Invited Review

NF- κ B and Therapeutic Approach

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Abstract – Since NF- κ B has been identified as a transcription factor associated with immune cell activation, groups of researchers have dedicated to reveal detailed mechanisms of nuclear factor of κ B (NF- κ B) in inflammatory signaling for decades. The various molecular components of NF- κ B transcription factor pathway have been being evaluated as important therapeutic targets due to their roles in diverse human diseases including inflammation, cystic fibrosis, sepsis, rheumatoid arthritis, cancer, atherosclerosis, ischemic injury, myocardial infarction, osteoporosis, transplantation rejection, and neurodegeneration. With regards to new drugs directly or indirectly modulating the NF- κ B pathway, FDA recently approved a proteasome inhibitor bortezomib for the treatment of multiple myeloma. Many pharmaceutical companies have been trying to develop new drugs to inhibit various kinases in the NF- κ B signaling pathway for many therapeutic applications. However, a gene knock-out study for IKK β in the NF- κ B pathway has given rise to controversies associated with efficacy as therapeutics. Mice lacking hepatocyte IKK β accelerated cancer instead of preventing progress of cancer. However, it is clear that pharmacological inhibition of IKK β appears to be beneficial to reduce HCC. This article will update issues of the NF- κ B pathway and inhibitors regulating this pathway.

Keywords: NF- κ B, IKK β , Inflammation, Cancer, Transglutaminase 2, NF- κ B inhibitor

NF- κ B AND I- κ B PROTEINS

There are five mammalian reticuloendotheliosis family/nuclear factor of κ B (REL/NF- κ B) proteins that belong to two groups: the first one that does not require proteolytic processing and the second one that requires proteolytic processing (Fig. 1). The first group includes RELA (p65), c-REL and RELB. The second group includes NF- κ B1 (p105) and NF- κ B2 (p100). p105 and p100 are processed to produce the mature p50 and p52 proteins, respectively. These two groups form dimers - the most commonly detected NF- κ B dimer is p65-p50. Due to the presence of a strong transcriptional activation domain, p65 is responsible for most of NF- κ B transcriptional activity. NF- κ B dimers are regulated by interactions with inhibitor of κ B (I- κ B) proteins. NF- κ B binding with I- κ B causes their cytoplasmic retention unless I- κ B is degraded in proteasome. RELB

associates with p100 in B-cells. The p100-RELB dimers exist exclusively in cytoplasm. Proteolytic processing of p100 results in the release of p52-RELB dimers, which translocate to the nucleus. RELB can have both activating

Fig. 1. NF- κ B and I- κ B proteins. RHD: rel homology domain, LZ: leucine zipper. The arrows point to the C-terminal residues of p50 and p52 (following processing of p105 and p100, respectively).

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and repressing functions. All NF- κ B proteins contain a REL homology domain (RHD) which mediates their dimerization as well as binding to DNA. The RHD also contains, at its carboxyl terminus, a nuclear localization signal (NLS) and is recognized by the I- κ B proteins, the binding of which to the RHD interferes with the function of the NLS. I- κ B proteins include I κ B α , I κ B β and I κ B ϵ that trap NF- κ B dimers in the cytoplasm. BCL3 acts as a transcriptional co-activator for p50 and p52 homodimers. Ubiquitinated BCL3 translocates into nucleus and enhances NF- κ B activation. Its activation is inhibited by CYLD. All I- κ Bs contain 6-7 ankyrin repeats, which mediate their binding to RHDs. The C-terminal halves of p105 and p100 are similar in sequence, structure and function to the I- κ Bs. Like the I- κ Bs, the C-terminal portions of p105 and p100 prevent nuclear entry, and are removed by ubiquitin-dependent degradation. Whereas the processing of p105 is constitutive, the processing of p100 is subject to regulation.

The NF- κ B activation pathways are classified into canonical and noncanonical pathways; the canonical pathway leads to the degradation of I- κ B, whereas the non-canonical pathway involves the processing of p100 to the mature subunit p52 (Pomerantz and Baltimore, 2002). The canonical pathway is activated by most NF- κ B stimulatory ligands, including proinflammatory cytokines such as tumor necrosis factor receptor (TNFR) and interleukin-1 β (IL-1 β), and microbial ligands such as bacterial lipopolysaccharides (LPS) and viral nucleic acids. These ligands bind to their receptors and trigger distinct signaling pathways that converge on a large kinase complex consisting of the catalytic subunits IKK α and IKK β (I κ B kinase α and β) and an essential regulatory subunit NEMO (NF- κ B essential modulator, also known as IKK γ or IKKAP). The IKK complex phosphorylates I κ Bs and targets these inhibitors for polyubiquitination and subsequent degradation by the proteasome. The liberated NF- κ B enters the nucleus to turn on the transcription of target genes.

The noncanonical pathway is activated by a subset of receptors in B cells, such as CD40 and B-cell activating factor receptor (BAFF-R). These receptors initiate a signaling cascade leading to activation of IKK α , which phosphorylates p100. Phosphorylated p100 is polyubiquitinated, and then its C-terminus is selectively degraded by the proteasome, sparing the N-terminal Rel homology domain to generate the mature p52 subunit. p52 forms a dimer with RelB, and the dimeric complex enters the nucleus to turn on the expression of genes that are important for B-cell maturation and activation.

In both canonical and noncanonical pathways, IKK is a key molecule to NF- κ B activation. Ubiquitination and deu-

biquitination play a central role in IKK regulation by diverse NF- κ B signaling pathways (Krappmann and Scheidereit, 2005).

NF- κ B ACTIVATION SIGNALING

Interleukin-1 receptor/Toll-like receptors (IL-1R/TLR)

NF- κ B is activated by infection that leads to the induction of IL-1 β , which potentiates NF- κ B activation. IL-1 β is an inflammatory cytokine that activates NF- κ B that is associated with effective immune responses against microbial infection (Dunne and O'Neill, 2003). IL-1R contains an intracellular signaling domain that is homologous to the intracellular domain of TLRs, which is a class of pattern-recognition receptors that recognize conserved microbial molecules, such as LPS and viral nucleic acids. Upon stimulation of cells with IL-1 β or LPS, the intracellular signaling domain, termed Toll and IL-1 Receptor (TIR) domain, recruits the adaptor protein MyD88 (myeloid differentiation primary response gene 88) containing a TIR domain. Consequently, MyD88 recruits the IL-1 receptor associated kinases (IRAK) such as IRAK4 and IRAK1. The recruited IRAK4 phosphorylates IRAK1, releasing IRAK1 into the cytosol, where it forms a complex with TIFA (TRAF-interacting protein with a forkhead associated domain) and TRAF6. Genetic experiments have shown that TRAF6 is essential for NF- κ B and MAP kinase activation by IL-1, CD40, LPS, and other TLR agonists. Self-polyubiquitination of TRAF6 events activates IKK activators 1 and 2 (TRIKA1/2). TRIKA1 is an ubiquitin-conjugating enzyme complex while TRIKA2 consists of the TGF- β -activated kinase (TAK1) and TAB1/2 (TAK1 binding protein1/2). TRIKA1 triggers poly-ubiquitination, which turns on TAK1 activation. TAK1 activation phosphorylates IKK β , which results in the activation of IKK complex (Deng *et al.*, 2000).

Tumor necrosis factor receptor (TNFR)

TNFR binds to two members of the TNF receptor superfamily, TNFR1 and TNFR2, initiate signaling cascades leading to activation of NF- κ B and MAP kinases, as well as apoptosis (Tartaglia and Goeddel, 1992). The signaling pathways initiated by TNFR1 have been most extensively studied. Upon binding to trimeric TNFR, TNFR1 becomes trimerized and recruits the adaptor protein, TRADD (TNFR1-associated death domain). TRADD further recruits the RING domain ubiquitin ligases TRAF2, TRAF5, cIAP1, and cIAP2, as well as the protein kinase RIP1 (receptor interacting protein kinase 1). RIP1 is rapidly polyubiquitinated at a specific lysine (K377) following TNFR stimulation (Ea *et al.*, 2006). Polyubiquitinated RIP1 acti-

vates IKK in a manner independent of its kinase activity. A point mutation of RIP1 at K377 abolishes its ubiquitination as well as its ability to recruit the TAK1 and IKK complexes to TNFR1, and it prevents IKK activation. Recent studies suggest that cIAP1 and cIAP2, ubiquitin ligases, may be more directly involved in the ubiquitination of RIP1 to activate NF- κ B signaling. On the contrary, there are opposite functional destination of cIAP that cIAP1 and cIAP2 target NIK for degradation. Consequently, IAP antagonists can induce NIK stabilization and lead to strong induction of classical and alternative NF- κ B signaling (Varfolomeev *et al.*, 2007). Therefore, the detailed mechanism of cIAP function remains to be elucidated.

T-cell receptors (TCRs)

T-cells, which are the central mediator of adaptive immune responses, are activated through the engagement of T-cell receptors (TCRs) by antigenic peptides presented by major histocompatibility complexes (MHCs) on the surface of antigen-presenting cells. Stimulation of TCRs activates a tyrosine kinase cascade, leading to activation of the serine/threonine kinase PKC θ (protein kinase θ). PKC θ triggers the formation of a complex termed CBM, which contains CARMA1 (caspase recruitment domain-containing membrane-associated guanylate kinase 1), BCL10 (B-cell lymphoma 10), and MALT1 (mucosa-associated lymphoid tissue lymphoma translocation gene 1) (Rawlings *et al.*, 2006). The CBM complex is essential for the activation of IKK through a mechanism involving K63 polyubiquitination. MALT1 contains binding sites for TRAF2 and TRAF6, and the binding of MALT1 to TRAF6 promotes the oligomerization of TRAF6, leading to activation of its ubiquitin E3 activity (Sun *et al.*, 2004). TRAF6 then functions together with Ubc13/Uev1A to catalyze K63 polyubiquitination of target proteins including BCL10, MALT1, NEMO, and TRAF6 itself (Wu and Ashwell, 2008). One or more of these polyubiquitination events activates the TAK1 kinase complex, which in turn activates IKK.

RIG-I-like receptors (RLRs)

Retinoic acid inducible gene I (RIG-I) is a cytosolic RNA helicase that binds to viral RNA and activates a signaling cascade leading to the induction of type-I interferons, such as IFN- β (Yoneyama and Fujita, 2008). Members of the RIG-I-like receptor (RLR) family include melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2). RIG-I and MDA5 contain N-terminal tandem CARD domains that activate the transcription factors NF- κ B, AP1, and IRF3, which form an enhanceosome complex to induce IFN- β . A key adaptor pro-

tein that links RIG-I and MDA5 to the downstream signaling cascade is MAVS (mitochondrial antiviral signaling; also known as IPS-1, VISA, or CARDIF), a CARD domain protein localized to the mitochondrial outer membrane. MAVS contains binding sites for TRAF2, TRAF3, and TRAF6. While TRAF2 and TRAF6 are likely to be important for IKK activation, TRAF3 mediates the activation of the IKK-like kinases, TBK1 and IKK β , which phosphorylate and activate IRF3 (Saha *et al.*, 2006).

DNA damage

DNA damage is known to activate NF- κ B, which promotes cell survival and provides a time window for DNA repair to proceed. NF- κ B activation by DNA damage may also contribute to resistance of cancer cells to chemo- and radiation therapies. Recent studies show that DNA damage, which occurs in the nucleus, activates the IKK complex in the cytosol through a complex mechanism involving modification of NEMO by ubiquitin and small ubiquitin-like modifier-1 (SUMO-1; Fig. 2). Sumoylated NEMO is phosphorylated by the kinase ATM (ataxia telangiectasia mutated) at Ser-25. Phosphorylated NEMO is then mono-ubiquitinated by unknown ubiquitination enzymes at the

Fig. 2. NF- κ B pathway. IL-1R/TLR: interleukin-1 receptor/Toll-like receptors, LPS: lipopolysaccharide, TLR: toll and IL-1 receptor, IRAK: IL-1 receptor associated kinases, TAK1: TGF- β -activated kinase, TAB: TAK1 binding protein, TNFR: tumor necrosis factor receptor, TRADD: TNFR1-associated death domain, RIP1: receptor interacting protein kinase 1, TCR: T-cell receptors, MHC: major histocompatibility complexes, MALT1: mucosa-associated lymphoid tissue lymphoma translocation gene 1, RIG-I: retinoic acid inducible gene I, ATM: ataxia telangiectasia mutated.

same lysines used for sumoylation (Wu *et al.*, 2006). Ubiquitinated NEMO together with ATM enters the cytoplasm, where they associate with IKK α , IKK β , and ELKS (a protein rich in glutamate, leucine, lysine, and serine). IKK is then activated by monoubiquitinated NEMO and ATM, but the biochemical mechanism remains to be elucidated.

Transglutaminase 2

Many reports have shown that transglutaminase 2 (TGase 2) expression is increased in the inflammatory diseases (Suh *et al.*, 2006). TGase 2 is a cross-linking enzyme that is widely used in many biological systems for general tissue stabilization purposes or immediate defenses for infection. Many reports showed that TGase 2 is aberrantly activated in tissues and cells and contributes to a variety of diseases, including neurodegenerative diseases, autoimmune diseases, and cancers (Kim, 2006; Lee *et al.*, 2006b). In most cases, TGase 2 appears to be a factor in the formation of inappropriate proteinaceous aggregates that may be cytotoxic enough to trigger inflammation and/or apoptosis. In some other cases such as celiac disease and rheumatoid arthritis, TGase 2 is also involved in the progress of inflammation as well as in the generation of autoantibodies. We discovered that increase of TGase 2 activity triggers NF- κ B activation without I- κ B kinase signaling (Lee *et al.*, 2004) (Fig. 3). TGase2 induces the polymerization of I- κ B through N^ε-(γ -glutamyl)-lysine cross-linking rather than stimulating I- κ B kinase (Park *et al.*, 2006). This polymerization of I- κ B results in direct activation of NF- κ B in various cell lines. We also found that TGase inhibition reverses NF- κ B activation. Interestingly, it coincides with reverse of inflammatory conjunctivitis, lung inflammation, encephalitis, and cystic fibrosis using specific TGase 2 inhibitors. Furthermore, TGase 2 inhibition resulted in increased sensitivity to chemotherapeutic drugs, which may represent a potentially useful treatment of certain cancers (Kim *et al.*, 2006b).

NEGATIVE REGULATION OF NF- κ B

CYLD

The cylindromatosis gene *Cyld* encodes a tumor suppressor protein involved in the development of familial cylindromatosis, a benign skin tumor (Trompouki *et al.*, 2003). The CYLD protein contains a USP domain at the C-terminus, and many of the tumor-associated mutations identified in human patients impair the deubiquitination activity of CYLD. Overexpression of CYLD inhibits IKK activation, whereas reducing CYLD expression has the opposite effect (Trompouki *et al.*, 2003). CYLD contains three

Fig. 3. TGase 2 activation exacerbates NF- κ B activation. TGase 2 can be induced by various stresses including LPS, oxidative stress, UV, calcium ionophores, retinoic acid, inflammatory cytokines, and viral infection. Intermediates of oxidative stress and reactive oxygen have been shown to increase TGase 2 expression. TGase 2 expression appears to be induced directly by NF- κ B activation because the TGase 2 promoter has an NF- κ B binding motif. This is an intriguing finding because many stimuli that trigger NF- κ B activation, such as viral infection, UV, oxidative stress, and inflammatory cytokines, in addition to LPS (see review Kim, 2006).

CAP-Gly domains (cytoskeleton-associated proteins glycine rich domains) at the N-terminus, which mediate binding to TRAF2 and NEMO. CYLD specifically cleaves K63-linked polyubiquitin chains from target proteins including NEMO, TRAF2, and TRAF6. CYLD also inhibits viral induction of type-I interferons by removing K63 polyubiquitin chains from RIG-I (Friedman *et al.*, 2008).

A20

A20 is rapidly induced by NF- κ B, and it potently inhibits NF- κ B to provide a negative feedback loop such as I- κ B α (Krikos *et al.*, 1992). It has been proposed that the ovarian tumor-type proteases (OUT) domain of A20 inhibits IKK by removing K63 polyubiquitin chains from target proteins including RIP1 and TRAF6 (Boone *et al.*, 2004). A second mechanism of IKK inhibition by A20 is mediated through its C-terminal zinc finger domains, which function as an E3 to promote K48-linked polyubiquitination of RIP1 and target it for degradation by the proteasome (Wertz *et al.*, 2004). Genetic deletion of A20 in mice causes persistent activation of NF- κ B in response to TNFR and TLR stimulation,

leading to multi-organ inflammation, cachexia, and neonatal lethality (Lee *et al.*, 2000).

CANCER AND NF-κB

An important signaling pathway that is often altered in human cancers is the I-κB kinase (IKK)/nuclear factor κB (NF-κB) signaling. Aberrant NF-κB regulation has been observed in many cancers, including both solid and hematopoietic malignancies (Karin, 2006). NF-κB affects all hallmarks of cancer through the transcriptional activation of genes associated with self-sufficiency in proliferative growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, acquisition of limitless replicable potential, induction of angiogenesis, induction of invasion and meta-

Fig. 4. NF-κB target genes involved in cancer development and progression. A20: zinc finger protein A20 (also TNFAIP3), BCL2: B-cell lymphoma protein 2, BCL-XL: also known as BCL2-like 1, BFL1: also known as BCL2A1, CDK2: cyclin-dependent kinase 2, COX2: cyclooxygenase 2, CXCL: chemokine (C-X-C motif) ligand, ELAM1: endothelial adhesion molecule 1, FLIP: also known as CASP8, GADD45β: growth arrest and DNA-damage-inducible protein β, HIF1α: hypoxia-inducible factor 1α, ICAM1: intracellular adhesion molecule 1, IEX-1L: radiation-inducible immediate early gene, IL: interleukin, iNOS: inducible nitric oxide synthase, KAL1: Kallmann syndrome 1 sequence, MCP1: monocyte chemoattractant protein 1 (also CCL2), MIP2: macrophage inflammatory protein 2, MMP: matrix metalloproteinase, MnSOD: manganese superoxide dismutase (also SOD2), TNF: tumour necrosis factor, TRAF: TNF receptor-associated factor, uPA: urokinase plasminogen activator, VCAM1: vascular cell adhesion molecule 1, VEGF: vascular endothelial growth factor, XIAP: X-linked inhibitor of apoptosis protein.

stasis (Fig. 4). Various mouse models of cancer in which IKK/NF-κB activation has been blocked by genetic means have highlighted the key role of NF-κB as a crucial promoter of inflammation linked cancers (Karin, 2008).

Constitutive NF-κB activation

In normal cells, NF-κB activity is under tight control, in that its nuclear translocation and activation is transient, due to negative feedback inhibition by de novo synthesis of the I-κB and precursor molecules (e.g. p105, p100). However, persistent NF-κB activation has been reported in many human cancers, including multiple myeloma, chronic lymphocytic leukemia, Hodgkin's disease, and solid tumors (Jost and Ruland, 2007). Such persistent NF-κB activity could derive from genetic mutations or over-expression in the NF-κB signaling components in tumor cells, which bypass the negative feedback inhibition, as suggested by recent oncogenomic studies on multiple myeloma cell lines and patient samples (Keats *et al.*, 2007). Persistent NF-κB activation contributes to the tumorigenic process by inducing the expression of cell cycle proteins, anti-apoptotic molecules, autocrine and paracrine cyto-

Anti-Cancer Drug Activates NF-κB

Anti-cancer drug	Cell lines	Reference
Irinotecan	HeLa, MCF-7, HT1080, Colon cancer	Bottero <i>et al.</i> , 2001
Doxorubicin	HeLa	Bottero <i>et al.</i> , 2001
Etoposide	BxPC-3, PT45-P1, Capan-1	Arlt <i>et al.</i> , 2001
Gemcitabine	A549, H157	Denlinger <i>et al.</i> , 2004
Sodium butyrate & Suberoylanilide Hydroxamic acid	U937	Gao <i>et al.</i> , 2004
Suberoylanilide Hydroxamic acid	A549, H157, H358, H460, H1299	Rundall <i>et al.</i> , 2004
Cisplatin	293T	Sanchez-Perez <i>et al.</i> , 2002
Daunorubicin, Vinblastine	A549	Das <i>et al.</i> , 1997
Irinotecan, SN38 Etoposide Actinomycin D	ACH-2, CEM T	Piret and Piette, 1996
Daunorubicin	HT1080	Wang <i>et al.</i> , 1996
Vinblastine	HBL100, HeLa S3	Rosette and Karin, 1995
Sodium butyrate & Trichostatin A	H157, H358, A549	Mayo <i>et al.</i> , 2003
Sodium butyrate & Trichostatin A	HeLa	Adam <i>et al.</i> , 2003
Doxorubicin	Ikk1/Ikk2 -/- MEF	Tergaonkar <i>et al.</i> , 2003
Paclitaxel, Gemcitabine	MIA-PaCa 2	Fahy <i>et al.</i> , 2004

Fig. 5. NF-κB activation by anti-cancer drug treatment in various cancer cell lines.

kines, chemokines, and adhesion molecules. These collective changes support tumor growth survival, migration, angiogenesis, and drug resistance. Cumulative studies in B cell tumors have also shown that different tumor subsets may utilize either the "classical" or "alternative" NF- κ B pathway for survival and drug resistance. Therefore, it will be necessary to develop therapies for both pathways to be tailored to specific tumor subset. This implicates that we have to evaluate very carefully the NF- κ B activation in cancer. Sometimes it progresses together with inflammation, but NF- κ B activation in cancer is not always associated with classical inflammatory pathogenesis. That could be an answer why chronic inflammation by viral infection does not effect on triggering mice cancer as efficient as it does in human. Most cancer does not require chronic inflammation for long period although inflammatory disease appears to be responsible for certain types of cancers. However, NF- κ B activation is common phenomena in the most cancers without inflammation (Fig. 5). Therefore, we believe that constitutive activation of NF- κ B may have its own novel paradigm independent of normal physiological paradigm.

NF- κ B induction by viral infection

There are several mechanisms by which NF- κ B transcription factors are uncoupled from their normal modes of regulation, and these have been associated with cancer. For example, the avian REV-T oncovirus produces the constitutively active v-REL oncoprotein, which causes rapidly progressing lymphomas and leukaemias (Gilmore, 1999). The TAX oncoprotein of human T-cell leukaemia virus (HTLV)-1 has been shown to directly interact with and constitutively activate the IKK complex, which results in the activation of both NF- κ B signalling pathways (Xiao *et al.*, 2001). Other viral oncoproteins have also been shown to activate NF- κ B by means of different mechanisms (Mosialos, 1997).

Genetic mutation in NF- κ B pathway

In general, loss of suppressor function against NF- κ B activation such as loss of I- κ B α function dominates in the most cancer cases. The loss of function does not fully count on mutation of suppressor genes that covers about 10% of total NF- κ B dysfunction. Gain of function mutations was identified in genes encoding positive regulators of NF- κ B signaling, including NIK, NFKB1 (encoding p105/p50), and NFKB2 (encoding p100/p52). Three receptors of the TNF receptor (TNFR) superfamily, CD40, LT β receptor (LTBR) and TACI (also known as TNFRSF13B), were also identified. Loss of function mutations occurs in

genes encoding negative regulators of NF- κ B activity, including TNFR associated factor 2 (TRAF2; a negative regulator of the alternative NF- κ B pathway but a positive regulator of the classical NF- κ B pathway), TRAF3, cIAP1/2 and Cyld. Most of these genetic alterations result in the activation of both the classical and alternative NF- κ B pathways as evaluated by p100 processing, p52 nuclear localization, I- κ B α phosphorylation, increased p65, p50, RelB and p52 DNA binding activity, and increased NF- κ B target gene expression (Keats *et al.*, 2007).

A lesson from paradox in transgenic mouse study

An association between inflammation and cancer has been noted by more recent epidemiological data that led to the estimate that approximately 20% of cancer deaths are linked to chronic infections and persistent inflammation (Kuper *et al.*, 2000). Primary examples are gastric cancer and *Helicobacter pylori* infections (Roder, 2002), hepatocellular carcinoma (HCC) and viral hepatitis (Fattovich *et al.*, 2004) and colitis-associated cancer (CAC) (Ekbom, 1998).

One of the most common inflammation-linked cancers, thought to be the third leading cause of cancer deaths is HCC. Although liver-targeted oncogenes can induce HCC development in mice (Lewis *et al.*, 2005), a chemical carcinogen, diethyl nitrosamine (DEN), also easily induce highly penetrate HCC in mice whose gene expression profile is very similar to that of aggressive human HCC (Thorgeirsson and Grisham, 2002). Therefore if IKK β pathway could be blocked, less HCC induction would be expected. Surprisingly, Ikk $\beta^{\Delta\text{hep}}$ mice (mice lacking hepatocyte IKK β) were found to develop more and faster growing HCCs than control mice (Maeda *et al.*, 2005). Although inactivation of IKK β in hepatocytes enhanced development of HCC by increasing the extent of carcinogen-induced liver injury, inactivation of IKK β in inflammatory cells inhibited the development of HCC, as with CAC (Maeda *et al.*, 2005).

NF- κ B and chemotherapy

Although chemotherapeutic agents have been successfully used in treating patients with many different types of cancer, acquisition of resistance to the cytotoxic effects of chemotherapy has emerged as a significant impediment to effective cancer treatment. Most chemotherapy agents trigger the cell-death process through activation of the tumor suppressor protein p53. However, NF- κ B is also activated in response to treatment with cytotoxic drugs, such as taxanes, *Vinca* alkaloids and topoisomerase inhibitors (Fig. 5).

The NF- κ B pathway impinges on many aspects of cell

growth and apoptosis. For example, in HeLa cells, the topoisomerase I inhibitor SN38 (7-ethyl-10-hydroxycamptothecin), which is an active metabolite of irinotecan, and the topoisomerase II inhibitor doxorubicin both induce NF- κ B nuclear translocation and activation of NF- κ B target genes directly through mobilization and stimulation of the IKK complex, but not through the secondary production of NF- κ B activators such as cytokines, leading to cell survival (Bottero *et al.*, 2001).

NEW NF- κ B PARADIGM

TGase 2 pathway

As you see in Fig. 5, NF- κ B activation following to chemotherapy is mostly due to constitutive activation of IKK instead of constitutive expression of upstream signaling molecules such as IL-6 or TNF. Therefore, many reports suggest that there may be alternative pathways to trigger IKK activity without phosphorylation/ubiquitination (Lee *et al.*, 2004). However, we introduced one novel mechanism that is mediated by TGase 2, in order to explain the mechanism for activation of NF- κ B without phosphorylation/ubiquitination. TGase 2 induces the polymerization of I- κ B rather than stimulating I- κ B kinase (Park *et al.*, 2006). This polymerization of I- κ B results in direct activation of NF- κ B in various cell lines. We also found that TGase inhibition reverses NF- κ B activation. Furthermore, TGase 2 inhibition resulted in increased sensitivity to chemotherapeutic drugs, which may represent a potentially useful treatment of certain cancers (Kim *et al.*, 2006b; Kim *et al.*, 2009c).

Warburgh effect

Both basal level expression of p53 and activation of p53 by genotoxic stress lead to inhibition of the kinase activities of IKK α and IKK β and the transcriptional activity of NF- κ B. Deficiency of p53 enhances NF- κ B-dependent glucose metabolism, which is partially dependent on GLUT3. Moreover, in cells that evade a p53-mediated tumor surveillance system, oncogene induced cell transformation requires NF- κ B activation. Therefore, the positive-feedback loop by which glycolysis drives IKK-NF- κ B activation may have an important role in oncogenesis. p53 restricts IKK-NF- κ B activation and subsequently suppresses glycolysis through down-regulation of NF- κ B-dependent genes including GLUT3, down-regulation of PGM18 and up-regulation of TIGAR (Bensaad *et al.*, 2006), and increases mitochondrial respiration through up-regulation of SCO2 (Matoba *et al.*, 2006). When p53 function is lost, oncogene expression increases the rate of glycolysis and decreases

the rate of mitochondrial respiration - the Warburg effect - in transformed cells. So how does p53 suppress the kinase activities of IKK α and IKK β ? Although it is possible that IKKs activities are directly suppressed by a p53 target gene(s), an alternative explanation is that p53 restricts the activation of the IKK-NF- κ B pathway through suppression of aerobic glycolysis.

NF- κ B AS A DISEASE TARGET

Chronic inflammation has been involved in the pathogenesis of cancer, acute lung injury, muscle wasting diseases, insulin resistance and type 2 diabetes mellitus, neurodegenerative disorders, inflammatory bowel disease, autoimmune diseases (e.g. rheumatoid arthritis), atherosclerosis and various symptoms of aging. Infiltrating leukocytes and activated stromal cells are the major sources for inflammatory cytokines in chronic inflammation.

Cancer

NF- κ B, as an antiapoptotic factor, crucially affects the outcomes of cancer treatments, being one of the major causes of resistance to chemotherapy. One of the famous drugs in targeted therapy of cancer, Gleevec also developed the drug resistance via NF- κ B activation. IKK2 inhibitor AS602868 prevented survival of BaF cells expressing either wild-type, M351T or T315I imatinib-resistant mutant forms of Bcr-Abl in a murine xenograft model (Lounnas *et al.*, 2009). Dehydroxymethylepoxyquinomicin (DHMEQ) inhibited the constitutively activated NF- κ B in JCA-1 cells and, given intraperitoneally (i.p.), it suppressed re-established JCA-1 subcutaneous tumor growth in nude mice (Kuroda *et al.*, 2005). Pristimerin, a dual inhibitor of IKK and proteasome is potently and selectively lethal to primary myeloma cells (IC₅₀ < 100 nM) and inhibits xenografted plasmacytoma tumors in mice, thereby providing the rationale for pharmaceutical development of triterpenoid dual-function proteasome/NF- κ B inhibitors as therapeutics for human multiple myeloma (Tiedemann *et al.*, 2009).

It is also alternatively proposed that the inhibition of TGase 2 is one of novel mechanisms for blocking activation of NF- κ B (Kim *et al.*, 2006b). When one of well-known TGase 2 inhibitors, KCC009, was co-administered with the chemotherapeutic N, N'-bis(2-chloroethyl)-N-nitrosourea (BCNU) to glioblastoma xenografted mice, the co-treatment decreased the size of subcutaneously implanted glioblastoma tumors in mice by 50% compared to controls treated with BCNU only (Choi *et al.*, 2005).

Inflammatory bowel disease

The origin of some chronic inflammatory disorders, such as inflammatory bowel disease (IBD) or Crohn's disease, is unknown. IBD is characterized by chronic inflammation of the intestinal mucosa and is a risk factor for the development of colon cancers. Hyperactivated NF- κ B in intestinal epithelial cells, lymphocytes and myeloid cells is accompanied by uncontrolled inflammatory cytokine production and colon tissue damage. The role of NF- κ B as the positive regulator of colon inflammation and colon cancer development is shown in the azoxymethane- and dextran sulfate-induced colitis-associated cancer models in mice. In case of IBD, NF- κ B activation is observed in mutation of NOD1 (Strober *et al.*, 2006). Simvastatin prohibits NF- κ B signaling in intestinal epithelial cells and improves acute murine colitis (Lee *et al.*, 2007b).

Diabetes

Inflammation is also related to the metabolic disorders of insulin resistance and type 2 diabetes mellitus. Using knockout mouse models, both IKK2/NF- κ B have been shown to be implicated in the etiology of insulin resistance and type 2 diabetes mellitus (Arkan *et al.*, 2005). It has been hypothesized that obesity, type 2 diabetes and inflammation are inter-connected. Eldor *et al.* (2006) showed that conditional and specific NF- κ B blockades nearly completely protect against multiple low-dose streptozocin-induced diabetes with reduced intra-islet lymphocytic infiltration.

Rheumatoid arthritis

The canonical NF- κ B pathway is constitutively activated in synovial tissue of rheumatoid arthritis (RA) patients (Handel *et al.*, 1995). In animal models NF- κ B activation drives the induction of proinflammatory genes and synovial hyperplasia through its anti-apoptotic properties (Miagkov *et al.*, 1998). Oral administration of ML102B, a potent, small molecule inhibitor of IKK-2, suppressed both clinical and histopathologic manifestations of disease. In vivo imaging data showed that NF- κ B activity in inflamed arthritic paw was inhibited by ML120B, resulting in significant suppression of multiple genes in the NF- κ B pathway such as TNF α , IL-1 β , IL-6, inducible nitric oxide synthase, cyclooxygenase 2, matrix metalloproteinase 3, cathepsin B and cathepsin K (Izmailova *et al.*, 2007).

Asthma

Several lines of evidences imply enhanced NF- κ B pathway activation in asthmatic tissues. Peripheral blood mononuclear cells (PBMCs) of adult uncontrolled, severe

and moderate asthmatics have higher levels of NF- κ B p65 protein expression, I- κ B phosphorylation and IKK- β protein levels than normal individuals (Gagliardo *et al.*, 2003). Also in children, NF- κ B p65 protein is more abundant and I- κ B phosphorylation level is higher in moderate asthmatic PBMCs than in normal individuals (La Grutta *et al.*, 2003). The effect of inhibiting IKK-2 was assessed in stimulated, cultured, primary human airway smooth muscle cells and an antigen-driven rat model of lung inflammation. In an in vivo antigen-driven model of airway inflammation, the IKK-2 inhibitor blocked NF- κ B nuclear translocation, which was associated with a reduction in inflammatory cytokine gene expression, airway eosinophilia, and late asthmatic reaction, in a similar magnitude to that obtained with budesonide (Birrell *et al.*, 2005). Desmet *et al.* (2004) showed the efficacy of NF- κ B decoy oligonucleotides in ovalbumin (OVA) induced allergic airway inflammation model. The selective IKK- β inhibitor TCPA-1 reduced TNF- α and IL-1 β , eotaxin, and Th2 cytokines IL-4, IL-5 and IL-13 induced by OVA in a rat model of allergen sensitisation and challenge (Birrell *et al.*, 2005).

Gastritis & systemic lupus erythematosus

Activation of I- κ B kinase and NF- κ B is essential for *Helicobacter pylori*-induced chronic gastritis in Mongolian gerbils. Treatment with IKK β inhibitor resulted in milder inflammation in gerbils with *H. pylori* gastritis and infiltration of neutrophils and mononuclear cells decreased markedly in the animals treated with IKK β inhibitor IMD-0354 (Yanai *et al.*, 2008). Systemic lupus erythematosus (SLE) is a multisystem disease that is caused by antibody production and complement fixing immune complex deposition that leads to tissue damage (Kurylowicz *et al.*, 2008). Patients with SLE show constitutively high plasma levels of the B cell activating factor (BAFF) that is known to induce a non-canonical NF- κ B signaling pathway. DCB-3503, a synthetic compound derived from a natural product that inhibits NF- κ B nearly abrogated inflammatory skin disease in MRL/Fas(lpr) murine model of systemic lupus erythematosus (SLE) (Choi *et al.*, 2006a).

Sepsis

LPS released by gram-negative bacteria is the most frequent cause of septic shock, affecting ~ 400,000 patients in the United States annually. LPS is known to activate complexes of proteins which bind to NF- κ B-regulatory DNA sequences. Members of the NF- κ B family, induced by cytokines or directly by reactive oxygen intermediates, are believed to play a central role in LPS-mediated lethality (Zuckerman *et al.*, 1991). Cell-permeable dominant in-

hibitor of NF- κ B activation, termed I- κ B α -(DeltaN)-MTS, contains a 12 amino acid MTS motif attached to the COOH-terminal region of a nondegradable inhibitor protein [I- κ B α -(DeltaN)]. Inhibition of NF- κ B by I- κ B α -(DeltaN)-MTS in the endotoxin model attenuated physiological responses to endotoxemia (Mora *et al.*, 2005). Most recently, two selective IKK β inhibitors have been studied more extensively *in vivo*. Oral administration of ML120B in mice was shown to inhibit NF- κ B activation in different organs such as lung, liver and lymphoid organs upon LPS stimulation.

Multiple sclerosis

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) in which myelin and myelin-forming cells (oligodendrocytes) become the target of an inflammatory response, resulting in their drainage from the MS plaque. In active MS lesions, double-label immunohistochemistry with oligodendrocyte markers showed up-regulation of the nuclear staining of both NF- κ B and JNK on a large proportion of oligodendrocytes located at the edge of active lesions and on microglia/macrophages throughout plaques (Bonetti *et al.*, 1999). Pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF- κ B activation, markedly inhibited the *in vivo* activation of NF- κ B in the spinal cord of EAE rats and attenuated the clinical symptoms of allergic encephalomyelitis (EAE), an animal model of MS, from the onset of the disease (Pahan and Schmid, 2000). A fusion peptide corresponding to the NEMO-binding domain (NBD) of IKK β and the cell permeability domain can be used as IKK inhibitors (May *et al.*, 2000), because prevention of NEMO binding to IKK β and regular complex formation results in inactivation of canonical NF- κ B signaling. Indeed, the NBD peptide was shown to inhibit inflammation *in vivo* in experimental allergic encephalomyelitis (Gilmore and Herscovitch, 2006).

Ischemia

Several lines of evidence illustrate that cerebral ischemia is associated with the infiltration of inflammatory cells into the ischemic lesion, which is preceded by increased expression of proinflammatory mediators such as cytokines, chemokines, and adhesion molecules (Sironi *et al.*, 2006). Proinflammatory genes are mainly controlled by NF- κ B, which is also upregulated in experimental stroke, although its role in neurodegeneration is still controversial (Nurmi *et al.*, 2004). Daily intraperitoneal injections of IMD-0560 (5 mg/kg), a novel inhibitor of IKK, significantly improved cardiac function in a rat myocardial ischemia model, which is indicated by the preservation of fractional

shortening and lower serum brain natriuretic peptide level. Histological analysis demonstrated that IMD-0560 treatment suppressed thinning in the infarcted area compared with vehicle-treated hearts (Wakatsuki *et al.*, 2008).

INHIBITORS TARGETING NF- κ B ACTIVITY

As shown in above, the roles of NF- κ B in cancers and chronic inflammatory disorders implicate that blocking NF- κ B signaling may be useful in the treatment of these disorders. The ability of more than 200 different stimuli to activate NF- κ B and the large number of target genes turned on by NF- κ B in many cell types make the design of pathway-specific inhibitors a difficult task. More than 700 compounds have been reported to inhibit NF- κ B activation (Gilmore and Herscovitch, 2006). These NF- κ B inhibitors may be classified into those that block signalling upstream of IKK, at the IKK step, at the I- κ B degradation step, and the nuclear function of NF- κ B subunits. We adopted the classifications of NF- κ B inhibitors by Gilmore in 2006 and added new inhibitors (approximately 50 compounds) since 2006. Typical inhibitors and their target sites are summarized in Fig. 6.

Fig. 6. Schematic representation of inhibitory compounds of NF- κ B activation.

Inhibitors that act on upstream targets of IKK complex

Agents that affect upstream targets of the IKK complex include inhibitors of receptor activation by natural ligand, inhibitors of adaptor protein recruitment to the receptor and inhibitors of IKK-activating kinases. In most cases, the IKK complex is the first common node in the integration of many NF- κ B activating pathways. Therefore, one strategy for inhibiting activation of NF- κ B is to block a signal before

it activates IKK. These inhibitors include medicinal chemistry-based synthetic compound, purified compounds from natural sources, and proteins or peptide from biochemical approaches.

Interestingly, PMX464, a novel thiol-reactive quinol, was originally found to inhibit the Trx/TrxR system. Later, PMX464 turned out to inhibit IL-1 β -induced NF- κ B DNA binding, nuclear translocation of NF- κ B p65 subunit and

Table I. Upstream NF- κ B inhibitors

Molecule (type)	Point of inhibition	Molecule (type)	Point of inhibition
Acacetin (N)	PI3K/Akt/IKK and MAPK (Pan <i>et al.</i> , 2006)	Fisetin (N)	TAK-1 (Sung <i>et al.</i> , 2007)
CM-glucosamine (S)	MAPK (Rajapakse <i>et al.</i> , 2008)	Lutein (N)	PI3K/PTEN/Akt (Kim <i>et al.</i> , 2008d)
Cis-9,trans-11-conjugated linoleic acid (N) 	IKK & PI3K-Akt (Hwang <i>et al.</i> , 2007)	Morusin (N)	PDK1 (Lee <i>et al.</i> , 2008)
Denbinobin (N)	TAK-1 (Sánchez-Duffhues <i>et al.</i> , 2009)	PMX464 (N)	Upstream of IKK (Callister <i>et al.</i> , 2008)
Desmethylanhydroicaritin (N)	PI3K/PTEN/Akt (Kim <i>et al.</i> , 2009d)	SH-5 (S)	Akt (Sethi <i>et al.</i> , 2008b)
Ergolide (N)	PKC-alpha (Chun <i>et al.</i> , 2007)	Tubocapsanolide A (N)	TAK1 (Pan <i>et al.</i> , 2009)
Gambogic acid (N)	Inhibition of TAK1/TAB1 (Pandey <i>et al.</i> , 2007)	WDR34 (P) 	TAK1 (Gao <i>et al.</i> , 2009)

N: natural product, P: protein, S: synthetic, CM glucosamine: carboxybutyrylated glucosamine.

factors involved in NF- κ B activation; specifically, I- κ B α degradation, I- κ B phosphorylation and IKK activity in A549 (Callister *et al.*, 2008). Incensole acetate (IA) and its non-acetylated form, incensole (IN) from *Boswellia* resin inhibited TAK/TAB-mediated IKK activation loop phosphorylation, resulting in the inhibition of cytokine and lipopolysaccharide-mediated NF- κ B activation. It had no effect on IKK activity in vitro (Moussaieff *et al.*, 2007).

The finding that transferrin receptor is reported to be a new player in NF- κ B signaling pathway is elucidated by the mechanism of Gambogic acid (GA) against NF- κ B activation. That is GA, a xanthone derived from the resin of the *Garcinia hanburyi*, suppressed NF- κ B activation induced by various inflammatory agents and carcinogens. The NF- κ B activation induced by TNFR1, TRADD, TRAF2, NIK, TAK1/TAB1, and IKK β was also inhibited. The effect of GA mediated through transferrin receptor as down-regulation of the receptor by RNA interference reversed its effects on NF- κ B and apoptosis (Pandey *et al.*, 2007).

Other several inhibitors were reported to act on upstream targets of IKK complex, as listed in Table I.

Inhibitors of IKK complex activities

IKK has been considered as a prime target for the development of NF- κ B signaling inhibitors, in part due to its central role in funneling upstream signals into the NF- κ B activation pathways and in part due to other successes in developing kinase inhibitors for therapeutic applications (Gilmore and Herscovitch, 2006). There are over 170 compounds that have been shown to inhibit activation of NF- κ B at the IKK step based either on the lack of I- κ B α phosphorylation or on the lack of stimulus-induced IKK activity in immune complex kinase assays. In an overall view, IKK inhibitors developed from medicinal chemistry-based approach display superior inhibitory activities, whereas IKK inhibitors from natural products show diverse chemical skeletons which give new insights to medicinal chemists.

PHA-408 was identified from a tricyclic pyrazole chemical series with potent activity against recombinant human (rh) IKK-2. PHA-408 was described as a highly selective IKK-2 inhibitor with 350-fold selectivity over rhIKK-1 and no inhibitory activity against a panel of over 30 tyrosine and serine/threonine kinases (Mbalaviele *et al.*, 2009). NSC 676914 which was originally identified in a high-throughput cell-based assay for activator protein-1 (AP-1) inhibitors using synthetic compound libraries and the National Cancer Institute (NCI) natural product repository, is shown to repress 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced I- κ B α phosphorylation and translocation of p65/50 to the nucleus but not the processing of p52 from p100,

suggesting the inhibition of NF- κ B regulator IKK β rather than IKK α (Kang *et al.*, 2009). BOT-64 inhibits NF- κ B activation with an IC₅₀ value of 1 μ M by blocking I- κ B kinase β in LPS-activated RAW 264.7 macrophages, resulting in sequential prevention of downstream events, including proteolytic degradation of I- κ B, DNA binding ability, and transcriptional activity of NF- κ B (Kim *et al.*, 2008a). KINK-1, a novel class of small-molecule substances, was derived from a large-scale high-throughput screening. KINK-1 specifically inhibited the kinase activity of human recombinant IKK β with a K_i of 2 nM for ATP and 4 nM for the GST-I- κ B α substrate (Schon *et al.*, 2008).

17-Acetoxyjolkolide B from *Euphorbia fischeriana* Steud (Euphorbiaceae) kept IKK in its phosphorylated form irreversibly. This irreversible modification of IKK inactivated its kinase activity, leading to its failure to activate NF- κ B. The effect of 17-AJB on IKK was specific (Yan *et al.*, 2008). Humulone, a bitter acid derived from hop (*Humulus lupulus* L.), blunted TPA-induced activation of IKK in mouse skin, which accounts for its suppression of phosphorylation and subsequent degradation of I- κ B α . An in vitro kinase assay revealed that humulone could directly inhibit the catalytic activity of IKK β (Lee *et al.*, 2007a). Artemisolide was isolated as a NF- κ B inhibitor from a plant of *Artemisia asiatica* and is a typical inhibitor of IKK β , resulting in inhibition of LPS-induced NF- κ B activation in RAW 264.7 macrophages. Moreover, the effect of Artemisolide on IKK β activity was completely abolished by substitution of Cys-179 residue of IKK β to Ala residue, indicating direct targeting site of Artemisolide (Kim *et al.*, 2007). The semi-synthetic triterpenoid 1-[2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oyl]imidazole (CDDO-lm) dose dependently inhibited the in vitro kinase activity of IKK β (Yore *et al.*, 2006).

Tautomycetin (TC) was isolated from *Streptomyces grieseochromogenes* as an antifungal antibiotic. TC inhibited T-loop phosphorylation of IKK α and IKK β , thereby preventing degradation of the I- κ B α (Mitsuhashi *et al.*, 2008). The molecule mentioned here and other several inhibitors of IKKs were listed in Table II.

Agents that stabilize I- κ B or block its degradation

Several molecules are known to inhibit NF- κ B by maintaining a high level of I- κ B proteins in the cytoplasm, thereby preventing NF- κ B nuclear translocation. Among these molecules, some promote synthesis of I- κ B α , or inhibit I- κ B α ubiquitination, while others block I- κ B α degradation (Gilmore and Herscovitch, 2006). The successful story of bortezomib incites researchers to develop proteasome inhibitors.

Table II. IKK activity and I- κ B phosphorylation inhibitors

Molecule (type)	Point of inhibition	Molecule (type)	Point of inhibition
17-Acetoxyjolkynolide B (N)	IKK (Yan <i>et al.</i> , 2008)	Miyabenol A (N)	IKK (Ku <i>et al.</i> , 2008)
ABIN-1 (P)	IKK γ (Mauro <i>et al.</i> , 2006)	NBD (P)	IKK γ (Dave <i>et al.</i> , 2007)
Delphinidin (N)	IKK γ (Hafeez <i>et al.</i> , 2008)	NSC 676914 (S)	IKK β (Kang <i>et al.</i> , 2009)
Eupatilin (N)	Hsp90, IKK α , IKK γ (Kim <i>et al.</i> , 2009e)	Piinitol (N)	IKK α (Sethi <i>et al.</i> , 2008a)
Ginsenoside Rp1 (N) R ₁ = Glc-Glc- R ₂ = Glc	Downregulation of IKK (Kim <i>et al.</i> , 2009a)	^a Seliciclib (S)	IKK (Dey <i>et al.</i> , 2008)
Humulone (N)	IKK β (Lee <i>et al.</i> , 2007a)	^b TPCA-1 (S)	IKK (Kondo <i>et al.</i> , 2008)
PHA-408 (S)	IKK (Sommers <i>et al.</i> , 2009)	Tautomycetin (N)	IKK (Mitsuhashi <i>et al.</i> , 2008)
PF-184 (S)	IKK (Sommers <i>et al.</i> , 2009)	Flavokavain A (N)	IKK (Folmer <i>et al.</i> , 2006)
*IMD-0560 (S)	IKK (Wakatsuki <i>et al.</i> , 2008)	^c BOT-64 (S)	IKK (Kim <i>et al.</i> , 2008a)

Table II. Continued

Molecule (type)	Point of inhibition	Molecule (type)	Point of inhibition
Incensole acetate (N)	IKK (Moussaieff <i>et al.</i> , 2007)	Artemisolide (N)	IKK (Kim <i>et al.</i> , 2007)
Isoliquiritigenin (N)	IKK (Kim <i>et al.</i> , 2008e)	Ertiprotafib (S)	IKK (Shrestha <i>et al.</i> , 2007)
KINK-1 (S)	IKK (Schon <i>et al.</i> , 2008)	CDDO-Im (S)	IKK β (Yore <i>et al.</i> , 2006)

N: natural product, P: protein, S: synthetic, *IMD-0560: N-[2,5-bis-trifluoromethylphenyl]-5-bromo-2-hydroxybenzamide, ^aSeliciclib: R-Roscovitine, CYC202, ^bTPCA-1: [5-(p-fluorophenyl)-2-ureido]thiophene-3-carboxamide, ^cBOT-64: Benzoxathiole (6,6-dimethyl-2-phenylimino)-6,7-dihydro-5H-benzo-[1,3]oxathiol-4-one.

Table III. Inhibitors of I- κ B degradation, proteasome and protease

Molecule (type)	Point of inhibition	Molecule (type)	Point of inhibition
Antiprotealide (N)	Proteasome (Manam <i>et al.</i> , 2009)	Pristimerin (N)	IKK, proteasome (Tiedemann <i>et al.</i> , 2009)
Carfilzomib (S)	Proteasome (Kuhn <i>et al.</i> , 2007)	*GS143 (S)	Ubiquitinylation (Nakajima <i>et al.</i> , 2008)
		ChlaDub1 (P)	I- κ B α ubiquitination & degradation (Le Negrat <i>et al.</i> , 2008)

N: natural product, P: protein, S: synthetic, *GS143: (4-{3-benzyl-4-[5-(2-fluoro-phenyl)-furan-2-ylmethylene]-5-oxo-4,5-dihydro-pyrazol-1-yl}-benzoic acid).

A novel, irreversible proteasome inhibitor, carfilzomib potently specifically binds and inhibits the chymotrypsin-like proteasome and immunoproteasome activities, resulting in accumulation of ubiquitinated substrates.

Carfilzomib showed increased efficacy compared with bortezomib and was active against bortezomib-resistant multiple myeloma cell lines and samples from patients with clinical bortezomib resistance (Kuhn *et al.*, 2009). A

small-molecule inhibitor, GS143, which was obtained from screening of approximately 50,000 compounds and suppressed I- κ B α ubiquitylation (Nakajima *et al.*, 2008).

Pristimerin, a naturally occurring triterpenoid, rapidly (<90 minutes) and specifically inhibits chymotrypsin-like proteasome activity at low concentrations (<100 nM) and causes accumulation of cellular ubiquitinated proteins. Pristimerin also inhibits NF- κ B activation via inhibition of IKK β or IKK α . By inhibiting both IKK and the proteasome, pristimerin causes overt suppression of constitutive NF- κ B activity in myeloma cells (Tiedemann *et al.*, 2009). Antiprotealide, a natural product metabolite of *Salinispora tropica*, revealed comparable IC₅₀ values for the in vitro inhibition of purified rabbit 20S proteasome chymotrypsin-like, trypsin-like, and caspase-like activities, as well as for inhibiting the cellular proliferation of the human multiple myeloma cell line RPMI 8226 (Manam *et al.*, 2009). The molecules mentioned here and several others are listed in Table III.

Blockers of I- κ B polymerization

TGase 2 was shown to activate NF- κ B via an IKK-independent pathway involving I- κ B α polymerization (Lee *et al.*, 2004; Park *et al.*, 2006; Kim *et al.*, 2008c). Since treatment of cancer cells with TGase 2 inhibitors reduced NF- κ B activity in a dose-dependent manner, blocking of

TGase 2 activity will be suggested as a novel strategy to ameliorate NF- κ B-mediated cancer progression. TGase 2 inhibitors can be divided into 3 classes based upon their mechanism of inhibition. These 3 classes are (1) competitive amine inhibitors, (2) reversible inhibitors, and (3) irreversible inhibitors. A recent review about TGase 2 inhibitors was reported by Siegel *et al.* (2007). Therefore, we added new TGase 2 inhibitors since 2007 in Table IV.

Competitive peptidic TGase 2 amino donor and amine acceptor pseudo-substrates suppress TGase 2 activity by deterring it from the natural protein substrate. Sohn *et al.* (2003) constructed a recombinant peptide containing sequences from human pro-elafin, a well-known acyl donor (glutamine) and acceptor (lysine) TGase 2 substrate for anchoring pro-elafin onto matrix proteins, which was a competitive reversible inhibitor of TGase 2. This compound was effective in a rabbit model of allergic conjunctivitis as expected. In accordance with the finding that an increase of TGase 2 expression and activity is associated with inflammatory processes (Kim *et al.*, 2006b), Suh *et al.* (2006) demonstrated that TGase 2 was a novel therapeutic target in a study with a peptidic inhibitor (the octapeptide KVLGQDP) in a model of LPS-induced lung injury in BALB/c mice.

Recently, Pardin reported the series of 15 cinnamoyl tri-

Table IV. Transglutaminase 2 inhibitors

Molecules (type)	Point of inhibition	Molecules (type)	Point of inhibition
*Sulfonium peptidyl-methylketones (S) 4a-m	Active site irreversible (Griffin <i>et al.</i> , 2008)	Tyrphostin 47 (S)	Thiol-dependent (Lai <i>et al.</i> , 2008)
Glucosamine (N)	Competitive substrate (Kim <i>et al.</i> , 2009b)	Vitamine K (N)	Thiol-dependent (Lai <i>et al.</i> , 2008)
Ac-PQP-(DON)-LPF-Aldehyde (S)	Active site irreversible (Siegel and Khosla, 2007)	ZM 39923 (S)	Thiol-dependent (Lai <i>et al.</i> , 2008)
p-nitrocinnamoyl triazoles (S)	Reversible & competitive (Pardin <i>et al.</i> , 2008)	ZM 449829 (S)	Thiol-dependent (Lai <i>et al.</i> , 2008)

N: natural product, S: synthetic, *Sulfonium peptidylmethylketones: N-benzyloxycarbonyl-L-prolinyl-6-dimethylsulphonium-5-oxo-L-norleucine bromide.

azole derivatives as inhibitors of guinea-pig TGase 2. Several compounds exhibited activity as reversible inhibitors that were competitive with acyl donor transglutaminase substrates (Pardin *et al.*, 2008). Griffing reported dipeptide-based sulfonium peptidylmethylketones derived from 6-diazo-5-oxo-L-norleucine (DON) as potential water-soluble inhibitors of extracellular TGase 2. One of typical compounds, proline derivative showed good water solubility which may be a contributing factor for targeting only the extracellular TGase 2 (Griffin *et al.*, 2008). Siegel *et al.* (2007), reported PQP-(DON)-LPF-aldehydes as TGase 2 inhibitors. Glucosamine which is used as pop-

ular alternative therapy for arthritis is also reported to inhibit TGase 2 (Kim *et al.*, 2009b).

The finding that TGase 2 acts via cysteine protease has led to the generation of various active-site directed, irreversible TGase 2 inhibitors (Lorand and Graham, 2003). Recent results showed ZM 39923, ZM 449829, Tyrphostin 47 and Vitamine K were found as TGase 2 inhibitors in part a thiol-dependent mechanism (Lai *et al.*, 2008). The competitive peptide substrate, dipeptide carbobenzyloxy-Lglutaminyglycine (Cbz-Gln-Gly) has been modified to generate inhibitors possessing different properties (Folk and Cole, 1965). It is well known that a Cbz-protecting

Table V. I- κ B upregulators, and NF- κ B nuclear translocation, expression, DNA binding and transactivation inhibitors

Molecule (type)	Point of inhibition	Molecule (type)	Point of inhibition
*CP-1158 (S)	Nuclear translocation (Kim <i>et al.</i> , 2006a)	11-Hydrotephrosin (N)	NF- κ B expression (Dat <i>et al.</i> , 2008)
Deguelin (N)	NF- κ B expression (Dat <i>et al.</i> , 2008)	R1, R2=OH Licochalcone A (N)	NF- κ B transactivation & p65 phosphorylation (Furusawa <i>et al.</i> , 2009)
R1, R2= H Deoxydopodophyllotoxin (N)	DNA binding (Jin <i>et al.</i> , 2008)	a4-OMGA (N)	DNA-binding (Lee <i>et al.</i> , 2006)
Diarctigenin (N)	DNA binding (Kim <i>et al.</i> , 2008b)	Tephrosin (N)	NF- κ B expression (Dat <i>et al.</i> , 2008)
Ellagic acid (N)	DNA binding (Edderkaoui <i>et al.</i> , 2008)	R1=OH, R2=H	

N: natural product, P: protein, S: synthetic, *CP-1158: 6-Hydroxy-7-methoxychroman-2-carboxylic acid (3-nitrophenyl) amide, a4-OMGA: 4-O-methylgallic acid.

group is required to confer affinity to TGase 2 substrates (Chica *et al.*, 2004). Analogs of Cbz-Gln-Gly modified with the active pharmacophore 1,2,4-thiadiazole ring were reported to inactivate guinea pig TGase 2 *in vitro* (de Macedo *et al.*, 2002).

Downregulation of NF- κ B nuclear functions: nuclear translocation, DNA binding and transcriptional activation

Direct inhibition of NF- κ B-specific transactivation could involve blocking either nuclear translocation, DNA binding or transactivation by NF- κ B dimers. In many studies, these last steps of NF- κ B activation have been used as indicators of NF- κ B inhibition (Table V) (Gilmore and Herscovitch, 2006). However, inhibition of nuclear activities can also occur by maintaining a pool of active I- κ B proteins in the cytoplasm to thus reduce nuclear NF- κ B activity. Most of compounds in this category are from natural products.

Tephrosin, 11-hydroxytephrosin, and deguelin from *Amorpha fruticosa* showed IC₅₀ values of 0.11, 0.19, and 0.22 μ M in inhibiting NF- κ B DNA binding activity (Dat *et al.*, 2008). Deoxypodophyllotoxin, a naturally occurring flavolignan from *Anthriscus sylvestris*, potently suppressed both reporter gene activity and DNA binding activity of NF- κ B (Jin *et al.*, 2008). Diarctigenin, an inhibitor of nitric oxide (NO) production in macrophages from the seeds of *Arctium lappa*, inhibited the transcriptional activity and DNA binding ability of NF- κ B in zymosan-activated macrophages but did not affect the degradation and phosphorylation of I- κ B proteins (Kim *et al.*, 2008b). 4-OMGA, a major metabolite of gallic acid abundant in red wine, directly blocked the binding activity of NF- κ B to its consensus DNA oligonucleotide, when pre-incubated with the nuclear extract from TNF- α -stimulated HUVECs (Lee *et al.*, 2006a). Licochalcone A, a major component of *Glycyrrhiza inflata* markedly inhibited the phosphorylation of p65 at serine 276. As a result, it reduced NF- κ B transactivation by preventing the interaction of p65 with p300 (Furusawa *et al.*, 2009).

Miscellaneous NF- κ B inhibitors

Antioxidant, anti-inflammatory drugs and Immunosuppressive agents: In many, but not all cell types, NF- κ B can be activated by direct treatment with oxidants, such as hydrogen peroxide; in addition, the induction of NF- κ B activity in response to several stimuli (e.g., IL-1 β , LPS, TNF α) in some cell types can be inhibited by antioxidants (Gloire and Herscovitch, 2006; Gilmore *et al.*, 2006). Thus, many antioxidant compounds such as thiol antioxidants (e.g., NAC, PDTC), calcium chelators (e.g., EGTA, laci-

pine), vitamin C and E derivatives, and α -lipoic acid have been used to inhibit hydrogen peroxide- or stimulus-induced NF- κ B activation.

Several nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin (sodium salicylate), ibuprofen and indomethacin, can suppress activation of NF- κ B in cells (Gilmore and Herscovitch, 2006). Glucocorticoids can also inhibit activation of NF- κ B. Several well-known immunosuppressants target NF- κ B. Cyclosporin A (CycA) can also inhibit NF- κ B induction. *In vitro*, CycA has been reported to act as a non-competitive inhibitor of the chymotrypsin-like activity of the proteasome, thereby enabling it to block LPS-induced I- κ B degradation and p105 processing *in vivo* (Meyer *et al.*, 1997). CycA is able to block the activation of IL-2 and IL-8 gene expression by NF- κ B in T cells (Nishiyama *et al.*, 2005). FK506 (Tacrolimus) is an immunosuppressant that acts as a potent blocker of B- and T-cell proliferation. At least in part, FK506, like CycA, acts to block the activity of calcineurin. FK506 can specifically block c-Rel nuclear translocation (but not p50/RelA) upon treatment of cells with phorbol esters and ionomycin (Venkataraman *et al.*, 1995).

NF- κ B inhibitors encoded or produced by viruses and bacteria: Several microorganisms and viruses encode proteins that can block NF- κ B (Gilmore and Herscovitch, 2006). Viruses have developed several mechanisms to block NF- κ B signaling (see Hiscott *et al.*, 2006). African swine fever virus (ASFV), rabbit myxoma virus, and insect *Microplitis demolitor* bracovirus encode I- κ B-like inhibitors of NF- κ B (Gilmore and Herscovitch, 2006). In addition, several viruses have adaptor-like or small proteins that inhibit IKK activity (Hiscott *et al.*, 2006). Interestingly, the MC160 protein from *Molluscum contagiosum* virus (Nichols and Shisler, 2006) and the NS5B protein from hepatitis C virus (Choi *et al.*, 2006b) seem to be specific for IKK α , and thereby, may provide prototypes for inhibitors of the non-canonical NF- κ B pathway. The YopJ protein from the enteropathogen *Yersinia pseudotuberculosis* blocks NF- κ B activation via deubiquitinating I κ B α . Therefore, eukaryotic cells infected with YopJ-expressing *Yersinia* become damaged in NF- κ B-dependent cytokine expression (Schesser *et al.*, 1998). The *Salmonella typhimurium* AvrA protein, a homolog YopJ, also suppresses NF- κ B activation, although the mechanism may be different from that of YopJ (Collier-Hyams *et al.*, 2002).

CONCLUSIONS

Although some responsible pathways that bridge inflammation and cancer remain to be revealed, there is one

advantage of targeting the NF- κ B pathway in cancer. Normal cells are genetically tolerable and unlikely to develop drug resistance as easily as the genetically unstable cancer cells. Anti-NF- κ B therapy is likely to be the most effective in combination with conventional cancer therapy instead of single application with NF- κ B inhibitors. NF- κ B activation also contributes greatly to the pathogenesis of chronic inflammatory disease, however, it is not the only one responsible target to be cured. Immunological reaction is much more complicated than innate immune system because it is an inter-tissue complication matter together with different signaling pathways. Although multiple factors are involved in chronic inflammation, NF- κ B inhibition indeed reduces inflammation. Therefore, we believe that combination therapy may provide a benefit to patient by ameliorating cytotoxic effect of anti-cancer drugs or other side effects of anti-inflammatory drug, possibly by reducing amount of daily uptake due to additive or synergistic effects.

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