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Methylated *NEUROD1* Promoter is a Marker for Chemosensitivity in Breast Cancer

Heidi Fiegl,[1,2] Allison Jones,[1] Cornelia Hauser-Kronberger,[6] Georg Hutarew,[6] Roland Reitsamer,[7] Robin L. Jones,[8] Mitch Dowsett,[8] Elisabeth Mueller-Holzner,[3] Gudrun Windbichler,[3] Günter Daxenbichler,[3] Georg Goebel,[4] Christian Ensinger,[5] Ian Jacobs,[1] and Martin Widschwendter[1]

Abstract

Purpose: Chemotherapy can be an integral component of the adjuvant management strategy for women with early stage breast cancer. To date, no tool is available to predict or monitor the efficacy of these therapies. The aim of this proof-of-principle study was to assess whether *NEUROD1* DNA methylation is able to predict the response to neoadjuvant and adjuvant chemotherapy.

Experimental Design: Recently, we showed that *NEUROD1* DNA is differentially methylated in neoplastic versus nonneoplastic breast tissue samples. In this study, we used MethyLight and analyzed *NEUROD1* methylation in (*a*) 74 breast cancer tissue samples, (*b*) two independent sets of pretreatment core biopsies of 23 (training set) and 21 (test set) neoadjuvantly treated breast cancer patients, and (*c*) pretherapeutic and posttherapeutic serum samples from 107 breast cancer patients treated with adjuvant chemotherapy.

Results: High-grade tumors showed higher *NEUROD1* methylation levels. Estrogen receptor–negative breast cancers with high *NEUROD1* methylation were 10.8-fold more likely to respond with a complete pathologic response following neoadjuvant chemotherapy. Patients with positive serum pretreatment *NEUROD1* methylation, which persisted after chemotherapy, indicated poor relapse-free and overall survival in univariate and multivariate analyses (relative risk for relapse, 6.2; 95% confidence interval, 1.6-24; $P = 0.008$, and relative risk for death, 14; 95% confidence interval, 1.6-120; $P = 0.02$).

Conclusions: These data support the view that *NEUROD1* methylation is a chemosensitivity marker in estrogen receptor–negative breast cancer.

Breast cancer is the most frequent malignancy among women in the industrialized world. To date, the presence or absence of metastatic involvement in the axillary lymph nodes is still the most powerful prognostic factor available for patients with primary breast cancer (1), although this is just an indirect measure reflecting the tendency of the tumor to spread. Recently, we showed that *RASSF1A* DNA methylation in serum is a marker of poor prognosis in women with breast cancer (2) and that this cancer-specific DNA alteration allows the monitoring of adjuvant tamoxifen therapy (3), which is applied mainly in estrogen receptor (ER)–positive tumors. To date, no tool is available to sufficiently predict or monitor the efficacy of neoadjuvant or adjuvant systemic chemotherapy which is frequently applied in ER-negative breast cancer.

We recently reported that stem cell polycomb group targets (PCGT) are up to 12-fold more likely to have cancer-specific promoter DNA hypermethylation compared with nontargets (4). This supports the idea of a stem cell origin of cancer whereby reversible gene repression is replaced by permanent silencing, forcing the cell into a perpetual state of self-renewal and therefore increasing the possibility for subsequent malignant transformation (4). A large number of *PCGT* genes have not yet been described to play a role in cancer and this could explain why non–tumor suppressor genes are found to be frequently hypermethylated in adult epithelial cancers. In a recent study, we analyzed the methylation status of 61 genes in breast cancer and nonneoplastic breast tissues of 15 patients and 15 healthy controls, respectively. *NEUROD1* DNA methylation was the best discriminator between these different groups (4). In this proof-of-principle study, we focused on the role of *NEUROD1* methylation in breast

Authors' Affiliations: [1]Department of Gynecological Oncology, UCL Institute for Women's Health, University College London, London, United Kingdom; [2]Tyrolean Cancer Research Institute, Departments of [3]Gynecology, [4]Medical Statistics, Informatics, and Health Economics, and [5]Pathology, Medical University, Innsbruck, Austria; Departments of [6]Pathology and [7]Gynecology, General Hospital and Paracelsus University Salzburg, Salzburg, Austria; and [8]Department of Medicine, Royal Marsden Hospital, London, United Kingdom

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Requests for reprints: Martin Widschwendter, Department of Gynecological Oncology, Institute for Women's Health, University College London, EGA Hospital, 2nd Floor Huntley Street, London WC1E 6DH, United Kingdom. Phone: 44-20-7380-9140; Fax: 44-20-7380-9033; E-mail: M.Widschwendter@ucl.ac.uk.

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Table 1. Association of *NEUROD1* methylation in 74 primary breast cancer patients with clinicopathologic features

		n	*NEUROD1* methylation values (percentage of methylated reference)		*P*
			Median	25th; 75th percentile	
Size	T_1	14	19	0.7;52	0.6
	$T_{2/3/4}$	60	26	5.4;61	
LN	Negative	23	18	3.2;48	0.8
	Positive	46	26	7.0;65	
	NA	5			
Grade	Grade 1	31	13	3.2;37	0.03
	Grade 2/3	41	34	8.9;75	
	NA	2			
MP	Premenopausal	18	24	5.6;39	0.8
	Postmenopausal	56	19	4.5;63	
ER	Negative	27	25	6.9;40	1.0
	Positive	47	18	3.6;71	
PR	Negative	31	25	3.6;52	0.7
	Positive	43	19	6.9;75	
HER2	Score 0/+	49	16	4.0;54	0.1
	Score ++/+++	23	34	13;62	
	NA	2			

Abbreviations: LN, lymph node status; MP, menopausal status; ER, estrogen receptor status; PR, progesterone receptor; NA, not available.

cancer biology. We analyzed tumor samples, pretreatment core biopsies, and pretherapeutic and posttherapeutic serum samples by means of MethyLight, a sensitive fluorescence-based real-time PCR technique (5). We found that *NEUROD1* methylation is a marker for chemosensitivity in breast cancer.

Materials and Methods

Patients and samples. The following samples were analyzed:

1. Frozen breast tissue samples from 74 patients with breast cancer. All samples were collected during surgery at the Department of Obstetrics and Gynecology of the Innsbruck Medical University, Austria in compliance with and approved by the Institutional Review Board. Breast cancer specimens were obtained immediately after resection of the breast or lumpectomy. Specimens were brought to our pathologist, and a part of the tissue was pulverized under cooling with liquid nitrogen and stored at -70°C. Patients were 35 to 90 years old (mean age at diagnosis, 62 years). Other clinicopathologic features are shown in Table 1.
2. Paraffin-embedded pretreatment core biopsies (formalin-fixed 16-gauge cores) from patients with breast cancer. Samples were obtained from the Departments of Pathology and Gynecology, General Hospital and Paracelsus University Salzburg (training set samples); the Department of Obstetrics and Gynecology, Medical University Innsbruck, Austria; and the Royal Marsden Hospital, London, United Kingdom (test set samples). All samples were collected at diagnosis prior to chemotherapy in compliance with and approved by the Institutional Review Boards. In the training set, we analyzed samples from 23 patients who received six cycles of anthracycline-based therapy. Twenty-one of 23 samples yielded sufficient amounts of DNA. Seven of 21 patients showed a complete pathologic response (CR; disappearance of the invasive cancer in the breast). Clinicopathologic features are shown in Table 2A. For further evaluation, we analyzed samples from an independent test set from 21 patients. One patient received three cycles of a combination of cyclophosphamide, methotrexate, and 5-fluorouracil, 10 patients received four cycles, 9 patients received six cycles, and 1 patient received three cycles of an anthracycline-based therapy. Clinicopathologic features are shown in Table 2B.
3. Pretherapeutic and posttherapeutic serum samples from 107 patients with breast cancer, treated at the Department of Gynecology and Obstetrics, Medical University Innsbruck, Innsbruck, Austria, with primary non–metastatic breast cancer. Serum samples were recruited from all patients diagnosed with breast cancer between September 1992 and February 2002 who met all the following criteria: (*a*) primary breast cancer without metastasis at diagnosis, (*b*) adjuvant treatment with chemotherapy (41 patients received an anthracycline-based therapy, 64 patients received a combination of cyclophosphamide, methotrexate, and 5-fluorouracil, and 2 patients received another kind of chemotherapy), (*c*) availability of serum samples at diagnosis and 1 year after treatment (a time when the patient has completed her chemotherapy), and (*d*) alive after 1 year. Hormone receptor status was determined by either radioligand binding assay or immunohistochemistry. Clinicopathologic features are shown in Table 3. Patients' blood samples were drawn before or 1 year after therapeutic intervention. Blood was centrifuged at 2,000 × *g* for 10 min at room temperature and 1 mL aliquots of serum samples were stored at -30°C.

DNA isolation, bisulfite modification, and MethyLight analysis. Genomic DNA from fresh-frozen tissue samples or paraffin-embedded tissue samples were isolated using the DNeasy Tissue Kit (Qiagen) according to the manufacturer's protocol.

DNA isolation from serum samples, bisulfite modification, and MethyLight analysis was done as described previously (2). Briefly, two sets of primers and probes, designed specifically for bisulfite-converted DNA, were used; a methylated set for the gene of interest and a reference set, actin β (*ACTB*), to normalize for input DNA. The specificity of the reactions for methylated DNA was confirmed separately using *Sss*I-treated (New England Biolabs) human WBC

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DNA (heavily methylated). The percentage of fully methylated molecules at a specific locus was calculated by dividing the GENE/ACTB ratio of a sample by the GENE/ACTB ratio of *Sss*I-treated human WBC DNA and multiplying by 100. Primers and probes for *NEUROD1* have been described recently (6).

Statistics. Descriptive analysis of obtained data was done and the median as well as interquartile ranges were given. Data from parametric distributed variables were shown as mean and SD. Differences in the percentage of methylated reference values between groups were analyzed by means of a two-sided Mann-Whitney *U* test. Survival analysis was done by using univariate Kaplan-Meier curves and Cox regression models. All statistical analyses were done with SPSS Software 10.0.

Results

Based on our recent study, *NEUROD1* methylation is the best discriminator between breast cancer and nonneoplastic tissue samples (ref. 4; Supplementary Table S1). To further explore the role of *NEUROD1* methylation in primary breast cancer, in this study, we first analyzed *NEUROD1* methylation in 74 frozen primary breast cancer specimens. High-grade tumors showed higher *NEUROD1* methylation levels ($P = 0.03$), whereas no other clinicopathologic feature was associated with *NEUROD1* methylation (Table 1). The promoter of *NEUROD1* is occupied by repressive regulators in human embryonic stem cells (7), which would be consistent with *NEUROD1* DNA methylation marking cancer stem cells in the tumor. Although there is a highly significant increase in *NEUROD1* methylation from nonneoplastic to breast cancer tissue (Supplementary Table S1), with higher levels in high-grade tumors (Table 1), surprisingly, *NEUROD1* methylation in breast cancer is not an indicator of tumor aggressiveness, which is shown in a lack of association of *NEUROD1* methylation and lymph node metastasis (Table 1) or survival (Table 4). This rather surprising finding led us to the hypothesis that *NEUROD1* methylation may be associated with other tumor features like responsiveness to systemic treatment in breast cancer.

To test this hypothesis, we used two *in vivo* experiments: *NEUROD1* methylation analysis in core breast cancer biopsies taken prior to preoperative chemotherapy with complete pathologic response as the end point (model 1) and seroconversion of *NEUROD1* methylation in serum DNA during adjuvant chemotherapy with survival as the end point (model 2). For model 1, we first analyzed DNA from pretreatment core biopsies from 23 patients with breast cancer (training set). Twenty-one of 23 samples yielded sufficient DNA and 7 of 21 patients showed a CR (Table 2A). Patients with a CR showed significantly higher *NEUROD1* methylation levels in their pretreatment cancer cores (Fig. 1A). To exclude

Table 2. Characteristics of patients with neoadjuvantly treated primary breast cancer

(A) Clinicopathologic features of the training set		***n***
Age, y (±SD)	46.9 (±10.1)	
Histologic type	Invasive ductal	17
	Invasive lobular	4
ER	Negative	9
	Positive	12
HER2	Score 0/+	15
	Score ++/+++	5
	NA	1
Pathologic response	Partial response	14
	Complete response	7
Percentage of tumor cells in sample (±SD)	51 (±24.6)	
Type of chemotherapy	Anthracyclines	21
Cycle number of chemotherapy	6	21
(B) Clinicopathologic features of the test set		***n***
Age, y (±SD)	50 (±10.3)	
Histologic type	Invasive ductal	18
	Other	3
ER	Negative	21
	Positive	0
HER2	Score 0/+	11
	Score ++/+++	10
Pathologic response	Partial response	10
	Complete response	11
Type of chemotherapy	Anthracyclines	20
	Cyclophosphamide, methotrexate, fluorouracil	1
Number of chemotherapy cycles	3	2
	4	10
	6	9

NOTE: Core biopsy samples of the (A) training and (B) test sets.

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Table 3. Characteristics of patients with adjuvantly treated primary non–metastatic breast cancer

(A) Clinicopathologic features		***n***
Age at diagnosis (y)	55.5	
SD	11.3	
Size	T_1	40
	$T_{2/3/4}$	66
	NA	1
LN	Negative	27
	Positive	78
	NA	2
Grade	Grade 1	16
	Grade 2/3	89
	NA	2
MP	Premenopausal	38
	Postmenopausal	69
ER	Negative	57
	Positive	50
PR	Negative	55
	Positive	52
OP-Mode	TE	38
	ME	68
	NA	1
Endocrine therapy	No	55
	Tamoxifen	52
Radiation therapy	No	44
	Yes	63
Type of chemotherapy	Anthracyclines	41
	Cyclophosphamide, methotrexate, fluorouracil	64
	Others	2

(B) Clinicopathologic features		***n***
Age at diagnosis (y)	57.6	
SD	10.7	
Size	T_1	9
	$T_{2/3/4}$	11
	NA	1
LN	Negative	5
	Positive	15
	NA	1
Grade	Grade 1	4
	Grade 2/3	17
MP	Premenopausal	3
	Postmenopausal	18
PR	Negative	18
	Positive	3
OP-Mode	TE	6
	ME	14
	NA	1
Endocrine therapy	No	18
	Tamoxifen	3
Radiation therapy	No	7
	Yes	14
Type of chemotherapy	Anthracyclines	7
	Cyclophosphamide, methotrexate, fluorouracil	14

NOTE: Serum samples from all patients (A) and from 21 ER-negative patients (B) with positive NEUROD1 methylation in pretreatment serum. Abbreviations: LN, lymph node status; MP, menopausal status; ER, estrogen receptor status; PR, progesterone receptor status; NA, not available; ME, mastectomy; TE, tumorectomy.

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Table 4. Univariate survival analysis of 74 patients with primary breast cancer

(A) Overall survival

		No. of patients (died/total)	RR of death (95% CI)	*P*
Size	T_1	4/14	1.8 (0.6-5.2)	0.3
	$T_{2/3/4}$	26/60		
LN	Negative	6/23	2 (0.8-5.1)	0.1
	Positive	21/46		
Grade	Grade 1	14/31	0.9 (0.4-1.8)	0.7
	Grade 2/3	16/41		
MP	Premenopausal	6/18	1.5 (0.6-3.7)	0.4
	Postmenopausal	24/56		
HR	Negative	7/24	1.7 (0.7-4.0)	0.2
	Positive	23/50		
Chemotherapy	No	16/38	0.9 (0.5-1.9)	0.8
	Yes	14/36		
Endocrine therapy	No	10/28	1.4 (0.7-3.0)	0.4
	Tamoxifen	20/46		
Radiation therapy	No	12/29	0.7 (0.4-1.6)	0.4
	Yes	18/45		
NEUROD1	Low methylation	16/37	0.8 (0.4-1.7)	0.6
	High methylation	14/37		

(B) Relapse-free survival

		No. of patients (relapsed/total)	RR of relapse (95% CI)	*P*
Size	T_1	3/14	1.7 (0.5-5.7)	0.4
	$T_{2/3/4}$	18/60		
LN	Negative	2/23	5.7 (1.3-24.4)	0.02
	Positive	19/46		
Grade	Grade 1	8/31	1.1 (0.5-2.8)	0.8
	Grade 2/3	13/41		
MP	Premenopausal	6/18	1.0 (0.40-2.6)	1.0
	Postmenopausal	15/56		
HR	Negative	5/24	1.5 (0.5-4.0)	0.5
	Positive	16/50		
Chemotherapy	No	4/38	4.0 (1.3-11.8)	0.01
	Yes	17/36		
Endocrine therapy	No	5/28	1.9 (0.7-5.2)	0.2
	Tamoxifen	16/46		
Radiation therapy	No	5/29	1.3 (0.5-3.6)	0.6
	Yes	16/45		
NEUROD1	Low methylation	10/37	0.8 (0.3-1.8)	0.6
	High methylation	11/37		

Abbreviations: RR, relative risk; LN, lymph node status; MP, menopausal status; HR, hormone receptor status.

the possibility that this association was merely a reflection of cellularity in the core, we adjusted for the percentage of tumor cells (reviewed by G. Hutarew, a pathologist who was blinded for the chemotherapy response) and still observed a significant ($P = 0.006$) association between pretreatment core *NEUROD1* methylation and response to neoadjuvant chemotherapy.

As ER-negative tumors are more likely to respond to neoadjuvant chemotherapy (8–10), we analyzed the association of CR and *NEUROD1* methylation separately in ER-negative and ER-positive tumor samples. Although the numbers are small, the association between *NEUROD1* methylation and response to neoadjuvant chemotherapy was retained in ER-negative cancers (Mann-Whitney *U* test; $P = 0.02$; Fig. 1B).

In order to validate these finding and to calculate the predictive potential of *NEUROD1* methylation, we analyzed an independent test set of 21 core biopsies taken prior to the start of neoadjuvant chemotherapy from patients with ER-negative breast cancer (Table 2B). We classified *NEUROD1* methylation as low ($n = 11$) or high ($n = 10$) using the median percentage of methylated reference value (PMR = 2.18) as the cutoff. Eight of 10 (80%) women with high *NEUROD1* methylation and 3 of

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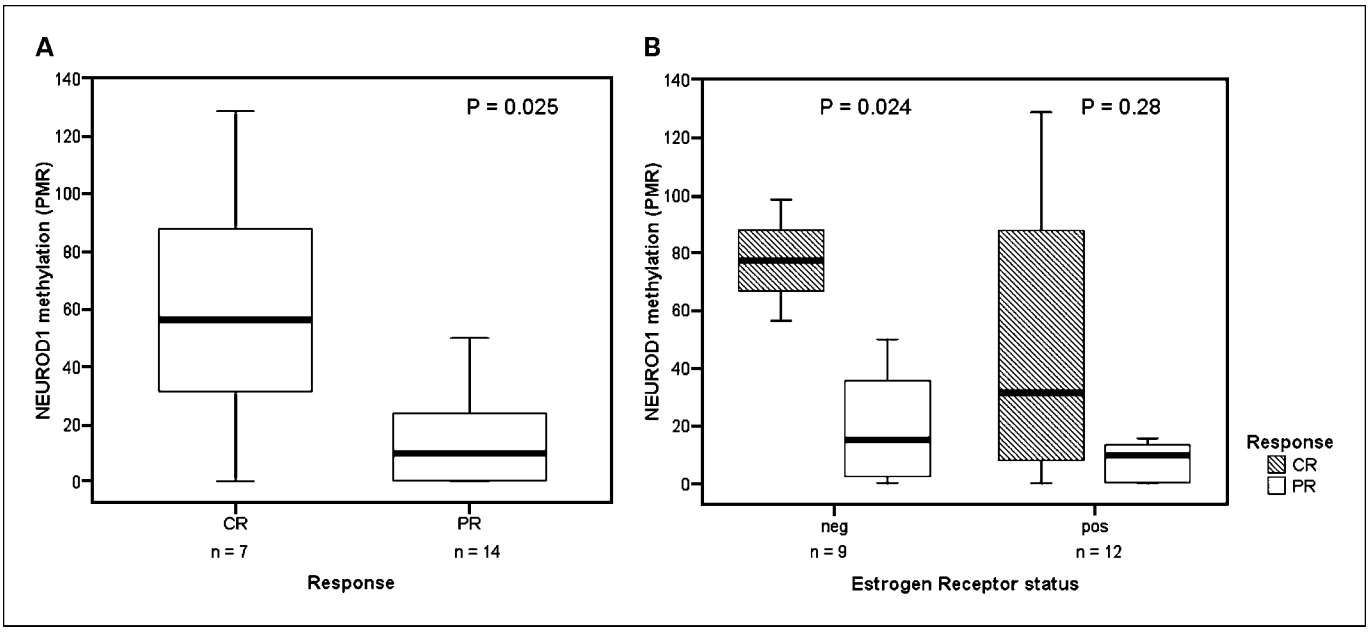

Fig. 1. *NEUROD1* DNA methylation in the pretreatment breast cancer core biopsies of the training set. *A,* samples stratified by response (*PMR*, percentage of methylated reference; *CR,* complete pathologic response; *PR,* partial response; Mann-Whitney *U* test, $P = 0.025$). *B,* samples stratified by ER status (Mann-Whitney *U* test, $P = 0.024$ for ER-negative samples, $P = 0.28$ for ER-positive samples).

11 (27%) women with low *NEUROD1* methylation in their core biopsy had a CR. Using a logistic regression model, and adjusting for age and HER2 status, high *NEUROD1* methylation in ER-negative pretreatment breast cancer biopsies was associated with a 10.8-fold increased likelihood for a CR following neoadjuvant chemotherapy [95% confidence interval (95% CI), 1.1-106.4; $P = 0.042$]. This means that *NEUROD1* methylation had a sensitivity of 80% (44.4-96.3) and a specificity of 72% (39.0-92.0) to predict complete pathologic response in women treated with neoadjuvant chemotherapy. In our second model, we assessed whether serum *NEUROD1* methylation was able to predict the response to adjuvant chemotherapy in patients with primary breast cancer. In previous articles, we have shown that DNA methylation of specific genes in circulating serum DNA is a marker for poor prognosis (2) and a tool to monitor adjuvant tamoxifen treatment (3). If our hypothesis is true that *NEUROD1* methylation is a marker for chemosensitivity in breast cancer, we would expect that women whose serum *NEUROD1* methylation was positive before but not detectable after adjuvant chemotherapy have an improved relapse-free and overall survival as their chemosensitive tumor cells have been eliminated. We identified 107 patients who received adjuvant chemotherapy due to primary non–metastatic breast cancer and from whom we have stored both pretreatment and postchemotherapy serum samples. The characteristics of these patients are shown in Table 3A. Pretreatment *NEUROD1* serum DNA methylation was more prevalent in postmenopausal women, whereas no difference in any of the other clinicopathologic features could be observed (data not shown). In the group of 21 ER-negative patients with positive pretreatment *NEUROD1* methylation in their serum, the persistence of *NEUROD1* DNA methylation after chemotherapy indicated poor overall and relapse-free survival in the univariate analysis (Fig. 2; Table 5). The characteristics of these patients are shown in Table 3B. Using a Cox multiple-regression analysis which included tumor size, grade, lymph node metastasis, and menopausal status, the persistence of methylated *NEUROD1* serum DNA was the only predictor of poor outcome (relative risk for relapse, 6.2; 95% CI, 1.6-24; $P = 0.008$; relative risk for death, 14; 95% CI, 1.6-120; $P = 0.02$). No association between serum *NEUROD1* DNA methylation and response to adjuvant chemotherapy could be observed for patients with ER-positive breast cancer (data not shown).

Discussion

Neoadjuvant chemotherapy has been widely used prior to surgery for locally advanced breast cancer (11, 12). Response to this kind of therapy has been shown to be a valid surrogate marker of survival and facilitates breast-conserving surgery (13–15). However, current clinical and pathologic markers poorly predict response to neoadjuvant chemotherapy. In our study, ER-negative breast cancers with high *NEUROD1* methylation were more likely to respond with a complete pathologic response following neoadjuvant chemotherapy.

Predictive factors in adjuvant breast cancer therapy are limited to ER, progesterone receptor, and HER-2/neu. These markers are used to predict response to hormonal treatment and herceptin, respectively (16, 17). Recently, HER-2/neu in serum was shown to be a significant predictor of response to neoadjuvant anthracycline-based chemotherapy for breast cancer, whereas the HER-2/neu status of tumor tissue did not correlate with response to treatment (18). Furthermore, HER-2/neu overexpression was identified as a major prognostic factor in patients with stage II and III breast cancer treated with a neoadjuvant docetaxel and epirubicin combination (19). Despite these findings, a more extensive range of predictive markers is highly needed in order to extend the

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range of individualized therapies for patients with breast cancer.

The biological characteristics of circulating tumor cells are poorly understood despite their potential contribution towards the formation of distant metastases. Up until recently, only a limited number of reports examined the occurrence of circulating tumor cells in the context of systemic therapy for primary or metastatic breast cancer. It has been shown that circulating tumor cells are present in a substantial fraction of breast cancer patients undergoing systemic therapy (20). These circulating tumor cells are usually nonproliferative, and a fraction of these cells seem to be resistant to chemotherapy (20). Only very limited data is available regarding specific characterization of these circulating tumor cells. In our proof-of-principle study, we described *NEUROD1* methylation, a marker which may act as a surrogate for breast cancer cells which are responsive to chemotherapy. Expression of cyclooxygenase-2 has recently been shown to be a marker of doxorubicin-resistant breast cancer (21). In addition, inhibitors of cyclooxygenase-2 increase doxorubicin-induced cytotoxicity (22), and this is at least in part due to cyclooxygenase-2–mediated up-regulation of MDR1/P-glycoprotein (MDR1/P-gp; refs. 23, 24), an energy-dependent

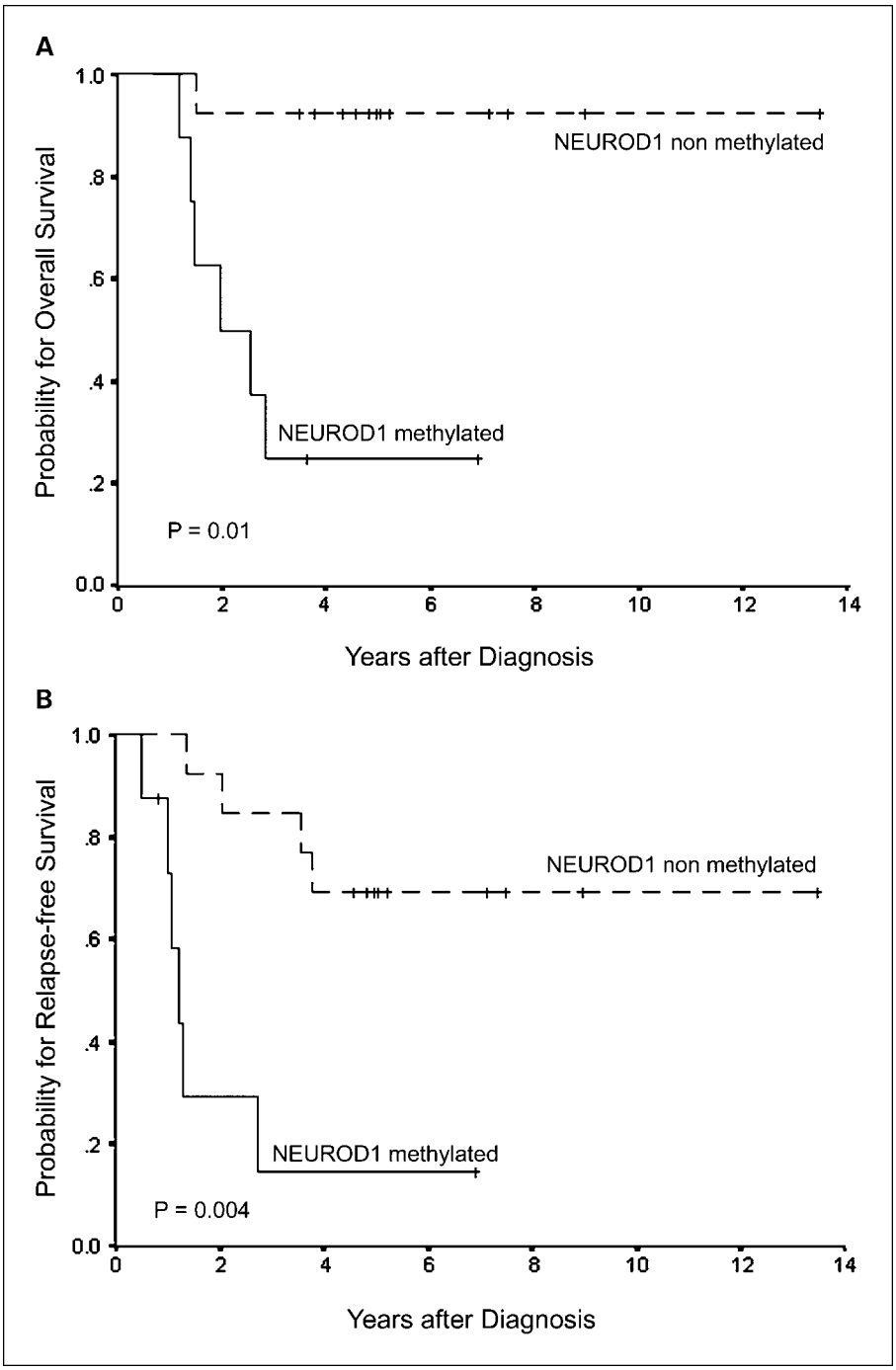

Fig. 2. Kaplan-Meier survival curves and *NEUROD1* DNA methylation status in serum samples. Overall (*A*) and relapse-free survival (*B*) of 21 ER-negative primary breast cancer patients with positive *NEUROD1* methylation in pretreatment serum. Broken lines, negative serum *NEUROD1* methylation after chemotherapy; continuous lines, positive serum *NEUROD1* methylation after chemotherapy.

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Table 5. Univariate analysis of 21 patients with ER-negative primary breast cancer with positive *NEUROD1* methylation in pretreatment serum

(A) Overall survival

		No. of patients (died/total)	RR of death (95% CI)	*P*
Size	T_1	2/9	2.4 (0.5-12.6)	0.3
	$T_{2/3/4}$	5/11		
LN	Negative	2/5	0.6 (0.1-3.4)	0.6
	Positive	4/15		
Grade	Grade 1	1/4	1.8 (0.2-14.5)	0.6
	Grade 2/3	6/17		
MP	Premenopausal	1/3	1.2 (0.2-10.2)	0.9
	Postmenopausal	6/18		
PR	Negative	7/18	0.04 (0.0-196)	0.5
	Positive	0/3		
OP-Mode	TE	1/6	2.5 (0.3-22)	0.4
	ME	5/14		
Radiation	No	3/7	0.7 (0.2-3.0)	0.6
	Yes	4/14		
NEUROD1	Negative after chemotherapy	1/13	15 (1.8-125)	0.01
	Positive after chemotherapy	6/8		

(B) Relapse-free survival

		No. of patients (relapsed/total)	RR of relapse (95% CI)	*P*
Size	T_1	4/9	1.5 (0.4-5.4)	0.5
	$T_{2/3/4}$	6/11		
LN	Negative	4/5	0.4 (0.1-1.3)	0.1
	Positive	5/15		
Grade	Grade 1	1/4	2.3 (0.3-18.5)	0.4
	Grade 2/3	9/17		
MP	Premenopausal	2/3	0.6 (0.1-2.8)	0.5
	Postmenopausal	8/18		
PR	Negative	9/18	0.5 (0.1-3.7)	0.5
	Positive	1/3		
OP-Mode	TE	4/6	0.6 (0.2-2.4)	0.5
	ME	5/14		
Radiation	No	4/7	0.6 (0.2-2.0)	0.4
	Yes	6/14		
NEUROD1	Negative after chemotherapy	4/13	6.9 (1.9-26)	0.004
	Positive after chemotherapy	6/8		

Abbreviations: RR, relative risk; LN, lymph node status; MP, menopausal status; PR, progesterone receptor status; ME, mastectomy; TE, tumorectomy.

pump that participates in multidrug resistance. In addition, cyclooxygenase-2–derived prostaglandin E_2 protects embryonic stem cells from apoptosis (25). Interestingly, we observed a strong inverse correlation of cyclooxygenase-2 expression and *NEUROD1* methylation in ER-negative breast cancer specimens (correlation coefficient $r = -0.4$; $P = 0.03$; Supplementary Fig. S1), which supports our hypothesis that *NEUROD1* methylation is a surrogate for the status of the cell associated with chemosensitivity. Further studies will be needed to show whether *NEUROD1* upstream regulators (e.g., members of the polycomb repressor complex) have a particular effect on the responsiveness of tumor cells to chemotherapy.

In summary, this is the first study describing a DNA-based marker which is able to predict the response to neoadjuvant as well as adjuvant chemotherapy in a solid tumor independent of gene transcription and the source of DNA analyzed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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