Expression of Endothelin-1, Endothelin-A, and Endothelin-B Receptor in Human Breast Cancer and Correlation with Long-Term Follow-Up[1]

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ABSTRACT

Purpose: **Endothelin-1 (ET-1) is overexpressed in breast carcinomas and stimulates tumor cell growth in an autocrine and paracrine fashion via its receptors, ET_AR and ET_BR. In this study, we evaluated the expression of ET-1 and ET receptors in breast carcinomas and determined its clinical and prognostic significance.**

Experimental Design: **We analyzed expression of ET-1, ET_AR, and ET_BR in 176 breast carcinomas using a semiquantitative immunohistochemical approach. Statistical analysis of clinicopathological variables such as pT stage, pN stage, hormone receptor status, Her-2/neu amplification, histological grade, and long-term follow-up data were performed.**

Results: **We observed a moderate to strong cytoplasmic staining for ET-1 in 69 (43.1%), for ET_AR in 74 (46.5%), and for ET_BR in 86 (53.4%) cases of primary breast cancer. A correlation was found between increased ET-1 expression and its receptors with several clinicopathological parameters that characterize aggressive types of breast cancer, with the exception of increased ET_AR and ET_BR expression with positive estrogen receptor status. Elevated expression of ET-1, ET_AR, and ET_BR was more common in breast carcinomas of patients with lower disease-free survival time and overall survival. In addition, a statistically significant correlation was observed between ET_AR expression and reduced disease-free survival time ($P = 0.041$). Interestingly, the prognostic impact of ET_AR expression was shown to be more pronounced in the subgroup of patients with a putative favorable prognosis according to classic prognostic factors.**

Conclusions: **Therefore, analysis of ET_AR expression may improve the prediction of relapse and death and facilitate an individually based risk-directed adjuvant therapy in breast cancer patients.**

INTRODUCTION

ETs,[3] ET-1, ET-2, and ET-3, are potent vasoactive 21-amino acid peptides first isolated from vascular endothelial cells (1). Various tumor cell lines, including several breast carcinoma cell lines release immunoreactive ET-1 (2). Increased expression of ET-1 has been shown in various human malignancies such as ovarian, colorectal, and prostate cancer (3–6). In human breast carcinomas, ET-1 expression was demonstrated applying radioimmunoassays (7), IHC (8, 9), and quantitative reverse transcription-PCR (8). The physiological role of ET-1 in human breast cancer has not been well characterized. Several studies have reported a much higher expression of ET-1 in breast carcinomas than in normal breast tissue (7, 8), indicating that the ET system may be associated with breast cancer. ET-1 staining of ductal carcinomas *in situ* had intensities between carcinomas and normal tissues. This suggests a possible involvement of ET expression in tumor progression to invasive phenotypes. Upregulation of ET-1 may occur in response to cell activation by various stimuli, including hypoxia, growth factors, and cytokines (10). ET-1 acts as a mitogen for human breast fibroblasts (11, 12) and human breast cancer cells (13) involving prostaglandin E_2. The tumor-promoting activity of ET-1 is mediated through autocrine and paracrine effects on tumor cell proliferation (14), invasion (15, 16), angiogenesis (4, 6, 15), and neovascularization (4, 17).

ET-1 is effective through two types of G protein-coupled receptors, ET_AR and ET_BR. ET_AR binds ET-1 and ET-2 with high affinity and ET-3 with low affinity, whereas ET_BR is nonselective with equal affinity for the three subtypes (18). It has been proposed that the influence of ET-1 on cell proliferation and migration is mediated through activation of ET_BR (19, 20). In contrast, the synthesis of vascular endothelial growth factor as well as angiogenic and mitogenic effects are stimulated by ET-1 predominantly through ET_AR (4, 21).

In cultured human breast epithelial and stromal cells, ET receptor expression and biochemically induced responsiveness to ET were observed only in the stromal cells (22). These

Received 11/14/02; revised 3/28/03; accepted 4/21/03.
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[1] This study was supported, in part, by Abbott Laboratories, Abbott Park, IL.
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[3] The abbreviations used are: ET, endothelin; IHC, immunohistochemistry; DFST, disease-free survival time; OS, overall survival; CI, confidence interval; PR, progesterone receptor; RR, relative risk.

findings provide evidence that ET-1 released from the breast epithelial cells may, in part, act in a paracrine fashion on neighboring stromal tissue. The only study of ET receptor expression in breast cancer tissue reported that ET_AR mRNA was not detected in any tissue, whereas ET_BR mRNA was present in the normal breast tissue and was overexpressed in carcinomas (8). To the best of our knowledge, there are no data about protein expression of the ET receptors available.

Several preclinical and clinical studies in various malignancies suggest that ET-1, ET_AR, and ET_BR may represent interesting targets for chemoprevention and chemotherapy. A selective ET_BR antagonist (BQ788) was found to inhibit the growth of human melanoma cell lines and to reduce human melanoma tumor growth in a nude mouse model (23). Also, in advanced prostate cancer, treatment with an ET_A-selective receptor antagonist (ABT-627) delayed disease progression in patients with hormone-refractory prostate cancer (24). Another interesting aspect is the previously observed fall of ET-1 plasma levels in breast cancer patients during use of antiestrogens as adjuvant therapy, indicating that antiestrogens may inhibit the synthesis of ET-1 *in vivo* (25).

Thus far, the prognostic value of ET expression in breast cancer is unclear. Previous studies failed to find a significant correlation of ET-1 expression with clinicopathological variables (7–9, 26). Also, conflicting data exist on whether ET-1 expression in breast cancer is associated with relapse-free and overall survival (9, 26). At present, there are no data available linking ET receptor status with clinicopathological data and disease outcome. In human breast tumors, ET-3 expression has only been investigated by Alanen *et al.* (8). As it is known that ET-3 has a much lower affinity to the ET_A receptor than ET-1 (18), this study focused on the expression of ET-1. Thus, we investigated the immunohistochemical expression of ET-1, ET_AR, and ET_BR in 176 samples of human breast cancer. Extensive histopathological, clinical, and follow-up data were available for all breast carcinoma samples. Results were correlated with this data to evaluate the prognostic value of ET-1, ET_AR, and ET_BR in breast cancer.

MATERIALS AND METHODS

Patients. We analyzed a consecutive series of 176 patients who underwent lumpectomy or mastectomy for invasive breast cancer between 1993 and 1997 at the Department of Gynecology, University of Münster (Munster, Germany). Formalin-fixed and paraffin-embedded tumor samples of these patients were obtained from the archives of the Gerhard-Domagk-Institute of Pathology, University of Münster. Among the 176 carcinomas, 92 (52.3%) were ductal invasive, 40 (22.7%) were lobular, 22 (12.5%) were of mixed histological differentiation, 12 (6.8%) were tubular, 4 (2.3%) were medullary, 3 (1.7%) were mucinous, and 1 (0.6%) was scirrhoes. Tumor stages were classified according to the tumor-node-metastasis classification of 1997. Different pT stages were represented, including pT_1 in 75 (42.6%), pT_2 in 57 (32.4%), pT_3 in 12 (6.8%), and pT_4 in 32 (18.2%) cases, respectively. Tumor diameter, which was recorded in the initial pathology description, ranged from 3 to 190 mm (27.4 ± 24.6 mm). Information on the number of lymph nodes showing metastases was available in 172 (97.7%) patients [pN_0, $n = 95$ (54%), pN_1, $n = 65$ (37.8%), and pN_2, $n = 12$ (6.9%)]. Histological grading according to the BRE score showed 19 (10.9%) well differentiated (G1), 93 (53.1%) moderately differentiated (G2), and 63 (36%) poorly differentiated (G3) tumors. For evaluation of prognostic value, clinicopathological parameters, including tumor size, lymphonodal involvement, histological type and grade, lymphovascular invasion, hormone receptor status, Her-2/neu status, and proliferation index (MIB-1-LI) were used. Furthermore, detailed information regarding operative therapy, neoadjuvant or adjuvant cytotoxic chemotherapy, and hormonal therapy were available for these patients. These data and information on long-term follow-up were registered in a database. Study subject follow-up was generally followed using the German guidelines for treatment and follow-up of breast cancer patients with gradually increasing intervals between visits. Thus, clinical examination and tumor marker controls were performed every 3 months for 3 years, every 6 months for 2 years, then annually. Imaging aftercare (including mammography and sonography of breast and axillary region) was initially performed every 6 months for 3 years, then annually. Follow-up data were evaluated until May 2002. Mean DFST was 68.1 months (range, 3–111 months; median, 76.7 months; SD, 34.8 months), and mean OS was 75.2 months (range, 4–111 months; median, 86.7 months; SD, 30.1 months).

IHC for ET-1, ET_AR, and ET_BR Expression. Consecutive 4-μm sections were cut from formalin-fixed and paraffin-embedded tissue blocks. Immunohistochemical staining for ET-1, ET_AR, and ET_BR was performed in a multistep semiautomatic procedure (Dako-Autostainer). For ET-1, a monoclonal mouse antibody at a 1:500 dilution (25 min) was used (Antiendothelin-1 MAb; Alexis Biochemicals Corporation, Lausen, Switzerland). Two sheep polyclonal antibodies (ET_A-Receptor Antiserum, ET_B-Receptor Antiserum; Alexis Biochemicals Corporation) at a 1:100 dilution (30 min) for ET_AR and ET_BR.

The positive controls used were prostate cancer tissue known to express ET_AR and smooth muscle tissue with ET_B receptor activity. Omission of primary antibodies served as negative control. After nuclear counterstaining with hematoxylin, cytoplasmic immunostaining intensity was scored semiquantitatively into different grades on an arbitrary four-tiered scale of 0–3+ (4). Grade 0 represented cases with no detectable immunostaining of tumor cells. Tissue sections graded as 1+ showed a weak staining of the majority of tumor cells. Cases with a moderate or strong staining intensity were scored as 2+ or 3+, respectively. We defined these samples with a moderate (2+) or strong (3+) cytoplasmic immunostaining intensity to have an elevated ET-1, ET_AR, or ET_BR expression and thus to be positive (Fig. 1). Each case was independently scored by two investigators.

Data Analysis. Semiquantitative analysis of staining results was performed in blind-trial fashion without knowledge of the clinical data for the corresponding case. Correlations between ET expression and clinicopathological parameters were tested for statistical significance by a χ^2 test using SPSS Version 10.0. For analysis of survival data related to ET expression, Kaplan-Meier-survival estimates were generated and compared

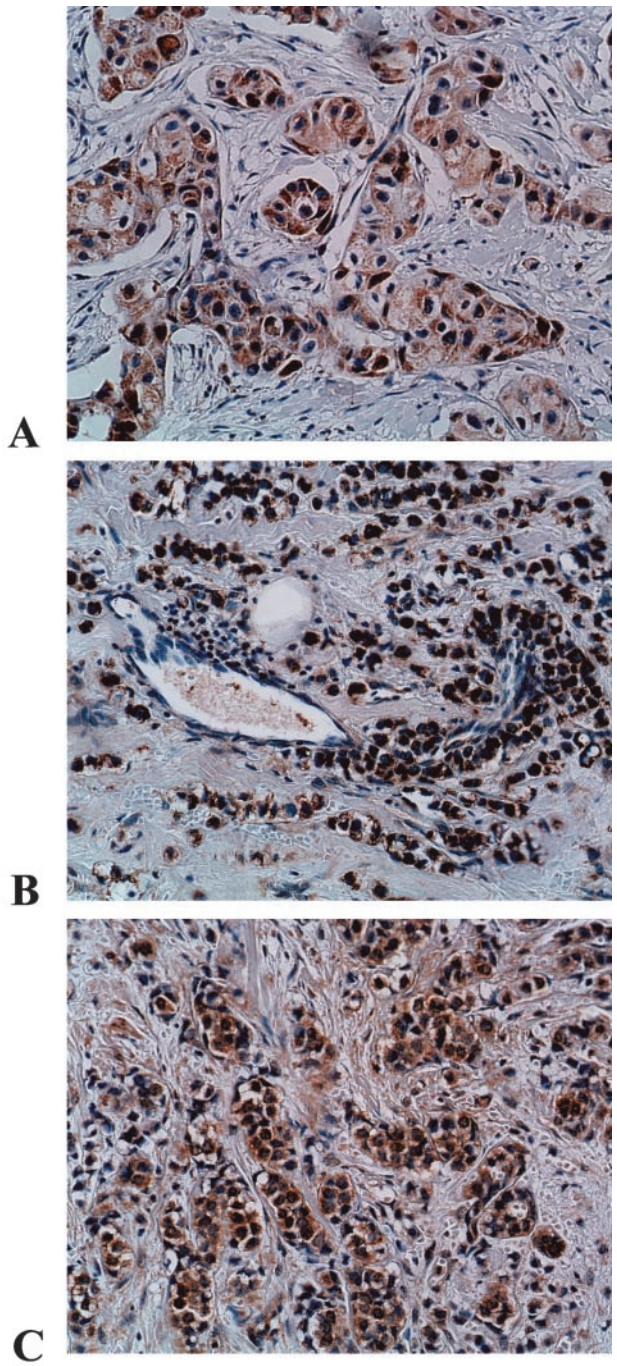

Fig. 1 Three breast cancer samples with strong expression (3+) of ET-1 (*A*), ET_AR (*B*), and ET_BR (*C*) as assessed by IHC.

with the log-rank test. DFST was calculated as the time from the date of diagnosis to the occurrence of locoregional or distant metastasis or death from cancer. Patients with local recurrence ($n = 5$) or metachronous breast cancer ($n = 9$) were excluded for analysis of OS. Patients with distant metastasis at the time of diagnosis ($n = 19$) were additionally excluded for calculation of DFST. Multivariate analysis was performed using Cox's proportional hazards regression model.

Table 1 Immunohistochemical analysis of ET_AR, ET_BR, and ET-1 expression in primary human breast carcinomas

	n (%)		
Score	ET_AR ($n = 159$)	ET_BR ($n = 161$)	ET-1 ($n = 160$)
0	39 (24.5)	37 (23)	31 (19.4)
1+	46 (28.9)	38 (23.6)	60 (37.5)
2+	46 (28.9)	38 (23.6)	44 (27.5)
3+	28 (17.6)	48 (29.8)	25 (15.6)
Negative (score 0–1)	85 (53.5)	75 (46.6)	91 (56.9)
Positive (score 2–3)	74 (46.5)	86 (53.4)	69 (43.1)

RESULTS

Immunoreactivity for ET-1, ET_AR, and ET_BR. ET-1 expression could be analyzed in 160 cases of primary breast cancer. Of these 69 (43.1%) cases displayed, moderate to strong cytoplasmic immunostaining for ET-1 referred to as ET-1-positive cases and 91 (56.9%) showed negative or weak immunostaining (Table 1). The stromal tissue was mostly negative. Cytoplasmic ET_AR expression was detected at an intermediate or high level in epithelial cells in 74 of 159 (46.5%) evaluable primary breast cancers. Negative or weak specific immunostaining for ET_AR was observed in 85 (53.5%) of these tumors. Stromal staining for ET_AR was frequently detected in ET_AR-positive tumors but not in ET_AR-negative ones. The staining pattern for ET_BR was also cytoplasmic. In invasive breast cancer, 86 (53.4%) of 161 cases showed moderate or strong ET_BR expression (ET_BR positive), whereas 46.6% were ET_BR negative. Similar to ET_AR, immunostaining for ET_BR was frequently observed in stromal tissue of ET_BR-positive tumors.

Association of ET-1, ET_AR, and ET_BR with Clinicopathological Variables. Results on analysis of correlation between ET-1, ET_AR, and ET_BR expression and clinicopathological variables in patients with primary breast cancer are summarized in Tables 2 and 3. Elevated expression of ET-1 was more common in tumors with larger size (pT_1 *versus* pT_{2-4}: $P = 0.018$), high histological grade ($P = 0.0001$), and presence of lymphovascular invasion ($P = 0.084$). Furthermore, 7 of 10 inflammatory carcinomas were ET-1 positive ($P = 0.008$). ET_AR-positive tumors correlated significantly with poor histological differentiation ($P = 0.009$), Her-2/neu overexpression ($P = 0.003$), presence of lymphovascular invasion ($P = 0.030$), and positive estrogen receptor status ($P = 0.038$). A significant association was also found with incidence of distant metastasis ($P = 0.013$) or local recurrence ($P = 0.047$). All correlations between ET_AR status and these clinicopathological variables were found to be linear. Statistical analysis of ET_BR expression showed a significant association with tumor size ($P = 0.049$). Similar to ET_AR, ET_BR was weakly correlated with positive ER status ($P = 0.079$). Additionally, ET_BR-positive tumors showed a trend toward a lower 5-year DFST, although this difference was not statistically significant ($P = 0.086$). Taking all cases ($n = 176$), including 14 patients with local recurrence or metachronous breast cancer into consideration, the same associations between ET_AR, ET_BR and ET-1 expression and clinicopathological variables were observed.

Table 2 Association of increased ET_AR expression with clinicopathological variables in primary breast cancer patients

Clinicopathological variables	ET_AR staining *n* positive/total (%)	*P*[a]
PT stage		
≤pT_2	53/120 (44.2)	NS[b]; 0.642
≥pT_3	21/39 (53.9)	
Lymph node status		
Negative	35/87 (40.2)	NS; 0.137
Positive	36/69 (52.2)	
Histologic grading		
I–II	38/98 (38.8)	0.009
III	36/60 (60.0)	
ER status		
Positive	52/96 (54.2)	0.038
Negative	21/57 (36.8)	
PR status		
Positive	36/73 (49.3)	NS; 0.760
Negative	37/79 (46.8)	
Her-2/neu status		
Negative	63/143 (44.0)	0.003
Positive	10/11 (90.9)	
MIB-1-labeling index		
≤20%	45/99 (45.5)	NS; 0.590
>20%	27/54 (50.0)	
Lymphovascular invasion		
No	51/122 (41.8)	0.030
Yes	23/37 (62.2)	
Local recurrence		
No	60/138 (43.5)	0.047
Yes	14/21 (66.7)	
Distant metastasis		
No	46/114 (40.3)	0.013
Yes	28/45 (62.2)	
5-yr DFST		
Yes	36/94 (38.3)	0.030
No	24/41 (58.5)	

[a] χ^2 test.
[b] NS, not significant.

Table 3 Association of ET-1 expression with clinicopathological variables in primary breast cancer patients

Clinicopathological variables	ET-1 status *n* positive/total (%)	*P*[a]
pT stage		
pT_1	22/68 (32.4)	0.036[b]
pT_2	26/52 (50.0)	
pT_3	5/11 (45.5)	
pT_4	16/29 (55.2)	
Histologic grading		
I–II	31/99 (31.3)	<0.0001
III	37/60 (61.7)	
Lymphovascular invasion		
No	48/122 (39.3)	NS[c]; 0.030
Yes	21/38 (55.3)	
Distant metastasis		
No	42/114 (36.8)	0.012
Yes	27/46 (58.7)	

[a] χ^2 test.
[b] χ^2 test for linear trend.
[c] NS, not significant.

Association of ET-1, ET_AR, and ET_BR with Survival. Results on survival analysis with respect to ET-1, ET_AR, and ET_BR expression are summarized in Fig. 2. Statistical analysis of survival data revealed that elevated ET_AR expression was significantly associated with decreased DFST. This was evident when ET_AR-positive patients ($n = 60$) were compared with ET_AR-negative patients ($n = 75$; $P = 0.041$). Mean DFST was 74 ± 6 months (95% CI, 62–85) in ET_AR-positive patients and 90 ± 4 months (95% CI, 81–98) in ET_AR-negative patients, respectively. Interestingly, the prognostic impact of ET_AR expression was shown to be different in various subgroups of patients (Table 4). ET_AR had significant prognostic value for DFST in patients with ER-positive ($P = 0.011$) or Her-2/neu-negative tumors ($P = 0.035$) but not in the ER-negative or Her-2/neu-positive tumors. Elevated ET_AR expression also predicted decreased DFST in patients with moderately differentiated tumors ($P = 0.036$) and in tumors with a low proliferation rate identified by MIB-1-labeling index ($P = 0.002$). Furthermore, significant prognostic impact of ET_AR was observed in tumors without lymphovascular invasion ($P = 0.033$), whereas the association was not significant in those with lymphovascular invasion. A significant association of poor DFST and increased ET_AR expression was present in breast cancer specimens with a histological type other than ductal or lobular invasive ($P = 0.003$). Correlating DFST with ET_BR expression, 71 ET_BR-positive cases had a mean DFST of 80 ± 5 months (95% CI, 70–90), whereas 65 ET_BR-negative cases had a DFST of 86 ± 5 months (95% CI, 76–96). This difference was statistically not significant (NS; $P = 0.445$). Analysis of ET-1 expression also revealed a trend toward decreased DFST in ET-1-positive cases. Mean DFST was 77 ± 6 months (95% CI, 65–88) in ET-1-positive cases ($n = 54$) and 87 ± 4 months (95% CI, 78–95) in ET-1-negative cases ($n = 81$; NS; $P = 0.224$).

No significant differences were observed when associations between OS and expression of ET_AR, ET_BR, and ET-1 were analyzed. Mean OS was 88 ± 4 months (95% CI, 79–97) in ET_AR-positive patients ($n = 70$) and 94 ± 4 months (95% CI, 87–101) in ET_AR-negative patients ($n = 81$; NS; $P = 0.385$). Eighty-one cases with an increased ET_BR expression had an OS of 90 ± 4 months (95% CI, 82–97) and 72 cases that were ET_BR-negative had an OS of 92 ± 4 months (95% CI, 84–100; NS; $P = 0.625$). Survival analysis in patients that were positive for ET-1 ($n = 64$) showed an OS of 86 ± 5 (95% CI, 77–95) and an OS of 94 ± 3 (95% CI, 88–101) in ET-1-negative patients ($n = 88$; NS; $P = 0.166$).

The number of patients, as well as time and frequency of follow-up assessment, was similar in both of the subgroups of ET-negative and -positive patients.

Multivariate Analysis. To evaluate the independent prognostic value of ET_AR expression for DFST multivariate survival analysis was performed. ET_AR expression (positive *versus* negative), pT stage (pT_1 *versus* ≥pT_2), lymph node status (pN_0 *versus* $pN_{1–3}$), histological grading (G1 + 2 *versus* G3), and PR status (positive *versus* negative) were entered as covariates. The model revealed only PR status as an independent prognostic factor (RR; 95% CI, 2.19–4.40; $P = 0.027$), whereas ET_AR expression and the other covariates mentioned above did not add significant independent prognostic information.

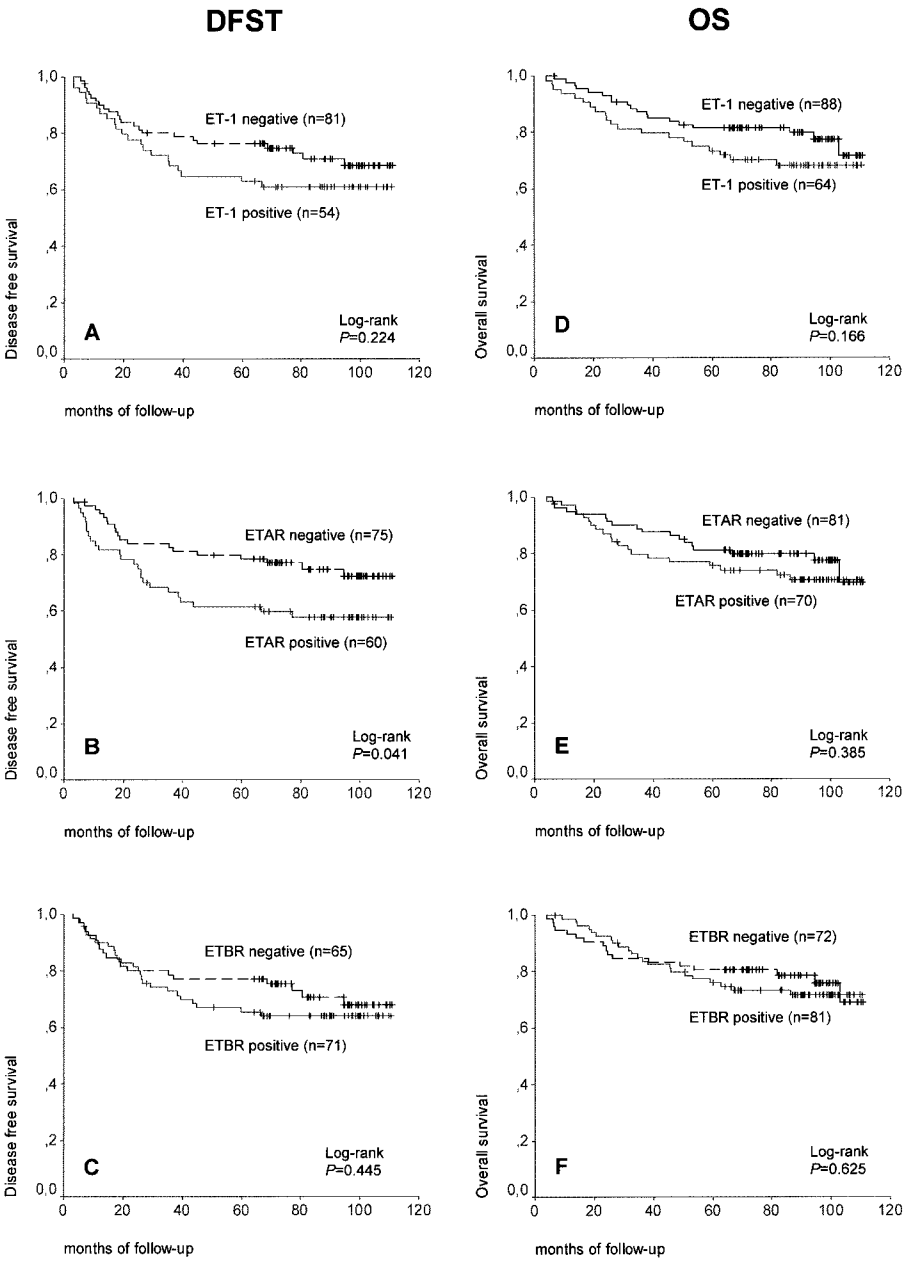

Fig. 2 Survival curves of patients with primary breast cancer. Kaplan-Meier estimates for DFST stratified by ET-1 (*A*), ET_AR (*B*), and ET_BR (*C*) expression. OS with respect to ET-1 (*D*), ET_AR (*E*), and ET_BR (*F*) expression.

DISCUSSION

To date, the presence or absence of axillary lymph node metastasis is the most valuable single prognostic attribute in breast cancer patients. New prognostic markers are requested, which differentiate breast cancer patients for individually based risk-directed therapy.

In our study, elevated levels of ET-1 expression were detected in 43.1% of primary breast cancers. In addition, 46.5% of breast carcinomas were ET_AR positive and 53.4% showed enhanced ET_BR expression. Expression of ET-1 mRNA and protein in breast cancer specimens has been reported previously (7–9, 26), but the small number of tumors studied and the different methods used make the comparison of these data with our findings difficult. The amount of ET-1-positive tumors in our series is consistent with the reported 40.0% breast cancers with positive immunoreactivity for ET-1 in the study of Kojima and Nihei (9) but contradictory to Alanen *et al.* (8) who described a moderate to strong staining in 72% carcinomas. The latter study further reported that ET_BR mRNA was present in normal breast tissue and was augmented in breast carcinomas, whereas no evidence of ET_AR mRNA could be detected either in normal or in the neoplastic breast tissue. In our data, increased ET_AR and ET_BR expression was observed only in a subset of breast carcinomas. ET_AR-positive carcinomas frequently showed stromal staining for ET_AR in interstitial cells such as fibroblasts. This stromal immunoreactivity corresponds with studies showing that ET-1 predominantly acts in an endocrine/

Table 4 Correlation of ET_AR expression with DFST according to clinicopathological variables[a]

Clinicopathological variables	ET_AR status	DFST Months (95% CI)	P[b]
Tumor size			
≤2 cm	Negative	97 (87–106)	NS[c]; 0.141
	Positive	82 (66–98)	
Tumor size			
>2 cm	Negative	82 (69–96)	NS; 0.194
	Positive	67 (51–83)	
Lymph node negative	Negative	95 (85–104)	NS; 0.424
	Positive	86 (72–101)	
Lymph node positive	Negative	80 (64–95)	NS; 0.145
	Positive	61 (45–76)	
Invasive ductal	Negative	88 (77–100)	NS; 0.296
	Positive	74 (60–87)	
Invasive lobular	Negative	73 (54–92)	NS; 0.331
	Positive	90 (64–115)	
Special	Negative	105 (94–116)	0.003
	Positive	62 (40–84)	
Histologic grade I–II	Negative	96 (87–93)	0.046
	Positive	78 (64–93)	
Histologic grade III	Negative	71 (52–91)	NS; 0.837
	Positive	67 (49–86)	
ER positive	Negative	96 (85–106)	0.011
	Positive	71 (58–85)	
ER negative	Negative	76 (63–89)	NS; 0.922
	Positive	75 (54–96)	
PR positive	Negative	95 (84–106)	NS; 0.090
	Positive	79 (64–95)	
PR negative	Negative	76 (64–88)	NS; 0.327
	Positive	66 (50–83)	
Her-2/neu negative	Negative	88 (80–97)	0.035
	Positive	71 (58–83)	
Her-2/neu positive	Negative	79 (56–102)	NS; 0.627
	Positive	Censored	
MIB-1 labeling index ≤20%	Negative	92 (82–102)	0.002
	Positive	64 (50–78)	
MIB-1 labeling index >20%	Negative	82 (65–99)	NS; 0.503
	Positive	86 (68–104)	
Lymphovascular invasion absent	Negative	93 (84–101)	0.033
	Positive	75 (62–88)	
Lymphovascular invasion present	Negative	65 (41–88)	NS; 0.965
	Positive	66 (44–88)	
No inflammatory component	Negative	90 (81–99)	NS; 0.059
	Positive	75 (63–86)	
Inflammatory component	Negative	77 (40–115)	0.045
	Positive	8	

[a] Primary breast cancers only.
[b] χ^2 test.
[c] NS, not significant.

paracrine fashion because breast cancer epithelial cells express ET-1, whereas stromal breast fibroblasts possess ET receptors (11, 22, 27). However, we observed ET_AR and ET_BR protein expression in breast cancer epithelial cells, which is in contrast to the previously reported lack of ET receptors in breast epithelial cells (22).

According to our data, elevated expression of ET-1, ET_AR, and ET_BR was more common in breast cancers of patients with reduced disease-free survival and OS. Expression of ET_AR correlated statistically significant with reduced DFST. We also observed an association between ET_AR-positive tumors and some clinicopathological markers for poor prognosis such as high histological grade, Her-2/neu overexpression, and lymphovascular invasion as well as with incidence of local recurrence or distant metastasis. However, increased expression of ET_AR and ET_BR correlated with positive ER status of tumors. We also found a significant association of ET_BR expression and tumor size. Enhanced ET-1 expression correlated significantly with poor histological differentiation and incidence of distant metastasis. Moreover, a linear correlation with tumor size was detected. Lymphovascular invasion and an inflammatory component was more common in tumors positive for ET-1 staining. Hence, it may be followed from our data that increased expression of ET-1, ET_AR, and ET_BR is correlated with various parameters that characterize aggressive types of breast cancer. The only exception to this trend, however, was the association

of these factors with positive ER status. The reason for this finding is open to speculation. Nevertheless, the use of ET-1, ET_AR, and ET_BR as biological markers in breast carcinomas could be helpful for characterization of more aggressive and less differentiated tumor groups and therefore may add significant prognostic information to standard prognostic variables. In contrast to our data, no significant correlation of ET-1 expression with clinicopathological parameters was found in previous studies (7–9, 26), whereas increased ET-1 immunoreactivity predicted for recurrence and distant metastasis (9).

Our main finding is that elevated levels of ET_AR expression are associated with decreased disease-free survival in patients with primary breast cancer. Because in the subgroup of patients with a putative favorable prognosis according to classic prognostic factors those patients with ET_AR-positive tumors had a significantly decreased disease-free survival probability, analysis of ET_AR expression may improve the prediction of relapse and death. ET_AR may therefore help to identify patients who may profit from adjuvant therapy.

In conclusion, our findings suggest that analysis of ET_AR expression as an additional prognostic marker in breast cancer should be included in future validation studies. Ease of measurement and good reproducibility of IHC may permit routine measurements of ET_AR expression in paraffin-embedded tumor tissues from routine histopathological specimens. ET_AR may serve as a parameter for prognostic classification that facilitates a risk-directed therapy in breast cancer patients. In addition, the clinical relevance of ET_AR as a target for the prevention and treatment of breast cancer should be evaluated in future clinical trials.

ACKNOWLEDGMENTS

We thank Inka Buchroth and Judith Obernüfemann for excellent technical assistance.

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Clin Cancer Res 2003;9:4125-4131.