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News and Commentary

Arresting NF-κB by β-arrestin2

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Any activity without restriction can be dangerous for the proper function of an organism. The same is true for NF-κB, a ubiquitous transcription factor governing the expression of genes involved in cell-to-cell communication, cell-to-cell interaction, cell migration, cell cycle, and cell growth regulation during both normal physiological conditions and pathological circumstances.[1] A number of endogenous inhibitors that restrict the activation or activity of NF-κB have been identified recently, in addition to IκB family proteins. The most remarkable regulatory mechanism of NF-κB activation is its association with the endogenous inhibitors, the IκB family proteins that retain it in the cytoplasm.[2] In response to extracellular signals, the inhibitory proteins are first phosphorylated through the activation of kinase cascades, such as IκB kinase complexes (IKK), and then degraded in a ubiquitin–proteasome-dependent manner.[2]

The reputation of NF-κB is notorious because of its involvement in inflammatory and/or carcinogenic diseases in human beings.[1] Therefore, inhibition of NF-κB may slow down disease processes. Many strategies to ablate NF-κB have been suggested based on the stabilization of endogenous inhibitors – IκB proteins and/or the precursors of NF-κB p50 and p52 proteins. These strategies include protection of these inhibitors from proteasomal degradation or processing, and kinase inhibition to prevent the phosphorylation, a prerequired step for ubiquitin conjugation of these inhibitors.[2] In addition to exogenous agents that include proteasome inhibitors, nonsteroidal anti-inflammatory drugs and antioxidants, a few endogenous molecules have been identified as potential blockers of IKK kinase activation or IκB protein ubiquitination and degradation. The first identified endogenous molecule eliciting an inhibitory effect on IKK is cyPG15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2).[2] 15d-PGJ2 inhibits IKK activity by direct covalent modification of IKKβ at cysteine 179 within the activation loop of this kinase.[2] Another molecule, CYLD, a product of a tumor suppressor gene, has been recently identified as a negative regulator of NF-κB activation signaling.[3,4] Mutation of the CYLD gene results in predisposition to cylindromatosis in which benign tumors arise in hair follicles and in cells of the sweat and scent glands due to persistent NF-κB activation. The CYLD prevents NF-κB activation by deubiquitinating lysine 63 polyubiquitin chain from TRAF2, and possibly TRAF6 or NEMO, causing dissociation of the TRAF2–NEMO complex that would otherwise activate NF-κB signaling through the assembly of the IKK complex.

The work by Pei and co-workers[5] describes a new endogenous inhibitor, β-arrestin2, that blocks signal-induced IκBα degradation and subsequent activation of NF-κB transcription factor. β-arrestin2 is ubiquitously expressed in virtually all types of cells, where it interacts with seven-membrane-spanning receptors after their phosphorylation by G-protein-coupled receptor (GPCR) kinases.[6] Originally discovered as a desensitization molecule for GPCRs, mainly the β2-adrenergic receptor (β_2AR) and angiotensin II receptor, increasing evidence suggests that β-arrestin2 also serves as a modulator of a number of intracellular signaling pathways, including p53, MAPK, TGFβ, IGF1, PI3K-Akt, Wnt5a, etc. In both the yeast two-hybrid and immunoprecipitation assays, Pei and co-workers[5] demonstrated a direct interaction of β-arrestin2 with IκBα protein. Such interaction substantially stabilized IκBα protein and decreased TNFα-induced NF-κB activation. A series of deletion mutant experiments indicated that the N-terminal 1–60 amino acid of β-arrestin2 is critical for the interaction with the C-terminal 276–317 region of IκBα protein, where a PEST domain is located.

The main function of β-arrestin2 is to desensitize GPCR-mediated (especially β_2AR-mediated) second-messenger signaling.[6] It is possible, therefore, that the interaction of β-arrestin2 with IκBα may be regulated by agonists of the GPCR. Indeed, treatment of HEK293 cells with a β_2AR agonist, isoproterenol, induced the interaction of β-arrestin2 with IκBα, whereas pretreatment of the cells with a β_2AR antagonist blocked such an effect.[5] Now the question was how does β-arrsetin2 stabilize IκBα? The stability of IκBα protein, either free or associated with NF-κB heterodimer, is dependent on the phosphorylation of Ser32 and Ser36 of IκBα by activated IKK. The phosphorylation leads to conjugation of lysine 48 polyubiquitin chain on lys21 and/or lys22, followed by proteasomal degradation.[1,2] Alternatively, the C-terminal PEST region of IκBα is phosphorylated by either IKK[7] or casein kinase 2 (CK2),[8] which facilitates both basal and inducible turnover of IκBα protein. The alternative mechanism is mainly for the unassociated IκBα protein, which is possibly independent of the ubiquitin–proteasome system, and may depend on the calpain proteases.[9]

So how does β-arrestin2 fit into these processes? The authors[5] determined the effect of recombinant β-arrestin2 on the activity of IKK in an *in vitro* kinase activity assay. Taking advantage of the fact that IKK phosphorylates the conserved $DS^{32}GLDS^{36}$ motif in the N-terminus of IκBα protein, they monitored the phosphorylation status of N-terminal 1–54 region or full-length IκBα protein.[5] This experiment led to two conclusions. First, β-arrestin2 appears to be ineffective in interfering with the IKK kinase activity that phosphorylates the $DS^{32}GLDS^{36}$ motif at the N-terminus of IκBα, suggesting β-arrestin2 acts

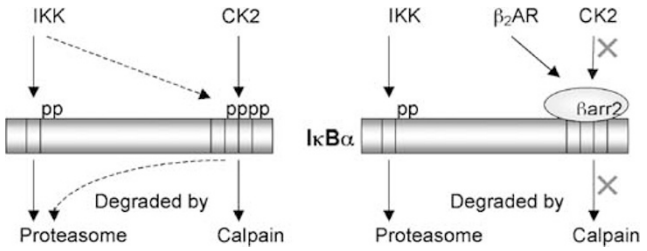

Figure 1 Possible effect of β-arrestin2 on IκBα degradation. Only the proteasome and calpain degradation pathways are shown. 'p' denotes phosphorylation by IKK or CK2. Solid arrows: main pathway; dashed arrows: possible alternative pathways; red 'X': pathways that have been blocked; β_2AR: β_2-adrenergic receptor; βarr2: β-arrestin2

downstream of IKK. Second, β-arrestin2 substantially decreases the phosphorylation of full-length IκBα induced by IKK kinase or CK2. Both IKK and CK2 have been implicated in the phosphorylation of the PEST domain of IκBα in response to certain stress signals, such as UV or oxidative stress.[7,8] Thus, β-arrestin2 may impair the pathway of IκBα degradation that requires C-terminal phosphorylation of IκBα protein.

The finding that β-arrestin2 inhibits NF-κB by Pei and co-workers[5] is interesting, because it not only reveals a novel regulatory circuit for NF-κB signaling but also provides new mechanistic insight into the crosstalk between the sympathetic nervous system and the immune system. However, this finding also poses several questions. First and foremost, how IκBα degradation was prevented by the interaction between β-arrestin2 and IκBα. We know quite a bit about the degradation of IκBα by the ubiquitin–proteasome system. However, the knowledge about alternative degradation pathways, especially the degradation mediated by the C-terminus of IκBα protein, is very limited. Based on the fact that β-arrestin2 is purely cytosolic[10] and is able to interact with the C-terminal region of IκBα where a PEST domain is located, it is highly likely that the association of β-arrestin2 with the C-terminus of IκBα will make the PEST domain inaccessible for either IKK or CK2. Therefore, the likely mechanism for β-arrestin2 regulation of NF-κB is by preventing the degradation of unassociated cytosolic IκBα protein (Figure 1), rather than the IκBα protein that associates with the NF-κB heterodimer in either cytosol or nuclei as proposed by the authors.[5] The second question is whether β-arrestin2 also affects the partial degradation of p105 and p100, precursors of NF-κB p50 and p52 subunits, respectively, that serve as inhibitors for NF-κB. A similar PEST domain or the $-(S/T)X_{4\text{-}5}(S/T)$ motif can also be found in the C-terminus of p105 or p100.[1] Although the authors were not in a position to clarify this issue, such a possibility is worth exploring in the future. Lastly, does β-arrestin2 interaction with IκBα affect cell apoptosis? Since NF-κB has been generally considered as a pivotal antiapoptotic transcription factor, impairment of IκBα degradation by β-arrestin2 supposedly renders preapoptotic responses of the cells due to the inhibition of NF-κB activation. However, a considerable number of reports indicate an antiapoptotic response following the activation β_2AR signaling.[11–14] Thus, future work will be required to reconcile such controversy.

Note added in proof

A recent report by Witherow *et al.*[15] suggested that both β-arrestin2 and β-arrestin1 can stabilize IκBα protein.

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