Tumor Biol 1991;12:321–329

1010–4283/91/0126–0321$2.75/0

Expression of the Epitope Recognized by the Monoclonal Antibody 44-3A6 during Human Fetal Development[1]

J.A. Radosevich[a], *S.G. Combs*[b], *S.T. Rosen*[a]

Department of Medicine/[a]Cancer Center and [b]Pathology, Lakeside Medical Center, Northwestern University/Veterans Administration, Chicago, Ill., USA

Key Words. MAb 44-3A6 · Immunohistochemistry · Human fetal development · Monoclonal antibodies

Abstract. In this report we describe the expression of the adenocarcinoma associated antigen recognized by the monoclonal antibody 44-3A6, in various tissues during normal human fetal development. Conventional, formalin-fixed and paraffin-embedded sections of normal organs were examined from fetuses ranging from 9 to 42 weeks of gestation. Immunohistochemical localization of antigen-antibody complexes was accomplished using the avidin-biotin complex (ABC) method using horseradish peroxidase. The monoclonal antibody (MAb) 44–3A6 detects a cell surface 40 kD protein which is frequently expressed by adenocarcinomas and by select normal glandular tissues. Detectable expression of this protein was seen at different time periods during fetal development depending on the tissue. This expression was confined to a relatively small range of cell types and tissues; immunostaining was noted in select epithelial cells of the aerodigestive tract, exocrine pancreas, neural tissues, renal tubules, and transitional urothelium, as well as in other tissues. This immunostaining generally, but not invariably, corresponded with patterns previously reported in benign and/or malignant neoplasms of adult tissues. In most instances, once expression occurred within a tissue, it continued through gestation. These data show that this tumor associated gene product is differentially expressed in a broad range of normal developing human fetal tissues.

Introduction

The expression of 'normal' fetal antigens by malignant neoplasms has long been appreciated, while their possible roles in carcinogenesis remain in question [1, 2]. It is unclear as to whether these substances may cause the cancer to express its malignant properties, or if they are otherwise associated with the transformation process. Their

[1] This work is dedicated in the memory of S.G. Combs. It was supported by a Veterans Administration Merit Review grant to J.A.R.

existence has led to a search for additional fetal antigens to aid in understanding the biology of tumors, to distinguish normal from malignant growth processes, and to classify subtypes of tumors.

Oncofetal gene products have been implicated in tumor growth mechanisms because they are expressed in maturing and rapidly growing fetal tissues. The developing fetus shares many characteristics associated with malignant tumors e.g., rapid growth, differentiation of cell types, escape from destruction by the immune system, and the expression of select gene products [3–6]. Therefore, an improved understanding of the expression patterns of genes which are regulated at various points in the developmental program of the human fetus may provide an insight into their aberrant expression during neoplastic growth.

The expression of the antigen recognized by MAb 44-3A6 is well preserved in formalin-fixed paraffin-embedded tissues. This MAb has been shown by RIA and Western blot analysis to react specifically with a 40 kD cell surface protein expressed by the human adenocarcinoma cell line, A549 [7]. This antigen has been found to have select reactivity among human tumor cell lines, primary pulmonary neoplasms, and other normal and neoplastic tissues. In light of these findings, a systematic immunohistochemical study was performed in order to define the distribution of this adenocarcinoma-associated antigen in human fetal tissues at various gestational stages. The mapping of this gene product during fetal development may provide clues as to which organs or cell types may express this antigen in malignancies other than those already known to express this antigen.

Materials and Methods

Hematoxylin and eosin-stained tissue sections from conventionally formalin-fixed and paraffin-embedded autopsied fetal specimens were examined. Only cases less than 12 h post-mortem were used to minimize artifactual destruction and autolysis, and to improve antigen preservation. Tissue specimens that revealed poor cellular detail or anomalies were discarded. Twenty-five human fetal autopsies of gestational ages of 9, 18, 19, 20, 21, 22, 23–24, 26, 26–27, 27, 28, 29, 30, 35, 37, and 42 weeks were studied. Tissues from 12 males and 12 females were examined including: brain, trachea, lung, heart, esophagus, stomach, duodenum, ileum, colon, pancreas, liver, adrenal gland, kidney, prostate, bladder, ovary, uterus, cervix, testes, thymus, bone marrow, and thyroid. The gender of the 9-week-old fetus could not be established.

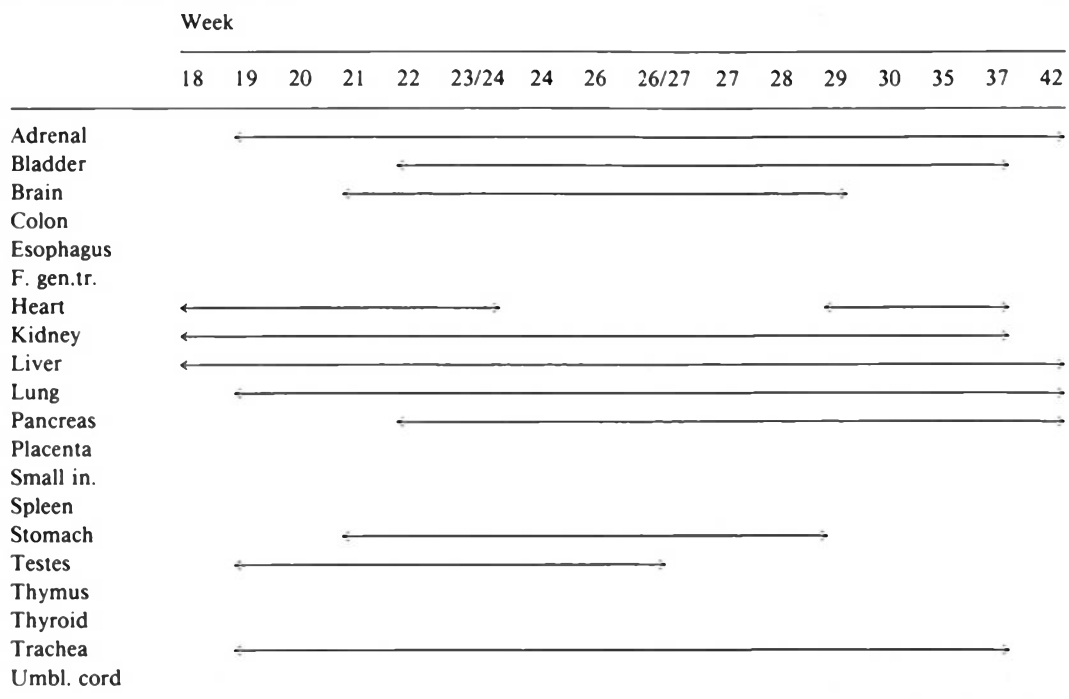
Immunoperoxidase staining was performed on 6-μm sections using the avidin-biotin complex technique (Vector Laboratories, Burlingame, Calif.) as previously described [7, 8]. Culture supernatant containing the MAb 44-3A6 was used undiluted and contained 1–2 μg/ml of antibody. Visualization of antigen-antibody complexes was accomplished using 3,3′-diaminobenzidine tetrahydrochloride; sections were subsequently counterstained with hematoxylin for 2 min. Controls included the omission of primary antibody, and the use of a nonspecific antibody. In addition, a known positive tumor was simultaneously stained as an internal positive control. The production and characterization of the MAb 44-3A6 has been reported elsewhere, as well as the detailed descriptions of all methods and materials [7].

Results

In all instances, serial sections were stained with the MAb 44-3A6, no primary, or an isotypic identical MAb.

Immunostaining of various organ systems with MAb 44-3A6 was noted beginning as early as 18 weeks and continuing to birth (38–42 weeks) and is summarized in table 1. Immunoreactivity was noted in various epi-

Table 1. Expression of the antigen recognized by MAb 44–3A6 during human gestation



Immunoreactivity was seen in those tissues during the times indicated by the arrows. Blanks represent weak or no staining.

thelial cell types of the gastrointestinal tract. This system also showed a continuum of staining throughout development for select cell types. In the esophagus, no immunoreactivity was evident except in 1 rare case in which the squamous epithelium weakly stained at 22 weeks of gestation. Parietal and chief cells of the stomach showed strong cytoplasmic staining from 21 weeks to 29 weeks (fig. 1). In the small bowel, there was only rare weak staining of enterocytes at weeks 22–24 and no staining was observed in the colon.

Hepatocytes showed immunostaining beginning at 18 weeks, and continuing to 38–42 weeks. However, the staining pattern within these tissues was variable. Bile duct epithelium showed cytoplasmic-like immunoreactivity; however, staining was only seen at 18 weeks. Immature white blood cell precursors in hematopoietic islands in the liver showed intense imunoreactivity. Staining within the pancreas was noted in acinar, islet, and ductal cells. They appeared as uniformly stained cells, and were observed at week 22 to weeks 38–42 (fig. 2). The strongest immunoreactivity was noted in acinar cells.

Immunostaining in the genitourinary system was frequently seen. Within the kidney,

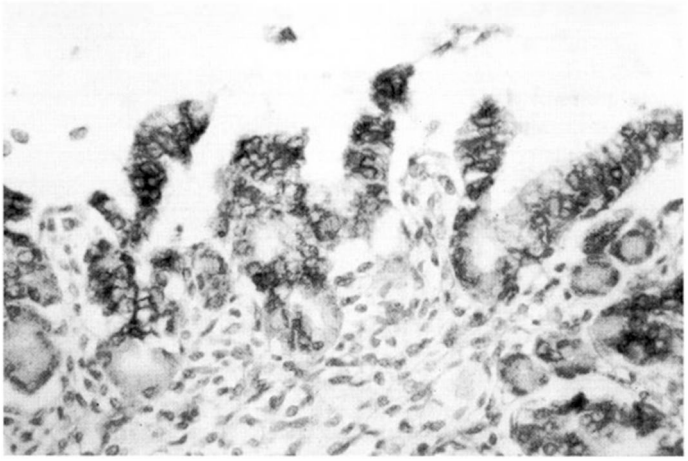


Fig. 1. Strong immunoreactivity is noted in the chief cells of the fetal stomach while weaker staining is seen in a few parietal cells at 21 weeks. × 112.

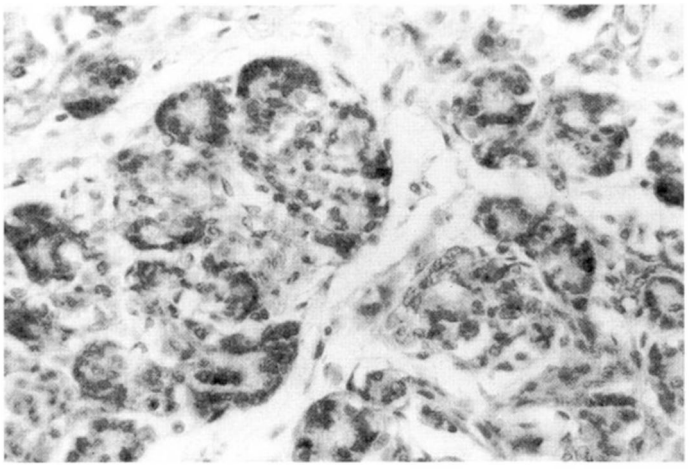


Fig. 2. Strong immunoreactivity is noted within the pancreatic acinar cells at 26 weeks. × 112.

apparent cytoplasmic reactivity was noted in the cells of the proximal tubules. Cells within the collecting ducts also showed immunoreactivity which was more intense than that noted in the proximal tubules. This reactivity began at 18 weeks and continued to week 35 (fig. 3). The glomeruli were not immunostained throughout the developmental time periods studied. Transitional cell epithelium of the renal pelvis and bladder showed strong cytoplasmic and/or membrane staining during these time periods (fig. 4, 5).

In the testis, immunoreactivity in the interstitial cells was noted beginning at week 19 and continuing up to week 26. Immunostaining was rarely seen in the seminiferous tubules at 22 weeks. Female genital tract tissue displayed no staining except in ovarian oocytes. Tissues from the uterus and cervix showed no immunoreactivity throughout the developmental periods studied.

Immunoreactivity in the respiratory tract was limited to cytoplasmic-like staining of bronchial epithelial cells, alveolar lining

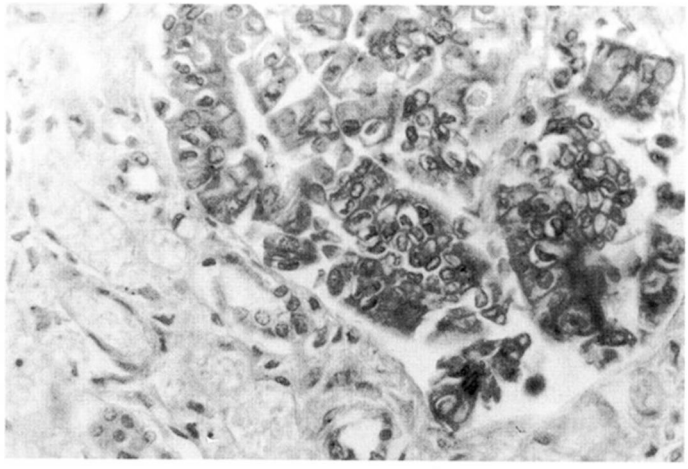


Fig. 3. Collecting ducts of the kidney show strong immunostaining of the tubular epithelium at 28 weeks. × 112.

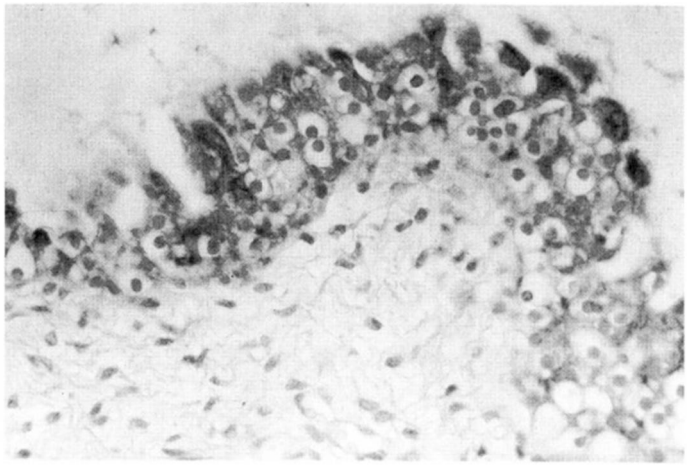


Fig. 4. Transitional cells of the renal pelvis showing strong immunoreactivity. × 112.

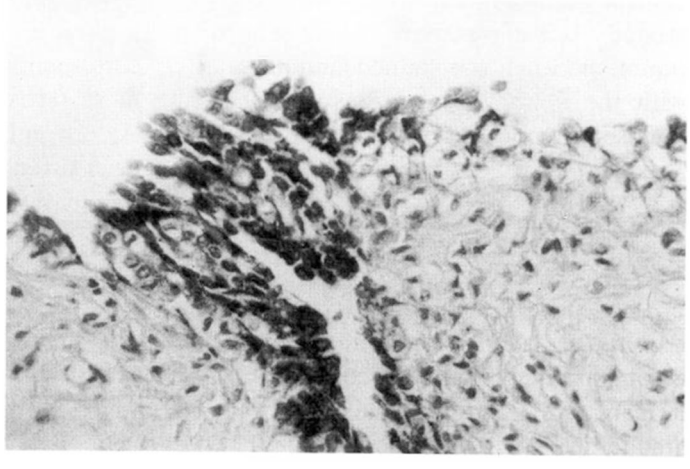


Fig. 5. Strong immunostaining of the transitional cells of the bladder epithelium. × 112.

cells, and type II pneumocytes, beginning at week 19 and continuing to maturity (fig. 6). In addition, bronchial cells of the trachea showed strong immunoreactivity, while only weak and focal staining was noted in the peritracheal seromucinous glands. In the endocrine system, the distribution and intensity of immunostaining varied. Thyroid tissues showed spotty focal cytoplasmic-like reactivity in follicular cells; this staining pattern was seen at 22, 26, and 42 weeks. In contrast, adrenal cortical tissue showed cytoplasmic-like staining, especially within the zona fascicularis and reticularis, beginning at 19 weeks and continuing to maturity. Adrenal medullary cells showed no immunoreactivity.

Immunostaining in cardiac muscle cells was noted, beginning at weeks 18–24, and then from week 29 to 37. Endothelial cells showed no immunoreactivity.

Tissue from the central nervous system showed immunoreactivity in ependymal cells lining the ventricles, and in the choroid plexus (fig. 7, 8). Peripheral nerves did not stain.

In adjacent serial sections of all the aforementioned samples, both the no primary control and isotypic identical MAbs demonstrated a lack of reactivity. A known positive tumor specimen was stained simultaneously with the fetal tissue sections and showed intense immunoreactivity.

Discussion

Immunohistochemical analysis of human fetal tissues with 44-3A6 demonstrated that a comparatively limited number of normal human tissues expressed this tumor-associated antigen. These results indicate that the gene encoding this protein is differentially expressed during the normal developmental program of the human fetus, and that the expression is limited to select cell types within various tissues.

Recently, we have reported that the extracellular concentration of calcium can influence the intracellular free calcium concentrations within the human adenocarcinoma cell line, A549 [9]. These studies demonstrated that calcium can influence the expression of this antigen, in that increased concentrations of calcium resulted in the increased expression of this antigen. It is not clear at the present time, whether or not this is a direct effect, or if it is an epiphenomenon. However, it is interesting to note that the expression of this antigen is most frequently seen in cell types which are associated with glandular functions [10]. This is true in both fetal and adult tissues, with the exception of neural, urinary tract, and cardiac tissues. All of these tissues however, have unique physiological processes which are calcium-linked.

Previous immunohistochemical studies of tumors arising in human breast and lung tissue have demonstrated that select histological subtypes of these tumors express detectable levels of this antigen, and that their corresponding normal adult tissues have a more restricted pattern of expression [10]. Within pulmonary neoplasms, which have been extensively studied, adenocarcinomas frequently express this antigen, as well as other select subtypes [11–14]. Among those subtypes which express this antigen are the large cell carcinomas. In a study of this subgroup of pulmonary neoplasms, we confirmed that the expression of this antigen is frequently seen in these tumors, and that there is a very good correlation with the

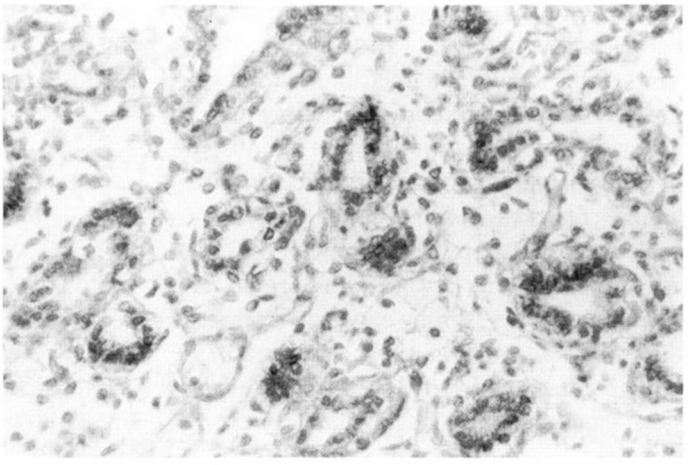


Fig. 6. Immunostaining of the alveolar lining cells of the fetal lung at 21 weeks. × 112.

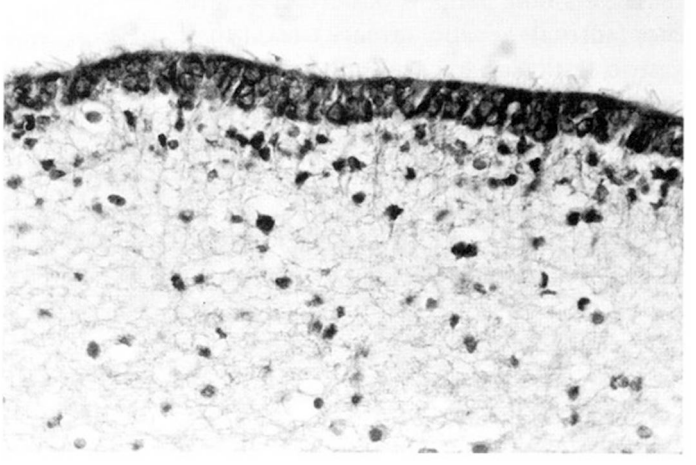


Fig. 7. Ependymal cells lining the brain ventricle showing strong immunoreactivity. × 112.

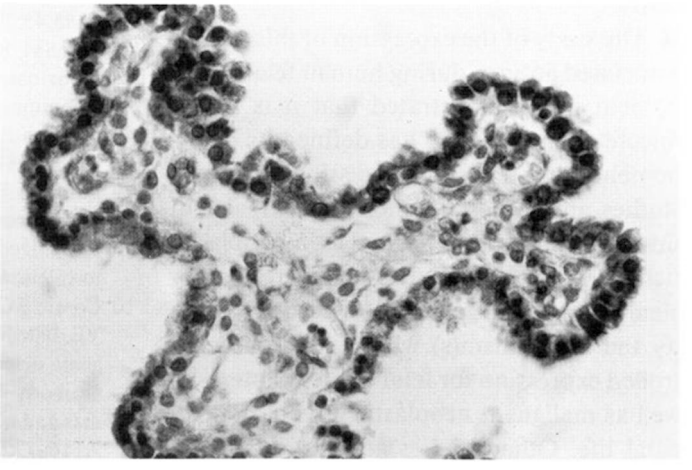


Fig. 8. Strong immunoperoxidase staining of the cells of the choroid plexus. × 112.

expression of this antigen and those large cell carcinomas which also express exocrine features. This suggests a relationship between antigen expression and the expression of exocrine differentiation, despite the absence of conventional light microscopic and histological features seen in adenocarcinomas such as glands, papillae, or mucus [12]. The expression of this antigen in normal fetal and adult tissues lacking exocrine features is therefore not surprising and supports the thought that in addition to exocrine expression, calcium may also regulate its expression.

The staining patterns of normal fetal tissues (adrenal, hepatic, urinary tract, neural, gastric, testicular, tracheal, and renal), noted in this study, may be useful in predicting which tumors or subsets of tumors arising in these tissues may also express this antigen. The predictive pattern of negative findings for tumors arising in the esophagus, ovary, uterus, colon, prostate, and bone marrow correlates well with the finding reported herein [unpubl. data: 10]. In addition, preliminary studies on the expression of the antigen in tumors arising in the stomach are consistent with the expression found in this study.

The study of the expression of this tumor-associated antigen during human fetal development has demonstrated that it is a true oncofetal antigen, and has defined its developmental pattern of expression. Ongoing studies are addressing molecular aspects of this gene in various normal and neoplastic tissues. Molecular studies of the regulatory element(s) of this gene will be needed to clarify the mechanism(s) which allow the controlled expression for fetal tissues in utero, as well as malignant neoplastic growth in postnatal life. Other studies are directed at assessing the expression pattern of tumors arising in tissues not yet studied, but which frequently express this antigen during fetal development.

References

1 Cohn SL, Lincoln ST, Rosen ST: Present status of serum tumor markers in diagnosis, prognosis, and evaluation of therapy. Cancer Invest 1986;4:305–327.

2 Nishi S, Hirai H: in Fishman WH; Sell SA (eds): Onco-Developmental Gene Expression. New York, Academic Press, 1976.

3 Fishman WH (ed): Oncodevelopmental Markers: Biologic, Diagnostic, and Monitoring Aspects. New York, Academic Press, 1983.

4 Rosenberg SA, Parker GA, Thorpe WP: Expression of oncofetal antigens by murine and human cells in tissue culture. Isr J Med Sci 1978;14:98–104.

5 McIntyre JA, Faulk WP: Cross-reactions between cell surface membrane antigens of human trophoblast and cancer cells. Placenta 1980;1:197–207.

6 Alexander P: Escape from immune destruction by the host through shedding of surface antigens: Is this a characteristic shared by malignant and embryonic cells? Cancer Res 1974;34:2077–2082.

7 Radosevich JA, Ma Y, Lee I, Salwen HR, Gould VE, Rosen ST: Monoclonal antibody 44-3A6 as a probe for a novel antigen on human lung carcinomas with glandular differentiation. Cancer Res 1985;45:5808–5812.

8 Hsu SM, Raine L, Fanger HJ: Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase technique: A comparison between ABC and unlabeled antibody PAP procedures. Histochem Cytochem 1981;29:577–580.

9 Siddiqui FS, Iqbal Z, Radosevich JA: Changes in the expression of the tumor associated antigen recognized by the MCA 44-3A6 in A549 cells due to calcium, submitted.

10 Combs SG, Radosevich JA, Ma Y, Lee I, Gould VE, Battifora H, Rosen ST: Expression of the antigenic determinant recognized by the monoclonal antibody 44-3A6 on select human adenocarcinomas and normal human tissues. Tumor Biol 1988; 9:116–122.

11 Lee I, Radosevich JA, Rosen ST, Ma Y, Combs SG, Gould VE: Immunohistochemistry of lung carcinomas using monoclonal antibody 44-3A6. Cancer Res 1985;45:5813–5817.
12 Piehl MR, Radosevich JA, Gould VE, Ma Y, Warren WH, Lee I, Rosen ST: Immunohistochemical identification of exocrine and neuroendocrine subsets of large cell lung carcinomas. Pathol Res Pract 1988;183:675–682.
13 Spagnolo DV, Witaker D, Carrello S, Radosevich JA, Rosen ST, Gould VE: The use of monoclonal antibody 44-3A6 in cell blocks in the diagnosis of lung carcinoma, carcinomas metastatic to lung and pleura, and pleural malignant mesothelioma. Am J Clin Pathol 1991;95:322–329.
14 Radosevich JA, Noguchi M, Rosen ST, Shimosato Y: Immunocytochemical analysis of human adenocarcinomas and bronchioloalveolar carcinomas of the lung using the monoclonal antibody 44-3A6. Tumor Biol 1990;11:181–188.

Received: March 18, 1991
Accepted: July 3, 1991

James A. Radosevich, PhD
Department of Medicine (Tarry 3-707)
303 E. Chicago Avenue
Chicago, IL 60611 (USA)