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REVIEW

The NF-κB/IL-6 pathway in metastatic androgen-independent prostate cancer: new therapeutic approaches?

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Abstract The nuclear factor of kappa beta (NF-κB) transcription factor regulates the transcription of numerous genes including that of interleukin 6 (IL-6). The IL-6 acts as an autocrine and paracrine growth factor of androgen-independent prostate cancer. An aberrant expression of the IL-6 gene and an increase in IL-6 expression are detected in bone metastatic and hormone-refractory prostate cancer. IL-6 has been suggested to have a crucial role in the resistance to chemotherapy or hormonal therapy involving apoptotic cell death. The NF-κB/IL-6 dependent pathways promote tumour-cell survival and in most situations protect cells against apoptotic stimuli. These data provide a rational framework for targeting NF-κB and IL-6 activity in novel biologically based therapies for aggressive and androgen independent prostate cancers.

Keywords Prostate cancer · NF-κB · IL-6 · New therapies · Androgen independence · Metastasis

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Introduction

The nuclear factor of kappa beta (NF-κB) regulates the transcription of numerous genes including interleukin 6 (IL-6). The IL-6, in addition to its role as an immunomodulatory cytokine, acts as an autocrine and paracrine growth factor for prostate cancer cells. Elevated serum level of IL-6 has been found in hormone refractory prostate cancer also referred to as androgen-independent prostate cancer [1–3]. Furthermore, an aberrant expression of the IL-6 gene and an increased production of IL-6 are associated in the advance of bone metastases and their morbidity [4–7] as well as in resistance to chemotherapy [8]. Thus, it is easy to understand the rationale of attempt to use the NF-κB/IL-6 pathway as a potential therapeutic target in hormone-refractory metastatic prostate cancer wherein growth, proliferation or apoptosis resistance are supported and mediated by this signalling.

Biology of NF-κB

In most cell types, the activated form of NF-κB is a heterodimer composed of two NF-κB/Rel family members, p65 (or Rel A) protein and a p50 sub-unit. The p65 and p50 can be categorically subdivided into modules of differing functional domains; whereas the two members contain N-terminal region with structural information needed to localize in the nucleus and bind to definable DNA response elements, only p65 have a transactivation domain in its C-terminal region that is accountable for the transcriptional activator

functions of the NF-κB heterodimer. Other NF-κB members exist, such as Rel B, C and Rel p52 and can form various dimers that provide activation of a large array of genes as well as a means to regulate the major NF-κB dimer, p65/p50 [9]. Despite its nuclear localization sequence (NLS), NF-κB is usually retained in the cytoplasm where it cannot act as a transcription factor. This observation has been mostly attributed to the presence of inhibitory proteins so called IκBs that bind NF-κB which results in masking the NLS sequence and blocking the NF-κB nuclear import. Importantly, stability of these inhibitors is regulated by phosphorylation. For instance, upon various stimuli and/or under pathologic condition, IκB kinase complex also named IKK complex and composed of at least three subunits (IKK-α? IKK-β and IKK-γ), phosphorylates IκBα at serine residues within its N-terminal region. Phosphorylated form of IκBα attracts the β-tranducine repeat containing protein (β-TrCP) a subunit of an ubiquitin ligase complex that promotes ubiquitination of IκBα rendering it an avid target for proteolysis by proteosomes. Down-regulation of the IκB protein level enables then the newly free NF-κB complexes to translocate into the nucleus and activate a wide spectrum of genes. In addition to its role in normal development and homeostasis, by initiating the transcription of cytokine genes such as IL-6 and IL-8, cell adhesion molecules (E and P selectin, intercellular adhesion molecules ICAM1, vascular cell adhesion molecule 1), stress response genes (COX-2), growth factor (FGF-8), anti-apoptotic proteins such as Bcl-xL, Bcl-2, A1/BFL1, IEX1 and XIAP, CIAP1 and cIAP2, NF-κB particularly in hyperactive conditions, guarantees the survival of tumour cells [9–11] and, therefore, has been postulated to participate in initiation and progression of several human malignancies including haematological malignancies as well as solid tumours [12–17]. It seems nevertheless that under some circumstances, NF-κB could also trigger apoptosis by regulating p53-mediated apoptosis and by up-regulating the expression of FAS, a pro-apoptotic factor in cells [18, 19]. Markedly, it has been shown that NF-κB promotes the growth of tumour cells by activating the transcription of genes that code for cyclinD1 which in turn, promotes phosphorylation of the Rb protein (pRb) and the entry of cells into the S phase of the cell cycle [20]. One additional target of NF-κB that may regulate cell growth and proliferation is C-MYC. As well, NF-κB could serve to stimulate angiogenesis and metastasis by inducing the expression of genes such as MMP9, uPA, IL-8 and VEGF [21].

NF-κB in prostate cancer

The over-expression of NF-κB in the nucleus of prostatic tumour cells appeared to be correlated with resistance to chemotherapy, the advanced stage of the tumour, the biochemical recurrence of PSA and the pre-surgical serum level of PSA [22, 23] (Fig. 1). Recent evidences give reasons to believe that hyperactive NF-κB observed in prostate cancer may occur through constitutive activation of IKK, therefore, resulting in heavily phosphorylated and a faster turnover of IκB alpha protein inhibitor that facilitates dissociation of IκB from NF-κB, which then translocates into the nucleus [24, 26]. Mechanisms that cause such IKK activation are still unclear and clearly need to be further investigated. Among the potential candidates that mediate this event are activation of upstream kinases, such as MEKK1, MEKK2, NIK or either other kinase related signalling pathways [25–27]. Caution should be exercised in considering the complex role of NF-κB in prostate cancer as has been covered by an excellent review to which the reader is referred [27] and wherein as mentioned before in non-prostate cells, authors discussed the potential pro-apoptotic role of NF-κB with respect to its relation with FAS and p53 protein studied in recent works [28, 29]. Indeed contradicting results could be explained partly by the relative expression of NF-κB in each cell types and by genetic modifications that intervene during cancer progression. Additionally, in vivo, it should consider the changes that occur in the tumour environment in which metastatic or non-metastatic cancer cells develop. In vivo models nicely provided some evidence for the implication of NF-κB in metastasis progression of prostate cancer. Thus compared to mock-transfected cells, prostate cancer PC3 cells that stably express an inactive form of IκBα when orthotopically injected in nude mice seemed to loose a large part of their metastatic features and ability to promote angiogenesis both of which are key steps in tumour progression. In vitro NF-κB expression level seemed inversely correlated with the expression of the androgen receptor (AR) suggesting a means for some cancer cell clones to override the androgen-dependent apoptosis when patients are treated by hormone therapy. Conversely it was demonstrated that hyperactivated NF-κB bind to consensus NF-κB binding sites in the PSA promoter and enhance PSA expression suggesting an elegant mechanism by which tumour cells could reactivate transcription of certain AR target genes in a low androgen environment to sustain their growth. In a xenograft model (LAPC-4), the same authors further found that the androgen-independent status of tumours was correlated with hyperactivity of NF-KB as compared to androgen responsive tumours [30]. These data demonstrate the potential influence of NF-κB on tumour progression. Interestingly, as compared to the hormone-insensitive prostate tumour cell lines PC-3 and DU-145 wherein, NF-κB is constitutively activated, in the hormone responsive LNCaP cell line, activity of NF-κB is low. Nevertheless this activity

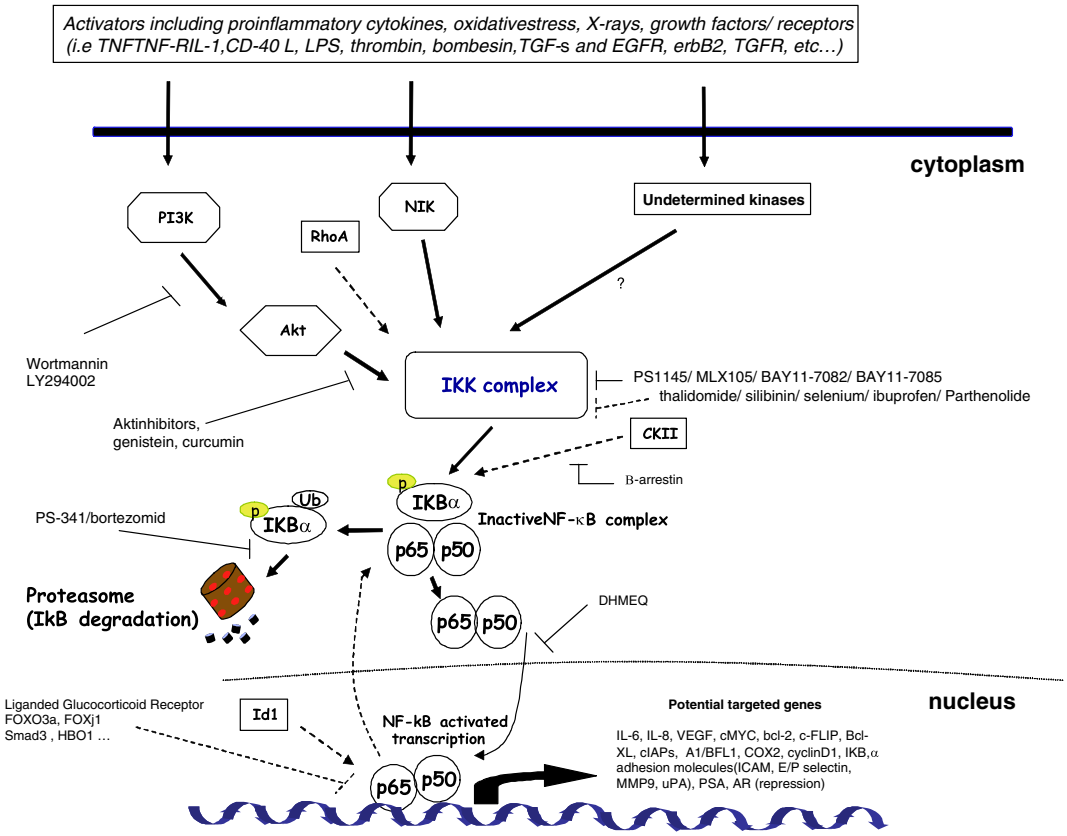

Fig. 1 Schematic representation of the classical NF-κB signalling and its regulation in prostate cancer cells. Activation of NF-κB results from various stimuli that activate kinases, and subsequently the IKK complex driving the proteasomal degradation of the NF-κB inhibitor (IκB). Activation of NF-κB, concomitant to its translocation in the nucleus, allows the onset of transcription of a wide spectrum of genes. Some of them are noted above. Disruption of the NF-κB activity can occur by the use of compounds able to target the kinase cascades that phosphorylate the IKK complex, compounds that inhibit the activity of IKK, prevent the proteasomal degradation of IκB or either the translocation of NF-κB in the nucleus. Finally endogenous repressors can act on NF-κB in the cytoplasm or in the nucleus. These molecules might be the subject of new therapeutic approaches

can be stimulated by cytokines and, therefore, it can be anticipated that this condition may represent an important step for cancer progression and development [31]. Additional factors may activate NF-κB to ensure cancer progression such as Id1 [32], tumour necrosis factor (TNF) [33], the Thrombin (through its liganded receptor PAR-1) [34] and the (TGF)-β family factors. TGF-β acts as a potent growth inhibitor of normal prostate epithelial cells, but in tumours, aberrant function of their receptor (type I and II) correlates with tumour and aggressiveness suggesting a switch of the TGF-β role in prostate tumourigenesis. Importantly, TGF-β is secreted by various types of tumour cell lines that exhibit constitutively active NF-κB. Lu et al. [35] recently showed that TGF-β2 potently stimulates the activation of NF-κB and that disruption of this interplay killed prostate cancer PC3 cells.

Intracellular and serum TGF-β1 levels are elevated in prostate cancer patients. A recent work demonstrated that TGF-β1 activates interleukin IL-6, via multiple signalling pathways including Smad2, NF-κB, JNK and Ras [36]. More striking for this review is that authors observed that the IL-6 inactivation restored the sensitivity to TGF-β1-mediated growth arrest and apoptosis. This indication suggests that elevated IL-6 level in prostate cancer might reflect a condition for the cells to disregard negative effect of the circulating TGF-β1. This work concluded in proposing that expression of IL-6 could contribute to the oncogenic switch of TGF-β1 role for prostate tumourigenesis, in part by counteracting its growth suppression function.

As demonstrated by others, herein it is particularly interesting to understand that the constitutive activation of NF-κB may account at least in part for elevated IL-6 expression by prostate cancer cells [37, 38]. This hyperactivity of NF-κB induces IL-6 expression and its soluble receptor, the serum level of which is a predictive factor in the biological recurrence of PSA. Interestingly, NF-κB inhibition seems to block the activation of signal transducers and activators of transcription 3 (STAT3) and helps to repress the promoter of the IL-6 gene mediated in androgen sensitive and androgen independent cells [38, 39].

Deregulation of IL-6 gene

The expression of transcriptional factors such as activator protein 1 (AP-1) [37], members of the Fra-1 family and the GBx2 gene appears to coincide with an increase in the IL-6 expression through its promoter regulation in prostate cancer cells. The GBx2 gene is over-expressed in prostate cancer cell cultures. It codes for a transcription factor that belongs to the homeobox gene family that recognizes and binds to specific DNA sequences. These factors are active against target genes such as IL-6 localized to chromosome 7p21-14. Diminished expression of the GBx2 gene is associated with a decreased tumourigenicity and growth rate, as well as a down-regulation of IL-6 in prostate cancer cells in vitro [40]. Of note, exogenous addition of recombinant of IL-6 protein restored the growth of tumour cells that had been knocked out for GBx2 with antisens suggesting that IL-6 is an important mediator of prostate cancer cell growth in the advance of prostate cancer.

IL-6 signalling pathways

The cell membrane IL-6 binding site includes a specific IL-6 receptor (IL-6R) or gp80 (alpha sub-unit) and the corresponding transduction element, which forms the gp130 chain (beta sub-unit). Bound to its receptor, IL-6 triggers formation of a multimeric complex containing IL-6R and two gp130 molecules. An initial activation pathway passes through the Janus kinase (JAKs) family. Indeed the newly formed complexes afford autophosphorylation of the JAKs (JAK-1, JAK-2 and JAK-3) which are able to phosphorylate gp130**.** Phosphorylated gp130 are able to recruit STAT to the complex, resulting in their phosphorylation. Then phosphorylated STAT proteins form homo- or heterodimers that translocate into the nucleus where they bind to definable DNA response elements and regulate the transcription of genes. A second pathway results in the activation of a transcriptional factor, NF-IL-6, which acts in the nucleus.

Biochemical and genetic studies have also identified the STAT 3-interacting-protein (STIP1) protein as a regulator in the STAT3 signalling pathway [41]. It combines with the inactivated, non-phosphorylated form of STAT3 and blocks its IL-6-dependent activation, its translocation in the nucleus and the induction of the STAT-3 dependent gene. It is also involved in the interaction between the Janus kinase group and its STAT3 substrate [42].

IL-6 functions in prostate cancer cell

The IL-6 is considered as a positive growth factor in prostatic tumour cells (Fig. 2). Its receptor (IL-6R) is expressed in many cell lines including LNCaP [43, 44] and it acts as a paracrine growth factor in androgen-sensitive LNCaP prostate cancer cells (1,2,3) as well as an autocrine growth factor for tumour cells on androgen-independent DU145 and PC3 cell lines [45]. However, it should be noted that the above findings remain controversial because IL-6 has the ability to act as a growth inhibitor in LNCaP cell line, highlighting the dual functionality of IL-6 [46]. In numerous systems, IL-6 can stimulate or inhibit tumour proliferation notably via activated STAT3 (PSTAT3) [42, 47, 48]. Indeed, pSTAT3 plays a key role in the transition of the G1 phase into the G2 phase of the cell cycle and the concomitant decrease of p21 and p27 (cell cycle inhibitor) via cdc 25A [48]. The p27 inhibition has been linked with tumour aggressiveness and with reduced survival rates. Mori et al. [49] have shown that IL-6 blocks the cell cycle in the G1 phase of the LNCaP cell line, reduces cdk 2, 4, 6 cyclin dependent kinases and conversely augments expression of the p27 cell cycle inhibitor that resulted in giving the tumour cells a neuroendocrine-like phenotype that is known to be implicated in progression of prostate cancer to an hormone resistant status. According to these observations Spiotto et al. reported that STAT3 mediates neuroendocrine transdifferentiation of LNCaP under IL-6 exposure [50]. During inhibition of the cell cycle, pSTAT3 has been reported to bind to the C/EBP delta gene promoter. The over-expression of this gene in the LNCaP cells suppresses cell growth and plays a role in the survival of tumour cells and their resistance to chemotherapy [51]. The dual function of IL-6 observed in the LNCaP cells emerges in three successive stages: the tumour cells are initially inhibited by IL-6 and that must be concomitant with acquisition of resistance to different therapeutic agents, then, after a persistent effort by IL-6, the inhibitory effect is abolished switching to a final stage where a positive growth effect [52] allows at least some populations of the tumour cells, known to be genetically unstable, to grow more rapidly in vivo. Interestingly, another published work showed that under IL-6-unstimulated conditions, in LNCaP cells (so called LNCaP-IL6-), the hypophosphorylated Rb (activated form) represses the E2F transcription factor known to be essential for entry into S phase of the cell cycle. Whereas in long term IL-6-treated cells (LNCaP-IL6+), accumulation of the inactive hyperphosphorylated form of Rb together with the down-regulation of p27 protein level might explain how these cells can escape the inhibitory effects of IL-6 observed under short term IL-6 exposure. Finally, it was observed that LNCaP-IL-6+ cells abolished IL-6 mediated activation of STAT, but conversely up-regulated expression of MAPK (the activation of which can be regulated by IL-6 as will be discussed below) suggesting an alternative mechanism independent of STATs through which IL-6 can influence behaviour of prostate cancer cells and their gene expression profile [53].

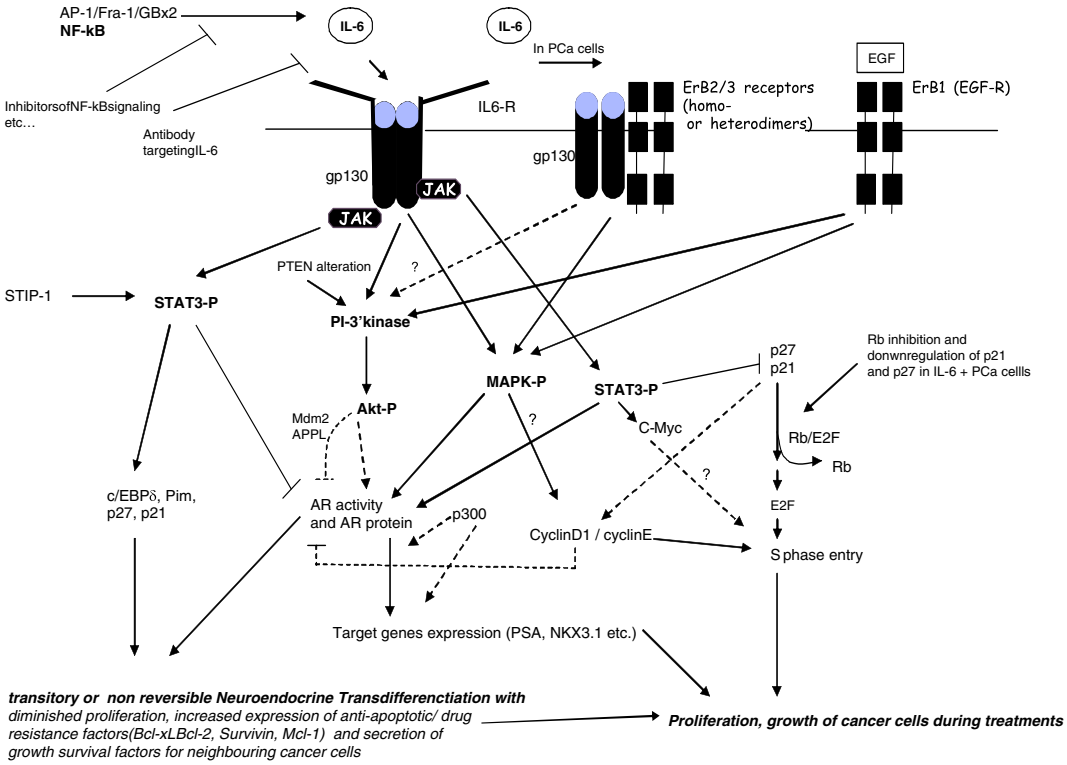

Fig. 2 Schematic representation of the multiple modes through which signal transduction by IL-6 can influence the cell behaviour in prostate cancer. IL-6 exposure activates MAPK, PI3K/Akt and STAT3 pathways. Several studies have now showed that IL-6 induces neuroendocrine transdifferentiation of prostate cancer cells a phenotype characterized by a low rate of proliferation and acquisition of anti-apoptotic features that allow the cells to survive treatment with most chemotherapic agents, as well as endocrine and radiation treatments. Furthermore, neuroendocrine cancer cells secrete neuropeptides and growth factors that are believed to sustain the growth of neighboring and still "epithelial-like" cancer cells and metastatic cancer cells in a low androgen environment thereby allowing progression of androgen-refractory prostate cancer. Activated IL-6 signalling can also directly maintain the growth and augment the proliferation rate of certain prostate cancer cells. Yet it's still unclear as to which cells differentially respond to IL-6 exposure. Cellular protein kinases levels and activities as well as signal transduction molecules are critical in regulating these events. Likewise differential modulation of AR expression and activity by IL-6 would explain at least in part these differing effects. It is also important to underline the seemingly influence of mutual crosstalks between IL-6 signalling and growth factor mediated signalling pathways that are especially pertinent to prostate cancer progression

In accordance with the anti-apoptotic role of IL-6, Shirogane et al. [54] have identified Pim 1 and Pim 3 proto-oncogenes as STAT3 targets. Pim 1 induces the expression of anti-apoptotic bcl-2 protein. bcl-2 is a protein that intervenes in prostate cancer resistance to hormone therapy. Previous works also indicate that NF-κB can enhance expression of bcl-2 by directly activating the promoter region of BCL2 gene [55]. bcl-xL, a member of the bcl2 family, is involved in the chemotherapy resistance of IL-6-dependent tumour cells. Finally, in osteoblastic cells, p21 is a downstream target of IL-6 signalling that mediates the anti-apoptotic and differentiating effects of the cells [56].

IL-6 and AR

The AR protein, a unique member of the nuclear steroid receptor gene family with a molecular mass of approximately 110 kDa in size that preferentially utilizes dihydrotestosterone (DHT) as its natural ligand (Fig. 2). Ligand binding induces a conformational transition in the AR protein that facilitates dissociation of cytoplasmic AR from its chaperone, translocation of the protein into the nucleus where it homo-dimerizes and binds to definable DNA response elements. Whereas the spectrum of gene products regulated by AR-mediated transcription is not fully characterized there are some very well characterized targets such as the human prostate specific antigen (PSA) gene. Indeed, recent evidence identified alternative molecular paradigms that influence or modify the activity of the canonical androgen signalling pathway in prostate cancer cells. These alternative mechanisms occasionally involve a direct interaction between the AR protein and other proteins (referred to as co-activators or co-repressors, depending upon the end effect on the androgen signalling process) [57] or an interaction with alternate signalling pathways, such as those

driven by Erb and IGF receptors that selectively target certain amino acid residues of the AR protein for phosphorylation (thus modifying its transcriptional activity).

More recently other example of such regulation was illustrated by responses induced by IL-6/IL6R activated pathway [58–60]. A correlation has been established between IL-6-induced AR activation and tumour proliferation [7]. However, studies on the effect of IL-6 on prostate cancer cell growth and transcriptional activation of AR have showed contradictory results and this is likely to rely to previous observations regarding the positive or negative effects of IL-6 on cell proliferation. It seemed that in AR-negative and hormone independent DU145 cells, the transcriptional activity of transfected AR was not affected by IL-6 treatment. Besides, the observation that inhibitor of the inhibition of PI3K (LY294002) potentiates IL-6 activation of AR transcription in the presence of androgens demonstrates that presumably various pathways stimulated by IL-6 differentially mediate the AR activity [61]. In androgen sensitive LNCaP tumour cells expressing the AR protein, it was also described that IL-6 can boost the expression and activation of AR, regardless of its ligand, via the P13K/AKT pathway, the MAPK and STAT pathways [34, 58–60]. The observed increase in AR transactivation upon IL-6 treatment is blocked by the MAPK inhibitors PD98059 and U0126, suggesting that the IL-6-MAPK pathway is required for enhancement of AR activity. This activation of the STAT3 and the MAPK pathways makes tumour cells hypersensitive to androgens and this could account for the transition from an androgen-dependent into an androgen-independent phenotype that occurs during prostate cancer progression [62, 63].

Aside from this initial description on the positive effect of IL-6 on AR activity perhaps the most intriguing observation came from new evidence that the PI3K/Akt pathway appears to have an a negative effect on AR signalling in prostate cancer cells. It has been suggested that activated form of Akt, mediates degradation of AR through a proteasome dependent pathway [64]. The effect of Akt on AR would also be mediated by the Akt bridging protein (APPL) that inhibits DHT-induced transcription through a mechanism dependent on Akt activity [65]. It is also interesting to note that Jia et al. [66] have seen that IL-6 inhibition of AR was not mediated by MAPK pathway and was abrogated to some extent by down-regulation of IL6-mediated induction of STAT3 signalling. Even if this work did not find correlation with the Akt regulation, the authors further showed that IL-6 prevents the recruitment of p300 protein to co-activate AR transcription. In another published work, investigators found that the p300 co-activator was necessary, downstream of the MAPK pathway, for AR activation by IL-6 [57]. Moreover, cells treated with long term exposure to IL-6 (LNCaP-IL6+), that do not express detectable AR protein, seemed to respond positively to exogenous p300 overexpression by up-regulating several AR target genes such as PSA or NKX3.1 in an androgen and anti-androgen independent manner [67]. As well, a co-activator effect of p300 is observed on AR when AR is co-transfected with p300 in the absence of androgens. From a very recent survey came also evidence that p300 is regulated by androgens in the sense that androgen deprivation elevated the p300 protein level while addition of androgens lowered this level in the lines [68]. Overall these findings are especially pertinent to prostate cancer during hormone therapy where level of circulating androgens is reduced because in both situations, i.e. where AR is overexpressed or barely detectable, the p300 factor could control certain androgen target genes. Yet it is still unclear why p300 protein is down-regulated in LNCaP-IL6+ compared to LNCaP-IL6− cells [67] and genetic changes, related or not to the pleiotropic effects of IL-6 as well as the multifaceted interaction between kinases, such as STAT3, MAPK or PI3k/Akt for which the activities may vary in the time, play a role in p300 regulation. Finally, Heemers et al. have recently showed the propensity of NF-κB signalling to positively regulate the p300 protein. Once again this strengthens the relevance of the IL6/NF-κB pathway in metastatic and hormone-resistant cancer [68].

The divergent effects of the hyperactive Akt on AR pathway must be caused by differences in cell line passage numbers, culture conditions, as well as signals that modulate the balance between MAPK, STAT and PI3K stimulation, which is directly or indirectly influenced by IL-6 [69].

Since elevated phosphorylation of Akt is also believed to be a characteristic of aggressive prostate cancers it has to be related to increase expression and auto- or paracrine-action of IL-6 during the prostate cancer progression [70]. It is established that IL-6 increases the interaction between the p85 subunit of PI3K and gp130 and enhances p85 phosphorylation [46]. Then inhibition of IL-6-induced PI3K activity by wortmannin causes apoptosis in LNCaP cells, suggesting that this pathway may contribute to prostate cancer cell survival. Furthermore, it was demonstrated that down-regulation of AR signalling as well as IL-6 mediated activation of PI3K/Akt pathway were associated with neuroendocrine (NE)-transdifferentiation in LNCaP cells [71–73]. Collectively, these results have significant implications regarding to the role of IL-6 and there are reasons to believe that tumour sub-clones that undergo, transitory or stably, into a NE-transdifferentiation process in vivo, by down-regulating AR dependent transcription may become resistant to apoptosis and as well facilitate the growth, metastatic behaviour and therapeutic resistance of surrounding and more distant tumour cells possibly by stimulating their AR activity independently or in a low androgen level environment [74, 75]. Another appealing possible role of NE

transdifferentiation is supported by recent evidence that neuroendocrine factors such as the Bombesin can stimulate NF-κB activity along with increased IL-8 and VEGF mRNA expression and protein secretion [76]. Moreover, a previous work that ascertained the molecular alterations found in prostate cancer progression using the transgenic TRAMP mouse model nicely showed the potential relationship between activation PI3K/Akt and NF-κB signallings in conjunction with expression of anti-apototic and pro-angiogenic factors [77].

IL-6 and EGF/R and HER2 receptors

Transforming growth factor alpha (TGF α) and epidermal growth factor (EGF) are autocrine or paracrine growth factors for normal and tumour epithelial prostatic cells. The effects of these growth factors are mediated through the epidermal growth factor receptor (EGFR). The latter belongs to the family of tyrosine-kinase receptors (TKR), which includes HER-2 (CerbB2) HER-3 (CerbB3) and HER-4 (CerbB4). It is also of interest to note that members of this family can form homo- or heterodimer with each other. In prostate cancer, the expression of EGF-R is generally found to increase in patients that have progressed to a hormone refractory state suggesting that this receptor play a critical role in tumour maintenance and progression [78]. Furthermore, HER-2 expression, which is present in between 16 and 34% of prostate cancers, is correlated with an advanced stage of the disease with a high grade of differentiation. Evidence is accumulating that HER-2 may have a role in the advance towards hormone resistance and diminished AR antagonist effects being that HER-2 activates AR mediated transcription in the presence of residual androgens and can promote both the growth and survival of androgen-independent tumour cells via two signalling pathways: the PI3K/Akt and the MAP kinase (MAPK) pathways, which likely activate the AR regardless of its ligand [79, 80].

In several prostate cancer cell lines, IL-6 selectively activates phosphorylation of HER-2 and HER-3 tyrosine kinases. Indeed it was shown that IL-6 facilitates the association between the IL-6 receptor subunit gp130 and Her2 forming an IL-6 dependent complex**.** This results in the phosphorylation of MAPK and hence specific activation of the MAP kinase pathway. Importantly, HER-2 inhibition blocked MAPK signalling and inhibited AR activation [81]. Recent findings have implicated, respectively, phosphoinositol-3-kinase (PI3K) and casein kinase II (CKII) in EGFR- and Her2/Neu-mediated constitutive NF-κB activation using prostate cancer cell line models. Thus suggesting additional ways for the cancer cells to activate NF-κB possibly by regulating IKK complex and that may result in elevated IL-6 level and NF-κB targeted gene products [82].

IL-6, NF-κB and bone metastases of prostate cancer

The bone metastases of prostate cancer have a significant morbidity: bone pain, risk of fracture, bone marrow compression and, more rarely, hypercalcaemia (Fig. 3). These bone metastases are typically bone-condensing, but histomorphometric and biochemical studies have revealed concomitant osteoclastic bone absorption [83]. The prostatic tumour cells in the bone express osteoclastic bone absorption factors, such as MCS-F, PTH-rP, IL-1 and IL-6. Bone osteoblastic stromal cells express RANK-L, which can interact with the RANK receptor (a membrane- bound NF-KB-activating receptor) expresses by osteoclast precursors. RANKL is a member of TNF cytokine family, the expression of which is regulated by factors triggering osteoclastic bone resorption: vitamin D 3, glucocorticoids, IL-1, IL-6 and TNFα [84]. Importantly, formation of the complex RANKL/RANK results in the activation of NF-κB that induced the maturation of the osteoclast precursors [85]. Indeed tumour cells, themselves, such as C4-2B and LNCaP cells can express RANKL or a soluble form of RANKL that activate osteoclastogenesis. Interestingly, a molecule that interferes with the complex RANKL/RANK, the osteoprotegerin (OPG) will in turn inhibit both the bone destruction and tumour cell growth [86]. This underlines the importance of the RANKL/RANK binding complex and its downstream targets such as NF-κB in these processes. Abnormally high expression of the osteoclast-activating cytokine IL-6 is observed in bone metastatic/androgen independent PC-3 cells that show elevated activity of NF-κB. Conversely, PC-3-mIκB cells that stably express a mutant of IκB have lost the capacity to invade and activate bone resorption in conjunction with the down-regulation of IL-6 reinforcing the relevance of a NF-κB/IL-6 signalling pathway in bone metastasic disease [87]. Androgenic ablation increases osteoclastic bone resorption via IL-6. As well the parathormone (PTH-rP) increases IL-6 level in vivo and this augmentation is correlated with an increase in the biochemical markers of bone turnover. However, this event may be temporary since recent evidence demonstrated that the PSA, a serine protease, triggers the cleavage of PHT-rP and that the remaining fragments may stimulate bone formation by their positive action on the osteoblasts [88]. For that reason it can speculate that the level of active PSA could mediate in part, by its action on PHT-rP and IL-6 level, the balance of bone formation versus bone destruction observed during the progression of mestastatic prostate cancer.

IL-6, a predictive relapse factor following radical prostatectomy

In clinically localized prostate cancer treated by radical prostatectomy, there is a clear-cut link between TGF-β1

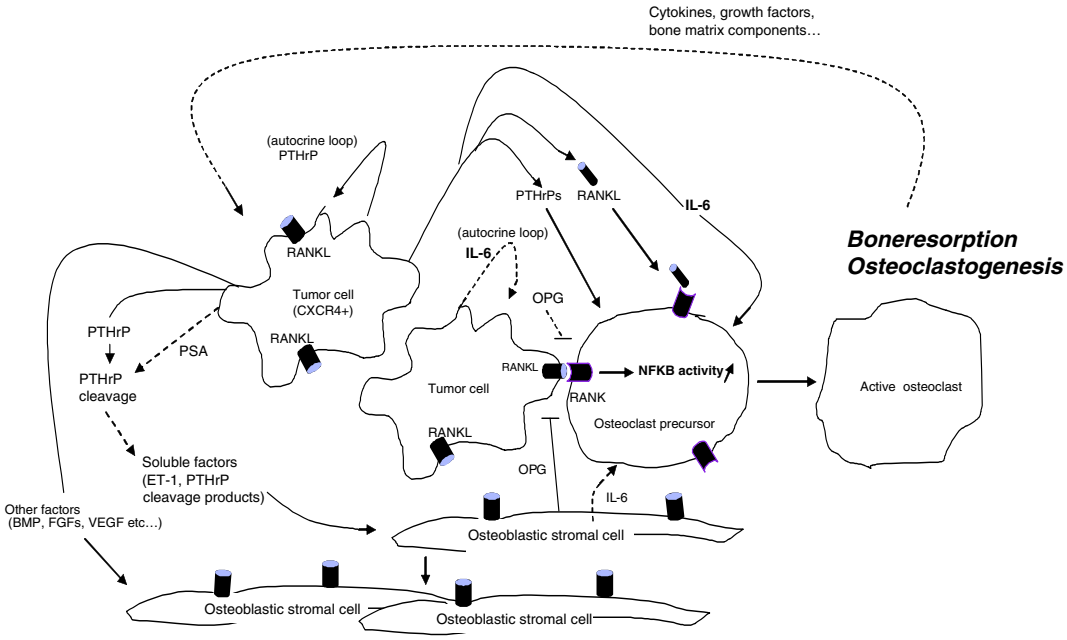

Fig. 3 Model illustrating the potential role of IL-6, NF-κB and other critical factors in the development of bone metastasis. Prostate cancer cells may release soluble pro-osteolytic factors (RANKL, IL-6, PTHrP) that promote osteoclastogenesis and bone resorption. Such molecules, like IL-6, often act as pro-survival factors of tumour cells enhancing the resistance to therapy. When activated by RANKL/RANK signalling, NF-κB is an important inducer of the osteoclast maturation that may further enable the release of growth factors (not clearly determined yet) stored in the bone matrix that in turn could activate tumour proliferation. Tumour cells, presumably in response to hypoxia and their new microenvironment, start then to produce osteoblastic factors such as the bone morphogenic proteins (BMP), the vascular endothelial factor (VEGF), the fibroblast growth factors (FGFs), endothelin-1 (ET1) and PTHrP derived peptides that in turn counteract the osteoclastic phase and facilitate the entry into an osteoblastogenesis phase. Osteoprotegerin (OPG) by blocking the RANK ligand seems to inhibit both the osteolytic and osteoblastic tumours underlining the importance of the osteoclastic phase in prostate cancer. PSA via its action on PHT-rP might regulate the balance between bone formation versus bone destruction. Finally, although not illustrated in the figure, most of the above-mentioned factors are now actively targeted by new agents for potential therapeutic benefits

and IL6-Rs serum levels and the histological characteristics of prostate tumour, the presence of occult metastases, their potential advance and the Gleason score. A nomogram including TGFβ1 and IL-6R serum levels was developed by Kattan et al. [89] to predict the biochemical progression of PSA in clinically localized prostate cancers. Similarly, the over-expression of NF-κB in the prostate tumour is also a predictive relapse factor following radical prostatectomy [23].

Therapeutic implications

NF-κB inhibitors

Physiological, endogenous inhibitors

Endogenous inhibitors negatively regulate the activity or activation of NF-κB. These inhibitors include A20, CYLD, Foxj1, and Twist and β-arrestin proteins. The antagonist activity of these molecules towards NF-κB could be the object of therapeutic development [90].

Proteasome inhibitors

Proteasome is a multicatalytic proteinase complex present throughout the cell, both in the cytoplasm and the nucleus, and it is fundamentally responsible for the degradation of most intracellular proteins including regulatory proteins in the cell cycle such as cyclin B1, cyclin-dependent kinase inhibitors (p21 and p27), and tumour-suppressor genes such as p53 and NF-κB [91]. The proteasome is the final step of a pathway called "the ubiquitin proteasome pathway". In this system, ubiquitin is the marking agent that covalently links the protein and presents it to the proteasome structure. Ubiquitin effectively tags proteins, marks them for presentation to the proteasome, where the proteins are digested in peptides of 3–22 amino-acids in size. It was demonstrated that IκBα stabilization by a proteosome inhibitor such as bortezomid increases the sensitivity of tumour cells to chemotherapy, and their apoptosis [92, 93]. This compound acts as a competitive but reversible inhibitor and forms a tight complex at the active site of the proteasome. Herein, it is of interest to note that, although not fully understood, compared to the normal "non-transformed cells", the cancer

cell is uniquely sensitive to proteasome inhibition and sensitive in the form of driven to apoptosis that may this drug an ideal tool for targeting tumour cells.

Given the role of proteosome in IκB degradation, proteosome inhibitors trigger apoptosis by stabilizing IκB in the cytoplasm and blocking the translocation of NF-κB in the nucleus. Bortezomid induces the apoptosis of androgen-dependent and -independent cell lines (PC-3 and DU 145), inhibits angiogenesis and metastases and increases anti-tumour activity in PC3 prostate cancer xenografts [94]. Apoptosis is all the more marked in LNCaP cells that over-express bcl2 [95] giving indications that the proteasome inhibition could overcome bcl-2 mediated-resistance to apoptosis. Pre-clinical trials reveal a synergy between bortezomid and chemotherapy with reversion of NF-κB-induced chemoresistance [22, 96]. A Phase I clinical trial has demonstrated the interest of bortezomid in patients presenting with hormone-resistant prostate cancer [97]. The results of this Phase I clinical trial showed that the toxicity was very mild and put some evidence of anti-tumoural activity and symptom improvement. Several Phase II trials combining docetaxel or mitoxantrone and bortezomid are currently underway [98]. The NF-κB inhibitors combined with chemotherapy would diminish the risk of recurrence following RP in patients at high risk of relapse. Moreover, selective inhibition of NF-κB blocks in-vivo osteoclastogenesis and would prevent the complications of bone metastases combined with bisphosphonates.

IKK inhibitors

PS1145 and its analogues are IκK complex inhibitors that are active in malignant, non-Hodgkins, large, B-cell lymphomas that depend on NF-κB activity for their survival and proliferation [99]. Their properties could be used in the treatment of hormone-resistant, metastatic, prostate cancers. Hormone-dependent (LNCaP) and hormone-independent (PC-3 and DU-145) prostate cancer lines have been exposed to docetaxel alone or combined with the NF-κB inhibitor PS-1145. PC-3 and DU-145 cells had higher NF-κB activity, secreted more IL-6 and were more resistant to docetaxel than LNCaP cells. NF-κB activity was induced by docetaxel. Co-treatment with docetaxel and PS-1145 prevented docetaxel induced NF-κB activation, reduced IL-6 production and increased docetaxel effects on cell in PC-3 and DU-145 cells, but not in LNCaP [100].

Thalidomide

Anti-tumoural activity of thalidomide includes inhibition of NF-κB activity through suppression of I–kappa beta kinase activity and inhibition of the cyclooxygenase 1 and 2 enzymes [101, 102]. Thalidomide reduces PSA levels in 20 to 25 % of patients with hormone-refractory prostate cancer. Combined with docetaxel, 53% of patients presented with a PSA decrease of more than 50% [103, 104].

Glucocorticoids

IL-6 is a proinflammatory cytokine. Recent work has shown that IL-6 produced by tumour infiltrating T cells, dentritic cells and epithelial cells drives the growth of colitis-associate cancer induced by administration of the procarcinogen azoxymethane and the irritant dextrane sulphate salt (DSS) [105]. Administration of DSS results in an inflammatory response that leads to activation of transcription factor NF-κB [106]. A better understanding of such interactions will pave the way for new therapies that will either prevent activation of inflammatory cells by cancer derived products or block the ability of growth factors produced by inflammatory cells to stimulate tumour angiogenesis, growth and survival. Interestingly, the activation of NF-κB is blocked by glucocorticoids. The complex formed from glucocorticoid and its receptor binds to the p65 subunit of NF-κB and prevents it from binding to the NF-κB response elements. Glucocorticoids could also increase the transcription of the IκB alpha gene, the endogenous inhibitor of activated NF-κB [107]. These mechanisms would explain the anti-inflammatory and anti-tumoural activity of glucocorticoids in androgen-independent prostate cancer either alone or combined with mitoxantrone [108].

Genistein

Genistein, an isoflavones isolated from soy, has been shown to inhibit the activity of NF-κB and the growth of various cancer cells without causing systemic toxicity. Moreover, there are reasons to believe that the effect of genistein may rely on its intrinsic tyrosine kinase inhibitor activity and/or its effect on Akt activity [109]. Genistein pretreatment inactivates NF-κB and may contribute to increased growth inhibition and apoptosis induced by cisplatin, docetaxel, and doxorubicin in prostate, breast, lung, and pancreatic cancer cells. Genistein also inhibits radiation-induced activation of NF-κB in prostate cancer cells by promoting apoptosis and G2/M cell cycle arrest [110]. These results warrant carefully designed clinical studies investigating the combination of soy isoflavones and commonly used chemotherapeutic agents for the treatment of prostate cancers. The observed effect of the anti-tumoural activity of docetaxel by genistein in the SCID-human xenograft model of experimental bone metastasis could be mediated by regulation of OPG/RANK/RANKL/MMP-9 signalling, resulting in the inhibition of osteoclastic bone resorption and prostate cancer bone metastasis. From these results, genistein could be a promising non-toxic agent to

improve the treatment outcome of metastatic prostate cancer with docetaxel [111].

The IL-6 signalling pathway represents a privileged, therapeutic target

An IL-6 receptor blockade by means of IL-6R antagonists might reduce the growth of certain tumour types and therefore delay the progression of the disease [112]. Given that the IL6/IL6-R pathway subdivides into a large spectrum of phosphorylation cascade pathways with distinct outcomes, downstream effectors of those may also be relevant targets for specific therapies as exemplified by recent evidence that inhibition of activated STAT3 suppresses prostate cancer progression [113], reduces the expression of STAT3 target genes, such as VEGF, Bcl-XL and cyclin D1, and triggers apoptosis [114].

Conclusion

Major modes through which NF-κB/IL-6 dependent pathway might promote in vivo tumour-cell survival, stimulates growth or protects against apoptotic stimuli were described. Although differing findings were reported in the literature, this pathway is critical in controlling differentiation processes both in the tumour cells and other cell types that may regulate tumour progression and metastasis. More importantly, as discussed in this work, it seems that the overactivity of the NF-κB/IL-6 pathway is a pattern of aggressive, recurrent and hormone resistant prostate cancers. Thus, these results provide the framework for targeting NF-κB and the downstream effect of IL-6 in novel biologically based therapies. Targeting such factors gave promising effects on various pathologies including non-malignant and malignant such as prostate cancer. It can be speculated that as more cell survival pathways will be defined, more the specific targeting by newly designed drugs will be efficient and paying off. The pro-apoptotic agents such as bortezomid, which observed effects seem strongly linked to its ability to down-regulate NF-κB activity highlights the potential benefits of these types of compounds. Interestingly, such drugs have apparently shown partial or strong ability to overcome drug resistance and to synergize with more classical therapies as illustrated by the fact that tumour cells was sensitized to chemotherapy. It can be speculated that other therapeutic agents that interfere with the HER-2 receptor, osteoclastic bone resorption or VEGF pathway will probably work and give benefits, at least in part, as components of combinational therapy for prostate cancer. In the near future, the search for new therapies, aimed to target specifically tumour cells and/or their environment, may further increase the chances of survival for patients with advanced disease as well as androgen refractory diseases that are today considered incurable.

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