IL-6, IL-10 and HSP-90 Expression in Tissue Microarrays from Human Prostate Cancer Assessed by Computer-assisted Image Analysis

MARIA ROSARIA CARDILLO[1] and FLORA IPPOLITI[2]

[1]Section of Pathologic Anatomy, Service of Uropathology and [2]Section of Immunology, Department of Experimental Medicine and Pathology, University "La Sapienza", Rome, Italy

Abstract. *The interleukins (IL)-6 and -10 and heat shock proteins (HSP) have an important role in the host-tumor interaction and in tumor bulk. HSP-90 may have a regulatory role in cytokine biosynthesis and prognostic value in some tumors. To define the role of IL-6, IL-10 and HSP-90 in prostate cancer progression the immunohistochemical expressions of these proteins were analyzed in 168 prostatic carcinomas. IL-6, IL-10 and HSP-90 immunoreactivity was higher in prostatic carcinoma (CaP) and intra-epithelial prostatic neoplasia (PIN) than in normal prostatic tissue (NAP) adjacent to neoplasia. In the epithelium, IL-6, IL-10 and HSP-90 expressions increased from NAP to PIN to CaP. In the stroma, IL-6 and IL-10 expressions decreased significantly from NAP to PIN to CaP ($p<0.01$ by Chi-square test), while HSP-90 expression increased. In the epithelium of PIN and CaP, IL-6 immunoreactivity was significantly lower than IL-10 and HSP-90. In neoplastic acini HSP-90 levels were significantly higher than those of IL-6 and IL-10 ($p<0.01$ by Chi-square test). In the stroma of NAP and PIN, but not of CaP, HSP-90 immunoreactivity was significantly lower than that of IL-6 and IL-10 ($p<0.01$). Our results indicate that the IL-6 and IL-10 cytokine balance differs in pathological and normal prostate, thus suggesting that certain cytokines are specific to the neoplastic prostate. Changes in the expressions of IL-6, IL-10 and HSP-90 in human prostate carcinoma samples could be used as a prognostic marker of disease progression.*

Correspondence to: Dr. Maria Rosaria Cardillo, MD, Department of Experimental Medicine and Pathology, Section of Pathologic Anatomy, Service of Uropathology, University "La Sapienza", Policlinico Umberto I, 324 Viale Regina Elena, 00161 Rome, Italy. Tel: 0039.06.49972417, Fax: 0039.06.4454820, e-mail: mariarosaria.cardillo@uniroma1.it / 2494@mclink.it

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Despite intense research over the years, the mechanisms underlying the incidence and progression of prostate cancer are unclear. The development, growth and differentiation of the prostate gland are regulated by steroid hormones, growth factors and cytokines mediating stromal-epithelial interactions (1). Little is known about the relationship between prostate cancer and cytokines (2). The observation that certain cytokines are specific to the pathologically altered prostate, suggests that the cytokine balance differs in pathological and normal prostate (3). Increasing interest focuses on the interleukins (IL)-6 and -10. Owing to their pleiotropic actions, both have a regulatory role in the inflammation, immune responses and pathogenesis of various neoplasia (4-8) including prostate cancer (9). Given that IL-10 is secreted by regulatory T cells and inhibits dendritic cell function, the presence of IL-10 in many tumors may influence the native antitumor response, thereby inducing a response against tumor cells (10). Genetic alterations in cytokine genes may influence tumor progression by acting on pathways of tumor angiogenesis (11, 12). The cytokine IL-10 inhibits angiogenesis and has anti-inflammatory actions (13, 14). It is a T-helper subset 2 cytokine and down-regulates macrophage proinflammatory cytokines such as IL-6. It also influences the humoral immune response by promoting B-cell activation and regulates immunoglobulin (Ig) class switching (15).

In patients with hormone-refractory prostate cancer, high circulating serum levels of IL-6 and IL-10, correlate with progression, prognosis, tumor metastases and patient morbidity (2, 16). The mechanisms underlying the development of hormone resistance are poorly defined, but this process leads to several molecular changes (16). Experimental studies demonstrated that IL-6 acts as a paracrine growth factor for human LNCaP androgen-sensitive prostate cancer cells, and as an autocrine growth factor for human PC3 androgen-insensitive prostate cancer cells. IL-6 activates androgen receptor (AR)-mediated gene expression by activating the AR, regulates the

expression of androgen-responsive genes in an androgen-independent manner and induces androgen-independent growth of androgen-dependent human cancer cells. AR activity is induced by IL-6 in a ligand-independent and synergistic manner in cell lines (16-21). Before ligand binding, AR exists in a complex with HSP-90 and other co-chaperones: this interaction maintains the AR in a high-affinity ligand-binding conformation, which is necessary for an efficient response to hormones (22). In addition, the mutation of certain oncoproteins reflects an increased requirement of heat shock protein (HSP)-90 for their efficient folding (22). HSP-90 is expressed at high levels in tumor cells, suggesting that it may be important for tumor cell growth or survival, or for both (23, 24). Cytokine biosynthesis is regulated by HSPs (22). IL-6 and HSP-90 seem to play an important role in the survival response of cancer cells exposed to the toxic effects of chemotherapy or radiotherapy (22-24). The ability of these chaperone proteins to protect cells under stress condition suggests a novel diagnostic or prognostic approach using immunohistochemistry in human prostatic specimens.

As candidate interleukins for this study, IL-6 and IL-10 cytokines were selected and as candidate co-chaperone HSP-90 was chosen, because few studies analysed its expression immunohistochemically in human prostatic tissue. Most studies examined IL-6 and IL-10 expression, mainly in human prostatic cell lines, culture supernatant and peripheral blood, with various methods [immunoassay, ELISA, CAT assays , Western Blot, immunohistochemistry, and reverse-transcript polymerase chain reaction (RT-PCR) analysis] (12, 21, 25-28).

In this study, the role of the cytokines IL-6 and IL-10 and HSP-90 were investigated in the progression of human prostate cancer. The epithelial and stromal expressions of these proteins in normal adjacent prostate (NAP), prostatic intra-epithelial neoplasia (PIN) and prostate carcinoma (CaP) tissues from 168 patients who had undergone radical prostatectomy were characterized by immunohistochemistry, and immunohistochemical levels were quantified by computer-assisted image analysis. The possible association of IL-6, IL-10 and HSP-90 immunoreactivity with prostate tumor grade and stage was determined.

Materials and Methods

Patients and specimens. One hundred sixty-eight tumor samples were obtained from 168 patients (age range 51 to 88 years, mean age 68.11 years) with clinically localized and advanced CaP, who had undergone radical prostatectomy as monotherapy (no hormonal or radiation therapy) in the Department of Urology "U. Bracci", University "La Sapienza" of Rome, Italy, between 1990 and 2000. Prostate tumors were graded with the Gleason system: one tumor was Gleason score 5; 5 were score 6; 24 score 7; 7 score 8; and 1 was score 9. Pathological stage was determined using the TNM staging system: 28 tumors were organ-confined diseases (pT2) and 10 extraprostatic diseases (pT3). The samples analyzed were then arbitrarily divided into three histological groups: low (score 5 and 6), intermediate (score 7) and high (score 8 and 9) Gleason score tumors, and into two pathological stages: pT2 and pT3. PIN areas were graded as low-grade and high-grade. The reported values refer only to high-grade PIN.

Tissue MaxArrays (TMA) construction. High-density TMAs were assembled using the manual tissue puncher/array (Beecher Instruments, Silver Springs, MD, USA). The device consists of thin-walled stainless steel needles with an inner diameter of approximately 600 μm and a stylet used to transfer and empty the needle contents. The assembly was held in an X-Y position guide that was manually adjusted by digital micrometers. Small biopsy specimens were retrieved from selected regions of donor tissue and precisely arrayed in a new paraffin block. Tissue cores were 0.6 mm in diameter and ranged in length from 1.0 to 3.0 mm depending on the depth of tissue in the donor block. Multiple replicate core samples of NAP, high-grade PIN and CaP tissue were acquired from each case. The cores were inserted into a 45 X 20 X 12-mm recipient block and spaced 0.8 mm apart. The study used 6 high-density TMAs composed of NAP, PIN and CaP prostate cancer.

Immunohistochemical staining. Samples were immunostained with the anti-IL6 mouse purified recombinant human IL-6 (supplied by Genzyme, Diagnostics, Cambridge, MA, USA); anti-IL10 rabbit recombinant human IL-10 (supplied by Genzyme, Diagnostics) and anti-HSP90 (clone D-19) rabbit polyclonal antibody IgG1/K fraction (supplied by Novocastra, Laboratories Ltd., Newcastle-upon-Tyne, UK), at 1:100 dilution and incubated overnight at 4°C as previously reported (6). The sections were previously microwave oven treated for 5 min at 750 w (two cycles). All reactions included appropriate positive controls (breast tissue) and negative controls (the primary antibody was replaced by normal swine serum) and carried out using the avidin-biotin-peroxidase complex (ABC) (reagents from Dako S.p.A., Milan, Italy) and as chromogen substrate diaminobenzidin-hydrogen peroxidase (DAB) (Sigma-Alderich, S.R.L., Milan, Italy).

Immunohistochemical evaluation by computer-assisted image analysis. To quantify the IL-6, IL-10 and HSP-90 immunoreactivity in tissue sections and reduce operator subjectivity, computer-assisted image analysis was used. Three representative areas of each sample (assessed by IHC and *in situ* analysis) were randomly selected for each tumor tissue sample from the three areas (NAP, PIN, CaP) under a light microscope Olympus Uplan FI (20x objective) and were captured with a digital camera (Nikon). Areas of interest were quantified with IMAGE-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA) using the histogram function of the software, as already described (29). In the equal areas, the expressions of IL-6, IL-10 and HSP-90 positive areas and the average positive signal optical density (average positive dense) were detected respectively. The immunohistochemical staining positive index (PI) was equal to the positive area X average positive dense. The results are expressed in pixel and represent an average of the 3 visual fields. The error bars represent standard error (SE). Data are given as optical density per unit surface area (1 mm^2). For each slide, the means±SE of colorPercent area (ColorPercent=ColorArea/tissueAreaX100) was calculated.

Statistical analysis. The results are reported as mean±standard error (SEM) and are expressed in arbitrary densitometric units. The data were analyzed by the Multistat program (Biosoft, Cambridge, 1988). The paired Student's *t*-test and Chi-square test were used to analyze differential expressions of IL-6, IL10 and HSP-90 proteins in the epithelial and stromal compartments of the three areas examined. A possible correlation between the various proteins in the three neoplastic and non-neoplastic areas was determined by linear regression analysis using rank correlation. The Chi-square test was used to determine the association IL-6, IL-10 and HSP-90 ligand levels with Gleason histological grade and TNM clinical stages. *P* values less than 0.05 were considered to indicate statistical significance.

Results

IL-6 protein expression. The IL-6 protein was detected in the cytoplasm of epithelial cells of NAP, PIN and CaP prostatic neoplastic acini (Figure 1A and 1B), as well as in the stromal tissue (mononuclear, fibroblasts and endothelial cells) around NAP, PIN and in single stromal cells adjacent to CaP areas (Figure 1C). IL-6 expression was higher in the epithelium than in the stroma, the difference reaching significance in preneoplastic and neoplastic areas (PIN, $p=0.033$; and CaP, $p=0.011$, by Chi-square test) (Table I). In normal peritumoral prostatic epithelium, IL-6 showed scarce and weak immunoreactivity in basal cells and diffuse immunoreactivity in the superficial luminal layers (Figure 1A). The percentage of IL-6-positive cells was higher in PIN and in adenocarcinoma glands than in benign glandular epithelium (Table I). Intensity of labelling was stronger in CaP than PIN and NAP, and immunoreactivity concentrated in single neoplastic cells infiltrating the stroma (Figure 1B and 1C). While IL-6 expression increased from NAP to PIN to CaP in the epithelial compartment, it decreased in the stroma (Figure 2A and 2B).

IL-10 protein expression. IL-10 immunostaining was cytoplasmic and localized in epithelial and stromal cells (Figure 1D, 1E and 1F) and immunoreactivity was significantly higher in the epithelium than in the stroma of PIN ($p=0.030$) and CaP ($p=0.0015$) (Table I). IL-10 expression in the epithelium increased from NAP to PIN to CaP and in the stroma decreased from NAP to PIN to CaP (Figure 2A and 2B).

HSP-90 protein expression. HSP-90 immunostaining was cytoplasmic. HSP-90 immunoreactivity was significantly higher in the epithelium than in the stroma of NAP ($p=0.0005$), PIN ($p=0.0001$) and CaP ($p=0.001$) and its expression increased from NAP to PIN to CaP (Figure 1G, 1H and 1I). Epithelial and stromal expression of IL-10 and HSP-90 differed in the three areas. In the epithelium of PIN and CaP, IL-6 levels were significantly lower than those of IL-10 and HSP-90 (PIN, $p=0.013$; CaP, $p=0.0121$ by Chi-square analysis) (Figure 2A). In contrast, in the stromal tissue of NAP and PIN, HSP-90 levels were significantly lower than those of IL-10 and IL-6, and in the stromal cells around neoplastic acini, HSP-90 levels were significantly higher than those of IL-6 and IL-10 ($p=0.0001$ by Student's *t*-test) (Figure 2B).

Although the comparison of IL-6, IL-10 and HSP-90 levels with Gleason histological grade and TNM staging showed elevated levels of all three antibodies investigated in high-grade and in more advanced TNM stage tumors, no correlation was found between Gleason score, TNM stages and IL-6, IL-10 and HSP-90 immunoreactivity in the epithelium and stroma (Table II).

Linear regression showed a negative correlation between IL-6 and IL-10 in the epithelium of NAP ($r=-0.161$; $p=0.041$) and a positive correlation between IL-10 and HSP-90 in the epithelium and stroma of NAP ($r=0.170$, $p=0.031$; $r=0.335$, $p=0.000$), PIN ($r=0.471$, $p=0.00001$; $r=0.3829$, $p=0.0001$) and CaP ($r=0.534$, $p=0.0001$) and between IL-6 and HSP-90 in epithelium and stroma of PIN ($r=0.471$, $p=0.0001$; $r=0.382$, $p=0.0001$) and CaP ($r=0.534$, $p=0.0001$; $r=0.693$, $p=0.0001$) .

Discussion

In this *in vivo* study designed to investigate the role of IL-6 and IL-10 cytokines and HSP-90 in the progression of human prostate cancer, all three proteins studied were found in the epithelium and stroma of neoplastic and normal peritumoral tissues investigated. All proteins were more strongly expressed in CaP and PIN than in NAP. No correlation was found between Gleason histological grade, TNM stages and IL-6, IL-10 and HSP-90 immunoreactivity in the epithelium and stroma, although elevated levels of all three antibodies investigated were found in high-grade and in more advanced TNM stage tumors.

Given that the behavior of prostate tumors depends on the interaction between the stromal and epithelial compartments, an important finding was that our immunohistochemical study showed that prostatic epithelium and stroma both expressed IL-6. In NAP, PIN and CaP, IL-6 was immunolocalized to the stromal cells and to the luminal cytoplasm of epithelial cells, suggesting that these cells secrete IL-6. IL-6 expression increased in the epithelium and decreased in the stroma as the prostatic malignancy progressed (from NAP to PIN to CaP). These data, in agreement with other findings (25, 26), indicate that the prostatic mesenchymal compartment of NAP secretes IL-6 in large amounts, suggesting an autocrine role for IL-6 in stromal cells of NAP. Stromal cells secrete high amounts of IL-6 into culture supernatants, whereas basal cells are the main epithelial source of IL-6 in the benign prostate (25). Although epithelial cells from benign hyperplastic prostate do not respond to IL-6, in prostatic carcinoma cell lines IL-

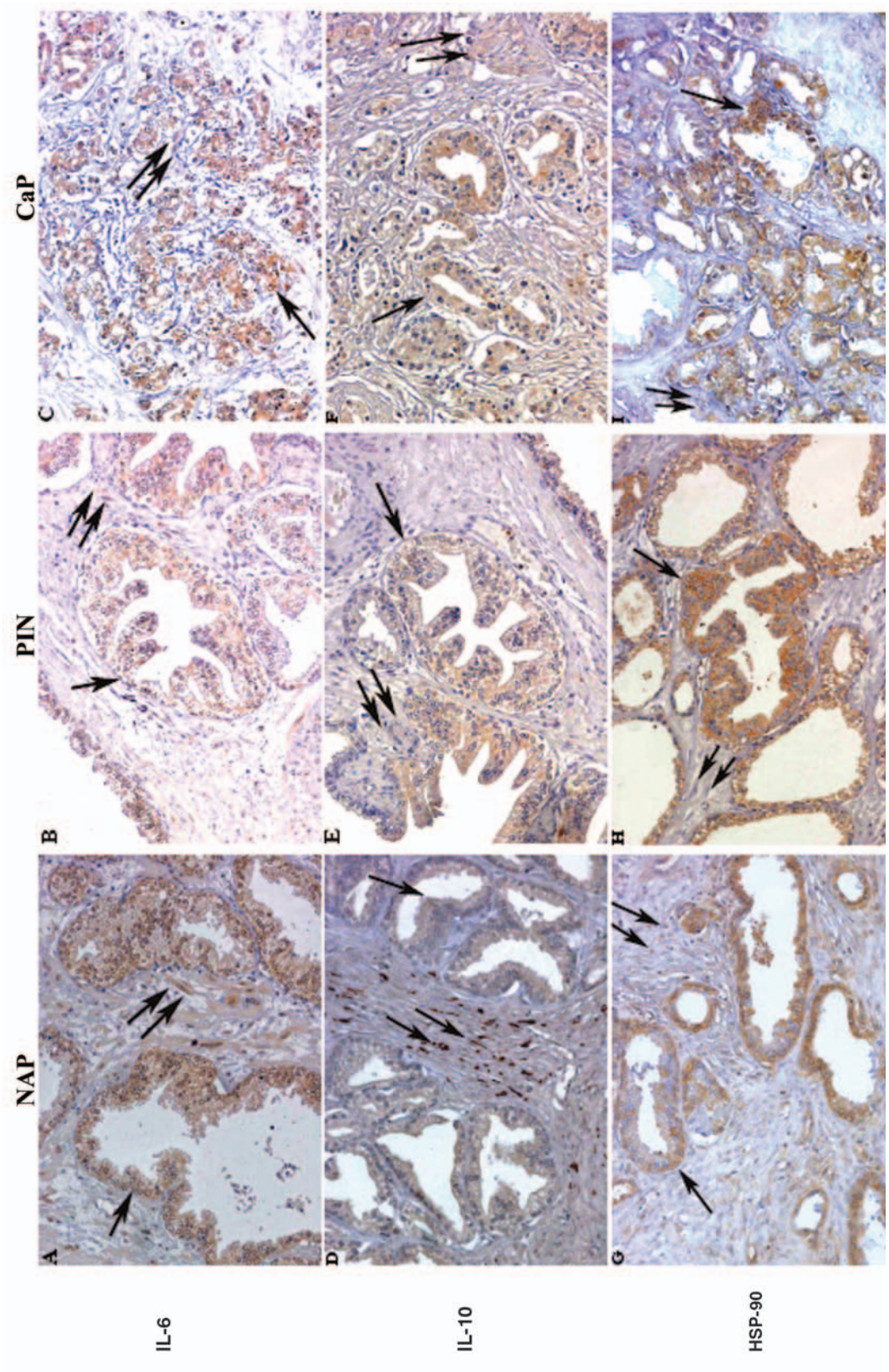

Figure 1. *Interleukin (IL)-6, -10 and heat shock protein (HSP)-90 immunoreactivity in epithelium and stroma of normal prostate (NAP), prostatic intra-epithelial neoplasia (PIN) and prostatic carcinoma (CaP). The anti-IL-6 reactivity in: A) area of NAP was relatively scarce and weak in basal cells and diffuse in the secretory luminal cells (arrow). The stroma contained smaller amounts of IL-6 than epithelial cells (double arrows); B) area of PIN shows diffuse staining pattern in luminal cells and discontinuous staining in the basal cell layer (arrow). PIN epithelial cells stained more strongly than stromal cells (double arrows); C) area of CaP, shows staining pattern concentrated in single neoplastic cells infiltrating the stroma prostatic neoplastic acini (arrow) and also in the stromal cells (double arrows). Anti-IL-10 reactivity in: D) area of NAP, IL-10 immunostaining was localized in the cytoplasm of epithelial luminal cells (arrow) and immunoreactivity was significantly more intense in epithelium than in the stroma (double arrows); E) area of PIN showing cytoplasmic staining, localized on luminal cells (arrow), and IL-10 levels were lower in the stroma than in the epithelium (double arrows); F) area of CaP showing IL-10 luminal staining (arrow) concentrated in the cytoplasm of neoplastic acini: the intensity of staining was higher in malignant epithelium than in the stroma (double arrows). Anti-HSP-90 immunoreactivity in: G) area of NAP showing immunostaining mainly in luminal cells (arrow). HSP-90 immunostaining was weaker in stromal than in epithelial cells (double arrows); H) area of PIN shows HSP-90 cytoplasmic staining, localized on luminal cells (arrow). The surrounding stroma (double arrows) contained lower HSP-90 levels than epithelium; I) area of CaP, the cytoplasmic HSP-90 immunoreactivity was intense in neoplastic acini (arrow) and weaker in the surrounding stroma (double arrows).*

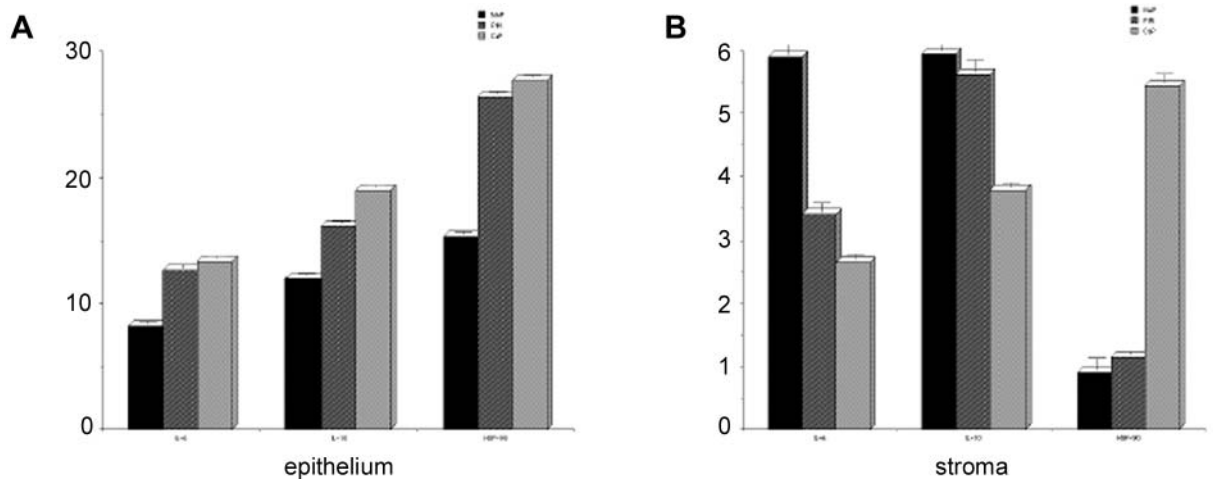

Figure 2. *IL-6, IL-10 and HSP-90 proteins in 168 radical prostatectomies: comparison between epithelium and stroma of normal prostate (NAP), prostatic intra-epithelial neoplasia (PIN) and prostatic carcinoma (CaP) tissues. (A) In the epithelium IL-6 and IL-10 and HSP-90 expression increased from NAP to PIN to CaP. IL-6 levels were significantly lower than IL-10 and HSP-90 (PIN, $p<0.01$; CaP, $p<0.01$ by Chi-square test). In the neoplastic acini HSP-90 was significantly more strongly expressed than IL-6 and IL-10 ($p<0.01$ by Chi-square test). (B) In the stroma IL-6 and IL-10 significantly ($p<0.01$) decreased from NAP to PIN to CaP. In contrast HSP-90 expression increased from NAP to PIN to CaP. In NAP and PIN, HSP-90 was significantly less intensely expressed than IL-6 and IL-10, while in stromal tissue around neoplastic acini HSP-90 was more strongly expressed than IL-6 and IL-10 ($p<0.01$ by Student's t-test).*

Table I. *IL-6, IL-10 and HSP-90 protein expressions in epithelium and stroma of normal adjacent prostate (NAP), prostate intra-epithelial neoplasia (PIN) and prostate carcinoma (CaP) from 168 radical prostatectomies by computer-assisted quantitative image analysis. IL-6 and IL-10 epithelial expressions were significantly higher than in the stroma, in PIN ($p<0.05$) and CaP ($p<0.01$) by Chi-square analysis, HSP-90 epithelial expression was significantly higher than in the stromal in NAP, PIN and CaP ($p<0.01$) by Chi-square analysis.*

	NAP			PIN			CaP		
	Total area mean±SE	immunostaining positive index mean±SE	*Chi-square* test (*p*)	Total area mean±SE	immunostaining positive index mean±SE	*Chi-square* test (*p*)	Total area mean±SE	immunostaining positive index mean±SE	*Chi-square* test (*p*)
IL-6									
epithelium	26635.6±1311.52	8.20±0.16	0.128	31454.62±1798	12.63±0.201	4.593	30432.03±924.16	13.36±0.21	6.376
stroma	13965.72±903.35	5.90±0.09	(0.719)	30218.80±3785.66	3.405±0.13	(0.032)	21790.68±999.60	2.66±0.09	(0.011)
IL-10									
epithelium	26486.94±1319.68	11.99±0.13	1.548	25104.98±1220	16.15±0.18	4.681	54239±6719.32	19.01±0.20	10.027
stroma	15741±665.02	5.96±0.14	(0.213)	15637±1372.07	5.62±0.19	(0.030)	19043.94±1388.27	3.78±0.08	0.0015
HSP-90									
epithelium	23526±585.07	15.29±0.19	12.049	23178±2310.11	26.45±0.16	24.819	29453±1327.99	27.69±0.18	16.336
stroma	16753.68±647.45	0.90±0.05	(0.0005)	21867.00±807.83	1.15±0.04	(0.000)	21714.25±814.09	5.44±0.15	(0.001)

by Chi-square test
*$p<0.05$

6 acts through an IL-6-mediated autocrine or paracrine growth mechanism or through both (21). Notwithstanding the intense investigation into lymphocytic infiltration and T-cell-derived cytokines such as growth factors, the local factors fine-tuning intraprostatic immune responses are poorly known. Recent studies reported that activated T cells in benign prostatic hyperplasia express IL-17 that up-regulates IL-6 production of prostatic stromal cells (3). The progressive increase in IL-6 levels found in the epithelial and stromal compartments of PIN and CaP, indicates that this cytokine could have a growth-promoting role during the early stages of prostate carcinogenesis. Stromal IL-6 might therefore affect prostatic growth and differentiation in autocrine and paracrine loops.

Table II. *IL-6, IL-10 and HSP-90 expressions in the prostatic carcinoma samples according to Gleason histological grade and TNM stage.*

	No. of cases	IL-6		IL-10		HSP-90	
		Epithelium	Stroma	Epithelium	Stroma	Eepithelium	Stroma
Gleason score							
Group 1 (GS 3-5)	20	8.53±0.39	1.26±0.18	14.69±0.44	2.24±0.14	24.14±0.75	2.50±0.31
Group 2 (GS 6-7)	117	13.41±0.15	2.46±0.07	19.16±0.19	3.77±0.07	27.98±0.10	5.39±0.14
Group 3 (GS 8-10)	31	15.71±0.15	4.09±0.12	20.96±0.15	4.44±0.10	28.94±0.35	7.67±0.13
Total	168						
TNM							
Group 1 (pT1)	20	9.11±0.49	1.29±0.20	15.28±0.41	2.34±0.15	24.36±0.72	2.72±0.36
Group 2 (pT2)	91	13.57±0.20	2.70±0.10	19.43±0.19	3.81±0.07	27.88±0.10	5.66±0.17
Group 3 (pT3)	57	14.67±0.28	3.15±0.14	19.94±0.30	4.30±0.16	28.67±0.29	6.13±0.23
Total	168						

In our samples from patients undergoing radical prostatectomy, IL-6 expression was also localized in the cytoplasm of epithelial neoplastic acini and we found a higher percentage of immunolabeled cells in high-grade Gleason and in more advanced TNM stage tumors. Our data agree with previous immunohistochemical studies on human specimens (25, 26) and also with *in vitro* studies reporting secretion of IL-6 and its receptor by tumor cells (9, 30) and a correlation of high IL-6 serum concentrations with a worse outcome in prostatic carcinoma (5, 27). In patients with advanced stage cancer, treatment with monoclonal antibody to IL-6 improves the disease, suggesting that anti-IL-6 therapy may also be useful at an earlier stage of the disease (5). Collectively, our immunohistochemical results again confirm the role of IL-6 in the growth, development and progression of prostate cancer (9).

With regard to the other cytokine studied, IL-10, our findings refer to protein expression, not in human prostatic cell lines (18-20, 28, 31, 32) but in specimens from patients who had undergone radical prostatectomy. Genetic alterations in cytokine genes may lead to high or low production of certain cytokines that may influence native antitumor immune responses or tumor progression by acting on pathways of tumor angiogenesis (11-12). In primary human prostate tumor lines, IL-10 inhibits angiogenesis by inhibiting MMP-9 production (28, 31, 32). Stroma cells associated with the malignant tumors may be responsible for the over-production of MMP-9 (29). In this study, IL-10 expression in epithelial and stromal cells and its immunoreactivity in epithelium significantly increased from NAP to PIN to CaP, whereas in the stroma it decreased as prostate cancer advanced. The progressive epithelial increase in this Th2 cytokine IL-10 from NAP to CaP presumably prevents the cytotoxic action of locally infiltrating Th1 lymphocytes against the tumor cells themselves. Conversely, the stromal decrease in IL-10 that inhibits MMP-induced angiogenesis and metastasis could favor prostate cancer progression. Our findings support the hypothesis that IL-10 acts as a Th2 cytokine favoring carcinogenesis, thus, inhibiting the cytotoxic (Th1) response against the transformed tumor cells. Conversely, decreased stromal levels of IL-10 favor tumor metastasis insofar as IL-10 inhibits MMP-9 production and blocks MMP-2 in primary human prostate tumor (14). We detected elevated levels of IL-10 antibody in the epithelium and stroma of high Gleason grade and more advanced TNM stage prostatic tumors. In renal cell carcinoma, IL-10 down-regulates the secretion of pro-inflammatory cytokines including IL-6 (33). IL-10 may therefore play a major role in suppressing immune and inflammatory responses. IL-6 and IL-10 probably intervene in modulating the host-tumor interaction both locally and systemically by eliciting alterations in the physiological, biochemical and immunological status of the host. In our previous studies on the prostatic samples (29), we reported increased MMP expression as CaP progressed, suggesting that IL-10 is ineffective in inhibiting the action of MMPs but leaves the action of MMPs unchanged.

As a result, in this study we found a negative correlation between IL-6 and IL-10 in the epithelium of NAP and a positive correlation between IL-10 and HSP-90 in the epithelium and stroma of NAP and CaP and between IL-6 and HSP-90 in the epithelium and stroma of PIN and CaP. The positive correlation between IL-6 and IL-10 and IL-6 and HSP-90 suggests that the proteins studied are dependently regulated.

The role of HSP-90 in the progression of human prostate cancer was further defined by the finding that like IL-6 and IL-10, HSP-90 was expressed in epithelial and stromal cells of NAP, PIN and CaP and its expression in both compartments significantly increased as the malignancy progressed. The ability of these chaperone

proteins to protect cancer cells under conditions of stress indicates a novel immunohistochemical diagnostic or prognostic approach in human prostatic specimens. Although we found no correlation of tumor grade and stage with IL-6, IL-10 and HSP-90 immunoreactivity, the higher levels of all three antibodies we found in high-Gleason grade and more advanced TNM stage prostatic carcinomas might be interpreted as evidence for autonomous (autocrine) growth of invasive malignancy. They also suggest that these proteins may be useful as markers of tumor progression in prostate carcinoma. In line with previous studies (23, 24), the significantly higher immunoreactivity to HSP-90 than IL-6 and IL-10 detected in high-grade and advanced stage tumors ($p<0.01$), suggests that therapy with anti-HSP-90 could be effective in patients with advanced prostate cancer.

Conclusion

Our immunohistochemical findings provide a basis for further studying on the potential role of the examined proteins as prognostic indicators of prostatic carcinoma and also suggest the cytokines IL-6 and IL-10 and HSP-90 as targets for immunotherapy.

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