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REVIEW ARTICLE

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USP3: Key deubiquitylation enzyme in human diseases

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Abstract

Ubiquitination and deubiquitylation are pivotal posttranslational modifications essential for regulating cellular protein homeostasis and are implicated in the development of human diseases. Ubiquitin-specific protease 3 (USP3), a member of the ubiquitin-specific protease family, serves as a key deubiquitylation enzyme, playing a critical role in diverse cellular processes including the DNA damage response, cell cycle regulation, carcinogenesis, tumor cell proliferation, migration, and invasion. Despite notable research efforts, our current understanding of the intricate and context-dependent regulatory networks governing USP3 remains incomplete. This review aims to comprehensively synthesize existing published works on USP3, elucidating its multifaceted roles, functions, and regulatory mechanisms, while offering insights for future investigations. By delving into the complexities of USP3, this review strives to provide a foundation for a more nuanced understanding of its specific roles in various cellular processes. Furthermore, the exploration of USP3's regulatory networks may uncover novel therapeutic strategies targeting this enzyme in diverse human diseases, thereby holding promising clinical implications. Overall, an in-depth comprehension of USP3's functions and regulatory pathways is crucial for advancing our knowledge and developing targeted therapeutic approaches for human diseases.

KEYWORDS
cancer, deubiquitylation, DNA damage, posttranslation modification, USP3

1 | INTRODUCTIONS

Cellular protein homeostasis is intricately regulated, with proteasome-mediated degradation and lysosomal-mediated proteolysis serving as prominent pathways for eliminating abnormal or damaged proteins.[1] Over the past decades, the ubiquitin-proteasome system (UPS) has emerged as a therapeutic target for various human cancers.[2–9] The pivotal role of UPS in cancer has been extensively documented,[10,11] and a growing body of research suggests that targeting the UPS holds promise as an approach to overcome resistance to anticancer

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drugs.[12,13] Deubiquitinating enzymes (DUBs), integral components of the UPS, play a crucial role in cleaving ubiquitin chains from substrate proteins, thereby counteracting ubiquitin-mediated proteasomal degradation. The significance of DUBs, particularly in the context of cancer, has garnered substantial attention, positioning them as potential targets and biomarkers in cancer research.[14]

Ubiquitin-specific protease 3 (USP3) has recently emerged as a key player in diverse cellular processes, including DNA damage response (DDR), cell cycle regulation, epithelial–mesenchymal transition (EMT), tumorigenesis, tumor cell proliferation, migration, and invasion. Its intricate role, particularly in DDR, underscores its potential as both a biomarker and a therapeutic target for chemotherapy and radiotherapy.

This review comprehensively explores the multifaceted role of USP3 in various cellular processes, delving into the molecular mechanisms that underlie its functions and discussing its potential clinical applications in human diseases. With a specific focus on its relevance to human cancers, we highlight the need for further studies to assess the efficacy and safety of targeting USP3 in cancer therapy. We anticipate that ongoing research in this area will continue to unveil exciting prospects in the years to come.

2 | UBIQUITINATION AND DEUBIQUITYNATION

2.1 | Ubiquitination system

Ubiquitination stands as a critical protein posttranslational modification, exerting a determining influence on various cellular processes, including DDR, signal transduction, cell cycle progression, apoptosis, immune response, and tumorigenesis.[2,15,16] This process involves an ATP-dependent cascade of enzymatic reactions, typically and sequentially regulated by three ubiquitin-specific enzymes: E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin ligase. Currently, the human genome is anticipated to harbor two E1 activating enzymes, approximately 50 E2 conjugating enzymes, and more than 600 E3 ligases.[17] The ubiquitination system's diversity and specificity predominantly hinge on the variety of E2 and E3 enzymes.[18]

Polyubiquitin chain formation, contingent upon different lysine residues of ubiquitin linkages, yields distinct physiological roles. For instance, aside from K48-linked chains, K11-linked chains also earmark proteins for proteasomal degradation.[19] K27- and K29-linked chains have been implicated in autophagy and mitophagy, respectively,[20,21] while K33-linked chains serve as crucial regulators in DDR pathways. The versatility of ubiquitination, governed by specific linkages and enzymes, underscores its multifaceted contributions to cellular homeostasis and responses to diverse stimuli.

2.2 | Deubiquitination system

Ubiquitination modification is a reversible process, with DUBs playing a crucial role in reversing this modification by cleaving ubiquitin molecules.[22,23] The orchestrated interplay between ubiquitinating and deubiquitinating enzymes selectively governs cellular protein homeostasis.[16,24] Currently, the human genome encodes approximately 100 DUBs, with a substantial portion of them still not thoroughly elucidated in terms of their functions or characteristics.[2,23,25] Deubiquitinating enzymes exhibit a diverse range of activities, including recycling ubiquitin, reversing ubiquitin conjugations, cleaving polyubiquitin chains, trimming ubiquitin modification forms, and processing ubiquitin precursors or linear fusion products.[2,14,23,26]

The ubiquitin-binding domain within the structure of DUBs is pivotal, comprising three components: the ubiquitin-associated domain (UBA domain), ubiquitin-interacting motif (UIM), and zinc finger domain (ZnF domain).[23,26,27] Deubiquitinating enzymes are characterized by substrate specificity and specificity for different types of ubiquitin chains. Previous studies have indicated that the ZnF domain plays a decisive role in determining the specificity of the target protein for DUBs.[23,26]

Structurally and sequence-wise, DUBs are classified into six families, including ubiquitin-specific proteases (USPs), ovarian-tumor proteases, JAMM/MPN domain-associated metallopeptidases, monocyte chemotactic protein-induced protein, Machado–Joseph disease protein domain proteases, and ubiquitin carboxy-terminal hydrolases.[2,25] The roles of DUBs in various diseases, such as cancer, COVID-19, brain diseases, and central nervous system autoimmunity, have been elucidated in previous studies.[2,14,23,25,28–30] These findings underscore the significance of DUBs in diverse physiological and pathological processes, highlighting their potential as therapeutic targets in various disease contexts.

3 | UBIQUITIN-SPECIFIC PROTEASE 3

Ubiquitin-specific protease 3 stands as a pivotal member within the USP family, distinguishing itself as the second known USP efficiently cleaving a ubiquitin–proline bond. Initially discovered, characterized, and named by Sloper-Mould et al. in 1999[31] the *USP3* gene is a singular entity located at chromosome 15q22.3. Its complete cDNA sequence spans 2.3 kb, featuring an ORF extending from a start codon at 100 bp to a stop codon at 1666 bp. USP3 RNA comprises two transcripts, a 2.45 kb variant and a 5.8 kb variant, both expressed in human tissues in approximately equal proportions.[31] As per data from The Human Protein Atlas, USP3 shows low tissue and cell type specificity. The protein generated by the ORF weighs approximately 59 kDa and encompasses two conserved structural domains, a USP catalytic domain and a ZnF ubiquitin-binding domain (UBP)[32] (Figure 1). Nicassio et al.[32] reported that the USP3 protein, with its ZnF-UBP domain, is localized in the chromatin fraction within the nucleus, mediating interaction with uH2A. Other research indicates that the USP catalytic domain is responsible for interacting with its substrates.[33–35]

In human tissues, the primary function of USP3 revolves around deubiquitylation, preventing the degradation of substrate proteins and maintaining their functional integrity. Over the past decades, several studies have delineated the role of USP3 in various diseases,

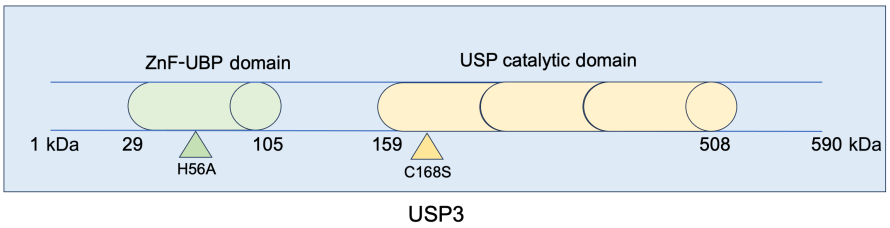

FIGURE 1 Schematic overview of human ubiquitin-specific protease 3 (USP3) protein structure. H56A is the mutant of the zinc finger ubiquitin-binding (ZnF-UBP) domain, and C168S is an enzymatic inactive mutant of the USP catalytic domain. Both domains are required for USP3 interaction with ubiquitin substrates.

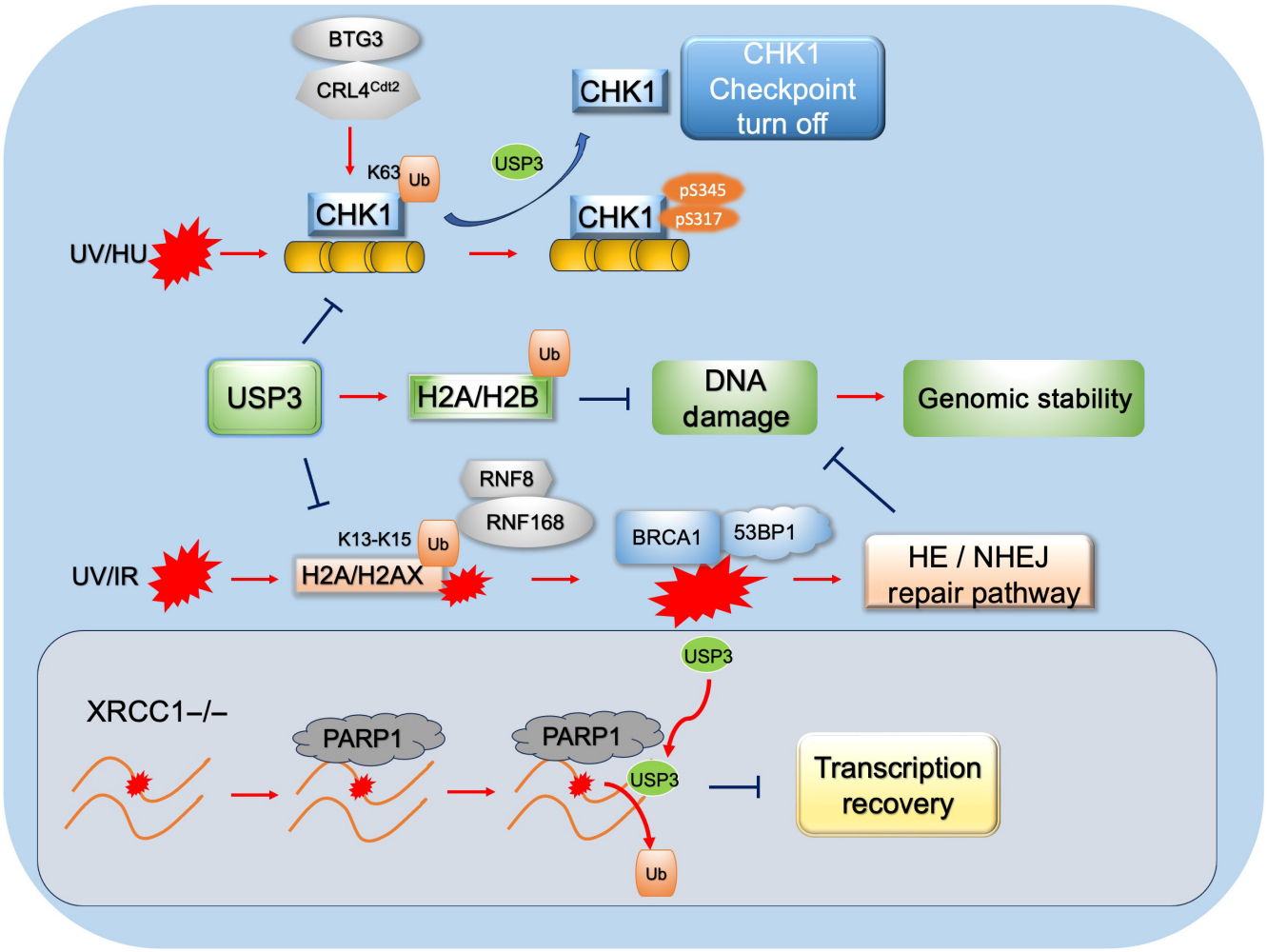

FIGURE 2 Substrates and mechanism of ubiquitin-specific protease 3 (USP3) in DNA damage response. USP3 safeguards the genomic stability and integrity by eliminating H2A/H2B ubiquitination. When the genome is subjected to DNA damage agents, USP3 disrupts the repair of damaged DNA. This occurs either by deactivating the CHK1 checkpoint or impairing the recruitment of DNA damage repair factors BRCA1 and 53BP1, ultimately inducing cell apoptosis. In situations where cells lack XRCC1 and experience impaired base excision repair, there is an abnormal accumulation of USP3 due to low but persistent PARP1 activity. The excess USP3 then deubiquitylates uH2A/H2B at nearby or damaged sites, leading to prolonged transcriptional repression. IR, ionizing radiation.

particularly its involvement in critical biological processes such as DDR, cell cycle progression, and gene transcription regulation. Furthermore, emerging evidence suggests that USP3 contributes to the pathogenesis of diverse diseases, including inflammation, neurodegeneration, and cancers. These observations position USP3 as a promising diagnostic and therapeutic target for a spectrum of pathological conditions.

3.1 | Function of USP3 in DNA damage

Figure 2 provides a summary of the substrates modulated by USP3 in DDR. The involvement of USP3 in DDR was first reported in 2007 by Nicassio et al.[32] According to their findings, USP3 is essential for the complete deubiquitylation of uH2A/H2B and

γ-H2AX through its enzymatic catalytic activity. The downregulation of USP3 significantly elevates uH2A and uH2B protein levels, leading to functional consequences such as replication stress, abnormal DNA replication and/or recombination, delayed S-phase progression, accumulation of DNA breaks, and hyperactivation of ATR/ATM-regulated checkpoint responses. The absence of USP3 results in checkpoint hyperactivation, consequently causing abnormalities in replication and recombination, ultimately leading to genotoxicity.[32]

Additionally, a study has revealed that USP3 interacts with checkpoint kinase 1 (CHK1), counteracting the effects of BTG3-mediated K63-linked polyubiquitination of CHK1.[36] This interaction turns off CHK1 phosphorylation and activation by upstream kinases, facilitating CHK1 release from chromatin to exert its kinase activity on other substrates. Depletion of USP3 leads to prolonged CHK1 checkpoint activation, promoting cell resistance to cell death induced by hydroxyurea (HU), and enhancing genome stability.[36] These investigations shed light on the crucial role of USP3 in DNA damage checkpoints, where USP3 serves as a regulator of checkpoint activation.

Histones, particularly H2A and H2B, are rich in monoubiquitin covalent modifications, playing a crucial role in governing gene expression and chromatin dynamics.[37] Consequently, the deubiquitylation of H2A and H2B has garnered significant attention. Sharma et al. reported that USP3 counters RNF168/RNF8-dependent ubiquitination of H2A and γ-H2AX at the K13–15 site of H2A and γ-H2AX, as well as at the K118–119 sites of γ-H2AX. This counteraction impairs the accumulation of breast cancer susceptibility gene 1 (BRCA1) and 53BP1 at DNA damage sites, suggesting a negative regulatory role of USP3 in DDR in the presence of DNA damage agents. This hypothesis is substantiated by the observed increase in γ-H2AX levels, FK2 foci, and uH2A foci following USP3 knockdown under UV exposure.[38] Conversely, Das et al. report that the upregulation of USP3 stimulates the DDR, evident from a substantial increase in γ-H2AX following USP3 overexpression. Notably, the oncogene Cdc25A, a substrate of USP3, accumulates, leading to DNA replication stress and damage.[39] Adamowicz et al.[40] reported that deletion of XRCC1 impaired base excision repair, leading to aberrant accumulation of USP3 by low but persistent PAP1 activity. This excessive protein deubiquitylates uH2A/H2B at nearby or damage sites, leads to prolonged transcriptional repression. These contrasting results underscore the context-dependent nature of USP3′s role in DDR.

While the aforementioned studies primarily elucidate the significant role of USP3 in vitro, Lancini et al.'s work sheds light on its critical in vivo functions. Their findings reveal that the depletion of USP3 results in an increase in spontaneous chromosomal breaks and mitotic recombination in mouse embryonic fibroblasts (MEFs) compared to WT MEFs. Moreover, the loss of USP3 leads to a preference for hematopoietic stem cells (HSCs) to endure more DNA damage and accumulate spontaneous DNA damage. This work suggests a role for USP3 in preventing chronic genotoxic stress by controlling chromatin ubiquitination. The absence of USP3 may be strongly linked to tumorigenesis and a decline in adult tissue function.[41]

The DDR and repair network not only contribute to carcinogenesis but also play a pivotal role in cancer therapy and resistance. Accumulated evidence suggests that an enhanced DDR system serves as a common underlying mechanism for cancer therapy resistance.[42,43] Moreover, there is a growing notion that a small population of cancer stem cells (CSCs) primarily drives tumor initiation, proliferation, metastasis, and even cancer resistance, leading to cancer relapse and a poor prognosis. Cancer stem cells possess an enhanced efficiency of DDR, resulting in resistance to chemotherapy or radiotherapy.[44]

Tu et al.[33] reported the positive impact of USP3 on glioblastoma (GBM) radiation resistance by activating Claspin-dependent ATR-Chk1 signaling (Figure 2C, left). Claspin plays a crucial role in the activation of the ATR-CHK1 signal, and USP3 attenuates the degradation of Claspin by deubiquitinating the K48-linked polyubiquitination. This process activates the ATR-CHK1 signal pathway, contributing to the survival of GBM cells in response to ionizing radiation (IR)-induced DNA damage. This survival is evidenced by increased phosphorylation of Chk1 and ATR, and decreased γ-H2AX levels after IR. In essence, the presence of USP3 enhances the survival of DNA-damaged GBM cells by promoting DDR. This suggests that USP3 could be a promising therapeutic target to overcome chemotherapy or radiotherapy resistance. Furthermore, exploring the role of USP3 in CSCs warrants further research, considering its potential implications in the context of CSC biology.

3.2 | Dual functions of USP3 in cancer

In addition to its functions in DDR, numerous studies have focused on the role of USP3 in cancer. These investigations reveal that USP3 targets a spectrum of tumor suppressors or promoters, thereby participating in various aspects of tumor progression. By counteracting the effects of these substrates, USP3 is implicated in processes crucial to tumor advancement, including EMT, tumor proliferation, invasion, metastasis, cell apoptosis, and cell cycle regulation. A comprehensive summary of these findings is presented in Figures 3–5 and Table 1.

3.2.1 | Tumor suppressor role of USP3

Lancini et al.[41] generated transgenic USP3-KO mice and observed that the depletion of USP3 did not impact normal embryogenesis or postnatal development. However, it results in a shortened lifespan and a decline in HSC function. Notably, these mice show increased spontaneous tumor occurrence and decreased tumor-free survival compared to WT mice. The authors attribute these phenotypes to chronic genotoxic stress and a decline in tissue function. Another study indicates that USP3 is upregulated during TPA-mediated differentiation of leukemia cells. Through the regulation of H2AK119ub, USP3 promotes leukemia cell differentiation, suggesting its potential therapeutic role in leukemia.[45] The

work of Wang et al. unveiled the crucial role of the USP3 3′-UTR in distal metastasis and prognosis of colorectal cancer (CRC). They reported that by sponging microRNA (miR)-224, the USP3 3′-UTR positively regulates the expression of SMAD4, inhibiting CRC cell metastasis. Loss of USP3 promotes cell migration and invasion in vitro, along with lung metastasis in vivo, highlighting the anti-metastatic capacity of USP3 in CRC.[46]

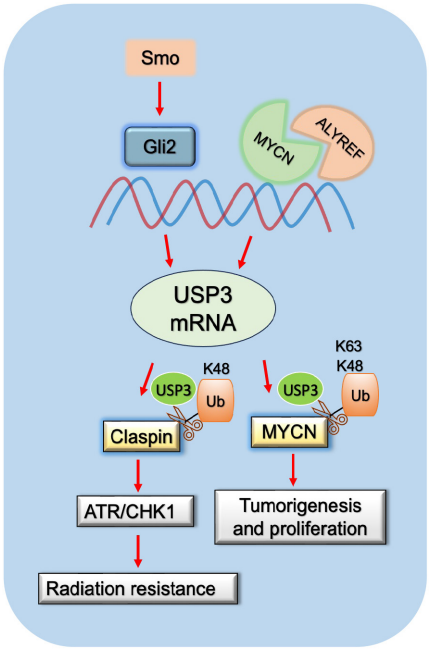

FIGURE 3 Signaling of ubiquitin-specific protease 3 (USP3) is involved in tumor progression. USP3 is positively regulated by Smoothened (Smo) and the ALYREF-MYCN complex at the transcriptional level. This leads to the stabilization of Claspin and MYCN, resulting in radiation resistance in glioblastoma (GBM) and neuroblastoma tumorigenesis, respectively.

Known for its role in maintaining genome integrity, p53 is primarily regulated by the E3 ligase MDM2.[47] Ubiquitin-specific protease 3 functions as a DUB of p53, deubiquitinating K48-linked Ub chains of p53 and thereby enhancing the stability of the p53 protein. Knockdown of USP3 significantly increases cell proliferation and colony formation, and promotes normal fibroblast cell transformation.[48] These findings underscore the intricate role of USP3 in various cellular processes and its potential implications in cancer biology.

3.2.2 | Tumor promoter role of USP3 in gastrointestinal tumors

In recent years, the oncogenic functions of USP3 in cancer have also been reported. Fang et al. detailed the positive role of USP3 in gastric cancer (GC).[49] Their findings indicate that USP3 overexpression accelerates GC cell proliferation by promoting G1–S transition and upregulating cyclins D and E. Additionally, USP3 promotes GC cell migration and invasion by positively regulating the EMT process. These results are validated in a mouse xenograft model. Consistent results have been reported in other studies regarding USP3's role in GC, demonstrating its ability to promote GC cell proliferation, invasion, metastasis[34,50–52] and EMT.[34,50] For example, through interactions with and removal of the ubiquitin chain of SUZ12, a critical component of the Polycomb PRC2-HMTase complex, USP3 stabilizes SUZ12, which is implicated in maintaining stemness.[50] Furthermore, USP3 relieves the K48-linked ubiquitination of COL9A3 and COL6A5, maintaining their stability. As members of the collagen family, COL9A3 and COL6A5 function as the structural framework of the ECM.[34] Additionally, USP3 mediates the deubiquitylation of Snail1, stabilizing its protein levels.[52] Overall, USP3 primarily functions as a tumor promoter in GC, driving GC cell proliferation, invasion, migration, and the EMT process.

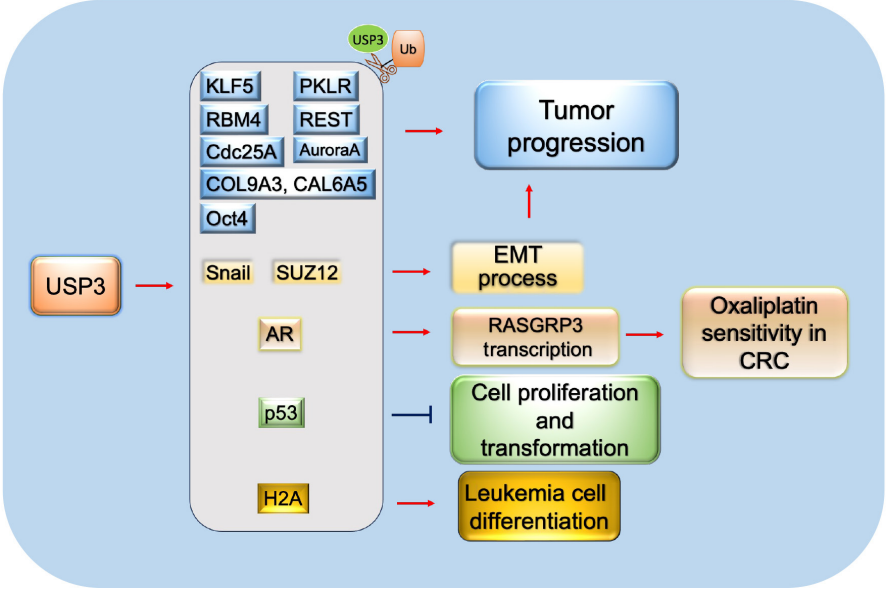

FIGURE 4 Substrates and functions of ubiquitin-specific protease 3 (USP3) in human cancers. USP3 serves as a deubiquitinase (DUB) for various substrates in human cancers, contributing to tumor progression, the advancement of epithelial–mesenchymal transition (EMT), increased sensitivity to oxaliplatin in colorectal cancer (CRC), as well as enhanced cell proliferation and transformation.

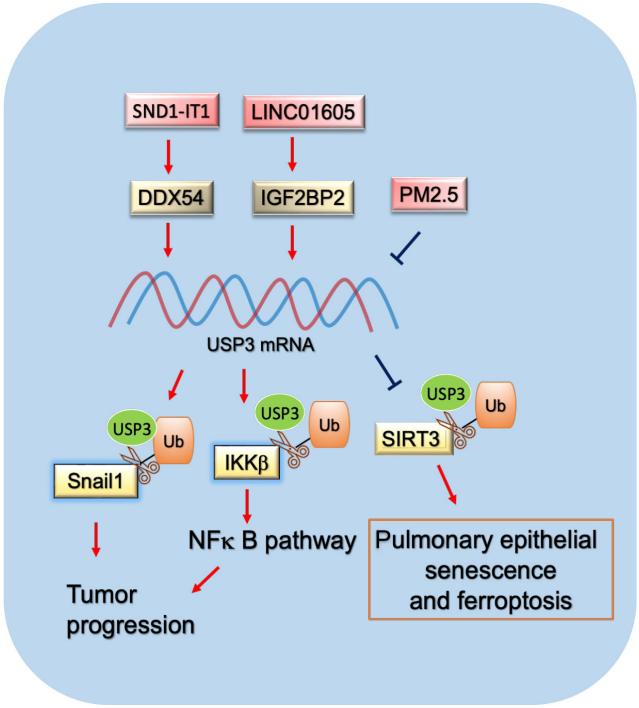

FIGURE 5 Regulation of ubiquitin-specific protease 3 (USP3) at the mRNA level involves various cellular processes. The long noncoding RNA (lncRNA) SND1 intronic transcript 1 (SND1-IT1) is present in exosomes secreted by gastric cancer (GC) cells. This lncRNA exhibits the capability to recruit DDX54, thereby stabilizing the mRNA level of USP3. This stabilization is implicated in Snail1 deubiquitination and contributes to tumor progression. Additionally, LINC01605 recruits IGF2BP2 to stabilize USP3 mRNA levels, resulting in the activation of the nuclear factor-κB (NF-κB) pathway. The exposure to PM2.5 inhibits USP3 mRNA expression, reduces sirtuin 3 (SIRT3) expression, and triggers pulmonary inflammation.

Ubiquitin-specific protease 3 has also been implicated in esophageal squamous cell carcinoma (ESCC). Through the deubiquitination of the K143 residue of Aurora A, a serine/threonine kinase associated with centrosomes and mitosis, USP3 promotes invasion and metastasis in ESCC cells.[53] In gallbladder cancer, Liang et al. reported that USP3 deubiquitinates and stabilizes PKLR, a critical regulator of glycolysis. Consequently, this stabilization promotes cell proliferation, glycolysis, and tumor growth in vivo.[54] Notably, this marks the first instance of USP3 being implicated in tumor metabolism, although further investigation is warranted to fully understand the role of USP3 in this context.

3.2.3 | Tumor promoter role of USP3 in neural tumors

Fan et al. reported that USP3 promotes the EMT program in glioblastoma (GBM) by stabilizing Snail protein expression through its DUB activity, thereby facilitating GBM tumor growth, migration, and invasion. Depletion of USP3 significantly represses mouse xenograft tumor growth.[55] Another study revealed a similar role for USP3 in promoting EMT and progression in GBM, but in the context of long noncoding RNA (lncRNA). The authors identified lncRNA HOXA-AS3, upregulated in GBM and associated with poor survival rates, cell proliferation, and invasion. They further discovered that lncRNA HOXA-AS3 executes its oncogenic function mainly through upregulation of USP3 expression by competitively sponging with miR-455-5p.[56] Ubiquitin-specific protease 3 has also been reported to promote GBM radiation resistance through the deubiquitylation of Claspin.[33]

Neuroblastoma, a common malignant pediatric tumor, is also influenced by USP3. Nagy et al. reported that USP3 promotes neuroblastoma cell viability, proliferation, and xenograft tumor growth by cleaving K48- and K63-linked ubiquitin chains of MYCN (Figure 2C, right). Tumor-bearing mice with downregulation of USP3 showed longer median survival.[57] The repressor element-1 silencing transcription factor (REST), a master transcriptional repressor, plays a significant role in neuroblastoma tumors, influencing neural stem cell self-renewal, differentiation, and neurogenesis.[58–61] Karapurkar et al. reported that USP3 deubiquitinates and stabilizes REST. Depletion of USP3 impedes neuroblastoma cell clone formation, self-renewal capability, and tumor growth.[58] These studies unveil the significant role of USP3 in neural tumors, from occurrence to radiation resistance, identifying the potential therapeutic target of USP3 in neural tumors.

3.2.4 | Tumor promoter role of USP3 in breast cancer and cervical cancer

Krüppel-like factor 5 (KLF5) is a critical transcription factor highly expressed in basal subtype breast cancers, with previous studies establishing its role as an oncogene that promotes breast cancer cell proliferation, migration, invasion, and stemness.[62–65] Wu et al. report that USP3 acts as an oncogene in breast cancer in a KLF5-dependent manner. Specifically, USP3 decreases the polyubiquitination levels of the KLF5 protein, maintaining its stability and thereby increasing the proliferation of triple-negative breast cancer (TNBC) cells in vitro, as well as xenograft tumor growth.[66]

Cdc25A, a key regulator of cell cycle G_1/S and G_2/M progression by dephosphorylating activated CDKs, is identified as a substrate of USP3. By reducing the ubiquitination levels of Cdc25A, increased Cdc25A protein facilitates cell cycle progression. The knockout of USP3 decreases HeLa cell clone formation, cell viability, invasion, and migration, as well as decreasing tumor growth in vivo.[39] These findings underscore the multifaceted role of USP3 in cancer, influencing key regulatory pathways involved in cell cycle progression and transcription factor stability.

3.2.5 | Tumor promoter role of USP3 in lung cancer and nasopharyngeal carcinoma

The oncogenic role of USP3 has also been reported in non-small-cell lung cancer (NSCLC). RNA binding motif 4 (RBM4), responsible

TABLE 1 Major mechanisms and functions of ubiquitin-specific protease 3 (USP3) acting in diversity tumorigenesis and progression.

Cancer	Role	Mechanisms and functions
HSC	Tumor suppressor	USP3 maintain HSC homeostasis. Its depleted inducing DDR defective, chromosomal unintegrity, spontaneous DNA damage and mutations, which caused various tumorigenesis[41]
CRC	Tumor suppressor	USP3 3′-UTR positively regulates SMAD4 expression at transcription level in a miR-224-dependent manner, which formed a symmetrical ceRNA network in CRC cells. USP3 knockdown promoting CRC tumor progression and invasion in vitro, and lung metastasis in vivo[46]
Leukemia	Tumor suppressor	USP3 increases TPA-induced leukemia cell differentiation by regulating H2AK119ub[45]
GC	Oncogene	USP3 accelerates GC proliferation, invasion, and migration in vitro and in vivo by positively regulating cyclins D and E, MMP2, and EMT,[49] deubiquitinating SUZ12[50] and COL9A3/COL6A5[34] hsa_circ_0017639 positively regulates USP3 through sponged miR-224-5p to execute promoting GC cell proliferation and migration function[51] Through absorbed miR-1245b-5p, exosome-mediated lncRNA SND1-IT1 promoted USP3 transcription, the later deubiquitinated SNAIL1 to increase GES-1 cell malignant transformation[52]
GBM	Oncogene	USP3 can increase GBM growth, invasion, and migration possibly by promoting EMT by stable the Snail expression through deubiquitinating activity, the latter is a core transcription factor that triggers the EMT program[55] USP3 transcription can promote by Smo through Gli2, thereby increasing Claspin deubiquitinating, activating the Claspin-dependent ATR-CHK1 signal, leading to radiation resistance by promoting radiation-induced DDR and significantly lessening cell apoptosis[33] LncRNA HOXA-AS3 sponged miR-455-5p to upregulate USP3 expression to promote GBM progression and EMT[56]
Breast cancer	Oncogene	USP3 deubiquitinates KLF5 to accelerate the breast cancer cell proliferation, growth, and tumorigenesis[66]
NSCLC	Oncogene	USP3 negative regulates RBM4 to stimulate NSCLC cell proliferation[67]
HeLa cells	Oncogene	Through deubiquitinating oncogene Cdc25A, USP3 facilitates Cdc25A-mediated cell cycle transition, reducing cell apoptosis. USP3 KO impedes HeLa cell colony formation, significantly reduces cell viability, invasion, and migration, and suppresses xenograft tumor growth[39]
Neuroblastoma	Oncogene	MYCN and ALYREF formed a transcriptional coactivator complex that can promote USP3 transcription; the later, in turn, as a deubiquitinating enzyme of oncogene MYCN to increase the stability of MYCN protein at levels driving neuroblastoma tumorigenesis and proliferation[57]
Neuroblastoma	Oncogene	USP3 positive regulates neuroblastoma cell self-renewal, proliferation, tumorigenesis, tumor differentiation, and growth by counteracting transcriptional repressor REST ubiquitination[58]
ESCC	Oncogene	Through deubiquitylation of Aurora-A, USP3 promotes ESCC proliferation and metastasis[53]
GBC	Oncogene	USP3 stabilizes tumor driver gene *PKLR*, thus promotes GBC cells expansion in vitro and xenograft tumor growth in vivo[54]
NPC	Oncogene	USP3 indirective activates nuclear factor-κB signaling by negative regulating IKKβ protein proteasome degradation, thus promotes NPC tumor growth[68]

Abbreviations: ceRNA, competing endogenous RNA; CRC, colorectal cancer; DDR, DNA damage response; EMT, epithelial–mesenchymal transition; ESCC, esophageal squamous cell carcinoma; GBC, gallbladder cancer; GBM, glioblastoma; GC, gastric cancer; HSC, hematopoietic stem cell; KLF5, Krüppel-like factor 5; miR, microRNA; NPC, nasopharyngeal carcinoma; NSCLC, non-small-cell lung cancer; RBM4, RNA binding motif 4.

for gene splicing, is negatively regulated by USP3 at both the protein and mRNA levels. Elevated USP3 levels contribute to increased NSCLC cell proliferation.[67]

In nasopharyngeal carcinoma (NPC), Zhao et al. reported that USP3 is positively regulated by LINC01605 at the transcriptional level. In turn, USP3 mediates IKKβ protein deubiquitylation and enhances protein stability, promoting NPC development by activating the nuclear factor-κB (NF-κB) pathway. This activation is evidenced by strengthened cell viability and proliferation, reduced cell apoptosis, and increased tumor volume in vivo. Silencing USP3 significantly reduces IKKβ protein levels.[68] These findings shed light on the diverse mechanisms through which USP3 contributes to the oncogenic processes in different cancer types.

3.2.6 | Role of USP3 in cancer therapy resistance

Cancer therapy resistance has become a focal point of research due to its urgency in clinical settings. While Cdc25A deficiency has been reported to confer resistance to ATR inhibitors in mammalian cells,[69] interestingly, USP3 KO HeLa cells show ATR inhibitor resistance at high doses, indicating a unique role for USP3 in this context. The reconstruction of WT USP3, but not the catalytically inactive USP3CS, resensitizes cells to ATR inhibitors.[39]

In a related context, Zheng et al. investigated the role of USP3 in oxaliplatin resistance in CRC.[70] They observed that the ectopic expression of lncRNA AC092894.1 is associated with increased oxaliplatin sensitivity. Further experiments indicated that

AC092894.1 can recruit USP3 to promote the deubiquitylation of androgen receptor (AR). The elevated levels of AR then facilitated the transcription of RASGRP3, ultimately enhancing the sensitivity of HCT116 resistance cells to oxaliplatin both in vitro and in vivo.[70] These two studies shed light on additional mechanisms by which USP3 contributes to cancer therapy resistance beyond the previously mentioned DNA damage mechanism.[33] This suggests that understanding the function of USP3 in overcoming anticancer therapy resistance warrants further attention in future research.

3.3 | Multifaceted roles of USP3 beyond cancer

Beyond its role in cancer, the other biological functions of USP3 have captured researchers' attention. Rhie et al.'s work showed the USP3 as a necessary regulator for maintaining the pluripotency of human embryonic stem cells (Figure 6). Through its DUB activity, USP3 stabilizes the key transcription factor Octamer-binding factor 4 protein (Oct4).[71] Additionally, Lancini et al. reported that USP3 is required for preserving HSC repopulation potential and self-renewal.[41] These studies underscore the indispensable function of USP3 in stem cell maintenance and self-renewal, offering valuable insights into the potential role of USP3 in CSC.

Innate immunity and inflammasome homeostasis constitute a robust defense against various diseases, and the involvement of USP3 is depicted in Figure 6. The reported findings suggest a dual role for USP3 in virus infection. While it promotes infection through the inhibition of the type I interferon signaling pathway,[35,72] Zhao et al. presented contrasting evidence, indicating that USP3 inhibits HIV replication by enhancing the host defensive factor AG3.[73] Additionally, Zhuang et al. proposed that USP3 enhances ASC stability by eliminating the K-48 linked ubiquitin chain. Notably, USP3 overexpression is linked to increased activation of NLRP3, AIM2, and NLRC4, leading to elevated interleukin (IL)-1β secretion in three mouse models.[74]

The research undertaken by Zhou et al. highlights USP3's role in preventing tumor necrosis factor receptor-associated factor 6 (TRAF6)-induced NF-kB activation, thereby safeguarding chondrocytes from IL-1-induced apoptosis and senescence through TRAF6 deubiquitination (Figure 6).[75,76] Jia et al.'s[77] findings suggest that USP3 overexpression alleviates elevated IL-6 levels induced by miR-146b-5p mimics in primary chondrocyte cells. Furthermore, Li et al. reported that decreased USP3 mRNA levels induced by particulate matter 2.5 (PM2.5) exacerbate inflammatory injury in pulmonary epithelial cells through increased sirtuin 3 (SIRT3) ubiquitination[78] (Figure 5). These studies collectively underscore the significant role of USP3 in both virus infection and inflammation.

In summary, while the primary focus has been on revealing USP3's role in human cancers, notable research underscores its involvement in conferring resistance to anticancer therapies. Consequently, USP3 emerges as a potential therapeutic target for anticancer interventions. Investigations into its participation in other biological processes hint at a potential regulatory link between USP3 and the tumor immune microenvironment, indicating a promising avenue for future research.

3.4 | Regulation of USP3

Understanding the upstream regulators of USP3 is crucial for devising targeted interventions. Tu et al. identified Smoothened (Smo), a component of the Hedgehog pathway, as an upstream regulator of USP3 in GBM radiation resistance (Figure 3, left). Smoothened positively regulates USP3 gene transcription by facilitating Gli2 binding to the USP3 promoter region, specifically at positions −1355 to −1344. This enhancement leads to increased deubiquitylation of

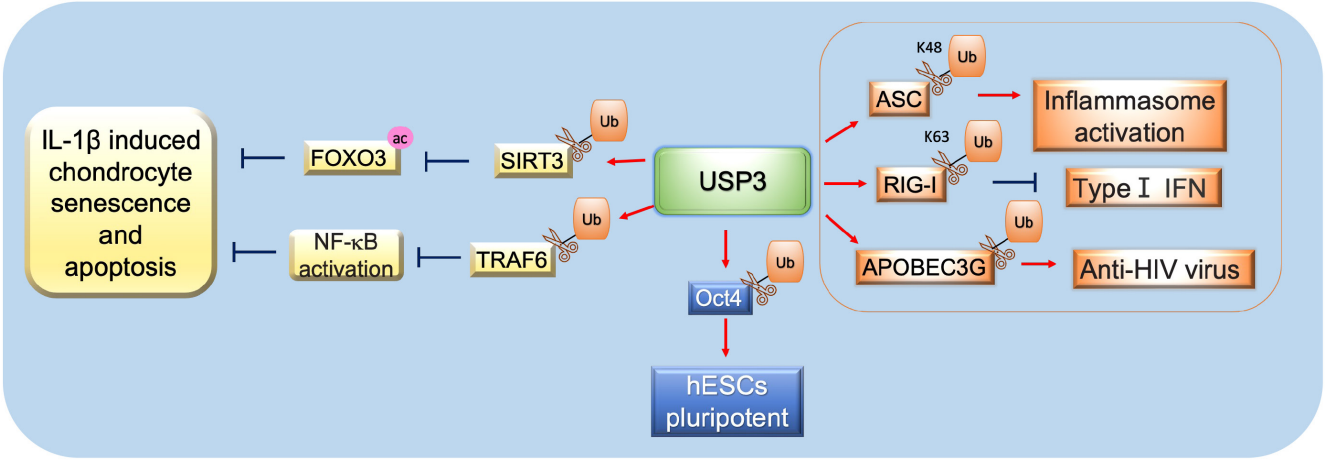

FIGURE 6 Substrates and functions of ubiquitin-specific protease 3 (USP3) extend to various cellular process of human disease. By deubiquitinating different substrates, USP3 plays crucial roles in innate immunity, inflammasome activation, and multipotential stem cells. hESC, human embryonic stem cell; IFN, interferon; IL-1β, interleukin-1β; NF-κB, nuclear factor-κB; SIRT3, sirtuin 3; TRAF6, tumor necrosis factor receptor-associated factor 6.

Claspin protein and activation of the ATR/CHK1 signaling pathway, contributing to GBM radiation resistance.[33] Another study revealed that ALYREF, a nuclear chaperone protein, forms a transcription activator complex with MYCN, promoting USP3 transcription (Figure 3, right). Elevated USP3 levels further increase MYCN protein levels, contributing to neuroblastoma tumorigenesis.[57] Additionally, Wu et al. reported that transforming growth factor-β induces USP3 expression in gastric cancer cells in a dose- and time-dependent manner, although the specific mechanism remains undisclosed, leading to the promotion of EMT.[50]

Moreover, USP3 has been identified as a downstream target of various noncoding RNAs. Chen et al. reported that lncRNA HOXA-AS3 upregulates USP3 expression by sequestering miR-445-5p, which negatively regulates USP3 by binding to its 3′-UTR, ultimately promoting the EMT process in GBM cells.[56] Similarly, Li et al. show that hsa_circ_0017639 positively regulates USP3 expression by acting as a sponge against miR-224-5p, an upstream negative regulator of USP3.[51] During influenza A virus infection, miR-26a directly targets and negatively regulates USP3.[72] Zhang et al. revealed that miR-146-5p overexpression reduces the mRNA and protein levels of USP3 in a human pulmonary artery endothelial cell line under hypoxic conditions.[79] In osteoarthritis, Jia et al. found that miR-146b-5p directly targets the 3′-UTR of USP3, leading to decreased protein levels.[77] Jin et al.[52] found that the mRNA level of USP3 is significantly upregulated by lncRNA SND1-IT1 overexpression and decreased by miR-1245b-5p overexpression. They further discovered that USP3 mRNA stability is promoted by SND1-IT1 through recruitment of RNA binding protein DDX54.[52] In NPC, USP3 mRNA stability is stabilized by RNA binding protein IGF2BP2, which is recruited by LINC01605, activating the NF-κB signaling pathway and promoting NPC development.[68] Li et al.[78] report that PM2.5 can inhibit USP3 expression in mRNA and protein levels, resulting in pulmonary inflammation.

In summary, lncRNAs and circular RNAs predominantly act as positive regulators of USP3 expression, whereas miRNAs typically serve as negative regulators. Notably, the Smo-Gli-USP3 axis in GBM radiation resistance suggests that targeting Smo or USP3 might be a viable strategy to overcome resistance in this context.

4 | ANIMAL MODELS OF USP3

The use of animal models with USP3 deficiency is crucial for comprehending the molecular mechanisms of USP3 in various diseases. Lancini et al.[41] generated a *Usp3* gene knockout mouse model to investigate the impact of dynamic ubiquitination removal on genome maintenance in vivo. Deubiquitylation by USP3 is deemed critical for preserving genome integrity and stability. The depletion of the *Usp3* gene in mice resulted in several phenomena, including: (1) a significantly shortened lifespan compared to WT littermates, and (2) a predisposition to spontaneous tumorigenesis due to a general decline in tissue function and the progressive accumulation of mutations caused by spontaneous DNA damage. These findings underscore the critical role of USP3 in chromatin ubiquitination, genomic stress response, and tissue function.

In addition to the transgenic mouse model deficient in the *Usp3* gene, other animal models have been used to study the role of USP3 in various diseases. For instance, Wang et al.[46] generated a metastasis mouse model by intravenously injecting USP3 knockdown LoVo cells. The results indicated that USP3 knockdown led to more lung metastases than the control group, highlighting the metastatic suppressive capacity of USP3 in CRC.

Similarly, a xenograft mouse model was established by subcutaneously injecting cells with USP3 knockdown in GC research. Tumors in the USP3 knockdown group showed slow growth, decreased mitotic activity, and lower expression of Ki-67 compared with the control group.[49] In contrast, injection of cells either overexpressing[49,50] or knocking down[34] USP3 into the tail vein resulted in USP3 promoting lung metastasis.

Furthermore, an intracranial xenograft tumor model of GBM was constructed by injecting luciferase-encoding cells depleted of USP3.[55] Mice bearing USP3-depleted tumors showed lower tumor formation and invasive activity, contributing to better survival rates. Additionally, Wu et al. constructed a USP3 knockdown xenograft tumor model of breast cancer, demonstrating that a decrease in USP3 significantly inhibited tumor growth compared with the control group.[66] Das et al. generated a xenograft model of cervical cancer by subcutaneously transplanting HeLa cells with USP3 KO or mock control. The results showed that tumor growth is significantly suppressed by USP3 KO compared to the mock control group.[39] Liang et al. established gallbladder cancer (GBC) cell xenograft tumors by subcutaneously injecting GBC-SD cells overexpressing USP3. The results indicated that tumor volume and size significantly increased with USP3 overexpression, while they decreased with USP3 knockdown.[54] Karapurkar et al. created a neuroblastoma xenograft tumor by subcutaneously injecting USP3 KO cells and USP3 KO cells re-overexpressing USP3 or REST. The results indicated that compared to the mock control, USP3 KO significantly decreased tumor volume and weight, while this effect could be reversed by USP3 or REST re-expression.[58]

5 | CLINICAL APPLICATION OF USP3

Several studies have suggested that USP3 holds promise as a prognostic indicator for various tumors, as summarized in Table 2. The downregulation of USP3 expression has been associated with poor prognosis in several cancers, including CRC, GC, breast cancer, lung cancer,[45] and urothelial cancer.[44] In CRC tissues, USP3 expression is lower compared to matched normal tissues. Reduced USP3 levels indicate distal metastasis, advanced tumor stage, and shorter overall survival (OS) and disease-free survival (DFS) rates for CRC patients.[46]

However, like two sides of a coin, USP3 has also been identified as a poor prognostic biomarker for GC in several studies.[34,49,50] These studies report higher expression of USP3 in GC tissues compared to adjacent normal gastric tissues. Patients with higher USP3

TABLE 2 Prediction of ubiquitin-specific protease 3 (USP3) in clinical prognosis of different cancers.

Cancer	USP3 level	Clinical prognosis	Reference
Colorectal cancer	Low mRNA levels	Distant metastasis. Shortened OS and DFS. Advanced tumor stages	46
Urothelial cancer	Low levels	Low survival rate	45
Gastric cancer	High levels	Worse clinicopathologic characteristics. Inferior OS and DFS	49
Gastric cancer	High levels	Gastric cancer cell differentiation. Advanced AJCC stage and lymph node metastasis. Poor survival	50
Glioblastoma	High levels	Worse OS and DFS. Worse prognosis	55
Breast cancer	High mRNA levels	Shorten relapse-free survival and distant metastasis-free survival	66
Lung cancer (NSCLC)	High levels	Worse prognosis. Advanced pathological stages	67
Breast cancer Acute myeloid leukemia Pancreatic adenocarcinoma	High levels	Poorer survival	39
Glioblastoma multiforme	High levels	Better survival	39
Neuroblastoma	High mRNA levels	Poorer prognosis	57,58

Abbreviations: AJCC, American Joint Committee on Cancer; DFS, disease-free survival; NSCLC, non-small-cell lung cancer; OS, overall survival.

expression levels show worse clinicopathologic characteristics, DFS, and OS.[34,49,50] In GBM, USP3 is overexpressed in tumor tissues and cell lines. Aberrant expression of USP3 predicts worse DFS and OS, indicating its potential as a prognostic biomarker for GBM patients.[55] Ubiquitin-specific protease 3 was also reported as a prognostic biomarker in neuroblastoma, where higher USP3 mRNA levels indicate poor OS and vice versa.[58] Wu et al.[66] reported that USP3 is highly expressed in clinical TNBC samples by immunohistochemical staining, and high expression of USP3 is positively correlated with shorter distance metastasis-free survival and DFS based on The Cancer Genome Atlas database analysis. Das et al. found that USP3 levels correlated with worse survival in breast cancer and proposed USP3 as a potential cancer biomarker for predicting prognosis.[39] Another study reported that USP3 could serve as a prognostic bio-indicator of NSCLC. They observed significantly higher expression of USP3 in NSCLC tissues than paracarcinoma tissues, which is associated with worse prognosis and pathological stages of NSCLC patients.[67]

In addition to its prognostic value, the critical role of USP3 in various cancers and DDR suggests its potential as a therapeutic target in cancer treatment. Ubiquitin-specific protease 3 plays a critical role in promoting cancer growth, invasion, and metastasis, making it an attractive target for developing novel anticancer therapies. Further research is needed to better understand the underlying molecular mechanisms of USP3 in cancer development and progression, providing a solid foundation for developing targeted therapies against USP3.

Moreover, due to its crucial role in DDR, USP3 inhibitors have gained attention as promising sensitizers for enhancing the efficacy of radiation and chemotherapy drugs used in cancer treatment. Ubiquitin-specific protease 3 inhibitors might also synergize with PARP inhibitors, a class of drugs that specifically target cancer cells with defective DNA repair mechanisms, such as those carrying *BRCA* mutations. This suggests that USP3 inhibitors could be a valuable addition to current cancer treatment strategies, offering new opportunities for improving patient outcomes. However, no USP3 inhibitor has been reported to date.

6 | PERSPECTIVES

As a member of the USP DUB family, the biological functions of USP3 have not been fully elucidated. Through its ubiquitin molecular cleavage function, USP3 regulates various substrates involved in tumorigenesis, development, and DDR to maintain genomic integrity.

6.1 | Controversial functions of USP3

However, the detailed underlying mechanisms of USP3 remain ambiguous. For instance, the research conducted by Lancini et al.[41] found that *Usp3* gene knockout mice showed a higher incidence of

spontaneous cancer compared to WT mice (32.3% vs. 3.8%). They also observed adult tissue function decline, spontaneous chromosomal breaks, and susceptibility to DNA damage in USP3 KO mice. Although the works of Nicassio et al.,[32] Sharma et al.,[38] and Yu-Che et al.[36] uncovered some mechanisms underlying how USP3 affects the DNA damage response, further investigation is needed to fully understand the functions and mechanisms of USP3 in maintaining genomic integrity. It is crucial to differentiate the role of USP3 in physiological conditions, where it contributes to genomic integrity and stability, from its role in the presence of DNA damage agents, where it affects DDR.

6.2 | Role of USP3 in oncobiology

The roles of USP3 in oncobiology seem to depend on the function of its substrates, which include both tumor suppressors and oncogenes. The specific characteristics of substrates binding to USP3 remain unknown. Lancini et al.'s study[41] underscores the significant role of USP3 in DDR and tumorigenesis, suggesting the potential of targeting USP3 in cancer therapy. Notably, USP3 has been implicated in radiotherapy resistance in GBM[33] and increased sensitivity to oxaliplatin in CRC cells,[70] yet no USP3 inhibitor has been reported.

6.3 | Ubiquitin-specific protease 3 as a therapeutic target in cancers

Despite the critical role of DUBs in maintaining cellular protein stability, only those DUBs deubiquitinating oncoproteins are considered as potential targets for small molecule inhibitors. Collaborating proteolysis-targeting chimeras (PROTACs) with DUBs could be a promising solution, replacing E3 ligase recruiting ligand with DUB recruiting ligand. This approach forms a trimeric complex, stabilizing and elevating the targeting tumor suppressor protein levels selectively and specifically, thereby enhancing tumor suppressor function.

Moreover, we propose the druggability of USP3 as a therapy target. While most reported substrates of USP3 are oncoproteins, the ambiguous function of USP3 in both cancers and DNA damage prompts us to consider what strategy would be appropriate to target USP3. We suggest a competitive peptide against USP3 binding with a specific substrate as a potential strategy. This small competitive peptide could occupy the binding site of substrates, preventing USP3 degradation or deubiquitination of the target substrates. This approach could significantly decrease the toxicities associated with classical pure DUB inhibitors, overcoming off-target effects and drug resistance.

6.4 | Transgenic USP3 animal models

Considering the complex functions of USP3 reported, creating sophisticated USP3 genetically engineered animal models, such as USP3 KO mice or organ-specific conditional knockouts, would be a better approach to establishing the role of USP3 in cancers and DDR. These models can contribute to unraveling the complex biology of USP3 in human diseases and shed light on its full potential in cancer diagnosis, treatment, and management.

AUTHOR CONTRIBUTIONS

Ceshi Chen: Funding acquisition; supervision; writing – review and editing. **Hongyan Zhang:** Writing – original draft. **Wenjing Liu:** Writing – review and editing. **Yingying Wu:** Supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Ceshi Chen is an editorial board member of *Cancer Science*. The other authors declare no conflict of interests.

ETHICS STATEMENT

Approval of the research protocol by an institutional review board: N/A.

Informed consent: N/A.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

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REFERENCES

1. Liu J, Shaik S, Dai X, et al. Targeting the ubiquitin pathway for cancer treatment. *Biochim Biophys Acta*. 2015;1855:50-60.

2. Hanpude P, Bhattacharya S, Dey AK, Maiti TK. Deubiquitinating enzymes in cellular signaling and disease regulation. *IUBMB Life*. 2015;67:544-555.
3. Ciechanover A. Intracellular protein degradation: from a vague idea through the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting. *Bioorg Med Chem*. 2013;21:3400-3410.
4. Çetin G, Klafack S, Studencka-Turski M, Krüger E, Ebstein F. The ubiquitin-proteasome system in immune cells. *Biomol Ther*. 2021;11:11.
5. Bhat SA, Vasi Z, Adhikari R, et al. Ubiquitin proteasome system in immune regulation and therapeutics. *Curr Opin Pharmacol*. 2022;67:102310.
6. Aliabadi F, Sohrabi B, Mostafavi E, Pazoki-Toroudi H, Webster TJ. Ubiquitin-proteasome system and the role of its inhibitors in cancer therapy. *Open Biol*. 2021;11:200390.
7. Park J, Cho J, Song EJ. Ubiquitin-proteasome system (UPS) as a target for anticancer treatment. *Arch Pharm Res*. 2020;43:1144-1161.
8. Lu X, Xu H, Xu J, et al. The regulatory roles of the E3 ubiquitin ligase NEDD4 family in DNA damage response. *Front Physiol*. 2022;13:968927.
9. Csizmadia T, Lőw P. The role of deubiquitinating enzymes in the various forms of autophagy. *Int J Mol Sci*. 2020;21:4196.
10. Han D, Wang L, Jiang S, Yang Q. The ubiquitin-proteasome system in breast cancer. *Trends Mol Med*. 2023;29:599-621.
11. Voutsadakis IA, Papandreou CN. The ubiquitin-proteasome system in prostate cancer and its transition to castration resistance. *Urol Oncol*. 2012;30:752-761.
12. Okubo K, Isono M, Asano T, et al. Ubiquitin-proteasome system is a promising target for killing cisplatin-resistant bladder cancer cells. *Anticancer Res*. 2021;41:2901-2912.
13. Narayanan S, Cai CY, Assaraf YG, et al. Targeting the ubiquitin-proteasome pathway to overcome anti-cancer drug resistance. *Drug Resist Updat*. 2020;48:100663.
14. Poondla N, Chandrasekaran AP, Kim KS, Ramakrishna S. Deubiquitinating enzymes as cancer biomarkers: new therapeutic opportunities? *BMB Rep*. 2019;52:181-189.
15. Nandi D, Tahiliani P, Kumar A, Chandu D. The ubiquitin-proteasome system. *J Biosci*. 2006;31:137-155.
16. Welchman RL, Gordon C, Mayer RJ. Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nat Rev Mol Cell Biol*. 2005;6:599-609.
17. Ye Y, Rape M. Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol*. 2009;10:755-764.
18. Metzger MB, Hristova VA, Weissman AM. HECT and RING finger families of E3 ubiquitin ligases at a glance. *J Cell Sci*. 2012;125:531-537.
19. Leznicki P, Kulathu Y. Mechanisms of regulation and diversification of deubiquitylating enzyme function. *J Cell Sci*. 2017;130:1997-2006.
20. Piccirillo R, Goldberg AL. The p97/VCP ATPase is critical in muscle atrophy and the accelerated degradation of muscle proteins. *EMBO J*. 2012;31:3334-3350.
21. Zhang CF, Gruber F, Ni C, et al. Suppression of autophagy dysregulates the antioxidant response and causes premature senescence of melanocytes. *J Invest Dermatol*. 2015;135:1348-1357.
22. Vucic D, Dixit VM, Wertz IE. Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death. *Nat Rev Mol Cell Biol*. 2011;12:439-452.
23. Tanguturi P, Kim KS, Ramakrishna S. The role of deubiquitinating enzymes in cancer drug resistance. *Cancer Chemother Pharmacol*. 2020;85:627-639.
24. Wilkinson KD. Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. *Semin Cell Dev Biol*. 2000;11:141-148.
25. Fraile JM, Quesada V, Rodriguez D, Freije JM, Lopez-Otin C. Deubiquitinases in cancer: new functions and therapeutic options. *Oncogene*. 2012;31:2373-2388.
26. Komander D, Clague MJ, Urbé S. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol*. 2009;10:550-563.
27. Dikic I, Wakatsuki S, Walters KJ. Ubiquitin-binding domains – from structures to functions. *Nat Rev Mol Cell Biol*. 2009;10:659-671.
28. Clemente V, D'Arcy P, Bazzaro M. Deubiquitinating enzymes in coronaviruses and possible therapeutic opportunities for COVID-19. *Int J Mol Sci*. 2020;21:3492-3508.
29. Ruan J, Schlüter D, Wang X. Deubiquitinating enzymes (DUBs): DoUBle-edged swords in CNS autoimmunity. *J Neuroinflammation*. 2020;17:102.
30. Lim KH, Joo JY, Baek KH. The potential roles of deubiquitinating enzymes in brain diseases. *Ageing Res Rev*. 2020;61:101088.
31. Sloper-Mould KE, Eyre HJ, Wang XW, Sutherland GR, Baker RT. Characterization and chromosomal localization of USP3, a novel human ubiquitin-specific protease. *J Biol Chem*. 1999;274:26878-26884.
32. Nicassio F, Corrado N, Vissers JH, et al. Human USP3 is a chromatin modifier required for S phase progression and genome stability. *Curr Biol*. 2007;17:1972-1977.
33. Tu Y, Chen Z, Zhao P, et al. Smoothened promotes glioblastoma radiation resistance via activating USP3-mediated claspin Deubiquitination. *Clin Cancer Res*. 2020;26:1749-1762.
34. Wu X, Wang H, Zhu D, et al. USP3 promotes gastric cancer progression and metastasis by deubiquitination-dependent COL9A3/COL6A5 stabilisation. *Cell Death Dis*. 2021;13:10.
35. Cui J, Song Y, Li Y, et al. USP3 inhibits type I interferon signaling by deubiquitinating RIG-I-like receptors. *Cell Res*. 2014;24:400-416.
36. Cheng YC, Shieh SY. Deubiquitinating enzyme USP3 controls CHK1 chromatin association and activation. *Proc Natl Acad Sci USA*. 2018;115:5546-5551.
37. Zhang Y. Transcriptional regulation by histone ubiquitination and deubiquitination. *Genes Dev*. 2003;17:2733-2740.
38. Sharma N, Zhu Q, Wani G, He J, Wang QE, Wani AA. USP3 counteracts RNF168 via deubiquitinating H2A and γH2AX at lysine 13 and 15. *Cell Cycle*. 2014;13:106-114.
39. Das S, Chandrasekaran AP, Suresh B, et al. Genome-scale screening of deubiquitinase subfamily identifies USP3 as a stabilizer of Cdc25A regulating cell cycle in cancer. *Cell Death Differ*. 2020;27:3004-3020.
40. Adamowicz M, Hailstone R, Demin AA, et al. XRCC1 protects transcription from toxic PARP1 activity during DNA base excision repair. *Nat Cell Biol*. 2021;23:1287-1298.
41. Lancini C, van den Berk PC, Vissers JH, et al. Tight regulation of ubiquitin-mediated DNA damage response by USP3 preserves the functional integrity of hematopoietic stem cells. *J Exp Med*. 2014;211:1759-1777.
42. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer*. 2008;8:193-204.
43. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature*. 2009;461:1071-1078.
44. Zhao J. Cancer stem cells and chemoresistance: the smartest survives the raid. *Pharmacol Ther*. 2016;160:145-158.
45. Chae YC, Jung H, Kim JY, Lee DH, Seo SB. Ubiquitin-specific peptidase 3 induces TPA-mediated leukemia cell differentiation via regulating H2AK119ub. *Anim Cells Syst (Seoul)*. 2019;23:311-317.
46. Wang Z, Yang J, Di J, et al. Downregulated USP3 mRNA functions as a competitive endogenous RNA of SMAD4 by sponging miR-224 and promotes metastasis in colorectal cancer. *Sci Rep*. 2017;7:4281.
47. Kruse JP, Gu W. Modes of p53 regulation. *Cell*. 2009;137:609-622.
48. Fu S, Shao S, Wang L, et al. USP3 stabilizes p53 protein through its deubiquitinase activity. *Biochem Biophys Res Commun*. 2017;492:178-183.

49. Fang CL, Lin CC, Chen HK, et al. Ubiquitin-specific protease 3 overexpression promotes gastric carcinogenesis and is predictive of poor patient prognosis. *Cancer Sci*. 2018;109:3438-3449.
50. Wu X, Liu M, Zhu H, et al. Ubiquitin-specific protease 3 promotes cell migration and invasion by interacting with and deubiquitinating SUZ12 in gastric cancer. *J Exp Clin Cancer Res*. 2019;38:277.
51. Li B, Jin M, Cao F, et al. Hsa_circ_0017639 expression promotes gastric cancer proliferation and metastasis by sponging miR-224-5p and upregulating USP3. *Gene*. 2020;750:144753.
52. Jin G, Zhang J, Cao T, Chen B, Tian Y, Shi Y. Exosome-mediated lncRNA SND1-IT1 from gastric cancer cells enhances malignant transformation of gastric mucosa cells via up-regulating SNAIL1. *J Transl Med*. 2022;20:284.
53. Shi K, Zhang JZ, Yang L, et al. Protein deubiquitylase USP3 stabilizes Aurora a to promote proliferation and metastasis of esophageal squamous cell carcinoma. *BMC Cancer*. 2021;21:1196.
54. Liang RP, Zhang XX, Zhao J, et al. Ubiquitin-specific protease 3 facilitates cell proliferation by deubiquitinating pyruvate kinase L/R in gallbladder cancer. *Lab Investig*. 2022;102:1367-1376.
55. Fan L, Chen Z, Wu X, et al. Ubiquitin-specific protease 3 promotes glioblastoma cell invasion and epithelial-mesenchymal transition via stabilizing snail. *Mol Cancer Res*. 2019;17:1975-1984.
56. Chen W, Li Q, Zhang G, Wang H, Zhu Z, Chen L. LncRNA HOXA-AS3 promotes the malignancy of glioblastoma through regulating miR-455-5p/USP3 axis. *J Cell Mol Med*. 2020;24:11755-11767.
57. Nagy Z, Seneviratne JA, Kanikevich M, et al. An ALYREF-MYCN coactivator complex drives neuroblastoma tumorigenesis through effects on USP3 and MYCN stability. *Nat Commun*. 2021;12:1881.
58. Karapurkar JK, Kim MS, Colaco JC, et al. CRISPR/Cas9-based genome-wide screening of the deubiquitinase subfamily identifies USP3 as a protein stabilizer of REST blocking neuronal differentiation and promotes neuroblastoma tumorigenesis. *J Exp Clin Cancer Res*. 2023;42:121.
59. Gao Z, Ure K, Ding P, et al. The master negative regulator REST/NRSF controls adult neurogenesis by restraining the neurogenic program in quiescent stem cells. *J Neurosci*. 2011;31:9772-9786.
60. Lunyak VV, Rosenfeld MG. No rest for REST: REST/NRSF regulation of neurogenesis. *Cell*. 2005;121:499-501.
61. Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S. REST maintains self-renewal and pluripotency of embryonic stem cells. *Nature*. 2008;453:223-227.
62. Qin J, Zhou Z, Chen W, et al. BAP1 promotes breast cancer cell proliferation and metastasis by deubiquitinating KLF5. *Nat Commun*. 2015;6:8471.
63. Jia L, Zhou Z, Liang H, et al. KLF5 promotes breast cancer proliferation, migration and invasion in part by upregulating the transcription of TNFAIP2. *Oncogene*. 2016;35:2040-2051.
64. Liu R, Shi P, Nie Z, et al. Mifepristone suppresses basal triple-negative breast cancer stem cells by Down-regulating KLF5 expression. *Theranostics*. 2016;6:533-544.
65. Chen CH, Yang N, Zhang Y, et al. Inhibition of super enhancer downregulates the expression of KLF5 in basal-like breast cancers. *Int J Biol Sci*. 2019;15:1733-1742.
66. Wu Y, Qin J, Li F, et al. USP3 promotes breast cancer cell proliferation by deubiquitinating KLF5. *J Biol Chem*. 2019;294:17837-17847.
67. Liao XH, Wang Y, Zhong B, Zhu SY. USP3 promotes proliferation of non-small cell lung cancer through regulating RBM4. *Eur Rev Med Pharmacol Sci*. 2020;24:3143-3151.
68. Zhao W, Xin L, Tang L, Li Y, Li X, Liu R. A positive feedback loop between LINC01605 and NF-κB pathway promotes tumor growth in nasopharyngeal carcinoma. *RNA Biol*. 2022;19:482-495.
69. Ruiz S, Mayor-Ruiz C, Lafarga V, et al. A genome-wide CRISPR screen identifies CDC25A as a determinant of sensitivity to ATR inhibitors. *Mol Cell*. 2016;62:307-313.
70. Zheng Z, Wu M, Li H, et al. Downregulation of AC092894.1 promotes oxaliplatin resistance in colorectal cancer via the USP3/AR/RASGRP3 axis. *BMC Med*. 2023;21:132.
71. Rhie BH, Antao AM, Karapurkar JK, et al. Ubiquitin-specific protease 3 deubiquitinates and stabilizes Oct4 protein in human embryonic stem cells. *Int J Mol Sci*. 2021;22:5584-5599.
72. Gao S, Li J, Song L, Wu J, Huang W. Influenza a virus-induced downregulation of miR-26a contributes to reduced IFNα/β production. *Virol Sin*. 2017;32:261-270.
73. Zhao S, Zheng B, Wang L, et al. Deubiquitinase ubiquitin-specific protease 3 (USP3) inhibits HIV-1 replication via promoting APOBEC3G (A3G) expression in both enzyme activity-dependent and -independent manners. *Chin Med J*. 2022;135:2706-2717.
74. Zhuang W, Zhang L, Zheng Y, et al. USP3 deubiquitinates and stabilizes the adapter protein ASC to regulate inflammasome activation. *Cell Mol Immunol*. 2022;19:1141-1152.
75. 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;514:482-489.
76. Zhou Q, Wang W, Wu J, et al. Ubiquitin-specific protease 3 attenuates interleukin-1β-mediated chondrocyte senescence by deacetylating forkhead box O-3 via sirtuin-3. *Bioengineered*. 2022;13:2017-2027.
77. Jia H, Duan L, Yu P, Zhou Y, Liu R, Wang H. Digoxin ameliorates joint inflammatory microenvironment by downregulating synovial macrophage M1-like-polarization and its-derived exosomal miR-146b-5p/Usp3&Sox5 axis. *Int Immunopharmacol*. 2022;111:109135.
78. Li N, Xiong R, Li G, Wang B, Geng Q. PM2.5 contributed to pulmonary epithelial senescence and ferroptosis by regulating USP3-SIRT3-P53 axis. *Free Radic Biol Med*. 2023;205:291-304.
79. Zhang W, Qi Y, Wu B. MicroRNA-146-5p promotes pulmonary artery endothelial cell proliferation under hypoxic conditions through regulating USP3. *Dis Markers*. 2021;2021:3668422.

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