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Decreased Expression of Integrin Adhesive Protein Receptors in Adenocarcinoma of the Breast

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The integrin superfamily represents a major class of receptors mediating cell-substrate adhesion. Our recent study of the tissue distribution of the $\alpha_2\beta_1$ integrin, a cell-surface collagen receptor, revealed that high levels of receptor expression were associated with orderly, regulated epithelial cell proliferation. Those observations prompted the present investigation of $\alpha_2\beta_1$ and other integrins in adenocarcinoma of the breast. The $\alpha_2\beta_1$ integrin was highly expressed on the epithelium of the ducts and ductules of normal breast tissue. Normal or nearly normal levels of the receptor were expressed in fibroadenomas. In contrast, markedly decreased or undetectable $\alpha_2\beta_1$ expression was typical of poorly differentiated adenocarcinomas. Well-differentiated lesions exhibited intermediate levels of expression. Similar, but less extensive, decreases in expression were observed for the $\alpha_5\beta_1$ (fibronectin receptor) and $\alpha_v\beta_3$ (vitronectin receptor). Significant expression of the β_1 subunit on even poorly differentiated tumors suggests that the expression of other undefined members of the β_1 family is not reduced to the same low level as $\alpha_2\beta_1$ and $\alpha_5\beta_1$. Expression of the $\alpha_2\beta_1$ integrin was highly correlated with estrogen-receptor expression. Decreased expression of $\alpha_2\beta_1$ and other integrin adhesive protein receptors probably contributes to the altered adhesive properties of tumor cells characteristic of the malignant phenotype. (Am J Pathol 1990, 137: 863–870)

Recent studies carried out independently in many laboratories have revealed that the integrin superfamily of heterodimeric adhesive protein receptors plays a major role in mediating the adhesive behavior of cells.[1-3] Studies carried out in our laboratory, and independently in other laboratories, established the role of the $\alpha_2\beta_1$ integrin as a cell-surface receptor for collagen.[4-9] Studies demonstrating the apparent identity of the $\alpha_2\beta_1$ integrin, the very late activation antigen 2 (VLA-2) on T cells, the platelet membrane glycoprotein Ia-IIa complex, and the extracellular matrix receptor II (ECMRII) on fibroblasts resulted in a convergence of previously distinct avenues of investigation.[5,6,8,10,11] These observations also indicated that the $\alpha_2\beta_1$ integrin mediates the adhesive properties of diverse cell types.

Recently we established that the $\alpha_2\beta_1$ receptor is widely distributed in normal human tissues.[12] In addition to its presence on fibroblasts and endothelial cells, high levels of $\alpha_2\beta_1$ expression were observed on many epithelial cells, including keratinizing and nonkeratinizing stratified squamous epithelia, ciliated columnar epithelium of the respiratory tract, and the epithelia of the gastrointestinal and urinary tracts. Studies of epithelial cell lines in culture also indicated the widespread distribution of the $\alpha_2\beta_1$ integrin.[13]

A recurrent finding in our earlier histologic studies was the increased expression of the $\alpha_2\beta_1$ integrin at sites of epithelial proliferation and the association of enhanced $\alpha_2\beta_1$ expression with orderly, regulated cellular proliferation.[12] These observations prompted our study of this adhesive receptor in neoplasia. In this report we describe the results of our studies concerning the expression of $\alpha_2\beta_1$ and related integrins in benign and malignant neoplasms of the breast.

Materials and Methods

Antibodies

Monoclonal antibody 12F1, which is directed against the α_2 subunit of the $\alpha_2\beta_1$ integrin, was provided by Dr. Virgil

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Woods, Jr., of the University of California, San Diego. The characterization and specificity of this antibody have been described earlier.[10,14] The monoclonal antibody 7E3, which reacts in a complex-specific manner with both members of the β_3 integrin family, the platelet membrane glycoprotein IIb-IIIa complex ($\alpha_{IIb}\beta_3$), and the vitronectin receptor ($\alpha_v\beta_3$), was provided by Dr. Barry S. Coller, of the State University of New York, Stonybrook.[15,16] Dr. John A. McDonald, Washington University School of Medicine, of St. Louis, Missouri, provided us with A33, an affinity-purified polyclonal antiserum directed against the carboxyterminal, cytoplasmic domain of the α_5 subunit of the $\alpha_5\beta_1$ integrin, the fibronectin receptor.[17] The monoclonal antibody P1D6, specific for the α_5 subunit, was purchased from Telios Pharmaceuticals, Inc.(San Diego, CA). The 4B4 monoclonal antibody reactive with the β_1 subunit (CD 29) common to all members of the β_1 integrin family was purchased from Becton-Dickinson (Mountain View, CA).

Immunohistochemistry

Breast tissue was obtained from material submitted to the surgical pathology division of the Department of Pathology, Washington University School of Medicine. The tissue was embedded in OCT compound (Miles Laboratories, Elkhart, IN), snap frozen in liquid nitrogen-cooled isopentane, and stored at −70°C. Frozen sections (6 μ thick) were cut, fixed briefly in acetone, and held at −20°C before staining by the immunoperoxidase technique using either 12F1, 7E3, A33, P1D6, or 4B4 reagents as the primary antibody at a concentration of 5 to 10 μg/ml. Detection was achieved with a biotinylated anti-mouse IgG for 12F1, 7E3, P1D6, and 4B4 or with a biotinylated goat anti-rabbit IgG for A33 and avidin-biotin-peroxidase complex (Vector, Burlingame, CA), as previously described.[12,18] Peroxidase activity was detected by incubation with a solution of 10 mg of 3, 3′ diaminobenzidine tetrahydrochloride (Sigma Chemical, St. Louis, MO) in 15 ml TRIS buffer containing 12 μl of 30% H_2O_2. Sections were counterstained with methyl green. A semiquantitative estimate of cell-surface and cytoplasmic-staining intensity (1+, 2+, 3+, 4+) was performed independently by two of us (MMZ, GM). In the occasional instance of disagreement, the slide was reviewed by a third observer and a consensus opinion obtained.

The immunohistochemical localization of nuclear estrogen receptors on frozen sections was performed with the ERICA kit (Estrogen Receptor Immunocytochemical Assay, Abbott Laboratories, North Chicago, IL).

Results

As described in our previous report[12] and illustrated in Figure 1a and b, high levels of the $\alpha_2\beta_1$ integrin are expressed in the ducts and ductules of normal breast tissue. The most intense staining was observed in the region of cell contact with the basement membrane and at sites of cell–cell contact (Figure 1b). Expression by the basal cell layer of the bilayered cuboidal epithelium was greater than that of the more superficial layer.

The level of $\alpha_2\beta_1$ expression on the nonmalignant epithelium of the fibroadenomas was comparable to or only slightly less than the level of expression observed on normal breast tissue (Figure 2a and b). In marked contrast, $\alpha_2\beta_1$ was expressed at extremely low or undetectable levels (0 to 1+) by poorly differentiated adenocarcinomas (Figure 4a and b, Table 1). The presence of $\alpha_2\beta_1$ on the endothelium of vessels within the tumor served as a useful internal positive control in these poorly differentiated tumors. The striking difference between levels of $\alpha_2\beta_1$ expression on normal and malignant cells is best illustrated in sections containing both normal breast elements and invasive adenocarcinoma (Fig. 5). Most well-differentiated and intraductal lesions showed intermediate levels (2 to 3+) of $\alpha_2\beta_1$ expression (Figure 3a and b).

These findings and the results of additional studies summarized in Table 1 indicate that $\alpha_2\beta_1$ expression is markedly reduced in adenocarcinomas of the breast. The more profound decreases in expression were observed in the poorly differentiated lesions. To determine whether these changes were unique to the $\alpha_2\beta_1$ integrin or were shared with other members of the integrin superfamily of extracellular matrix receptors, we examined the same lesions for expression of other selected integrins. The fibronectin receptor ($\alpha_5\beta_1$) and the vitronectin receptor ($\alpha_v\beta_3$) were studied. We also used an antibody directed against the β_1 subunit to assess the presence of any members of the β_1 integrin subfamily.

The vitronectin receptor ($\alpha_v\beta_3$) was only weakly expressed (1 to 2+) on ducts and ductules of normal breast tissue (Figure 1c). The receptor was localized primarily along the basement membrane and at sites of cell–cell contact. Faint cytoplasmic staining also was observed. The vitronectin receptor was undetectable in all examples

Figures 1 to 4. *The ducts and ductules of normal breast tissue* **(1A–E)**, *a fibroadenoma* **(2A–E)**, *a well-differentiated carcinoma of the breast* **(3A–E)**, *and a poorly differentiated carcinoma of the breast* **(4A–E)** *are shown. Sections were stained with hematoxylin and eosin* **(A)** *or immunohistochemically with monoclonal antibody 12F1 directed against the* $\alpha_2\beta_1$ *integrin* **(B)**, *monoclonal antibody 7E3 directed against the* $\alpha_v\beta_3$ *vitronectin receptor* **(C)**, *antiserum A33 directed against the carboxyterminal cytoplasmic domain of the* α_5 *subunit of the* $\alpha_5\beta_1$ *fibronectin receptor* **(D)**, *or monoclonal antibody 4B4 directed against the* β_1 *integrin subunit* **(E)**. *All magnifications 400×.*

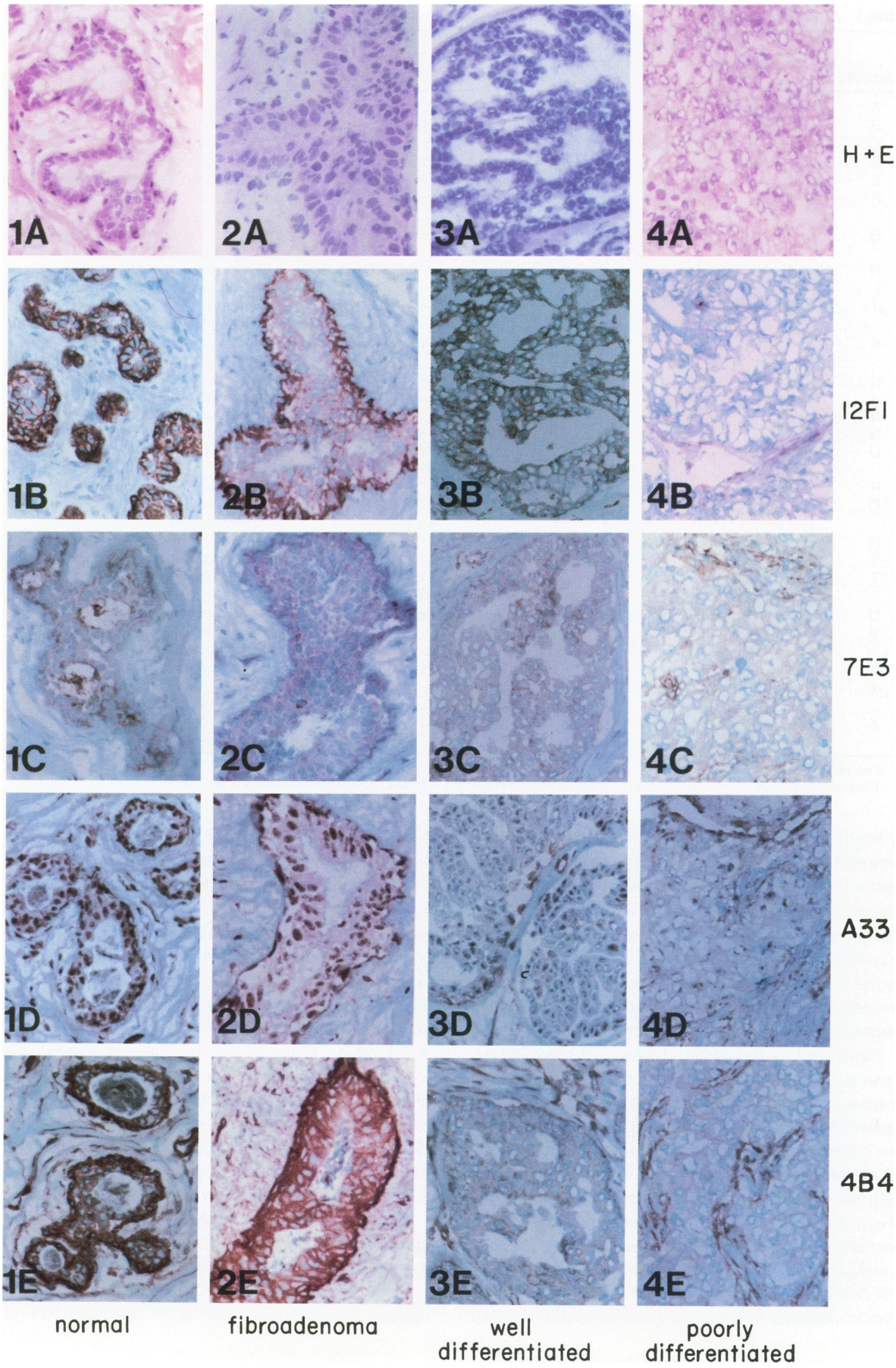
1A
2A
3A
4A
H+E
1B
2B
3B
4B
12F1
1C
2C
3C
4C
7E3
1D
2D
3D
4D
A33
1E
2E
3E
4E
4B4
normal
fibroadenoma
well differentiated
poorly differentiated

Table 1. *Integrin Expression in Adenocarcinoma of the Breast*

Specimen	Antibody α_2	α_5	β_1	α_v	Estrogen receptor†	Histology
A	++++	++++	++++	++	nd*	Normal breast (n = 7)
B	++++	++++	++++	++	nd	Fibroadenoma
C	++++	+++−++++	++++	nd	nd	Fibroadenoma
D	++++	++++	++++	nd	nd	Benign papilloma
E	++	++	+++	+	+++ (>75%)	Invasive ductal, well differentiated
F	++−+++	++	+++	+	+++ (>75%)	Intraductal and invasive ductal, well differentiated
G	+++	++−+++	++	nd	++ (>75%)	Intraductal and invasive ductal, well differentiated
H	+++	+++	+++	nd	+++ (>75%)	Invasive ductal, well differentiated
I	+++	+++	+++	nd	+++ (>75%)	Invasive celloid, well differentiated
J	++	0−+	+	+	++−+++ (>75%)	Focal intraductal and invasive ductal, moderately differentiated
K	+	++	+++	+	+ (25%–50%)	Intraductal, well to moderately differentiated
L	+	++	++	nd	+++ (>75%)	Invasive ductal with focal intraductal, moderately differentiated
M	++	+++	++	nd	+++ (>75%)	Invasive ductal, moderately differentiated
N	+−++	++	+++	nd	++ (50%–75%)	Invasive ductal, moderately differentiated
O	0−+	++−+++	++	nd	+ (25–50%) ++ (25%)	Intraductal and moderately differentiated
P	+−++	+−++	+−++	nd	0	Invasive ductal, moderately differentiated
Q	++	++	+	nd	+ (50%–75%) ++ (>25%)	Invasive and intraductal, moderately differentiated
R	0−+	++	++	0−+	+ (25%–50%)	Invasive ductal, poorly differentiated
S	+	++	+++	+	0	Invasive ductal, poorly differentiated
T	−	+	++	−	+ (50%–75%) ++ (25%)	Invasive ductal, poorly differentiated
U	−	++	+	−	++ (25%)	Invasive lobular
V	−	++	++	−	0	Invasive ductal, poorly differentiated
W	+	++	++	0−+	0	Invasive ductal, poorly differentiated
X	0	+−++	+++	nd	0	Invasive ductal, poorly differentiated
Y	++	++−+++	++	nd	0	Metastatic, carcinoma, lymph node, moderately differentiated
Z	++	+−++	+++	nd	nd	Metastatic carcinoma, brain, poorly differentiated

* nd, not determined.
† Values in parentheses give the percentage of malignant cells exhibiting the indicated level of estrogen-receptor expression.

of poorly differentiated, invasive adenocarcinomas that were studied (Figure 4c, Table 1). In the well-differentiated lesions, staining was negative to weakly positive (1+) (Figure 3c). Thus, as observed with $\alpha_2\beta_1$, the level of expression of the vitronectin receptor ($\alpha_v\beta_3$) also decreased to undetectable levels in poorly differentiated adenocarcinomas. However, because the vitronectin receptor is expressed at lower levels than $\alpha_2\beta_1$ on normal cells, the magnitude of the decrease was much less.

Staining for the fibronectin receptor ($\alpha_5\beta_1$) revealed intense staining along the basement membrane, strongly positive staining of cell membranes, and moderate cytoplasmic staining in normal breast epithelium (Figure 1d). The pattern of staining was similar to that of $\alpha_2\beta_1$, except for dotlike positivity in the nuclear region observed for $\alpha_5\beta_1$ when using the polyclonal antisera A33. Expression of $\alpha_5\beta_1$ also decreased in carcinomas and, in general, the decreases paralleled those observed for $\alpha_2\beta_1$ and $\alpha_v\beta_3$. A slight decrease in $\alpha_5\beta_1$ expression was observed in the well-differentiated lesions (Figure 3d) and a moderate to marked decrease in expression was observed with the poorly differentiated lesions (Fig. 4d). In no case was a complete loss of $\alpha_5\beta_1$ expression observed (Table 1).

Staining with an antibody directed against the integrin β_1 subunit gave similar results. High levels of expression were observed in normal breast tissue and in the fibroadenomas (Figures 1e and 2e). A slight decrease in expression was observed in the well-differentiated carcinomas (Figure 3e), with more marked decreases observed in the poorly differentiated lesions (Figure 4e). The decrease in β_1 expression, however, was less than that observed for $\alpha_2\beta_1$ and $\alpha_5\beta_1$. For example, in cases in which $\alpha_2\beta_1$ was undetectable and $\alpha_5\beta_1$ was only weakly expressed, significant although reduced β_1 expression was observed (Table 1 and Figure 4e). This observation suggests that expression of some other, as yet undefined, β_1 integrins is not reduced to the same low levels as $\alpha_2\beta_1$ and $\alpha_5\beta_1$.

Immunohistochemical analysis of estrogen-receptor status was performed on the same tissue blocks used for the analysis of integrin expression to facilitate a comparative analysis (Table 1). In each case in which the estrogen receptor was judged to be 2 to 3+ in at least 50% of

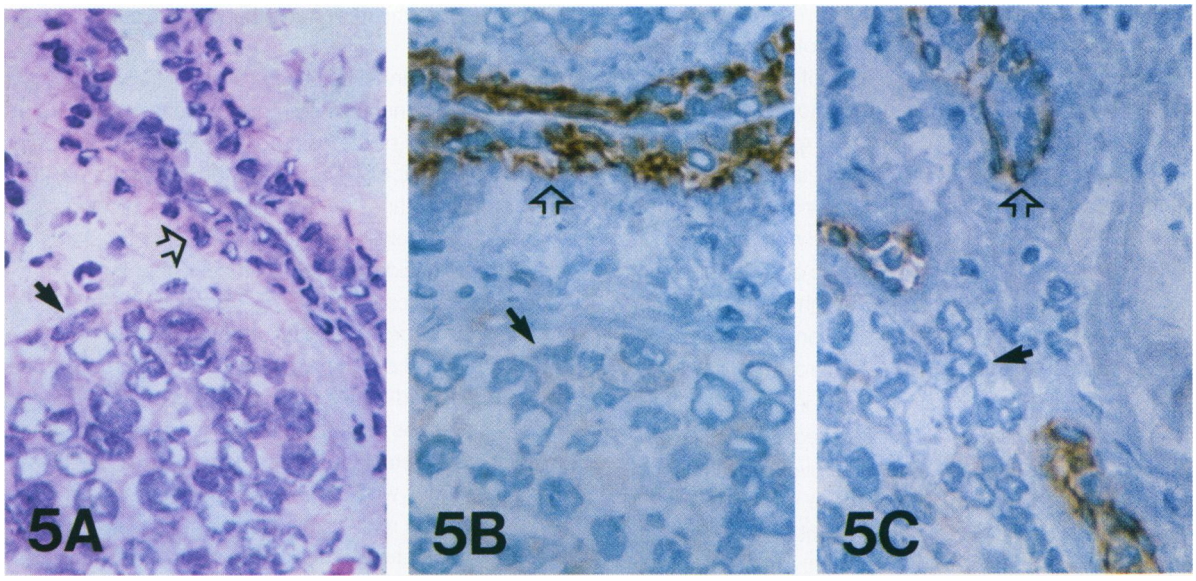

Figure 5. *Elements of normal breast tissue (open arrow) located adjacent to a focus of invasive poorly differentiated carcinoma (solid arrow). Sections were stained with hematoxylin and eosin* (A), *or the monoclonal antibody 12F1, which is directed against the* $\alpha_2\beta_1$ *collagen receptor* (B, C). *Magnifications, 400×.*

the tumor tissue, the $\alpha_2\beta_1$ and $\alpha_5\beta_1$ integrins also were expressed at high levels (2 to 3+). In those cases in which estrogen-receptor expression was weak or absent, staining for the integrin adhesive receptors also was markedly diminished or absent. Thus, in this limited series, there appears to be a clear-cut correlation of estrogen-receptor status with integrin expression.

Discussion

Although the genetic mechanisms underlying malignant transformation are complex and only incompletely understood at present, it is clear that acquisition of the malignant phenotype is associated with impaired regulation of cellular proliferation and attainment of the ability to invade and metastasize. During the complex multistep processes of invasion and metastasis, tumor cells interact with components of the extracellular matrix.[19-22] Transformed cells in culture exhibit altered adhesive properties that are thought to reflect the invasive and metastatic potential of these cells *in vivo*. The many studies that have documented the diminished association of extracellular matrix molecules such as fibronectin, collagen, and laminin with transformed cells in culture have been the subject of several excellent reviews.[23-25] These changes, however, do not appear to adequately account for the altered adhesive behavior of transformed cells.

Hynes[26] previously argued that a defect in cell-surface receptors for matrix components would be more likely to produce altered cell behavior *in vivo* than would a reduction in matrix synthesis because the altered cell would have access to the matrix secreted by its normal neighbors. In the last few years considerable progress has been made in understanding the molecular basis of cell-substrate adhesion. Many adhesive activities appear to be mediated by members of the integrin superfamily of cell-surface heterodimeric adhesive protein receptors.[1-3] Studies in this and other laboratories have established the role of the $\alpha_2\beta_1$ integrin as a cell-surface receptor for collagen.[4-9] Recent data suggest that, in contrast to the absolute specificity for collagen exhibited by $\alpha_2\beta_1$ on platelets and fibroblasts, $\alpha_2\beta_1$ derived from other cell types may exhibit a broader range of ligand specificity.[27,28]

Increased levels of $\alpha_2\beta_1$ expression on proliferating cells have been observed in several systems, both *in vitro* and *in vivo*.[13,29,30] Our recent immunohistochemical survey of the normal tissue distribution of the $\alpha_2\beta_1$ integrin revealed high levels of expression at several sites of epithelial proliferation.[12] Those observations led us to suggest that increased expression of the $\alpha_2\beta_1$ integrin was associated with orderly, regulated cellular proliferation[12] and prompted the study of $\alpha_2\beta_1$ expression in breast carcinoma described in this report.

Immunoperoxidase staining revealed that the $\alpha_2\beta_1$ integrin is highly expressed on the epithelium of the ducts and ductules of normal breast tissue. Markedly diminished or undetectable levels of $\alpha_2\beta_1$ were typical of poorly differentiated adenocarcinomas. Intermediate levels of expression were seen on the more well-differentiated adenocarcinomas. Normal or nearly normal levels of expression were observed on fibroadenomatous tissue.

These reductions in integrin expression, which were associated with adenocarcinomas of the breast and the magnitude of which correlated with the degree of morpho-

logic differentiation of the neoplasm, were not unique to the $\alpha_2\beta_1$ integrin. Similar but less profound decreases also were observed in the expression of $\alpha_5\beta_1$ (fibronectin receptor) and $\alpha_v\beta_3$ (vitronectin receptor) in breast neoplasms. It does not appear that the expression of other members of the β_1 family of integrins is reduced to the same low levels as $\alpha_2\beta_1$ and $\alpha_5\beta_1$ in poorly differentiated tumors. Expression of the β_1 subunit was detected on these lesions, suggesting the presence of as-yet unidentified β_1 integrins. It is possible, although unprecedented, that an integrin β subunit would exist on a cell surface in the absence of an appropriate $\alpha\beta$ heterodimer.

It seems likely, but remains to be experimentally established, that the semiselective loss of integrin adhesive protein receptors contributes to the invasive and metastatic potential of tumor cells. Loss of these receptors, which function to anchor cells to matrix, might be a key step in attaining the malignant phenotype. Although at present such a reduction in integrin expression has only been documented in carcinoma of the breast, it is likely to be a more general phenomenon. The recent *in vitro* studies of Plantefaber and Hynes[31] lend credence to this hypothesis. They demonstrated that oncogenic transformation of rodent fibroblasts with Rous sarcoma virus encoding the *src* oncogene or murine sarcoma viruses encoding *ras* oncogenes led to reductions in the expression of $\alpha_5\beta_1$ and at least two other undefined integrin receptors. Expression of $\alpha_3\beta_1$ was retained by the transformed cells. Reduced integrin expression was shown to be a consequence of decreased integrin synthesis. The mechanism resulting in decreased integrin expression by the malignant cells of breast carcinomas remains to be established.

It is unlikely that any single cell-surface alteration alone is responsible for the invasive and metastatic behavior of tumor cells. Such behavior requires a balance between diminished cell-substrate and cell–cell adhesive reactions, which tend to maintain cells in their normal locale on one hand, and on the other, retention or even upregulation of adhesive receptors needed to facilitate tumor cell localization at sites of metastasis. For example, Madin-Darby canine kidney epithelial cells, in an *in vitro* model of transformation, acquired a 'more malignant,' ie, invasive phenotype, with blockade or removal of the uvomorulin cell–cell adhesion receptor.[32] An extensively characterized laminin receptor has been shown to be expressed at higher levels on more invasive tumor cells.[33–37] Cheresh[38] recently described a vitronectin receptor on lung adenocarcinomas, pancreatic carcinomas, and melanoma that contained a β subunit that differed from that of the more extensively characterized vitronectin receptor, $\alpha_v\beta_3$. Diminished expression of integrin adhesive receptors such as we have observed, as well as alterations in other adhesive molecules previously described by others, probably all contribute to the malignant phenotype exhibited by tumor cells.

We also observed that expression of integrin adhesive receptors in adenocarcinoma of the breast closely paralleled expression of the estrogen receptor as judged immunohistochemically. The underlying molecular basis of this correlation, and the pathologic and clinical implications of the correlation with estrogen receptor status remain to be elucidated. Many studies now have established a correlation between the presence of estrogen receptors (and progesterone receptors) and a more favorable clinical prognosis, as well as the presence of a well-differentiated tumor cell morphology.[39,40] Estrogens and antiestrogens have been shown to alter the adhesive, invasive, and metastatic properties of several breast carcinoma cell lines when assayed *in vitro* or *in vivo* in nude mice.[37,41–49] The relationship between invasiveness and metastatic potential in these models and integrin expression remains to be elucidated. The findings described in this report suggest that diminished integrin expression may be a significant determinant giving rise to altered adhesive properties of tumor cells.

References

1. Hynes RO: Integrins: A family of cell surface receptors. Cell 1987, 48:549–554
2. Ruoslahti E, Pierschbacher MD: New perspectives in cell adhesion: RGD and integrins. Science 1987, 238:491–497
3. Buck CA, Horwitz AF: Cell surface receptors for extracellular matrix molecules. Ann Rev Cell Biol 1987, 3:179–205
4. Santoro SA: Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. Cell 1986, 46:913–920
5. Santoro SA, Rajpara SM, Staatz WD, Woods VL Jr: Isolation and characterization of a platelet surface collagen binding complex related to VLA-2. Biochem Biophys Res Commun 1988, 153:217–223
6. Staatz WD, Rajpara SM, Wayner EA, Carter WG, Santoro SA: The membrane glycoprotein Ia-IIa (VLA-2) complex mediates the Mg^{++}-dependent adhesion of platelets to collagen. J Cell Biol 1989, 108:1917–1924
7. Wayner EA, Carter WG: Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique α and β subunits. J Cell Biol 1987, 105:1873–1884
8. Kunicki TJ, Nugent DJ, Staats SJ, Orchelowski RP, Wayner EA, Carter WG: The human fibroblast class II extracellular matrix receptor mediates platelet adhesion to collagen and is identical to the platelet glycoprotein Ia-IIa complex. J Biol Chem 1988, 262:4516–4519
9. Coller BS, Beer JH, Scudder LE, Steinberg MH: Collagen-platelet interactions: Evidence for a direct interaction of collagen with platelet GP Ia/IIa and an indirect interaction with

platelet GP IIb/IIIa mediated by adhesive proteins. Blood 1989, 74:182–192
10. Pischel KD, Bluestein HG, Woods VL Jr: Platelet glycoproteins Ia, Ic and IIa are physicochemically indistinguishable from the very late activation antigens adhesion-related proteins of lymphocytes and other cell types. J Clin Invest 1988, 81:505–513
11. Takada Y, Wayner EA, Carter WG, Hemler ME: Extracellular matrix receptors ECMRII and ECMRI for collagen and fibronectin correspond to VLA-2 and VLA-3 in the VLA family of heterodimers. J Cell Biochem 1988, 37:385–393
12. Zutter MM, Santoro SA: Widespread histologic distribution of the $\alpha_2\beta_1$ integrin cell surface collagen receptor. Am J Pathol 1990, 137, 113–120
13. Wayner EA, Carter WG, Piotrowicz RS, Kunicki TJ: The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: Preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. J Cell Biol 1988, 107:1881–1891
14. Pischel KD, Hemler ME, Huang C, Bluestein HG, Woods VL Jr: Use of the monoclonal antibody 12F1 to characterize the differentiation antigen VLA-2. J Immunol 1987, 138:226–233
15. Coller BS: A new murine monoclonal antibody reports on activation-dependent change in the conformation and/or microenvironment of the platelet GPIIb/IIIa complex. J Clin Invest 1985, 76:101–110
16. Charo IF, Bekeart LS, Phillips DR: Platelet glycoprotein IIb-IIIa-like proteins mediate endothelial cell attachment to adhesive proteins and the extracellular matrix. J Biol Chem 1987, 262: 9935–9938
17. Roman J, LaChance RM, Broekelmann TJ, Kennedy CJR, Wayner EA, Carter WG, McDonald JA: The fibronectin receptor is organized by extracellular matrix fibronectin: Implications for oncogenic transformation and for cell recognition of fibronectin matrices. J Cell Biol 1989, 108: 2529–2543
18. Hsu SM, Raine L, Fanger H: The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
19. Poste G, Fidler I: The pathogenesis of cancer metastases. Nature 1980, 282:139–146
20. Liotta LA, Rao CN, Barsky SH: Tumor invasion and the extracellular matrix. Lab Invest 1983, 49:639–649
21. Liotta LA: Tumor invasion and metastasis—Role of the extracellular matrix. Cancer Res 1986, 46:1–7
22. Juliano RL: Membrane receptors for extracellular matrix molecules: Relationship to cell adhesion and tumor metastasis. Biochim Biophys Acta 1987, 907:261–278
23. Hynes RO: Surfaces of normal and malignant cells. John Wiley and Sons. 1979. New York
24. Hynes RO, Yamada KM: Fibronectins: Multifunctional modular glycoproteins. J Cell Biol 1982, 95:369–377
25. Yamada KM: Cell surface interactions with extracellular materials. Ann Rev Biochem 1983, 52:761–799
26. Hynes RO: Fibronectin and its relation to cellular structure and behavior. *In* Hay ED, ed. The Cell Biology of the Extracellular Matrix. Plenum, New York, 1981, pp 295–311
27. Languino LR, Gehlsen KR, Wayner E, Carter WG, Engvall E, Ruoslahti E: Endothelial cells use $\alpha_2\beta_1$ integrin as a laminin receptor. J Cell Biol 1989, 109:2455–2462
28. Elices MJ, Hemler ME: The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others. Proc Natl Acad Sci USA 1989, 86:9906–9910
29. Hemler ME, Jacobson JG: Cell matrix adhesion-related proteins VLA-1 and VLA-2: Regulation of expression on T cells. J Immunol 1987, 138:2941–2948
30. Peltonen J, Lanjava H, Jaakkola S, Gralnick H, Akiyama SK, Yamada SS, Yamada KM, Uitto J: Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. Variable expression in basal and squamous cell carcinomas. J Clin Invest 1989, 84:1916–1923
31. Plantefaber LC, Hynes RO: Changes in integrin receptors on oncogenically transformed cells. Cell 1989, 56:281–290
32. Behrens J, Mareel MM, Van Roy FM, Birchmeier W: Dissecting tumor cell invasion: Epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. J Cell Biol 1989, 108:2435–2447
33. Terranova VP, Rao CN, Kalebic T, Margulies IMK, Liotta LA: Laminin receptor on human breast carcinoma cells. Proc Natl Acad Sci USA 1983, 80:444–451
34. Rao CN, Barsky SH, Terranova VP, Liotta LA: Isolation of a tumor cell laminin receptor. Biochem Biophys Res Commun 1983, 111:804–808
35. Malinoff H, Wicha MS: Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. J Cell Biol 1983, 96:1475–1479
36. Horan HP, Thor A, Schlom J, Rao CH, Liotta L: Expression of laminin receptor in normal and carcinomatous human tissues as defined by a monoclonal antibody. Cancer Res 1985, 45:2713–2719
37. Castronovo V, Taraboletti G, Liotta LA, Sobel ME: Modulation of laminin receptor expression by estrogen and progestins in human breast cancer cell lines. J Natl Cancer Inst 1989, 81:781–788
38. Cheresh DA, Smith JW, Cooper HM, Quaranta V: A novel vitronectin receptor integrin ($\alpha_v\beta_x$) is responsible for distinct adhesive properties of carcinoma cells. Cell 1989, 57: 59–69
39. Clark GM, McGuire WL: Steroid receptors and other prognostic factors in primary breast cancer. Sem Oncol 1988, 15:20–25
40. Berger U, Wilson P, McClelland RA, Davidson J, Coombes RC: Correlation of immunocytochemically demonstrated estrogen receptor distribution and histopathologic features in primary breast cancer. Human Pathol 1987, 18:1263–1267
41. Engel LW, Young NA: Human breast carcinoma cells in continuous culture: A review. Cancer Res 1978, 38:4327–4336
42. Albini A, Grof J, Kitten GT, Kleinmann HK, Martin GR, Veillette A, Lippman ME: 17β-Estradiol regulates and v-Ha-ras transfection constitutively enhances MCF-7 breast cancer cell interactions with basement membrane. Proc Natl Acad Sci USA 1986, 83:8182–8186
43. Butler WB, Kirkland WL, Gargola TL, Goran N, Kelsey WH, Berlinski PJ: Steroid stimulation of plasminogen activator

production in a human breast cancer cell line (MCF-7). Cancer Res 1983, 43:1637–1641
44. Kao RT, Stern R: Collagenases in human breast carcinoma cell lines. Cancer Res 1986, 46:1349–1354
45. Morisset M, Capony F, Rochefort H: The 52-kDa estrogen-induced protein secreted by MCF-7 cells is a lysosomal acidic protease. Biochem Biophys Res Commun 1986, 138:102–109
46. Westley BR, May FEB: Oestrogen regulates cathepsin D mRNA levels in oestrogen responsive human breast cancer cells. Nucleic Acids Res 1987, 15:3773–3786
47. Millon R, Nicora F, Muller D, Eber M, Klein-Soyer C, Abecassis J: Modulation of human breast cancer cell adhesion by estrogens and antiestrogens. Clin Expl Metastasis 1989, 7:405–415
48. Osborne CK, Hobbs K, Clark GM: Effect of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. Cancer Res 1985, 45:584–590
49. Shafie SM, Liotta LA: Formation of metastasis by human breast carcinoma cells (MCF-7) in nude mice. Cancer Letters 1980, 11:81–87