Biochemical and Biophysical Research Communications 378 (2009) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mini Review

NF-κB functions in osteoclasts

N.S. Soysa [a,*], N. Alles [b]

[a] Division of Pharmacology, Faculty of Dental Sciences, University of Peradeniya, Peradeniya, Sri Lanka
[b] Section of Pharmacology, Graduate School, Tokyo Medical and Dental University,1-5-45, Yushima, Bunkyo-ku, Tokyo, Japan

ARTICLE INFO

Article history:
Received 17 October 2008
Available online 6 November 2008

Keywords:
Osteoclast
NF-κB
Canonical pathway
Non-canonical pathway

ABSTRACT

NF-κB is a pleiotropic transcription factor, which regulates osteoclast formation, function, and survival. The finding that the deletion of both NF-κB p50 and p52 subunits resulted in osteopetrosis due to the absence of osteoclasts was followed by the observation that NF-κB is essential for RANK-expressing osteoclast precursors to differentiate into TRAP+ osteoclasts in response to RANKL and other osteoclastogenic cytokines. Thus, inhibitors of NF-κB should prevent osteoclast formation induced directly or indirectly by RANKL or TNF. In this mini review, we discuss the research findings that revealed essential roles of NF-κB signaling in osteoclasts.

Osteoclasts (OCs) are bone-resorbing cells of hematopoitic origin. Study of osteopetrosis (a family of diseases characterized by a marked increase in bone mass due to OC absence or dysfunction) and osteopetrotic mutant mice led to the identification of genes that regulate osteoclast progenitor/precursor (OCP) differentiation and the means by which OCs degrade bone. An essential role for NF-κB in OC formation was discovered unexpectedly when NF-κB p50 and p52 double-knockout (dKO) mice were generated and found to have severe osteopetrosis [1,2]. NF-κB p50/p52 dKO mice do not form OCs, whereas OC formation in p50 or p52 single-knockout mice is normal. The dKO mice have increased numbers of $CD11b^{+}/RANK^{+}$ OCP in their spleens, indicating that p50/p52 expression is required for progression of these cells along the OC differentiation pathway.

The pleiotropic NF-κB transcription factor is a family consisting of five members and originally named because they were considered as essential regulators of B-cell Ig κ light chain expression. The subfamily, 'Rel' proteins includes Rel A (p65), Rel B, and c-Rel (Rel) that contain transcription activation domain (TAD) and synthesized as mature proteins (Fig 1). Unlike Rel A and c-Rel, Rel B has a leucine zipper domain (LZ) which is required for full transcriptional activity. NF-κB proteins consist of NF-κB1 (p105/p50) and NF-κB2 (p100/p52). NF-κB1 and NF-κB2 are synthesized as large precursors, p105 and p100, respectively, with long C-terminal domains that contain multiple ankyrin repeats (Fig. 1). These NF-κB proteins become shorter following post-translational processing into p50 and p52, respectively. The Rel-homology domain (RHD) which is shared by all NF-κB proteins contains a nuclear localization sequence and is involved in dimerization, DNA binding, and interaction with IκB inhibitory proteins. Whereas the processing of p105 is constitutive upon activation, p100 processing is tightly regulated and highly inducible. NF-κB proteins, p50 and p52 are generally not activators of transcription unless they form dimers with Rel family members. NF-κB family proteins can form homodimers or heterodimers *in vivo* with the exception of Rel B which only forms heterodimers. Of the 15 possible dimers at least 12 are able to bind to 9–10 bp DNA sites (κB sites) [3].

In most cells, NF-κB dimers are retained in the cytoplasm by inhibitory IκB proteins. These interactions cover the nuclear localization signal of the dimers and prevent nuclear localization and DNA binding. IκB proteins include IκBα, IκBβ, and IκBε and contain ankyrin repeats that mediate binding of the RHD. There are two major signaling pathways named canonical (classical) and non-canonical (alternative) (Fig 2).

Canonical/classical NF-κB pathway

Canonical NF-κB pathway is typically activated through binding of ligands to their receptors on the cell surface, like RANK, TNFR, and IL-1R which in turn activates the IKK complex consisting of catalytic subunits IKKα, IKKβ, and the regulatory subunit IKKγ (NEMO) (Fig 2). Recent studies identified some additional components of the IKK complex like ELKS and the heat shock protein Hsp90/Cdc37 chaperone complex. ELKS seems to act as a regulatory subunit by recruiting IκB to the IKK complex for phosphorylation [4]. IKKγ in contrast acts as a scaffold protein to assemble the IKK complex and mediate NF-κB-inducing signals to the activation of catalytic subunits. The activated IKK complex catalyzes its phosphorylation triggering its ubiquitination and subsequent degrada-

* Corresponding author. Fax: +94 81 2388948.
E-mail addresses: hnsnit@yahoo.com, niroshanis@pdn.ac.lk (N.S. Soysa).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.10.146

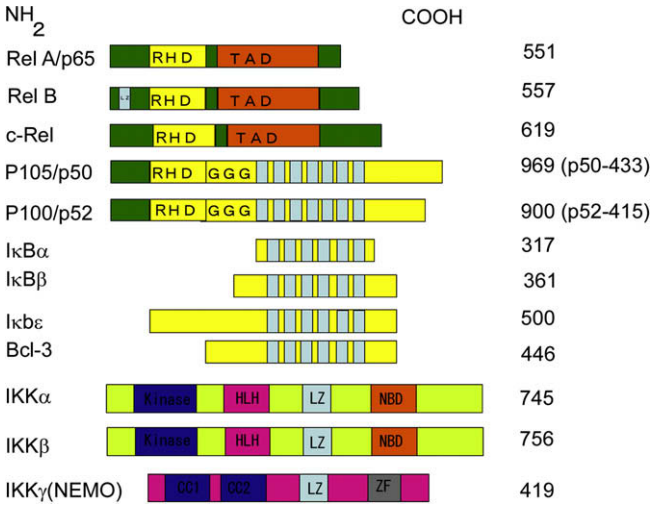

Fig. 1. NF-κB family members.

tion of IκB by the 26S proteasome which in turn releases the NF-κB dimmers (mostly p50-Rel A) facilitating their nuclear translocation, DNA binding, and gene transcription. Phosphorylation of p65 stabilizes and inhibits it from binding to IκBα. Despite the similarities, IKKα and IKKβ play distinct roles within the canonical NF-κB pathway. A recent report shows that TNF-induced NF-κB activation occurs via a NEMO-IKKβ dependent pathway, while IL-1-induced IκBα degradation occurs through NEMO-IKKβ or NEMO-IKKα complexation and leads to nuclear translocation and DNA binding of the classical NF-κB p50:p65 heterodimers [5]. There is evidence to suggest that IKKα plays a crucial role in maintaining the full classical pathway response that is independent of its function as an IκB kinase in macrophages [6]. In this regard, IKKα has a negative regulatory role in macrophage activation and inflammation. The authors further observed that IKKα-induced NF-κB suppression is through accelerating the turnover of the subunits Rel A and c-Rel and their removal from pro-inflammatory gene promoters.

The canonical pathway is rapidly activated by a large number of stimuli and is regulated by auto-regulatory feedback mechanisms to ensure transient activation. This is done by replenishing the pool of IκB proteins via NF-κB activation itself. The newly synthesized IκB proteins enter the nucleus and transport NF-κB dimers back to the cytoplasm. In addition, inactivation of IKK catalytic activity prevents degradation of newly synthesized IκB proteins.

Alternative NF-κB pathway

In contrast to the canonical pathway which depends on IKKβ–IKKγ, IKKα is required for activation of the alternative pathway. This pathway is activated by TNF cytokine family members, including RANKL, B-cell activating factor (BAFF), CD40L, TWEAK, and lymphotoxin-β, but not by TNF-α itself. Activation of NF-κB-inducing kinase (NIK) results in activation of IKKα homodimers and the phosphorylation and proteasome-induced processing of p100 (Fig 2). Ubiquitinated p100 is not completely degraded, instead it is cleaved to generate an active p52 product. Hence this process is slower than the activation of the classical pathway and leads to delayed activation and nuclear translocation of p52-Rel B under physiological conditions.

Possible crosstalk exists linking the canonical and alternative pathways at the level of both IKKs and transcription factors. For example, activation of the classical NF-κB pathway may feed the alternative pathway to ensure sufficient synthesis of p100 and production of its cleaved product p52 [7]. In addition, p52 homodimers could down-regulate the transcription of the *nfκb2* gene under the control of p50/p65. Also, there is evidence that p52

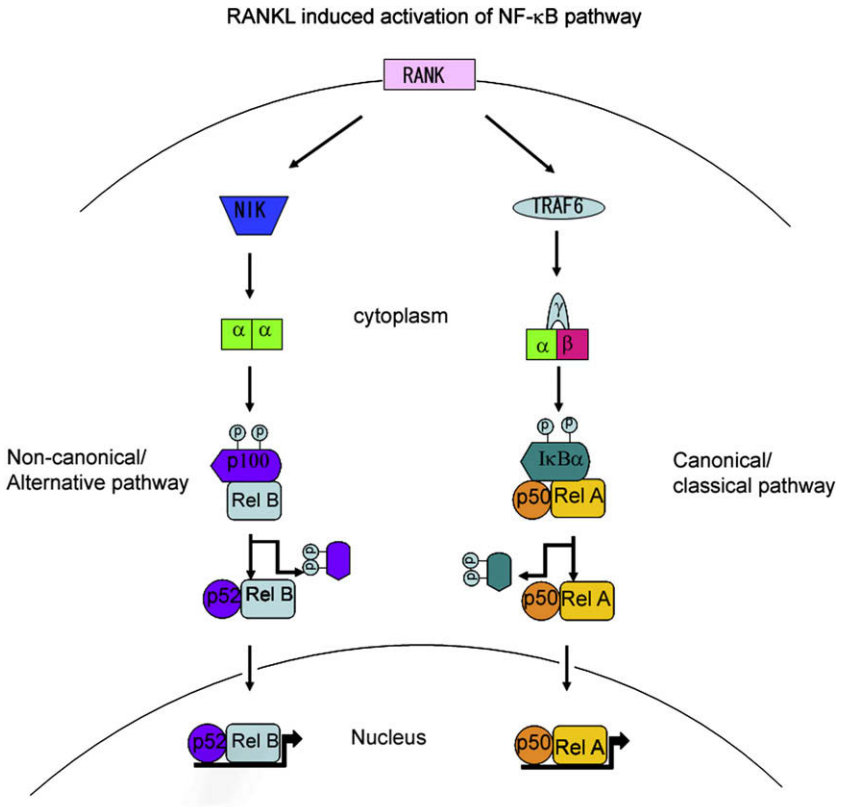

Fig. 2. The main NF-κB pathways in osteoclast.

pre-bound with p65 or c-Rel is activated via the classical pathway suggesting a hybrid pathway for activation of p52/p65 and p52/c-Rel [7]. Further evidence of inter-pathway cross talk comes from NIK-deficient mice. In $NIK^{-/-}$ cells, p100 is shown to bind to a p50:Rel A complex and prevents its nuclear translocation [8].

What can we learn from knockout and transgenic models?

Formation of a multitude of homodimers and heterodimers by NF-κB family members results in a high degree of complexity within this family of transcription factors. Targeted disruption of family members demonstrated the importance of different proteins, which play distinct biological roles even though the possible existence of functional redundancy among the family members has yet to be clarified.

IKKβ KO mice

IKKβ kinase of the IKK complex activates Rel A, c-Rel, and p50-containing dimers. Absence of IKKβ leads to mouse embryonic death as a result of TNF-α-induced hepatocyte apoptosis. Conditional IKKβKO ($IKK\beta^{\Delta}$) mice have an osteopetrotic bone phenotype with reduced OC numbers *in vivo*. These mice have increased numbers of trabeculae with obliterated bone marrow. IKKβ deficient bone marrow (BM) progenitors do not form OCs *in vitro* in response to RANKL or when co-cultured with osteoblasts and this cannot be complemented by adding TNF-α or IL-1, thus revealing the importance of IKKβ in OC formation [9]. Further it has been shown that a small peptide encoding the NEMO binding domain of IKKβ could disrupt IKKβ-NEMO binding and thus prevent RANKL-induced osteoclastogenesis and function [10], suggesting a critical role for IKKβ in osteoclastogenesis and inflammation-induced bone loss. The importance of NF-κB signaling in OC activity was reported by Soysa et al. [11]who used the same peptide against IKKβ showing that inhibition of NF-κB suppresses the pit forming activity of OC through inhibiting osteoclast actin ring formation, migration, and by reducing bone resorption-mediated gene expression.

IKKα KO mice

$IKK\alpha^{-/-}$ mice die at birth as a result of severe epidermal defects. Analysis of embryonic OCs (E18.5 dpc) revealed reduced numbers of multinucleated OCs with altered morphology. $IKK\alpha^{-/-}$ fetal liver cells cultured in the presence of RANKL and CSF-1 generated few multinucleated OCs. This correlated with the lack of p100 processing into p52. In addition no difference in GM-CSF colony formation assay revealed that the lack of OCs is due to an intrinsic defect in OCs and is not due to a lack in myeloid progenitors. Addition of TNF-α or TGF-β could rescue the osteoclastogenesis in these experiments suggesting that RANKL in combination with TNF-α/TGF-β could overcome the inhibition of OC differentiation by p100 [12]. These results were further confirmed by the study of Ruocco et al. [9] who using mice homozygous for a knock-in mutant allele, $IKK\alpha^{AA}$ showed that IKKα has a redundant role in basic osteoclastogenesis *in vivo* and in inflammation-induced bone loss which could be due to the other factors that activate IKKβ-driven classical NF-κB pathway.

NIK KO mice

The conversion of p105 to p50 is predominantly constitutive, while the processing of p100 to p52 is tightly controlled by NIK. Even though the defect in RANKL-mediated osteoclastogenesis *in vitro* in $NIK^{-/-}$ cultures is profound, NIK-deficient mice show no major bone phenotype *in vivo* in the basal state (they have a small, but significant increase in bone volume compared to littermate controls) and are resistant to stimulated osteoclastogenesis in response to injection of RANKL or parathyroid hormone [8]. In the absence of NIK, NF-κB signaling in OCP is initially intact. However once sufficient p100 accumulates in the cytosol it binds to Rel A/p50 dimers as they are released from IκBα. This cytoplasmic retention of NF-κB combined with the lack of p52 reduces NF-κB-mediated transcription in the nucleus and inhibits osteoclastogenesis. Reduced binding of Rel B to κB sites in the nucleus further contributes to reduced osteoclastogenesis. On the other hand, other factors in the bone microenvironment allow OC differentiation to occur at a normal basal rate in $NIK^{-/-}$ mice, but these cannot compensate for the inhibitory influence of accumulated p100 induced by exogenous RANKL or PTH. Hence it seems that NIK/p100 effects on osteoclastogenesis are important in clinical conditions of increased osteoclastogenesis. This was confirmed by the observation that $NIK^{-/-}$ mice showed reduction in bone erosion, but not inflammation in a serum transferred arthritis model (STA) [13].

NF-κB p50, p52, and double KO mice

Mice lacking p50 (*nfkb1*$^{-/-}$) show no basal bone phenotype [1] and co-culture studies showed reduced OC numbers in $p50^{-/-}$ mice. IL-1-induced *in vivo* and *ex vivo* osteoclastogenesis was not impaired in these mice compared to control mice [14]. Campbell et al. [15] reported that nfkb1$^{-/-}$ mice had a markedly diminished response to methylated BSA-induced acute arthritis and collagen-induced arthritis. Bone degradation in these mice was significantly reduced compared to that of Wt mice.

Similarly p52-deficient mice (*nfkb2*$^{-/-}$) also show no basal bone phenotype [1]. Despite unaltered osteoclastogenesis in $p52^{-/-}$ splenocytes in co-culture, BM from these mice showed enhancement of RANKL-mediated osteoclastogenesis *in vitro* [8]. However, IL-1-induced *in vivo* and *ex vivo* osteoclastogenesis in $p52^{-/-}$ is similar to $p50^{-/-}$ or wild-type mice [14]. Subtle changes in bone phenotype and slightly reduced *in vitro* osteoclast differentiation were observed in $p50^{-/-}/p52^{-/+}$ mice indicating that only a minimal number of functional osteoclasts are enough for bone remodeling and that osteoclast precursors in these mice may produce sufficient numbers of OCs for bone resorption [14]. FACS analysis of dKO mice showed a threefold increase in RANK-expressing splenocytes suggesting that NF-κB p50 and p52 is not required for RANK-expressing progenitor formation, but is necessary for RANKL-RANK-induced osteoclastogenesis [16]. Interestingly, IL-1-induced *in vivo* as well as *ex vivo* osteoclastogenesis was drastically reduced in $p50^{-/-}p52^{-/+}$ mice. Unlike single KO mice, the nfkb1$^{-/-}$ and nfkb2$^{-/-}$ dKO mice are osteopetrotic and show growth retardation, craniofacial abnormalities with unerupted incisor teeth. Incomplete rescue of these mice with marrow transplantation hints at an additional defect in the marrow microenvironment in addition to hematopoietic cell defects [1]. A similar study using the dKO mice showed that adoptive transfer of hematopoietic precursors from wild-type mice rescued osteopetrosis in these mice implying that the mice harbor a defect that tracked with the OC lineage rather than the osteoblast lineage [2]. Even though this phenotype mirrors that of c-fos-deficient mice, immunocytochemistry demonstrated that c-fos could be readily induced in $p50^{-/-}/p52^{-/-}$ splenocytes raising the possibility of failure in expression of c-fos at the right time in the appropriate cell type. This deduction may be acceptable, as a recent study showed that neither RANKL nor TNF could induce c-fos in dKO M-CSF dependent splenocytes [17]. Hence, the overall phenotype of the dKO mice and the *in vitro* data narrow the defect closely to the point in macrophage-OC differentiation control by c-fos [1,17].

Recently, characterization of mice with a novel mutation in nfkb2 has been reported [18]. This mutant allele encodes a non-processible form of p100 ($p100^{Lym1}$), preventing p52 formation. Histomorphometric analysis of homozygous mice ($Nfkb2^{Lym1/Lym1}$) revealed a mild osteopetrotic phenotype with significantly increased trabecular bone volume and number, indicating a defect in basal osteoclastogenesis. *In vitro* RANKL-induced osteoclastogenesis was significantly reduced in $Nfkb2^{Lym1/+}$ and $Nfkb2^{Lym1/Lym1}$ mice. However, no obvious defect in tooth eruption was observed in $Nfkb2^{Lym1/Lym1}$ mice. The similarities between $NIK^{-/-}$ and $Nfkb2^{Lym1/Lym1}$ mice suggest that NIK function is mainly responsible for p100 processing. Furthermore, nuclear transport of Rel A and p50 in response to LPS stimulation through the canonical pathway appeared to be inhibited in $Nfkb2^{Lym1/Lym1}$ cells suggesting the inability to process p100 ^{Lym1} in $Nfkb2^{Lym1/Lym1}$ cells results in a "super repressor" form of p100 that not only prevents the formation of p52, but also continually inhibits Rel A activation and nuclear translocation, regardless of stimulation with ligands that activate either the canonical or non-canonical pathway [18].

Rel A KO mice

Although knockout of p65 was embryonic lethal because of massive TNF-induced hepatocyte apoptosis [19], this phenotype was rescued by the generation of p65/TNFR-1 dKO mice, which lack TNF signaling [20] and seem to have no skeletal defect. In order to analyze the role of Rel A in osteoclast biology, radiation chimeras were generated by injecting bone marrow cells from $rela^{-/-}$/$tnfr^{-/-}$ to C57BL/6J mice. Histomorphometric analysis of these chimeric mice revealed a 50% reduction in osteoclast numbers and surface compared to Wt mice, although bone volume was not changed in these mice. In addition, RANKL-induced osteoclastogenesis was blunted in these mice *in vivo*. Further *in vitro* analysis demonstrated that Rel A is necessary for OCP survival, but not for OC differentiation [21,22]. Bone marrow macrophages (BMM) of $rela^{-/-}$ mice cultured in the presence of a JNK inhibitor (SP600125) and siRNA for Bid (siBid2) could rescue the OC differentiation defect, suggesting that Rel A opposes a pro-apoptotic pathway mediated by JNK and Bid in OCPs. The retroviral expression of Rel B did not affect the levels of apoptosis and did not rescue osteoclastogenesis in $rela^{-/-}$ BMM, despite the ability of this construct to rescue the $relb^{-/-}$ BMM defect [22]. Activation of the alternative pathway in these mice was not affected as RANKL-induced RelB-κB binding activity was similar in the presence or absence of Rel A. Furthermore, fetal liver cells from p65/p50 dKO and p65/c-Rel dKO mice have been used to reconstitute lethally irradiated mice, and there has been no mention of bone abnormalities in these reconstituted animals [23,24].

Rel B KO mice

Rel B, which was originally identified as an immediate-early gene in growth factor-induced fibroblasts, [25] acts in the late phase of RANKL-induced osteoclastogenesis. Rel B-deficient mice have multi organ inflammation, myeloid hyperplasia and splenomegally due to extramedullary hematopoiesis [26]. In $relb^{-/-}$ mice there is a small but significant increase in trabecular bone volume compared with wild-type littermates at base-line [22]. $relb^{-/-}$ mice show no difference in OC number or OC surface. In contrast $relb^{-/-}$ BMMs fail to differentiate into OCs in the presence of RANKL *in vitro*, and TNF-induced inflammatory osteolysis is blunted in $relb^{-/-}$ mice, suggesting an important role of Rel B in inflammatory-induced osteoclastogenesis.

It has been reported that $relb^{-/-}/p50^{-/-}$ mice have markedly increased myeloid hyperplasia in bone marrow and spleen compared to single KO $relb^{-/-}$ animals [27]. Heterozygous $relb^{-/+}$ mice that also lack $p50^{-/-}$ show qualitatively similar but moderate pathological changes supporting the notion that the lack of Rel B is partially compensated by other p50-containing complexes [27]. This could be the reason for the absence of a marked bone phenotype in $relb^{-/-}$ mice at the basal level.

c-Rel KO mice

Abbadie et al. [28] reported that over expression of c-Rel-induced apoptosis of avian BM cells. A study of murine models of acute and chronic arthritis using $c\text{-}Rel^{-/-}$ mice showed that these mice did not develop CIA, whereas they did develop acute arthritis comparable to Wt mice [15]. Inflammation-induced cartilage and bone degradation was similar in both Wt and $c\text{-}Rel^{-/-}$ mice. Therefore data from this study suggested that c-Rel is needed for systemic but not local joint disease. EMSA revealed presence of p50/p65 heterodimers and p50 homodimers in nuclear extracts of cells isolated from acutely inflamed joints of c-Rel-deficient mice. A recent study revealed that the κB DNA binding activity of c-Rel is unchanged in $rela^{-/-}$ BMM cultures compared to that of $rela^{+/+}$ cultures suggesting that c-Rel does not perform the same function as Rel A in osteoclasts [21].

Conclusion

The importance of NF-κB in osteoclastogenesis and function has been highlighted by several mouse models. Given the fact that NF-κB pathway is necessary for most cells, the inhibition of this pathway may result in undesirable side effects as a result of non-specific inhibition. Therefore, selective inhibition of its members may be a good approach in pharmacological drug development. Alternative pathway is a more attractive drug target as it is limited to a subset of TNF family of cytokines. Moreover more studies are required to see the feasibility of IκB like inhibitor p100 as a drug target.

Acknowledgement

The authors would like to thank Professor Keiichi Ohya and Dr. Kazuhiro Aoki (Section of Pharmacology, Graduate School, Tokyo Medical and Dental University) for their valuable comments during the preparation of this manuscript.

References

[1] V. Iotsova, J. Caamano, J. Loy, Y. Yang, A. Lewin, R. Bravo, Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2, Nat. Med. 3 (1997) 1285–1289.
[2] G. Franzoso, L. Carlson, L. Xing, L. Poljak, E.W. Shores, K.D. Brown, A. Leonardi, T. Tran, B.F. Boyce, U. Siebenlist, Requirement for NF-kappaB in osteoclast and B-cell development, Genes Dev. 11 (1997) 3482–3496.
[3] A. Hoffmann, D. Baltimore, Circuitry of nuclear factor kappaB signaling, Immunol. Rev. 210 (2006) 171–186.
[4] J.L. Ducut Sigala, V. Bottero, D.B. Young, A. Shevchenko, F. Mercurio, I.M. Verma, Activation of transcription factor NF-kappaB requires ELKS, an IkappaB kinase regulatory subunit, Science 304 (2004) 1963–1967.
[5] L.A. Solt, L.A. Madge, J.S. Orange, M.J. May, Interleukin-1-induced NF-kappaB activation is NEMO-dependent but does not require IKKbeta, J. Biol. Chem. 282 (2007) 8724–8733.
[6] T. Lawrence, M. Bebien, G.Y. Liu, V. Nizet, M. Karin, IKKalpha limits macrophage NF-kappaB activation and contributes to the resolution of inflammation, Nature 434 (2005) 1138–1143.
[7] E. Dejardin, The alternative NF-kappaB pathway from biochemistry to biology: pitfalls and promises for future drug development, Biochem. Pharmacol. 72 (2006) 1161–1179.
[8] D.V. Novack, L. Yin, A. Hagen-Stapleton, R.D. Schreiber, D.V. Goeddel, F.P. Ross, S.L. Teitelbaum, The IkappaB function of NF-kappaB2 p100 controls stimulated osteoclastogenesis, J. Exp. Med. 198 (2003) 771–781.
[9] M.G. Ruocco, S. Maeda, J.M. Park, T. Lawrence, L.C. Hsu, Y. Cao, G. Schett, E.F. Wagner, M. Karin, I{kappa}B kinase (IKK){beta}, but not IKK{alpha}, is a critical

mediator of osteoclast survival and is required for inflammation-induced bone loss, J. Exp. Med. 201 (2005) 1677–1687.
[10] E. Jimi, K. Aoki, H. Saito, F. D'Acquisto, M.J. May, I. Nakamura, T. Sudo, T. Kojima, F. Okamoto, H. Fukushima, K. Okabe, K. Ohya, S. Ghosh, Selective inhibition of NF-kappa B blocks osteoclastogenesis and prevents inflammatory bone destruction in-vivo, Nat. Med. 10 (2004) 617–624.
[11] N.S. Soysa, N. Alles, H. Shimokawa, E. Jimi, K. Aoki, K. Ohya, Inhibition of the classical NF-kB pathway prevents osteoclast bone-resorbing activity, J. Bone Miner. Metab. 27 (2009).
[12] M.L. Chaisson, D.G. Branstetter, J.M. Derry, A.P. Armstrong, M.E. Tometsko, K. Takeda, S. Akira, W.C. Dougall, Osteoclast differentiation is impaired in the absence of inhibitor of kappa B kinase alpha, J. Biol. Chem. 279 (2004) 54841–54848.
[13] K. Aya, M. Alhawagri, A. Hagen-Stapleton, H. Kitaura, O. Kanagawa, D.V. Novack, NF-(kappa)B-inducing kinase controls lymphocyte and osteoclast activities in inflammatory arthritis, J. Clin. Invest. 115 (2005) 1848–1854.
[14] L. Xing, L. Carlson, B. Story, Z. Tai, P. Keng, U. Siebenlist, B.F. Boyce, Expression of either NF-kappaB p50 or p52 in osteoclast precursors is required for IL-1-induced bone resorption, J. Bone Miner. Res. 18 (2003) 260–269.
[15] I.K. Campbell, S. Gerondakis, K. O'Donnell, I.P. Wicks, Distinct roles for the NF-kappaB1 (p50) and c-Rel transcription factors in inflammatory arthritis, J. Clin. Invest. 105 (2000) 1799–1806.
[16] L. Xing, T.P. Bushnell, L. Carlson, Z. Tai, M. Tondravi, U. Siebenlist, F. Young, B.F. Boyce, NF-kappaB p50 and p52 expression is not required for RANK-expressing osteoclast progenitor formation but is essential for RANK- and cytokine-mediated osteoclastogenesis, J. Bone Miner. Res. 17 (2002) 1200–1210.
[17] T. Yamashita, Z. Yao, F. Li, Q. Zhang, I.R. Badell, E.M. Schwarz, S. Takeshita, E.F. Wagner, M. Noda, K. Matsuo, L. Xing, B.F. Boyce, NF-kappaB p50 and p52 regulate receptor activator of NF-kappaB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1, J. Biol. Chem. 282 (2007) 18245–18253.
[18] E. Tucker, K. O'Donnell, M. Fuchsberger, A.A. Hilton, D. Metcalf, K. Greig, N.A. Sims, J.M. Quinn, W.S. Alexander, D.J. Hilton, B.T. Kile, D.M. Tarlinton, R. Starr, A novel mutation in the Nfkb2 gene generates an NF-kappa B2 "super repressor", J. Immunol. 179 (2007) 7514–7522.
[19] A.A. Beg, W.C. Sha, R.T. Bronson, S. Ghosh, D. Baltimore, Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B, Nature 376 (1995) 167–170.
[20] M.E. Rosenfeld, L. Prichard, N. Shiojiri, N. Fausto, Prevention of hepatic apoptosis and embryonic lethality in RelA/TNFR-1 double knockout mice, Am. J. Pathol. 156 (2000) 997–1007.
[21] S. Vaira, M. Alhawagri, I. Anwisye, H. Kitaura, R. Faccio, D.V. Novack, RelA/p65 promotes osteoclast differentiation by blocking a RANKL-induced apoptotic JNK pathway in mice, J Clin Invest 118 (2008) 2088–2097.
[22] S. Vaira, T. Johnson, A.C. Hirbe, M. Alhawagri, I. Anwisye, B. Sammut, J. O'Neal, W. Zou, K.N. Weilbaecher, R. Faccio, D.V. Novack, RelB is the NF-kappaB subunit downstream of NIK responsible for osteoclast differentiation, Proc. Natl. Acad. Sci. USA 105 (2008) 3897–3902.
[23] B.H. Horwitz, M.L. Scott, S.R. Cherry, R.T. Bronson, D. Baltimore, Failure of lymphopoiesis after adoptive transfer of NF-kappaB-deficient fetal liver cells, Immunity 6 (1997) 765–772.
[24] M. Grossmann, D. Metcalf, J. Merryfull, A. Beg, D. Baltimore, S. Gerondakis, The combined absence of the transcription factors Rel and RelA leads to multiple hemopoietic cell defects, Proc. Natl. Acad. Sci. USA 96 (1999) 11848–11853.
[25] R.P. Ryseck, P. Bull, M. Takamiya, V. Bours, U. Siebenlist, P. Dobrzanski, R. Bravo, RelB, a new Rel family transcription activator that can interact with p50-NF-kappa B, Mol. Cell. Biol. 12 (1992) 674–684.
[26] F. Weih, G. Warr, H. Yang, R. Bravo, Multifocal defects in immune responses in RelB-deficient mice, J. Immunol. 158 (1997) 5211–5218.
[27] F. Weih, S.K. Durham, D.S. Barton, W.C. Sha, D. Baltimore, R. Bravo, p50-NF-kappaB complexes partially compensate for the absence of RelB: severely increased pathology in p50(−/−)relB(−/−) double-knockout mice, J. Exp. Med. 185 (1997) 1359–1370.
[28] C. Abbadie, N. Kabrun, F. Bouali, J. Smardova, D. Stehelin, B. Vandenbunder, P.J. Enrietto, High levels of c-rel expression are associated with programmed cell death in the developing avian embryo and in bone marrow cells in-vitro, Cell 75 (1993) 899–912.