Review Article | Basic and Mechanism Sciences

Literature review: nuclear factor kappa B (NF-κB) regulation in human cancers mediated by ubiquitin-specific proteases (USPs)

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Background and Objective: The nuclear factor kappa B (NF-κB) consists of a group of transcription factors of which its dysregulation is responsible for diseases such as inflammation and cancer. Ubiquitin-specific proteases (USPs) are the most prominent group among the deubiquitinases (DUBs). Their functions include control of protein stability and regulation of signaling transduction. The association between NF-κB activity and human cancer progression is evident. Still, the role of USPs in the NF-κB regulation in human cancers, especially prostate cancer, is not well understood. This review discusses on the role of USP-mediated regulation of the canonical NF-κB signaling pathway in human cancers and provides a prospect of future studies in prostate cancers.

Methods: Within the biomedical literature database, PubMed, our review team searched for keywords including USP, NF-κB signaling pathway, cancer, prostate cancer, and specific USPs such as USP1, USP2, USP3, etc. These keywords were used individually or in combinations. After screening, only mechanistic studies and articles reporting the subsequent changes in cellular behaviors were included for full-text review.

Key Content and Findings: Most USPs function primarily as DUBs to regulate the canonical NF-κB signaling pathway. The typical K48- and K63-linked DUB activities of USPs are the best understood. These USPs are positive and negative regulators of the NF-κB activity. However, their DUB activities against polyubiquitin chains with atypical linkages have not yet been extensively studied. Furthermore, some USPs can regulate the canonical NF-κB signaling pathway via ubiquitin-independent mechanisms.

Conclusions: In the regulation of the canonical NF-κB pathway, the USPs function primarily as DUBs, but they also regulate the p65/p50 by ubiquitin-independent mechanisms. Generally, in human cancer models, USP-mediated elevation and suppression of p65/p50 activity lead to more or less malignant cellular behaviors, respectively. Given the currently unbalanced focus on K48- and K63-linked DUB activities and the context-dependent function of USPs, future research of USP-mediated NF-κB regulation in human cancers should invest more in the DUB activities against the atypical polyubiquitin chains and test known mechanisms in different cancer models.

Keywords: Nuclear factor kappa B (NF-κB); human cancer; prostate cancer; ubiquitin-specific proteases (USPs)

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Introduction

Background

Cancer is one of the most lethal diseases in humans. In 2020, there were 1,414,259 new cases of prostate cancer and 375,304 related deaths reported globally, and age-standardized incidence and mortality rates were increasing in 65 and 19 countries, respectively (1). There is an urgent need to improve understanding of the development and progression of prostate cancer and identify potential novel treatments for it. Examining signaling pathways widely conserved in different cancer models may provide insight into potential druggable targets. The canonical nuclear factor kappa B (NF-κB) signaling pathway is one of them. It promotes cell survival, proliferation, invasion, and migration in multiple cancers. Thus, the regulatory mechanisms of the canonical NF-κB signaling pathway may provide opportunities for the development of novel treatments. Regulation of signaling molecules is often actuated through post-translational modifications, including phosphorylation, ubiquitination, acetylation, and methylation. This review will focus on the role of ubiquitination and deubiquitination systems in the NF-κB activity regulatory mechanisms, especially ubiquitin-specific proteases (USPs)' role in regulating NF-κB signaling.

Rationale and knowledge gap

Firstly, although the role of deubiquitinases (DUBs) such as tumor necrosis factor alpha-induced protein 3 (TNFAIP3, A20) and cylindromatosis lysine 63 DUB (CYLD) in DUB-mediated NF-κB regulation has been reviewed, a general understanding of the functions of USPs (the largest family of DUBs) in the NF-κB regulation has not been established (2). A literature review can provide a general understanding of the current knowledge in USP-mediated regulation of the canonical NF-κB signaling pathway in human cancers and potentially guide the future study of NF-κB regulation in certain cancers.

Secondly, knowledge gaps exist between the conventional belief and novel findings in the ubiquitination and deubiquitination systems. Ubiquitin was discovered in the 1970s (3). The linkage of polyubiquitin chains determines the function of the target protein. Traditionally, it is widely accepted that the lysine-48 (K48)-linked polyubiquitin chain leads to proteasomal degradation, and the K63-linked polyubiquitin chain serves as a scaffold for the recruitment of downstream signaling molecules (*Figure 1*) (4,5). These two typical linkages of polyubiquitin chains are the most extensively studied, but the remaining atypical polyubiquitin chains are not well understood. A literature review involving USP-mediated NF-κB regulation in human cancers can find out the size of this knowledge gap from the angle of the deubiquitination system in this specific area of cancer research and provide potential directions for future studies.

Objective

This review aims to summarize the research progress on the NF-κB regulating mechanisms mediated by USPs in human cancers and their influences on cellular behaviors. Based on summarized regulatory mechanisms and the knowledge gaps identified during this process, this review will provide a prospect for future research on NF-κB regulation mediated by USPs in human cancers. We present this article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-24-32/rc).

Methods

A preliminary search was conducted in the biomedical literature database PubMed. Keywords included ubiquitin-specific proteases (USP), NF-κB signaling pathways, cancer, prostate cancer, and specific USPs such as USP1, USP2, USP3, etc. (*Table 1*). These keywords were used individually or in combinations. Results from the preliminary search were screened for full-text review. Only the ones with precise mechanisms and those demonstrating the influences of USP-mediated NF-κB regulation on cellular behaviors passed the screening (*Table 1*). During the full-text review, studies were excluded if the regulatory mechanisms involve proteins that are not ubiquitously expressed.

Key content and findings

Conventional understanding and novel findings about ubiquitination and deubiquitination

Ubiquitin is a small protein consisting of 76 amino acids, equivalent to a molecular weight of approximately 8.5 kDa. Seven of these amino acids are lysine (K) residues that determine the linking pattern of polyubiquitin chains and functions of the ubiquitinated protein (*Figure 1*). It is conventionally accepted that K48-linked polyubiquitination is associated with proteasomal degradation, while K63-linked polyubiquitination is generally

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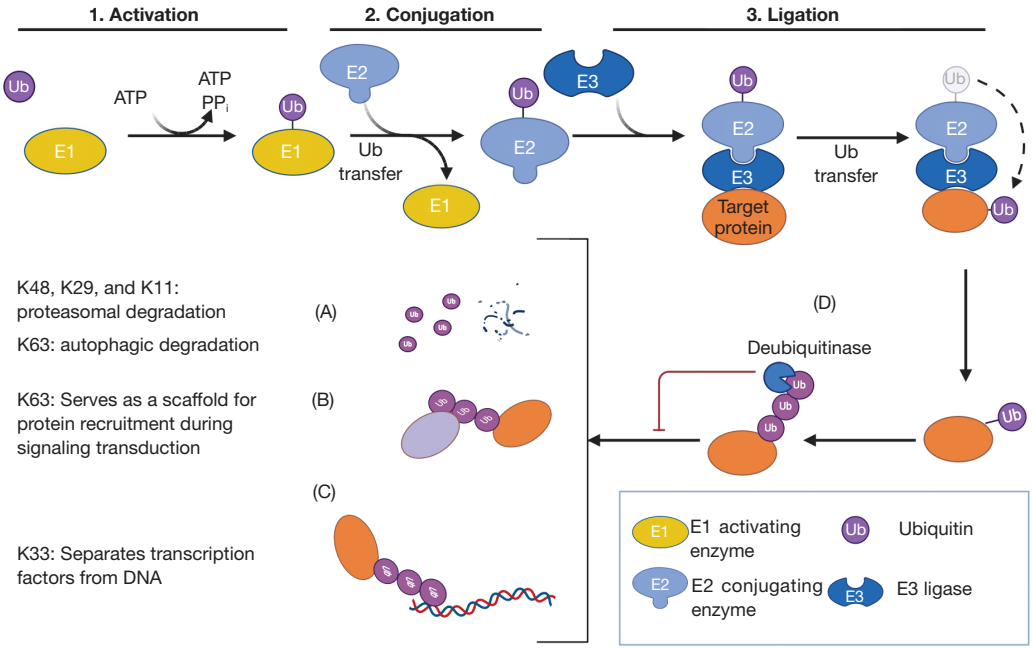

Figure 1 Summary of ubiquitination and deubiquitination. Each molecule of ubiquitin is activated by the E1-activating enzyme, using 1 ATP. The activated ubiquitin is conjugated with the E2-conjugating enzyme. Then, E3 ligase facilitates the transfer of ubiquitin from the E2-conjugating enzyme to the target protein. At this moment, the target protein is monoubiquitinated. If this process is repeated multiple times, the target protein will be polyubiquitinated and have a chain of many ubiquitin molecules. (A) If the polyubiquitin chain is linked by the lysine-48, 29, and 11 (K48, K29, and K11) residue in the ubiquitin, the polyubiquitinated protein will be recognized and digested by the proteasome. If the polyubiquitin chain is linked by the lysine-63 (K63) residue, it is subject to autophagic degradation. (B) If the polyubiquitin chain is linked by the lysine-63 (K63) residue in the ubiquitin, the polyubiquitinated protein will serve as a scaffold for protein recruitment during signaling transduction. (C) The K33-linked polyubiquitin chain can inhibit the protein-DNA interaction. (D) The target protein with a polyubiquitin chain can be recognized by DUBs. As a result, polyubiquitination can be reversed, and events described in (A-C) can be inhibited by the DUBs. ATP, adenosine triphosphate; AMP, adenosine monophosphate; PPi, (inorganic) pyrophosphate group; DUBs, deubiquitinases; Ub, ubiquitin.

Table 1 The search strategy summary

Items	Specification
Date of search	Dec. 1, 2023
Databases and other sources searched	PubMed
Search term used	Ubiquitin specific protease (USP), NF-κB signaling pathway, cancer, prostate cancer, and specific USPs such as USP1, USP2, USP3, etc. These keywords were used individually and in combinations
Timeframe	2003–2023
Inclusion and exclusion criteria	Inclusion criteria:
	• Original research articles must explain the mechanisms on the molecular level
	• Regulatory targets of USPs must be ubiquitously expressed
	Exclusion criteria:
	• The substrate of USPs is only expressed in limited types of cells or tissue
Selection process	The first author, K.S., conducted the selection independently. Consensus was achieved by discussion between the first author and corresponding author

NF-κB, nuclear factor kappa B.

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involved in signal transduction (4,5). Recent studies have unraveled the functions of some atypical polyubiquitin chains on the molecular level. Similar to K48, K11- and K29-linked polyubiquitination account for proteasomal degradation as well (*Figure 1A*) (6). Besides its role in signaling transduction, K63-linked polyubiquitination is also associated with protein degradation via a proteasome-independent pathway during autophagy (both mechanisms are summarized in *Figure 1*) (6). The K33-linked polyubiquitin chain is responsible for the separation of the signal transducer and activator of transcription 3 (STAT3) from DNA (6). In other words, the K33-linked polyubiquitin chain can inhibit protein-DNA interaction. Besides the lysine-dependent polymerization, ubiquitin molecules can also be linked linearly (in an N-terminus-to-C-terminus manner) in the context of NF-κB regulation (7). Ubiquitination requires three enzymes—E1 activating enzyme, E2 conjugating enzyme, and E3 ligase. The collaboration of E1, E2, and E3 enzymes facilitates the covalent bonding between ubiquitin and the target protein (*Figure 1*). On the other hand, the reverse of ubiquitination, which is deubiquitination, involves a class of enzymes named DUBs (*Figure 1D*).

A total of 102 DUBs in humans can be divided into two classes—cysteine (also known as thiol proteases) and metalloproteases (8). Cysteine proteases consist of six superfamilies—the USPs, the ovarian tumor (OTU) superfamily, the Machado-Josephin domain (MJD) superfamily, the ubiquitin C-terminal hydrolases (UCHs), the K48-specific MIU-containing novel DUBs (MINDYs), and the K63-specific zinc finger with ubiquitin fold modifier 1 (UFM1)-specific peptidase domain protein (ZUSFP) (8). The USPs make up the most prominent family of DUBs with over 60 members (9,10). They remove polyubiquitin chains from their substrates, including the proteins involved in the regulation of the canonical NF-κB signaling pathways.

The basics of NF-κB

NF-κB is a family of five transcription factors—NF-κB1 (p50/p105), NF-κB2 (p52/p100), p65 (RelA), c-Rel, and RelB, where p105 and p100 are precursors of p50 and p52 respectively (11). In the canonical NF-κB pathway (*Figure 2*), the inactivated form of p65-p50 heterodimer is bound to the inhibitor of κB (IκB) in the cytoplasm. Upon phosphorylation by the IκB kinase (IKK) complex, a complex consisting of IKKα, IKKβ, and NF-κB essential modulator (NEMO, or IKKγ), IκB is ubiquitinated and subject to proteasomal degradation. The degradation of IκB releases p65-p50 dimer, which is phosphorylated (on the p65 subunit) and translocated into the nucleus to activate the transcription of target genes involved in inflammation and oncogenesis (11). In the non-canonical pathway, the NF-κB inducing kinase (NIK) forms a complex with two IKKα subunits to phosphorylate the p100 subunit in the RelB/p100 heterodimer, inducing its cleavage (p100-p52 conversion) and activation (11). This review will focus on the regulation of the canonical NF-κB pathway (p65/p50) by the USPs.

NF-κB in human cancer

Alterations of the canonical NF-κB signaling pathway are detected in multiple solid tumors, including pancreatic, lung, cervical, prostate, breast, and gastric carcinoma (12). High levels of p65 are found in non-small cell lung carcinoma and breast cancer (13,14). IκB deficiency is detected in breast, colon, ovarian, pancreatic, bladder, prostate cancers, and melanoma. Constitutively active IKK is found in colorectal carcinomas (15).

Upstream inducing pathways are subject to regulation by USPs

Even though the NF-κB family exclusively consists of intracellular proteins, their activation is often induced by extracellular signals via surface receptors such as tumor necrosis factor receptor (TNFR), toll-like receptor (TLR), and interleukin 1 receptor (IL-1R) (*Figure 2*). The signaling cascades following the activation of these surface receptors can usually be altered and, therefore, become regulatory sites for the activity of the canonical NF-κB signaling pathways (7,16-18).

TNFR-induced p65/p50 activation is subject to USP-mediated regulation

TNFR can induce the activation of the canonical NF-κB signaling pathway from its upstream, and proteins in this upstream pathway are subject to regulation mediated by USPs. Upon stimulation of TNF-α (*Figure 2A*), TNFR1 recruits receptor interacting protein 1 (RIP1) and TNFR-associated factor (TRAF) 2 (an E3 ligase) to form a receptor complex using its TNFR type 1-associated DEATH domain (TRADD) (7,16). TRAF2 conjugates the K63-linked polyubiquitin chain to RIP1 and itself with the presence of Ubc13 and UeV1A (both are E2 conjugating enzymes)

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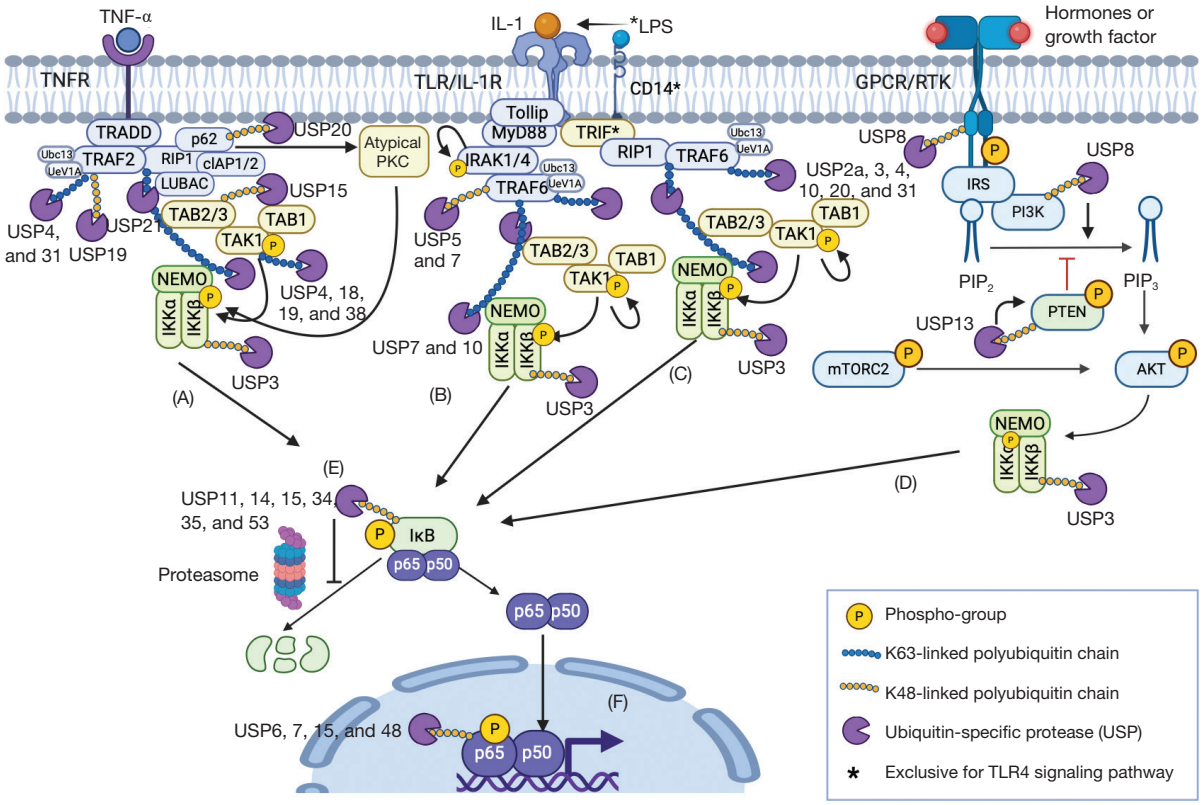

Figure 2 USPs function as deubiquitinases in the regulation of the TNFR-, TLR-, IL-1R-, and PI3K/AKT-induced p65/p50 activity. (A) During the TNFR-induced p65/p50 activation, USPs can remove K63-linked polyubiquitin chains from TRAF2, RIP1, TAK1, and NEMO. They can also remove K48-linked polyubiquitin chains from p62, TAB2/3, and IKKβ. (B,C) Everything from and downstream of TRAF proteins during IL-1R- and TLR-induced p65/p50 activation are shared with the TNFR-induced pathway depicted in (A). The asterisk (*) in (C) indicates the labeled molecules belong to the TLR-4 pathway exclusively. (D) As an example for RTKs, during the EGFR-induced p65/p50 activation, USPs remove K48-linked polyubiquitin chains from the surface receptor (EGFR), PI3K, and PTEN to regulate the p65/p50. Note that the stabilization of PI3K and PTEN will result in opposite effects on p65/p50 activity. (E) IκB is phosphorylated by the IKK complex. Phosphorylated IκB is conjugated with a K48-linked polyubiquitin chain and is subject to proteasomal degradation. USPs can stabilize IκB by removing this polyubiquitin chain. (F) Nuclear p65 can be conjugated with K48-linked polyubiquitin chains. This process can be reversed by the USPs listed above. TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRADD, TNFR type 1-associated DEATH domain; TRAF, TNFR-associated factor; USP, ubiquitin-specific protease; RIP1, receptor interacting protein 1; LUBAC, linear ubiquitin chain assembly complex; TAK1, transforming growth factor-β-activated kinase 1; TAB, TAK1-binding protein; NEMO, NF-κB essential modulator; IKK, IκB kinase; PKC, protein kinase C; TLR, toll-like receptor; IL-1R, interleukin 1 receptor; MyD88, myeloid differentiation primary response 88; IRAK, IL-1 receptor associated kinase; PI3K, phosphoinositide 3-kinase; mTORC2, mechanistic target of rapamycin complex 2; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; GPCR, G-protein-coupled receptor; RTK, receptor tyrosine kinase; EGFR, epithelial growth factor receptor.

to recruit the transforming growth factor-β-activated kinase 1 (TAK1) complex [a heterotrimer of TAK1, TGF-beta activated kinase 1 and MAP3K-7 binding protein 1 (TAB1), and TAB2/3] and the IKK complex (7,16). The TRAF2-RIP1 complex recruits cIAP1/2, a ubiquitin ligase and scaffold protein for the linear ubiquitin chain assembly complex (LUBAC), which later synthesizes a K63-linked or linear polyubiquitin chain that links to the TAB2/3 subunit in the TAK1 complex and the NEMO subunit in the IKK complex to recruit them (7,16). The recruited TAK1

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complex undergoes autophosphorylation and activates the IKK complex by phosphorylation of IKKβ. The activated IKK complex phosphorylates IκB to induce its K48-linked polyubiquitination and proteasomal degradation (7,16,18). Eventually, the degradation of IκB causes the release of p65/p50 dimer, which translocates into the nucleus and leads to subsequent transcriptional activities (7,16,18). Meanwhile, IKK can also be activated in a TAK1-independent manner (19). RIP1 in the receptor complex can interact with p62 to activate atypical protein kinase C (aPKCs), which in turn phosphorylates and activates the IKK complex (19).

In the TNFR-induced p65/p50 activation, the signaling cascades downstream of TRAF2 are shared by the TLR and IL-1R pathways (*Figure 2B*,*2C*), which is covered in upcoming sections of this review. Proteins unique to the TNFR pathway include TNFR, TRAF2, RIP1, and the aPKC. They can be regulatory sites for TNFR-induced NF-κB activity.

RIP1 can be a target for USP-mediated regulation. An *in vitro* study in cervical cancer cells shows that USP21 can remove K63-linked polyubiquitin chains from RIP1 (*Figure 2A*, *Table 2*). As a result, p65/p50 activation is inhibited (20).

Similar to RIP1, TRAF2 is another regulatory site specific to the TNFR-p65/p50 pathway. Although USP4, 19, and 31 all interact with TRAF2 in the TNFR-p65/p50 pathway, the outcomes are different (*Figure 2A*) (21-23). A study in the pulmonary carcinoma model reveals that USP4 interacts with K63-linked TRAF2 and deubiquitinates it to inhibit TNF-α-induced p65/p50 activation (22). In this case, USP4-knockdown pulmonary carcinoma has a significantly higher migration rate than its control upon treatment of TNF-α (22). This inhibitory mechanism was also discovered in a study of USP31 conducted in the HEK293T cell line (21). On the other hand, a study in cardiac cells indicates that USP19 stabilizes TRAF2 by removing its K48-linked polyubiquitin chain (23). In this case, the stabilized TRAF2 promotes the p65/p50 activation, which results in elevated cell viability (23).

The p62 protein is also a regulatory site specific to the TNFR-p65/p50 pathway (*Figure 2A*). It is reported that USP20 removes the K48-linked polyubiquitin chains on p62 to rescue it from proteasomal degradation (24). Consequently, TNF-α-induced p65/p50 activity is promoted (24). This study was conducted in a cervical cancer model and uncovered that USP20-mediated p62 stabilization and subsequent upregulation of p65/p50 activity contribute to cancer cell survival (24).

IL-1R and TLR-induced p65/p50 activation are subject to USP-mediated regulation

In a similar manner as TNFR, IL-1R and TLRs share a pathway that induces activation of the IKK complex and, therefore, the activation of p65/p50 heterodimer in the canonical NF-κB signaling pathway (*Figure 2B*). This pathway provides opportunities for USPs to regulate the downstream p65/p50 activity indirectly. IL-1R is stimulated by IL-1, and the TLRs are stimulated by various ligands such as lipopolysaccharides (LPS) and viral DNA (17,18,62-67). Upon stimulation, the receptors recruit myeloid differentiation primary response 88 (MyD88), toll-interacting protein (TOLLIP), IL-1 receptor associated kinase 1 (IRAK1), IRAK4, and TRAF6 to form a complex (16-18). At this stage, IRAK1 undergoes autophosphorylation and phosphorylates IRAK4 to trigger the dissociation of the IRAK1-TRAF6 complex from the receptor complex (19). Meanwhile, with the assistance of Ubc13 and Uev1A, TRAF6 undergoes K63-linked autoubiquitination and uses a K63-linked polyubiquitin chain to recruit the TAK1 complex via the TAB2/3 subunit and IKK complex via the NEMO subunit (18). From this point and beyond, the IL-1R, TLRs, and TNFR share the same mechanism to activate the canonical NF-κB signaling pathway (7,16-18). For TLR-4, an extensively studied TLR, there is also a MyD88-independent pathway upstream of TRAF6 in addition to the MyD88-dependent pathway described above (*Figure 2C*). Upon stimulation of LPS trafficked by cluster of differentiation 14 (CD14), it uses TRIF [TIR (toll/IL-1R) domain-containing adaptor protein inducing interferon beta; also known as TICAM-1 (TIR-containing adaptor molecule-1)] to recruit RIP1 and TRAF6 (16-18). MyD88, TOLLIP, IRAK1/4, and TRAF6 are specific to the IL-1R/TLR-induced p65/p50 activating mechanism, and TRIF is a protein specific to TLR-4. All these proteins can be targets for indirect regulation of p65/p50 activation.

TRAF6 is shared by IL-1R and TLRs to induce p65/p50 activation, making it an effective regulatory site for mechanisms involving these receptors (*Figure 2B*,*2C*) (16,18). It is a target for USPs with proteasome-dependent and proteasome-independent DUB activities. Mechanistic studies demonstrate that USP2a, 3, 4, 10, 20, and 31 deactivate TRAF6 by removing the K63-linked polyubiquitin chain from it (*Table 2* and *Figure 2*) (21,22,25-29). Investigations on the cellular behaviors imply that the USP-mediated K63-linked deubiquitination of TRAF6 leads to suppressed malignant behaviors of cancer

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Table 2 The summary of USP-mediated NF-κB regulation

Target	NF-κB activating mechanism	Ubiquitin chain linkage	Regulating USPs	Effects on the target	Effects on NF-κB activity	Effects on cellular behaviors	Cell/tissue types	Reference
RIP1	TNFR activation	K63	USP21	Deactivated	Negative	N/A	Cervical cancer	(20)
TRAF2	TNFR activation	K63	USP31	Deactivated	Negative	N/A	Immortalized embryonic kidney cells	(21)
TRAF2	TNFR activation	K63	USP4	Deactivated	Negative	Inhibited cell migration	Pulmonary carcinoma	(22)
TRAF2	TNFR activation	K48	USP19	Stabilized	Positive	Increased cell viability, protection against mitochondrial dysfunction	Mouse cardiac cells	(23)
p62	TNFR activation	K48	USP20	Stabilized	Positive	Contribution to cell survival	Cervical cancer	(24)
TRAF6	TRAF6 activation	K63	USP31	Deactivated	Negative	N/A	Immortalized embryonic kidney cells	(21)
TRAF6	TNFR activation	K63	USP4	Deactivated	Negative	Inhibited cell migration	Pulmonary carcinoma	(22)
TRAF6	Viral infection	K63	USP2a	Deactivated	Negative	N/A	Colorectal carcinoma	(25)
TRAF6	IL-1R activation	K63	USP3	Deactivated	Negative	Inhibited apoptosis	Chondrocytes	(26)
TRAF6	HTLV-1 infection IL-1R activation	K63	USP20	Deactivated	Negative	Inhibited oncogenesis	HTLV-1 transformed cells (T-cell leukemia)	(27)
TRAF6	IL-1R activation	K63	USP20	Deactivated	Negative	Inhibited cell growth and proliferation	Smooth muscle cells	(28)
TRAF6	Genotoxic stress	K63	USP10	Deactivated	Negative	N/A	Immortalized embryonic kidney cells	(29)
TRAF6	N/A	N/A	USP1	Stabilized	Positive	Inhibited pyroptosis	Osteoblasts	(30)
TRAF6	TNFR activation	N/A	USP4	Stabilized	Positive	Increased malignancy	Pancreatic cancer	(31)
TRAF6	N/A	K48	USP5	Stabilized	Positive	Increased expression of pro-inflammatory cytokine	Rheumatoid arthritis-fibroblast-like synoviocytes	(32)
TRAF6	LPS-TLR4 interaction	K48	USP7	Stabilized	Positive	Increased inflammation	Immortalized mouse macrophages	(33)

Table 2 (*continued*)

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Table 2 (*continued*)

Target	NF-κB activating mechanism	Ubiquitin chain linkage	Regulating USPs	Effects on the target	Effects on NF-κB activity	Effects on cellular behaviors	Cell/tissue types	Reference
TRAF6	Viral infection	N/A	USP25	Stabilized (by a ubiquitin-independent mechanism)	Positive	Increased expression of pro-inflammatory cytokine	Mouse lung fibroblasts, plasmacytoid dendritic cells, and bone marrow derived dendritic cells	(34)
EGFR & PI3K	EGFR activation PI3K/AKT	N/A	USP8	Stabilized	Positive	Cancer cell growth, proliferation, metastasis, and docetaxel resistance	Androgen-independent prostate cancer	(35)
PTEN	PI3K/AKT	N/A	USP13	Stabilized	Negative	Inhibited proliferation and metastasis	Bladder cancer	(36)
TAK1	N/A	K63	USP18	Deactivated	Negative	N/A	Immortalized embryonic kidney cells, cervical cancer cells, leukemia monocytes	(37)
TAK1	TNFR activation	K63	USP4	Deactivated	Negative	N/A	Cervical cancer	(38)
TAK1	Doxorubicin	K63	USP4	Deactivated	Negative	N/A	Cervical cancer, immortalized embryonic kidney cell, and mouse embryonic fibroblast	(39)
TAK1	LPS-TLR4 interaction CpG-TLR9 interaction	K63	USP18	Deactivated	Negative	Inhibited expression of proinflammatory cytokines	Mice model	(40)
TAK1	TNFR activation IL-1R activation	K63 K27	USP19	Deactivated; interaction with TAB2/3 is disrupted	Negative	Inhibited expression of proinflammatory cytokines	Embryonic kidney cells	(41)
TAK1	N/A	N/A	USP18	N/A	Negative	Inhibited proliferation and increase apoptosis	Leukemia, multiple myeloma, B-cell lymphoma, and cervical cancer cells	(42)

Table 2 (*continued*)

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Table 2 (*continued*)

Target	NF-κB activating mechanism	Ubiquitin chain linkage	Regulating USPs	Effects on the target	Effects on NF-κB activity	Effects on cellular behaviors	Cell/tissue types	Reference
TAK1	N/A	N/A	USP38	Elevated phosphorylation level	Positive	Promoted post-myocardial-infarction inflammation	Mouse heart	(43)
TAB2	TNFR activation IL-1R activation	K48	USP15	Stabilized (with both ubiquitin-dependent and ubiquitin-independent mechanisms)	Positive	N/A	Immortalized embryonic kidney cells	(44)
TAB3	TNFR activation IL-1R activation	N/A	USP15	Stabilized (by inhibiting NBR1-induced autophagic degradation)	Positive	N/A	Immortalized embryonic kidney cells	(44)
NEMO	Genotoxic stress	K63	USP10	Deactivated	Negative	N/A	Immortalized embryonic kidney cells	(29)
NEMO	N/A	N/A	USP18	Deactivated (physically shielded from K63-linked poly-ubiquitination)	Negative	N/A	Immortalized embryonic kidney cells, cervical cancer cells, leukemia monocytes	(37)
NEMO	N/A	K63	USP7	Deactivated	Negative	N/A	Colorectal carcinoma	(45)
NEMO	Genotoxic stress	linear	USP10	Deactivated	Negative	Promoted apoptosis	Immortalized embryonic kidney cells and mouse intestine tissue	(46)
NEMO	LPS-TLR4 interaction	N/A	USP10	Stabilized	Positive	Promoted inflammatory response	Leukemia monocytes and mouse macrophages	(47)
IKK-β	TNFR activation	N/A	USP3	Stabilized	Positive	Promoted tumor growth	Nasopharyngeal carcinoma	(48)
IκB-α	TNFR activation	K48	USP11	Stabilized	Negative	N/A	Immortalized embryonic kidney cells	(49)
IκB-α	TNFR activation	N/A	USP15	Stabilized	Negative	N/A	cervical cancer	(50)
IκB-α	N/A	N/A	USP34	Stabilized	Negative	Inhibited differentiation	Immortalized mouse macrophages	(51)

Table 2 (*continued*)

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Table 2 (*continued*)

Target	NF-κB activating mechanism	Ubiquitin chain linkage	Regulating USPs	Effects on the target	Effects on NF-κB activity	Effects on cellular behaviors	Cell/tissue types	Reference
IκB-α	LPS-TLR4 interaction	K48	USP39	Stabilized	Negative	Inhibited secretion of proinflammatory cytokines	Leukemia monocytes	(52)
IκB-α	N/A	N/A	USP53	Stabilized	Negative	Inhibited cell proliferation, invasion and migration	Renal cancer cells	(53)
IκB-α	IL-1R activation	N/A	USP14	USP14 interacts with and deubiquitinates IκB-α, but suppresses its expression level	Positive	Inhibited differentiation	Chondrocytes	(54)
IκB-α	N/A	N/A	USP14	USP14 interacts with and deubiquitinates IκB-α, but suppresses its expression level	Positive	Inhibited apoptosis, promoted EMT, colony formation, invasion, and metastasis	Endometrial carcinoma	(55)
p65	N/A	N/A	USP7	Stabilized	Positive	Upregulated transcription	Immortalized mouse macrophages	(56)
p65	N/A	N/A	USP15	Stabilized	Positive	Inhibited apoptosis	Multiple myeloma	(57)
p65	TNFR activation	K48	USP48	Stabilized	Positive	N/A	Cervical cancer and embryonic kidney cells	(58)
p65	Basal NF-κB activity TNFR activation	N/A	USP48	Destabilized (via unknown mechanism)	Negative	N/A	Retinal pigment epithelial cells	(59)
p65	N/A	N/A	USP6	Increased phosphorylation (with the presence of IKKα and IKKβ)	Positive	Promoted tumorigenesis	Cervical cancer, mouse osteoblasts, and mouse embryonic fibroblasts	(60)
ABIN2	TNFR activation	N/A	USP35	Stabilized	Negative	Inhibited cell proliferation (*in vitro*) and tumor formation (*in vivo*)	Non-small cell lung carcinoma and androgen-sensitive prostate adenocarcinoma	(61)

USP, ubiquitin-specific protease; NF-κB, nuclear factor kappa B; RIP1, receptor interacting protein 1; TRAF, TNFR-associated factor; TNFR, tumor necrosis factor receptor; EGFR, epithelial growth factor receptor; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; TAK1, transforming growth factor-β-activated kinase 1; TAB, TAK1-binding protein; NEMO, NF-κB essential modulator; IKK, IκB kinase; ABIN, A20-binding inhibitor of NF-κB; IL-1R, interleukin 1 receptor; HTLV-1, human T-lymphoma virus 1; K63, lysine-63 residue (linked polyubiquitin chain); K48, lysine-48 residue (linked polyubiquitin chain); K27, lysine-27 residue (linked polyubiquitin chain); N/A, not applicable; LPS, lipopolysaccharides; TLR, toll-like receptor; EMT, epithelial-to-mesenchymal transition.

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cells such as pulmonary carcinoma and human T-lymphoma virus 1 (HTLV-1) transformed T-cell leukemia (22,27). On the other hand, the proteasome-associated DUB activity of USPs stabilizes TRAF6 to promote NF-κB signaling. USP1, 4, 5, and 7 stabilize TRAF6 through their DUB activity (30-33). USP4-mediated TRAF6 stabilization was studied in pancreatic cancer models (MIA PaCa-2 and AsPC-1) (31). This study supports the idea that USP-mediated TRAF6 stabilization and subsequently upregulated NF-κB signaling are responsible for increased malignancy (31).

Usually, USPs stabilize proteins using DUB activity, which protects proteins from proteasomal degradation. Yet, there is an exception. In the case of viral infection, USP25 elevates the expression level of TRAF6 in a USP-independent manner to promote NF-κB signaling (34).

Crosstalk between phosphoinositide 3-kinase (PI3K)-AKT and the canonical NF-κB pathway is regulated by USPs

Surface receptors such as G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) can also activate p65/p50 dimer via an upstream inducing pathway—PI3K-AKT axis (*Figure 2D*) (35,36,68,69). Upon stimulation of hormones (via GPCR) or growth factors (via RTK), PI3K is activated to convert phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3). Subsequently, PIP3 activates the pyruvate dehydrogenase kinase 1 (PDK1) and the mechanistic target of rapamycin (mTOR) complex 2 (mTORC2) to phosphorylate AKT at T308 and S473, respectively, to convert AKT into the active form, pAKT (68,69). The active pAKT can cause the activation of the NF-κB signaling pathway by phosphorylating IKKα (69). On the other hand, the conversion from PIP2 to PIP3 is reversed by phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a tumor suppressor protein (36,68,69). Proteins specific to the crosstalk between PI3K/AKT and the canonical NF-κB signaling pathway include the surface receptor, PI3K, mTORC2, and PDK. They can be potential sites for indirect regulation of p65/p50 activation.

Epithelial growth factor (EGF) receptor (EGFR) (a type of RTK) and PI3K can be deubiquitinated and stabilized by USPs (*Figure 2D*). A mechanistic study in androgen-independent prostate cancer models (PC3 and DU145) unravels that USP8 catalyzes the deubiquitination of both EGFR and PI3K to stabilize them, which in turn activates IKKα to upregulate the p65/p50 activity (35). Subsequently, upregulated p65/p50 activity results in an increase in N-cadherin and a decrease in E-cadherin [epithelial-to-mesenchymal transition (EMT) marker], cleaved caspase 3, and cleaved caspase 9 (apoptosis markers) (35). The *in vitro* functional study indicates that USP8 is responsible for the inhibition of apoptosis, increase in cell proliferation and migration, and the development of docetaxel resistance of prostate cancer (35).

In contrast, USP13 reverses the effects of EGF-induced p65/p50 activity by interacting and stabilizing PTEN to suppress the p65/p50 activity (*Figure 2D*) (36). The *in vitro* functional study reveals that USP13 knockdown exacerbates the colony formation, proliferation, invasion, and migration of bladder cancer cells, which can be reversed by re-expression of PTEN (36). The *in vivo* rescue experiments also indicate that re-expression of USP13 or PTEN can partially restore tumor growth due to PTEN or USP13 knockout, respectively (36).

Signaling cascades shared by upstream inducing pathways are regulated by USPs

The regulatory mechanisms presented above are unique to each upstream inducing pathway. These pathways share some signaling cascades that eventually converge into the IKK-p65/p50 axis (*Figure 2E,2F*), and their signaling molecules are potentially USP's substrates. The TNFR, IL-1R, and TLR pathways share everything downstream of the TRAF proteins (TRAF2 and TRAF6, *Figure 2A-2C*), and the PI3K/AKT pathway shares the IKK-p65/p50 axis with all three of them (*Figure 2D*) (7,16-18,68,69).

The TAK1 complex is regulated by USPs

Shared by the TNFR, IL-1R, and TLR pathways, the TAK1 complex is a regulatory site where USPs can indirectly control the p65/p50 activity (7,16-18). Given the recruiting and activating mechanisms (K63-linked polyubiquitin chains), the K63-linked DUB activity of USPs can inhibit the TAK1 activity and subsequent p65/p50 activation. Mechanistic studies demonstrate that USP4, 18, and 19 dissociate the K63-linked polyubiquitin chain from the TAK1 subunit (*Table 2* and *Figure 2A-2C*) (37-41). In the study of USP19-mediated regulation p65/p50 activity, evidence also supports that USP19 cleaves off the K27-linked polyubiquitin chain as well, and the TAK1-TAB2/3 binding was disrupted (41). A different study

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involving various *in vitro* cancer models provides evidence that USP18-mediated p65/p50 inhibition causes inhibited proliferation and increased apoptosis in leukemia, multiple myeloma, B-cell lymphoma, and cervical cancer cells (42).

Another TAK1-regulating protein is USP38 (*Table 2*). However, USP38-mediated TAK1 regulation does not involve the DUB activity of USP38. A study on USP38-mediated post-myocardial-infarction (post-MI) inflammation reveals that USP38 physically interacts with TAK1 and the conditional knockout of USP38 in mouse heart leads to a decrease in the phosphorylation level of TAK1 (pTAK1) and subsequent decrease in p65 activation (43). Meanwhile, the TAK1 (native protein, not phosphorylated) level remains constant (43).

As a building block and the scaffold for recruitment of the entire functional complex, the expression level of the TAB2/3 subunit can also influence the TAK1 activity and the downstream p65/p50 activity. Thus, stabilizing TAB2/3 by USP using DUB activities associated with proteasomal degradation is another feasible option to potentiate the p65/p50 activity. A mechanistic study conducted in the HEK293T cell line reveals that USP15 stabilizes the TAB2 and TAB3 subunits of the TAK1 complex (*Table 2*) (44). USP15 uses its K48-linked DUB activity to rescue TAB2 from proteasomal degradation (*Figure 2A-2C*), and USP15 also stabilizes TAB2 in a ubiquitin-independent mechanism—it inhibits the lysosomal degradation of TAB2 (44). On the other hand, USP15 stabilizes TAB3 without using its DUB activity at all. Instead, it inhibits NBR1 (neighbor of BRCA1 gene 1, a selective autophagy receptor)-mediated autophagic degradation of TAB3 (44).

NEMO is regulated by USPs

Similar to the TAK1 complex, the IKK complex is also recruited to the surface receptors (TNFR, IL-1R, and TLRs) via a K63-linked polyubiquitin chain attached to its regulatory subunit—NEMO. Given this fact, it is potentially another regulatory site where USPs can indirectly control the p65/p50 activity. Studies indicate that the ubiquitination of the NEMO subunit is associated with both proteasomal degradation and signal transduction (*Table 2*) (29,37,45-47). USP7 and USP10 remove the K63-linked polyubiquitin chain from NEMO to deactivate the IKK complex, resulting in the downregulation of p65/p50 activity (*Figure 2A-2C*) (29,45). USP18 is essentially the same in terms of function but slightly different in terms of mechanism. Instead of cleaving off the K63-linked polyubiquitin chain, it physically shields NEMO from K63-linked polyubiquitination (37). Besides the K63-linked polyubiquitin chain, USP10 also removes linear polyubiquitin chains from the NEMO subunit in the IKK complex (46). Removing the linear polyubiquitin chains from NEMO downregulates the p65/p50 activity to promote apoptosis *in vitro* and *in vivo* (46). In addition, USP10-mediated deubiquitination of NEMO inhibits NEMO degradation and promotes p65/p50 activity in human leukemia monocytes and mouse macrophages (47).

The common IKK-p65/p50 axis is regulated by USPs

Unlike the NEMO subunit, the IKKα and IKKβ subunits are involved in all four NF-κB inducing mechanisms described above (*Figure 2*). These two subunits are where all those inducing pathways converge into the canonical NF-κB signaling pathway. Proteins in this common IKK-p65/p50 axis are subject to regulation mediated by USPs.

IKKβ is regulated by USP3

As described in *Table 2*, it has been reported that ubiquitination of IKKβ is associated with proteasomal degradation (48). USP3 can remove the polyubiquitin chain from IKKβ to stabilize it (48). As a result, NF-κB activity is upregulated, and promoted tumor growth is detected in nasopharyngeal carcinoma (48).

The expression level of IκB is altered by USPs by various means

The IκB-p65/p50 complex is immediately downstream of the IKK complex described above, and its degradation is induced by IKK-mediated phosphorylation, making it another effective regulatory site where USPs can function as DUBs (*Figure 2E*) (16,18). Deubiquitination of IκB can result in the stabilization of IκB and subsequent inhibition of p65/p50 activity. As summarized in *Table 2*, USP11, 15, 34, 39, and 53 interact with IκB-α and function as its DUBs to inhibit the degradation, and the USP-mediated IκB stabilization leads to the downregulation of p65/p50 activity (49-53). The study of USP34-mediated IκB-α stabilization was conducted in immortalized mouse macrophages, where cell differentiation was inhibited (51). In the study of USP53-mediated IκB stabilization, researchers found that USP53 inhibits the proliferation, invasion, and migration of renal cancer cells *in vitro* and *in vivo* (53).

It is widely agreed that deubiquitination of IκB leads to its stabilization and subsequent inhibition of p65/p50 activity. This is not necessarily true with USP14-IκB

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interaction (*Table 2*). A mechanistic study of IL-1β-induced NF-κB activity in human chondrocytes demonstrated that the p65 phosphorylation is positively correlated with USP14 despite USP14's DUB activity upon IκB-α (54). A study on USP14-mediated NF-κB activation in endometrial carcinoma also confirms this result with a more readily visible negative correlation between the expression levels of USP14 and IκB-α (55). The USP14-mediated p65/p50 activation inhibits apoptosis and increases malignant behaviors such as migration, invasion, and EMT of endometrial carcinoma *in vivo* and *ex vivo* (55).

USPs interact with p65 and regulate p65 activity

Upon release from IκB, the p65/p50 heterodimer is facing its destination—the nucleus (*Figure 2F*). The p65 subunit at this stage functions as a regulatory site for the canonical NF-κB signaling pathway. Deubiquitination of p65 by USP7, 15, and 48 can stabilize p65 and upregulate the NF-κB activity (*Table 2*) (56-58). USP15 in multiple myeloma models accounts for inhibited apoptosis of cancer cells (57).

It is essential to clarify that USP48 specifically stabilizes nuclear p65 in HeLa and HEK293 cells when it is associated with COP9 signalosome (CSN), and USP48 preferably trims the K48-linked polyubiquitin chain instead of completely removing it (58). Another study on USP48-mediated p65/p50 regulation conducted in retinal pigment epithelial cells unveils that knockdown of USP48 causes destabilization of both cytoplasmic and nuclear p65 and downregulates NF-κB activity despite the interaction between USP48 and p65 (59).

Another mechanism of USP-mediated p65 regulation is to induce its phosphorylation. The p65 phosphorylation requires USP6 to physically interact with both IKKα and IKKβ subunits in the IKK complex (60). Results from xenografts support the idea that USP6-mediated p65 phosphorylation is associated with tumorigenesis *in vivo* (60).

Uncommon regulatory mechanisms

The p65/p50 activity is inhibited by USP35-mediated A20-binding inhibitor of NF-κB 2 (ABIN2) stabilization

ABIN2 is not a part of the NF-κB signaling pathway. Instead, it negatively regulates TNF-induced NF-κB signaling by competing with RIP1 for the NEMO subunit in the IKK complex (70). Thus, USPs that function as DUBs of ABIN2 can stabilize it and inhibit the NF-κB activity. A study involving multiple cancer models revealed that USP35 inhibits TNF-α-induced NF-κB signaling by stabilizing ABIN2 with DUB activity (61). The NF-κB inhibition through USP35-ABIN2 interaction leads to the inhibition of cancer cell proliferation in non-small cell lung carcinoma, cervical cancer, and prostate adenocarcinoma (61). *In vivo* experiments also indicate that the USP35 inhibits tumor formation (61).

Phosphorylation of p65 is induced by USP6

Although most USPs function as DUBs in NF-κB regulation, existing studies have identified a few exceptions. In addition to the ubiquitin-independent mechanisms of USP14 and USP25 mentioned above, USP6 can also regulate the NF-κB activity with a ubiquitin-independent method (*Table 2*). Despite its physical interaction with IKKα and IKKβ, USP6 does not influence the IκB expression level, which is immediately downstream of and regulated by the IKK complex (60). Instead, USP6 promotes phosphorylation of p65 with the presence of both IKKα and IKKβ. Knockdown of either subunit will result in a lower level of p65 in the nucleus (60). Mice allografts stably expressing USP6 and its vector demonstrate that USP6-mediated NF-κB upregulation promotes tumorigenesis and tumor vascularization (60).

Discussion

Known mechanisms and missing parts

In most studies summarized in *Table 2*, USPs primarily function as DUBs to regulate the canonical NF-κB signaling pathway by removing polyubiquitin chains from signaling molecules in the TNFR-, TLR-, IL-1R-, and PI3K/AKT-induced canonical NF-κB pathway (*Figure 2*). The K48-linked DUB activity of USPs usually inhibits proteasomal degradation and stabilizes the target proteins, and K63-linked DUB activity usually downregulates signal transduction. K48-linked deubiquitination and stabilization of NF-κB's positive regulators, including TRAF proteins, TAK1, IKKα, IKKβ, and p65, lead to upregulation of p65/p50 activity. In contrast, stabilizing the negative regulators, including IκB-α and PTEN, can downregulate the p65/p50 activity. K63-linked deubiquitination and deactivation of NF-κB's positive regulators also downregulate the p65/p50 activity. TRAF proteins, TAK1, and NEMO undergo K63-linked deubiquitination by USPs to inhibit NF-κB activation. These mechanisms are validated by studies conducted in different cancer models with different USPs. Thus, the existing studies have already covered USPs'

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function as DUBs in protein degradation and signaling transduction. Nevertheless, there are more questions that need to be addressed.

Firstly, it is essential to point out that USP-mediated p65/p50 regulation is context-dependent. Different proteins or the same proteins with different polyubiquitin chains can be the substrates of identical USPs. Thus, USPs have multifaced roles in the regulation of the canonical NF-κB pathway. USP4 sets up a good example. Studies demonstrate that USP4's substrates include TAK1, TRAF2, and TRAF6 (*Table 2*) (22,31,38,39). USP4 can detach the K63-linked polyubiquitin chains from all these proteins to downregulate the p65/p50 activity in multiple cell lines and inhibit cell migration of pulmonary carcinoma (22,38,39). It can also stabilize TRAF6 by deubiquitination, promoting p65/p50 activity and malignant cellular behaviors in the pancreatic cancer cell lines (31). In other words, despite the widely conserved regulatory mechanisms on the molecular level, the comprehensive effects of USP-mediated p65/p50 regulation on cellular behaviors depend on the context (for instance, the cell line or type of cancer). Thus, it is necessary to investigate the influence of USP-mediated NF-κB regulation individually in different cancer models.

Secondly, most of the mechanistic studies focus on the DUB activities against K48- and K63-linked polyubiquitin chains, and novel findings beyond the scope of these typical polyubiquitin chains are very limited. Only K27-linked polyubiquitinated TAK1 and linearly polyubiquitinated NEMO have been studied in USP-mediated p65/p50 regulation (41,46). K11- and K29-linked polyubiquitin chains are also associated with proteasomal degradation, and K63-linked polyubiquitin chains are responsible for autophagic degradation (*Figure 1*). Many studies involving protein degradation in the p65/p50 regulation did not specify the linkage of the polyubiquitin chain (denoted by "N/A" in *Table 2*), leaving an unneglectable space for further investigation.

Thirdly, no USPs with K63-linked DUB activity have been identified as regulators of the TAB2/3 subunit in the TAK1 complex. Among the published studies, the only TAB2/3-regulating USP is USP15, and it only inhibits the degradation of TAB2 by K48-linked DUB activity (*Table 2*). Furthermore, USP15 more often regulates the degradation of TAB2 and TAB3 via ubiquitin-independent mechanisms. Given the fact that K63-linked polyubiquitination of TAB2/3 subunit is essential for TAK1 activation, this potential regulatory site deserves further investigation by screening USPs with K63-linked DUB activity.

Similar to TAB2/3, RIP1's activity relies on the K63-linked polyubiquitin chain, and it is essential for TNFR-induced p65/p50 activation. Existing evidence only supports that the K48-linked DUB activity of USP21 inhibits the proteasomal degradation of RIP1 (20). None of the studies have identified a USP with K63-linked DUB activity against it, leaving a broad area for future research.

Another problem is the rarity of studies complicated with the lack of data on USP-mediated ubiquitin-independent regulatory mechanisms. It is also practical for the canonical NF-κB pathway to be regulated by the USPs in a ubiquitin-independent manner. Existing studies demonstrate that USP14 suppresses the expression level of IκB-α despite its ability to interact with and deubiquitinate IκB-α (*Table 2*) (54,55). This paradoxical result implies that there must be a ubiquitin-independent mechanism by which the USP14 regulates the IκB-α, and this mechanism must override the stabilizing effect of USP14's DUB activity upon IκB-α. USP25 also promotes the RNA virus-induced p65/p50 activity by stabilizing TRAF6 through a ubiquitin-independent mechanism. Despite the physical interaction between USP25 and TRAF6, Western blot analysis indicates that USP25 has neither K48- nor K63-linked DUB activity against TRAF6 (34). However, USP25-positive cells maintain a higher level of TRAF6 and p-IκB-α (34). USP6 is also a ubiquitin-independent positive regulator of the canonical NF-κB signaling pathway. It facilitates the phosphorylation of p65 by interacting with IKKα and IKKβ, promoting the p65/p50 activity and subsequent tumorigenesis (60). In these cases, ubiquitin is not involved. Whether the remaining USPs can carry out identical regulatory mechanisms for the canonical NF-κB signaling pathway remains unknown and requires further investigation. In addition, the studies listed above can only demonstrate the quantitative correlations between the USPs and their targets but cannot explain the mechanisms clearly. For instance, no kinases interacting with USP6 were identified for p65 phosphorylation in the study of USP6-mediated p65/p50 regulation (60). For another example, no quantitative polymerase chain reaction (qPCR) data of IκB were provided in the study of USP14-mediated p65/p50 regulation to explain whether IκB is transcriptionally upregulated when USP14 is overexpressed (54). These studies are rare, even though they offer certain insights into ubiquitin-independent regulatory mechanisms of the canonical NF-κB signaling pathway.

Last but not least, there are no published studies on certain USPs' role in the regulation of the canonical NF-

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κB signaling pathway. As they are not listed in *Table 2*, the functions of USP16, 17, 22–24, 26–30, 36, 37, 40–47, and 49–52 have not been studied in the USP-mediated p65/p50 regulation, leaving a major space for future exploration.

Implications in human cancers

As reviewed in 2005, the alterations of canonical NF-κB signaling pathway were found in lung, breast, ovarian, pancreatic, bladder, prostate cancer, and melanoma (15). In this review, none of the mechanistic studies on USP-mediated p65/p50 regulation were conducted in breast cancer, ovarian cancer, and melanoma. Instead, many of the studies were conducted in leukemia monocytes and cervical cancer cells (*Table 2*). In addition to the cancers listed above, USP-mediated p65/p50 regulation is also found in colorectal carcinoma, renal cancer, endometrial carcinoma, and multiple myeloma (*Table 2*).

USP-mediated inhibition of p65/p50 activity in human cancers is usually associated with decreased malignant behaviors such as proliferation, invasion, migration, tumor formation, EMT, chemotherapy resistance, and inhibition of apoptosis. In contrast, USP-mediated promotion of p65/p50 activity in human cancers is associated with increased malignancy. These results are congruent with the findings in a review of NF-κB in human cancer (15).

If we narrow the scope down to specific cancers, such as prostate cancer only, it is apparent that there are still many investigations to be done. The four inducing pathways upstream of the IKK complex have their unique regulatory sites subject to USPs' regulation, and they converge into the IKK-p65/p50 axis (*Figure 2*). Despite the number of potential regulatory sites, only two studies on USP-mediated p65/p50 regulation were done in prostate cancer models, and these regulatory sites are very uncommon (35,61). Thus, for future prostate cancer research in this area, it will be promising to aim for the remaining potential regulatory sites (TRAF proteins, TAK1 complex, IKK complex, etc.) and screen the USPs for each of them. This direction may also apply to future research in breast cancer, ovarian cancer, and melanoma.

Conclusions

According to the mechanistic studies, regulation of the canonical NF-κB signaling pathway mediated by USPs is primarily actuated through the K48-linked and K63-linked DUB activities against signaling molecules involved in the TNFR-, TLR-, and IL-1R-induced p65/p50 activation. In a few cases, the USPs can interfere with the crosstalk between PI3K/AKT and the canonical NF-κB pathway by deubiquitinating and stabilizing EGFR, PI3K, and PTEN. It has also been reported that linear polyubiquitin chains attached to NEMO and K27-linked polyubiquitin chains from TAK1 can be removed by USPs. Thus, USPs primarily function as DUBs in NF-κB regulation.

In addition, USPs regulate the canonical NF-κB signaling pathway with ubiquitin-independent mechanisms. Current studies indicate that these mechanisms involve phosphorylation of p65, ubiquitin-independent degradation of TAB2/3, and suppression of IκB expression level. These ubiquitin-independent regulatory mechanisms involving USPs are yet to be extensively studied. Given their influence on cancer cells, there is a growing need for further investigations in this area.

Based on existing findings of mechanistic studies, it is safe to draw a conclusion that future studies can be done in these aspects—removal of the K63-linked polyubiquitin chain from TAB2/3 and RIP1 by USPs, removal of atypical polyubiquitin chains from the regulatory sites (*Figure 1*), identifying the p65/p50-associated regulatory roles of USPs not listed in *Table 2*, and ubiquitin-independent regulation of the canonical NF-κB signaling pathway mediated by USPs.

The general trend is that USP-mediated inhibition of p65/p50 activity by K63-linked DUB activity often leads to less malignant cellular behaviors of human cancers. In contrast, USP-mediated promotion of p65/p50 activity by positive-regulator-stabilization accelerates tumorigenesis (*in vivo*), colony formation, cell proliferation, invasion, and migration (*in vitro*). These changes in phenotypes due to USP-mediated p65/p60 regulation have been extensively studied in leukemia monocytes and cervical cancer models but not as extensively studied in lung, breast, ovarian, pancreatic, bladder, prostate cancers, and melanoma.

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References

1. Wang L, Lu B, He M, et al. Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019. Front Public Health 2022;10:811044.
2. Mooney EC, Sahingur SE. The Ubiquitin System and A20: Implications in Health and Disease. J Dent Res 2021;100:10-20.
3. Carroll EC, Marqusee S. Site-specific ubiquitination: Deconstructing the degradation tag. Curr Opin Struct Biol 2022;73:102345.
4. Grice GL, Nathan JA. The recognition of ubiquitinated proteins by the proteasome. Cell Mol Life Sci 2016;73:3497-506.
5. Madiraju C, Novack JP, Reed JC, et al. K63 ubiquitination in immune signaling. Trends Immunol 2022;43:148-62.
6. Yang Q, Zhao J, Chen D, et al. E3 ubiquitin ligases: styles, structures and functions. Mol Biomed 2021;2:23.
7. Hayden MS, Ghosh S. Regulation of NF-κB by TNF family cytokines. Semin Immunol. 2014;26:253-66.
8. Zhou Z, Song X, Kang R, et al. The Emerging Role of Deubiquitinases in Cell Death. Biomolecules 2022;12:1825.
9. Lange SM, Armstrong LA, Kulathu Y. Deubiquitinases: From mechanisms to their inhibition by small molecules. Mol Cell 2022;82:15-29.
10. Chen S, Liu Y, Zhou H. Advances in the Development Ubiquitin-Specific Peptidase (USP) Inhibitors. Int J Mol Sci 2021;22:4546.
11. Taniguchi K, Karin M. NF-κB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol 2018;18:309-24.
12. Rasmi RR, Sakthivel KM, Guruvayoorappan C. NF-κB inhibitors in treatment and prevention of lung cancer. Biomed Pharmacother 2020;130:110569.
13. Zhang L, Ludden CM, Cullen AJ, et al. Nuclear factor kappa B expression in non-small cell lung cancer. Biomed Pharmacother 2023;167:115459.
14. Zheng W, Wang X, Yu Y, et al. CircRNF10-DHX15 interaction suppressed breast cancer progression by antagonizing DHX15-NF-κB p65 positive feedback loop. Cell Mol Biol Lett 2023;28:34.
15. Dolcet X, Llobet D, Pallares J, et al. NF-kB in development and progression of human cancer. Virchows Arch 2005;446:475-82.
16. Yu H, Lin L, Zhang Z, et al. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. Signal Transduct Target Ther 2020;5:209.
17. Yu C, Wang D, Yang Z, et al. Pharmacological Effects of Polyphenol Phytochemicals on the Intestinal Inflammation via Targeting TLR4/NF-κB Signaling Pathway. Int J Mol Sci 2022;23:6939.
18. Wang J, Wu X, Jiang M, et al. Mechanism by which TRAF6 Participates in the Immune Regulation of Autoimmune Diseases and Cancer. Biomed Res Int 2020;2020:4607197.

19. Verstrepen L, Bekaert T, Chau TL, et al. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. Cell Mol Life Sci 2008;65:2964-78.
20. Xu G, Tan X, Wang H, et al. Ubiquitin-specific peptidase 21 inhibits tumor necrosis factor alpha-induced nuclear factor kappaB activation via binding to and deubiquitinating receptor-interacting protein 1. J Biol Chem 2010;285:969-78.
21. Tzimas C, Michailidou G, Arsenakis M, et al. Human ubiquitin specific protease 31 is a deubiquitinating enzyme implicated in activation of nuclear factor-kappaB. Cell Signal 2006;18:83-92.
22. Xiao N, Li H, Luo J, et al. Ubiquitin-specific protease 4 (USP4) targets TRAF2 and TRAF6 for deubiquitination and inhibits TNFα-induced cancer cell migration. Biochem J 2012;441:979-86.
23. Dhingra R, Rabinovich-Nikitin I, Rothman S, et al. Proteasomal Degradation of TRAF2 Mediates Mitochondrial Dysfunction in Doxorubicin-Cardiomyopathy. Circulation 2022;146:934-54.
24. Ha J, Kim M, Seo D, et al. The Deubiquitinating Enzyme USP20 Regulates the TNFα-Induced NF-κB Signaling Pathway through Stabilization of p62. Int J Mol Sci 2020;21:3116.
25. He X, Li Y, Li C, et al. USP2a negatively regulates IL-1β- and virus-induced NF-κB activation by deubiquitinating TRAF6. J Mol Cell Biol 2013;5(1):39-47. doi:http://dx.doi.org/10.1093/jmcb/mjs024
26. Zhou Q, Xiao Z, Zhou R, et al. Ubiquitin-specific protease 3 targets TRAF6 for deubiquitination and suppresses IL-1β induced chondrocyte apoptosis. Biochem Biophys Res Commun 2019;514:482-9.
27. Yasunaga J, Lin FC, Lu X, et al. Ubiquitin-specific peptidase 20 targets TRAF6 and human T cell leukemia virus type 1 tax to negatively regulate NF-kappaB signaling. J Virol 2011;85:6212-9.
28. Zhang L, Wu JH, Jean-Charles PY, et al. Phosphorylation of USP20 on Ser334 by IRAK1 promotes IL-1β-evoked signaling in vascular smooth muscle cells and vascular inflammation. J Biol Chem 2023;299:104911.
29. Wang W, Huang X, Xin HB, et al. TRAF Family Member-associated NF-κB Activator (TANK) Inhibits Genotoxic Nuclear Factor κB Activation by Facilitating Deubiquitinase USP10-dependent Deubiquitination of TRAF6 Ligase. J Biol Chem 2015;290:13372-85.
30. Sun D, Peng Y, Ge S, et al. USP1 Inhibits NF-κB/NLRP3 Induced Pyroptosis through TRAF6 in Osteoblastic MC3T3-E1 Cells. J Musculoskelet Neuronal Interact 2022;22:536-45.
31. Wang Y, Zhou L, Lu J, et al. Ubiquitin-specific protease 4 predicts an unfavorable prognosis and promotes malignant behaviors in vitro in pancreatic cancer. Exp Cell Res 2020;396:112317.
32. Luo XB, Xi JC, Liu Z, et al. Proinflammatory Effects of Ubiquitin-Specific Protease 5 (USP5) in Rheumatoid Arthritis Fibroblast-Like Synoviocytes. Mediators Inflamm 2020;2020:8295149.
33. Zhao XB, Ji FY, Li HR, et al. P22077 inhibits LPS-induced inflammatory response by promoting K48-linked ubiquitination and degradation of TRAF6. Aging (Albany NY) 2020;12:10969-82.
34. Lin D, Zhang M, Zhang MX, et al. Induction of USP25 by viral infection promotes innate antiviral responses by mediating the stabilization of TRAF3 and TRAF6. Proc Natl Acad Sci U S A 2015;112:11324-9.
35. Islam MT, Chen FZ, Chen HC, et al. Knockdown of USP8 inhibits prostate cancer cell growth, proliferation, and metastasis and promotes docetaxel's activity by suppressing the NF-kB signaling pathway. Front Oncol 2022;12:923270.
36. Man X, Piao C, Lin X, et al. USP13 functions as a tumor suppressor by blocking the NF-kB-mediated PTEN downregulation in human bladder cancer. J Exp Clin Cancer Res 2019;38:259.
37. Yang Z, Xian H, Hu J, et al. USP18 negatively regulates NF-κB signaling by targeting TAK1 and NEMO for deubiquitination through distinct mechanisms. Sci Rep 2015;5:12738.
38. Fan YH, Yu Y, Mao RF, et al. USP4 targets TAK1 to downregulate TNFα-induced NF-κB activation. Cell Death Differ 2011;18:1547-60.
39. Liang L, Fan Y, Cheng J, et al. TAK1 ubiquitination regulates doxorubicin-induced NF-κB activation. Cell Signal 2013;25:247-54.
40. Hu B, Ge C, Zhu C. Ubiquitin-specific peptidase 18 negatively regulates and inhibits lipopolysaccharide-induced sepsis by targeting transforming growth factor-β-activated kinase 1 activity. Int Immunol 2021;33:461-8.
41. Lei CQ, Wu X, Zhong X, et al. USP19 Inhibits TNF-α- and IL-1β-Triggered NF-κB Activation by Deubiquitinating TAK1. J Immunol 2019;203:259-68.
42. Mao H, Wang M, Cao B, et al. Interferon-stimulated gene 15 induces cancer cell death by suppressing the NF-κB

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signaling pathway. Oncotarget 2016;7:70143-51.
43. Gong Y, Kong B, Shuai W, et al. USP38 regulates inflammatory cardiac remodeling after myocardial infarction. Clin Sci (Lond) 2023;137:1665-81.
44. Zhou Q, Cheng C, Wei Y, et al. USP15 potentiates NF-κB activation by differentially stabilizing TAB2 and TAB3. FEBS J 2020;287:3165-83.
45. Li T, Guan J, Li S, et al. HSCARG downregulates NF-κB signaling by interacting with USP7 and inhibiting NEMO ubiquitination. Cell Death Dis 2014;5:e1229.
46. Niu J, Shi Y, Xue J, et al. USP10 inhibits genotoxic NF-κB activation by MCPIP1-facilitated deubiquitination of NEMO. EMBO J 2013;32:3206-19.
47. Tang X, Weng R, Guo G, et al. USP10 regulates macrophage inflammation responses via stabilizing NEMO in LPS-induced sepsis. Inflamm Res 2023;72:1621-32.
48. Zhao W, Xin L, Tang L, et al. A positive feedback loop between LINC01605 and NF-κB pathway promotes tumor growth in nasopharyngeal carcinoma. RNA Biol 2022;19:482-95.
49. Sun W, Tan X, Shi Y, et al. USP11 negatively regulates TNFalpha-induced NF-kappaB activation by targeting on IkappaBalpha. Cell Signal 2010;22:386-94.
50. Schweitzer K, Bozko PM, Dubiel W, et al. CSN controls NF-kappaB by deubiquitinylation of IkappaBalpha. EMBO J 2007;26:1532-41.
51. Li Q, Wang M, Xue H, et al. Ubiquitin-Specific Protease 34 Inhibits Osteoclast Differentiation by Regulating NF-κB Signaling. J Bone Miner Res 2020;35:1597-608.
52. Quan J, Zhao X, Xiao Y, et al. USP39 Regulates NF-κB-Mediated Inflammatory Responses through Deubiquitinating K48-Linked IκBα. J Immunol 2023;210:640-52.
53. Gui D, Dong Z, Peng W, et al. Ubiquitin-specific peptidase 53 inhibits the occurrence and development of clear cell renal cell carcinoma through NF-κB pathway inactivation. Cancer Med 2021;10:3674-88.
54. Li M, Zhao J, Jia L. USP14-mediated IκBα degradation exacerbates NF-κB activation and IL-1β-stimulated chondrocyte dedifferentiation. Life Sci 2019;218:147-52.
55. Gong X, Jia L, Zhou L, et al. USP14 predicts poorer survival outcomes and promotes tumor progression in endometrial carcinoma by activating NF-κB signaling. Aging (Albany NY) 2023;15:12120-35.
56. Colleran A, Collins PE, O'Carroll C, et al. Deubiquitination of NF-κB by Ubiquitin-Specific Protease-7 promotes transcription. Proc Natl Acad Sci U S A 2013;110:618-23.
57. Zhou L, Jiang H, Du J, et al. USP15 inhibits multiple myeloma cell apoptosis through activating a feedback loop with the transcription factor NF-κBp65. Exp Mol Med 2018;50:1-12.
58. Schweitzer K, Naumann M. CSN-associated USP48 confers stability to nuclear NF-κB/RelA by trimming K48-linked Ub-chains. Biochim Biophys Acta 2015;1853:453-69.
59. Mirra S, Sánchez-Bellver L, Casale C, et al. Ubiquitin Specific Protease USP48 Destabilizes NF-κB/p65 in Retinal Pigment Epithelium Cells. Int J Mol Sci 2022;23:9682.
60. Pringle LM, Young R, Quick L, et al. Atypical mechanism of NF-κB activation by TRE17/ubiquitin-specific protease 6 (USP6) oncogene and its requirement in tumorigenesis. Oncogene 2012;31:3525-35.
61. Liu C, Wang L, Chen W, et al. USP35 activated by miR let-7a inhibits cell proliferation and NF-κB activation through stabilization of ABIN-2. Oncotarget 2015;6:27891-906.
62. Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci 2021;78:1233-61.
63. Niebuhr M, Schorling K, Heratizadeh A, et al. Staphylococcal α-toxin induces a functional upregulation of TLR-2 on human peripheral blood monocytes. Exp Dermatol 2015;24:381-3.
64. Mcheik S, Al-Akl NS, Abdelnoor AM. The Effect of Denatured Flagellin on Toll-Like Receptor-5 (TLR-5) in Mice. Endocr Metab Immune Disord Drug Targets 2018;18:412-6.
65. Wallach T, Raden M, Hinkelmann L, et al. Distinct SARS-CoV-2 RNA fragments activate Toll-like receptors 7 and 8 and induce cytokine release from human macrophages and microglia. Front Immunol 2022;13:1066456.
66. Jin Y, Zhuang Y, Dong X, et al. Development of CpG oligodeoxynucleotide TLR9 agonists in anti-cancer therapy. Expert Rev Anticancer Ther 2021;21:841-51.
67. Chattopadhyay S, Sen GC. dsRNA-activation of TLR3 and RLR signaling: gene induction-dependent and independent effects. J Interferon Cytokine Res 2014;34:427-36.
68. Fattahi S, Amjadi-Moheb F, Tabaripour R, et al. PI3K/AKT/mTOR signaling in gastric cancer: Epigenetics and beyond. Life Sci 2020;262:118513.

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69. Glaviano A, Foo ASC, Lam HY, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol Cancer 2023;22:138.

70. Verstrepen L, Carpentier I, Verhelst K, et al. ABINs: A20 binding inhibitors of NF-kappa B and apoptosis signaling. Biochem Pharmacol 2009;78:105-14.

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