

## Short Communication

# Genetic Polymorphisms of the *Transforming Growth Factor-β1* Gene and Breast Cancer Risk: A Possible Dual Role at Different Cancer Stages

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## Abstract

Transforming growth factor-β (TGF-β) inhibits the proliferation of carcinomas in early stages of breast cancer, whereas it promotes tumor growth and metastasis in later stages of cancer. We evaluated a possible association between *TGF-β1* gene polymorphisms and breast cancer risk in a population-based case-control study of Chinese women living in Shanghai, which included 1,127 breast cancer cases and 1,228 population controls. Two polymorphisms, C-509T and T+29C, were in strong linkage disequilibrium. There were no overall differences in the genotype distribution of T+29C polymorphisms of the *TGF-β1* gene among cases and controls. However, the distribution of the high-activity C allele of T+29C polymorphisms differed by cancer stages ( $P_{\text{trend}} = 0.02$ ). This allele was associated with decreased risk

of early-stage breast cancer [stages 0 and I; odds ratio (OR), 0.73; 95% confidence interval (95% CI), 0.54-0.99], and the OR was further reduced to 0.66 (95% CI, 0.45-0.96) for those homozygous for this allele ( $P_{\text{trend}} = 0.03$ ). On the other hand, the same allele was associated with nonsignificantly increased risk of breast cancer with advanced stages III and IV (OR, 1.33; 95% CI, 0.81-2.18), which differed significantly from that observed for early-stage cancer ( $P = 0.04$ ). This result suggests a possible dual effect of *TGF-β1* shown by *in vitro* experiments and provides an explanation for some of the inconsistent findings from previous epidemiologic studies that did not evaluate this association by cancer stage. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1567-70)

## Introduction

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that regulates cellular processes such as cell division, differentiation, motility, adhesion, and death (1). It has been shown that TGF-β is a potent inhibitor for cell cycle progression of epithelial cells (2). In early stages of breast cancer, TGF-β inhibits the proliferation of carcinomas (3). TGF-β also mediates the cytostatic and chemopreventive actions of antiestrogens, such as tamoxifen (4). In later stages of cancer, however, the role of TGF-β shifts to that of a tumor promoter, suggesting a dual role for TGF-β during the progression of cancer (3). TGF-β1 is the predominant isoform of TGF-β in mammary epithelial areas of breast cancer (5), and increased serum levels of TGF-β1 have been related to lymph node metastasis, poor histology grades, and advanced cancer stage (6).

Two polymorphisms of the *TGF-β1* gene, T+29C at codon 10 and C-509T, are in strong linkage disequilibrium, and the T allele of C-509T and C allele of T+29C are associated with increased TGF-β1 serum levels (7, 8). The results of association studies on these polymorphisms and breast cancer risk, however, have been inconsistent. Two studies reported that the T allele of the T+29C polymorphism was associated with increased risk of breast cancer in Caucasian women ages 65

years or older (9) and in Japanese women (10). In contrast, a Korean study and a European study have both shown an increased risk of breast cancer in women carrying the C allele (11, 12), and four studies suggested no association between this polymorphism and breast cancer risk (13-16). Given the dual role of TGF-β1 in carcinogenesis, we hypothesized that the alleles associated with elevated TGF-β1 levels (T in C-509T and C in T+29C) may reduce the risk of early-stage breast cancer, while promoting the progression of late-stage breast cancer. We evaluated this hypothesis in the Shanghai Breast Cancer Study, a large population-based case-control study conducted among Chinese women in Shanghai.

## Materials and Methods

Detailed study design and data collection procedures for the Shanghai Breast Cancer Study have been described elsewhere (17). Briefly, cases were permanent Shanghai residents between the ages of 25 and 64 who were newly diagnosed with breast cancer between August 1996 and March 1998. Through a rapid case ascertainment system, supplemented by the population-based Shanghai Cancer Registry, 1,602 eligible breast cancer cases were identified and 1,459 (91.1%) completed in-person interviews using a structured questionnaire. The initial cancer diagnoses for all patients were confirmed by two senior pathologists through a review of pathologic slides. Information on clinical cancer characteristics, including tumor-node-metastasis stage, treatment for cancer, and estrogen and progesterone receptor status, was obtained by medical record review using a standard protocol. The major reasons for nonparticipation were refusal (109 cases, 6.8%), death before interview (17 cases, 1.1%), and inability to locate (17 cases, 1.1%).

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**Table 1. Association of *TGF-β1* gene polymorphisms with breast cancer risk, the Shanghai Breast Cancer Study**

Genotypes	Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
C-509T				
CC	260 (23.3)	260 (21.6)	1.0	1.0
CT/TT	858 (76.7)	946 (78.4)	0.91 (0.75-1.10)	0.90 (0.74-1.10)
CT	559 (50.0)	628 (52.0)	0.89 (0.72-1.09)	0.89 (0.72-1.10)
TT	299 (26.7)	318 (26.4)	0.94 (0.74-1.19)	0.93 (0.73-1.17)
T + 29C				
TT	258 (23.2)	255 (21.5)	1.0	1.0
TC/CC	856 (76.8)	934 (78.5)	0.91 (0.74-1.10)	0.90 (0.73-1.10)
TC	554 (49.7)	615 (51.7)	0.89 (0.72-1.10)	0.88 (0.71-1.09)
CC	302 (27.1)	319 (26.8)	0.94 (0.74-1.18)	0.92 (0.72-1.17)

\*Adjusted for age, education, age at menarche, menopausal status, age at menopause, age at first live birth, waist-to-hip ratio, and physical activity during the preceding 10 years.

Eligible controls were randomly selected from the Shanghai Resident Registry (which contains demographic information for all residents of urban Shanghai) and were frequency matched by age at 5-year intervals to the predetermined age distribution of the cases reported to the Shanghai Cancer Registry from 1990 to 1993. Of the 1,734 eligible controls, 1,556 (90.3%) completed interviews. The major reasons for nonparticipation of the eligible controls were refusal (166 controls, 9.6%) and death or a prior cancer diagnosis (2 controls, 0.1%).

The structured questionnaire used for this study included information on demographic factors, menstrual and reproductive history, hormone use, previous disease history, family history of cancer, physical activity, tobacco and alcohol use, and usual dietary habits. All participants were measured for current weight, circumferences of the waist and hips, and sitting and standing height. In addition to the in-person interviews and anthropometric measurements, 10 mL blood samples were collected from 1,193 (82%) cases and 1,310 (84%) controls. These samples were processed on the same day and stored at  $-70^{\circ}\text{C}$ .

Detailed genotyping methods for *TGF-β1* polymorphisms have been previously described (18). Briefly, genomic DNA was extracted from buffy coat fractions and used for genotyping assays with the PCR-RFLP method. The PCR was done in a PTC-200 Peltier Thermal Cycler (MJ Research, Inc., Waltham, MA). The PCR products were digested with restriction endonucleases to determine the genotype of each subject. For the C-509T polymorphism, the PCR products were digested with the *Eco81I* restriction endonucleases. For the T+29C polymorphism, the T→C substitution creates a *MspA1I* restriction site. The laboratory staff was blind to the identity of the subjects. Quality control samples were included in the genotyping assays. Each 96-well plate contained one water blank, two CEPH 1347-02 DNA, two unblinded quality control DNA, and two blinded quality control DNA. Quality control samples were distributed across separate 96-well plates. The blinded and unblinded quality control samples were taken from the second tube of samples included in the study. The agreement rates for C-509T polymorphism (50 quality control samples) and for T+29C polymorphism (51 quality control samples) were both 98%. Genotypes for polymorphisms of C-509T were successfully determined for 1,118 cases and 1,206 controls, and genotypes for T+29C polymorphisms were determined for 1,114 cases and 1,189 controls.

The  $\chi^2$  test and *t* test were used for comparing characteristics of cases and controls. Odds ratios (OR) and 95% confidence intervals (CI) were derived using unconditional logistic regression models and polytomous logistic models. All *P* values presented in this paper are two-sided. SAS software was used for statistical analysis (version 9.1; SAS Institute, Cary, NC).

## Results

The distribution of demographic characteristics and known breast cancer risk factors of the 1,127 cases and 1,228 controls was similar to our previous reports (data not shown; ref. 17). Reproductive risk factors, such as early menarche, late menopause, and late age at the first live birth, were related to increased breast cancer risk. Cases were also more likely than controls to have higher body mass index, waist-to-hip ratio, or to have had breast fibroadenomas, and were less likely to have exercised regularly during the past 10 years. The case-control difference was not statistically significant for age, education, menopausal status, or family history of breast cancer.

Table 1 shows the genotype distributions among cases and controls of the two polymorphisms of the *TGF-β1* gene under study. The frequencies of the CC, CT, and TT genotypes of C-509T were 21.6%, 55%, and 26.4% in controls, respectively. The frequencies of the TT, TC, and CC genotypes of T+29C were 21.5%, 51.7%, and 26.8% in controls, respectively. The genotype frequency of these two polymorphisms in controls did not deviate from the Hardy-Weinberg equilibrium and the two polymorphisms were in strong linkage disequilibrium ( $D' = 0.989$ ,  $P < 0.001$ ).

Overall, there was no association between polymorphisms and breast cancer risk. Further analyses stratified by age at disease onset, menopausal status, body mass index, and waist-to-hip ratio did not find any association between these polymorphisms and breast cancer risk (data not shown).

We stratified genotype frequencies by stage in Table 2. Among 1,127 cases, stage information for 77 patients (6.8%) was missing. Only 29 patients were stage 0; therefore, we combined those patients with stage I. A higher percentage of patients with advanced breast cancer carried the C allele in the T+29C polymorphism than patients with an earlier-stage cancer ( $P = 0.02$ ). In stages 0 and I, the C allele was associated with decreased breast cancer risk (OR, 0.73; 95% CI, 0.54-0.99) in a dose-response manner ( $P_{\text{trend}} = 0.03$ ), with the lowest OR observed for those homozygous for this allele (OR, 0.66; 95% CI, 0.45-0.96; Table 3). On the other hand, the same allele was associated with an increased risk in advanced stages III and IV of breast cancer (OR, 1.31; 95% CI, 0.80-2.15), although the OR was not statistically significant perhaps due to a small sample size. However, when the OR for the association of the C allele with early stage cancer (stages 0 and I) was compared with that for advanced stage cancer (III and IV), the difference was statistically significant ( $P = 0.04$ ).

## Discussion

Grainger et al. (7) showed that the serum concentration of *TGF-β1* was correlated with the C-509T polymorphism of the *TGF-β1* gene. The presence of the T allele at  $-509$  bp was

**Table 2. Association of the *TGF-β1* gene polymorphisms with breast cancer stage, the Shanghai Breast Cancer Study**

Genotypes	Stages			<i>P</i>
	0, I	IIa, IIb	III, IV	
C-509T				
<i>n</i>	279	640	122	
CC (%)	26.9	22.0	18.8	
CT (%)	50.5	50.3	52.5	
TT (%)	22.6	27.7	28.7	0.03
T+29C				
<i>n</i>	277	638	122	
TT (%)	26.7	21.9	17.2	
TC (%)	50.5	49.8	53.3	
CC (%)	22.7	28.2	29.5	0.02

**Table 3. Association of *TGF-β1* gene polymorphisms and breast cancer risk by breast cancer stage, the Shanghai Breast Cancer Study**

Genotypes	Controls (n)	Stage 0 or I (n)	Adjusted OR* (95% CI)	Stage II (n)	Adjusted OR* (95% CI)	Stage III or IV (n)	Adjusted OR* (95% CI)
C-509T							
CC	255	75	1.0	141	1.0	23	1.0
CT/TT	934	204	0.74 (0.54-0.99)	499	0.97 (0.76-1.23)	99	1.19 (0.74-1.93)
CT	615	141	0.77 (0.56-1.06)	322	0.95 (0.74-1.21)	64	1.18 (0.71-1.95)
TT	319	63	0.67 (0.46-0.98)	177	1.01 (0.76-1.33)	35	1.22 (0.70-2.13)
<i>P</i> <sub>trend</sub>			0.04		0.92		0.50
T+29C							
TT	255	74	1.0	140	1.0	21	1.0
TC/CC	934	203	0.73 (0.54-0.99) <sup>†</sup>	498	0.96 (0.76-1.22)	101	1.31 (0.80-2.15) <sup>†</sup>
TC	615	140	0.77 (0.56-1.07)	318	0.93 (0.73-1.20)	65	1.29 (0.77-2.17)
CC	319	63	0.66 (0.45-0.96)	180	1.01 (0.76-1.33)	36	1.34 (0.76-2.36)
<i>P</i> <sub>trend</sub>			0.03		0.92		0.35

\*Derived from polytomous logistic regression models adjusted for age, education, age at menarche, menopausal status, age at menopause, age at first live birth, waist-to-hip ratio, and physical activity during the preceding 10 years.

<sup>†</sup>The OR (1.31) for advanced cancer differs statistically significant from that (0.73) for early-stage cancer ( $P = 0.04$ ).

associated with higher serum concentrations of TGF-β1, and serum concentration is higher among individuals homozygous for T than among those who are heterozygous (7). T+29C polymorphism results in an amino acid change from leucine to proline in codon 10. The serum concentration of TGF-β1 has been shown to be significantly higher in individuals with the CC genotype than in those with the TC or TT genotypes (8, 19), and the amounts of TGF-β1 secreted from CMV-P (Pro)-transfected cells is consistently greater than from CMV-L (Leu)-transfected cells (12). However, these two polymorphisms are in strong linkage disequilibrium and it seems reasonable to consider T+29C a functional polymorphism because leucine, which possesses a hydrophobic aliphatic side chain, favors the formation of α-helices, whereas proline introduces breakage in the α-helical portion of the peptide backbone (8, 20).

TGF-β acts as a growth inhibitor through the down-regulation of genes involved in cellular proliferation, such as cyclin-dependent kinases, the retinoblastoma susceptibility product (pRB), the c-Myc oncoprotein, and the NF-κB/Rel family of transcription factors (21). Binding TGF-β to TGF-β receptors activates intracellular signal transducers, Sma and mothers against decapentaplegic homologue (SMAD; ref. 2). Once activated, SMAD proteins are translocated into the nucleus and regulate various pathways related to growth inhibition (1). In advanced stages of breast cancer, however, the TGF-β/SMAD signaling pathway is inhibited, resulting in resistance to TGF-β-induced growth inhibition (1, 22, 23). Tumor cells themselves also produce inactive TGF-β and plasmin. Plasmin, which is converted from plasminogen by urokinase-type plasminogen activator produced by advanced breast cancer cells, may activate latent TGF-β (4). Tumor-derived TGF-β may affect cell-to-cell and cell-to-substrate interaction, resulting in a tendency for invasion, angiogenesis, and metastasis. Tumor-derived TGF-β also may act as an immune suppressor and enhance the ability of tumor cells to escape immune surveillance (2, 4).

Our results may also explain some of the inconsistent findings from previous epidemiologic studies on *TGF-β1* gene polymorphisms and breast cancer risk. Ziv et al. (9) reported decreased breast cancer risk in women with the TT or TC genotypes of the T+29C polymorphism from a study in which 70% of breast cancer patients were stages 0 or I. Krippel et al. (14) reported that the C allele of T+29C was more common in patients with lymph node metastasis. For prostate cancer, Ewart-Toland et al. (24) reported an association of the T allele of the C-509T polymorphism with the risk of advanced-stage cancer, but not with the risk of early-stage cancer, suggesting this variant may play different roles over the course of prostate

carcinogenesis. In contrast, in a case-control study with 1,123 breast cancer cases and 2,314 controls, the authors found no difference in the association of *TGF-β1* gene polymorphism with cancer by various stages (15). Our previous report on breast cancer patient survival and *TGF-β1* gene polymorphisms showed that the C allele of the T+29C polymorphism was related to poorer disease-free survival (18). The 5-year disease-free survival rates were 85.2% and 77.5% for breast cancer patients who carried the TT and TC/CC genotypes, respectively ( $P < 0.01$ ; ref. 18). If the C allele of the T+29C polymorphism is responsible for the invasiveness and metastasis of breast cancer, via the promotion of TGF-β1 expression, it is reasonable to assume that patients with the C allele are more likely to experience recurrences or metastases, followed by poorer disease-free survival.

In conclusion, our results are in agreement with data from many *in vitro* experiments indicating that functional polymorphisms of the *TGF-β1* gene may play a possible dual role in breast carcinogenesis. Our findings also suggest that epidemiologic studies evaluating the association of *TGF-β1* gene polymorphisms with breast cancer risk need to take tumor stage into consideration.

## References

- Kretzschmar M. Transforming growth factor-β and breast cancer: Transforming growth factor-β/SMAD signaling defects and cancer. *Breast Cancer Res* 2000;2:107-15.
- Yue J, Mulder KM. Transforming growth factor-β signal transduction in epithelial cells. *Pharmacol Ther* 2001;91:1-34.
- Benson JR. Role of transforming growth factor β in breast carcinogenesis. *Lancet Oncol* 2004;5:229-39.
- Reiss M, Barcellos-Hoff MH. Transforming growth factor-β in breast cancer: a working hypothesis. *Breast Cancer Res Treat* 1997;45:81-95.
- Gorsch SM, Memoli VA, Stukel TA, Gold LJ, Arrick BA. Immunohistochemical staining for transforming growth factor β1 associates with disease progression in human breast cancer. *Cancer Res* 1992;52:6949-52.
- Sheen-Chen SM, Chen HS, Sheen CW, Eng HL, Chen WJ. Serum levels of transforming growth factor β1 in patients with breast cancer. *Arch Surg* 2001;136:937-40.
- Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type β1. *Hum Mol Genet* 1999; 8:93-7.
- Yokota M, Ichihara S, Lin TL, Nakashima N, Yamada Y. Association of a T29-C polymorphism of the transforming growth factor-β1 gene with genetic susceptibility to myocardial infarction in Japanese. *Circulation* 2000;101: 2783-87.
- Ziv E, Cauley J, Morin PA, Saiz R, Browner WS. Association between the T29-C polymorphism in the transforming growth factor β1 gene and breast cancer among elderly white women: The Study of Osteoporotic Fractures. *JAMA* 2001;285:2859-63.
- Hishida A, Iwata H, Hamajima N, et al. Transforming growth factor B1 T29C polymorphism and breast cancer risk in Japanese women. *Breast Cancer* 2003;10:63-9.

11. Lee KM, Park SK, Hamajima N, et al. Genetic Polymorphisms of *TGF-β1* & *TNF-β* and Breast Cancer Risk. *Breast Cancer Res Treat.* 2005;90:149–55.
12. Dunning AM, Ellis PD, McBride S, et al. A transforming growth factor β1 signal peptide variant increases secretion *in vitro* and is associated with increased incidence of invasive breast cancer. *Cancer Res* 2003;63:2610–5.
13. Jin Q, Hemminki K, Grzybowska E, et al. A. Polymorphisms and haplotype structures in genes for *transforming growth factor-β1* and its receptors in familial and unselected breast cancers. *Int J Cancer* 2004;112:94–9.
14. Krippel P, Langsenlehner U, Renner W, et al. The L10P polymorphism of the *transforming growth factor-β1* gene is not associated with breast cancer risk. *Cancer Lett* 2003;201:181–4.
15. Le Marchand L, Haiman CA, van den BD, Wilkens LR, Kolonel LN, Henderson BE. T29C polymorphism in the *transforming growth factor β1* gene and postmenopausal breast cancer risk: the Multiethnic Cohort Study. *Cancer Epidemiol Biomarkers Prev* 2004;13:412–5.
16. Sigurdson AJ, Hauptmann M, Chatterjee N, et al. Kin-cohort estimates for familial breast cancer risk in relation to variants in DNA base excision repair, BRCA1 interacting and growth factor genes. *BMC Cancer* 2004;4:9.
17. Gao YT, Shu XO, Dai Q, et al. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. *Int J Cancer* 2000;87:295–300.
18. Shu XO, Gao YT, Cai Q, et al. Genetic polymorphisms in the *TGF-β1* gene and breast cancer survival: a report from the Shanghai Breast Cancer Study. *Cancer Res* 2004;64:836–9.
19. Yamada Y, Miyauchi A, Goto J, et al. Association of a polymorphism of the *transforming growth factor-β1* gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. *J Bone Miner Res* 1998;13:1569–76.
20. Karvonen MK, Pesonen U, Koulu M, et al. Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. *Nat Med* 1998;4:1434–7.
21. Sovak MA, Arsura M, Zanieski G, Kavanagh KT, Sonenshein GE. The inhibitory effects of transforming growth factor β1 on breast cancer cell proliferation are mediated through regulation of aberrant nuclear factor-κB/Rel expression. *Cell Growth Differ* 1999;10:537–44.
22. Jeruss JS, Sturgis CD, Rademaker AW, Woodruff TK. Down-regulation of activin, activin receptors, and Smads in high-grade breast cancer. *Cancer Res* 2003;63:3783–90.
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.
24. Ewart-Toland A, Chan JM, Yuan J, Balmain A, Ma J. A gain of function *TGFB1* polymorphism may be associated with late stage prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2004;13:759–64.