tions. However, the potential toxicities that may be associated with immunotherapy targeted at angiogenic agents should not dampen our enthusiasm for moving these strategies through preclinical studies, but rather should ignite the desire to move forward quickly with a watchful eye toward rationally designed clinical trials.

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Is NF-κB2/p100 a direct activator of programmed cell death?

Transcription factor NF-κB has been implicated in cancer development due to its ability to upregulate expression of genes with potentially prooncogenic functions, such as cell cycle regulators and antiapoptotic proteins (Karin et al., 2002). A recent report by Wang et al. (2002) suggests that a structural motif, a death domain (DD), present in one of the mammalian NF-κB proteins, NF-κB2/p100, allows it to directly activate cell death in a transcription-independent manner. Further, it is suggested that loss of the proapoptotic function of NF-κB2/p100 is directly linked to its oncogenic activity in lymphomas.

Nuclear factor of κB (NF-κB) defines a family of related homo- and heterodimeric transcription factors (Ghosh and Karin, 2002). It includes proteins that are synthesized in their mature form and proteins that are synthesized as large precursors that undergo proteolytic processing. The first group encompasses RelA, RelB, and c-Rel, which have an N-terminal Rel homology domain (RHD), required for DNA binding and dimerization, and a C-terminal region with transactivating properties (Figure 1). These proteins are held in the cytoplasm by "Inhibitors of κB" (IκB) proteins, characterized by 6–7 ankyrin repeats that are required for binding to the RHD of Rel proteins and masking their nuclear localization sequences. Upon cell stimulation, the IκBs are phosphorylated and undergo ubiquitin-dependent degradation. The liberated NF-κB dimers enter the nucleus and act as sequence-specific transcription factors. The second group consists of NF-κB1/p105 and NF-κB2/p100 and their products p50 and p52, respectively. The precursors contain an N-terminal RHD as well as a C-terminal region that contains ankyrin repeats similar to those in IκBs (Figure 1). In their C terminus, the precursors contain a death domain (DD). By virtue of their ankyrin repeats and their ability to dimerize with other Rel proteins, p105 and p100 act like IκBs (Mercurio et al., 1993). While p105 processing is largely constitutive, processing of p100 is triggered by its phosphorylation and ubiquitination in a manner akin to IκB degradation (Xiao et al., 2001). Limited proteolysis results in generation of the mature NF-κB2/p52 transcription factor consisting of the N-terminal RHD.

The pathways that lead to activation of NF-κB and processing of p100 depend on components of the IκB kinase (IKK) complex, which contains two catalytic subunits, IKKα and IKKβ, and a regulatory subunit IKKγ/NEMO (Rothwarf et al., 1998). After activation by stimuli like the proinflammatory cytokine TNFα or bacterial products such as LPS, IKKβ phosphorylates the IκBs, leading to their degradation and nuclear entry of NF-κB dimers. This pathway, named the canonical NF-κB activation pathway, applies primarily to NF-κB dimers composed of RelA, c-Rel, and p50. An alternative NF-κB signaling pathway involving IKKα leading to p100 processing has recently been described (Senftleben et al., 2001). This pathway is triggered upon occupancy of BAFF (B cell activating factor) receptor (BAFF-R) and CD40 on B cells and lymphotoxin β receptor (LTβR) on stromal cells. Activation of this pathway results in disappearance of p100 and nuclear translocation of p52:RelB dimers (Dejardin et al., 2002; Senftleben et al., 2001; Xiao et al., 2001). Processing of p100 depends on the catalytic activities of IKKα and another kinase, NIK, which probably acts upstream of IKKα (Senftleben et al., 2001). The function of this pathway is still not well defined, but it is likely to be required for B cell maturation and formation of secondary lymphoid organs (Senftleben et al., 2001). The DD of p100 is also involved in processing, as its removal leads to constitutive processing as well as inhibition of NIK-dependent phosphorylation and processing (Fong et al., 2002). Notably, mutant forms of NF-κB2 were detected in B and T cell lymphomas, where they are caused by chromosomal translocations that result in truncations of the regulatory C-terminal

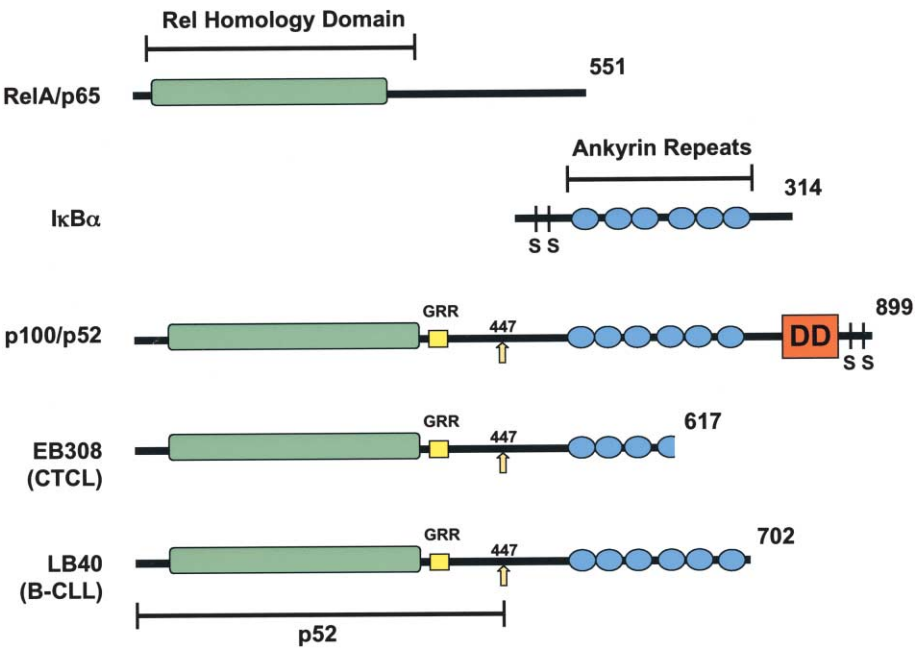

Figure 1. NF-κB and IκB proteins

Schematic representation of the NF-κB proteins RelA/p65, NF-κB2/p100, NF-κB2/p52, IκBα, and tumor-derived mutants of NF-κB2. The mutants, EB308 and LB40, were found in a cutaneous T cell lymphoma (CTLC) and a B cell chronic lymphocytic leukemia (B-CLL), respectively (Migliazza et al., 1994). DD, death domain; GRR, glycine rich region. Established (IκBα) and putative (p100) serine phosphoacceptor sites (S) are indicated.

domain, including the DD (Migliazza et al., 1994). Invariably, these mutations result in constitutive p52 production and reduction of p100 (Figure 1). It has been proposed that the constitutive production of p52 and nuclear entry of p52:RelB dimers lead to aberrant growth regulation and/or suppression of apoptosis in lymphocytes, thereby promoting lymphomagenesis (Karin et al., 2002). This mechanism should be dependent on the transcriptional activity of p52:RelB dimers.

A different explanation for the transforming potential of this chromosomal rearrangement based on the elimination of NF-κB2/p100 rather than p52 production is proposed by Wang et al. (2002), who suggest that recruitment of p100 via its DD to death receptors, such as the type I TNF receptor (TNFRI) or FAS, leads to caspase 8 and caspase 3 activation and cell death. According to this model, it is the loss of p100-mediated cell death that contributes to lymphomagenesis. Unfortunately, several technical and conceptual problems cast shadows on this attractive scenario. Curiously, the starting point for this work was the use of a cell-free system to study induction of p53-dependent cell death by ionizing radiation (IR). Partially purified cell extracts from irradiated p53 wt cells (depleted of caspase 8) were screened for their ability to activate caspases 8 and 3 in extracts of p53-deficient cells. A series of chromatographic purification steps yielded in a (still crude) fraction that induced caspase 8 and 3 activation. This fraction contained p100, in addition to many other polypeptides. Depletion of p100 from this fraction, but not from the initial whole cell extract, reduced caspase 3 activation. To study the functional significance of this finding, the authors undertook a series of overexpression and underexpression experiments using wt p100 and C-terminal truncations thereof. Overexpression of p100 conferred sensitivity to apoptosis triggered by ligation of either TNFR1 or FAS, as long as the DD in p100 was present. Surprisingly, IR (whose mechanism of induced cell death was the starting point for this work) was not included in these experiments as one of the proapoptotic stimuli. Next, recruitment of p100 into TNFRI and FAS signaling complexes was investigated. Again, dependent on its DD, p100 was found to be recruited to activated TNFRI and FAS. Strikingly, overexpression of the isolated DD of p100 was sufficient to induce cell death. To examine the relevance of all of this to cancer, the authors show that wt p100, but not its mature and DD-lacking form p52, reduces RAS-induced transformation of NIH3T3 fibroblasts. In conclusion, the authors suggest that p100 is an integral part of a death-inducing signaling complex (DISC), acting independently of its ability to give rise to the p52 transcription factor and to sequester RelB in the cytoplasm.

As mentioned above, both problems inherent to this work and results obtained by others question the physiological relevance of this novel mechanism. First, it is well established that IR and FAS or TNFRI ligation trigger apoptosis through entirely different pathways (Kumar and Vaux, 2002). Thus, it is difficult to understand why p100, recruited to TNFRI or FAS, should be an active and essential ingredient of an apoptosis-triggered extract prepared from irradiated p53wt cells. In vivo, caspase 8 is required for apoptosis triggered by death receptors, but not by DNA damaging agents, whose proapoptotic action was recently suggested to depend on caspase 2 activation (Lassus et al., 2002). Another limitation is that depletion of p100 reduces the activity of a partially purified fraction but does not reduce the caspase activation ability of the unfractionated extract. Therefore, at best, the complex containing p100 is one of several activities that promote caspase activation, whose physiological role in IR-mediated apoptosis has not been investigated. It is also not examined whether reduced p100 expression inhibits FAS-induced apoptosis. Given that p100 may associate with a variety of proteins and that some of these associations may occur after the cells are homogenized, it is possible that immunodepletion of p100 may remove proapoptotic mediators, although p100 itself does not have such an activity, as suggested by the analysis of p100-deficient lymphocytes (see below). A different but just as important limitation of this work is that it is impossible to separate the DD from p100 without affecting its processing to p52, apart from experiments in which the DD is investigated in isolation. As outlined above, deletion of the DD leads to constitutive generation of p52 and elimination of the IκB-like C-terminal portion of p100. Thus, it is difficult to understand why Wang et al. did not detect enhanced p52 production from

the truncated versions of p100 and instead found that these polypeptides can still inhibit NF-κB. It is possible that expression of different amounts of p100 and its truncation mutants may result in different and unpredictable outcomes, ranging from induction of transcription through p52:RelB dimers to inhibition of NF-κB-dependent transcription of anti-apoptotic genes through nonphysiological titration of RelA or c-Rel. The observed recruitment of p100 into the DISCs, generated by TNFRI and FAS, further complicates interpretation of the original findings with the cell-free system, as membrane-bound receptors and their DISCs are expected to be excluded from the cytosolic S100 extract. Therefore, a different, non-membrane-bound, p100-containing signaling complex must be postulated to be activated by IR. Even more difficult to explain is the apoptotic activity of the isolated NF-κB2 DD. As DDs can only engage in bipartite homotypic interactions (Hofmann, 1999), it is difficult to envision how a single DD can bridge two signaling molecules leading to caspase activation through the well-established induced proximity mechanism (Salvesen and Dixit, 1999). In fact, an isolated DD can only be expected to function as a dominant negative inhibitor of DD-mediated signaling (Hofmann, 1999). Thus, it might be possible that expression of the p100 DD leads to inhibition of p100 processing and the accumulated p100 nonspecifically titrates off RelA and c-Rel.

Given the complexities of the experimental approach used by Wang et al., one needs to consider what happens to cells unable to express NF-κB2/p100. Fortunately, three different mouse mutants that lack p100 due to either a complete knockout of the NF-κB2 gene (Franzoso et al., 1998), removal of its 3′ half (Ishikawa et al., 1997), or ENU-induced mutation (Miosge et al., 2002) were described. According to Wang et al., all of these mutations should invariably result in suppression of apoptosis, regardless of what happens to p52. However, such an aberration has not been described in mice lacking both p100 and p52. On the contrary, detailed analysis of xdr/xdr mice, which lack p100 and p52, reveals a B cell deficiency similar to the one caused by defective signaling through the antiapoptotic BAFF-R (Miosge et al., 2002). These aberrations are very similar to those caused by the absence of IKKα or NIK in B cells (Senftleben et al., 2001). Rather than causing their proapoptotic effect through accumulation of p100, the NIK and IKKα deficiencies are likely to sensitize B cells to apoptosis by interfering with BAFF-R signaling (Claudio et al., 2002). Most likely, the chromosomal rearrangements affecting the human NF-κB2 locus promote lymphomagenesis through a similar mechanism.

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