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Monoclonal Antibody 44-3A6 as a Marker for Breast Carcinoma[1]

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Abstract. The monoclonal antibody (MAb) 44-3A6 detects a 40-kD cell surface protein on adenocarcinomas and may serve as an effective marker for glandular differentiation. Immunohistochemical analysis of 123 paraffin-embedded malignant breast tissue specimens, 27 normal or benign breast disease specimens and 10 atypical hyperplasia specimens from patients without breast cancer was performed with MAb 44-3A6. The antigen was identified in 76% of breast cancer specimens, 0% of normal or benign breast disease specimens and 88% of the atypical hyperplasia specimens. MAb 44-3A6 also detected this antigen on adjacent normal breast ductal cells in 88% of the breast cancer specimens. There was no statistically significant correlation between immunoreactivity and histological mitotic or nuclear grade, recurrence or overall survival. This study suggests that the cell surface antigen detected by the MAb 44-3A6 may serve as an important marker in the differentiation of normal breast epithelium into an atypical or malignant lesion.

Introduction

Breast cancer is the second leading cause of death in women in the USA. American women now have a one-in-ten lifetime risk for the development of this heterogeneous disease [1]. The specific etiology is unknown, but breast cancer may be the result of a complex interaction of genetic, endocrine and environmental factors.

The utilization of immunohistochemistry as a routine method to assess the development of human neoplasms has increased significantly over the past several years. This has been primarily due to the production of monoclonal antibodies (MAbs) which are di-

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rected against a wide range of tumor-associated antigens. These antigens cover a broad range of biological components and include oncogene products, aberrant blood group expression, oncofetal gene products and other associated tumor antigens.

The IgG1 murine MAb 44-3A6 is directed against a 40-kD cell surface protein which is expressed by the human lung adenocarcinoma cell line A549 [2]. This antigen is well preserved in formalin-fixed, paraffin-embedded tissues. The MAb 44-3A6 has been applied in a number of studies directed at determining the frequency and pattern of expression of this antigen in normal human tissues as well as malignant specimens [3–9]. Expression of this antigen has also been found in routinely preserved cytological material, further allowing the use of this antibody in the study of clinical material [4, 8].

Previous studies have indicated that this antigen is associated with glandular differentiation of malignant tumors and that it is selectively expressed by tumors which are considered to be adenocarcinomas [3, 6, 9]. This present study is directed at determining the expression of the antigen recognized by the MAb 44-3A6 in a spectrum of breast tissues ranging from normal to benign to neoplastic histologies. In addition, this study was designed to determine if the expression of this antigen correlated as a prognostic indicator for the progression of disease for node-negative breast cancer patients.

Materials and Methods

Tissues

Paraffin-embedded tissues were obtained for 123 malignant breast tumors, 27 normal or benign breast tumors and 10 atypical hyperplasia breast tumors from the Department of Pathology, Northwestern Memorial Hospital (NMH), Chicago, Ill. Histological review of all specimens was performed by the study pathologist and revealed 116 infiltrating ductal carcinomas, 4 colloid carcinomas and 3 medullary carcinomas. The benign lesions consisted of 13 fibroadenomas, 9 fibrocystic disease specimens and 3 normal breast epithelium specimens. Five specimens each of atypical ductal hyperplasia and atypical lobular hyperplasia were also included in this series. None of the 37 patients with normal, benign or atypical hyperplasia biopsies had a previous or present history of breast cancer.

Patient Demographics

The charts of 123 female patients with node-negative breast cancer diagnosed from 1976 to 1981 at NMH were reviewed for demographic information which included age at diagnosis, recurrence and overall survival. The median follow-up was 88 months. The patients with normal breast tissue, benign breast disease or atypical hyperplasia were diagnosed from 1988 to 1989 and no further clinical review was performed at this time.

MAb 44-3A6 Production

The MAb 44-3A6 was produced by the classic hybridoma technology and characterized as previously described [2, 10].

Immunohistochemical Analysis

All tissues were fixed in 10% buffered formalin and embedded in paraffin following the surgical excision of the lesion. Conventional deparaffinization techniques were performed and 6 sections were stained by the avidin-biotin-peroxidase complex technique [11]. Controls included no primary antibody and an irrelevant antibody of the same isotype. The slides were reviewed by the study pathologist and scored for intensity: 0 (none), + (weak), ++ (moderate), or +++ (strong) staining, and for percent cell uptake: + (less than 25%), ++ (25–50%), +++ (50–75%) or ++++ (greater than 75%). Benign and normal ductal cells were scored for absence, focal or intense staining.

Statistical Evaluation

The statistical evaluations were performed by the χ^2 analysis.

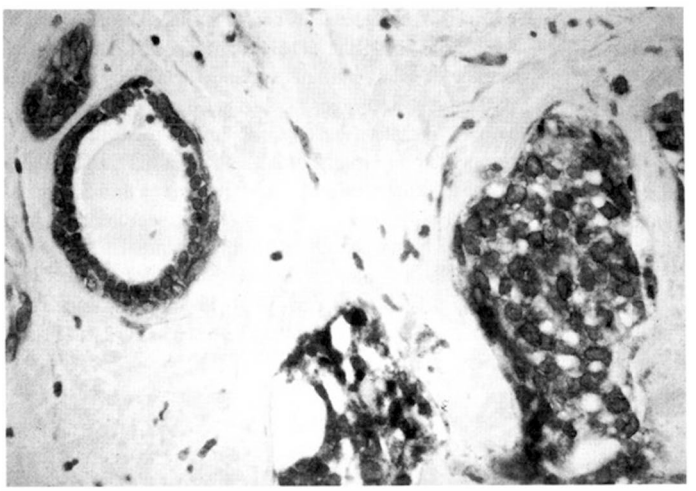

Fig. 1. Infiltrating ductal carcinoma of the breast demonstrating strong immunoreactivity with MAb 44-3A6 in both the normal ductal cells and the malignant ductal cells. × 275.

Results

Of the 123 malignant breast tumors selected, 113 had sufficient malignant cells available for immunohistologic staining analysis and 90 of these specimens also contained adjacent normal ductal cells. Review of the 10 specimens of atypical hyperplasia revealed 8 specimens with remaining abnormal atypical cells and all specimens contained normal epithelial cells for comparison. All 27 benign breast disease specimens had breast ductal cells present for immunohistologic evaluation.

The staining of the malignant cells by the MAb 44-3A6 was noted in the cytoplasm with cell membrane enhancement in 85 (75%) of 113 specimens. Eighty-one (76%) of 106 of the infiltrating ductal carcinomas, 3 (100%) of the 3 medullary carcinomas and 1 (25%) of the 4 colloid carcinoms demonstrated immunoreactivity with MAb 44-3A6. The intensity of immunoreactivity was + in 15 tumors, ++ in 31 tumors and +++ in 39 tumors. The percent cell uptake was + in 36 tumors, ++ for 21 tumors, +++ for 11 tumors and ++++ for 17 tumors. Fifty-eight percent of all malignant tumors had 25% or more of the cells staining with the MAb 44-3A6.

Of the 113 slides from malignant specimens, 90 had normal ductal cells available for analysis with this antibody. Of these 90 specimens, 79 (88%) with adjacent normal ductal cells present exhibited immunoreactivity with MAb 44-3A6, 59% of which had an intense stain. There were 81 matched sets of malignant and adjacent normal ductal cells per slide. Among the 69 malignant tumors which demonstrated immunoreactivity with the MAb, 65 (94%) has normal cells also expressing the antigen. Within the group of 12 malignant tumors that did not express immunoreactivity with MAb 44-3A6, 5 (42%) specimens with adjacent normal ductal cells demonstrated immunoreactivity with this antibody (fig. 1).

Histologically benign tumors and normal breast tissue specimens from 27 patients with no previous history of breast cancer revealed no immunoreactivity of the ductal

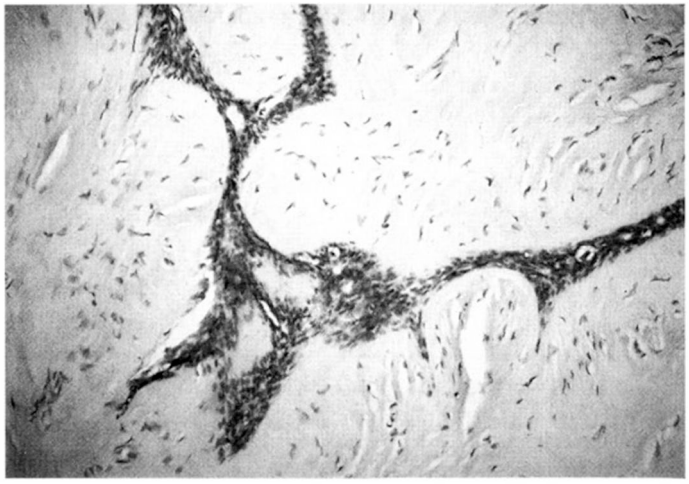

Fig. 2. Fibroadenoma of the breast from a patient without breast cancer demonstrating no staining with the MAb 44-3A6. × 275.

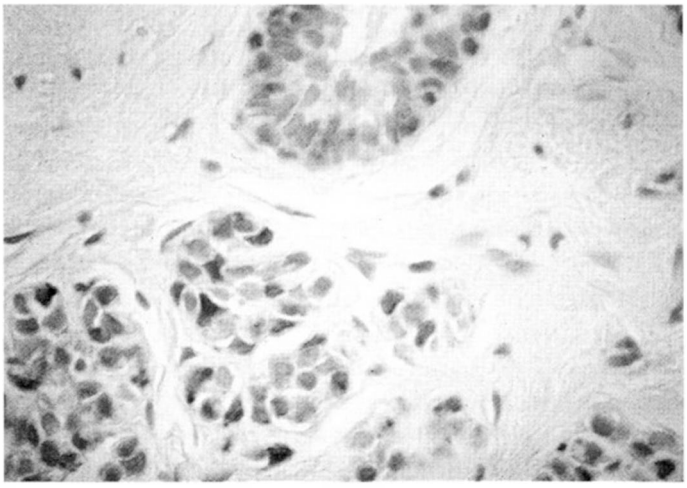

Fig. 3. Atypical lobular hyperplasia of the breast demonstrating strong immunoreactivity with the MAb 44-3A6 in both normal and abnormal cells. × 440.

cells with the MAb 44-3A6 (fig. 2). Four specimens revealed focal uptake in the myoepithelial cells, but there was no cytoplasmic or cell membrane staining in any of these tissues.

Specimens from the 10 patients with atypical hyperplasia revealed intense staining of the normal ductal cells in the specimen, as well as variable degrees of cytoplasmic and membrane staining for 7 of the 8 specimens with atypical cells present (fig. 3). In no case did normal or benign ductal cells demonstrate immunoreactivity with MAb 44-3A6 unless associated with malignant ductal cells or atypical hyperplastic ductal cells. Table 1 summarizes the immunohistochemical reactivity of breast epithelial cells with MAb 44-3A6.

Clinical information was assessed and there was no statistically significant correla-

Table 1. Immunohistochemical analysis of breast tissue specimens with MAb 44-3A6

Breast tissue	Number of specimens with positive uptake	Total specimens evaluable	Percent staining
Infiltrating ductal carcinoma	81	106	76
Medullary carcinoma	3	3	100
Colloid carcinoma	1	4	25
Adjacent-normal[a]	79	90	88
Atypical hyperplasia	7	8	88
Benign-normal[b]	0	27	0

[a] This group represents the normal ductal cells present on the histological slides from specimens with malignant cells.

[b] These specimens are from breast biopsies with no patient history of atypical or malignant breast disease. They include normal, fibrocystic and fibroadenoma histological diagnoses.

tion of staining with age at the time of diagnosis, histological, mitotic or nuclear grade of the tumor. Recurrence and overall cancer survival was known for 111 patients. Those patients with a positive immunoreactive tumor had a 29% (24/84) recurrence rate while those with a negative staining tumor had a 15% (4/27) recurrence rate. Although the trend suggests a higher recurrence of disease for the patients whose tumor expresses the antigen reactive with MAb 44-3A6, the difference is not statistically significant. The 5-year overall survival for patients whose tumor demonstrated immunoreactivity with the MAb 44-3A6 was 80% compared to 86% for those patients with nonimmunoreactive tumors, a statistically insignificant difference.

Discussion

The MAb 44-3A6 has been previously shown to recognize a cell surface antigen in the glandular epithelium of various adenocarcinoma tumors. In the study by Combs et al. [6], focal staining in normal breast ductal cells adjacent to malignant ductal cells was observed but not analyzed. In this present study, 76% of all malignant breast tumors demonstrated reactivity with MAb 44-3A6 and 94% of the malignant tumors with evaluable normal cells had adjacent normal ductal cells expressing this antigen.

Despite the observation that normal breast ductal cells adjacent to the carcinoma demonstrated immunoreactivity with MAb 44-3A6, none of the benign or normal ductal cells without an associated malignancy did react with this antibody. This striking difference suggests that the MAb 44-3A6 recognizes an antigen that may serve as a marker for the development of breast cancer. Detection of this antigen in histologically nonmalignant cells may signal the malignant potential of the cell. It may have significant clinical importance for determining which proliferative breast diseases confer an increased risk for the later development of breast cancer. In order to study the relationship of MAb 44-3A6 to the development of breast

malignancy further several cases of atypical hyperplasia without associated in situ or invasive breast carcinoma were examined. Atypical hyperplasia has been found in 7% of all breast biopsies and confers at least a 4-fold increased risk of later developing breast cancer [12]. Immunoreactivity with the MAb 44-3A6 was demonstrated to varying degrees in 7 of 8 specimens with atypical hyperplasia and was also demonstrated in the adjacent normal ductal cells. This observation supports the theory of a continuum from histologically benign through atypical to malignant cells. Although the histological criteria for atypical hyperplasia are well established, there are certain cases in which the diagnosis is difficult. Additional studies examining the relationship of the MAb 44-3A6 with atypical hyperplasia and malignancy may confirm the utility of MAb 44-3A6 for the diagnosis of malignant lesions as well as those with malignant potential.

The antigen recognized by the MAb 44-3A6 is not associated with any of the histological features examined, including grade, degree of architectural differentiation, nuclear pleomorphism or mitotic rate. It is expressed in the majority of the malignant specimens examined and does not appear to be a prognostic indicator for the clinical progression of the breast cancer. A recent study using fluorescence-activated cell sorter analysis (immunostaining/DNA content measurements) has demonstrated that the antigen is expressed throughout the cell cycle. This would suggest that the expression of this antigen is not solely a consequence of cell proliferation [13].

The MAb 44-3A6 may be an important marker in breast malignancy as it recognizes an antigen that appears to be associated with the differentiation of normal breast epithelial cells into atypical or malignant cells. This antigen can be detected with the MAb 44-3A6 prior to any apparent histologically identifiable change in the breast ductal cell. Further studies to identify the antigen in other breast diseases and studies to characterize this antigen, clone the gene and determine its function are currently in progress.

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