B7-H4 Is Highly Expressed in Ductal and Lobular Breast Cancer

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ABSTRACT

Purpose: **This study was designed to investigate the expression of B7-H4 protein, a member of the B7 family that is involved in the regulation of antigen-specific immune responses, in normal breast and in primary and metastatic breast carcinomas.**

Experimental Design: **Archival formalin-fixed tissue blocks from breast cancers and normal somatic tissues were evaluated for B7-H4 expression by immunohistochemistry with manual and automated image analysis. The proportion of B7-H4-positive cells and the intensity of B7-H4 staining were compared with histologic type, grade, stage, hormone receptor status, and HER-2/*neu* status.**

Results: **B7-H4 was detected in 165 of 173 (95.4%) primary breast cancers and in 240 of 246 (97.6%) metastatic breast cancers. B7-H4 staining intensity was greater in invasive ductal carcinomas [24.61 relative units (RU)] and in invasive lobular carcinomas (15.23 RU) than in normal breast epithelium (4.30 RU, $P = 0.0003$). Increased staining intensity was associated with negative progesterone receptor status ($P = 0.014$) and history of neoadjuvant chemotherapy ($P = 0.004$), and the proportion of B7-H4-positive cells was associated with negative progesterone receptor ($P = 0.001$) and negative HER-2/*neu* ($P = 0.024$) status. However, there was no statistically significant relationship between the proportion of B7-H4-positive cells or staining intensity and grade, stage, or other clinicopathologic variables. Low levels of B7-H4 expression were also detected in epithelial cells of the female genital tract, lung, pancreas, and kidney, but B7-H4 was generally absent in most other normal somatic tissues.**

Conclusions: **The nearly ubiquitous expression of B7-H4 in breast cancer, independent of tumor grade or stage, suggests a critical role for this protein in breast cancer biology.**

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INTRODUCTION

Numerous therapeutic modalities are available for the adjuvant treatment of advanced breast cancer including radiotherapy, conventional chemotherapy with cytotoxic antitumor agents, hormone therapy (aromatase inhibitors, luteinizing-hormone releasing-hormone analogues), bisphosphonates, and signal-transduction inhibitors (1). The current approach to the optimal treatment selection for breast cancer is multidisciplinary and based on several factors, including clinical stage, biological characteristics of the cancer, disease recurrence, patient's age and preferences, as well as risks and benefits associated with each treatment protocol, which help clinicians to stratify patients for appropriate treatment decisions. However, despite the great variety of adjuvant treatment options, many patients either respond poorly or not at all to any of the above-described therapeutic modalities. Thus, there is a need to identify new molecular markers for breast cancer that could provide further therapeutic targets for patients that are unlikely to respond to current treatment options.

We initially identified and characterized DD-O110 as a novel gene encoding a predicted membrane glycoprotein that is overexpressed in breast and ovarian cancer with relatively little expression in normal somatic tissues, by quantitative PCR analysis of over 200 human tissue samples.[3] Based on the predicted amino acid sequence, we subsequently determined that DD-O110 is homologous to B7-H4 (also known as B7x or B7S1), a recently discovered B7 family member. B7 family members and their receptors play critical roles in the regulation of antigen-specific immune responses (2). B7-H4 ligation to its receptor BTLA on T lymphocytes results in inhibition of T-cell activation, cytokine secretion, and the development of cytotoxicity (3–6). B7-H4 mRNA but not protein expression has been detected in a wide range of normal somatic tissues, including liver, skeletal muscle, kidney, pancreas, and small bowel (3, 5). However, cell surface expression of B7-H4 protein was induced upon stimulation of T cells, B cells, monocytes, and dendritic cells in addition to a constitutive B7-H4 protein expression in lung and ovarian cancer (5). The significance of B7-H4 expression in normal or malignant nonhematopoietic cell populations has not been determined.

The present study was designed to test the hypothesis that B7-H4 protein is consistently overexpressed in primary and metastatic breast cancer and to determine if B7-H4 expression is dependent on histologic type, grade, stage, estrogen receptor (ER), progesterone receptor (PR) or HER-2/*neu* status, or with other clinical variables.

MATERIALS AND METHODS

Tissue Samples. Tissues were obtained from 173 patients with primary breast cancer who underwent surgery at the

[3] Papkoff et al., submitted for publication.

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University of Colorado Hospital, Denver, CO. Tissue blocks were assembled from the archival collections from the Department of Pathology and included 155 invasive ductal carcinomas (152 cases of ductal carcinoma of the usual type and 3 cases of invasive tubular carcinoma) and 18 lobular carcinomas of the breast. Cases with mixed patterns of histologic differentiation were excluded from the analysis. The mean age of patients at the time of diagnosis was 55.5 years (±12.8; range, 29-89 years). The tumors were classified as American Joint Committee on Cancer pathologic stage I (90 cases), stage IIa (35 cases), stage IIb (20 cases), stage IIIa (16 cases), stage IIIb (5 cases), stage IIIc (5 cases), and stage IV (2 cases). The study also included 246 breast cancer–positive lymph nodes from a subset of 27 patients who were part of the primary study population. We also evaluated normal breast tissue from women (n = 15) undergoing reduction mammoplasty but with no history of breast cancer. In addition, a broad spectrum of normal adult and fetal somatic tissues (n = 314) was evaluated for B7-H4 expression to confirm the specificity of the B7-H4 protein to breast cancer cells (Table 3).

Information on ER, PR, and HER-2/*neu* status was collected from the original surgical pathology reports. HER-2/*neu* status was determined by fluorescence *in situ* hybridization analysis (ACIS; Chromavision, San Juan Capistrano, CA). Patient survival data was provided by the University Hospital Tumor Registry for all patients. These data reported patients that had expired following the diagnosis of breast cancer but did not include information regarding disease recurrence or cause of death. This study was reviewed by the Colorado Multiple Institutional Review Board (Protocol 00-1094).

Development and Characterization of the A57.1 Antibody Directed Against B7-H4. Monoclonal antibody production and characterization was done at diaDexus (South San Francisco, CA). Seven to 8-week-old BALB/c mice were immunized twice weekly over a 5- to 6-week period with 10 μg of the recombinant B7-H4 protein, corresponding to the complete extracellular domain of the native protein. Lymphocytes were subsequently isolated and fused with P3x63Ag8.653 cells (7) to form a hybridoma using standard techniques. Hybridoma supernatants were screened by ELISA for reactivity against B7-H4 and for the absence of cross-reactivity with an unrelated recombinant protein. B7-H4-positive hybridomas were cloned by single-cell sorting using a Coulter EPICS Elite-ESP Flow Cytometer (Beckman-Coulter, Miami, FL). The A57.1 monoclonal antibody was selected for use in subsequent studies.

Western Blot Analysis. SKBR3, MCF-7, and RK3E cells were obtained from the American Type Culture Collection (Manassas, VA). RK3E cells were infected with a recombinant retrovirus expressing either B7-H4 or alkaline phosphatase used as a control. Twenty-five micrograms of protein extracts were separated on a precast 4% to 12% SDS polyacrylamide minigel (Nupage; Invitrogen, Carlsbad, CA) and transferred to an Immobilon-P polyvinylidene difluoride membrane (Invitrogen). The membrane was blocked for 1 hour at room temperature using 5% nonfat dry milk and incubated overnight with the A57.1 antibody (1 μg/mL). The blot was developed using a horseradish peroxidase linked goat anti-mouse immunoglobulin (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA; 1:10,000) for 1 hour at room temperature and subsequently visualized using enhanced chemiluminescence reagent per manufacturer's directions (Amersham Biosciences, Piscataway, NY).

Immunohistochemical Staining. Formalin-fixed, paraffin-embedded tissue blocks were sectioned to 5 μm and mounted on charged glass slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA). Endogenous peroxidase activity was blocked with 3.0% hydrogen peroxide for 15 minutes. Antigen retrieval was done in a citrate buffer [20 mmol/L (pH 6.0)] at 120°C for 10 minutes. Staining was conducted on a DAKO autostainer (DakoCytomation, Carpinteria, CA) using an indirect avidin-biotin immunoperoxidase method (Vector Laboratories, Burlingame, CA). Sections were incubated at 25°C for 60 minutes with the A57.1 antibody (0.8 μg/mL). Negative controls were run on all sections at 0.8 μg/mL of a subclass-matched IgG1κ (BD PharMingen, San Diego, CA), generated against unrelated antigens. B7-H4 staining was visualized using 3,3′-diaminobenzidine (DakoCytomation). Specificity of B7-H4 staining was confirmed by a blocking experiment with preincubation of the A57.1 antibody with the full-length B7-H4 protein (7.80 ng/mL) at 25°C for 60 minutes, before immunohistochemical processing.

Evaluation of B7-H4 Staining. The proportion of B7-H4-positive cells for each case was scored on a scale from 0% to 100%. Results represent the average proportion of B7-H4-positive cells within the entire tumor area of a single representative tissue block (0-10% positive cells, >10-50% positive cells, >50-80% positive cells, and >80-100% positive cells). The B7-H4 stained slides were digitally scanned using a Zeiss Axioskop 50 microscope fitted to a Syncroscan imaging system (Syncroscopy, Cambridge, United Kingdom). Image manipulation and preparation was done using Adobe Photoshop 6.0 and image analysis of tumor and normal breast epithelium was done using Media Cybernetics Optimas 6.5 (Media Cybernetics, Silver Spring, MA). Median delta base 10 intensity values (derived from 256 Grayscale median pixel Luminosity) were corrected by subtraction of hematoxylin-based background staining and recorded as relative units (RU).

Statistical Analysis. The association of proportion of B7-H4-positive cases and proportion of B7-H4-positive cells with categorical clinicopathologic characteristics was assessed by the Fisher's Exact test or the χ^2 test where appropriate. The differences between median staining intensity and clinicopathologic variables were evaluated by the Wilcoxon rank sum test or the Kruskal-Wallis test where appropriate. Statistically significant univariate relationships were further evaluated by multivariate analysis. A log-rank test was used to test for differences in overall patient survival. Ps ≤ 0.05 were considered statistically significant. Statistical analyses were done using SAS v8.1 (SAS Institute, Cary, NC).

RESULTS

Characterization of B7-H4 Antibody. Specificity of the A57.1 antibody for B7-H4 protein was confirmed by Western blot analysis (Fig. 1). The A57.1 antibody recognized a major protein form with a diffuse band at ~60 to 80 kDa as well as several minor species of lower molecular weight in RK3E B7-H4 cells

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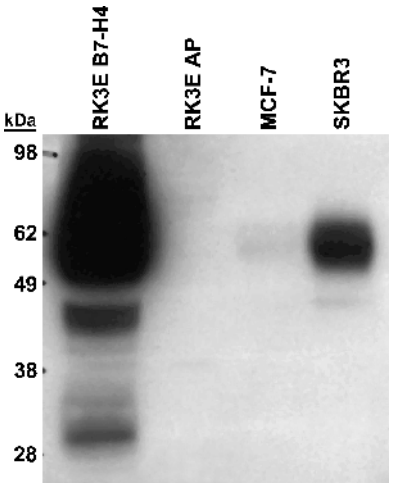

Fig. 1 Western blot analysis. The A57.1 antibody detected a major protein band at ~60 to 80 kDa and several minor bands of lower molecular weight in a RK3E cell line overexpressing B7-H4 (RK3E B7-H4). A single band of similar size was found in two breast cancer cell lines (MCF-7, SKBR3) expressing native B7-H4 mRNA but was not identified in control RK3E cells (RK3E AP).

overexpressing human B7-H4 protein, but did not detect B7-H4 protein in negative control RK3E alkaline phosphatase cells expressing alkaline phosphatase. Similar protein bands were noted in extracts of both MCF-7 (low-level B7-H4 mRNA expression) and SKBR3 (high-level B7-H4 mRNA expression) breast cancer cells. In other experiments, we have shown that the size heterogeneity observed for B7-H4 proteins in tumor tissues and cell lines is due to variable N-linked glycosylation.[4] Preincubation of the B7-H4.A57.1 antibody with the full-length recombinant B7-H4 protein completely blocked the staining in histologic sections.

Primary Breast Tumors. B7-H4 expression was detected in invasive breast cancers, including 147 of 155 (94.8%) cases of invasive ductal carcinoma and 18 of 18 (100%) cases of invasive lobular carcinoma (Table 1). In almost all cases of invasive ductal (Fig. 2*A*) and lobular (Fig. 2*B*) carcinomas, B7-H4 expression was present diffusely throughout the cytoplasm with a pronounced membranous component. However, in rare cases of ductal carcinoma ($n = 7$), the tumor cells showed only localized, incomplete cytoplasmic, and membranous staining. There was no significant association observed between B7-H4 status (positive cases versus negative cases) and any clinicopathologic variables or overall patient survival ($P = 0.910$) using the log-rank test.

The proportion of B7-H4-positive cells in most cases of ductal and lobular carcinomas was >80% of the tumor cells. However, in a small subset of B7-H4-positive carcinoma cases, <10% of the tumor cells were positive (Table 1). When the cancer cases were grouped by proportions of B7-H4-positive cells (Table 2), only PR and HER-2/*neu* were significantly associated by univariate analysis. A multivariate analysis found that grade was an effect modifier in the relationship of PR to percentage of B7-H4-positive cells. A negative PR status was a significant predictor of increasing staining intensity only in grade 3 carcinomas. HER-2/*neu* was not significant at any grade level.

The median B7-H4 staining intensity was greater in invasive ductal carcinomas (24.61 RU) and in invasive lobular carcinomas (15.23 RU) than in normal breast epithelium (4.30 RU) and the differences between these three groups were statistically significant (Table 1; Fig. 3). Univariate analysis showed that increasing B7-H4 staining intensity was associated with a negative PR status and with a history of neoadjuvant chemotherapy (azidothymidine, Adriamycin, taxotere, and cytoxan). No other statistically significant associations were observed. Multivariate analysis found that grade was again an effect modifier. A significant relationship between increasing B7-H4 staining intensity was found only in grade 3 carcinomas for those with chemotherapy. Negative PR status approached significance in grade 3 carcinomas ($P = 0.059$).

Lymph Node Metastases. B7-H4 expression was detected in tumor cells of 240 of 246 (97.6%) breast cancer–positive lymph nodes from 27 patients with nodal metastases. Within the metastatic foci, B7-H4 expression was cytoplasmic and predominantly circumferential membranous in distribution (Fig. 2*C*). The B7-H4 expression pattern of metastatic cells was always identical between individual lymph nodes from the same patient. Furthermore, B7-H4 expression in tumor cells of nodal metastases was identical to that observed in the corresponding primary tumors. Within B7-H4-negative lymph nodes with metastatic carcinoma ($n = 6$), five were from the same patient. In that patient, the primary tumor showed B7-H4 expression in only 5% of the tumor cells. The other B7-H4-negative lymph node was from a patient whose primary tumor showed B7-H4 expression in only 10% of the tumor cells. Three other lymph nodes from that same patient showed B7-H4 expression in a very low proportion of metastatic tumor cells. Focal membranous and granular cytoplasmic B7-H4 expression was also detected in scattered follicular dendritic cells of hyperplastic lymphoid follicles of lymph nodes from patients with metastatic carcinoma but was never seen in lymph nodes from patients that were negative for carcinoma.

Normal Somatic Tissue. Predominantly apical, luminal membranous B7-H4 expression was observed in ductal and lobular epithelial cells in 15 of 15 (100%) cases of normal breast tissue (Table 1; Fig. 2*D*). In one case, however, there was circumferential membranous B7-H4 expression, equivalent to that seen in breast carcinomas. B7-H4 expression was never identified in myoepithelial cells or in other cellular components of normal breast tissue.

A broad spectrum of normal adult and fetal somatic tissues was evaluated to test for the expression of B7-H4 in other cell types (Table 3). The confluent circumferential membranous pattern of expression, as seen in breast cancer cases, was never observed in normal adult somatic tissues of any anatomic site. However, apical membranous expression was noted in fallopian tubal epithelium (17 of 17), endometrial glandular epithelium (19 of 25), and occasionally in endometrial luminal surface epithelium. In addition, uniform

[4] Papkoff et al., submitted for publication.

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Table 1 B7-H4 expression (no. positive cases, proportion of positive cells, and median staining intensity) in primary breast cancer and normal breast tissue

Histological diagnosis	No. positive cases (%)	No. cases (%) grouped by proportion of B7-H4-positive cells					Staining intensity	
		0-10%	>10-50%	>50-80%	>80-100%	P*	Image analysis median RU (range)	P†
Invasive ductal carcinoma‡	147/155 (94.8)	26 (17)	15 (10)	11 (7)	103 (66)	0.132	24.61 (0-75.00)	0.0003
Invasive lobular carcinoma	18/18 (100)	2 (11)	0	3 (17)	13 (72)		15.23 (0.39-55.08)	
Normal breast tissue	15/15 (100)	1 (7)	1 (7)	5 (33)	8 (53)		4.30 (1.95-13.67)	

*χ^2 test.
†Kruskal-Wallis Test.
‡Including three cases subclassified as tubular carcinoma; due to rounding, percentages in parentheses may not add up to 100%.

cytoplasmic expression without a membranous component was observed in endocervical glands (10 of 10). Focal membranous expression was detected in the bronchial epithelium of the lung (4 of 4), the columnar epithelium of the gallbladder (1 of 5), the ductal and occasionally acinar epithelium of the pancreas (10 of 10), the distal convoluted tubules of the kidney (5 of 11), and the transitional epithelium of the ureter (2 of 3) and the urinary bladder (4 of 4). Focal cytoplasmic B7-H4 expression was also noted in the pars intermedia of 1/4 sections of normal pituitary. B7-H4 cytoplasmic expression was further detected in the squamous epithelium of the larynx (2 of 3), as well as the cortex and cuticle of hair shafts and in the inner zone of the outer root sheath of hair follicles (7 of 7). All other normal somatic tissues were consistently negative for B7-H4 expression.

Within fetal tissue, B7-H4 expression was noted in the bronchial epithelium of the lung, the distal convoluted tubules and collecting ducts of the kidney, the hair follicles, the amniotic epithelium, and in cytotrophoblast cells of chorionic villi of first trimester placentas. By contrast, chorionic villi from term placentas were always negative for B7-H4 expression (Table 3).

DISCUSSION

Despite the use of a wide range of adjuvant treatment options, including radiotherapy, conventional chemotherapy with cytotoxic antitumor agents alone or in combination with endocrine therapy, bisphosphonates, and HER-2/*neu* directed therapy (trastuzumab; ref. 1), over 40,000 women will die from breast cancer in the United States in 2004 (8). Thus, new molecular targets must be defined as a first step leading to the development of novel therapeutic strategies for the treatment of breast cancer.

The current study is the first to examine the expression of B7-H4 protein in primary and metastatic breast cancer. In

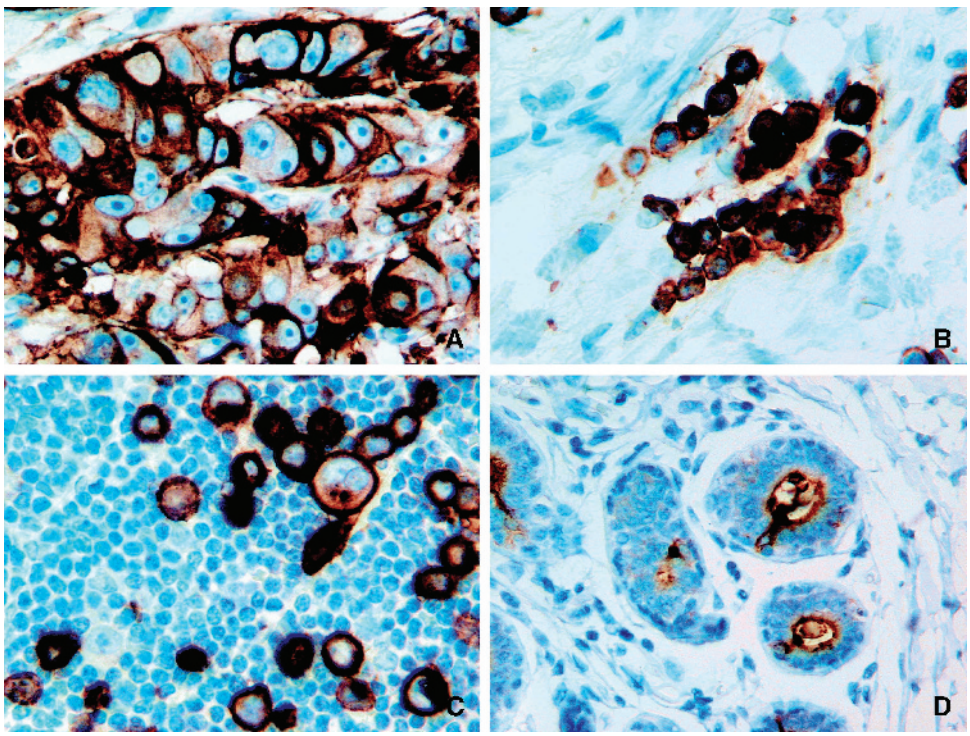

Fig. 2 Immunohistochemical detection of B7-H4 expression in breast cancer and normal breast tissue. Note strong cytoplasmic and circumferential membranous B7-H4 expression in both invasive ductal (*A*) and lobular (*B*) breast cancers. An identical pattern of B7-H4 expression is also present in metastatic breast cancer of an axillary lymph node (*C*). By contrast, predominantly apical, luminal membranous B7-H4 expression is observed in normal breast epithelium (*D*). *A*, *B*, and *C,* original magnification ×600; *D,* original magnification ×400.

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Table 2 Proportion of B7-H4-positive cells and median staining intensity of 173 invasive breast cancer cases compared with clinicopathologic variables

			No. cases (%) grouped by proportion of B7-H4-positive cells					Staining intensity	
		No. cases	0-10%	>10-50%	>50-80%	>80-100%	*P**	Image analysis median RU (range)	*P*
Grade†	1	29	3 (10)	5 (17)	0	21 (72)	0.088	19.92 (0-69.92)	0.205‡
	2	56	13 (23)	7 (13)	4 (7)	32 (57)		16.60 (0-75.00)	
	3	70	10 (14)	3 (4)	7 (10)	50 (71)		26.17 (0-74.22)	
Receptor status	ER+	137	22 (18)	16 (12)	10 (7)	87 (64)	0.255	17.58 (0-75.00)	0.099§
	ER−	36	4 (11)	1 (3)	2 (6)	29 (81)		30.08 (0.78-74.22)	
	PR+	120	22 (18)	17 (14)	10 (8)	71 (59)	0.001	16.41 (0-75.00)	0.014§
	PR−	53	6 (11)	0	2 (4)	45 (85)		30.86 (0-74.22)	
	HER-2/*neu*+	25	1 (4)	2 (8)	5 (20)	17 (68)	0.024	24.61 (0-54.69)	0.924§
	HER-2/*neu*−	148	27 (18)	15 (10)	7 (5)	99 (67)		19.92 (0-75.00)	
Tumor size (cm)	≤2	108	17 (16)	15 (14)	9 (8)	67 (62)	0.063	19.92 (0-74.22)	0.120‡
	>2-5	50	11 (22)	1 (2)	2 (2)	36 (72)		16.60 (0-75.00)	
	>5	15	0	1 (7)	1 (7)	13 (87)		35.94 (8.59-63.67)	
No. lymph nodes with metastatic carcinoma	0	97	15 (15)	9 (9)	8 (8)	65 (67)	0.965	17.58 (0-69.92)	0.066‡
	1-3	31	6 (19)	2 (6)	2 (6)	21 (68)		23.44 (0-59.38)	
	>3	21	2 (10)	1 (5)	1 (5)	17 (81)		30.08 (0.78-74.22)	
	Unknown†	24	6 (25)	2 (8)	5 (21)	11 (46)		20.70 (0-66.02)	
Stage	I	90	14 (16)	13 (14)	5 (6)	58 (64)	0.082	19.92 (0-69.92)	0.194‡
	IIa	35	6 (17)	2 (6)	3 (9)	24 (69)		18.36 (0-75.00)	
	IIb	20	6 (30)	0	1 (5)	13 (65)		13.04 (0-59.38)	
	IIIa	16	0	2 (13)	0	14 (88)		36.52 (0.78-74.22)	
	IIIb+	12	2 (17)	0	3 (25)	7 (58)		16.80 (0-68.75)	
Age at diagnosis (y)	≤50	69	11 (16)	9 (13)	6 (9)	43 (62)	0.542	23.44 (0-74.22)	0.921§
	>50	104	17 (16)	8 (8)	6 (6)	73 (70)		20.90 (0-75.00)	
Neoadjuvant chemotherapy	Yes	17	1 (6)	0	2 (12)	14 (82)	0.235	41.80 (6.64-74.22)	0.004§
	No	156	27 (17)	17 (11)	10 (6)	102 (65)		19.18 (0-75.00)	

NOTE. Cases with an unknown lymph node status were excluded from the statistical analysis.
Due to rounding, percentages in parentheses may not add up to 100%.
*Fisher's exact test.
†Ductal carcinoma including three cases subclassified as tubular carcinoma.
‡Kruskal-Wallis test.
§Wilcoxon rank sum test.

addition, we evaluated B7-H4 protein expression in normal breast tissue and in a wide range of normal adult and fetal somatic tissues. B7-H4 circumferential membranous and cytoplasmic expression was observed in >95% of invasive breast cancer cases and was also detected in most nodal metastases. Univariate analysis showed a significant correlation between the proportion of B7-H4-positive cells and a negative status of PR and HER-2/*neu*. A significant association was also observed between B7-H4 staining intensity and negative PR status, and a history of treatment with neoadjuvant chemotherapy but not with other clinicopathologic variables or overall patient survival. The observed relationship between B7-H4 staining intensity level and negative PR status in primary breast cancer was not anticipated and could not be attributed to an indirect relationship with tumor grade. Thus, further studies are warranted to determine if this inverse association is due to other confounding clinicopathologic variables or could reflect a mechanistic link between B7-H4 expression and PR status.

This study provided pivotal data but not definitive evidence to support the concept that B7-H4 could be a diagnostic marker or therapeutic target for breast cancer. Both HER-2/*neu* and B7-H4 are associated with the cell surface, an important consideration for potential antibody therapeutic targets (9, 10). B7-H4 overexpression was detected in most breast carcinomas, including cases that were not candidates for hormonal or trastuzumab (Herceptin) therapy due to negative ER/PR and HER-2/*neu* status. By contrast, only weak apical cell surface expression of B7-H4 was seen in normal ductal and lobular breast epithelial cells. Focal apical membranous B7-H4 expression was also observed in the distal convoluted tubules of the kidney, ductal cells, and rare acinar cells of the pancreas, endometrial glands, and in few other normal somatic tissues. Previous studies have indicated that HER-2/*neu* is also expressed in some normal somatic tissues, including renal tubular epithelium, pancreatic acinar cells, and endometrial glands (11–14). Thus, the limited expression of B7-H4 in a subset of normal tissues does not necessarily rule out a potential role for this protein as a therapeutic target for patients with breast cancer.

Our findings that B7-H4 is not expressed in liver, small bowel, colon, and skeletal muscle are consistent with previous observations by Sica et al. (3) and Choi et al. (5). In contrast with our current study, however, Choi et al. reported that B7-H4 is not expressed in the lung, gallbladder, pancreas, kidney, ureter, urinary bladder, pituitary, or breast. The antibody used by Choi et al. was used at a dilution of 1:100 (final concentration not specified) and was noted to be reactive only with tissue frozen sections (5). By contrast, the A57.1

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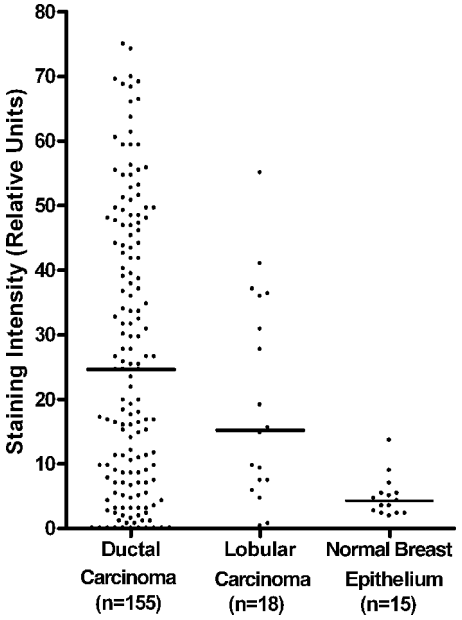

Fig. 3 Median B7-H4 staining intensity values in primary invasive ductal carcinoma, invasive lobular carcinoma, and normal breast tissue. Horizontal bars, median staining intensity within each diagnostic category.

monoclonal antibody in our study was used at a dilution of 1:2,000 (final concentration, 0.8 μg/mL) and was reactive with both frozen sections and sections from archival formalin-fixed tissue blocks. Thus, the basis for the discrepancy in the detection of B7-H4 in some normal tissues from our study compared with previous observations by Choi et al. could be due to differences in the sensitivity of the immunohistochemical staining protocols or to differences in the B7-H4 antibodies that were used.

Although the role of B7-H4 expression in malignant transformation or tumor progression has not been determined, B7 family members and their receptors are known to regulate antigen-specific immune response through inhibition of T-cell activation, cytokine secretion, and the development of cytotoxicity (2–6). Extensive laboratory and histopathologic data indicate that T-cell immune reactivity is a favorable prognostic indicator in nonmetastatic breast cancer but that the suppression of cell-mediated immunity could be critically involved in breast cancer progression (15–17). Thus, it is reasonable to hypothesize that B7-H4 overexpression could provide a mechanism for tumors to avoid detection by immune surveillance. In this light, we are currently focusing on experiments to determine if an antibody approach could inhibit tumor cell growth and/or reverse the postulated antitumor effects of B7-H4 on the immune system.

In conclusion, this study showed that B7-H4 is consistently expressed in most primary and metastatic breast carcinomas. Although B7-H4 detection was associated with negative progesterone receptor status, negative HER-2/*neu* status, and history of neoadjuvant chemotherapy, B7-H4 expression was independent of tumor grade, stage, or other clinicopathologic variables. The nearly ubiquitous expression of B7-H4 in breast carcinomas suggests that B7-H4 could be involved in breast cancer pathogenesis or tumor progression. Further studies, however, are indicated to evaluate the potential role of B7-H4 as a diagnostic marker or therapeutic target.

Table 3 B7-H4 expression (no. positive cases) in 314 normal adult and fetal somatic tissue samples

	B7-H4 positive (%)*
Normal adult tissue	
Breast (ductal and lobular cells)	16/16 (100)
Ovary	0/22 (0)
Fallopian tubal epithelium	17/17 (100)
Endometrial glands	19/25 (76)
Myometrium	0/25 (0)
Endocervical glands	10/10 (100)
Ectocervix	0/10 (0)
Thyroid	0/5 (0)
Parathyroid	0/3 (0)
Adrenal gland	0/3 (0)
Pancreas/chronic Pancreatitis	10/10 (100)
Salivary gland	0/1 (0)
Pituitary	1/4 (25)
Heart	0/5 (0)
Larynx	2/3 (67)
Lung	4/4 (100)
Esophagus	0/5 (0)
Stomach	0/5 (0)
Duodenum	0/4 (0)
Ileum	0/7 (0)
Colon/cecum	0/4 (0)
Liver	0/4 (0)
Gallbladder	1/5 (20)
Kidney (distal convoluted tubules)	5/11 (45)
Ureter	2/3 (67)
Urinary bladder mucosa	4/4 (100)
Testis	0/5 (0)
Prostate	0/5 (0)
Abdominal peritoneum	0/1 (0)
Skin	0/5 (0)
Hair follicle	7/7 (100)
Thrombus	0/4 (0)
Skeletal muscle	0/4 (0)
Synovial cyst	0/1 (0)
Bone marrow	0/5 (0)
Lymph node	0/5 (0)
Thymus	0/4 (0)
Spleen	0/5 (0)
Cerebral cortex	0/3 (0)
Cerebellum	0/3 (0)
Spinal cord	0/5 (0)
Eye	0/1 (0)
Normal fetal tissue	
Amnion	2/8 (25)
Chorion	0/8 (0)
Placental villi	2/6 (33)
Heart	0/1 (0)
Lung	1/1 (100)
Small bowel	0/1 (0)
Kidney	1/1 (100)
Skin	0/3 (0)
Hair follicle	3/3 (100)
Skeletal muscle	0/1 (0)
Cartilage	0/2 (0)
Adipose tissue	0/1 (0)

*Cases with any detectable staining (minimal focal staining or greater) were scored as B7-H4 positive.

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