*Molecular and Cellular Biochemistry* **234/235:** 169–176, 2002.


# NF-κB, a pivotal transcription factor in silica-induced diseases

Fei Chen and Xianglin Shi

*The Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, USA*

## Abstract

Inhalation of silica in a number of occupational settings can result in debilitating and costly lung disease. It is thought that the pathological replacement of functional lung tissue with fibrotic lesions in silica-induced lung disease is the result of chronic inflammation mediated by products of the silica-exposed alveolar macrophage. In particular, inflammatory cytokines, growth factors and reactive oxygen species have been implicated in many acute and chronic inflammatory lung diseases. Pharmacological intervention to modify the production of these mediators has been shown to ameliorate several of these disease processes. Recent studies have demonstrated that the production of these inflammatory mediators is altered as a result of the activation of nuclear factor-κB (NF-κB). NF-κB is a pivotal transcription factor activated by silica in macrophages and other types of lung cells. The understanding of how silica induces NF-κB activation and what signaling pathways are involved in this silica-induced NF-κB activation is important and should provide valuable new information related to both the etiology and potential treatment of silica-related lung diseases. This review summarizes the molecular mechanisms involved in silica-induced NF-κB activation and discusses the importance of NF-κB as a critical transcription factor in mediating silica-induced lung diseases. (Mol Cell Biochem **234/235**: 169–176, 2002)

*Key words*: silica, NF-κB, apoptosis, cancer

## Introduction

It has been 6 years since the discovery that silica is a potent inducer for nuclear factor κB (NF-κB) [1, 2], a pivotal eukaryotic transcription factor governing the expression of early response genes involved in inflammation and cancer [3–5]. Recent studies indicate that NF-κB is also an important transcription factor regulating processes of viral replication, embryonic development, control of cell proliferation and apoptosis, and tissue degeneration [6, 7]. Therefore, it is not surprising that silica has long been recognized as one of the most potent inducers for proliferative lung diseases, such as lung fibrosis and possibly cancer.

NF-κB is an ubiquitous transcription factor that governs the expression of genes encoding cytokines, chemokines, growth factors, cell adhesion molecules and some acute phase proteins [3, 7]. This transcription factor was first identified as a B cell nuclear factor and named NF-κB based on its ability to bind to an intronic enhancer of the immunoglobulin κ-light chain gene [8]. Since then, NF-κB has been identified in numerous cell types and found to be activated by a wide range of inducers including UV irradiation, cytokines, inhaled occupational particulates, and bacterial and viral products [3]. In resting cells, NF-κB is sequestered in the cytoplasm in an inactive form by a group of inhibitory proteins known as IκB of which IκBα, IκBβ, and IκBε appear to be the key members [9, 10]. Upon cellular activation by extracellular stimuli, IκB is phosphorylated and proteolytically degraded or processed by proteasomes and other proteases. Following these processes, NF-κB is activated and translocated into the nucleus. In nuclei, NF-κB can initiate or regulate early response gene transcription by binding to decameric motifs GGGRNNYYCC [κB motif] found in the promoter or enhancer regions of specific genes.

The degradation of IκBα is a key step required for the activation of NF-κB. This occurs through signal-induced phosphorylation of two serines (Ser32 and Ser36) on IκBα molecule [6]. Replacement of Ser32 and Ser36 by threonine or alanine

*Address for offprints*: X. Shi, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA (E-mail: Xshi@cdc.gov)

residues significantly decreases signal-induced phosphorylation and degradation of IκBα protein [11]. Phosphorylation of these conserved S residues in response to inducers leads to the immediate polyubiquitination of IκB proteins by SCF-β-TrCP complex [12, 13], a step which has been shown recently to be inhibited by the nonpathogenic *Salmonella* bacteria in gut epithelial cells [14]. This modification subsequently targets IκB proteins for rapid degradation by the 26S proteasome [15]. A major breakthrough in the studies of NF-κB signaling in the last few years has been the identification of a high-molecular-weight IκB kinase (IKK) complex that phosphorylates IκBα or IκBβ [16, 17]. This complex contains two catalytic subunits, IKKα and IKKβ, and a structural component named NEMO/IKKγ/IKKAP [5]. An earlier report by Cohen *et al*. [18] suggested the presence of a scaffold protein named IKK complex-associated protein (IKAP) in the IKK complex, which could not be confirmed in other studies. A recent study by the group, who originally identified IKAP, indicated that the observed association of IKAP with IKK was due to a non-strict elusion condition of chromatographic extracts during the purification of IKK [19]. Recently, two groups independently reported the identification of a novel protein, CIKS/Act1, associated with the IKK complex and suggested that CIKS/Act1 functions as an anchoring protein in the assembly of the IKK complex and in providing a possible connection between IKK and c-Jun-N-terminal kinase (JNK) signaling [20, 21]. IKKα and IKKβ share 52% sequence homology in their kinase domain. Both proteins contain an amino terminal kinase domain, a carboxy terminal region with a leucine zipper, and a helix-loop-helix domain. *In vitro* or *in vivo* studies indicate that although both IKKα and IKKβ are capable of phosphorylating IκBα on ser32 and ser36, IKKβ is more potent in IκBα phosphorylation induced by proinflammatory stimuli [5]. Intriguingly, recent studies suggested a kinase- or NF-κB-independent effect of IKKα on the differentiation of keratinocytes [22]. A distinct IKK complex, named IKKi/ε that does not contain IKKα, β or γ, was also identified recently in T cells [23–25]. IKKi/ε shares 27% homology with IKKα and IKKβ and possibly mediates NF-κB-activating kinase (NAK) signaling, PMA/PKCε-induced S36 phosphorylation of IκBα, and NF-κB activation [26, 27].

Although silica has been recognized as a potent inducer for NF-κB [1, 28, 29] and the signaling pathways leading to the activation of NF-κB have been well defined [3, 5], there has been no information on how silica stimulates the signaling pathways leading to the activation of NF-κB. Recent publications describing the mechanism of action of other NF-κB inducers, such as cytokines [30], LPS [31], HTLV Tax protein [32, 33], and CD28 signal [34], have been focused on the role of the IKK, or other upstream kinases possibly participating in the activation of IKK. However, no evidence has been presented to demonstrate or indicate that silica, or reactive oxygen species (ROS) generated by silica-mediated reactions, can affect the activation or activity of these kinases. While silica-induced lung diseases have been considered a world-wide health problem in terms of both the quality of life for the worker and the national expenditure for lost production, health care costs and disability compensation, only a relatively small number of researchers are investigating the mechanistic aspects of silica-induced lung diseases. In view of the fact that activation of NF-κB plays a key role in the initiation and progression of silica-induced lung diseases, elucidation of molecular mechanisms determining silica-induced NF-κB activation and its various functions may lead to the development of novel preventive and therapeutic strategies for diseases resulting from silica inhalation.

## Silica activates NF-κB

Silica exposure causes macrophages to release a number of inflammatory mediators including PGE2 [35], nitric oxide [36], TNFα [37], IL-1 [37] and IL-6 [38]. At the DNA level, a common structural characteristic of the genes responsible for the generation of these mediators is the presence of one or several NF-κB binding sites in the promoter or 5′-flanking region [4]. This raises the possibility that silica-induced release of divergent inflammatory mediators may be dependent on a common mechanism, NF-κB activation. Indeed, the earlier work conducted by Chen *et al.* [1] indicated that silica could induce the nuclear translocation of NF-κB in murine macrophages. Analysis of the composition of the nuclear translocated NF-κB subtypes induced by silica suggested that both the p50/p50 homodimer and the p50/p65 or p52/p65 heterodimer of NF-κB are induced at either earlier or later time points. In contrast, only a heterodimer composed either p50/p65 or p52/p65 could be induced in the earlier time points by LPS. These results indicate that the mechanisms for silica-induced NF-κB activation may differ from that for LPS-induced NF-κB activation. Dose-response experiments *in vitro* suggested that NF-κB activation by silica follows a bell-shaped dose-response curve with peak activity for silica at 50 to 100 μg/ml. At relatively high doses of silica (200–400 μg/ml), the activation of NF-κB was decreased, which may explain why some reports have suggested that silica had no effect on the release of inflammatory mediators in certain experimental systems.

Previously, an oxidative stress model was proposed for the activation of NF-κB by diverse agents [39]. It is reasonable to speculate that ROS derived either from the interaction of the surface of silica particles with aqueous medium or as a result of chronic respiratory burst activity in the lung is responsible for silica-induced NF-κB activation [40, 41]. This hypothesis was based on several lines of evidence. First, ROS

have been shown to be increased in some cell types in response to silica exposure [41]. Second, direct incubation of certain cell lines with $H_2O_2$ activates NF-κB [42]. Third, addition of compounds possessing antioxidant properties, such as N-acetyl-L-cysteine (NAC), catalase, deferoxamine, ascorbate, and formate, can inhibit signal-induced NF-κB activation [43].

The question is whether ROS, resulting form silica exposure, activates NF-κB through mechanisms similar to those of IL-1, TNFα, or LPS. Up to now, there is no evidence indicating that silica and its ROS derivatives stimulate the IKK kinase activity, suggesting a possible IKK-independent mechanism. There are a number of other pathways by which silica may activate NF-κB. Both silica and ROS have been implicated in the activation of mitogen-activated protein kinases (MAPKs), such as Erk, p38 and JNK [44, 45]. It is possible that certain molecules in MAPK signaling pathway may have the potential to stimulate the IKK kinase. The oxidative damage or modification of a protein or DNA might be another measure for the effects of silica on NF-κB. It has been proposed that oxidation and reduction could affect the assembly of both kinase complexes and NF-κB-DNA binding complexes [46].

## Silica induces different proteolytic system for the degradation of IκBα

Two major proteolytic pathways have been studied with regard to their role in signal-induced NF-κB activation: proteasomes and calpains [3]. Evidence suggests that the ubiquitin-proteasome pathway plays a major role in the degradation of IκBα protein and consequently in the activation of NF-κB transcription factor [4, 6]. Several recent studies have shown that the calpain system might also be involved under certain circumstances in basal or signal-induced degradation of IκBα and the activation of NF-κB [28, 29, 47–50].

Calpains are calcium-dependent cysteine proteinases present in a variety of cells [51]. Two major groups of calpains, termed ubiquitous calpains and tissue-specific calpains, have been identified. The ubiquitous calpains include calpain 1 (μ-calpain) and calpain 2 (m-calpain), which require micromolar and millimolar concentrations of calcium for their activation, respectively. The tissue specific calpains, mainly calpain 3 and calpain 4, are found in skeletal muscle and smooth muscle, respectively. Only nanomolar concentrations of calcium are required for the activation of calpain 3 and calpain 4. Experiments using synthetic inhibitors of calpains have shown that calpains are pivotal proteases participating in a limited proteolytic reaction of a number of cellular structural or regulatory proteins, such as cytoskeletal proteins [52], kinases [53], cytokines [54] and the tumor suppressing protein, p53 [55–58]. However, the reliability of the use of synthetic pharmacological inhibitors to delineate the role of calpains has been compromised due to the low cellular permeability and poor specificity of these inhibitors. These inhibitors include calpain inhibitor I/II, the family of E64 compounds and leupeptin [51, 59].

The most direct evidence demonstrating that calpain system contributes to silica-induced IκBα degradation and subsequent activation of NF-κB is provided by the use of ectopic expression of an endogenous calpain inhibitor, calpastatin, in both a murine macrophage cell line and in human bronchial epithelial cells [29]. Whereas a potent proteasome inhibitor, MG132, failed to abrogate silica-induced IκBα degradation in macrophages, transient overexpression of calpastatin, a specific endogenous inhibitor for calpain, resulted in an appreciable inhibition of IκBα degradation induced by silica [29]. *In vitro* digestion of recombinant IκBα by purified calpain or cytosolic extracts from silica-stimulated cells demonstrated further that calpain was capable of degradating IκBα protein by the cleavage of several leucine rich domains**.** In an independent study, Han *et al.* showed that calpain provides a parallel proteolytic pathway to the ubiquitin-proteasome pathway for TNF-α-induced IκBα degradation in human HepG2 cells [47]. In WEHI231 immature B cells, a rapid degradation of IκBα was insensitive to proteasome inhibitors, but was substantially inhibited by calpain inhibitors [49]. More recently, Baghdiguian and associates [48] provided direct evidence demonstrating that patients with an autosomal muscular dystrophy caused by calpain 3 deficiency exhibited a substantial impairment of IκBα degradation and NF-κB activation in muscle cells.

The evidence for involvement of calpain in silica-induced IκBα degradation and NF-κB activation warrant future studies on the mechanisms of calpain activation, consequences of calpain deficiency or overfunction, cross-talk with proteasome and other proteolytic systems, and the feasibility of targeting calpains to interfere with silica-related disease processes.

## Silica, NF-κB and cancer

Whether silica is a carcinogen is still a debatable issue [60–64]. While epidemiological data indicate links between silica exposure and cancer [63], only limited laboratory or animal evidence exists for the carcinogenic effects of silica [62, 64]. The carcinogenic effect of many known carcinogens inducing transformation is believed to be exerted through a mutagenic effect of these agents on the genome. No substantial evidence, however, indicates that silica is genotoxic [62]. Thus, if silica is carcinogenic, it is most likely that it acts through epigenetic processes, such as an alteration of intracellular sig-

nal transduction mechanisms that affect the regulation of cell cycle progression, DNA repair, protein ubiquitination, transcription factor activation, or oncogenic gene expression. The ability of silica to activate NF-κB indicates a potential link between silica and cancer, since increased activation of NF-κB has been observed in a number of human cancers [65], including breast cancer, non-small cell lung carcinoma, thyroid cancer, T- or B-lymphocyte leukemia, melanoma, colon cancer, bladder cancer, and several virally-induced tumors.

One of the most important mechanisms linking NF-κB to the processes of carcinogenesis is the anti-apoptotic role of NF-κB [3, 4]. Fausto *et al.* found that NF-κB is required for the liver regeneration and hepatocyte proliferation after partial hepatectomy [66]. A NF-κB relA gene knockout mouse model exhibits massive liver cell apoptosis and embryonic lethal phenotype [67]. Treatment of RelA deficient [$RelA^{-/-}$] mouse embryonic fibroblasts and macrophages with TNFα resulted in a significant reduction in viability, while $RelA^{+/+}$ cells from a wild type mouse were unaffected. Reintroduction of RelA into $relA^{-/-}$ fibroblasts resulted in an enhanced survival [67]. It is believed that the protective role of NF-κB against apoptosis may be through the induction of anti-apoptotic genes including cIAP1, cIAP2 [68], xIAP [69], IEX-1L [70], Bcl-xl [71], Bcl-2 homolog Bfl-1/A1 [72] and zinc finger protein A20 [73]. It has been demonstrated that the transcriptional regulation of NF-κB on the genes encoding zinc finger protein A20 and a Bcl-2 family member, Bfl-1/A1 [72], is through one or two NF-κB binding sites located in their promoter regions [73].

Increased expression of many antiapoptotic genes, especially bcl-xl and survivin, has been frequently observed in a number of human tumors [74]. Bcl-xl was first identified using a murine bcl-2 cDNA probe under low stringency conditions to identify bcl-2-related genes in chicken lymphoid cells. The product of bcl-xl gene, Bcl-xl, has been shown to protect cells from apoptosis induced by a wide range of agents that also activate NF-κB transcription factor in a variety of cell lines [75]. Bcl-xl is transiently expressed in immature intermediate cells such as pro- and pre-B cells and double-positive T cells [76], and is known to be up-regulated as a consequence of antigen receptor cross-linking, an important extracellular signal leading to the activation of NF-κB. In macrophages, Bcl-xl was up-regulated by IFN-γ and LPS, two well-known NF-κB activators [77]. Bcl-xl was predominantly expressed in malignant cells in which NF-κB was usually over-activated [78, 79].

It appears plausible to speculate that NF-κB and its regulated expression of antiapoptotic genes, especially bcl-2 family of genes, may account for the potential carcinogenic effect of silica. Indeed, analysis of protein expression for Bcl-2 family proteins in macrophages indicated that the expression of Bcl-xl is increased in response to silica and the level of expression of Bcl-xl is correlated with the status of NF-κB activation [71]. The contribution of NF-κB in this silica-induced bcl-xl expression was further confirmed by both promoter activity assay and NF-κB DNA binding analysis, which showed that enhancement of NF-κB activity increased the bcl-xl promoter reporter gene activity through at least three putative NF-κB binding sites within the 5′-flanking region of bcl-xl gene [71].

Tumorigenesis or oncogenesis is a multistep process and the steps reflect defects in regulatory circuits that govern normal cell proliferation, differentiation, and death [80]. While abnormal activation or function of NF-κB has been clearly demonstrated in the initiation or facilitation of oncogenesis, the central question that has to be answered is: how many and what steps are influenced by NF-κB? NF-κB has been shown to antagonize the function of p53 as demonstrated by several reports [81–83]. Obviously, this antagonism of p53 by NF-κB will result in the escape of cells from stress-induced cell cycle arrest and/or programmed cell death and consequently sensitize the cells for genomic instability. Furthermore, NF-κB could promote cell cycle transition by a direct transcriptional upregulation of the cyclin D1 gene [84, 85]. Although it remains to be confirmed, this increased expression of cyclin D1 may possibly provide cells with an uncontrolled or limitless replicative potential. Upregulation of anti-apoptotic genes, such as cIAP1, cIAP2, XIAP, and bcl-xl, by NF-κB [3] is an additional mechanism for cells to escape from or resist to signal-induced apoptosis. Other NF-κB-regulated genes include those encoding intercellular adhesion molecule-1 (ICAM-1), extracellular matrix protein tenascin-C, vascular endothelial growth factor (VEGF), chemokines, and cyclooxygenase-2 [4]. These gene products are directly associated with tumor cell metastasis and tumor tissue angiogenesis.

The key role that NF-κB plays on multiple steps of oncogenesis makes this factor a central and favorable target for therapeutic intervention of cancer, especially, certain types of leukemia or lymphomas [86]. Indeed, experimental data suggest that inhibition of NF-κB by antisense oligonucleotides to relA, degradation resistant IκBα, and aspirin or nonsteroidal anti-inflammatory drugs could enhance the efficacy of cancer chemotherapies and radiation [7, 87]. Studies by Wang *et al.* [88] showed that inhibition of NF-κB by infecting the cells with an adenovirus carrying a modified form of IκBα (super-repressor IκBα) leads to dramatically enhanced apoptosis of HT1080 fibrosarcoma cells in response to ionizing radiation or daunorubicin treatment. Consistent with these reports, working with pancreatic cancer cell lines exposed to VP16 or doxorubicin, Arlt and colleagues [89] demonstrated recently that NF-κB inhibition by pharmacological proteasome inhibitors or transfection of the cells with a N-terminal truncated IκBα variant efficiently reduces chemoresistance of these cells. Using similar or different approaches to inhibit NF-κB, this effect has also been noted in a variety

of other cell types including non-small cell lung cancers [90], head and neck squamous carcinomas [91], human myeloblastic leukemia cells [92], colorectal cancer [93], and bladder cancer cells [94]. In spite of these encouraging observations, however, care has to be taken when using different approaches to inhibit NF-κB. Indeed, different approaches for the inhibition of NF-κB do not necessary lead to the same extent of inactivation of NF-κB, since NF-κB exists as functionally and stoichiometrically different complexes that respond to different activation signals [3, 4]. Also, the inhibitory effects of NF-κB inhibitors can vary considerably among different cell types because of unique simultaneous or asynchronous events triggered by these inhibitors in any given cell type [65].

## Summary

The detailed molecular mechanisms by which the NF-κB transcription factor contributes to cell growth control, such as cell apoptosis, cell cycle transition and oncogenesis, remain to be determined. One of the major challenges in understanding mechanisms of cell growth regulation by NF-κB in response to silica is the elucidation of how signal transduction pathways are activated and how signaling cross-talk and specificity are achieved when several signaling pathways that elicit different cellular responses are activated simultaneously by silica. For instance, why does activation of the NF-κB, an anti-apoptotic transcription factor, coincide with obvious apoptotic features in cells undergoing silica-induced stress responses? Since many stress inducers and their mediators are highly reactive but nonspecific, activation of only one specific signaling pathway is hard to achieve in the cells in response to a particular inducer. Even in a single signaling pathway, because of their highly reactive and nonspecific characteristics, certain stress inducers and their mediators can in principle induce conflicting signals by affecting signaling molecules at different levels. A good example is the effects of oxidative stress on the NF-κB signaling pathway. It has been frequently observed that in certain types of cells oxidative stress amplified or potentiated NF-κB activation, whereas at the same time oxidation of IKK or NF-κB proteins inhibited NF-κB function. Translating the knowledge gained by studying the connections among NF-κB activation, cell apoptosis, cell cycle regulation, and oncogenesis may aid in identifying novel preventive and therapeutic measures for silica-related diseases.

## Acknowledgement

We thank our colleagues in the Health Effects Laboratory Division for critical reading of this manuscript. We apologize to the authors whose pioneering work in the study of silica-related diseases could not be cited because of space constraints. Dr. Fei Chen thanks the Health Effects Laboratory Division/National Institute for Occupational Safety and Health for support for a Career Development Award through a cooperative agreement between the Association of Teachers of Preventive Medicine and the Centers for Disease Control and Prevention of the United States (E-mail: lfd3@cdc.gov).

## References

1. Chen F, Sun SC, Kuhn DC, Gaydos LJ, Demers LM: Essential role of NF-κB activation in silica-induced inflammatory mediator production in macrophages. Biochem Biophys Res Commun 214: 985–992, 1995
2. Chen F, Kuhn DC, Sun SC, Gaydos LJ, Demers LM: Dependence and reversal of nitric oxide production on NF-κB in silica and lipopolysaccharide-induced macrophages. Biochem Biophys Res Commun 214: 839–846, 1995
3. Chen F, Castranova V, Shi X, Demers LM: New insights into the role of nuclear factor-κB, a ubiquitous transcription factor in the initiation of diseases. Clin Chem 45: 7–17, 1999
4. Baldwin AS Jr: The NF-κB and IκB proteins: New discoveries and insights. Annu Rev Immunol 14: 649–683, 1996
5. Karin M, Delhase M: The IκB kinase [IKK] and NF-κB: Key elements of proinflammatory signalling. Semin Immunol 12: 85–98, 2000
6. Karin M, Ben-Neriah Y: Phosphorylation meets ubiquitination: The control of NF-κB activity. Annu Rev Immunol 18: 621–663, 2000
7. Mayo MW, Baldwin AS: The transcription factor NF-κB: Control of oncogenesis and cancer therapy resistance. Biochim Biophys Acta 1470: M55–62, 2000
8. Sen R, Baltimore D: Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. Cell 47: 921–928, 1986
9. Kopp EB, Ghosh S: NF-κB and rel proteins in innate immunity. Adv Immunol 58: 1–27, 1995
10. Zhang G, Ghosh S: Toll-like receptor-mediated NF-κB activation: A phylogenetically conserved paradigm in innate immunity. J Clin Invest 107: 13–19, 2001
11. Chen ZJ, Parent L, Maniatis T: Site-specific phosphorylation of IκBα by a novel ubiquitination-dependent protein kinase activity. Cell 84: 853–862, 1996
12. Orian A, Gonen H, Bercovich B, Fajerman I, Eytan E, Israel A, Mercurio F, Iwai K, Schwartz AL, Ciechanover A: SCF-β-TrCP ubiquitin ligase-mediated processing of NF-κB p105 requires phosphorylation of its C-terminus by IκB kinase. EMBO J 19: 2580–2591, 2000
13. Strack P, Caligiuri M, Pelletier M, Boisclair M, Theodoras A, Beer-Romero P, Glass S, Parsons T, Copeland RA, Auger KR, Benfield P, Brizuela L, Rolfe M: SCF-β-TrCP and phosphorylation dependent ubiquitinationof IκB alpha catalyzed by Ubc3 and Ubc4. Oncogene 19: 3529–3536, 2000
14. Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL: Prokaryotic regulation of epithelial responses by inhibition of IκBα ubiquitination. Science 289: 1560–1563, 2000
15. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T: The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. Cell 78: 773–785, 1994
16. Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV: IκB kinase-beta: NF-κB activation and complex formation with IκB kinase-α and NIK. Science 278: 866–869, 1997

17. Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, Young DB, Barbosa M, Mann M, Manning A, Rao A: IKK-1 and IKK-2: Cytokine-activated IκB kinases essential for NF-κB activation. Science 278: 860–866, 1997
18. Cohen L, Henzel WJ, Baeuerle PA: IKAP is a scaffold protein of the IκB kinase complex. Nature 395: 292–296, 1998
19. Krappmann D, Hatada EN, Tegethoff S, Li J, Klippel A, Giese K, Baeuerle PA, Scheidereit C: The IκB kinase [IKK] complex is tripartite and contains IKK gamma but not IKAP as a regular component. J Biol Chem 275: 29779–29787, 2000
20. Leonardi A, Chariot A, Claudio E, Cunningham K, Siebenlist U: CIKS, a connection to IκB kinase and stress-activated protein kinase. Proc Natl Acad Sci USA 97: 10494–10499, 2000
21. Li X, Commane M, Nie H, Hua X, Chatterjee-Kishore M, Wald D, Haag M, Stark GR: Act1, an NF-κB-activating protein. Proc Natl Acad Sci USA 97: 10489–10493, 2000
22. Hu Y, Baud V, Oga T, Kim KI, Yoshida K, Karin M: IKKα controls formation of the epidermis independently of NF-κB. Nature 410: 710–714, 2001
23. Peters RT, Liao SM, Maniatis T: IKKε is part of a novel PMA-inducible IκB kinase complex. Mol Cell 5: 513–522, 2000
24. Shimada T, Kawai T, Takeda K, Matsumoto M, Inoue J, Tatsumi Y, Kanamaru A, Akira S: IKK-i, a novel lipopolysaccharide-inducible kinase that is related to IκB kinases. Int Immunol 11: 1357–1362, 1999
25. Pomerantz JL, Baltimore D: NF-κB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. Embo J 18: 6694–6704, 1999
26. Israel A: The IKK complex: An integrator of all signals that activate NF-κB? Trends Cell Biol 10: 129–133, 2000
27. Tojima Y, Fujimoto A, Delhase M, Chen Y, Hatakeyama S, Nakayama K, Kaneko Y, Nimura Y, Motoyama N, Ikeda K, Karin M, Nakanishi M: NAK is an IκB kinase-activating kinase. Nature 404: 778–782, 2000
28. Chen F, Lu Y, Kuhn DC, Maki M, Shi X, Sun SC, Demers LM: Calpain contributes to silica-induced IκBα degradation and nuclear factor-kappa B activation. Arch Biochem Biophys 342: 383–388, 1997
29. Chen F, Demers LM, Vallyathan V, Lu Y, Castranova V, Shi X: Impairment of NF-κB activation and modulation of gene expression by calpastatin. Am J Physiol Cell Physiol 279: C709–716, 2000
30. Baud V, Liu ZG, Bennett B, Suzuki N, Xia Y, Karin M: Signaling by proinflammatory cytokines: Oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. Genes Dev 13: 1297–1308, 1999
31. Guha M, Mackman N: LPS induction of gene expression in human monocytes. Cell Signal 13: 85–94, 2001
32. Carter RS, Geyer BC, Xie M, Acevedo-Suarez CA, Ballard DW: Persistent activation of NF-κB by the tax transforming protein involves chronic phosphorylation of IκB kinase subunits IKKβ and IKKγ. J Biol Chem 26: 26, 2001
33. Jeang K: Functional activities of the human T-cell leukemia virus type I Tax oncoprotein: Cellular signaling through NF-κB. Cytokine Growth Factor Rev 12: 207–217, 2001
34. Lin X, Cunningham ET Jr, Mu Y, Geleziunas R, Greene WC: The proto-oncogene Cot kinase participates in CD3/CD28 induction of NF-κB acting through the NF-κB-inducing kinase and IκB kinases. Immunity 10: 271–280, 1999
35. Demers LM, Kuhn DC: Influence of mineral dusts on metabolism of arachidonic acid by alveolar macrophage. Environ Health Perspect 102 Suppl 10: 97–100, 1994
36. Huffman LJ, Judy DJ, Castranova V: Regulation of nitric oxide production by rat alveolar macrophages in response to silica exposure. J Toxicol Environ Health A 53: 29–46, 1998
37. Davis GS, Pfeiffer LM, Hemenway DR: Persistent overexpression of interleukin-1β and tumor necrosis factor-α in murine silicosis. J Environ Pathol Toxicol Oncol 17: 99–114, 1998
38. Gosset P, Lassalle P, Vanhee D, Wallaert B, Aerts C, Voisin C, Tonnel AB: Production of tumor necrosis factor-alpha and interleukin-6 by human alveolar macrophages exposed *in vitro* to coal mine dust. Am J Respir Cell Mol Biol 5: 431–436, 1991
39. Imbert V, Rupec RA, Livolsi A, Pahl HL, Traenckner EB, Mueller-Dieckmann C, Farahifar D, Rossi B, Auberger P, Baeuerle PA, Peyron JF: Tyrosine phosphorylation of IκBα activates NF-κB without proteolytic degradation of IκBα. Cell 86: 787–798, 1996
40. Shi X, Dong Z, Huang C, Ma W, Liu K, Ye J, Chen F, Leonard SS, Ding M, Castranova V, Vallyathan V: The role of hydroxyl radical as a messenger in the activation of nuclear transcription factor NF-κB. Mol Cell Biochem 194: 63–70, 1999
41. Shi X, Ding M, Dong Z, Chen F, Ye J, Wang S, Leonard SS, Castranova V, Vallyathan V: Antioxidant properties of aspirin: Characterization of the ability of aspirin to inhibit silica-induced lipid peroxidation, DNA damage, NF-κB activation, and TNFα production. Mol Cell Biochem 199: 93–102, 1999
42. Garban HJ, Bonavida B: Nitric oxide disrupts $H_2O_2$-dependent activation of nuclear factor κB. Role in sensitization of human tumor cells to tumor necrosis factor-alpha-induced cytotoxicity. J Biol Chem 276: 8918–8923, 2001
43. Chen F, Lu Y, Demers LM, Rojanasakul Y, Shi X, Vallyathan V, Castranova V: Role of hydroxyl radical in silica-induced NF-κB activation in macrophages. Ann Clin Lab Sci 28: 1–13, 1998
44. Ding M, Shi X, Dong Z, Chen F, Lu Y, Castranova V, Vallyathan V: Freshly fractured crystalline silica induces activator protein-1 activation through ERKs and p38 MAPK. J Biol Chem 274: 30611–30616, 1999
45. Shukla A, Timblin CR, Hubbard AK, Bravman J, Mossman BT: Silica-induced activation of c-Jun-NH2-terminal amino kinases, protracted expression of the activator protein-1 proto-oncogene, fra-1, and S-phase alterations are mediated via oxidative stress. Cancer Res 61: 1791–1795, 2001
46. Li N, Karin M: Is NF-κB the sensor of oxidative stress? FASEB J 13: 1137–1143, 1999
47. Han Y, Weinman S, Boldogh I, Walker RK, Brasier AR: Tumor necrosis factor-α-inducible IκBα proteolysis mediated by cytosolic m-calpain. A mechanism parallel to the ubiquitin-proteasome pathway for nuclear factor-κB activation. J Biol Chem 274: 787–794, 1999
48. Baghdiguian S, Martin M, Richard I, Pons F, Astier C, Bourg N, Hay RT, Chemaly R, Halaby G, Loiselet J, Anderson LV, Lopez de Munain A, Fardeau M, Mangeat P, Beckmann JS, Lefranc G: Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IκBα/NF-κB pathway in limb-girdle muscular dystrophy type 2A. Nat Med 5: 503–511, 1999
49. Miyamoto S, Seufzer BJ, Shumway SD: Novel IκBα proteolytic pathway in WEHI231 immature B cells. Mol Cell Biol 18: 19–29, 1998
50. Shumway SD, Maki M, Miyamoto S: The PEST domain of IκBα is necessary and sufficient for *in vitro* degradation by μ-calpain. J Biol Chem 274: 30874–30881, 1999
51. Wang KK, Yuen PW: Calpain inhibition: An overview of its therapeutic potential. Trends Pharmacol Sci 15: 412–419, 1994
52. Potter DA, Tirnauer JS, Janssen R, Croall DE, Hughes CN, Fiacco KA, Mier JW, Maki M, Herman IM: Calpain regulates actin remodeling during cell spreading. J Cell Biol 141: 647–662, 1998
53. Tanabe F, Cui SH, Ito M: Ceramide promotes calpain-mediated proteolysis of protein kinase Cβ in murine polymorphonuclear leukocytes. Biochem Biophys Res Commun 242: 129–133, 1998
54. Kavita U, Mizel SB: Differential sensitivity of interleukin-1α and -β precursor proteins to cleavage by calpain, a calcium-dependent protease. J Biol Chem 270: 27758–27765, 1995

55. Gonen H, Shkedy D, Barnoy S, Kosower NS, Ciechanover A: On the involvement of calpains in the degradation of the tumor suppressor protein p53. FEBS Lett 406: 17–22, 1997
56. Kubbutat MH, Vousden KH: Proteolytic cleavage of human p53 by calpain: A potential regulator of protein stability. Mol Cell Biol 17: 460–468, 1997
57. Pariat M, Carillo S, Molinari M, Salvat C, Debussche L, Bracco L, Milner J, Piechaczyk M: Proteolysis by calpains: A possible contribution to degradation of p53. Mol Cell Biol 17: 2806–2815, 1997
58. Zhang W, Lu Q, Xie ZJ, Mellgren RL: Inhibition of the growth of WI-38 fibroblasts by benzyloxycarbonyl-Leu-Leu-Tyr diazomethyl ketone: Evidence that cleavage of p53 by a calpain-like protease is necessary for G1 to S-phase transition. Oncogene 14: 255–263, 1997
59. Balcerzak D, Cottin P, Poussard S, Cucuron A, Brustis JJ, Ducastaing A: Calpastatin-modulation of m-calpain activity is required for myoblast fusion. Eur J Cell Biol 75: 247–253, 1998
60. McDonald AD, McDonald JC, Rando RJ, Hughes JM, Weill H: Cohort mortality study of North American industrial sand workers. I. Mortality from lung cancer, silicosis and other causes. Ann Occup Hyg 45: 193–139, 2001
61. Steenland K, Sanderson W: Lung cancer among industrial sand workers exposed to crystalline silica. Am J Epidemiol 153: 695–703, 2001
62. Hessel PA, Gamble JF, Gee JB, Gibbs G, Green FH, Morgan WK, Mossman BT: Silica, silicosis, and lung cancer: A response to a recent working group report. J Occup Environ Med 42: 704–720, 2000
63. Cherry NM, Burgess GL, Turner S, McDonald JC: Crystalline silica and risk of lung cancer in the potteries. Occup Environ Med 55: 779–785, 1998
64. Checkoway H, Franzblau A: Is silicosis required for silica-associated lung cancer? Am J Ind Med 37: 252–259, 2000
65. Bours V, Bentires-Alj M, Hellin AC, Viatour P, Robe P, Delhalle S, Benoit V, Merville MP: Nuclear factor-κB, cancer, and apoptosis. Biochem Pharmacol 60: 1085–1089, 2000
66. Fausto N, Laird AD, Webber EM: Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. FASEB J 9: 1527–1536, 1995
67. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D: Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. Nature 376: 167–170, 1995
68. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS Jr: NF-κB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281: 1680–1683, 1998
69. Stehlik C, de Martin R, Kumabashiri I, Schmid JA, Binder BR, Lipp J: Nuclear factor NF-κB-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor alpha-induced apoptosis. J Exp Med 188: 211–216, 1998
70. Wu MX, Ao Z, Prasad KV, Wu R, Schlossman SF: IEX-1L, an apoptosis inhibitor involved in NF-κB-mediated cell survival. Science 281: 998–1001, 1998
71. Chen F, Demers LM, Vallyathan V, Lu Y, Castranova V, Shi X: Involvement of 5'-flanking κB-like sites within bcl-x gene in silica-induced Bcl-x expression. J Biol Chem 274: 35591–35595, 1999
72. Lee HH, Dadgostar H, Cheng Q, Shu J, Cheng G: NF-κB-mediated up-regulation of Bcl-x and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes. Proc Natl Acad Sci USA 96: 9136–9141, 1999
73. Sarma V, Lin Z, Clark L, Rust BM, Tewari M, Noelle RJ, Dixit VM: Activation of the B-cell surface receptor CD40 induces A20, a novel zinc finger protein that inhibits apoptosis. J Biol Chem 270: 12343–12346, 1995
74. Hinz M, Loser P, Mathas S, Krappmann D, Dorken B, Scheidereit C: Constitutive NF-κB maintains high expression of a characteristic gene network, including CD40, CD86, and a set of antiapoptotic genes in Hodgkin/Reed-Sternberg cells. Blood 97: 2798–2807, 2001
75. Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G, Thompson CB: Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74: 597–608, 1993
76. Fang W, Weintraub BC, Dunlap B, Garside P, Pape KA, Jenkins MK, Goodnow CC, Mueller DL, Behrens TW: Self-reactive B lymphocytes overexpressing Bcl-xL escape negative selection and are tolerized by clonal anergy and receptor editing. Immunity 9: 35–45, 1998
77. Okada S, Zhang H, Hatano M, Tokuhisa T: A physiologic role of Bcl-xL induced in activated macrophages. J Immunol 160: 2590–2596, 1998
78. Xerri L, Parc P, Brousset P, Schlaifer D, Hassoun J, Reed JC, Krajewski S, Birnbaum D: Predominant expression of the long isoform of Bcl-x [Bcl-xL] in human lymphomas. Br J Haematol 92: 900–906, 1996
79. Bargou RC, Daniel PT, Mapara MY, Bommert K, Wagener C, Kallinich B, Royer HD, Dorken B: Expression of the bcl-2 gene family in normal and malignant breast tissue: Low bax-α expression in tumor cells correlates with resistance towards apoptosis. Int J Cancer 60: 854–859, 1995
80. Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 100: 57–70, 2000
81. Wadgaonkar R, Phelps KM, Haque Z, Williams AJ, Silverman ES, Collins T: CREB-binding protein is a nuclear integrator of nuclear factor-κB and p53 signaling. J Biol Chem 274: 1879–1882, 1999
82. Yang JP, Hori M, Takahashi N, Kawabe T, Kato H, Okamoto T: NF-κB subunit p65 binds to 53BP2 and inhibits cell death induced by 53BP2. Oncogene 18: 5177–5186, 1999
83. Shao J, Fujiwara T, Kadowaki Y, Fukazawa T, Waku T, Itoshima T, Yamatsuji T, Nishizaki M, Roth JA, Tanaka N: Overexpression of the wild-type p53 gene inhibits NF-κB activity and synergizes with aspirin to induce apoptosis in human colon cancer cells. Oncogene 19: 726–736, 2000
84. Joyce D, Bouzahzah B, Fu M, Albanese C, D'Amico M, Steer J, Klein JU, Lee RJ, Segall JE, Westwick JK, Der CJ, Pestell RG: Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-κB-dependent pathway. J Biol Chem 274: 25245–25249, 1999
85. Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS Jr: NF-κB controls cell growth and differentiation through transcriptional regulation of cyclin D1. Mol Cell Biol 19: 5785–5799, 1999
86. Schwartz SA, Hernandez A, Mark Evers B: The role of NF-κB/IκB proteins in cancer: Implications for novel treatment strategies. Surg Oncol 8: 143–153, 1999
87. Baldwin AS: Control of oncogenesis and cancer therapy resistance by the transcription factor NF-κB. J Clin Invest 107: 241–246, 2001
88. Wang CY, Cusack JC Jr, Liu R, Baldwin AS Jr: Control of inducible chemoresistance: Enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-κB. Nat Med 5: 412–417, 1999
89. Arlt A, Vorndamm J, Breitenbroich M, Folsch UR, Kalthoff H, Schmidt WE, Schafer H: Inhibition of NF-κB sensitizes human pancreatic carcinoma cells to apoptosis induced by etoposide [VP16] or doxorubicin. Oncogene 20: 859–868, 2001
90. Jones DR, Broad RM, Madrid LV, Baldwin AS Jr, Mayo MW: Inhibition of NF-κB sensitizes non-small cell lung cancer cells to chemotherapy-induced apoptosis. Ann Thorac Surg 70: 930–936; discussion 936–937, 2000
91. Kato T, Duffey DC, Ondrey FG, Dong G, Chen Z, Cook JA, Mitchell JB, Van Waes C: Cisplatin and radiation sensitivity in

human head and neck squamous carcinomas are independently modulated by glutathione and transcription factor NF-κB. Head Neck 22: 748–759, 2000

92. Romano MF, Lamberti A, Bisogni R, Tassone P, Pagnini D, Storti G, Del Vecchio L, Turco MC, Venuta S: Enhancement of cytosine arabinoside-induced apoptosis in human myeloblastic leukemia cells by NF-κB/Rel-specific decoy oligodeoxynucleotides. Gene Ther 7: 1234–1237, 2000
93. Cusack JC Jr, Liu R, Baldwin AS Jr: Inducible chemoresistance to 7-ethyl-10-[4-[1-piperidino]-1-piperidino]-carbonyloxycamptothecin [CPT-11] in colorectal cancer cells and a xenograft model is overcome by inhibition of nuclear factor-kB activation. Cancer Res 60: 2323–2330, 2000
94. Sumitomo M, Tachibana M, Ozu C, Asakura H, Murai M, Hayakawa M, Nakamura H, Takayanagi A, Shimizu N: Induction of apoptosis of cytokine-producing bladder cancer cells by adenovirus-mediated IκBα overexpression. Hum Gene Ther 10: 37–47, 1999