IκB-α: At the crossroad between oncogenic and tumor-suppressive signals (Review)

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Abstract. Nuclear factor κB (NF-κB) is an essential component of tumorigenesis and resistance to cancer treatments. NFKB inhibitor α (IκB-α) acts as a negative regulator of the classical NF-κB pathway through its ability to maintain the presence of NF-κB in the cytoplasm. However, IκB-α is also able to form a complex with tumor protein p53, promoting its inactivation. Recently, we demonstrated that IκB-α is able to mediate p53 nuclear exclusion and inactivation in chronic myeloid leukemia, indicating that IκB-α can modulate either oncogenic or tumor-suppressive functions, with important implications for cancer treatment. The present review describes the role of IκB-α in cancer pathogenesis, with particular attention to hematological cancers, and highlights the involvement of IκB-α in the regulation of p53 tumor-suppressive functions.

Contents

1. Introduction

Nuclear factor κB (NF-κB) proteins comprise a family of transcription factors that includes RelA (also known as p65), RelB, c-Rel, p50 (which originates from the p105 precursor) and p52 (which originates from the p100 precursor) (1,2). NF-κB transcription factors have essential roles in numerous physiological and pathological processes, which have been extensively reviewed (3-5). NF-κB members form homodimeric and heterodimeric complexes that are involved in signaling through canonical and non-canonical pathways (Fig. 1). In particular, the NF-κB canonical pathway is based on the p65/p50 dimer, which is physiologically trapped in the cytoplasm by the inhibitor protein NFKB inhibitor α (IκB-α) (Fig. 1A) (6). Various stimuli, including inflammatory cytokines [e.g. tumor necrosis factor α (TNF-α)], radiation, stress signals and cell surface receptors, induce the activation of the IκB-kinase (IKK) complex, which is in turn able to promote the serine phosphorylation of IκB-α (7). This event primes IκB-α for proteasomal degradation (8) and, as a consequence, free NF-κB dimers are allowed to migrate into the nucleus, where they regulate gene expression, leading to cellular proliferation and resistance to apoptosis (1). Notably, one of the first genes to be transcribed is *NFKBIA*, the gene that encodes IκB-α, allowing the generation of new IκB-α protein in order to terminate NF-κB signaling through its removal from the nucleus (9,10).

The non-canonical NF-κB activation pathway promotes the activation of the p50 and p52 NF-κB subunits (Fig. 1B). This pathway, also known as the alternative pathway, requires the activation of NF-κB-inducing kinase, which is able to promote IKK-α-mediated p100 degradation (11). Similarly to IκB-α, p100 prevents the translocation of RelB/p50 and RelB/p52 into the nucleus.

These two pathways are involved in numerous biological and pathological processes, ranging from immunological responses to cancer pathogenesis. Due to the key role of NF-κB in such processes, NF-κB signaling has been extensively investigated from a therapeutic standpoint (12). In addition to the development of several selective inhibitors of the kinases involved in NF-κB signaling (e.g. IKK) (13), the most relevant and clinically used therapeutic approach is to prevent the degradation of IκB-α through proteasome inhibitors (14). In particular, bortezomib is routinely used for the treatment of multiple myeloma and other cancers, due to its ability to prevent IκB-α degradation and therefore block NF-κB signaling (15,16). Clinical experiences with proteasome inhibitors have demonstrated that IκB proteins act as essential mediators of NF-κB signaling; however, the true

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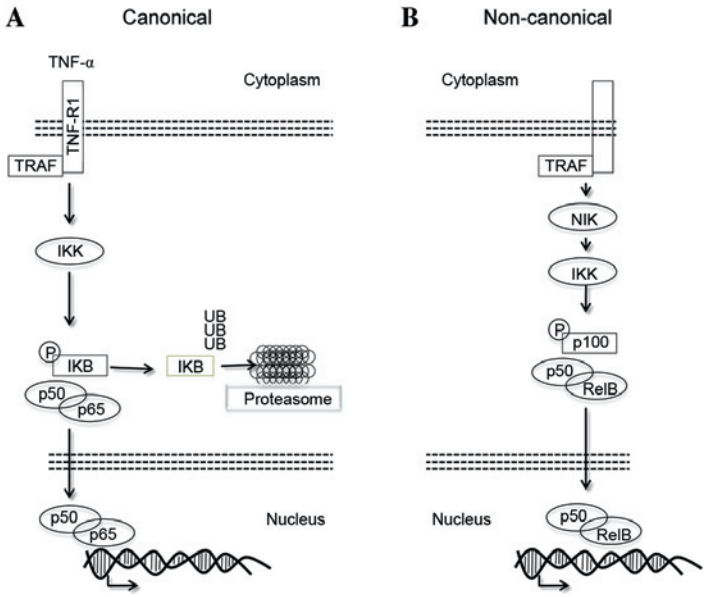

Figure 1. NF-κB signaling. The figure shows a schematic representation of (A) canonical and (B) non-canonical NF-κB signaling. NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor α; TNF-R1, tumor necrosis factor receptor 1; TRAF, TNF receptor-associated factor; IKK, IκB kinase; P, phosphate; IKB, NFKB inhibitor; UB, ubiquitin; NIK, NF-κB-inducing kinase.

contribution of IκB proteins to cancer pathogenesis is far from being exhaustively investigated.

2. IκB proteins

IκB proteins comprise a family of proteins that includes the typical IκBs expressed in the cytoplasm, which undergo stimuli-dependent phosphorylation, degradation and re-synthesis; the atypical IκBs (such as B-cell CLL/lymphoma 3), which are barely expressed in basal conditions, and are upregulated upon stimulation and translocated into the nucleus; and the precursor proteins p100 and p105 (7). The typical IκBs include IκB-α, IκB-β and IκB-ε, and are characterized by the presence of six ankyrin repeats (7,17). It has been generally reported that IκB proteins mask the nuclear localization signal of NF-κB (18,19). However, a lack of IκBs only partially promotes the translocation of p65 into the nucleus, due to the compensation of other IκB proteins, suggesting that IκBs are primarily involved in the regulation and inhibition of basal NF-κB activation (20,21). IκB-α is a 37-kDa protein, which is phosphorylated in response to Toll-like receptor, TNF-α, interleukin 1 and other stimulatory signals, and is predominantly able to bind to heterodimers containing p50, p65 and c-Rel. IκB-α is the product of the *NFKBIA* gene at chromosome 14 (22).

3. IκB-α-knockout murine models

IκB-α-knockout mice are apparently normal at birth, but inevitably die within few days due to skin defects and extensive granulopoiesis. The bone marrow cells exhibit increased NF-κB activity, and the concurrent deletion of p50 partially rescues the phenotype (20). Similar data have been observed in a different knockout model (21). In particular, IκB-α homozygous null mice are normal at birth but die by day 10 due to the development of dry, flaky skin. In this model, the authors observed an increase in myelopoiesis in the spleen, which was considered to be of reactive origin (21).

4. IκB-α involvement in cancer from a genetic perspective

The role of IκB-α in murine models suggests that IκB-α acts as a tumor suppressor. In line with these phenotypes, IκB-α has been suggested to act as a tumor suppressor in various types of cancer. In particular, ~25% of glioblastomas harbor heterozygous *NFKBIA* gene deletions at chromosome 14q13 (23,24). Notably, the loss of *NFKBIA* has also been associated with shortened patient survival time; patients with *NFKBIA* loss had outcomes similar to those observed in patients with EGFR amplification, which are poorer than those observed in patients with normal levels of NFKBIA and EGFR, suggesting that *NFKBIA* deletion is a prognostic factor in glioblastoma (23). In Hodgkin lymphoma, *NFKBIA* was found to be mutated in rare cases, indicating a role of *NFKBIA* as a tumor suppressor in this lymphoid cancer (25). In 16% of lung cancer specimens, immunohistochemical analysis revealed an absence of IκB-α protein expression. In particular, *NFKBIA* appeared to be silenced in cases with wild-type epidermal growth factor receptor/wild-type RAS and in the absence of echinoderm microtubule-associated protein-like 4/anaplastic lymphoma kinase gene rearrangement (26). However, the *NFKBIA* gene has not been reported to be mutated/deleted in lung cancer.

5. NF-κB/IκB-α pathway in cancer

Aside from the genetic inactivation of IκB-α in some cases of Hodgkin lymphoma and glioblastoma, IκB-α has not been found to be recurrently mutated or deleted in other cancer types. However, NF-κB/IκB-α signaling has been shown to play essential roles in various types of hematological cancers, which have been extensively reviewed (27). In particular,

NF-κB was demonstrated to have a key role in acute myeloid leukemia (AML), via canonical (28,29) and non-canonical pathways (30), and via different modalities in chronic myeloid leukemia (CML) (31). In particular, during the chronic phase of CML, NF-κB was found to be active, although not as much as in AML blasts; in the blast phase of CML, NF-κB activity appeared to substantially increase, as reviewed recently (31). More recently, the IκB-α kinase, IKK-α, was revealed to be essential in CML pathogenesis, suggesting that IκB-α may also play a key role in this disease (32). The fact that *NFKBIA* is occasionally mutated/deleted in various cancer types, and in particular in AML/CML, while IκB-α has an essential therapeutic role when stabilized by proteasome inhibitors, suggests that IκB-α may have NF-κB-independent functions in the context of cancer.

6. IκB-α expression pattern in CML

While investigating the level of expression of IκB-α in CML samples, we discovered that, surprisingly, IκB-α is highly expressed in primary CML cells and that it retained a marked cytosolic compartmentalization (33). This observation prompted us to investigate other IκB-α functions in this particular cancer. CML is a BCR-ABL-driven myeloproliferative disorder (34,35), which is effectively treated with BCR-ABL inhibitors, although tyrosine kinase inhibitors are unable to fully eradicate this disease (36). In the search for NF-κB-independent IκB-α functions, previous studies clearly demonstrated that IκB-α is able to bind with either the p65 subunit of NF-κB, as is well known, or p53 (37-40). However, while these findings were described by independent groups, the IκB-α/p53 interaction has not been observed and analyzed in the setting of primary cancer cells. As reported in our recent study, in CML, BCR-ABL is able to interact with IκB-α, which is in turn able to bind to p53 (33). This complex forces p53 to become localized in the cytoplasm, with consequent loss of the nuclear pool; this is responsible for the majority of p53 tumor-suppressive functions. Notably, inactivation of BCR-ABL is associated with the re-localization of p53 into the nucleus. Finally, the study also demonstrated that IκB-α promotes the inactivation of p53 as a transcriptional factor in the setting of CML (33).

7. Discussion

IκB-α is well-established to negatively regulate the NF-κB canonical pathway through its ability to prevent p50/p65 translocation into the nucleus (1,2). However, IκB-α is also able to constrain p53 to the cytoplasm, thereby counteracting the tumor-suppressive functions of p53 (33,37-40). IκB-α appears to act as a traffic light at the crossroads between oncogenes and tumor suppressors. Further investigations are necessary to better understand how, when and in which cellular context IκB-α is able to modulate these two opposing signals. At least in CML, where oncogenic signals are constitutively active due to the presence of BCR-ABL, IκB-α appears to be stably associated with p53, promoting its inactivation as a transcriptional factor, due to its re-localization to the cytoplasm (33). In our opinion, the IκB-α-mediated functional inactivation of p53 is a challenging opportunity for cancer therapy, particularly for cancers without effective molecularly targeted therapies. The concept of functional inactivation of wild-type tumor suppressors has been extensively studied for *PTEN* (41,42), and in particular in CML (43,44). While genetically mutated/deleted tumor suppressors cannot be specifically targeted, the identification of functionally inactivated tumor suppressors may offer the potential to design therapies that will reactivate them, leading to cancer-selective induction of apoptosis.

In summary, in addition to promoting the loss of p53 tumor-suppressive functions via changes in cellular compartmentalization, IκB-α may also orchestrate p53 cytoplasmic functions. Therefore, further analyses must also investigate the role of the IκB-α/p53 complex on p53 cytoplasmic functions.

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