

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

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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 Prairie, WI).

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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 μ m) 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

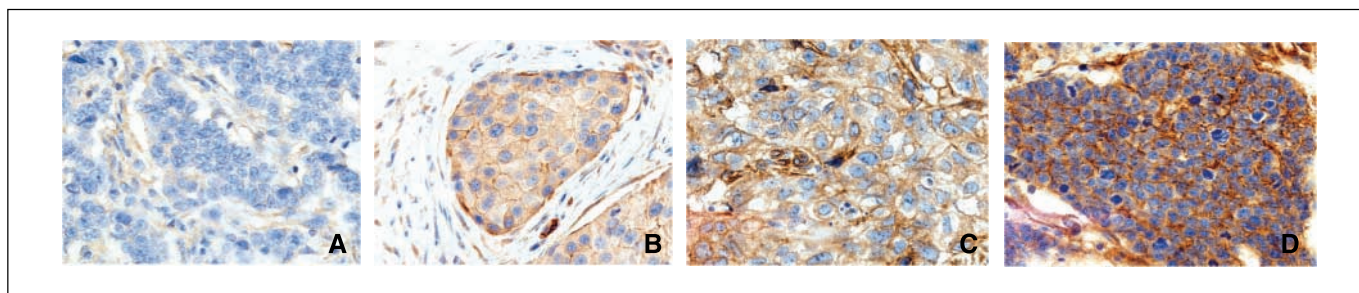


Figure 1. Breast cancer specimens were scored for membranous β_1 integrin signal intensity detected by immunohistochemistry of paraffin-embedded tissue. A, the absence of signal was scored as "0." B, low intensity signal was scored as "1." C, moderate intensity signal was scored as "2." D, high intensity 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* P | 1.000 | | | | | |
| β_1 integrin percentage | K-tau P | 0.707 0.000 [†] | 1.000 | | | | |
| ECM FN intensity | K-tau P | 0.170 0.055 | 0.167 0.075 | 1.000 | | | |
| ECM FN pattern | K-tau P | 0.186 0.031 [†] | 0.176 0.055 | 0.913 0.000 [†] | 1.000 | | |
| ECM LN intensity | K-tau P | 0.006 0.948 | −0.025 0.794 | 0.231 0.006 [†] | 0.221 0.007 [†] | 1.000 | |
| ECM LN pattern | K-tau P | 0.039 0.663 | 0.022 0.821 | 0.196 0.019 [†] | 0.200 0.014 [†] | 0.868 0.000 [†] | 1.000 |

*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) | <i>P</i> | No. patients | HR (95% CI) | <i>P</i> |
| 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

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

| Factor | HR (95% CI) | <i>P</i> |
|---|------------------|----------|
| 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 |

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.

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

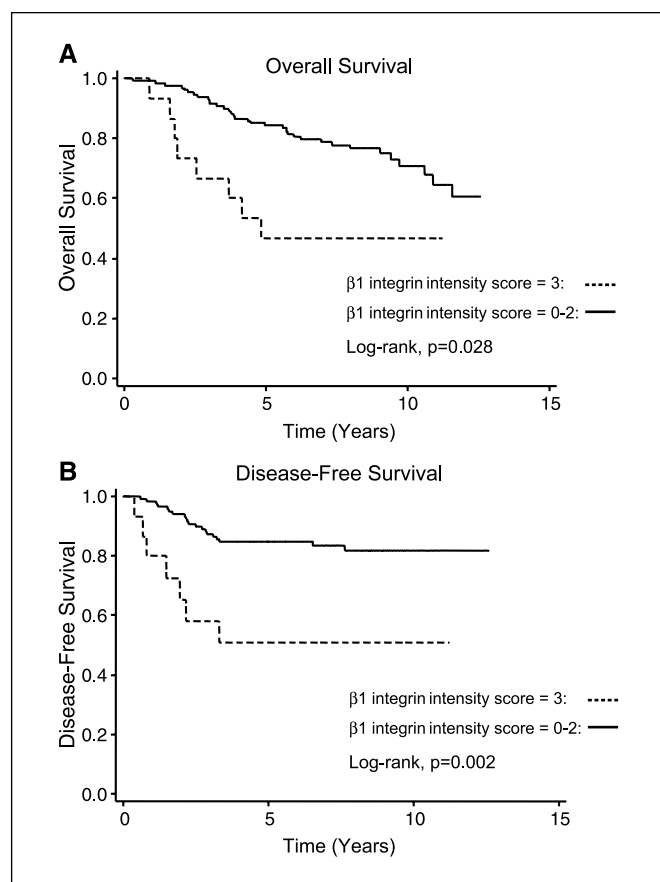


Figure 2. High β_1 integrin intensity score is associated with decreased overall and recurrence-free survival. **A**, Kaplan-Meier survival curves show a significantly decreased overall survival among patients with β_1 integrin intensity score of 3 (dashed line) compared with patients with a score of 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.

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.

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