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MINI REVIEW

Ubiquitin-mediated NFκB degradation pathway

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The nuclear factor κB (NFκB) transcription factor plays critical roles in inflammation and immunity. The dysregulation of NFκB is associated with inflammatory and autoimmune diseases and cancer. NFκB activation is negatively regulated by the ubiquitin-dependent proteasomal degradation pathway. In the present review, we discuss recent advances in our understanding of how ubiquitin ligases regulate the NFκB degradation pathway.
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Nuclear factor κB (NFκB) was identified approximately 20 years ago by Dr David Baltimore as a transcription factor that binds to the intronic enhancer of the kappa light chain gene (the κB site) in B cells.[1] The NFκB family is composed of several members including p50, p52, p65/RelA, c-Rel and RelB and plays a central role in cell growth, inflammation, immunity and apoptosis. NFκB activation is dependent on the stability of the inhibitor IκBα. IκBα stabilizes the NFκB complex so that after its degradation the remaining subunits translocate from the cytoplasm to the nucleus.[2] The phosphorylation of IκBα is catalyzed by IκB kinase (IKK), a complex composed of three subunits: IKKα/IKK1, IKKβ/IKK2, and IKKγ/NEMO. IKK1 and IKK2 are the catalytic subunits, whereas IKKγ serves as a non-enzymatic regulator.[3–5] The IKKs/NFκB pathway is activated by extracellular stimuli such as cytokines, ultraviolet irradiation, free radicals, bacterial or viral antigens and oxidized low density lipoprotein.[3,4] In the absence of persistent upstream stimuli, NFκB transcriptional activity is terminated by the NFκB/IκBα negative feedback loop.[4]

The activation of NFκB in immune or stromal cells causes a pro-inflammatory response, and persistent tissue inflammation has been linked to inflammation-associated cancer.[6] NFκB activation is negatively regulated by ubiquitin-dependent proteasomal degradation.[7–9] The ubiquitin-proteasome system comprises a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). Ubiquitin E3 ligases play a critical role in substrate recognition and poly-ubiquitination by recruiting E2 ubiquitin-conjugating enzymes to specific substrates.[10] SOCS-1 (suppressor of cytokine signaling) is one of the components of the EC_2S (Elongin BC-CUL2-SOCS-box protein) ubiquitin ligase complex that mediates JAK2 (Janus kinase 2) ubiquitination and degradation.[11,12] Similar to JAK2, NFκB/p65 is also a substrate of the EC_2S ubiquitin ligase complex in both mice and cancer cells.[13] Pin1 increases NFκB/p65 stability and transactivation, while the loss of Pin1 leads to SOCS-1-mediated p65 ubiquitination and degradation.[13] This offers a new mechanism for NFκB-mediated pathogenesis.

NFκB is also activated by viral infections. In immunodeficiency virus-1-infected $CD4^+$ lymphocytes, NFκB activation is terminated by COMMD1 (MURR1), resulting in the inhibition of immunodeficiency virus-1 growth in unstimulated or cytokine-stimulated $CD4^+$ T cells.[14] This study demonstrated that COMMD1 decreases NFκB transcriptional activity in T cells by inducing the ubiquitin-dependent proteasomal degradation of p65, but did not identify the mechanism of p65 degradation.[14] Marine *et al.*[15] found that COMMD1 promoted the ubiquitination and degradation of nuclear NFκB/p65 in cancer cell lines through its interaction with the EC_2S multisubunit ubiquitin ligase complex. As a component of the EC_2S ubiquitin ligase complex, COMMD1 serves as a cofactor of the EC_2S ubiquitin ligase complex and promotes the degradation of nuclear p65.[15] The main controversy is that while COMMD1 is predominantly localized to the cytoplasm, it induces the ubiquitination and degradation of nuclear p65. It is unknown whether COMMD1 can affect the stability of cytoplasmic p65 protein. In addition, it is unclear whether COMMD1 induces NFκB/p65 degradation in $CD4^+$ T cells by the same EC_2S multisubunit ubiquitin ligase. Therefore, multiple issues remain to be resolved.

Interestingly, the histone acetyltransferase GCN5 serves as a cofactor for COMMD1 to promote NFκB/p65 ubiquitination

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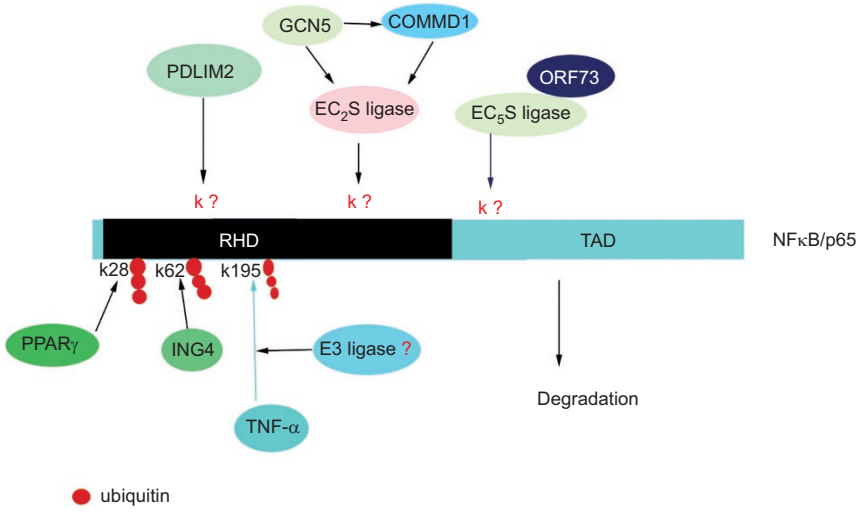

Figure 1 NFκB/p65 ubiquitination and degradation signaling. NFκB contains RHD and TAD domains. NFκB/p65 undergoes ubiquitination and degradation by the ubiquitin ligases EC_2S, EC_5S, PDLIM2, PPARγ or ING4. GCN5 or COMMD1 serves as a cofactor for EC_2S ubiquitin ligase-mediated p65 degradation. Ubiquitin is attached to the lys-195, lys-28 or lys-62 residue on p65 in response to ubiquitin ligase. NFκB, nuclear factor κB.

and degradation in cancer cell lines, independent of its enzymatic activity.[16] GCN5 induces p65 ubiquitination depending on the phosphorylation state of the Ser 468 residue of p65.[16] The interaction between GCN5, COMMD1 and the other components of the EC_2S ubiquitin ligase promotes NFκB/p65 degradation.[16] These observations leave some unanswered questions: do other GCN5 cofactors induce NFκB/p65 degradation by the GCN5/COMMD1/EC_2S complex; and do any other proteins hijack the EC_2S ubiquitin ligase to induce p65 ubiquitination and degradation?

NFκB plays a crucial role in the innate immune response against microbial and viral infections. The inhibition of NFκB activation has been observed in various viral infections. The lymphotropic gammaherpesvirus MuHV-4 (murid herpesvirus-4) ORF73 protein inhibits host NFκB transcriptional activity by inducing NFκB/p65 ubiquitination and degradation.[17] The SOCS-box motif of ORF73 acts by forming one component of the EC_5S (ElonginC/Cullin5/SOCS) ubiquitin ligase complex. The genetic deletion of the SOCS-box of ORF73 suppresses MuHV-4 expansion in germinal center B cells and prevents persistent MuHV-4 infection in mice.[17] This study suggests that the virus escapes host immune surveillance by using the ubiquitin-dependent proteasomal degradation pathway. In addition to degrading NFκB/p65 *via* the $EC_{2/5}S$ ubiquitin ligase complex, PDLIM2 alone can act as a ubiquitin ligase to induce the ubiquitination and degradation of nuclear p65 in T cells and macrophages.[18] PDLIM2 is associated with several malignancies including breast cancer and adult T-cell leukemia. As a nuclear protein, PDLIM2 contains a LIM domain similar to those of the RING family of ubiquitin ligases.[18] However, there is no direct evidence demonstrating PDLIM2 ubiquitin ligase activity in *in vitro* ubiquitination assays.[18] As stated above, COMMD1 and PDLIM2 induce the ubiquitination and degradation of nuclear p65, although it is unknown whether they act in the same cell types and in response to the same stimuli.

In the ubiquitin–proteasome pathway, ubiquitin is attached to the lysine residue of a substrate. The ubiquitin-tagged proteins are recognized and degraded by the proteasome pathway. Although PDLIM2 and COMMD1 induce p65 ubiquitination and degradation, the identity of the ubiquitin-modified lysine residue of p65 is still unclear.[14,18] Saccani *et al.*[7] were the first to identify that NFκB/p65 undergoes ubiquitination and degradation in cancer cells in response to TNF-α. Consistent with these findings, Fan *et al.*[19] found that the lysine-195 residue of p65 was critical for TNF-α-induced ubiquitination by an unknown ubiquitin E3 ligase. Because GCN5 and COMMD1 can induce p65 degradation in response to TNF-α, the possibility that they are responsible for the TNF-α-mediated p65 ubiquitination and degradation needs to be further studied.

Like PDLIM2 and COMMD1,[14,18] peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG) acts as a ubiquitin E3 ligase to induce the ubiquitination and degradation of both cytoplasmic and nuclear p65.[8] This proteasome-mediated degradation requires the lysine-28 residue of p65.[8] PPARγ contains two zinc-finger domains and does not have a typical RING domain. The two zinc-finger domains of PPARγ cooperate in its enzymatic activity by interacting with UbcH3 (an ubiquitin-conjugating enzyme). This is an important prerequisite for the ubiquitin ligase to transfer activated ubiquitin from E2 to the substrate.[8] The glitazone receptor PPARγ is implicated in numerous diseases including obesity, diabetes, atherosclerosis, and cancer.[20–23] PPARγ inhibits NFκB activation in response to bacterial stimuli,[24] oxidized low density lipoprotein[25] and TNF-α.[26]

Recently, a new mechanism for the PPARγ-mediated inhibition of NFκB signaling has been revealed,[8] and another ubiquitin E3 ligase involved in NFκB degradation was identified.[9]

Inhibitor of growth 4 (ING4) has ubiquitin E3 ligase activity but lacks the typical RING domain.[9] ING4 belongs to the ING family and inhibits tumor growth *via* the suppression of NFκB activation.[27,28] ING4 is present in plant and animal transcriptional regulatory pathways, and has a highly conserved PHD (plant homeodomain) zinc finger domain that can act as a ubiquitin E3 ligase.[27–30] Consistent with this, the PHD of ING4 functions as a ubiquitin ligase and induces the degradation of cytoplasmic and nuclear p65. Importantly, the lysine-62 residue of p65 is essential for proteasomal degradation.[9] These observations indicate that either PPARγ or ING4 promote p65 degradation in the cytoplasm and nucleus,[8,9] distinct from PDLIM2 and COMMD1-mediated nuclear p65 degradation.[14,18] It is unclear whether other PPAR family members (PPARα or PPARβ) or INGs[1–5] have E3 ligase activity.

In summary, NFκB activation is negatively regulated by the ubiquitin-dependent proteasomal degradation pathway (Figure 1), which can modulate inappropriate or excessive activity during NFκB-mediated inflammatory disease and in cancer. The NFκB degradation pathway provides a novel therapeutic target in these diseases.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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