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NF-κB Activation in Human Prostate Cancer: Important Mediator or Epiphenomenon?

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Abstract The NF-κB family of transcription factors has been shown to be constitutively activated in various human malignancies, including leukemias, lymphomas, and a number of solid tumors. NF-κB is hypothesized to contribute to development and/or progression of malignancy by regulating the expression of genes involved in cell growth and proliferation, anti-apoptosis, angiogenesis, and metastasis. Prostate cancer cells have been reported to have constitutive NF-κB activity due to increased activity of the IκB kinase complex. Furthermore, an inverse correlation between androgen receptor (AR) status and NF-κB activity was observed in prostate cancer cell lines. NF-κB may promote cell growth and proliferation in prostate cancer cells by regulating expression of genes such as *c-myc*, cyclin D1, and IL-6. NF-κB may also inhibit apoptosis in prostate cancer cells through activation of expression of anti-apoptotic genes, such as *Bcl-2*, although pro-apoptotic activity of NF-κB has also been reported. NF-κB-mediated expression of genes involved in angiogenesis (IL-8, VEGF), and invasion and metastasis (MMP9, uPA, uPA receptor) may further contribute to the progression of prostate cancer. Constitutive NF-κB activity has also been demonstrated in primary prostate cancer tissue samples and suggested to have prognostic importance for a subset of primary tumors. The limited number of samples analyzed in those studies and the relative lack of NF-κB target genes identified in RNA expression microarray analyses of prostate cancer cells suggest that further studies will be required in order to determine if NF-κB actually plays a role in human prostate cancer development, and/or progression, and to characterize its potential as a therapeutic target. J. Cell. Biochem. 91: 100–117, 2004.

Key words: prostate cancer; NF-κB; transcription; apoptosis

The NF-κB/Rel proteins are a family of transcription factors that regulate expression of genes involved in immune and inflammatory responses, cell growth, differentiation, and apoptosis (reviewed in Ghosh et al., 1998; Karin and Ben-Neriah, 2000; Ghosh and Karin, 2002).

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NF-κB was originally identified as a B cell-specific nuclear factor that bound to an enhancer element in the immunoglobulin kappa (κ) light chain gene. NF-κB was later found to be ubiquitously expressed in various cell types and to function as an important transcription factor governing many aspects of cellular and organismal physiology.

Activation of NF-κB involves induction of its nuclear localization and transcriptional activation potential, leading to the expression of a large number of target genes (reviewed in May and Ghosh, 1998). Many genes encoding a large array of cytokines and chemokines are transcriptionally activated by NF-κB, contributing to immune and inflammatory responses. NF-κB regulates the expression of adhesion molecules promoting cell migration and cellular interactions. Other NF-κB target genes encode proteins with anti-apoptotic activity promoting cell survival. Expression of genes encoding cell cycle

regulators (including c-myc and cyclin D1) is also induced by NF-κB, contributing to cellular growth and proliferation. NF-κB also serves to induce a variety of genes encoding proteins that induce cellular stress responses promoting cell survival and damage repair.

As suggested by the vast array of target genes, the importance of NF-κB in normal development and physiology was manifested by the phenotypes of targeted gene deletions of NF-κB/Rel family members in knockout mice (reviewed in Baeuerle and Baltimore, 1996; Ghosh et al., 1998). Deletion of the RelA/p65 subunit of NF-κB resulted in embryonic lethality associated with massive apoptosis of hepatocytes, demonstrating that NF-κB governs expression of important anti-apoptotic proteins required for hepatocyte development. c-Rel knockout mice exhibited impaired responses of T and B cells in lymphocyte activation. Multiple organ inflammation and thymic atrophy were observed in RelB knockout mice. Therefore, NF-κB is indispensable for normal development and immune and inflammatory responses to infection, cellular stress, and injury.

There is growing evidence that NF-κB/Rel proteins play important roles in the development and progression of a number of human malignancies (reviewed in Rayet and Gélinas, 1999; Baldwin, 2001; Karin et al., 2002). Not only are alterations of NF-κB/Rel genes associated with a series of leukemias and lymphomas, but NF-κB/Rel gene products have also been shown to have important pro-proliferative and anti-apoptotic activities that could contribute to the development, progression, and resistance to therapy of non-lymphoid tumor cells. In the past few years, there has been increasing interest in a possible role of NF-κB in prostate cancer initiation and/or progression. This review will present recent findings demonstrating constitutive NF-κB activity in the prostate cancer cell lines as well as in prostate cancer patient tissue samples, and consider possible roles for NF-κB in the growth, survival, and biological behavior of human prostate cancer.

NF-κB/REL PROTEINS: STRUCTURE AND REGULATION

NF-κB/Rel proteins refer to a family of transcriptional regulators, existing in a variety of homo-and hetero-dimeric forms, that has been evolutionarily conserved in both overall structural and regulation from fruit flies to humans. There are five mammalian family members (Fig. 1A): c-Rel, RelA (p65), RelB, NF-κB1 (p50/p105), and NF-κB2 (p52/p100) (reviewed in Ghosh et al., 1998; Karin and Ben-Neriah, 2000; Ghosh and Karin, 2002). The NF-κB/Rel proteins all have an N-terminal approximately 300 amino acid region known as the Rel homology domain (RHD), which contains DNA binding domains, a dimerization domain, and a nuclear localization signal (NLS). The major NF-κB dimer in most cell types is a p65 (RelA)/p50 (NF-κB1) heterodimer. The c-Rel, RelA, and RelB proteins have C-terminal transactivation domains responsible for function as transcriptional activators. In contrast, NF-κB1 and NF-κB2 are produced as precursor forms, p105 and p100, respectively, whose C-terminal domains contain a series of ankyrin repeats (similar to those found in the IκB inhibitors, see below) in place of a transactivation domain. Either by co-translational or post-translational processing, the p105 and p100 precursor proteins undergo proteolytic processing removing C-terminal sequences to generate final products, p50 NF-κB1 and p52 NF-κB2, respectively. Due to the absence of C-terminal transactivation domain, p50 and p52 homodimers are believed to function as transcriptional repressors, blocking access of other NF-κB dimers to the target gene promoters, while heterodimers of p50 or p52 with transactivation domain-containing Rel proteins lead to activation of target gene transcription.

In most cell types, NF-κB is normally retained in the cytoplasm in an inactive form, bound by inhibitory proteins referred to as inhibitors of κBs (IκBs) (Fig. 1A). The IκB family proteins contain five to seven ankyrin repeats of 30–33 amino acids, through which they bind to the RHD in NF-κB proteins, as exemplified by IκBα, the prototypical member of the family (reviewed in Ghosh et al., 1998; Karin and Ben-Neriah, 2000). The interactions between IκB proteins and NF-κB have been classically understood to result in masking of the NLS in the RHD in NF-κB proteins, thereby blocking nuclear localization of NF-κB. By virtue of having ankyrin repeats, the precursor forms of NF-κB1 and NF-κB2, p105 and p100, respectively, also function as IκB proteins, retaining NF-κB in the cytoplasm. IκBα, IκBβ, and IκBε contain an N-terminal regulatory domain that is important

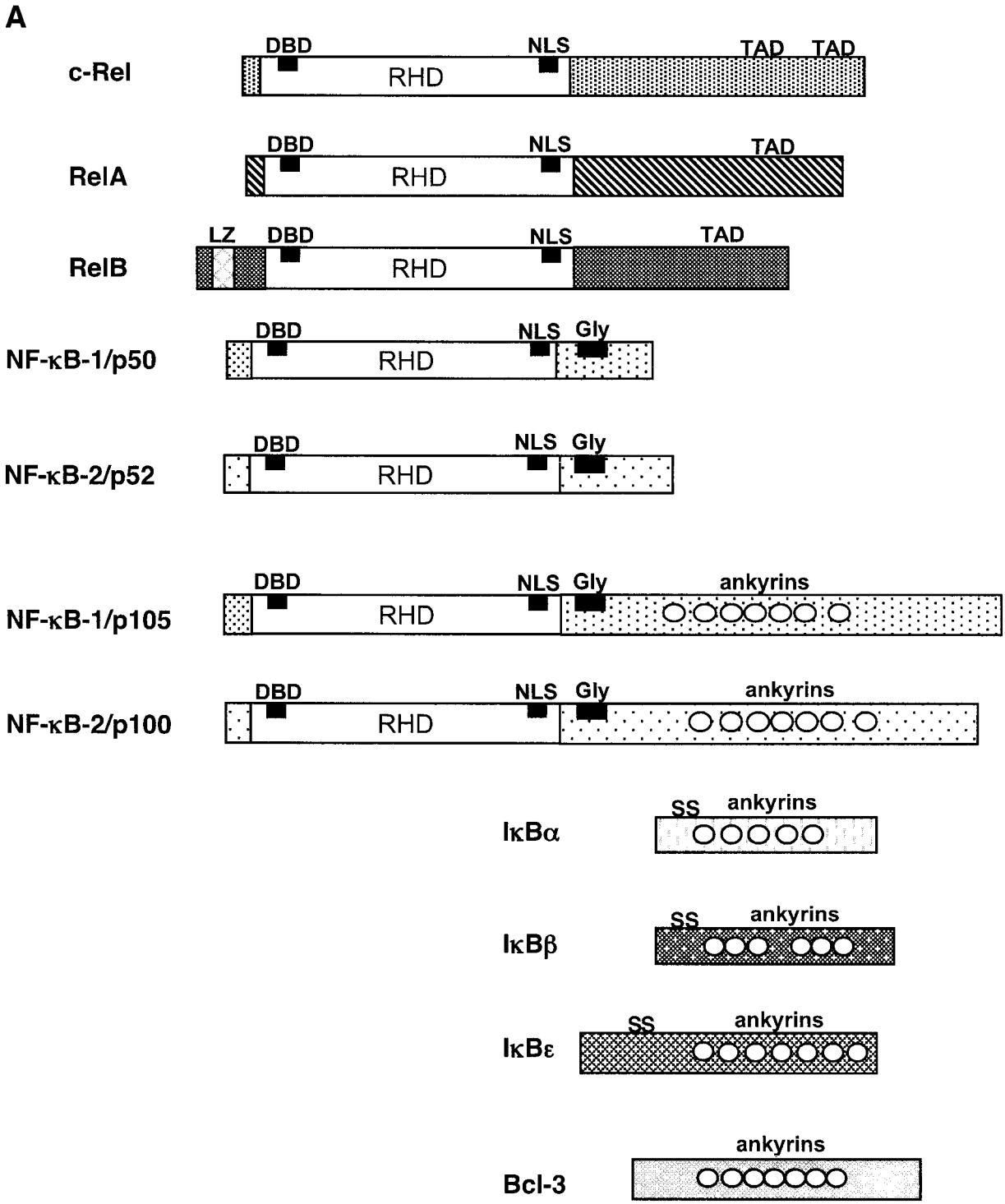

Fig. 1. Structure and regulation of NF-κB/Rel proteins. **A**: Structures of the mammalian NF-κB/Rel and IκB proteins. The positions of the Rel Homology Domain (RHD), DNA binding domains (DBD), nuclear localization sequence (NLS), glycine rich hinge regions (Gly), transactivation domains (TAD), leucine zipper domain (LZ), and ankyrin repeats are indicated. **B**: NF-κB regulatory pathways. In the classical pathway, proinflammatory cytokines (TNF-α and IL-1) and bacterial products (LPS) activate the IKKβ subunit of the IKK complex, leading to phosphorylation, ubiquitination, and proteasome-mediated degradation of IκBα, and nuclear accumulation of p50/RelA heterodimers. In the non-canonical pathway, in response to LTβ, BAFF/Blys, and CD40L, the IKKα subunit of the IKK complex is activated by NIK, leading to processing of NF-κB2/p100 to p52, and generation of p52/RelB heterodimers.

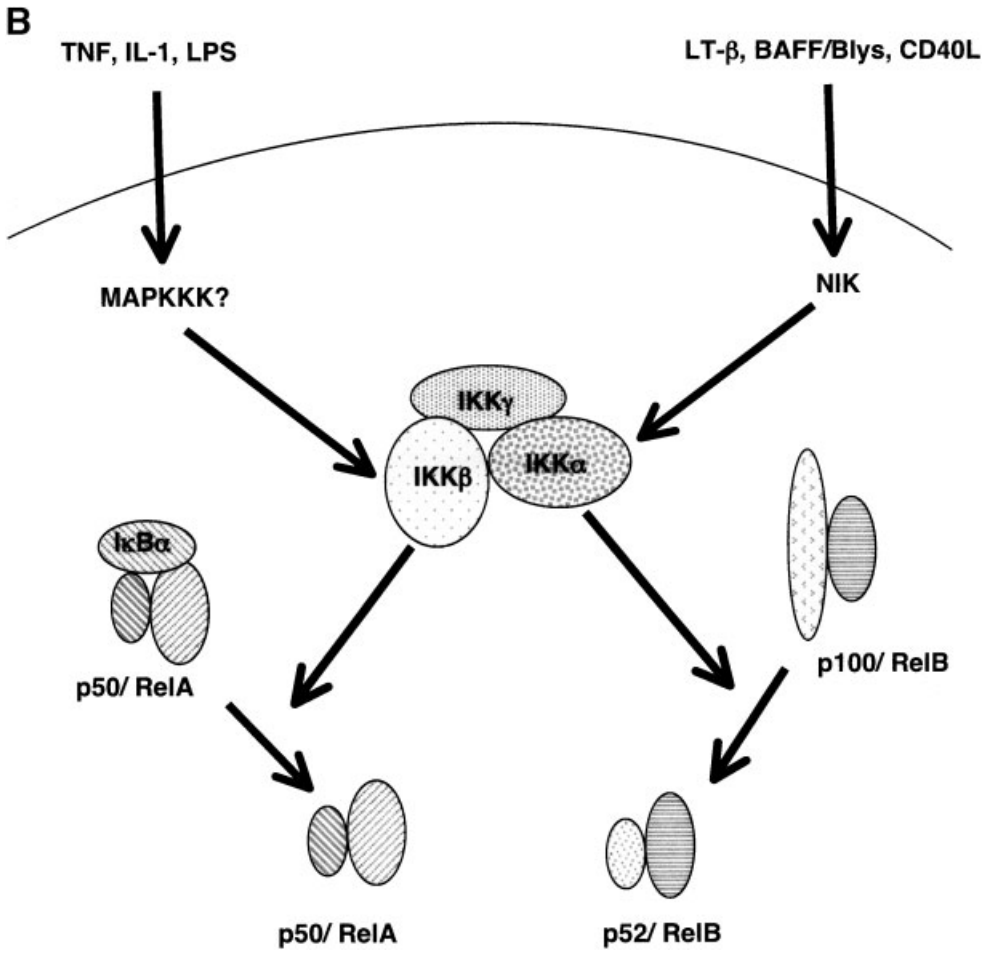

Fig. 1. (*Continued*)

for stimulation-induced degradation of IκB proteins. IκBα and IκBβ share an additional C-terminal domain called the PEST (proline-, glutamic acid-, serine-, and threonine-rich) domain that is involved in casein kinase 2 (CK2)-mediated phosphorylation and subsequent basal turnover of these IκB proteins. Bcl3 is a unique member of the IκB family, which is predominantly nuclear and forms complexes with either p50 or p52 homodimers, allowing p50 and p52 homodimers to function as transcriptional activators.

It was initially believed that the cytoplasmic localization of NF-κB in unstimulated cells was strictly due to inhibition of NF-κB nuclear import. There is growing evidence that the NF-κB/IκBα complex is more dynamic than originally suggested (reviewed in Ghosh and Karin, 2002). Shuttling of the NF-κB/IκBα complex in and out of nucleus has been observed; nuclear import occurs due to the fact that IκBα blocks only the NLS in p65 of p65/p50 heterodimers, without masking the other NLS present in p50 protein. The presence of a nuclear export signal (NES) present in the N-terminal region of the IκBα protein brings the complex back to cytoplasm, and cytoplasmic retention of NF-κB is postulated to occur because of the dominant effect of the IκBα NES over the NLS.

NF-κB is activated to induce nuclear localization and transcriptional activation by a wide variety of different stimuli, including proinflammatory cytokines (TNF-α, IL-1), growth factors, bacterial and viral products, double-stranded RNA, phorbol esters, oxidative stress, and ultraviolet (UV) light (Figs. 1B and 2) (reviewed in Ghosh et al., 1998). In the classic pathway of NF-κB activation, NF-κB-inducing stimuli lead to phosphorylation of the IκB proteins at two conserved serine residues in the N-terminal regulatory domain by the IκB kinase (IKK) complex. Phosphorylated IκB proteins are recognized by β-TrCP, a subunit

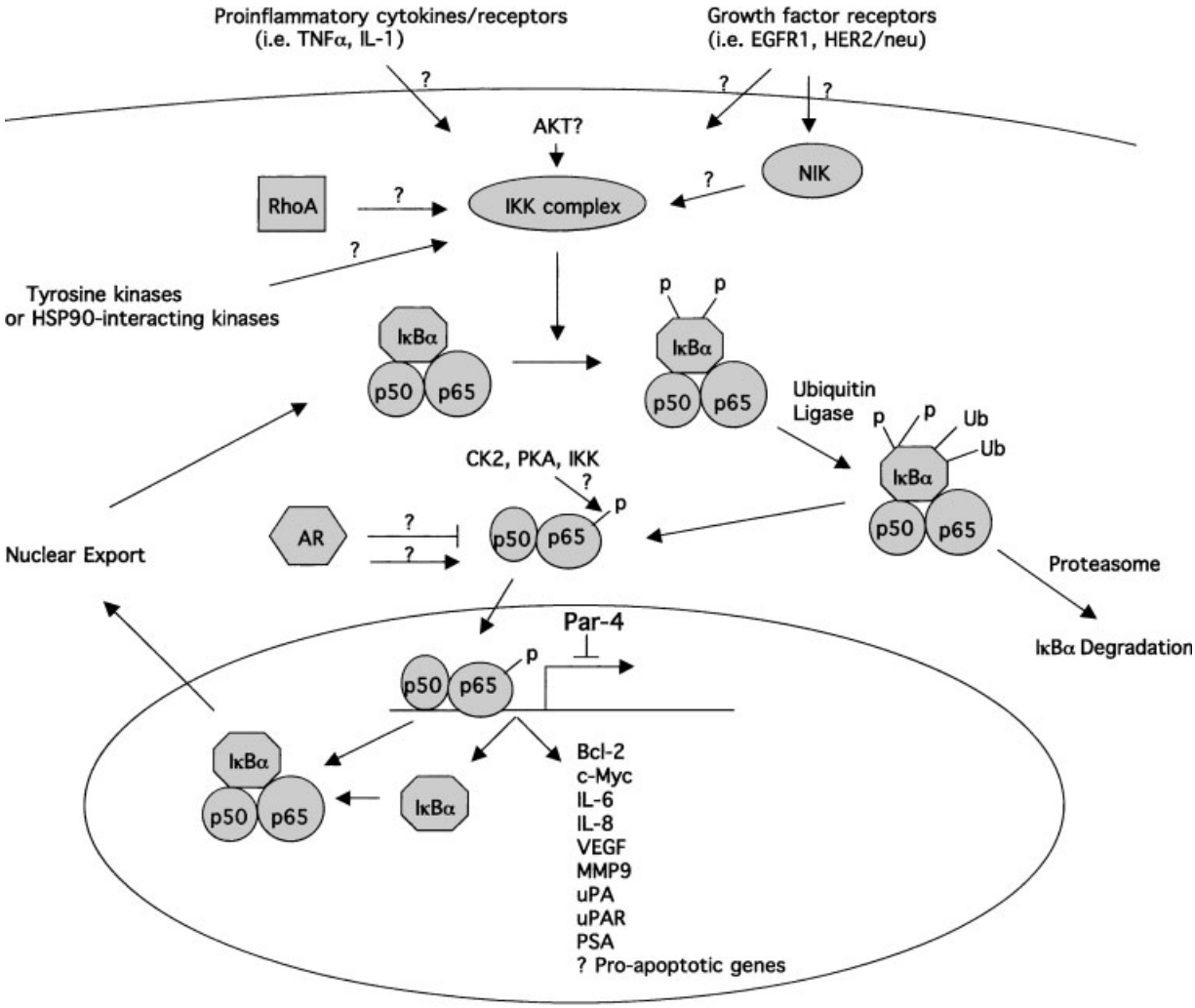

Fig. 2. Regulation of NF-κB activity and NF-κB target genes in prostate cancer cells. Schematic representation of the classical pathway of NF-κB activation indicating possible activating and regulatory stimuli in prostate cancer cells. Potential roles for growth factor receptors, NIK, RhoA, HSP90, and tyrosine kinases, as well as kinases that directly phosphorylate RelA are described in the text, as are the possible effects of the androgen receptor (AR) and Par-4. NF-κB target genes suggested to be biological effectors in prostate cancer are indicated, including those affecting cell growth and proliferation (c-myc and IL-6), angiogenesis (IL-8 and VEGF), invasion and metastasis (MMP9, uPA, and uPAR), anti-apoptotic genes (Bcl-2), prostate cancer markers (PSA), and potentially as yet uncharacterized pro-apoptotic target genes.

of the SCF (Skp-1/Cul/F box) ubiquitin ligase complex, and subsequently polyubiquitinated and degraded by the 26S proteasome (reviewed in Karin and Ben-Neriah, 2000). NF-κB dimers can then translocate into, and accumulate in the nucleus, activating transcription through binding to a decameric DNA sequence, the κB motif (GGGRNNYYCC) present in enhancers/promoters of target genes. One of these target genes is IκBα. Newly synthesized IκBα can bind NF-κB dimers in the nucleus, blocking DNA binding, and promoting nuclear export of NF-κB, completing a negative feedback loop. IKK-independent pathways of NF-κB activation have been reported to occur in response to oxidative stress and UV light. Reoxygenation of hypoxic cells was shown to induce phosphorylation of the IκBα protein at tyrosine 42 leading to dissociation of IκBα proteins from NF-κB [Imbert et al., 1996] in the absence of proteasome-mediated degradation of IκBα [Beraud et al., 1999]. UV irradiation-induced NF-κB activation was also demonstrated to be mediated by proteasome-dependent degradation of IκBα in the absence of IκBα phosphorylation [Li and Karin, 1998].

The IKK complex is a 700–900 kDa protein kinase complex composed of catalytic (IKKα

and IKKβ) and regulatory (IKKγ) subunits (reviewed in Karin, 1999; Zandi and Karin, 1999). IKKα and IKKβ are highly homologous; both proteins contain an N-terminal catalytic kinase domain, and leucine zipper and helix-loop-helix motifs at their C-termini. IKKα and IKKβ can form homo- or heterodimers with each other through their leucine zippers. Activation of these kinases requires phosphorylation of two serine residues in the activation loops present in each kinase, via either trans-autophosphorylation or by upstream kinases, which mediate the effects of extracellular activation signals.

Based on the high degree of structural similarity, the two catalytic subunits of the IKK complex were initially thought to have similarly important functions in NF-κB activation. However, mutations of two serine residues in the activation loop of IKKα were found to have no effect on NF-κB activation induced by proinflammatory cytokines such as TNFα and IL-1, whereas mutations of corresponding residues of IKKβ impaired NF-κB activation [Delhase et al., 1999]. Indeed, genetic knockout experiments revealed different roles of IKKα and IKKβ not only in NF-κB activation but also in normal development (reviewed in Zandi and Karin, 1999). IKKβ knockout mice died between embryonic day 12.5 and 14 due to apoptotic liver cell death, a similar phenotype as that observed for RelA/p65 knockout mice. Furthermore, embryonic fibroblast cells from these mice showed markedly reduced TNFα- and IL-1-induced NF-κB activation, suggesting that IKKβ is required for cytokine-induced NF-κB activation. In contrast, IKKα knockout mice showed a very different phenotype, exhibiting defective limb and skeletal patterning and impaired epidermal differentiation. Proinflammatory cytokine-induced NF-κB activation was not affected in the IKKα-deficient embryonic fibroblasts, indicating that IKKα is dispensable for NF-κB activation in response to cytokines.

Even though IKKα is not required for the classical pathway for NF-κB activation, its unique role has been found in a non-canonical NF-κB activation pathway regulating the processing of the NF-κB2 (p100) precursor [Senftleben et al., 2001]. Levels of p52, the processed product of p100, were dramatically decreased in IKKα-deficient B lymphocytes, and IKKα was shown to be required for B cell maturation and formation of secondary lymphoid organs. Physiological inducers responsible for IKKα-mediated processing of p100 were later found to be members of the TNF family, LTβ [Dejardin et al., 2002], CD40 ligand (CD40L) [Coope et al., 2002], and B cell-activating factor (BAFF)/B lymphocyte stimulator (Blys) [Claudio et al., 2002]. IKKα phosphorylates the C-terminus of p100, leading to ubiquitination-mediated processing of p100 into p52. Therefore, two catalytic subunits of the IKK complex, IKKα and IKKβ, have distinct roles in NF-κB activation pathway, the former functioning to process p100 precursor to p52 via a non-canonical pathway, and the latter activating NF-κB in response to infections and inflammation via the classical pathway (Fig. 1B).

The regulatory subunit of the IKK complex, IKKγ/NEMO, is a 419-amino-acid-long, glutamine-rich protein that contains several coiled-coil protein interaction motifs, including a leucine zipper motif (reviewed in Karin and Ben-Neriah, 2000). IKKγ is not only necessary for proper assembly of the IKK complex, but is also needed to connect the IKK complex to upstream activators. The importance of IKKγ as an essential component of the IKK complex was shown by the embryonic lethality of IKKγ-deficient mice generated by gene targeting [Rudolph et al., 2000]. Mutant embryos died at embryonic day 12.5–13 due to severe apoptosis of hepatocytes, similar to observations made in *p65* and *IKKβ* gene targeting studies. Embryonic fibroblasts from the mutant embryos also showed defective NF-κB activation in response to TNFα, IL-1, and LPS.

In addition to IKKα, IKKβ, and IKKγ, the IKK complex was recently found to contain additional components including heat shock protein 90 (HSP90) and Cdc37 [Chen et al., 2002a]. When geldanamycin was used to inhibit HSP90, the IKK complex was no longer activated upon TNFα stimulation. HSP90 and Cdc37 were required to recruit the IKK complex to the TNF receptor. Therefore, HSP90 and Cdc37 serve as essential components of the IKK complex required for TNFα-induced activation of the IKK complex.

Activation of the IKK complex by upstream inducing stimuli appears to be mediated through a variety of protein kinases. In a stimuli- and cell type-specific manner, the IKK complex may be activated by multiple members of the MAPKKK family and MAPKKK-related kinases, including NF-κB-inducing kinase (NIK), MEKK1, MEKK2, MEKK3, Tpl2/Cot1,

and TAK1 (reviewed in Karin and Ben-Neriah, 2000; Ghosh and Karin, 2002). Other kinases, including PKCθ, PKB/AKT, NAK/TBK1/T2K, and PKR, were also shown to activate NF-κB in certain settings. All of the above kinases were shown to be able to activate the IKK complex when overexpressed, and the dominant negative mutant forms of these kinases, with the exception of PKR, were also able to suppress NF-κB activation induced by various stimuli. However, it should be noted that only the loss of MEKK3 expression by gene targeting [Yang et al., 2001] or reduced expression of TAK1 by small interfering RNA [Takaesu et al., 2003] resulted in impaired activation of NF-κB in response to pro-inflammatory cytokines such as TNFα. Therefore, it remains unclear which members of upstream kinases actually mediate activation of the IKK complex.

Several recent studies have demonstrated that signal-induced post-translational modifications, including phosphorylation and acetylation, of the RelA/p65 subunit, are essential for the induction and duration of NF-κB-dependent transcription. Protein kinase A [Zhong et al., 1997] and casein kinase 2 [Bird et al., 1997; Wang and Baldwin, 1998; Wang et al., 2000] have been shown to phosphorylate the RelA/p65 subunit at serine 276 and 529 in response to LPS and pro-inflammatory cytokines, respectively. PKB/AKT has also been reported to induce IKK complex-mediated phosphorylation of RelA/p65 at serine 535 in response to IL-1 [Madrid et al., 2001]. Phosphorylation of RelA/p65 results in increased transactivation function of NF-κB, for example, by promoting the interaction of RelA/p65 with transcriptional co-activators, p300/CBP [Zhong et al., 1998]. Another mode of post-translational modification shown to be important for NF-κB-dependent transcription is acetylation of RelA/p65 proteins [Chen et al., 2002b]. RelA/p65 proteins were reported to be acetylated in the nucleus, possibly by the p300/CBP histone acetylase complex, and the acetylated RelA/p65 proteins were found to be refractory to the binding of newly synthesized IκBα. Deacetylation of RelA/p65 proteins by histone deacetylase 3 (HDAC3), which directly binds RelA/p65 proteins, allows IκBα to form a complex with RelA/p65-contaning NF-κB complex and to shuttle back to cytoplasm. Therefore, acetylation of Rela/p65 serves as a factor that prolongs the duration of NF-κB-dependent transcription.

NF-κB AND CANCER

A role for NF-κB in the initiation and/or progression of various types of cancers has been attributed to the target genes of NF-κB, which contribute to many aspects of tumorigenesis, including cell growth and proliferation, anti-apoptosis, angiogenesis, and metastasis (reviewed in Rayet and Gélinas, 1999; Baldwin, 2001; Karin and Lin, 2002; Karin et al., 2002). Cyclin D1 and *c-myc* are NF-κB target genes, both of which have important roles in cell growth and proliferation. Some NF-κB target genes, such as *c-IAP1*, *c-IAP2*, *c-FLIP*, *Traf1*, *Traf2*, *A20*, *Bfl1/A1*, and *Bcl-xL*, provide tumor cells with anti-apoptotic machinery, which allows them to evade apoptotic cell death, important for oncogenesis and resistance to cancer therapies. NF-κB also contributes to tumor development by regulating the expression of genes (*VEGF*, *IL-8*, *uPA*, and *MMP9*) involved in angiogenesis, and tumor invasion and metastasis. Thus, it is not surprising that many types of cancer exhibit constitutive NF-κB activity resulting from either genetic alterations of genes encoding NF-κB and IκB proteins or from changes in regulatory cascades, inducing abnormal NF-κB activation.

Hematologic malignancies provide the clearest evidence for the roles of NF-κB/Rel proteins in oncogenesis. The v-Rel oncoprotein, the viral homologue of c-Rel, encoded by the highly oncogenic avian reticuloendotheliosis virus strain T (Rev-T) retrovirus (reviewed in Gilmore, 1999) induces aggressive lymphomas and leukemias in chickens and induces T-cell lymphomas in transgenic mice [Carrasco et al., 1996]. Chromosomal amplification and rearrangement of genes encoding NF-κB/Rel and IκB proteins have been well documented in human hematological malignancies. For example, rearrangements of the NF-κB-2 gene (chromosome 10q24) have been identified in cutaneous T-cell lymphoma [Thakur et al., 1994; Neri et al., 1996] leading to production of C-terminally truncated forms of the p100, with the loss of the inhibitory IκB-like activity of p100. Constitutive activation of NF-κB is also seen in Hodgkin's disease, in which inactivating mutations have been observed in the *IκBα* gene [Cabannes et al., 1999]. Most Hodgkin's disease cell lines and patients express the wild type IκBα protein, implying that other mechanisms, such as constitutive IKK activation also induce NF-κB

activity in this disease [Krappmann et al., 1999]. Constitutive activation of the IKK complex was also reported in activated B cell-like diffuse large B cell lymphoma (DLBCL), a subtype of DLBCL, and was shown to be associated with poor clinical prognosis [Davis et al., 2001].

As compared to lymphoma and leukemia, genetic alterations of the NF-κB/Rel proteins and IκBs are rare in solid tumors. However, constitutive NF-κB activity is nonetheless common and there is growing evidence supporting the importance of NF-κB in development of a number of solid tumors. Constitutive NF-κB activity has been demonstrated in in vitro cell lines of head and neck squamous cell carcinoma (HNSCC) [Ondrey et al., 1999], pancreatic adenocarcinoma [Wang et al., 1999], melanoma [Shattuck-Brandt and Richmond, 1997], and breast cancer [Nakshatri et al., 1997; Sovak et al., 1997]. Nuclear localization of RelA/p65, which is indicative of NF-κB activation, has also been observed in tumor samples from patients of pancreatic adenocarcinoma [Wang et al., 1999], melanoma [Dhawan and Richmond, 2002], hepatocellular carcinoma [Tai et al., 2000], thyroid C-cell carcinoma [Ludwig et al., 2001], and breast cancer [Sovak et al., 1997], further confirming that observations made in in vitro cell lines are unlikely to be artifactual. Conflicting observations have been made as to the subunit composition of NF-κB detected in the nuclei of breast cancer cells as well as the correlation between estroger receptor (ER) status and NF-κB activation [Cogswell et al., 2000]. Cogswell et al. observed the presence of c-Rel, p50/NF-κB1, p52/NF-κB2, and Bcl-3 in the nuclei of breast cancer tissue samples. Furthermore, in this study, a previously detected inverse correlation between ER status and NF-κB activity [Nakshatri et al., 1997] was not observed; high levels of NF-κB activity were observed in both ER-positive and ER-negative breast cancer cells [Cogswell et al., 2000]. Although the reasons for these discrepant results are not entirely clear, these studies confirm constitutive NF-κB activation in breast cancer cells.

A possible functional role for activated NF-κB in cancer cell lines has been suggested through both genetic and pharmacologic inhibition. Inhibition of NF-κB activity can be effectively achieved by using superrepressor IκBα (SR-IκBα), a non-degradable form of IκBα in which serines 32 and 36 are replaced by alanines [Brown et al., 1995; Traencker et al., 1995]. Blocking NF-κB activity in HNSCC cells stably transfected with an expression plasmid encoding SR-IκBα resulted in reduced cell growth and survival [Duffey et al., 1999]. A contribution of NF-κB to metastasis of pancreatic cancer cells was also demonstrated by showing that suppression of NF-κB activity by SR-IκBα resulted in decrease in liver and peritoneal metastases of tumor cells orthotopically injected in mice [Fujioka et al., 2003]. Inhibition of NF-κB activity by SR-IκBα caused decreased tumorigenicity of both melanoma and ovarian cancer cells in mice as well as reduced angiogenesis and metastasis [Huang et al., 2000a,b]. In breast cancer cells, suppression of NF-κB activity by SR-IκBα resulted in apoptotic cell death induced by a chemotherapeutic and microtubule-stabilizing agent, paclitaxel [Patel et al., 2000]. These studies demonstrate that suppression of NF-κB renders cancer cells sensitive to apoptotic inducing agents, providing a potential target for effective chemotherapeutic regimens to treat these diseases.

In summary, NF-κB has been shown to be constitutively activated in hematopoietic malignancies and a variety of solid tumors, either by genetic alterations of the genes encoding members of the NF-κB/Rel and IκB families or by altered signal transduction pathways leading to increased activity of the NF-κB activation pathways.

CONSTITUTIVE NF-κB ACTIVITY IN PROSTATE CANCER CELL LINES

The observed activation and biological activities of NF-κB in several different human cancer cell lines raised the question of the possible roles of NF-κB in human prostate cancer. In a series of studies from several laboratories, NF-κB activity was shown to be constitutively activated in a series of human prostate cancer cell lines and prostate carcinoma xenografts [Palayoor et al., 1999; Gasparian et al., 2002; Suh et al., 2002]. Similar to findings reported in breast cancer cell lines, there was inverse correlation between androgen receptor (AR) status and constitutive activation of NF-κB. Prominent constitutive NF-κB activation (as detected by electrophoretic mobility shift assays, reporter gene assays, and expression of NF-κB target genes), was observed in prostate cancer cell lines lacking AR expression (PC-3,

DU145, and Du-Pro), whereas only very low levels of NF-κB activity were seen in androgen-responsive cell lines expressing detectable levels of AR (LNCaP and CWR22Rv1). The DNA binding activity of NF-κB in CL2 cells, androgen-independent cells derived from androgen-sensitive LNCaP, was found to be higher than in the parental LNCaP cells [Gasparian et al., 2002]. These data suggest either that the presence of AR actually inhibits NF-κB activity in prostate cancer cells or alternatively, that constitutive activation of NF-κB may correlate with AR loss and may contribute to compensatory cellular changes allowing cell survival and growth in the absence of AR activation.

The relationship between steroid hormone receptor expression and NF-κB activation has been of considerable interest in both prostate and breast adenocarcinomas. While initial studies of breast cancer cell lines and tumors also strongly suggested an inverse correlation between NF-κB activation and estrogen receptor expression [Nakshatri et al., 1997], this correlation has not been seen by all investigators [Cogswell et al., 2000]. With respect to the AR, mutually antagonistic effects of NF-κB and the AR were demonstrated in transiently transfected Cos-1 cells [Palvimo et al., 1996]. Although androgen treatment enhanced this effect, inhibition of NF-κB-directed transcription was observed even in the absence of ligand, possibly due to physical association between the two proteins [Palvimo et al., 1996]. However, Suh et al. [2002] observed that transient transfection of AR-negative prostate cancer cell lines (PC-3 and DU145) with an AR-expression plasmid actually resulted in increased NF-κB-dependent transcription in the absence of the synthetic AR ligand, R1881. Although addition of ligand blunted the AR-associated increase, it did not actually inhibit NF-κB activity in these cells. Thus, contrary to the transfected Cos-1 cells, it is unlikely that a simple model of direct physical interactions between AR and RelA, irrespective of the presence of ligand, is responsible for decreased NF-κB activity in AR-expressing prostate cancer cells. A different model for AR-mediated suppression of NF-κB activity has been proposed by Keller et al. [1996], who demonstrated that 5α-dihydrotestosterone (DHT)-activated AR inhibited PMA-induced NF-κB activation by maintaining IκBα levels in LNCaP cells. Although a precise mechanism by which AR achieves maintenance of IκBα levels has not been elucidated, this study provides a further evidence for antagonistic effect of AR on NF-κB activity in prostate cancer cells.

Based on the findings demonstrating antagonistic effects of AR on NF-κB activity and an inverse correlation of AR expression and constitutive NF-κB activity in prostate cancer cell lines, it is tempting to speculate that constitutive activation of NF-κB, known to induce potent anti-apoptotic effects, may play a role in the progression of prostate cancer and contribute to prostate cancer cell survival following androgen withdrawal. In this regard, Chen et al. have observed markedly higher NF-κB activity in an androgen-independent prostate cancer xenograft models than in its androgen-dependent counterpart. NF-κB activated expression of the prostate specific antigen (PSA) gene in androgen-independent prostate cancer cells [Chen and Sawyers, 2002], supporting the possibility that NF-κB may compensate for the loss of AR activity by activating expression of a subset of AR target genes, contributing to androgen-independent prostate cancer cell growth in the absence of the androgen-AR signaling pathway.

Contrary to the antagonistic effects of AR on NF-κB activity described above, there are several studies demonstrating enhancement of NF-κB activity by AR in prostate cancer cells. DHT treatment of LNCaP cells increased NF-κB DNA binding activity, potentially contributing to the anti-apoptotic role of androgens in these cells [Coffey et al., 2002]. Similarly, R1881 treatment increased NF-κB DNA binding activity in LNCaP cells [Ripple et al., 1999]. In view of these multiple and conflicting observations, further studies will be required to clarify the effects of AR on NF-κB activity in prostate cancer cells, as well as to evaluate the possible relationship between NF-κB activation and androgen independence in primary human prostate cancers.

CONSTITUTIVE NF-κB ACTIVITY IN PROSTATE CANCER TISSUE SAMPLES

Despite the well-documented activation of NF-κB observed in a number of human cancer cell lines, including prostate cancer cell lines as described above, evidence of constitutive activation of NF-κB in primary patient tumor samples

of human solid malignancies, either by EMSA or by immunohistochemical demonstration of nuclear localization of NF-κB proteins, has been more limited (see above).

Immunohistochemistry has been utilized to demonstrate nuclear localization of RelA in primary prostate cancer patient tissue samples [Gasparian et al., 2002; Suh et al., 2002; Lessard et al., 2003]. The pattern of nuclear staining of p65 was variable between different patients, and within an individual patient appeared to be focal and heterogeneous, consistent with the well-known heterogeneity of prostate cancer for other genetic alterations [Mirchandani et al., 1995]. It is interesting that nuclear staining of p65 was observed in organ-confined, primary tumor samples, raising the possibility that constitutive NF-κB activation may be an early event in prostate cancer development. RelA staining was also detected in epithelial cells in regions of benign hyperplastic prostatic glands [Suh et al., 2002]. Gasparian et al. [2002] reported that nuclear staining for p65 was observed in approximately 24% of cells within cancerous regions, but in only about 10% of cells in adjacent normal prostate epithelium. The most extensive study has been conducted by Lessard et al. [2003], who observed nuclear p65 staining in 18 out of 45 tissue specimens derived from prostate cancer patients. By itself, NF-κB staining did not correlate with the Gleason grade, however, when nuclear NF-κB positivity was combined with Gleason grade to reclassify tumor samples, nuclear RelA staining predicted a poor clinical outcome with disease progression in intermediate Gleason grade tumors [Lessard et al., 2003]. These in vivo data suggest that the activation of RelA-containing NF-κB complexes observed in the prostate cancer cell lines may be reflective of the biological properties of subsets of prostate cancer cells in vivo and not merely an artifact of in vitro cell growth during the derivation of specific prostate cancer cell lines. Furthermore, NF-κB activation in prostate cancer in vivo may be associated with a poorer prognosis in a subset of patients, however, further studies will be required to confirm and extend these observations. It is also not yet clear if the in vivo NF-κB activation correlates with proliferative activity and/or responses to inflammatory or other stresses of both benign and malignant prostate epithelial cells, or whether the observed activation plays a specific role in prostate cancer progression.

MECHANISMS FOR CONSTITUTIVE NF-κB ACTIVITY IN PROSTATE CANCER CELLS

The detailed mechanisms responsible for constitutive NF-κB activation in prostate cancer cells remain unclear, as is true for many human cancers in which NF-κB activation is detected. Possible regulatory pathways in prostate cancer cells are illustrated in Figure 2. At one level, it has been clearly shown that NF-κB activation in these cells is due to increased activity of the IKK complex [Palayoor et al., 1999; Gasparian et al., 2002; Suh et al., 2002]. As a result, the half-life of the IκBα protein is greatly reduced in prostate cancer cells with constitutive NF-κB activity as compared to the cells with basal activity. Detection of phosphorylated IκBα in those cells with high NF-κB activity confirms that the IκBα proteins may be degraded at a higher rate, due to the IKK complex-mediated phosphorylation of the IκBα proteins. Accordingly, NF-κB activity was suppressed by a dominant negative mutant form of IKKβ in the transient transfection reporter assay [Gasparian et al., 2002; Suh et al., 2002]. These studies of the mechanisms responsible for constitutive NF-κB activation in the prostate cancer cell lines strongly implicate inappropriate, constitutive activation of the IKK complex, resulting in enhanced IκBα degradation, consistent with data from other, non-prostate tumor cell lines [Krappmann et al., 1999; Ludwig et al., 2001; Romieu-Mourez et al., 2001; Yang and Richmond, 2001].

Clearly an important question remaining to be addressed is the nature of the alterations in upstream signaling pathways that result in constitutive activation of the IKK complex and NF-κB in prostate cancer cells. Members of the MAPKKK family of protein kinases have been reported to provide signals that activate IKK kinase activity. Using a dominant negative kinase approach, our group was able to demonstrate that inhibition of NIK resulted in inhibition of NF-κB mediated transcription in the prostate cancer cells containing high NF-κB activity [Suh et al., 2002]. In contrast, dominant negative forms of other MAPKKK family members, MEKK1 and MEKK2, which have been shown to regulate NF-κB activity in certain settings [Lee et al., 1997; Zhao and Lee, 1999], failed to suppress NF-κB activity, implying that a specific signal transduction pathway(s) involving NIK may be constitutively activated

in prostate cancer cells. Interestingly, recent studies have also suggested an important role for NIK in constitutive NF-κB activation in melanoma cells [Dhawan and Richmond, 2002]. However, unlike melanoma cells where overexpression of NIK was positively correlated with high levels of NF-κB activity, we failed to detect significant difference in the levels of NIK protein expression in the prostate cancer cell lines. Therefore, other mechanism(s) may cause activation of NIK in the prostate cancer cells. It should be noted, however, that these studies do not conclusively prove a role for NIK in NF-κB activation in the prostate cancer cells. Even though overexpression of the kinase-dead mutant form of NIK can block cytokine-mediated activation of NF-κB, gene targeting studies revealed that NF-κB activation by cytokines, including TNFα and IL-1, was not lost in the absence of NIK [Yin et al., 2001], and NIK has been particularly implicated as an upstream activator of IKKα in the non-canonical pathway for NF-κB activation (see above). Thus, while NIK may play a role in constitutive activation of NF-κB in prostate cancer cells, it is also possible that other as yet undetermined kinases may be more important.

A possible role for NIK in growth factor-induced NF-κB activation in breast cancer cells was suggested by the observations that NIK interacts with the EGF receptor when overexpressed in 293 EBNA cells, and that a high level of EGFR expression induced activation of NF-κB in breast cancer cells [Habib et al., 2001]. Most growth factors transduce their signals at least in part through activation of tyrosine kinases, either as through receptor or non-receptor tyrosine kinases. In this regard, treatment of prostate cancer cells with a tyrosine kinase inhibitor, herbimycin A, resulted in suppression of NF-κB-mediated transcriptional activation [Suh et al., 2002]. A different tyrosine kinase inhibitor, genistein, was also shown to suppress NF-κB activity in PC-3 and LNCaP cells [Davis et al., 1999]. Therefore, as yet undetermined tyrosine kinase pathways may lead to NF-κB activation in the prostate cancer cell lines, through activation of the IKK complex. While consistent with possible effects on tyrosine kinases, the inhibitory effects of herbimycin A on NF-κB in prostate cancer cells could also be a result of direct inhibition of the IKK complex, by targeting Hsp90 to destabilize Hsp90-binding proteins.

Despite numerous studies of multiple different signal transduction pathways, it is still unclear as to which cellular protein kinases and signal transduction molecules may activate the IKK complex to induce constitutive NF-κB activity in prostate cancer cells. RhoA activity was reported to be necessary for increased NF-κB activity in a highly invasive variant of PC-3 cells [Hodge et al., 2003]. Although this study was performed using a variant of PC-3 cells and did not identify how RhoA exerts its effects on NF-κB, it still presents an important alternative pathway that may enhance constitutive NF-κB activation in prostate cancer cells.

One candidate kinase that may be involved in NF-κB activation in prostate cancer cells is PKB/AKT, a downstream effector of the PI3 kinase (PI3K). PKB/AKT may function to activate NF-κB activity in other types of cancer cells, including HNSCC [Bancroft et al., 2002], melanoma [Dhawan et al., 2002], and breast cancer [Biswas et al., 2000; Pianetti et al., 2001]. Given that a loss of expression of PTEN, an inhibitor of the PI3K-PKB/Akt pathway, is a hallmark of prostate cancer [Suzuki et al., 1998; Wang et al., 1998], the absence of PTEN may explain constitutive activation of NF-κB induced by PI3K-PKB/AKT pathway. However, no direct correlation between NF-κB activity and loss of PTEN expression was observed in prostate cancer cell lines. Neither PC-3 cells (high constitutive NF-κB) nor LNCaP cells (low NF-κB) express PTEN, while DU145 cells retain PTEN expression but have high NF-κB activity, suggesting that the absence of PTEN expression is unlikely to lead to constitutive NF-κB activity in prostate cancer cells. It remains possible, however, that elevated expression of the AKT3 isoform observed in both PC-3 and DU145 cells as compared to LNCaP cells [Nakatani et al., 1999], may allow PKB/AKT protein to evade restraints imposed by PTEN, functioning to activate NF-κB in a constitutive manner. Alternatively, the PI3K-PKB/AKT pathway may be downstream effector of growth factor receptors, including EGFR1 and HER2/neu, activating NF-κB in prostate cancer cells.

The effects of nuclear regulation of the transcriptional activating function of NF-κB have not been extensively studied in prostate cancer cells, yet represent a second important level of NF-κB regulation. Preliminary studies (Suh et al., in preparation) suggest that CK2-induced modulation of RelA transcriptional activation

may be markedly enhanced, in conjunction with increased NF-κB nuclear localization, in androgen-independent prostate cancer cell lines, contributing to increased activation of NF-κB target genes in these cells. Furthermore, the effect of Par-4 in inhibiting RelA phosphorylation that mediates transcriptional activation of anti-apoptotic target genes in androgen-independent prostate cancer cell lines (see below), likely plays an important role in the pro-apoptotic functions of this protein [El-Guendy and Rangnekar, 2003].

ROLE OF NF-κB ACTIVITY IN PROSTATE CANCER BIOLOGY

NF-κB has been postulated to contribute to initiation and progression of various types of human cancer by regulating the expression of genes important for many steps of tumorigenesis. Suppression of NF-κB activity has been shown to repress growth of a variety of cancer cells both in vitro cell culture and in mice. Furthermore, the anti-apoptotic activity of NF-κB plays a role in the resistance of tumor cells to chemotherapeutic reagents and radiation therapy. For example, inhibition of NF-κB activity potentiates TNFα- and chemotherapy-induced apoptosis [Wang et al., 1996]. The roles of NF-κB in many of these processes have been studied in prostate cancer cells in culture and in tumor xenografts, and some of the implicated target genes and pathways are illustrated in Figure 2.

NF-κB clearly has potent anti-apoptotic activity in many different cells, and this activity has been demonstrated repeatedly in prostate cancer cells, particularly in androgen-insensitive cells with high NF-κB activity, such as PC-3 cells. Suppression of NF-κB activity by using SR-IκBα has been shown to sensitize prostate cancer cells to apoptosis induced by TNFα [Herrmann et al., 1997]. Curcumin has also been shown to suppress NF-κB activity, rendering prostate cancer cells sensitive to TNFα- [Mukhopadhyay et al., 2001], or chemotherapeutic agent-induced [Hour et al., 2002] apoptotic cell death. Other groups have shown that PDTC- [Sumitomo et al., 1999], silibinin- [Dhanalakshmi et al., 2002], and zinc- [Uzzo et al., 2002] mediated suppression of NF-κB activity led to an increased apoptotic response of prostate cancer cells to TNFα treatment. When a specific inhibitor of the IKK complex, BAY 11-7082, was employed to suppress NF-κB activity, prostate cancer cells became more sensitive to N-(4-hydroxyphenyl)retinamide-mediated apoptosis [Shimada et al., 2002]. A novel NF-κB inhibitor, DHMEQ, was also shown to be able to induce apoptosis of androgen-independent prostate cancer cells [Kikuchi et al., 2003]. Suppression of NF-κB activity by infection with an adenoviral vector expressing SR-IκBα decreased clonogenicity of PC-3 cells [Pajonk et al., 1999], and growth of DU145 xenografts in mice was impaired when NF-κB activity was suppressed by dexamethasone [Nishimura et al., 2001]. These studies all suggest a possible role for NF-κB in the viability of prostate cancer cells and in resistance to TNF- and chemotherapeutic agent-induced cell death. This is consistent with the hypothesis that NF-κB may also help mediate survival of tumor cells in the face of growth factor or androgen withdrawal.

The anti-apoptotic activity of NF-κB in prostate cancer cells has been attributed to its ability to induce *Bcl-2* gene expression. Catz et al. showed that *Bcl-2* gene expression in prostate cancer cells is mediated through NF-κB binding to sites in the Bcl-2 P2 promoter [Catz and Johnson, 2001]. Interestingly, this NF-κB-mediated activation of Bcl-2 expression in LNCaP cells was found to be enhanced in response to hormone withdrawal, suggesting the possibility that Bcl-2 expression induced by NF-κB may allow prostate cancer cells to transition from androgen-dependence to androgen-independence, possibly by blocking apoptosis induced by androgen ablation therapy. A role for NF-κB in regulating apoptosis in androgen-independent prostate cancer cells has also been suggested by the fact that Par-4, an endogenous inducer of apoptosis in these cells, inhibits NF-κB nuclear transcriptional activation as part of its pro-apoptotic effect [Chakraborty et al., 2001]. Overexpression of Par-4 has also been shown to sensitize radio-resistant PC-3 cells to radiation-induced apoptosis by inhibiting NF-κB activity and subsequent induction of *Bcl-2* gene expression [Chendil et al., 2002]. Interestingly, Par-4 fails to induce apoptosis in LNCaP cells, which have only low constitutive levels of NF-κB [Chakraborty et al., 2001], highlighting important potential differences in apoptotic regulation between androgen-dependent and -independent prostate cancer cells.

A possible role of NF-κB activity in mediating differential sensitivity to apoptosis in androgen-dependent and -independent prostate cancer cells has also been raised in studies in androgen-dependent LNCaP cells, which have low basal levels of NF-κB activity. Suppression of NF-κB by SR-IκBα in these cells actually decreased TNFα and γ-irradiation-mediated apoptosis, suggesting a pro-apoptotic role of NF-κB following certain stimuli in androgen-dependent prostate cancer cells [Kimura and Gelmann, 2002]. In this study, NF-κB was found to be necessary for enhanced Fas expression and activation of pro-apoptotic serine proteases contributing to cell death. NF-κB activity was also shown to be necessary for 2-methoxyestrdiol (2-ME)-induced apoptosis in LNCaP cells [Shimada et al., 2003]. 2-ME was reported to induce *p53* gene expression through NF-κB activation, and inhibition of NF-κB abrogated 2-ME-mediated apoptosis, further supporting a role of NF-κB in apoptosis of prostate cancer cells. It is not yet clear, however, if a pro-apoptotic role for NF-κB is limited to androgen-dependent prostate cancer cells and/or to those prostate cancer cells with low basal NF-κB activity (i.e., LNCaP), as described in these studies. It remains also possible that this apparently opposite role of NF-κB in apoptosis may be contingent upon other cellular changes associated with prostate cancer cell development, such as the status of p53 and the PTEN/AKT pathway.

The direct contributions of NF-κB to various steps in prostate cancer cell tumorigenesis have been demonstrated in in vivo mouse models [Huang et al., 2001]. When a highly metastatic derivative of PC-3 cells was stably transfected with the expression plasmid encoding SR-IκBα and orthotopically injected into nude mice, angiogenesis, invasion, and metastasis of highly aggressive prostate cancer cells were dramatically decreased, and this was associated with reduced expression of VEGF, IL-8, and MMP9. Therefore, NF-κB-regulated expression of genes such as IL-8, VEGF, MMP-9, uPA, and uPA receptor may be important for tumorigenicity, angiogenesis, and metastasis of prostate cancer cells.

Another NF-κB target of potential importance in prostate oncogenesis is the cytokine, IL-6. IL-6 has been suggested to function as a possible paracrine or autocrine growth factor for human prostate carcinoma cells [Okamoto and Oyasu, 1997], and circulating serum levels of IL-6 were found to be elevated in hormone refractory prostate cancer patients [Drachenberg et al., 1999]. Indeed, NF-κB activity was shown to be essential for constitutive expression of IL-6 in androgen-independent prostate cancer cells [Zerbini et al., 2003].

Thus, as shown in Figure 2, NF-κB may contribute to prostate cancer initiation and/or progression by regulating expression of genes important for various aspects of tumor development, including enhanced tumor cell growth and survival, angiogenesis, invasion, and metastasis. The functional effects of NF-κB activation may differ in different prostate cancer cells, particularly in those exhibiting altered basal levels of NF-κB and altered androgen sensitivity. Nonetheless, despite this extensive analysis in tissue culture and in animal model systems, a definitive role of NF-κB in human prostate cancer development and progression remains hypothetical. Further studies of NF-κB expression and that of its target genes in patient samples, as well as of the potential effects of NF-κB inhibitors in patients will be required to confirm its functional roles in human tumors.

One approach to addressing possible functional roles of NF-κB in primary human prostate cancers is afforded by the rapidly accumulating large-scale gene expression data derived from human prostate cancer tissues and adjacent normal prostate, through the use of microarray experiments to quantitate RNA expression [Dhanasekaran et al., 2001; Luo et al., 2001; Magee et al., 2001; Welsh et al., 2001; Ernst et al., 2002; Luo et al., 2002; Rhodes et al., 2002; Singh et al., 2002]. These analyses have identified multiple genes associated with prostate oncogenesis and disease progression, such as those associated with the emergence of androgen-independent disease. Based on the tissue culture and animal studies described above, one might have predicted that a number of classic NF-κB target genes would have been detected if NF-κB activation was a significant factor in prostate cancer progression. Surprisingly, very few classically described NF-κB target genes were identified among the genes whose transcription was elevated in prostate cancer and/or associated with disease progression. In fact, c-Myc was the only immediately recognizable NF-κB target identified as associated with prostate cancer. *c-Myc* gene

expression was shown to be upregulated in prostate cancer cells in both primary analysis [Dhanasekaran et al., 2001], and in a meta-analysis of four different array studies [Rhodes et al., 2002]. The apparent lack of target genes in the list of differentially regulated genes in prostate cancer samples suggests that NF-κB may not play a significant role in prostate cancer development and/or progression. Nonetheless, it remains possible that some of the genes whose expression is upregulated in prostate cancer may contain previously unidentified κB response elements in their promoter/enhancer regions and thus be authentic NF-κB targets, or that some of the as yet uncharacterized genes (represented as ESTs and unknown open reading frames) may also be NF-κB targets.

CONCLUSIONS

In a series of studies of prostate cancer cell lines, NF-κB activity has been shown to be constitutively activated in AR-negative prostate cancer cells due to increased activity of the IKK complex. An inverse correlation in these cell lines between AR status and NF-κB activity, coupled with the known anti-apoptotic activities of NF-κB in many different cell types, has raised the hypothesis that activation of NF-κB may be a prosurvival factor allowing prostate cancer cell growth following androgen depletion. Furthermore, in studies of both these cell lines, and of the tumors that they induce in immunodeficient mice, NF-κB activation appears to contribute to prostate cancer cell growth, survival, tumorigenicity, and resistance to therapy.

Despite fairly extensive study of cultured cell lines, the extent and functions of NF-κB activation in primary human prostate cancers remains unclear. Although three studies have identified nuclear RelA, indicative of NF-κB activation, in prostate cancer tissue, only one examined a sufficient number of patients with known clinical outcomes to provide evidence that such activation may predict a worse clinical course in subset of patients. Furthermore, RNA expression microarray experiments have failed to demonstrate generalized activation of known NF-κB target genes in human prostate cancers. In view of the on-going development of clinical therapeutic agents that target NF-κB for inhibition, and the continued difficulty in successfully treating advanced prostate cancer, there remains an urgent need to more carefully and clearly define any possible role that the NF-κB transcription factors may play in prostate cancer and their potential utility as therapeutic targets in this disease.

REFERENCES

Baeuerle PA, Baltimore D. 1996. NF-κB: Ten years after. Cell 87:13–20.

Baldwin AS. 2001. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 107:241–246.

Bancroft CC, Chen Z, Yeh J, Sunwoo JB, Yeh NT, Jackson S, Jackson C, Van Waes C. 2002. Effects of pharmacologic antagonists of epidermal growth factor receptor, PI3K and MEK signal kinases on NF-kappaB and AP-1 activation and IL-8 and VEGF expression in human head and neck squamous cell carcinoma lines. Int J Cancer 99:538–548.

Beraud C, Henzel WJ, Baeuerle PA. 1999. Involvement of regulatory and catalytic subunits of phosphoinositide 3-kinase in NF-kappaB activation. Proc Natl Acad Sci USA 96:429–434.

Bird TA, Schooley K, Dower SK, Hagen H, Virca GD. 1997. Activation of nuclear transcription factor NF-kappaB by interleukin-1 is accompanied by casein kinase II-mediated phosphorylation of the p65 subunit. J Biol Chem 272:32606–32612.

Biswas DK, Cruz AP, Gansberger E, Pardee AB. 2000. Epidermal growth factor-induced nuclear factor kappa B activation: A major pathway of cell-cycle progression in estrogen-receptor negative breast cancer cells. Proc Natl Acad Sci USA 97:8542–8547.

Brown K, Gerstberger S, Carlson L, Franzoso G, Siebenlist U. 1995. Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. Science 267: 1485–1488.

Cabannes E, Khan G, Aillet F, Jarrett RF, Hay RT. 1999. Mutations in the *IκBα* gene in Hodgkin's disease suggest a tumour suppressor role for IκB. Oncogene 18:3063–3070.

Carrasco D, Rizzo CA, Dorfman K, Bravo R. 1996. The v-rel oncogene promotes malignant T-cell leukemia/lymphoma in transgenic mice. EMBO J 15:3640–3650.

Catz SD, Johnson JL. 2001. Transcriptional regulation of bcl-2 by nuclear factor kappa B and its significance in prostate cancer. Oncogene 20:7342–7351.

Chakraborty M, Qiu SG, Vasudevan KM, Rangnekar VM. 2001. Par-4 drives trafficking and activation of Fas and Fasl to induce prostate cancer cell apoptosis and tumor regression. Cancer Res 61:7255–7263.

Chen CD, Sawyers CL. 2002. NF-kappa B activates prostate-specific antigen expression and is upregulated in androgen-independent prostate cancer. Mol Cell Biol 22:2862–2870.

Chen G, Cao P, Goeddel DV. 2002a. TNF-induced recruitment and activation of the IKK complex require Cdc37 and Hsp90. Mol Cell 9:401–410.

Chen LF, Mu Y, Greene WC. 2002b. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. EMBO J 21:6539–6548.

Chendil D, Das A, Dey S, Mohiuddin M, Ahmed MM. 2002. *Par-4*, a pro-apoptotic gene, inhibits radiation-induced NF kappa B activity and Bcl-2 expression leading to induction of radiosensitivity in human prostate cancer cells PC-3. Cancer Biol Ther 1:152–160.

Claudio E, Brown K, Park S, Wang H, Siebenlist U. 2002. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. Nat Immunol 3:958–965.

Coffey RN, Watson RW, O'Neill AJ, Mc Eleny K, Fitzpatrick JM. 2002. Androgen-mediated resistance to apoptosis. Prostate 53:300–309.

Cogswell PC, Guttridge DC, Funkhouser WK, Baldwin AS, Jr. 2000. Selective activation of NF-kappa B subunits in human breast cancer: Potential roles for NF-kappa B2/p52 and for Bcl-3. Oncogene 19:1123–1131.

Coope HJ, Atkinson PG, Huhse B, Belich M, Janzen J, Holman MJ, Klaus GG, Johnston LH, Ley SC. 2002. CD40 regulates the processing of NF-kappaB2 p100 to p52. EMBO J 21:5375–5385.

Davis JN, Kucuk O, Sarkar FH. 1999. Genistein inhibits NF-kappa B activation in prostate cancer cells. Nutr Cancer 35:167–174.

Davis RE, Brown KD, Siebenlist U, Staudt LM. 2001. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med 194:1861–1874.

Dejardin E, Droin NM, Delhase M, Haas E, Cao Y, Makris C, Li ZW, Karin M, Ware CF, Green DR. 2002. The lymphotoxin-beta receptor induces different patterns of gene expression via two NF-kappaB pathways. Immunity 17:525–535.

Delhase M, Hayakawa M, Chen Y, Karin M. 1999. Positive and negative regulation of IkappaB kinase activity through IKKbeta subunit phosphorylation. Science 284: 309–313.

Dhanalakshmi S, Singh RP, Agarwal C, Agarwal R. 2002. Silibinin inhibits constitutive and TNFalpha-induced activation of NF-kappaB and sensitizes human prostate carcinoma DU145 cells to TNFalpha-induced apoptosis. Oncogene 21:1759–1767.

Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta KJ, Rubin MA, Chinnaiyan AM. 2001. Delineation of prognostic biomarkers in prostate cancer. Nature 412:822–826.

Dhawan P, Richmond A. 2002. A novel NF-kappa B-inducing kinase-MAPK signaling pathway up-regulates NF-kappa B activity in melanoma cells. J Biol Chem 277: 7920–7928.

Dhawan P, Singh AB, Ellis DL, Richmond A. 2002. Constitutive activation of Akt/protein kinase B in melanoma leads to up-regulation of nuclear factor-kappaB and tumor progression. Cancer Res 62:7335–7342.

Drachenberg DE, Elgamal AA, Rowbotham R, Peterson M, Murphy GP. 1999. Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. Prostate 41:127–133.

Duffey DC, Chen Z, Dong G, Ondrey FG, Wolf JS, Brown K, Siebenlist U, Van Waes C. 1999. Expression of a dominant-negative mutant inhibitor-κBα of nuclear factor-κB in human head and neck squamous cell carcinoma inhibits survival, proinflammatory cytokine expression, and tumor growth in vivo. Cancer Res 59: 3468–3474.

El-Guendy N, Rangnekar VM. 2003. Apoptosis by Par-4 in cancer and neurodegenerative diseases. Exp Cell Res 283:51–66.

Ernst T, Hergenhahn M, Kenzelmann M, Cohen CD, Bonrouhi M, Weninger A, Klaren R, Grone EF, Wiesel M, Gudemann C, Kuster J, Schott W, Staehler G, Kretzler M, Hollstein M, Grone HJ. 2002. Decrease and gain of gene expression are equally discriminatory markers for prostate carcinoma: A gene expression analysis on total and microdissected prostate tissue. Am J Pathol 160:2169–2180.

Fujioka S, Sclabas GM, Schmidt C, Frederick WA, Dong QG, Abbruzzese JL, Evans DB, Baker C, Chiao PJ. 2003. Function of nuclear factor kappaB in pancreatic cancer metastasis. Clin Cancer Res 9:346–354.

Gasparian AV, Yao YJ, Kowalczyk D, Lyakh LA, Karseladze A, Slaga TJ, Budunova IV. 2002. The role of IKK in constitutive activation of NF-kappaB transcription factor in prostate carcinoma cells. J Cell Sci 115: 141–151.

Ghosh S, Karin M. 2002. Missing pieces in the NF-kappaB puzzle. Cell 109:S81–S96.

Ghosh S, May MJ, Kopp EB. 1998. NF-kappaB and Rel proteins: Evolutionarily conserved mediators of immune responses. Ann Rev Immunol 16:225–260.

Gilmore TD. 1999. Multiple mutations contribute to the oncogenicity of the retroviral oncoprotein v-Rel. Oncogene 18:6925–6937.

Habib AA, Chatterjee S, Park SK, Ratan RR, Lefebvre S, Vartanian T. 2001. The epidermal growth factor receptor engages receptor interacting protein and nuclear factor-kappa B (NF-kappa B)-inducing kinase to activate NF-kappa B. Identification of a novel receptor-tyrosine kinase signalosome. J Biol Chem 276:8865–8874.

Herrmann JL, Beham AW, Sarkiss M, Chiao PJ, Rands MT, Bruckheimer EM, Brisbay S, McDonnell TJ. 1997. Bcl-2 suppresses apoptosis resulting from disruption of the NF-kappa B survival pathway. Exp Cell Res 237: 101–109.

Hodge JC, Bub J, Kaul S, Kajdacsy-Balla A, Lindholm PF. 2003. Requirement of RhoA activity for increased nuclear factor kappaB activity and PC-3 human prostate cancer cell invasion. Cancer Res 63:1359–1364.

Hour TC, Chen J, Huang CY, Guan JY, Lu SH, Pu YS. 2002. Curcumin enhances cytotoxicity of chemotherapeutic agents in prostate cancer cells by inducing p21(WAF1/CIP1) and C/EBPbeta expressions and suppressing NF-kappaB activation. Prostate 51:211–218.

Huang S, DeGuzman A, Bucana CD, Fidler IJ. 2000a. Nuclear factor-kappaB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice. Clin Cancer Res 6:2573–2581.

Huang S, Robinson JB, Deguzman A, Bucana CD, Fidler IJ. 2000b. Blockade of nuclear factor-kappaB signaling inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor and interleukin 8. Cancer Res 60: 5334–5339.

Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. 2001. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. Oncogene 20:4188–4197.

Imbert V, Rupec RA, Livolsi A, Pahl HL, Traenckner BM, Mueller-Diechmann C, Farahifar D, Rossi B, Auberger P, Baeuerle PA, Peyron J. 1996. Tyrosine phosphorylation of IκB-α activates NF-κB without proteolytic degradation of IκB-α. Cell 86:787–798.

Karin M. 1999. The beginning of the end: IkappaB kinase (IKK) and NF-kappaB activation. J Biol Chem 274: 27339–27342.

Karin M, Ben-Neriah Y. 2000. Phosphorylation meets ubiquitination: The control of NF-κB activity. Annu Rev Immunol 18:621–663.

Karin M, Lin A. 2002. NF-kappaB at the crossroads of life and death. Nat Immunol 3:221–227.

Karin M, Cao Y, Greten FR, Li ZW. 2002. NF-kappaB in cancer: From innocent bystander to major culprit. Nat Rev Cancer 2:301–310.

Keller E, Chang C, Ershler W. 1996. Inhibition of NF-κB activity through maintenance of IκBα levels contributes to dihydrotestosterone-mediated repression of the interleukin-6 promoter. J Biol Chem 271:26267–26275.

Kikuchi E, Horiguchi Y, Nakashima J, Kuroda K, Oya M, Ohigashi T, Takahashi N, Shima Y, Umezawa K, Murai M. 2003. Suppression of hormone-refractory prostate cancer by a novel nuclear factor kappaB inhibitor in nude mice. Cancer Res 63:107–110.

Kimura K, Gelmann EP. 2002. Propapoptotic effects of NF-kappaB in LNCaP prostate cancer cells lead to serine protease activation. Cell Death Differ 9:972–980.

Krappmann D, Emmerich F, Kordes U, Scharschmidt E, Dorken B, Scheidereit C. 1999. Molecular mechanisms of constitutive NF-kappaB/Rel activation in Hodgkin/Reed–Sternberg cells. Oncogene 18:943–953.

Lee FS, Hagler J, Chen ZJ, Maniatis T. 1997. Activation of the IkappaB alpha kinase complex by MEKK1, a kinase of the JNK pathway. Cell 88:213–222.

Lessard L, Mes-Masson AM, Lamarre L, Wall L, Lattouf JB, Saad F. 2003. NF-kappa B nuclear localization and its prognostic significance in prostate cancer. BJU Int 91:417–420.

Li N, Karin M. 1998. Ionizing radiation and short wavelength UV activate NF-kappaB through two distinct mechanisms. Proc Natl Acad Sci USA 95:13012–13017.

Ludwig L, Kessler H, Wagner M, Hoang-Vu C, Dralle H, Adler G, Bohm BO, Schmid RM. 2001. Nuclear factor-kappaB is constitutively active in C-cell carcinoma and required for RET-induced transformation. Cancer Res 61:4526–4535.

Luo J, Duggan DJ, Chen Y, Sauvageot J, Ewing CM, Bittner ML, Trent JM, Isaacs WB. 2001. Human prostate cancer and benign prostatic hyperplasia: Molecular dissection by gene expression profiling. Cancer Res 61: 4683–4688.

Luo JH, Yu YP, Cieply K, Lin F, Deflavia P, Dhir R, Finkelstein S, Michalopoulos G, Becich M. 2002. Gene expression analysis of prostate cancers. Mol Carcinog 33:25–35.

Madrid LV, Mayo MW, Reuther JY, Baldwin AS, Jr. 2001. Akt stimulates the transactivation potential of the RelA/p65 subunit of NF-kappa B through utilization of the Ikappa B kinase and activation of the mitogen-activated protein kinase p38. J Biol Chem 276:18934–18940.

Magee JA, Araki T, Patil S, Ehrig T, True L, Humphrey PA, Catalona WJ, Watson MA, Milbrandt J. 2001. Expression profiling reveals hepsin overexpression in prostate cancer. Cancer Res 61:5692–5696.

May MJ, Ghosh S. 1998. Signal transduction through NF-kappa B. Immunol Today 19:80–88.

Mirchandani D, Zheng J, Miller GJ, Ghosh AK, Shibata DK, Cote RJ, Roy-Burman P. 1995. Heterogeneity in intratumor distribution of p53 mutations in human prostate cancer. Am J Pathol 147:92–101.

Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. 2001. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. Oncogene 20:7597–7609.

Nakatani K, Thompson D, Barthel A, Sakaue H, Liu W, Weigel RJ, Roth RA. 1999. Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines. J Biol Chem 274: 21528–21532.

Nakshatri H, P B-N, Martin DA, Goulet RJ, Sledge GW. 1997. Constitutive activation of NF-κB during progression of breast cancer to hormone-independent growth. Mol Cell Biol 17:3629–3639.

Neri A, Fracchiolla NS, Migliazza A, Trecca D, Lombardi L. 1996. The involvement of the candidate proto-oncogene NFKB2/lyt-10 in lymphoid malignancies. Leuk Lymphoma 23:43–48.

Nishimura K, Nonomura N, Satoh E, Harada Y, Nakayama M, Tokizane T, Fukui T, Ono Y, Inoue H, Shin M, Tsujimoto Y, Takayama H, Aozasa K, Okuyama A. 2001. Potential mechanism for the effects of dexamethasone on growth of androgen-independent prostate cancer. J Natl Cancer Inst 93:1739–1746.

Okamoto M, Oyasu R. 1997. Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells in vitro. Cancer Res 57:141–146.

Ondrey FG, Dong G, Sunwoo J, Chen Z, Wolf JS, Crowl-Bancroft CV, Mukaida N, Van Waes C. 1999. Constitutive activation of transcription factors NF-(kappa)B, AP-1, and NF-IL6 in human head and neck squamous cell carcinoma cell lines that express pro-inflammatory and pro-angiogenic cytokines. Mol Carcinog 26:119–129.

Pajonk F, Pajonk K, McBride WH. 1999. Inhibition of NF-κB, clonagenicity, and radiosensitivity of human cancer cells. J Natl Cancer Inst 91:1956–1960.

Palayoor ST, Youmell MY, Calderwood SK, Coleman CN, Price BD. 1999. Constitutive activation of IkappaB kinase alpha and NF-kappaB in prostate cancer cells in inhibited by ibuprofen. Oncogene 18:7389–7394.

Palvimo JJ, Reinikainen P, Ikonen T, Kallio PJ, Moilanen A, Janne OA. 1996. Mutual transcriptional interference between RelA and androgen receptor. J Biol Chem 271: 24151–24156.

Patel NM, Nozaki S, Shortle NH, Bhat-Nakshatri P, Newton TR, Rice S, Gelfanov V, Boswell SH, Goulet RJ, Jr., Sledge GW, Jr., Nakshatri H. 2000. Paclitaxel sensitivity of breast cancer cells with constitutively active NF-kappaB is enhanced by IkappaBalpha super-repressor and parthenolide. Oncogene 19:4159–4169.

Pianetti S, Arsura M, Romieu-Mourez R, Coffey RJ, Sonenshein GE. 2001. Her-2/neu overexpression induces NF-kappaB via a PI3-kinase/Akt pathway involving calpain-mediated degradation of IkappaB-alpha that can be inhibited by the tumor suppressor PTEN. Oncogene 20:1287–1299.

Rayet B, Gélinas C. 1999. Aberrant *rel/nfkb* genes and activity in human cancer. Oncogene 18:6938–6947.

Rhodes DR, Barrette TR, Rubin MA, Ghosh D, Chinnaiyan AM. 2002. Meta-analysis of microarrays: Interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer. Cancer Res 62:4427–4433.

Ripple MO, Henry WF, Schwarze SR, Wilding G, Weindruch R. 1999. Effect of antioxidants on androgen-induced AP-1 and NF-kappaB DNA-binding activity in prostate carcinoma cells. J Natl Cancer Inst 91:1227–1232.

Romieu-Mourez R, Landesman-Bollag E, Seldin DC, Traish AM, Mercurio F, Sonenshein GE. 2001. Roles of IKK kinases and protein kinase CK2 in activation of nuclear factor-kappaB in breast cancer. Cancer Res 61:3810–3818.

Rudolph D, Yeh WC, Wakeham A, Rudolph B, Nallainathan D, Potter J, Elia AJ, Mak TW. 2000. Severe liver degeneration and lack of NF-kappaB activation in NEMO/IKKgamma-deficient mice. Genes Dev 14:854–862.

Senftleben U, Cao Y, Xiao G, Greten FR, Krahn G, Bonizzi G, Chen Y, Hu Y, Fong A, Sun SC, Karin M. 2001. Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. Science 293:1495–1499.

Shattuck-Brandt RL, Richmond A. 1997. Enhanced degradation of I-kappaB alpha contributes to endogenous activation of NF-kappaB in Hs294T melanoma cells. Cancer Res 57:3032–3039.

Shimada K, Nakamura M, Ishida E, Kishi M, Yonehara S, Konishi N. 2002. Contributions of mitogen-activated protein kinase and nuclear factor kappa B to N-(4-hydroxyphenyl)retinamide-induced apoptosis in prostate cancer cells. Mol Carcinog 35:127–137.

Shimada K, Nakamura M, Ishida E, Kishi M, Konishi N. 2003. Roles of p38- and c-jun NH2-terminal kinase-mediated pathways in 2-methoxyestradiol-induced p53 induction and apoptosis. Carcinogenesis 24:1067–1075.

Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, Tamayo P, Renshaw AA, D'Amico AV, Richie JP, Lander ES, Loda M, Kantoff PW, Golub TR, Sellers WR. 2002. Gene expression correlates of clinical prostate cancer behavior. Cancer Cell 1:203–209.

Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, Sonenshein GE. 1997. Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. J Clin Invest 100:2952–2960.

Suh J, Payvandi F, Edelstein LC, Amenta PS, Zong WX, Gélinas C, Rabson AB. 2002. Mechanisms of constitutive NF-kappaB activation in human prostate cancer cells. Prostate 52:183–200.

Sumitomo M, Tachibana M, Nakashima H, Murai M, Miyajima A, Kimura F, Hayakawa M, Nakamura H. 1999. An essential role for nuclear factor kappa B in preventing TNF-alpha-induced cell death in prostate cancer cells. J Urol 161:674–679.

Suzuki H, Freije D, Nusskern DR, Okami K, Cairns P, Sidransky D, Isaacs WB, Bova GS. 1998. Interfocal heterogeneity of *PTEN/MMAC1* gene alterations in multiple metastatic prostate cancer tissues. Cancer Res 58:204–209.

Tai DI, Tsai SL, Chang YH, Huang SN, Chen TC, Chang KS, Liaw YF. 2000. Constitutive activation of nuclear factor kappaB in hepatocellular carcinoma. Cancer 89:2274–2281.

Takaesu G, Surabhi RM, Park KJ, Ninomiya-Tsuji J, Matsumoto K, Gaynor RB. 2003. TAK1 is critical for IkappaB kinase-mediated activation of the NF-kappaB pathway. J Mol Biol 326:105–115.

Thakur S, Lin HC, Tseng WT, Kumar S, Bravo R, Foss F, Gélinas C, Rabson AB. 1994. Rearrangement and altered expression of the *NFKB-2* gene in human cutaneous T-lymphoma cells. Oncogene 9:2335–2344.

Traencker EB, Pahl HL, Henkel T, Schmidt KN, Wilk S, Baeurle PA. 1995. Phosphorylation of human IκB-α on serines 32 and 36 controls IκB-α proteolysis and NF-κB activation in response to diverse stimuli. EMBO J 14:2876–2883.

Uzzo RG, Leavis P, Hatch W, Gabai VL, Dulin N, Zvartau N, Kolenko VM. 2002. Zinc inhibits nuclear factor-kappa B activation and sensitizes prostate cancer cells to cytotoxic agents. Clin Cancer Res 8:3579–3583.

Wang D, Baldwin AS, Jr. 1998. Activation of nuclear factor-kappaB-dependent transcription by tumor necrosis factor-alpha is mediated through phosphorylation of RelA/p65 on serine 529. J Biol Chem 273:29411–29416.

Wang CY, Mayo MW, Baldwin AS, Jr. 1996. TNF- and cancer therapy-induced apoptosis: Potentiation by inhibition of NF-kappaB. Science 274:784–787.

Wang SI, Parsons R, Ittmann M. 1998. Homozygous deletion of the PTEN tumor suppressor gene in a subset of prostate adenocarcinomas. Clin Cancer Res 4:811–815.

Wang W, Abbruzzese JL, Evans DB, Larry L, Cleary KR, Chiao PJ. 1999. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. Clin Cancer Res 5:119–127.

Wang D, Westerheide SD, Hanson JL, Baldwin AS, Jr. 2000. Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. J Biol Chem 275:32592–32597.

Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson HF, Jr., Hampton GM. 2001. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. Cancer Res 61:5974–5978.

Yang J, Richmond A. 2001. Constitutive IkappaB kinase activity correlates with nuclear factor-kappaB activation in human melanoma cells. Cancer Res 61:4901–4909.

Yang J, Lin Y, Guo Z, Cheng J, Huang J, Deng L, Liao W, Chen Z, Liu Z, Su B. 2001. The essential role of MEKK3 in TNF-induced NF-kappaB activation. Nat Immunol 2:620–624.

Yin L, Wu L, Wesche H, Arthur CD, White JM, Goeddel DV, Schreiber RD. 2001. Defective lymphotoxin-beta receptor-induced NF-kappaB transcriptional activity in NIK-deficient mice. Science 291:2162–2165.

Zandi E, Karin M. 1999. Bridging the gap: Composition, regulation, and physiological function of the IkappaB kinase complex. Mol Cell Biol 19:4547–4551.

Zerbini LF, Wang Y, Cho JY, Libermann TA. 2003. Constitutive activation of nuclear factor kappaB p50/p65 and Fra-1 and JunD is essential for deregulated interleukin 6 expression in prostate cancer. Cancer Res 63:2206–2215.

Zhao Q, Lee FS. 1999. Mitogen-activated protein kinase/ERK kinase kinases 2 and 3 activate nuclear factor-kappaB through IkappaB kinase-alpha and IkappaB kinase- beta. J Biol Chem 274:8355–8358.

Zhong H, SuYang H, Erdjument-Bromage H, Tempst P, Ghosh S. 1997. The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. Cell 89:413–424.

Zhong H, Voll RE, Ghosh S. 1998. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell 1:661–671.