Role of transforming growth factor-β1 in breast carcinogenesis

Vesna Ivanović, Koviljka Krtolica, Milena Krajnović, Bogomir Dimitrijević

ABSTRACT

The main objective of this presentation is to review current knowledge regarding molecular mechanisms of Transforming Growth Factor-β1 (TGF-β1) action in breast carcinogenesis. In addition, our recent results will be presented on TGF-β1 gene polymorphism and its relationship to TGF-β1 secretion in breast cancer (BC) patients. Special focus will be made on potential clinical applicability of TGF-β1 as a putative diagnostic, prognostic or predictive tool in BC detection and treatment. TGF-β1 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-β1 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-β1 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-β1 gene polymorphism, at codon 10 due to Leu10Pro substitution in the signal peptide, with the risk of developing BC. The somatic mutations approach, provides an explanation for the TGF-β1 overexpression in advanced BC through mutations acquired in the components of Smad-mediated TGF-β1 signalling pathway. The available results indicate decreased TßRII (TGF-β1 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-β1 (TGF-β1) 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-β1 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-β1 as a putative diagnostic, prognostic or predictive tool in BC detection and treatment.

TGF-β1 ACTS BOTH AS TUMOUR SUPPRESSOR AND AS TUMOUR PROMOTER

TGF-β1 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-β1 signalling pathways also have an important role in human mammary carcinogenesis revealing dual function of TGF-β1 in this process (4). In healthy tissue, premalignant, and early-transformed states, TGF-β1 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-β1 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-β1, which has been correlated with a more malignant phenotype and impaired clinical outcome. Therefore, a major challenge remains to precisely define TGF-β1 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-β1 polymorphism with risk of developing breast cancer.
ing evidence that common variants of TGF-$\beta_1$ gene may affect the production, secretion, or activity of this cytokine. Now, five different TGF-$\beta_1$ gene polymorphisms have been identified with respect to BC risk (7). Among these, the most extensively studied is the TGF-$\beta_1$ gene polymorphism at codon 10 due to Leu10Pro substitution in the signal peptide (8-10).

LEU10PRO TGF-$\beta_1$ POLYMORPHISM

Previous reports have analyzed relationship between the Leu10Pro TGF-$\beta_1$ polymorphism versus progressive BC stages or survival of BC patients. The obtained results indicate that the Pro10 homoygotes have an increased incidence of invasive BC (9) and significantly decreased BC patients’ survival (10). In addition, increased plasma levels of TGF-$\beta_1$, protein were observed in Pro10 homoygotes when compared to Leu10 homoygotes or Leu10Pro heterozygotes in a general population (11). Moreover, Dunning and co-workers (9) have described in the Legend to Figure 1.

Concentration of TGF-$\beta_1$ in plasma was analyzed by the TGF-$\beta_1$ receptor-type II (T(II))-based TGF-$\beta_1$, ELISA kit as previously described (12). Our results indicate that plasma TGF-$\beta_1$, levels of Stage I11 disease (mean value: 1.01 ± 0.16 ng/ml; range: 0.17-1.94 ng/ml; n=10, > 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-$\beta_1$, gene fragment of 485 bp, including the exon 1 and neighbouring parts of the surrounding sequences as described in the Legend to Figure 1.

The data reveal one Leu10Pro heterozygous and two Leu10 homoygous BC patients relative to one Leu10 homoygous HD and two Leu10Pro heterozygous HD. Our results indicate the presence of Leu10 Pro variant in both stage III patients and healthy donors 3. The obtained genotype for an early BC and HD might explain unchanged TGF-$\beta_1$, secretion in plasma of these subjects. Although obtained on small number of subjects, our results suggest that plasma TGF-$\beta_1$, levels may not warrant it as useful biomarker for early BC stages.

TGF-$\beta_1$ SIGNALING PATHWAYS

During the past 4-5 years, there have been some important advances in the understanding of postreceptor signal transduction for TGF-$\beta_1$, (5). Currently, two signalling mechanisms have been identified including the Smad-mediated TGF-$\beta_1$, 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-$\beta_1$, ligand, TGF-$\beta_1$, 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 TjRII, TjRII, Smad2, Smad3, and Smad4 as intracellular mediators in the following

Table 1. A summary of data on Leu10Pro polymorphism of TGF-$\beta_1$, gene for three early stage BC patients relative to healthy donors (HD), as detected from DNA sequencing profiles illustrated in Figure 1

<table>
<thead>
<tr>
<th>SUBJECT’S CHARACTERISTICS</th>
<th>DNA POLYMORPHISM</th>
</tr>
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<tbody>
<tr>
<td>BC patient</td>
<td>Healthy donor</td>
</tr>
<tr>
<td>number prognosis</td>
<td>Menopausal status</td>
</tr>
<tr>
<td>low high risk</td>
<td>premn.*</td>
</tr>
<tr>
<td>Case 1</td>
<td>+</td>
</tr>
<tr>
<td>Case 2</td>
<td>+</td>
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<td>Case 3</td>
<td>+</td>
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<tr>
<td>HD 1</td>
<td>+</td>
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<td>HD 2</td>
<td>+</td>
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<td>HD 3</td>
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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 Leu10Pro substitution in the signal peptide of TGF-$\beta_1$, with two of the types variants. Table 1 illustrates the distribution of TGF-$\beta_1$, Leu10Pro genotype in BC patients (n=3) and healthy donors (n=3), with respect to subjects characteristics.

Figure 1. Sequence analysis illustrating presence of Leu10Pro 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 Leu10Pro heterozygote (Case 1); and Panel b, arrow pointing a single T peak at the 29 nucleotide position of codon 10, representing Leu10 homoygote (Case 2). Genomic DNA was isolated from tumour tissue obtained after surgery. Polymorphic region of TGF-$\beta_1$, exon 1, 485 bp in length, was then amplified using specific primers, whose sequences were as follows: 5’ – TTGCGCTCTCTGGAAGT – 3’ (forward) and 5’ – TTCTTCGGCACTAGCTCCTACCC – 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°C, 1 min at 65°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.
cascade of events: The TGF-β1 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-β1 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-b1 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-β1, tumour suppressor and tumour promoter activities depend on crosstalk between Smad and MAPK pathways (4).

POTENTIAL CLINICAL APPLICABILITY OF TGF-β1 IN BREAST CANCER

The ability to define alterations in the TGF-β, 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-β1 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 TβRII receptor expression was observed as well as rare TβRII gene mutations, but no mutations in Smad2 and Smad4 genes were detected (Table 2).

Likewise, in prostate cancer (another hormonal tumour), complete loss of TβRII 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 TβRII levels (5).

Numerous studies have revealed the potential clinical prognostic or predictive utility of TGF-β1 or TβRII levels (4,5). Among others, the tumour promoting role of TGF-β1, has been supported by the demonstration of increased TGF-β1 levels in human BC - production is increased with advanced stages of tumour (14); decreased TGF-β1 levels after surgical resection (15); persistently elevated levels after surgical resection in correlation with lymph node metastasis or residual tumour (15); and elevated TGF-β1 levels conferring a poorer prognosis for BC patients (16). Consistent with these findings, we have previously determined significantly elevated plasma TGF-β1, 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-β1, is a biomarker of a poor prognosis (17). Therefore, in clinical scenarios involving increased TGF-β1 activity, attempts to decrease or abrogate TGF-β1 signalling could be used as a therapy for advanced or metastatic disease. Attempts to block the effects of excessive TGF-β1, activity has so far involved agents that inhibit TGF-β1, binding to its receptor including natural TGF-β1 inhibitors (e.g., decoy), neutralizing TGF-β1, antibodies, and soluble extracellular domain of TβRII receptor (4).

CONCLUSION

TGF-β1 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-β1, 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-β1, signalling pathways. Although Smad-mediated TGF-β1, 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-β1, signal-related components will be possible. Consequently, further research involving the manipulation of TGF-β1, 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-β1, strategies are more appropriate for the metastatic disease.

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REFERENCES


