Advances in Brief

Association between Keratin and Vimentin Expression, Malignant Phenotype, and Survival in Postmenopausal Breast Cancer Patients[1]

Patricia A. Thomas,[2] Dawn A. Kirschmann, James R. Cerhan, Robert Folberg, Elisabeth A. Seftor, Thomas A. Sellers, and Mary J. C. Hendrix[3]

University of Iowa Cancer Center [P. A. T., D. A. K., J. R. C., R. F., E. A. S., M. J. C. H.], Departments of Pathology [P. A. T., R. F.], Ophthalmology [R. F.], Anatomy and Cell Biology [D. A. K., E. A. S., M. J. C. H.], Preventive Medicine and Environmental Health [J. R. C.], The University of Iowa, Iowa City, Iowa 52242-1109 and Health Sciences Research [T. A. S.], Mayo Clinic, Rochester, Minnesota 55905

Abstract

Pathology observational reports and experimental data suggest that keratin and vimentin intermediate filament (IF) coexpression in breast cancer confers a more aggressive "interconverted" phenotype, expressing both epithelial and mesenchymal markers. In this study, we extended previous observations by measuring the expression of keratin and vimentin, in relation to other selected biomarkers of disease progression, in postmenopausal women with breast cancer. Using immunohistochemical analysis of 54 archival, formalin-fixed, paraffin-embedded invasive breast cancers from a well-defined cohort, we examined relative IF (keratin and vimentin) expression in a semiquantitative fashion and compared these results with other biological markers and survival. By univariate analysis, we found that vimentin expression was inversely associated with keratin expression alone ($P = 0.0089$) and directly related to histological grade ($P = 0.017$), nuclear grade ($P = 0.027$), Ki67 growth fraction ($P = 0.024$), and epidermal growth factor receptor immunostaining ($P = 0.019$). The relative expression of keratin and vimentin in approximately similar amounts characterized tumors with the poorest prognosis, as compared with keratin-high/vimentin-negative or keratin-low/vimentin-positive tumors. These latter two groups demonstrated similar Kaplan-Meier survival curves; the former group (keratin and vimentin in approximately similar amounts) demonstrated a poorer survival, with a hazard ratio of 2.1 (95% confidence interval, 0.5–9.6). These data suggest that relative keratin and vimentin IF expression is more indicative of prognosis and tumor phenotype than either IF marker detected independently.

Introduction

IF[4] proteins are best known for their cell type specificity and their static structural role as components of the vertebrate cell cytoskeleton. There is growing evidence for IF involvement in a variety of dynamic cellular functions, including intercellular and cell-to-extracellular matrix signal transduction, cellular motility, and tumor cell invasiveness (1–5).

Infidelity of IF expression, *i.e.*, expression of the mesenchymal marker vimentin by an epithelial cell, or expression of more than one type of IF (coexpression) has been observed *in vitro* in certain tumor cell lines and in cellular and tissue samples of certain human malignancies (6–13). Recently, an *in vitro* model of keratin and vimentin coexpression in breast cancer cells (developed as stable transfectants) has revealed that these keratin/vimentin IF-positive cells display increased proliferation rates, invasive potential, clonogenicity, and tumorigenicity when compared with keratin-positive/vimentin-negative controls displaying low invasive potential (12). Furthermore, the keratin/vimentin-positive, transfected breast cancer cells demonstrated networks of well-formed IFs, which appear as interwoven keratin and vimentin filamentous networks. The *in situ* appearance of vimentin immunoreactivity in human breast cancer cells, which normally express only the epithelial IF marker keratin, has been reported in up to 61% of human breast cancers (10, 13–19). The immunohistochemical detection of vimentin expression in invasive breast cancer has also been associated with biomarkers of poor prognosis and adverse clinical outcomes (14–19). In contrast, increased expression of certain keratins has been associated with a favorable outcome (20).

In this study, we postulated that expression of both keratin and vimentin IFs is associated with a more aggressive phenotype in human breast cancers, compared with tumors expressing either IF alone. Furthermore, we propose that the relationship or relative amounts of these two IF proteins are more important than the mere aberrant expression of the mesenchymal marker vimentin. We therefore undertook a retrospective study using immunohistochemical analysis of keratin and vimentin IF pro-

Received 7/9/99; accepted 8/24/99.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by NIH/National Cancer Institute Minority Supplement 3R01CA59702-06S1 (to P. A. T.), CA59702 (to M. J. C. H.), CA39712 (to T. A. S.), and the Search to Prevent Blindness Senior Scientific Investigator Award (to R. F.).
[2] Present address: Department of Pathology and Laboratory Medicine, Kansas University Medical Center, 3901 Rainbow Boulevard, Kansas City, Kansas 66160-7281.
[3] To whom requests for reprints should be addressed, at Department of Anatomy and Cell Biology, 1–100 BSB, The University of Iowa, College of Medicine, 51 Newton Road, Iowa City, IA 52242-1109. Fax: (319) 335-7770; E-mail: mary-hendrix@uiowa.edu.

[4] The abbreviations used are: IF, intermediate filament; FNAB, fine-needle aspiration biopsy.

teins in invasive breast cancers from a well-defined patient cohort of postmenopausal women and assessed the relative expression of these IF protein markers in tumor tissue sections. Vimentin expression was also compared with other known prognostic markers for disease progression. We identified statistically significant associations between vimentin expression and histological grade, nuclear grade, Ki67, epithelial growth factor receptor, and keratin expression. In addition, poorer survival of breast cancer patients was observed when tumor cells expressed both keratin and vimentin IFs in approximately equal ratios. The data derived from this study may provide important translational significance to the utility of using vimentin:keratin ratios in the diagnosis of breast pathology, including fine-needle aspirates.

Materials and Methods

Patients and Breast Cancer Tissues. Diagnostic breast cancer tissues from patients participating in the Iowa Women's Health Study were obtained from the University of Iowa Hospitals and Clinics and the Iowa Methodist Hospital. Breast cancer was diagnosed from 1986 to 1993, and follow-up through the Iowa Cancer Registry was complete through December 31, 1996. None of the patient population was lost to follow-up in this study. Cause of death was determined from the death certificate (ICD 9 code), and the primary outcome variable was time-to-death (all causes). Death attributable to breast cancer was also evaluated.

Paraffin-embedded tissue blocks of any histological subtype of invasive breast cancer, original H&E-stained slides, and surgical reports were collectively requested from University of Iowa Hospitals and Clinics and Iowa Methodist Hospital. Cases were excluded if H&E-stained slides were not available for review, tissue blocks were not available, no invasive tumor remained in the block, or if insufficient material was present for block sectioning. A total of 63 cases were received, of which 54 fit the inclusion criteria.

Immunohistochemical Staining. The appropriate tissue blocks were chosen for processing based on review of the original H&E-stained slides. Four sections, each 4-μm in thickness, were cut and placed on poly-L-lysine-coated slides for immunohistochemistry. A total of 60 sequential sections were prepared per block. The sections used for the immunohistochemical studies were deparaffinized in xylene and rehydrated in graded alcohols, and the first and last sections of each tissue block were stained with H&E. The remaining sections were used for immmunohistochemical analysis. Monoclonal antibodies against keratin or BDK (CAM5.2) and vimentin (V9 clone) were purchased from Becton Dickinson (San Jose, CA) and BioGenex (San Ramon, CA), respectively. Other antibodies used were as follows: estrogen receptor (Dako, Carpinteria, CA), progesterone receptor (Nova Castra, Vector Labs, Burlingame, CA); Ki67 (MIB1 clone; Immunotech, West Brook, ME); c-erbB2 (Signet, Dedham, MA); p53 (Dako); epidermal growth factor receptor (BioGenex); and cathepsin (Signet). All antibodies were applied after antigen retrieval, as per manufacturer's instructions. Labeled streptavidin-biotin and diaminobenzidine were used in the detection reactions. Color photographic images were recorded using an Olympus AH-2 photomicroscope and a Kodographic digital color printer.

Table 1 Semiquantitative immunohistochemical analysis criteria

None *vs.* some staining	<10% *vs.* ≥10%	<50% *vs.* ≥50%
Vimentin	Estrogen receptor	Keratin
p53	Progesterone receptor	
Cathepsin	c-erbB2	
	Epithelial growth factor receptor	

Histological sections were examined at ×40 to identify areas of maximum tumor staining. At ×400, 200 cells were analyzed for staining, and the percentage of positive cells was recorded. This procedure was repeated, and the two percentages were averaged. On the basis of the known biological significance of the markers and sample size considerations, immunostaining was *a priori* categorized into the above three groups for data analysis.

Semiquantitative Immunohistochemical Scoring. Each histological section was examined at ×40 to identify areas of maximum tumor staining. At ×400, 200 cells were analyzed (in the areas of maximum tumor staining), and the percentage of positive cells was recorded. This procedure was repeated, and the average of the two percentages was recorded. These averaged values were originally stratified into four scoring groups: (*a*) no immunopositive cells identified; (*b*) <10% positive tumor cells; (*c*) 10–50% positive tumor cells; and (*d*) >50% positive tumor cells. On the basis of known biological significance of the markers analyzed and sample size considerations, immunostaining was *a priori* further subcategorized into three groups for data analysis: (*a*) no staining *versus* some staining; (*b*) <10% staining *versus* ≥10% staining; and (*c*) <50% *versus* ≥50% staining (Table 1).

Histological Grade and Nuclear Grade. Histological grade was determined using the histological criteria described previously by Elston and Ellis (21). Nuclear grade criteria were derived from the modified Scarff-Bloom-Richardson method (22). For both histological and nuclear grade, a scale of 1 to 3 was used; grade 1 corresponds to well-differentiated or low grade, grade 2 to moderately differentiated or intermediate grade, and grade 3 to poorly differentiated or high grade.

Statistical Methods. χ^2 analysis (or Fisher's exact test, as appropriate) was used to test for associations between vimentin staining and other biological markers. To evaluate the association between vimentin and keratin staining and survival, Kaplan-Meier curves and Cox proportional hazards regression were used (23). Analyses were conducted using SAS (Carey, NC) and EGRET (Seattle, WA).

Results

Tumor and Patient Characteristics. The histopathological characteristics of tumor samples derived from 54 women diagnosed with breast cancer were assessed. All patients were postmenopausal, and the mean age at diagnosis was 66.3 years (range, 56.1–76.1). The stage at presentation was localized for 72% of the patients. Tumor size was 2.0 cm or less in 44% of the patients, >2.0 cm in 36% of the patients, and unreported for 20% of the patients. The majority of tumors were of low to intermediate histological and nuclear grade, 78 and 60%, re-

Table 2 Correlation of vimentin and other breast cancer progression marker expression

		Vimentin		
	n^a (53)	Negative	Positive[b]	P^c
Keratin				
<50%	7	0%[d]	100%	
≥50%	37	59%	41%	0.0089
Stage at diagnosis				
Local	38	53%	47%	
Regional/Distant	15	53%	47%	0.96
Tumor size (cm)				
<1.0	6	67%	33%	
1–1.9	18	39%	61%	
>2.0	19	58%	42%	0.37
Histological grade				
Low/Intermediate	42	62%	38%	
High	10	20%	80%	0.017
Nuclear grade				
Low/Intermediate	31	65%	35%	
High	21	33%	67%	0.027
Estrogen receptor				
<10%	12	33%	67%	
≥10%	41	59%	41%	0.12
Progesterone receptor				
<10%	28	50%	50%	
≥10%	18	56%	44%	0.71
Ki67 (MIB-1)				
<10%	35	63%	37%	
≥10%	17	29%	71%	0.024
c-*erb*B2				
<10%	17	53%	47%	
≥10%	35	51%	49%	0.92
p53				
Negative	37	54%	46%	
Positive	15	47%	53%	0.63
Epithelial growth factor receptor				
<10%	48	58%	42%	
≥10%	5	0%	100%	0.019
Cathepsin				
Negative	9	44%	56%	
Positive	44	55%	45%	0.58

[a] *n*, number of cases; number of cases may not sum to the total of 53 due to missing values on the variable of interest (vimentin data was not obtainable in one case).
[b] Any vimentin staining was counted as positive.
[c] Statistically significant at $P < 0.05$.
[d] Numbers represent the percentage of tumors with positive or negative staining for each parameter.

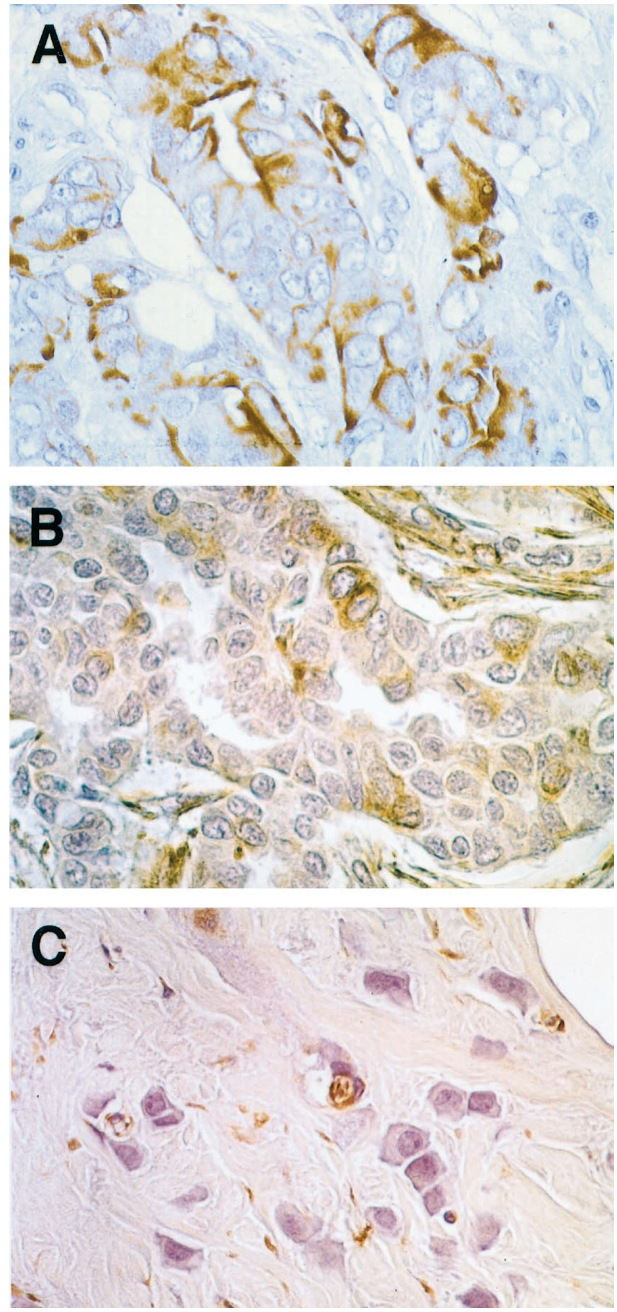

Fig. 1 Keratin and vimentin expression in breast cancer tissues. Expression of keratin IFs (*A*) and vimentin IFs (*B*) in ductal breast carcinomas and vimentin IFs (*C*) in lobular breast carcinomas was evaluated by immunoperoxidase analysis, as described in "Materials and Methods." ×160.

spectively (shown in Table 2). Seventy-eight % of the tumors showed >10% of tumor cells positive for estrogen receptor (determined immunohistochemically). These findings are consistent with expected tumor characteristics in this patient population (24–26).

Semiquantitative Immunohistochemical Analysis of Keratin and Vimentin IFs in Breast Tumor Tissues. To determine the relative expression of keratin and vimentin IFs in primary breast cancers, immunohistochemical staining for keratin and vimentin was assessed semiquantitatively, as described in Table 1. Keratin immunostaining was interpretable in 44 cases and vimentin in 53 cases. Poor tissue preservation precluded assessment in some cases. Of the 44 cases for which keratin staining could be assessed, 100% of these showed some degree of keratin positivity; in 7 of 44 cases (16%), keratin positivity was seen in ≤50% of the tumor cells. Characteristic immunostaining patterns for keratin are illustrated in Fig. 1*A*. Vimentin immunopositivity was seen in 25 of 53 cases (47%). In 4 of these 25 cases (16%), vimentin staining was present in >50% of the tumor cells, as shown in Fig. 1*B*. Vimentin positivity was also observed in lobular breast carcinomas, as demonstrated in Fig. 1*C*. These data suggest that keratin expres-

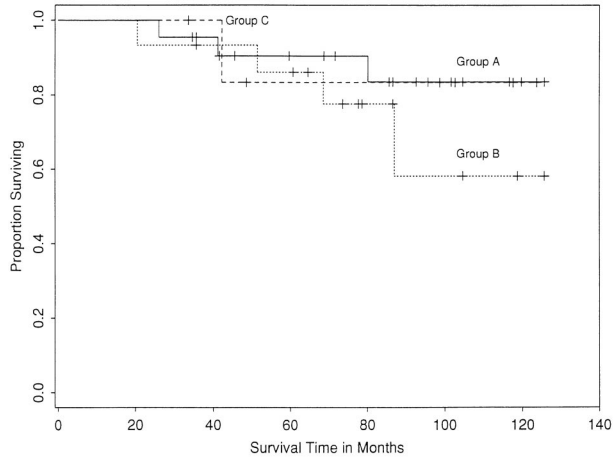

Fig. 2 Keratin and vimentin coexpression in breast cancer tissues and patient survival. Kaplan-Meier survival curves for breast cancer patients with tumors immunostaining: *A*, >50% keratin staining *versus* no vimentin staining; *B*, >50% keratin staining *versus* any vimentin staining; and *C*, <50% keratin staining *versus* any vimentin staining.

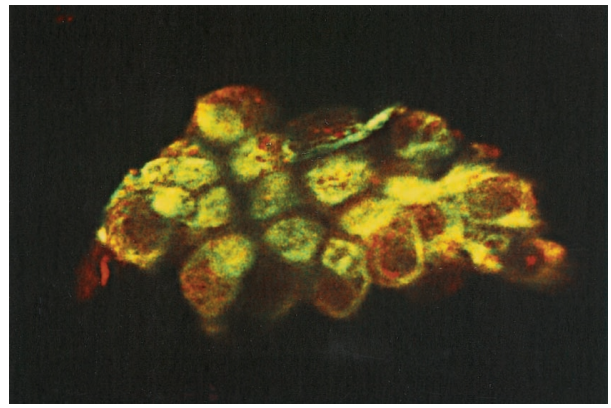

Fig. 3 Keratin and vimentin coexpression in a FNAB. A FNAB specimen from a 45-year-old female with a family history of breast cancer and presenting with a benign, but atypical, indistinct breast nodule was stained with keratin (FITC) and vimentin (rhodamine) monoclonal antibodies as described in "Materials and Methods."

sion is consistently expressed in breast tumors, confirming the epithelial origin of the tumors, whereas vimentin expression occurs to a variable degree in a subset of both lobular and ductal carcinomas.

Comparison of Vimentin Expression with Other Breast Tumor Biomarkers. The presence of vimentin IFs was compared with keratin staining and other biomarkers to determine the validity of using vimentin as an independent prognostic marker for breast cancer metastatic potential. All of the tumors containing <50% keratin expression were immunopositive for vimentin, whereas only 41% of the tumors with >50% keratin expression were vimentin immunopositive ($P = 0.0089$), as illustrated in Table 2. Vimentin immunopositivity was also significantly associated with high histological grade ($P = 0.017$), high nuclear grade ($P = 0.027$), large Ki67 growth fractions ($P = 0.024$), and greater epidermal growth factor receptor immunostaining ($P = 0.019$), presented in Table 2. Conversely, vimentin immunostaining did not significantly correlate with tumor stage, size, or immunostaining for progesterone receptor, c-*erb*B2, cathepsin, or p53. There was suggestive evidence that vimentin immunostaining was inversely correlated with immunostaining for estrogen receptor ($P = 0.12$). These data suggest that the level of keratin and vimentin expression in breast cancers is associated with other known prognostic markers in a somewhat specific manner of the biomarkers selected for study.

IF Expression and Survival Analysis. Survival curve analyses were performed to determine a possible relationship between patient outcome and keratin and vimentin expression in the breast tumors examined. Through December 31, 1996, 11 of the 54 women (20%) had died from any cause. The median follow-up time was 86.1 months (range, 20.5 to 125.6 months). Fig. 2 illustrates the Kaplan-Meier survival curves obtained from patients with tumors classified by keratin and vimentin expression. Patients, whose primary breast tumors were both keratin and vimentin positive (group B), had a poorer survival outcome (hazard ratio, 2.1; 95% confidence interval, 0.5–9.6) than patients whose primary breast tumors were only keratin positive (group A) or only vimentin positive (group C: hazard ratio, 1.0; 95% confidence interval, 0.1–10). When survival analysis was performed using death attributable to breast cancer as an outcome ($n = 7$), group B continued to have a poorer survival outcome compared with group A (hazard ratio, 5.9; 95% confidence interval, 0.7–53; data not shown). There were no deaths attributable to breast cancer in group C. Although the survival differences are not statistically different from each other, there is a reasonable trend showing that these data support the hypothesis that coexpression of keratin and vimentin are associated with poorer overall survival.

Demonstration of Keratin and Vimentin in a FNAB. FNAB is a technique widely used as a safe and economical first-line biopsy procedure for the diagnosis of palpable and nonpalpable breast masses (27–29). Immunohistochemical analysis of keratin and vimentin IFs in mammary tissues obtained from FNABs was investigated to determine whether IF immunostaining can be used as a prognostic indicator of aggressive breast cancer in a technique routinely used for diagnosis. Fig. 3 shows keratin and vimentin immunostaining of an FNAB specimen from a 45-year-old female with a family history of breast cancer and presenting with a benign, but atypical, indistinct breast nodule. This particular type of atypical lesion is associated with an increased risk for the subsequent development of cancer (30, 31). Keratin (FITC stain) and vimentin (rhodamine stain) IFs were colocalized in this specimen, demonstrating that keratin and vimentin immunohistochemical analysis can be performed on FNAB specimens. On the basis of family history, keratin and vimentin IF coexpression, and the correlation of keratin and vimentin IF coexpression with a poorer survival rate, these clinical data collectively suggest that this individual may be at high risk for developing invasive and metastatic breast cancer.

Discussion

Axillary lymph node status remains perhaps the most accepted prognostic factor for human breast cancer (32–34). However, detection of positive lymph nodes occurs late in tumor progression, and negative lymph nodes do not preclude aggressive disease or subsequent distant metastases. Therefore, identification of new tumor markers that can predict the most aggressive behavior at an early point in tumor development and progression is of paramount importance.

Because alterations in cellular IF expression occur as a key feature in the transition from the benign to the metastatic phenotype in several tumor types, there is strong rationale to pursue the validation of this family of cell type-specific proteins as putative prognostic markers in breast tumor progression. Previous reports evaluating prognostic markers for breast cancer aggressiveness *in vitro* have presented strong direct evidence correlating coexpression of vimentin and keratin IFs with an invasive and metastatic phenotype (5, 6, 8, 12). In this present study of postmenopausal breast cancer tissues, we sought to further test the hypothesis that vimentin, combined with keratin immunohistochemical staining, could provide useful prognostic information of disease and, possibly, survival status.

The results indicate that vimentin immunopositivity in breast cancer tissues is inversely related to keratin expression ($P = 0.0089$) and to a certain extent, estrogen receptor expression ($P = 0.12$), and directly correlated with histological grade ($P = 0.017$), nuclear grade ($P = 0.027$), Ki67 growth fraction ($P = 0.024$), and epidermal growth factor receptor expression ($P = 0.019$). The demonstration of a relationship between IF expression and histological and nuclear grade of breast cancer tissues extend and confirm our previous experimental evidence of the relationship between IF expression and tumor cell phenotype (6, 8, 9, 12, 13). In addition, the observed relationship between vimentin IF expression and Ki67 growth fraction also substantiates the experimental finding of increased proliferation rates of primary tumors comprised of interconverted breast cancer cells (18).

Our study further suggests that it is the ratio of vimentin and keratin IF coexpression that predicts patient survival, rather than the presence of either IF alone. This observation is consistent with the morphological appearance of the more invasive, interconverted, vimentin/keratin expressing breast carcinoma cell transfectants, which show coexpression of interwoven IFs (12). These results, in conjunction with previous studies, strongly suggest that further evaluation of IF expression in the prognostic evaluation of human breast cancers is warranted. In fact, recent work has demonstrated the powerful approach of combining tumor arrays and cDNA arrays to evaluate the predictive power of using vimentin as a molecular marker in the screening of renal cell carcinoma (35). It is highly likely that the vimentin/keratin data generated from the preliminary clinical finding in postmenopausal breast cancer samples can also be adapted in a screening method for disease status, either in tissue samples or in FNABs. Indeed, beneficial information regarding the prediction of breast cancer disease progression and perhaps even patient survival would be fruitful outcomes from this work. Much larger studies are obviously needed to assess vimentin and keratin coexpression in disease-free survival.

Acknowledgments

We thank Dr. Richard G. Lynch for his support and encouragement for this research study, and Christine Bromley, Jan Rodgers, and Mary Sturm for immunohistochemical staining expertise and assistance.

References

1. Steinert, P. M., and Liem, R. K. Intermediate filament dynamics. Cell, *60:* 521–523, 1990.

2. Skalli, O., and Goldman, R. D. Recent insights into the assembly, dynamics, and function of intermediate filament networks. Cell Motil., *19:* 67–79, 1991.

3. Chu, Y-W., Runyan, R. B., Oshima, R. G., and Hendrix, M. J. C. Expression of complete keratin filaments in mouse L cells augments cell migration and invasion. Proc. Natl. Acad. Sci. USA, *90:* 4261–4265, 1993.

4. Fuchs, E., and Wever. K. Intermediate filaments: structure, dynamics, function, and disease. Annu. Rev. Biochem., *63:* 345–382, 1994.

5. Hendrix, M. J. C., Seftor, E. A., Chu, Y-W., Trevor, K. T., and Seftor, R. E. B. Role of intermediate filaments in migration, invasion and metastasis. Cancer Metastasis Rev., *15:* 507–525, 1996.

6. Ramaekers, F. C. S., Haag, D., Kant, A., Moesker, O., Hap, P. H. K., and Vooljs, G. P. Coexpression of keratin and vimentin type intermediate filaments in human metastatic carcinoma cells. Proc. Natl. Acad. Sci. USA, *80:* 2618–2622, 1983.

7. Sommers, C. L., Walker-Jones, D., Heckford, S. E., Worland, P., Valverius, E., Clark, R., McCormick, F., Stampfer, M., Abularach, S., and Gelmann, E. P. Vimentin rather than keratin expression in some hormone-independent breast cancer cell lines and in oncogene-transformed mammary epithelial cells. Cancer Res., *49:* 4258–4263, 1989.

8. Hendrix, M. J. C., Seftor, E. A., Chu, Y-W., Seftor, R. E. B., Nagle, R. B., McDaniel, K. M., Leong, S. P. L., Yohem, K. H., Leibovitz, A., Meyskens, F. L., Conoway, D. H., Welch, D. R., Liotta, L. A., Stetler-and Stevenson, W. G. Coexpression of vimentin and keratin by human melanoma tumor cells: correlation with invasive and metastatic potential. J. Natl. Cancer. Inst., *84:* 165–174, 1992.

9. Azumi, N., and Battifora, H. The distribution of vimentin and keratin in epithelial and nonepithelial neoplasms. Am. J. Clin. Pathol., *88:* 286–296, 1987.

10. Athanassiadou, P., Athanassiades, P., Kyrkou, K., Giahnaki, E., Giannioti, E., and Nanas, S. Expression of vimentin and epidermal growth factor receptor in effusions from patient with breast cancer: correlation with oestrogen and progesterone receptor status. Cytopathology, *4:* 91–98, 1993.

11. Ioakim-Liossi, A., Karakitsos, P., Markopoulos, C. H., Aroni, K., Gogas, J., and Kyrkou, K. DNA ploidy and vimentin expression in primary breast cancer. Cytopathology, *6:* 325–330, 1995.

12. Hendrix, M. J. C., Seftor, E. A., Seftor, R. E. B., and Trevor, K. T. Experimental co-expression of vimentin and keratin intermediate filaments in human breast cancer cells results in phenotypic interconversion and increased invasive behavior. Am. J. Pathol., *150:* 483–495, 1997.

13. Raymond, W. A., and Leong, A. S. Co-expression of cytokeratin and vimentin intermediate filament proteins in benign and neoplastic breast epithelium. J. Pathol., *157:* 299–306, 1989.

14. Santini, D., Ceccarelli, C., Taffurelli, M., Pileri, S., and Marrano, D. Differentiation pathways in primary invasive breast carcinoma as suggested by intermediate filament and biopathological marker expression. J. Pathol., *179:* 386–391, 1996.

15. Bell, C. D., Tischler, E. M., and Laroye, G. J. The relationship of cytoplasmic intermediate filaments and membrane antigens with hormone receptors, nuclear staining density, and mode of stromal invasion in human breast cancer. Breast Cancer Res. Treat., *33:* 147–162, 1995.

16. Domagala, W., Markiewski, M., Kubiak, R., Barkowiak, J., and Osborn, M. Immunohistochemical profile of invasive lobular carcinoma of the breast: predominantly vimentin and p53 protein negative, cathepsin D and oestrogen receptor positive. Virchows. Arch. A Pathol. Anat. Histopathol., *423:* 497–502, 1993.

17. Domagala, W., Striker, G., Szadowska, A., Dukowicz, A., Harezga, B., and Osborn, M. p53 protein and vimentin in invasive ductal NOS breast carcinoma: relationship with survival and sites of metastases. Eur. J. Cancer, *30A:* 1527–1534, 1994.

18. Domagala, W., Lasota, J., Bartowiak, J., Weber, K., and Osborn, M. Vimentin is preferentially expressed in human breast carcinomas with low estrogen receptor and high Ki-67 growth fraction. Am. J. Pathol., *136:* 219–227, 1990.

19. Domagala, W., Wozniak, L., Lasota, J., Weber, K., and Osborn, M. Vimentin is preferentially expressed in high-grade ductal and medullary but not in lobular breast carcinomas. Am. J. Pathol., *137:* 1059–1064, 1990.

20. Schaller, G., Fuchs, I., Pritze, W., Ebert, A., Hermann, H., Pantel, K., Wetzel, H., and Lengyel, E. Elevated keratin 18 protein expression indicates a favorable prognosis in patients with breast cancer. Clin. Cancer Res., *2:* 1879–1885, 1996.

21. Elston, C. W., and Ellis, I. O. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology, *16:* 403–410, 1991.

22. Le Dousal, V., Tubiana-Hulin, M., Friedman, S., Hacene, K., Spyratos, F., and Brunet, M. Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson (SBR): an improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. Cancer (Phila.), *64:* 1914–1921, 1989.

23. Bennett, S. Analysis of survival data by the proportional odds model. Stat. Med., *2:* 273–277, 1983.

24. Lippman, M. Growth regulation of breast cancer. Prog. Clin. Biol. Res., *320:* 111–119, 1989.

25. Bronzert, D. A., Davidson, N., and Lippman, M. Estrogen and antiestrogen resistance in human breast cancer cell lines. Adv. Exp. Med. Biol., *196:* 329–345, 1986.

26. Cowan, K., and Lippman, M. Steroid receptors in breast cancer. Arch. Intern. Med., *142:* 363–366, 1982.

27. Thomas, P. A., Raab, S. S., and Cohen, M. B. Is the fine-needle aspiration biopsy diagnosis of proliferative breast disease feasible? Diag. Cytopathol., *11:* 301–306, 1994.

28. Thomas, P. A., Cangiarella, J., Raab, S. S., and Waisman, J. Fine needle aspiration biopsy of proliferative breast disease. Mod. Pathol., *8:* 130–136, 1995.

29. de Paredes, E. S., Langer, T. G., and Cousins, J. Interventional breast procedures. Curr. Probl. Diag. Radiol., *27:* 133–184, 1998.

30. Page, D. L., and Dupont, W. D. Anatomic markers of human premalignancy and risk of breast cancer. Cancer (Phila.), *66:* 1326–1335, 1990.

31. Dupont, W. D., Parl, F. F., Hartmann, W. H., Brinton, L. A., Winfield, A. C., Worrell, J. A., Schuyler, P. A., and Plummer, W. D. Breast cancer risk associated with proliferative breast disease and atypical hyperplasia. Cancer (Phila.), *71:* 1258–1265, 1993.

32. Weidner, N., Cady, B., and Goodson, W. H., III. Pathologic prognostic factors for patients with breast carcinoma. Which factors are important? Surg. Oncol. Clin. N. Am., *6:* 415–462, 1997.

33. Pinder, S. E., Ellis, I. O., and Elston, C. W. Prognostic factors in primary breast carcinoma. J. Clin. Pathol., *48:* 981–983, 1995.

34. Fisher, E. R., Anderson, S., Redmond, C., and Fisher, B. Pathologic findings from the National Surgical Adjuvant Breast Project protocol B-06. 10-year pathologic and clinical prognostic discriminants. Cancer (Phila.), *71:* 2502–2514, 1993.

35. Moch, H., Scraml, P., Bubendorf, L., Mirlacher, M., Kononen, J., Gasser, T., Mihatsch, M. J., Kallioniemi, O. P., and Sauter, G. High-throughput tissue microarray assay to evaluate genes uncovered by cDNA microarray screening in renal cell carcinoma. Am. J. Pathol., *154:* 981–986, 1999.

Clinical Cancer Research

Association between Keratin and Vimentin Expression, Malignant Phenotype, and Survival in Postmenopausal Breast Cancer Patients

Patricia A. Thomas, Dawn A. Kirschmann, James R. Cerhan, et al.

Clin Cancer Res 1999;5:2698-2703.