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Beyond NF- κ B activation: nuclear functions of I κ B kinase α

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Abstract

I κ B kinase (IKK) complex, the master kinase for NF- κ B activation, contains two kinase subunits, IKK α and IKK β . In addition to mediating NF- κ B signaling by phosphorylating I κ B proteins during inflammatory and immune responses, the activation of the IKK complex also responds to various stimuli to regulate diverse functions independently of NF- κ B. Although these two kinases share structural and biochemical similarities, different sub-cellular localization and phosphorylation targets between IKK α and IKK β account for their distinct physiological and pathological roles. While IKK β is predominantly cytoplasmic, IKK α has been found to shuttle between the cytoplasm and the nucleus. The nuclear-specific roles of IKK α have brought increasing complexity to its biological function. This review highlights major advances in the studies of the nuclear functions of IKK α and the mechanisms of IKK α nuclear translocation. Understanding the nuclear activity is essential for targeting IKK α for therapeutics.

Keywords

Nuclear IKK α , NF- κ B, Gene transcription, Tumor progression

Introduction

I κ B kinase (IKK)/Nuclear factor kappa B (NF- κ B) family signaling mediates the expression of hundreds of genes involved in inflammation, immune response, cell survival, and cancer [1-4]. NF- κ B proteins are part of a molecular cascade that begins with signals outside the cell and culminates in the nucleus by binding to DNA and activating gene expression. The best-known form of NF- κ B consists of the DNA-binding subunit p50 and a transcription activator, p65 (also known as Rel A). In the absence of specific extracellular signals, NF- κ B inhibitors, such as I κ B, p105, and p100 proteins, tether to NF- κ B in the cytoplasm to prevent NF- κ B-mediated gene transcription [1]. When cells receive appropriate stimuli, such as tumor-necrosis factor- α (TNF- α), a ternary IKK complex consisting of IKK α , IKK β and NEMO (IKK γ) induces I κ B phosphorylation, leading to I κ B ubiquitination and proteasomal degradation that are required for liberation of NF- κ B in the nucleus where it binds to specific promoter elements to activate gene expression [1,5].

Although IKK α and IKK β share structural and biochemical similarities, different phenotypes between IKK α and IKK β knockout mice imply distinct physiological roles of the IKK isoforms [6]. IKK β deficiency results in embryonal death and shows the defective response to inflammatory cytokines and liver cell apoptosis [7]. IKK α knockout mice display the defective proliferation and differentiation of keratinocyte and the abnormalities of limb and skeleton, suggesting the requirement of the IKK α subunit in morphogenesis [8]. Importantly, these studies of gene knockout have shown that IKK α is dispensable for I κ B degradation although both IKK α and IKK β are critical for NF- κ B-mediated gene expressions. Instead of its role in phosphorylating I κ B α in classic NF- κ B activation, IKK α homodimer has been shown to mediate the processing of p100 precursor to p52 by the noncanonical NF- κ B pathway [5]. IKK α - and IKK β -deficient mouse embryo fibroblasts exhibit different patterns of β -catenin cellular localization in which IKK β decreases β -catenin-dependent transcriptional activation while IKK α increases β -catenin-dependent transcriptional activity [9]. Differential requirements for IKK α and IKK β were also found in primary human osteoarthritic chondrocyte differentiation [10].

In addition to phosphorylating distinct substrates in the cytoplasm [6,11], sub-cellular distribution of IKK α is also different from that of IKK β , further indicating that these two related signaling kinases are functionally different. Many studies have indicated that IKK α can be detected in both the cytoplasm and the nucleus whereas IKK β is detected predominantly in the cytoplasm [12-16]. The observation of nuclear/cytoplasm shuttling of IKK α led to the discovery of the first nuclear role of IKK α in phosphorylating histone H3, which results in NF- κ B-mediated gene expression [12,16]. These studies provided an explanation of why IKK α is dispensable for I κ B α degradation but remains essential for NF- κ B-dependent transcription. Aside from nuclear regulation of NF- κ B-dependent gene transcription through chromatin modification in response to pro-inflammatory stimuli, nuclear IKK α also functions in apoptosis, cell cycle, and tumor progression in colorectal [17,18], breast [19,20], pancreatic [21], gastric [22], osteosarcoma [23], and prostate [24] cancers. The current understandings of nuclear translocation and functions of IKK α are discussed below.

Nuclear IKK α regulates NF- κ B-dependent gene transcription and inflammation

Nuclear expression and functions of IKK α were first discovered based on the observation of different patterns of β -catenin activation in IKK α - and IKK β -deficient mouse embryonic fibroblast (MEF) cells by Lamberti *et al.* in 2001 [9]. Their study showed β -catenin-dependent transcription was decreased by IKK β but increased by IKK α that is likely due to the differential abilities of IKK α and IKK β to bind to and phosphorylate β -catenin. Unlike the predominantly cytoplasmic IKK β , IKK α has been detected in both the nucleus and the cytoplasm of MEF cells at resting state [9,25]. The constitutive shuttling of IKK α between cytoplasm and nucleus was further confirmed by accumulation of IKK α in the nucleus of HeLa cells in the presence of a nuclear export blocking agent, leptomycin B (LMB) [13]. Therefore, the protein binding and phosphorylation of specific pools of β -catenin by IKK α in the nucleus have been proposed to explain the contradictory effect of the IKK isoforms on β -catenin-dependent transcription [9]. However, the phosphorylation sites and detailed mechanisms that account for the differential regulation of β -catenin by IKK isoforms remain to be explored.

IKK complex is activated in response to various stimuli involving inflammation, apoptosis, immune response, and cancer. These physiological and pathological stimuli have been reported to enhance the nuclear levels of IKK α (Figure 1). TNF- α , a critical pro-inflammatory cytokine, was the first stimulant for IKK α nuclear translocation identified [12,16]. Other inducers of alternative NF- κ B pathway, including lymphotoxin β and CD40, also elicit nuclear IKK α signaling [15]. In addition to TNF- α , *Helicobacter pylori* (HP) can also trigger nuclear translocation of IKK α via its virulence factor CagA protein to induce cytokine production for appropriate inflammatory responses [22]. Similarly, overexpression of Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded viral FLICE inhibitory protein K13 [26] and hepatitis B virus-encoded X (HBx) protein [14] also induce IKK α nuclear translocation to regulate NF- κ B activity. The requirement of cytoplasmic/nuclear shuttling and chromatin association of IKK α for NF- κ B-dependent gene regulation in a TAK1-dependent manner in TLR4-activated antigen-presenting cells [27] and activated neutrophils [28,29] further support that nuclear IKK α functions as a common and important regulator for NF- κ B activity in response to various immune and inflammatory stimuli (Figure 1).

Figure 1 Nuclear IKK α -dependent molecular regulations of NF- κ B-mediated gene transcription. In response to a variety of stimuli, including proinflammatory cytokines, pathogens, and growth factors, IKK α translocates into the nucleus and bind to DNA in association with CBP to phosphorylate histone H3 at Ser10, CBP at Ser1382/1386, and p65 at S536. Nuclear IKK α also removes repressive HDAC3/SMRT complex from NF- κ B-dependent gene expression through phosphorylating SMRT at Ser2410. These events facilitate the formation of transcriptional enhanceosome to increase NF- κ B-dependent gene expression. On the other hand, nuclear IKK α also contributes to the termination of NF- κ B-mediated gene transcriptions by phosphorylating p65 at Ser536 and PIAS at Ser90 to facilitate the turnover of p65 in response to TNF- α or LPS stimulation.

As illustrated in Figure 1, TNF- α -induced nuclear IKK α mediates NF- κ B-dependent gene transcription, regardless of I κ B α degradation, by enhancing transactivation [25] and DNA binding [30] of p65 as well as chromatin regulation through its interaction CREB-binding protein (CBP), a histone acetyltransferase [15,16]. Phosphorylation of serine residues within the transactivation domains (TA1 and TA2) of p65 is responsible for transcriptional

activation of the NF- κ B target genes [31]. By employing the Gal4 DNA binding domain fused to the p65 transactivation portion in a heterologous luciferase reporter assay, nuclear IKK α was shown to transduce NF- κ B-inducing kinase (NIK)-dependent p65 TA1 transcriptional activity, suggesting that IKK α phosphorylates the TA1 domain of p65 in the nucleus [25]. In addition, using the IKK α mutant lacking an intact nuclear localization sequence (NLS), p65 chromatin immunoprecipitation assays further revealed that nuclear IKK α plays a role in binding activity of NF- κ B/p65 to some but not all NF- κ B-target promoters by removing histone deacetylase 3 (HDAC3), a negative regulator of NF- κ B-dependent transcription, from these promoters [30]. Even though several earlier studies have implicated the function of IKK α in chromatin, the role of nuclear IKK α as a chromatin modifier was not reported until later by Baldwin's and Gaynor's group [12,16]. They showed that IKK α functions as a chromatin kinase in the nucleus and targets histone H3 at Ser10 for activation of NF- κ B-directed gene expression (Figure 1). The nuclear import, chromatin association, histone phosphorylation of IKK α relies on its kinase activation by the upstream NIK kinase in response to both TNF- α and endotoxin lipopolysaccharide (LPS) [32]. However, the correlation between chromatin-bound IKK α and histone H3 phosphorylation on NF- κ B-target genes was not found in human prostate carcinoma DU145 cells [18], indicating that cell type- and target gene-specificity exists in IKK α -dependent histone phosphorylation.

Gaynor's group further showed that nuclear IKK α interacts with the transactivation domain of CBP. The IKK α /CBP complex in conjunction with p65 is recruited to the NF- κ B responsive promoters to mediate cytokine-induced phosphorylation and subsequent acetylation of specific residues in histone H3 [16]. These findings suggested that IKK α not only targets on NF- κ B but also functions as a key epigenetic regulator to initiate sequential chromatin modifications. Our studies also demonstrated that nuclear IKK α binds directly to CBP and phosphorylates its HAT domain at Ser1382 and Ser1386 to enhance the enzymatic activity of CBP on histone acetylation [15] (Figure 1). CBP and its homolog, p300, are transcriptional coactivators that function in the communication between transcription factors and the transcriptional machinery [33]. Since the availability of these coactivators is limited due to their essentiality for large number of transcription factors, competition between different transcription factors for CBP or p300 has been proposed to play a role in the coordination of gene expression and the appropriate execution of many biological processes [34]. Our study further demonstrated that the IKK α -dependent CBP phosphorylation enhances NF- κ B-mediated gene expression and suppresses p53-mediated gene expression by switching the binding preference of CBP from p53 to NF- κ B, thereby promoting cell growth [15]. Similar to IKK α , IKK γ /NEMO has also been shown to shuttle between the cytoplasm and the nucleus and to compete with p65 and IKK α for binding to the N-terminus of CBP. Even though IKK γ /NEMO is essential for the kinase activity of IKK complex in the cytoplasm, it seems to act as a negative regulator of nuclear IKK α and inhibit CBP-dependent transcriptional activation in the nucleus [35].

CBP and p300 mediate acetylation of histones as well as many transcription factors, including p65, for their transcriptional potential [33]. Acetylation of p65 is critical for its DNA binding and transactivation activity [36,37]. In contrast, deacetylation of p65 by HDACs, including HDAC1, HDAC2, HDAC3, and SIRT1, has been reported to repress its transcriptional activity [38,39]. The enzymatic activities of HDACs are regulated by their ability to associate with co-repressor proteins, such as SMRT and NCoR [40]. Chromatin-associated HDAC3/SMRT complex tethered by p50 homodimers on NF- κ B-target *cIAP* and *IL-8* promoters of unstimulated cells is responsible for the basal suppression of NF- κ B-regulated gene transcriptions [18]. In addition to enhancing histone acetylation by targeting

CBP, IKK α was also found to relieve the suppression of NF- κ B-mediated transcription by removing the HDAC3/SMRT repressor complex from target promoters [18,30,41]. Chromatin immunoprecipitation analysis demonstrated that upon attachment to laminin, the induction of chromatin-associated IKK α protein and acetylated histone is accompanied by a decrease in chromatin-bound HDAC3/SMRT complex. Direct phosphorylation of SMRT at Ser2410 by IKK α on chromatin also stimulates nuclear export and proteasomal degradation of the HDAC3/SMRT complex by recruiting TBL1/TBLR1, Ubc5, and 14-13-3 ϵ proteins [18]. The removal of HDAC3/SMRT by IKK α allows the active p50-RelA/p65 heterodimer of NF- κ B to bind and potentiate transcription (Figure 1). Although the SMRT corepressor returns to the chromatin-bound NF- κ B complex almost immediately after the active p50-RelA/p65 heterodimer binds to the promoter, IKK α remains associated with the chromatin and phosphorylates both p65 at Ser536 and SMRT at Ser2410 to prevent the recruitment and chromatin association of HDAC3 at the NF- κ B-regulated promoter. Displacement of HDAC3 from active NF- κ B allows p300 to load and subsequently acetylate p65 at Lys310, which is required for full NF- κ B transcription [41]. Thus, IKK α -mediated derepression of SMRT is an initial step critical for NF- κ B transcription and survival in response to laminin attachment. Interestingly, cigarette smoke extract (CSE) was recently reported to induce the translocation of IKK α from cytoplasm to nucleus in mouse lung tissue in a dose-dependent manner. CSE-activated nuclear IKK α mediates the pro-inflammatory gene transcription through phospho-acetylation of RelA/p65 and histone H3 [42], suggesting nuclear IKK α -targeted histone H3, SMRT, and CBP/p300 play a role in CSE-induced NF- κ B activation.

In contrast to activating NF- κ B in response to proinflammatory stimuli, IKK α kinase activity has been reported to be required for the termination of NF- κ B activation [43]. In response to the systemic challenge with the Gram-positive human pathogen group B *Streptococcus* (GBS), transgenic mice expressing the inactivatable variant of IKK α (AA) showed higher bacterial clearance but accelerated mortality compared with the wild-type mice. The exacerbated inflammatory phenotype was believed to be associated with this paradoxical result. Indeed, after administration of the *Escherichia coli*-derived LPS, transcripts of several pro-inflammatory and antiapoptotic NF- κ B-target genes were higher in IKK $\alpha^{AA/AA}$ mice than in the littermate controls, indicating a role of IKK α in terminating the activation of classical NF- κ B pathway in response to LPS-induced Toll-like receptor (TLR) signaling. It was then demonstrated that IKK α activity is required to accelerate the removal of RelA/p65 and c-Rel from pro-inflammatory gene promoters and the turnover of these NF- κ B subunits by specifically phosphorylating p65 within its transactivation domain at Ser536. These events terminate LPS-induced NF- κ B activation, leading to the negative regulation of macrophage activation and inflammation [43] (Figure 1). While much attention has focused on pro-inflammatory signaling, this study explored an opposing but complimentary role of IKK α in resolving inflammation. Since IKK α also showed nuclear function in histone phosphorylation in LPS-treated macrophage [32], this raises the possibility that LPS-activated IKK α also phosphorylates p65 at Ser536 in the nucleus to terminate the transcriptional activity of NF- κ B. However, this hypothesis seems contradictory to the previous finding that TNF- α -induced NIK/IKK α complex phosphorylates p65 at Ser536 in the nucleus to enhance NF- κ B activity [25]. These findings suggest that the transcriptional activity of NF- κ B is not determined merely by p65 Ser536 phosphorylation and other regulatory factors are required. The work by Liu *et al.* further demonstrated that protein inhibitor of activated STAT1 (PIAS1), a gene-specific transcriptional repressor with SUMO E3 ligase activity, is involved in the IKK α -mediated negative regulation of NF- κ B and inflammation restriction [44]. In response to various inflammatory stimuli, including TNF- α and LPS, PIAS is rapidly phosphorylated at Ser90, and this phosphorylation is mediated by IKK α and required for its

association with chromatin and enzymatic activity to repress promoter-binding and transcriptional activities of NF- κ B [44]. It would be interesting to further address the relationship between p65 Ser536 phosphorylation and PIAS Ser90 phosphorylation in the IKK α -mediated negative regulation of NF- κ B activity (Figure 1).

Nuclear functions of IKK α in NF- κ B-independent gene transcription regulation

Nuclear IKK α -mediated histone H3 phosphorylation is involved in c-fos upregulation in a NF- κ B-independent manner in response to EGF [45] and UV [46] stimulation (Figure 2). Dong *et al.* demonstrated that Ser32 phosphorylation, which is required for c-Fos protein stability and promoter recruitment of c-Fos, requires the kinase activity of nuclear IKK α [46], indicating that the chromatin regulation by nuclear IKK α is not limited to NF- κ B-targeted genes but also affects gene transcriptions regulated through targeting various transcription factors or cofactors (Figure 2). For example, IKK α -mediated suppression of SMRT is required not only for NF- κ B activation [18] but also Notch-dependent transcription [17], implying that nuclear IKK α may function as a common epigenetic regulator for gene transcription. Our recent study further indicated that nuclear IKK α may also derepress Notch-dependent transcription by diminishing the gene expression of NUMB [47], which targets Notch1 for lysosomal degradation through protein-protein interaction [48]. We identified forkhead box protein A2 (FOXA2) as the transcription factor for NUMB gene transcription and demonstrated that IKK α reduces the transcriptional activity of FOXA2 via binding to and phosphorylating it at Ser107 and Ser111 [47]. Therefore, nuclear IKK α may also enhance Notch-dependent gene transcription by suppressing FOXA2/NUMB signaling pathway (Figure 2). In addition to Notch, IKK α but not IKK β was found to be involved in estrogen receptor (ER)-mediated gene transcription by binding to the estrogen-responsive elements (EREs)-containing promoters and phosphorylating histone H3, ER α , or coactivators such as AIB1/SRC-3. Coordinated promoter recruitment of ERs and specific coactivators, such as SRC-1, AIB1/SRC-3, GRIP1, CBP/p300, PCAF, CARM1, and PRMT1, is required for estrogen-regulated transcriptional activation. By forming a transcriptional complex with ER α and AIB/SRC3, IKK α mediates histone H3 phosphorylation on the promoters of several estrogen-responsive genes. In addition, nuclear IKK α also enhances the activities of ER α and AIB1/SRC-3 through phosphorylation of their Ser118 and Ser857 respectively (Figure 2), leading to the increase in cyclin D1 and Myc expression and estrogen-mediated breast cancer cell growth [19]. Since IKK α -activated SRC3 is also required for transcriptional activity of NF- κ B [49], SRC-3 coactivator, in addition to histone H3 and SMRT, may also be another target of nuclear IKK α to increase general gene transcriptions.

Figure 2 The roles of nuclear IKK α in the regulation of NF- κ B-independent gene transcription. Nuclear IKK α enhances Notch-dependent gene transcriptional by phosphorylating and removing co-repressor SMRT from target gene promoters. IKK α also contributes to Notch transcriptional activity through phosphorylating and inactivating FOXA2, which subsequently leads to NUMB suppression. By direct target on transcription factors, nuclear IKK α also increases AP-1, ER α , and E2F-mediated gene transcription. Phosphorylation of SRC3 at Ser857 by nuclear IKK α also contributes to ER α transcriptional activity.

In response to estrogen stimulation, IKK α also regulates cell cycle progression through modulating E2F1-dependent transcription [20]. The Rb/E2F pathway controls G1/S phase transition by activating expression of genes required for DNA replication. Silencing of IKK α

but not IKK β significantly reduced estrogen-induced cell cycle progression and transcription of the E2F1 gene as well as other E2F1-responsive genes, including thymidine kinase 1, proliferating cell nuclear antigen, cyclin E, and cdc25A, indicating that IKK α plays a critical role in regulating E2F-dependent gene transcription. Through association with E2F1, IKK α is recruited to E2F-1 responsive promoters and potentiates the ability of p300/CBP-associated factor to acetylate E2F1 for transcriptional activation in response to estrogen treatment (Figure 2). These findings suggest that nuclear IKK α influences estrogen-mediated cell cycle progression by modulating E2F1 at both the transcriptional and posttranscriptional levels [20]. Taken together, IKK α appears to modulate various epigenetic signaling pathways to regulate specific sets of genes.

Regulation of apoptosis by nuclear IKK α

NF- κ B is activated to control apoptosis upon exposure to various cytotoxic stimuli, including reactive oxygen species (ROS). Recent evidence suggests a negative regulatory role of activated NF- κ B in ROS-elicited JNK signaling to antagonize apoptosis [50]. Interestingly, the Src-dependent Tyr42 phosphorylation but not IKK-mediated Ser32/36 phosphorylation of I κ B α contributes to ROS-induced NF- κ B activation in a proteolysis-independent mechanism [51,52]. Although hydrogen peroxide stimulation has been shown to induce IKK α activity and thus its nuclear translocation, it had no effect on NF- κ B activation [53], suggesting that IKK α is not involved in the ROS-induced NF- κ B activation. In contrast to the pro-survival role of NF- κ B, nuclear IKK α plays an opposite function in ROS-mediated apoptosis through modulating p53 transcriptional activity (Figure 3). In the nucleus, IKK α enhances p53-mediated GADD45 and BAD gene expressions by phosphorylating p53 at Ser20 [53] and stabilizing p53 protein levels [54], leading to the induction of apoptosis in response to ROS exposure. In response to DNA damage, both p53 and its homolog p73 function against NF- κ B in deciding cell fate. Treatment with cisplatin induces IKK α nuclear translocation in human osteosarcoma-derived U2OS cells and hepatocellular carcinoma HepG2 cells in an ATM-dependent manner [23,55]. As shown in Figure 3, nuclear IKK α also stabilizes p73 protein through physical interaction in response to cisplatin [23]. Although the exact residue of p73 phosphorylated by IKK α has not yet been identified, a study showed that nuclear IKK α phosphorylates p73 within its N-terminal region, which may protect p73 from ubiquitination and proteasomal degradation [23]. These findings suggest an indispensable role of IKK α in cisplatin sensitivity. Different from ROS-induced nuclear IKK α on p53 stability in ROS-treated human MOLT-4 and HL-60 leukemia cells, cisplatin-activated IKK α nuclear translocation did not lead to p53 stabilization in HepG2 cells [53,54], suggesting that IKK α -dependent protein stabilization is cell type- and stimulus-specific.

Figure 3 Nuclear IKK α targets p53 and p73 to mediate apoptosis. In response to DNA damage induced by ROS and cisplatin, nuclear IKK α stabilizes p53 and p73 protein level respectively to promote apoptosis.

Nuclear IKK α is essential for cell cycle arrest and differentiation of keratinocyte in the epidermis and the morphogenesis of skeletal and craniofacial morphogenesis

Unlike its well-established role in anti-apoptosis, the involvement of NF- κ B in regulating cell cycle progression remains unclear. NF- κ B activation is required for cell cycling in fibroblast [56], regenerating liver cells [57], breast cancer cells [58], and HeLa cells whereas NF- κ B

inhibition impairs cell cycle progression and retardation of G1/S transition [59]. During cell cycle, D-type cyclins (cyclin D1, D2, and D3) are critical for G1 to S phase progression. By phosphorylating the retinoblastoma tumor suppressor protein (pRb), cyclin D with its partner cyclin-dependent kinases (CDKs), releases the E2F family of transcription factors to activate the expression of cyclin E and several other genes required for the cell cycle progression [60,61]. The direct binding of NF- κ B on the promoter region of cyclin D1 gene and the pronounced reduction of cyclin D1 expression by NF- κ B inhibition provide additional evidence for the involvement of cyclin D1 transcription in NF- κ B-mediated cell cycle progression [62-64]. However, several studies showed contradictory results in which NF- κ B activation by overexpression of p65 or c-Rel causes cell-cycle arrest and induces cells to commit to terminal differentiation [65,66]. For instance, the G1-arrest by p65 occurs in pro-B but not in mature B cells [66], suggesting that this event depends on cell developmental stage. Interestingly, mouse with IKK α gene inactivation also had an unexpected excessive proliferation of the skin basal layer due to the absence of epidermal differentiation [67,68]. The specific roles of NF- κ B in IKK α -mediated cell cycle arrest and subsequent differentiation in keratinocyte was initially proposed based on the observation that NF- κ B activation is not detectable in keratinocytes from IKK α -null mouse skin [69]. However, a subsequent study pointed out that IKK α controls epidermal keratinocyte differentiation independently of NF- κ B activation but regulates cyclin D1 protein stability in which cyclin D1 is overexpressed and predominantly localized in the nucleus of IKK α ^{-/-} MEF cells compared with parental MEF cells [70]. *In vitro* binding and kinase assays showed that IKK α directly binds cyclin D1 and phosphorylates it at Thr286. The cytoplasmic expression and increased degradation of cyclin D1 by reconstitution of IKK α in knockout cells further suggest that this phosphorylation by IKK α is required for nuclear export and turnover of cyclin D1 [71]. The predominantly nuclear localized cyclin D1 implies that IKK α may phosphorylate cyclin D1 in the nucleus and regulates its nuclear export. The potential nuclear function of IKK α in facilitating cyclin D1 protein degradation but not in NF- κ B activation may be attributed to IKK α -mediated cell cycle arrest and differentiation of keratinocytes (Figure 4). Indeed, keratinocyte differentiation is associated with increased nuclear distribution of IKK α . Inactivation of the NLS by site-directed mutagenesis prevents IKK α from entering the nucleus without affecting its kinase activity and blocks the IKK α -induced differentiation of primary cultured IKK α ^{-/-} keratinocyte, supporting an essential role of nuclear IKK α in the keratinocyte differentiation [8]. Likewise, Marinari *et al.* also found that nuclear IKK α can act as a tumor suppressor in stratified epithelia [72]. After stimulation with TGF β , IKK α accumulates in the nucleus of keratinocytes and occupies the promoter of genes responsive to TGF β -SMAD signaling to mediate TGF β -induced *Ovo11* and *Mad1* upregulation and *Myc* downregulation (Figure 4). Such activity of nuclear IKK α is important for the anti-proliferative TGF β pathway. In contrast, the expression and nuclear localization of IKK α are gradually reduced during malignant progression of squamous cell carcinoma (SCC) and acquisition of an invasive phenotype [72], which supports the tumor suppressive role of nuclear IKK α . However, the function of TGF β -induced nuclear IKK α seems to counter its metastatic role in breast cancer cells [73] (please see below), suggesting a keratinocyte-specific role of nuclear IKK α in suppressing cell proliferation.

Figure 4 Regulations of cell cycle progression by nuclear IKK α . In the nucleus, IKK α is involved in cell cycle arrest at G1/S transition by increasing Smad transcriptional activity, facilitating cyclin D1 proteasomal degradation, and FGF gene expression. Nuclear IKK α also promotes G2/M phase progression by increasing kinase activity of Aurora A and by de-repressing 14-3-3 σ gene expression through preventing DNA and histone methylation on the promoter.

Besides the failure of epidermal differentiation, IKK α -deficient mice also exhibit abnormalities in skeletal and craniofacial morphogenesis [70,74], which is not observed in mice with systemic inhibition of NF- κ B [75]. The results from these studies further support the dispensable role of NF- κ B in nuclear IKK α -mediated keratinocyte differentiation. By introducing an epidermal-specific IKK α transgene into IKK α -deficient mice, most of these morphological abnormalities were completely rescued, suggesting that nuclear IKK α -dependent epidermal differentiation control skeletal and craniofacial morphogenesis [8]. In addition to targeting cyclin D1 protein degradation, another potential mechanism by which nuclear IKK α affects keratinocyte differentiation and craniofacial and skeletal morphogenesis is through repression of the fibroblast growth factor (FGF) family members [8], which bind to FGF receptor (FGFR) to antagonize bone morphogenic protein (BMP) signaling [76]. Since reintroduction of a catalytically inactive form of IKK α in IKK α -/- mice is still able to rescue epidermal differentiation and skeletal morphogenesis, the developmental functions of IKK α have been proposed to be independent of its protein kinase activity [76]. Therefore, nuclear IKK α may also contribute to the suppression of FGF transcription through a kinase-independent manner, hence excluding its involvement in phosphorylating histone H3 (Figure 4). Exploration of the kinase-independent roles of nuclear IKK α awaits further studies.

In addition to G1/S transition, IKK α also has a role in regulating the M phase of cell cycle as shown in Figure 4. Progression through the M phase of cell cycle is dependent on several mitotic kinases, including those of the Aurora families [77]. Aurora A localizes to the centrosome and functions in centrosome maturation and separation [77], and knock down of Aurora kinase A by siRNA increased the percentage of mitotic cells with high levels of Plk1 and cyclin B1 [78]. In a similar pattern to Aurora A siRNA knockdown, Prajapati *et al.* showed that silencing of IKK α but not IKK β by siRNA also increased the number of HeLa cells at the G2/M phase and the levels of Plk1 and cyclin B1 [79]. These results further revealed that IKK α is associated with Aurora A in the centrosome and directly phosphorylates Aurora A at Thr288 [79], suggesting a nuclear function of IKK α in regulating the M phase of the cell cycle through Aurora A phosphorylation. In addition to targeting Aurora A, chromatin-bound IKK α also maintains the progression of G2/M phase during the cell cycle by preventing the silencing of 14-3-3 σ , a check point protein for G2/M phase transition [80]. In IKK α -deficient keratinocytes that showed cell cycle arrest at the G2/M phase, the SUV39h1 histone trimethyltransferase and the Dnmt3a DNA methyltransferase were found to associate and methylate histone H3 lysine-9 (K9) and 14-3-3 σ locus DNA, respectively, which then silenced 14-3-3 σ expression. Reintroduction of wild-type (WT) IKK α , but not its chromatin-unbound mutants bearing defects within the leucine zipper domain and helix-loop-helix motif, restored the 14-3-3 σ expression by preventing the association of SUV39h1 and Dnmt3a with the 14-3-3 σ locus, indicating that chromatin-associated IKK α prevents 14-3-3 σ from hypermethylation (Figure 4). Interestingly, the kinase activity of IKK α is dispensable for blocking 14-3-3 σ hypermethylation [80], suggesting that nuclear IKK α may protect 14-3-3 σ from hypermethylation through an unexplored kinase-independent mechanism.

Nuclear function of IKK α in tumorigenesis and metastasis

Constitutive activation of NF- κ B has been found in many types of tumor cells. Most of these studies report an increased IKK activity that results in phosphorylation of I κ B α ; however, some have found little or no changes in the subcellular localization of p65 in some of the tumor cells. For instance, Fernández-Majada *et al.* reported that the increased IKK activity in colorectal cancer (CRC) cell lines and primary CRC is concomitant with undetectable levels

of nuclear p65 and p52, which is consistent with the absence of p65 and p52 on different promoters of NF- κ B-target genes detected by ChIP analysis. These results indicate that NF- κ B activation may not be the main consequence of IKK activity in colorectal tumors, reflecting the substrate specificity of different IKK complexes. By immunohistochemistry staining and subcellular fractionation followed by Western blot analysis, they also showed that IKK α is present in the nucleus of most primary colorectal tumor tissues and CRC cell lines but not in HS27 or HEK-293 control cells. The increase in nuclear IKK activity in colorectal tumors is significantly correlated with SMRT phosphorylation at Ser2410 and its cytoplasmic translocation (Figure 5). At the chromatin level, the association of IKK α to specific Notch target promoters results in the release of chromatin-bound SMRT and thus activating *hes1*, *hes5*, or *herp2/hrt1* transcription, which promotes cell proliferation by repressing transcription of the cyclin-dependent kinase inhibitor p27^{Kip1} [17]. In addition, we reported that enhancement of Notch transactivation by IKK α through inhibition of FOXA2/NUMB signaling is also likely to contribute to inflammation-mediated liver cancer progression [47]. Our other study also indicated that constitutively activated IKK α , found in certain human cancers, including lung, liver, pancreatic, and ovarian cancers, can phosphorylate and direct CBP to bind preferentially to NF- κ B but not p53, thereby favoring proliferation and survival over p53-dependent apoptosis [15].

Figure 5 Nuclear IKK α and tumor progression. Nuclear IKK α promotes tumor growth by enhancing NF- κ B- and Notch-dependent gene transcriptions and suppressing FOXA2-mediated gene expression. By promoting Smad and STAT3 transcriptional activity and suppressing maspin gene expression, nuclear IKK α contributes to cancer metastasis.

The epithelial to mesenchymal transition (EMT) is a crucial step in tumor progression in many tumor types. Independently of NF- κ B activation, nuclear IKK α has been implicated in EMT by enhancing gene expression of SNAIL and SLUG transcription factors to downregulate expression of the adherens junction protein E-cadherin. As shown in Figure 5, IKK α enters the nucleus and regulates gene expression of SNAIL and SLUG by interacting with SMAD3 and controlling SMAD complex formation on the promoters of these two transcription factors in response to TGF β activation, leading to metastasis of breast cancer cells [73]. Tumor-infiltrating immune cells expressing lymphotoxin- β [81] and RANKL [24] have also been found to induce activation and nuclear localization of IKK α in prostatic epithelial tumor cells (Figure 5). After castration, activated STAT3 has been reported to promote the transcriptional activity of unliganded androgen receptor in prostate cancer cells [82]. Lymphotoxin β -induced nuclear IKK α , in conjunction with STAT3, contributes to the emergence of castration resistance and enhances hormone-free survival and metastasis of prostate cancer by an NF- κ B-independent, cell autonomous mechanism [81]. By targeting histone H3 Ser10 on the promoter of maspin, nuclear IKK α was also proposed to mediate the repression of maspin, a critical suppressor of metastasis, through an unidentified mechanism, which then commits malignant prostatic epithelial cells to a metastatic fate [24]. Similarly, we found that overexpression of HBx reduced maspin expression in Hep3B cells, and expression of wild-type IKK α but not its NLS mutant suppressed maspin expression in Hep3B cells, indicating that nuclear IKK α likely plays a role in HBx-mediated cell migration and invasion via suppressing maspin expression [14]. Although nuclear IKK α has been proposed to suppress maspin expression via histone H3 Ser10 phosphorylation [24] (Figure 5), it is still unclear how this histone phosphorylation reduces the promoter activity of maspin. Taken together, these findings indicate that a specific set of genes regulated by nuclear IKK α plays a critical role in tumorigenesis and metastasis. The detailed molecular mechanisms await further investigations.

Potential mechanisms of IKK α nuclear translocation

Exploration of various nuclear IKK α functions raised a fundamental question of how IKK α travels from the cytoplasm to the nucleus. It is believed that, for a majority of proteins, NLS-bearing molecules are transported into the nucleus by forming a complex with importin α/β [83] or importin β alone [84]. A lysine-rich motif, Lys235, Lys236, and Lys237 within the kinase domain of IKK α has been shown to contain the NLS. Mutation of these residues attenuated the spontaneous nuclear import of IKK α but did not interfere with its kinase activity or binding to IKK γ [8]. We also characterized the signaling peptide for IKK α nucleo-cytoplasmic shuttling in response to HBx overexpression and found that in addition to these three lysines, two additional lysines (233 and 240) are also required for the nuclear translocation of IKK α [14]. The energy for the importin-based nuclear transport is provided by the small Ras family GTPase, Ran [85]. A dominant negative mutant of Ran has been reported to inhibit IKK α nuclear translocation [25], suggesting that the nuclear import of IKK α requires importins. However, the specific molecules that are involved in NLS-mediated IKK α nuclear translocation remain to be investigated.

Likewise, nuclear export signals (NES), which are recognized by a soluble export receptor (also known as Exportin 1 or CRM1), mediate nuclear export [86]. A study in 2002 by Birbach *et al.* showed that presence and incubation of LMB, an inhibitor of CRM1, enhanced the levels of IKK α in the nucleus [13]. This raises the possibility that IKK α can shuttle out of the nucleus through the CRM1 pathway and contains an NES to allow for the recognition and binding of CRM1 receptor. Based on the consensus NES sequence, which typically has a leucine-rich consensus sequence in the form of LX₁₋₃LX₂₋₃LXL (L=leucine and X=any amino acid) [86], there are two putative NESs (Leu601 ~ Leu612 and Leu714~Leu724) located at the C-terminus of IKK α . Leucine or isoleucine substitution within the motif containing residues 714–724 enhanced nuclear accumulation of IKK α , thereby supporting the presence of an NES for IKK α nuclear export [14].

Since IKK α can enter the nucleus in response to diverse stimuli, including TNF- α [12,16], *Helicobacter pylori* [13], estrogen [20], EGF [45], and cisplatin [55], it is likely that signaling pathways, in addition to NLS and NES, are also critical for regulating IKK α nucleo-cytoplasmic shuttling (Figure 6). A kinase-dead mutant of IKK α (IKK α -K44M) has been shown to have lower nuclear accumulation than the wild type form, indicating that kinase activation is required for IKK α to translocate into the nucleus [13]. Activation of IKK complex usually involves trans-autophosphorylation by the catalytic domains of IKK α and IKK β . However, knockdown of IKK β by siRNA had no effect on *H. pylori*-induced IKK α nuclear translocation [22]. This suggests that IKK α can bypass the classic IKK complex activation pathway to enter the nucleus. As IKK β does not exist in the nucleus, the distinct mechanisms by which the kinases are regulated may have a role in controlling the nuclear translocation of IKK α . Indeed, Akt, a mitogen-activated survival factor, has been shown to increase the activity of IKK α but not IKK β by phosphorylating it at Thr23 in response to TNF- α [87]. Intriguingly, the signals that stimulate IKK α nuclear import, including HBx, EGF, HER2, and TNF- α , also commonly induce Akt activation. Akt-enhanced nuclear expression of IKK α is further augmented by overexpression of ubiquitin, suggesting that ubiquitination plays a role in Akt-regulated IKK α nuclear transportation [14]. Further investigations are necessary to identify the E3 ligase and the ubiquitination sites of IKK α .

Figure 6 Molecular mechanisms of IKK α nuclear transportation. Ran GTPase activity is required for the nuclear transport of IKK α through interacting with importin- α . In response to HBx overexpression and cisplatin treatment, phosphorylations of IKK α at Thr23 and Ser473 by Akt and ATM respectively promote its nuclear translocation. The ubiquitination of IKK α is essential for the Akt-regulated IKK α nuclear import. Under exposure to ROS, activated PKC δ also enhances the nuclear accumulation of IKK α .

In response to cisplatin-induced DNA damage, ATM has been shown to activate and phosphorylate IKK α at Ser473 in an *in vitro* kinase assay. Treatment with ATM inhibitors blocked the nuclear IKK α accumulation by cisplatin, suggesting that ATM plays a role in the nuclear translocation of IKK α . In addition, the active form of ATM was shown to colocalize with IKK α in the nucleus to mediate cisplatin-induced p73 protein stabilization and apoptosis [55]. These findings suggest that Ser473 phosphorylation by ATM may be a critical posttranslational modification for IKK α nuclear import and functions in response to cisplatin treatment. Similarly, in response to ROS exposure, PKC δ has also been demonstrated to increase the kinase activity and nuclear translocation of IKK α through protein-protein interaction (Figure 6). PKC-activated nuclear IKK α promotes the stability of p53 protein and mediates ROS-induced apoptosis [53]. However, the phosphorylation site mediated by ROS-activated PKC remains unclear. It would be of interest to further address whether phosphorylation of IKK α by Akt, ATM, or PKC δ at different residues affects its substrate preference in the nucleus.

Conclusion

Since the first observation of nuclear localization of IKK α more than a decade ago, the field has gained tremendous insight into the distinct regulation and functions of nuclear IKK α . Other than I κ B protein in the cytoplasm, these studies added histone and transcriptional co-factors as nuclear targets of IKK α for activation of NF- κ B-dependent transcription. By targeting a growing list of substrates in the nucleus, IKK α has also been implicated in a variety of biological functions, including apoptosis, tumor suppression, immune functions, cell proliferation, and chromatin remodeling in an NF- κ B-independent manner. Dysregulation of nuclear IKK α has been further linked to diabetes [88]. Contextual conditioned fear memory may also transduce IKK α to the nucleus of hippocampus for transcriptional regulation after memory recall [89]. These findings uncovered functional diversity of nuclear IKK α and other probable roles worthy of further exploration. For example, the involvement of nuclear IKK α in the termination of NF- κ B signaling is an attractive yet under-developed area. The exploration of the nuclear role of IKK α in terminating NF- κ B activity could lead us to understand how inflammation is resolved. The kinase-independent function of nuclear IKK α , which has been shown to control FGF suppression during epidermal differentiation and skeletal morphogenesis, is also another interesting area requiring further investigations. Addressing the kinase-independent functions of IKK α will likely provide more comprehensive explanation for the distinct roles between IKK α and its homologue, IKK β .

In the past decade, there has been increased interest in the therapeutic disruption of the IKK/NF- κ B pathway by using various approaches ranging from genetic manipulation to the development of pharmacologic inhibitors of IKK for inflammatory and autoimmune diseases [90,91]. Preclinical studies have also suggested IKK α/β as a therapeutic target for inhibition of NF- κ B activity in various types of cancer[91], but translation of this mechanistic

knowledge to clinically relevant therapeutic is much more difficult than researchers' expectation. Instead, much of the effort toward the development of IKK β and other NF- κ B inhibitors has come from the pharmaceutical industry [90]. The limitation of clinical development of IKK β inhibitors is probably due to the detrimental effects of excessive and prolonged NF- κ B inhibition by IKK β inhibition on innate immunity [92]. Although potent IKK α -specific inhibitors have not yet been described, the dispensability of IKK α in classic NF- κ B activation and the unique roles of nuclear IKK α in modulating NF- κ B-independent pathological activity, which are important for tumor progression, indicate that the chromatin-associated IKK α might be a promising target for therapeutic intervention in cancer. An ideal inhibitor designed to abrogate nuclear IKK α functions involved in a particular disease is therefore anticipated to minimize systemic toxicity and avoid general suppression of innate immunity, and may provide a more specific and safer therapeutic efficacy for cancer therapy.

Abbreviations

BMP, Bone morphogenic protein; CBP, CREB-binding protein; CDK, Cyclin-dependent kinase; CRC, Colorectal cancer; CSC, Squamous cell carcinoma; CSE, Cigarette smoke extract; EMT, Epithelial to mesenchymal transition; ER, Estrogen receptor; ERE, Estrogen-responsive element; FGF, Fibroblast growth factor; GBS, Gram-positive human pathogen group B Streptococcus; HBx, Hepatitis B virus-encoded X protein; HDAC, Histone deacetylase; IKK, I κ B kinase; KSHV, Kaposi's sarcoma-associated herpesvirus; LMB, Leptomycin B; LPS, Lipopolysaccharide; MEF, Mouse embryonic fibroblast; NES, Nuclear export signal; NF- κ B, Nuclear factor kappa B; NIK, NF- κ B-inducing kinase; NLS, Nuclear localization sequence; PIAS1, Protein inhibitor of activated STAT1; pRB, Retinoblastoma tumor suppressor protein; ROS, Reactive oxygen species; TLR, Toll-like receptor; TNF- α , Tumor-necrosis factor alpha

Competing interests

These authors declare no competing conflict.

Authors' contributions

WCH designed the concept, collected information, and prepared the manuscript and figures. WCH and MCH wrote the manuscript. All authors read and approved the final manuscript.

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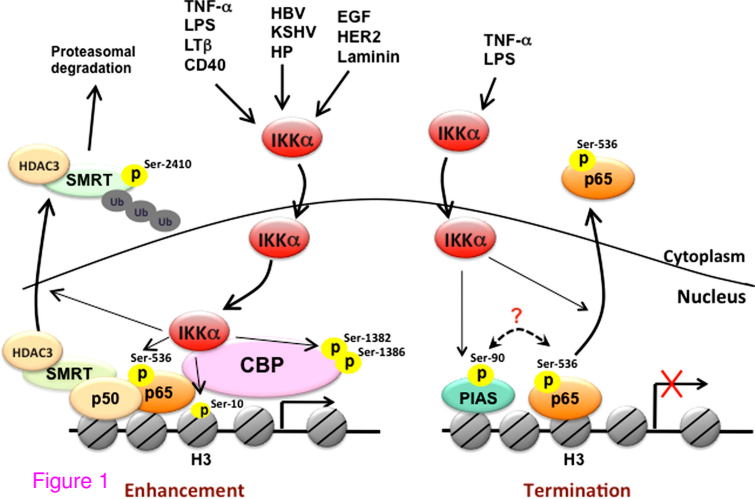


Figure 1

Enhancement

Termination

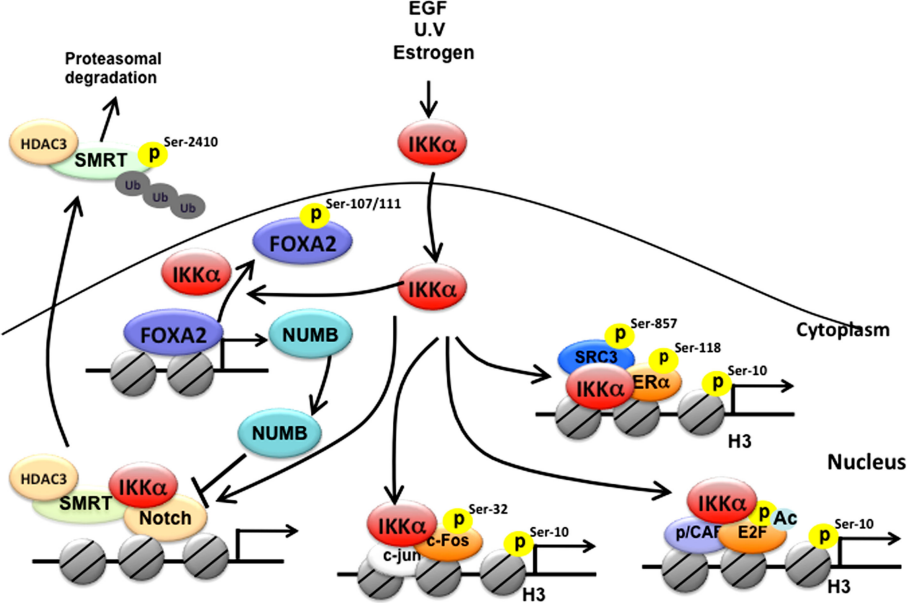


Figure 2

AP-1

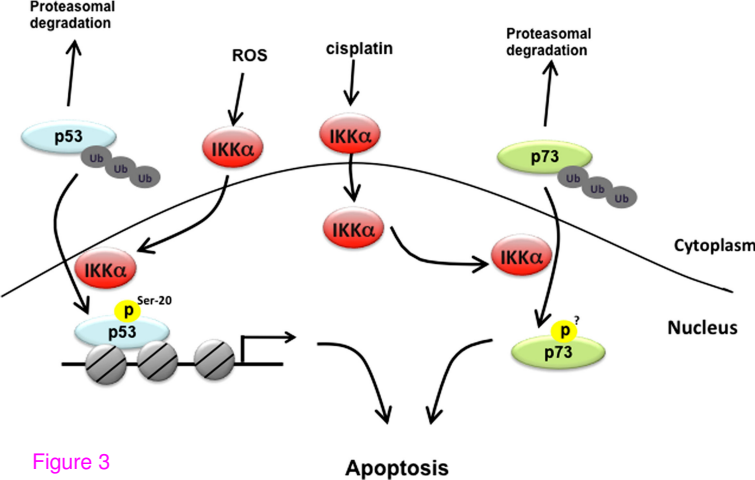
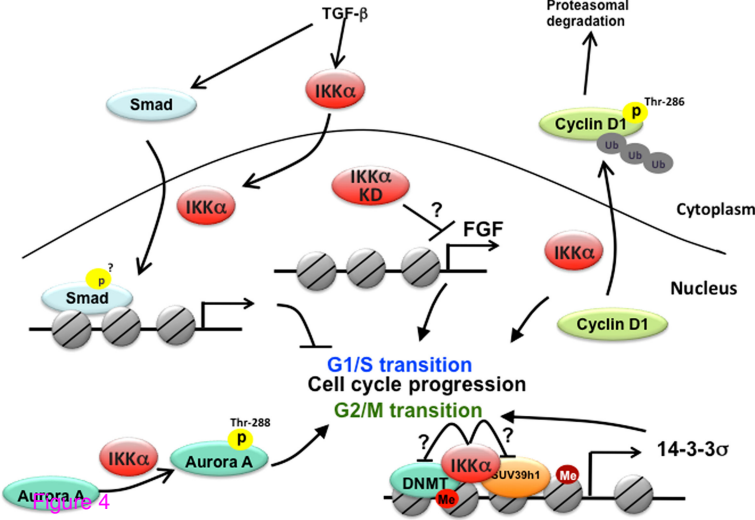
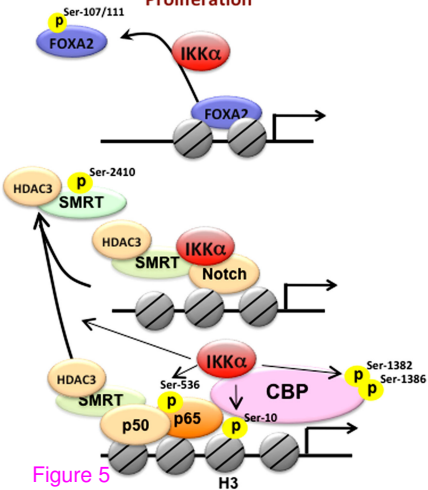


Figure 3



Proliferation



Metastasis

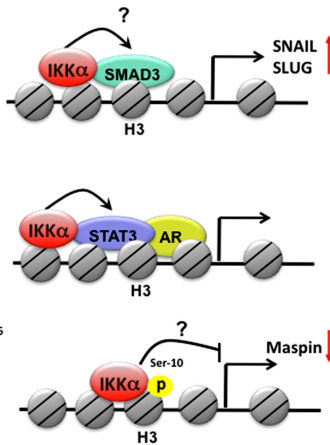


Figure 5

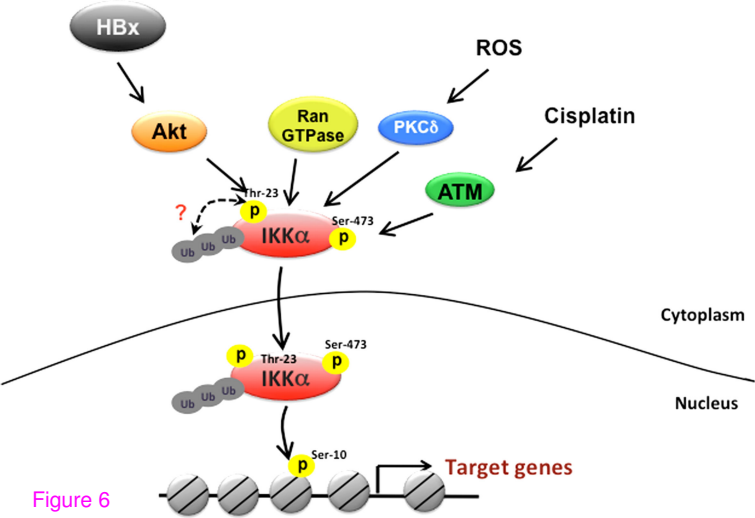


Figure 6