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

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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.

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-β1.
associated with the conversion from a benign state to a malignant state: 1) a reduction or loss of the sensitivity to the inhibitory effects of TGF-β and 2) an increase in the ability to elevate TGF-β expression levels, which in turn promotes carcinogenesis by stimulating extracellular matrix production, promoting angiogenesis, and inhibiting the host immune system.

To evaluate whether the expression levels of TGF-β1 in prostate cancer cells are associated with prognosis, as predicted by the TGF-β elevation in carcinogenesis, we investigated a putative correlation between TGF-β gene expression and Gleason score, pathological stage, and PSA serum level.

PATIENTS AND METHODS

Patients

This study was conducted using surgical specimens from 79 patients with clinically localized PCa who underwent radical prostatectomy in our institution between 1993 and 2007; patients with a minimum follow-up of five years were randomly selected from a frozen tumor tissue database (Table 1). Benign tissue samples from an additional 10 patients with PCa who also underwent radical prostatectomy were included in this group. Patients who had undergone other treatments for PCa were excluded. All of the subjects provided their informed consent to participate in the study and to allow their biological samples to be genetically analyzed. Approval for the study was given by the Institutional Board of Ethics (n:0453/08).

We correlated the expression levels of the TGF-β1 genes in each sample with the patient’s Gleason score, pathologic stage according to the TNM 2002 staging system, and serum PSA level (in ng/ml). For the analysis, the pathologic stage was considered as an organ-confined (pT2) or non-organ-confined (pT3) disease. The Gleason score was classified as low grade (<7) or high grade (≥7), and preoperative PSA was used to identify patients at high (≥10 ng/ml) or low risk (<10 ng/ml) for disease recurrence. In addition, at a mean follow-up time of 60 months, we analyzed the gene expression levels in patients with biochemical recurrence, which was defined as a PSA level >0.4 ng/ml.

The control group consisted of tissue specimens from 11 patients with benign prostate hyperplasia (BPH) who presented with lower urinary tract symptoms and had an interest in undergoing surgery (mean age 64 ± 6.0 years).

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

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean</th>
<th>Min - Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA (ng/ml)</td>
<td>Mean</td>
<td>Min - Max</td>
</tr>
<tr>
<td>Stage</td>
<td>pT2 n (%)</td>
<td>38 (48)</td>
</tr>
<tr>
<td>Gleason Score</td>
<td>&lt;7 n (%)</td>
<td>32 (41.7)</td>
</tr>
<tr>
<td>≥7 n (%)</td>
<td>46 (58.3)</td>
<td></td>
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</tbody>
</table>
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-β1 contributes to the development of prostate cancer by acting as an inhibitor of cell proliferation and an inducer of apoptosis. Furthermore, we identified higher TGF-β1 expression levels in tumors with higher Gleason scores, suggesting that this gene may play a role in PCA progression and prognosis.

Soulitzis et al also reported a decrease in TGF-β expression in PCa. TGF-β is generally a growth inhibitor of both benign prostatic epithelial cells and prostate cancer cells *in vitro* and has been shown to inhibit proliferation and induce apoptosis in prostatic epithelial cells.

Moreover, TGF-β stimulates the synthesis of collagen, fibronectin, and integrins, and it inhibits matrix degradation through the downregulation of metalloproteinases such as collagenses, stromelysins, and plasminogen activators. Paired with its downregulation of metalloproteinases, TGF-β upregulates metalloproteinase inhibitors such as the following endogenous matrix metalloproteinases (MMP) inhibitors: the tissue inhibitor of metalloproteinase-1 (TIMP-1) and plasminogen activator inhibitor-1. 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).
Interestingly, a quantitative analysis revealed a positive association between increased TGF-β1 mRNA levels and elevated Gleason scores ($p=0.002$). Although controversial, this finding is consistent with that of a previous study in which TGF-β1 mRNA levels were higher in men with PCA who died 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 tissues. 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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REFERENCES


