Report

Specific polypeptide differences in normal versus malignant human breast tissues by two-dimensional electrophoresis

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Summary

Postmitochondrial and cytosolic polypeptides were extracted from human breast tumors and non-malignant breast tissue and analyzed using high resolution two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Approximately 800–1000 postmitochondrial and 600–800 silver stained cytosolic polypeptides were detected over the pH range of 4.8 to 7.5 and molecular weight range of 18–120 kDa. The 2D-PAGE patterns of polypeptides from normal and malignant tissue were very similar, although both qualitative and quantitative polypeptide differences were noted. Six cytosolic polypeptides (pI/molecular weight $\times 10^{-3}$) 5.20/80 kDa, 5.75/43, 6.25/40, 5.43/35, 5.45/34.5, 5.50/34 and 6.15/24 were expressed only in malignant tissues. One constitutive polypeptide, 7.25/52, was not detected in any of the malignant tissue samples. Quantitatively, marked differences in spot density were noted in polypeptides localized mainly in the molecular weight ranges of 22–40 kDa and pI ranges of 5.65–7.00. A general increase in polypeptide expression was noted in malignant tissues as compared to normal. Twenty-two polypeptides were significantly and consistently increased in tumor samples while only one polypeptide was decreased. One polypeptide, p24 (6.15/24) was expressed in greatest concentrations in tumors which also expressed the greatest estrogen receptor content. Expression of p24 was markedly reduced in normal tissue and malignant tissues expressing low levels of estrogen and progesterone receptors.

Introduction

In both human and animal carcinogenesis studies, the growth and development of mammary tumors are, to some extent, under endocrine control and can be manipulated both *in vivo* and *in vitro*. In human breast cancer, analysis of cytoplasmic estrogen receptor (ER) and progesterone receptor (PgR) content of human breast tumors is now recognized as an extremely useful clinical tool for evaluating the prognosis and the sensitivity of tumors to endocrine therapy [1–3]. Tumors lacking

ER respond infrequently to endocrine treatment [1], whereas 50–60% of those tumors positive for ER and approximately 80% of those which are positive for both ER and PgR are responsive to endocrine therapy [4].

Although these receptor assays are extremely useful diagnostically, numerous inherent problems associated with steroid-receptor binding studies in general have limited their usefulness, and have prompted searches for additional biochemical markers of transformation-associated changes in human breast tumors in order to detect, diagnose,

and treat this form of cancer as early as possible [5–11]. In the present study we have used the high resolving power of 2D-PAGE, in combination with computer assisted qualitative and quantitative analysis of between 600–800 polypeptides from cellular homogenates and cytosolic preparations from human breast tumors and from non-malignant breast tissue samples, in an attempt to define sets of cellular polypeptides which may be involved in (or associated with) the malignant transformation of human breast tissue.

Material and methods

Materials

The monoclonal antibody G3.1, specific for the M_r 24,000 protein of MCF-7 human breast cancer cells, was kindly provided by Drs. Dean P. Edwards and William L. McGuire of the Division of Oncology, Department of Medicine, University of Texas Health Science Center, San Antonio TX, under the auspices of NIH CA11378.

Tissue samples

Samples of malignant and non-pathological breast tissue were obtained from the Surgical Department of the University Hospital of Iceland. Malignant tumors were immediately put in ice-cold saline in the operating room. Samples were washed as free of blood as possible with ice-cold saline before and after mincing. Tumors were either quick-frozen on dry ice (CO_2) and kept at -70°C until use, or, if used fresh, were immediately cooled to 0°C , homogenized, and fractionated.

Eight non-pathological samples were obtained from breasts removed by radical mastectomy. Fat was excised as completely as possible macroscopically, and samples were histochemically inspected for fat and malignant growth. Samples used contained up to approximately 30% fat. Non-pathological samples were treated in the same way as tumor samples. No differences in the hexokinase isoenzyme patterns were observed between freshly

prepared samples and previously frozen ones [11].

Preparation of cytosolic fractions

All work on sample preparation was done at $0-4^\circ\text{C}$. The procedure for fractionation of tissue was based on the method used by Drouin [12]. Briefly, samples which had been frozen were thawed in isotonic saline in an ice-bath. Tissues were minced, washed in a homogenization buffer consisting of 10 mM Tris-HCl, pH 7.4, 1.5 mM EDTA, 0.5 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride as a protease inhibitor, and homogenized with a Polytron^R homogenizer for 3×8 sec at 1 min intervals to allow cooling of the specimen in an ice-bath. Homogenates were spun at $1,000 \times g$ for 10 min to remove cell debris, nuclei, and whole cells. The supernatant was spun at $15,000 \times g$ for 15 min to yield a mitochondrial pellet and postmitochondrial supernatant. The postmitochondrial supernatant was spun at $105,000 \times g$ for 1 hr to yield the microsomal pellet and a supernatant fraction which is referred to as the cytosolic fraction. It should be pointed out that some of the membrane-bound or embedded soluble proteins may not be observed in these cytosolic preparations. Postmitochondrial and cytosolic fractions (5–10 mg/ml) were lyophilized to dryness for concentration and stored desiccated at -70°C prior to electrophoresis. No difference in hexokinase isozyme patterns was detected before and after lyophilization [11].

Cytosolic receptor assay

ER and PgR content were determined by the dextran-coated charcoal (DCC) method of McGuire [13], using Insta-Gel as aqueous counting scintillant. ER and PgR concentration of more than 8 fmol/mg protein was designated positive. Protein concentration was measured by the method of Bradford using bovine serum albumin as a standard [14].

Two-dimensional electrophoresis

2D-PAGE analysis of postmitochondrial and cytosolic polypeptides from normal and malignant breast tissues was performed as described by O'Farrell [15] and modified by Wirth *et al.* [16]. Equilibrium pH gradient electrophoresis was used in the first dimension and SDS-PAGE in the second dimension. Ampholytes (LKB Instruments, Rockville, MD) were used at concentrations of 1.6%, pH range 5–8, and at 0.4%, pH range 3.5–10.0. Lyophilized protein samples were dissolved in O'Farrell's lysis buffer A [15] to a final concentration of 10 µg protein/µl lysis buffer A and 100 µg of protein loaded at the basic end of the first dimension isoelectrofocusing tube gels (inside diameter, 2.0 mm × 160 mm). Greater protein loads resulted in gel overloading and decreased resolution of individual polypeptides. Samples were electrophoresed at 750 V for 16 hr at room temperature and finally at 1000 V for 1 hr (13,000 V-hr total). SDS-PAGE was performed at constant current (20 mA/gel) using 0.75 mm thick 10% polyacrylamide gels. Following electrophoresis, gels were silver stained using the procedure of Morrissey [17], and the wet gels were scanned and analyzed using a semiautomated computer analysis system as previously described [16]. For quantitative purposes and to correct for slight variations in protein loading on gels, each polypeptide spot has been expressed (normalized) as a percent (%) of the total integrated density (summed intensities of all polypeptide spots) on each gel.

Western blotting

Polypeptides were transferred from 2D-PAGE gels to 0.45 µm nitrocellulose membranes (Schleicher and Schuell, Keene, NH) using a Trans-Blot apparatus (Hoefer Scientific Instruments, San Francisco, CA) at 60 V and 0.2 amps at 4° C using a transfer buffer containing 20 mM Tris-HCl, 150 mM glycine, and 20% (v/v) methanol [18]. After transfer overnight, the nitrocellulose paper was treated with a buffer consisting of 20 mM Tris-HCl and 500 mM NaCl, pH 7.4 (TBS), for 10 min at

room temperature with gentle shaking, and was then placed in TBS buffer containing 3% gelatin and 0.05% Tween 20 (TTBS) for 30 min at room temperature. The nitrocellulose was incubated in TBS containing 1% gelatin and anti-M_r 24,000 protein antibody at a dilution of 1:1000 for 2 hr at room temperature. The nitrocellulose was washed twice with TTBS for 10 min at room temperature and incubated in TBS containing 1% gelatin and peroxidase conjugated goat anti-mouse IgG (Bio-Rad Laboratories, Richmond, CA) for 1 hr at room temperature. The antibody was used at a 1:2000 dilution. The paper was washed twice with TTBS, each time for 10 min at room temperature with gentle shaking. For development, the paper was immersed in 60 ml of chilled [4° C] development solution [50 ml TBS containing 0.05% 4-chloro-1-naphthol (Bio-Rad), 0.03% hydrogen peroxide, and 10 ml methanol]. Development was stopped when red-purple spots appeared on the paper.

Results

Figure 1 illustrates the 2D-PAGE separation of silver stained polypeptides from tissue homogenate samples (15,000 × g supernatant) from human mastectomy tissue with infiltrating ductal carcinoma (Fig. 1B) and from non-involved normal breast tissue obtained from the same patient (Fig. 1A). Approximately 800–1000 polypeptides over the pH range of 4.8 to 7.5 and the molecular weight range of 18–120 kDa were readily visible on each electrophoretogram. The electrophoretic patterns of polypeptides from non-pathological and malignant breast tissue were very similar; however, analysis of polypeptide patterns from malignant and normal tissue revealed both qualitative and quantitative polypeptide differences. Due to the complexity of the electrophoretic patterns and as an aid in the analysis of polypeptide differences between normal and malignant breast tumors, tissue samples were further fractionated using differential centrifugation, and analysis was limited to changes in cytosolic (105,000 × g supernatant) polypeptides.

Fig. 1. Two dimensional electrophoretic patterns of silver stained polypeptides from human tissue homogenate samples ($15,000 \times g$ supernatant) from normal non-malignant breast tissue (A) and from breast tissue with infiltrating ductal carcinoma (B). Polypeptides were separated in the first dimension by isoelectric focusing between the pH range of 4.75 (on the left) and 7.50 (on the right). Procedures for preparation of tissue samples for electrophoresis, modifications of the 2D-PAGE procedure, fixing of gels, and silver staining of polypeptides are as described in "Materials and Methods". Abscissa, pH range; ordinate, molecular weight $\times 10^{-3}$.

Qualitative polypeptide differences between normal breast tissue and malignant tumor tissue

Malignant breast biopsy tissue was obtained from eight individual patients along with normal non-malignant tissue from non-involved sites from the same individuals. Table 1 summarizes the histologi-

cal evaluation as well as the PgR and ER content of each tumor. The tumors were primarily infiltrating ductal carcinoma, some with metastases. Two tumors (94TC and 97TC) were positive for both PgR and ER, and one tumor (130TC) was negative for both receptors.

Fig. 2 illustrates the 2D-PAGE separation of

Fig. 2. Two dimensional electrophoretic patterns of cytosolic polypeptides from normal (A) and malignant (B) breast tissue illustrating polypeptides p80, p43, p40, p35, p34.5, p34, and p52. Open circles have been positioned on appropriate gels at expected molecular weight and pI values for missing spots. In each panel, boxes enclose areas in which major quantitative differences in polypeptide expression are observed. Amount of protein loaded in the first dimension was 100 μ g.

cytosolic polypeptides from normal breast (Fig. 2A) and malignant tumor tissue (Fig. 2B) from one individual. Approximately 600–800 polypeptides were readily detected on each electrophoretograms. The 2D-PAGE patterns of cytosolic polypeptides from normal and from malignant breast tissue were very similar, although both qualitative and quantitative polypeptide differences were observed. Albumin (pI 6.4/MW 66 kDa) was the ma-

ior cytosolic polypeptide in both normal and malignant tissues, accounting for approximately 20–25% of the total protein concentration. No significant differences in expression of albumin were observed between normal and malignant tissues or among the individual tumor samples (data not shown).

Analysis of polypeptide patterns from eight individual tumor samples revealed seven common qualitative polypeptide differences when com-

Table 1. ER and PgR content of malignant tumor tissue.

Sample	Histology	ER fmol/mg	PgR fmol/mg
94TC ^a	intraductal carcinoma, invasive	43	34
97TC	intraductal carcinoma, invasive no metastases to axillary lymph gland	43	57
100TC	intraductal carcinoma, invasive	192	N.D. ^b
110TC	intraductal carcinoma, invasive	128	N.D.
111TC	inflammatory carcinoma with metastases	23	N.D.
117TC	intraductal carcinoma, invasive	9	N.D.
125TC	adenocarcinoma	38	N.D.
130TC	adenocarcinoma with metastases to axillary lymph gland	N.D.	N.D.

^a TC: Tumor, malignant breast tissue; ^b N.D. Not detected (<8 fmol/mg protein).

pared to normal non-malignant breast samples. These are illustrated on Fig. 2 with the numbered arrows. To aid in comparison, open circles have been placed at the expected pI and molecular weight values for the missing polypeptides. Six polypeptides were expressed in all eight tumors that were not expressed in any of the corresponding normal tissues. Quantitatively, polypeptides p80 (5.2/80 kDa) and p43 (5.75/43 kDa) were expressed as relatively major polypeptides representing 0.12–0.37% and 0.37–0.44%, respectively, of the total integrated density of the tumor polypeptides. Polypeptides p40 (6.2/40 kDa) and p34 (5.50/34 kDa) were expressed at intermediate concentrations, while p35 (5.43/35 kDa) and p34.5 (5.46/34.5 kDa) were expressed in relatively minor concentrations. One constitutive polypeptide, p52 (7.25/52 kDa), was not detected in any of the malignant tissue samples.

Quantitative polypeptide differences between normal and malignant breast tissues

In addition to these qualitative changes, more numerous quantitative polypeptide differences were observed. In contrast to the qualitative changes, which occurred at all pI and molecular weight regions, these quantitative changes occurred in polypeptides localized mainly in the pI ranges of 5.65–

7.00 and molecular weight ranges between 22 and 40 kDa, and are illustrated in Fig. 2 with the enclosed boxes. These quantitative differences are further highlighted in Fig. 3, which illustrates mosaic enlargements of this region from four representative normal and malignant tissue samples. Quantitative comparison of polypeptides in this region revealed a general up-regulation of polypeptide expression in malignant tissues as compared to those from normal tissues.

Distribution of quantitative polypeptide differences

Figure 4 illustrates a computer drawn composite map of total paired polypeptides between normal breast tissue and malignant tissue (panel A). Polypeptides which show significant modulation are outlined in panel B and are summarized in Table 2. Twenty-two polypeptides are significantly and consistently increased in malignant samples while only one polypeptide (603; 6.35/45 kDa) is decreased in malignant samples as compared to normal breast tissues.

Included among these polypeptides is one polypeptide, p24 (6.15/24 kDa), which is expressed in greatest concentrations in two tumors which also exhibit the greatest ER content, namely 100T and 110T. Expression of p24 is markedly reduced in normal tissue and in malignant tissues expressing

Fig. 3. Comparison of two-dimensional polypeptide patterns among four individual normal and malignant breast tissue samples in the pI range of 5.65–7.00 and molecular weight range 22–40 kDa. NC, normal cytosol; TC, tumor cytosol.

low levels of PgR/ER content and is illustrated in Fig. 5. McGuire and coworkers have recently reported the expression of a M_r 28,000 protein (originally termed 24K owing to its apparent molecular weight on a one-dimensional (1-DE) SDS-PAGE gel) in cytosolic fractions from MCF-7 human breast cancer cells, an established cell line which retains functional levels of ER and PgR, and this protein appears to be a very good marker for differentiation in human breast cancer [7, 19]. Immunoblot analysis using G3.1, a specific monoclonal antibody against this 24K marker protein, and co-electrophoresis of cytosolic preparations with purified 24K protein (kindly supplied by Dr. W.L.

McGuire) revealed this 24K protein to be distinct from the p24 polypeptide. As illustrated in Fig. 6, monoclonal antibody G3.1 is immunoreactive with one major and three minor polypeptides from malignant breast tissues. The major immunoreactive polypeptide corresponds to p1126 (6.30/26 kDa). Similar patterns of immunoreactivity against G3.1 were observed in all eight cytosolic preparations (data not shown).

In a further attempt to identify the p24 protein, we compared the electrophoretic characteristics of p24 with those of the major milk proteins, casein complex and α -lactalbumin. The p24 did not migrate with either of these proteins (data not shown).

Discussion

We have previously shown that 2D-PAGE can be used quite reliably and accurately to separate and quantitate changes in both known and as yet unidentified polypeptide markers for hepatocarcinogenesis [16, 20, 21]. We have now extended these studies to the analysis of polypeptide changes during human breast carcinogenesis.

In the present study we have compared both

qualitative and quantitative changes in cytosolic polypeptides found in human breast cancer with polypeptide patterns observed in normal breast tissue, by using 2D-PAGE in combination with computerized densitometry. Although the 2D-PAGE polypeptide patterns from malignant tissue and normal tissue were remarkably similar with regard to overall spot patterns and number of polypeptides detected, analysis of approximately 600–800 cytosolic polypeptides from normal (Fig. 2A)

Table 2. Qualitative and quantitative polypeptide differences between normal breast and malignant breast tumor tissue.

Polypeptides	pI	MW	Tissue	
			Normal	Malignant
p80	5.20	80,000	N.D. ^a	0.124–371 ^b
p52	7.25	52,000	0.065–0.085	N.D.
p43	5.75	43,000	N.D.	0.370–0.440
p40	6.25	40,000	N.D.	0.140–0.202
p35	5.43	35,000	N.D.	0.060–0.080
p34.5	5.46	34,500	N.D.	0.060–0.075
p34	5.50	34,000	N.D.	0.204–0.303
p24	6.15	24,000	0.020–0.040	0.040–0.360
603 ^c	6.35	45,000	0.130–0.180	0.059–0.076
658	6.20	40,000	0.204–0.370	0.652–1.220
679	6.60	43,000	0.058–0.070	0.100–0.303
691	6.90	43,000	0.019–0.025	0.107–0.201
712	7.15	41,000	0.026–0.052	0.070–0.101
791	6.05	36,000	0.225–0.358	0.546–0.777
808	7.30	38,000	0.050–0.058	0.080–0.148
863	5.35	34,500	0.049–0.055	0.152–0.170
866	5.70	33,000	0.258–0.386	0.475–0.677
920	5.25	33,000	0.052–0.069	0.090–0.255
940	6.40	32,000	0.138–0.277	0.249–0.450
942	6.42	31,500	0.087–0.108	0.266–0.451
986	5.50	29,000	0.330–0.395	0.390–0.614
992	6.05	29,000	0.200–0.240	0.300–0.777
1068	7.40	25,000	0.056–0.098	0.164–0.307
1096	6.45	26,000	0.028–0.060	0.091–0.211
1126	6.30	26,000	0.090–0.188	0.187–0.338
1128	6.90	26,000	0.050–0.070	0.120–0.166
1132	6.70	26,000	0.052–0.074	0.109–0.339
1145	6.00	25,000	0.020–0.068	0.080–0.150
1165	6.80	25,000	0.012–0.030	0.074–0.326
1219	6.15	24,000	0.049–0.098	0.104–0.176
1292	6.45	20,000	0.016–0.050	0.098–0.162

^a Not detected.

^b Values represent the range of intensities for each polypeptide expressed in the eight individual tissue samples. Individual polypeptide intensities are expressed as a per cent of total integrated polypeptide spot density for all spots in the gel.

^c Computer assigned polypeptide spot number.

Fig. 4. Computer drawn composite map highlighting polypeptides significantly altered during mammary carcinogenesis. Panel A illustrates a contour map of commonly expressed cytosolic polypeptides in normal breast tissue and malignant tumors. Panel B illustrates polypeptides which exhibit significant modulation during mammary carcinogenesis. As an aid for polypeptide location and orientation, albumin, as well as the polypeptides p80, p43, p40, p35, p34.5, p34, and p24, are also illustrated. An open circle has been placed at the expected pI and molecular weight values for p52.

and malignant (Fig. 2B) tissue from eight individual patients revealed both qualitative and quantitative polypeptide differences. Six relatively acidic polypeptides (5.20/80 kDa, 5.75/43, 6.20/40, 5.50/34, 5.46/34.5, and 5.43/35) were detected only in tumor samples, while one constitutive polypeptide (7.25/52) was lost during cancer development.

In addition to these qualitative changes we have identified twenty-three additional polypeptides

whose concentrations are significantly modulated during carcinogenesis (Table 2 and Fig. 4). In all cases but one, a relative increase in protein expression was observed during malignant transformation, and these changes occurred in polypeptides localized in the molecular weight ranges of 22–40 kDa. No significant quantitative modulation in proteins occurring at higher molecular weights (>45 kDa) were observed.

Fig. 5. Mosaic composite of areas of gels from eight individual tumor samples illustrating the expression of p24.

At present neither the identity nor the biologic function of any of these polypeptides is known. Several investigators have reported the appearance of apparently new or markedly increased polypeptides both in primary breast tumor samples and in established breast tumor cell lines using both 1D and 2D-PAGE [6–8, 10, 11, 22–27]. None of these new or increased polypeptides corresponds with respect to molecular weight and pI to any of these previously described polypeptides.

We have identified one polypeptide, p24 (6.15/24 kDa), which is expressed in greatest concentra-

tions in the tumor samples exhibiting greatest ER content, namely 100TC and 110TC (Table 1). The expression of p24 is markedly reduced in normal tissues and in malignant tissues expressing low levels of PgR/ER (e.g. 111TC and 117TC) (Table 2). McGuire and co-workers have isolated and characterized a 24,000 molecular weight cytoplasmic protein originally termed 24K (later shown to be a 28,000 dalton protein with a pI 5.9–6.0 [19]) that is regulated by estrogen in the MCF-7 human breast cancer line, is found in human breast tumors which retain both ER and PgR receptors [7, 8], and has

Fig. 6. Western blot analysis using monoclonal antibody G3.1 and 2D-PAGE of 24K protein. Polypeptides immunoreactive with G3.1 have been circled in panel A. Panel B illustrates 2D-PAGE separation of purified 24K protein. The position of p24 has been illustrated in panel A with an arrow.

been proposed as a marker for differentiation in breast cancer [28]. 2D-PAGE separation of this protein (generous gift from Dr. McGuire) revealed one major polypeptide spot. Western transfer and immunoblot analysis of human breast tumor polypeptides with G3.1, a monoclonal antibody specific against this 24K protein, revealed a cross reactivity with one major and three minor polypeptides, molecular weight of 26–28,000 (Fig. 6). The major immunoreactive polypeptide corresponded to p1126 (6.30/26) in the present study and none corresponded to p24. Furthermore p24 did not coelectrophoresis with any of the known major milk proteins, namely casein complex and α -lactalbumin (data not shown). We therefore conclude that the p24 polypeptide is not one of the major milk proteins.

Polypeptides p34, p34.5, and p35 probably represent charge shift modifications of constitutively expressed polypeptides in normal breast tissue rather than newly synthesized polypeptides, because the shift in position (toward higher molecular weight-acidic region) and the distance of shift in relation to their position among surrounding non-changing polypeptides is near that expected for a single, double, or triple charge difference between polypeptides [15, 29]. Such charge-shift modifications could result as differences in either phosphorylation, glycosylation, or the sialylation of constitutive proteins. In order to investigate the possibility that p34, p34.5, and p35 represent polypeptides which contain variable amounts of sialic acid, we are currently in the process of incubating protein samples of tumor tissue in the presence of neuraminidase prior to 2D-PAGE analysis.

In addition to these qualitative differences, more numerous quantitative differences in polypeptide expression were noted between normal and malignant tissue. Interestingly, a general increase in polypeptide expression was noted in all malignant tissues, and only one polypeptide (603; 6.35/45) was decreased in cancerous tissue as compared to normal breast (Table 2). These increases were not due to artifactual proteolysis of higher molecular weight proteins with concomitant increases in lower molecular weight species, since no significant changes in higher or lower molecular weight proteins were observed between normal tissue and tumors (Fig. 2). Furthermore, to eliminate artifactual proteolysis during sample preparation and electrophoresis, all tissue manipulations were performed in the presence of the protease inhibitor phenylmethylsulfonyl fluoride, and at 0°C. It is interesting to note that no consistent polypeptide differences, either qualitatively or quantitatively, were noted between the two samples which were also positive for the PgR receptor, namely 94TC and 97TC (Table 1), and the other malignant samples which were negative for PgR.

It should be emphasized, however, that the biopsy samples analyzed contain a heterogeneous population of cell types including both transformed tumor cells and normal non-malignant tissue cell types. Therefore, it is not known with certainty

whether the sets of polypeptides identified here and which are closely associated with human mammary carcinogenesis are tumor-specific or merely cell-specific. This work represents a preliminary investigation of polypeptide differences between malignant and non-malignant tissues. Subsequent cell fractionation along with isolation, purification, and characterization of these proteins either through the use of specific monoclonal antibodies or partial amino acid sequencing, may reveal specific biologic functions in relation to hormonal dependency of mammary tumors. Development of monoclonal or polyclonal antibody preparations that recognize these tumor-associated antigens may provide useful tools for the early diagnosis and potential treatment of human mammary cancers.

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