands with 64% tumor staining from the two systems. Light microscopic features of the various neoplasms did not predict the pattern of immunoreactivity. The positive staining in normal tissues within the gastrointestinal tract, genitourinary tract, breast and endocrine glands did help predict which tumors would show positive staining patterns in the majority of cases; the notable exception being the staining patterns seen in normal and neoplastic pancreatic tissues.

Several recently described monoclonal antibodies [8, 9] also bind to adenocarcinomas and demonstrated heterogeneous staining patterns. Monoclonal antibody B73.2 recognizes a cell surface glycoprotein with a molecular weight of 200,000–400,000. Monoclonal antibodies KS 1/17 and KS 1/4 recognize cell surface glycoproteins with a molecular weight of 40,000 while KS 1/9 recognizes a cell surface glycolipid of unknown molecular weight [10]. Though KS 1/17 and KS 1/4 react with an antigen of similar molecular weight to that recognized by 44-3A6, the reported binding spectrum is different. These antibodies along with 44-3A6 represent a panel of reagents that may help distinguish subsets of adenocarcinomas.

MCA 44-3A6 recognizes an antigen present on adenocarcinomas from varied organ sites. The epitope has a pleotropic expression. The reason for this varied expression both within a given adenocarcinoma type and between adenocarcinomas from different sites is not clear. However, this pattern has been noted with many tumor-associated antigens. Current studies are now directed at the purification of this antigen from the A549 cell line which is known to express this antigen. In addition, another study is in progress to analyze various normal and neoplastic tissues which express this antigen using both Western blot analysis and immunoperoxidase staining. It is hoped that these investigations will identify the molecular nature of the antigen detected in these tissues. The heterogeneous expression of tumor markers signifies biological and/or clinical differences in tumor cell populations

and may potentially aid in our understanding of the molecular nature of these cancers.

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