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Perioperative changes in TGF-β1 levels predict the oncological outcome of cryoablation-receiving patients with localized prostate cancer

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ABSTRACT

Purpose: To evaluate the cytokine transforming growth factor beta 1 (TGF-β1) as a predictor of oncological outcomes in patients after cryoablation.
Materials and methods: Perioperative blood samples from prostate cancer (PC) patients who underwent total gland cryoablation between October 2011 and March 2013 were collected prospectively. Plasma TGF-β1 levels were quantified using magnetic bead immunoassay. The perioperative change in TGF-β1 was defined as the change in TGF-β1 from before surgery to 1–2 months after surgery. Biochemical recurrence (BCR) was defined according to the Phoenix criteria. The Mann–Whitney U, Kruskal–Wallis rank sum, and Chi-square test were used to compare the clinical characteristics of the subsets. The Cox proportional hazard model was applied for the comparison of recurrence risk among the groups.
Results: A total of 75 PC patients were included. During a median follow-up period of 12 months (range: 2.5–47 months), 11 patients had BCR, and 64 patients did not. Significantly greater changes in the perioperative TGF-β1 levels (median: 470.3 vs. 78.9 pg/ml) were observed in patients with than without BCR ($p < 0.05$). According to the changes in TGF-β1 levels, the patients were further divided into 4 groups, which were determined in the quartile categories of perioperative TGF-β1 levels. Group 4 (≥430) predicted the worst BCR outcome.
Conclusions: Perioperative plasma TGF-β1 levels were associated with BCR after prostate cryoablation for localized PC. Increase in postoperative plasma TGF-β1 may be a novel predictor for poor oncological outcomes and prompt a more aggressive follow-up or earlier salvage treatment.

1. Introduction

Prostate cancer (PC) is the second most common cancer among men worldwide [16]. Over the past few decades, PC has also become one of the leading cancers in Asian male populations [21] with an incremental increase in mortality [14]. Therapeutic methods for the treatment of localized PC have evolved over time. Cryoablation has been recognized as a definitive therapy for controlling localized PC as it destroys tumor tissues in a minimally invasive and efficient way [2,10,11,20,26,27]. Although previous studies have identified the initial clinical stage, biopsy Gleason sum, and prostate-specific antigen (PSA) measurements (total PSA and PSA doubling time) as important prognostic factors for predicting the likely outcomes of prostate total gland salvage cryoablation therapy [15,17,19,25], other potential factors need to be evaluated to improve the prediction of patient outcomes with localized PC.

Transforming growth factor beta 1 (TGF-β1) is known to be a multifunctional modulator of cellular differentiation and

Abbreviations: TGF-β1, transforming growth factor beta 1; PC, prostate cancer; PSA, prostate-specific antigen; EMT, epithelial–mesenchymal transition; BF, biochemical failure.

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proliferation, with a potent activity in regulating extracellular matrix production and degradation while exerting positive and negative effects at different stages of malignant progression [7,8,13,22,23,29]. Although TGF-β1 functions as a powerful tumor suppressor in the initial phases of cancer progression [1], it induces epithelial–mesenchymal transition (EMT), invasion and angiogenesis in tumor cells [6,12], and suppresses the immune system at the later stages of cancer progression [9], thereby enhancing tumor recurrence and metastasis [30].

In a recent initial report, Tang et al. reported that cryoablation induced TGF-β1 expression in CWR-22RV, a PC cell line [18]; however, the clinicopathological significance of changes in plasma TGF-β1 levels in patients with localized PC after cryoablation has not been explored. Because the induction of TGF-β1 expression has been shown to be associated with the transition from benign prostatic hyperplasia to PC [3] and TGF-β1 is induced by cryoablation [18], the aim of this study was to evaluate the clinicopathological significance of plasma TGF-β1 levels in PC patients receiving total gland cryoablation.

2. Materials and methods

2.1. Patient population

A total of 75 patients with localized PC who were treated with total gland cryoablation were prospectively recruited from October 2011 through March 2013. The study was approved by the Research Ethics Committee of National Taiwan University Hospital in September 2011. This study has also been registered at the ClinicalTrials.gov (NCT01454037).

2.2. Eligibility criteria

The inclusion criteria were as follows: (1) age $\geq$ 20 years; (2) histopathologically proven, non-metastatic, and localized prostate adenocarcinoma; (3) curative and definitive PC treatments chosen by the subjects prior to enrollment into the study; and (4) subjects willing to sign informed consent and agree to comply with the study procedures.

The exclusion criteria were as follows: (1) chronic use (>2 weeks) of >10 mg/day of prednisone or prednisolone within 2 months of screening (topical or inhalational corticosteroids were permitted); (2) concurrent use of immunosuppressive therapy, including cyclosporine, antithymocyte globulin, or tacrolimus, within 3 months of study entry; and (3) other conditions, which the investigators thought might affect subject compliance.

2.3. Prostate cryoablation technique

This technique has been described in detail in the previous literature [4,5].

2.4. Study design and sample collection

Preoperative blood samples were obtained for baseline parameter determination. Postoperative blood samples were collected at predetermined time points, i.e., 1–2, 3, 6, and 12 months after cryoablation. All subjects were followed for at least 24 months after treatment. Approximately 8 ml of peripheral whole blood was obtained in heparinized tubes (BD Vacutainer™ CPT). The blood samples were separated by centrifugation into plasma and peripheral blood mononuclear cells for further analysis, divided into aliquots, and frozen at −80 °C. Plasma immunocytokine levels were determined using the method described below. Perioperative clinical characteristics, including the Gleason sum, clinical stages, preoperative PSA value, D'Amico risk group, seminal vesicle invasion (SVI), prostate volume, and cryoprobe number were recorded according to the study protocol.

During follow-up, regular PSA tests (at least every 3 months in the first 12 months and every 6 months in the following period), physical examination, and radiographic examinations were performed according to routine practice. The endpoints of the study included PSA recurrence-free survival, prostate biopsy results, clinical/radiological evidence of recurrence, and quality of life.

2.5. TGF-β1 measurement

The concentration of TGF-β1 was quantified using a commercialized Milliplex MAP Human Cytokine/Chemokine Panel (#TGFBMAG-64K-03, Merck Millipore, Darmstadt, Germany). The assay was performed according to the manufacturer's instructions. Standards and samples were analyzed in duplicates on a MAGPIX® device (Luminex Corporation, Texas, United States) using xPONENT® software. The perioperative changes in TGF-β1 levels were determined by subtraction the preoperative levels from postoperative levels at 1–2 (Δ1), 3 (Δ2), 6 (Δ3), and 12 (Δ4) months.

2.6. Statistical analysis

All analyses were performed using standard statistical software, including IBM® SPSS® Statistics, version 22 (Armonk, New York, USA), and SigmaPlot™, version 12.5 (San Jose, CA, USA). The Mann–Whitney U/Kruskal Wallis rank sum test was used to compare median values among the groups. Contingency tables were constructed using data subjected to Chi-square testing. All tests were two-tailed. A p-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Patient demographics

During a median follow-up period of 12 months, 11 (15%) patients had biochemical failure (BF) based on the Phoenix criteria and 64 (85%) patients had not developed recurrence (non-BF) until December 2015. On comparing the clinical and preoperative characteristics (Table 1), preoperative PSA levels and the Gleason sum were determined to be statistically higher in the BF group than in the non-BF group. No statistically significant differences were detected between BF and non-BF patients in terms of age, clinical stages, D'Amico risk classification, SVI, prostate volume, cryoprobe number, and baseline TGF-β1 level. Nevertheless, a greater proportion of stage T3a-b tumors and a lower proportion of stage T1c-2a tumors was observed in the BF patients than the non-BF patients. Furthermore, the BF patients had a greater proportion of tumors with a Gleason sum of $\geq$8 than the non-BF patients (55% vs. 22%). On combining the clinicopathological characters, a greater proportion of high-risk tumors, as defined by the D'Amico risk classification, were noted in the BF group than in the non-BF group. SVI was diagnosed from prostate cryoablation biopsy samples more frequently in the BF patients than in the non-BF patients (45% vs. 19%). The median preoperative TGF-β1 levels in the BF patients were less than those in the non-BF patients (387.4 vs. 510.7 pg/ml) (Tables 2–4).

3.2. Change of TGF-β1 level after cryoablation

Following prostate cryoablation, the median PSA nadir was significantly higher in the BF patients than in the non-BF patients ($p = 0.0004$). Eighteen per cent of the BF patients acquired a high