

## E2F1 Expression Predicts Outcome in Korean Women Who Undergo Surgery for Breast Carcinoma

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### ABSTRACT

**Background.** The transcriptional factors E2F1 and E2F2 have been reported to be associated with improved chemosensitivity in various cancers. We aimed to investigate whether E2F1 and E2F2 can be used as predictors of chemosensitivity in hormone-receptor-negative breast cancers (HRNBCs), which are common in Korean women.

**Methods.** A total of 183 patients with primary breast cancer who had undergone surgical resection were evaluated on the basis of hormonal status, age, histological subtype and grade, tumor size, lymph node metastasis, and stage. The immunohistochemical expressions of E2F1 and E2F2 were analyzed for these histopathological data and patient survival.

**Results.** E2F1 expression was associated with low histological grade (grade 1) and larger tumor size (>2 cm), while E2F2 expression was correlated only with large tumor size (>2 cm). The E2F1-positive group had less tumor recurrences, lymph node metastases during follow-up, and distant metastases than the E2F1-negative group; E2F1 expression was found to be an independent predictive factor of more favorable survival among HRNBC patients on univariate and multivariate analyses, but E2F2 expression was not.

**Conclusions.** E2F1 may be a potential prognostic and predictive factor for clinical outcome and therapeutic results following adjuvant chemotherapy in HRNBC patients.

Currently, breast cancer is the most common carcinoma and the fifth most common cause of cancer-related death among Korean women.<sup>1</sup> Breast cancer in Asian women reveals a high proportion of hormone-receptor-negative breast cancers (HRNBC, 47–58%) compared with in Western countries (30–40%). HRNBCs are characterized by high histological grade and poorly differentiated tumors and are associated with poor median survival (i.e., 18–24 months); less than 5% of patients survive longer than 5 years.<sup>2–6</sup>

Because HRNBCs do not respond to hormone therapies such as tamoxifen or aromatase inhibitors, adjuvant chemotherapy is essential for these patients.<sup>7,8</sup> Therefore, it is important to evaluate predictive factors for chemotherapeutic response. Although a study establishing a predictive factor for chemotherapy warranted for Asian women has been carried out, data on patients with HRNBC in Asian countries are limited.<sup>2</sup>

E2F1 and E2F2 are transcriptional factors that play a key role in G1 to S phase transition by attracting numerous upstream signals.<sup>9</sup> E2F1 and E2F2 belong to the activators of the E2F family. There are eight subtypes in the E2F family (i.e., E2F1–E2F8), functionally divided into two groups: activating transcription factors, which predominantly comprise E2F1, E2F2, and E2F3b, and transcriptional repressors, which predominantly comprise E2F4 and E2F5.<sup>10,11</sup> E2F6, E2F7, and E2F8 have only recently been detected, and their functions are not yet clear.<sup>11</sup> E2F1 is especially distinct from other E2F family members, because its bimodal effect determines whether a cell will advance through the cell cycle or die via apoptosis. Its action vis-à-vis proliferation versus apoptosis depends on the cell type and microenvironmental stimulation.<sup>12</sup>

E2F1 expression is known to be associated with chemosensitivity in various tumors. E2F1 expression has

been found to be associated with improved survival in patients treated with adjuvant chemotherapy for gastric cancer and colon cancer.<sup>13,14</sup> However, its expression was associated with poor survival in patients with breast cancer.<sup>15</sup> Nonetheless, the role of E2F1 as a predictor of chemosensitivity and improved survival outcome in breast cancer remains largely uncertain.

The role of E2F2 as a predictive factor has not been deeply investigated. So far, only one study on breast cancer has demonstrated the promoting effects of E2F2.<sup>16</sup> However, the mechanism by which E2F2 influences breast cancer progression has not been elucidated.

This study compares prognosis on the basis of hormone receptor status and investigates the correlation between E2F1/E2F2 expression and patient survival. We also evaluated whether E2F1 and E2F2 could function as predictive factors for successful therapeutic outcomes in HRNBC patients who had undergone adjuvant chemotherapy.

## MATERIALS AND METHODS

### *Patients and Histological Evaluation*

The study group included patients who had undergone surgical resection with primary breast cancer between 1997 and 2002. Patients with distant metastasis or any T4 tumor at the time of surgery were excluded. Immunohistochemical staining slides for hormone receptors, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) were re-evaluated in all cases. Immunohistochemical staining was done with labeled streptavidin biotin complex (LSAB) technique. Primary antibodies for estrogen receptor alpha (NCL-ER-6F11), progesterone receptor A (NCL-PgR-312, clone 16), and c-erbB-2 oncoprotein internal domain (CB11) were used. ER and PR were considered positive if at least 5% of tumor cells showed nuclear staining. HER2 was considered positive if 2+ or 3+ was found. Hormone receptor status was divided into two groups: both ER and PR negative, defined as hormone-receptor-negative breast cancer (HRNBC), and ER and/or PR positive, defined as a hormone receptor (HR)-positive tumor.

Medical reports were also reviewed to retrieve clinical information regarding demographics, treatment details, and patient outcome. Most adjuvant chemotherapies were composed of six cycles of AC (adriamycin 60 mg/m<sup>2</sup>, cyclophosphamide 600 mg/m<sup>2</sup>), CMF (cyclophosphamide 500 mg/m<sup>2</sup>, methotrexate 40 mg/m<sup>2</sup>, fluorouracil 600 mg/m<sup>2</sup>), and FEC (fluorouracil 700 mg/m<sup>2</sup>, epirubicin 75 mg/m<sup>2</sup>, cyclophosphamide 700 mg/m<sup>2</sup>) in all HRNBC patients. Oral tamoxifen (30 mg) was given daily for 5 years in all HR-positive patients. Each group consisted of patients

treated with the same therapeutic schedule. Finally, a total of 183 patients were enrolled.

Tumors were staged according to the International Union against Cancer's tumor-node-metastasis (TNM) classification criteria. Histological grade was divided into grades 1, 2, and 3 according to a modified Bloom and Richardson grading system. Patient survival was assessed via outpatient follow-up and telephone interview, and disease recurrence was confirmed by radiological studies and biopsy. Local recurrence was defined as regional recurrence in the primary site. Lymph node metastasis at follow-up was defined as contralateral or any lymph node metastasis during the follow-up period after complete surgical resection. Distant metastasis was defined as metastasis to the bone, liver, lung, brain, bone marrow, or pleural or pericardial cavity.

### *Tissue Microarray Block Manufacture*

After a case review for diagnostic confirmation, the tissue microarray (TMA) was constructed. The representative area was enclosed in a drawn circle. Each paraffin-embedded block relevant to hematoxylin and eosin slides was punched out by using a TMA manufacture tool (Quick-Ray<sup>TM</sup>; Unitma, Seoul, South Korea). A 3-mm-diameter punch size was used. One core from each paraffin-embedded block was punched out, and 25 cores were embedded in each TMA block, in a 5 × 5 arrangement. To evaluate the order of cores, a punch of palatine tonsil was embedded ahead of each TMA block.

### *Immunohistochemical Staining*

Histological sections (4 μm) of eight tissue microarray blocks of 10% formalin-fixed, paraffin-embedded materials were used in this study. The TMA sections were deparaffinized in xylene and rehydrated through descending concentrations of ethanol. Antigen retrieval was achieved through 2 min of microwave treatment in 0.01 mol/l citrate buffer (pH 6.0) and 2 h of cooling. Immunohistochemical staining was performed using a Dako ChemMate kit (Dako, Glostrup, Denmark), according to the manufacturer's instructions. The two primary antibodies used were E2F1 (monoclonal, 1:100 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) and E2F2 (L-20, 1:50 dilution; Santa Cruz Biotechnology). The sections were incubated with primary antibodies at room temperature for 1 h. Each section was treated sequentially with biotinylated secondary antibody for 20 min, followed by streptavidin peroxidase for 20 min.

We used a Dako catalyzed signal amplification (CSA) system, after incubation with E2F1 and E2F2 primary antibodies for 1 h. The sections were treated with chromogen 3,3'-diaminobenzidine tetrahydrochloride, and thereafter subjected to hematoxylin counterstain treatment.

A gastrointestinal stromal tumor section previously proven to have strongly positive staining was processed simultaneously, as a positive control for E2F1 and E2F2.

#### Analyses and Interpretations of Immunohistochemical E2F1 and E2F2 Expression

The two authors (Nam, ES and Cho, SJ) interpreted the staining results, and there was close agreement (>90%) between the investigators. In case of disagreement, final grading was determined by consensus.

With regard to E2F1 expression, only nuclear staining was considered positive. Although E2F2 expression was occasionally found in the nucleus, only cytoplasmic staining was considered positive. E2F1 was considered to be positive when >10% of cancer cell nuclei showed immunostaining, whereas E2F2 was considered to be positive when >10% of cancer cell cytoplasm showed immunostaining. These cutoff values were based on previous studies of breast cancers.<sup>15,16</sup>

#### Statistical Analysis

A Pearson's  $\chi^2$  test was used to evaluate the relationships among clinicopathological variables, hormone receptor status, and E2F1/E2F2 expression, as well as the correlation between tumor recurrence and E2F1, E2F2, and hormone-receptor status. The association between E2F1/E2F2 expression, hormone receptor status, and overall or disease-free survival rates was estimated using both a log-rank and generalized Wilcoxon test. Disease-free survival was defined as the time from surgery to the first relapse of breast cancer, the occurrence of a second primary tumor, or death by any cause. The time of analysis for disease-free survival was December 2008. Multivariate survival analyses were performed using the Cox proportional hazard regression model. All calculations were performed using the Statistical Package for the Social Sciences (SPSS) software (version 12; SPSS Inc., Chicago, IL), and results were considered statistically significant when *P* values were <0.05.

## RESULTS

#### Clinicopathological Features

The clinical and pathological features of the patients are listed in Table 1. The median age was 51 years (range, 25–87 years). The age of 147 patients (80.3%) was <60 years, while the age of 36 patients (19.7%) was ≥60 years; the majority of patients were in their 1950s. Histological diagnosis was invasive ductal carcinoma (IDC) in 163

**TABLE 1** Patient characteristics

	Total <i>n</i> = 183 (%)	HR (+) <i>n</i> = 62 (%)	HR (–) <i>n</i> = 121 (%)	<i>P</i> value
Age (years)				0.059
Median (range)	51 (25–87)			
<60, <i>n</i> (%)	147 (80.3)	45 (30.6)	102 (69.4)	
≥60, <i>n</i> (%)	36 (19.7)	17 (47.2)	19 (52.8)	
Histological subtype				0.252
IDC	163 (89.1)	56 (34.4)	107 (65.6)	
ILC	11 (6.0)	5 (45.5)	6 (54.5)	
Other <sup>a</sup>	9 (4.9)	1 (11.1)	8 (88.9)	
Histological grade				0.001 <sup>b</sup>
Grade 1	34 (18.6)	21 (61.8)	13 (38.2)	
Grade 2	103 (56.3)	34 (33.0)	69 (67.0)	
Grade 3	46 (25.1)	7 (15.2)	39 (84.8)	
T stage, <i>n</i> (%)				0.790
T <sub>1</sub> (≤2 cm)	62 (33.9)	20 (32.3)	42 (67.7)	
T <sub>2</sub> (>2 cm, ≤5 cm)	113 (61.7)	40 (35.4)	73 (64.6)	
T <sub>3</sub> (>5 cm)	8 (4.4)	2 (25)	6 (75)	
N stage, <i>n</i> (%)				0.169
N <sub>0</sub>	122 (66.7)	44 (36.1)	78 (63.9)	
N <sub>1</sub>	24 (13.1)	4 (16.7)	20 (83.3)	
N <sub>2</sub>	37 (20.2)	14 (37.8)	23 (62.2)	
Stage, <i>n</i> (%)				0.648
I	42 (23.0)	15 (35.7)	27 (64.3)	
IIA	87 (47.5)	30 (34.5)	57 (65.5)	
IIB	18 (9.8)	4 (22.2)	14 (77.8)	
IIIA	36 (19.7)	13 (36.1)	23 (63.9)	

HR (+) hormone receptor status with either estrogen receptor or progesterone receptor positive, HR (–) hormone receptor status with both estrogen receptor and progesterone receptor negative

<sup>a</sup> Mixed invasive ductal and lobular carcinoma = 3, micropapillary carcinoma = 2, medullary carcinoma = 2, mucinous carcinoma = 2

<sup>b</sup> Statistically significant, *P* value < 0.05

(89.1%), invasive lobular carcinoma (ILC) in 11 (6.0%), and minor histological subtypes in 9 (4.9%). Regardless of histological type, 34 tumors (18.6%) were grade 1, 103 (56.3%) were grade 2, and 46 (25.1%) were grade 3, according to a modified Bloom and Richardson grading system.

Follow-up information was available in all 183 patients, with the follow-up period ranging from 8 to 130 months. The 5-year survival rate was 69.62%, and the 10-year survival rate was 56.63%. Overall, 116 of the 183 patients were still alive.

HRNBCs constituted a high proportion (65.6%) of IDCs and were grade 3 tumors in 84.8% (*P* = 0.001). However, there was no statistical difference in histological subtypes

between HRNBCs and HR-positive tumors ( $P = 0.252$ ). The other parameters of age, T stage, N stage, and clinical stage were not statistically significant in terms of hormone status ( $P = 0.059$ ,  $P = 0.790$ ,  $P = 0.169$ , and  $P = 0.648$ , respectively).

#### Correlation of E2F1 Expression with Clinicopathological Variables

The clinicopathological characteristics of the patients with E2F1-positive tumors and E2F1-negative tumors are summarized in Table 2. Age (<60 vs.  $\geq 60$  years) was not significantly different between the two groups ( $P = 0.088$ ). Of the 163 IDCs, 73 (39.8%) were positive for E2F1 expression, and 4 ILCs (36.4%) were positive. E2F1 expression was observed in 58.8% (20/34), 43.7% (45/103), and 30.4% (14/46) of the grade 1, grade 2, and grade 3 tumors, respectively. E2F1 expression was higher in grade 1 than grade 2 and 3, and its expression tended to decrease as the grade increases ( $P = 0.040$ ).

The expression level of E2F1 was higher in large tumors (>2 cm) than in small tumors ( $\leq 2$  cm) ( $P = 0.000$ ). On

the other hand, E2F1 expression was not correlated to histological type or tumor stage ( $P = 0.370$  and  $P = 0.822$ , respectively). Lymph node metastasis was observed in 61 of 183 patients (33.3%) at the time of surgery. There were no significant differences in E2F1 expression between the patients in the node-negative and node-positive groups ( $P = 0.916$ ) or by hormonal status ( $P = 0.099$ ).

#### Correlation of E2F2 Expression with Clinicopathological Variables

E2F2 expression was associated with age (<60 vs.  $\geq 60$  years) ( $P = 0.001$ ); E2F2 expression was lower in the patients who were  $\geq 60$  years than in those who were <60 years. E2F2 expression did not vary statistically significantly with histological grade ( $P = 0.687$ ). E2F2 expression tended to increase with increasing tumor size ( $P = 0.007$ ), whereas its expression did not vary statistically significantly with histological type, tumor stage, lymph node metastasis or hormonal status

**TABLE 2** Clinicopathological features associated with E2F1 and E2F2 expression

	Total <i>n</i> = 183 (%)	E2F1 (+) <i>n</i> = 79 (%)	E2F1 (-) <i>n</i> = 104 (%)	<i>P</i>	E2F2 (+) <i>n</i> = 66 (%)	E2F2 (-) <i>n</i> = 117 (%)	<i>P</i>
Age (years)				0.088			0.001 <sup>a</sup>
<60	147 (80.3)	68 (46.3)	79 (53.7)		62 (42.2)	85 (57.8)	
$\geq 60$	36 (19.7)	11 (30.6)	25 (69.4)		4 (11.1)	32 (88.9)	
Histological type				0.370			0.145
IDC	163 (89.1)	73 (39.8)	90 (55.2)		56 (34.4)	107 (65.6)	
ILC	11 (6.0)	4 (36.4)	7 (63.6)		4 (36.4)	7 (63.6)	
Others	9 (4.9)	2 (22.2)	7 (77.8)		6 (66.7)	3 (33.3)	
Histological grade				0.040 <sup>a</sup>			0.687
Grade 1	34 (18.6)	20 (58.8)	14 (41.2)		12 (35.3)	22 (64.7)	
Grade 2	103 (56.3)	45 (43.7)	58 (56.3)		35 (34.0)	68 (66.0)	
Grade 3	46 (25.1)	14 (30.4)	32 (69.6)		19 (41.3)	27 (58.7)	
Stage				0.822			0.234
I-IIA	129 (70.5)	55 (42.6)	74 (57.4)		43 (33.3)	86 (66.7)	
IIB-III	54 (29.5)	24 (44.4)	30 (55.6)		23 (42.6)	31 (57.4)	
Tumor size (cm)				0.000 <sup>a</sup>			0.007 <sup>a</sup>
$\leq 2$	62 (33.9)	15 (24.2)	47 (75.8)		14 (22.6)	48 (77.4)	
>2	121 (66.1)	64 (52.9)	57 (47.1)		52 (43.0)	69 (57.0)	
Lymph nodes				0.916			0.514
Positive	61 (33.3)	26 (42.6)	35 (57.4)		24 (39.3)	37 (60.7)	
Negative	122 (66.7)	53 (43.4)	69 (56.6)		42 (34.4)	80 (65.6)	
Hormone receptor				0.099			0.907
ER (+) or PR (+)	62 (33.9)	32 (51.6)	30 (48.4)		22 (35.5)	40 (64.5)	
ER (-) and PR (-)	121 (66.1)	47 (38.8)	74 (61.2)		44 (36.4)	77 (63.6)	

IDC invasive ductal carcinoma, ILC invasive lobular carcinoma, ER estrogen receptor, PR progesterone receptor

<sup>a</sup> Statistically significant,  $P$  value <0.05

**TABLE 3** Associations among E2F1/E2F2 expression and hormone receptor status with disease recurrence

	Total <i>n</i> = 183 (%)	E2F1 (+) <i>n</i> = 79 (%)	E2F1 (-) <i>n</i> = 104 (%)	<i>P</i>	E2F2 (+) <i>n</i> = 66 (%)	E2F2 (-) <i>n</i> = 117 (%)	<i>P</i>	HR (+) <i>n</i> = 62 (%)	HR (-) <i>n</i> = 121 (%)	<i>P</i>
Local recurrence				0.039 <sup>a</sup>			0.900			0.432
Yes	16 (8.7)	3 (18.8)	13 (81.2)		6 (37.5)	10 (62.5)		4 (25)	12 (75)	
No	167 (91.3)	76 (45.5)	91 (54.5)		60 (35.9)	107 (64.1)		58 (34.7)	109 (65.3)	
LN metastasis at follow-up				0.005 <sup>a</sup>			0.248			0.001 <sup>a</sup>
Yes	36 (19.7)	8 (22.2)	28 (77.8)		10 (27.8)	26 (72.2)		4 (11.1)	32 (88.9)	
No	147 (80.3)	71 (48.3)	76 (51.7)		56 (38.1)	91 (61.9)		58 (39.5)	89 (60.5)	
Distant metastasis				0.001 <sup>a</sup>			0.305			0.470
Yes	38 (20.8)	7 (18.4)	31 (81.6)		11 (28.9)	27 (71.1)		11 (28.9)	27 (71.1)	
No	145 (79.2)	72 (49.7)	73 (50.3)		55 (37.9)	90 (62.1)		51 (35.2)	94 (64.8)	

LN lymph node, HR hormone receptor

<sup>a</sup> Statistically significant, *P* value <0.05

(*P* = 0.145, *P* = 0.234, *P* = 0.514, and *P* = 0.907, respectively) (Table 2).

#### *Correlation of E2F1 and E2F2 Expression and Hormone-Receptor Status with Disease Recurrence*

During follow-up, 16 patients (8.7%) had local recurrence, 36 (19.7%) had lymph node metastases during the postsurgery follow-up period, and 38 (20.8%) had distant metastases (Table 3). Interestingly, the E2F1-expression group had less local recurrence (*P* = 0.039), lymph node metastasis during the follow-up period (*P* = 0.005), and distant metastases (*P* = 0.001) than the E2F1-negative group.

However, E2F2 expression was not correlated with local recurrence, lymph node metastasis during the follow-up period, or distant metastasis (*P* = 0.900, *P* = 0.248, and *P* = 0.305, respectively).

The HRNBC patients experienced more lymph node metastasis during postsurgery follow-up, whereas the patients with HR-positive tumors had less lymph node metastasis (*P* = 0.001). Hormone receptor status was not statistically associated with local recurrence or distant metastasis (*P* = 0.432 and *P* = 0.470, respectively).

#### *Relationship of E2F1 and E2F2 Expressions and Hormone Receptor Status with Disease-Free and Overall Survival Rates on Univariate Analysis*

The variables tested at the univariate level included E2F1 and E2F2 expression and hormone receptor status (Table 4). Wilcoxon statistical survival curves were constructed to assess the prognostic significance of E2F1 and E2F2 expressions and HR negativity. The E2F1-positive group was significantly associated with better disease-free

survival compared with the E2F1-negative group: the patients in the E2F1-positive group had longer disease-free survival periods (median 120 months) than those in the E2F1-negative group (median 58 months) (*P* = 0.0014) (Figs. 1, 2a). The overall survival periods of patients in the E2F1-positive breast cancer group (median 120 months) were significantly better than those of patients in the E2F1-negative breast cancer group (median 89 months) (*P* = 0.0021) (Fig. 2b).

There was no statistically significant association between E2F2 expression and disease-free or overall survival (*p* = 0.1584 and *p* = 0.0872, respectively), whereas hormone-receptor-positive status was associated with better and longer disease-free and overall survival than in HRNBC patients (*P* = 0.0219 and *P* = 0.0022, respectively) (Figs. 2c, d).

#### *Correlation Between E2F1 and E2F2 Expression and Outcome of Adjuvant Chemotherapy on Multivariate Analyses*

Using backward stepwise Cox regression, E2F1 and E2F2 expressions were tested in terms of their influence on disease-free and overall survival in HRNBC patients (Table 5). E2F1 was the sole independent predictive factor for clinical outcome of hormone-receptor-negative patients treated with adjuvant chemotherapy. E2F1 expression was associated with more favorable disease-free survival and overall survival rates, compared with E2F1 negativity [*P* = 0.001, hazard ratio = 0.327, 95% confidence interval (95% CI) 0.179–0.598; and *P* = 0.001, hazard ratio = 0.273, 95% CI 0.137–0.547, respectively]. However, E2F2 expression did not have any effect on disease-free or overall survival (*P* = 0.923 and *P* = 0.671, respectively).

**TABLE 4** Univariate analyses of disease-free and overall survival

Parameters	Total, <i>n</i> (%) <i>n</i> = 183	Disease-free survival <i>P</i>	Overall survival <i>P</i>
E2F1		0.0014 <sup>a</sup>	0.0021 <sup>a</sup>
Positive	79 (43.2)		
Negative	104 (56.8)		
E2F2		0.1584	0.0872
Positive	66 (36.1)		
Negative	117 (63.9)		
Hormone receptor		0.0219 <sup>a</sup>	0.0022 <sup>a</sup>
Positive	62 (33.9)		
Negative	121 (66.1)		

<sup>a</sup> Statistically significant, *P* value <0.05

## DISCUSSION

The main prognostic factors associated with breast cancer are the number of lymph nodes involved, tumor size, histological grade, and hormone receptor status.<sup>17</sup> However, after determining the stage, histological grade, and hormone receptor status, the tumor can behave in an unexpected manner, and the prognosis can therefore be changed.<sup>17</sup> Other prognostic and predictive factors have been studied in an effort to explain this phenomenon; we found that E2F1 could be used as a predictive factor for patient survival outcome following adjuvant chemotherapy. The promise of this predictive factor is especially significant for HRNBC patients, because they have few or no adjuvant systemic treatments other than chemotherapy.

In the E2F family, only E2F1 seems to be involved in a dual promoting and apoptotic effect.<sup>11,12</sup> In normal cellular physiology, E2F1 modulates diverse cellular functions such as DNA synthesis, mitosis, and apoptosis.<sup>10–12</sup> In tumorigenesis, E2F1 acts either as an oncogene or as a tumor suppressor gene, depending on the tumor type and predominant signal.<sup>10–12</sup> Overexpression of E2F1 is significantly associated with higher tumor proliferation and

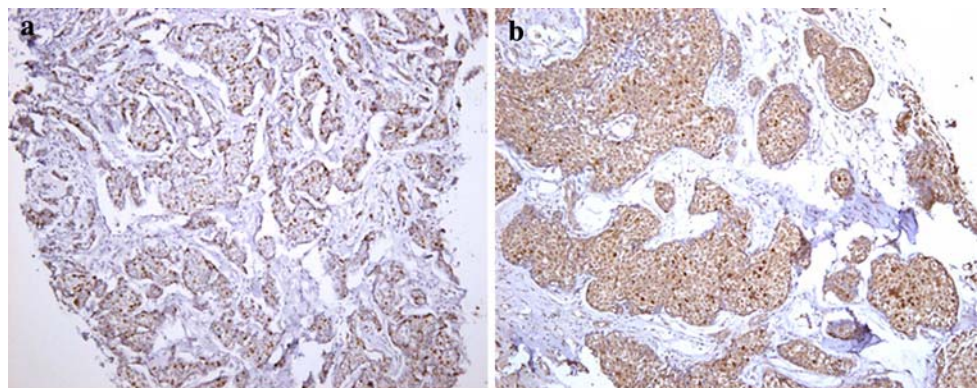
greater invasive ability. In previous studies, E2F1 has been shown to act as an oncogene, and it has a tumor-promoting effect in small cell lung cancer and breast, thyroid, and ovarian epithelial cancers.<sup>17–20</sup> On the other hand, its tumor-suppression effect has been studied in colon and bladder cancers.<sup>21,22</sup>

In an earlier study, E2F1 was thought to act as a tumor suppressor in breast cancer, and its downregulation was considered likely to be associated with the metastatic process.<sup>23</sup> With advances in molecular methods, many studies have since demonstrated that E2F1 acts as an oncogene in breast cancer.<sup>24</sup> In our study, E2F1 expression increased with tumor size; this explained the fact that E2F1 displays proliferation activity in the tumorigenesis of breast cancer.

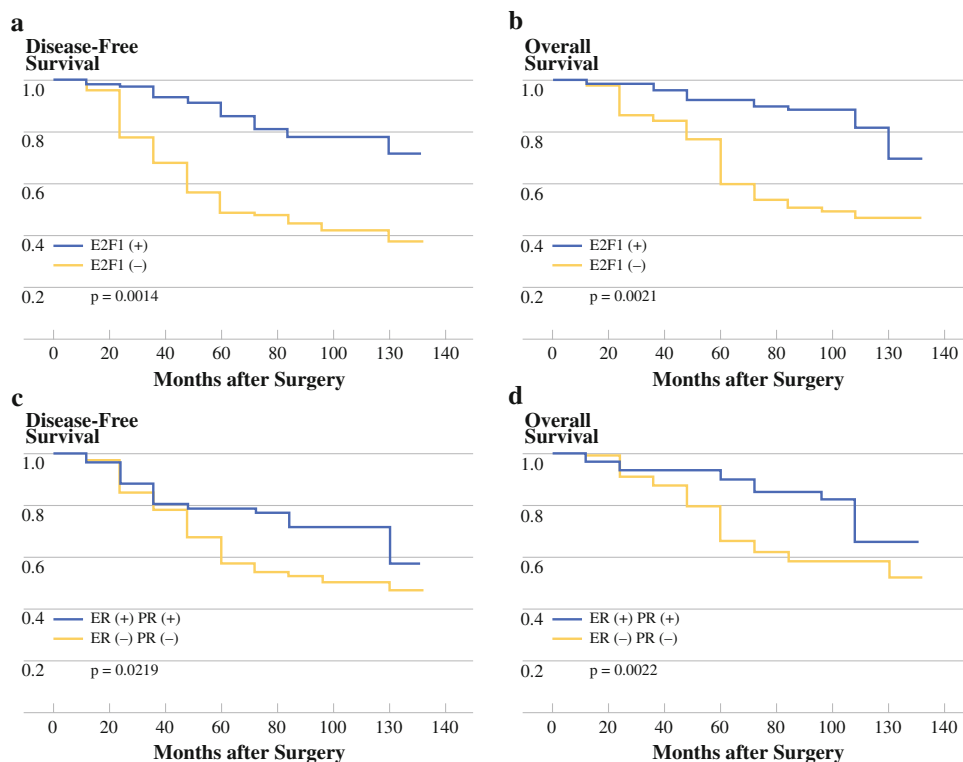
E2F1 expression has been associated with chemosensitivity in several kinds of tumors, e.g., fibrosarcoma, pancreatic cancer, and gastric cancer, and it would be expected that E2F1-expressed tumors would respond to chemotherapy better than E2F1-negative tumors.<sup>13,25,26</sup> Also the patients with E2F1-expressed tumors showed better survival or therapeutic outcomes, suggesting that the apoptotic effect of E2F1 and chemotherapy produce a synergistic effect against tumors.<sup>13</sup> The current study also found that the E2F1-expressed group experienced less disease recurrence during the postsurgery follow-up period and enjoyed more favorable prognoses than those with E2F1-negative tumors. In multivariate analyses, E2F1 expression was an independent factor predicting favorable survival outcomes among HRNBC patients treated with adjuvant chemotherapy.

Previous studies related to E2F1 expression and patient survival after chemotherapy showed controversial results. Lee et al.<sup>13</sup> revealed that E2F1 immunopositivity predicted a more favorable survival rate in a study of 467 gastric cancer patients treated with adjuvant chemotherapy. Han et al.<sup>15</sup> also presented data proving poor survival rates among 165 low-E2F1-expression patients with lymph-node-positive breast cancer undergoing adjuvant chemotherapy. However, they did not differentiate the effect of

**FIG. 1** E2F1 and E2F2 expression in breast cancer. **a** Immunohistochemical staining for E2F1 shows nuclear positivity in invasive ductal carcinoma of breast. **b** Immunohistochemical staining for E2F2 shows cytoplasmic positivity and a few positive nuclei in invasive ductal carcinoma of breast



**FIG. 2** Correlation of E2F1 expression and hormone receptor status with disease-free and overall survival. Disease-free survival (a) and overall survival (b) curves for the E2F1-positive group showed longer survival and more favorable clinical outcomes than for the E2F1-negative group. Disease-free survival (c) and overall survival (d) curves for the group with either hormone receptor positive showed longer survival and more favorable clinical outcomes than the group with both hormone receptors negative



**TABLE 5** Multivariate analyses of disease-free and overall survival of patients with hormone receptor-negative breast cancer

	Disease-free survival			<i>P</i>	Overall survival			<i>P</i>
	Hazard ratio	95% CI			Hazard ratio	95% CI		
		Lower	Upper			Lower	Upper	
E2F1	0.327	0.179	0.598	0.001	0.273	0.137	0.547	0.001
E2F2	0.972	0.546	1.730	0.923	0.875	0.473	1.620	0.671

*CI* confidence interval

E2F1 on HRNBCs from that on hormone-receptor-positive tumors. In the current study, the correlation of E2F1 with large tumor size is considered to be caused by the proliferative activity of E2F1 as an oncogene; meanwhile, the action of E2F1 as a predictor of favorable survival outcome after chemotherapy leads to the hypothesis that E2F1-expressing tumor cells are more sensitive to chemotherapy and increase apoptotic activity. These dual proliferative and apoptotic activities of E2F1 were consistent with the findings of previous *in vitro* and *in vivo* studies.<sup>27</sup>

E2F2 expression was more predominant among young patients and those with large tumors. Unlike E2F1, E2F2 did not predict tumor recurrence or survival outcome. E2F2 is one of the activators of the E2F family, and it is associated with tumor progression in breast cancer; however, E2F2 is known not to have an apoptotic capacity.<sup>28</sup> Interestingly, in the current study, E2F2 was related to age. The tumors arising in young patients (i.e., <60 years) expressed

more E2F2. This E2F2–age correlation is inexplicable; we had presumed that E2F2 would be related to tumor progression among younger patients. Because there have been few studies on the effects of E2F2, further investigation of the role of E2F2 in breast cancer is needed.

In conclusion, we recommend E2F1 as a potential prognostic and predictive factor that predicts clinical outcome and therapeutic results following adjuvant chemotherapy in HRNBC patients. Further large cohort studies into E2F1 and chemosensitivity are required with respect to HRNBCs.

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## REFERENCES

1. Lee JH, Yim SH, Won YJ, Jung KW, Son BH, Lee HD, et al. Population-based breast cancer statistics in Korea during 1993–

- 2002: incidence, mortality, and survival. *J Korean Med Sci.* 2007;22:S11–6.
2. Argarwal G, Pradeep PV, Aggarwal V, Yip CH, Ceung P. Spectrum of breast cancer in Asian women. *World J Surg.* 2007;31:1031–40.
  3. Greenberg PA, Hortobagyi GN, Smith TL, et al. Long-term follow-up of patients with complete emission following combination chemotherapy for breast cancer. *J Clin Oncol.* 1996;14:2197–205.
  4. Chopra R. The Indian scene. *J Clin Oncol.* 2001;19:(18)106–11.
  5. Khan SA, Rogers MA, Khurana KK, Mequid MM, Numann PJ. Estrogen receptor expression in benign breast epithelium and breast cancer risk. *J Natl Cancer Inst.* 1998;90:37–42.
  6. Kushlinskii NE, Gershtein ES. Modern prospects for molecular-biochemical methods for evaluating biological “behavior” of breast neoplasms. *Vestn Ros Akad Med Nauk.* 2001;9:65–70.
  7. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years’ adjuvant treatment for breast cancer. *Lancet.* 2005;365:60–2.
  8. Winer EP, Hudis C, Burstein HJ, Wolff AC, Pritchard KI, Ingle JN, et al. American society of clinical oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer: status report 2004. *J Clin Oncol.* 2005;23:619–29.
  9. Sherr CJ. Cancer cell cycles. *Science.* 1996;274:1672–7.
  10. Stevens C, La Thangue NB. E2F and cell cycle control: a double-edged sword. *Arch Biochem Biophys.* 2003;412:157–69.
  11. Attwooll C, Lazzarini Denchi E, Helin K. The E2F family: specific functions and overlapping interests. *EMBO J.* 2004;23:4709–16.
  12. La Thangue NB. The yin and yang of E2F1: balancing life and death. *Nat Cell Biol.* 2003;5:587–9.
  13. Lee JY, Park CK, Park JO, Lim T, Park YS, Lim HY, et al. Impact of E2F1 expression on clinical outcome of gastric adenocarcinoma patients with adjuvant chemoradiation therapy. *Clin Cancer Res.* 2008;14:82–8.
  14. Belvedere O, Puglisi F, Di Loreto C, Cataldi P, Guglielmi A, Aschele C, et al. Lack of correlation between immunohistochemical expression of E2F1, thymidylate synthase expression and clinical response to 5-fluorouracil in advanced colorectal cancer. *Ann Oncol.* 2004;15:55–8.
  15. Han SH, Park KM, Bae BN, Kim KH, Kim HJ, Kim YD, et al. E2F1 expression is related with the poor survival of lymph node-positive breast cancer patients treated with fluorouracil, doxorubicin and cyclophosphamide. *Breast Cancer Res Treat.* 2003;82:11–6.
  16. Kim EY, Jo HJ, Lee MJ. Expressions of E2F4 and E2F2 transcription factors in breast carcinoma. *Korean J Pathol.* 2005;39:301–6.
  17. Saiz AD, Olvera M, Rezk S, Florentine BA, McCourty A, Brynes RK. Immunohistochemical expression of cyclin D1, E2F1, and Ki-67 in benign and malignant thyroid lesions. *J Pathol.* 2002;198:157–62.
  18. Eymis B, Gazzeri S, Brambilla C, Brambilla E. Distinct pattern of E2F1 expression in human lung tumours: E2F1 is up-regulated in small cell lung carcinoma. *Oncogene.* 2001;20:1678–87.
  19. Zhang SY, Liu SC, Al-Saleem LF, Holloran D, Babb J, Guo X, et al. E2F1: a proliferative marker of breast neoplasia. *Cancer Epidemiol Biomarkers Prev.* 2000;9:395–401.
  20. Kim JY, Kim HW, Choi KU, Lee CH, Sol MY, Kang HJ, et al. Alteration of G1/S cell cycle regulatory proteins in ovarian epithelial tumors. *Korean J Pathol.* 2006;40:274–81.
  21. Zacharators P, Kotsinas A, Evangelou K, Karakaidos P, Vassiliou LV, Rezaei N, et al. Distinct expression patterns of the transcription factor E2F1 in relation to tumour growth parameters in common human carcinomas. *J Pathol.* 2004;203:744–53.
  22. Rabbani F, Richon VM, Orlov I, Lu ML, Drobnyak M, Dudas M, et al. Prognostic significance of transcription factor E2F-1 in bladder cancer: genotypic and phenotypic characterization. *J Natl Cancer Inst.* 1999;91:874–81.
  23. Ho GH, Calvano JE, Bisogna M, Van Zee KJ. Expression of E2F1 and E2F4 is reduced in primary and metastatic breast carcinomas. *Breast Cancer Res Treat.* 2001;69:115–22.
  24. Verlinden L, Vanden Bempt I, Eelen G, Drijkoningen M, Verlinden I, Marchal K, et al. The E2F-regulated gene Chk1 is highly expressed in triple-negative estrogen receptor-/progesterone receptor-/HER2- breast carcinomas. *Cancer Res.* 2007;67:6574–81.
  25. Pruschy M, Wirbelauer C, Glanzmann C, Bodis S, Krek W. E2F1 has properties of a radiosensitizer and its regulation by cyclin A kinase is required for cell survival of fibrosarcoma cells lacking p53. *Cell Growth Differ.* 1999;10:141–6.
  26. Elliott MJ, Farmer MR, Atienza C Jr, Stilwell A, Dong YB, Yang HL, et al. E2F1 gene therapy induces apoptosis and increases chemosensitivity in human pancreatic carcinoma cells. *Tumour Biol.* 2002;23(2):76–86.
  27. Yamasaki L. Balancing proliferation and apoptosis in vivo: the goldilocks theory of E2F/DP action. *Biochim Biophys Acta.* 1999;1423:M9–15.
  28. Yamasaki L, Jacks T, Bronson R, Goillot E, Harlow E, Dyson NJ. Tumor induction and tumor atrophy in mice lacking E2F1. *Cell.* 1996;85:537–48.