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Clinical significance of both tumor and stromal expression of components of the IL-1 and TNF-α signaling pathways in prostate cancer

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ABSTRACT

IL-1 and TNF-α, the two major proinflammatory cytokines, have been involved in initiation and progression of several malignancies. They could influence the biological behavior of prostatic tumors and patient outcome, and could be useful as prognostic factors. This study evaluated the prognostic capability for biochemical progression after radical prostatectomy of expression of IL-1, TNF-α and related signaling components, in the tumor and surrounding stroma, as well as its correlation with other clinicopathological features. Expression of IL-1α, IL-1β, IL-1Ra, IL-1RI, IL-1RII, IRAK-1, TRAF6, TNF-α, TNFRI and TRAF2 was analyzed by immunohistochemistry in radical prostatectomy samples from 93 prostate cancer patients. Spearman's test, Kaplan–Meier curves, and univariate and multivariate Cox proportional hazard regression analyses were performed. Expression of TNF-α, TNFRI, TRAF2, ILRI, IRAK-1 and TRAF6 correlated with at least one clinicopathological feature (clinical T stage, pathological T stage, preoperative serum PSA or Gleason score). Increased tumor expression of TNF-α, TNFRI and IL-1RI, and reduced tumor expression of IRAK-1 were significantly correlated with a poor prognosis in univariate analysis. Reduced stromal expression of IL-1β and IL-1RII, and increased stromal expression of IRAK-1 were also adverse prognostic factors in univariate analysis. Remarkably, tumor IL-1β and stromal IL-1RII and IRAK-1 remained as independent prognostic factors after adjustment for preoperative serum PSA, pathological T stage and Gleason score in multivariate Cox models. Our results suggest that prostatic expression of TNF-α, IL-1β and related signaling proteins (TNFRI, IL-1RI, IL-1RII and IRAK-1) predicts clinical outcome in prostate cancer, and support the involvement of TNF-α and IL-1β signaling in prostate cancer progression.

1. Introduction

Prostate cancer is the second most frequent cancer in men and a leading cause of cancer-related death [1]. Radical prostatectomy constitutes the first line treatment for localized prostate cancer. However, after definitive therapy disease frequently progresses as evidenced by elevations in serum prostate-specific antigen (PSA) levels, a phenomenon called biochemical progression [2].

Early identification of patients at high risk to progress can be useful to anticipate therapy. Some predictors for biochemical recurrence exist, being Gleason score, preoperative serum PSA and pathologic stage, alone or in combination, the most important prognostic markers [3]. However, the accuracy of prediction could be improved by incorporating new prognostic markers into clinical practice.

Evidences from epidemiological, histopathological, genetic and molecular studies strongly support a relation between inflammation and prostate cancer [4]. Tumor necrosis factor (TNF)-α and interleukin (IL)-1 are two major proinflammatory cytokines which have been proposed as molecular links between inflammation and cancer. It has been shown that TNF-α secreted in the tumor microenvironment is able to activate signaling pathways that promote tumor initiation and progression [5]. On the other hand, endogenous interleukin (IL)-1 has been demonstrated to exert both pro and antitumor actions [6].

The IL-1 family has two major agonistic ligands, namely IL-1α and IL-1β, and the IL-1 receptor antagonist (IL-1Ra) [6]. All three ligands bind to both IL-1 receptor of type (IL-1R) I and IL-1RII.

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While IL-1α and IL-1β are able to transmit signaling cascades by binding to IL-1RI, IL-RII acts as a decoy receptor. Thus, IL-1Ra and IL-1RII attenuate the signals elicited by IL-1α/β [6]. After binding of IL-1α/β to IL-1RI, IL-1 receptor-associated kinase (IRAK)-1 and TNF receptor-associated factor (TRAF) 6 are recruited to the cytoplasmic domain of the receptor and transmit a signal which ultimately leads to modification of gene expression, mainly through nuclear factor-κB (NF-κB) activation [7]. Naturally occurring mature IL-1α and IL-1β are thought to have differentiated functions which rely on the form in which are presented by the cells, which in turn has been shown to influence their role in cancer. Most IL-1β remains intracellular and is secreted in limited amounts. Mature L-1α is preferentially presented bound to the plasma membrane and is secreted in a much lesser extent than IL-1β [6].

Binding of TNF-α to TNF receptor (TNFR) I, the most known TNFR, triggers the recruitment of several proteins at the cytoplasmic domain of the latter, including TRAF2, which constitutes a point of divergence of both pro and antiapoptotic signals. Thus, TRAF2 participates in the activation of mitogen-activated protein kinases (MAPKs) and the antiapoptotic transcription factor nuclear factor-κB (NF-κB) [7].

The predictive value of members of the TNF-α and IL-1 signaling pathways has been assessed in several human malignancies. IL-1β gene variants have been shown to predict outcome, for instance, in colon cancer [8], and have been associated with risk of lung [9] and gastric [10] cancer. Polymorphisms in the gene encoding for IL-1Ra have been associated, for instance, with colon [8] and gastric [10] cancer prognosis; and serum levels of IL-1Ra protein predicted outcome in patients with cervical [11] and bone [12] cancer. Serum levels and genetic variants of TNF-α have also been proposed as prognostic markers for several malignancies [13–15].

To the best of our knowledge there are no reports studying the significance for prostate cancer patient outcome of prostatic expression of IL-1 or TNF-α. In this work, we ought to assess the prognostic value for biochemical progression of expression of components of the IL-1 and TNF-α signaling pathways (IL-1α, IL-1β, IL-1Ra, IL-1RI, IL-1RII, IRAK-1, TRAF6, TNF-α, TNFRI and TRAF2), in the tumor and surrounding stroma, as well as to evaluate its correlation with classic clinicopathological markers of prostate cancer.

2. Materials and methods

2.1. Patients

All the procedures were examined and approved by the University of Alcalá and Principe de Asturias Hospital Ethics Committees (reference number SAF2007-61928) and were in accordance with the ethical standards of the Committee for Human Experimentation, with the Helsinki Declaration of 1975 (revised in Tokyo 2004) and the Committee on Publication Ethics guidelines.

The present study included 93 men who were diagnosed with prostate cancer and experienced radical prostatectomy as definitive therapy between 1992 and 1999, without receiving presurgical treatment, or postsurgical therapy before biochemical recurrence. Prostate cancer was detected by serum PSA screening and rectal examination, and diagnostic was confirmed by histopathological examination of needle biopsy cores. The median age (range) at time of surgery was 66 (51–74). Patients were generally scheduled to have a serum PSA measure every 3 months for the first year and every 6 months thereafter. Median follow-up (range) time of the cohort was 75.9 (15.6–159.2) months, being defined as the time between the surgery and the endpoint of the study or the last record. Clinicopathological features of patients are shown in Table 1. Data on node involvement were not available in 10 patients.

Table 1
Clinicopathological features of patients.

	Median (range)
Age (years)	66 (51–74)
Preoperative serum PSA (ng/ml)	10.5 (0.0–167.0)
	% (*n*)
Preoperative serum PSA	
<10 ng/ml	39.8 (37)
⩾10 ng/ml	60.2 (56)
Pathological T stage	
II	71.0 (66)
III	24.7 (23)
IV	4.3 (4)
Clinical T stage	
I	51.6 (48)
II	48.4 (45)
Gleason score	
⩽6	22.6 (21)
7	54.8 (51)
⩾8	22.6 (21)
Perineural invasion (yes)	7.5 (7)
Node involvement (yes)	8.4 (7)
Positive surgical margins	31.2 (29)
Biochemical progression (yes)	41.9 (39)
Survival (yes)	81 (87.1)

2.2. Reagents

Total serum PSA was measured by the AxSYM system (Abbott, IL). All primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit antihuman IL-1α, IL-1β, IL-1Ra and IL-1RI were used at a 1:150 dilution; rabbit antihuman IRAK-1 and TNF-α were diluted 1:200; mouse antihuman IL-1RII, TRAF2 and TRAF6 were diluted 1:100, 1:250 and 1:200 respectively; and goat antihuman TNFRI was diluted 1:100. Biotin-conjugated antibodies (DAKO, (Barcelona, Spain)) and Vectastain ABC (avidin–biotin complex) kit (Vector Labs (Barcelona, Spain)) were at a 1:500 dilution.

2.3. Immunohistochemical analysis and scoring

Immediately after surgery prostate tissues were fixed, dehydrated, and embedded in paraffin, and 5 μm thick sections were processed following the avidin–biotin–peroxidase complex (ABC) method as described previously [16].

Specificity controls for immunohistochemistry were performed as previously published [17–19]. Briefly, for negative controls tissues were incubated with blocking peptides or preimmune serum (Santa Cruz Biotechnology). As external positive controls, histologic sections of human lymph nodes, skin or thymus were incubated with the same antibodies. Additionally, in some samples, one part of the prostate tissues was frozen in liquid nitrogen immediately after surgery and maintained at −80 °C, to be later used for Western blotting analysis in order to test antibody specificity. In this portion, cryostat sections were stained with toluidine blue to confirm the histopathological diagnosis.

Immunostaining was evaluated at both cancerous epithelium and surrounding stroma level by two independent pathologists (P.M.-O. and G.O.), blinded for the endpoint, in five randomly selected fields per section and six sections per patient. First, patients were stratified as positive (those showing staining in more than 5% of the surface of the corresponding compartment) or negative. For those cases in which <10% of patients showed negative immunoreactions (for tumor TNF-α, TNFR1, IL-1α, IL1-RI and TRAF6) a score combining both intensity and percentage of immunostained surface was assigned to each sample, and accordingly patients were stratified as having "negative", "low", "intermediate", or "high" immunostaining.