

REVIEW

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# Exploiting E3 ubiquitin ligases to reeducate the tumor microenvironment for cancer therapy

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## Abstract

Tumor development relies on a complex and aberrant tissue environment in which cancer cells receive the necessary nutrients for growth, survive through immune escape, and acquire mesenchymal properties that mediate invasion and metastasis. Stromal cells and soluble mediators in the tumor microenvironment (TME) exhibit characteristic anti-inflammatory and protumorigenic activities. Ubiquitination, which is an essential and reversible posttranscriptional modification, plays a vital role in modulating the stability, activity and localization of modified proteins through an enzymatic cascade. This review was motivated by accumulating evidence that a series of E3 ligases and deubiquitinases (DUBs) finely target multiple signaling pathways, transcription factors and key enzymes to govern the functions of almost all components of the TME. In this review, we systematically summarize the key substrate proteins involved in the formation of the TME and the E3 ligases and DUBs that recognize these proteins. In addition, several promising techniques for targeted protein degradation by hijacking the intracellular E3 ubiquitin-ligase machinery are introduced.

**Keywords** Tumor microenvironment, Ubiquitination, Deubiquitinases, Cancer therapy, SUMOylation

## Background

The tumor microenvironment (TME) refers to a complex ectopic ecosystem composed of multiple cell types (immune cells, fibroblasts, endothelial cells), as well as extracellular components, including extracellular matrix (ECM) and secreted mediators (growth factors and cytokines, chemokines) [1]. Bidirectional communication

between tumor cells and the TME is not only directly responsible for the genesis, progression and metastasis of tumors but is also closely related to the therapeutic efficacy and long-term prognosis of patients [2–4]. Although nonmalignant stromal cells in the TME have the same origin as adjacent normal tissue cells, they can be hijacked by tumor cells to create a protumorigenic microenvironment along with tumor cells. Various signaling pathways, transcription factors, and key enzymes are involved in adjusting the TME, which is a highly complex network.

Ubiquitination is the most common posttranscriptional modification after phosphorylation, in which 76-amino acid ubiquitin molecules are covalently conjugated to the substrate protein [5, 6]. Ubiquitin (Ub), which is a highly conserved modifier, is attached to substrate proteins by a cascade of three enzymes: E1 Ub-activating enzyme, E2 Ub-conjugating enzyme, and E3 ubiquitin ligase [7]. First, E1 activates ubiquitin in an ATP-dependent manner and

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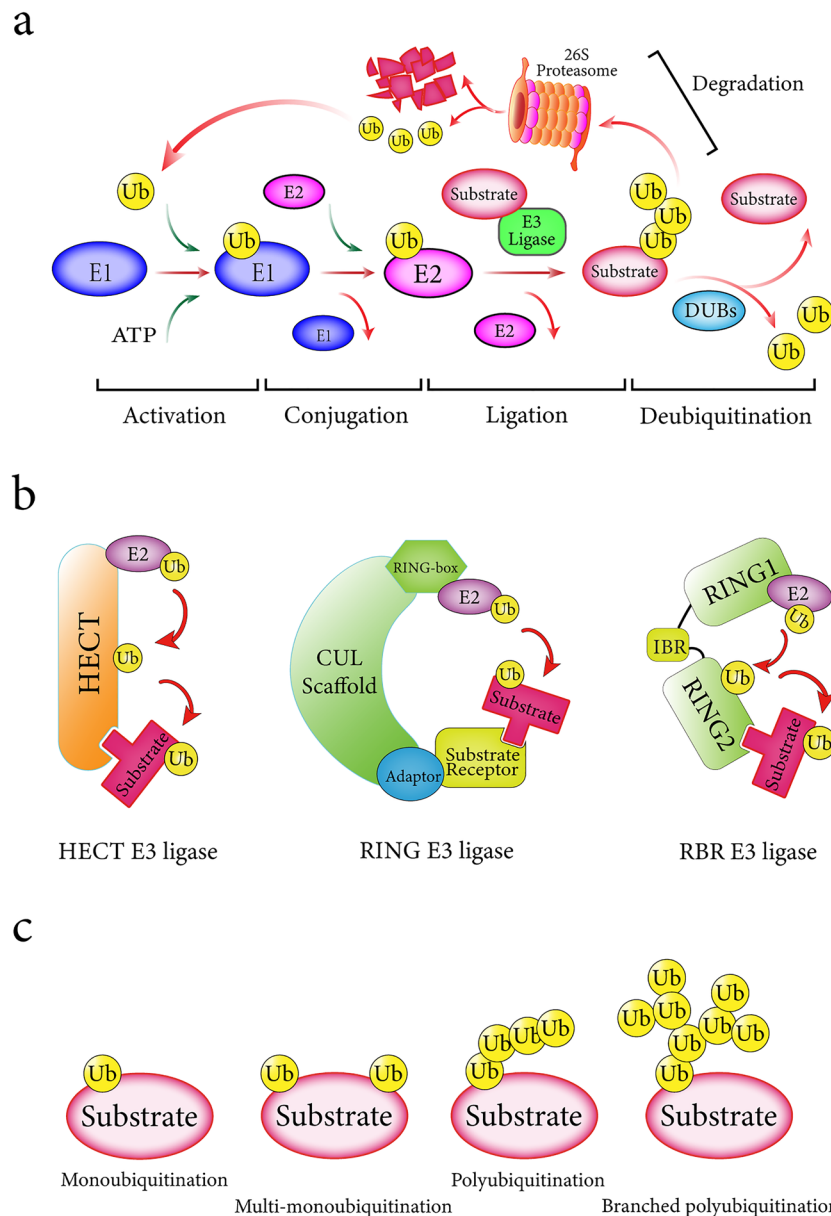
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then transfers it to E2 via a transesterification reaction. Finally, E3 bridges the Ub-loaded E2 and target protein to facilitate the binding of ubiquitin with a substrate lysine (K), resulting in protein ubiquitination. E2 ligases are more than just carriers, showing specificity in E2-E3 pairings and regulating the progressivity and topology of ubiquitin chain assembly [8, 9]. Ubiquitin molecules can

be cleaved from ubiquitin-modified proteins by a super-family of DUBs (Fig. 1a) [10].

By regulating the quantity and quality of modified proteins, ubiquitination mediates almost all vital biological processes in eukaryotic cells, such as embryonic development, cell cycle control, and immunity [11]. If the ubiquitination process is abnormal, the disruption of protein



**Fig. 1** The ubiquitination process and ubiquitin code. **a.** The cascade of ubiquitin modification. Ubiquitin modification involves three successive steps catalyzed by three types of enzymes: (1) E1 ubiquitin-activating enzyme activates ubiquitin in an ATP-dependent manner; (2) the activated ubiquitin protein is transferred to E2 ubiquitin- conjugating enzyme via a transesterification reaction; (3) E3 ubiquitin ligase bridges the ubiquitin-loaded E2 and targeted protein to facilitate the binding of ubiquitin with a substrate lysine. These attached ubiquitin molecules can be cleaved by deubiquitinases from substrates. Polyubiquitin chains represented by the K48 linkage will lead to 26S proteasome-mediated proteolysis. **b.** Differential catalytic domains and transfer modes of three classes of E3 ligases. **c.** Topological versatility in protein ubiquitination

homeostasis can ultimately lead to cellular dysfunction or even carcinogenesis [12, 13]. Notably, studies indicate that ubiquitination is widely involved in the regulation of the TME [14, 15]. Given the complexity and significance of the TME and ubiquitination, insights into the exact mechanisms by which ubiquitination regulates the TME would not only shed light on tumorigenesis but also help identify potential therapeutic targets. The present review first summarizes how E3 ligases and DUBs dynamically regulate a variety of key cellular proteins that play critical roles in the TME. Then, the latest and attractive techniques that take full advantage of the inherent ubiquitin–proteasome system (UPS) to degrade targeted oncoproteins and reeducate the TME are introduced.

### E3 ubiquitin ligases and degrons within substrate proteins

There are over 600 identified E3 ligases, and these proteins are the largest and most critical components of the three classes of enzymes, determining the specificity and efficiency of substrate ubiquitination [16]. Increasing evidence suggests that abnormalities in E3 activity and subcellular localization are closely associated with various human diseases [12, 17]. Based on differences in the catalytic domains and process of ubiquitin transferring, E3 ligases are generally divided into three broad categories: RING (really interesting new gene), HECT (homologous to E6-AP carboxyl terminus), and RBR (ring-between-ring) E3 ligases (Fig. 1b) [18]. As the largest subgroup, RING E3 ligases can recruit Ub-loaded E2 ligases and substrates together to mediate the direct transfer of ubiquitin molecules [19]. In contrast, HECT E3 ligases possess a specific HECT domain containing a catalytic cysteine residue, which catalyzes the transesterification reaction of ubiquitin charged by E2 to form a transient Ub-E3 intermediate before transferring ubiquitin to the substrate [20]. Recently, hybrid RING/HECT E3 ligases known as RBR E3 ligases were found to simultaneously exhibit the functional characteristics of both RING and HECT types. Typically, RBR E3 ligases consist of two RING domains, in which the N-terminal RING1 domain can recruit Ub-loaded E2 in a RING E3 ligase-dependent manner, and the C-terminal RING2 domain possesses a catalytic cysteine similar to HECT E3 ligases [21].

Substrates are selectively recognized by E3 ligases in a key-lock manner. Therefore, what confers the specificity of the substrate? Degrons are a group of short peptide motifs that serve as the minimal but sufficient elements to allow recognition by the degradation mechanism within substrate proteins [22]. E3 ligases typically target the degrons of damaged, misfolded, mislocalized or redundant proteins for proteolysis via the UPS, which maintains protein homeostasis in cells [22, 23]. Intriguingly, fusing a degron element with a long-lived protein

significantly reduces its half-life and confers instability, suggesting the potential transferability of degrons [24]. Degrons tend to be concentrated at both terminals of proteins rather than in the middle regions due to their intrinsically disordered property [25]. Although the first characterized degrons were the N-degrons at the N-terminals of short-lived proteins, an increasing number of C-terminal degrons have been identified [26–28]. Post-transcriptional modifications of degron, which are most commonly kinase-mediated phosphorylation, significantly alter the affinity of E3-degron binding, which can be exploited to integrate upstream signals and time protein degradation [29–31].

### The ubiquitin code and fate of proteins

Ubiquitin labeling of proteins is a sophisticated process known as the ubiquitin code that involves initial substrate lysine selection and ubiquitin chain elongation (Fig. 1c). Sometimes, one or more ubiquitin molecules are individually attached to substrate lysine residues in a one-to-one manner, which is defined as monoubiquitination or multi-monoubiquitination [32, 33]. As the simplest ubiquitination type, monoubiquitination was previously thought to regulate cellular processes, including DNA damage repair, histone functions, chromatin remodeling and receptor endocytosis, by affecting protein activity, localization and interaction [34, 35]. However, emerging evidence suggests an underestimated proteolytic role [36]. After monoubiquitination, another ubiquitin can use the different lysine residues or N-terminal methionine of linked ubiquitin as an anchor point, forming polyubiquitin chains. Because each ubiquitin molecule contains seven lysine residues and a particular N-terminal methionine (K6, K11, K27, K29, K33, K48, K63, and M1, respectively), insights into the diversity of ubiquitin linkages will help to further elucidate the relationship between ubiquitin encoding and functional consequences [37].

As the two most abundant linkage types, Lys48-linked polyubiquitination typically serves as a degradation signal for the 26S proteasome, while Lys63-linked chains play a nonproteolytic role in cellular processes [38]. Lys11-linked polyubiquitination participates in degradation of cell-cycle proteins during mitosis [39]. Similar to Lys63, Lys6-linked polyubiquitination is related to the DNA damage repair process but is not involved in degradation [40]. In addition to polyubiquitination, recent studies have identified the widespread presence of branched polyubiquitination, that is, the formation of branching chains on the basis of polyubiquitination. K11/K48-linked branched polyubiquitination is catalyzed by the APC/C E3 ligase complex, thereby promoting protein degradation to regulate the mitotic process

[41, 42]. Notably, polyubiquitination chains exhibit different conformational flexibilities: extended or compact conformations, which potentially affect the fate of substrate proteins [43, 44].

### **SUMOylation, neddylation and the deubiquitination process**

Since the first discovery of ubiquitin in the 1970s, a series of ubiquitin-like (UBL) homologous small molecules have also been discovered, most notably SUMO and NEDD8. Each of the UBL modifiers can be catalyzed by an enzymatic cascade reaction similar to ubiquitination to covalently bind to substrate proteins [45]. Alternatively, ubiquitin-like proteins are able to mix in the polyubiquitin chain, which further increases the structural complexity of polyubiquitin chains [6]. In the human proteome, the SUMO family includes five SUMO paralogs (SUMO1-5), among which SUMO1, 2 and 3 exhibit ubiquitin-like protein modification functions [46]. Although E3 SUMO ligases are far fewer in number than E3 ubiquitin ligases, there are more than 500 SUMO substrates [47]. By regulating the stability, activity, and localization of these downstream substrates, SUMOylation orchestrates various vital facets of cellular processes [46]. Analogously, NEDD8-mediated posttranscriptional modification is known as neddylation, and cullin-family members are the best-known neddylation substrates. As the key subunit of Cullin-RING ligases (CRLs), the neddylation of cullin proteins can stabilize and activate the ubiquitination activity of CRLs [48, 49].

Similar to other posttranscriptional modifications, such as phosphorylation and acetylation, cellular protein ubiquitination appears to be a dynamic and reversible process that involves antagonism between E3 ligases and DUBs to precisely regulate intracellular protein homeostasis [50]. As a specific kind of protease, DUBs are capable of hydrolyzing the iso-peptide bond on polyubiquitin chains or ubiquitylated substrates and the particular peptide bond between ubiquitin and the N-terminal Met of another ubiquitin [51]. According to their sequence and domain homology, more than 100 DUBs in the human genome can be categorized into seven major families: USP, UCH, OTU, MJD, ZUFSP, MINDY, and JAMM [52]. In addition to reversing or reediting ubiquitin signals, DUBs play a vital role in processing ubiquitin precursors and cleaving polyubiquitin chains to maintain an adequate free ubiquitin pool [53]. Coincidentally, some cysteine proteases can remove UBL molecules from substrates; sentrin/SUMO-specific proteases (SENPs) can selectively target SUMO conjugation in humans [54].

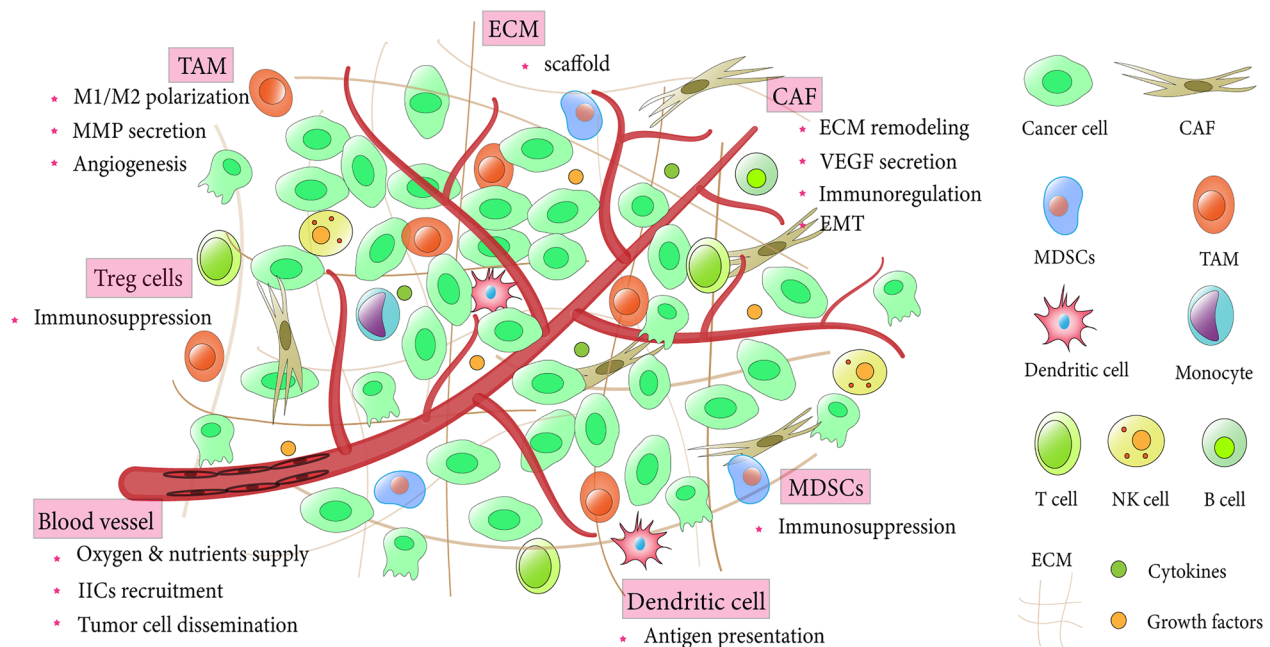
### **Major components of the tumor microenvironment**

Although cancer-related studies have previously focused on alterations in downstream proto-oncogenic pathways due to aberrant genetic mutations in cancer cells, the contribution of the TME to tumor phenotypes has received increasing attention in the last two decades. In addition to malignant tumor cells, the TME is composed of multiple stromal cells and complex non-cellular constituents, which are nourished by a limited or poorly differentiated vasculature [2]. Stromal cells mainly include infiltrating immune cells (IICs), cancer-associated fibroblasts (CAFs), and cancer-associated endothelial cells (CAEs); ECM and other secreted mediators such as growth factors and cytokines are the most representative acellular components (Fig. 2) [1]. In the TME, bidirectional communication between tumor cells and their surrounding stroma collectively reconstructs the ectopic and tumorigenic organoid niches that not only participate in primary tumor growth and survival but also facilitate tumor progression, metastasis and drug resistance [3, 55].

### **Cancer-associated endothelial cells**

In 1971, Judah Folkman first proposed a revolutionary viewpoint that neoplasm growth depended on angiogenesis [56]. Angiogenesis is involved in the activation of cancer-associated endothelial cells from the preexisting vasculature to form new blood vessels to meet the cellular demands for oxygen and nutrients under physiological and pathological conditions. As the tumor expands (in diameter), the incompatibility between vascular supply and tumor metabolism leads to hypoxia in the TME, which in turn induces the synthesis of a series of proangiogenic factors in tumor cells and the surrounding stroma [57]. Among these proangiogenic factors, vascular endothelial growth factor-A (VEGF-A), a potent proangiogenic factor, directly initiates proangiogenic signaling pathways in vascular endothelial cells by binding to its receptor VEGFR2 (VEGF receptor 2) [58, 59]. However, excessive and sustained VEGF-VEGFR2 signaling in malignancies leads to architecturally abnormal angiogenesis, which is characterized by irregular shape, poor differentiation, loose interendothelial contact, a defective basement membrane and reduced pericyte coverage [60]. These features are not only detrimental to adequate vascular perfusion of the tumor area but also greatly impair the vascular barrier, which allows blood extravasation and metastatic dissemination of cancer cells under high interstitial fluid pressure.





**Fig. 2** Major components of the tumor microenvironment. The tumor microenvironment refers to a complex and tumorigenic ecosystem comprised of multiple cell types (immune cells, fibroblasts, endothelial cells), as well as extracellular components including extracellular matrix (ECM) and secreted mediators (growth factors and cytokines, chemokines). Tumor cells can communicate bidirectionally with the surrounding tumor microenvironment and are nourished by limited or poorly differentiated blood vessels

### CAFs

As a major stromal cell type, CAFs can remodel the TME through the synthesis and degradation of its components, release diverse growth factors and cytokines to drive angiogenesis and tumor progression, and interfere with therapeutic responses by secreting soluble mediators [61]. Histopathological studies have indicated an association between CAF abundance and adverse clinical outcomes among a variety of human malignancies [62, 63]. CAFs are initially derived from local tissue fibroblasts that are activated by diverse tumor-derived stimuli and can be further amplified via cell proliferation, resulting in the dominant source of CAFs [64]. In addition, the differentiation of mesenchymal stem cells and the conversion from resident epithelial or endothelial cells via epithelial-mesenchymal transition (EMT) or endothelial-mesenchymal transition (EndMT) has also been observed [65, 66]. Recently, the role of CAFs in immunoregulation has been gradually appreciated. By secreting various cytokines, such as CXCL12, and expressing specific surface molecules, CAFs participate in the recruitment, accumulation and maturation of Treg cells, thus inhibiting T effector responses [67]. Despite the potential immune-promoting effects of CAFs, their superior immunosuppressive effect has been widely accepted.

### Immune cells

As early as 1863, Rudolf Virchow observed leukocyte infiltration in neoplastic tissues and further speculated the inflammatory origin of cancer [68]. Since then, a myriad of studies have been dedicated to elucidating the association between chronic inflammation and tumors, which are referred to as “wounds that do not heal”. The chronic inflammatory response suggests that the immune system does not ignore the tumor as a heterogeneous new organism but attempts to restore tissue homeostasis instead by damaging tumor cells [69]. These inflammatory environments contain a wide spectrum of IICs, which can be roughly divided into innate and adaptive immune cells. Among these IICs, tumor-associated macrophages (TAMs) are the most abundant innate immune cells, originating from bone marrow-derived monocytes and resident macrophages [70]. Currently, the subtype classification of TAMs proposed by Mills’ group is widely accepted, in which TAMs are categorized into M1 (classically activated) and M2 (alternatively activated) subtypes according to different polarization states [71]. M1 macrophages are induced by Th1 cytokines, particularly IFN- $\gamma$ , and are involved in Th1-type immune responses, as they can mediate antigen presentation and secrete nitric oxide, reactive oxygen species (ROS), interleukins, and TNF- $\alpha$ , which are proinflammatory and antitumorigenic.

In contrast, M2 macrophages, which are activated by Th2 cytokines such as IL-4, IL-10 and TGF- $\beta$ , participate in Th2-type immune responses and show widespread anti-inflammatory and protumorigenic effects [72–74].

A critical hallmark of cancer is that immune cells in the TME become defective in response to tumor cells, and the host's immune system becomes ineffective. Regulatory T (Treg) cells called suppressive T cells are a small class of specific CD4+ T cells with high CD25 expression, constituting 5–10% of human peripheral CD4+ T cells [75, 76]. Under physiological conditions, Treg cells are responsible for inhibiting the activation of self-reactive lymphocytes and maintaining tissue homeostasis via peripheral immune tolerance. However, Treg cell infiltration into the TME is thought to prevent tumor-associated antigen presentation and suppress the antitumor immune response [77]. In 2003, three laboratories simultaneously identified Forkhead Box P3 (Foxp3) as a core transcription factor in Treg cells, which dominates Treg cell development and immunosuppressive function [78–80]. Noticeably, deletions or mutations in Foxp3 genes cause vigorous peripheral infiltration of T effector cells in mice and severe autoimmune disease in humans [80, 81]. Similar to Treg cells, myeloid-derived suppressor cells (MDSCs) also compromise major mechanisms of immunosurveillance to promote tumor progression; these cells are a heterogeneous population of precursors of macrophages, dendritic cells, and granulocytes [82].

#### **Role of ubiquitination and SUMOylation in TME modulation**

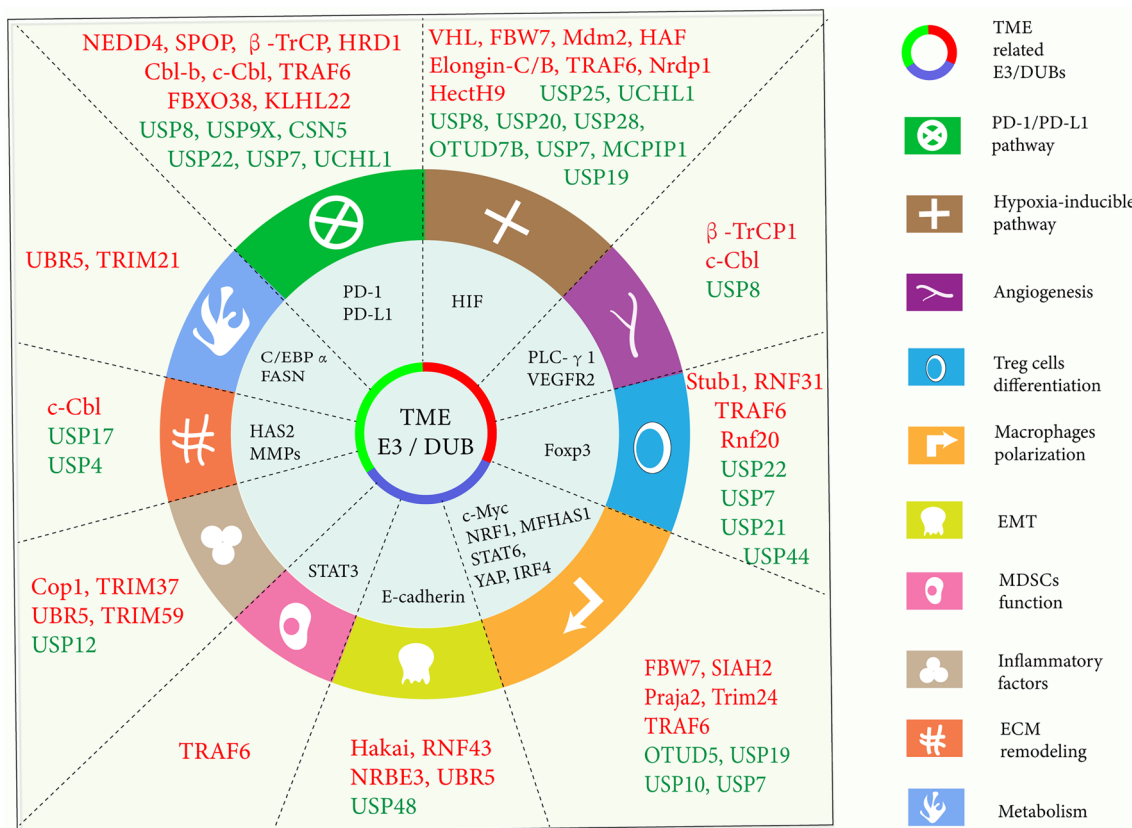
Abundant evidence has indicated that the tumorigenesis and progression are related to a variety of protumorigenic processes in the TME, including immune escape, angiogenesis, hypoxia, and the degeneration of immune cells and stromal cells. The complex interaction between the TME and tumor cells contributes to these biological changes in multiple components of the TME. Although the mechanism of the malignant transformation of the TME is complex, it often requires a series of core intracellular and extracellular factors, enzymes and transcription factors mediating signaling pathways to control the function of TME. Thus, identifying these key proteins and understanding the factors that affect their protein levels can provide ideas for studying the mechanism of TME formation and provide clues for subsequent drug discovery. Ubiquitin ligase-induced ubiquitination, which is the most important pathway that regulates protein degradation in cells, cooperates with DUBs to maintain protein homeostasis. Many studies have shown that the UPS closely regulates these biological processes and maintains the TME in an immunosuppressive state (Fig. 3). The E3 ubiquitin ligases and DUBs that regulate

these key proteins, especially in specific TME, are summarized (Table 1).

#### **PD-1/PD-L1 pathway**

Immune responses mediated by T-lymphocytes are indispensable means against tumor cells, involving the formation of CD8+ cytotoxic T cells and the acquisition of helper functions by CD4+ cells [2]. Unfortunately, certain tumors have evolved various escape mechanisms to evade immune surveillance, one of which involves the activation of inhibitory receptors known as immune checkpoints on the surface of these T-effector cells [83, 84]. The binding of related ligands to immune checkpoints can negatively regulate T-cell migration, proliferation and antitumor activity, which is characterized by an exhausted phenotype [85]. In the TME, the PD-1/PD-L1 axis is an important representative immune checkpoint pathway; its potential clinical value has attracted considerable attention in recent years [86, 87]. PD-L1 (programmed death-ligand 1) is widely expressed on the surface of cancer cells, ICCs including myeloid cells and T cells, and even tumor-associated nerves, and its expression is higher than that in adjacent normal tissues [88, 89].

Increasing evidence has revealed that multiple E3 ligases play critical roles in regulating PD-1/PD-L1 levels via ubiquitination [90, 91]. In bladder cancer cells, NEDD4, a HECT-type E3 ligase, can be phosphorylated by fibroblast growth factor receptor 3 to increase its Lys48-linked polyubiquitination activity for PD-L1 [92]. PD-L1 also serves as a substrate for the adaptor protein SPOP in Cullin3-Ring E3 ligases. Intriguingly, PD-L1 abundance is dynamically regulated during cell cycle progression because the Cyclin D-CDK4-mediated phosphorylation of SPOP effectively protects SPOP from degradation by APC/CCdh1 E3 ligase [30]. Skp1-Cul1-F-box (SCF) E3 ligase complexes, a well-characterized class of CRLs, need to target typical phospho-degrons in substrate proteins for ubiquitination. As the receptor subunits of SCF complexes, variable F-box proteins are able to selectively bind a distinct subset of substrates [93]. Phosphorylation mediated by glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) is required to induce the recognition of PD-L1 by  $\beta$ -TrCP, a classic F-box protein. The glycosylation of PD-L1 can suppress contact between PD-L1 and GSK3 $\beta$ , indirectly stabilizing the PD-L1 protein [94]. In particular, PD-L1 in the endoplasmic reticulum (ER) can be phosphorylated at its S195 residue in a metformin-induced manner, which obstructs ER-to-Golgi translocation of PD-L1. Subsequently, aberrant ER accumulation enhances polyubiquitination-dependent proteasomal degradation of PD-L1 via the ER-associated degradation (ERAD) pathway, in which HRD1 plays an important role



**Fig. 3** The role of E3 ligases and DUBs in the TME. The UPS deeply regulates the immunosuppressive TME, involving a variety of biological processes such as immune escape, angiogenesis, hypoxia, EMT, and degeneration of immune cells and stromal cells. Mechanistically, E3 ligases (shown in red) and DUBs (shown in blue) collectively target core factors, enzymes and transcription factors in these pro-tumorigenic processes

as an ERAD E3 ligase [95]. These four ubiquitin ligases all control PD-L1 protein levels by directly affecting the proteolysis of ubiquitin-modified PD-L1. However, Cbl-b and c-Cbl, which are monomeric RING-type E3 ligases, indirectly downregulate PD-L1 expression by inhibiting the activation of STAT3/AKT/ERK signaling in lung cancer [96]. In contrast, TRAF6 ligase positively regulates PD-L1 stability by catalyzing K63-linked polyubiquitination rather than the degradable K48-type linkage, which is abrogated by USP8 [97].

It is worth noting how DUBs reversely regulate PD-L1 polyubiquitination. Among them, USP9X, CSN5, USP22 and USP7 are responsible for the deubiquitination and stabilization of PD-L1 in tumor cells [98–101]. As a mechanism by which TNF- $\alpha$  inhibits T-cell surveillance, the TNF- $\alpha$ -activated transcription factor p65 mediates the transcriptional activation of CSN5, thereby inhibiting the ubiquitination and proteolysis of PD-L1 [99]. In particular, the UCH subgroup’s DUB UCHL1 promotes PD-L1 transcription through the AKT-p65 axis, in which p65 recognizes and activates the promoter of the PD-L1 gene [102]. Analogously, various E3 ligases, including

c-Cbl, FBXO38 and KLHL22, are involved in the K48-link ubiquitination of PD-1 (programmed death protein-1). In tumor-infiltrating T cells, these E3 ligases are markedly reduced and T-cell function is significantly suppressed [103–106].

**Hypoxia-inducible factor 1 (HIF-1)**

In response to characteristic hypoxic stress in the TME, tumor cells trigger a variety of signaling pathways in which oxygen-sensitive HIF-1, a crucial transcriptional activator, can upregulate the transcription of over 100 hypoxia-inducible genes. These genes include VEGF, erythropoietin, lactate dehydrogenase A and glucose transporters, which orchestrate angiogenesis, erythropoiesis, anaerobic metabolism, tumor immune evasion, and other events in the tumor region [107–109]. HIF-1 functions as a heterodimer composed of a regulatory HIF-1 $\alpha$  subunit and a constitutive HIF-1 $\beta$  subunit. Under normoxic conditions, HIF-1 $\alpha$  is readily hydroxylated on its conserved Pro402/Pro564 residues and is subsequently ubiquitinated by VHL E3 ligase for proteolysis [110]. However, prolyl-hydroxylation of HIF-1 $\alpha$  is

**Table 1** Summary of E3 ligases and deubiquitinases involved in the tumor microenvironment

| Abbreviation    | Full name  | Substrate                          | Refs                     |
|-----------------|--|------------------------------------|--------------------------|
| E3 ligases      |  |                                    |                          |
| NEDD4           | Neuronal precursor cell expressed developmentally down-regulated 4 | PD-L1                              | [92]                     |
| SPOP            | Speckle-type BTB/POZ protein                                       | PD-L1                              | [30]                     |
| β-TrCP          | β-transducin repeat-containing protein                             | PD-L1, VEGFR2                      | [94, 135]                |
| HRD1            | HMG-coA reductase degradation protein 1                            | PD-L1                              | [95]                     |
| Cbl-b           | Casitas B lymphoma-b   |                                    | [96]                     |
| c-Cbl           | c-Caritas B cell lymphoma  | PD-1, PLC-γ1, MMP2                 | [96, 103, 139, 203]      |
| TRAF6           | Tumor necrosis factor receptor-associated factor 6                 | PD-L1, HIF-1α, STAT6, Foxp3, STAT3 | [97, 118, 149, 160, 188] |
| FBXO38          | F-box protein 38   | PD-1                               | [104, 105]               |
| KLHL22          | Kelch-like family member 22  | PD-1                               | [106]                    |
| VHL             | Von Hippel-Lindau  | HIF-1α                             | [110]                    |
| Siah1/2         | Seven in absentia homologue 1/2                                    | PHDs                               | [111]                    |
| FBW7            | F-box and WD repeat domain-containing 7                            | HIF-1α, c-Myc                      | [112, 145]               |
| Mdm2            | Mouse double minute 2  | HIF-1α                             | [114, 115]               |
| HAF             | Hypoxia-associated factor  | HIF-1α                             | [116]                    |
| Nrdp1           | Neuregulin receptor degradation protein-1                          | USP8                               | [123]                    |
| HectH9          |  | USP7                               | [125]                    |
| β-TrCP1         | β-transducin repeat-containing protein 1                           | VEGFR2                             | [134]                    |
| SIAH2           | Seven in absentia homolog 2  | NRF1                               | [146]                    |
| Praja2          |  | MFHAS1                             | [147]                    |
| Trim24          | Tripartite motif containing protein 24                             | CBP                                | [148]                    |
| Stub1           | STIP1 homology and U-box-containing protein 1                      | Foxp3                              | [157]                    |
| RNF31           | Ring finger protein 31   | Foxp3                              | [158]                    |
| Rnf20           | Ring finger protein 20   | Foxp3                              | [161]                    |
| Hakai           |  | E-cadherin                         | [177]                    |
| RNF43           | RING finger protein 43   | E-cadherin                         | [179]                    |
| NRBE3           | Novel RB E3 ubiquitin ligase                                       | RB                                 | [180]                    |
| UBR5            | ubiquitin protein ligase E3 component n-recognin 5                 | Snail1, Twist, FBP1                | [182, 204]               |
| TRIM21          | Tripartite motif containing protein 21                             | FASN                               | [206]                    |
| Deubiquitinases |  |                                    |                          |
| USP8            | Ubiquitin-specific protease 8                                      | PD-L1, HIF-1α, VEGFR2              | [97, 121, 136]           |
| USP9X           | Ubiquitin-specific peptidase 9, X-linked                           | PD-L1                              | [98]                     |
| CSN5            | COP9 signalosome 5   | PD-L1                              | [99]                     |
| USP22           | Ubiquitin-specific protease 22                                     | PD-L1, Foxp3                       | [100, 161]               |
| USP7            | Ubiquitin-specific protease 7                                      | PD-L1, HIF-1α, Foxp3, Tip60        | [101, 125, 162, 163]     |
| USP28           | Ubiquitin-specific protease 28                                     | HIF-1α                             | [113]                    |
| USP25           | Ubiquitin-specific protease 25                                     | HIF-1α                             | [119]                    |
| UCHL1           | Ubiquitin C-terminal hydrolase L1                                  | HIF-1α                             | [120]                    |
| USP20           | Ubiquitin-specific protease 20                                     | HIF-1α                             | [122]                    |
| MCPIP1          | Monocyte chemoattractant protein-induced protein 1                 | HIF-1α                             | [126]                    |
| USP19           | Ubiquitin-specific protease 19                                     | NLRP3                              | [127, 152]               |
| OTUD5           | ovarian tumor deubiquitinase 5                                     | YAP                                | [151]                    |
| USP10           | Ubiquitin-specific protease 10                                     | NLRP7                              | [153]                    |
| USP44           | Ubiquitin-specific protease 44                                     | Foxp3                              | [164]                    |
| USP21           | Ubiquitin-specific protease 21                                     | Foxp3                              | [165]                    |
| USP48           | Ubiquitin-specific protease 48                                     | TRAF2                              | [181]                    |
| USP17           | Ubiquitin-specific protease 17                                     | HAS2                               | [199]                    |
| USP4            | Ubiquitin-specific protease 4                                      | HAS2                               | [199]                    |



significantly inhibited during hypoxia, partly due to the degradation of HIF-1 $\alpha$ -targeting prolyl-hydroxylase proteins (PHDs) by the RING E3 ligases Siah1/2 [111].

Given the hypoxic state of tumors, it would be valuable to gain insight into how E3 ligases ubiquitinate HIF-1 $\alpha$  through oxygen-independent mechanisms. Under hypoxic conditions, phosphorylated HIF-1 $\alpha$  is catalyzed by GSK3 $\beta$  and can be ubiquitinated by FBW7 E3 ligase and degraded, and this effect is antagonized by USP28 to increase the half-life of HIF-1 $\alpha$  [112, 113]. The E3 ligase Mdm2 is also enhanced by p52 to ubiquitinate HIF-1 $\alpha$  during hypoxia [114]. Noticeably, Mdm2-mediated ubiquitination may occur only in the cytoplasm; activation of PI3K/AKT signaling induces the translocation of Mdm2 into the nucleus to interfere with ubiquitination [115]. HAF E3 ligase can control the basal levels of HIF-1 $\alpha$  via ubiquitination independent of cellular oxygen tension [116]. In addition, receptor for activated protein C kinase 1 (RACK1) serves as an anchoring protein for HIF-1 $\alpha$  to specifically recruit Elongin-C/B complex to HIF-1 $\alpha$ , which that can be inhibited due to competitive binding between HSP90 and RACK1 [117]. Different from these degradation outcomes, RING E3 ligase TRAF6 can form a K63-linked polyubiquitin chain in an oxygen-independent condition, which contributes to the stability of HIF-1 $\alpha$  [118].

As the only E3 ligase that mediates ubiquitination of HIF-1 $\alpha$  in an oxygen-dependent manner, the VHL complex appears to be antagonized by several DUBs, including USP25 [119], UCHL1 [120], USP8 [121], and USP20 [122]. However, in the extremely hypoxic tumor micro-environment of pancreatic ductal adenocarcinoma, USP25 still deubiquitinate HIF-1 $\alpha$  to abrogate its degradation [119]. Given that the mechanism of USP25 antagonism under this hypoxic condition was not described, whether there are other E3 ligases targeting HIF-1 $\alpha$  that can be antagonized by USP25 in pancreatic ductal carcinoma deserves further investigation. In addition, Nrdp1 E3 ligase targets USP8 for degradation and can indirectly inhibit the deubiquitination of HIF-1 $\alpha$ , thus destabilizing HIF-1 $\alpha$  in ischemic neurons [123]. Surprisingly, Lys11-specific DUB OTUD7B stabilizes HIF-1 $\alpha$  in the presence of VHL through chaperone-mediated autophagy rather than hydroxylation-dependent proteolysis [124]. In addition, the DUBs USP7, MCP1P1, and USP19 have been shown to deubiquitinate HIF-1 $\alpha$  and increase its stability [125–127]. Among these factors, HectH9 E3 ligase in the HECT family can catalyze the K63-linked polyubiquitination of USP7 to increase its deubiquitinating functions, especially under the condition of intracellular hypoxia [125], and USP19 alleviates HIF-1 $\alpha$  degradation independently of its catalytic activity [127]. Finally, SUMOylation of HIF-1 $\alpha$  could replace prolyl-hydroxylation to promote

VHL-mediated ubiquitination and proteolysis under hypoxia, and this effect could be reversed by SENP1 to regulate EPO production [128].

#### **VEGF-A/VEGFR2 pathway**

The activation of VEGFR2, a powerful receptor tyrosine kinase, is the most critical mechanism by which VEGF mediates and promotes tumor angiogenesis [129, 130]. After binding to VEGF-A, VEGFR2 triggers its own dimerization and catalyzes the autophosphorylation of multiple tyrosine residues in its intracellular kinase region, which is essential for the induction of pivotal downstream effectors such as phospholipase C- $\gamma$ 1 (PLC- $\gamma$ 1) [131]. Subsequently, activated VEGFR2 is rapidly removed from the cell surface and internalized by clathrin-mediated endocytosis [132]. Interestingly, it has been suggested that the ubiquitination of autophosphorylated VEGFR2 is involved in regulating its internalization and proteolysis [133]. Meyer et al. found that VEGFR2 contained an unstructured PEST sequence rich in serine and threonine residues, which is a potential recognition site for  $\beta$ -TrCP1 E3 ligases. In response to VEGF, the PEST sequence is phosphorylated at Ser1188/Ser1191, which is required for  $\beta$ -TrCP1-induced degradable polyubiquitination [134]. In another study,  $\beta$ -TrCP1 was shown to specifically recognize the C-terminal phosphodegrons of VEGFR2 in a casein kinase  $\delta$ -dependent manner [135].

The balance between ubiquitination and deubiquitination is responsible for the intracellular trafficking, signal conduction and proteolysis of VEGFR2. The deubiquitinating enzyme USP8 can cleave the K48- and K63-type polyubiquitin chains of VEGFR2 to influence these processes [136]. USP8 depletion can significantly increase the retention of VEGFR2 in early endosomes and reduce its recycling to the plasma membrane. As a result, ubiquitinated VEGFR2 in early endosomes is subject to proteolysis, which impairs the activation of downstream Akt and ERK1/2 effectors, but not p38 MAPK or PLC $\gamma$ 1 [136]. In addition, SUMOylation was shown to preserve VEGFR2 localization in the Golgi apparatus, which could be reversed by SENP1-mediated deSUMOylation. SENP1-deficient endothelial cells fail to express sufficient VEGFR2 on their cell surface, which is not conducive to VEGFR2-mediated angiogenesis [137].

Peculiarly, tyrosine phosphorylation of PLC- $\gamma$ 1 induced by VEGFR2 in endothelial cells is required for cell proliferation by activating the downstream PKC/ERK pathway. On the one hand, the autophosphorylation of Tyr1173 on activated VEGFR2 is a major docking site for PLC- $\gamma$ 1 recruitment [138]. On the other hand, the autophosphorylation of Tyr1052 and Tyr1057 on VEGFR2 can directly bind to c-Cbl and phosphorylate its tyrosine to enhance ubiquitin-ligase activity [139]. In the tertiary complex,

full activation of c-Cbl distinctly enhances the ubiquitination level of PLC- $\gamma$ 1 in a proteolysis-independent manner, which prevents tyrosine phosphorylation of PLC- $\gamma$ 1 [139, 140].

#### **M1/M2-like macrophage polarization**

M1- and M2-like macrophages in the TME exhibit two contradictory functional characteristics. However, macrophage polarization only indicates the activation state at a specific time [141]. When the stimulus in tissue microenvironment changes, the polarization state of macrophages will also be altered due to cellular plasticity [142, 143]. Mechanistically, a variety of key transcription factors can stimulate upstream signaling pathways to induce different macrophage phenotypes by regulating the transcriptional activity of different genes [144]. The UPS has been reported to target different transcription factors to regulate the polarization states of macrophages. For example, FBW7 is a tumor suppressor that inhibits macrophage transformation to the M2 type by degrading the oncoprotein c-Myc [145]. As a master transcription factor of mitochondria-related genes, nuclear respiratory factor 1 (NRF1) can also be ubiquitinated by the hypoxia-activated E3 ligase SIAH2. Subsequently, the downregulation of nuclear-encoded mitochondrial gene expression due to NRF1 degradation contributes to M2-like polarization, which may be associated with metabolic remodeling [146].

In addition, E3 ligase-mediated ubiquitination regulates macrophage polarization in a nondegradable manner. Praja2 polyubiquitinates and stabilizes the oncoprotein MFHAS1, which is critical for inducing M1-like macrophage polarization by upregulating the TLR2/JNK/p38/NF- $\kappa$ B pathway [147]. Moreover, STAT6 has been recognized as a pivotal transcription factor that drives macrophage M2 polarization [144]. Acetyltransferase CREB-binding protein (CBP) can be ubiquitinated by Trim24 E3 ligase to promote its binding to STAT6, which is required for STAT6 acetylation. Acetylation of STAT6 dramatically inhibits its transcriptional activity towards M2 macrophage-specific genes [148]. During IL-4-induced activation of M2 macrophages, the E3 ligase TRAF6 promotes K63-linked ubiquitination of STAT6 and inhibits K48-linked ubiquitination of STAT6, ultimately avoiding the degradation of STAT6. Unexpectedly, K63 ubiquitination mediated by TRAF6 is dispensable for STAT6 stability, and the suppression of K48 ubiquitination is independent of its E3 ligase activity [149]. As another key transcription factor in IL-4-induced activation of M2 macrophages, KLF4 can be SUMOylated to increase its transcriptional activity [150].

In recent years, we have also recognized that multiple DUBs promote macrophage M2 polarization through

different mechanisms. Among these DUBs, OTUD5 of the OTU subfamily deubiquitinates Yes-associated protein (YAP) to maintain its stability in macrophages. As a significant transcriptional coactivator in the Hippo pathway, YAP was first shown to be involved in M2 macrophage polarization, which further contributed to the invasive properties of triple-negative breast cancer (TNBC) [151]. As for the transcription factor interferon regulatory factor 4 (IRF4) in M2 macrophages, USP19 can competitively block the binding between IRF4 and p62 by deubiquitinating and stabilizing NLRP3, thus protecting IRF4 from p62-mediated selective autophagic degradation [152]. Furthermore, USP10-mediated stabilization of NLRP7 promotes TAM polarization through NF- $\kappa$ B pathway-induced CCL2 secretion [153]. By screening 51 DUB genes, USP7 was shown to be abundant in M2 but not M1 macrophages. Specific silencing of USP7 can effectively upregulate the M1-related p38 MAPK pathway, resulting in reeducating TAMs to the M1 type [154].

#### **Foxp3 in Treg cells**

The differentiation and immunosuppressive capabilities of Treg cells are characteristically programmed by a specific gene expression profile, which is predominantly governed by the transcription factor Foxp3. The phenotypes of Treg cells and effector T cells are unstable and can be switched according to intracellular Foxp3 abundance [78, 80]. Multiple posttranscriptional modification mechanisms regulate Foxp3 activity, stability and localization, among which ubiquitination plays an important role [155, 156]. To date, three E3 ubiquitin ligases have been identified that specifically ubiquitinate Foxp3: Stub1, RNF31, and TRAF6. As the first E3 ligase that was shown to mediate Foxp3 ubiquitination, Stub1 is upregulated and translocates from the cytoplasm to the nucleus in response to multiple cytokines and LPS-mediated inflammatory stimuli. In the Foxp3 complex, Hsp70 is an essential mediator that can recruit stress-activated Stub1 to target its partner Foxp3 for degradation [157]. RNF31, a component of the linear ubiquitin chain assembly complex, is responsible for catalyzing multi-monoubiquitination of Foxp3 at eight lysine residues [158]. This atypical ubiquitination modification contributes to the stability of Foxp3, which is indispensable for maintaining the immunosuppressive phenotype of tumor-infiltrating Treg cells [158, 159]. Similar to the positive regulation of Foxp3 by RNF31, TRAF6 can catalyze K63-type polyubiquitination at Lys262, thereby cancelling the perinuclear isolation of Foxp3 and promoting its translocation to nuclear transcription sites [160].

In a CRISPR-based gene knockout screen of approximately 500 nuclear factors, the deubiquitinase USP22 and

E3 ligase Rnf20 were recognized as reciprocal ubiquitin switches that positively and negatively regulate Foxp3 expression in mouse primary Treg cells, respectively [161]. In addition, USP7, USP21 and USP44 can directly recognize Foxp3 and remove its K48-linked polyubiquitin chains to maintain Foxp3 homeostasis. When Foxp3 is deubiquitinated, USP7 can also deubiquitinate and stabilize the histone/protein acetyltransferase Tip60, which is required for the acetylation, dimerization and function of Foxp3 [162, 163]. Notably, TGF- $\beta$ -induced USP44 exhibits an obvious synergistic effect with USP7, and the coexpression of USP44 and USP7 almost completely eliminates Foxp3 polyubiquitination [164]. A total of 7 lysine residues on Foxp3 are potential targets of USP21-mediated deubiquitination [165]. Furthermore, stable Foxp3 can recognize the promoter of the USP21 gene and upregulate its transcriptional activity, thus forming a positive feedback loop between Foxp3 and USP21 [166, 167]. Similar to the master transcription factor Foxp3, the basic leucine zipper transcription repressor BACH2 is required for maintaining the differentiation and immunosuppressive activity of Treg cells by repressing effector T-cell-specific transcriptional programs [168]. DeSUMOylation of BACH2 is mediated by the ROS-SEN3-BACH2 axis, facilitates the nuclear localization of BACH2 and maintains Treg cell-specific gene signatures [169].

#### **EMT associated with cancer stem cells and CAFs**

EMT is a reversible process characterized by epithelial cells acquiring mesenchymal properties. Phenotypically, epithelial cells undergoing EMT lose cell junctions and apical-basal polarity and reshape their cytoskeleton to gain a new shape and better motility [170]. During tumorigenesis, the formation of cancer stem cells and some CAFs has been confirmed to require EMT induction. Cancer stem cells, which are a rare population of tumor cells, have the potential for self-renewal and multilineage differentiation, which are closely related to the initiation and relapse of multiple human tumors [171–173]. The most significant molecular hallmark of EMT is the disappearance of the transmembrane protein E-cadherin that mediates epithelial cell adhesions and preserves epithelial integrity [174]. In response to cytokines such as TGF- $\beta$  and multiple stress cues, EMT-related pathways can be hijacked to activate downstream EMT-inducing transcription factors (EMT-TFs) [175]. These EMT-TFs include the Snail family members Snail1 and Snail2 (also known as Snail and Slug), the Zeb family members Zeb1 and Zeb2, and Twist1, all of which have the ability to inhibit E-cadherin transcription [175, 176].

In epithelial cells, the ubiquitination of E-cadherin is catalyzed by the E3 ligase Hakai and can be endocytosed, which may eventually lead to its recycling back to the cell

membrane or destruction [177, 178]. Before being ubiquitinated by Hakai, E-cadherin needs to be tyrosine phosphorylated by the tyrosine kinase c-Src [177]. Similarly, c-Src-mediated tyrosine phosphorylation is indispensable for RNF43-mediated ubiquitination of E-cadherin in lung adenocarcinoma [179]. As an E3 ligase targeting retinoblastoma protein (RB), NRBE3 negatively regulates E-cadherin transcription in breast cancer mainly through RB-dependent mechanisms [180]. The deubiquitinase USP48 is an indirect regulator of E-cadherin expression. In response to TNF- $\alpha$ , GSK3 $\beta$ -mediated serine phosphorylation can further enhance the deubiquitination activity of USP48, which in turn stabilizes the second messenger adaptor TRAF2 in the TNF- $\alpha$  pathway and ultimately suppresses E-cadherin expression [181]. In addition, the E3 ubiquitin ligase UBR5 not only regulates the growth, metastasis and immune response of TNBC but also affects the expression of E-cadherin. Deletion of UBR5 reduces intracellular E-cadherin levels and the aberrant EMT phenotype. After excluding the effect of UBR5 on promoter hypermethylation of the E-cadherin gene, the degradation of EMT-TFs including Snail1 and Twist is thought to be a potential mechanism by which UBR5 positively regulates E-cadherin [182].

It is worth noting that the imbalance in the dynamic regulation in EMT-TFs by ubiquitin modification is an important factor that induces EMT. In epithelial cells, most EMT-TFs, which are short-lived proteins, are susceptible to rapid UPS-mediated degradation and thus remain at very low levels [183]. Numerous F-box proteins have been shown to participate in EMT by targeting EMT-TFs degradation [184]. Broader E3 ligases and DUBs have also been comprehensively described in several reviews [185–187]. Therefore, further elucidation of the specific mechanisms of the UPS-DUBs system in EMT-TFs degradation and how it fails in tumorigenesis can help identify attractive targets for inhibiting tumor metastasis.

#### **Mediator production, ECM and cancer metabolism**

In MDSCs, the E3 ligase TRAF6 catalyzes K63-linked polyubiquitination of the core transcription factor STAT3, which is a prerequisite for the phosphorylation and activation of STAT3. With the substantially increased expression of TRAF6 in tumor-infiltrating MDSCs, abnormally activated STAT3 signaling impairs the maturation of myeloid progenitor cells and promotes the proliferation and immunosuppressive activity of MDSCs [188]. Moreover, the specific STAT3 phosphatase CD45 in MDSCs can be SUMOylated to attenuate its phosphatase activity and maintain the phosphorylation and activation of STAT3, which can be reversed by SENP1 [189]. In addition, chronic inflammation in the TME

can activate multiple inflammatory pathways in tumor cells, tumor stromal cells, and ICCs, thereby promoting the release of inflammatory mediators, including growth factors, cytokines, chemokines, and proangiogenic factors [190, 191]. The ubiquitin ligases Cop1, TRIM37, and UBR5 have been shown to modulate the production of key chemokines and cytokines in tumor cells to promote the recruitment and activation of immunosuppressive macrophages [192–194]. Downregulation of the deubiquitinase USP12 in lung cancer cells promotes the hyperactivation of NF- $\kappa$ B, a positive regulator of inflammation, and generates a tumor-promoting secretome by reducing the stability of PPM1B [195]. In particular, the ubiquitin ligase TRIM59 can be transported from tumor cells to macrophages via exosomes to increase the secretion of IL-1 $\beta$  by macrophages [196].

As an acellular component of the TME, ECM produced by stromal cells possesses an intricate fiber network that plays a key role in tumor growth, invasion and metastasis [197]. The activity of hyaluronan synthase 2 (HAS2), which is responsible for hyaluronan synthesis, in CAFs is associated with monoubiquitination at Lys190 [198]. The ubiquitination of HAS2 was also shown to be removed by diverse DUBs, among which USP17 and USP4 effectively remove polyubiquitin chains and monoubiquitin molecules from HAS2, respectively [199]. In solid tumors, the ECM often becomes highly dysregulated, resulting in the loss of normal matrix organization and homeostasis [200]. As a major class of ECM-remodeling proteinases, matrix metalloproteinases (MMPs) can extensively degrade matrix proteins and are closely related to the malignant migration of tumor cells and the selective release of bound signaling mediators [201]. With the continuous in-depth research on the regulatory mechanism of MMPs, a variety of E3 ubiquitin ligases have been shown to target MMPs [202]. For example, c-Cbl facilitates tumor invasion and metastasis by upregulating MMP2 expression in human glioma [203].

Finally, UPS-mediated regulation of tumor metabolism can respond to metabolic stress in the TME and meet the growth and survival needs of cancer cells. In response to characteristic nutrient deprivation and hypoxia in the TME, pancreatic cancer cells fully utilize aerobic glycolysis for energy in an UBR5-dependent manner. Mechanistically, upregulated UBR5 ubiquitinates the transcription factor C/EBP $\alpha$  of fructose-1,6-bisphosphatase (FBP1) for proteolysis and reduces the levels of the rate-limiting enzyme FBP1 in gluconeogenesis to promote aerobic glycolysis [204]. Hexokinase 2, which is the first rate-limiting enzyme of glycolysis, is expressed in aggressive tumors and can bind to the outer membrane of mitochondria to promote glycolysis and reduce mitochondrial respiration [205]. The affinity of hexokinase 2 to mitochondria

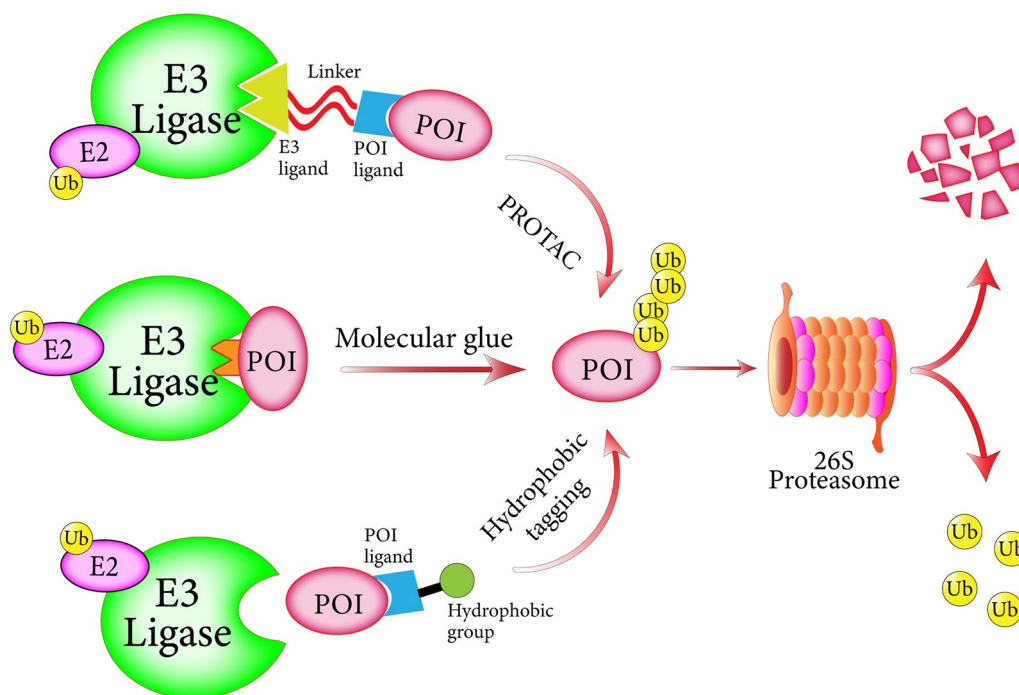
can be regulated by SUMOylation, and de-SUMOylated hexokinase 2, which is catalyzed by SENP1, preferably binds to mitochondria [205]. TRIM21 can ubiquitinate and degrade acetylated fatty acid synthase (FASN). FASN is a rate-limiting enzyme complex that catalyzes de novo lipogenesis, and its level is frequently increased in human hepatocellular carcinoma, which is accompanied by diminished FASN acetylation and is conducive to tumor cell growth [206].

#### **Potential strategies for reeducating the TME through the UPS**

Increasing evidence has shown that reforming the TME into an anticancer environment is a promising therapeutic strategy, given the plasticity of the multiple stromal cells and its importance in cancer progression and drug resistance [1, 207–210]. Most drugs currently targeting the TME, such as immunotherapies and antiangiogenic drugs, are not very effective and typically only benefit one subset of patients [211–213]. In addition, a variety of oncoproteins, especially transcription factors lacking enzymatic activity, have been regarded as undruggable targets according to traditional occupancy-driven pharmacology. As accumulating evidence points to the critical role of ubiquitination in the TME, targeting key mediators with the full use of the inherent UPS seems to be an attractive strategy for anticancer drug development. Notably, extensive examination of small-molecule degraders has made it possible to hijack intracellular E3 ubiquitin-ligase machinery for targeted protein degradation (Fig. 4) [214, 215].

Since the innovative concept was first proposed in 2001, proteolysis targeting chimeras (PROTACs) have received considerable attention as a powerful tool with the potential to revolutionize pharmaceutical research [216, 217]. Typically, PROTACs are heterobifunctional small-molecule degraders whose two ligands, which are bridged by a short linker, can simultaneously recruit a specific E3 ligase and a POI (protein of interest). PROTAC technology, which relies on the formation of the E3-PROTAC-POI ternary complex, can theoretically achieve ubiquitination and subsequent proteolysis of any protein target by merely changing the POI ligand. Due to their stable structure, PROTACs can be recycled for continuous POI degradation [218]. In addition, a unique feature and advantage of PROTACs lies in its inherent selectivity of intracellular E3 ligases. The E3 ligases VHL, MDM2, CRBN, and IAP have been reported to be successfully recruited by PROTACs, and the potential of other E3 ligases as candidates needs to be further verified [219, 220]. Given that E3 ligase activity is related to expression levels in different tumor cell types and subcellular localization, the selection of appropriate E3





**Fig. 4** Small-molecule degraders hijacking the inherent UPS. PROTACs, molecular glues and hydrophobic tags all hijack intracellular E3 ligases and recruit targeted proteins for ubiquitination and subsequent degradation. Both PROTACs and hydrophobic tags are heterobifunctional small-molecule degraders containing a ligand of a protein of interest, which can recruit E3 ligase through specific ligand and hydrophobic group, respectively. Molecular glues, a class of proximity-inducing small molecules, can modify the substrate-recognizing site of E3 ligase by direct contact, allowing it interaction with neosubstrates

ligands based on different tumor types provides a feasible strategy for specific recognition of tumor tissues and maximum protection of other normal tissues in follow-up PROTACs design [215]. The latest advance in the PROTAC technology field involves the orally bioavailable PROTAC drugs ARV-110 and ARV-471, which have entered phase I clinical trials and target androgen and estrogen receptors, respectively; these drugs have shown encouraging efficacy, safety, and pharmacokinetic profiles in the treatment of prostate and breast cancer [221–223]. With the extensive research of PROTAC technology in cancer treatment, PROTACs present unique advantages over traditional inhibitors and antibodies by targeting TME pathways [224, 225].

Analogous to PROTACs, hydrophobic tags are heterobifunctional small-molecule degraders that link POI ligands to highly hydrophobic groups [226]. Different from ligand-mediated E3 ligase recruitment, hydrophobic tagging technology takes advantage of hydrophobic groups to mimic a misfolded state and induce the unfolded protein response. Under physiological conditions, the exposure of hydrophobic side-chains can activate the chaperone machinery that assists in the refolding of misfolded proteins [227]. However, the hydrophobic

groups in hydrophobic tags cannot be repaired by chaperones, eventually leading to UPS-mediated POI clearance. Molecular glues, which are a class of proximity-inducing small molecules, can modify the substrate-recognizing site of an E3 ligase through direct contact, allowing for interactions with neosubstrates. Thalidomide and its derivatives, which are known as immunomodulatory drugs, have been widely researched as molecular glues through which substrate-receptor CRBN can recognize as many as 13 neosubstrates, which plays a critical role in the treatment of multiple myeloma [228–231]. It should be pointed out that extracellular proteins in the TME cannot be disregarded due to their levels and importance, but the targeted degradation methods introduced above seem inadequate. To address this challenge, Bertozzi's team and Spiegel's team proposed Lysosomal-Targeting Chimeras (LYTACs) and Molecular Degraders of Extracellular proteins through the Asialoglycoprotein receptor (MoDE-As) technology, respectively [232, 233]. Mechanistically, LYTACs and MoDE-As drive the internalization and subsequent lysosomal degradation of extracellular proteins through recycling receptors.



## Conclusions and perspectives

In the present review, we mainly summarized the proteolytic ubiquitination signals in the tumor microenvironment and tumorigenesis. Ubiquitin signaling is involved in most steps in the TME, and its dysregulation eventually leads to tumorigenesis and tumor progression. Thus, targeting the ubiquitin system has provided good therapeutic strategies for cancer treatments and have achieved some FDA-approved POIs, including small molecule PROTACs. PROTAC has several advantages, such as good cell permeability, solubility, and easy for oral administration. Until now, only several E3 ligases have been used in PROTACs, and more promising candidate E3 ligases are being explored to expand the way of cancer treatment. However, the specific role of ubiquitination in immune cells, such as T cells and macrophages, is still unclear, especially regarding immunotherapies targeting ubiquitination. On the other hand, we do not completely understand the structural analysis of target proteins and the dynamic process of tumorigenesis, and most of these POIs work well in cell culture or animal models but not well enough in clinical trials. Thus, in-depth understanding the functions of the UPS in the TME can expand and diversify the range of novel anticancer strategies, and more clinical studies should be conducted in the future.

## Abbreviations

|                 |   |
|-----------------|---|
| CAEs            | Cancer-associated endothelial cells   |
| CAFs            | Cancer-associated fibroblasts   |
| CRLs            | Cullin-RING ligases   |
| DUBs            | Deubiquitinases   |
| ECM             | Extracellular matrix  |
| EMT             | Epithelial-mesenchymal transition   |
| EMT-TFs         | EMT-inducing transcription factors  |
| EndMT           | Endothelial-mesenchymal transition  |
| ER              | Endoplasmic reticulum   |
| ERAD            | ER-associated degradation   |
| FASN            | Fatty acid synthase   |
| FBP1            | Fructose-1,6-bisphosphatase   |
| Foxp3           | Forkhead box P3   |
| GSK3 $\beta$    | Glycogen synthase kinase 3 $\beta$  |
| HAS2            | Hyaluronan synthase 2   |
| HECT            | Homologous to E6-AP carboxyl terminus   |
| HIF-1           | Hypoxia-inducible factor 1  |
| IICs            | Infiltrating immune cells   |
| IRF4            | Interferon regulatory factor 4  |
| LYTACs          | Lysosomal-targeting chimeras  |
| MDSCs           | Myeloid-derived suppressor cells  |
| MMPs            | Matrix metalloproteinases   |
| MoDe-As         | Molecular degraders of extracellular proteins through the asialoglycoprotein receptor |
| NRF1            | Nuclear respiratory factor 1  |
| PD-1            | Programmed death protein-1  |
| PD-L1           | Programmed death-ligand 1   |
| PHDs            | Prolyl-hydroxylase proteins   |
| PLC- $\gamma$ 1 | Phospholipase C- $\gamma$ 1   |
| POI             | Protein of interest   |
| PROTACs         | Proteolysis targeting chimeras  |
| RACK1           | Receptor of activated protein C kinase 1  |
| RB              | Retinoblastoma protein  |
| RBR             | Ring-between-ring   |

|            |                                      |
|------------|--------------------------------------|
| RING       | Really interesting new gene          |
| ROS        | Reactive oxygen species              |
| SCF        | Skp1-Cul1-F-box                      |
| SENP       | Sentrin/SUMO-specific proteases      |
| TAMs       | Tumor-associated macrophages         |
| TME        | Tumor microenvironment               |
| TNBC       | Triple-negative breast cancer        |
| Treg cells | Regulatory T cells                   |
| Ub         | Ubiquitin                            |
| UBL        | Ubiquitin-like                       |
| UPS        | Ubiquitin-proteasome system          |
| VEGF-A     | Vascular endothelial growth factor-A |
| VEGFR2     | VEGF receptor 2                      |
| YAP        | Yes-associated protein               |

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## Author contributions

LXM, ZYZ and WHL searched the related literature LXM, WHL, and ZYZ drafted the manuscript YX and WHL made the figures and Tables XQD, ZP, WSG, WHL and HJ participated in the design of the review, LXM, WHL, and HJ wrote and finalized the manuscript. All authors read and approved the final manuscript.

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All data generated during this study are included in this article.

## Declarations

### Ethics approval and consent to participate

Not applicable.

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The authors declare that they have no competing interests.

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