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Vimentin Is Preferentially Expressed in Human Breast Carcinomas with Low Estrogen Receptor and High Ki-67 Growth Fraction

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Vimentin expression, growth fractions (GF), and estrogen receptor (ER) levels were determined for 90 untreated primary breast carcinomas. Coexpression of keratin and vimentin was found in approximately 20% of the tumors regardless of menopausal status. Vimentin was expressed preferentially in tumor cells of high-grade ductal breast carcinomas (15 of 28 histologic grade 3 vs. 0 of 40 grades 1 and 2). Vimentin expression was found preferentially in tumors with high GF (>15% Ki-67 positive by immunoperoxidase staining) and low ER levels (<60 fmols/mg protein by a monoclonal enzyme immunoassay). Sixty-eight percent of tumors in this group were vimentin positive and 88% of all vimentin-positive tumors fell into this category. More than 50% of the tumor cells coexpressed vimentin and keratin. Thus, vimentin expression may be helpful in identifying a substantial subset of ER-independent breast carcinomas with poor prognostic indicators. (Am J Pathol 1990, 136: 219–227)

The coexpression of vimentin and keratin in tumor cells of human breast carcinomas has been described in both histologic biopsies[1–3] and cytologic aspirates.[4] In these studies, the percentage of carcinomas showing vimentin expression ranged from 12%[1] to 60%.[3] The biologic and clinical significances of this phenomenon are not understood. Vimentin expression has been found in some estrogen-independent breast cancer cell lines,[5] and a positive correlation between vimentin and estrogen receptor (ER) negative, epidermal growth factor receptor (EGFR) positive human breast carcinomas has been reported.[2] Experimental evidence further suggests that, at least in certain cell lines in culture, vimentin mRNA levels are growth regulated.[6–8] In the past, several studies have provided evidence for an inverse correlation between ER status and the proliferative activity of breast carcinomas,[9–14] although exceptions also have been noted.[15] Proliferative activity usually is measured either by determining the thymidine labeling index (TLI) or the growth fraction (GF) with antibodies to the proliferation-associated nuclear antigen Ki-67.[16,17]

Both ER status and proliferative rate are regarded as important determinants of the pathologic features[13] and clinical behavior[12] of breast carcinomas and thus seem to have prognostic value in predicting recurrence-free survival. It therefore seemed worthwhile to test whether there was a correlation between vimentin, ER levels, and Ki-67 growth fraction in human breast carcinomas.

Materials and Methods

Specimens

Ninety consecutive and unselected patients with primary breast cancer previously untreated underwent radical mastectomy with lymph node dissection. Aspiration biopsy was performed on mastectomy specimens. Samples for Ki-67 and vimentin expression were obtained by aspiration biopsy of those portions of breast carcinomas that were subsequently excised and sent for ER monoclonal enzyme immunoassay (EIA). Fine-needle aspiration was performed by a trained cytologist as described by Koss, Woyke, and Olsewski.[18] The needle (22-gauge, 0.6 mm in diameter) was fitted to a 20-ml disposable syringe mounted in a syringe holder that facilitated easy aspiration of cellular material from different portions of the tumor by changing the direction of the needle during aspiration.

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Table 1. *Vimentin Expression in Breast Carcinomas of Different Histologic Types*

Histologic type	No. of cases	V+ >50%	V+ <5%
Invasive ductal NOS	68		
grade I	4	0/4	0/4
grade I/II	2	0/2	0/2
grade II	34	0/34	2/34
grade III	28	15/28	2/28
Invasive lobular	15	0/15	0/15
Medullary	3	1/3	0/3
Mucinous	1	0/1	0/1
Secretory	1	1/1	0/1
Apocrine	1	0/1	0/1
Male breast	1	0/1	0/1
Total	90	17/90	4/90

NOS, not otherwise specified.

The cellular material was expelled from the needle onto clean glass slides. Smears were prepared and immediately frozen on dry ice (for Ki-67) or fixed in 96% ethanol for 15 to 30 minutes at 4 C (for vimentin and keratin). They were then stored on dry ice and tested for Ki-67 within 1 week and for vimentin and keratin within 8 weeks. A parallel tissue sample was processed routinely for morphologic evaluation on paraffin sections. Histologic typing was performed according to the guidelines recommended by the World Health Organization and histologic grading was according to Bloom and Richardson.[19] The histologic diagnosis for each tumor is given in Table 1.

Estrogen Receptor

The ER EIA was run as recommended by the manufacturer (Abbot Laboratories, Chicago, IL). ER concentrations were expressed in fmol/mg cytosol protein according to the routine procedure. They were quantified in the range of 0 to 500 fmol/ml cytosol.

Immunocytochemistry

For measurement of the GF, smears were removed from dry ice storage and immediately fixed for five minutes in acetone at 4 C and then dried for 30 to 60 seconds at room temperature. Ki-67 (anti-human proliferative cell antibody, Dako, Klostrup, Denmark) was applied at a dilution of 1:50 for 45 minutes followed by a routine peroxidase-anti-peroxidase (PAP) procedure. Smears were lightly counterstained with hematoxylin.

Double-label immunofluorescence was performed with the mouse monoclonal KL1 antibody (a broad-specificity keratin antibody obtained from Dianova, Hamburg, FRG), used at a 1+10 dilution, and guinea pig vimentin antibody. This antibody was affinity purified on vimentin bound to sepharose 4B and used at a concentration of approximately 30 μg/ml. Both first antibodies were applied simultaneously. After 45 minutes at 37 C, the samples were washed with phosphate-buffered saline (PBS) and the two second antibodies were applied simultaneously. The second antibodies used were fluorescein isothiocyanate (FITC)-labeled goat anti-mouse and rhodamine-labeled goat anti-guinea pig antibodies (Cappell Laboratories, Cochranville, PA). The second antibodies were absorbed before use on unlabeled IgGs of the other species to eliminate unwanted cross-reactions. After an additional wash with PBS, the smears were counterstained with Hoechst 33258 for five minutes at room temperature to facilitate identification of nuclei under the fluorescence microscope and then mounted with no further wash in Moviol 4-88 (Hoechst, Frankfurt, FRG). Controls included tests in which a single first antibody was used together with both second antibodies to check for nonspecific cross-reactions, as well as tests in which the first antibodies were replaced by buffer on different parts of the same slide.

Statistical Analysis

Descriptive statistics were used to determine the means ($\bar{x}$) and standard deviation (S). Statistical differences of ER and Ki-67-GF values between the subgroups of breast carcinomas were determined by the Kolmogorow-Smirnoff test. The chi-square test was used to compare vimentin expression in subgroups of breast carcinomas.

Results

In view of the known heterogeneity of morphologic and immunocytochemical features of tumors one might expect an uneven distribution of vimentin-positive tumor cells within tumor tissue. To reduce the sampling error as much as possible, cellular material for study with the vimentin and Ki-67 antibodies was obtained by fine-needle aspiration biopsy taken from multiple sites in the part of the tumor used subsequently for the ER assay. In this way, tumor cells studied for the presence of vimentin and Ki-67 antigen were derived from the part of the tumor analyzed for ER and the results were more representative than those that could have been obtained from several histologic sections taken from the area of the tumor neighboring that sent for ER. We chose to assay ER by EIA because immunocytochemical assays have shown that the distribution of ER in tissue is heterogeneous[20] and this could influence the comparative analysis.

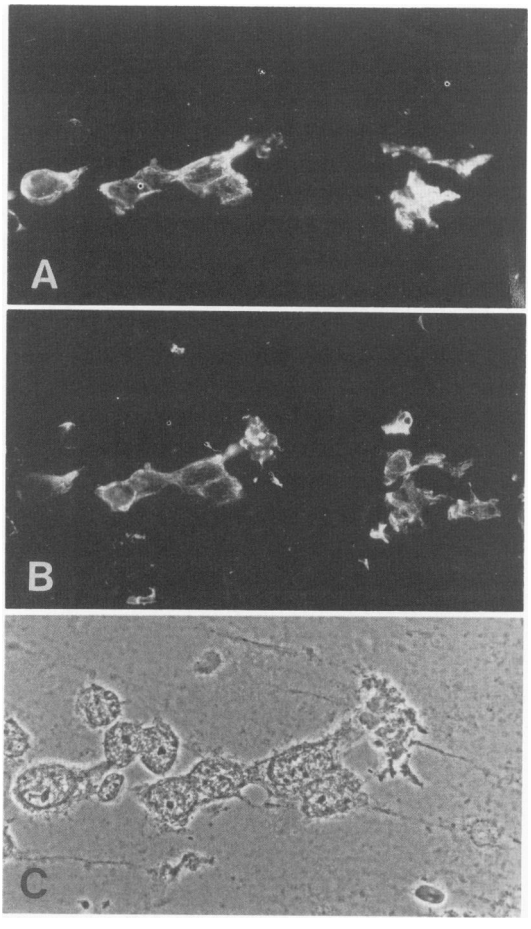

Figure 1. *Fine-needle aspirate of a breast carcinoma that coexpresses vimentin and keratin.* **A–C:** *Tumor cells labeled in double immunofluorescence microscopy by antibody to keratin* **(A)** *and antibody to vimentin* **(B).** *In this smear almost all carcinoma cells coexpress keratin and vimentin (×380).* **C:** *Phase-contrast of the cells shown in left side of Figures 2A and 2B at higher magnification (×600).*

Ninety breast carcinomas were tested for vimentin and keratin. In smears examined by immunofluorescence microscopy, more than 95% of tumor cells from all breast carcinomas were positive with the broad specificity keratin antibody (KL1). Double immunofluorescence staining on the same smears with keratin and vimentin antibodies revealed that in 21 fo 90 (23%) of the breast carcinomas some tumor cells coexpressed keratin and vimentin, whereas in the other tumors only keratin expression was seen in tumor cells. The proportion of tumor cells revealing a true coexpression of keratin and vimentin, as judged by double immunofluorescence staining, ranged from 50% to 100% in 17 cases (19%) (Figure 1). In one case, 1% to 5% of the tumor cells showed a true coexpression and in three additional cases <1% of cells coexpressed vimentin and keratin. In the remaining 69 cases, tumor cells expressed only keratin and did not show vimentin (Figure 2).

Because we cannot be sure at the less-than-1% level whether we are dealing exclusively with tumor cells (and not, for instance, with myoepithelial cells that coexpress keratin and vimentin), we have considered only tumors in which >5% of cells express vimentin as vimentin positive. Using this definition, we find that in our series, 19% of the tumors coexpress vimentin and keratin. The number of vimentin-positive breast carcinomas divided according to histologic type is shown in Table 1. The data demonstrate a strong inverse correlation between vimentin expression and histologic grade of the tumor. Vimentin was found preferentially in grade 3 invasive ductal NOS (not otherwise specified) carcinomas (15 of 28), whereas none of the grade 1 ductal NOS (0 of 4) or invasive lobular carcinomas (0 of 15) expressed vimentin (Table 1). When lymph node metastases were considered, 59% (10 of 17) of vi-

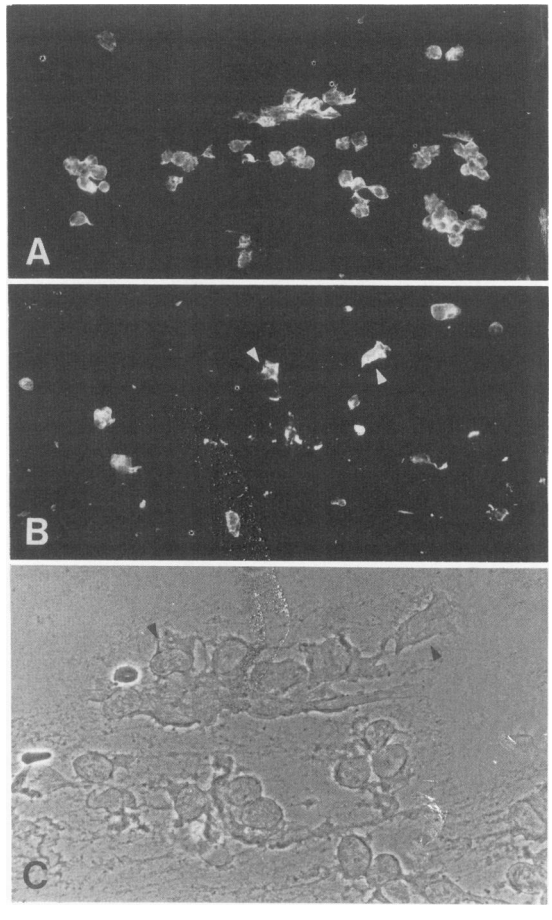

Figure 2. *Fine-needle aspirate of a breast carcinoma that shows no coexpression of keratin and vimentin.* **A–B:** *Groups of cells seen in double immunofluorescence microscopy labeled by antibody to keratin* **(A)** *and antibody to vimentin* **(B).** *The tumor cells are keratin positive* **(A)** *and vimentin negative* **(B).** *The vimentin-positive cells in* **B** *include macrophages, lymphocytes and connective tissue cells (double immunofluorescence staining on the same smear, ×240).* **(C)** *Phase-contrast of tumor cells shown in the center of A. Note two macrophages (arrow heads) keratin negative* **(A)** *vimentin positive* **(B)** *with kidney-shaped nuclei (×600).*

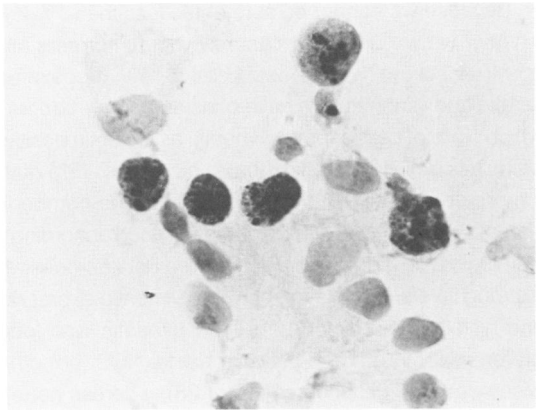

Figure 3. *Fine-needle aspirate of a breast carcinoma. A group of tumor cells labeled with Ki67 antibody. Prominent staining of nuclei and nucleoli is present in some tumor cells (immunoperoxidase, ×940).*

mentin-positive cancers had metastasized to the regional lymph nodes *vs.* 51% (37 of 73) of vimentin-negative cancers. A slightly increased number of vimentin-positive tumors was found among breast carcinomas with one (19%), two or more (23%), and three or more (28%) metastatic lymph nodes as opposed to those without lymph node involvement (16%). Vimentin was expressed in tumors as small as 1 cm in diameter. It was present in 19% (14 of 73) of tumors with diameters of 2 cm and in 17% (6 of 36) of those 3 cm in diameter. Larger tumors were underrepresented in this series.

All breast carcinomas also were tested for Ki-67 by immunocytochemical methods. Ki-67 positivity was restricted to the cell nuclei. Use of the peroxidase technique and immunofluorescence microscopy showed that Ki-67 often was concentrated in the nuclei and nucleoli (Figure 3), (cf. 21,22) a finding that we also noted when examining Ki-67-positive cell lines. The exact staining pattern seen with Ki-67 antibody is cell cycle dependent. The distribution of positive cells in smears was heterogeneous. Immunoreactive tumor cells could be distinguished easily from unreactive tumor cells as well as from lymphocytes and macrophages. One thousand tumor cells were counted in each smear, and the result expressed as the percentage of tumor cells labeled with the Ki-67 antibody. All tumor cells in which a brownish color, characteristic of the peroxidase stain, could be detected in nuclei or nucleoli were scored as positive regardless of staining intensity. The percentage of Ki-67-positive tumor cells varied from 1% to 56% (Figure 4). Other studies have shown that immunostaining of Ki67 antigen on both histologic and cytologic material from breast cancer yielded similar results, although growth fractions were slightly lower in cytologic specimens.[23]

ER EIA assay and GF results are summarized in Table 2. Vimentin was expressed in low ER (<60 fmols/mg protein, $\bar{x}$ 15.5 ± 14.9) and high GF (>15% Ki-67 positive, $\bar{x}$ 32.3 ± 12.3) breast carcinomas, whereas vimentin-negative breast cancers had high ER levels and low GF. This trend remained unchanged when premenopausal and postmenopausal patients were separately analyzed (Table 2). Vimentin expression did not correlate with menopausal status ($\chi^2 = 0.5$). ER levels, GF, and relevant clinical data for 17 vimentin-positive breast carcinomas and four carcinomas with less than 5% vimentin-positive tumor cells are summarized in Table 3. The results from all assays are presented in Figure 4, where the percentage of Ki-67 positive tumor cells in each tumor is plotted against the estrogen receptor level. Vimentin content is superimposed on the figure by the use of different symbols indicating 50% to 100%, 0% to 5%, or 0% vimentin positive tumor cells.

The most striking feature of the data in Figure 4 is the positive correlation of vimentin positivity (filled squares) with low ER and high GF. Vimentin is expressed predominantly in ER-negative (EIA less than 10 fmols/mg protein) and in low (<60 fmols/mg protein) ER breast cancers that have high GF. No high ER-positive breast carcinomas (EIA > 400 fmols/mg cytosol protein) expressed vimentin. Only one breast carcinoma with an ER level above 60 fmol/mg cytosol protein expressed vimentin in more than 5% of tumor cells.

The cut-off value of 60 fmol/mg cytosol protein was chosen to divide high and low ER cancers (see Discussion), and 15% Ki-67 positive tumor cells was used to divide tumors with high and low GF. The average in this series of 90 cases was 14.3%; the averages were 15.3% and 16.6% in other series comprising 154 and 160 breast carcinomas, respectively.[10,24] Thus, the breast carcinomas in Figure 4 can be divided into four groups—Group A, ER-high, GF-low (52 cases); Group B, ER-low, GF-low (11 cases); Group C, ER-low, GF-high (22 cases); Group D, ER-high, GF high (5 cases). Such demarcation is supported by strong statistically significant differences of ER and Ki-67 found between group C (which contains almost all vimentin positive cells) and group A, as well as differences of ER between groups A and B (Table 4). There also is a significant correlation between vimentin expression and ER EIA <60 fmols/mg protein ($\chi^2 = 32.9$; 15.3 for premenopausal and 21.0 for postmenopausal groups). A similarly strong correlation was found between vimentin expression and GF ≥ 15% ($\chi^2 = 37.3$; 8.8 for premenopausal and 25.6 for postmenopausal groups).

Discussion

That tumor cells in breast carcinomas invariably express keratin has been noted often.[25-28] In contrast, vimentin coexpression has been documented only recently.[1-4] In

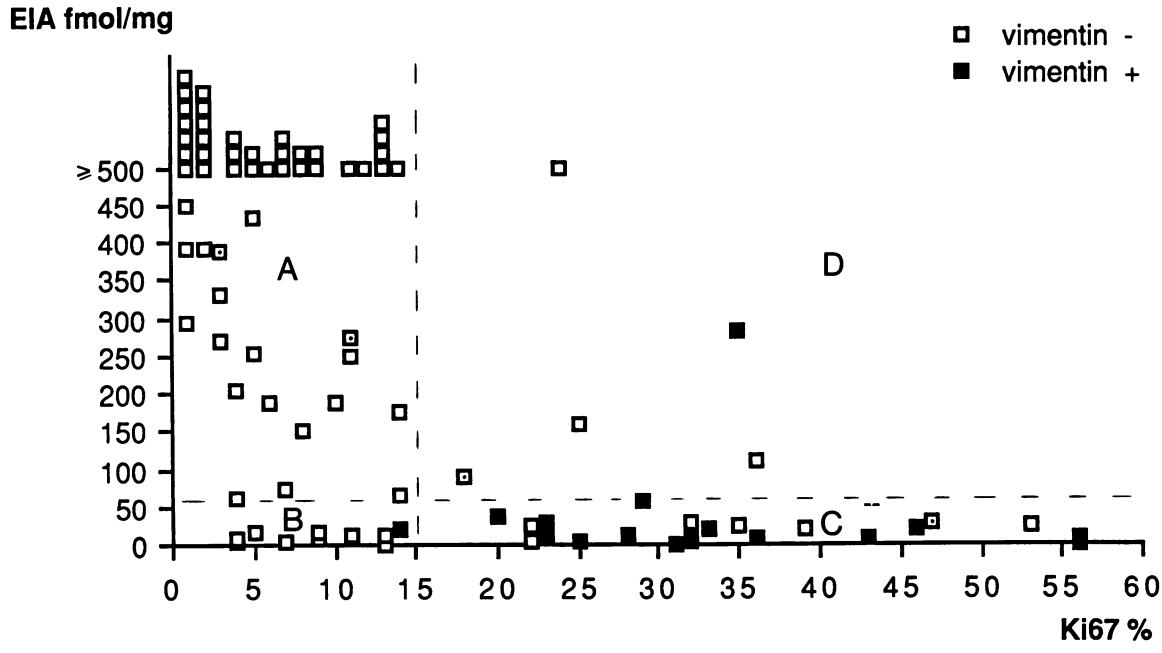

Figure 4. *ER-EIA, GF, and vimentin in breast carcinomas. Open squares: vimentin negative; filled squares: vimentin positive (>5% vimentin-positive tumor cells); open squares with a dot: <5% vimentin positive tumor cells. A, B, C, D denote the four groups into which the tumors were divided by ER and GF values (see text).*

most studies it is restricted to a minority of cases, whereas in others no vimentin expression was found.[25,27] In our series of breast carcinomas, 19% coexpress vimentin and keratin. Three other laboratories have reported values of 12% (5 of 43),[7] 25% (49 of 196)[2] and 60% (38 of 63).[3] If cases where less than 10% of the tumor cells were vimentin positive are excluded from the study by Raymond and Leong,[3] the percentage of vimentin-positive breast carcinomas is reduced to 16%. Thus, these four studies are in reasonable agreement even though different fixation methods were used (alcohol by Azumi and Battifora[1] and our study, frozen sections by Catoretti et al,[2] and microwave irradiation by Raymond and Leong[3]). In our series of primary tumors obtained before initiation of any adjuvant treatment, and in that of Raymond and Leong,[3] vimentin seems to be expressed preferentially in high-grade infiltrating ductal carcinomas and therefore is not independent of conventional histologic criteria used to grade breast carcinomas (Table 5). Our data show that vimentin expression is not an obligatory feature of tumors with regional lymph node involvement, or of larger, primary tumors. Data on a large series of T1NoMo tumors suggets that vimentin expression is found rarely in early cases.[28]

Table 2. *Ki67-GF and ER in Vimentin-Positive and -Negative Breast Carcinomas*

Breast Carcinomas	n (%)	ER-EIA (fmols/mg protein) Mean ± SD	Ki67-GF (%) Mean ± SD
All	89		
V−	73 (92)	305.9 ± 209.6	10.3 ± 11.0
V+	16 (18)*	15.5 ± 14.9	32.3 ± 12.3
Premenopausal	28	199.4 ± 196.8	18.6 ± 18.1
V−	22 (79)	250.0 ± 193.2	13.2 ± 15.7
V+	6 (21)	13.7 ± 13.4	38.0 ± 12.8
Postmenopausal	61	281.7 ± 224.1	12.4 ± 11.4
V−	51 (84)	331.1 ± 213.6	9.1 ± 8.1
V+	10 (16)	16.5 ± 16.5	28.9 ± 11.3

* Case 063 with unusually high ER-EIA (284) is excluded.
ER-EIA, estrogen receptor monoclonal enzyme immunoassay.
GF, growth fraction as measured by percentage of Ki67 positive cells.
SD, standard deviation.

Vimentin and Estrogen Receptor

We found that breast carcinomas with high ER values did not express vimentin. Among low ER breast carcinomas, one half expressed vimentin (16 of 33) whereas the other half did not (17 of 33). This correlates with the finding that only one half of the breast carcinomas found to be estrogen negative by an ER immunocytochemical assay (9 of 22) express vimentin.[2] Interestingly, estrogen-dependent (ER-positive) breast carcinoma cell lines (eg, MCF-7) do not express vimentin, whereas in estrogen-independent (ER-negative) breast cancer cell lines, some express vimentin and others do not.[5]

It has been suggested that vimentin expression may mark the progression from hormone dependence to independence in certain human breast cancer cell lines.[5] Data

Table 3. *Vimentin-Positive Breast Carcinomas*

Case	Age/menop. status	Histol. diagnosis	Tumor size (cm)	No. of inv. lymph nodes	~% V+	ER-EIA fmols/mg	Ki67-GF %+
014	33/pre*	m†	4	3	>70	0.0	31
029	28/pre	d3‡	1.5	35	100	6.9	43
031	45/pre	d3	3	6	50	37.6	20
074	33/pre	d3	2.5	1	100	8.9	56
078	35/pre	d3	2.5	0	50	20.6	46
117	39/pre	d3	8	0	90	8.6	32
018	62	d3	3	10	50	6.3	22
028	73	d3	3	5	100	11.5	28
055	65	d3	2.5	0	80	8.7	36
062	63	d3	1.0	1	70	9.8	23
063	52	d3	>10	3	100	284.0	35
065	62	d3	4	0	100	21.7	23
070	46	d3	4	0	50	20.3	33
086	69	se§	3.5	0	70	5.8	25
111	55	d3	2.5	1	90	19.9	14
116	61	d3	2.5	0	70	59.1	29
127	63	d3	—	10	100	1.5	56
025	39/pre	d2	7	0	<1	27.4	47
085	45/pre	d2	4	0	<5	387.0	3
075	65	d3	4	0	<1	275.5	11
110	66	d3	2.5	1	<1	89.7	18

* premenopausal, †medullary carcinoma, ‡grade 3 ductal carcinoma, §secretory carcinoma.
ER-EIA, estrogen receptor monoclonal enzyme immunoassay.
GF, growth factor as measured by percentage of Ki67 positive cells.

on induction of vimentin in other cell lines suggest an involvement of external influences such as cell density,[8] hormones,[29] and TPA[30] on vimentin expression. Our results show that the proportion of vimentin-positive breast cancers increases as the ER content decreases. ER-negative tumors are not always vimentin positive, however. Within this context, tumors with vimentin expression in less than 10% of tumor cells (in the study by Raymond and Leong[3] and this series) are of special interest. Do these small populations of vimentin-positive tumor cells belong to emerging clones of tumor cells that acquire new characteristics that endow them with a growth advantage? Or are such cells merely local phenomena with no major impact on the biology of the tumor?

Vimentin, Estrogen Receptor and Growth Fractions

We found vimentin to be expressed preferentially in ER-negative and low ER breast carcinomas with high proliferative activity, ie, high GF. Other data indirectly suggest that vimentin expression is a feature of low ER, high GF breast cancers. Thus, in one study nearly all EGFR-positive breast cancers were ER negative[31] and a coordinate expression of EGFR and vimentin was found on ER-negative (by ERICA) or low ER (by dextran-coated charcoal method [DCC]; $\bar{x}$ 82 fmols/mg protein for vimentin, and $\bar{x}$ 75 for EGFR) tumors.[2] Tumors that express higher amounts of EGFR may have an enhanced proliferation rate per unit of released growth factor because EGF-receptor complexes that remain on the cell surface are essential for the generation of a mitotic response. Transforming growth factor-beta (TGFβ), which is produced in large amounts by some ER-negative, vimentin-positive breast carcinoma cell lines, causes a rapid increase in the number of EGF receptors and in NRK fibroblasts alters the down-regulation of EGF receptor in response to the ligand.[32] Such a mechanism may explain in part the coordinate expression of vimentin and EGFR in ER-negative tumors[2] and vimentin expression in low ER, high GF breast cancers in our series.

Table 4. *Ki67-GF, ER Levels and Vimentin in Subgroups of Breast Carcinomas*

Group	Levels	n	ER* (fmols/mg protein) Mean ± SD)	Ki67-GF† (%) Mean ± SD	V+
A	ER ≥ 60 & Ki67 < 15	52	411.2 ± 140.3	6.0 ± 4.4	0/52
B	ER < 60 & Ki67 < 15	11	10.4 ± 6.2	8.8 ± 3.8	1/11
C	ER < 60 & Ki67 ≥ 15	22	18.4 ± 13.2	34.4 ± 11.7	15/22
D	ER ≥ 60 & Ki67 ≥ 15	5	229.3 ± 169.0	27.6 ± 7.7	1/5

* Differences between groups A and C and A and B are significant ($P < 0.001$).
† Differences between groups A and C and B and C are significant ($P < 0.001$).
ER, estrogen receptor. SD, standard deviation.

Table 5. *Vimentin Expression and Histologic Grading of Invasive Ductal Breast Carcinomas*

	I	II	III
Raymond and Leong[3]	1/16 (6%)	2/23 (9%)	5/11 (45%)
This study	0/4 (0%)	0/34 (0%)	15/28 (54%)

I, II, III: Bloom and Richardson histologic grade,[19] with I being most and III the least differentiated.

Number of vimentin-positive cases (>10% vimentin-positive tumor cells)/number of cases tested.

In some systems vimentin seems to be growth regulated.[6–8] In rat model systems used to study kidney regeneration it has been shown that vimentin is expressed during the regrowth phase that accompanies tubule regeneration,[33] but that expression ceases once the tubular epithelium is regenerated. Perhaps vimentin coexpression in carcinomas is indicative of a faulty growth regulation that favors replication of the tumor cells.

Estrogen Receptor and Growth Fraction

Our results are in agreement with an inverse correlation between GF or TLI and ER level also revealed in other studies.[9–14] We found large differences between the mean GFs of the low and high ER tumors. Low ER breast cancers were by no means homogeneous, however. They belonged to high and low GF subgroups (with vimentin-positive cases found almost exclusively in the high Ki-67 group) (Table 4). Similarly, Meyer et al[13] noted that although the differences between mean TLIs of the ER-positive and ER-negative carcinomas were large, some carcinomas in the ER-negative group had very low TLI and others in the low ER-positive group (10 to 49 fmols/mg protein by DCC method) had high TLI. A proportion of exceptional cases, ie, those with a positive ER status and a large or moderately large GF, has been noted by others[10,14] or can be inferred from the analysis of published data.[28] These data from the literature can be used to support our division of breast cancer into four main subgroups with regard to high or low ER and Ki-67 GF. In analyzing these data, two points have to be kept in mind. First, the ER status seems to depend on the proliferation rate, because the prognostic use of ER status appears to result from its correlation with TLI.[12] In addition, MCF-7 breast carcinoma cells that proliferate slowly accumulate more than twice as much ER activity as those that proliferate at a faster rate.[34] Second, measurement of TLI shows that higher values are found for recurrent carcinomas than for primary breast carcinomas.[12]

Cut-off Level for High vs. *Low ER*

Although opinions differ,[35,36] it seems that for optimal management of breast cancer it is important not only to distinguish between ER-negative and ER-positive tumors, but also to quantitate the amount of ER in tumor tissue.[35] A level of 5 or 10 fmols/mg protein generally has been used as the cut-off level, and therefore a significant number of low ER tumors, which may behave like ER-negative ones,[37,38] will be placed into the ER-positive category (see Figure 4 in Silvestrini and coworkers[14] and Figure 4 in this article). Indeed, only about 60% of ER-positive breast cancers respond to hormone therapy.[39] The few discrepancies found in the otherwise excellent correlation of results of the DCC and ER EIA assays[35] showed that 53 fmols/mg cytosol protein was the highest ER EIA level designated as ER negative by DCC. Therefore, in our study, we deliberately chose a higher cut-off value of 60 fmols/mg for ER, and correlated vimentin and Ki-67 GF using this value. We also noted that the ER EIA assay is more sensitive and gives slightly higher absolute values of ER in the lower range of receptor concentrations.[35] Thus, by adopting the higher cut-off ER value, we can compare our results more easily with the majority of previous reports on proliferative activity and ER in breast cancers, which used the less sensitive DCC method and 10 fmols/mg as the cut-off level.

Vimentin and Prognosis

Data on vimentin expression in breast carcinomas can be summarized as follows. Vimentin has been shown to be preferentially expressed in ER-negative and low ER breast carcinomas (Catoretti et al[2] and this study). It also is correlated with high GF (this study), with EGFR positive breast carcinomas,[2] and with carcinomas with high histologic grade (Raymond and Leong[3] and this paper). Thus, vimentin expression seems to be strongly associated with poor prognostic indicators in breast carcinomas. (The association between high proliferative rate, positive EGFR, negative ER status, and poor prognosis has been documented.[9,12,23,31,40,41]) Recently, a correlation between vimentin expression and high nuclear grade has been found in renal cell carcinomas,[42,43] with a particularly unfavorable course for vimentin-positive nuclear grade 3 tumors. Of the breast carcinoma groups defined in Figure 4 and Table 4, Group A (high ER and low GF) would be expected to have the best prognosis because it is associated with two good prognostic indicators. Group C (low ER and high GF) would be expected to have the worst prognosis because it is associated with at least two unfavorable prognostic indicators. Whether there are biologic differences between vimentin-positive and vimentin-negative group C tumors in terms of prognosis and response to treatment remains to be examined.

References

1. Azumi N, Battifora H: The distribution of vimentin and keratin in epithelial and nonepithelial neoplasms: A comprehensive immunohistochemical study on formalin- and alcohol-fixed tumors. Am J Clin Pathol 1987, 88:286–296
2. Catoretti G, Andreola S, Clemente C, D'Amato L, Rilke F: Vimentin and p53 expression on epidermal growth factor receptor-positive, oestrogen receptor-negative breast carcinomas. Br J Cancer 1988, 57:353–357
3. Raymond WA, Leong A S-Y: Coexpression of cytokeratin and vimentin intermediate filament proteins in benign and neoplastic breast epithelium. J Pathol 1989, 157:299–306
4. Domagala W, Lasota J, Chosia M, Szadowska A, Weber K, Osborn M: Diagnosis of major tumor categories in fine needle aspirates is more accurate when light microscopy is combined with intermediate filament typing: A study of 403 cases. Cancer 1989, 63:504–517
5. Agnor C, Walker-Jones D, Valverins E, Heckford S, Wilding G, Clark R, McCormick F, Stampfer M, Gelmann EP: Vimentin expression characterizes hormone-independent breast cancer cell lines and mammary epithelial cells transformed by two oncogenes (abstr). Proc Am Assoc Cancer Res 1987, 28:11
6. Ferrari S, Bettini R, Kaczmarek L, Rittling S, Lalabretta B, de Riel JK, Philiponis V, Wei J-F, Baserga R: Coding sequence and growth regulation of the human vimentin gene. Mol Cell Biol 1986, 6:3614–3620
7. Connel ND, Rheinwald JG: Regulation of the cytoskeleton in mesothelial cells: Reversible loss of keratin and increase in vimentin during rapid growth in culture. Cell 1983, 34:245–253
8. Ben-Ze'ev A: Differential control of cytokeratins and vimentin synthesis by cell-cell contact and cell spreading in cultured epithelial cells. J Cell Biol 1984, 99:1424–1433.
9. Charpin C, Andrac L, Vacheret H, Habib MC, Devictor B, Lavaut MN, Toga M: Multiparametric evaluation (SAMBA) of growth fraction (monoclonal Ki67) in breast carcinoma tissue sections. Cancer Res 1988, 48:4368–4374
10. Gerdes J, Pichertz M, Brotherton J, Hammerstein J, Weitzel M, Stein H: Growth fractions and estrogen receptor in human breast cancers as determined *in situ* with monoclonal antibodies. Am J Pathol 1987, 129:486–492
11. McGurrin JF, Doria MI, Dawson PJ, Karrison T, Stein MO, Franklin WA: Assessment of tumor cell kinetics by immunohistochemistry in carcinoma of breast. Cancer 1987, 59: 1744–1750
12. Meyer JS, Lee JY: Relationships of S-phase fraction of breast carcinoma in relapse to duration of remission, estrogen receptor content, therapeutic responsiveness and duration of survival. Cancer Res 1980, 40:1890–1896
13. Meyer JS, Prey MN, Babcock DS, McDivitt RW: Breast carcinoma cell kinetics, morphology, stage and host characteristics: A thymidine labeling study. Lab Invest 1986, 54:41–51
14. Silvestrini R, Daidone MG, Di Fronzo G: Relationship between proliferative activity and estrogen receptors in breast cancer. Cancer 1979 44:665–670
15. Barnard NJ, Hall PA, Lemoine NR, Kadar N: Proliferative index in breast carcinoma determined *in situ* by Ki67 immunostaining and its relationship to clinical and pathological variables. J Pathol 1987, 152:287–295
16. Kamel DW, Franklin WA, Ringus JC, Meyer JS: Thymidine labeling index and Ki-67 growth fraction in lesions of the breast. Am J Pathol 1989, 134:107–113
17. Gerdes J, Schwab U, Lemke H, Stein H: Production of a mouse monoclonal antibody reactive with cell proliferation. Int J Cancer 1983, 31:13–20
18. Koss LG, Woyke S, Olsewski W: Aspiration Biopsy: Cytologic Interpretation and Histologic Bases. Tokyo, Igaku-Shoin, 1984
19. Bloom HJG, Richardson WW: Histologic grading and prognosis in breast cancer. Br J Cancer 1957, 11:359–377
20. Charpin C, Martin PM, Jacquemier J, Lavaut MN, Pourreau-Schneider N, Toga M: Estrogen receptor immunocytochemical assay (ER-ICA) computerized image analysis system, immunoelectron microscopy and comparisons with estradiol binding assays in 115 breast carcinomas. Cancer Res 1986, 46:4271–4277
21. Verheijen R, Knijpers HJM, Schlingemann RO, Boehmer ALM, van Driel R, Brakenhoff GJ, Ramaekers FCS: Ki67 detects a nuclear matrix-associated proliferation-related antigen: I. Intracellular localization during interphase. J Cell Sci 1989, 92:123–130
22. van Dierendonck JH, Keijzer R, van de Velde CJH, Cornelisse CJ: Nuclear distribution of the Ki-67 antigen during the cell cycle: Comparison with growth fraction in human breast cancer cells. Cancer Res 1989, 49:2999–3006
23. Lellé RJ, Heidenreich W, Stauch G, Gerdes J: Growth fraction as determined in cytologic specimens of breast carcinomas: A study with the monoclonal antibody Ki-67, New Frontiers in Cytology: Modern Aspects of Research and Practice. Edited by K Goerttler, GE Feihter, S Witte. Berlin, Springer-Verlag, 1988, pp 212–215
24. Lellé RJ, Heidenreich W, Strauch G: The correlation of growth fraction with histologic grading and lymph node status in human mammary carcinoma. Cancer 1987, 59:83–88
25. Gabbiani G, Kapanci Y, Barazzone P, Franke WW: Immunochemical identification of intermediate-sized filaments in human neoplastic cells. Am J Pathol 1981, 104:206–216
26. Altmannsberger M, Osborn M, Shauer A, Weber K: Antibodies to different intermediate filament proteins: Cell-type specific markers on paraffin-embedded human tissues. Lab Invest 1981, 45:427–434
27. Guelstein VI, Tchypysheva TA, Ermilova VD, Litvinova LV, Troyanovsky SM, Bannikov GA: Monoclonal antibody mapping of keratins 8 and 17 and of vimentin in normal human mammary gland, benign tumors, dysplasias and breast cancer. Int J Cancer 1988, 42:147–153
28. Schauer A, Rothe H, Balzer I, Bergholz M, Brehler R, Fiebig I, Rauschecker H, Nagel G, Blossey HC, Weber K, Osborn M, Gabius MJ, Cramer F, Aus HM, Harms H, Sauer R, Scheurlen H, Schumacher M: Monoclonal antibodies for improved evaluation of breast tumor prognosis, Breast Cancer: Scientific and Clinical Progress. Edited by MA Rich, JC Hager, DM Lopez. Boston, Kluwer Academic Publishers, 1987, pp 299–320

29. Schmid E, Schiller DL, Grund C, Stadler J, Franke WW: Tissue type-specific expression of intermediate filament proteins in a cultured epithelial cell line from bovine mammary gland. J Cell Biol 1983, 96:37–50
30. Giese G, Traub P: Induction of vimentin synthesis in a murine myeloma cell line by TPA is strongly dependent on the composition of the cell culture medium. Eur J Cell Biol 1988, 47: 291–299
31. Sainsbury JR, Farndon JR, Needham GK, Malcolm AJ, Harris AL: Epidermal-growth-factor receptor status as predictor of early recurrence of and death from breast cancer. Lancet 1987, i:1398–1402
32. Assoian RK, Frolik CA, Roberts AB, Miller DM, Sporn MB: Transforming growth factor-β controls receptor levels for epidermal growth factor in NRK fibroblasts. Cell 1984, 36:35–41
33. Gröne H-J, Weber K, Gröne E, Helmchen K, Osborn M: Coexpression of keratin and vimentin in damaged and regenerating tubular epithelia of the kidney. Am J Pathol 1987, 129:1–8
34. Jakesz R, Smith CA, Aitken S, Huff K, Schnette W, Schackney S, Lippman M: Influence of cell proliferation and cell cycle phase on expression of estrogen receptor in MCF-7 breast cancer cells. Cancer Res 1984, 44:619–625
35. Thorpe SM: Monoclonal antibody technique for detection of estrogen receptors in human breast cancer: Greater sensitivity and more accurate classification of receptor status than the dextran-coated charcoal method. Cancer Res 1987, 47: 6572–6575
36. Barnes DM, Fentiman IS, Mills RR, Rubens RD: Who needs steroid receptor assays? Lancet 1989, i:1126–1127
37. Osborne CK, Yochmowitz MG, Knight WA, McGuire WC: The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 1980 46:2884–2888
38. Heuson JC, Longeral E, Mattheiem WM, Deboel ML, Sylvester RJ, Leclercq G: Significance of quantitative assessment of estrogen receptors for endocrine therapy in advanced breast cancer. Cancer 39:1971–1977, 1977
39. McGuire WL: Current status of estrogen receptors in human breast cancer. Cancer 1975, 36:638–644
40. Silverstrini R, Diadone MG, Casparini G: Cell kinetics as a prognostic marker in node-negative breast cancer. Cancer 1985, 56:1982–1987
41. Thorpe SM, Rose C, Rasmussen BB, Mouridsen HT, Bayer T, Keiding N, on behalf of the Danish Breast Cancer Cooperative Group. Prognostic value of steroid hormone receptors: Multivariate analysis of systematically untreated patients with node negative primary breast cancer. Cancer Res 1987, 47:6126–6133
42. Donhuijsen K, Schulz S: Prognostic significance of vimentin positivity in formalin-fixed renal cell carcinomas. Pathol Res Practice 1989, 184:287–291
43. Medeiros LJ, Michie SA, Johnson DE, Warnke RA, Weiss LM: An immunoperoxidase study of renal cell carcinomas: Correlation with nuclear grade, cell type, and histologic pattern. Human Pathol 1988, 19:980–987