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Review

TNF-α/IL-1/NF-κB transduction pathway in human cancer prostate

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Summary. TNFα exerts apoptosis throughout an intracellular transduction pathway that involves the kinase proteins TRAF-2 (integration point of apoptotic and survival signals), ASK1 (pro-apoptotic protein), MEK-4 (p38 activator and metastasis suppressor gene), JNK (stress mitogen activated protein kinase) and the transcription factor AP-1. TNFα also exerts proliferation by p38 activation, or when TRAF-2 simultaneously induces the transcription factor NF-κB by NIK. NIK and p38 may also be activated by IL-1. P38 activated several transcription factors such as Elk-1, ATF-2 and NF-κB. NIK also may activate NF-κB.

The aim of the present article was to evaluate the different components of this TNFα/IL-1 transduction pathway in human prostate carcinoma (PC) in comparison with normal human prostate. In prostate cancer, pro-apoptotic TNFα/AP-1 pathway is probably inactivated by different factors such as p21 (at ASK-1 level) and bcl-2 (at JNK level), or diverted towards p38 or NIK activation. IL-1α enhances proliferation through IL-1RI that activates either NIK or p38 transduction pathway. P38 and NIK activate different transcription factors related with cell proliferation and survival such as ATF-2, Elk-1 or NF-κB.

In order to search a possible target to cancer prostate treatment we proposed that inhibition of several proinflamatory cytokines such as IL-1 and TNFα might be a possible target for PC treatment, because decrease the activity of all transduction pathway members that activate transcription factors as NF-κB, Elk-1 or ATF-2.

Key words: Prostate carcinoma; IL-1, TNF-α, NF-κB, NIK, p38

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Introduction

Prostate cancer is the most common cancer in men in the Western world and the second most common cause of male cancer death. The development and progression of prostate cancer is controlled by complex mechanisms that are still not completely understood. Inflammation may be a causal factor in several human tumors, including prostate cancer. In the inflammatory process several factors such as cytokines appear to play crucial roles in these processes, but its role is poorly understood. TNFα and IL-1 are two pro-inflammatory cytokine families with multiple biological properties and are involved in prostate cancer development (Muenchen et al., 2000; Ricote et al., 2004).

The TNFα superfamily includes several members such as: TNFα, TNFß, CD40L (CD40 ligand), CD30L (CD30 ligand), CD27L (CD27 ligand), FasL (Fas ligand), TRAIL (TNF- related apoptosis ligand), VEGF (vascular endothelial growth factor) and APRIL (a proliferation-inducing ligand) among other members (Zhang, 2004). Tumor necrosis factor-α (TNFα) has important roles in immunity and cellular remodelling, as well as in influencing apoptosis and cell survival (Szlosarek and Balkwill, 2003). TNFα is produced by numerous immune cell types but also by several non-immune cells and many kinds of tumour cells (Bazzoni and Beutler, 1996). At present, it is assumed that TNFα has paradoxical roles in the evolution and treatment of malignant diseases.

Two different TNFα receptors, named TNFRI and TNFRII, have been identified. The binding of TNFα to TNFRI or TNFRII induces receptor trimerization and recruitment of several downstream signaling proteins to their cytoplasmic domains. TNFRI is the major mediator of TNFα activities, including programmed cell death (apoptosis), fibroblast proliferation and antiviral activity (Tartaglia et al., 1993). Binding of TNFα/ TNFRI

complex to TRADD proteins (TNF receptor associated death domain) activates TRAF-2 (one of the six members of the TNF receptor associated factor), which represents an integration point of pro- and anti- apoptotic signals (Wajant and Scheurich, 2001). TRAF-2 activation might stimulate two different pathways. One is initiated by TRAF-2 and NIK (NF-κB-inducing kinase) interaction that activates NF-κB (nuclear factor-kB), which promote survival factors such as bcl-2 and bcl-XL (Tamatani et al., 1999). The other pathway activates the cascade ASK1 (signal regulating kinase), MEK-4 (mitogen activated protein kinase kinase 4) and JNK (Jun N-terminal kinase) (Ichijo et al., 1996); this latter phosphorylates AP-1 (activator protein-1), which stimulates apoptosis either directly or by p38 activation (Fig. 1).

In addition to TNFα (TNFα/AP1 pathway), Interleukin-1 (IL-1) is another physiological regulator of p38. IL-1 activates PAK-1 through its binding to two GTPases, called Cdc42 and Rac. These activate PAK-1, which induces MEK-6 activation that in turn activates p38 (Raingeaud et al., 1996) (Fig. 1).

P38 was first proposed as a mitogen activated protein kinase involved in apoptosis, although recent studies have suggested that p38 activation is a protective apoptotic protein (Lüschen et al., 2004). P38 regulates gene expression by phosphorylation of some transcription factors, including the activating transcription factor-2 (ATF-2), the Ets-domain protein (Elk-1) (Rao and Reddy, 1994), the cAMP-response element binding protein (CREB) and the nuclear factor-κB (NF-κB).

The Interleukin-1 (IL-1) family includes two bioactive ligands (IL-1α and IL-1ß), two types of transmembrane receptors (IL-1RI and IL-1RII), and a specific receptor antagonist (IL-1Rα) (Dinarello, 1998). The affinity of IL-1α, IL-1ß and IL-1Rα to receptors IL-1RI and IL-1RII differs according to the ligand. IL-1α and IL-1Rα present higher affinity to IL-1RI than IL-1ß, while IL-1ß displays higher affinity to IL-1RII (Boraschi et al., 1996). IL-1RI has an intracellular domain that is responsible for the initiation of the signal transduction mechanism leading to cell proliferation activation. IL-1RII presents an extremely short intracellular domain that is unable to initiate signal transduction. It has been suggested that this receptor is a natural inhibitor of IL-1 action. IL-1Rα neutralizes IL-1 action by binding to IL-1RI and IL-1RII (Boraschi et al., 1996).

IL-1α and IL-1ß bound to their receptors (RI and RII) promote the association of IL-1 receptor-associated kinase (IRAK). In humans, four different IRAK molecules have been described: IRAK-1, -2, -M, and -4 (Cao et al., 1996). They all comprised four domains: an N-terminal death domain, a linker region, a centrally located classical serine/threonine kinase domain, and a C-terminal domain. Whereas IRAK-1 and IRAK-4 have been identified in all tissues, IRAK-2 and IRAK-M have a narrower cellular distribution (Rosati and Martin, 2002). Genotyping methods have shown that IRAK-1 and IRAK-4 are associated with prostate cancer risk (Sun et al., 2006). IRAK-1, the primarily described member of the IRAK family, is a membrane proximal serine-threonine kinase that, when it is phosphorylated, forms a complex with TRAF-6 (TNF receptor associated factor-6) (Cao et al., 1996). TRAF-6 interacts with the C-terminus of IRAK-1, which contains three TRAF-6 binding sites, leading to the activation of several downstream signaling pathways, such as the activation of NF-κB inducing kinase termed NIK, which is a MAP3K-related kinase that activates the IKK complex composed of IKK-α and IKK-ß (DiDonato et al., 1997). NIK stimulate IKK-ß, which induces IKK-α degradation. IKK complex phosphorylates IκB, following its ubiquitination and rapid degradation, causing the nuclear translocation of NF-κB (Fig. 1), which, in turn, activates target genes involved in carcinogenesis: tumor initiation, malignant transformation and metastasis (Wu and Kral, 2005). Using the prostate carcinoma cell lines LNCaP, DU45 and PC3, Gasparian et al. (2002) found that increased IKK activation leads to the activation of NF-κB. A potential role of NF-κB in the development of different tumors such as breast (Sovak et al., 1997), colon (Dejardin et al., 1999), pancreas (Wang et al., 1999) and prostate (Ross et al., 2004; Domingo-Domenech et al., 2005; Lessard et al., 2006) have been reported.

The aim of this review was to elucidate the possible involvement of TNFα/IL-1 transduction pathway in prostate cancer development and its role in the breakdown of the apoptosis-proliferation equilibrium. We also discuss the possible use of some members of this pathway as a potential therapeutic factor.

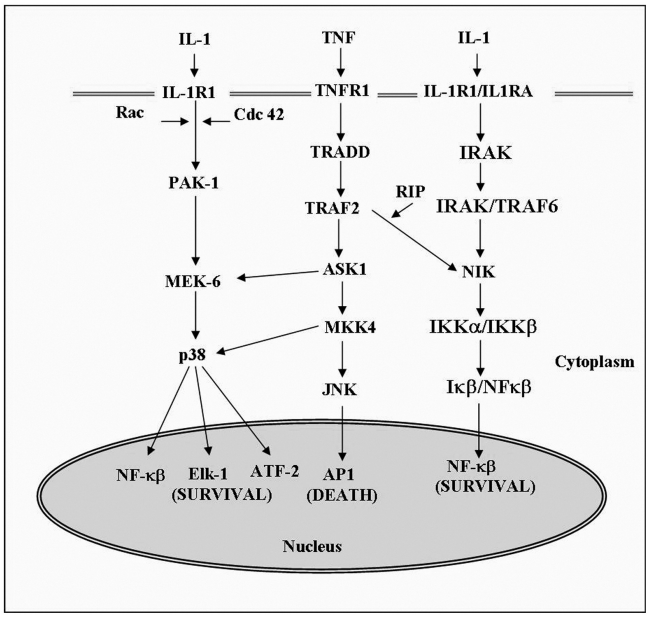

Fig. 1. TNFα/IL-1 transduction pathway.

TNFα family

Tumor necrosis factor α (TNFα) seems to exert a key role in the promotion of many tumors such as prostate cancer. TNFα is a 17 kDa polypeptide that was first described as a serum-derived substance that causes tumor cell death (Carswell et al., 1975). It can induce tumor necrosis by affecting tumor vascularization and initiating apoptotic cell death, but paradoxically, it also can promote cell proliferation (Szlosarek and Balkwill, 2003).

Wise et al. (2000) presented no significant different in TNFα serum levels between normal patients compared with hormone sensitive and refractory prostate cancer. Other author described high circulating levels of TNFα in prostate cancer patients (Perambakam et al., 2005; Li et al., 2006) but also in other prostate diseases as the chronic prostatitis (Razumov et al., 2003; Li et al., 2006) or in several conditions such as after transurethral prostatic resection (Irie et al., 1999). Increased levels of these cytokines were not correlated with PSA levels (Pfitzenmaier et al., 2003), the most successfully and widely used cancer serum marker. Stamey (2001) suggested that PSA levels have little correlation with cancer grade or with long-term outcome. Nevertheless, as a functional product of normal prostatic epithelial tissue, PSA levels will reflect changes due to inflammation, trauma or benign proliferation (Roscigno et al., 2004). The presence of high serum TNFα levels has been associated with extent of disease (Michalaki et al., 2004), poor prognosis and resistance to therapy (Foa et al., 1990). These data suggest an increase of both proliferation and apoptosis processes, which could lead to a deregulated control cycle which favors the increment of mutation chances and deregulates the control proliferation/apoptosis equilibrium.

We have previously reported that the immunoexpression of TNFα (Fig. 2A) and its receptors (TNFRI and TNFRII) in the cytoplasm of epithelial cells of normal prostate samples. Immunoexpressions of TNFα (Fig. 2B), TNFRI (Fig. 2C), and TNFRII were also located in the cytoplasm of epithelial cells of cancer prostate samples but are increased in comparison with normal prostates (de Miguel et al., 2000). The expression and action of TNFα and its receptors have been reported in several tumors as esophageal (Hubel et al., 2000), follicular thyroid (Zubelewicz et al., 2002), ovarian (Rzymski et al., 2005) and breast (Garcia-Tuñon et al., 2006) cancers. Several studies revealed that endogenous TNFα is involved in tumour-cell growth and stromal interactions that facilitate tumour invasion and metastasis (Kollias et al., 1999). TNFα can also provide a survival signal for cancer cells and, hence, it has been referred to as a tumour-promoting factor (Balkwill, 2002).

Inflammation has been suggested as a causal factor in several human tumors, including prostate cancer. In inflammatory process several factors such cytokines has been described, and the role of proinflammatory cytokines, such as TNFα, has been well established in prostate cancer. In this way, TNFα was the first cytokine to be used in humans for cancer therapy. However, its role in the treatment of cancer patients is debated (Bertazza and Mocellin, 2008). Its systemic application in patients with advanced tumor diseases provokes toxicities, rare tumor remissions and organ failure (Wiedenmann et al., 1989; Pilati et al., 2008). On the other side, TNFα clearly posses antitumor effect when we analyzed preclinical models or clinical setting (Mocellin and Nitti, 2008). TNFα inhibitors represent a new class of biological agents that specifically target key in inflammatory process (Chang and Girgis, 2007) but also immunotherapy may provide an alternative treatment for cancer prostate and the use of vaccination with different antigens such as TNFα is considered attractive (García-Hernández et al., 2007). Further investigations of this important topic are clearly warranted. A better understanding of the members that participate in the different signalling pathway activated by TNFα may improve clinical management and provide new targets for therapy in prostate cancer patients.

TNFα/AP-1 pathway

The unbalanced between proliferation and apoptosis in human normal prostates is broken in prostate cancer. The proliferation and apoptosis indexes were higher in prostate cancer than in normal prostate (De Miguel et al., 2000). This uncontrolled proliferation is characteristic of cancer pathology. These data suggest that several factors which regulate proliferation/apoptosis equilibrium are altered in pathologic conditions. In this way, cells have different mechanisms for detecting environmental changes and developing a response.

In all normal human prostates, positive immunoreactions to TRAF-2, ASK1 MEK-4 and JNK were found, although immunoreaction to AP-1 (p-c-jun) was negative (De Miguel et al., 2000; Ricote et al., 2003). TRAF-2 (Fig. 2D) and ASK1 (Fig. 2E) were located in the cytoplasm of epithelial cell in normal prostate. ASK1 is associated with apoptosis (Ricote et al., 2003). MEK-4 preferentially accumulates in the nucleus (Fig. 2F) and -at low levels- in the cytoplasm (Kim et al., 2001; Ricote et al., 2003). The mechanism that accounts for the nuclear location of this protein is unclear, since MEK-4 is activated by a cytoplasmic protein and phosphorylates JNK in the cytoplasm. However, MEK-4 function may not be restricted to the JNK signal transduction pathway because MEK-4 also phosphorylates and activates p38, and this latter is prelocalized in the nucleus and is rapidly exported to the cytoplasm upon activation (Ben-Levy et al., 1998). With these data, Ricote et al. (2003) suggest that MEK-4 is not involved in the JNK/AP-1 pathway, although it might be involved in the p38 activation pathway. This hypothesis agrees with the high p38 levels found in normal prostate in our laboratory (Royuela et al., 2002). Therefore, the intermediate proteins (TRAF-2, ASK1

and MEK-4) seem to be mainly involved in other transduction pathways such as NF-κB activation or p38 pathway (Fig. 1).

In human prostate cancer, the TNFα cascade seems to be over-stimulated, since TNFα receptors (TNFRI and TNFRII) present high immunoexpression (De Miguel et al., 2000). However, the transduction pathway from TRAF-2 to AP-1 seems to be inactive; since immunoreaction (in the same place described in normal prostate) to TRAF-2 (Fig. 2G), ASK-1 (Fig. 2H) and MEK-4 (Fig. 2J) decreased and no immunoreaction to AP-1 (Fig. 2J) was even found (Ricote et al., 2003). In this pathology there must be several extracellular or intracellular factors that are blocking the activation of this transduction pathway in different steps. ASK1 might be a critical blockage point of this transduction pathway.

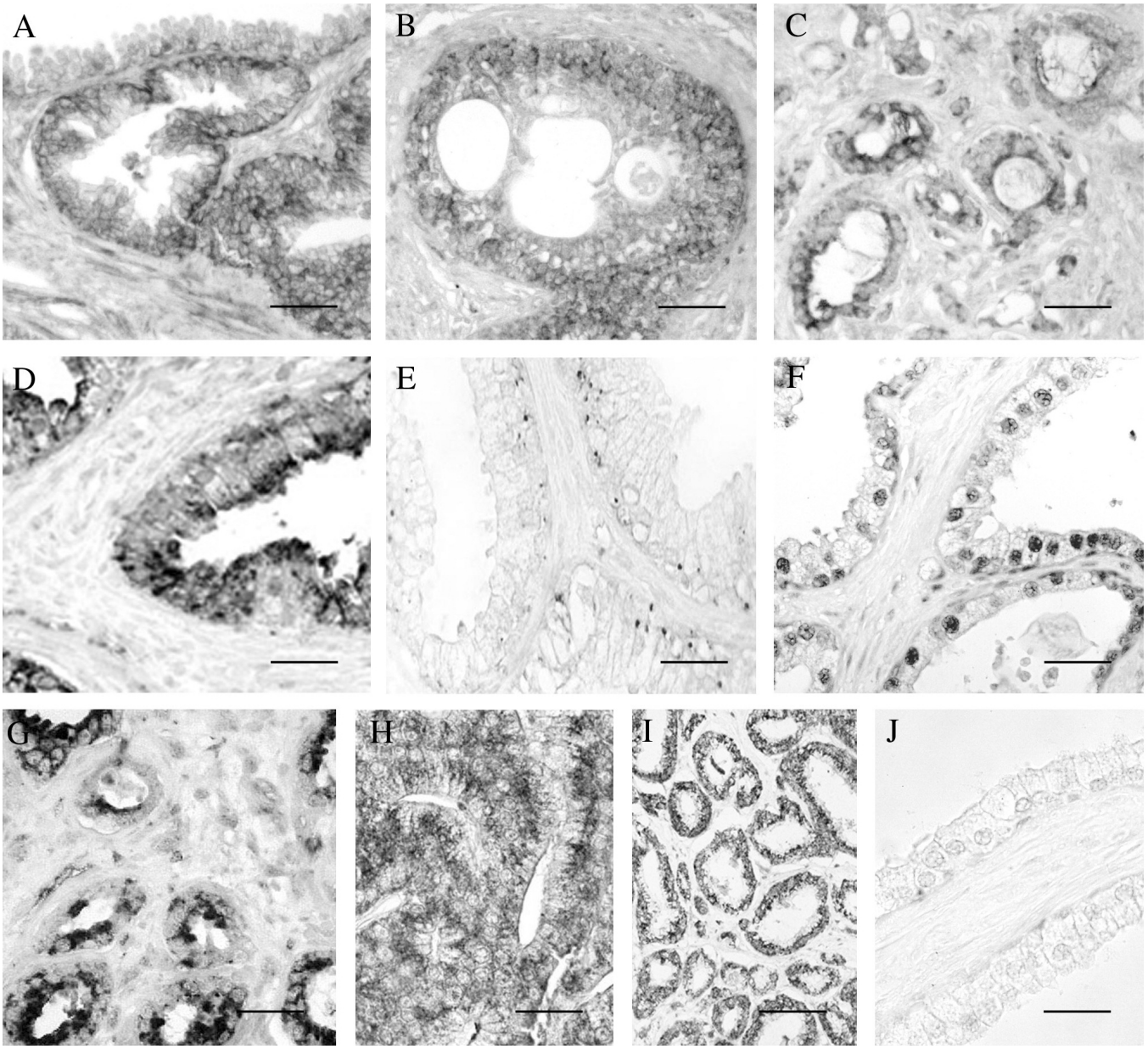

Fig. 2. TNFα immunostaining in prostate from normal men **(A)** and prostatic adenocarcinoma (**B**). TNFRI **(C)** in prostate cancer. TRAF-2 **(D)** and ASK1 **(E)** were found in cytoplasm of epithelial cells of normal samples whereas MEK-4 **(F)** was found in the nucleus of epithelial cells. TRAF-2 **(G)**, ASK-1 **(H)** and MEK-4 **(I)** were observed in epithelial cells of prostate cancer. Ap-1 **(J)** was negative in prostate cancer. Scale bars: A, B, 25 μm; C-G, J, 20 μm; H, 15 μm; I, 30 μm

P21 has been reported as an ASK1 inhibitor and has been found significantly associated with a high Gleason score (Aaltomaa et al., 1999; Royuela et al., 2001). Bcl-2 has been postulated as a potential modulator of JNK activation in fibroblasts. Since an increase of bcl-2 has been reported in prostate cancer specimens (Royuela et al., 2000), bcl-2 might be another potential inhibitor of JNK in prostate cancer.

IL-1 family

In normal human prostate, only IL-1ß and IL-1RI were found but not IL-1α (Fig. 3A), IL-1RII and IL-1Rα (Ricote et al., 2004). IL-1ß and IL-1RI have also been reported in normal nervous tissue, where their presence has been associated with the synthesis and secretion of NGF (neuronal growth factor) (Juric and Carman-Krzan, 2001). Hacham et al. (2002) found differential expression between IL-1α (in organs with abundant lymphoid cells such as lungs, small intestine, spleen and liver) and IL-1ß (in organs with scanty lymphoid cells such as heart, brain, muscle or kidney). In normal human prostate, IL-1ß is located in the nuclei of both epithelial basal cells and stromal cells. This molecule possesses a small seven-aminoacid sequence, which is specific for nuclear translocation (Grenfell et al., 1991). Labriola-Tompkins et al. (1991) described that the surface of the IL-1ß molecule presents seven non-contiguous aminoacids, which correspond to the IL-1RI binding site. This interaction provokes the endocytosis of the IL-1ß/L-1RI complex. While IL-1ß enters into the nucleus to develop its biological action, the receptor returns to the cell surface (Lowenthal and MacDonald, 1986).

There have been some reports on IL-1α in prostatic tissue. Epithelial cells in tissue cultures of human benign prostate hyperplasia (BPH), showed an overproduction of IL-1α, which has been related to the increase of proliferation (Giri and Ittmann, 2000). In prostatic cancer (PC), androgen-independent PC cell lines (PC-3 and DU-145) showed IL-1α expression (Hoosein, 1998), which has been related to a neuroendocrine phenotype but also to growth and differentiation (Abdul and Hoosein, 2002).

IL-1ß immunoreaction in prostate group (Fig. 3C) appeared in stromal cell nuclei and in both the nucleus and cytoplasm of all epithelial cells (basal cells and tall

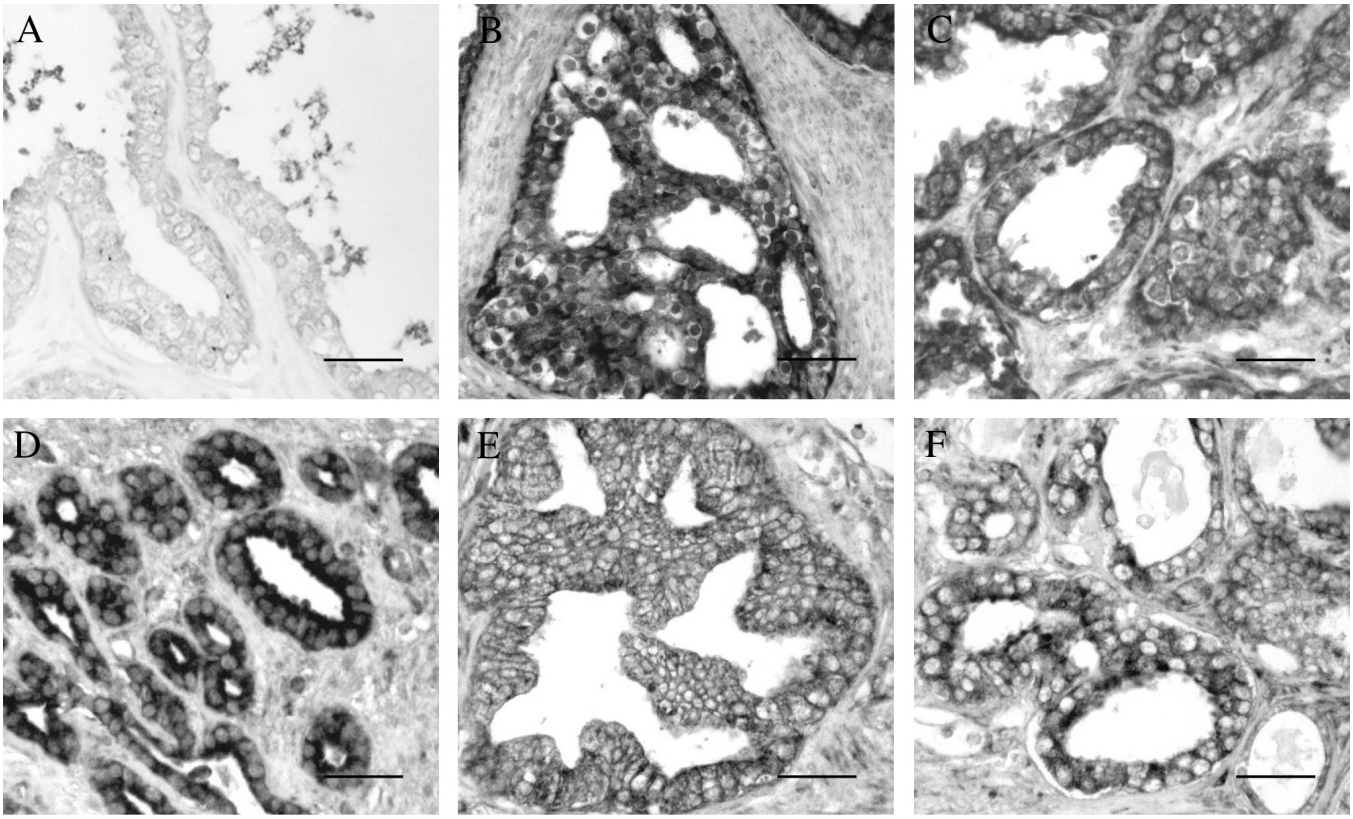

Fig. 3. IL-1 family immunostaining in human prostate. IL-1α **(A)** was negative in normal samples. PC samples showing intense immunoreaction to IL-1α **(B)** in the epithelial cell cytoplasm. Immunoreaction to IL-1ß **(C)** in PC samples appears in both the nucleus and cytoplasm of epithelial cells. Immunostaining to IL-1RI **(D)**, IL-1RII **(E)** and IL-1Rα **(F)** in PC samples appeared in the cytoplasm of epithelial cells. Scale bars: A, C-F, 20 μm; B, 15 μm

secretory cells), but immunoexpression is lower in this group than in normal prostate group and decreasing with Gleason grade (not found in high Gleason). The most cancer patients studied presented immunoreaction to IL-1α in the epithelial cell cytoplasm (Fig. 3B); IL-1RI in both epithelial and stromal cytoplasm cells (Fig. 3D); IL-1RII (Fig. 3E) only in the periphery of epithelial cells; and IL-1Rα in the cytoplasm of epithelial cells (Fig. 3F) (Ricote et al., 2004). Interaction between IL-1α and IL-RI would be involved with the high proliferation degree of these tumors. Endogenous IL-1α production was associated with resistance to exogenous anti-proliferative stimuli and the increase in the constitutive production of IL-6 in autocrine manner (Lázár-Molnár et al., 2000). In previous results made in our laboratory we report increased IL-6 expression in prostatic epithelial cells in high Gleason grade PC (Royuela et al., 2004). Since increased IL-6 has been associated with bad prognosis in PC (Twillie et al., 1995; Shariat et al., 2001), evaluation of IL-1α as IL-6 inductor might be important to assess malignancy.

Patients with cancer prostate displaying poor differentiation presented a complete absence of IL-1ß expression in high Gleason cancer (Ricote et al., 2004). This finding has also been reported in other cancers including lung (Matanic et al., 2003) or breast (Singer et al., 2006). Expression of IL-1RII in prostate cancer might be related to the ability of this receptor as a natural inhibitor of IL-1 function, and this expression could be an effort to counteract IL-1α function. Furthermore, a complete absence of IL-1Rα (the antagonist receptor) expression has been described (Ricote et al., 2004). AP-1 site appears to be the most crucial binding site for IL-1Rα promoter activity (La et al., 2002). A previous study in our laboratory (Ricote et al., 2003) reported the absence of AP-1 expression in prostate cancer samples and suggests a possible genetic regulation mechanism of cancer progression. It has been reported that local overproduction of IL-1α and/or underproduction of IL-1Rα predispose to the development of cancer, and the therapeutic administration of IL-1Rα, in experimental models, is effective in preventing tissue damage such as leukemia, kidney diseases and cancer (Arend, 2002). This specific antagonist has been studied in clinical trials tested in several tumor cell lines (Dinarello, 1998) and animals (corneal angiogenesis in rat) (Coxon et al., 2002), in which administration of IL-1Rα inhibits IL-1 function and might help to prevent the aggressiveness or even to provoke tumor regression of prostate tumors. In this way, Lewis et al. (2006) proposed the use of IL-1 inhibition as a novel therapeutic approach in the treatment of solid organ malignancies. In our knowledge, at the present there are no reports with clinical studies on IL-1 family and cancer prostate.

P38 transduction pathway

It has been proposed that, among other cellular responses, TNFα induces cell death, but also cell proliferation by activation of p38. It has also been reported that IL-1α favors cell proliferation by p38 activation (Ricote et al., 2006).

In normal human prostate, immunoexpression to p38, p-Elk-1 and p-ATF-2 (the downstream executors of the p38 pathway) were present in epithelial cells, but no immunoreaction to PAK-1 (Fig. 4A) and MEK-6 (the upstream executors of IL-1/p38 pathway) were found (Ricote et al., 2006). This suggests that activation of Elk-1 and ATF-2 is triggered by TNFα/MEK-4/p38 but not by IL-1 (Fig. 1), since in addition to the presence of TNFα (De Miguel et al., 2000), immunoexpressions of p38 (Royuela et al., 2002) and MEK-4 (Ricote et al., 2003) have been demonstrated in normal prostates. Previous reports indicate that localization of p38 is related to their phosphorylation: inactivated (non-phosphorylated) is located in the cytoplasm, while phosphorylated p38 is found in the nuclei (Zheng and Guan, 1994; Chen et al., 1997). When MAPKs are translocated to the nucleus and are phosphorylated, they activate transcription factors and other target proteins (Cano and Mahadevan, 1995).

In human prostate cancer, intense immnoreaction was observed in the cytoplasm of epithelial cells to PAK-1 (Fig. 4B), MEK-6 (Fig. 4C) and p38 (Fig. 4D); p-Elk-1 (Fig. 4E) immunoreaction was nuclear in 18.5% of patients, cytoplasmic in 51.85% of patients, and both nuclear and cytoplasmic in 29.6% of patients; and, p-ATF-2 (Fig. 4F) immunostaining was nuclear in 25.9% of patients and cytoplasmic in 59.2% of patients (Ricote et al., 2006). In several patients, p-Elk-1 and p-ATF-2 change its location from the nucleus to the cytoplasm. This fact may be related with its biological function. In mammalian cells, endogenous p38 is present in the nucleus but it can be exported to the cytoplasm upon activation (Royuela et al., 2002). In the nucleus, p38 phosphorylates Elk-1, ATF-2 and also NF-κB (Raingeaud et al., 1996). ATF-2 (Raingeaud et al., 1996) and Elk-1 (Xiao et al., 2002) are not only a target of p38, but also a target for JNK. Since immunoreaction to JNK was found in normal human prostate, but not in prostate cancer, is reasonable to suggest that the activation of ATF-2 and Elk-1 are the consequence of p38 pathway activation (Ricote et al., 2006). Proapoptotic effects of TNFα/AP-1 pathway decrease, because this pathway is inhibited by p21 at ASK1 step (Ricote et al., 2003). Cell proliferation stimulation triggered by TNFα via p38 occurs, since intense immunoreaction to PAK-1 and MEK-6 was found (Ricote et al., 2006), but previous studies have shown elevated levels of IL-1 (Ricote et al., 2004) and p38 (Royuela et al., 2002). When LNCaP cell cultures were treatment with the p38 inhibitor, increase the frequency of apoptosis indicating that p38 exerts an important role in prostatic tumour promotion by TNF-α stimulation. Hence, down-regulation of p38 activity by specific pharmacological inhibitors may represent a strategy to clinical improve efficacy of TNF-α in androgen-dependent prostatic cancer (Ricote et al., 2006). In our knowledge, at the present there are no reports with clinical studies on p38 and prostate cancer.

NIK transduction pathway

Several executors of the TNFα/AP-1 transduction pathway connect with the NIK transduction pathway triggered by IL-1 (Raingeaud et al., 1996).

In normal prostates, the cytoplasm of epithelial cells was immunostained to TRAF-2, IRAK (Fig. 5A), TRAF-6 (Fig. 5B), NIK, Ikkα/ß, IkBα, p-IkB (Fig. 5C) and NF-κB-p50 (Nuñez et al., 2008). NIK seems to be triggered by TNF/TRAF-2 or IL-1/IRAK/TRAF-6 (Fig. 1), since the presence of TNF, TNFRI and TRAF-2 has been described (de Miguel et al., 2000; Ricote et al., 2003), but also the presence of IL-1 family members (Ricote et al., 2004). Activation of NF-κB requires the successive action of NIK, IKK complex and IkB. The transduction pathway from NIK to NF-κB seems to be inactive because immunoreactions to IKK, IkB and NF-κB/p50 were scanty and no immunoreaction to NF-κB/p65 (see Fig. 5D) was found. The scanty expression of NF-κB/p50 was localized in the cytoplasm of epithelial cells, because the low expression of p-IkB is unable to activate the degradation to IkB-α required for the translocation of NF-κB to the nucleus.

In PC, the proapoptotic effect of the TNFα/AP-1 pathway decreases, because this pathway is inhibited by p21 (at the ASK-1 step), diverging towards the p38 pathway (Ricote et al., 2003). However, TRAF-2 might be involved in the NIK activation pathway, although immunoexpression to TRAF-2 was detected in a low number of cases (decreasing with Gleason grade), at the same time that the most of theses patients were positives to NF-κB/p50 and NF-κB/p65 (Nuñez et al., 2008). These data, in addition to the elevated immunoexpressions also observed in the cytoplasm of epithelial cells to IL-1, IRAK, TRAF-6 (Fig. 5E) and NIK (Fig. 5F), compared with normal prostate, suggest that NIK is stimulated by IL-1.

NF-κB/p50 changes its location from the cytoplasm to the nucleus in PC patients at the same time that NF-κB/p65 (Fig. 5I) is also expressed in the nucleus. This is related with the increase of IkB-α (Fig. 5G) and p-IkB (Fig. 5H) observed in PC. Shukla et al. (2004) in human prostate described a progressive increase in the expression of NF-κB/p65 (but not of NF-κB/p50) in PC compared to benign samples, and this increase is correlated to increasing levels of IkBa and its phosphorilation. When NF-κB is located in the nucleus (the active form) may promote cell growth and proliferation in prostate cancer cells by regulating the expression of c-myc, cyclin D1 or IL-6 (Chen et al.,

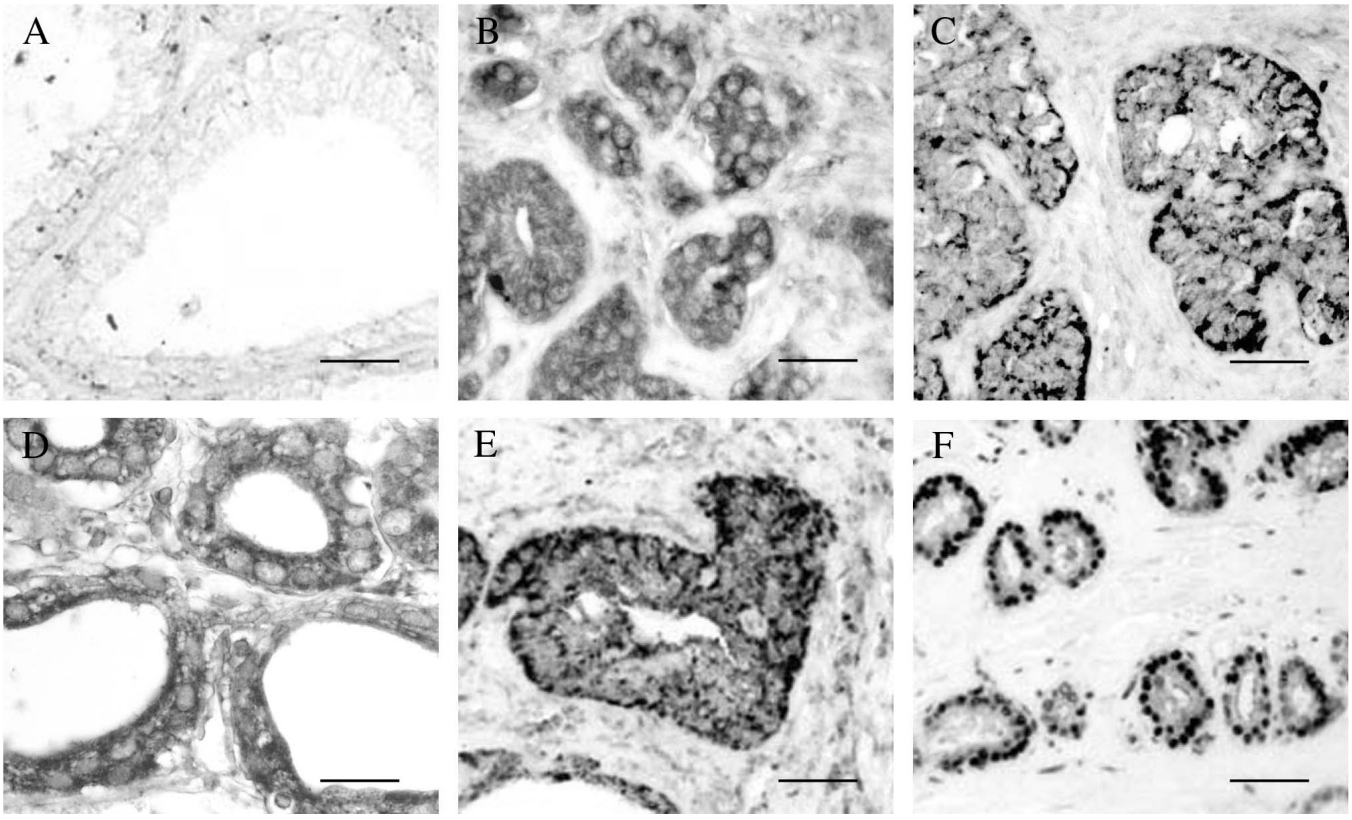

Fig. 4. α-PAK was negative in normal prostate **(A)** but positive in the cytoplasm of epithelial cells in PC **(B)** samples. Immunoreaction to MEK-6 **(C)**, p38 **(D)** and p-Elk-1 **(E)** appeared in the cytoplasm of epithelial cells of PC samples. p-ATF-2 immunostaining was localised in the nuclei of epithelial cells in PC **(F)**. Scale Bar: A, C, 20 μm; B, D, 15 μm; E, F, 25 μm

2002; Suh and Rabson, 2004); but also by the up-regulation of the expression of several anti-apoptotic proteins, including Bcl-xL (Chen et al., 2002), the inhibitor of apoptosis protein (IAP) (Stehlik et al., 1998) or TRAF- 1 and 2 (Wang et al., 1998). Nuclear location of NF-κB/p65 has been described by several authors (Ross et al., 2004; Domingo-Domenech et al., 2005; Lessard et al., 2006), although their results are discrepant. Whereas Domingo-Domenech et al. (Domingo-Domenech et al., 2005) indicate that nuclear localization of NF-κB is an independent prognostic factor in PC, other authors (Ross et al., 2004; Lessard et al., 2006) concluded that NF-κB is a new predictive marker of prostate cancer, and several authors have proposed the use of NF-κB inhibitors as therapeutic agents, either alone or combined with other agents. In human prostate cancer cell lines LNCaP and DU-145, Chen et al. (2002) described that NF-κB favors prostate cancer progression because it is required to activate PSA (prostate specific antigen) transcription. This

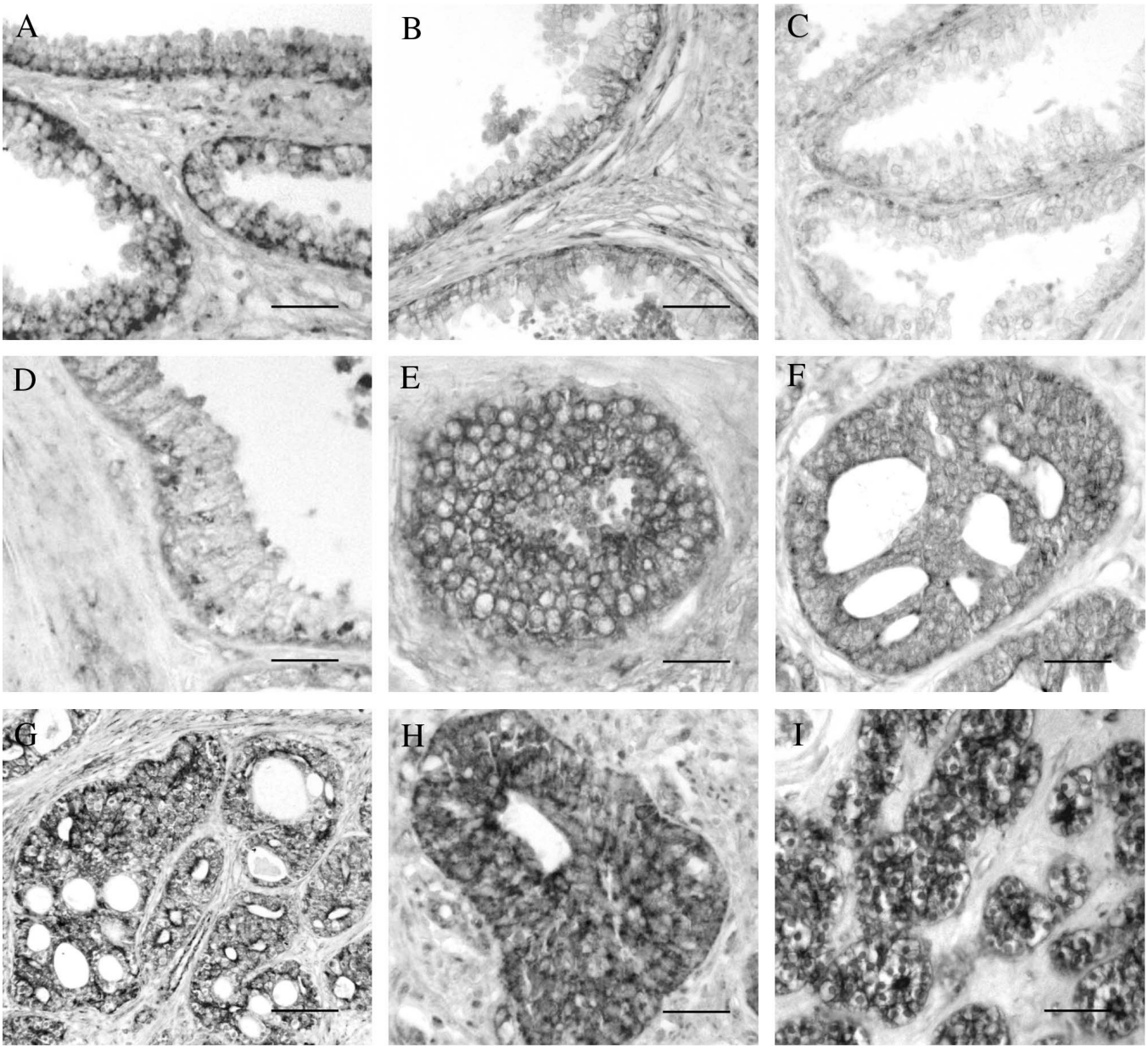

Fig. 5. In normal prostate samples, the cytoplasm of epithelial cells presented positive immunoreaction to IRAK **(A)**, TRAF-6 **(B)**, p-IkB **(C)** and NF-κB-p50 **(D)**. TRAF-6 **(E)**, NIK **(F)**, IkB-a **(G)** and p-IkB **(H)** immunostaining appeared in the epithelial cells of PC samples. In PC, NF-κB-p65 **(I)** was localized in the nuclei but also in the cytoplasm of epithelial cells. Scale bars: A, B, H, 25 μm; C, D, F, 20 μm; E, I, 15 μm; G, 30 μm

relationship between serum PSA levels and NF-κB expression could not be confirmed by Shukla et al. (2004) in vivo patients. PSA level in serum is a clinical marker for BPH and prostate cancer progression; however, this level is poorly specific, since it can be influenced by prostatic disease, urologic manipulations, pharmacological treatments, ejaculation, etc (Polascik et al., 1999). In this way, Andreakos et al., (2006) considered NIK as a potent adjuvant strategy that offers great potential for genetic vaccine development in mouse myeloid cells. NF-κB is involved in cell proliferation triggered by IL-1/NIK but also by TNF/NIK.

New perspectives

TNF/TRAF-2 pathway may diverge to NIK but also towards Ap-1, and this latter pathway may also diverge towards p38, which activates different cell proliferation transcription factors such as NF-κB, ATF-2 and Elk-1. P38 activation is also stimulated by IL-1. The overexpression of both ATF-2 and Elk-1 are also related to enhanced cell proliferation and survival (Ricote et al., 2006). These data suggest that inhibition of IL-1α might be a possible target to cancer treatment. Nevertheless, in addition to components of the TNFα/IL-1 transduction pathway (NF-κB, ATF-2 and Elk-1), several proteins involved in the cell cycle, including IFNγ, mutated p53, cell proliferation antigen Ki-67, ERK, p21, Rb, mcl-1 or estrogen receptors are overexpressed in PC.

Translocation of NF-κB to the nucleus in PC might be due the overexpression of several components of the IL-1/NIK/NF-κB or TNF/NF-κB (NIK or p38) pathways. Since nuclear localization of NF-κB was observed only in PC patients, it may be considered as a marker for predicting PC. In order to search a dominant target for therapy, it should be taken into account that PC is a heterogeneous disease in which multiple transduction pathways may interact in the uncontrolled apoptosis/cell proliferation. Since we observed that immunoexpresion of several proinflamatory cytokines such as IL-1α or TNFα are increased in PC (de Miguel et al., 2000; Ricote et al., 2004), inhibition of these cytokines might be a possible target for PC treatment, because this inhibition would decrease all the transduction pathway members that activate several transcription factors such as NF-κB, Elk-1 or ATF-2. Additional fundamental research with different TNF/IL pathway members and their correlation with clinical experimentation will be required in the cancer prostate field.

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