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Original Article

Immune profiling of human prostate epithelial cells determined by expression of p38/TRAF-6/ERK MAP kinases pathways

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Abstract The aim of the present work was to study the immune profiling of prostate epithelial cells by the expression of ASK-1/p38 and Raf-1/ERK MAP Kinases signaling pathways mediated by TRAF-6. Immunohistochemical and Western blot analyses for TRAF-6, ASK-1, MEK-6, p38, Raf-1, MEK-1, ERK-1, ERK-2 and PSA were carried out in 5 samples of normal prostate gland, 24 samples of BPH and 19 samples of PC. Immunoreaction to TRAF-6 was found in the cytoplasm of epithelial cells of BPH and tumor cells of PC samples. For patients with the profile (TRAF-6+), optical densities revealed a weak immunoexpression of ASK-1 in PC compared to BPH patients. Whereas, immunoexpression to Raf-1 was higher in PC than in BPH. According to the expression of ASK-1 and Raf-1, two main profiles were identified: (TRAF-6+, ASK-1+, Raf-1+) and (TRAF-6+, ASK-1+, RAF-1−) in both BPH and PC. In addition, ASK-1/p38 axis expression was increased in BPH. Raf-1/ERK signaling pathway was increased in PC samples. On the other hand, representing of individual signaling protein expression enclosing each of p38 and ERK MAP Kinases according to TRAF-6+ showed a qualitative behavior of ASK61/p38 and Raf-1/ERK signaling pathways and a dynamic expression of PSA associated with immune and inflammatory process. These findings suggest that prostate epithelial cell could able an immune and inflammatory setting.

Conflicts of interest: All authors declare no conflicts of interest.

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Introduction

The link between chronic inflammation and cancer has been well recognized, inducing several forms of human cancer including prostate cancer [1]. Benign prostatic hyperplasia (BPH) is an inflammatory disease mediated by immune responses, and the presence of chronic inflammation is associated with benign prostate tissue and prostate cancer (PC) [2–4]. Currently, prostate specific antigen (PSA) in the serum is a clinically biomarker for PC screening. However, PSA lacks specificity in discriminating between BPH and PC and is unable to distinguish between aggressive and less aggressive tumors [5,6]. In consequence of the high heterogeneity of PC, it is important to study molecular and cellular pathways involved in the development and progression of prostatic diseases [7].

In human prostate tissue, as in the case of immune system cells infiltrating, epithelial cells are acquired to recognize bacterial products through the Toll like receptors (TLRs) and to release pro-inflammatory cytokines leading to the initiation of an immune response, inflammation and cancer carcinogenesis [8–11].

The Tumor necrosis factor receptor (TNF-R) associated factor 6 (TRAF-6) is a crucial adaptor molecule signaling transducer that regulates a diverse physiological processes, including immunity and inflammation [12]. TRAF-6 is required for signaling by TLRs and pro inflammatory cytokines including IL-1 and IL17, activating NF-κB and MAPK transduction pathways [13,14]. A strong link has been established between the p38 and ERKMAP Kinases pathways, inflammation, apoptosis and proliferation [15–17]. Furthermore, in responses to TLRs and Il-17 stimulations, TRAF-6 is recruited to activate ASK-1/p38 and Raf-1/ERK MAP Kinases signaling pathways [12].

Although the role of infiltrating immune cells in prostate inflammation has been well studied, little is known about the contribution of prostate epithelial cells to an immune setting and the prostate immune-mediated signaling pathways. In the present study, we investigated the immune profiling of prostate epithelial cells by immunoexpression of TRAF-6 and the upstream components of p38 (ASK-1, MEK-6) and ERK (Raf-1, MEK-1) transduction pathways, its modifications in BPH and PC and demonstrate that prostate epithelial cells would contribute to the immune and inflammatory responses.

Materials and methods

Prostates were obtained from a) transurethral resections from 24 men (aged from 61 to 85 years) diagnosed clinically and histopathologically with BPH, b) radical prostatectomy from 19 men (aged from 57 to 88 years) diagnosed with prostate cancer (PC) and 5 histologically normal prostates (NP) obtained at autopsy (8–10 h after death) from 6 men (aged from 21 to 40 years) without histories of reproductive, endocrine or related diseases. All pathological, clinical and personal data were anonymized and separated from any personal identifiers. All the procedures followed were examined and approved by the Hospital of La Rabta of Tunis and the Military Hospital of Tunis (HMPIT). All applicable international, national, and institutional guidelines for the care and use of human samples were followed. The primary antibodies used were mouse anti-human TRAF-6, MEK-6, p38, MEK-1 and ERK-2, goat anti-human ASK-1, Raf-1, rabbit anti-human ERK-1 (Santa Cruz Biotechnology, CA, USA) and PSA mouse anti human (Dako, Barcelona, Spain).

Western blotting

For Western blot analysis of each prostate tissues were homogenized in the extraction buffer (0.005 M Tris–HCl, pH 8) with addition of a cocktail of protease inhibitors (10 mM iodoacetamide, 100 mM phenylmethyl sulphonic fluoride, 0.01 mg/mL of soybean trypsin inhibitor and 1 μL/mL of leupeptin) and phosphates inhibitors (10 mM sodium fluoride and 1 mM sodium orthovanadate) in the presence of 0.5% Triton X-100. After centrifugation, supernatants were equilibrated with loading buffer (10% SDS in Tris/HCl pH 8 containing 50% glycerol, 0.1 mM 2-beta-mercaptoethanol and 0.1% bromophenol blue). Then, the mixture was denatured for 5 min at 100 °C and aliquots of 10 μL of homogenate were separated in SDS polyacrylamide slab minigels (15% gradient gels). Separated proteins were transferred to nitrocellulose membranes in the transfer buffer (25 mM Tris–HCl, 192 mM glycine, 0.1% SDS and 20% methanol). Membranes (0.2 μm), were blocked for 1 h in TBS containing 1% donkey serum, and incubated overnight at room temperature with the primary antibodies at 1:50 (ASK-1), 1:100 (MEK-6, p38, Raf-1, MEK-1, ERK-1, ERK-2) and 1:400 (TRAF6) in TBS with 5% bovine serum albumin (BSA). After extensive washing with TBS/Tween-20 (TBST), the membranes were incubated with swine anti-rabbit (ERK1), rabbit anti-goat (ASK-1, Raf-1) and rabbit anti-mouse (TRAF-6, MEK-6, p38, MEK-1, ERK-2 and PSA) biotinylated immunoglobulins (Dako, Barcelona, Spain) for 1 h at 1:4000 dilution in TBS with 5% BSA; and then washed and incubated with the avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA) at 1:10.000 dilution. For detection of immunoreactive protein, we used the ECL system (Amersham, Buckinghamshire, UK).

Immunohistochemistry

Immunochemical procedure specificity was checked using negative controls. For negative controls, tissues of each type (normal, BPH, and PC) were incubated with blocking peptides (Santa Cruz Biotechnology, CA, USA) at the same immunoglobulin concentration used for each antibody. For immunohistochemistry analysis, tissues were fixed with 10% formaldehyde, dehydrated and embedded in paraffin. Sections (5 mm thick) were processed following the avidin–biotin–peroxidase complex (ABC) method. After deparaffinization, sections were hydrated, incubated for 30 min in 0.3% H_2O_2 diluted in methanol to reduce endogenous activity. To retrieve the antigen, the sections were incubated with 0.1 M citrate buffer (pH 6) for 2 min in a conventional pressure cooker. After incubation with TBS containing 3% donkey serum, the primary antibodies were applied at a dilution of 1:200 (TRAF-6), 1:50 (MEK-6, p38, Raf-1, ERK-1, ERK-2), 1:25 (ASK-1) and 1:100 (PSA) in TBS at 37 °C overnight. Afterwards, the sections were washed twice

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