Increased β_1 Integrin Is Associated with Decreased Survival in Invasive Breast Cancer

Evelyn S. Yao,[1] Hui Zhang,[5] Yunn-Yi Chen,[2] Brian Lee,[1] Karen Chew,[3] Dan Moore,[4] and Catherine Park[1]

Departments of [1]Radiation Oncology and [2]Pathology, [3]Comprehensive Cancer Center and Department of Laboratory Medicine, and [4]Department of Epidemiology and Biostatistics, University of California at San Francisco, San Francisco, California and [5]Lawrence Berkeley National Laboratory, Berkeley, California

Abstract

Aberrant microenvironments and loss of balance in cell-extracellular matrix signaling are associated with breast cancer invasion, metastasis, and resistance to therapy. We have recently shown that increased β_1 integrin signaling is involved in malignant progression and that inhibitory antibody to β_1 integrin leads to selective apoptosis and decreased proliferation in three-dimensional cultures and in xenograft models of breast cancer *in vivo*. To investigate the clinical importance of these findings, in the present study we examined the expression of β_1 integrin and extracellular β_1 integrin ligands fibronectin and laminin-1 in a cohort of 249 breast cancer patients who had a median follow-up of 8.4 years. Among the 149 scorable cases, the highest β_1 integrin intensity score (3+ versus 0–2+) was associated with significantly decreased 10-year overall survival of 48% versus 71% ($P < 0.03$) and decreased disease-free survival of 50% versus 80% ($P < 0.05$). Importantly, high fibronectin expression was associated with decreased overall and disease-free survival on univariate analysis ($P < 0.04$) and β_1 integrin intensity score was significantly correlated with fibronectin expression (Kendall's tau-b = 0.19; $P = 0.03$). In a multivariate Cox proportional hazards model, β_1 integrin intensity score remained a significant independent predictor of overall survival [hazard ratio (HR), 1.69; 95% confidence interval (95% CI), 1.19–2.38; $P < 0.003$] and disease-free survival (HR, 1.87; 95% CI, 1.21–2.88; $P < 0.005$). These findings show that β_1 integrin expression has potential prognostic value in invasive breast cancer and that coexpression of fibronectin may help identify patients with more aggressive tumors who may benefit from targeted therapy. [Cancer Res 2007;67(2):659–64]

Introduction

Malignant breast tumors are fundamentally characterized by loss of normal tissue architecture and cell-extracellular matrix (ECM) interactions (1). β_1 integrins mediate cell-ECM signaling that affects diverse cell behavior, including proliferation, apoptosis, and survival (2, 3). Although β_1 integrin has not been viewed as a classic proto-oncogene, increasing evidence points to its critically important role in tumorigenesis in cell culture models (4–6) and, more recently, in a transgenic murine model (7). We have shown previously that inhibition of β_1 integrins using inhibitory antibodies selectively enhances apoptosis and decreases proliferation in three-dimensional cultures of breast cancer (8). Strikingly, these effects were also observed in breast cancer xenografts with no toxicity to the host *in vivo* (8). In addition, β_1 integrins have been shown to mediate resistance to cytotoxic chemotherapy (9, 10) and radiation (11, 12) in several human cancers, illustrating its potentially multifaceted role as a therapeutic target and predictive factor.

β_1 integrin signaling involves several steps, including binding to key extracellular ligands, such as fibronectin and laminin-1. Indeed, ECM ligands have been shown to facilitate and promote growth of several solid tumors, perhaps by providing a more permissive microenvironment (13, 14). Fibronectin and laminin-1 expression alone have each been shown to correlate with features of aggressive breast cancer (15–17), and the coexpression of β_1 integrin with fibronectin and laminin-1 has been associated with decreased survival following treatment for lung cancer (18). β_1 integrin alone has been studied extensively in the biology of solid tumors, and its expression has been shown to correlate with poor prognosis in cancers of the lung (19), pancreas (20), and cutaneous melanoma (21). Among studies of human breast cancer, β_1 integrin has been implicated in progression and metastasis (22, 23); however, its value as a prognostic marker alone or in conjunction with its major ligands has not been shown. Here, we report the first evidence that β_1 integrin expression is associated with decreased disease-free and overall survival among patients with invasive breast cancer. In addition, we show that ECM components were expressed in a significant proportion of tumors and that coexpression of β_1 integrin and fibronectin was significantly correlated. These findings have important implications for understanding the role of the microenvironment in breast cancer and identifying breast cancer patients that may benefit from targeted therapy against β_1 integrin.

Materials and Methods

Patient population. Formalin-fixed, paraffin-embedded invasive breast tumors from 249 patients who were treated at the University of California at San Francisco (UCSF)/Mt. Zion Comprehensive Cancer Center or at the California Pacific Medical Center (San Francisco, CA) from 1974 to 1999 formed the basis for this correlative outcomes study, which was approved by the UCSF Committee on Human Research. All tumor specimens were reviewed by a breast pathologist. Three tissue microarrays (TMA) were created from these cases (see below for methods). Follow-up time was calculated from the time of initial pathologic diagnosis to last contact date (minimum, 5 years; mean, 7.7 years; median 8.2 years for those without recurrence). Overall survival was determined by subtracting the date of death or date of last known contact from the date of diagnosis. Disease-free survival was determined by the status of any recurrence (local-regional recurrence, distant metastasis, or no evidence of disease) at the last known follow-up date.

Tissue specimens. TMAs were prepared by the Tissue Core of the UCSF Cancer Center using the Beecher Instruments Tissue Arrayer (Sun Prarie, WI).

Requests for reprints: Catherine Park, Department of Radiation Oncology, University of California at San Francisco/Mt. Zion Cancer Center, 1600 Divisadero Street H1031, San Francisco, CA 94143-1708. Phone: 415-353-7186; Fax: 415-353-9883; E-mail: Catherine.park@ucsf.edu.

doi:10.1158/0008-5472.CAN-06-2768

Several cores of 0.6 mm in diameter were taken from tumor areas of donor paraffin blocks, and those containing the most representative sample were inserted into the TMA block. Three tissue arrays were constructed at different times, consisting of patients with predominantly early-stage invasive breast cancer (one array was composed of cases collected between 1974 and 1987 and two arrays were composed of cases collected between 1990 and 1999). Patients were followed prospectively. The three TMAs contained a total of 249 samples, of which we included 149 cases that had high quality tissue spot that could be read for β_1 integrin. Specimens were not usable if there was insufficient tumor tissue within the core, artefactual distortion of the tissue, or high background (see section on Scoring). This eliminated 100 cases from the original 249. To determine whether this significantly biased the study, we compared the 149 cases with the 100 cases for the most important prognostic factors. This analysis revealed that there was a significant difference in the number of patients with estrogen receptor–positive tumors (78% versus 65%; $P = 0.03$) and the use of hormone therapy (32% versus 49%; $P = 0.01$) in the two groups. However, neither of these factors was significant with respect to β_1 integrin expression or overall or disease-free survival. In addition, there were no differences in overall or disease-free survival between the two groups (log-rank test: $P = 0.84$ for disease-free survival and $P = 0.22$ for overall survival).

Immunohistochemistry. Sections (5 μm) were obtained from three TMAs. Each slide was baked at 60°C for 30 min, dewaxed in xylene, and rehydrated through graded alcohols. For β_1 integrin, slides were placed in methanol/30% hydrogen peroxide (H_2O_2) for 20 min at room temperature, microwaved for 3.5 min in 10 mmol/L sodium citrate (pH 6.0), and cooled at room temperature for 20 min. The microwaving and cooling steps were repeated thrice. Sections were blocked using an avidin/biotin blocking kit (Vector Laboratories, Burlingame, CA) and 10% normal horse serum. Primary antibody (1:50; Oncogene Research Products, San Diego, CA) was incubated for 1.5 h. For laminin-1 and fibronectin, antigens were retrieved by incubating the samples in 0.1% trypsin-PBS (pH 7.6) at 37°C for 15 min. Slides were washed and incubated in 3% H_2O_2 for 5 min and then blocked in 10% horse serum. Primary antibodies against fibronectin (1:55; Sigma, St. Louis, MO) or laminin-1 (1:75; Sigma) were incubated overnight at 4°C. Antibody detection was done using the avidin-biotinylated enzyme (Vector Laboratories), and color was developed with diaminobenzidine. Sections were counterstained in Harris' hematoxylin. Primary antibody was withheld from negative controls, which were incubated in diluent alone.

Scoring. All arrays were scored in a blinded fashion by a study pathologist (Y-Y.C.). Each sample was scored for overall quality based on the degree of background staining (0 = heavy, 1 = light, 2 = none) and the integrity of the tissue (0 = mostly nonepithelial tissue, 1 = <25% tumor cells, 2 = 25–75% tumor cells, 3 = >75% tumor cells). Only those samples that had an overall additive quality score of ≥3 were used in the study, and samples with heavy background staining were excluded from the analysis. For β_1 integrin, each sample was scored based on the intensity of signal (0–3) and the percentage of positive cells (0 = <10%, 1 = 10–25%, 2 = 25–50%, 3 = >50%; representative samples are shown in Fig. 1). For fibronectin and laminin-1, a score was assigned for the intensity and percentage of cytoplasmic staining and for intensity and pattern [negative = 0, focal (<25%) = 1, moderate (25–75%) = 2, diffuse (>75%) = 3] of extracellular staining. All scoring methods were assigned based on a review of the literature and review of sample cases before reading any slides; scoring was not recategorized based on any analysis with outcomes.

Statistical methods. To estimate the role of β_1 integrin in breast cancer prognosis, survival curves were calculated and plotted according to the Kaplan-Meier method. For univariate comparisons of overall and disease-free survival with clinicopathologic variables, a Cox proportional hazards model was fit to data to assess the significance of predictors of recurrence.

To investigate the associations among β_1 integrin, fibronectin, and laminin-1, multiple pairwise comparisons were made using nonparametric Kendall's tau-b as a measure of association. This statistic was selected because it is more robust than Pearson's correlation statistic when there are many tied values and the scoring is ordered but may not be linear (e.g., a score of 2 is not necessarily twice that of a score of 1).

To determine which variables may be jointly predictive of outcome, multivariate models were tested where predictors, which were significant at $P < 0.10$ in univariate analysis, were entered into a Cox proportional hazards model simultaneously and then eliminated stepwise one at a time until all remaining predictors were significant at $P < 0.05$.

Means for continuous variables were compared using unpaired two-sided t tests. Categorical data were compared using χ^2 statistics. All calculations were carried out in Stata version 9 (Stata Corp., College Station, TX).

Results

β_1 integrin and ligands fibronectin and laminin-1 are expressed in a significant proportion of breast cancers. β_1 integrin signal intensity was scored on a scale of 0 to 3 (Fig. 1). Of the 149 patients included in the study, 117 (79%) scored positively for expression of β_1 integrin, and of these, 12 of 117 (13%) had the highest intensity score of 3 (Table 1). The percentage of β_1 integrin–expressing cells were also scored, and staining was found to be diffusely present in the vast majority (114 of 117, 96%) of cases (Table 1). Extracellular fibronectin was similarly scored and found to be present in 55 of 134 (41%) of cases; of these, fibronectin was characterized by low intensity and diffuse staining in 46 of 55 (84%) and in 33 of 55 (60%) of cases, respectively. In addition, extracellular laminin-1 was found to be expressed in 43 of 135 (32%) of cases; laminin-1 showed moderate intensity staining in 27 of 43 (63%) of cases and 29 of 44 (66 %) cases showed a restricted, focal pattern of expression (Table 1).

β_1 integrin and extracellular fibronectin expression are significantly correlated. β_1 integrin mediates cues from its major extracellular ligands, fibronectin and laminin-1, to elicit major signaling effectors downstream. To determine if there were significant patterns of expression between these major components of the β_1 integrin signaling axis, multiple pairwise tests were done among β_1 integrin, fibronectin, and laminin-1 among the cases that

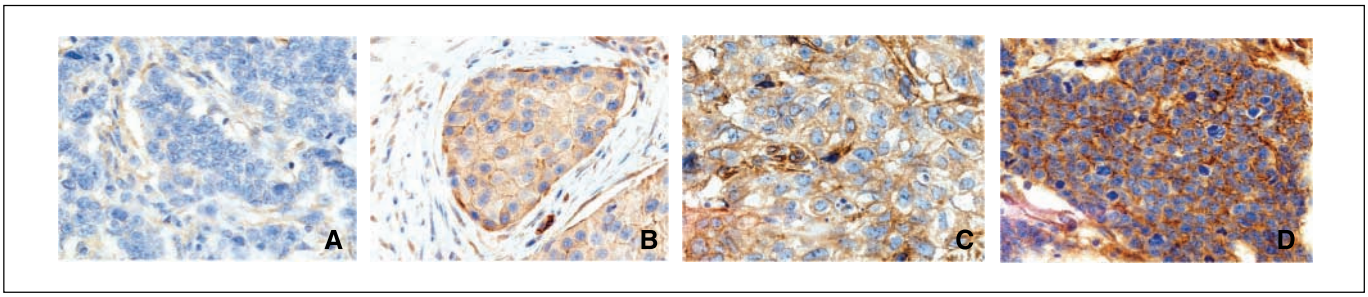

Figure 1. Breast cancer specimens were scored for membranous β_1 integrin signal intensity detected by immunohistochemistry of paraffin-embedded tissue. *A,* the absence of signal was scored as "0." *B,* low intensity signal was scored as "1." *C,* moderate intensity signal was scored as "2." *D,* high intensity signal was scored as "3."

Table 1. Expression pattern for β_1 integrin, extracellular fibronectin, and laminin-1 in breast cancer

Factor	No. patients	Score, *n* (%)			
		0	1	2	3
β_1 integrin intensity	149	32 (21.5)	75 (50.3)	27 (18.1)	15 (10.1)
β_1 integrin percentage	147	31 (21.1)	2 (1.4)	2 (1.4)	112 (76.2)
ECM FN intensity	134	79 (59.8)	46 (34.8)	7 (5.3)	2 (1.5)
ECM FN pattern	134	79 (59.8)	8 (6.1)	14 (10.6)	33 (25.0)
ECM LN intensity	135	92 (68.1)	10 (7.4)	27 (20.0)	6 (4.4)
ECM LN pattern	135	91 (67.4)	29 (21.5)	6 (4.4)	9 (6.7)

NOTE: β_1 integrin intensity score (based on membranous signal): 0 = none, 1 = light, 2 = moderate, 3 = heavy; β_1 integrin percentage score (based on the percentage of tumor cells stained in the sample): 0 = <10%, 1 = 10% to 25%, 2 = 25% to 50%, 3 = >50%; ECM fibronectin intensity score: 0 = none, 1 = light, 2 = moderate, 3 = heavy; ECM fibronectin pattern score (of extracellular staining): negative = 0, focal (<25%) = 1, moderate (25–75%) = 2, diffuse (>75%) = 3; ECM laminin-1 intensity score: 0 = none, 1 = light, 2 = moderate, 3 = heavy; ECM laminin-1 pattern score (of extracellular staining): negative = 0, focal (<25%) = 1, moderate (25–75%) = 2, diffuse (>75%) = 3.
Abbreviations: FN, fibronectin; LN, laminin-1.

were scored for β_1 integrin. We found no significant correlation between β_1 integrin expression and laminin-1. However, there was a significant association between β_1 integrin intensity score and fibronectin pattern (Kendall's tau-b = 0.19; P = 0.03) and an association that approached significance between β_1 integrin intensity score and fibronectin intensity score (Kendall's tau-b = 0.17; P < 0.06; Table 2).

Factors associated with outcomes. We analyzed β_1 integrin staining for associations with overall and disease-free survival. β_1 integrin intensity was significantly associated with overall and disease-free survival [with hazard ratio (HR) of 2.89; 95% confidence interval (95% CI), 1.08–7.72; P = 0.026 for overall survival and HR of 4.1; 95% CI, 1.31–13.0; P = 0.016 for disease-free survival, respectively; Table 3]. Compared with a β_1 integrin intensity score of 0, patients with scores of 1, 2, or 3 had incrementally increasing HR, which became significant with the highest score of 3 for overall and disease-free survival (Table 3). In addition, among the cases that were scored for β_1 integrin (n = 149), the highest fibronectin intensity score of 3 was observed in a single case and was found to be significantly associated with overall and disease-free survival (HR, 8.53; 95% CI, 1.08–67.13; P = 0.042 for overall survival and HR, 13.76; 95% CI, 1.63–115.91; P = 0.016 for disease-free survival, respectively; Table 3).

To determine whether known prognostic factors influenced survival, several other clinical and pathologic variables were tested for associations with outcomes (Table 3). Among these, the factors that were significantly associated with overall survival included American Joint Committee on Cancer (AJCC) stage (HRs of 1.93 and 6.17 for stages II and III compared with stage I, respectively), number of axillary nodes positive (HRs of 1.75 and 5.13 for 1–3 and >3 positive nodes compared with 0 nodes, respectively), tumor size (HRs of 1.01, 1.68, and 5.73 for 10–19 mm, 20–29 mm, and ≥30 mm compared with 0–9 mm, respectively), and Scharff-Bloom-Richardson (SBR) grade (HRs of 1.66 and 2.40 for grades 2 and 3 compared with grade 1, respectively; Table 3). These factors showed a significant trend with increasing values (Table 3).

Table 2. Multiple pairwise Kendall tau-b correlations among β_1 integrin, ECM fibronectin, and ECM laminin-1

	Statistic	β_1 integrin intensity	β_1 integrin percentage	ECM FN intensity	ECM FN pattern	ECM LN intensity	ECM LN pattern
β_1 integrin intensity	K-tau*	1.000					
	P						
β_1 integrin percentage	K-tau	0.707	1.000				
	P	0.000 †					
ECM FN intensity	K-tau	0.170	0.167	1.000			
	P	0.055	0.075				
ECM FN pattern	K-tau	0.186	0.176	0.913	1.000		
	P	0.031 †	0.055	0.000 †			
ECM LN intensity	K-tau	0.006	−0.025	0.231	0.221	1.000	
	P	0.948	0.794	0.006 †	0.007 †		
ECM LN pattern	K-tau	0.039	0.022	0.196	0.200	0.868	1.000
	P	0.663	0.821	0.019 †	0.014 †	0.000 †	

*K-tau is a nonparametric measure of correlation.
†Statistically significant if P < 0.05.

Table 3. Univariate analysis for overall and disease-free survival

Variable	Overall survival			Disease-free survival		
	No. patients	HR (95% CI)	*P*	No. patients	HR (95% CI)	*P*
β_1 integrin intensity	146	1.43 (1.04–1.97)	0.03*	146	1.48 (0.98–2.21)	0.06*
0	32	1		32	1	
1	72	1.17 (0.51–2.70)	0.71	74	1.23 (0.43–3.50)	0.69
2	27	1.65 (0.63–4.33)	0.31	27	0.72 (0.17–3.01)	0.65
3	15	2.89 (1.08–7.72)	0.03	15	4.13 (1.31–13.0)	0.02
ECM fibronectin intensity	108	1.03 (0.65–1.65)	0.87*	105	1.40 (0.72–2.71)	0.32*
0	66	1		64	1	
1	35	0.87 (0.40–1.92)	0.73	34	0.73 (0.37–3.18)	0.88
2	6	1.20 (0.28–5.17)	0.80	6	0.80 (0.13–7.97)	0.98
3	1	8.53 (1.08–67.1)	0.04	1	13.76 (1.63–115.9)	0.02
ECM laminin-1 intensity	133	0.95 (0.69–1.31)	0.76*	105	0.94 (0.57–1.57)	0.83*
0	89	1		24	1	
1	10	1.20 (0.42–3.45)	0.73	35	0.62 (0.08–4.80)	0.51
2	28	0.61 (0.27–1.41)	0.25	28	0.80 (0.23–2.85)	0.80
3	6	2.08 (0.64–6.83)	0.22	18	1.17 (0.15–9.02)	0.87
Age	145	1.02 (1.003–1.04)	0.03*	145	0.97 (0.94–0.99)	0.02*
AJCC stage	144	2.43 (1.51–3.91)	<0.001*	144	3.05 (1.69–5.51)	<0.001*
1	57	1		57	1	
2	72	1.93 (0.93–3.98)	0.07	72	3.37 (1.12–10.11)	0.03
3	15	6.17 (2.51–15.15)	<0.001	15	9.56 (2.77–33.0)	<0.001
Axillary nodes positive	144	2.26 (1.58–3.23)	<0.001	144	1.62 (1.06–2.50)	0.03*
0	91	1		91	1	
1–3	26	1.75 (0.69–4.68)	0.24	26	2.24 (0.88–5.69)	0.24
≥3	27	5.13 (2.55–10.33)	<0.001	27	2.58 (1.05–6.32)	0.44
Tumor size (mm)	148	2.07 (1.48–2.88)	<0.001	146	2.44 (1.57–3.78)	<0.001
0–9	19	1		18	1	
10–19	62	1.01 (0.36–2.83)	0.99	61	N/A†	N/A
20–29	34	1.68 (0.53–5.30)	0.38	34	N/A	N/A
≥30	33	5.73 (2.03–16.13)	<0.001	33	N/A	N/A
Estrogen receptor positive	120	0.60 (0.288–1.27)	0.18	120	0.62 (0.28–1.64)	0.40
Progesterone receptor positive	140	0.58 (0.31–1.09)	0.09	138	0.62 (0.28–1.34)	0.22
SBR score	141	1.52 (1.00–2.31)	0.05*	141	2.25 (1.20–4.23)	0.01*
1	28	1		27	1	
2	54	1.66 (0.68–4.25)	0.29	53	4.19 (0.53–33.1)	0.17
3	59	2.40 (0.96–6.00)	0.06	61	7.89 (1.04–59)	0.04
Radiation therapy	143	1.26 (0.675–2.36)	0.46	141	1.13 (0.52–2.43)	0.76
Chemotherapy	144	1.12 (0.58–2.15)	0.73	142	1.59 (0.74–3.39)	0.22
Hormone therapy	143	1.27 (0.664–2.43)	0.47	141	0.99 (0.45–2.21)	0.98

*First HR is for factor considered as a continuous variable.
†Not available due to 0 recurrence in baseline group.

β_1 integrin is associated with overall and disease-free survival in breast cancer. Among this cohort with predominantly early-stage invasive breast cancer, patients with tumors expressing high β_1 integrin signal intensity had significantly decreased overall survival at 5 years compared with patients with tumors that had none to moderate intensity of expression (48% versus 85%; $P = 0.028$, log-rank test). Moreover, this difference persisted at 10 years (48% versus 71%; Fig. 2). In addition, β_1 integrin expression was significantly associated with worse disease-free survival at 5 and 10 years ($P = 0.002$, log-rank test; Fig. 2). Importantly, all deaths and recurrences among patients with high β_1 integrin–expressing tumors occurred within the first 5 years.

Multivariate analyses of variables associated with outcomes. Next, we wished to determine which factors associated with outcome were jointly predictive. All factors analyzed in Table 3 that had a significance level of at least $P < 0.10$ were included in a reverse stepwise multivariate analysis to determine the best-fit model for overall and disease-free survival. For overall survival, β_1 integrin intensity score, axillary nodal status, and tumor size were all jointly significant predictors of outcome with HRs of 1.73, 1.84, and 1.98, respectively (Table 4). For disease-free survival, the factors that were significantly associated with improved disease-free survival were age, β_1 integrin intensity score, tumor size, grade based on SBR score, and adjuvant chemotherapy (Table 4).

Discussion

The increasing importance of cell-ECM interactions (14) and the tumor microenvironment in cancer progression have led to the development of novel biomarkers and molecular targets for cancer

therapy (24). β_1 integrin signaling has been shown to critically mediate breast cancer progression in culture models (4, 25) and is essential for tumorigenesis *in vivo* (7). We have shown previously that β_1 integrin inhibitory antibodies selectively enhance apoptosis and decrease proliferation in three-dimensional cultures of breast cancer and *in vivo* (8), indicating that β_1 integrin is a promising therapeutic target; however, clinical correlation of these findings has been lacking. In the present study, we report that high β_1 integrin signal intensity (3+ versus 0–2+) was significantly associated with decreased overall survival, 48% versus 85% at 5 years and 48% versus 71% at 10 years, among patients with invasive breast cancer. Moreover, high β_1 integrin signal intensity was associated with decreased disease-free survival of 50% versus 85% at 5 years and 50% versus 80% at 10 years. β_1 integrin remained an independent factor associated with decreased overall survival (HR, 1.69; 95% CI, 1.19–2.38; $P \leq 0.003$) and disease-free survival (HR, 1.87; 95% CI, 1.21–2.88; $P \leq 0.005$) on multivariate regression analysis. This is the first clinical evidence implicating β_1 integrin expression with poor outcome after treatment for breast cancer. Importantly, extracellular fibronectin expression was also associated with decreased overall and disease-free survival on univariate analysis (HR, 8.53; 95% CI, 1.08–67.12; $P < 0.042$ and HR, 13.76; 95% CI, 1.63–115.9; $P = 0.016$) and β_1 integrin expression was significantly correlated with fibronectin ($P < 0.03$). These findings implicate specific integrin-ligand interactions that may be associated with more aggressive disease and may help identify subsets of patients who may benefit from targeted therapy.

Table 4. Multivariate model for overall and disease-free survival

Factor	HR (95% CI)	*P*
Overall survival, best-fit model		
Tumor size (T1, T2, T3)	1.98 (1.31–3.01)	0.001
Axillary node status (0, 1–3, ≥4)	1.84 (1.21–2.81)	0.004
β_1 integrin intensity score (0, 1, 2, 3)	1.73 (1.22–2.44)	0.002
Disease-free survival, best-fit model		
Tumor size (T1, T2, T3)	3.86 (2.18–6.81)	0.000
SBR score (1, 2, 3)	2.24 (1.10–4.53)	0.025
β_1 integrin intensity score (0, 1, 2, 3)	1.88 (1.20–2.95)	0.006
Age	0.95 (0.92–0.99)	0.007
Chemotherapy	0.33 (0.12–0.89)	0.030

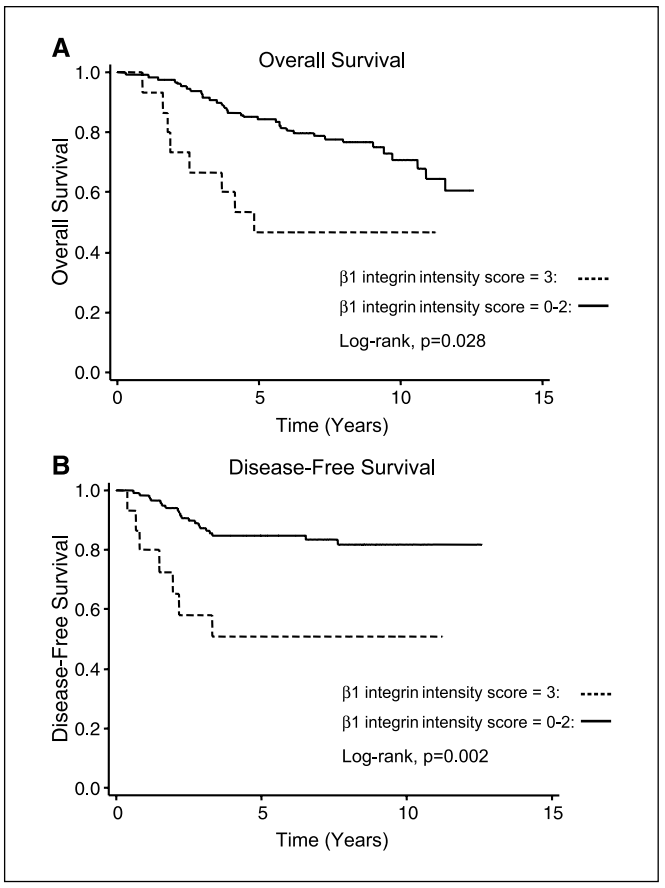

Figure 2. High β_1 integrin intensity score is associated with decreased overall and recurrence-free survival. *A,* Kaplan-Meier survival curves show a significantly decreased overall survival among patients with β_1 integrin intensity score of 3 (*dashed line*) compared with patients with a score of 0 to 2 (*solid line*). $P = 0.028$, log-rank test. *B,* Kaplan-Meier survival curves show a significantly reduced recurrence-free survival among patients with β_1 integrin intensity score of 3 (*dashed line*) compared with patients with a score of 0 to 2 (*solid line*). $P = 0.002$, log-rank test.

The normal tissue architecture is reflective of a homeostatic balance among key receptors that govern cell-ECM interactions, including β_1 integrin. This balance is disrupted in malignant tissue, where receptors and ligands are aberrantly expressed. It is plausible that a relative loss or gain of β_1 integrin expression in malignant tissue could occur relative to the normal intact epithelium. Previous studies of human breast tumors have reported that, compared with expression in normal tissue seen primarily in the myoepithelial cell layer of breast ducts, decreased β_1 integrin expression was associated with characteristics of more aggressive disease (26–29). Conversely, increased β_1 integrin signaling has been shown to promote tumorigenesis by facilitating growth factor receptor activity (4, 30) and inhibition of β_1 integrin has been shown to abrogate metastasis (31, 32). We hypothesized that overexpression of β_1 integrin in tumors represents a distinct biology that may benefit from targeted therapy. Thus, we chose to score β_1 integrin intensity staining based on the level of signal and percentage of tumor cells expressing the signal. This approach, as well as differences in immunohistochemical techniques and antibodies used, may account for disparate results between institutional studies. Notably, among studies in lung (33) and periampullary (20) carcinomas, where scoring and antibodies used were similar to this study, high β_1 integrin expression in the tumors was associated with poor outcomes.

β_1 integrin signaling depends on binding to extracellular ligands, including fibronectin and laminin-1. Fibronectin has been shown previously to act as poor prognostic factor, and the coexpression of integrin receptors has been shown to enhance resistance to chemotherapies in the treatment of lung cancer (18). We found that fibronectin was associated with decreased overall and disease-free survival on univariate analysis and that β_1 integrin was significantly coexpressed with fibronectin in a subset of breast tumors. The fact that fibronectin was not an independent prognostic factor in the multivariable model indicates that its interaction with β_1 integrin is more important than the expression of fibronectin itself. Previous studies have shown that fibronectin-mediated cell adhesion associated with $\alpha_5\beta_1$ and $\alpha_v\beta_3$ receptors has been associated with tumor invasion and angiogenesis (34). The promise of such studies has led to the development of several

small-molecule inhibitors designed to disrupt integrin and fibronectin binding (35–37).

In the present study, there was no significant association between β_1 integrin expression and chemotherapy, hormone treatment, and radiation therapy. However, increasing evidence supports the role of β_1 integrin signaling in mediating resistance to chemotherapy and radiotherapy in lung, breast cancer, and hematologic malignancies (38–41). Thus, although β_1 integrin was not found to be a predictive factor in this small series, further investigation is warranted to clarify this issue.

We acknowledge the potential drawbacks of testing a novel potential biomarker in clinical tissue specimens. Although the immunoreactivity of a particular receptor or molecule may be associated with outcome, it may not reflect the biological activity of the receptor. In addition, although the present cohort has significant follow-up time, the relatively modest sample size necessitates validation studies using larger numbers of patients.

In conclusion, we report that high β_1 integrin expression is associated with decreased overall and disease-free survival among patients with invasive breast cancer. These findings are consistent with previous reports in different types of cancer (20, 33); however, they need to be validated in a larger cohort of breast cancer patients. In addition, our results indicate that both expression of the β_1 integrin receptor and its association with fibronectin may be useful in identifying subsets of patients who may benefit from targeted therapies.

Acknowledgments

Received 7/27/2006; revised 10/5/2006; accepted 11/14/2006.

Grant support: NIH P50 Specialized Program of Research Excellence grant CA CA58207-08 (C. Park) and Bay Area/UCSF Breast Oncology Program Tissue Bank.

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.

We thank Mina Bissell for advice and critical reading of the manuscript and Loretta Chan for expert technical assistance.

References

1. Bissell MJ, Radisky DC, Rizki A, Weaver VM, Petersen OW. The organizing principle: microenvironmental influences in the normal and malignant breast. Differentiation 2002;70:537–46.
2. Hannigan G, Troussard AA, Dedhar S. Integrin-linked kinase: a cancer therapeutic target unique among its ILK. Nat Rev Cancer 2005;5:51–63.
3. Boudreau N, Werb Z, Bissell MJ. Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. Proc Natl Acad Sci U S A 1996;93:3509–13.
4. Wang F, Hansen RK, Radisky D, et al. Phenotypic reversion or death of cancer cells by altering signaling pathways in three-dimensional contexts. J Natl Cancer Inst 2002;94:1494–503.
5. Weaver VM, Petersen OW, Wang F, et al. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and *in vivo* by integrin blocking antibodies. J Cell Biol 1997;137:231–45.
6. Zutter MM, Santoro SA, Staatz WD, Tsung YL. Re-expression of the $\alpha_2\beta_1$ integrin abrogates the malignant phenotype of breast carcinoma cells. Proc Natl Acad Sci U S A 1995;92:7411–5.
7. White DE, Kurpios NA, Zuo D, et al. Targeted disruption of β_1-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. Cancer Cell 2004;6:159–70.
8. Park CC, Zhang H, Pallavicini M, et al. β_1 integrin inhibitory antibody induces apoptosis of breast cancer cells, inhibits growth, and distinguishes malignant from normal phenotype in three dimensional cultures and *in vivo*. Cancer Res 2006;66:1526–35.
9. Damiano JS. Integrins as novel drug targets for overcoming innate drug resistance. Curr Cancer Drug Targets 2002;2:37–43.
10. Hazlehurst LA, Damiano JS, Buyuksal I, Pledger WJ, Dalton WS. Adhesion to fibronectin via β_1 integrins regulates p27kip1 levels and contributes to cell adhesion mediated drug resistance (CAM-DR). Oncogene 2000;19:4319–27.
11. Cordes N, Beinke C. Fibronectin alters cell survival and intracellular signaling of confluent A549 cultures after irradiation. Cancer Biol Ther 2004;3:47–53.
12. Cordes N, Beinke C, Plasswilm L, van Beuningen D. Irradiation and various cytotoxic drugs enhance tyrosine phosphorylation and β(1)-integrin clustering in human A549 lung cancer cells in a substratum-dependent manner *in vitro*. Strahlenther Onkol 2004;180:157–64.
13. Muschler J, Levy D, Boudreau R, Henry M, Campbell K, Bissell MJ. A role for dystroglycan in epithelial polarization: loss of function in breast tumor cells. Cancer Res 2002;62:7102–9.
14. Singh J, Itahana Y, Knight-Krajewski S, et al. Proteolytic enzymes and altered glycosylation modulate dystroglycan function in carcinoma cells. Cancer Res 2004;64:6152–9.
15. Viacava P, Naccarato AG, Collecchi P, Menard S, Castronovo V, Bevilacqua G. The spectrum of 67-kD laminin receptor expression in breast carcinoma progression. J Pathol 1997;182:36–44.
16. Molino A, Pedersini R, Micciolo R, et al. Prognostic significance of laminin, laminin receptor, and bone marrow micrometastases in breast cancer patients: are these markers of aggressive behavior and metastatic potential? Appl Immunohistochem Mol Morphol 2003;11:311–8.
17. Ioachim E, Charchanti A, Briasoulis E, et al. Immunohistochemical expression of extracellular matrix components tenascin, fibronectin, collagen type IV, and laminin in breast cancer: their prognostic value and role in tumour invasion and progression. Eur J Cancer 2002;38:2362–70.
18. Sethi T, Rintoul RC, Moore SM, et al. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance *in vivo*. Nat Med 1999;5:662–8.
19. Oshita F, Kameda Y, Hamanaka N, et al. High expression of integrin β_1 and p53 is a greater poor prognostic factor than clinical stage in small-cell lung cancer. Am J Clin Oncol 2004;27:215–9.
20. Bottger TC, Maschek H, Lobo M, Gottwohl RG, Brenner W, Junginger T. Prognostic value of immunohistochemical expression of β-1 integrin in pancreatic carcinoma. Oncology 1999;56:308–13.
21. Nikkola J, Vihinen P, Vlaykova T, Hahka-Kemppinen M, Heino J, Pyrhonen S. Integrin chains β_1 and α_v as prognostic factors in human metastatic melanoma. Melanoma Res 2004;14:29–37.
22. Jonjic N, Lucin K, Krstulja M, Iternicka Z, Mustac E. Expression of β-1 integrins on tumor cells of invasive ductal breast carcinoma. Pathol Res Pract 1993;189:979–84.
23. Gui GP, Wells CA, Browne PD, et al. Integrin expression in primary breast cancer and its relation to axillary nodal status. Surgery 1995;117:102–8.
24. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. Oncogene 2000;19:6550–65.
25. Weaver VM, Lelievre S, Lakins JN, et al. β_4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. Cancer Cell 2002;2:205–16.
26. Gonzalez MA, Pinder SE, Wencyk PM, et al. An immunohistochemical examination of the expression of E-cadherin, α- and β/γ-catenins, and α_2- and β_1-integrins in invasive breast cancer. J Pathol 1999;187:523–9.
27. Lanzafame S, Emmanuele C, Torrisi A. Correlation of $\alpha_2\beta_1$ integrin expression with histological type and hormonal receptor status in breast carcinomas. Pathol Res Pract 1996;192:1031–8.
28. Pignatelli M, Cardillo MR, Hanby A, Stamp GW. Integrins and their accessory adhesion molecules in mammary carcinomas: loss of polarization in poorly differentiated tumors. Hum Pathol 1992;23:1159–66.
29. Mechtersheimer G, Munk M, Barth T, Koretz K, Moller P. Expression of β_1 integrins in non-neoplastic mammary epithelium, fibroadenoma, and carcinoma of the breast. Virchows Arch A Pathol Anat Hist 1993;422:203–10.
30. Wang F, Weaver VM, Petersen OW, et al. Reciprocal interactions between β_1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. Proc Natl Acad Sci U S A 1998;95:14821–6.
31. Elliott BE, Maxwell L, Arnold M, Wei WZ, Miller FR. Expression of epithelial-like markers and class I major histocompatibility antigens by a murine carcinoma growing in the mammary gland and in metastases: orthotopic site effects. Cancer Res 1988;48:7237–45.
32. Fujita S, Suzuki H, Kinoshita M, Hirohashi S. Inhibition of cell attachment, invasion, and metastasis of human carcinoma cells by anti-integrin β_1 subunit antibody. Jpn J Cancer Res 1992;83:1317–26.
33. Oshita F, Kameda Y, Ikehara M, et al. Increased expression of integrin β_1 is a poor prognostic factor in small-cell lung cancer. Anticancer Res 2002;22:1065–70.
34. Jia Y, Zeng ZZ, Markwart SM, et al. Integrin fibronectin receptors in matrix metalloproteinase-1-dependent invasion by breast cancer and mammary epithelial cells. Cancer Res 2004;64:8674–81.
35. Juarez JC, Guan X, Shipulina NV, et al. Histidine-proline-rich glycoprotein has potent antiangiogenic activity mediated through the histidine-proline-rich domain. Cancer Res 2002;62:5344–50.
36. Shannon KE, Keene JL, Settle SL, et al. Anti-metastatic properties of RGD-peptidomimetic agents S137 and S247. Clin Exp Metastasis 2004;21:129–38.
37. Stoeltzing O, Liu W, Reinmuth N, et al. Inhibition of integrin $\alpha_5\beta_1$ function with a small peptide (ATN-161) plus continuous 5-FU infusion reduces colorectal liver metastases and improves survival in mice. Int J Cancer 2003;104:496–503.
38. Cordes N, Seidler J, Durzok R, Geinitz H, Brakebusch C. β_1-integrin-mediated signaling essentially contributes to cell survival after radiation-induced genotoxic injury. Oncogene 2006;25:1378–90.
39. Aoudjit F, Vuori K. Integrin signaling inhibits paclitaxel-induced apoptosis in breast cancer cells. Oncogene 2001;20:4995–5004.
40. Damiano JS, Dalton WS. Integrin-mediated drug resistance in multiple myeloma. Leuk Lymphoma 2000;38:71–81.
41. Damiano JS, Hazlehurst LA, Dalton WS. Cell adhesion-mediated drug resistance (CAM-DR) protects the K562 chronic myelogenous leukemia cell line from apoptosis induced by BCR/ABL inhibition, cytotoxic drugs, and γ-irradiation. Leukemia 2001;15:1232–9.

Increased β_1 Integrin Is Associated with Decreased Survival in Invasive Breast Cancer

Evelyn S. Yao, Hui Zhang, Yunn-Yi Chen, et al.

Cancer Res 2007;67:659-664.