Review article
UDC: 618.19-006:577.11:616-037

Arch Oncol 2006;14(3-4):122-5.
DOI: 10.2298/AOO0604122I

Role of transforming growth factor-β_1 in breast carcinogenesis

Vesna Ivanović, Koviljka Krtolica, Milena Krajnović, Bogomir Dimitrijević

Institute of Nuclear Sciences "Vinča", Laboratory for Radiobiology and Molecular Genetics, Belgrade, Serbia; Address correspondence to: Dr. Vesna Ivanović, 080 Laboratory for Radiobiology and Molecular Genetics, The Institute of Nuclear Sciences "Vinča", P.O. Box 522, 11001 Belgrade, Serbia; E mail: vesnai@EUnet.yu; The manuscript was received: 21.09.2006, Provisionally accepted: 23.10.2006, Accepted for publication: 27.10.2006

© 2007, Institute of Oncology Sremska Kamenica, Serbia

ABSTRACT

The main objective of this presentation is to review current knowledge regarding molecular mechanisms of Transforming Growth Factor-β_1 (TGF-β_1) action in breast carcinogenesis. In addition, our recent results will be presented on *TGF-β_1 gene polymorphism and its relationship to TGF-β_1 secretion in breast cancer (BC) patients. Special focus will be made on potential clinical applicability of TGF-β_1 as a putative diagnostic, prognostic or predictive tool in BC detection and treatment. TGF-β_1 has a complex multifunctional profile, with tumour suppressive effects in early stages of breast carcinogenesis, but progressive dominance of tumour promoting effects with transition to more advanced malignant states. Clarification of molecular mechanisms that control parallel processing of these opposing TGF-β_1 activities might suggest new approaches for shifting the balance in favour of net tumour suppression. Now, a major challenge remains in more precisely defining TGF-β_1 signalling pathways and their cancer-related alterations. Current dogma views human tumorigenesis as a molecular disruption of normal physiology through genetic, epigenetic, or somatic alterations. The genetic model offers biological plausibility to epidemiological studies that link the TGF-β_1 gene polymorphism, at codon 10 due to Leu^{10}Pro substitution in the signal peptide, with the risk of developing BC. The somatic mutations approach, provides an explanation for the TGF-β_1 overexpression in advanced BC through mutations acquired in the components of Smad-mediated TGF-β_1 signalling pathway. The available results indicate decreased TßRII (TGF-β_1 receptor-type II) expression, rare TßRII gene mutations, but no mutations in Smad2 and Smad4 genes, in advanced BC patients.*

KEYWORDS: *Receptors, Transforming Growth Factor beta; Breast Neoplasms; Tumor Markers, Biological; Prognosis*

INTRODUCTION

Despite increased awareness and earlier detection, large percentage of breast cancer (BC) diagnosed women die from metastatic disease each year. Furthermore, treatment regimens for fighting advanced disease have significant side effects including cardiotoxicity, neurotoxicity, and secondary cancers. A better understanding of BC biology and the mechanisms of drug therapies should allow for more selective and less toxic treatments(1). Due to the morbidity associated with chemotherapy, there is a demand for molecular markers that can provide a more accurate prognosis and predict response to therapy (2,3). At the present time Transforming Growth Factor-β_1 (TGF-β_1) is being evaluated as potential candidate for such biomarker, although its diagnostic role in BC has not been established yet (4). This communication covers literature survey on current knowledge regarding TGF-β_1 molecular mechanisms of action in breast carcinogenesis. In addition, some of the recent results from our laboratory will be presented. Special focus will be made on potential clinical applicability of TGF-β_1 as a putative diagnostic, prognostic or predictive tool in BC detection and treatment.

TGF-β_1 ACTS BOTH AS TUMOUR SUPRESSOR AND AS TUMOUR PROMOTER

TGF-β_1 has an important role in normal mammary biology as a potent regulator of mammary epithelial proliferation, mammary ductal and alveolar development, and postlactation involution of the mammary gland. The TGF-β_1 signalling pathways also have an important role in human mammary carcinogenesis revealing dual function of TGF-β_1 in this process (4). In healthy tissue, premalignant, and early-transformed states, TGF-β_1 might act mainly as an epithelial growth inhibitor. As cells progress along the neoplastic continuum, these regulatory mechanisms become compromised because of a loss of negative cell signalling or because of a fundamental change in the TGF-β_1 switch. The net result of these pathophysiological changes is a loss of growth inhibition and concomitant stimulation of growth promotion in the process of tumour progression. Consequently, tumours that are further advanced generally express more TGF-β_1 , which has been correlated with a more malignant phenotype and impaired clinical outcome. Therefore, a major challenge remains to precisely define TGF-β_1 molecular mechanisms of action in the process of carcinogenesis (5). Current dogma views human tumorigenesis as a molecular disruption of normal physiology through genetic, epigenetic, or somatic alterations (4-6). The genetic model offers biological plausibility to epidemiological studies that link *TGF-β_1* polymorphism with risk of developing breast cancer. There is grow-

ing evidence that common variants of *TGF-ß1* gene may affect the production, secretion, or activity of this cytokine. Now, five different *TGF-ß1* gene polymorphisms have been identified with respect to BC risk (7). Among these, the most extensively studied is the *TGF-ß1* gene polymorphism at codon 10 due to Leu^{10}Pro substitution in the signal peptide (8-10).

LEU^{10}PRO TGF-β1 POLYMORPHISM

Previous reports have analyzed relationship between the Leu^{10}Pro TGF-ß$_1$ polymorphism versus progressive BC stages or survival of BC patients. The obtained results indicate that the Pro^{10} homozygotes have an increased incidence of invasive BC (9) and significantly decreased BC patients' survival (10). In addition, increased plasma levels of TGF-ß$_1$ protein were observed in Pro^{10} homozygotes when compared to Leu^{10} homozygotes or Leu^{10}Pro heterozygotes in a general population (11). Moreover, Dunning and co-workers (9) have shown that the allele encoding Pro^{10} is associated with increased rates of TGF-ß$_1$ secretion in advanced BC patients. However, this and other studies (8) have excluded cases with early stages of BC whose genetic susceptibility might considerably contribute to the evaluation of plasma TGF-ß$_1$ as a prognostic factor for the Stage I/II disease.

Therefore, we have investigated the role of plasma TGF-ß$_1$ in prognosis of early (Stage I/II) BC patients and possible relevance of genetic variants that affect TGF-ß$_1$ production and secretion. Concentration of TGF-ß$_1$ in plasma was analyzed by the TGF-ß$_1$ receptor-type II (TβRII) -based TGF-ß$_1$ ELISA kit as previously described (12). Our results indicate that plasma TGF-β_1 levels of Stage I/II disease (mean value: 1.01 ± 0.16 ng/ml; range: 0.17-1.94 ng/ml; n=10; p> 0.1) tended to be unchanged with respect to normal donors (mean value: 1.45 ± 0.15 ng/ml; range 0.39-4.93 ng/ml; n=37). Based on clinical parameters obtained after surgery, we have selected three early stage patients presented in Table 1, one with low risk (Case 1) and two with high risk prognosis (Cases 2 and 3). DNA was isolated from their malignant tissue samples as well as from full blood of three healthy donors(HD) used as controls. PCR was used to amplify for the *TGF-ß1* gene fragment of 485 bp, including the exon 1 and neighbouring parts of the surrounding sequences as described in the Legend to Figure 1.

Table 1. A summary of data on Leu^{10}Pro polymorphism of *TGFß1* gene for three early stage BC patients relative to healthy donors (HD), as detected from DNA sequencing profiles illustrated in Figure 1

SUBJECT'S			CHARACTERISTICS				DNA POLYMORPHISM	
BC patient			Healthy donor					
number	prognosis			Menopausal status *		Age	*TGF*β*1* Leu^{10} Pro genotype	
	low risk	high risk		premn.	postmn.	(years)		zygocity
Case 1	+			+		40	Leu^{10}Pro	hetero
Case 2		+			+	55	Leu^{10}	homo
Case 3		+			+	75	Leu^{10}	homo
			HD 1		+	58	Leu^{10}	homo
			HD 2	+		51	Leu^{10}Pro	hetero
			HD 3		+	57	Leu^{10}Pro	hetero

* Abbreviations: premn., premenopausal; postmn., postmenopausal.

Sequencing profiles and the respective DNA sequences for the described gene fragment were almost identical for all of the above samples, indicating lack of mutation in this genetic locus. Exception was observed at nucleotide position 29 of the amplicon. As shown in Figure 1, our results reveal polymorphism at codon 10 due to Leu^{10}Pro substitution in the signal peptide of TGF-ß$_1$ with the two types of variants. Table 1 illustrates the distribution of *TGF-ß1* Leu^{10}Pro genotype in BC patients (n=3) and healthy donors (n=3), with respect to subjects characteristics.

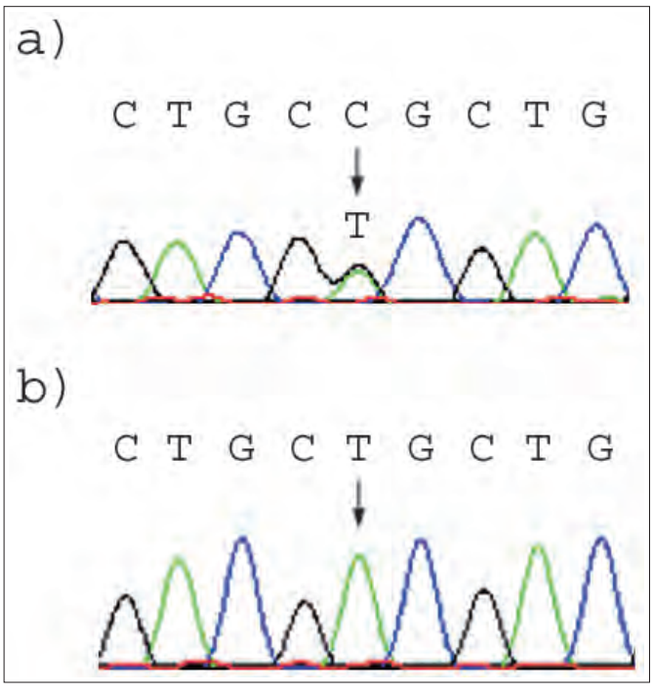

Figure 1. Sequence analysis illustrating presence of Leu^{10}Pro polymorphism in two BC patients with early stages of disease: Panel a, with arrow pointing double C/T peak at the 29 nucleotide position of codon 10, representing Leu^{10}Pro heterozygote (Case 1); and Panel b, arrow pointing a single T peak at the 29 nucleotide position of codon 10, representing Leu^{10} homozygote (Case 2). Genomic DNA was isolated from tumour tissue obtained after surgery. Polymorphic region of TGF-ß$_1$ / Exon 1, 485 bp in length, was then amplified using specific primers, whose sequences were as follows: 5' – TTCGCGCTCTCGGCAGTG – 3' (forward) and 5' – TTCTTCTGCCAGTCACTTCCTACCC – 3' (reverse). The PCR mixture contained 300 ng of genomic DNA, 0.2 mM dNTP, and 0.4μM of specific primers in PCR buffer solution in a 50μl final volume. Reaction was hot-started at 94°C for 5 min, 1.25 units of Taq polymerase was added and amplification carried out for 35 cycles (1 min at 94^{0}C, 1 min at 65^{0}C, and 1 min at 72°C, followed by a final extension for 10 min at 72°C). Ten μl of each PCR reaction was directly loaded onto 1.6% agarose gels, stained with ethidium bromide and visualised under UV illumination. PCR products were then purified and sequenced (DNA sequencer ABI 310, Applied Biosystems) with the same primers as those in PCR.

The data reveal one Leu^{10}Pro heterozygous and two Leu^{10} homozygous BC patients relative to one Leu^{10} homozygous HD and two Leu^{10}Pro heterozygous HD. Our results indicate the presence of Leu^{10}Pro or Leu^{10} variants in both stage I/II patients and healthy donors but not the Pro^{10} variant responsible for the increased TGF-β_1 secretion (9,11). The obtained genotype for an early BC and HD might explain unchanged TGF-β_1 secretion in plasma of these subjects. Although obtained on small number of subjects, our results suggest that plasma TGF- β_1 levels may not warrant it as useful biomarker for early BC stages.

TGF-β_1 SIGNALING PATHWAYS

During the past 4-5 years, there have been some important advances in the understanding of postreceptor signal transduction for TGF-ß$_1$ (5). Currently, two signalling mechanisms have been identified including the Smad-mediated TGF-ß$_1$ pathway and the mitogen-activated protein kinase (MAPK) pathway (4). Generally, these pathways are less complex than expected and involve finite number molecules including TGF-ß$_1$ ligand, TGF-ß receptors and intracellular mediators that convey signals directly from cell-surface receptors to gene transcription sites.

The molecular mechanism of Smad-mediated pathway has been completely elucidated. It involves TβRI, TβRII, Smad2, Smad3, and Smad4 as intracellular mediators in the following

cascade of events: The TGF-β_1 ligand binds to TβRII directly. Once bound to the ligand, TβRII recruits, binds, and transphosphorylates TβRI, thereby stimulating its protein kinase activity. The activated TβRI phosphorylates intracellular transducer Smad2 (or Smad3), which binds to Smad4. The resulting Smad complex translocates into the nucleus and interacts in a cell-specific manner with transcription factors to regulate specifically the transcription of a multitude of TGF-β-responsive genes. TGF-β_1 signalling is regulated by the level and duration of TβRII receptor activation (5).

Current evidence suggests that MAPK signalling pathway involves transcription factors such as c-FOS/c-JUN complexes, which mediate TGF-β_1 autoinduction (13). Other molecular details of the MAPK pathway are not elucidated yet (4). There are suggestions that activation of both Smad and MAPK pathways depends on the amount of input from the TβRII receptor and that decreased TβRII receptor expression changes the relative flux through the two parallel pathways. Moreover, it has been speculated that balance between TGF-$ß_1$ tumour suppressor and tumour promoter activities depend on crosstalk between Smad and MAPK pathways (4).

POTENTIAL CLINICAL APPLICABILITY OF TGF-$ß_1$ IN BREAST CANCER

The ability to define alterations in the TGF-$ß_1$ signalling pathways at a molecular level in an individual's tumour will allow the matching of targeted therapies developed for these alterations to make individualized cancer treatment a less toxic and more effective reality (5). Current findings suggest that selective cancer-specific somatic mutations of Smad-mediated signalling pathway might be responsible for the observed TGF-$ß_1$ overexpression in advanced stages of various malignancies. As examples, four of the most prevalent human cancers have been selected: BC, cancer of the prostate, lung , and colon with mutational analysis of their Smad-mediated components presented in Table 2. The data reveal that in BC, decreased TßRII receptor expression was observed as well as rare TßRII gene mutations, but no mutations in Smad2 and Smad4 genes were detected (Table 2).

Table 2. Mutation of Components of the Smad-mediated TGF-β_1 Signaling Pathway in Cancer (**)

Cancer	TβRII Gene	Smad2 Gene	Smad4 Gene
Breast	Frequently downregulated, rarely mutated	No mutations detected in ~100%	No mutations detected in ~ 100%
Prostate	TβRII protein not detected in 24%	No mutation detected in ~ 100%	No mutation detected in ~ 100%
Lung	Frequently downregulated, rarely mutated	Mutated in 2%	Mutated in 7%
Colon	Mutated in 58-82%	Mutated in 6%	Mutated in 20%

** Modiffied from Elliott and Blobe (5)

Likewise, in prostate cancer (another hormonal tumour), complete loss of TßRII protein was observed in 24% cases and also no mutations in Smad2 and Smad4 genes were detected (Table 2). Whereas, in lung cancer the decreased expression of TβRII receptor and mutations of the Smad2 (2%) and Smad4 (7%) genes were observed. In contrast, colon cancer reveals selective mutations of the TβRII receptor, which result in a non-functional receptor in 58-82% of the cases and mutated/partially inactivating Smad2 (6%) and mutated or deleted Smad4 (20%) genes (Table 2). Thus, in the clinical scenarios involving decreased receptor expression, an increased expression of the receptor may be a reasonable therapeutic target with variety of agents such as bortezomib etc. These agents potentially could be used in conjunction with standard adjuvant therapy for BC, which exhibits frequently decreased TßRII levels (5).

Numerous studies have revealed the potential clinical prognostic or predictive utility of TGF-$ß_1$ or TβRII levels (4,5). Among others, the tumour promoting role of TGF-$ß_1$ has been supported by the demonstration of increased TGF-$ß_1$ levels in human BC - production is increased with advanced stages of tumour (14); decreased TGF-$ß_1$ levels after surgical resection (15); persistently elevated levels after surgical resection are in correlation with lymph node metastasis or residual tumour (15); and elevated TGF-$ß_1$ levels conferring a poorer prognosis for BC patients (16). Consistent with these findings, we have previously determined significantly elevated plasma TGF-$ß_1$ levels in advanced BC patients (12). Moreover, we have observed that this elevation was correlated with decreased survival of metastatic BC patients, thus providing direct evidence that plasma TGF-$ß_1$ is a biomarker of a poor prognosis (17). Therefore, in clinical scenarios involving increased TGF-$ß_1$ activity, attempts to decrease or abrogate TGF-$ß_1$ signalling could be used as a therapy for advanced or metastatic disease. Attempts to block the effects of excessive TGF-$ß_1$ activity has so far involved agents that inhibit TGF-$ß_1$-binding to its receptor including natural TGF-$ß_1$ inhibitors (e.g., decorin), neutralizing TGF-$ß_1$ antibodies, and soluble extracellular domain of TβRII receptor (4).

CONCLUSION

TGF-$ß_1$ has a complex multifunctional profile, with tumour suppressive effects in early stages of breast carcinogenesis, but progressive dominance of tumour promoting effects with transition to more advanced malignant states. Clarification of molecular mechanisms that control parallel processing of these opposing TGF-$ß_1$ activities might suggest new approaches for shifting the balance in favour of net tumour suppression (18). Currently, a major challenge remains in more precisely defining TGF-$ß_1$ signalling pathways.

Although Smad-mediated TGF-$ß_1$ signalling is well established, the mechanisms of MAPK signalling and other pathways remain to be elucidated. Once these pathways are established, more specific targeting of the TGF-$ß_1$ signal-related components will be possible. Consequently, further research involving the manipulation of TGF-$ß_1$ expression in a temporal and stage-dependent manner will help elucidate how and when therapeutic agents should be applied for chemoprevention and treatment of an early BC and whether anti-TGF-$ß_1$ strategies are more appropriate for the metastatic disease.

Acknowledgement

Financial support for this work, under projects # 143010 (to BD), was provided by the Ministry of Sciences and Environmental Protection of the Republic of Serbia.

REFERENCES

1. Al-Mansouri LJ, Alokail MS. Molecular basis of breast cancer. Saudi Med J 2006;27:447-54.
2. Esteva FJ, Hortobagyi GN. Prognostic molecular markers in early breast cancer. Breast Cancer Res 2004;6:109-18.
3. Masood S. Prognostic/Predictive Factors in Breast Cancer. Clin Lab Med 2005;25:809-25.
4. Benson JR. Role of transforming growth factor β in breast carcinogenesis. Lancet Oncol 2004;5:229-39.
5. Elliott RL, Blobe GC. Role of Transforming Growth Factor Beta in Human Cancer. J Clin Oncol 2005;23:2078-93.
6. Kim IY, Kim MM, Kim SJ. Transforming Growth Factor-β: Biology and Clinical Relevance. J Biochem Mol Biol 2005;38:1-8.
7. Kaklamani VG, Baddi L, Liu J, Rosman D, Phukan S, Bradley C, et al. Combined Genetic Assessment of Transforming Growth Factor-β Signaling Pathway Variants May Predict Breast Cancer Risk. Cancer Res 2005;65:3454-61.
8. Ziv E, Cauley J, Morin PA, Saiz R, Browner WS. Association Between the T29→C Polymorphism in the Transforming Growth Factor-β_1 Gene and Breast Cancer Among Elderly White Women. JAMA 2001;285:2859-63.
9. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, 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.

10. Shu XO, Gao YT, Cai Q, Pierce L, Cai H, Ruan ZX, et al. Genetic Polymorphisms in the *TGF-β_1* Gene and Breast Cancer Survival: A Raport from the Shanghai Breast Cancer Study. Cancer Res 2004;64:836-9.

11. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, et al. Genetic control of the circulating concentration of transforming growth factor type β1. Hum Mol Genet 1999;8:93-7.

12. Ivanovic V, Todorovic-Rakovic N, Demajo M, Neskovic-Konstantinovic Z, Subota V, Ivanišević-Milovanović O, et al. Elevated plasma levels of transforming growth factor-β_1 (TGF-β_1) in patients with advanced breast cancer: Association with disease progression. Eur J Cancer 2003;39:454-61.

13. Kim SJ, Angel P, Lafyatis R, Hattori K, Kim KY, Sporn MB, et al. Autoinduction of Transforming Growth Factor-β1 Is Mediated by the AP-1 Complex. Mol Cell Biol 1990;10:1492-7.

14. Gorsch SM, Memoli VA, Stukel TA, Gold LI, Arrick BA. Immunocytochemical staining for transforming growth factor-β1 associates with disease progression in human breast cancer. Cancer Res 1992;52:6949-52.

15. Kong FM, Anscher MS, Murase T, Abbott BD, Iglehart JD, Jirtle RL. Elevated plasma for transforming growth factor-β1 levels in breast cancer patients decrease after surgical removal of the tumor. Ann Surg 1995;222:155-62.

16. Ghellal A, Li C, Hayes M, Prognostic significance of TGF beta 1 and TGF beta 3 in human breast carcinoma. Anticancer Res 2000;20:4413-8.

17. Ivanović V, Demajo M, Krtolica K, Krajnović M, Konstantinović M, Baltić V, et al. Elevated plasma TGF-β_1 levels correlate with decreased survival of metastatic breast cancer patients. Clin Chem Acta 2006;371:191-3.

18. Wrana JL, Attissano L, Rotraund W. Mechanism of activation of the transforming growth factor-β receptor. Nature 1994;370:341-6.