Chapter

E3 Ubiquitin Ligases in Cancer and Their Pharmacological Targeting

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Abstract

Ubiquitination plays many critical roles in protein function and regulation. Consequently, mutation and aberrant expression of E3 ubiquitin ligases can drive cancer progression. Identifying key ligase-substrate relationships is crucial to understanding the molecular basis and pathways behind cancer and toward identifying novel targets for cancer therapeutics. Here, we review the importance of E3 ligases in the regulating the hallmarks of cancer, discuss some of the key and novel E3 ubiquitin ligases that drive tumor formation and angiogenesis, and review the clinical development of inhibitors that antagonize their function. We conclude with perspectives on the field and future directions toward understanding ubiquitination and cancer progression.

Keywords: E3 ubiquitin ligase, cancer, pharmacological targeting

1. Introduction

The regulation and turnover of proteins is an essential aspect of cell homeostasis and one that is commonly disrupted in cancer cells [1]. Regulation of a protein's levels, activity, or localization is affected by ubiquitination, a posttranslational modification that involves the covalent attachment of a 76 amino acid ubiquitin molecule onto a substrate protein [2, 3]. Depending on the cellular context, ubiquitinated proteins can affect a myriad of cellular processes, including signaling [4], epigenetics [5], endosome trafficking [6], DNA repair [7] and protein stability via the 26S-proteasome [8].

The outcome of protein ubiquitination is affected primarily by two properties: what kind of ubiquitin linkage and how many ubiquitin molecules are present [2]. Ubiquitin is usually covalently attached to its substrate via a nucleophilic lysine residue on the substrate and the ubiquitin carboxy terminus. Ubiquitin itself can serve as a nucleophile via one of seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) [9, 10] though K48- and K63-linkages seem to be the most abundant and are the most well-studied. In some cases, the N-terminal amide of the initiator methionine (M1) of the substrate can serve as the nucleophile [11, 12]. If one of the lysine residues or the initiator methionine of ubiquitin serves as the nucleophile for another ubiquitin molecule, a polyubiquitin chain is formed. A K48-linked polyubiquitin chain of four or more ubiquitin molecules is typically enough to target the substrate for 26S-proteasome mediated degradation [13]. Meanwhile, poly-K63 linkages are involved in many processes, including endocytic trafficking, inflammation, and DNA repair [5, 6, 14]. Other ubiquitin linkages [11], combinations of

linkages (mixed or branched chains) [15–17], monoubiquitination [5, 18], and multi-monoubiquitination [19, 20] events have other diverse functions within the cell.

Ubiquitination occurs in three main steps [21, 22]. First, the E1 ubiquitin-activating enzyme (two in the human genome) covalently attaches to a ubiquitin molecule via a thioester bond in an ATP-dependent process. Next, the E1 enzyme transfers ubiquitin onto an E2 ubiquitin-conjugating enzyme (about 40 in the human genome). Finally, the E2 enzyme binds a substrate-bound E3 ligase (about 600 in the human genome) to transfer ubiquitin onto a lysine residue of the substrate. Repeating the cycle creates a polyubiquitin chain.

E3 ligases can function either as single peptides (like Parkin), simple complexes (e.g.: hetero/homodimers, like MDM2/MDMX or XIAP), or as large complexes (like Cullin-RING-ligase complexes or the anaphase promoting complex/cyclosome). There are two main classes of E3 ligases [23]: HECT (about 30 in the human genome) and RING ligases (including RING and RING-like ligases and their accessory proteins, about 600 in the human genome).

HECT ligases contain a C-terminus HECT domain that accepts the ubiquitin molecule from an E2 conjugating enzyme via a thioester bond before transferring the ubiquitin to the substrate [24]. RING ligases contain a zinc finger domain, and these proteins allow the E2 to transfer ubiquitin directly onto the substrate [25]. A subclass of RING ligases known as RING-between-RING (RBR) ligases contain two RING domains that have elements of both HECT and RING ligases: one RING domain binds the charged E2, while the other RING domain accepts the ubiquitin molecule before transferring it onto the substrate [26].

As E3 ligases ultimately determine the target of the ubiquitination machinery, they play a critical role in cell regulation. They regulate key players in processes like apoptosis (caspases), cell senescence and growth (p53, p21, p27; Hippo and

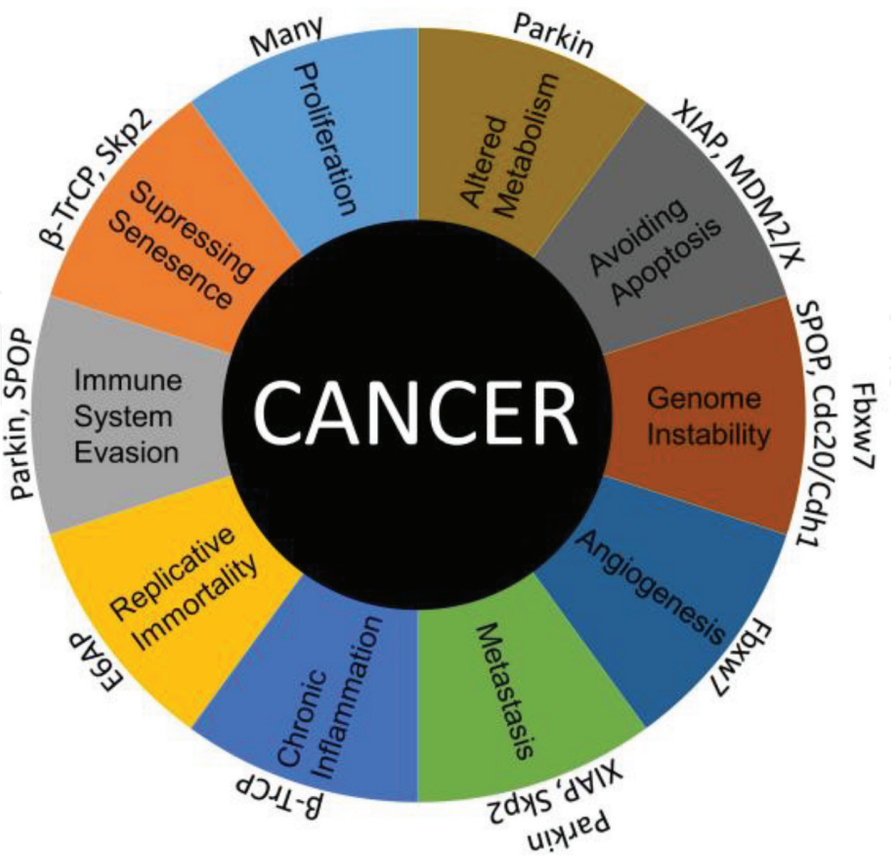

Figure 1.
E3 ubiquitin ligases (outer circle) regulate hallmarks of cancer (inner circle) to drive cancer progression.

Hedgehog signaling), proliferation and genomic stability (c-Myc, cyclins), immune system evasion (PD-L1), inflammation (NFκB), and metastasis and angiogenesis (Wnt signaling) (**Figure 1**). Misregulation or mutation of E3 ligases can lead to overexpression of oncogenes or downregulation of tumor suppressor genes, leading to cancer progression. Consequently, understanding the molecular targets and functions of E3 ligases serves as the basis for designing new cancer therapies.

Here, we describe some central and novel E3 ligases related to cancer development, pharmacological targeting of those ligases, and perspectives on understanding the role of E3 ligases in cancer progression.

2. E3 ligases and cancer progression

2.1 TP53

The tumor protein p53 (TP53) is a transcription factor that serves as one of the principal regulators of cell function and survival (reviewed in [27]), mediating cellular responses to proliferation, cell cycle control, DNA damage response pathways, and apoptosis. Consequently, it is mutated in approximately 50% of all cancer types. Thus, regulators of p53 serve as ideal candidates to understand and address cancer cell progression (**Table 1**).

E6AP (Ube3a) is a 100 kDa HECT domain ligase discovered for mediating the interaction between human papillomavirus protein E6 and p53 [28]. Neither E6AP nor E6 alone have a strong affinity for p53, but together, the E6/E6AP complex binds to p53 and changes the substrate specificity of E6AP [28], allowing E6AP to ubiquitinate p53 at the N-terminal DNA binding domain and target it for

E3 ligase		Notable substrates and binding partners	Expression in cancer	Cancer types
TP53	E6AP	p53	Gain of function via HPV E6	Cervical, breast [38, 166]
	MDM2/X	p53	Overexpressed	Many; liposarcomas [48, 167]
SCF	Skp2	p21, p27	Overexpressed	Many [95, 168]
	Fbxw7	Cyclin E, mTOR	Downregulated or dominant-negative mutant	Many; endometrial, cervical, blood [64, 67, 169]
	β-TrCP	IkB, β-catenin, Wee1, Cdc25a/b	Overexpressed (in some tissues)	Many [60, 168]
APC/C	Cdc20	Cyclin A/B, securin	Overexpressed	Pancreatic, lung, gastric [95, 168, 170]
	Cdh1	Cdc20, Plk1, Aurora kinase A/B	Underexpressed	Many [171]
Other	XIAP	Caspases 3, 7, 9	Overexpressed	Many [98, 99]
	Park2	Cyclin D/E, Cdc20/Cdh1, tubulin	Underexpressed	Breast, pancreatic, colorectal, ovarian [172]
	SPOP	PD-L1, androgen and estrogen receptor	Downregulated or dominant-negative mutant	Prostate, endometrial, kidney [139, 141, 150]

Table 1.
E3 ligases and cancer progression.

degradation [29]. Consequently, E6AP may play a role in HPV-mediated cervical cancers [30], particularly for those mediated by high-risk HPV16 strain, as E6 proteins from lower-risk strains of HPV lack the ability to degrade p53 [31].

The E6/E6AP complex plays other roles in cancer cell progression. Neither E6 nor E6AP alone can activate the hTERT promoter, but together, the E6/E6AP complex can activate the hTERT promoter, perhaps via interactions with c-Myc and NFX-1 to respectively activate and repress promoter activity [32]. The E6/E6AP complex has also been implicated in the ubiquitination of apoptosis-inducing proteins Bak [33], Fas [34], and TNFR1 [35]. Independent of E6 binding, endogenous E6AP targets include the tumor suppressor PML [36]; cell cycle regulators p27 [36], Cdk1, Cdk4; cell proliferation regulator MAPK1 [37];, and guanine nucleotide exchange factor ECT2 [38]. A published list of 130 likely substrates of E6AP includes β-catenin and PRMT5, proteins involved in cancer progression [37].

MDM2 is best known as a regulator of p53. MDM2 is a RING ligase [39] that forms stable heterodimers with a homolog, **MDMX** (MDM4), via their RING domains [40]. MDM2 localizes primarily in the nucleus bound to p300/CBP [41]. When complexed to p53, MDM2 inhibits p53 activity in two ways: first, MDM2 binds the N-terminal transactivation domain [42], inhibiting p53-mediated transcription [43]; secondly, MDM2 modulates p53 protein levels via ubiquitination near the C-terminus [44]. After MDM2 monoubiquitinates p53, p300 and CBP catalyze the polyubiquitination of p53, leading to p53 degradation [8, 41, 45]. Overexpression of MDM2 [46, 47], seen in many cancers where p53 is not mutated [48], leads to a loss of p53 activity.

During p53 activation, p53 is phosphorylated by multiple serine/threonine kinases at residues near the N-terminus, disrupting p53/MDM2 binding and stabilizing p53. For example, ATM kinase phosphorylates p53 at S15 [49] to promote p53-mediated transcription. Additionally, ATM phosphorylation of MDM2 on S395 disrupts the MDM2/p53 complex, allowing p53 to accumulate [50].

2.2 SCF complexes

The SCF complex is a multimeric ubiquitination complex with multiple roles in cell regulation (**Table 1**). The main scaffold of the SCF complex, Cullin 1 (Cul1), recruits the substrate to be ubiquitinated at the N-terminus and the charged ubiquitin at the C-terminus. Rather than bind the substrate directly, Cul1 uses two adaptor proteins: Cul1 binds directly to Skp1, which then binds to one of about 70 F-box proteins [51] that directly bind their substrates. At the C-terminus, Cul1 binds an adaptor protein, either Rbx1 or Rbx2 (also known as Roc1 or Roc2), that will bind a charged E2 ubiquitin conjugating enzyme [52, 53] .

Skp2 (Fbxl1) is a F-box protein that is most active during S-phase [54]. During S phase, Skp2 binds and ubiquitinates phosphorylated p27 [55] by binding the Cdk2-cyclin E complex [56]. Degradation of p27 frees inhibition of Cdk2-cyclinA/E complexes, allowing for progression into S-phase and entry into mitosis [57]. Other targets of Skp2 include p21 [58] and E-cadherin [59]. In some cases, Skp2 requires an accessory protein Cks1 to enhance binding to the substrate [60]. Skp2 both enhances c-Myc transcriptional activity and promotes c-Myc degradation [61]. Interestingly, p300-mediated acetylation of Skp2 changes the localization of Skp2 from nuclear to cytoplasmic, increasing cellular proliferation, motility, and tumorigenesis [59]. Skp2 is commonly overexpressed in a variety of cancers [62], including blood, colorectal, stomach, ovarian, and cervical cancers [60].

Fbxw7 (in yeast, Cdc4) contains a homodimerization domain, an F-box domain that binds Skp1, and eight WD40 repeats that form a beta-propeller structure to bind substrates [63]. Substrate binding is dependent on interaction between the

arginine residues of the Fbxw7 WD40 domains and phosphorylated residues of the substrate in a recognition motif termed the Cdc4 phosphodegron (CPD) [63]. Mutations that disrupt substrate binding, especially point mutations of the arginine residues of the WD40 region, are commonly found in tumor samples [64]. Because Fbxw7 homodimerizes, these mutations may have a dominant-negative effect [65], as wild-type Fbxw7-mutant Fbxw7 dimers are able to effectively bind but not ubiquitinate their substrates [66]. Fbxw7 is deleted [67] or mutated in many cancers, with mutations being especially common in cancers of the bile duct and blood [68].

One well-characterized substrate of Fbxw7 is cyclin E [69]. The ubiquitination and degradation of cyclin E is dependent on phosphorylation of by Cdk2 and glycogen synthase kinase 3 (GSK3) [70]. Dimerization of Fbxw7 can also change its affinity for cyclin E as well as other substrates [71]. Other substrates of Fbxw7 include transcription factors c-Myc [72]; c-JUN, Notch 1; DNA-binding protein DEK [73]; and nutrient sensing protein mTOR [74]. Interestingly, the SV40 large T antigen contains a decoy CPD that can mislocalize Fbxw7 and inhibit Fbxw7-mediated degradation of cyclin E [75].

β-TrCP (BTRC), **Fbxw1a** (β-TrCP1) and **Fbxw11** (β-TrCP2) are protein homologs that appear to have redundant roles [76]. These F-box proteins can form homo- and hetereodimers with each other [76] and use WD40 domains to bind a DSG phosphodegron motif (such as DpSGXXpS) [60]. Overexpression of β-TrCP is seen in various types of cancers, including colorectal, pancreatic, breast, ovarian and melanomas [77].

β-TrCP plays an important role as a regulator of Cdk1. One substrate of β-TrCP is Wee1, a kinase that inhibits Cdk1 activity [78]. Phosphorylation of Wee1 at S53 and S123 by Plk1 and Cdk1 respectively allow β-TrCP to bind to and ubiquitinate Wee1, activating Cdk1 during G2 to promote rapid entry into mitosis. Similarly, in prophase, β-TrCP also ubiquitinates Emi1, an inhibitor of the APC/C [79]. Consequently, β-TrCP accelerates mitotic progression both by increasing Cdk1 activity and activating the APC/C. In the case of DNA damage, checkpoint proteins hyperphosphorylate Cdc25a [80], a phosphatase that activates Cdk1 by removing repressive phosphorylation events. β-TrCP binds to and ubiquitinates hyperphosphorylated Cdc25a, deactivating Cdk1 and delaying the cell cycle. β-TrCP also ubiquitinates Cdc25b [81], a phosphatase that activates Cdk2/cyclin A and Cdk1/cyclin B to progress through the G2/M transition [82]. Other β-TrCP substrates that are linked to cancer progression include the IkB family [83], β-catenin [76] and MDM2 [84].

2.3 APC/C

Proper cell cycling and successful mitotic events rely on the coordinated accumulation and destruction of cyclins [85]. Disruption of this coordination can lead to aberrant mitotic events, aneuploidy, and cancer [86] (**Table 1**). While entry into mitosis is mediated by activation of Cdk1/2, progression through and exit from mitosis is mediated principally by the anaphase promoting complex or cyclosome (**APC/C**).

The APC/C is a 1.2 megadalton complex whose activity is necessary for entry to and exit from mitosis [87]. The structure of the human APC/C was solved via cryoEM to 7.4 angstrom resolution, allowing for the identification of 20 subunits of the APC/C and a mechanistic understanding of its function [88]. APC/C ubiquitin ligase activity depends on two activating subunits, **Cdc20** or **Cdh1** (coded by gene FRZ1; not to be confused with the gene CDH1, which codes for E-cadherin), which are necessary for APC/C binding to substrate and subsequent degradation [89] via

K11 ubiquitin linkages [90]. In early mitosis, APC/C-Cdc20 degrades proteins such as cyclins A and B and Securin, the inhibitor of separase [91]. In later stages of mitosis and early G1, APC/C-Cdh1 degrades Cdc20, mitotic kinases like Plk1 and Aurora kinases A/B, and the contractile ring protein Anillin to ensure exit from mitosis and proper transition into G1 [92]. Binding of the substrate to APC/C is mediated by two main modalities [93]: for some substrates, Cdc20/Cdh1 binds the substrate through a KEN box motif; for others, both the APC/C subunit Apc10 and Cdc20/Cdh1 "sandwich" the substrate at the substrate's D box. Some substrates have both and/or additional motifs to bind the APC/C and Cdc20/Cdh1 [92].

Cdc20 is found overexpressed in many cancers, including lung, oral, liver, and colon cancers [94, 95]. Cdh1 is generally a tumor suppressor, as downregulation of Cdh1 is found in some aggressive cancer cell types [95], and loss of Cdh1 sensitizes cells to DNA damage [96].

2.4 Other

X-linked inhibitor of apoptosis protein (**XIAP**) is a IAP family E3 ligase characterized by three N-terminal baculovirus IAP repeat domains and a C-terminal RING domain [97]. Like other IAPs, XIAP plays a central role in mediating the cell's response to apoptosis. XIAP is overexpressed in many cancer cell lines, particularly in kidney and skin cancers [98, 99].

The linker region of XIAP between BIR1 and BIR2 binds to the active site and inhibits caspase 3 and caspase 7 [100]. The BIR3 domain of XIAP also binds to caspase 9, inhibiting caspase 9 dimerization and activity [101]. Moreover, XIAP ubiquitinates caspase 3 [102], caspase 9 [103], and caspase 7 [104] and targets them for degradation. As a final level of regulation, in addition to its ubiquitin E3 ligase role, XIAP can also function as a neddylation E3 ligase, neddylating and inhibiting the activity of caspases [105].

XIAP also plays important roles in cell motility. On one hand, XIAP degrades COMMD1 [106], a regulator of NFκB [107] and copper homeostasis. XIAP also binds to MAP3K7IP1, an event that activates kinase MAP3K7 to phosphorylate substrates leading to removal of NFκB inhibition [108]. XIAP also binds to survivin [109], activating NFκB signaling and encouraging cell metastasis by activating cell motility kinases Fadk1 and Src [110]. Conversely, XIAP has also been show to inhibit cell migration by binding to and ubiquitinating c-RAF to direct another ubiquitin ligase (CHIP) to degrade c-RAF [111]. Under non-stressed conditions, XIAP ubiquitinates and degrades MDM2, stabilizing p53 and inhibiting autophagy [112]. XIAP also binds to and monoubiquitinates TLE3, allowing β-catenin to activate Wnt-mediated transcription [113]. Finally, in addition to inflammation involving the NFκB pathway, XIAP suppresses TLR-based inflammation [114].

Park2 (PARKIN) is an RBR-E3 ligase with both RING and HECT ligase characteristics [115]. The Park2 locus is commonly deleted in cancers [116]. In mouse models, loss of Park2 causes spontaneous liver cancer [117] and contributes to colorectal cancer in mouse models [118]. Additionally, Park2 plays a central role in mitophagy [119], which may affect cell redox state [120], proliferation, and metastasis [121].

Park2 plays a prominent role in regulating cyclin levels. Park2 degrades cyclins D [122] and E [123] in a Cul1-dependent manner [124]. Park2 mutations found in cancer lead to stabilization of these G1/S-phase cyclins, an increase in the number of cells in S and G2/M phase [123, 124], and increased rates of cellular proliferation [122]. Moreover, Park2 associates with Cdc20 and Cdh1 during mitosis in an APC/C-independent manner and regulates the levels of many APC/C substrates including mitotic kinases and mitotic cyclins [125]. Park2 regulates microtubules

and the mitotic spindle, cytokinetic bridge [126], cell motility [127], and invasion [128]. Park2 ubiquitinates and degrades HIF-1α to contribute to cell migration, and loss of Park2 leads to tumor metastasis in mouse models [129].

In Park2 knock-out mouse models, the resulting oxidative stress and the Warburg effect [130] caused an increase in the mRNA of Aim2, a protein involved in cytokine production [131]. In these mouse models, activation of Aim2 ultimately led to upregulation of PD-L1 in pancreatic tumors and lower rates of survival, an effect seen in human pancreatic tumors and patients [131]. Thus, Park2's roles in metabolism may affect the ability of the immune system to regulate cancer progression.

SPOP is a Cul3 substrate adaptor mutated in about 10% of prostate cancers and some kidney cancers [132]. SPOP has three basic domains: an N-terminal MATH domain for substrate recognition [133], a BTB domain for dimerization and interaction with Cul3 [134], and a BACK domain which assembles SPOP dimers into oligomers [134], a mechanism which increases SPOP binding to and ubiquitination of the substrate [135]. As SPOP regulates many proteins responsible for maintaining cell integrity, mutations in the MATH domain that disrupt binding to substrate encourage cancer progression [136].

SPOP plays a role in immunotherapy by ubiquitinating and degrading PD-L1 [137]. SPOP binding mutants cannot ubiquitinate PD-L1, resulting in larger tumor growth and fewer tumor-infiltrating lymphocytes compared to tumors harboring wild-type SPOP in mouse models [137]. Similarly, pancreatic cancer samples with mutant SPOP had higher levels of PD-L1, demonstrating a role for SPOP in immune system invasion [137].

Other notable SPOP substrates include the apoptotic protein Daxx [138, 139], deSUMOlyase SENP7 [140], c-Myc [141], HDAC6 [142], Cdc20 [143], proto-oncogene DEK [144], phosphatases PTEN and Dusp7 [139], hedgehog pathway proteins Gli2 and Gli3 [145, 146], and BET transcriptional coactivators BRD2–4 [147–149]. SPOP is also closely tied to hormone-activated pathways, as steroid receptor coactivator SRC-3 [150], androgen receptor (AR) [151], enhancer of AR-mediated transcriptional activity TRIM24 [144], and estrogen receptor α (ERα) [136] are all substrates of SPOP. Finally, wild-type, but not mutant SPOP degrades ERG [152]. Interestingly, in some prostate cancer samples, some tumors expressed a fused ERG protein due to genome rearrangements, a phenotype driven by SPOP mutation [153]. Unlike wild-type ERG, these ERG-fusions lack an SPOP binding site, contributing to cancer progression [154].

3. E3 ligases and their inhibitors

One ubiquitin-proteasome inhibitor has already found use in the treatment of cancer: Bortezomib is a 26S-proteasome inhibitor approved for treating certain types of myeloma and lymphoma that binds to and inhibits the proteasome from degrading other proteins [155]. Another compound still in clinical development is MLN4924 (Pevonedistat), an inhibitor of the Nedd8-activating enzyme and thus of Cullin RING ligase complexes [155]. As ubiquitination plays many important roles in cell regulation, these broad inhibitors can affect many cellular pathways, not just those that are therapeutically useful. As E3 ligases are specific for their substrates, E3 ligases serve as precise targets for therapeutic intervention (**Table 2**). Inhibition of E3 ligases will hopefully minimize off-target effects. Moreover, as some E3 ligases have many oncogenes as their substrates, targeting E3 ligases may serve to be more efficient than targeting individual substrates.

While most inhibitors have been identified via high throughput screens, the most clinically relevant inhibitors have been derived from structure–function

analyses of E3 ligases complexed to their substrates. For example, the crystal structure of MDM2 bound to p53 allowed for the identification of the MDM2-p53 binding pocket and the design of small molecules [156] (like Nutlins and their derivatives) and stapled peptides [157] that bind to MDM2 and inhibit p53 binding. Similarly, the structure of the IAP family of E3 ligases and their endogenous inhibitors, the SMAC peptides, allowed for the development of higher affinity peptides [158] and peptidomimetics and the discovery of one small molecule inhibitor, Embelin [159]. Of the inhibitors mentioned here, MDM2 and XIAP inhibitors have advanced the farthest in clinical trials. A crystal structure of the SPOP substrate binding domain was also used to develop an SPOP inhibitor, suggesting that structural studies may greatly enhance development of small molecule inhibitors [160].

Most inhibitors disrupt E3 ligase-substrate binding by blocking the binding pocket of the E3 ligase. However, because HECT domains first transfer the ubiquitin molecule to themselves via a thioester bond [24], HECT ligases have an

E3 ligase		Therapeutic	Mechanism	Model			In clinical trials
				In vitro assay	Cell culture	Mouse model	
TP53	E6AP	CM-11 peptides [161]	Binds HECT domain	X	X		
		Compound 9 [173]	Binds HPV E6	X	X		
	MDM2/X	Nutlins [156], RG7112 [174]	Binds p53 binding site	X	X	X	
		Idasanutlin (RG7388) [175]		X	X	X	X
		MI-888 [176], SAR405838 [151]	Binds p53 binding site	X	X	X	X
		AMG-232 [177]	Binds p53 binding site	X	X	X	X
		NVP-CGM097 [178], HDM201 [179]	Binds p53 binding site	X	X	X	X
		JNJ-26854165 (Serdemetan)	Assumed to bind to RING domain of MDM2 [180]		X	X	X
		ALRN-6924 [157]	Stapled peptide binds MDM2 and MDMX at p53 binding site	X	X	X	X
SCF	Skp2	Compound #25 [181]	Binds Skp1 binding site	X	X	X	
		C1, C2, C16, C20 [163, 182]	Presumed: Binds Skp2, Cks1 at p27 binding site	X	X		
		CpdA [165]	Inhibits Skp2-Skp1 binding	X	X		
		NSC689857, NSC681152 [164]	Inhibits Skp2-Cks1 binding	X			
	Fbxw7	Oridonin [183]	Stabilizes Fbxw7, increases the activity of kinase Gsk-3	X	X		

E3 ligase		Therapeutic	Mechanism	Model			In clinical trials
				In vitro assay	Cell culture	Mouse model	
	β-TrCP	Erioflorin [184]	Inhibits β-TrCP1 binding to substrate	X	X		
		GS143 [185]	Presumed: Inhibits binding of β-TrCP1 and p-IkBa	X	X		
APC/C	Cdc20	Apcin [186]	Binds to D-box binding site of Cdc20	X	X		
	Cdc20/ Cdh1	ProTAME [187]	Inhibits formation of APC/C-Cdc20, -Cdh1	X	X	X	
Other	XIAP	LCL161 [158]	Binds to BIR3 domain of XIAP [188]	X	X	X	X
		AEG 35156 [189]	XIAP antisense oligonucleotide		X		X
	SPOP	Palbociclib [137]	Cdk4 phosphorylates SPOP, destabilizes PD-L1	X	X	X	*
		Compound 6b [160]	Binds to substrate pocket	X	X	X	

**Palbociclib is clinically approved for treatment of breast cancer.*

Table 2.
E3 ligases and their inhibitors.

additional mode of pharmacological inhibition. The CM-11 peptides (E6AP inhibitors) are one such therapy that takes advantage of this step to inhibit or disrupt the HECT-Ubiquitin transthiolation reaction [161]. Future work may focus on designing small molecules that disrupt this function of the HECT domain.

To degrade its most clinically relevant targets p21 and p27, Skp2 functions with an adaptor protein, Cks1 [162]. At least two classes of inhibitors (NSC689857/NSC681152 [163] and the C1/2/16/20 compounds [164]) have been developed that disrupt the Skp2-Cks1 interaction. Similarly, the SCF ligase complex is only active upon the binding of an F-box protein to Skp1. CpdA inhibits Skp2-Skp1 binding [165]. These results suggest that another method of inhibitor design may focus on disrupting crucial activators and binding partners of E3 ligases instead of merely disrupting E3 ligase-substrate binding.

Upon phosphorylation by Cdk4, SPOP protein levels are stabilized, and PD-L1 expression levels decrease [137]. To improve the efficiency of anti-PD-L1 immunotherapies, mice treated with both Cdk4/6 inhibitors (to destabilize SPOP and thus stabilize PD-L1) and anti-PD-L1 immunotherapy showed improved survival when compared to untreated mice or mice with each individual treatment [137]. In this case, stabilization of an oncogenic protein led to improved efficacy of a complimentary therapy. Whether a similar combination of therapies can be used to improve the overall survival rate in other pathways remains to be seen.

4. Conclusions and perspectives

Recent research has highlighted the role of ubiquitination in cell regulation, division, and cancer cell progression. While much work has advanced the identification of E3 ubiquitin ligases and their substrates, untangling how these ligases act upon interconnected pathways remains a challenge in cancer cell biology. For example, understanding in which contexts certain E3 ligases are tumor-supportive or tumor-suppressive (like β-TrCP) is still not clear. Genome-wide analyses and advancements in systems biology have aided in and will continue to contribute to addressing these issues.

The tumor microenvironment has established itself as a central component in understanding and treating cancer progression. The macro-level questions of tumors—how cancers induce angiogenesis, interact with the immune system and cytokines, interact with the microbiome, and metastasize—are some questions that are best addressed with research in animal models, not human cell culture models. For example, the recent discoveries that both SPOP and Park2 play a role in mediating PD-L1 stability demonstrate the need to study the roles of E3 ligases in animal models. Given the recent success of immuno-oncology and CAR-T cell therapy, a further understanding how E3 ligases affect macro-level phenotypes like tumor sensitivity to immunotherapies may influence the design of clinical therapies.

While many E3 ligase inhibitors are being identified via high-throughput small molecule screens that assess inhibition of E3 ligase-substrate binding or ubiquitination activity, the most clinically advanced inhibitors have been refined from structural analysis of the E3 ligase binding pocket. The structures of many E3 ligases have already been determined (for example, all 11 ligases discussed here have at least a partial structure), so further pharmacological development may involve identifying binding pockets and designing inhibitors to perturb ligase function, and optimizing already identified inhibitors. On the other hand, E3 ligases are often redundant, so inhibition of one ligase may not completely stabilize a beneficial substrate. Nonetheless, the early clinical success of some E3 ligase inhibitors suggests that ubiquitin ligase inhibition is a promising venue for therapeutic intervention in cancer patients.

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