Journal of Cellular Biochemistry 66:9–15 (1997)

Nuclear Matrix Proteins in Well and Poorly Differentiated Human Breast Cancer Cell Lines

Shanti K. Samuel, Travis M. Minish, and James R. Davie*

Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

Abstract The nuclear matrix, besides providing the structural support of the nucleus, is involved in various cellular functions of the nucleus. Nuclear matrix proteins (NMPs), which are both tissue- and cell type–specific, are altered with transformation and state of differentiation. Furthermore, NMPs have been identified as informative markers of disease states. Here, the NMP profiles from human breast cancer cell lines and breast tumours were analyzed using two-dimension gel electrophoresis. We identified NMPs that are associated with well and poorly differentiated human breast cancer cells in vitro and in vivo. Five NMPs (NMBC 1–5) were found to be exclusive for well-differentiated human breast cancer cells, while one NMP (NMBC-6) was found to be present only in poorly differentiated human breast cancer cells. The identification of these proteins suggests the potential use of nuclear matrix proteins as prognostic indicators. J. Cell. Biochem. 66:9–15, 1997. © 1997 Wiley-Liss, Inc.

Key words: breast cancer; well/poorly differentiated human breast cancer cells; estrogen receptor; nuclear matrix proteins; diagnostic indicators

Breast cancer is the most common form of cancer among women. While it is the second leading cause of cancer mortality among females, the pathogenesis of the disease remains unclear [Kuller, 1995; Ernster et al., 1996]. In the malignant progression of breast cancer, breast tumours progress from hormone-dependent growth to a more aggressive phenotype characterised by hormone-independent growth, resistance to endocrine therapy, and a frequently widespread metastases [Leonessa et al., 1992; Ruiz Cabello et al., 1995].

A cellular landmark in the pathological diagnosis of cancer is the nucleus [Pardee, 1989]. Nuclear alterations are so prevalent in transformed cells that it is commonly used as a pathological marker for malignancy. The nucleus of a transformed cell is commonly enlarged, has an abnormal chromatin pattern, and is irregular in shape. Nuclear shape is a reflection of internal nuclear structure and processes and is determined in part by the nuclear matrix [Replogle and Pienta, 1996].

The nuclear matrix provides the structural support of the nucleus and is involved in various cellular functions of the nucleus, including regulation of transcription, replication, and DNA repair. Nuclear matrix proteins (NMPs), which are tissue- and cell type–specific, are altered with transformation and state of differentiation [Bidwell et al., 1994a,b; Replogle-Schwab et al., 1996]. They have been identified as informative markers of disease states [Khanuja et al., 1993; Keesee et al., 1994]. Informative NMPs have been identified for bladder, breast, colon, prostate, head, and neck cancers [Getzenberg et al., 1991a, 1996; Khanuja et al., 1993; Keesee et al., 1994; Donat et al., 1996]. For example, the nuclear matrix protein PC-1 is found in the NMPs from prostate carcinoma but not in the nuclear matrix from normal prostate or benign prostatic hyperplasia [Getzenberg et al., 1991a]. Of potential importance is the demonstration that NMPs can be detected in the serum and urine of cancer patients, thus suggesting that the detection of specific nuclear matrix proteins may be of value in breast

Contract grant sponsor: Medical Research Council of Canada, contract grant number PG-12809; contract grant sponsors: H.J. Simmons Memorial, N.W. Simmons Memorial, and Natalie Riedle.

*Correspondence to: J.R. Davie, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Manitoba, 770 Bannatyne Ave., Winnipeg, MB R3E OW3, Canada.

Received 18 April 1997; accepted 24 April 1997

cancer diagnosis and monitoring [Miller et al., 1992; Replogle-Schwab et al., 1996].

Although the most influentially used prognostic marker for human breast cancer is metastasis to the axillary lymph nodes, the presence of estrogen receptors (ERs) in breast tumours has been used as one of the major characteristics to identify well and poorly differentiated epithelial cells in the malignant progression of breast cancer [Godolphin et al., 1981; Aamdal et al., 1984; McGuire et al., 1986; Clarke et al., 1990]. Studies have shown that, both in vivo and in vitro, human breast cancer cells that lack ER are poorly differentiated, estrogen-independent, and resistant to anti-estrogen therapy and express vimentin (an intermediate filament protein) [Raymond and Leong, 1989a,b; Clarke et al., 1990; Thompson et al., 1992]. Conversely, cells that express ER are generally well or moderately differentiated, estrogen-dependent, and sensitive to anti-estrogen therapy and lack vimentin. The presence of ER, independent of axillary lymph node status [Godolphin et al., 1981], would therefore predict longer disease-free and overall survival.

In this study, the nuclear matrix protein profiles of a variety of human breast cancer cell lines were examined by two-dimension gel electrophoresis. These breast cancer cell lines were ER+/hormone-dependent, ER−/hormone-independent, and ER+/hormone-independent. MCF-10A1, a spontaneously, immortalized human breast epithelial cell line, was used as a control. Using these cell lines, we have identified specific NMPs exclusive to ER status. Furthermore, the presence of these specific NMPs has also been detected in human breast tumours.

MATERIALS AND METHODS

Cell Culture

The human breast carcinoma cell lines used in this study were ER+, hormone-dependent (MCF-7, T47D, ZR-75), and ER−, hormone-independent (MDA-MB 231 and BT-20). MCF-10A1, the closest representative of normal breast epithelia, was used as a control. All cell lines were maintained at 37°C (humidified atmosphere, 5% CO_2/95% air) on 150 × 20 mm tissue culture dishes (Nunc, Naperville, IL) in culture medium containing Dulbecco's modified Eagle medium (DMEM) (Gibco-BRL, Grand Island, New York) supplemented with 1% (v/v) L-glutamine, 1% (v/v) glucose, 1% (v/v) penicillin/streptomycin, and 5% (v/v) fetal calf serum (FCS) (Gibco) except for T5-PRF [Coutts et al., 1996], which was cultured in medium containing Dulbecco's modified Eagle medium–Phenol Red Free (Sigma, St. Louis, MO) and 5% (v/v) twice charcoal stripped fetal calf serum and supplemented as mentioned above. At ~90% confluence, cells were removed from the plates with a rubber policeman and frozen as pellets containing 1×10^7 cells at −70°C.

Tumour Samples

Individual frozen ER+ and ER− breast cancer tumours (0.5 g) were obtained from the NCIC-Manitoba Breast Tumour Bank [Watson et al., 1996]. The tumours were minced finely and processed according to the protocol described below.

Purification of Nuclear Matrix Proteins

Nuclear matrices were prepared according to a procedure previously reported [Samuel et al., 1997]. In a previous study we found that the partitioning of nuclear matrix proteins was the same regardless of whether the cells were fresh or frozen [Chen et al., 1996]. In brief, nuclei from cell pellets (1×10^7) and breast tumour samples were resuspended to a concentration of 20 A_{260}/ml and digested with DNase I (D5025; Sigma) for 20 min at room temperature. Ammonium sulphate (final concentration of 0.25 M) was added, and the nuclear matrix (NM-IF) pellet was obtained by centrifugation. The NM-IF pellet was resuspended in buffer with 1 mM PMSF and extracted by adding 4 M NaCl to a final concentration of 2 M. The pellet (NM2-IF) was collected by centrifugation and reextracted with 2 M NaCl. The resulting pellet was resuspended in disassembly buffer (8 M urea, 20 mM 2[N-morpholino] ethane sulfonic acid, pH 6.6, 1 mM EGTA, 1 mM PMSF, 0.1 mM $MgCl_2$, 1% (v/v) β-mercaptoethanol) and dialysed overnight at room temperature against two litres of assembly buffer (0.15 M KCl, 25 mM imidazole pH 7.1, 5 mM $MgCl_2$, 2 mM DTT, 0.125 M EGTA, 0.2 mM PMSF). The IFs were removed by ultracentrifugation and the resulting supernatant containing NMPs removed. The NMP containing supernatant was then dialysed against double-distilled H_2O for 8 h to reduce the salt concentration and then lyophilized. Lyophilized samples were resuspended in appropriate volumes of 8 M urea, aliquoted, and frozen at −20°C. Before two-dimension gel

electrophoresis, protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad, Richmond, CA) with bovine serum albumin as a standard.

Two-Dimension Gel Electrophoresis

Two-dimension gel electrophoresis was done as described previously [Samuel et al., 1997]. Molecular masses and isoelectric points were determined using two-dimensional SDS-PAGE standards (Bio-Rad, Menlo Park, CA; Pharmacia BioTech, Uppsala, Sweden) and carbamylated carbonic anhydrase (Pharmacia BioTech). Gels were stained using the Pharmacia Silver Stain Kit for protein detection. The gels were then dried between sheets of gel-drying film (Promega Corporation, Madison, WI).

The silver-stained two-dimension gel patterns were scanned using a PDI 325OE densitometer (PDI, Kingston Station, NY) and the data analyzed using the Image Master System (Pharmacia BioTech). All studies were carried out using three to four preparations from each cell line and six preparations of human breast tumours.

RESULTS

Nuclear matrix proteins (NMPs) from estrogen receptor (ER)+/hormone-dependent and ER−/hormone-independent human breast cancer cell lines were analyzed to identify NMPs exclusive to receptor status. The human breast cancer cell lines used were T47D, MCF-7, and ZR-75 (ER+/hormone-dependent), MDA MB231 and BT-20 (ER−/hormone-independent), and T5-PRF (ER+/hormone-independent). A nontumorigenic, spontaneously immortalized human breast epithelial cell line known as MCF-10A1 (ER−/hormone-independent) obtained from a reduction mammoplasty specimen was chosen as the closest representative of normal breast epithelia. Each cell line was cultured and consecutively passaged at least three times. NMPs were extracted from each passage and electrophoresed. The two-dimension NMP patterns shown in this study are representative of each cell line.

To align these NMPs, several internal markers were used. Carbamylated carbonic anhydrase (30 kDa, pl 4.8–6.7) was used as an internal standard for the determination of molecular mass and pI. Two-dimension SDS-PAGE standards (Bio-Rad) were used to calibrate the pI and molecular masses of the NMPs. Lamins A and C enriched in the nuclear matrix fraction were identified based on molecular mass and pI, as shown previously [Fey and Penman, 1988; Partin et al., 1993; Mattern et al., 1996].

While analysis of the NMP composition of these epithelial cell lines revealed similarities between MCF-10A1, T5-PRF, ER+/hormone-dependent (T47D, ZR-75, MCF-7), and ER−/hormone-independent (BT-20, MDA MB231), specific notable differences were detected. Many of the common abundant proteins were likely heterogenous nuclear ribonucleoproteins (hnRNPs) [Mattern et al., 1996]. HnRNP-K (60 kDa, pI 5.2–5.4), a transcription factor [Michelotti et al., 1996], was found in all two-dimension gel patterns of NMPs. Several NMPs were found in either ER+ or ER− breast cancer cell lines but not in the "normal" MCF-10A1. Using the nomenclature proposed by Khanuja et al. [1993], we refer to these proteins as NMBCs (nuclear matrix proteins in breast cancer). Five NMBCs (1–5) exclusive to the ER+ cell lines (Fig. 1) and one NMBC (6) exclusive to the ER− cell lines were identified (Fig. 2). The molecular masses and pIs, respectively, of NMBCs 1–6 were as follows: NMBC 1, 57 kDa, 5.5; NMBC 2, 62 kDa, 5.1; NMBC 3, 40 kDa, 5.4; NMBC 4, 41 kDa, 5.3; NMBC 5, 39 kDa, 5.5; NMBC 6, 52 kDa, 5.7. NMBCs 1–5 but not NMBC 6 were also detected in T5-PRF, an ER+/hormone-independent breast cancer cell line (Fig. 1). This would suggest that NMBCs 1–5 were exclusive to ER+ status. The NMBCs were not found in nonmatrix nuclear fractions (data not shown). NMBC-Z reported by Khanuja et al. [1993] was consistently identified in all the human breast cancer cell lines except in T5-PRF and MCF-10A1. However, NMBC-X and -Y were not found in any of the NMP preparations from the cell lines.

It has been reported that the extracellular environment can alter cellular morphology as well as the protein composition of the cytoskeletal and nuclear matrix compartments [Getzenberg et al., 1991b; Pienta et al., 1991]. To ascertain if these proteins identified as exclusive to the ER status in immortalized human breast cancer cell lines in vitro were present in vivo, we extracted NMPs from 0.5 g of individual ER+ and ER− breast tumours (Table I). Prior to extraction, adipose tissue surrounding the tumour was removed. It was vital that the adipose tissue be removed for efficiency of NMP

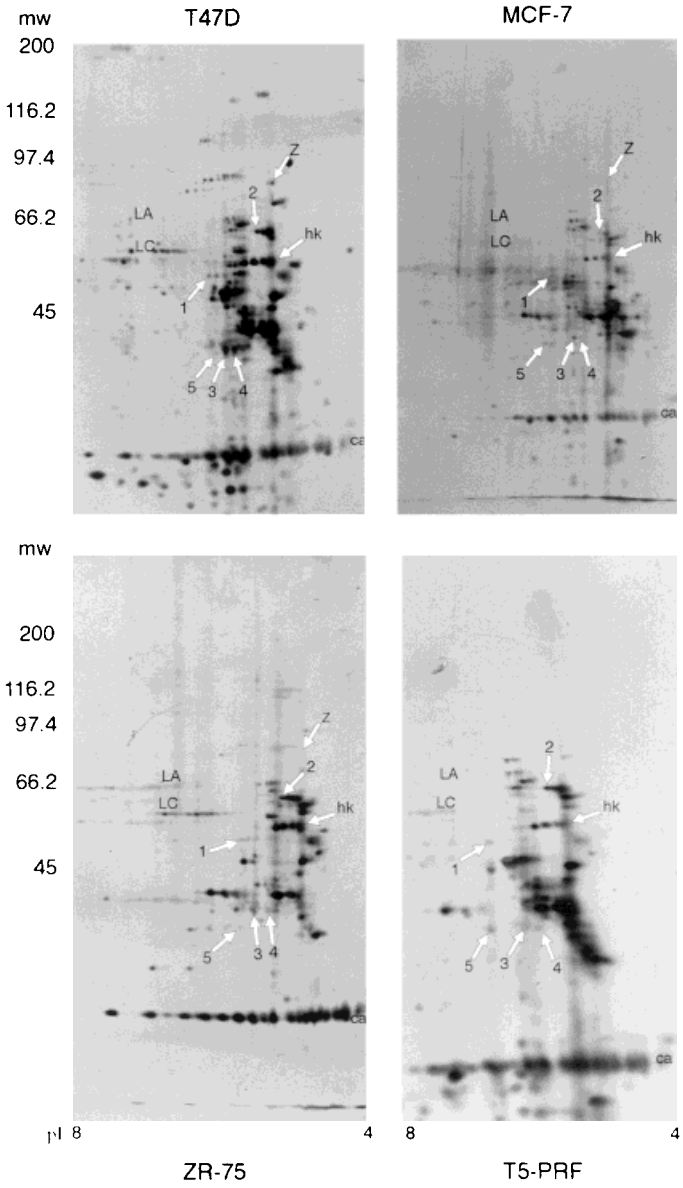

Fig. 1. Nuclear matrix proteins of ER+, hormone-dependent human breast cancer cell lines and ER+, hormone-independent human breast cancer cell line T5-PRF. Protein (40 µg) was electrophoretically resolved on two-dimension gels. The gels were stained with silver. The position of the carbamylated forms of carbonic anhydrase is indicated by ca. The position of the molecular weight standards (in thousands) is shown on the left side of each two-dimension gel pattern. LA and LC show the position of lamin A and C, respectively. The white arrows show the location of five NMBCs (1–5) unique to the ER+ cell lines and NMBC-Z (Z). The location of hnRNP-K is indicated as hK.

extraction. Figure 3 shows representative two-dimension NMP profiles of ER+ and ER− breast tumours. We found all NMBCs (1–5) exclusive to ER+ status in human breast cancer cell lines to be present in the ER+ breast tumours, while NMBC-6 was always not detectable. Although the quality of the two-dimension gel patterns of ER− tumour NMPs was inferior to that of the patterns from ER+ tumours, we always observed the presence of NMBC-6, while NMBCs 1–5 were undetectable.

DISCUSSION

The progression of normal breast epithelial cells to a malignant phenotype may be dependent on cellular genetic events and failure of

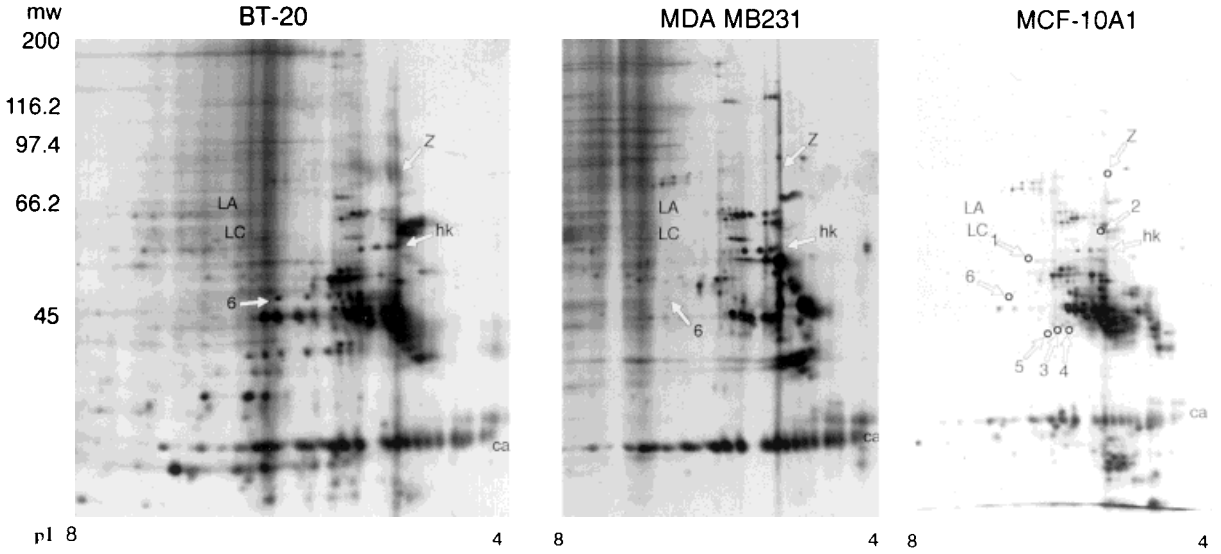

Fig. 2. Nuclear matrix proteins of ER− human breast cancer cell lines and spontaneously immortalized normal breast epithelial cell line. Protein (40 μg) was electrophoretically resolved on two-dimension gels. The gels were stained with silver. The position of the carbamylated forms of carbonic anhydrase is indicated by ca. The position of the molecular weight standards (in thousands) is shown on the left side of each two-dimensional gel pattern. LA and LC show the position of lamin A and C, respectively. The location of hnRNP-K is indicated as hK. The white arrows show the location of the NMBC-6 (6), unique to the ER− cell lines and NMBC-Z. The circles show the absence of the NMBCs (1–6, Z) proteins in the normal breast cell line.

TABLE I. Patient and Breast Tumour Characteristics

Tumour number	Age of patient (years)	Operation[a]	ER[b]	PR[c]	Lymph nodes[d]
11173	65	MRM	52	87	P
12702	52	Biopsy	31	16.3	?
12797	52	MRM	6.1	13.0	P
12431	49	Biopsy	2.7	2.1	?
9859	46	Lumpectomy	2.0	9.1	N
8896	72	MRM	0.5	7.6	N

[a]MRM, modified radical mastectomy.
[b]ER, estrogen receptor reported as femtomoles per milligram of protein, positive >3.
[c]PR, progesterone receptor reported as femtomoles per milligram of protein, positive >15.
[d]P, positive; N, negative.

host mechanisms [Replogle and Pienta, 1996]. While prognostic markers do exist for breast cancer, more effective indicators of malignant progression are needed. Identifying such intermediate biomarkers will aid in the development of more specific cancer treatments and prevention strategies.

It has long been realized and accepted that an abnormal nuclear shape and the presence of abnormal chromatin pattern are characteristic of a cell exhibiting a transformed phenotype [Replogle-Schwab et al., 1996]. While the molecular and structural changes that occur during transformation of a cell are not well elucidated, it has been shown that the nuclear matrix does undergo changes with transformation [Berezney et al., 1979; Getzenberg et al., 1991a; Samuel et al., 1997]. Recently, our group has reported that NMP composition was radically altered in highly metastatic oncogene-transformed mouse fibroblasts [Samuel et al., 1997]. Interestingly, highly metastatic *ras*-transformed 10T½ cells and highly metastatic *fes*-transformed NIH 3T3 cells had a similar set of NMPs that were not seen in poorly metastatic or nontumorigenic parental mouse fibroblast cell lines. Clearly, this study shows a correlation between the NMP profile and the metastatic potential.

A previous study by Khanuja and coworkers [1993] revealed the presence of several nuclear matrix proteins (X, Y, Z) as specific to malignant breast tissue. In our study, we identified NMBC-Z in all the breast cancer cell lines except T5-PRF. In the isolation of nuclear matrix, we extract nuclease-digested nuclei with 0.25 M ammonium sulfate followed by several extractions with 2 M NaCl. The Khanuja et al. [1993] protocol has a salt extraction of 0.25 M ammonium sulfate prior to nuclease digestion. We found that the high salt extraction step is re-

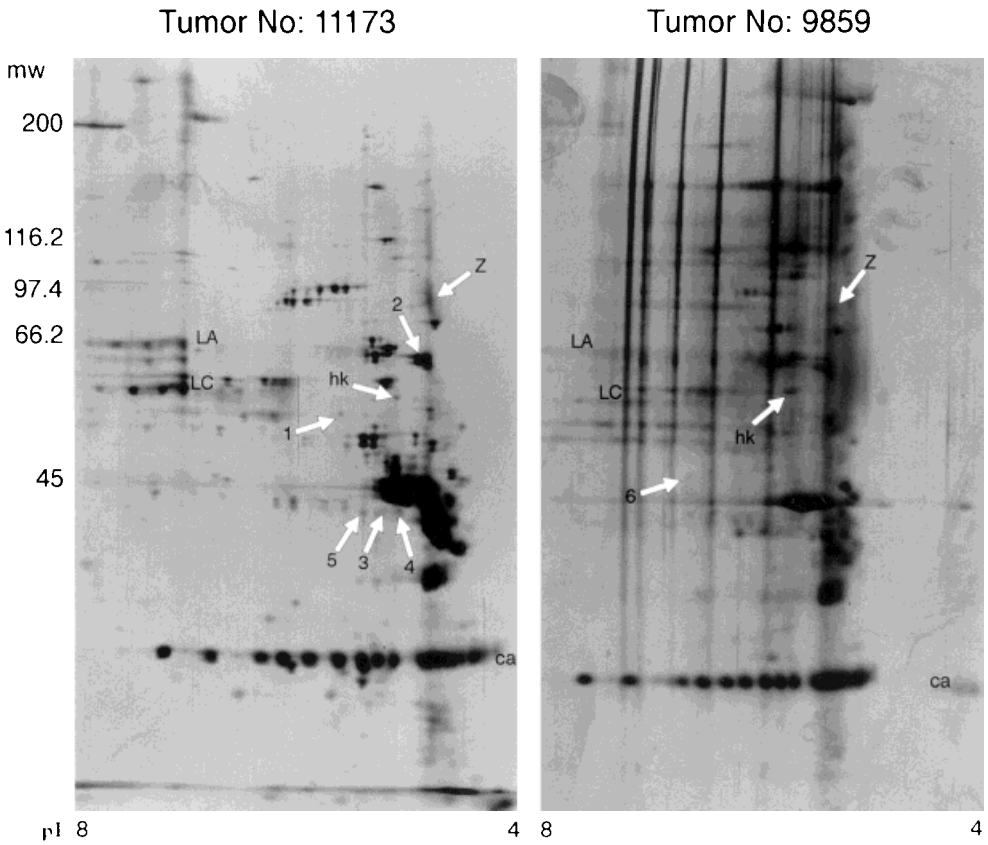

Fig. 3. Representative NMP profiles of ER+ and ER− human breast tumours. NMPs from 0.5 g of a tumour were electrophoretically resolved on two-dimension gels. The gels were stained with silver. The position of the carbamylated forms of carbonic anhydrase is indicated by ca. The position of the molecular weight standards (in thousands) is shown on the left side of each two-dimensional gel pattern. LA and LC show the position of lamin A and C, respectively. The white arrows in the gel pattern of NMPs from the ER+ tumour show the location of NMBCs 1–5 (1–5) unique to the ER+ human breast cancer cell lines and NMBC-Z (Z) being expressed in vivo. The white arrows in the gel pattern of NMPs from the ER− tumour show the location of NMBC-6 (6) unique to the ER− human breast cancer cell lines and NMBC-Z. The location of hnRNP-K is indicated as hK.

quired to remove histones and other nonmatrix nuclear proteins from the nuclear matrix preparation [Coutts et al., 1996]. This rigorous extraction procedure provides confidence that the proteins remaining with the nuclear matrix preparation are NMPs. As NMBCs X and Y were not observed in our NMPs, these proteins were likely extracted from the nuclear matrices, suggesting that NMBCs X and Y were either not nuclear matrix proteins or were proteins not tightly associated with the nuclear matrix.

In summary, we have identified five NMBCs (1–5) exclusive to ER+ breast cancer cell lines (T47D, ZR-75, MCF-7, T5-PRF) and NMBC (6) exclusive to ER−/hormone-independent cell lines (BT-20, MDA MB231). The presence of NMBCs 1–5 in T5-PRF suggests that these proteins are exclusive to ER status and not to hormone dependence. None of these proteins were found in MCF-10A1. Furthermore, using ER+ and ER− human breast tumours, we were able to confirm the presence of NMBCs 1–5 (exclusive to ER+ cell lines) in ER+ tumours and NMBC 6 (exclusive to ER− cell lines) in ER− tumours. The results of this study reinforce the observations that NMPs are potentially excellent prognostic indicators. The presence or absence of NMPs may be identified with further characterization as biomarkers of malignant progression.

ACKNOWLEDGMENTS

The Natalie Riedle, J. Simmons Memorial, and N.W. Simmons Memorial were awarded to T.M. We thank Dr. Janice Safneck for reviewing this manuscript.

REFERENCES

Aamdal S, Bormer O, Jorgensen O, Host H, Eilassen G, Kaalhus O, Pihl A (1984): Estrogen receptor and long term prognosis in breast cancer. Cancer 53:2525–2529.

Berezney R, Basler J, Hughes BB, Kaplan SC (1979): Isolation and characterization of the nuclear matrix from Zajdela ascites hepatoma cells. Cancer Res 39:3031–3039.

Bidwell J, Van Wijnen A, Banerjee C, Fey E, Merriman H, Penman S, Stein J, Lian J, Stein G (1994a): Parathyroid-responsive modifications in the nuclear matrix of ROS 17/2.8 rat osteosarcoma cells. Endocrinology 134:1738–1744.

Bidwell JP, Fey EG, van Wijnen AJ, Penman S, Stein JL, Lian JB, Stein GS (1994b): Nuclear matrix proteins distinguish normal diploid osteoblasts from osteosarcoma cells. Cancer Res 54:28–32.

Chen HY, Sun J-M, Hendzel MJ, Rattner JB, Davie JR (1996): Changes in the nuclear matrix of chicken erythrocytes that accompany maturation. Biochem J 320:257–265.

Clarke R, Dickson RB, Brunner N (1990): The process of malignant progression in human breast cancer. Ann Oncol 1:401–407.

Coutts AS, Davie JR, Dotzlaw H, Murphy LC (1996): Estrogen regulation of nuclear matrix-intermediate filament proteins in human breast cancer cells. J Cell Biochem 63:174–184.

Donat TL, Sakr W, Lehr JE, Pienta KJ (1996): Unique nuclear matrix protein alterations in head and neck squamous cell carcinomas: Intermediate biomarker candidates. Otolaryngol Head Neck Surg 114:387–393.

Ernster VL, Barclay J, Kerlikowske K, Grady D, Henderson C (1996): Incidence of and treatment for ductal carcinoma in situ of the breast. JAMA 275:913–918.

Fey EG, Penman S (1988): Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci U S A 85:121–125.

Getzenberg RH, Pienta KJ, Huang EY, Coffey DS (1991a): Identification of nuclear matrix proteins in the cancer and normal rat prostate. Cancer Res 51:6514–6520.

Getzenberg RH, Pienta KJ, Huang EY, Murphy BC, Coffey DS (1991b): Modifications of the intermediate filament and nuclear matrix networks by the extracellular matrix. Biochem Biophys Res Commun 179:340–344.

Getzenberg RH, Konety BR, Oeler TA, Quigley MM, Hakam A, Becich MJ, Bahnson RR (1996): Bladder cancer–associated nuclear matrix proteins. Cancer Res 56:1690–1694.

Godolphin W, Elwood JM, Spinelli JJ (1981): Estrogen receptor quantitation and staging as complementary prognostic indicators in breast cancer: A study of 583 patients. Int J Cancer 28:677–683.

Keesee SK, Meneghini MD, Szaro RP, Wu Y-J (1994): Nuclear matrix proteins in human colon cancer. Proc Natl Acad Sci U S A 91:1913–1916.

Khanuja PS, Lehr JE, Soule HD, Gehani SK, Noto AC, Choudhury S, Chen R, Pienta KJ (1993): Nuclear matrix proteins in normal and breast cancer cells. Cancer Res 53:3394–3398.

Kuller LH (1995): The etiology of breast cancer—from epidemiology to prevention. Public Health Rev 23:157–213.

Leonessa F, Boulay V, Wright A, Thompson EW, Brunner N, Clarke R (1992): The biology of breast tumor progression. Acquisition of hormone independence and resistance to cytotoxic drugs. Acta Oncol 31:115–123.

Mattern KA, Humbel BM, Muijsers AO, de Jong L, van Driel R (1996): HnRNP proteins and B23 are the major proteins of the internal nuclear matrix of HeLa S3 cells. J Cell Biochem 62:275–289.

McGuire WL, Clark GM, Dressler LG, Owens MA (1986): Role of steroid hormone receptors as prognostic factors in primary breast cancer. NCI Monogr 19–23.

Michelotti GA, Michelotti EF, Pullner A, Duncan RC, Eick D, Levens D (1996): Multiple single-stranded *cis* elements are associated with activated chromatin of the human c-*myc* gene in vivo. Mol Cell Biol 16:2656–2669.

Miller TE, Beausang LA, Winchell LF, Lidgard GP (1992): Detection of nuclear matrix proteins in serum from cancer patients. Cancer Res 52:422–427.

Pardee AB (1989): Principles of cancer biology: Biochemistry and cell biology. In Devita VT, Hellman (eds): "CANCER, Principles and Practices of Oncology." Philadelphia: J.B. Lippincott.

Partin AW, Getzenberg RH, CarMichael MJ, Vindivich D, Yoo J, Epstein JI, Coffey DS (1993): Nuclear matrix protein patterns in human benign prostatic hyperplasia and prostate cancer. Cancer Res 53:744–746.

Pienta KJ, Murphy BC, Getzenberg RH, Coffey DS (1991): The effect of extracellular matrix interactions on morphologic transformation in vitro. Biochem Biophys Res Commun 179:333–339.

Raymond WA, Leong AS (1989a): Vimentin—a new prognostic parameter in breast carcinoma? J Pathol 158:107–114.

Raymond WA, Leong AS (1989b): Co-expression of cytokeratin and vimentin intermediate filament proteins in benign and neoplastic breast epithelium. J Pathol 157:299–306.

Replogle TS, Pienta KJ (1996): Role of the nuclear matrix in breast cancer. In Dickson R, Lippman M (eds): "Mammary Tumor Cell Cycle, Differentiation, and Metastasis." Boston/Dordrecht/London: Kluwer Academic Publishers, pp 127–140.

Replogle-Schwab TS, Pienta KJ, Getzenberg RH (1996): The utilization of nuclear matrix proteins for cancer diagnosis. Crit Rev Eukaryot Gene Expr 6:103–113.

Ruiz Cabello J, Berghmans K, Kaplan O, Lippman ME, Clarke R, Cohen JS (1995): Hormone dependence of breast cancer cells and the effects of tamoxifen and estrogen: 31P NMR studies. Breast Cancer Res Treat 33:209–217.

Samuel SK, Minish TM, Davie JR (1997): Altered nuclear matrix protein profiles in oncogene transformed fibroblasts exhibiting high metastatic potential. Cancer Res 57:147–151.

Thompson EW, Paik S, Brunner N, Sommers CL, Zugmaier G, Clarke R, Shima TB, Torri J, Donahue S, Lippman ME, Martin GR, Dickson RB (1992): Association of increased basement membrane invasiveness with absence of estrogen receptor and expression of vimentin in human breast cancer cell lines. J Cell Physiol 150:534–544.

Watson PH, Snell L, Parisien M (1996): The NCIC-Manitoba breast tumour bank: A resource for applied cancer research. Can Med Assoc J 155:281–283.