Akt Regulates Basal and Induced Processing of NF-κB2 (p100) to p52*

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NF-κB is a family of transcription factors important for innate and adaptive immunity. NF-κB is restricted to the cytoplasm by inhibitory proteins that are degraded when specifically phosphorylated, permitting NF-κB to enter the nucleus and activate target genes. Phosphorylation of the inhibitory proteins is mediated by an IkB kinase (IKK) complex, which can be composed of two subunits with enzymatic activity, IKKα and IKKβ. The preferred substrate for IKKβ is IkBα, degradation of which liberates p65 (RelA) to enter the nucleus where it induces genes important to innate immunity. IKKα activates a non-canonical NF-κB pathway in which p100 (NF-κB2) is processed to p52. Once produced, p52 can enter the nucleus and induce genes important to adaptive immunity. This study shows that Akt binds to and increases the activity of IkKα and thereby increases p52 production in cells. Constitutively active Akt augments non-canonical NF-κB activity, whereas kinase dead Akt or inhibition of phosphatidylinositol 3-kinase 3-kinase have the opposite effect. Basal and ligand-induced p52 production is reduced in mouse embryob fibroblasts deficient in Akt1 and Akt2 compared with parental cells. These observations show that Akt plays a role in activation of basal and induced non-canonical NF-κB activity.

The NF-κB family of transcription factors regulates development of the immune system and immunity and promotes cell viability (1–3). NF-κB is composed of dimers of subunits, among which are p105/p50 (NF-κB1), p100/p52 (NF-κB2), p65 (RelA), RelB, and c-Rel (4). In unstimulated cells, binary complexes of subunits are restricted to the cytoplasm by interaction with inhibitors of κB, IkB proteins. Cytokines, or UV radiation, promote the phosphorylation and degradation of the IkB proteins, permitting NF-κB to move into the nucleus and alter gene expression. Phosphorylation of IkBαs is mediated by an IkB kinase (IKK) complex composed of IKKa (IKK1), IKKB (IKK2), and IKKγ (NEMO) (5). IKKα and IKKB phosphorylate IkBαs, whereas IKKγ is a scaffolding protein essential for function of the complex. Homozygous deletion of IKKB diminishes cytokine-induced NF-κB activation and results in embryonic lethality in mice due to apoptosis of the liver (6–8). Cytokine-induced NF-κB activity is modestly reduced or unaffected in cells from IKKa knock-out animals that die shortly after birth due to skin and bone defects (9–11). Both kinases phosphorylate IkBα proteins, however, IKKB does this more effectively than IKKa (12–15). Thus, IKKa and IKKB have different substrate specificities and functions.

IKKB plays a predominant role in TNF- and interleukin-1-induced phosphorylation of IkBα, whereas IKKa is largely dispensable for this process. The IKKB-mediated pathway that leads to IkBα degradation and translocation of RelA/p50 heterodimers into the nucleus is the canonical pathway that plays an essential role in innate immunity (1, 3). Non-canonical NF-κB activation depends on processing of p100 (NF-κB2) to p52 (16–18). p100 contains C-terminal IkB-like ankyrin motifs that retain it in the cytoplasm (19, 20). The N-terminal product of p100 degradation, p52, does not contain ankyrin motifs, binds transcriptionally active Rel proteins, particularly RelB, and can enter the nucleus (19–22). Processing of p100 to p52 appears independent of IKKB and NEMO (23, 24); however, IKKa is obligate for this process, as p52 is absent in IKKa-deficient MEFs (18). Non-canonical NF-κB is important to B-cell maturation, lymphoid organ development, and adaptive immunity (16, 17).

NF-κB subunits and signaling events associated with the induction of the canonical and non-canonical pathways play important roles in immunity, cell viability and, when hyperactivated, in tumor development and survival of cancers (25, 26). Highly activated NF-κB is detected in many transformed cells and primary tumors, contributes to angiogenesis and metastasis, is activated by chemotherapy and radiation therapy, and contributes to their failure by activation of survival genes (25, 26). Production of p52 is important to immunity and the development and progression of cancer. In mice, overexpression of p52 in the absence of p100 leads to lymphocyte hyperplasia and transformation (27), and tumor-associated truncations of p100 have transforming effects in murine fibroblasts (28). In humans, chromosomal translocations that cause NγκB2 gene rearrangements and constitutive processing of p100 lead to B- and T-cell lymphomas (29). Deregulated p100 processing is associated with T-cell transformation by the human T-cell leukemia virus type I (30) and constitutive activation of NF-κB2 with breast (31) and skin cancers (32).

Because IKKa is necessary for non-canonical NF-κB activity (18, 33), identification of kinases that activate IKKa is of consequence. Akt, a downstream effector of PI 3-kinase (34), activates canonical NF-κB through cell type-specific mechanisms (35–40) and acts, at least in part, through IKKa (35). This led us to test whether Akt might play a role in activation of non-canonical NF-κB. Here we demonstrate that Akt promotes processing of p100 to p52 and therefore regulates non-canonical NF-κB activity.

MATERIALS AND METHODS

Reagents—Recombinant human TNF was from Genentech Inc. Antibodies to IKKa, IKKB, IKKγ, p52, and RelB were from Santa Cruz Bio-
technology. Anti-phospho-Akt and anti-Akt were from Cell Signaling, Inc., anti-RelA from Upstate Biotechnology, and anti-p50 from Geneka Biotechnology. An agonist, monoclonal antibody to the murine LTB2R was from Apotech Inc.

Cell Culture—Cells were grown in Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, penicillin/streptomycin, and l-glutamine at 37°C under 5% CO2. Spontaneously immortalized wild-type MEFs and Akt1−/−, Akt2−/− MEFs were a gift from Dr. Morris Birnbaum.

Transfections, Immunoprecipitations, and in Vitro Kinase Assays—293 cells were transfected using the calcium phosphate procedure and MEFs with Fugene-6. After transfection, cells were lysed and immunoprecipitations were conducted by modification of our previously described procedure (41). Cells were washed twice with ice-cold phosphate-buffered saline and lysed in 50 mM HEPES, pH 7.0, 150 mM NaCl, 10% glycerol, 1.2% Triton X-100, 1.5 mM MgCl2, 1 mM EGTA, 1 mM EDTA, 10 mM sodium pyrophosphate, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 0.15 unit/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 1 mM sodium orthovanadate, and 1 mM dithiothreitol. After lysates were centrifuged (13,000 rpm, 4°C) for 5 min, equal amounts of supernatant protein were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P. For immunoprecipitations, 1 μg of specified antibody was absorbed to Protein A/G Plus-agarose. Lysate was added to the antibody/bead conjugates, which were shaken for 2 h at 4°C. Immunoprecipitates were washed three times in lysis buffer, fractionated by SDS-PAGE, and transferred to Immobilon-P. For in vitro kinase assays, immunoprecipitates were washed twice with kinase assay buffer (10 mM HEPES, pH 7.4, 1 mM MnCl2, 5 mM MgCl2, 12.5 mM β-glycerophosphate, 1 mM sodium orthovanadate, 2 mM NaF, and 1 mM dithiothreitol). Immunoprecipitates in 15 μl of kinase assay buffer were incubated with 0.25 μCi/μl [γ-32P]ATP and 1 μg of GST-IkBa-(1–51) at 30°C for 30 min before the reaction was stopped.

Nuclear and Cytoplasmic Fractions—MEFs were scraped into 1 ml of phosphate-buffered saline and centrifuged, and the supernatant was aspirated. The cells were washed with ice-cold buffer A (10 mM HEPES, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 4 μg/ml aprotinin, 2 μg/ml leupeptin, 0.8 μg/ml pepstatin A, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride) and lysed in buffer B, which contained 0.1% Nonidet P-40 during a 5-min incubation on ice. Lysates were centrifuged at 4,500 rpm for 3 min, and cytosolic supernatants were retained. Pellets were washed twice with buffer A, which contained 1.7 M sucrose, being centrifuged at 12,000 rpm for 10 min after each wash. The nuclear fraction was isolated by incubating the pellet for 30 min at 4°C in 50 mM HEPES, pH 7.4, 10% glycerol, 400 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and the protease inhibitors in buffer A.

Gene Reporter Assays—MEFs were transiently co-transfected with 5xNF-kB reporter and Rous sarcoma virus β-galactosidase plasmids. Forty-eight hours after transfection, cells were incubated with 1 mM TNF or vehicle for 6 h, and luciferase activity was divided by β-galactosidase activity to normalize for variations in transfection efficiencies.

RNA Extraction and RT-PCR—Total RNA was isolated from MEFs using TRIzol. 1 μg of RNA was assayed using Superscript One-Step RT-PCR (Invitrogen). Primer pairs for mouse cIAP-2 are sense, 5’-GGGGAGAAATTTGACCTCGG-3’, and antisense, 5’-GTCGCGACCTGTCGG-3’. The expected size for mouse cIAP2 is 259 bp. The primer pairs for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are sense, 5’-GAGGACCGAGGTGTCTCC-3’, and antisense, 5’-CTCTGAGGGCCATGTAGG-3’. This yields a product of 232 bp. RT-PCR products were fractionated on 1% agarose gels and stained with ethidium bromide.

Confocal Microscopy—MEFs were treated with vehicle or LY294002 for 8 h, fixed, permeated with 0.1% Triton X-100, blocked with phosphate-buffered saline/2% bovine serum albumin, probed with anti-RelB, and then incubated with a Texas red-conjugated sheep anti-mouse secondary antibody. Syto 16 stain detected the cell nucleus. Excitation was performed using a Bio-Rad MRC 1024 krypton/argon laser confocal imaging system, and cells were visualized at 60× magnification.

To assay the fraction of nuclear RelB, the fluorescence intensity of nuclear and cytoplasmic staining was analyzed using ImageJ 1.33u software (National Institutes of Health). The nucleus of each cell in a field was highlighted, and its density was measured. Then the cytoplasm of each cell was highlighted, and its density was likewise determined. This was repeated for each cell in two fields for each experimental condition described in the Fig. legends. The graphical representation of the data is shown as a bar graph and depicts the average ratios of the nuclear to cytoplasmic mean fluorescence intensities (MFI (N)/MFI (C)) of all the cells in two fields for each condition tested.

RESULTS

Akt Induces p100 Processing to p52—To determine whether Akt activates non-canonical NF-κB, HEK 293 (Fig. 1, A and B) or HeLa (Fig. 1C) cells were transfected with CA-Akt or KD-Akt and p52 expression was assayed. CA-Akt, but not KD-Akt, increased p52 production. Evaluation of the effect of CA-Akt in replicate experiments showed this to be significant in 293 cells (Fig. 1B). Increased expression of p52 in HeLa cells was comparable to that in 293 cells (Fig. 1C).

Activated IκKα phosphorylates p100, initiating its degradation to p52, leading us to test whether Akt activates IκKα. An in vitro kinase assay revealed that Akt increases IκKα activity (Fig. 2, A and B). We also tested whether Akt and IκKα associate by immunoprecipitating IκKα, using MEFs

FIGURE 1. Akt induces p100 processing to p52. A, 293 cells were transfected with empty vector, CA-Akt, or KD-Akt, incubated in 2.5% fetal bovine serum and then harvested 48 h later. A Western blot prepared from cell lysates was probed with antibodies to p100/p52, Akt, and GAPDH. B, a bar graph showing results from three experiments conducted as described under A; errors bars represent the standard deviation of the mean. C, a representative experiment conducted as described under A using HeLa cells.

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which binds IKKα and IKKβ, from lysates of wild type (WT) MEFs or MEFs deficient in Ikkα or Ikkβ. Immunoblots show that WT MEFs contain a low level of IKKα relative to Ikkβ−/− MEFs and that Akt and IKKα co-immunoprecipitate from both cell types (Fig. 3). Akt was not immunoprecipitated from Ikkα−/− MEFs, showing that it does not complex IKKβ. These observations provide evidence that Akt complexes and activates IKKα and induces processing of p100 to p52.

NF-κB Activation in IKKα and IKKβ Knock-out Cells—We extended our use of cells deficient in Ikkα or Ikkβ to investigate p100 processing to p52 first by characterizing the capacity of the each cell type to activate canonical or non-canonical NF-κB. Activation of canonical NF-κB is initiated by degradation of IκBα, whereas non-canonical NF-κB activity results from processing of p100 to p52. TNF induced degradation of IκBα in WT and Ikkα−/− but not in Ikkβ−/− MEFs (Fig. 4A), showing that IKKβ plays a predominant role in activation of canonical NF-κB. Spontaneously immortalized MEFs were used for these experiments, because responses of these cells were less variable than those of primary MEFs.4 WT MEFs express a low level of p52, which is undetectable in Ikkα−/− cells but highly expressed in Ikkβ−/− MEFs (Fig. 4B) showing the necessity of IKKα for processing of p100 to p52. Because IKKα homo-oligomers predominate in the IKKβ knock-out cells, p100 degradation to p52 is the predominant pathway activated, whereas in WT MEFs canonical and non-canonical NF-κB activity can be induced. p100 complexes RelB and restricts it to the cytoplasm, whereas p52 shuttles RelB into the nucleus. The basal activity of IKKα led us to test whether it could induce production of p52 and affect RelB localization in cells not stimulated with a cytokine or factor previously associated with activation of non-canonical NF-κB. RelB, or p100/p52, was immunoprecipitated from cytoplasmic or nuclear fractions of actively growing MEFs, in which processing of p100 to p52 was not observed, RelB was cytoplasmic. Confocal microscopy shows significant nuclear RelB in Ikkβ−/− MEFs and less in WT or Ikkα−/− MEFs (Fig. 6A). The mean fluorescence intensity of stained RelB in two independent microscopic fields was also assayed as described under “Materials and Methods.” The

4 Q. Li and I. Verma, unpublished observations.
results presented in Fig. 6B show that more RelB is nuclear in IKKβ−/− cells than WT MEFs, which in turn contain more nuclear RelB than IKKα−/− cells. Thus, the greater fraction of nuclear RelB in Ikkβ−/− compared with WT MEFs correlates with elevated p52 in these cells. Also, in cells that express IKKα basal NF-κB2 activity promotes translocation of RelB into the nucleus. TNF stimulates canonical but not non-canonical NF-κB as it activates IKKβ, which promotes IκBα degradation, but not NIK, which is essential for p100 processing to p52 (42). Gene reporter assays using serum-starved MEFs and a consensus reporter that assays canonical and non-canonical activity show that TNF increased NF-κB transactivation 3.5- and 2.2-fold in WT and Ikkα−/− cells, respectively, but did not increase NF-κB activity in Ikkβ−/− cells (Fig. 7A, left). The -fold stimulation of NF-κB gene reporter activity in WT MEFs reported here is comparable to that found by Sizemore et al. (43) using the same cell type. By determining NF-κB activity without comparison to control (Fig. 7A, right) one finds that TNF activated NF-κB in WT and Ikkα−/− cells; however, basal activity was 10-fold greater in WT than in Ikkα−/− cells. TNF did not stimulate NF-κB in Ikkβ−/− cells, but basal transactivation in Ikkβ−/− cells was 3 times greater than in WT and 200 times greater than in Ikkα−/− cells.

To validate the gene reporter assays we assayed expression of the mRNA for an NF-κB target gene, cIAP2, in control- and TNF-stimulated MEFs by RT-PCR (Fig. 7B). TNF induced c-IAP2 mRNA in WT and Ikkα−/−, but not in Ikkβ−/−, MEFs. The basal level of c-IAP2 mRNA in Ikkβ−/− cells was higher than in WT cells and much higher than in Ikkα−/− cells. As these latter cells were deficient in IKKβ, expression of c-IAP2 mRNA must have resulted from activation of non-canonical NF-κB by IKKα. Thus, p100 processing to p52 and gene induction associated with non-canonical NF-κB is observed due to basal IKKα activity in growing cells. Because p52/RelB binds DNA and activates genes, we postulated that by increasing p52, Akt would augment NF-κB transactivation. To test the effect of p52 on NF-κB, we assayed transactivation in WT and Ikkα−/− MEFs transfected with p52 or empty vector. p52 elevated
NF-κB transactivation. These results show that factors that activate IKKα and induce p52 diminish the response to TNF (Fig. 7C). This suggests that NF-κB activity in WT MEFs is a composite of the canonical and non-canonical pathways. A unique site in the promoters of some genes specifically binds p52/RelB (44). Other sites are activated rapidly by p50/RelA and more slowly by p52/RelB (45, 46). This results from dimer exchange at promoter binding sites, and permits intersection of adaptive and innate immunity (44–46), which require activation of distinct and common genes in a temporally correct manner. That p52 expression diminishes the effect of TNF on a consensus NF-κB pro-
motor probably results from competition for binding and supports the view that signaling important to innate and/or adaptive immunity may induce distinct and common genes.

PI 3-Kinase/Akt Signaling Affects Non-canonical NF-κB Activity in IKKα Knock-out Cells—TNF and lymphotoxin β activate Akt in WT, Ikke−/−, and Ikkβ−/− MEFs (data not shown), permitting us to test for a role for PI 3-kinase/Akt signaling in production of p52. In MEFs treated with the PI 3-kinase inhibitor LY294002, p52 production was diminished (Fig. 8A). Detection of residual p52 in cells with impaired PI 3-kinase activity likely resulted from failure of p52 present at the initiation of the experiment to turn over. This experiment supports a role for Akt and IKKα in establishing the basal level of cellular p52.

The demonstration that Akt is important for p52 production led to the hypothesis that inhibition of PI 3-kinase function would impair nuclear localization of RelB and it did (Fig. 8B). Assay of the mean fluorescence intensity of RelB in Ikkβ−/− MEFs and Ikkβ−/− MEFs preincubated with LY294002 shows that inhibition of PI 3-kinase activity diminishes the amount of nuclear RelB (Fig. 8C).

These results led us to test whether KD-Akt, which acts as a dominant-negative to block Akt action (36, 47), would diminish NF-κB transactivation in WT or Ikkβ−/− MEFs, with a positive result (Fig. 8D). That this results from an effect of KD-Akt on NF-κB is supported by confocal microscopy showing that inhibition of PI 3-kinase/Akt in Ikkβ−/− MEFs restricts nuclear localization of RelB (Fig. 8D), which is mediated by its interaction with p52, and by the obligate role of IKKα in activation of non-canonical NF-κB (16, 17). These observations show a previously unappreciated relationship between PI 3-kinase/Akt signaling, p52 production, and RelB localization.

p100 Processing to p52 in Akt-deficient Cells—Experiments were conducted with MEFs or MEFs deficient in Akt1 and Akt2. First, we tested whether incubation of 293 cells or MEFs with an agonist antibody to the lymphotoxin β receptor (LTβR), which induces non-canonical NF-κB, could activate Akt with positive results (Fig. 9A, top). An average of results from three independent experiments, normalized to GAPDH, showed that the agonist antibody to the LTβR induced a 1.8-fold increase of phospho-Akt (active Akt) in 293 cells 60 min after the initiation of incubation (Fig. 9A, bottom). In MEFs, averaging results from two independent experiments and normalizing to GAPDH showed that activation of the LTβR induced a 2.1-fold increase of Akt phosphorylation 15 min after initiation of incubation. The magnitude of these effects is comparable to the increase of Akt activity induced by TNF in cells (35). The demonstration that the LTβR activates Akt led us to determine whether this was mediated by PI 3-kinase signaling. To test this, 293 cells were incubated in the absence or presence of LY294002 before stimulation with an agonist antibody to the LTβR (Fig. 9B). Inhibition of PI 3-kinase impaired the capacity of the activated LTβR to induce phosphorylation of Akt. Thus, PI 3-kinase/Akt signaling is induced by the LTβR.

To test whether Akt plays a role in LTβR-induced activation of NF-κB, we used the WT- and Akt-deficient MEFs. The agonist LTβR antibody increased p52 in WT cells, and this was attenuated in MEFs deficient in Akt−/− and Akt2−/− (Fig. 10A). The basal level of p52 was also diminished in Akt-deficient compared with WT cells. Evaluation of results from six independent experiments showed that basal processing of p100 to p52 was diminished in double knock-out compared with WT cells, and this was statistically significant (Fig. 10B). Fig. 10C shows that LTβR-induced p52 production was diminished by Akt deficiency as shown by analysis of results from five independent experiments.
DISCUSSION

Mitogen-activated protein kinases activate the IKK complex. One of these, NF-κB-inducing kinase (NIK) activates IKKα, which by phosphorylating p100 initiates its processing to p52 (18, 48). p100 processing to p52 is blocked by dominant negative IKKα (48, 49) and in cells from alpophasia (aly) mice, which contain a mutant NIK gene (48, 49) showing that IKKα and NIK are required for p100 processing. Also, by recruiting IKKα to p100 NIK organizes the complex that processes p100 to p52 (50).

Akt increases transactivation of p65, which induces p100 (51–53) and therefore increases the pool of p100 available for processing to p52. We show that Akt not only affects the amount of p100 available for processing, but the processing itself. IKK shows that Akt not only affects the amount of p100 available for processing to p52 (50).

NIK, which in turn interacts with and activates NIK (55, 56). Thus, the activation (55, 56). Downstream of the T-cell receptor Akt activates Cot, which associates with Cot/Tpl2, a mitogen-activated protein 3-kinase-receptor that act on distinct domains each promote activation of IKK. Additionally, blockade of PI 3-kinase/Akt with LY294002 diminishes expression of p52 in Ikkg/−/− cells. In these cells, the absence of Ikkb precludes increased transactivation of p65 (RelA) and induction of its target gene, p100, indicating that the effect of PI 3-kinase inhibition is on p100 processing and not its synthesis. A role for Akt in p52 production is also supported by observation of more nuclear RelB in Ikkb/−/− MEFs that express p52 than in Ikka/−/− MEFs in which NF-κB2 is not activated, inhibition of RelB nuclear localization in Ikkb/−/− MEFs treated with LY294002, and suppression of NF-κB2 transactivation in Ikkb/−/− MEFs by KD-Akt.

Additional support for a role for Akt in p100 processing to p52 comes from studies with Akt-deficient MEFs. The absence of Akt1 and Akt2 diminished the basal level of p52 in MEFs and the capacity of an agonist antibody to the LTβR to promote processing. These observations show that Akt affects basal expression of p52 and plays a role in induced processing of p100 to p52.

Little difference was observed in p100 expression in WT- and Akt-deficient MEFs, suggesting that Akt has a more substantial effect on p100 processing to p52 than on p100 production through increased transactivation of p65. The results presented here demonstrate that Akt is important to the induction of non-canonical NF-κB, whereas our previous observations showed that Akt plays a role in activation of canonical NF-κB (35). Considered together, our studies suggest that Akt affects innate and adaptive immunity and cell survival through induction of canonical and non-canonical NF-κB.

Akt phosphorylates threonine-23 in IKKα (35), whereas NIK phosphorylates serine-176 in the kinase loop of IKKα (54). How can kinases that act on distinct domains each promote activation of IKKα? Akt and NIK associate with Cot/Tpl2, a mitogen-activated protein 3-kinase-related serine-threonine kinase that induces NF-κB-dependent transcription (55, 56). Downstream of the T-cell receptor Akt activates Cot, which in turn interacts with and activates NIK (55, 56). Thus, the NIK-IKKɛp100 complex (50) may contain additional components. Also, phosphorylation by Akt may alter the tertiary structure of IKKα and affect its activity. Such regulation is found in the capacity of phosphorylations to affect access of substrates to the catalytic domains of Src family kinases and the ZAP-70 tyrosine kinase, thereby positively or negatively regulating their activities (57, 58).

Integration of IKKα into heterogeneous populations of signalosomes may affect its function. IKKα and IKKβ form heterodimers in which activation of IKKα is necessary for subsequent activation of IKKβ (59). This may provide a regulatory role for IKKα in innate immunity, in addition to its role in adaptive immunity. Homodimers and heterodimers of IKKα and IKKβ are in cells (12) and the proportion of IKKα to IKKβ varies among cell types (39). Thus, IKKα homodimers that activate non-canonical NF-κB, the IKKα-IKKβ heterodimers and IKKβ homodimers that activate canonical NF-κB, and the activities of canonical and non-canonical NF-κB are likely to vary with cell type.

Induction of p100 processing to p52 is receptor-specific (42). TNF induces NIK-independent recruitment of the IkB kinase complex to the type 1 TNF receptor; consequently, TNFR1 activates canonical, but not non-canonical NF-κB. Activation of CD27 by CD70 leads to recruitment of NIK, as well as the IkB kinase signalosome, to the receptor. Consequently, CD70 activates non-canonical as well as canonical NF-κB. Thus, access of receptors to signaling molecules (39) and whether or not the cytoplasmic domain of a receptor can interact with signaling molecules (42) determine whether a receptor can activate canonical NF-κB, non-canonical NF-κB, or both.

A recent study reported that interferon induces p100 processing to p52 that was dependent on NIK and TRAF2 but not PI 3-kinase (60). The observation that PI-3 kinase was not involved in activation of non-canonical NF-κB by interferon in the Daudi line of hematopoietic cells distinguishes it from the present investigation of the capacity of the LTβR to activate NF-κB in non-hematopoietic cells. The differences in these studies reinforce the conclusion that activation of canonical and non-canonical NF-κB by various receptors is dependent on the signaling proteins with which the receptor can interact (42) and the presence or absence of such proteins in the cell being studied (39).

Mice deficient in Nf-κB2, NIK, or aly/aly mice that express mutant NIK are characterized by the absence of lymph nodes, Peyer’s patches, disorganized splenic, and thymic architecture, defects in B-cell function, and immunodeficiency (61–64). Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis characterize mice lacking Akt1 and Akt2 (65); these animals die within hours of birth and retain Akt3, and their immune function has not been evaluated. However, support for a role for Akt in Nf-κB2 activation in addition to that presented here comes from other avenues of experimentation. Deregulation of PI 3-kinase/Akt signaling occurs in human cancers due to gene amplification, activating mutations, or loss of the PTEN tumor suppressor, which dephosphorylates the lipid mediators of activated PI 3-kinase (34). Deletion of Pten in T cells causes aggressive lymphomas, defective thymic negative selection, increased thymic cellularity, elevated B cell numbers, and autoantibody production; mammary deletion of Pten causes breast cancers (66–68). Mice heterozygous for a Pten null mutation tend to develop lymphoid hyperplasia, which can progress to T cell lymphoma (69, 70). PI 3-kinase is required for B- and T-cell proliferation, and aberrant activity is associated with a lymphoproliferative disorder that progresses to lymphoma when crossed with p53 null mice (71), autoimmunity, and leukemia (71–73). Knock-out of the regulatory subunit of PI 3-kinase, p58α, results in mice with altered splenic B-cell subsets, increased B-cell proliferation, and an autoimmune disorder (71–76). The Philadelphia chromosome BCR/Abl fusion that causes chronic myelogenous leukemia constitutively activates the Abl tyrosine kinase and the PI 3-kinase pathway (77). Mutants that do not activate PI 3-kinase are not transforming unless a constitutively active Akt transgene is present, showing the obligate role of PI 3-kinase/Akt signaling for transformation (78). In breast cancer, transforming events due to overexpression of HER-2/neu result from enhanced PI 3-kinase/Akt signaling (79, 80). Finally, the T-cell leukemia 1 oncprotein enhances Akt activity, is highly expressed in many human B-cell leukemias, and causes B-cell lymphomas in mice (81). Thus, activation of Akt by mutations associated with leukemia and breast cancer appears essential for the transforming activity of the oncogenes.
The similar pathologies and cellular derangements associated with alteration of previously known components of the NF-κB2 activation pathway and the PTEN/PI 3-kinase/Akt pathway are consistent with a role for Akt in activation of NF-κB2. The biochemical and genetic data presented here support this conclusion. Hyperactivation of PI 3-kinase/Akt signaling is common in cancers and supports tumorigenesis (82). The discovery that Akt plays a role in p52 production identifies a novel mechanism through which it may promote immune responses and provides another link between Akt and tumorigenesis (82).

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