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Advances in Brief

The Cell Cycle Inhibitor p27 Is an Independent Prognostic Marker in Small ($T_{1a,b}$) Invasive Breast Carcinomas

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Abstract

Breast carcinomas ≤1 cm in size ($T_{1a,b}$) are being detected more frequently as a result of screening. Because traditional prognostic parameters are either lacking (tumor size) or rare (nodal metastases), a marker(s) is needed to identify the subset of patients who could benefit from adjuvant therapy. A retrospective series of 202 patients with stage $T_{1a,b}$ invasive breast carcinomas was evaluated. The clinicopathological features (age, histological grade, extensive *in situ* carcinoma, hormone receptor status, and nodal metastasis) as well as microvessel density and the expression of c-erb-B2, p53, MIB-1/Ki-67, and *cdc25B* were assessed. In addition, expression of the cell cycle inhibitor p27 was evaluated.

Nineteen patients (18% of patients who had axillary dissection) had locoregional lymph node metastases. Forty-two % of them died of disease (median survival, 112 months), whereas mortality was 11% in node-negative patients (median survival, 168 months; $P = 0.0055$). Patients with low p27 expression had a median survival of 139 months (17% mortality) *versus* 174 months (9% mortality) in the group with high p27 expression ($P = 0.0233$). Lack of p27 was associated with poor prognosis when node-positive patients were excluded ($P = 0.0252$). Nodal status and low p27 were found to be the only independent prognostic parameters by both univariate and multivariate analysis, with relative risks of dying of disease of 4.9 ($P = 0.001$) and 3.4 ($P = 0.0306$), respectively.

Assessment of p27, which yields prognostic information in node-negative patients, could be useful to identify patients with small, invasive breast carcinomas who might benefit from adjuvant therapy.

Introduction

Recent trends in breast cancer detection have resulted in identification of progressively smaller invasive breast carcinomas, as well as increased numbers of *in situ* cancers. Several studies have shown that in small invasive tumors of ≤1 cm, the incidence of lymph node metastasis is very low, ranging from 3 to 13% (18% of patients who had axillary node dissection in this study; Ref. 1). At present, much controversy exists regarding the need for axillary lymph node dissection and/or adjuvant therapy following excision of small invasive primary tumors. In this study, we examined most pathological parameters shown previously to be important either in assessing a prognosis or in predicting a response to therapy. In addition, we examined some of the most important biological markers known to be associated with mammary oncogenesis (2–7).

Regulation of cell division cycle progression in mammalian cells depends on the sequential activation of a series of Cdks[3] (reviewed in Ref. 8). Their activation requires (*a*) association with cyclins; (*b*) dephosphorylation of a tyrosine and a threonine located in the ATP binding site controlled, at least in part, by a family of dual-specificity phosphatases called *cdc25* (for a review see Ref. 9); and (*c*) dissociation of the cyclin-Cdk complex from Ckis (reviewed in Ref. 10).

The Cki *p27* is a potential tumor suppressor. In fact, *p27*-deficient mice develop pituitary tumors and display increased body size (11–13). In addition, the adenoviral E1A (14) and the human papilloma viral E7[4] oncoproteins inactivate p27 by dissociating it from cyclin-Cdk complexes. However, in contrast to traditional antioncogenes such as the Ckis *p15* and *p16*, no homozygous deletions and only rare mutations of the *p27* gene have been found thus far in cell lines or in human tumors (15, 16). Because p27 abundance is regulated at the posttranscriptional level (17), we reasoned that, even in presence of a wild-type gene, tumors might obtain a growth advantage from lack of p27 due to an increase in its degradation, as we proposed previously in colorectal cancers (18). Therefore, we analyzed the expression of p27 protein to assess the relationship between lack of p27 expression and aggressive behavior in small invasive breast cancers.

Materials and Methods

Population Study. Two hundred two consecutive patients (mean follow-up, 72.3 months; median follow-up, 65.9 months) with stage T_{1a} and T_{1b} (defined as tumors ≤ 1 cm in greatest diameter as determined by macroscopic measurement and confirmed by remeasurement on the slide) invasive breast cancers diagnosed between 1969 and April 1994 were analyzed. Patients with distant metastases at the time of diagnosis, exclusive *in situ* disease, synchronous bilateral breast carcinomas or a history of previous malignancies were excluded from this study. Age at diagnosis, biochemical steroid hormone receptor levels (available in 133 patients), modalities of therapeutic interventions, time to recurrence or metastasis, length of overall survival, and cause of death were recorded. The mean age of the patients was 60.9 years (median, 62.0); 42 patients (21%) were less than 50 years of age. Follow-up data were obtained from patients' charts and Tumor Registry records. Death from cancer was accepted when confirmed by autopsy or when there was convincing evidence of disease by clinical, pathological, or radiographic parameters. Of the patients who were node negative or node indeterminate, 53 received adjuvant therapy (51% of which was radiation therapy alone). Of the 19 node-positive patients, 1 received chemotherapy (5%); 1 radiation therapy (5%); 3 hormonal therapy (16%); 7 radiation and chemotherapy (37%); 1 radiation and tamoxifen (5%); and 2 radiation, chemo-, and hormonal therapy (11%). Four patients (21%) received no adjuvant therapy. Twenty-three % of patients with ER-positive tumors in our series were treated with tamoxifen. The median tumor size was 0.66 cm (range, 0.1–1.0 cm). Tumors ≤5 mm in size (T_{1a}) made up 31% of the series. Distribution of different histological

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[3] The abbreviations used are: Cdk, cyclin-dependent kinase; Cki, Cdk inhibitor; ER, estrogen receptor; PR, progesterone receptor; MVD, microvessel density; EIC, extensive intraductal component.

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grades and presence or absence of an EIC in the patient population are outlined in Table 1. The majority of carcinomas were ductal type (139 or 69%), whereas 45 (22%) were tubular carcinomas, 11 (5%) were lobular, 6 (3%) were colloid carcinomas, and 1 was a metaplastic carcinoma. Eleven patients (5%) developed local disease recurrence, whereas 18 (9%) had radiologically or histopathologically documented distant metastases prior to death. Twenty-six patients (13%) died of the disease or complications related to it. One hundred two patients underwent axillary node dissection, and 19 of these had nodal metastases.

Immunohistochemistry. Specificity of the p27 staining was assessed by (*a*) preabsorption of the antibody with the protein used to generate it (resulting in abolished p27 staining), (*b*) a mixture comprising antibodies with no known human recognition site used as a negative control, and (*c*) positive staining of serum-starved MG-63 osteosarcoma cells (obtained from the American Type Culture Collection) in each immunoperoxidase run. Immunohistochemistry was performed on an automated processor (Ventana ES; Ventana Medical System, Tucson, AZ). Steps performed in the immunostainer include blocking with normal horse serum, application of the primary antibody, a biotinylated secondary antibody, and visualization with diaminobenzidine substrate, with standardized development time (allowing reproducible comparison between samples and between runs). The primary antibodies used were as follows: monoclonal anti-c-erb-B2 (Ciba-Corning, Alameda, CA), dilution 1:150, incubation time 20 min; monoclonal anti-factor VIII-related antigen (DAKO Corp., Carpinteria, CA), dilution 1:100, incubation time 16 min; monoclonal anti-hormone receptors (ER and PR; Ventana Medical Systems, Tucson, AZ), incubation time 32 min; monoclonal anti-p53 clone Ab-6 (Oncogene Science), dilution 1:500, incubation time 32 min; monoclonal anti-MIB-1, (Immunotech, Westbrook, ME), dilution 1:25, incubation time 16 min; and monoclonal anti-p27 (Transduction Laboratories, Lexington, KY), dilution 1:200, incubation time 24 min. A prior modified antigen retrieval procedure was employed by microwaving deparaffinized slides in citrate buffer (pH 6.1; Biogenex, San Ramon, CA) within a pressure cooker at power 10 (750-W oven) for 20 min (ER and PR), power 5 for 16 min (p53 and MIB1), and power 7 for 16 min (p27).

***In Situ* Hybridization.** *In situ* hybridization for *cdc25B* was performed on the automated processor (Ventana Gen II; Ventana Medical Systems, Tucson, AZ) on 154 cases in which there was adequate preservation of mRNA, as assessed by expression of glyceraldehyde-3-phosphate dehydrogenase. Digoxigenin-labeled probes were utilized for *in situ* hybridization as we described previously (19).

Scoring of Factors for Statistical Evaluation. Stained tumor sections were evaluated by estimating the percentage of invasive carcinoma cells stained positively by a primary antibody on the entire tissue section. p27 staining, for both immunochemistry and *in situ* hybridization, was evaluated in a coded manner (without knowledge of the clinical and pathological parameters or outcome) and scored independently for degree of p27 expression by two pathologists (M. L. and P. T.). At least 10 high-powered fields were counted. For c-erb-B2, a distinct brown membranous staining was scored as positive. For factor VIII, a distinct brown cytoplasmic staining in endothelial cells was considered positive. Areas with the highest vessel density were chosen at low magnification for counting. MVD was assessed by counting up to three fields at ×200 magnification. The counts were expressed as total number of microvessels in one ×200 field, as described previously (6). The range was 5–262 microvessels per ×200 microscopic field, with a median value of 50. This value was used as a cutoff point. For all the other markers, *i.e.*, p53, MIB-1, p27, ER, and PR, a distinct brown nuclear staining was scored as

Table 1 *Univariate analysis of prognostic markers*

Markers (total patients)	No. of patients	Death from disease *N* (%)	Cohort survival Median no. of months	*P* value
Tumor size (n = 202)				
T1a	62	8 (13%)	174	0.9802[a]
T1b	140	18 (13%)	168	0.7018[b]
Histological grade (n = 202)				
I	70	8 (11%)	162	0.9791[a]
II	89	13 (15%)	160	0.7550[b]
III	43	5 (12%)	174	
EIC (n = 202)				
Positive (≥25%)	141	15 (11%)	168	0.2755[a]
Negative (<25%)	61	11 (18%)	174	0.4226[b]
Lymph node metastases (n = 102)[c]				
Yes	19	8 (42%)	112	0.0006[a]
No	83	9 (11%)	168	0.0055[b]
p27 immunostaining (n = 202)				
<50%	100	17 (17%)	139	0.0042[a]
≥50%	102	9 (9%)	174	0.0233[b]
cdc25B score (n = 154)				
<3	122	13 (11%)	168	0.1803[a]
=3	32	5 (16%)	132	0.1348[b]
c-*erb*B-2 immunostaining (n = 200)				
<10%	177	23 (13%)	162	0.1015[a]
≥10%	23	2 (9%)	174	0.2463[b]
p53 immunostaining (n = 200)				
<10%	179	21 (12%)	174	0.1941[a]
≥10%	21	5 (24%)	168	0.4468[b]
MIB1 immunostaining (n = 199)				
<10%	193	26 (13%)	146	0.2948[a]
≥10%	6	0 (0%)	168	0.4415[b]
ER immunostaining (n = 196)				
<10%	56	5 (6%)	174	0.1650[a]
≥10%	140	21 (13%)	168	0.4104[b]
ER by cytosolic assay (n = 133)				
<10 fmol	78	10 (13%)	162	0.0806[a]
≥10 fmol	55	4 (7%)	168	0.0390[b]
PR immunostaining (n = 194)				
<10%	94	16 (17%)	174	0.0961[a]
≥10%	100	10 (10%)	168	0.1735[b]
MVD (n = 200)				
Low (<50)	124	13 (10%)	168	0.6409[a]
High (≥50)	76	12 (16%)	174	0.3963[b]

[a] Log-rank values.
[b] Wilcoxon values.
[c] Only patients who had axillary node dissection.

Table 2 *Clinicopathological features of study population in relation to p27 status*

Markers	p27 groupings <50% N	<50% %	≥50% N	≥50% %	Total N
Number of patients	100		102		202
Age (yr)					
<50	18	(18%)	24	(24%)	42
≥50	82	(82%)	78	(76%)	160
Tumor size (cm)					
T1a (≤0.5)	32	(32%)	30	(29%)	62
T1b (>0.5, ≤1.0)	68	(68%)	72	(71%)	140
Histological grade					
I	37	(37%)	33	(32%)	70
II	43	(43%)	46	(45%)	89
III	20	(20%)	23	(23%)	43
EIC (% of tumor size)					
Positive (≥25%)	68	(69%)	73	(72%)	141
Negative (<25%)	32	(31%)	29	(28%)	61
Local recurrence					
Yes	1	(1%)	10	(10%)	11
No	99	(99%)	92	(90%)	191
Lymph node metastases					
Yes	12	(12%)	7	(7%)	19
No	88	(88%)	95	(93%)	183
Lymph node metastases with axillary dissection					
Yes	12	(26%)	7	(13%)	19
No	35	(74%)	48	(87%)	83

positive. A cutoff value of ≤10% was used for p53, MIB-1, c-erb-B2, and ER/PR, and ≥50% for p27, as described previously (18). Fifty % of p27-positive tumor cells represented the median of this series. Cytosolic ER assays were performed as described previously (20). *cdc25B* expression was assessed by *in situ* hybridization. Because expression of *cdc25B*, when present, was found throughout the tumor, sections were evaluated for intensity of staining, which was scored as 0, 1, 2, and 3+. Only cases categorized as 3+ were considered overexpressors, and this group was compared to 0, 1+, and 2+ combined.

Statistical Analysis. The primary study outcome was survival, which was measured from the date of surgery to the date of last follow-up or death. Survival was censored if the patient was still alive or died from other causes. Survival curves were constructed using the Kaplan-Meier method. Univariate survival curves were compared using a Wilcoxon procedure, and differences between prognostic factors were tested for statistical significance with the log-rank analysis (Table 2). A Cox proportional hazards model for the risk ratio was used to assess the simultaneous contribution of the following baseline covariates: age, grade, nodal status, presence or absence of EIC (Cox model 1; Table 3), and expression of the various markers (Cox model 2; Table 3) or all parameters combined (not shown). Ki-67/MIB-1 was excluded from model 2 and the combined Cox because of (*a*) the scarcity of cases with ≥10% positive cells (6 of 199) and (*b*) the clustering of all deaths in the <10% group. All other covariates were retained in the model to illustrate a lack of effect in the presence of other significant factors. The distribution of p27 was compared to the distribution of each baseline covariate using the Jonckheere-Terpstra Test for doubly ordered data, the Kruskal-Wallis test was used for single ordered data, and Fisher's exact test was used for categorical data. A *P* value ≤0.05 was required for significance. Two-sided tests were performed throughout all analyses.

Results and Discussion

A cohort of 202 patients with invasive carcinomas that measured ≤1 cm ($T_{1a,b}$) were studied. All statistics were carried out with survival as an end point. Patients were examined for differences in tumor size (T_{1a} *versus* T_{1b}), histological grade (modified Bloom-Richardson), EIC, presence or absence of lymph node metastases in patients who had axillary node dissection (Table 1), local recurrences,

Table 3 *Multivariate analysis of prognostic markers by survivals, using Cox models*

Model 1

Clinicopathological parameters	Category	Risk ratio	95% confidence interval	*P* value[a]
Age (yr)	0 = <50, 1 = ≥50	1.508	0.598–3.802	0.3833
Positive lymph nodes	0 = No, 1 = Yes	4.877	1.900–12.523	0.0010
Tumor size	0 = T1a, 1 = T1b	1.034	0.4140–2.584	0.9421
Histological grade I	0 = III, 1 = I	1.125	0.334–3.784	0.8495
Histological grade II	0 = III, 1 = II	1.166	0.378–3.584	0.7901
EIC	0 = <25%, 1 = ≥25%	1.325	0.557–3.155	0.5241

Model 2

Biological parameters	Category	Risk ratio	95% confidence interval	*P* value[a]
c-*erb*B-2	0 = <10%, 1 = ≥10%	1.684	0.1846–15.385	0.6444
p53	0 = <10%, 1 = ≥10%	2.344	0.603–9.113	0.2190
p27	1 = ≥50%, 0 = <50%	3.401	1.121–10.309	0.0306
cdc25B	0 = <3, 1 = ≥3	1.740	0.532–5.687	0.3594
ER immunostaining	0 = <10%, 1 = ≥10%	4.158	1.000–17.289	0.0500
PR immunostaining	1 = <10%, 0 = ≥10%	3.289	1.079–10.101	0.0364
MVD	0 = <50, 1 = ≥50	2.260	0.813–6.281	0.1178

[a] *P* value is calculated by χ square.

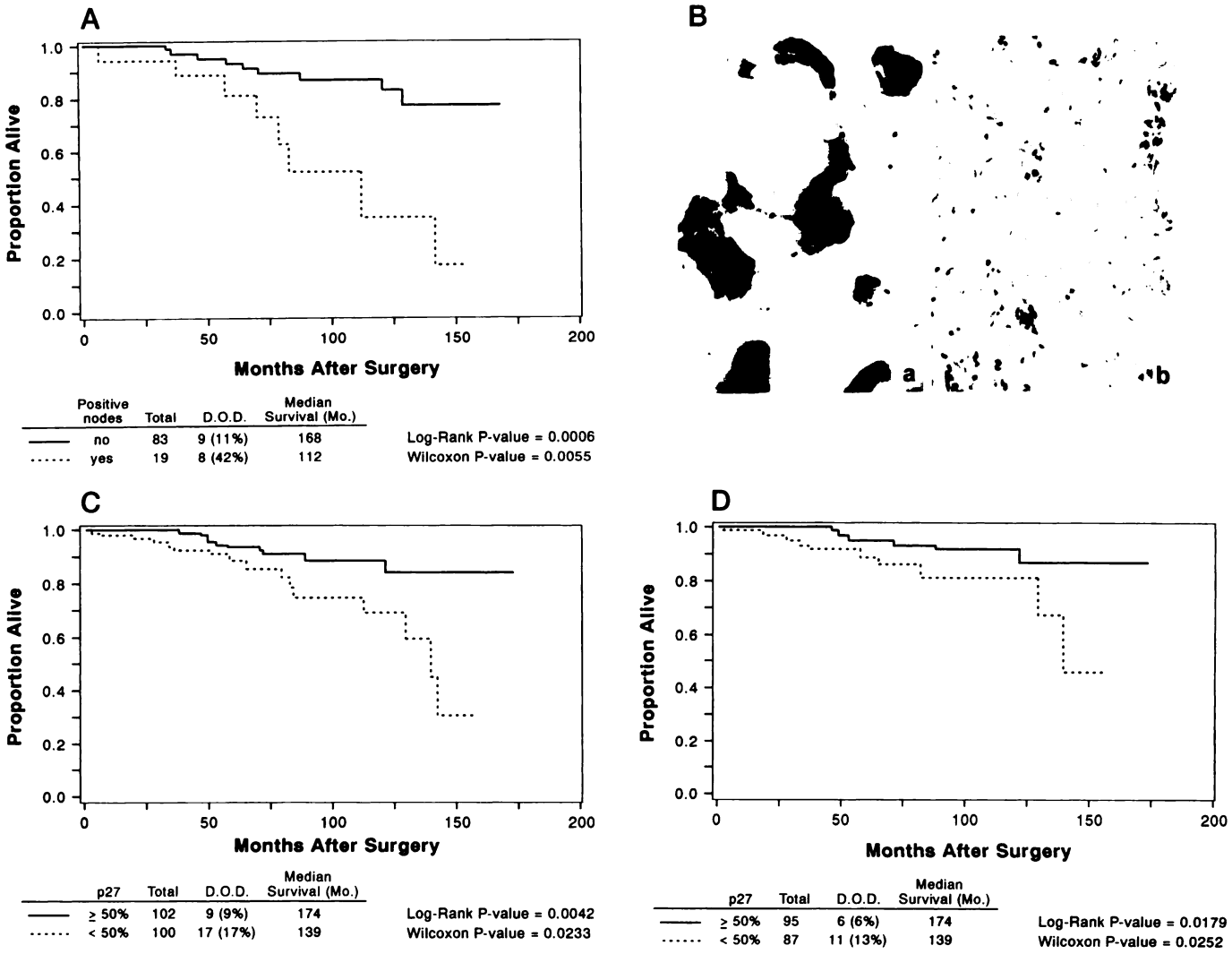

Fig. 1. *A*, actuarial survival curves in the 102 patients who had axillary node dissection stratified according to nodal status. *B*, nuclear localization of p27 by immunohistochemistry of a tumor with (*a*) ≥50% of positive tumor cells for p27 (high expresser), and (*b*) <50% of positive cells (low expresser). *Brown*, nuclear reaction; *methyl green*, counterstain. *C*, actuarial survival curve in the series of 202 patients stratified according to p27 expression; low, <50% of positive tumor cells; high, ≥50% of positive tumor cells. *D*, actuarial survival curve stratified according to p27 expression after exclusion of node-positive patients; low, <50% of positive tumor cells; high, ≥50% of positive tumor cells.

type of treatment (radiation therapy, chemotherapy, and/or tamoxifen), and type of surgery. We also evaluated and included in the analysis the expression of the proto-oncogene c-erb-B2, the tumor suppressor gene *p53*, the proliferation marker MIB-1/Ki-67, ER and PR, and MVD as a measure of angiogenesis. Because we had reported recently that *cdc25B* mRNA was associated with decreased disease-free survival when overexpressed in node-negative breast cancer patients (19), *cdc25B* was also assessed by *in situ* hybridization.

Twenty-six patients (13%) died of disease in this series of patients. Of all clinicopathological parameters considered, only patients with positive nodes had a significantly lower survival (Table 1). Specifically, of the 19 patients with positive nodes (18% of the patients who underwent axillary dissection), 8 (42%) died of disease (median survival, 112 months). In contrast, mortality was 11% in node-negative patients (median survival, 168 months) (log-rank $P = 0.0006$; Wilcoxon $P = 0.0055$; Fig. 1*A*). There was no significant difference in survival (174 *versus* 168 months) between patients who did and those who did not have axillary node dissection (log-rank $P = 0.8843$; Wilcoxon $P = 0.7998$).

ER, PR, p53, MVD, Ki-67/MIB-1, *cdc25B*, and c-erb-B2 were not significantly associated with survival by univariate analysis (Table 1). In contrast, the level of p27 expression (Fig. 1*B*) was associated significantly with survival by actuarial analysis, with a median survival of 174 months in patients whose tumors displayed high p27 (≥50% p27-positive cells) and 139 months in tumors that had low p27 expression (<50% p27-positive cells; log-rank $P = 0.0042$; Wilcoxon $P = 0.0233$; Fig. 1*C*). High and low p27 expression in patients was distributed equally in the positive and negative node groups (Table 2). Significance was maintained when node-positive patients were excluded (log-rank $P = 0.0179$; Wilcoxon $P = 0.0252$; Fig. 1*D*). Even in the small subgroup of patients who had axillary node dissection but were found to be node negative, the group with low p27 expression had a mortality of 14% compared to 8% in the group with high p27 expression, although this was not quite significant (log-rank $P = 0.0704$; Wilcoxon $P = 0.0526$). p27 expression was not associated directly (*i.e.*, was statistically independent or unrelated as a variable) with Ki-67/MIB-1, indicating that lack of p27 expression was not simply reflecting proliferation (data not shown).

When a Cox proportional hazards model was constructed with all of the clinicopathological variables (Table 3, model 1), positive nodal status was a significant covariate ($P = 0.0010$; relative risk, 4.9), whereas age, size (T_{1a} or T_{1b}), tumor differentiation, and EIC were

not. When a Cox proportional hazards model was constructed with all of the biological variables (Table 3, model 2), p27 down-regulation (<50% of tumor cells) and lack of PR (<10%) were significant covariates (for p27, $P = 0.0306$; relative risk, 3.4; for PR, $P = 0.0364$; relative risk, 3.3). In addition, ER expression (≥10% of tumor cells) was also significant, although with a rather wide confidence interval. Neither hormone receptor status showed significant association with survival by univariate analysis. Finally, *cdc25B,* p53, MVD, Ki-67/MIB1, and c-erb-B2 were not independent parameters.

Although the presence of nodal metastases was associated significantly with poor prognosis, with a 4.9-fold relative risk of death, these patients represented only a small portion of the population. Importantly, node-negative patients with poor outcome can be identified on the basis of their p27 status, thus providing a powerful prognostic tool that can be utilized in all patients. In contrast, whereas hormonal receptors were found to be weakly associated with prognosis by multivariate analysis, other biological markers, *i.e.,* c-erb-B2, p53, *cdc25B,* MVD, and Ki-67/MIB1, were not found to be significant predictors of survival in this group of patients.

In summary, p27 is a novel prognostic marker, the expression of which is associated with a better outcome in patients with $T_{1a,b}$ breast carcinomas who undergo potential curative surgery. Low p27 expression correlates with decreased survival and may be utilized in $T_{1a,b}$ node-negative patients to predict prognosis. Our study suggests that adjuvant therapy, which benefits patients with advanced breast cancer, may also be appropriate for patients with stage I disease whose tumors express little p27.

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References

1. Cady, B., Stone, M., Schuler, J., Thakur, R., Wanner, M., and Lavin, P. The new era in breast cancer: invasion, size and nodal involvement dramatically decreasing as a result of mammographic screening. Arch. Surg., *131:* 301–30, 1996.
2. Thor, A. D., Moore, D. H. II, Edgerton, S. M., Kawasaki, E. S., Reihsaus, E., Lynch, H. T., Marcus, J. N., Schwartz, L., Chen, L. C., Mayall, B. H., and Smith, H. S. Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancers. J. Natl. Cancer. Inst., *84:* 845–855, 1992.
3. Esteban, J. M., Ahn, C., Battifora, H., and Felder, B. Predictive value of estrogen receptors evaluated by quantitative immunohistochemical analysis in breast cancer. Am. J. Clin. Pathol., *102*(Suppl. 1)*:* S9–S12, 1994.
4. Slamon, D., Clark, G., Wong, S., Levin, W., Ullrich, A., and McGuire, W. Human breast cancer: correlation of relapse and survival with amplification of the *Her-2/neu* oncogene. Science (Washington DC), *235:* 177–182, 1987.
5. Pinder, S. E., Wencyk, P., Sibbering, D. M., Bell, J. A., Elston, C. W., Nicholson, R., Robertson, J. F., Blamey, R. W., and Ellis, I. O. Assessment of new proliferation marker MIB1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. Br. J. Cancer, *71:* 146–149, 1995.
6. Bosari, S., Lee, A., DeLellis, R., Wiley, B., Heatley, G., and Silverman, M. Microvessel quantitation and prognosis in invasive breast carcinoma. Hum. Pathol., *23:* 755–761, 1992.
7. Jacquemier, J., Kurtz, J. M., and Amalric, R. An assessment of extensive intraductal component as a risk factor for local recurrence after breast-conserving therapy. Br. J. Cancer, *61:* 873–876, 1990.
8. Lees, E. Cyclin dependent kinase regulation. Curr. Opin. Cell Biol., 773–780, 1995.
9. Coleman, T., and Dunphy, W. cdc2 regulatory factors. Curr. Opin. Cell Biol., *6:* 877–882, 1994.
10. Sherr, C., and Roberts, J. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev., *9:* 1149–1163, 1995.
11. Nakayama, K., Ishida, N., Shirane, M., Inomata, A., Inoue, T., Shishido, N., Horii, I., Loh, D., and Nakayama, K. Mice lacking $p27^{Kip1}$ display increased body size, multiple organ hyperplasia, retina dysplasia, and pituitary tumors. Cell, *85:* 707–721, 1996.
12. Kiyokawa, H., Kineman, R., Manova-Todorova, K., Soares, V., Hoffman, E., Onoi, M., Hayday, A., Frohman, D., and Koff, A. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of $p27^{Kip1}$. Cell, *85:* 721–732, 1996.
13. Fero, M., Rivkin, M., Tasch, M., Porter, P., Carow, C., Firpo, E., Tsai, L., Broudy, V., Perlmutter, R., Kaushansky, K., and Roberts, J. A syndrome of multi-organ hyperplasia with features of gigantism, tumorigenesis and female sterility in $p27^{Kip1}$-deficient mice. Cell, *85:* 733–744, 1996.
14. Mal, A., Poon, R., Howe, P., Toyoshima, H., Hunter, T., and Harter, M. Inactivation of p27kip1 by the viral E1A oncoprotein in TGFβ-treated cells. Nature (Lond.), *380:* 262–265, 1996.
15. Kawamata, N., Morosetti, R., Miller, C., Park, D., Spirin, K., Nakamaki, T., Takeuchi, S., Hatta, Y., Simpson, J., Wilczynski, S., Lee, Y., Bartram, C., and Koeffler, H. Molecular analysis of the cyclin-dependent kinase inhibitor gene *p27/kip1* in human malignancies. Cancer Res., *55:* 2266–2269, 1995.
16. Spirin, K. S., Simpson, J. F., Takeuchi, S., Kawamata, N., Miller, C. W., and Koeffler, H. P. *p27/Kip1* mutation found in breast cancer. Cancer Res., *56:* 2400–2404, 1996.
17. Pagano, M., Tam, S. W., Theodoras, A. M., Romero-Beer, P., Del Sal, G., Chau, V., Yew, R., Draetta, G., and Rolfe, M. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. Science (Washington DC), *269:* 682–685, 1995.
18. Loda, M., Cukor, B., Tam, S. W., Lavin, P., Fiorentino, M., Draetta, G., Jessup, J., and Pagano, M. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. Nat. Med., *3:* 231–234, 1997.
19. Galaktionov, K., Lee, A., Eckstein, J., Draetta, G., Meckler, J., Loda, M., and Beach, D. CDC25 phosphatases as potential human oncogenes. Science (Washington DC), *269:* 1575–1577, 1995.
20. Allred, D. C., Bustamante, M. A., Daniel, C. O., Gaskill, H. V., and Cruz, A. B. J. Immunocytochemical analysis of estrogen receptors in human breast carcinomas. Arch. Surg., *125:* 107–113, 1990.

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