CLINICAL SCIENCE

Tgf-β1 expression as a biomarker of poor prognosis in prostate cancer

Sabrina Thalita dos Reis, José Pontes-Júnior, Alberto Azoubel Antunes, Juliana Moreira de Sousa-Canavez, Daniel Kanda Abe, José Arnaldo Shiomi da Cruz, Marcos Francisco Dall’Oglio, Alexandre Crippa, Carlo Camargo Passerotti, Leopoldo A Ribeiro-filho, Nayara Izabel Viana, Miguel Srougi, Kátia Ramos Moreira Leite

1Laboratory of Medical Investigation (LIM55), Urology Department, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil. 2Uro-Oncology Group, Urology Department, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil. 3Genoa Biotechnology SA, São Paulo, Brazil.

OBJECTIVE: To evaluate the correlation between transforming growth factor beta (TGF-β1) expression and prognosis in prostate cancer.

PATIENTS AND METHODS: TGF-β1 expression levels were analyzed using the quantitative real-time polymerase chain reaction to amplify RNA that had been isolated from fresh-frozen malignant and benign tissue specimens collected from 89 patients who had clinically localized prostate cancer and had been treated with radical prostatectomy. The control group consisted of 11 patients with benign prostate hyperplasia. The expression levels of TGF-β1 were compared between the groups in terms of Gleason scores, pathological staging, and prostate-specific antigen serum levels.

RESULTS: In the majority of the tumor samples, TGF-β1 was underexpressed 67.0% of PCA patients. The same expression pattern was identified in benign tissues of patients with prostate cancer. Although most cases exhibited underexpression of TGF-β1, a higher expression level was found in patients with Gleason scores >7 when compared to patients with Gleason scores <7 (p = 0.002). Among the 26 cases of TGF-β1 overexpression, 92.3% had poor prognostic features.

CONCLUSIONS: TGF-β1 was underexpressed in prostate cancers; however, higher expression was observed in tumors with higher Gleason scores, which suggests that TGF-β1 expression may be a useful prognostic marker for prostate cancer. Further studies of clinical specimens are needed to clarify the role of TGF-β1 in prostate carcinogenesis.

KEYWORDS: Prostate cancer; Prognosis; Molecular markers; TGF-β.

INTRODUCTION

Prostate cancer (PCA) is the most common male malignancy and is the second-highest cause of death in many countries, including Brazil. Pathological staging, Gleason scores, and prostate-specific antigen (PSA) serum levels are the most reliable prognostic factors; however, even when combined, they do not perfectly identify patients who are at risk of progression. Therefore, research has been aimed at identifying molecular markers that can predict PCA predisposition and progression.

Transforming growth factor beta (TGF-β) is the prototype member of a superfamily that includes more than 25 members, including inhibin, bone morphogenetic protein, and Müllerian inhibiting substance. The TGF-β subfamily contains five members named TGF-β1 through β5. TGF-β1, -β2, and -β3 have been identified in mammals. Although TGF-β is a pleiotropic growth factor, it inhibits growth in most cell types, especially cells of epithelial lineage. TGF-β also inhibits prostatic epithelial cell proliferation and induces apoptosis. In addition to its numerous effects on cell proliferation, phenotype, and differentiation, TGF-β is an important transcriptional regulator of extracellular matrix components.

Malignant transformation is characterized by multiple genetic mutations that confer a growth advantage to transformed cells compared with benign cells. The development of prostate cancer follows this paradigm. TGF-β is involved in two critical events that are consistently
RNA Isolation and cDNA Synthesis

All of the tumor samples were obtained from surgical specimens and immediately frozen in liquid nitrogen at -170°C. A mirror image slide of the frozen fragment was stained with hematoxylin and eosin to verify that the tumor represented at least 75% of the fragment in the cancer patients and that no tumors were present in the control patients with BPH.

Total RNA was isolated using the RNeasy Tissue Kit (Applied Biosystems, CA, USA) according to the manufacturer’s instructions. The RNA concentration was determined by the ratio of the absorbance at 260 and 280 nm, using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The reactions were incubated at 25°C for 10 min, followed by 37°C for 120 min and 85°C for 5 min. The cDNA was stored at -20°C until use.

Table 1 - Age and clinical characteristics of 79 men who underwent radical prostatectomy to treat prostate cancer.

| Characteristics | Value |
|----------------|-------|
| Age (years)    | Mean: 63, Min - Max: 41 - 79 |
| PSA (ng/ml)    | Mean: 10.8, <10 ng/ml: 20 - 37.0, ≥10 ng/ml: 47 (52.5) |
| Stage          | pT2: 38 (48), pT3: 41 (52) |
| Gleason Score  | <7: 32 (41.7), ≥7: 46 (58.3) |

RESULTS

As shown in Figure 1, an analysis of the 79 patients with PCa showed that TGF-β1 was underepressed (median 6.07×10⁻⁴-fold) in malignant prostatic tissue as compared to the BPH samples.

The TGF-β1 expression levels in relation to the Gleason scores are shown in Table 2. The median TGF-β1 expression levels were significantly higher (p = 0.0065) among patients with a Gleason score ≥7 (9.4×10⁻¹-fold) compared with those patients with a Gleason score <7 (4.6×10⁻⁴-fold). With regard to the pathologic stage, the expression of TGF-β1 was similar (p = 0.2514) between stage pT3 patients (median 4.9×10⁻¹-fold) and stage pT2 patients (median 6.6×10⁻⁴-fold). When the results were evaluated according
to preoperative PSA values, the median TGF-β1 expression levels were also similar ($p = 0.5110$) between patients with PSA levels $\geq 10$ ng/ml ($6.7 \times 10^{-1}$-fold) and patients with PSA levels $<10$ ($5.5 \times 10^{-1}$-fold).

We found an overexpression of TGF-β1 in 26 cases, of which 21 (80.7%) had a Gleason score $\geq 7$, 13 (50%) had a PSA level $\geq 10$ ng/ml, 12 (46%) were staged as pT3 and 9 (35%) had biochemical recurrence. Only 2 (7.7%) of the patients with TGF-β1 overexpression exhibited no unfavorable prognostic factors.

We also evaluated the expression levels of TGF-β1 in patients with biochemical recurrence (which was defined as a PSA level above 0.4 ng/ml) at a mean follow-up period of 60 months. Median TGF-β1 expression levels were statistically similar ($p = 0.528$) among patients with and without recurrence ($5.4 \times 10^{-1}$ and $6.1 \times 10^{-1}$, respectively).

We performed a multivariate analysis, and the Gleason score was the unique independent prognostic factor in our series ($p = 0.022$).

Additionally, we tested the expression of TGF-β1 in the prostatic tissues from 10 patients with benign PCa and found the same expression patterns as observed in the malignant tissues (median $1.7 \times 10^{-1}$) ($p = 0.05$).

**DISCUSSION**

In this study, we found that TGF-β1 was underexpressed in malignant prostatic tissue as compared to BPH samples (median $7 \times 10^{-1}$-fold). Thus, we may assume that TGF-β contributes to the development of prostate cancer by acting as an inhibitor of cell proliferation and an inducer of apoptosis.$^8,9$ Furthermore, we identified higher TGF-β expression levels in tumors with higher Gleason scores, suggesting that this gene may play a role in PCa progression and prognosis.

Soulitzis et al$^{10}$ also reported a decrease in TGF-β expression in PCa. TGF-β is generally a growth inhibitor of both benign prostatic epithelial cells$^{11}$ and prostate cancer cells in vitro$^{12}$ and has been shown to inhibit proliferation and induce apoptosis in prostatic epithelial cells.$^8,13$

Moreover, TGF-β stimulates the synthesis of collagen, fibronectin, and integrins, and it inhibits matrix degradation through the downregulation of proteinases such as collagens, stromelysins, and plasminogen activators.$^{14}$ Paired with its downregulation of proteinases, TGF-β upregulates proteinase inhibitors such as the following endogenous matrix metalloproteinases (MMP) inhibitors: the tissue inhibitor of metalloproteinase-1 (TIMP-1)$^{15}$ and plasminogen activator inhibitor-1.$^{16}$ TGF-β also acts as a repressor of matrix metalloproteinase expression through the TGF-β inhibitory element (TIE), which is found in the 5'-flanking region of several genes in the MMP family, namely interstitial collagenase (MMP-1) and 92-kDa type IV collagenase (MMP-9).$^{17}$

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**Figure 1** - Quantitative expression of TGF-β1 messenger RNA in malignant prostatic tissue compared with BPH. Fold change in expression was calculated using the 2-ΔΔCT method.

**Table 2** - Relative expression of the TGF-β1 gene in the malignant prostatic tissues according to Gleason score, pathologic stage, PSA level and biochemical recurrence. Fold changes in gene expression were calculated using the ΔΔCT method (QRel $= 2^{-\Delta\Delta CT}$).

| TGF-β1 | Gleason Score | Pathologic Stage | PSA Level | Biochemical Recurrence |
|--------|--------------|-----------------|-----------|------------------------|
|        | $\leq 7$ ($n = 32$) | $>7$ ($n = 36$) | $<10$ ($n = 45$) | $\geq 10$ ($n = 33$) | Without ($n = 29$) | With ($n = 28$) |
| $p$-value | 0.46 (0.02 - 6.20) | 0.93 (0.02 - 33.3) | 0.65 (0.02 - 33.3) | 0.48 (0.04 - 18.9) | 0.55 (0.04 - 33.3) | 0.67 (0.02 - 14.3) |
| $\Delta\Delta CT$ | 0.006 | 0.249 | 0.508 | 0.508 |

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Since TGF-β contributes to the development of prostate cancer by acting as an inhibitor of cell proliferation and an inducer of apoptosis, we may assume that TGF-β plays a role in PCa progression and prognosis.
Interestingly, a quantitative analysis revealed a positive association between increased TGF-β mRNA levels and elevated Gleason scores (p = 0.002). Although controversial, this finding is consistent with that of a previous study in which TGF-β plasma levels were higher in men with PCA metastases than in healthy men.18 Shariat et al.19 also found a strong correlation between elevated plasma TGF-β and prostate cancer progression and the development of metastases in patients with locally advanced disease. Faria et al.20 demonstrated that among patients with PCa, the relative levels of TGF-β1 mRNA increased during the late stages of the disease.

Some studies have suggested that decreased expression of TGF-β is not entirely necessary, as some tumor cells might escape the inhibitory effects of TGF-β through mutations that could provide a growth advantage over their benign counterparts. It has also been established that both TGF-β receptors (TβR-I and TβR-II) are required for the proper transduction of TGF-β signaling,21 and prostate cancer cells might reduce the expression or function of either TβR-I or TβR-II to escape the growth-inhibiting effects of TGF-β. This loss of sensitivity to TGF-β by its receptors could induce a compensatory overexpression of TGF-β, thereby leading to more aggressive phenotypes.22

Research has suggested that TGF-β exerts a tumor-suppressive or oncogenic effect is contextual and/or depends upon the temporal stage of cellular transformation.23 A recent study reported that the activation of TGF-β signaling pathways might be responsible for mediating the epithelial-mesenchymal transition (EMT), thereby enhancing the invasiveness and survival of transformed cells.24 Moreover, TGF-β can alter the host-tumor interaction, thus facilitating tumor growth, promoting angiogenesis, and inhibiting the host immune system.9,25

By examining benign tissues from the removed prostates of patients with PCa who were treated by radical prostatectomy, we observed the same TGF-β expression pattern as in malignant tissue. This result is interesting because it allows us to propose the use of TGF-β as a tumor marker, thereby overcoming the sampling error that commonly occurs in conventional prostate biopsies; however, larger studies should be conducted to further validate our findings.

In conclusion, we showed for the first time in clinical specimens that decreased expression of TGF-β is a characteristic of prostate cancer and may be related to cancer initiation and promotion; the role of TGF-β as a regulator of cell growth and apoptosis should be confirmed in a wider series. On the other hand, TGF-β superexpression might be related to tumor progression, as it is more highly expressed in more aggressive tumors, and this finding could be explained by mutations that confer resistance to TGF-β receptors. This hypothesis also warrants further experimental studies to analyze the potential of TGF-β as a diagnostic or prognostic marker.

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