Jun Activation Domain Binding Protein 1 Expression Is Associated with Low p27\textsuperscript{Kip1} Levels in Node-Negative Breast Cancer

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**ABSTRACT**

**Purpose:** The purpose is to evaluate expression levels of Jun activation domain-binding protein 1 (JAB1) in breast cancer tissue and adjacent normal tissue, to determine whether JAB1 expression is associated with p27\textsuperscript{Kip1} expression in invasive breast carcinomas, and to evaluate the prognostic significance of JAB1 and p27\textsuperscript{Kip1} in node-negative breast cancer.

**Experimental Design:** JAB1 levels were measured in 10 matched pairs of invasive breast tumor tissue and adjacent normal tissue using Western blot analysis. We also investigated the immunoreactivity of JAB1 and p27\textsuperscript{Kip1} levels in paraffin-embedded tissue specimens from 220 patients with node-negative breast cancer who had not received adjuvant systemic therapy. The median follow-up was 15 years.

**Results:** JAB1 was expressed at higher levels in invasive tumors than in adjacent normal tissue (P = 0.01). JAB1 overexpression was observed in 57% of invasive breast cancers. Low levels of p27\textsuperscript{Kip1} were noted in 70% of the tumor specimens. We found an inverse correlation between JAB1 and p27\textsuperscript{Kip1} expression levels (P = 0.01). JAB1 overexpression was associated with patient age of at least 50 years (P = 0.03) and tumor size of ≤2 cm (P = 0.01). Elevated levels of p27\textsuperscript{Kip1} were associated with low nuclear grade (P = 0.01). At 5 years of follow-up, neither JAB1 nor p27\textsuperscript{Kip1} expression was related to disease-free survival.

**Conclusions:** These data indicate that JAB1 is commonly overexpressed in invasive breast carcinomas. JAB1 overexpression is associated with low levels of p27\textsuperscript{Kip1} in node-negative breast cancer. In this study, JAB1 and p27\textsuperscript{Kip1} were not independent prognostic factors.

**INTRODUCTION**

Mammalian cell cycle progression is regulated by the combined action of cyclins, CDKs, and CDK inhibitors. Any shift in the balance among these factors can result in abnormal cell proliferation, which may contribute to the development of cancer (1). The CDK inhibitor p27\textsuperscript{Kip1} is a potent inhibitor of the cyclin E-CDK2 and cyclin A-CDK2 complexes, both of which are involved in the regulation of the G\textsubscript{1}-S-phase transition of the cell cycle (1–3). Despite the putative role of p27\textsuperscript{Kip1} as a tumor suppressor, mutations in p27\textsuperscript{Kip1} are rarely seen in human tumors (4). However, in most types of cancer, a decrease in or the absence of p27\textsuperscript{Kip1} protein has been associated with tumor aggressiveness and poor patient survival rates (5–8), suggesting that disruption of p27\textsuperscript{Kip1} regulatory mechanisms contributes to neoplasia. The function of p27\textsuperscript{Kip1} is regulated by changes in its expression level and localization in the cell.

JAB1 is an activator protein-1 coactivator that interacts with and potentiates transactivation by c-Jun and promotes cellular proliferation (9). JAB1 has also been implicated recently in promoting cell proliferation by facilitating relocation of p27\textsuperscript{Kip1} from the nucleus to the cytoplasm, thereby accelerating the degradation of p27\textsuperscript{Kip1} by the ubiquitin/proteasome pathway (10).

There is a need to identify novel prognostic factors in patients with node-negative breast cancer (11). We hypothesized that JAB1 overexpression plays an important role in the pathogenesis of breast cancer. The purpose of this study was to evaluate the expression levels of JAB1 in invasive breast cancer and adjacent normal tissue and to define patterns of JAB1 expression in human breast cancer. The prognostic significance of JAB1 and p27\textsuperscript{Kip1} expression was evaluated in tissue specimens from 220 patients with node-negative breast cancer and long-term follow-up.

**MATERIALS AND METHODS**

**Western Blot Analysis.** We performed immunoblot analysis for JAB1 protein in matched pairs of fresh frozen tissue specimens of invasive breast cancer and adjacent normal tissue. Normal tissue was dissected from adjacent cancer at the time of lumpectomy or mastectomy at The University of Texas M. D. Anderson Cancer Center. All tumor and normal samples were...
examined by a pathologist. The fresh tissue was snap frozen in liquid nitrogen and stored at −80°C until use. Each tissue sample was thawed, and ∼0.5 g of tissue were homogenized and lysed in 2.5 ml of lysis buffer [20 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1% NP40, 2 mM EDTA, 200 μM Na3VO4, and 100 μM NaF, with 2 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 2 μg/ml aprogin added before using]. The lysates were subjected to centrifugation at 100,000 × g for 30 min at 4°C, and the supernatant was stored at −80°C until analysis. An equal amount of total protein (30–50 μg) was separated by 10% SDS-polyacrylamide gel and then transferred to nitrocellulose membranes (Nature Pure; Osmonics, Minnetonka, MN). The membranes were blocked using 5% nonfat dry milk in PBST (PBS solution plus 0.1% Tween 20) and then probed using the monoclonal antibodies directed against JAB1 (clone 4D11D8, diluted 1:1000; Zymed), p27Kip1 (clone SX53G8, diluted 1:1000; Dako Corp.), and β-actin (diluted 1:1000; Sigma Chemical Co., St. Louis, MO) in PBS solution containing 5% bovine serum. After several washes with Tris-buffered saline, membranes were probed with a horseradish peroxidase-conjugated goat antiamoige IgG (Dako Corp., Carpinteria, CA). Proteins were detected using enhanced chemiluminescence (Amersham, Piscatway, NJ). The expression of JAB1 was measured using densitometry and normalized to β-actin.

The human breast cancer cell lines MCF-7 and MDA-MB-231 were cultured in modified DMEM (DMEM:Ham’s F-12). Cells that had reached ∼80% confluence were lysed with lysis buffer, and Western blot analysis was conducted as described above.

Archival Tissue Specimens. Available tissue specimens from patients with node-negative invasive breast cancer treated at The University of Texas M. D. Anderson Cancer Center between July 1, 1978, and October 31, 1995, were subjected to immunohistochemical analysis. Tissue samples were obtained from surgical specimens at the time of definite surgery. All patients had early-stage breast cancer with no evidence of axillary lymph node involvement (T1N0, T2N0), and none of the patients received postoperative hormonal therapy or chemotherapy. The group of patients from whom the tissue samples were obtained includes all of the node-negative patients examined at our institution during the study period who did not receive systemic therapy and from whom a tumor tissue specimen was available. All available specimens had been fixed in formalin and embedded in paraffin. The study was approved by our institutional review board. Patient follow-up information was available from the beginning of the study period through November 31, 2001.

JAB1 and p27Kip1 Immunohistochemical Analysis. We investigated whether JAB1 affected the expression levels of the negative cell cycle regulator p27Kip1 in breast cancer tissue. Histopathological confirmation of the diagnosis of breast cancer was obtained using H&E stain on slides containing 5-μm specimens prepared from each block. Paraffin sections were deparaffinized and rehydrated. Antigens were retrieved using Target Retrieval Solution (Dako Corp.), and endogenous peroxidase activity was blocked using 3% hydrogen peroxide (12). After incubation with protein block solution (Dako Corp.) for 15 min to reduce nonspecific binding, the slides were incubated with primary antibody diluted with 1% BSA in PBS at room temperature for 1 h. Biotin-conjugated secondary antibody was applied for 20 min, and streptavidin-biotin-peroxidase complex (1:200, Strept-AB complex; Dako Corp.) complex was added for 20 min. 3,3’-Diaminobenzidine tetrahydrochloride was used as a chromogen, and hematoxylin was used as a counterstain. 4D11D8 monoclonal antibody (Zymed, San Francisco, CA) diluted 1:400 was used to detect JAB1 expression, and clone SX53G8 monoclonal antibody (Dako Corp.) diluted 1:200 was used to detect p27Kip1.

All slides stained for JAB1 and p27Kip1 immunohistochemical analysis were evaluated using light microscopy by workers without knowledge of the clinical data. On each slide, at least three areas with the highest degree of positive cells were selected for scoring. Tumor tissue sections previously found to exhibit strong staining were used as positive controls for JAB1 and p27Kip1 staining. In addition, the breast cancer cell line MDA-MB-231 (M. D. Anderson Cancer Center, Houston, TX) was used as a positive control for JAB1, and the MCF-7 cell line (Michigan Cancer Foundation, Detroit, MI) was used as a positive control for p27Kip1 (Fig. 1). Both cell lines were obtained from the American Type Culture Collection (Manassas, VA).

On nuclear staining of the cancer cells, JAB1 was recorded as absent (score of 0) or present (score of 1). p27Kip1 staining was scored on a four-point scale, with a score of 0 indicating absence of detectable staining (i.e., staining in <5% of cells), 1 indicating positive staining in at least 5% but <25% of the cells, 2 indicating positive staining in at least 25% but <50% of the cells, and 3 indicating positive staining in at least 50% of the cells. For the purposes of our comparative analysis, JAB1 protein expression was considered high (score of 1) or low (score of 0), and the degree of p27Kip1 protein expression was recorded as low (score of 0, 1, or 2) if <50% of the cells stained positive and high (score of 3) if at least 50% of the cells stained positive. The limits for p27Kip1 scoring were selected on the basis of previous immunohistochemical studies of p27Kip1 in breast cancer (5, 6, 13).

HER-2/neu and ER Immunohistochemistry. Immunohistochemical staining for HER-2/neu and ERs was performed as described previously (14–16). HER-2/neu protein was measured using the anti-HER-2/neu monoclonal antibody Ab-8 (clone e2-4001), which was purchased from Neomarkers (Freemont, CA). HER-2/neu expression was scored as the percentage
of cells with membranous staining and the intensity of the signal and was graded as negative (<10% complete membranous staining), weakly positive (≥10% weak to moderate complete membranous staining), and strongly positive (≥10% strong complete membranous staining).

The level of ERs was assessed by immunohistochemical staining using antibody 6F11 (Zymed). Nuclear staining of at least 10% of carcinoma cells was considered a positive finding of ERs.

Statistical Methods. The Cochran-Armitage test was used to test for trends in binomial proportions across levels of a single factor or covariate. The Wilcoxon rank-sum test was used to compare groups of independent continuous variables. The DFS time was defined as the interval from the date of surgery to the date of first recurrence or the date of the last follow-up visit. Patients who died of causes other than breast cancer were considered censored observations. Survival time was estimated using the Kaplan-Meier (product-limit) method. The log-rank test was used to test for associations between patient variables and survival. All P values presented are from two-sided analyses. Statistical analyses were carried out using SAS 8.02 (SAS, Cary, NC) and S-plus version 6 (Insightful Corp., Seattle, WA).

RESULTS

Overexpression of JAB1 in Human Breast Carcinomas. We performed immunoblot analysis for JAB1 protein in 10 matched pairs of specimens of invasive breast cancer and their adjacent normal tissue. The expression of JAB1 was measured using densitometry normalized to β-actin. High JAB1 protein expression was observed in 9 of the 10 invasive cancers (Fig. 2), whereas 3–8-fold lower expression levels were observed in adjacent normal tissue. In matched pairs of malignant tumor and normal tissues, the median normalized absorbance score for JAB1 was 0.075 in the benign tissue, compared with 0.36 in the malignant tissue (P = 0.01).

Immunohistochemistry Study: Patient Characteristics. Table 1 shows the clinical characteristics of the 220 patients whose tissue specimens were subjected to immunohistochemical analysis. The median patient age at the time of definite surgery was 59 years (range, 28–83 years). The median duration of follow-up was 15 years. During the course of follow-up, disease recurrence was noted in 60 patients (27%). The median DFS at 5 years was 80%. The 5-year DFS for patients with tumors measuring up to 2 cm in the largest dimension (T1N0, stage I) was 77%, compared with 66% for patients whose tumors were ≥2 cm (T2N0, stage II; P = 0.01).

Table 1 Characteristics of 220 patients whose breast cancer specimens were analyzed for JAB1 and p27Kip1 expression

<table>
<thead>
<tr>
<th>No. of patients (%)</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Patient age, years</td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>49 (22)</td>
</tr>
<tr>
<td>≥50</td>
<td>171 (73)</td>
</tr>
<tr>
<td>Histopathologic type</td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>196 (89)</td>
</tr>
<tr>
<td>Lobular</td>
<td>14 (6)</td>
</tr>
<tr>
<td>Other</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Tumor size (largest dimension), cm</td>
<td></td>
</tr>
<tr>
<td>≤1.0</td>
<td>30 (14)</td>
</tr>
<tr>
<td>&gt;1.0–2.0</td>
<td>101 (46)</td>
</tr>
<tr>
<td>&gt;2.0</td>
<td>89 (40)</td>
</tr>
<tr>
<td>Nuclear grade</td>
<td></td>
</tr>
<tr>
<td>I (well differentiated)</td>
<td>30 (14)</td>
</tr>
<tr>
<td>II (moderately differentiated)</td>
<td>132 (60)</td>
</tr>
<tr>
<td>III (poorly differentiated)</td>
<td>58 (26)</td>
</tr>
<tr>
<td>ER status</td>
<td></td>
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<tr>
<td>Positive</td>
<td>153 (70)</td>
</tr>
<tr>
<td>Negative</td>
<td>63 (29)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (2)</td>
</tr>
<tr>
<td>HER-2/neu status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
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<tr>
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JAB1 and p27Kip1 Expression Patterns in Node-Negative Breast Cancer. To investigate the relationship between JAB1 and p27Kip1 expression, we performed JAB1 immunohistochemistry on 220 paraffin-embedded, formalin-fixed breast cancer patient specimens. The p27 Kip1 level was measured in 207 of the 220 specimens (in the other 13 specimens, no invasive tumor remained after JAB1 analysis). As explained in “Materials and Methods,” the nuclear JAB1 expression level was assigned a score of 0 or 1, and the p27Kip1 was assigned a score of 0, 1, 2, or 3. Considering only the highest levels of expression as indicating positivity (i.e., specimens with a score of 1 for JAB1 and a score of 3 for p27Kip1), the incidence of expression was 57% (125 of 220) for JAB1 and 30% (62 of 207) for p27Kip1. Cytoplasmic JAB1 staining was noted in 46 (21%) of carcinomas. Cytoplasmic p27Kip1 staining (score, 3+) was noted in 21 (10%) of the carcinomas.

**Table 2** Association between JAB1 and p27Kip1 nuclear expression in 207 breast cancer specimens

<table>
<thead>
<tr>
<th>p27Kip1 expression scoreb</th>
<th>JAB1 expression scorec</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, 1, or 2</td>
<td>73 (83)</td>
<td>1 (n = 119)</td>
</tr>
<tr>
<td>3</td>
<td>15 (17)</td>
<td>48 (40)</td>
</tr>
</tbody>
</table>

* a P = 0.01, by Fisher’s exact test.
* b Nuclear staining for JAB1 was scored as follows: 0 indicated positive staining of <50% of cells; 1 indicated positive staining of at least 50% of cells.
* c Nuclear staining for p27Kip1 was scored as follows: 0, 1, or 2 indicated positive staining of <50% of cells; 3 indicated staining of at least 50% of cells.

JAB1 and p27Kip1 Expression Patterns in Node-Negative Breast Cancer. To investigate the relationship between JAB1 and p27Kip1 expression, we performed JAB1 immunohistochemistry on 220 paraffin-embedded, formalin-fixed breast cancer patient specimens. The p27Kip1 level was measured in 207 of the 220 specimens (in the other 13 specimens, no invasive tumor remained after JAB1 analysis). As explained in “Materials and Methods,” the nuclear JAB1 expression level was assigned a score of 0 or 1, and the p27Kip1 was assigned a score of 0, 1, 2, or 3. Considering only the highest levels of expression as indicating positivity (i.e., specimens with a score of 1 for JAB1 and a score of 3 for p27Kip1), the incidence of expression was 57% (125 of 220) for JAB1 and 30% (62 of 207) for p27Kip1. Cytoplasmic JAB1 staining was noted in 46 (21%) of carcinomas. Cytoplasmic p27Kip1 staining (score, 3+) was noted in 21 (10%) of the carcinomas.

**Associations of Nuclear JAB1 Expression with p27Kip1 Expression and Clinicopathological Factors.** We found an inverse correlation between nuclear JAB1 expression and nuclear p27Kip1 expression (Table 2). The expression of p27Kip1 was significantly lower in the invasive breast cancer cells in which JAB1 overexpression was found than in the non-JAB1-overexpressing cancers (Fig. 3). JAB1 nuclear expression was more frequent in patients aged at least 50 years (*P* = 0.03) than in younger patients and in patients with tumors measuring ≤2 cm in the largest dimension than in patients with larger tumors (*P* = 0.01). Analysis of the relationship between the nuclear grade of the tumors and JAB1 expression revealed that 106 of 190 (55%) of intermediate- and high-grade tumors expressed high levels of JAB1 in the nucleus. Compared with high-grade tumors, low-grade tumors had higher levels of p27Kip1 expression (*P* < 0.01).

**Fig. 3** JAB1 and p27Kip1 expression in human breast cancer tissue specimens. Representative tissue sections in a patient show high nuclear expression of JAB1 (A) and low p27Kip1 expression (B). Representative sections from another patient show low nuclear JAB1 expression (C) and high p27Kip1 expression (D).
The HER-2/neu oncogene is known to cause localization of p27 kip1 and JAB1 into the cytoplasm, thereby facilitating p27 kip1 degradation (17, 18). Thus, tissues were also examined for their HER-2/neu levels. No association was found between p27 kip1 and HER-2/neu overexpression. In contrast, JAB1 expression was associated with low levels of HER-2/neu expression ($P < 0.01$).

**Survival Analysis.** In the present case series of node-negative breast cancers, the 5-year DFS rate was 80%, a finding consistent with the results reported in most clinical studies (19, 20). The DFS rate at 5 years was significantly lower for patients with tumors larger than 2 cm than for patients with smaller tumors ($P = 0.01$). In contrast, at 5 years, neither the DFS nor the overall survival rate was related to JAB1 or p27kip1 expression, as determined both by statistical tests and by observation for trends across expression levels. Similarly, our analysis of patients grouped by ER and HER-2/neu status did not identify subgroups of patients with different probabilities of 5-year DFS. No association between DFS rates and JAB1 status was found among the ER-positive or ER-negative subgroups. We found a trend for poor DFS in the subgroup of patients with tumors $1 \text{ cm}$ that overexpressed JAB1, although the difference in the DFS rate was not statistically significant ($P = 0.01$). Notably, only 30 patients had tumors $1 \text{ cm}$.

**DISCUSSION**

We found that JAB1 is commonly expressed in human breast carcinomas and that its nuclear expression level correlates inversely with that of the cell cycle inhibitor p27kip1. These findings are consistent with those of in vitro studies showing that JAB1 is involved in the degradation of p27kip1 in cancer cells (10, 21). Experimental data indicate that p27kip1 mRNA levels do not fluctuate during the cell cycle (22) and that the p27kip1 protein levels are regulated at the translational and posttranslational levels (22), in part, by the ubiquitin/proteasome-mediated pathway (23–27). Another mechanism of p27kip1 inactivation was recently revealed when JAB1 nuclear export protein was shown to specifically interact with p27kip1, transport it from the nucleus to the cytoplasm, and promote its degradation (21). The expression and intracellular localization of JAB1 changes in association with the differentiation of tumor cells in a manner similar to that of p27kip1 (28). We showed that JAB1 is present in both the nucleus and the cytoplasm but is located predominantly in the nucleus. The accumulation of JAB1 and p27kip1 in the cytoplasm of breast carcinoma cells may be a result of p27kip1 exportation by JAB1 from the nucleus for degradation or inactivation by sequestration rather than a result of p27kip1 neosynthesis. Findings of relocalization of p27kip1 to the cytoplasm in association with the differentiation stage in breast cancer have been described by another group (12). These data, taken together with those from our present study, indicate that the level and intracellular localization of CDK inhibitors may play a role in determining the degree of differentiation of breast cancer cells and that this function of CDK inhibitors may be influenced by JAB1-mediated effects on p27kip1.

In our analysis of invasive cancers versus adjacent normal tissues, JAB1 was overexpressed in 90% of breast carcinomas. Our study involved a small number of samples but suggests that JAB1 may play a role in breast cancer pathogenesis. The sig-
nificance of high levels of JAB1 in invasive cancer versus surrounding normal tissue remains to be defined. As invasive cancers have already gone through numerous genetic events, the potential oncogenic role of JAB1 as an early or late event in breast tumor formation cannot be determined from this study but would require a detailed analysis of early breast cancers (such as ductal carcinoma in situ) versus invasive cancers. JAB1 overexpression may use multiple mechanisms to contribute to breast cancer progression because many growth factors and cell cycle proteins, including p27Kip1, p53, transforming growth factor β, hypoxia-inducible factor-1α, and c-jun are regulated by JAB1.

In our series, p27Kip1 immunoreactivity was low (i.e., <50% of cancer cells were immunoreactive) in 70% of the tumors. This finding is consistent with the levels of p27Kip1 protein reported in other studies (5, 8, 13, 32–35). We found a significant association between high p27Kip1 levels and low nuclear grade, a result that is also in agreement with findings in previous studies (7, 8, 36–38). Unlike Newman et al. (14), we did not find an association between p27Kip1 and HER-2/neu overexpression.

In the medical literature, the role of p27Kip1 expression as a prognostic factor is controversial (Table 3). In a study of 246 primary breast cancer specimens from women younger than 45 years, Porter et al. (33) found that low p27Kip1 expression was an independent predictor of poor overall survival. Catzavelos et al. (5) found a low level of staining for p27Kip1 in 56% of 168 evaluated tumors from patients with stage I–III breast cancer. These authors also reported that a low p27Kip1 expression level was an independent predictor of poor DFS rates among patients with lymph node involvement; low p27Kip1 levels appeared to have no effect on DFS among patients with node-negative breast cancer. Tan et al. (6) assayed p27Kip1 immunostaining in breast cancer specimens measuring up to 1 cm (T1a,b) from 202 patients; a low p27Kip1 level (i.e., positive staining in <50% of cells) was an independent prognostic factor associated with a poor DFS rate.

In contrast, other studies have failed to confirm the prognostic role of low p27Kip1 levels in breast cancer. After evaluating p27Kip1 expression in tumor specimens from 77 patients with node-negative breast cancers treated with local-regional therapy only, Reed et al. (32) found that a low level of p27Kip1 expression (i.e., nuclear immunoreactivity of <25% of cells) was not an independent prognostic factor. In the largest study published to date, Barbareysi et al. (8) investigated p27Kip1 expression in 512 patients with node-negative and node-positive breast cancer. Although low p27Kip1 levels were associated with high histopathological grade, p27Kip1 was not an independent prognostic factor. In contrast with the studies published by Tan et al. (6) and Porter et al. (33), the study by Barbareysi et al. (8) did not confirm a significant prognostic role for p27Kip1 in patients with small tumors (pT1) or in women younger than 50 years. Volpi et al. (34) evaluated the prognostic role of p27Kip1 expression in 286 patients with node-negative breast cancer treated with local-regional therapy alone (without adjuvant systemic therapy). Expression of p27Kip1 was defined as positive staining of >60% of cells; the level of p27Kip1 expression was not an independent prognostic marker (P = 0.1). Among patients with well-differentiated tumors (grade 1), Leong et al. (38) found that p27Kip1 levels were low, defined as positive staining of <75% of the cells, in 33% of the cases. These authors also found that p27Kip1 was not predictive of clinical outcome. Although Leivonen et al. (35) reported that p27Kip1 was a significant prognostic factor in patients with early-stage breast cancer at 5 years of follow-up, p27Kip1 levels did not have prognostic significance at 10 or 15 years of follow-up. Spataro et al. (39) found no evidence that DFS differed according to p27Kip1 expression levels in patient with node-negative breast cancer. Similarly, we did not find any prognostic value of p27Kip1 expression in our series of node-negative breast carcinomas. The discrepancies in the findings of the different studies may reflect technical differences (e.g., use of different antibodies and scoring systems) or patient selection criteria (e.g., node-negative versus node-positive disease and treatment with

| Table 3 | Literature identifying p27Kip1 as a prognostic factor in early-stage breast cancer |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| First author (reference no.) | Patient selection | Outcome | n | % with low p27Kip1 | Cut off | P |
| Catzavelos (5) | All patients | 5-year DFS | 168 | 56 | <50%, ≥50% | 0.0072 |
| T (6) | Node (−) | 5-year DFS | 75 | 48 | 0.22 |
| Porter (33) | Age <45 years | OS | 202 | 49 | <50%, ≥50% | 0.02 |
| Reed (32) | Node (−) | OS | 278 | 63 | Low/int/high | 0.001 |
| Wu (13) | All patients | 5-year DFS | 181 | 69 | ≥50%, >50% | 0.0001 |
| Tsuchiya (7) | Node (−) | 5-year DFS | 102 | 58 | ≥50%, >50% | 0.01 |
| Barbareysi (8) | Node (−) | 5-year DFS | 512 | 66 | <50%, ≤50% | 0.4 |
| Volpi (34) | Node (+) | 5-year DFS | 286 | 76 | ≥60%, >60% | 0.1 |
| Nohara (40) | Node (−) | 5-year DFS | 216 | 50 | ≥62%, >62% | 0.05 |
| Leivonen (35) | Node (−) | 10-year DFS | 197 | 75 | <50%, ≥50% | 0.67 |
| Liang (41) | N/A | 5-year DFS OS | 128 | 41 | ≤50%, >50% | N/A |
| Leong (38) | Grade I [node (−), node (+)] | DFS | 148 | 33 | ≥75%, >75% | N/A |
| Spataro (39) | Node (−) | DFS | 461 | 44 | <50%, ≤50% | 0.32 |
| | Node (−) | DFS | 461 | 44 | <50%, ≤50% | 0.32 |

* Percentages of cells positive on nuclear staining, as used to define negative and positive expression of p27Kip1 in each study.
* OS, overall survival; int, intermediate; NS, not statistically significant; N/A, data not available.

This percentage reflects the reported p27Kip1 expression level in the cytoplasm rather than the nuclei. (All other percentages reflect reported p27Kip1 expression levels in the nuclei.)

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versus without adjuvant systemic therapy). Alternatively, the conflicting results may indicate that p27Kip1 is not the only cell cycle inhibitor involved in breast cancer pathogenesis, invasion, and metastasis.

In this study, JAB1 was not an independent prognostic marker in node-negative breast cancer. It is possible that JAB1 may be involved in breast cancer initiation and be less important in the development of metastasis. Alternatively, JAB1 may, as with p27Kip1, be an important prognostic factor in specific subsets of patients such as patients with node-positive disease or patients treated with hormone therapy or chemotherapy (39).

In conclusion, our results indicate that JAB1 is commonly overexpressed in human breast carcinomas and is inversely correlated with p27Kip1 expression. JAB1 may play an important role in breast oncogenesis, although its role in the development of metastasis and prognosis is unclear. Future studies should be performed to define the clinical implications of JAB1 and its effects on p27Kip1 in breast cancer cells.

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