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Regulation of the Alternative NF-κb Pathway and Its Role in Cancer

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Abstract

NF-κB is regulated by two distinct pathways namely the Classical NF-κB pathway and the recently discovered alternative NF-κB pathway. While the classical NF-κB pathway has been extensively studied for its role in cancer, our understanding of the regulation of the alternative NF-κB pathway and its role in cancer has been limiting. Nevertheless, significant progress has been made recently that revolves around the regulation of NF-κB inducing kinase and its role in cancer. These recent developments will be discussed in this editorial.

The mammalian NF-κB family comprises five members including NF-κB1 (expressing p105 and the processed p50), NF-κB2 (expressing p100 and the processed p52), RelA (p65), cRel and RelB [1]. These members form different homo and heterodimers that regulate transcription of their respective target genes [1]. In resting cells, NF-κB heterodimers are inactive and are sequestered in the cytoplasm by the IκB family members such as IκBα [1]. Upon stimulation of different surface receptors, NF-κB is activated by two distinct pathways namely the "classical" and the "alternative" NF-κB pathways [1]. The classical pathway has been well studied and relies predominantly on IKKα-dependent phosphorylation and degradation of IκBα leading to the nuclear translocation of RelA-p50 [1]. The recently discovered alternative NF-κB pathway, on the other hand, relies on NF-κB inducing kinase (NIK) and IKKα dependent phosphorylation and processing of p100 to p52 resulting in the nuclear translocation of RelB-p52 [1]. As mentioned above, while the classical pathway has been well studied for its role in cancer, the regulation of the alternative NF-κB pathway and its role in cancer has hot been well studied.

Unlike the classical NF-κB (RelA-p50), which is predominantly inhibited by IκBα, the alternative NF-κB (RelB) is almost exclusively inhibited by p100 [1]. The presence of multiple ankyrin domains within the C-terminus of p100 enables it to bind to RelB and sequester the latter in the cytoplasm in the inactive form [1]. Upon stimulation of a unique set of Tumor Necrosis Factor Family of Receptors (TNFR) such as CD40 and BAFF-R etc., phosphorylation of p100 in a NIK and IKKα-dependent manner results in the limited proteolysis of the C-terminus of p100 leading to nuclear localization of RelB-p52 heterodimers [1-5]. Although the primary role of the full length p100 appears to sequester RelB in the cytoplasm and that regulation of its processing is largely a cytoplasmic event, a recent report indicated that degradation of nuclear p100 is essential for optimal activation of NF-κB and for the survival of Multiple Myeloma tumor cells [6,7]. Nevertheless, removal of C-terminus of p100 would result in constitutive activation of RelB-p52 [8,9]. In line with this notion, several mutations resulting in the C-terminal truncation of p100 were observed in different types of cancers including lymphoma, myeloma etc [1]. Moreover, several cancer causing viruses such as EBV, HTLV, herpes virus etc., are known to induce p100 processing and activate the alternative NF-κB pathway by expressing oncogenes such as LMP1, Tax and Tio respectively [10-13]. However, the exact function of RelB-p52 in the tumorigenesis initiated by the above viruses needs to be investigated.

While p100 processing to p52 is an important step in the activation of RelB-p52, recent reports indicated that the regulation of the NIK stability in resting Vs receptor stimulated cells is the most critical regulatory mechanism involved in the activation of the alternative NF-κB pathway [1,4,14]. It has been shown that the levels of NIK are almost invisible in resting cells due to its targeting for proteasomal degradation by a complex involving cIAP-TRAF2-TRAF3-NIK [4,14]. In this complex TRAF3 functions as a bridge between the cIAP-TRAF2 complex and NIK resulting in K-48 linked ubiquitination of NIK by cIAP (Figure 1) [4,14]. Owing to their redundant function, both cIAP1 and cIAP2 appear to be capable of ubiquitinating NIK [1,4,15]. However, upon stimulation of receptors such as CD40, BAFF-R etc., NIK escapes ubiquitination by cIAPs as stimulation of these receptors results in rapid proteasome dependent degradation of both TRAF3 and TRAF2 [4]. Interestingly, upon receptor stimulation cIAP (cIAP1 and/or cIAP2) targets TRAF3 for K-48 linked ubiquitination and subsequent proteasomal degradation (Figure 1) [4]. Therefore, the cellular levels of TRAF3 drop below a threshold leading to the loss of complex formation between cIAP-TRAF2 and NIK (Figure 1) [4]. Thus, NIK gets stabilized and activates IKKα, which in turn phosphorylates p100 leading p100 processing and nuclear localization of RelB-p52 (Figure 1) (For a thorough review please see [1]). While these reports indicated how NIK is regulated in resting Vs receptor stimulated cells, several unanswered questions remain. For instance how cIAPs target TRAF3 for ubiquitination upon CD40 stimulation is not completely clear although K63 linked ubiquitination of cIAPs appear to play a role in TRAF3 ubiquitination by cIAPs (Figure 1). In addition to the negative regulation of NIK by cIAPs, TRAF2 and TRAF3, additional players such as IKKα and the kinase TBK1 are reported to negatively regulate NIK by phosphorylating the latter and affect its stability (Figure 1) [16,17]. The existence of multiple negative regulatory mechanisms to achieve a balance NIK function suggested that uncontrolled activation of NIK is highly deleterious and could be tumorigenic. Indeed, the importance of having a tight negative control on NIK function has been illustrated by several mutations that lead to loss of control on NIK stability. First,

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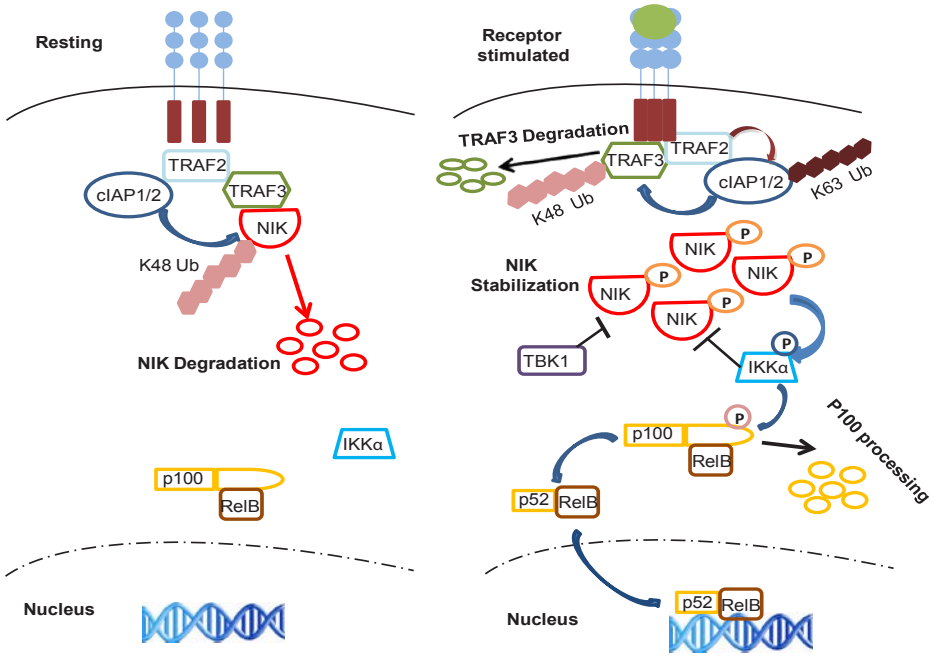

Figure 1: Regulation of the alternative NF-kB pathway in resting vs receptor stimulated cells. A tight control on NIK stability is essential to achieve controlled activation of the alternative NF-κB pathway upon activation of different receptors. In resting cells, a complex comprising cIAP-TRAF2-TRAF3-NIK induces constant degradation of NIK making its levels invisible. In this complex TRAF3 functions as a bridging factor between the cIAP-TRAF2 complex and NIK enabling cIAP to mediate K-48 linked ubiquitination of NIK leading to its degradation in a proteasome-dependent manner. In receptor-stimulated cells however, NIK escapes the ubiquitination by the cIAP-TRAF2-TRAF3 complex due to rapid degradation of TRAF3 by the proteasome machinery. Upon receptor stimulation, TRAF2-dependent K63-linked ubiquitination of cIAP seems to mediate cIAP-dependent K-48 linked ubiquitination of TRAF3 leading to the proteasome-dependent degradation of the latter. Once the TRAF3 levels drop below a threshold, NIK can no longer interact with the TRAF2-cIAP complex. As a consequence NIK gets stabilized and gets activated presumably due to transautophosphorylation. Activated NIK phosphorylates IKKa, which in turn phosphorylates p100 resulting in p100 processing and nuclear localization of RelB-p52 and their DNA binding. In receptor stimulated cells, IKKa and the kinase TBK1, phosphorylate NIK and induce its degradation to dampen the alternative NF-κB activity.

genetic deletion of TRAF3 or TRAF2 in mice leads to highly elevated levels of NIK and the high levels of NIK in these mice has been linked to the dramatic phenotypes that include postnatal lethality, atrophied thymus and spleen, enhanced survival of B-cells and autoimmune features [4]. Moreover, several recent reports indicated that loss of control on NIK stability due to deletion or mutations in the genes such as TRAF3, TRAF2 and combined deletion of both cIAP1 and cIAP2, is linked to hematological malignancies such as Lymphoma and Multiple Myeloma [1,18-21]. Collectively, it appears that a tight control on NIK function is highly important, as loss of control on NIK stability is highly deleterious. Therefore, development of small molecule inhibitors for this important kinase will have a significant impact on the therapy of cancer and other diseases that are associated with uncontrolled activation of NIK. Indeed, a recent report implicated the use of NIK specific inhibitors and their effect on the survival of lymphoma cells [22]. However, the efficacy of these inhibitors and their specificity towards NIK has not been clear from this study and needs further investigation.

While NIK seems to have several important functions in the immune system such as lymphoid organ development and lymphocyte development etc., surprisingly not many targets for its kinase function are known. To date, the major known target for NIK has been IKKα [1] although in a recent report it has been indicated that in MM cells NIK could directly activate IKKα [19]. This again is puzzling because NIK in many normal cells has been shown to be dispensable for the activation of classical NF-κB pathway mediated by IKKα [3,23]. Thus, it remains to be studied as to how NIK stabilization in MM cells leads to the activation of classical NF-κB in addition to constitutive activation of the alternative NF-κB pathway. Moreover, identification of novel NIK substrates is necessary and will be enhanced our understanding of the functions of this enigmatic kinase in the immune system, inflammation and cancer.

While a large number of studies have focused on the upstream kinases and cytoplasmic regulation of distinct NF-κB heterodimers, nuclear regulation of the NF-κB, especially as it relates to the regulation of RelB-p52 heterodimers has not been well established. Moreover, while NF-κB is largely appreciated as a transcriptional activator, the physiological significance of transcriptional repression by NF-κB in the context of tumor growth has not been established. Importantly, the mechanism by which RelB-p52 contributes to tumor growth has not been clear. It is thought that RelB-p52 might function as transcriptional repressors but the physiological impact of transcriptional repression by RelB-p52 in the context of tumor growth remains to be studied. Curiously, the association of RelB with an interesting factor "aryl hydrocarbon receptor nuclear translocator" (ARNT) seems to enhance the repressive function of RelB [24]. Inline with this notion, lack of ARNT resulted in loss of RelB dependent repression and enhanced transcriptional activation by the classical NF-κB [24]. However, the molecular mechanism by which ARNT enhances RelB dependent repression remains to be studied. Moreover, it is not clear whether ARNT constitutively interacts with RelB or if this interaction is signal regulated. Nevertheless, it is likely that RelB interacts with several nuclear factors that would bring out a fine tuned balance between the Transcriptional repression vs Transcriptional activation by RelB-p52. Thorough understanding of such novel RelB-complexes will enable us to target those complexes as a therapeutic means for cancer cure.

Owing to the increasingly important protumorigenic role of RelB and its novel nuclear complexes, it is essential to develop inhibitors targeting the functionality of specific and distinct RelB-complexes to achieve desired tumor inhibitory effects and minimize the side effects. However, unlike in the case of kinases, development of conventional small molecule inhibitors to target RelB- and/or other NF-κB nuclear complexes might be difficult. Therefore, it is necessary to develop alternative, peptide-based approaches that would disrupt the nuclear RelB-complexes. Such peptide-based disruption of protein-protein interactions has been a widely accepted approach especially when small molecule inhibitors are not available [25-27].

To conclude, hyper activation of the alternative NF-κB (RelB-p52) seems to be an important and integral aspect of many cancers as well as other disorders such as autoimmunity. Understanding the molecular mechanism by which nuclear RelB-p52 do regulated and identifying novel nuclear complexes comprise RelB-p52, and how these complexes contribute to tumor growth, would be beneficial in the development of novel therapeutic approaches for cancer cure.

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