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Expression of TGF- β signaling factors in invasive breast cancers: relationships with age at diagnosis and tumor characteristics

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Abstract

The transforming growth factor beta (TGF- β) pathway can play either a tumor-suppressing or a tumor-promoting role in human breast carcinogenesis. In order to determine whether expression of TGF- β signaling factors varies by age at onset and breast tumor characteristics that have prognostic significance, we undertook a study of 623 women with invasive breast carcinoma enrolled in a population-based case-control study conducted in Poland from 2000 to 2003. TGF- β signaling factors were analyzed by immunohistochemistry in tumor tissue microarrays. We found that most tumors expressed extracellular-TGF- β 1 (78%), TGF- β 2 (91%), TGF- β 3 (93%), TGF- β R2 (72%), and phospho-SMAD2 (61%), whereas intracellular-TGF- β 1 was expressed in 32% of tumors. Expression of TGF- β ligands (β 1, β 2, and β 3) was associated with prognostically favorable pathological features including small size, and low grade, and these associations were similar for ER-positive and negative tumors. On the contrary, expression of the receptor TGF- β R2 was primarily associated with small tumor size among ER-negative tumors, while expression of the transcription factor phospho-SMAD2 was associated with positive nodal status among ER-negative tumors. The greater frequency of expression of phospho-SMAD2 in cancers associated with lymph node metastases is consistent with a pro-progression role for TGF- β . In addition, expression of extracellular-TGF- β 1 ($P = 0.005$), TGF- β R2 ($P = 8.2E-11$), and phospho-SMAD2 ($P = 1.3E-8$) was strongly associated with earlier age at onset, independent of ER status. Our data provide evidence that TGF- β signaling patterns vary by age and pathologic features of prognostic significance including ER expression. These results warrant analysis in studies of clinical outcomes accounting for age, ER status and treatment.

Keywords

Transforming growth factor beta; Breast cancer; Estrogen receptor

Introduction

The transforming growth factor beta (TGF- β) pathway plays an important role in human breast development and carcinogenesis. The TGF- β pathway regulates essential cellular activities including differentiation, proliferation, and apoptosis [1–3]. In the canonical signaling pathway, the effects of the three TGF- β ligands (TGF- β 1, β 2, β 3) are mediated via binding to the TGF- β type II receptor (TGF- β R2), which in turn binds TGF- β type I receptor (TGF- β R1) to form a complex with serine/threonine kinase activity. This complex phosphorylates transcription factors SMAD2 and SMAD3, which translocate into the nucleus and activate transcription of numerous genes producing pleiotropic effects [3]. TGF- β s are synthesized as biologically latent forms that show complex patterns of post-translational regulation of bioavailability through binding to extracellular matrix components and other proteins that can modulate bioavailability [4].

Clinical and pre-clinical data support the concept that TGF- β signaling plays a dual role in cancer progression; activation of the TGF- β pathway in pre-neoplasia and early neoplasia has been associated with a reduced risk of progression, whereas in late-stage human cancers, over-expression of TGF- β ligands has been associated with an aggressive tumor phenotype and metastasis [5, 6]. Tumor suppressing effects of the TGF- β pathway are likely due to anti-proliferative and pro-apoptotic or pro-differentiating effects of TGF- β and the ability of TGF- β to maintain genomic stability. The tumor promoting effects of TGF- β are attributed to its ability to increase tumor cell migration and invasion, and to suppress immune surveillance and enhance angiogenesis [1, 2].

Clinical evidence for a tumor suppressor effect of TGF- β signaling in breast cancer is provided by data showing that the reduced expression of TGF- β R2 in breast epithelial hyperplasia is related to an increased risk for subsequent breast cancer [7], and that the reduced TGF- β R2 expression in ductal carcinomas in situ and invasive breast cancers is associated with a more aggressive histology and higher tumor grade [8]. In contrast, in other studies, ER-negative tumors, but not ER-positive tumors that express TGF- β R2 or demonstrate a TGF- β response transcript signature, have been associated with reduced overall survival [9] and increased lung metastasis [10], supporting a pro-progression role for the TGF- β pathway, at least in a subset of breast cancers.

Given that ER-negative tumors are more common among younger women, and that early onset tumors tend to have a worse prognosis than similar tumors occurring at older ages, it is plausible that altered TGF- β signaling might identify an etiologically distinctive subset of early onset clinically aggressive cancers [11]. However, analyses of this question have been limited by small sample sizes and examination of only a few components of the TGF- β pathway. Accordingly, to determine whether expression of TGF- β pathway components varies by breast tumor characteristics or age at diagnosis, we analyzed associations for a

panel of TGF- β signaling factors among 623 invasive breast carcinomas from a population-based case-control study conducted in Poland.

Materials and methods

Study population

The Polish population-based case-control study has been described previously [12]. In brief, eligible cases included female residents of Warsaw or Łódź, Poland in the age range of 20–74 years diagnosed with pathologically confirmed in situ or invasive breast cancer from 2000 to 2003. Eligible cases were identified either through a rapid ascertainment mechanism in participating hospitals (~90%) or cancer registries. Control women were randomly selected from population lists and frequency matched to breast cancer cases by the city of their residence and age (5-year intervals). In total, 2,386 cases (79% of eligible) and 2,502 control subjects (69% of eligible) participated in the study and provided informed consent as required by internal review boards at the National Cancer Institute and in Poland. Detailed demographic and risk factor data were collected through in-person interviews as previously described [12].

Pathology

Surgical pathology reports were used to acquire information on tumor size, histologic type, grade, and stage. Selected histologic slides were reviewed by a single pathologist in the U.S. (MES). Ninety percent of the cases were diagnosed as invasive. In this article, we analyze data for a subset of 842 invasive carcinomas prepared as tumor tissue microarrays (TMAs) [13, 14]. TMAs were prepared in the U.S. from 842 formalin-fixed paraffin-embedded tumor tissues processed in Poland according to local protocols [12–14]. As previously reported, tumors included in the TMA were slightly larger than those in the study overall [14]. Sections of TMA blocks were cut at 5- μ m thickness and placed on glass slides using a tape transfer method (Instrumedics, Hackensack, NJ). Cut sections were dipped in paraffin and stored at room temperature under gaseous nitrogen to limit loss of immunoreactivity prior to staining [13]. Results for ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), and other markers have been previously reported [14].

Immunohistochemistry and marker assessment

TMA sections were deparaffinized, and staining was performed using the i6000 Automated Staining System with research software (BioGenex, San Ramon, CA). Sections were treated with 6% hydrogen peroxide for 10 min to quench endogenous peroxidase activity, and nonspecific protein binding was blocked for 1 h with a solution containing 1% BSA and 5% goat serum. Sections for TGF- β ligand staining were also pretreated for 30 min with 1 mg/ml bovine testicular hyaluronidase (Sigma, Saint Louis, MO). Different anti-TGF- β 1 antibodies show different staining patterns [15] which likely correspond to different TGF- β 1 complexes. In order to account for this complexity, we employed two widely used anti-TGF- β 1 antibodies, one of which recognizes an intracellular form of TGF- β 1 and the other an extracellular form [15]. We evaluated immunohistochemical (IHC) expression of six

components of the TGF- β signaling pathway [intracellular-TGF- β 1, extra-cellular-TGF- β 1, TGF- β 2, TGF- β 3, TGF- β R2, and phosphorylated-SMAD2 (phospho-SMAD2)].

The entire staining was performed using primary rabbit polyclonal IgG antibodies which were applied for 2 h at room temperature. Antibodies recognizing intracellular (LC 1-30-1) and extracellular (CC 1-30-1) TGF- β 1 (applied at 5 and 0.25 μ g/ml, respectively) and TGF- β 3 (MPS 50-60-3) (applied at 1 μ g/ml) were prepared and used as described previously [15]. While CC 1-30-1 primarily picks up extracellular-TGF- β 1, there is some slight cross-reactivity with TGF- β 3 on Western blots [16]. All the other TGF- β antibodies are fully isoform specific. Affinity purified anti-TGF- β 2 (catalog no.sc-90) and anti-TGF- β R2 (catalog no.sc-220), were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and were used at 2.7 and 1 μ g/ml, respectively. The phospho-SMAD2 antibody, directed against the C-terminal phosphorylation sites, was used at a concentration of 1 μ g/ml (Millipore, Billerica, MA). Antigen-antibody complexes were detected using the Vectastain Elite rabbit ABC peroxidase kit (Vector Labs, Burlingame, CA) according to the manufacturer's instructions. After 30 min of incubation with the biotinylated secondary antibody followed by a 30-min incubation with ABC reagent, a 5-min reaction with diaminobenzidine/H₂O₂ was used to detect bound peroxidase. Sections were counterstained with Carazzi's hematoxylin. Slides were subsequently scanned using Aperio scanscope T2, and images were scored using AperioTMA Lab software version 8.2.1.1265.

Three pathologists independently scored two of the six stains. Intracellular-TGF- β 1 was a cytoplasmic mostly epithelial stain with some stromal and inflammatory cell staining; extracellular-TGF- β 1 was a mostly stromal stain; TGF- β 2 and TGF- β 3 were cytoplasmic epithelial stains; TGF- β R2 was a cytoplasmic and membranous stain; and phospho-SMAD2 was mostly a nuclear epithelial stain. Stains for extracellular-TGF- β 1, TGF- β R2, and phospho-SMAD2 were scored as negative, equivocal, weakly positive, or strongly positive; intracellular-TGF- β 1, TGF- β 2, and TGF- β 3 were scored as negative, equivocal, or positive. For all analyses except correlations, scores were dichotomized as negative (including equivocal) and positive (including weak and strong staining). For quality control purposes, 40 randomly selected spots were re-examined by the pathologists. Intra-observer agreement for all stains was satisfactory (weighted kappa = 75%). Six-hundred and twenty-three cases (74%) of the 842 in TMA, had expression data for all six of the TGF- β markers; 83 (10%) cases had expression data for five TGF- β markers; 18 (2%) had expression data for four; 25 (3%) had expression data for three; 13 (1%) had expression data for two; 47 (6%) had expression data for one; and 33 (4%) had expression data for none of the TGF- β markers of interest.

Statistical analysis

For tumors with two cores, we determined the maximum and average scores. Results for average and maximum scores showed similar patterns; we present results for maximum values to avoid redundancy. For tumors with a single satisfactory core, this result was used. Distributions of protein expression for each TGF- β marker were assessed among cases by tumor characteristics including size (≤ 2 cm, and >2 cm), histologic type (ductal, lobular), and grade (well, moderately, and poorly differentiated). Results were related to staining

intensity (0 = negative, 1 = weak, 2 = intermediate, and 3 = strong) and percentage of cells stained (0–100%) for ER, PR, HER2, and EGFR as previously reported [14]. ER and PR were classified as positive if the product of intensity and percentage was >10. We classified tumors as HER2-positive if intermediate or strong staining was identified in at least 20% of tumor cells, while tumors positive for EGFR were considered positive if any tumor cells were stained positive, as previously reported [14]. Further analyses with a more stringent definition of HER2 were performed, but did not change interpretation of results. Comparisons across strata were assessed by χ^2 . We assessed correlation between TGF- β markers and ER, PR, and HER2 markers using the Spearman rank correlation test. In order to evaluate which of several correlated tumor features were the most important in determining TGF- β IHC associations, we fitted logistic regression models with the TGF- β IHC expression as the outcome variable, and other tumor features as explanatory variables.

Associations between TGF- β IHC and age at diagnosis were assessed by comparing the percentage of positive tumors across age categories using a chi-squared or Fisher's exact test (when any cells had less than 5 counts), and a *t* test comparing the mean age for positive and negative tumors. Unless otherwise specified, statistical analyses were performed with STATA Version 9.1, Special Edition (STATA Corporation, College Station, Texas).

In order to estimate the age-incidence rates by ER and TGF- β markers, the number of incident breast cancer cases in the female populations of Warsaw and Łódź was estimated for each of ten 5-year age groups (25–29 to 70–74) as the total cases participating in the study divided by participation rate (number of participants divided by number of eligible women). We made use of ten 5-year age groups (25–29, 30–34, ...70–74) and plotted age-specific incidence rates on log Y and linear X scales such that a slope of 10 degrees approximated a change in rates of 1% per year of age [17]. Poisson regression models were used for examining the impact of biomarker expression, such as SMAD2-positive versus SMAD2-negative, on age-specific incidence rates, as previously described [18]. Under the null hypothesis of no difference in age-related effects with tumor marker expression, the age-specific incidence rate curves for positive and/or negative expression would be parallel on the log scale.

Results

Characteristics of cases

The median age of cases in this analysis was 54 years with 67% being postmenopausal at diagnosis, a proportion similar to that of the entire study population (data not shown). Compared with tumors not included in this study, tumors included in this analysis were modestly larger (50% of tumors evaluated measured >2 cm compared with 41% of those not analyzed; $P < 0.001$) and were more frequently associated with axillary lymph node metastases (44% of tumors analyzed compared with 34% of those not included; $P < 0.001$) (data not shown). The distributions of histologic type, grade, and ER, PR, HER2, and EGFR status for tumors in this analysis resembled those of the full study (data not shown [14]). In our study population, we had 197 (32%) cases that were ER-negative and 425 (68%) cases that were ER-positive (Table 1).

Expression of TGF- β pathway by pathologic features and expression of other markers

Staining images from the six tumor markers from one representative TMA tumor core are presented in Supplemental Fig. 1. Most tumors were positive for extracellular-TGF- β 1 (78%), TGF- β 2 (91%), TGF- β 3 (93%), and TGF- β R2 (72%), whereas expression of intracellular-TGF- β 1 was detected in 32% and phospho-SMAD2 in 61% of cases (Table 2). As expected, expression of ER and PR were directly correlated with each other and inversely with HER2 and EGFR (Supplemental Table 1). The TGF- β markers were significantly correlated with each other, while extracellular-TGF- β 1 and TGF- β 3 were positively correlated with ER (P value = 0.0316 and 0.0118, respectively), and extracellular-TGF- β 1 and TGF- β 2 were positively correlated with PR (P = 0.0088 and 0.0413, respectively). In particular, extracellular-TGF- β 1 and TGF- β 2 were highly correlated with phospho-SMAD2, suggesting that at least some of the TGF- β detected in this study is bioactive and eliciting a downstream signaling response in the tumor parenchyma. None of the other TGF-beta markers was significantly correlated with ER, PR, HER2 or EGFR expression, except for intracellular-TGF- β 1 and TGF β R2 with EGFR. (Supplemental Table 1).

In univariate analyses, expression of TGF- β ligands was variably associated with several correlated features of good prognosis, including: tumor size $\geq$ 2 cm (TGF- β 2 and TGF- β 3); lobular histology (extracellular-TGF- β 1 and TGF- β 2); well or moderate differentiation (extracellular-TGF- β 1, TGF- β 2, and TGF- β 3); and ER-positive and/or PR-positive status (extracellular-TGF- β 1 and TGF- β 2). TGF- β R2 was associated with smaller tumors and phospho-SMAD2 was significantly associated with positive nodal status. Although many of these relationships were statistically significant, the magnitude of differences between compared groups was generally small.

TGF- β marker expression by age at diagnosis

Intracellular-TGF- β 1-positive tumors were associated with older age (Table 3, P = 0.06). In contrast, expression of extracellular-TGF- β 1, TGF- β R2, and phospho-SMAD2 was strongly associated with early age at onset (P = 0.005, 8.2E-11, and 1.3E-8, respectively). The decline in the frequency of TGF- β R2-positive tumors with age was monotonic with 88% of tumors scored as positive among women younger than 40 years as compared to 50% of tumors among women 70 years or older (Table 3). A similar trend was identified for phospho-SMAD2; 84% of tumors among women less than 40 were positive compared to 58% of tumors among women 70 years and older (Table 3). As would be expected, the three markers that showed decreased expression among older cases were also significantly less frequently expressed among post- versus premenopausal women (data not shown). Stratified analyses showed fairly similar associations for ER-negative and ER-positive cancers, although that significant associations between TGF- β R2 expression and small tumor size (P = 0.002) and between phospho-SMAD2 expression and positive node status (P = 0.03) were restricted to ER-negative tumors (Supplemental Tables 2 and 3).

In order to identify pathologic features that are independently associated with expression of TGF- β markers, we analyzed logistic regression models, adjusting for age, histologic type, grade, tumor size, nodal status, and ER status. These models revealed intracellular-TGF- β 1-

positive tumors were associated only with older age ($P = 0.03$) while extracellular-TGF- β 1-positive tumors were significantly associated with ER-positive tumors ($P = 0.04$) and younger age ($P = 0.003$). TGF- β 2 expression was significantly related to smaller tumor size ($P = 0.02$). The TGF- β 3 expression was significantly lower among poorly differentiated tumors compared to those that were well or moderately differentiated ($P = 0.0002$). The TGF- β 2 expression was associated with smaller tumors ($P = 0.01$) and younger age ($P = 2.06 \times 10^{-8}$). Finally, phospho-SMAD2 expression was associated with younger age ($P = 7.17 \times 10^{-7}$) and positive nodal status ($P = 0.03$) (data not shown).

Age-specific incidence rate curves for ER, extracellular-TGF- β 1, TGF- β 2, and phospho-SMAD2

Age-specific incidence rates for breast cases in our population overall increased rapidly until the age of approximately 50 years, and then continued to rise at a slower rate (data not shown [19]). Age-specific incidence rates for ER-positive tumors mirrored the overall age-specific incidence rate pattern, whereas rates for ER-negative tumors plateau around age 50 years. The age-incidence patterns demonstrated significant age-related effects by ER expression (Fig. 1a) ($P = 4.77 \times 10^{-3}$ for null hypothesis of no different age-related effects by ER expression). Similarly, phospho-SMAD2 positive and negative tumors also showed differences in age-specific incidence rates (Fig. 1b, $P = 1.37 \times 10^{-11}$). Differences in age-specific rates by phospho-SMAD2 status were similar for ER-positive and ER-negative tumors (Fig. 1c, ER-positive tumors $P = 4.39 \times 10^{-9}$; Fig. 1d, ER-negative tumors $P = 1.00 \times 10^{-3}$). Age-specific incidence rate patterns for extracellular-TGF- β 1-positive and TGF- β 2-positive tumors were similar to phospho-SMAD2-positive cancers (data not shown).

Discussion

Our analysis of data from a large population-based case–control study provides evidence that TGF- β signaling patterns vary by age and pathologic features, including ER status. A key strength of this study was the simultaneous analysis of multiple markers on this complex signaling pathway in a relatively large number of tumors. Although several of the TGF- β pathway members that we analyzed were expressed by a high percentage of tumors, as previously suggested [8, 9, 20–23], we, nonetheless, found significant heterogeneity in the relationships between expression of individual markers and the clinical and pathological features of cases as well as age.

We identified contrasting relationships with pathologic features, although these disparities were generally small. Extracellular-TGF- β 1 expression was associated with ER-positive status, consistent with previous reports [24, 25] despite differences in the TGF- β 1 assays employed (including enzyme-linked immunosorbent assays). However, we did not confirm the prior finding that TGF- β 1 expression is more frequent in tumors with lymph node metastases although the percentage of extracellular-TGF- β 1-positive tumors was similar to the previous report [24]. We found extracellular-TGF- β 1, TGF- β 2, TGF- β 3, and TGF- β 2 were generally associated with several favorable prognostic features. TGF- β 2 expression was associated with small tumor size, low grade, and expression of and PR-positive status as suggested previously [26]. Similar to prior reports, TGF- β 2 was more strongly associated

with lobular histology. TGF- β 3 was also associated with several good prognostic features including small tumor size, low grade, and expression of ER-positive tumors. TGF- β 2 was associated with smaller tumors; however, this relationship was restricted to ER-negative tumors. In contrast, expression of phospho-SMAD2 was related to a higher frequency of lymph node metastasis among ER-negative tumors only. This last observation is consistent with previous observations that TGF- β pathway signaling is related to adverse clinical behavior among ER-negative tumors only [9, 10]. The expression of TGF- β 1, TGF- β 2, and TGF- β 3 was associated with ER-positive tumors showing less aggressive pathologic features with regard to size and grade, perhaps reflecting a prior effect to retard tumor development prior to clinical presentation. The heterogeneity of these associations provide evidence for multiple, complex biologic effects, and the modification of these relationships by ER status, supporting the view that the effects of TGF- β are interdependent on other tumor characteristics.

The frequency of expression of extracellular-TGF β 1, TGF- β 2 and phospho-SMAD2 was significantly higher among younger or premenopausal women compared to older or postmenopausal patients. The heterogeneity in expression of these three markers by age is a novel finding from this study and remained strongly significant after adjustment for other important tumor features. These findings are supported by a prior report that used enzyme-immunoassay to measure TGF- β 1 in human breast tumors, where higher values were seen among premenopausal compared to postmenopausal women [21]. Consequent to these expression patterns, the age-specific incidence rate patterns for the positive expression of three TGF- β markers (extracellular-TGF- β 1, TGF- β 2, and phospho-SMAD2) showed significant age-related differences that were independent of ER status. The age-related effects that we observed with phospho-SMAD2 in particular, provides evidence for biological differences of TGF- β effects that are non-uniform by age and not explained by ER expression. These observations may suggest that TGF- β effects may be a contributor to the findings seen in studies that suggest ER-positive tumors among younger women have a worse prognosis and are more difficult to treat than tumors with similar pathologic features among older women [27]. The potential tumor promoting or suppressing effects of TGF- β expression by ER and at younger and older ages warrant follow-up in further studies.

Our data show that all of the markers in the TGF- β pathway, apart from intracellular-TGF- β 1, generally showed strongly correlated expression. The relationship between extracellular-TGF- β 1 and phospho-SMAD2 being stronger than for intracellular-TGF- β 1 may suggest that the detection of the extracellular form is more relevant for pathway activation. Although SMAD2 can also be activated by other TGF- β superfamily members such as the activins [28], the strong correlation of phospho-SMAD2 with the TGF- β ligands and TGF- β 2 suggests that the TGF- β s are important drivers of SMAD2 activation in breast cancer. Further study to confirm and extend these results would be useful and may require the application of novel assays.

Strengths of our study include its relatively large sample size, population basis, comprehensive analysis of the main components of the TGF- β pathway and detailed pathologic molecular marker data. Given that immunohistochemical detection of TGF- β does not necessarily demonstrate bio-availability, the comprehensive analysis of the

pathway components including phospho-SMAD2 expression is another strength of this project. However, limitations include our inability to quantify levels of TGF- β ligands, and the inability of the current immunohistochemical methods to distinguish between biologically active and inactive forms of the ligands. In addition, although the study was population based, the tumors on TMAs tended to originate from larger tumors, thus not being totally representative of the source populations. Finally, although this is one of the largest studies performed to date evaluating the TGF- β pathway and breast cancer, all of these analyses would benefit from larger numbers and confirmation in different populations to establish generalizability.

In summary, expression of the TGF- β pathway showed striking age-specific expression patterns in invasive breast tumors, raising important questions about its potential role in age-related differences in breast cancer biology and differences by to pathologic features. Further examination of these findings in studies of clinical outcomes accounting for age, ER status, and treatment are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Pardali K, Moustakas A. Actions of TGF-beta as tumor suppressor and pro-metastatic factor in human cancer. *Biochim Biophys Acta*. 2007; 1775:21–62. [PubMed: 16904831]
2. Tan AR, Alexe G, Reiss M. Transforming growth factor-beta signaling: emerging stem cell target in metastatic breast cancer? *Breast Cancer Res Treat*. 2009; 115:453–495. [PubMed: 18841463]
3. Massague J. TGFbeta in cancer. *Cell*. 2008; 134:215–230. [PubMed: 18662538]
4. ten Dijke P, Arthur HM. Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol*. 2007; 8:857–869. [PubMed: 17895899]
5. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet*. 2001; 29:117–129. [PubMed: 11586292]
6. Wakefield LM, Roberts AB. TGF-beta signaling: positive and negative effects on tumorigenesis. *Curr Opin Genet Dev*. 2002; 12:22–29. [PubMed: 11790550]
7. Gobbi H, Dupont WD, Simpson JF, et al. Transforming growth factor-beta and breast cancer risk in women with mammary epithelial hyperplasia. *J Natl Cancer Inst*. 1999; 91:2096–2101. [PubMed: 10601380]
8. Gobbi H, Arteaga CL, Jensen RA, et al. Loss of expression of transforming growth factor beta type II receptor correlates with high tumour grade in human breast in situ and invasive carcinomas. *Histopathology*. 2000; 36:168–177. [PubMed: 10672063]
9. Buck MB, Fritz P, Dippon J, et al. Prognostic significance of transforming growth factor beta receptor II in estrogen receptor-negative breast cancer patients. *Clin Cancer Res*. 2004; 10:491–498. [PubMed: 14760070]
10. Padua D, Zhang XH, Wang Q, et al. TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. *Cell*. 2008; 133:66–77. [PubMed: 18394990]
11. Anderson WF, Jatoi I, Sherman ME. Qualitative age interactions in breast cancer studies: “Mind the gap”. *J Clin Oncol*. 2009 in press.
12. Garcia-Closas M, Brinton LA, Lissowska J, et al. Established breast cancer risk factors by clinically important tumour characteristics. *Br J Cancer*. 2006; 95:123–129. [PubMed: 16755295]
13. Sherman ME, Rimm DL, Yang XR, et al. Variation in breast cancer hormone receptor and HER2 levels by etiologic factors: a population-based analysis. *Int J Cancer*. 2007; 121:1079–1085. [PubMed: 17487843]

14. Yang XR, Sherman ME, Rimm DL, et al. Differences in risk factors for breast cancer molecular subtypes in a population-based study. *Cancer Epidemiol Biomark Prev.* 2007; 16:439–443.
15. Flanders KC, Thompson NL, Cissel DS, et al. Transforming growth factor-beta 1: histochemical localization with antibodies to different epitopes. *J Cell Biol.* 1989; 108:653–660. [PubMed: 2465297]
16. McCune BK, Mullin BR, Flanders KC, et al. Localization of transforming growth factor-beta isotypes in lesions of the human breast. *Hum Pathol.* 1992; 23:13–20. [PubMed: 1544664]
17. Devesa SS, Donaldson J, Fears T. Graphical presentation of trends in rates. *Am J Epidemiol.* 1995; 141:300–304. [PubMed: 7840107]
18. Anderson WF, Rosenberg PS, Menashe I, et al. Age-related crossover in breast cancer incidence rates between black and white ethnic groups. *J Natl Cancer Inst.* 2008; 100:1804–1814. [PubMed: 19066264]
19. Anderson WF, Matsuno RK, Sherman ME, et al. Estimating age-specific breast cancer risks: a descriptive tool to identify age interactions. *Cancer Causes Control.* 2007; 18:439–447. [PubMed: 17216325]
20. Barlow J, Yandell D, Weaver D, et al. Higher stromal expression of transforming growth factor-beta type II receptors is associated with poorer prognosis breast tumors. *Breast Cancer Res Treat.* 2003; 79:149–159. [PubMed: 12825850]
21. Desruisseau S, Palmari J, Giusti C, et al. Determination of TGFbeta1 protein level in human primary breast cancers and its relationship with survival. *Br J Cancer.* 2006; 94:239–246. [PubMed: 16404434]
22. Gorsch SM, Memoli VA, Stukel TA, et al. Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer. *Cancer Res.* 1992; 52:6949–6952. [PubMed: 1458485]
23. Xie W, Mertens JC, Reiss DJ, et al. Alterations of Smad signaling in human breast carcinoma are associated with poor outcome: a tissue microarray study. *Cancer Res.* 2002; 62:497–505. [PubMed: 11809701]
24. Ghellal A, Li C, Hayes M, et al. Prognostic significance of TGF beta 1 and TGF beta 3 in human breast carcinoma. *Anti-cancer Res.* 2000; 20:4413–4418.
25. Mu L, Katsaros D, Lu L, et al. TGF-beta1 genotype and phenotype in breast cancer and their associations with IGFs and patient survival. *Br J Cancer.* 2008; 99:1357–1363. [PubMed: 18827819]
26. Soufla G, Porichis F, Sourvinos G, et al. Transcriptional deregulation of VEGF, FGF2, TGF-beta1, 2, 3 and cognate receptors in breast tumorigenesis. *Cancer Lett.* 2006; 235:100–113. [PubMed: 15949894]
27. Goldhirsch A, Gelber RD, Yothers G, et al. Adjuvant therapy for very young women with breast cancer: need for tailored treatments. *J Natl Cancer Inst Monogr.* 2001:44–51. [PubMed: 11773291]
28. Jeruss JS, Santiago JY, Woodruff TK. Localization of activin and inhibin subunits, receptors and SMADs in the mouse mammary gland. *Mol Cell Endocrinol.* 2003; 203:185–196. [PubMed: 12782414]

Figure 1.

Age-specific incidence rates for phospho-SMAD2 and ER expression among breast cancer cases in Poland. * Under the null hypothesis of no age-related effects by tumor marker, the age-specific incidence rate curves for positive and/or negative expression would be parallel on the log scale. Age-incidence rates and 95% confidence intervals are presented for: **a** ER-positive and negative tumors; **b** phospho-SMAD2 positive and negative tumors; **c** phospho-SMAD2 positive and negative tumors among ER-positive tumors only; and **d** phospho-SMAD2 positive and negative tumors among ER-negative tumors only

Table 1

Clinically important tumor characteristics among 623 breast cancer cases evaluated for TGF- β markers in the Polish breast cancer study

	<i>N</i>	%
Tumor size <i>N</i> = 621		
2 cm	311	50
>2 cm	310	50
Histological type <i>N</i> = 608		
Ductal	507	83
Lobular	101	17
Tumor grade <i>N</i> = 623		
Well differentiated	111	18
Moderately differentiated	347	56
Poorly differentiated	165	26
Axillary node metastasis <i>N</i> = 610		
Negative	344	56
Positive	266	44
ER <i>N</i> = 622		
Negative	197	32
Positive	425	68
PR <i>N</i> = 622		
Negative	291	47
Positive	331	53
HER2 <i>N</i> = 622		
Negative	552	89
Positive	70	11
EGFR <i>N</i> = 621		
Negative	511	82
Positive	110	18

Expression of TGF- β markers by clinically important tumor characteristics among 623 breast cancer cases participating in the Polish Breast Cancer Study

Table 2

	Intracellular-TGF- β 1			Extracellular-TGF- β 1			TGF- β 2			TGF- β 3			TGF- β R2			Phospho-SMAD2		
	N	%	P value	N	%	P value	N	%	P value	N	%	P value	N	%	P value	N	%	P value
Positive	198	32		485	78		564	91		580	93		450	72		378	61	
Tumor size N = 621																		
2 cm	94	30		244	78		289	93		298	96		242	78		191	61	
>2 cm	103	33	0.42	239	77	0.68	273	88	0.04	280	90	0.01	206	66	0.002	186	60	0.72
Histological type N = 608																		
Ductal	157	31		383	76		452	89		470	93		370	73		309	61	
Lobular	33	33	0.74	89	88	0.01	98	97	0.01	95	94	0.63	67	66	0.18	62	61	0.93
Tumor grade N = 623																		
Well differentiated	37	31		93	80		104	92		110	98		78	76		65	60	
Moderately differentiated	95	33		212	77		248	91		259	95		182	71		148	59	
Poorly differentiated	86	34	0.91	178	71	0.01	217	86	0.02	211	85	0.001	156	69	0.44	144	60	0.73
Axillary node metastasis = 610																		
Negative	108	31		264	77		306	89		319	93		240	70		196	57	
Positive	88	33	0.66	211	79	0.45	245	92	0.19	248	93	0.81	197	74	0.24	174	65	0.03
ER N = 622																		
Negative	62	31		143	73		172	87		176	89		145	74		129	65	
Positive	135	32	0.94	342	80	0.03	391	92	0.06	403	95	0.01	305	72	0.63	248	58	0.09
PR N = 622																		
Negative	90	31		213	73		256	88		265	91		204	70		173	59	
Positive	107	32	0.71	272	82	0.01	307	93	0.04	314	95	0.06	246	74	0.24	204	62	0.58
HER2 N = 622																		
Negative	175	32		432	78		497	90		515	93		398	72		328	59	
Positive	22	31	0.96	53	76	0.63	66	94	0.25	64	91	0.56	52	74	0.70	49	70	0.09
EGFR N = 621																		
Negative	152	30		401	78		463	91		478	94		365	71		302	59	
Positive	45	41	0.02	83	75	0.49	99	90	0.84	100	91	0.32	84	76	0.29	74	67	0.11

%, Percent positive tumors

P value for χ^2 test

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Table 3
Expression of TGF-β markers by age among 623 breast cancer cases participating in the Polish Breast Cancer Study

	Intracellular-TGF-β1			Extracellular-TGF-β1			TGF-β2			TGF-β3			TGF-βR2			Phospho-SMAD2		
	N	%	P value	N	%	P value	N	%	P value	N	%	P value	N	%	P value	N	%	P value
Overall Positive	198	32		485	78		564	91		580	93		450	72		378	61	
Age																		
<40 (N = 32)	7	22		28	88		27	84		31	97		28	88		27	84	
40–49 (N = 170)	43	25		142	84		152	89		159	94		138	81		120	71	
50–59 (N = 213)	80	38		170	80		198	93		203	95		164	77		135	63	
60–69 (N = 144)	41	28		94	65		126	88		130	90		88	61		59	41	
70+ (N = 64)	27	42	0.04*	51	80	0.002*	61	95	0.16**	57	89	0.22**	32	50	2.31E-07**	37	58	7.53E-08*
Age (mean)																		
Negative	54.5			57.2			53.4			58.2			59.4			57.8		
Positive	56.2		0.06***	54.5		0.005***	55.2		0.20***	54.8		0.05***	53.4		8.24E-11***	53.3		1.33E-08***

%, percent positive tumors

* P value for χ^2 test
** P value for Fisher's exact test
*** P value for t test