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Localization of recognition site between transforming growth factor-β_1 (TGF-β_1) and TGFβ receptor type II: possible implications in breast cancer

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Summary Although overexpression of TGF-β_1 protein has been demonstrated in advanced breast cancer(BC) patients, as well as in other solid tumours, the molecular mechanism of this process remains obscure. This paper proposes that a genetic/epigenetic alteration might occur in the TGF-β_1 gene, within the region coding for the recognition site with TGFβ receptor type II, leading to a disruption of the ligand–receptor interaction and triggering the TGF-β_1 cascade-related BC progression. To establish the operational framework for this hypothesis, in the present study, this recognition site was identified by the Informational Spectrum Method (ISM) to comprise two TGF-β_1 peptides (positions 47–66 aa and 83–112 aa) and one receptor peptide at positions 112–151 aa of the extracellular domain of the receptor (TβRII$_M$). The TβRII$_M$ locus was further evaluated by ISM-derived deletion analysis of the TβRII sequences. To provide experimental support for the proposed model, a pilot study of plasma TGF-β_1 analysis was performed in advanced BC patients ($n = 8$). Two commercial ELISA assays, one with specific αTGF-β_1 MAb (MAb) and other with TβRII$_M$ as the immobilized phase, revealed pronounced differences in the pattern of plasma TGF-β_1 elevation. In MAb-profile, the TGF-β_1 increase was detected in 7 of 8 patients, whereas analogous TβRII$_M$-profile revealed the elevation in 3 of 8 patients, taking a 50% of maximal elevation as the cut-off value. These findings are consistent with the proposed aberration of TGF-β_1 ligand within the TβRII recognition site. Summarizing, this model system is a good starting point for further genetic studies, particularly on genetic/epigenetic alterations of sequences involved in TGF-β_1 and TβRII$_M$ interaction, with putative prognostic value for breast cancer.

Introduction

Breast cancer is the leading cause of cancer-related death in women throughout the world.

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Current evidence suggests a prognostic significance of Transforming Growth Factor-β_1 (TGF-β_1) in this malignancy [1–3]. This cytokine is essential for normal human physiology and plays a critical role in growth regulation and development of the majority of cell types in various organs [4]. In the solid tumour development, overexpression of TGF-β_1 protein has been detected both in tumour tissue and plasma of advanced patients with breast [1–3,5–8], colon [9,10], prostate [11,12], and bladder cancers [13]. At the present time, the molecular mechanisms of this TGF-β_1 upregulation, in the course of tumour progression, are unknown [14].

Abnormalities in the expression of TGF-β_1 protein have been proposed to play a key role in the progression of carcinogenesis in a variety of solid tumours [15]. According to current views, interaction between TGF-β and TGF-β receptor type II (TβRII) is the initial and mandatory step for TGF-β to exert its pleiotropic response [16]. It is well established that TGF-β binds the TβRII to inhibit the growth of most epithelial tissues [17,18]. Recent data indicate that most human colon and gastric cancers have frameshift mutations in polynucleotide repeats within the TβRII coding region [19]. These mutations truncate the receptor protein and disable the serine/threonine kinase to produce resistance, thus implicating TβRII as a candidate tumour suppressor gene [20]. However, in breast cancer (BC) the TβRII gene mutations were not observed [21,22], suggesting that tumour progression through this pathway may be related to organ specificity [22]. Due to a lack of evidence implicating TβRII aberrations in breast cancer, we contemplate a reciprocal event of putative abnormalities on the TGF-β_1 side of the ligand–receptor interaction and propose for BC the following model system.

Hypothesis

We hypothesise that, in breast cancer development, a genetic/epigenetic alteration of the TGF-β_1 gene might occur within the region coding for the recognition site with TGFβ receptor type II. This aberration may lead to a disruption of the ligand–receptor interaction, thus triggering the TGF-β_1 cascade-related pathological pathway and implicating TGF-β_1 gene as a candidate tumour promoter gene in BC progression.

To establish operational framework for this hypothesis, in the present study the recognition site between human TGF-β_1 and TGF-β receptor type II (TGF-β_1*TβRII) site was identified by the Informational Spectrum Method (ISM).

Bioinformatic framework of the hypothesis

Informational spectrum method

The ISM, as a virtual spectroscopy technique, has been successfully applied in structure/function analysis of different protein and DNA sequences [23–26]. According to this approach protein sequences are transformed into signals by assignment of numerical values to each amino acid, representing the electron-ion interaction potential, EIIP [27]. The signal obtained is then decomposed in periodical functions by Fourier transform. The results are series of frequencies and their amplitudes. The obtained frequencies correspond to the distribution of structural motives with defined physico-chemical characteristics responsible for the biological function of the protein. When comparing interacting proteins, the technique allows detection of code/frequency pairs which is specific for their interaction [27–29]. Accordingly, in this study, targeting between interacting TGF-β_1 ligand and the extracellular domain of TβRII (TβRII$_M$) was determined by the dominant common frequency component(s) in their ISM spectra.

ISM derived localization of TGF-β_1 * TβRII$_M$ recognition site

To identify the common frequency component(s) corresponding to the interaction between TGF-β_1 and TβRII, the individual informational spectra (IS) for TGF-β_1, TβRII, and the TβRII$_M$ were obtained revealing $F(0.0586)$ as the common frequency component for the three proteins among numerous frequency bands with varying amplitudes (data not shown). Fig. 1 shows cross-spectra (CS) between the ligand–receptor moieities exhibiting the dominant frequency $F(0.0586)$ as the major common spectral characteristic in IS of TGF-β_1, either in combination with TβRII or with TβRII$_M$ polypeptide. Further computer scanning survey of primary structures of these two polypeptides was elucidated and the interacting ligand–receptor region(s) are presented in Table 1. Our results revealed that the putative TGF-β_1 region of the recognition site with receptor involves two polypeptide fragments, labeled fr.$_1$ and fr.$_2$, encompassing positions 47–66 aa and 83–112 aa, respectively (Table 1). The reciprocal TβRII$_M$ interacting region, presented in Table 1 as fr.$_3$, reveals the polypeptide chain from positions 112–151 aa as the predominant in TGF-β_1 recognition.

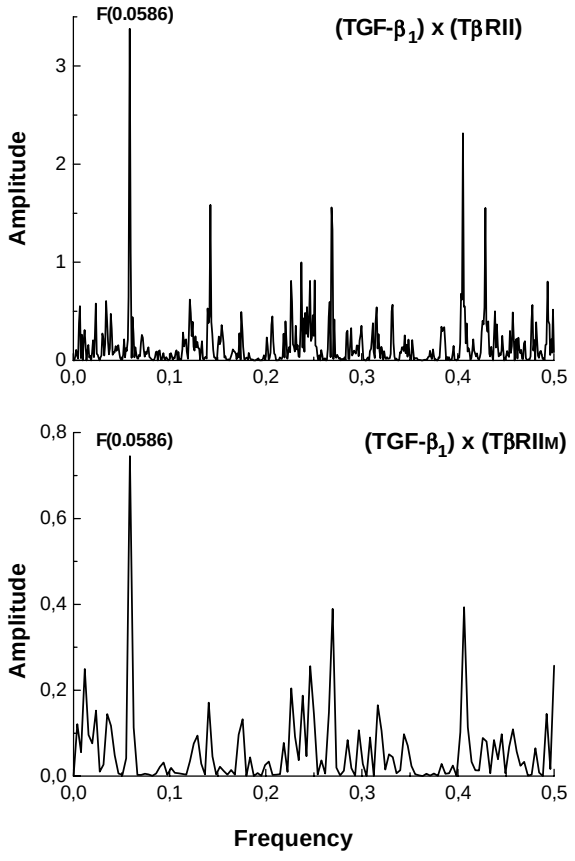

Figure 1 IMS-derived cross-spectrum between IS of TGF-β_1 (chain of 112 aa) and TβRII (chain of 573 aa) compared to cross-spectrum between TGF-β_1 and TβRII$_M$ (chain of 159 aa). The input amino acid sequences were obtained from protein database SWISS-PROT (release 41. 4, 2003) with accession codes: TGF1_HUMAN and TGR2_HUMAN for TGF-β_1 and TβRII, respectively. The identification of interacting TGF-β_1 and TβRII$_M$ sequences was performed by a computer scanning survey of short peptides (minimal length of 16 and maximal of 32 amino acids) of an individual macromolecule which exhibits a significant amplitude of the dominant frequency $F(0.0586)$. The frequencies represent the Fourier transform of the sequence of electron-ion interaction potential corresponding to the amino-acid sequence for the interacting proteins. The amplitudes, corresponding to each frequency component in the spectrum, are presented in arbitrary units.

It has been previously demonstrated through IS dominant frequency of a ligand—receptor interaction that location of a recognition site either overlaps with the direct binding regions or is situated very close to them [23,28,29]. Therefore, our recognition site loci are consistent with previously reported direct binding sites which revealed two TGF-β_1 peptides (positions 48—60 aa and 86—101 aa) as the binding sequences with TβRII$_M$ [30]. Moreover, our TβRII$_M$ core interacting region is in a close vicinity to the TβRII$_M$ sequence (84—101 aa position) previously reported by Demetriou et al. [31] as the counterpart TβRII$_M$ sequence involved in the ligand binding.

Role of TβRII$_M$ deletion mutations in TGF-β_1 ligand—receptor recognition

Recent results demonstrated an association between deletions in TβRII gene with clinical pathology-related progression of colon and gastric cancers [19]. These findings, along with previous experimental demonstration of specific TβRII$_M$ deletions responsible for the abolished TGF-β_1 binding [32,33], prompted us to undertake scanning-deletion analysis by ISM.

Fig. 2 reveals the amplitude of dominant frequency $F(0.0586)$ component as a function of specific type of amino acid deletions described previously [32]. They include TβRII$_M$ sequence with consecutive deletions of three amino acids at every five amino acid intervals as described in the legend to Fig. 2. Our results reveal the maximal amplitude of 0.75 in CS of TGF-β_1 with the wild-type TβRII$_M$ polypeptide, numbered as 1 in Fig. 2. Taking an amplitude of 0.40 as the cut-off value, our results showed that the sequence of TβRII$_M$ polypeptide may be classified into two subdomains: first region (positions 27—50 aa, and 148—150 aa) which is not critical for TGF-β_1 interaction, and second region (positions 53—120 aa, and 153—155 aa) which constitutes putative core interaction domain. It is of interest to note that among the critical deletions in Fig. 2 is the mutation numbered as 12 (Δ118—120)

Table 1 Identification of polypeptide sequences involved in the recognition between TGF-β_1 ligand and TβRII$_M$ receptor as determined by IS analysis derived from Fig. 1

Moiety	Fragment number	Region of gene expression	Amino acid	
			Positions	Sequence
TGF-β_1	fr.$_1$	Exon 1	47—66	PCPYIWSLDTQYSKVLALYN
	fr.$_2$	Exon 2	83—112	LEPLPIVYYVGRKPKVEQLSNMIVRSCKCS
TβRII receptor	fr.$_3$	Exon 3	112—151	ILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSE

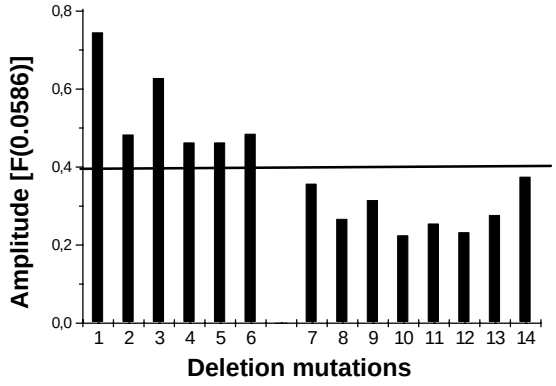

Figure 2 Effects of deletions in primary structure of $T\beta RII_M$ on the dominant frequency component $F(0.0586)$ in CS of TGF-$\beta_1 \times T\beta RII$. The amplitude value corresponding to the frequency $F(0.0586)$ with respect to: $T\beta RII_M$ - wild type sequence (1) and the $T\beta RII_M$ sequence with deletion mutations: $\Delta 27-29$ (2), $\Delta 32-34$ (3), $\Delta 38-40$ (4), $\Delta 48-50$ (5), $\Delta 148-150$ (6), $\Delta 58-60$ (7), $\Delta 63-65$ (8), $\Delta 68-70$ (9), $\Delta 78-80$ (10), $\Delta 103-105$ (11), $\Delta 118-120$ (12), $\Delta 53-55$ (13) and $\Delta 153-155$ (14).

which is positioned within the proposed $T\beta RII_M$ recognition site (positions $112-151$ aa) presented in Table 1. Summarizing, the deletion analysis provides a supporting evidence for the ISM predicted location of the $T\beta RII_M$ interaction site.

Experimental testing of the hypothesis

To provide experimental support for the hypothesis, determination of plasma TGF-β_1 levels was performed in advanced BC patients. Fig. 3 illustrates experimental data of comparative analysis of plasma TGF-β_1 using two commercial ELISA immunoassays as described before [3,8], one with the $T\beta RII_M$ ($T\beta RII_M$ ELISA) and the other with the specific αTGF-β_1 MAb (MAb ELISA) as the immobilized phase. Each histogram represents an individual plasma TGF-β_1 level for a single Stage III/IV patient $(n = 8)$. It is apparent that $T\beta RII_M$ ELISA reveals TGF-β_1 concentration range from 1.43 to 8.48 ng/ml, whereas MAb ELISA (range from 26.5 to 84.5 ng/ml) exhibits about 10-fold higher plasma TGF-β_1 values, presumably due to a detection of TGF-β_1-bound complexes as observed before [34]. Nevertheless, qualitatively, both assays exhibit elevated plasma TGF-β_1 levels in BC patients (Stage III/IV). The overall pattern, however, of pronounced TGF-β_1 elevation is different for the two ELISA systems, taking a 50% maximal TGF-β_1 concentration as the cut-off value (Fig. 3). The $T\beta RII_M$-detected elevation was observed in 3 out of 8 patients, numbered 1, 4 and 6 with respective values of 8.48, 6.10, and 4.40 ng/ml. On the other hand, the MAb-detected

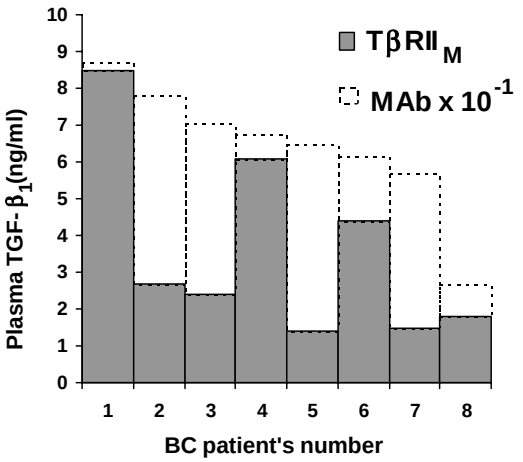

Figure 3 Comparative individualized plasma TGF-β_1 values for the Stage III/IV BC patients ($n = 8$) as monitored by the $T\beta RII_M$-ELISA versus MAb-ELISA. The study had received Institutional Review Board approval by the Institute of Oncology and Radiology of Serbia in Belgrade. The staging was based on AJCC/UICC TNM classification and an informed consent was obtained from each woman according to the National Health Regulation. Phlebotomy, plasma preparation and plasma TGF-β_1 analysis by MAb-based PREDICTA and $T\beta RII_M$ receptor-based Quantikine TGF-β_1 ELISA kits, were done as previously described [3,8]. The measurements were performed in separate experiments using fresh aliquots of the same plasma preparations. Special precautions were taken to prevent in vitro platelet activation. Moreover, platelet carryover in all plasma samples, as assessed by PF4 detection described before [3], was found to be insignificant.

TGF-β_1 elevation was exhibited in a much larger proportion of patients i.e. 7 out of 8 patients, numbered from 1–7, with the respective values ranging from 58.1–84.5 ng/ml (Fig. 3). Our subsequent $T\beta RII_M$ ELISA analysis revealed insignificant difference in plasma TGF-β_1 of Stage I/II BC patients prior to surgery (mean value: 1.01 ± 0.16 ng/ml; range: 0.17–1.94 ng/ml; $n = 10$; $p > 0.1$) when compared to healthy donors (mean value: 1.45 ± 0.15 ng/ml; range 0.39–4.93 ng/ml; $n = 37$). In contrast, the detection by MAb ELISA of Kong et al. [2] reveals 2- to 3-fold increase in plasma TGF-β_1 for a similar cohort of BC patients. Our data, therefore, are consistent with the proposed model and suggest that $T\beta RII_M$-negative plasma TGF-β_1 elevation is attributed to BC patients population with an aberrant TGF-β_1 ligand.

Conclusions

Since $T\beta RII_M$ gene mutations were not observed in breast cancer, this paper proposes a model of a

possible genetic/epigenetic alteration of the TGF-β_1 gene within the region coding for the recognition site with TGFβ receptor type II, thus leading to a disruption of the TGF-β_1★TβRII recognition site. To further elaborate on this concept, in the present study a recognition site between human TGF-β_1 and the core domain of human TβRII$_M$ has been identified by ISM to comprise two TGF-β_1 peptides (positions 47—66 aa and 83—112 aa) and one TβRII$_M$ peptide (positions 112—151 aa). This type of information is of considerable interest since it might provide insights into the molecular mechanism of TGF-β (miss)regulation and help in the development of a putative TGF-β antagonist.

Moreover, suggestive evidence as a proof of this concept was provided by determination of plasma TGF-β_1 levels in advanced BC patients. Despite the limited number of cases, our results revealed a marked reduction in the number of patients with plasma TGF-β_1 elevation when MAb was replaced by TβRII$_M$ ELISA detection. These findings are consistent with the proposed hypotheses, thus providing a preliminary support for further experimental designs.

Summarizing, we believe that this model is a good starting point for future studies in elucidating genetic/epigenetic alterations of TGF-β_1 ∗ TβRII recognition site, with putative specificity for malignancy of the breast. This research is currently in progress [35] and includes mutational analysis and hypermethylation of the pertinent genomic loci.

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