Emerging Roles of Non-proteolytic Ubiquitination in Tumorigenesis

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Ubiquitination is a critical type of protein post-translational modification playing an essential role in many cellular processes. To date, more than eight types of ubiquitination exist, all of which are involved in distinct cellular processes based on their structural differences. Studies have indicated that activation of the ubiquitination pathway is tightly connected with inflammation-related diseases as well as cancer, especially in the non-proteolytic canonical pathway, highlighting the vital roles of ubiquitination in metabolic programming. Studies relating degradable ubiquitination through lys48 or lys11-linked pathways to cellular signaling have been well-characterized. However, emerging evidence shows that non-degradable ubiquitination (linked to lys6, lys27, lys29, lys33, lys63, and Met1) remains to be defined. In this review, we summarize the non-proteolytic ubiquitination involved in tumorigenesis and related signaling pathways, with the aim of providing a reference for future exploration of ubiquitination and the potential targets for cancer therapies.

Keywords: ubiquitin, atypical ubiquitination, ubiquitin E2 conjugating enzyme, ubiquitin E3 ligase, tumorigenesis, ubiquitin-proteasome system

1 INTRODUCTION

1.1 The Ubiquitin-Proteasome System

Ubiquitination, also known as ubiquitylation, refers to the process by which ubiquitin (Ub, a small and highly conserved protein), with the help of a series of special enzymes, classifies proteins in cells, selects target proteins, and modifies those proteins (McDowell and Philpott, 2016; Seeler and Dejean, 2017; Rape, 2018). Ubiquitination plays fundamental roles in many cellular events such as cell proliferation (Kwon and Ciechanover, 2017; Werner et al., 2017; Senft et al., 2018; Song and Luo, 2019), cell cycle (Teixeira and Reed, 2013; Darling et al., 2017; Gilberto and Peter, 2017), DNA repair (Alpi and Patel, 2009; Abbas and Dutta, 2011), immune response (Heaton et al., 2016; Rudnicka and Yamauchi, 2016; Manthiram et al., 2017), transcription (Zhou et al., 2017; Imam et al., 2019; Sun et al., 2019), angiogenesis (Zhang et al., 2022), metastasis (Rossi and Rossi, 2022), and apoptosis (Xu et al., 2017; Zhou et al., 2017).

Protein ubiquitination requires three different enzymes: E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and E3 ubiquitin ligases (Heap et al., 2017; O’Connor and Huijbregts, 2017). E1 enzymes activate the ubiquitin polypeptide in an ATP-dependent manner, and the activated forms are then conjugated to E2 enzymes through the formation of thioester bonds (Wang et al., 2018). Finally, E3 ubiquitin ligases recognize both E2 enzymes and specific target substrates that confer specificity to the system, and then the Ub can be transferred from E2
enzymes to the target substrates to complete the ubiquitination process (Rittinger and Ikeda, 2017; Yau et al., 2017). The coordination of E1, E2, and E3 enzymes earmarks target proteins with a wide variety of ubiquitin modifications such that distinct ubiquitin modifications transmit different cellular signals (Khaminets et al., 2016; Venuto and Merla, 2019). Specific ubiquitin-binding domains (UBDs) can identify ubiquitylated substrate proteins (Kristariyanto et al., 2015; Kung et al., 2019; Polykratis et al., 2019; Spiliotopoulos et al., 2019). UBDs utilize diverse mechanisms to interact with various surface patches on ubiquitin molecules or different ubiquitin linkages (Hicke et al., 2005; Dikic et al., 2009; Komander and Rape, 2012). The hydrophobic surfaces of ubiquitin molecules, such as isoleucine 36 (Ile36) and isoleucine 44 (Ile44), are the structural basis for the recognition of ubiquitination signals. Different ubiquitin chains have various spatial structures and thus expose different hydrophobic surfaces, which can be recognized by specific UBDs. Proteins containing UBDs recognize and transmit the functional signals represented by ubiquitin chains (Buetow and Huang, 2016). Similar to many other protein post-translational modifications (PTMs), ubiquitination can be preserved through cleavage by deubiquitylating enzymes (DUBs) (Herhaus and Sapkota, 2014; Nishi et al., 2014; Clague et al., 2019) (Figure 1).

The versatility of ubiquitination is determined by the complex assembly pattern of ubiquitin molecules on the target protein. Ubiquitins can be attached to one or multiple lysine residues with either a single ubiquitin molecule (mono- and multi-mono-ubiquitination, respectively) or ubiquitin polymers (poly-ubiquitination) (Eger et al., 2010; Akutsu et al., 2016; Chitale and Richly, 2017; van der Heden van Noort et al., 2017). Poly-ubiquitin chains comprising only one single linkage are often assumed to be homotypic (Jeusset and McManus, 2017; Leestemaker and Ovaa, 2017), whereas heterotypic chains adopt multiple linkages within the same polymer (branched or non-branched) (Akturk et al., 2018). In a poly-ubiquitin chain, ubiquitin moieties can be linked through any of the seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) or N-terminal methionine (Met1) (Dittmar and Winklhofer, 2019; Spit et al., 2019; Liao et al., 2022), resulting in an almost unlimited number of poly-ubiquitin chain topologies (Varadan et al., 2004; Sadowski and Sarcevic, 2010; Dondelinger et al., 2016; O’Connor and Huibregtse, 2017; Padala et al., 2017). Further complexity is added to ubiquitination when the ubiquitin polypeptide is modified by phosphorylation (Dong et al., 2017) or acetylation (Choudhary et al., 2009; Ohtake et al., 2015). Given the sophisticated assembly of protein ubiquitination, it has been often referred to as the “ubiquitin code” (Komander and Rape, 2012). Proteomics studies have shown that Lys48-linked chains
are predominant in cells (>50% of all linkages), and Lys63-linked chains rank the second abundant chain form, however, researchers have begun to characterize the remain chain types, which were considered to be “atypical” ubiquitin modifications (linked through Lys6, Lys11, Lys27, Lys29, Lys33 and Met1) (Xu et al., 2009; Dammer et al., 2011; Kim et al., 2011; Wagner et al., 2011; Ziv et al., 2011).

1.2 Structural Features of Poly-Ubiquitin Chains
Poly-ubiquitin chains occur when a single ubiquitin molecule is repeatedly connected in series with another ubiquitin lysine residue. Substrate proteins can be distinguished by poly-ubiquitin chains by attaching between different types of deubiquitination formations, single mono-ubiquitination events, multiple mono-ubiquitinations events, homotypic ubiquitination events and heterotypic ubiquitination events (branched and non-branched ubiquitination) (Sokratos et al., 2014; Yau and Rape, 2016; Zhao et al., 2017). This also led to the formation of eight different homotypic chains. The key distinguishing feature of how this can be achieved is the specific combination of E2/E3 enzymes, thereby triggering distinct cellular fates of substrate proteins. However, some of the reported E2/E3 enzyme combinations were not 100% specific for targeted linkage. It has been reported that two E1 enzymes were selected for ubiquitin in humans: UBA1 and UBA6 (Barghout and Schimmer, 2021). Humans also encoded 40 E2 conjugation enzymes cooperate with approximately 600 E3 ligase enzymes (Hodson et al., 2014). The E3s were categorized into three groups: RING/U-box, HECT, and RING between RING (RBR) (Wang et al., 2020).

With the assistance of E1, E2, and E3, mono-ubiquitination occurs when a single ubiquitin is attached to its target proteins, then Ub molecules are added to the model in linear ways one by one (Torres et al., 2009; Tang et al., 2011). The sequential addition model of Ub on the substrate contributes to the elongation of the Ub lines. When secondary Ub molecules are connected to specific lysine residues, they are called homotypic chains. If any of the attached adjacent Ub molecules are linked to each other by different lysine residues (mixed or branched model), a heterotropic structure is formed. For homotypic chains, reports have found that different chain types are closely related to the confirmation of the structure, either “compact” or “open”. Generally, non-proteolytic ubiquitination Lys63 linkages and Met1 linkages, adopt “open” ones. Contrast to the aforementioned linkages, internal structural molecules interact with each other among degradable linkages, like Lys6, Lys11 and Lys48 linkages, and those linkages display “open” conformation. Furthermore, all ubiquitin moieties can be modified by acetylation or phosphorylation to add additional layers of complexity (Kane et al., 2014; Kazlauskaite et al., 2014; Koyano et al., 2014; Ordureau et al., 2014; Ohtake et al., 2015; Swaney et al., 2015) (Figure 1).

Numerous studies have found that ubiquitin acetylation inhibits poly-ubiquitination elongation, and phospho-ubiquitin leads to mitophagy. Any PTM chain can be changed to ubiquitin chains, which may prevent or facilitate ubiquitin interactions. Protein phosphorylation is linked to ubiquitination for proteasomal degradation. Reports have shown that the phosphorylation of ULK1 by MAPK1/3 kinase interacts with BTRC, which leads to subsequent proteasomal degradation and attenuates breast cancer bone metastasis (Deng et al., 2020). However, the stability of some proteins is also regulated by phosphorylation. Reports have also shown that Aurora B-mediated phosphorylation of ubiquitin specific protease 13 (USP13) at Serine 114 promoted the stability of Aurora B.

1.3 Encoding and Decoding the Ubiquitin Code
Encoding and decoding the ubiquitin code is performed by factors that recognize Ub chains and connect the substrate proteins to the downstream response (Ji and Kwon, 2017; Kwon and Ciechanover, 2017). Recognition of chains occurs through discrete domains and affinity binders with specificity for a particular Ub substrate and chain type (Fu et al., 2012; Suryadinata et al., 2014; Kniss et al., 2018; Michel et al., 2018). This complex system consists of the conjugation of diverse mono, multi-mono and polymeric chains (Rösner et al., 2015; Ji and Kwon, 2017). The interpretation of how, when, and why the ubiquitin codes are written, read, and erased emerged to be characterized. Ubiquitination is a powerful decoration process of proteins and is typically actualized by “ubiquitinase” (Zientara-Rytter and Subramani, 2019). Ubiquitin can be successfully linked to one of the seven lysine residues, all of which can be characterized as poly-ubiquitin chains (Laplantine et al., 2009; Regev et al., 2015; Bax et al., 2019). Poly-ubiquitin chains with different topologies depended upon the lysine residues (which were chosen to be attached) and the substantial chain length, determine the lucky chance of the target proteins and regulate diverse cellular processes, known as “ubiquitin code” (Dittmar and Selbach, 2017; Chatr-Aryamontri et al., 2018; Fottner et al., 2019; O’Donnell, 2019; Song and Luo, 2019). Recent discoveries have deepened our understanding of the whole picture of the ubiquitin code, the interplay between “writers” (E1/E2/E3s), “erasers” (DUBs) and “readers” (ubiquitin binding domain containing proteins) (Tanno and Komada, 2013; Heride et al., 2014; Rogerson et al., 2015; Di Lello and Hymowitz, 2016; Smeenk and Mailand, 2016). Studies have highlighted that mono-ubiquitination can be catalyzed by different E2 and E3 enzymes, acting either individually or together to determine specific substrates. Notably, the linkage specificity of E3s containing RING or U-box domains is likely dictated by E2. As for the HECT E3s class of enzymes, HECT domain swaps can activate the acceptor lysine and are sufficient to determine the linkage specificity. RBR E3s, however, are somewhat complex. RBR E3s display linkage specificity in multiple chains, Met1-, Lys63-, Lys48-, and Lys27-linked chains, as well as mono-ubiquitination, while cooperating with E2 to synthesize Lys-linked or Met1-linked chains. The Ub tag attached to a certain substrate, which is preferably achieved through the cooperation of specific E2/E3 enzyme pairs, represents a complex yet specific message encoded by the cell (Kim and Huibregtse, 2009; David
et al., 2009). To date, 55 ubiquitin speci-
downstream signaling components and/or subcellular
receptors for the transfer of the targeted substrate toward
speci-
Mevissen and Komander, 2017). These
In addition, ubiquitin-interacting proteins which served as
decoders of the ubiquitin message, participated in the
downstream regulation of the ubiquitinated substrate
(Heideker and Wertz, 2015; Leznicki and Kulathu, 2017;
Mevissen and Komander, 2017). These “decoders” may
specifically reverse the ubiquitination process or function as
receptors for the transfer of the targeted substrate toward
downstream signaling components and/or subcellular
compartments (Hu et al., 2002; Lin et al., 2008; Komander
et al., 2009). To date, 55 ubiquitin specific proteases (USPs),
14 ovarian tumor DUBs (OTUs), 10 JAMM family DUBs, 4
ubiquitin C-terminal hydrolases (UCHs) and 4 Josephin domain
DUBs have been identified. Encoding and decoding ubiquitin
codes are responsible for all levels of epigenetic changes, and by
changing substrate protein activities, they can also activate and
repress effects on gene transcription depending on their target
proteins and the ubiquitin chain types, all of which are
connectively related to the process of tumor proliferation (Kim
and Baek, 2006; Zheng et al., 2008; Fradet-Turcotte et al., 2013;
Yeh et al., 2018). Ubiquitin code signaling is frequently
dysregulated in numerous cancer types and can function as a
tumor suppressor or tumor promoter, suggesting a potential
target for cancer therapy (Loch and Strickler, 2012; Choudhry
et al., 2018; Emanuelli et al., 2019).

1.4 Physiological Functions of
Non-proteolytic Poly-Ubiquitin Chains
The simplest version of ubiquitination or mono-ubiquitination
confers non-degradative activities including protein localization
(Yang et al., 2017), endocytosis (Shih et al., 2002; Windheim et al.,
2008), trafficking, DNA repair (Xie et al., 2014; Whiteaker et al.,
2018), autophagy (Chen et al., 2017; Zheng et al., 2020; Leng
et al., 2021) and chromatin remodeling (Cole et al., 2021). When
mono-ubiquitination is further modified, multiple lysine residues
of the substrate are yielded to induce multi-mono-ubiquitination.
Emerging investigations have found that this process connects
ubiquitin with subcellular trafficking (Yin et al., 2010; Cooray
et al., 2011) and immune-suppressive functions (Zhu et al., 2018).
As for poly-ubiquitination, Lys48-linked poly-ubiquitination
leads to the degradation of substrates (Komander and Rape,
2012). In contrast, Lys63-linked poly-ubiquitination exerts
critical signaling functions in regulating protein stability,
including nuclear factor \(\kappa B\) (NF-\(\kappa B\)) signaling (Syed et al.,
2006; Wu et al., 2014; Gallo et al., 2014), endocytosis
(Galan and Haguaener-Tsipis, 1997), DNA damage
responses, and immune responses (Wu and Karin, 2015;
Hrdinka et al., 2016; Liu et al., 2017; Paul and Wang, 2017).
Emerging experiments have shown that incorrect regulation of
cellular processes (either tumour inhibitors or promoters)
contributes to cancer pathogenesis and progression ( Shirane
et al., 1999). Additionally, the cellular functions of atypical
ubiquitin linkages (except Lys11-linked ubiquitin) are
supposed to be non-degradable (Kulathu and Komander, 2012;
Iwai, 2014, 2015; Meza Gutierrez et al., 2018) and in most cases,
activities involved in Lys63-linked poly-ubiquitin chains are also
considered to be non-degradable (Liu et al., 2015).

Of note, other chains also regulate specific physiological functions: Lys6-linked poly-ubiquitin was supposed to be
indirectly linked to DNA damage response with the help of
heterodimeric ubiquitin E3 ligase BRCA1–BARD1 (Wu-Baer
et al., 2003; Wu-Baer et al., 2010). Linear (M1) chains can
regulate NF-kB activation (Behrends and Harper, 2011; Chen
et al., 2015; Borghi et al., 2018), whereas Lys11 linkage acts as a
powerful degradation signal in heterotypic ubiquitin conjugates
(Locke et al., 2014; Mevissen et al., 2016) and lys27 linkage can
prompt mitochondrial depolarization and mediate translocation
of the E3 ligase Parkin, which accumulates Lys27-linked
linkages on mitochondrial protein voltage-dependent anion-selective
channel protein1 (VDAC1). This exact Lys27-linked
translocation leads to Parkinson’s disease in the presence of
Parkin (Geisler et al., 2010; Glauser et al., 2011). Lys27 linkage
has also been demonstrated to be related with the DNA damage
response and innate immune response (Xue et al., 2018). The
propagation of Wnt/\(\beta\)-catenin signaling through Lys29-or Lys11-
linked ubiquitin chains is closely associated with cancer
pathogenesis and is involved in protein ubiquitination at
multiple levels (Hay-Koren et al., 2011). Several studies have
reported the functions of both Lys29- and Lys33-linked chains in
the regulation of AMPK-related protein kinases (Al-Hakim et al.,
2008), and the Lys33 linkage is negatively regulated by T-cell
antigen receptors (TCRs), which indirectly affect cellular
activities in tumors (Huang et al., 2010). In this review, we
will focus on recent progresses in non-degradable ubiquitin
chains in tumorigenesis and the essential activities involved in
non-proteolytic ubiquitination.

2 THE ROLES OF NON-PROTEOLYTIC
UBIQUITINATION IN TUMORIGENESIS
Non-proteolytic ubiquitination, including both mono-
ubiquitination and poly-ubiquitination (mainly K6-, K27-
K29-, K33-, K63-, and M1-linked poly-ubiquitination), has
become key regulators in a variety of cancers. How they
function as signaling entities in the pathogenesis and
progression of cancer will be beneficial to gain an in-depth
understanding of ubiquitination (Figure 2).

2.1 Mono-Ubiquitination
Mono-ubiquitination has been suggested to be even more
dynamic than previously thought, and its functions have been
deciphered by various ubiquitin-binding proteins. It has been demonstrated that the cellular functionality of ubiquitin is mediated by mono-ubiquitin and/or poly-ubiquitin. In fact, mono-ubiquitination is associated with tumorigenesis, not limited to membrane transportation, endocytosis, receptor internalization, degradation in lysosomes, and protein reprocessing (Haglund et al., 2003). BMI1 interacts with histone H2A through mono-ubiquitination, repressing multiple genes, such as INK4A/ARF, which function in the pRb and p53 pathways, thereby facilitating cancer progression (Lin et al., 2015). Interestingly, in the presence of UbE2E1, PRC1 catalyzes the mono-ubiquitination of H2A, contributing to cancer cell proliferation (Wheaton et al., 2017). Mono-ubiquitination is also involved in the process of USP22 regulating histone H2B and exhibits both oncogenic and tumor-suppressor roles in cancer development (Jeusset and McManus, 2017). In addition, reversible mono-ubiquitination activity plays an essential role in balancing TGF-β/SMAD signaling, which is involved in cancer initiation and progression (Xie et al., 2014). Mono-ubiquitination also regulates forkhead box O (FOXO) proteins, which control specific gene expression programs that are vital for slowing the onset of cancer in aging individuals (Greer and Brunet, 2008). Strikingly, FANCL cooperates with, UBE2T and catalyzes mono-ubiquitination, which participated in the regulation of Fanconi Anemia pathway, leading to chromosome instability and promoting tumorigenesis (Machida et al., 2006; Hodson et al., 2014; Miles et al., 2015; Sun et al., 2020; Wang S. et al., 2021) (Table 1, 2).

2.2 Linear (M1) Linkage

2.2.1 The LUBAC Complex Encodes the M1 Linkage

Emerging evidences connect Met1-linked ubiquitin chains to NF-κB signaling, which enables physiological regulation of inflammation and immune responses (Emmerich et al., 2011; Borghi et al., 2018). Indeed, mutations and deficiencies involved in the formation and dissolution of Met1-linked poly-ubiquitin chains have been extensively illustrated in immune-related disorders (Tokunaga and Iwai, 2012; Fiil et al., 2013; Fiil and Gyrd-Hansen, 2014). Until now, linear ubiquitin chain assembly complex (LUBAC) is the only known E3 ubiquitin ligase assembling this type of chain (Kirisako et al., 2006; Walczak et al., 2012). The multi-subunit E3 ligase comprises catalytically active hybrid organic-inorganic perovskite (HOIP, also known as RNF31) and two adaptor proteins, HOIP1L (also known as RBCK1) and SHANK-associated RH domain-interacting protein (SHARPIN) (Tokunaga et al., 2011). Both HoIL-1 and SHARPIN can co-operate with HOIP, which might be a central part of the LUBAC complex (Ikeda et al., 2011; Elton et al., 2015). The HOIP orthologue, linear ubiquitin E3 ligase (LUBEL), modifies Kenny with M1-linked linear ubiquitin chains in Drosophila and is...
| E2     | Alias | Accompanied E3 | Linkage | Phenotypic Characteristics | Neoplastic Implications | Substrate | Mechanism Summary |
|--------|-------|----------------|---------|-----------------------------|-------------------------|-----------|-------------------|
| UBE2N  | UBC13 | TRAF2/TRAF6?   | K63     | preventing tumor formation and metastasis | modulating breast cancer metastasis | NEMO?     | UBV1A, together with Ubc13, promote breast cancer metastasis through Lys63-linked polyubiquitination of target proteins and NF-κB-mediated MMP1 expression (Wu et al., 2014b) |
| UBE2N  | UBC13 | TRAF6          | K63     | DNA damage repair and protein kinase activation | metastatic spread and lung colonization by breast cancer cells | p38       | Ubc13 catalyzes K63-linked proteins, accompanied by TAK1-p38 activation, whose activity is essential for breast cancer metastasis (Wu et al., 2014a) |
| Ubc13; UBE2N; UBE2V1; Uev2 | RNF8 | K63           | DNA damage repair and cytokinesis | genomic instability in adult T-cell leukemia (ATL) | TAK1?IKK? | Ubc13/Uev1A and RNF8 interact with each other and generate K63-pUb, which is recognized by Tax, stimulating TAK1 and IKK activation (Ho et al., 2015) |
| Ubc13  | UBE2N | TRAF6          | K63     | activating NF-κB signaling | elicit anti-tumour responses | RANK?     | STAT3 negatively regulates Ubc1 involved K63-linked ubiquitination, and suppress pro-inflammatory cytokines by modulating NF-κB signaling (Zhang et al., 2014) |
| Ubc13  | UBE2N | RNF8           | K63     | DNA double-strand break (DSB) responses | BRCA1 Tumor Suppressor Recruitment | histone   | RNF8 stimulates Ubc13-dependent Lys-63 ubiquitination, which is pivotal for DNA damage response and recruitment of BRCA1 (Hodge et al., 2016) |
| Ubc13  | UBE2N | TRAF6          | K63     | innate and adaptive immunity | osteoclast differentiation | TRAF6 (autoubiquitination) | Ubc13/Uev1A interacts and binds to the active RING domain of TRAF6, which is essential for the formation of Lys63-linked poly-Ub, thus triggering NF-κB activation and osteoclast differentiation (Lamothe et al., 2007) |
| Ubc13/ Uev1A | UBE2N/UBE2V1 | TRAF6 | K63     | activating NF-κB signaling | osteoclast differentiation | TRAF6 (autoubiquitination) | Ubc13/Uev1A catalyzes TRAF6 auto-ubiquitination through Lys63-linked poly-Ub chains, which controls NF-κB signaling and osteoclast differentiation (Lamothe et al., 2007b) |
| Ubc13/ Uev1A | UBE2N/UBE2V1 | TRAF6 | K63     | spontaneous osteoclast differentiation | TRAF6 (auto-ubiquitination) | Ubc13/Uev1A interacts with Ubc13/Uev1A, facilitating Lys-linked auto-ubiquitination of TRAF in a RING domain-dependent fashion, and modulating downstream NF-κB signaling (Lamothe et al., 2007a) |
| UBE2O  | UBE2O | TRAF6          | K63     | activating NF-κB signaling | modulating NF-κB signaling associated cancers | TRAF6 (auto-polyubiquitination) | UBE2O negatively regulates the recruitment of TRAF6, inducing TRAF6 auto-ubiquitination through binding to K63 residue, and subsequently prevents NF-κB activation by the IL-1R/TLR complex (Zhang et al., 2013b) |

(Continued on following page)
### TABLE 1 (Continued) Summary of the combined E2/E3 enzymes in Tumorigenesis.

| E2   | Alias   | Accompanied E3 | Linkage   | Phenotypic Characteristics                      | Neoplastic Implications                        | Substrate | Mechanism Summary                                                                 |
|------|---------|----------------|-----------|-------------------------------------------------|------------------------------------------------|-----------|-----------------------------------------------------------------------------------|
| UBE2T| UBE2T   | FANCL          | monoubiquitination | maintenance of chromosome stability            | disrupting DNA repair pathways                   | FANCD2    | In the presence of FANCL, UBE2T stimulates monoubiquitination of FANCD2, which is vital for disrupting abnormal chromosomes and efficient DNA damage repair (Machida et al., 2006) |
| UBE2T| UBE2T   | FANCL          | automonoubiquitination | maintenance of chromosome stability            | disrupting DNA repair pathways                   | UBE2T     | Automonoubiquitination of UBE2T inhibits own conjugation activity (Machida et al., 2006) |
| UBE2T| UBE2T   | FANCL          | monoubiquitination | DNA repair                                      | leading to leukemia and bone marrow failure     | FANCD2    | FANCL interacts with UBE2T in an ELF-domain-dependent fashion, which regulates DNA damage-induced FANCD2 monoubiquitination (Miles et al., 2019) |
| UBE2T| UBE2T   | FANCL          | monoubiquitination | DNA interstrand crosslink repair                | genomic instabilities                           | FANCD2    | FANCL specifically interacts with UBE2T, leading to FANCD2 ubiquitination, which is involved in Fanconi Anemia pathway (Hodison et al., 2014) Rad8 facilitates DNA repair and stem cell gene transcription, through mediating H2B ubiquitination (Somasagara et al., 2017) |
| RAD6 | UBE2B   | in absence of E3 | K63?      | promoting DNA repair                            | promoting recurrence and metastasis in ovarian cancer | β-catenin | Rad8 ubiquitinates b-catenin through K63-linked ubiquitination, which regulates transcriptional activity in breast cancer (Shekhar et al., 2008) |
| RAD6B| UBE2B   | in absence of E3 | K63       | DNA repair and mutagenesis                      | h-catenin modification in breast cancer         | β-catenin |                                                                                   |
| UBE2B| UBE2B   | BRE1           | monoubiquitination | promoting the G1-S transition and cell proliferation | promoting G1-S transition and cell proliferation | H2B       | UBE2B modulates CCND1 transcription level by regulating the levels of H2B monoubiquitination, promoting cell cycle progression and proliferation (Cai et al., 2014) |
| Ube2w| UBE2W   | RNF4           | monoubiquitination | DNA damage repair                               | potential prostate, breast and lung cancer target | SUMO      | Ube2w associated with RNF4, mediating mono-ubiquitination of SUMO chains. Those chains can be further ubiquitinated through K63 chains in response to DNA damage (Maure et al., 2016) |
| Ubc13| UBE2N   | TRAF6?         | K63       | Autoimmunity and aberrant T cell activation     | modulating NF-κB associated cancer              | IKK       | Ubc13 conjugates K63-linked ubiquitin chains involving Ubc13-IKK signaling axis, which have a robust evidence in regulating T cell function (Chang et al., 2012) |
| UBC13| UBE2N   | Bcl110         | K63       | activating NF-κB pathway                        | modulating NF-κB associated cancer              | NEMO      | UBC13 is dependent in Bcl110 modulating NEMO lysine-63-linked ubiquitination, and subsequent NF-κB activation (Zhou et al., 2004) (Continued on following page) |
| E2 Alias | Accompanied E3 | Linkage | Phenotypic Characteristics | Neoplastic Implications | Substrate | Mechanism Summary |
|----------|----------------|---------|--------------------------|------------------------|-----------|------------------|
| UBE2N    | MEKK1          | K63     | embryonic survival       | promoting ES-cell differentiation and tumour formation | TAB1      | Together with UBE2N, MEKK1 could tag TAB1 with Lys63-linked poly-Ub, promoting ES-cell differentiation and tumourigenesis (Charlaftis et al., 2014) |
| UbcH6    | UBE2E1         | TRAF4   | DNA damage               | overcome chemotherapy in colorectal cancer | CHK1      | UbcH6 combined with TRAF4, which is critical for CHK1 K63-linked ubiquitination and essential for cell proliferation, colony formation (Yu et al., 2020b) |
| UBE2T    | UBE2T          | RNF8    | monoubiquitination       | conferring HCC radioresistance | H2AX      | Ubc2T/RNF8 complex, monoubiquitinated H2AX/γH2AX, facilitating cell cycle arrest activation, thus inducing HCC radioresistance (Sun et al., 2020) |
| Ubc13: Uev1A/UBE2N: Uev2/UBE2V1 | RNF8 | K63 | DNA damage repair and cytokinesis | genomic instability of ATL cells | NEMO and TAB2/3 | RNF8 and Ubc13:Uev1A/UBE2N:UBE2V1 assemble K63-pUb chains on NEMO and TAB2/3 respectively, allowing TAK1 and IKK activation (Ho et al., 2015) |
| Ube2E1   | PRC1           | monoubiquitination | maintenance of stem cell proliferation | promoting cancer cell proliferation | H2A       | Ube2E1 interacts with PRC1 complex, catalyzing monoubiquitination of H2A (Wheaton et al., 2017) |
| Ubc13    | UBE2N          | TRAF6   | regulating immune signaling | NF-κB signaling related cancer | AKT       | Ubc13, together with TRAF6, mediates K63-linked polyubiquitin signaling pathway, including NF-κB signaling (Lencor et al., 2021) |
| Uev1A/ Ubc13 | UBE2N/UBE2V1 | TRAF6   | regulating AKT signaling pathway | promoting breast cancer cell migration and EMT signaling | AKT       | Uev1A/Ubc13 interact with TRAF6, ubiquitinates AKT with K63-linked ubiquitination, which is required for AKT activation, promoting cell migration and EMT in breast cancer (Heu et al., 2021) |
| UBE2T    | FANCL          | monoubiquitination | involving in FA pathway-induced chromosome instability | functions in cancer predisposition | ID        | UBE2T/FANCL-FANCD2 complex remodeling the ID-DNA complex, preventing clamp opening after monoubiquitination (Wang et al., 2021a) |
| UbcH6    | NEDD4          | K63     | regulating cell-cell adhesion, mechanosensing and autophagy | involving in angiogenesis and tumor growth | IGPR-1    | NEDD4 and UbcH6 are involved in the K63-linked ubiquitination of IGPR-1, regulating different cellular activities, such as cell adhesion, autophagy, mechanosensing, angiogenesis and tumor growth (Sun et al., 2021) |
| Ubc13    | UBE2N          | RNF213  | angiogenic activity      | regulating cell mobility and invasion |           | RNF213 interacts with Ubc13 and promotes its own autoubiquitination, controlling inflammatory responses and angiogenic activities (Habu and Harada, 2021) |
| Type | DUBs | Linkage | Pheno-typic Characteristics | Neoplastic Implications | Substrate | Mechanism Summary |
|------|------|---------|-----------------------------|------------------------|-----------|-------------------|
| OTU  | TRABID (ZRANB1) | K63 | stem cell self-renewal or differentiation | Wnt-induced transcription in colorectal cancer cell | APC | Trabid preferentially binding to K63-linked ubiquitination chains, which is required for Wnt-induced transcription (Tran et al., 2009) |
| OTU  | TRABID (ZRANB1) | K29, K33 | inhibiting autophagy flux | Promoting autophagosome maturation and inhibiting hepatocellular carcinoma growth inhibiting breast cancer stemness and metastasis | UVRAG | TRABID preferentially binding to K63-linked ubiquitination chains from UVRAG, regulating autophagy system (Feng et al., 2019) |
| OTU  | OTUD1 | K33 | restricting the TGF-β signaling | Regulating tumorigenesis, cancer cell survival and chemoresistance | SMAD7 | OTUD1 directly deubiquitinates the SMAD7, shuts off TGF-β signals, thereby suppressing metastasis in breast cancer (Zhang et al., 2017) |
| OTU  | OTUD1 | K63 | regulating organ growth, tissue regeneration | Regulating tumorigenesis, cancer cell survival and chemoresistance | YAP | OTUD1 cleaves K63-linked polyUb from YAP, which is assembled by SKP2 E3 ligase, regulating tumorigenesis (Yao et al., 2018) |
| OTU  | OTULIN | M1 | activating NF-κB and promoting pro-inflammatory cytokines and restricting bacterial proliferation | Regulating NF-κB signaling and sensitizing cell death | RIPK1 | OTULIN interacts with LUBAC, balancing Met1-polyUb chains, thereby regulating NF-κB signaling (Keusekotten et al., 2013) |
| OTU  | OTULIN | M1 | activating NF-κB and promoting pro-inflammatory cytokines and restricting bacterial proliferation | Regulating NF-κB signaling and sensitizing cell death | RIPK1 | OTULIN interacts with LUBAC, balancing Met1-polyUb chains, thereby regulating NF-κB signaling (Keusekotten et al., 2013) |
| OTU  | OTUD1 | K63? | decreasing cell proliferation and increasing apoptosis | Regulating tumour-suppressor p53 | p53 | OTUD1 interacts with and stabilizes p53. Its overexpression significantly suppresses colony formation, and increases apoptosis (Pao et al., 2017) |
| OTU  | A20  | K63 | NF-κB transcriptional activity-mediated cell death and chronic inflammation | NF-κB signaling-related cancers | RIP | A20 erases K63-linked ubiquitin chains from RIP, and it also polyubiquitnates RIP with K48-linked ubiquitin chains in a carboxy-terminal-domain-dependent manner, which downregulates NF-κB signalling, (Wertz et al., 2004) |
| OTU  | A20  | K63 | downregulating NF-κB pathway | NF-κB signaling-related cancers | TRAF6/RIP | A20 display dual ubiquitin-editing functions, mediating both non-proteolytic Lys63-linked ubiquitin chains and degradative Lys48-linked ubiquitin chains, thus regulating NF-κB activities (Lin et al., 2008) |
| OTU  | OTUD7B | K63 | regulating mTORC2 signaling, thus relating to cell growth and metabolic disorders | activates Akt signaling and Kras-driven lung tumorigenesis in vivo mediating tumor suppression in both mice and humans | Gj8L | OTUD7B and TRAF2 regulate stability of Gj8L, which plays critical roles in mTORC2 signaling (Wang et al., 2017a) |
| UCH  | BAP1 | monoubiquitination | monoubiquitination | H2A | Inactivation of BAP1 causes apoptosis through regulating H2A monoubiquitination, regulating tumor suppression (He et al., 2019) |
| USP  | USP4 | K63 | activating inflammation and immune response | inhibiting TNFα-induced cancer cell migration | TRAF2/ TRAF6 | USP4 negatively regulates the TRAF2- and TRAF6-stimulated NF-κB activation, and inhibits cancer cell migration (Kiao et al., 2012) |
| USP  | CYLD | K63 | regulating NF-κB-mediated inflammation | associating the development of head and neck squamous cell carcinomas | NEMO | TRAF3/CYLD complex regulate NF-κB transcriptional level, which is associated with head and neck squamous cell carcinomas with HPV infection (Chen et al., 2017b) |
| USP  | CYLD | K63/M1 | regulating innate immune signaling | tumor suppressor | RIPK2 | CYLD counteracts Met1-Ub and Lys63-Ub conjugated to Ripk2, and this deubiquitnase activity plays an important role in innate immune regulation (Krithika et al., 2016) |

(Continued on following page)
| Type | DUBs | Linkage | Phenotypic Characteristics | Neoplastic Implications | Substrate | Mechanism Summary |
|------|------|---------|----------------------------|-------------------------|-----------|------------------|
| USP  | USP8 | K63     | DNA damage response        | genomic instability in cancer | BRIT1     | USP8 rescues BRIT1 from K63 ubiquitin and regulates its recruitment to DNA double-strand break sites (Ge et al., 2015) |
| USP  | USP10| K63     | controlling cell cycle     | promoting proliferation of chronic myeloid leukemia cells | Bcr-Abl   | SPK2 acts as co-regulator of K63-linked ubiquitination of Bcr-Abl for its activation. While USP10 deubiquitinates and stabilizes SKP protein levels and amplifies Bcr-Abl activation in chronic myeloid leukemia cells (Liao et al., 2019) |
| USP  | USP20| K63     | negatively regulating inflammation, cell proliferation, and apoptosis enhancing inflammation and promoting macrophage recruitment | promoting adult T cell leukemia (ATL) development | Tax       | USP20 targets and deubiquitinates TRAF6 and TAX, negatively regulating NF-κB signaling (Yasunaga et al., 2011) |
| USP  | USP17| K63     | enhancing inflammation and promoting macrophage recruitment | promoting lung cancer growth | clAP1/2   | USP17 interacted with and disrupted the TRAF2/TRAF3 complex through reducing K63-linked ubiquitination of TRAF2 and TRAF6. This activity positively drives stemness and inflammation in lung cancer (Lu et al., 2018) |
| USP  | CYLD | K63     | regulating inflammation    | promoting tumor growth   | TAK1      | Itch-Cyld complex sequentially cleaving K63-linked ubiquitin chain on Taki thus terminating the inflammatory response (Ahmed et al., 2011) |
| USP  | CYLD | K63     | negative regulate the NF-κB pathway | tumor suppressor         | E6        | HPV E6 suppresses the CYLD under hypoxic conditions, promoting unrestricted NF-κB activation and allowing for malignant progression of tumors (An et al., 2008) |
| USP  | CYLD | K63     | controlling inflammation  | inhibiting tumor formation | Bcl-3     | Cyld erases K63-linked polyubiquitin chains from Bcl-3, inactivating NF-κB signaling (Massoumi et al., 2006) |
| USP  | CYLD | K63     | controls survival and inflammation | inhibiting tumor cell proliferation | TRAF2     | Cyld regulates inflammation through deubiquitinating TRAF2 and blocking NF-κB pathway (Massoumi et al., 2006) |
| USP  | USP14| K63     | inflammation              | acute colitis and colitis-associated colon cancer development | p100/p52  | TRAM14 recruits USP14 to cleave K63-linked ubiquitin chains of p100/p52, regulating NF-κB-mediated autophagy and innate immunity. (Chen et al., 2020) |
| USP  | USP1 | K63     | regulating macroautophagy/autophagy | affecting breast cancer cell growth | ULK1      | USP1 modulates ULK1 K63-linked deubiquitination, and regulates autophagy, also affects breast cancer cell growth relying on autophagy (Raimondi et al., 2019) |
| USP  | USP1 | K63     | Double-strand breaks (DSBs) | potential tumour suppressor | histones  | USP1 actively destroys K63-linked poly-ubiquitin chains on histones. And its recruitment to damage sites has a close link with genome stability and double-strand breaks (Ha et al., 2017) |
| USP  | USP34| K63     | genome stability maintenance | promoting ES-cell differentiation and tumour formation | H2A       | USP34 stabilizes RNF168, recruiting repair proteins at DSBs, which is critical for genome stability (Sy et al., 2013) |
| USP  | CYLD | K63/M1  | DNA damage-induced apoptosis | enhancing sensitivity to chemodrug in cancer cells | NEMO      | CYLD downregulates K63-linked and linear ubiquitination of NEMO, promoting apoptosis (Niu et al., 2011) |
| USP  | CYLD | K63     | activating the cell death pathway | regulating ATLL cell death | RIPK1     | CYLD erases K63-linked ubiquitin chains from RIPK1, which activates the cell death pathway and activates CYLD and RIPK1-dependent tumor cell death in ATLL (Lu et al., 2020) |

(Continued on following page)
indispensable for inflammatory responses by activating Imd pathway (Aalto et al., 2019). Additionally, HOIP, with the help of cIAP1, is recruited to the linear ubiquitination of the TNFR2 signaling complex and activates canonical NF-κB, thereby facilitating cancer progression (Borghi et al., 2018). Multiple investigations found that HOIP, presumably through M1-linked ubiquitination, was proved to be connected with sorts of malignancies, including breast and prostate cancer (Guo et al., 2015; Zhu et al., 2016). Furthermore, previous experiments highlight that HOIL-1 interacts with HOIP, which adds a Mi-linked poly-ubiquitin chain to specific NF-κB signaling proteins, suggesting its link to a diversity of immune disorders, antiviral signaling (Elton et al., 2015), iron and xenobiotic metabolism (Elton et al., 2015), apoptosis (Emmerich et al., 2011; Lewis et al., 2015; Sasaki and Iwai, 2015), and cancer (Queisser et al., 2014; Taminiau et al., 2016). Another member of the LUBAC family, SHARPIN, is a novel component of the LUBAC complex. Spontaneous mutation of this tiny gene led to dysregulation of the NF-κB signaling pathway through linear ubiquitinate of NEMO (the key modulator of NF-κB). This systematic linear ubiquitination is obvious and can induce immune system disorders in SHARPIN-deficient mice (Tokunaga et al., 2011). Interestingly, SHARPIN-containing complexes can also interact with NEMO to activate NF-κB pathway (Ikeda et al., 2011). Moreover, NEMO was identified to be modified by LUBAC, generating M1-linked chains that were recognized by the UBAN domain of NEMO, causing conformational changes in the intertwined helices of NEMO dimers (Haas et al., 2009; Belgnaoui et al., 2012; Tokunaga and Iwai, 2012; Noad et al., 2017). NF-κB signaling regulates human cellular activities in different ways and is balanced by ubiquitination and deubiquitination (Adhikari et al., 2007; Lork et al., 2017) (Table 2).

### TABLE 2 | (Continued) Summary of the Identified DUBs Involving in Tumorigenesis.

| Type | DUBs | Linkage | Phenotypic Characteristics | Neoplastic Implications | Substrate | Mechanism Summary |
|------|------|---------|---------------------------|-------------------------|-----------|------------------|
| USP  | USP38 | K63     | regulating cancer cell response to genotoxic insults | HDAC1 | USP38 preferentially removed the K63-linked ubiquitin chains from HDAC1, regulating genomic stability (Yang et al., 2020) |
| USP  | UBQLN4 | K63/K48 | predictor of poor survival in various cancer entities | MRE11 | Overexpression of UBQLN4 represses homologous recombination activity through inhibiting MRE11 ubiquitination, thus presenting close relationship with survival rates in various cancer (Jacobowicz et al., 2019) |
| OTU  | ZRANB1 | K29/K33 | promoting autophagosome maturation | UVRAG | ZRANB1 specifically cleaves SMURF1-induced K29 and K3-linked polyubiquitin chains from UVRAG, regulating autophagosome maturation and HCC growth (Feng et al., 2019) |
| JAMM | POH1  | K63     | promoting tumour formation in human hepatocellular carcinomas (HCCs) | E2F1 | POH1 binds to and stabilizes the E2F1, upregulating Survivin and FOXM1 protein levels, accompanied by accelerating tumor growth (Wang et al., 2015) |
| USP  | USP30 | K6     | functions in hepatocellular carcinoma | RIPK1 | USP30 specifically cleaves the Ly6 linked ubiquitin chains, regulating mitophagy, apoptosis and tumorigenesis |
| OTU  | OTUD1 | K63     | suppressing intestinal inflammation | NF-κB signaling-related cancers | OTUD1 preferentially cleaves K63-linked polyubiquitin chains from RIPK1, inhibiting colonic inflammation and NF-κB signaling |
| USP  | CYLD  | K63     | regulating ERK activation | ERK1/2 | CYLD cleaves K63-linked ubiquitination mediated by TRIM1, regulating ERK signaling and the associated cancer development |
| USP  | USP10 | K63     | inhibiting NSCLC cell proliferation and migration | PTEN | USP10 suppresses NSCLC cell proliferation and migration through abolishing PTEN from K63-linked polyubiquitination mediated by TRIM25 |
| OTU  | A20   | K63     | anti-inflammatory effects | TBK1 | A20 inhibits TBK1 activation through reducing K63-linked ubiquitination of Nrp1, regulating inflammation |
## TABLE 3 Summary of the E3 enzymes in Tumorigenesis.

| E3   | Linkage | Phenotypic Characteristics                                                                 | Neoplastic Implications                        | Substrate | Mechanism Summary                                                                                                                                                                                                 |
|------|---------|-------------------------------------------------------------------------------------------|------------------------------------------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| RNF8 | K63     | regulating DNA double-strand break responses                                               | BRCA1 Tumor Suppressor Recruitment             | histone   | RNF8 interact with Ubc13, generating K63-linked ubiquitin chains on histone, which positively regulate DNA double-strand break and BRCA1 recruitment (Hodge et al., 2016) |
| RNF8 | K63     | DNA damage repair and cytokinesis                                                         | genomic instability of ATL cells               | Tax       | Stimulated by Tax, RNF8 and Ubc13:Uev1A function together, generating K63-pUb chains, which activated TAK and NF-κB signaling (Zhi et al., 2020)                                                                  |
| HOIL-1 | M1    | immune regulation                                                                        | NF-κB activation in cancers                    | NEMO      | HOIL-1 modifies NF-κB core proteins with linear ubiquitin chains, regulating NF-κB pathway signaling (Elton et al., 2015)                                                                                  |
| CHIP  | K6      | suppressing of cell death                                                                 | FADD                                           |           | CHIP triggers K6-linked polyubiquitlaytion of FADD, leading to the suppression of cell death (Seo et al., 2018)                                                                                               |
| BRCA1 | K6      | DNA damage response                                                                       | tumor suppressor                               | BRCA1 autoubiquitination                        |           | UBXN1 binds to BRCA1 active site and decorate it with K6-linked polyubiquitin chains in a UBA-domain-dependent manner and BRCA1/BARD1 complex is regulated by the ubiquitinate status of BRCA1 (Wu-Baer et al., 2010) |
| BRCA1 | K6      | DNA repair, transcriptional regulation, and cell cycle checkpoint control                  | tumor suppressor                               | BRCA1 autoubiquitination                        |           | BRCA1 mediates autoubiquitination by conjugating to K6-linked polymers, which impart cellular properties (Wu-Baer et al., 2003)                                                                       |
| BRCA1 | K6      | DNA double-stranded breaks repair                                                         | tumor suppressor                               | BRCA1 autoubiquitination?                       |           | BRCA1 recruits its autoubiquitination at DNA damage sites, which is dependended on K6-linked linkage. BRCA1:BARD1 enzyme activity is regulated by BRCA1 ubiquitin status. (Morris and Solomon, 2004) |
| BRCA1 | K6      | regulating DNA repair, transcriptional levels, cell cycle and cell apoptosis              | tumor suppressor                               | BRCA1 autoubiquitination                        |           | BRCA1:BARD1 regulate BRCA1 autoubiquitination by preferentially mediating K6-linked polyubiquitin chains (Nishikawa et al., 2004)                                                                    |
| Hectd3 | K27, K29 | leading to NF-κB activation                                                               | NF-κB associated cancer                        | Malt1     | Hectd3 promotes K27 and K29 polyubiquitlation on Malt1, regulating autoimmunity and other Th17-related diseases (Cho et al., 2019)                                                                                |
| WWP1  | K27     | suppressing the dimerization, membrane recruitment                                        | restoring tumor-suppressive activity           | PTEN      | WWP1 triggers K27-linked polyubiquitlation of PTEN to regulate subcellular localization cancer susceptibility syndromes (Lee et al., 2015c)                                                               |
| TRAF4 | K27, K29 | facilitating immune cell migration                                                        | promoting cancer cell invasion                | TrkA      | TRAF4 promotes K27 and K29-linked ubiquitin linkages on TrkA, facilitating prostate cell invasion (Singh et al., 2018)                                                                                      |
| RNF4  | K63     | DNA damage repair                                                                         | potential prostate, breast and lung cancer target | Trim5α    | Ube2w interacts with RNF4, promoting monoubiquitization of SUMO chains, which are further modified to form K63-linked ubiquitin chains (Maure et al., 2016)                                                  |
| RNF8  | K63     | DNA damage response                                                                       | breast cancer predisposition                  | H2A/H2AX  | RNF8 activated with Ubc13, promoting K63-linked polyubiquitin conjugation to histones H2A/H2AX, then contributing to breast cancer predisposition (Vuorela et al., 2011)                                              |
| Skp2  | K63     | promoting survival and Akt-mediated glycosylation                                         | restricting cancer cell progression            | Akt       | Skp2/SCF complex catalyzes K63-linked ubiquitination chains on Akt, which is required for glycosylation and cancer development (Chan et al., 2013)                                                             |
| Skp2  | K63     | controlling cell cycle                                                                    | promoting proliferation of chronic myeloid leukemia cells | Bcr-Abl   | SKP2 triggers K63-linked ubiquitination of Bcr-Abl, regulating downstream signaling, and is vital for chronic myeloid leukemia development and progression (Liao et al., 2019) |

(Continued on following page)
| E3         | Linkage | Phenotypic Characteristics                  | Neoplastic Implications                  | Substrate | Mechanism Summary                                                                 |
|------------|---------|---------------------------------------------|------------------------------------------|-----------|-----------------------------------------------------------------------------------|
| RNF113A    | K63     | DNA repair                                  | potentially associating with tumor       | BRR2      | RNF113A interacts with BRR2 through K63-linked polyubiquitin, mediating repairment of DNA alkylaton damage (Brickner et al., 2017) |
| TRAF2      | K63     | mediating several cell growth and metabolic pathways | facilitating tumorgenesis                | GjL       | TRAF2 promotes K63-linked polyubiquitination of GjL, and regulates mTORC2 signaling, thus mediating several cell growth and metabolic pathways (Wang et al., 2017a) |
| TRIM57     | mono-ubiquitination | regulating transcriptional repression | promoting transformation in breast cancer | H2A       | TRIM57 mono-ubiquitnates histone H2A, thus associating with transcriptional repression (Bhatnagar et al., 2014) |
| RNF8/ RNF168 | K63     | DNA double-strand breaks (DSBs)             | mediating ATM-dependent carcinogenesis   | H2A/H2AX  | RNF8 and RNF168 combined together to catalyze K63-linked poly-Ub chains on H2A/ H2AX, which is important for transcription and DNA double-strand breaks (Shanbhag et al., 2010) |
| HectH9     | K63     | regulating transcriptional activation and repression | tumor cell Proliferation                 | Myc       | HectH9 recruits 63-linked polyubiquitin chains to Myc, modulating cell proliferation in various tumor cells (Adhikary et al., 2005) |
| Bcl10      | K63     | activating the NF-κB pathway                | NF-κB associated cancer                  | NEMO      | UBC13 and Bcl10 function together inducing NEMO ubiquitination through lysine-63-linked ubiquitination, and subsequent NF-κB activation (Zhou et al., 2004) |
| RNF8       | K63     | DNA repair                                  | tumour-promoting                         | probably histone H1 | In p97–ATX3 activated conditions, RNF8 mediates K63-Ub at sites of DNA lesions, regulating genome instability, cell invasion and metastasis (Singh et al., 2019) |
| PARK2/ Parkin | K33     | fine-tune necroptosis and inflammation      | tumor suppressor/inflammation-associated tumorgenesis | RIPK3     | AMPK activated Parkin/RIPK3 complex through K33-linked polyubiquitination, which negatively regulates necroptosis and inflammation-associated tumorgenesis (Lee et al., 2019a) |
| TRAF6      | K63/K27 | maintaining nuclear genome integrity        | promoting cancer progression             | hDNA2     | hTRAF6 catalyzes the K27- and K63-linked polyubiquitination of hDNA2, maintaining nuclear genome integrity and the associated cancer biology (Meng et al., 2019) |
| HectH9     | K63     | integrating glycolysis activation and apoptosis resilience | regulating tumor metabolism and cancer stem cell expansion | HK2       | HectH9 catalyzes HK2’s K63-linked ubiquitination, regulating stem cell expansion and CSC-induced chemoresistance in prostate cancer (Lee et al., 2019a) |
| TRAF6      | K63     | regulating inflammation and immunity        | promoting liver tumorgenesis and correlates with poor prognosis | HDAC3     | TRAF6 ubiquitnates HDAC3 with K63-linked ubiquitin chains, regulating inflammation and malignant transformation and progression in HCC(Wu et al., 2020) |
| ITCCH      | K27     | immune response                             | promoting proliferation and invasion of melanoma cells | BRAF      | Activated ITCCH maintains BRAF activity and subsequent MEK/ERK signaling through Lysine 27-linked ubiquitination, enhancing proliferation and invasion of melanoma cells (Yin et al., 2019) |
| TRAF6      | K63     | immunity                                    | anti-tumor immunity in the cancer setting | FOXP3     | TRAF6 bind to and facilitates Regulatory T cells (Tregs) activities through K63-linked ubiquitination at lysine 262, acting as aTreg-stabilizing regulator and playing crucial roles in immune control and anti-tumor immunity (N et al., 2019) |
| SMURF1     | K29/K33 | promoting autophagosome maturation          | inhibiting cell growth in hepatocellular carcinoma | UVRAG     | SMURF1 mediates K29 and K33-linked polyubiquitin chains on UVRAG, promoting autophagosome maturation and inhibiting hepatocellular carcinoma growth (Feng et al., 2019) |

(Continued on following page)
TABLE 3 | (Continued) Summary of the E3 enzymes in Tumoregenesis.

| E3  | Linkage | Phenotypic Characteristics                                      | Neoplastic Implications                         | Substrate | Mechanism Summary                                                                                                                                 |
|-----|---------|---------------------------------------------------------------|-------------------------------------------------|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| TRAF6 | K63    | NF-κB activation and autophagy activation                     | cancer cell migration, cell invasion           | BECN1/TRA96 | PRDX1 negatively regulates TRAF6 ubiquitin-ligase activity, leading to NF-κB inactivation and autophagy activation (Min et al., 2018) |
| RNF8  | K63    | DNA double-strand break repair                                | regulating L3MBTL2 mutation in leukemia         | L3MBTL2   | MDC1 recruits L3MBTL2 to sites of DNA lesion and is then ubiquitylated by RNF8, promoting DNA DSB repair and regulating L3MBTL2-induced cancers (Nowsheen et al., 2018) |
| Itch  | K63    | regulating tissue patterning, stem cell maintenance           | modulating medulloblastoma tumorigenesis        | SuFu      | Itch/β-arrestin2 complex mediates Lys63-linked polyubiquitylation on SuFu, thus controlling Hedgehog signaling and medulloblastoma tumorigenesis (Infante et al., 2018) |
| FBXO32 | K63    | brain development                                             | promoting tumorigenicity and metastasis in humans | CtBP1     | FBXO32 directly ubiquitinates CSBP1 with K63-linked ubiquitin chains, and this interaction activity regulates downstream EMT signaling and is essential for tumor metastasis and brain development (Sahu et al., 2017) |
| Culins | K29    | promoting cell motility                                      | modulating cell migration                       | hnRNP A1  | SPSB1 catalyzes K29-linked polyUb chains on hnRNP A1, modulating cell migration and cell motility in EGf signaling (Wang et al., 2017b) |
| HUWE1 | K63    | preventing DNA damage accumulation                            | colonic tumour suppressor                       | Myc       | Huwe1 mediates MYC transactivation activity via K63-linked ubiquitination, inhibiting accumulation of DNA damage and preventing tumour initiation especially in colonic cancers (Myant et al., 2017) |
| HectH9 | K63    | relating to embryonic lethal                                  | promoting hypoxia-induced tumour progression    | HAUSP(USP7) | HectH9 mediates K63-polyubiquitin chains conjugated to HAUSP. HAUSP then deubiquitinates HIF-1α, promoting hypoxia-induced tumour progression (Wu et al., 2016) |
| RNF8  | K63    | conferring chemoresistance                                    | tumor-promoting function                        | Twist     | RNF8 activates and ubiquitinate Twist, leading to subsequent EMT and CSC functions, thus exerting tumor-promoting functions such as cell migration and invasion (Lee et al., 2016) |
| FBXW7 | K63    | genome integrity                                              | tumor suppressor                                | XRCC4     | FBXW7 firstly phosphorylated by ATM and then it ubiquitylates XRCC4 via K63-linkage, promoting NHEJ repair, which is closely related to DSB and genomic stability (Zhang et al., 2016) |
| Trim7 | K63    | regulating proliferation and apoptosis                        | promoting Ras-mediated lung adenocarcinoma      | RACO-1    | Trim7 catalyzes Lys63-linked ubiquitination of RACO-1 in response to RAS signaling, and Trim7 overexpression increases lung tumour burden while knockdown of Trim7 reduces tumour growth in xenografts models (Chakraborty et al., 2015) |
| Skp2  | K63    | regulating energy metabolism, proliferation, apoptosis, and cell polarity | tumor growth in HCC                            | LKB1      | Skp2-dependent activation of LKB1 through K63-linked Ubiquitination is essential for HCC tumor growth and related to poor survival outcomes (Lee et al., 2015) |
| TRAF6 | K63    | enhancing chemotherapeutic efficacy                           | promoting T-ALL progression                     | MCL1      | IRAK1/4 signaling activated TRAF6, mediating K63-linked ubiquitination of MCL1, promoting T-ALL progression (Li et al., 2015) |
| PELI1 | K63    | maintenance of autoimmunity                                   | promoting lymphomagenesis                       | BCL6      | PELI1 specifically binds to BCL6 and induces lysine 63-linked ubiquitin chains on BCL6, promoting lymphomagenesis, modulating the maintenance of autoimmunity through TLR and TCR signaling (Park et al., 2014) |

(Continued on following page)
| E3            | Linkage | Phenotypic Characteristics                      | Neoplastic Implications                  | Substrate          | Mechanism Summary                                                                                                                                 |
|--------------|---------|------------------------------------------------|------------------------------------------|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| MEKK1        | K63     | embryonic survival                             | promoting ES-cell differentiation and tumour formation | TAB1               | MEKK1 ubiquitylates TAB1 with Lys63-linked polyubiquitin in a PHD motif-dependent manner, inducing TAK1 and MAPK activation, which are crucial for ES-cell differentiation and tumour formation (Charlaftis et al., 2014) |
| TRAF4        | K63     | regulating immunity                            | driving Breast Cancer Metastasis         | TjIrI              | TjIrI-receptor TRAF4 interacts with each other, triggering Lys 63-linked TRAF4 polyubiquitylation and TAK1 activation, promoting cell migration and metastasis in breast cancer (Zhang et al., 2013a) |
| RNF8/RNF168  | K63     | maintaining genome stability                   | suppressing tumourigenesis               | BLM                | RNF8/RNF168 triggers BLM activation, leading to BLM recruiting to the ubiquitin-interacting motifs of RAP80, which is vital to maintain genome stability and suppressing tumourigenesis (Tikoo et al., 2013) |
| RFP          | K27     | inhibiting apoptosis and promoting cell survival and proliferation | tumor suppression                       | PTEN               | E3 ubiquitin ligase RFP interacts with PTEN through K27-linked ubiquitination diminishing the effect of AKT signaling, involving in tumor suppression regulation (Lee et al., 2013) |
| FAAP20       | K63     | DNA damage repair and genome maintenance       | leading to hematologic defects and cancer in patients | FANCA              | FAAP20 binds K-63-linked ubiquitin chains in a UBE2 domain-dependent manner, modulating DNA damage repair and genome maintenance (Ali et al., 2012) |
| LUBAC        | M1      | DNA damage-induced apoptosis                   | enhancing sensitivity to chemodrug in cancer cells | NEMO               | LUBAC-mediated NEMO linear ubiquitination promotes TAK1 and IKK activation, protecting cells from DNA damage-induced apoptosis (Niu et al., 2011) |
| TRAF4 (RNF83)| K63     | DNA damage                                     | overcome chemoresistance in colorectal cancer | CHK1               | CHK1 K63-linked ubiquitination is mediated by TRAF4, which is essential for CHK1 phosphorylation and activation during DNA damage response, and is close to cell proliferation, colony formation in colorectal cancer (Yu et al., 2020b) |
| LUBAC        | M1      | regulating cell activation and death           | promoting breast cancer                  | NEMO               | Epsins 1 and 2 interact with LUBAC, promoting NEMO linear ubiquitination and resulting in breast cancer development (Song et al., 2021) |
| DZIP3        | K63     | regulating cell cycle                          | promoting cancer cell growth, migration, and invasion | Cyclin D1          | DZIP3/HRUL138 stabilizes and ubiquitinated Cyclin D1 protein through K63-linked ubiquitination, and closely related with cell cycle progression, cancer cell growth, invasion, migration (Kolapalli et al., 2021) |
| RNF138       | K63     | driving NF-kB activation and innate immunity   | promoting NF-kB activation in lymphomas  | MYD88L265P         | RNF138 triggers K63-linked polyubiquitination of MYD88L265P, resulting in constitutive activation of NF-kB and the associated lymphomagenesis (Yu et al., 2020a) |
| RNF181       | K63     | endocrine resistance                           | facilitating breast cancer progression   | ERα protein        | RNF181 functions as E3 enzymes through K63-linked ubiquitination, which facilitates breast cancer progression (Zhu et al., 2020a) |
| TRIM11       | mono-ubiquitination | regulating estrogen-dependent gene expression | promoting cell growth and migration     | Erα                | TRIM11 interacts with the N terminal of ERα and maintains ERα stability through mono-ubiquitination, thus promoting cell growth and proliferation in breast cancer (Tang et al., 2020) |
| HUWE1        | K27-, K29- | regulating DNA damage response                | promoting radio-resistance of prostate cancer cells | JMJD1A             | HUWE1 mediates the K27- and K29-linked ubiquitination of JMJD1A, enhancing c-Myc activity, promoting DSB repair and (Continued on following page)
TABLE 3 | (Continued) Summary of the E3 enzymes in Tumoregenesis.

| E3 | Linkage | Phenotypic Characteristics | Neoplastic Implications | Substrate | Mechanism Summary |
|----|---------|---------------------------|------------------------|-----------|------------------|
| RNF6 | K63 | maintaining nuclear receptors | promoting cell proliferation | glucocorticoid receptor (GR) | sensitizing the response of prostate cancer (Fan et al., 2020) RNF6 stabilizes GR genes and enhances its transcriptional activity by catalyzing its K63-linked polyubiquitination, promoting MM cell proliferation and survival (Fan et al., 2020) TRIM27 regulates cell apoptosis, cell senescence through mediating the ubiquitination of p21 in breast cancer (Xing et al., 2020) |
| TRIM27 | K27 | suppressing cell senescence | cell cycle dysregulation, tumor cell proliferation and migration | p21 | |
| SPOP | K27 | increasing DNA replication stress | sensitizing cancer cells to ATR inhibition | Geminin | SPOP binding Geminin catalyzes K27-linked polyubiquitination of Geminin, preventing DNA replication over-firing and sensitizing cancer cells to ATR inhibition (Ma et al., 2021) |
| NF-X1 | K33 | regulating glycine metabolism | preventing glioma tumor growth | GLDC | Acetylation of GLDC inhibits its enzymes activity, and facilitates K33-linked ubiquitination by NF-X1, regulating glycine metabolism and tumorgenesis (Ji et al., 2021) |
| HUWE1 | K63 | promoting c-Myc activity | promoting retinoblastoma cell proliferation | c-Myc | HELZ2 triggers K63-linked ubiquitination activity of c-Myc by HUWE1 to mediate retinoblastoma tumorgenesis (Cao et al., 2021) |
| RNF8 | K63 | activating AKT pathway | promoting lung cancer cell proliferation and resistance to chemotherapy | Akt | RNF8 mediates K63-linked ubiquitination of Akt, promoting lung cancer cell proliferation and resistance to DNA damage (Ji et al., 2021) |
| TRIM31 | K63 | stabilizing and activating p53 | inhibiting breast cancer progression | p53 | TRIM31 directly ubiquitinates p53 with K63-linked ubiquitination through its RING domain, activating p53 pathway, suppressing breast cancer progression (Guo et al., 2021) |
| TRIM15 | K63 | activating NF-κB and Akt signaling pathway | regulating cancer cell growth, proliferation, migration | ERK1/2 | TRIM15 mediates K63-linked polyubiquitination of ERK1/2, then activating ERK signaling, leading to cell proliferation, migration and differentiation (Ji et al., 2021) |
| NEDD4 | K63 | regulating cell-cell adhesion, mechanosensing and autophagy | involving in angiogenesis and tumor growth | IGPR-1 | NEDD4 and UbcH6 are involved in the K63-linked ubiquitination of IGPR-1, regulating different cellular activities (Sun et al., 2021) |
| TRIM25 | K63 | activating AKT/mTOR signaling | promoting NSCLC cell survival and tumor growth | PTEN | TRIM25 directly interacts with PTEN and catalyzes its K63-linked ubiquitination, modulating PTEN signaling and involving in cell survival and tumor growth in NSCLC (He et al., 2021) |
| TRIM41 | K63 | innate antiviral response | NF-κB associated cancer | BCL10 | TRIM41 modifies K63-linked polyubiquitination of BCL10, activating NF-κB and TRK1 signaling pathway (Yu et al., 2021) |
| HECTD3 | K63 | regulating inflammation | NF-κB associated cancer | TRAF3 | HECTD3 interacts with TRAF3 via K63-linked polyubiquitination, reducing inflammation and facilitating NF-κB inflammation pathway (Zhou et al., 2021) |
| DZIP3 | K63 | driving cell cycle | promoting cancer progression | Cyclin D1 | DZIP3 stabilizes Cyclin D1 by promoting K63-linked ubiquitination of Cyclin D1, driving cell cycle and cancer progression (Kolapalli et al., 2021) |
| TRIM22 | K63 | activating NF-κB signaling | promoting glioblastoma tumor growth | IκKγ | TRIM22 promotes K63-linked ubiquitination of IκKγ, leading to degradation of IκBα and NF-κB activation (Ji et al., 2021) |
2.2.2 OTULIN Disassembles M1-Linked Poly-Ubiquitin Chains

OTULIN, a methionine 1 (M1)-specific deubiquitase (DUB), is a rare member of the OTU family of DUBs. Its proximal ubiquitin moiety cannot break down isopeptide linkages of ubiquitin chains, but can efficiently cleave peptide bonds present in the linear chains (Keusekotten et al., 2013; Rivkin et al., 2013). OTULIN presents negative regulation in the cellular process of immune homeostasis and inflammation (Damgaard et al., 2019).

Depletion of OTULIN resulted in an increase in the formation of linear Ub chains and demonstrated proteasome dysregulation as the cause of NF-kB positive activation, which in turn restricts bacterial proliferation (Takiuchi et al., 2014; van Wijk et al., 2017). Moreover, the deficiency of OTULIN led to the inability to remove M1-linked poly-ubiquitin signals, which are typically conjugated by the LUBAC, resulting in LUBAC degradation and dysregulation of TNF signaling and cell death (Tokunaga, 2013; Damgaard et al., 2016). Notably, the function of LUBAC is controlled by cytoplasmic HOIP, which interacts with the LUBAC core subunit HOIP to generate Met1-linked ubiquitin. However, this interaction can be weakened by the M1-Ub-specific deubiquitase OTULIN. In addition, through the deubiquitination function of OTULIN, LUBAC can regulate Met1-Ub to ensure an advisable response to innate immune activity (Elliott et al., 2016; Hrdinka et al., 2016). The CYLD/TRAF complex has also been reported to regulate NF-kB-mediated inflammation and interferon signaling, which defines a subset of head and neck cancers that harbor human papillomavirus (Chen T. et al., 2017).

Notably, accumulating evidence has manifested that linear ubiquitin chains play essential roles in ensuring appropriate activity of inflammatory responses and innate immune signaling (Tokunaga and Iwai, 2012; Tokunaga, 2013; Jing et al., 2017). The linear ubiquitination of cFLIP is directly induced by RNF31, a catalytic subunit of LUBAC, at Lys-351 and Lys-353, contributing to TNFα-induced apoptosis, thereby protecting cells from apoptosis (Tang et al., 2018) (Table 3).

2.2.3 Linear Ubiquitination in Tumorigenesis

M1-linked ubiquitination, specifically N-terminal Met1-linked ubiquitination, is able to form eight different inter-ubiquitin linkages via its N-terminal methionine (M1). It can be specifically catalyzed by LUBAC. For instance, EGFR recruits PKP2 to the plasma membrane and cooperates with LUBAC (HOIP), activating linear ubiquitination of NEMO, which is critical for tumor cell proliferation (Hua et al., 2021). Also, Epsins 1/2 promotes NEMO linear ubiquitination via LUBAC, driving breast cancer development (Song et al., 2021). Moreover, LUBAC (SHARPIN) regulated β-catenin activity through linear ubiquitination, promoting gastric tumorigenesis (Zhang et al., 2021). Interestingly, OTULIN exclusively cleaves M1-linked ubiquitination and exhibits a high affinity for linear ubiquitination. LUBAC and linear ubiquitination have been found to be relevant to TNF signaling. Interestingly, OTULIN was shown to remove linear ubiquitination from LUBAC-modified proteins, which is critical for various cellular activities (Draber et al., 2015). Moreover, the deubiquitinating enzyme, CYLD, was also identified for disassembly Met1-linked-Ub (mostly the immune system). A previous report demonstrated that modification of proliferating cell nuclear antigen (PCNA) induces apoptosis and inhibits tumor growth through the linear ubiquitin chain (Qin et al., 2018).

2.3 Lys63 Linkage

2.3.1 The Writer Enzymes for the Lys63 Linkage

It has been well established that Lys63 chains (ubiquitin chains topology lysine 63 poly-ubiquitin linkages) regulate and trigger distinct cellular signaling, including kinase activation, signal transduction, protein trafficking, endocytosis and DNA repair (Spence et al., 1995; Hofmann and Pickart, 1999; Komander and Rape, 2012; Wu and Karin, 2015; Hrdinka et al., 2016). The E2 conjugating enzyme complex Ubc13/Uev1A preferentially assembles the K63-pUb chain (Smith et al., 2013; Zhang et al., 2018). Furthermore, Tax can be recruited to K63-pUb by E3 ligase RNF8 and Ubc13/Uev1A, which allows the activation of TGFβ-activating kinase 1 (TAK1), followed by multiple downstream signaling pathways such as the IKK and JNK pathways. These ultimately lead to DNA damage repair, cytokinesis, and the genomic instability in ATL cells (Ho et al., 2015; Lee et al., 2017). Similarly, tumor necrosis factor receptor associated factor 6 (TRAF6) can interact with the E2 conjugation enzyme Ubc13/Uev1A in a RING-dependent manner, catalyzing Lys63-linked TRAF6 auto-ubiquitination. This activates IKK and NF-kB, thereby promoting TAK1 and IKK to trigger spontaneous osteoclast differentiation (Lamothe et al., 2007a; Lenoir et al., 2021).

Another report also showed that TRAF6, in a RING-dependent fashion, catalyzed auto-ubiquitination by conjugating with ubc13/Uev1A, activating the AKT pathway, and promoting cell migration in breast cancer (Lamothe et al., 2007b; Niu et al., 2021).

The Ubc13/Uev1A complex has been shown to conjugate Lys63-linked poly-ubiquitination of substrate proteins, which contribute to breast cancer metastasis via NF-kB signaling regulation (Wu Z. et al., 2014; 2017). It is also reported that Ubc13 can catalyze K63-linked protein poly-ubiquitination, which is indispensable for the activation of non-SMAD signaling by TAK1 and p38, whose activity controls breast cancer metastatic spread and lung colonization (Wu X. et al., 2014). Interacting with Ubc13, RNF213 mediates auto-ubiquitination and controls inflammatory responses and angiogenic activities (Habu and Harada, 2021). RNF8 was demonstrated to activate Ubc13 and recruit K63-linked poly-ubiquitin conjugation to histones H2A/H2AX, thus contributing to breast cancer predisposition (Vuorela et al., 2011). Moreover, RNF8 utilizes the RING domain, mediating Lys63-linked Ub chains, which is required for DNA double-strand break (DSB) signaling and the downstream BRCA1 tumor suppressor recruitment or lung cancer cell proliferation (Hodge et al., 2016; Xu et al., 2021). Inhibiting the Ubc13/Uev1A complex specifically, which is critical for Lys63-linked ubiquitination, promotes ubiquitin conjugation at the Lys147 site, thereby upregulating NF-kB signaling in multiple myeloma and other
cancers (Gallo et al., 2014). UbcH6 and NEDD4 regulate angiogenesis and tumor growth (Sun et al., 2021). Ube2w accompanies the E3 ligase RNF4 function in distinct DNA repair pathways through Lys63-linked chains and BRIC6 (also named BRUCE) acting on K63-linked ubiquitinylate in unstimulated cells, which regulates the DNA double-strand break response through bridging USP8 and BRCT-repeat inhibitor of hTERT expression (BRIT1) in a deubiquitination manner (Ge et al., 2015; Maure et al., 2016). Poly-ubiquitination of histone H1 depends on Ubc13 and RNF8, which prolong pre-existing ubiquitin modifications to K63-linked chains, thereby stimulating RNF8-Ubc13 mediated DNA damage response (Mandemaker et al., 2017). SKP2 triggers non-proteolytic K63-linked ubiquitination, which is crucial for cancer initiation and progression by positively regulating cancer cell survival and glycolysis. The depletion of SKP2 restricts cancer stem cell proliferation and survival (Chan et al., 2013). However, the non-proteolytic K63-linked ubiquitination triggered by SKP2 can be reversed and modulated by deubiquitination ovarian tumor domain-containing protein 1 (OTUD1) (Yao et al., 2018) and USP10 (Liao et al., 2019). USP10 has been identified as a novel deubiquitinase of SKP2 that modulates and stabilizes SKP2. Indeed, USP10 can recognize and remove Lys63-linked ubiquitin chains from Bcr-Abl, leading to positive activities in chronic myeloid leukemia cells. OTUD1 interacts with p53 and is essential for constant stabilization of p53. Its overexpression dramatically induces the cell cycle and apoptosis (Mevissen et al., 2013; Piao et al., 2017). In addition, several more DUBs have been defined as having linkage specificity for Lys63-linked ubiquitination. CYLD, a tumor suppressor, inhibits NF-κB signaling by cleaving K63-linked ubiquitination of NEMO/IKKγ, thus reducing its stability and averting the IKK complex from phosphorylation of IκB (Chen T. et al., 2017). Furthermore, CYLD was associated with the catalytic LUBAC subunit HOIP to counteract Lys63-Ub and Met1-Ub conjugation to receptor-interacting protein kinase 2 (RIPK2), leading to restriction of innate immune signaling and cytokine production (Hrdinka et al., 2016). TRAF-binding protein domain (TRABID) was demonstrated to bind and cleave Lys63-linked ubiquitin moieties on APC tumor suppressor substrates, which led to the disruption of APC and activation of Wnt signaling in colorectal cancer cell lines (Tran et al., 2008). The USP17/ TRAF2/TRAF3 complex acts to stabilize its client proteins, enhancing inflammatory responses and stemness in lung cancer cells (Lu et al., 2018). USP20 deubiquitinates TRAF6 and Tax, and may function as a key regulator of adult T cell leukemia (ATL) leukemogenesis through suppressing NF-κB activation (Yasunaga et al., 2011). In terms of actively removing Lys63-linked poly-ubiquitin chains on Gβ, OTUD7B appears to be the primary regulator in governing leukemia (ATL) leukemogenesis through suppressing NF-κB and Tax, and may function as a key regulator of adult T cell activation (Yasunaga et al., 2011). In terms of actively removing Lys63-linked poly-ubiquitin chains on Gβ, OTUD7B appears to be the primary regulator in governing leukemia (ATL) leukemogenesis through suppressing NF-κB and Tax, and may function as a key regulator of adult T cell activation (Yasunaga et al., 2011). In terms of actively removing Lys63-linked poly-ubiquitin chains on Gβ, OTUD7B appears to be the primary regulator in governing leukemia (ATL) leukemogenesis through suppressing NF-κB and Tax, and may function as a key regulator of adult T cell activation (Yasunaga et al., 2011). In terms of actively removing Lys63-linked poly-ubiquitin chains on Gβ, OTUD7B appears to be the primary regulator in governing leukemia (ATL) leukemogenesis through suppressing NF-κB and Tax, and may function as a key regulator of adult T cell activation (Yasunaga et al., 2011). In terms of actively removing Lys63-linked poly-ubiquitin chains on Gβ, OTUD7B ap
tumor progression (Ji et al., 2021; Xu et al., 2021; Yu et al., 2021; Zhou et al., 2021; Zhu et al., 2021; He et al., 2022). Lys63 poly-ubiquitin chains were also found to be involved in driving the cell cycle and promoting cancer progression (Kolapalli et al., 2021). In contrast, tripartite motif-containing 31 (TRIM31) directly ubiquitinates p53 via K63-linked ubiquitination, resulting in tumor-suppressing effects (Guo et al., 2021) (Table 2).

2.4 Lys6 linkages—Tumor Suppressors
Lys6-linked ubiquitin chains are less abundant in resting cells and their functional implications are unclear (Durcan et al., 2014; Michel et al., 2017). It has been reported that BRCA1 can be auto-ubiquitinated and then bound by UBX domain containing protein 1 (UBXN1) through K6-linked poly-ubiquitin chains. Interestingly, UBXN1 regulates BRCA1 expression upon ubiquitination. Auto-ubiquitinated forms of BRCA1 act as tumor suppressors and inhibit their enzymatic function (Wu-Baer et al., 2010). Moreover, BRCA1 auto-ubiquitination occurs in a way that the BRCA1/BARD1 complex conducts polymerization by conjugation with K6-linked polymers, which imparts cellular properties to its natural enzymatic substrates (Wu-Baer et al., 2003), further linking BRCA1 auto-ubiquitination to the tumor suppressor. C terminus HSC70-interacting protein (CHIP) was reported to bind to f-actin-associated protein with death domain (FADD) to induce K6-linked poly-ubiquitination of FADD, which was demonstrated to be essential for the prevention of cell death (Seo et al., 2018).

It has also been revealed that community-based learning collaborative (CBLC) assembles K6- and K11-linked poly-ubiquitin on EGFR and positively regulates its stability. The sustained activation of EGFR is largely dependent on CBLC-mediated ubiquitination, and its dysregulation is preferentially destined for membrane recycling, which plays an important role in non-small-cell lung carcinoma (NSCLC) progression (Hong et al., 2018).

Structural findings indicated that USP30 efficiently cleaves Lys6-linked Ub chains, which abrogates parkin-mediated Ub-chain formation in mitochondria. Dysfunction of this novel distal Ub-recognition mechanism is associated with physiological disorders such as hepatocellular carcinoma (Sato et al., 2017; Wang et al., 2022). Although an extraordinary progress has been made over the last 2 decades, the detailed functional consequences of Lys6 modifications require further investigation (Tables 2, 3).

2.5 Lys27 linkages—emerging Tumor Promoter
Emerging investigations have demonstrated that K27-linked poly-ubiquitination is crucial for promoting cancer development, such as facilitating cell proliferation, invasion, and metastasis (Peng et al., 2011; Yin et al., 2019). ITCH, an E3 ubiquitin ligase, was shown to generate Lys27-linked poly-ubiquitination of the transcription factor TIEG1, which inhibits TIEG1 nuclear translocation and subsequent Treg development (Peng et al., 2011). Additionally, TGF-β, in the presence of cytokine IL-6, can efficiently promote mono- and poly-ubiquitination of TIEG1 and modulate Treg/Th17 differentiation (Peng et al., 2011). Yet, it seems that tumor immunity in TIEG1−/− mice were apparently enhanced by hampering Treg development and increasing Th17 response, suggesting its pro-tumor effects (Peng et al., 2011). In the presence of proinflammatory cytokines, ITCH can catalyze BRAF to disrupt 14–3–3-mediated inhibition of BRAF kinase activity, resulting in MEK/ERK signaling activation. This ubiquitin function plays an essential role in supporting the proliferation and invasion abilities of melanoma cells (Yin et al., 2019). Another report connected Lys27-linked ubiquitination to melanoma cell invasive properties, in which HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1 (HACE1) decorated fibronectin with Lys27Ub moieties (El-Hachem et al., 2018). As mentioned in this study, upregulation of fibronectin in turn modulates the transcription levels of integrin subunit alpha V (ITGAV) and integrin δ1 (ITGB1), leading to an increased invasive power of melanoma cells (El-Hachem et al., 2018). TRAF4 was found to increase TrkA kinase activity through K27- and K29-linked ubiquitination upon nerve growth factor (NGF) stimulation, followed by the recruitment of downstream adaptor proteins and increased metastasis in prostate cancer (Singh et al., 2018).

Strikingly, reports have also revealed that Lys27-linked ubiquitination is implicated in the DNA damage response (DDR) and innate immunity. A previous study demonstrated that RNF168 can target the N-terminal tail of histones H2A/H2A.X, generating Lys27-linked ubiquitin chains. In response to DNA damage, K27 ubiquitination is the major source of PTMs that mark chromatin. Meanwhile, the activation of DDR can be inhibited by mutation of K27, which hinders the localization of 53BP1 and BRCA1 to DDR foci (Gatti et al., 2015). DDR is responsible for the recognition, signal transduction and repair of DNA damage. The inactivation of DDR will result in the accumulation of cell mutations and the increase of genomic instability, which play an essential role in cancer initiation (Klinakis et al., 2020). It is also indicated that K27 and the related DDR mediators may be the potential targets for the development of anti-tumor drugs. cGAS is subjected to K27 poly-ubiquitination by RNF185, facilitating cGAS-mediated innate immune response. TRIM-mediated ubiquitination by binding to residue K27 activates TBK1 recruitment to mitochondrial antiviral signaling (MAVS) and promotes innate immunity (Wang Q. et al., 2017). TRIM31 and TRIM40 can also mediate K27-linked ubiquitination, thereby regulating innate and adaptive immunity (Wang X. et al., 2021; Shen et al., 2021).

Specifically, TRIM40 interacts with Riol3, resulting in RIG-I and MDA5 degradation through K27-linked ubiquitination, and negatively regulates innate immunity (Shen et al., 2021). Nevertheless, TRIM31 catalyzes K27-linked ubiquitination of SYK, facilitating antifungal immunity (Wang X. et al., 2021). Additionally, auto-ubiquitination of TRIM23 through K27-linked ubiquitination was found to mediate autophagy via activation of TBK1 (Sparrer et al., 2017). Moreover, E3 ubiquitin ligase Hectd3 decorates Sta3 with non-degradative K27-linked poly-ubiquitin chains and Malt1 with K27/K29-linked poly-ubiquitin chains, leading to signaling-related ubiquitination in neuroinflammation (Cho
et al., 2019) (Table 3). In fact, the innate immune system plays a key role in the formation of tumor. The response of it is generally affected by a variety of immune cells and cytokines in tumor microenvironment (Wenbo and Wang, 2017). In this way, it is beneficial to clarify the regulatory effects of K27 on tumor innate immunity for understanding the mechanisms of cancer.

2.6 Lys29 and Lys33 linkage—possible Tumor Promoters

Recent proteomic data have identified the role of Lys29-and Lys33-linked ubiquitin chains in various biological processes, including the control of AMPK-mediated mitochondrial function and Wnt-induced transcription signaling (Jiang et al., 2015; Nusse and Clevers, 2017). AMPK-related kinases, AMPK-related kinase 5 (ARK5, also known as NUAK1) and microtubule-affinity-regulating kinase 4 (MARK4), which are mediated by unconventional Lys29/ Lys33 linkage, are involved in cell polarity and proliferation (Al-Hakim et al., 2008). One of the underlying mechanisms is that USP9X specifically identifies Lys29/Lys33-conjugated ubiquitin chains on NUAK1 and MARK4 (Al-Hakim et al., 2008). However, it should be noted that the ubiquitination of NUAK1 and MARK4 suppresses their phosphorylations rather than restores their stability and facilitates LKB1 activation. Interestingly, the TRABID core domain N-terminal Npl4-like zinc finger (NAZF1) preferentially hydrolyzes K29/K33-linked diUb, and this novel AnkUBD displays TRABID linkage specificity (Licchesi et al., 2011). Additionally, TRABID interacts with APC tumor suppressor protein, recruits TCF target genes, and activates their transcription in colorectal cancer cells (Tran et al., 2008). The OUT family DUB TRABID preferentially abolishes Smad ubiquitination regulatory factor 1-induced K29/K33-linked poly-ubiquitin chains from UV radiation resistance associated gene (UVRAG), thereby promoting autophagosome maturation and inhibiting cell proliferation in hepatocellular cancer (Feng et al., 2019). Considering its linkage specificity, it would be insightful to explore the potential positive regulation of TRABID in cancer tumorigenesis via these two pathways.

K29-linked ubiquitin chains play important roles in driving cancer invasion and metastasis, as well as in the positive regulation of immunity (Singh et al., 2018; Cho et al., 2019; Gao et al., 2021). Several studies have also manifested that, with the assistance of Cbl-b and ITCH, T cell receptor-zeta (TCR-zeta) was decorated with a K33 linage, accompanied by positively activated T cells and immune responses (Huang et al., 2010). In addition, OTUD1 directly deubiquitinates the inhibitor SMAD7 of TGF-β pathway and aborates Lys33-linked poly-ubiquitin chains, which inhibits cell stemness and suppresses metastasis (Zhang et al., 2017). Acetylation of GLDC inhibits its enzymatic activity and facilitates K33-linked ubiquitination by NF-X1, thereby suppressing glioma tumor growth (Liu et al., 2021). Ultimately, Lys29-and Lys33-linked ubiquitin chains appear to have more complicated cellular functions that remain to be characterized (Table 2, 3).

2.7 Mixed Linkage and Branched Poly-Ubiquitination

Mixed linkage chains send mixed signaling messages that can be identified by different linkage-specific receptors. Our understanding of the mixed linkages and branched poly-ubiquitin is limited. Previous reports have elucidated that Tax in combination with UbcH2, Uhc5c, or UbcH7, can catalyze the construction of free mixed-linkage poly-ubiquitin chains, which are responsible for IKK-NF-κB activation and induction of T cell transformation (Wang et al., 2016). Furthermore, Brcc 36 isopeptidase complex (BRISC), the JAMM/MPN + family of DUBs, preferentially cleaves K63 linkages within mixed-linkage chains. In addition, RING1B was found to generate atypical mixed poly-ubiquitin chains and mediate mono-ubiquitination of H2A (Ben-Sadon et al., 2006). A recent study showed that SPOP triggered mixed-linkage ubiquitination of Myd88 in human lymphoma cells and mouse HSCs, suggesting that the SPOP-Myd88 pathway plays a critical role in hematopoietic neoplasms (Jin et al., 2020).

3 CONCLUSION AND FUTURE PERSPECTIVES

Non-proteolytic ubiquitination, the molecular switch in cell fate regulation, plays a crucial role in post-translational protein modifications. Diverse ubiquitination enzymes are essential for ubiquitination linkages that are necessary for normal metabolism and physiological functions. Meanwhile, it is also the root cause of physiological disorders, such as cancer. The aberrant regulation of the UPS is typically achieved by ubiquitination enzymes, DUBs, 20S proteasome catalytic core particles and 19S proteasome regulatory particles (Rape, 2018). In general, specific ubiquitination enzymes determine ubiquitin linking with one of the seven lysine residues to form distinctive styles of poly-ubiquitin chains, deciding the fate of substrate proteins. It is noteworthy that the ubiquitin proteasome system functions as a theoretical target for drug screening, and the study of ubiquitination will provide more insights into the development of anti-tumor drugs. Recently, the quantities of E3s inhibitors have already been processed in preclinical models of cancer immunotherapy. It has been shown that E3 enzyme Smac mimetics (SMs) were the promising immune modulators for cancer therapy as the antagonists targeting E3 ligases IAPs (Cosu et al., 2019). The small molecule inhibitor AMG-232, targeting another E3-ubiquitin ligase oncogenic mouse double minute 2 homolog (MDM2), was shown to strengthen T cell killing of cancer cells, especially when combined with an anti-PD-1 monoclonal antibody (Sahin et al., 2020). Several other proteasome inhibitors have been confirmed to be clinically effective against malignancies as well. The neddylation (NAE) inhibitor pevonedistat has already been tested in multiple clinical trials and has shown positive effects in patients with AML or advanced solid tumors (Barghout and Schimmer, 2021). Small molecule inhibitors based on deubiquitinase have been widely used in experimental anti-tumor therapy, most of which are still
in the preclinical research stage. Due to dose-limiting toxicity, the first deubiquitinase inhibitor VXL1570 was terminated in the clinical trial phase. No other deubiquitinase inhibitors have been approved for clinical studies since then. Also, the discovered deubiquitinase inhibitors related to tumor treatment are mainly concentrated in the USP family, and the relationship between the inhibitors of non-USP family members and the treatment of malignant tumors needs to be further studied (Zhang et al., 2022).

Interestingly, the innovative approaches of proteolysis targeting chimeras (PROTACs) and molecular glues might facilitate clinical cancer therapy (Cruz Walma et al., 2022; Kung and Weber, 2022; Zhou and Xu, 2022). Consequently, it is essential further to investigate the role of ubiquitinization enzymes in tumorigenesis. Meanwhile, targeting different ubiquitination, including K6-, K27-, K29-, K33-, K63-, and M1-linked poly-ubiquitination, may also be one of the directions for cancer drug discovery. Although great progresses have been achieved with the development of anti-cancer drugs aimed at the UPS, numerous challenges still stand in the way. Some candidate inhibitors have emerged as drug resistant or have limited efficacy in patients. Despite these questions, the discovery of new drugs targeting single or multiple segments of the UPS is still worthy of future research.

**AUTHOR CONTRIBUTIONS**

Conceptualization, SJ and JH; writing original draft preparation, XY; writing review and editing, QL, FL, SJ and JH; visualization: XT and TY; supervision: SJ and JH; funding acquisition: SJ and XY. All authors have read and agreed to the published version of the manuscript.

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