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Review Article

Classical and Alternative NF-κB Activation Pathways and Their Roles in Lymphoid Malignancies

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Nuclear factor-κB (NF-κB) is a family of highly regulated dimeric transcription factors that play pivotal roles in inflammatory responses and immunological reactions. Although they are often activated concurrently, classical and alternative NF-κB activation pathways have distinct regulatory functions, producing secondary inflammatory responses and regulating lymphoid organ development, respectively. As NF-κB functions in the proliferation, differentiation, and survival of lymphocytes, increased activation also participates in the oncogenesis of many lymphoid malignancies. Aberrant NF-κB activation in these tumor cells results from genetic changes or the activation of NF-κB pathways by indirect mechanisms. Recent observations have suggested that NF-κB provides many of the requirements for cellular transformation. Bcl-3, a member of the IκB family, is overexpressed in t (2 ; 5)$^{+}$ anaplastic large cell lymphoma due to genetic and epigenetic alterations. The different contributions of the classical and alternative NF-κB pathways to tumorigenesis, however, are not well understood. The clinical importance of NF-κB is also being recognized, with the approval of the NF-κB inhibitor bortezomib for treatment of advanced multiple myeloma. A better understanding of the molecular pathways involving NF-κB will surely contribute to more sophisticatedly targeted treatments for malignancies in the future.

Key words NF-κB, lymphoma, two-stage carcinogenesis, Bcl-3, proteasome inhibitor

1. NF-κB/IκB family members and their physiological activities

Nuclear factor-κB (NF-κB) was first described as a transcription factor in B cells that binds to the enhancer element controlling immunoglobulin kappa light chain expression[1]. Since its discovery in 1986, NF-κB and its role in inflammatory responses, immune reactions, and tumorigenesis has been extensively studied.

In mammalian cells, the NF-κB/Rel family contains five members : RelA (p65), c-Rel, RelB, NF-κB1 (p50 ; p105), and NF-κB2 (p52 ; p100) (Fig. 1)[2]. These proteins possess a structurally conserved 300 amino acid sequence called the *REL* region, which contains the dimerization, nuclear localization, and DNA-binding domains. Three of the family members, RelA, c-Rel, and RelB, have a transactivation domain at the C-terminus. NF-κB1/p105 and NF-κB2/p100 are the inactive precursors of the p50 and p52 proteins, respectively ; in an unstimulated state, these proteins are localized to the cytoplasm. Proteolytic processing removes the C-terminal inhibitory domains, allowing the resulting proteins to enter the nucleus[3]. p50 and p52 usually form homodimers or heterodimers with one of the three proteins that has a transactivation domain. RelA and p50 exist in a wide variety of cell types, while c-Rel expression is confined to hematopoietic cells and lymphocytes. The expression of RelB is limited to highly specific sites, such as the thymus, lymph nodes, and Peyer's patches[2]. Although each NF-κB dimer has a different DNA-binding affinity for κB sites bearing the consensus sequence GGGRNNYYCC (R, purine : Y, pyrimidine : N, any base)[4], their functions often overlap. NF-κB complexes composed solely of family members lacking transactivation domains, such as p50 homodimers, are thought to impose transcriptional repression[5].

NF-κB is expressed in the cytoplasm of virtually all cell types, where its activity is controlled by a family of regulatory proteins, called inhibitors of NF-κB (IκB)[2,4]. IκBα, IκBβ, IκBε, and Bcl-3, members of the IκB family (Fig. 1), commonly possess 6 to 7 ankyrin repeats, which are 33 amino acid sequences that mediate binding to NF-κB dimers. The unprocessed NF-κB1/p105 and NF-κB2/p100 proteins also contain ankyrin repeats at their C-termini, which cause them to be included in this inhibitory family. IκB proteins were originally thought to retain NF-κB dimers in the cytoplasm by masking their nuclear localization sequences (NLSs). Recent

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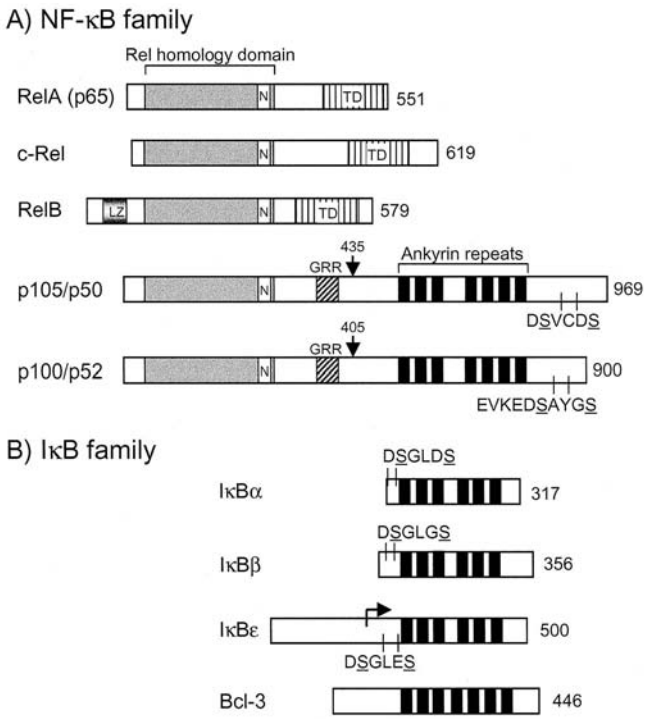

Fig. 1. Mammalian NF-κB and IκB family members. NF-κB family members possess a structurally conserved Rel-homology domain (RHD), which contains a nuclear localization domain (N), a dimerization motif, and a DNA-binding domain. RelA, c-Rel, and RelB also have a non-homologous transactivation domain (TD). RelB also contains a leucine-zipper motif (LZ). The IκB family members, including p105 and p100, are characterized by ankyrin repeats. The amino-acid sequences of the phosphorylation sites triggering their degradation/processing are designated. The glycine-rich region (GRR), which is required for the processing of p105 and p100, is also indicated.

observations, however, have indicated that both IκBα and IκBε shuttle between the nucleus and cytoplasm within NF-κB-IκB complexes ; these complexes are capable of displacing NF-κB from target DNA sites and transporting it back to the cytoplasm[6,7]. The expression of IκB proteins is regulated by NF-κB ; this feedback regulation is believed to contribute to the rapid shut down of NF-κB signaling. IκBβ expression, however, is not regulated by NF-κB. Instead, IκBβ is constitutively retained in the cytoplasm, indicating that it is not involved in the autoregulatory loop terminating NF-κB signaling. As seen for NF-κB, each IκB family member has both distinct and redundant actions.

NF-κB activation is tightly regulated by signals that degrade IκB. In the classical NF-κB signaling pathway (Fig. 2), IκB proteins are phosphorylated by an activated IκB kinase (IKK) complex at specific sites equivalent to Ser32 and Ser36 of IκBα. Phosphorylation triggers polyubiquitination at sites equivalent to Lys21 and Lys22 of IκBα and degradation by the 26S proteasome, releasing free NF-κB dimers[8]. The IKK complex is composed of the catalytic subunits IKKα and IKKβ and the regulatory subunit IKKγ, also known as NF-κB essential modulator (NEMO). IKKα and IKKβ, which are 52% identical, form homodimers or heterodimers. Although IKKα and IKKβ cooperate for IκB phosphorylation, these proteins differ in the signals that they mediate. The IKKβ component is essential for the signaling via the classical NF-κB pathway[9,10]. In a recently identified alternative pathway for NF-κB activation (Fig. 2), upstream NF-κB inducing kinase (NIK) activates an IKKα homodimer ; both IKKβ and IKKγ are dispensable in this signaling[11]. In the alternative pathway, NF-κB2/p100 is phosphorylated at two C-terminal sites by the IKKα homodimer and ubiquitinated. This modification targets the inhibitory C-terminus for proteasomal degradation, producing p52.

Although they are often activated concurrently, these two NF-κB activation pathways have distinct regulatory functions[3,4,12]. The classical pathway is typically triggered by ligand binding to tumor necrosis factor type 1/2 receptors (TNFR1/2), T-cell receptors (TCR), B-cell receptors (BCR), or the Toll-like receptor (TLR)—interleukin-1 receptor (IL-1R) superfamily members. This pathway terminates in the increased transcription of target genes encoding chemokines, cytokines, and adhesion molecules, perpetuating inflammatory responses, and promoting cell survival. In contrast, the alternative pathway is triggered by the activation of certain TNF receptor family members, including lymphotoxin β receptor (LTβR), B-cell-activating factor belonging to the TNF family receptor (BAFF-R), CD40, and CD30. Activation of the alternative pathway regulates the development of lymphoid organs and the adaptive immune system.

2 . Suggested oncogenetic roles played by NF-κB

In addition to its principal function in physiological immune reactions, substantial evidence indicates that NF-κB plays a pivotal role in the generation and maintenance of malignancies. The relationship of NF-κB and tumorigenesis is complicated, however, as this transcription factor can also suppress tumor growth in some situations. “Coley’s toxin”, which was used to treat cancer in the past, is actually lipopolysaccharide (LPS), a strong activator of NF-κB. Currently used chemotherapeutic agents, such as cytokines and radiation, also provoke NF-κB signaling. In contrast, LPS has also been shown to increase the growth of experimental metastases in a murine tumor model[13]. The presence of inflammation following surgery can promote the growth of metastases in patients[14,15], probably following a similar mechanism. These paradoxical effects of NF-κB are not well understood, but may partially explained by the differences between acute and chronic inflammation ; acute inflammation inhibits cancer growth, while chronic inflammation promotes tumor development[16]. The different spectrum of target genes en-

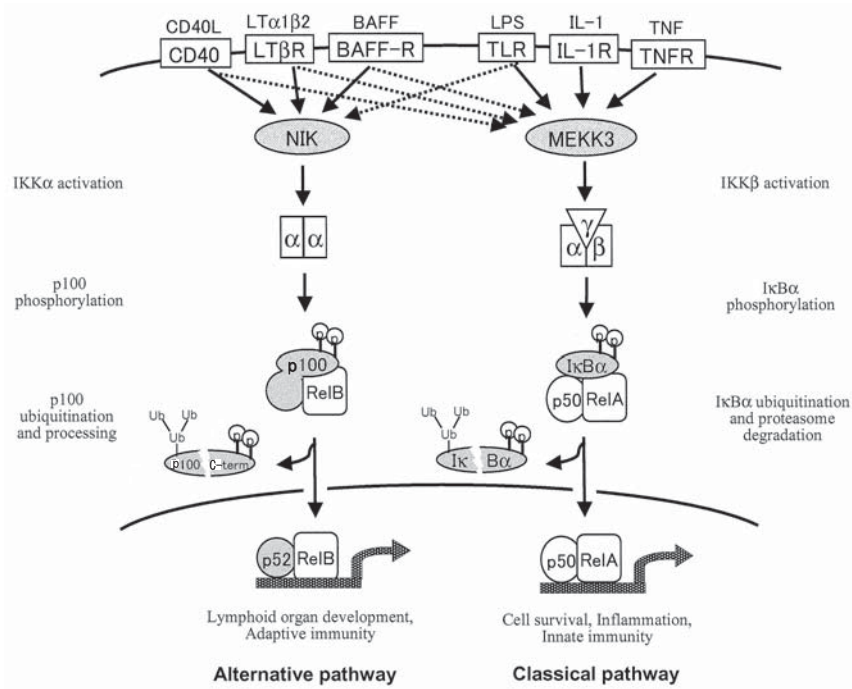

Fig. 2. Classical and alternative NF-κB activation pathways. In classical NF-κB activation, signaling is typically triggered through TNFR, IL-1R, or TLR. Signals mediated by MAP/ERK kinase kinase 3 (MEKK3) and IKKβ finally result in the degradation of IκBα and the translocation of the RelA/p50 homodimer to the nucleus. In the alternative NF-κB activation pathway, signals triggered via CD40, LTβR, or BAFF-R are mediated by NIK and IKKα, which leads to p100 processing and the translocation of p52 dimers into the nucleus.

hanced and cell types mobilized in these two situations may determine the effect of NF-κB activation on tumor outcome.

NF-κB targets many genes that facilitate tumor progression, inflammation, cellular immortality, cell survival, angiogenesis, proliferation, tumor promotion, and metastasis (Table 1).

In 2004, using a colitis-associated cancer model, Greten *et al*. demonstrated that deletion of IKKβ in intestinal epithelial cells (the source of the tumor cells) led to a significant decrease in tumor incidence, without an associated decrease in inflammation or suppression of tumor size. In contrast, deletion of IKKβ in myeloid cells (the surrounding cells) resulted in a considerable decrease in tumor size[18]. Pikarsky and Porat *et al*. examined Mdr-2 knockout mice, which spontaneously develop cholestatic hepatitis followed by the development of hepatocellular carcinomas. Blockade of NF-κB signaling from birth to 7 months of age with hepatocyte-specific inducible super-repressor IκB transgene (ΔN-IκB) had no effect on the course of hepatitis or the early phases of hepatocyte transformation[19]. Suppression of NF-κB in the later stages of tumor development, however, resulted in the prevention of progression to hepatocellular carcinoma. In a murine cancer model in which a colon adenocarcinoma cell line is used to generate lung metastases, Luo *et al*. reported that inhibition of NF-κB by the IκB super-repressor converted LPS-induced tumor growth, an effect mediated by TNF-α, to LPS-induced tumor regression, which was mediated by TNF-related apoptosis-inducing ligand (TRAIL)[20].

Table 1. NF-κB target genes related to the enhancement of tumor progression

Activity	Genes
Inflammation	TNF, IL-1, chemokines
Cellular immortality	telomerase
Cell survival	BCL-X_L, cIAP, XIAP, cFLIP
Angiogenesis	VEGF, TNF, IL-1, IL-8
Proliferation	TNF, IL-1, IL-6, cyclin D1, c-MYC
Tumor promotion	COX2, iNOS, MMP-9, uPA
Metastasis	ICAM-1, VCAM-1, ELAM-1

Abbreviations: cIAP, cellular inhibitors of apoptosis: XIAP, X chromosome-linked inhibitors of apoptosis: cFLIP, caspase 8-FAS-associated death domain (FADD)-like IL-1β-converting enzyme inhibitory protein: VEGF, vascular endothelial growth factor: COX2, cyclooxygenase 2: iNOS, inducible nitric oxide synthase: MMP-9, metalloproteinase-9: uPA, urokinase plasminogen activator: ICAM-1, intracellular adhesion molecule-1: VCAM-1, vascular cell adhesion molecule-1: ELAM-1, endothelial-leukocyte adhesion molecule-1.

These experiments effectively investigate the mechanisms by which NF-κB signaling participates in tumor development. The activation of NF-κB in both tumor cells and surrounding cells is important in oncogenesis, but the contributions of these two sites appears to be different.

From observations of the tumorigenetic processes generated experimentally in animals, the two-stage carcinogenesis model proposed that there are two critical steps in the malignant transformation of cells (Fig. 3)[16]. First, DNA is damaged by certain mechanisms, either spontaneously or by exposure to carcinogens. These "initiated cells" are long-lasting and accumulate throughout life. In the second step, repetitive stimulation acts as a "promoter", leading to proliferation. Increased numbers of mutations finally confer a growth advantage onto the cells. Usually initiators are DNA-reactive substances, while promoters are non-DNA-reactive. Encounter of initiators always precedes promoters ; promoters act over time until a tumor develops. Although the exact number of genetic events leading to cellular transformation has not been determined, several factors are thought to be required, which differ for each cell type and species[21]. NF-κB is notable in tumor generation, because it seems to be capable of functioning as both an initiator and a promoter. NF-κB may thus provide several independent requirements for malignant transformation. This may be the principal reason why aberrant activation of NF-κB is so closely linked to the generation of various tumor types.

3. Genetic and functional abnormalities of NF-κB in lymphoid malignancies

The NF-κB family plays a central role in regulating the expression of genes essential for the generation of both innate and adaptive immune responses. As NF-κB promotes the proliferation, differentiation, and survival of lymphocytes, aberrant activity contributes significantly to the pathogenesis of many lymphoid malignancies (Table 2).

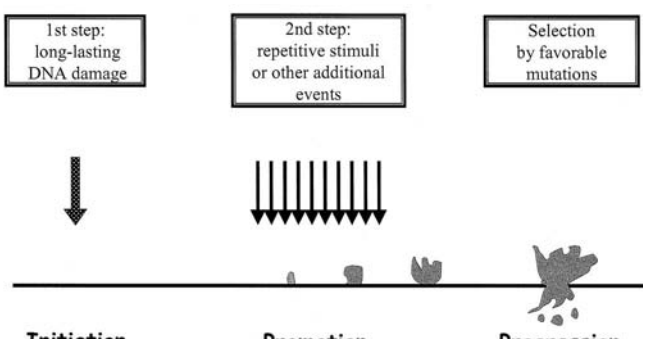

Fig. 3. The two-stage model of tumorigenesis initiation and promotion. Promotion is followed by a third stage of progression. This model was originally proposed on the basis of carcinogenetic experiments performed by Dr. Yamagiva (J Cancer Res 1918 ; 3 : 1-21), and is still well supported.

A subset of the NF-κB family members were originally recognized in chromosomal translocations of lymphoid neoplasms. *BCL3* was first identified as a gene involved in a recurrent translocation in certain cases of B-cell chronic lymphocytic leukemias (B-CLL)[22-24]. Recently, it was also reported to be overexpressed in $t(2;5)^{+}$ anaplastic large cell lymphomas (ALCL)[25]. The *NF-κB2/p100* gene is rearranged in a number of B-NHL (1-2%), B-CLLs, multiple myeloma, and mature T-cell tumors (14%), such as mycosis fungoides and Sézary syndrome[26-28]. Such rearrangements of *NF-κB2/p100* produce a C-terminal truncation that leads to constitutively active nuclear transactivation.

In contrast to rarity of genetic abnormalities of RelA[29], *C-REL* amplification is a recurrent genetic abnormality in lymphomas. The significance of this mutation remains controversial. Duplication of the 2p12-16 region has been described for multiple lymphoma subtypes, making *C-REL* the most frequently amplified gene of candidate genes mapped to this region[30-32]. Davis *et al*. reported that constitutive NF-κB activity was essential for the pathogenesis of activated B-cell-like (ABC) diffuse large B-cell lymphoma (DLBCL) cell lines[33], a subset of cells that exhibit expression of transcripts common to activated peripheral blood B lymphocytes[34]. In contrast, genomic amplification of *C-REL* occurs exclusively within germinal center B-cell-like (GCBL) DLBCLs[35], a subgroup that express transcripts common to germinal center B cells. While amplification of the *C-REL* locus can be seen in all DLBCL subtypes, *C-REL* copy number is not associated with the accumulation of active c-Rel, suggesting *C-REL* may not be the functional consequence of the 2p12-16 amplification[36].

Hodgkin lymphoma (HL) was the first hematopoietic tumor to be characterized as having clearly aberrant NF-κB activity[37] ; This tumor has served as a model in which to analyze the molecular mechanisms governing its deregulation. While c-Rel is the major form of NF-κB activated in B-cell lymphomas, Hodgkin and Reed-Sternberg (H-RS) cells typically possess abnormalities in the RelA/p50 complex. To date, several pathways have been suggested to induce aberrant signaling in H-RS cells, including expression of Epstein-Barr virus (EBV)-encoded latent membrane protein (LMP)-1[38], increased IKK activity[39], functional expression of receptor activator of NF-κB (RANK)[40], or ligand-independent signaling following overexpression of CD30[41]. Mutated *IκBα*, which encodes a defective IκBα, is estimated to be responsible for approximately 10-25% of cases[39,42-44]. Recently, inactivating *IκBε* mutations has also been suggested as a possible underlying cause for constitutive NF-κB activity in a subset of HL cases[45].

Aberrant activation of NF-κB can also be induced by indirect mechanisms. Extranodal marginal zone B-cell lym-

Table 2. Genetic abnormalities of NF-κB/IκB family members reported in lymphoid malignancies

Genes	Locus	Diagnosis	References
Translocation			
NF-κB2/p100	10q24	B-NHL, B-CLL, MM	26, 27, 28
BCL3	19q13	B-CLL (few cases of B-NHL)	22, 23, 24
Gene amplification			
C-REL	2p12-13	DLBCL, FL, HL	30, 31, 32
BCL3	19q13	t(2 ; 5)$^{+}$ ALCL[*1]	25
RELA	11q13	DLBCL	29
Mutation/deletion			
IκBα, *IκBε*	14q13, 6p11	HL	39, 42, 43, 44, 45
Indirect activation of NF-κB			
API2/MALT1,	t(11 ; 18) (q21 ; q21),	MALT	50, 51, 53
BCL10/IGH	t(1 ; 14) (p22 ; q32)		

Abbreviations : B-NHL, B-cell non-Hodgkin lymphoma : B-CLL, B-cell chronic lymphocytic leukemia : MM, multiple myeloma : DLBCL, diffuse large B-cell lymphoma : FL, follicular lymphoma : HL, Hodgkin lymphoma : ALCL, anaplastic large cell lymphoma : MALT, marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue.
*1. Experimental data in cell lines.

phoma of mucosa-associated lymphoid tissue (MALT lymphoma), a disease entity arising from marginal zone B cells, has several distinctive features. Malignant cells in MALT lymphomas originate from activated memory B cells that are produced during chronic inflammation, such as chronic gastritis or autoimmune diseases. Persistent activation of NF-κB is believed to be essential for disease development[46]. Gastric MALT lymphoma is the most peculiar subtype ; it has a marked relationship with, and often a dependency on, the chronic gastritis induced by *Helicobacter pylori*. Eradication of the bacteria with antibiotic treatment leads to regression in 70-80% of cases[47-49]. Gene rearrangements may also be involved in the pathogenesis of the disease ; the t(11 ; 18) (q21 ; q21) chromosomal translocation, which results in the production of the *API2-MALT1* fusion protein[50, 51], is found in approximately 30% of cases. Both the *API2* and *MALT1* genes are transcriptionally activated in lymphocytes upon PHA stimulation, suggesting this gene rearrangement is generated during chronic inflammation. The MALT1 protein forms a complex with Bcl-10 to mediate NF-κB activation following B-cell receptor signaling under physiological conditions. Although both API2 and MALT1 proteins turn over rapidly, the API2-MALT1 chimeric protein is very stable, causing the constitutive NF-κB activation[52]. The t(1 ; 14) (p22 ; q32) translocation is seen in approximately 3% of cases, which leads to the enhanced expression of *BCL10* driven by the juxtaposed *IGH* gene promoter[53]. Overexpression of *BCL10* activates the same signaling pathway triggered in API2-MALT1, resulting in the aberrant activation of NF-κB[54]. These gene rearrangements can replace the oncogenetic role played by *H. pylori*. Lymphomas with these genetic abnormalities can no longer be cured by antibiotic eradication therapy alone.

Persistent activation of NF-κB also functions in the pathogenesis of adult T-cell leukemia/lymphoma (ATLL)[55]. After a long latency period, ATLL develops in approximately 5% of individuals carrying human T-cell leukemia virus type 1 (HTLV-1). Although a multistep mechanism is proposed for ATLL tumorigenesis, as with other malignancies, the virally-encoded Tax protein plays a central role in the transformation of HTLV-1-positive T cells[56]. Tax exhibits a variety of activities, enhancing the expression of viral genes via the viral long terminal repeat (LTR), activating cellular signaling pathways mediated by NF-κB, cyclic AMP response element binding protein (CREB), serum responsive factor (SRF) and AP-1, and functionally inactivating $p16^{INK4a}$, p53, MAD1, and transforming growth factor β (TGF-β) signaling pathways that act as protective factors. Despite a substantial contribution to cellular transformation, Tax protein is frequently lost by several mechanisms once a tumor is established. As Tax is a major target of cytotoxic T lymphocytes (CTLs), this helps tumor cells escape the host immune system. The independence of ATLL on Tax is thought to result from the accumulation of alternative genetic and epigenetic changes by the completion of cellular transformation[57]. Although the mechanism is unclear, NF-κB activity remains high even after the down-regulation of Tax. Aberrant NF-κB activity thus plays an important role in both the pre-leukemic period and the establishment of a population of malignant ATLL cells.

In the pathogenesis of multiple myeloma, NF-κB activation is closely linked to the cellular interactions within the bone marrow (BM) microenvironment. While the exact

mechanism by which NF-κB signaling is enhanced is not clear, activators may include direct contact of myeloma cells with the extracellular matrix, bone marrow stromal cells (BMSCs), osteoblasts, or other cellular compartments in the BM. In addition, cytokines and growth factors, including IL-6, insulin-like growth factors (IGFs), IL-1α, IL-1β, hepatocyte growth factor (HGF), VEGF, stromal cell-derived factor-1 (SDF-1), TNF-α, and Notch family members released by BMSCs or myeloma cells, lead to the activation of pleiotropic proliferative/apoptotic signaling pathways that activate NF-κB in myeloma cells[58-61]. The proteasome is also constitutively induced in this microenvironment. NF-κB also triggers the differentiation and activation of osteoclasts[62]. Osteoclasts and their precursors, which express RANK, can interact with RANK ligand on BMSCs to create distinctive osteolytic lesions.

Although irrelevant activation of NF-κB is involved in a variety of lymphoid neoplasms, the roles played by classical and alternative NF-κB signaling seem to be varied. As the NF-κB proteins associated with the alternative pathway are preferentially involved in chromosomal translocations and blockade of classical NF-κB signaling leads to inactivation of NF-κB promoter in tumorigenesis[19], in a subset of tumors, aberrant activation of the alternative NF-κB pathway may function as a tumor initiator. In contrast, deregulated expression of the classical pathway may act as a tumor promoter. Regardless, these two pathways are usually activated concurrently in the actual tumorigenetic process. Further investigation is necessary to elucidate the exact contribution of each NF-κB family member in the generation and promotion of malignancies.

4. Role of Bcl-3 in lymphoid malignancies

Bcl-3 is categorized as a member of the IκB family based on structural similarity. In physiological activity, however, it is distinct from the other IκB proteins. Bcl-3 does not localize to the cytoplasm, but is expressed in the nucleus. With 2 NLSs in the N-terminus, Bcl-3 contains 7 ankyrin repeats, while the other major IκB proteins possess only 6. The Bcl-3 protein specifically interacts with p50 or p52 homodimers to activate the expression of NF-κB target genes.

BCL3 was originally identified as a putative oncogene in the cloning of the t(14; 19) (q32; q13) breakpoint, a recurrent chromosomal translocation in B-CLL[22-24]. Juxtaposition of the *BCL3* gene to the immunoglobulin gene locus results in enhanced expression of *BCL3*, which is believed to contribute to the pathogenesis of this tumor. Transgenic mice bearing an immunoglobulin Eμ-driven *BCL3* transgene exhibited splenomegaly and the accumulation of mature B cells in lymph nodes, the bone marrow, and the peritoneal cavity[63]. As is often the case with translocated genes, enhanced ex-

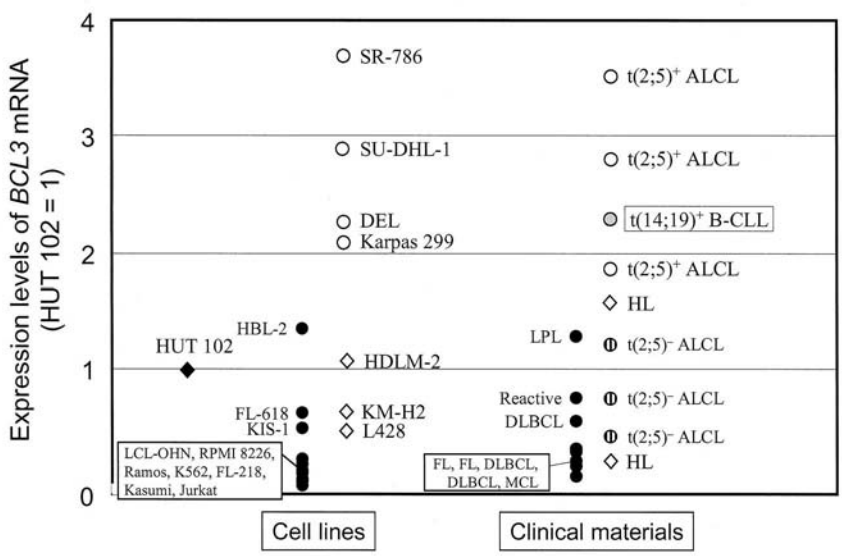

Fig. 4. *BCL3* mRNA levels in hematologic tumor cells measured by real-time quantitative RT-PCR. The *BCL3*/ 18s rRNA ratio in each test material was normalized to that of HUT 102, an ATLL cell line (=1). The results invariably demonstrate high *BCL3* expression in t(2; 5)$^+$ ALCL and t(14; 19)$^+$ B-CLL, in which high *BCL3* expression resulting from a translocation was previously observed. Karpas 299, SU-DHL-1, DEL, and SR-786 are cell lines derived from the t(2; 5)$^+$ ALCL. MCL, mantle cell lymphoma: LPL, lymphoplasmacytic lymphoma. Other abbreviations are as noted in Table 1.

pression of *BCL3* alone was not sufficient to generate malignancies.

Bcl-3 is also involved in the prevention of activation-induced cell death in T cells[64]. Microarray analysis comparing gene expression in T cells activated either by antigen alone or in the presence of adjuvant demonstrated that *BCL3* expression is increased in T cells stimulated with both antigen and adjuvant. These cells had a higher survival rate than those stimulated with antigen alone. Transduction of *BCL3* into activated T cells suggested that *BCL3* expression correlated well with the extent of both proliferation and survival in T cells.

BCL3 is overexpressed in t(2 ; 5) (p23 ; q35)-positive ALCL (Fig. 4)[25]. The t(2 ; 5) translocation fuses the nucleophosmin (*NPM*) gene to the anaplastic lymphoma kinase (*ALK*) gene, leading to the production of a NPM/ALK fusion protein with aberrant tyrosine kinase activity. Enhanced expression of *BCL3* in this lymphoma cell type did not result from a chromosomal translocation, but instead followed gene amplification or the methylation of the CpG island within the gene. The overexpressed Bcl-3 protein localized to the nucleus, where it bound to p50 homodimers in an unstimulated state. Stimulation of surface CD30 in t(2 ; 5)$^{+}$ ALCL cell lines resulted in further upregulation of Bcl-3 and hyperphosphorylation of the protein. In ALCL cells, stimulation of CD30 also activated the alternative NF-κB pathway, leading to the formation of a ternary complex between the Bcl-3 protein and p52 homodimer. Although its role in oncogenesis remains unclear, Bcl-3 appears to affect both the classical and alternative NF-κB signaling pathways in ALCL cells. The stimulation of CD30 causes apoptotic cell death in ALCL, but not in HL, cells. In addition, artificial expression of the Bcl-3 protein in HL cells did not result in either hyperphosphorylation or cell death upon CD30 stimulation (Cancer Science, *in press*). Thus, we speculate that hyperphosphorylation of Bcl-3 may impair cell survival. This hypothesis is compatible with past observations that the activity of Bcl-3 varies with different experimental settings and phosphorylation states.

As research to unveil the role of Bcl-3 in hematological malignancies is challenging, the physiological activity of Bcl-3 remains poorly understood. Bcl-3 will likely begin to receive more attention as a key molecule bridging normal lymphoid organ development to tumor generation.

5. Future directions

As the aberrant activation of NF-κB is associated with the generation of tumors as well as their maintenance and progression of a range of lymphoid malignancies, NF-κB has been expected as an attractive target for therapy. *In vitro* inhibition of NF-κB activity in lymphoma cell lines suggested this approach to be effective.

Recently, several agents inhibiting the ubiquitin-proteasome pathway have been developed. In addition to hindering IκB degradation, proteasome inhibitors induce apoptosis by affecting the levels of a variety of short-lived proteins, such as p53, Bax, and cyclin-dependent kinase inhibitors. Malignant cells appear to be more susceptible to proteasome inhibition than normal cells, making this a promising candidate therapy for the treatment of cancer[65,66].

The drug bortezomib (*N*-pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid), the first drug targeting the NF-κB pathway to enter clinical trials, is a promising anticancer agent. This substance rapidly inhibits proteasomal degradation in a reversible manner by blocking the enzymatic activity of the 20S proteasome. In a large multicenter phase II clinical trial, approximately one-third of patients with advanced multiple myeloma responded to bortezomib therapy (any response : 35%, complete or partial response : 27%)[67]. Common adverse events included gastrointestinal symptoms, fatigue, thrombocytopenia, and sensory neuropathy, most of which could be managed with standard approaches. The United States Food and Drug Administration (FDA) immediately approved this drug for the treatment of multiple myeloma patients who had relapsed after at least two prior treatment regimens and exhibited resistance to their last treatment. Currently, several clinical trials with bortezomib are ongoing, examining its efficacy against a range of hematological and non-hematological malignancies.

NF-κB is proving to be paramount in the clinical field. Research on NF-κB will surely provide more effective, directed, and sophisticated treatments for malignancies in the future.

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