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Review

Ubiquitin ligases in cancer: Functions and clinical potentials

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SUMMARY

Ubiquitylation, a highly regulated post-translational modification, controls many cellular pathways that are critical to cell homeostasis. Ubiquitin ligases recruit substrates and promote ubiquitin transfer onto targets, inducing proteasomal degradation or non-degradative signaling. Accumulating evidence highlights the critical role of dysregulated ubiquitin ligases in processes associated with the initiation and progression of cancer. Depending on the substrate specificity and biological context, a ubiquitin ligase can act either as a tumor promoter or as a tumor suppressor. In this review, we focus on the regulatory roles of ubiquitin ligases and how perturbations of their functions contribute to cancer pathogenesis. We also briefly discuss current strategies for targeting or exploiting ubiquitin ligases for cancer therapy.

INTRODUCTION

Ubiquitylation refers to the enzymatic post-translational modification in which the ubiquitin protein is covalently attached to cellular proteins (Hershko and Ciechanover, 1998). The core enzymes driving this process are the ubiquitin-activating enzyme (UAE or E1), the ubiquitin-conjugating enzyme (UBC or E2), and the ubiquitin ligase (E3).

E3s recruit substrates and thus determine the overall specificity for ubiquitylation. They constitute a wide class of proteins, with the human genome encoding more than 600 putative E3s (Li et al., 2008), further subdivided into several families based on their conserved structural domains (e.g., RING, HECT, UBOX, or RBR domains) (Buetow and Huang, 2016; Metzger et al., 2012). Among these, RING-finger ubiquitin ligases comprise the largest family, with members able to function as monomers, dimers, or multi-subunit complexes (Lipkowitz and Weissman, 2011; Petroski and Deshaies, 2005). Of the last, the best characterized subfamily is formed by ~230 E3s, known as cullin-RING ligases (CRLs). CRLs are modular complexes that contain a cullin scaffold, a RING-finger protein (RBX1 or RBX2) that serves as the site for E2 binding, and a variable/exchangeable substrate receptor (SR) subunit (Lydeard et al., 2013; Skaar et al., 2013). Mammals possess eight cullin proteins (CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5, CUL7, and CUL9), each of which interacts with a unique family of SRs that provides the substrate specificity (Cardozo and Pagano, 2004; Petroski and Deshaies, 2005; Skaar et al., 2013). F-box proteins, VHL/BC-box proteins, BTB proteins, DCAF proteins, and SOCS box proteins are the SRs of the CRL1, CRL2, CRL3, CRL4A/B, and CRL5 complexes, respectively. CRL7 uses only one SR (i.e., FBXW8), and it is not clear to date whether CRL9 binds any SR. CRL1 complexes are also known as SCF (SKP1, CUL1, F-box protein) complexes.

The large number of E3s and their targeted substrates connects the ubiquitin-proteasome system to diverse biological processes and human diseases, particularly cancer. Studies during the last 20 years have shown that deregulated E3s play a critical role in the development, progression, and therapeutic response of human cancers. Depending on their substrates, E3s themselves can play tumor-suppressive or pro-oncogenic roles, serving as therapeutic targets for anti-tumor drugs (Senft et al., 2018; Wang et al., 2014). This review will provide an overview of the current understanding of the functions of E3s in cancers, and discuss perspectives on cancer therapies that depend on inhibition or activation of ubiquitylation of target proteins.

Role of E3s in tumor development

The development of cancer is a multistep process through which normal cells evolve progressively into a neoplastic state and acquire features that Douglas Hanahan and Bob Weinberg defined as the "hallmarks of cancer," including increased capacity for proliferation, survival, invasion, and metastasis and the ability to evade immunosurveillance and destruction (Hanahan and Weinberg, 2011). Such a complex process requires the reprogramming of cellular signaling networks to meet the needs for malignant transformation. By controlling protein abundance and activity in a timely and specific manner, E3s serve as central regulatory nodes for many signaling pathways. It is therefore no surprise to observe that E3s and their substrates are frequently deregulated in human cancers (Qi and Ronai, 2015). Aberrant regulation of E3s can occur at the genetic, epigenetic, or post-translational level (Figure 1). These alterations in E3s can either convert proto-oncoproteins into oncoproteins or inactivate tumor suppressors. Moreover, some oncogenic viruses can alter or hijack E3 activity to modulate the abundance of cellular

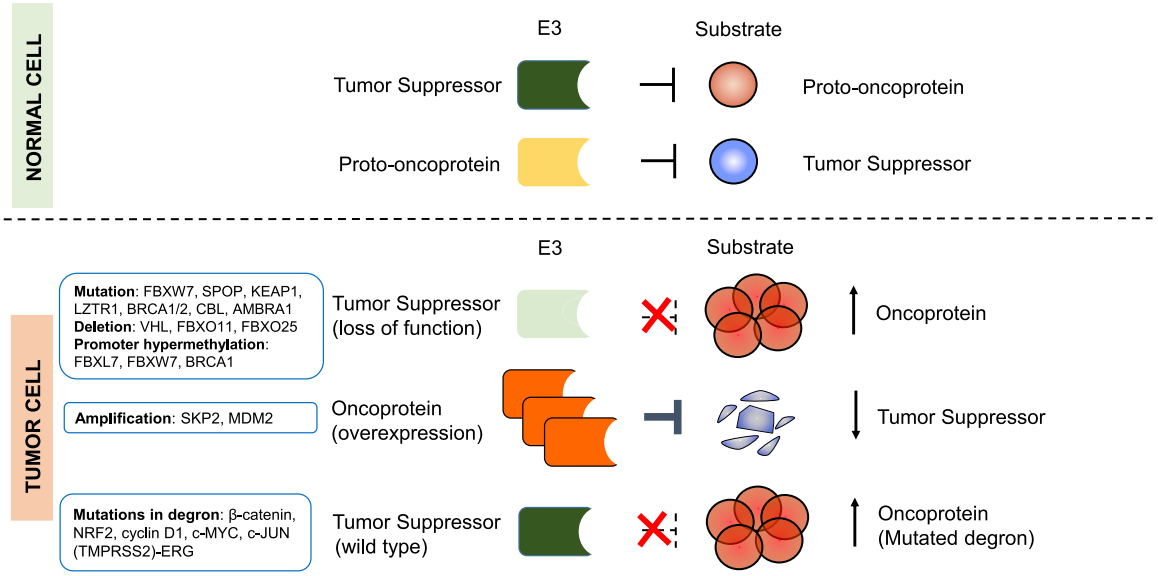

Figure 1. Deregulation of E3-mediated ubiquitylation in cancer

The illustration shows different modes of E3 deregulation, with well-established examples listed. Most deregulations of E3s arise as a consequence of genetic or epigenetic alterations, which can affect the abundance and/or activity of their substrates. For example, deletions, mutations, or promoter methylations can inactivate E3s that normally function as tumor suppressors, and lead to the overexpression of oncoprotein substrates (e.g., c-Myc, cyclin E, and ERG). Alternatively, overexpression of E3s (e.g., through gene amplification of *MDM2* and *SKP2* loci) that target tumor suppressors (p53 and p27, respectively) can promote tumor formation. In addition, mutations in the substrates that enable them to escape recognition by E3s can lead to their accumulation.

substrates for the benefit of viral replication, ultimately promoting tumor transformation.

In the following sections, we will discuss the critical roles of E3s in processes associated with the initiation and progression of cancers (Figure 2) and give examples on how deregulation of E3s affects these biological processes.

E3s and cell-proliferative signaling

One of the most fundamental traits of cancer cells is their ability to sustain constant proliferation. Under normal conditions, cellular proliferation signaling pathways are tightly controlled by the limited availability of growth factors and nutrients, contact inhibition, and various feedback mechanisms (Sever and Brugge, 2015). Cancer cells, by deregulating these signals, become masters of their own destinies and no longer depend on external stimuli to proliferate.

E3s modulate the abundance of regulatory proteins that participate in major hubs of the proliferative signaling. Thus, dysregulations of E3s may have profound effects on both upstream regulators and downstream effectors. As it is impossible to cover all the signaling molecules involved, we will focus primarily on major proliferative signaling pathways to illustrate the functions of E3s in monitoring essential signal transducers to ensure proper signaling dynamics. Constitutive hyperactivation of these signaling pathways can lead to excessive proliferation and ultimately tumorigenesis (Lavoie et al., 2020; Manning and Toker, 2017).

E3s mediate the ubiquitylation and downregulation of RTKs

Receptor tyrosine kinases (RTKs) function as entry points for many extracellular cues, initiating intracellular signaling cascades. Inappropriate activation of RTKs is associated with a large number of malignancies (Lemmon and Schlessinger, 2010). Among other mechanisms, escaping from ubiquitylation-mediated negative regulation is a central event in RTK deregulation. Activated RTKs are downregulated through endocytosis and subsequent intracellular degradation, and ubiquitylation either serves as a sorting signal for targeting RTKs to clathrin-coated pits for endocytosis or mediates the degradation of RTKs (Marmor and Yarden, 2004; Tomas et al., 2014). A well-documented example is the epidermal growth factor receptor (EGFR). Following ligand binding and receptor activation by phosphorylation, EGFR is modified by the E3 c-CBL, leading to its internalization by endocytosis and subsequent degradation via the lysosome (Levkowitz et al., 1999; Thien and Langdon, 2001). The CBL family of E3s also targets other receptors, including PDGFR, c-Fms/CSF-1R, c-Kit, and Met (Peschard and Park, 2003). Mutations in CBL or RTKs can impede their ubiquitylation and contribute to their hyperactivation. CBL mutations have been found in ~5% of a wide variety of myeloid neoplasms, and many of the mutations are missense mutations affecting its E3 activity (Kales et al., 2010). Moreover, RTK mutants impaired in their ability to bind c-CBL exhibit prolonged protein stability and enhanced activity (Grandal et al., 2007; Peschard and Park, 2003).

E3s regulate RAS-MAPK and PI3K-AKT signaling

Ligand-bound RTKs transduce signaling by activating downstream signaling pathways, including the RAS-mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K)-AKT-mTOR signaling cascades, to regulate various cytoplasmic and nuclear substrates that are responsible for cell growth and proliferation. RTKs recruit adaptor proteins, such as SOS, a RAS-specific guanine nucleotide exchange factor, to the plasma membrane. SOS enhances the exchange of

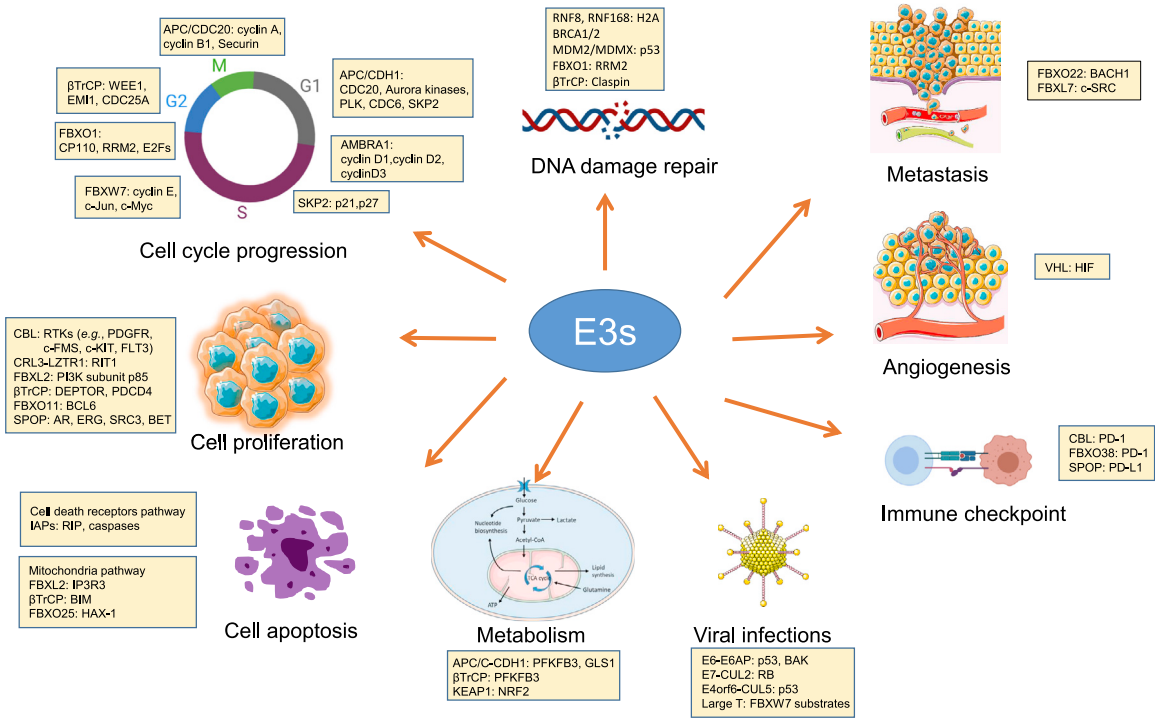

Figure 2. E3s in the regulation of the hallmarks of cancer

E3s are hubs of many cellular processes, including those playing key roles in cell proliferation, cell cycle, the DNA damage response, apoptosis, metabolism, metastasis, angiogenesis, immune checkpoint, and viral infections. Representative E3s with their best-defined substrates in these biological processes are listed.

GDP for GTP by the RAS small GTPase, resulting in RAS activation (Chardin et al., 1993; Lemmon and Schlessinger, 2010). The GTPases of the RAS superfamily are key signal transducers that activate kinase cascades, including the MAPKs, to regulate key pathways promoting cell proliferation. Ubiquitylation on several members of the RAS family has been observed, leading to their altered cellular localization and activity (de la Vega et al., 2011). LZTR1, the SR of a CRL3, has been proposed to mediate the ubiquitylation of RAS to control either its activity or its degradation (Abe et al., 2020; Bigenzahn et al., 2018; Steklov et al., 2018). Mutations in LZTR1 have been identified in human cancers and developmental diseases such as Noonan syndrome, a genetic disorder caused by germline mutations in genes involved in the RAS-MAPK pathway (Motta et al., 2019; Rauen, 2013). However, the role of LZTR1 in RAS ubiquitylation has not been confirmed by others, and an elegant study instead showed that $CRL3^{LZTR1}$ targets RIT1, a different small GTPase and proto-oncoprotein, for ubiquitin- and proteasome-mediated degradation (Castel et al., 2019). Pathogenic mutations in *LZTR1* affect its interaction with RIT1, leading to RIT1 accumulation and subsequent dysregulation of growth factor signaling responses (Castel et al., 2019), in agreement with what has been observed in human patients with LZTR1 mutations.

Another major downstream effector of RTKs is the PI3K, which catalyzes the production of phosphatidylinositol (3,4,5)-trisphosphate at the plasma membrane, resulting in the activation of AKT kinases (Hoxhaj and Manning, 2020). AKT activates numerous downstream effectors, including mTOR kinase and the forkhead box O family of transcription factors, to promote protein synthesis and cell proliferation. The PI3K-AKT signaling is regulated in multiple ways, including via E3-mediated ubiquitylation. PI3K is a dimeric enzyme composed of a catalytic subunit (p110α, p110β, or p110γ) and a regulatory subunit (p85α, p85β, p55α, p55γ, or p50α). Ubiquitylation on both subunits has been reported, with various effects on PI3K signaling. For example, FBXL2, a CRL1 SR, was shown to mediate the degradation of free p85 to prevent its competition with active PI3K (composed of p85-p110 heterodimers) for phospho-Tyr docking sites at the cell membrane (Kuchay et al., 2013). Via this mechanism, FBXL2 ensures efficient activation of the PI3K signaling cascade to promote cell proliferation and survival.

mTOR belongs to the PI3K-related kinases family and exists in two distinct complexes called complex 1 (mTORC1) and complex 2 (mTORC2). Both complexes respond to numerous environmental cues, especially those related to nutrient, energy, and oxygen levels, to modulate cell growth and survival (Liu and Sabatini, 2020). Within the mTORC, the DEPTOR protein binds mTOR and inhibits mTORC activity (Peterson et al., 2009). Upon mitogenic stimulation, $CRL1^{\beta TrCP}$ cooperates with mTOR and CK1 to induce the degradation of DEPTOR, generating an auto-amplification loop that promotes the full activation of mTOR (Duan et al., 2011; Gao et al., 2011; Zhao et al., 2011). A key function of activated mTOR is to regulate mRNA translation and promote protein synthesis, which is an energy-costly process. Under nutrient deprivation conditions, PDCD4 (a substrate of the mTOR pathway) inhibits the translation initiation factor eIF4A (Yang et al., 2003). Activated mTOR leads to the phosphorylation of PDCD4, promoting its interaction with βTrCP

and leading to its degradation (Dorrello et al., 2006). This event relieves PDCD4 repression of eIF4A, allowing efficient protein translation and cell growth. Hence, βTrCP coordinates increased protein synthesis with pro-survival signaling.

Other E3s linked to cell proliferation and cancer

E3s can of course regulate numerous other signaling pathways involved in cell proliferation. Notably, a number of E3s regulating cell proliferation are deregulated in specific types of cancers. For example, SPOP (Speckle-type POZ protein) is a CRL3 SR whose corresponding gene is mutated in 5%–15% of prostate cancer (Barbieri et al., 2012) and endometrial cancer (Le Gallo et al., 2012). CRL3-SPOP has been implicated in the regulation of key pro-oncogenic signaling proteins, including the androgen receptor (AR), SRC3 (also known as NCOA3), ERG, and BET proteins (BRD2, BRD3, and BRD4) (Clark and Burleson, 2020). In prostate cancer, *SPOP* mutations cluster within the N-terminal MATH domain, a region responsible for substrate recognition and ubiquitin transfer, thus leading to impaired E3 activity. Intriguingly, mutations in endometrial cancer occur in a distinct, uncharacterized region of the MATH domain and have the opposite effect in potentiating the degradation of BET proteins (Janouskova et al., 2017). Moreover, many SPOP substrates contain a SPOP-binding consensus motif (Zhuang et al., 2009), and mutations in this motif can disrupt their interaction with SPOP. For example, prostate cancer tumors harbor *SPOP* mutations and *TMPRSS2-ERG* gene fusions were found to occur in a mutually exclusive manner, with both alterations inhibiting $CRL3^{SPOP}$-dependent degradation of ERG (An et al., 2015; Gan et al., 2015).

Like SPOP, KLHL6 (Kelch-like protein 6) is a CRL3 SR, which is encoded by a gene mutated in human diffuse large B cell lymphoma (DLBCL) (Choi et al., 2018). Mutations in KLHL6 inhibit its ligase activity by disrupting the interaction with CUL3, leading to the stabilization of its substrate, the mRNA decay factor roquin-2, which subsequently promotes tumor cell growth.

Deletions and mutations in the gene encoding the F-box protein FBXO11 that lead to stabilization of the BCL6 oncoprotein have also been observed in ~10% of patients with DLBCL (Duan et al., 2012). BCL6 functions as a transcription factor in B cell development, differentiation, and activation (Ci et al., 2008). Thus, increased BCL6 expression drives the development of DLBCL.

E3s and cell-cycle progression

The transduction of mitogenic signals stimulates cell-cycle progression to promote proliferation. Eukaryotic cell-cycle progression is driven by oscillations in the activity of cyclin-dependent kinases (CDKs), which are activated by cyclins and inhibited by CDK inhibitors (CKIs) (Bassermann et al., 2014; King et al., 1996). Proteasomal degradation of cyclins and CKIs regulates CDK activity, and its dysregulation contributes to the sustained proliferation observed in cancer cells.

E3s in different phases of the cell cycle

Two multi-subunit families of E3s are crucial to cell-cycle progression, the CRL1 complexes and the APC/C (anaphase-promoting complex, also known as the cyclosome, which binds to one of its co-activators, CDC20 and CDH1) (Nakayama and Nakayama, 2006; Skaar and Pagano, 2009). APC/C^{CDC20} and APC/C^{CDH1} are active in mitosis and G1, respectively, while CRL1 complexes are active throughout the cell cycle. APC/C targets substrates with short linear sequence motifs, referred to as degradation motifs or degrons (e.g., D-box, KEN-box, and ABBA motifs) (Davey and Morgan, 2016). Some of the CRL1 SRs, such as βTRCP and FBXW7, trigger the degradation of cell-cycle regulatory proteins through conserved degrons only when these are phosphorylated.

Upon mitogen stimulation, cells commit to cell division and pass a restriction checkpoint in G1. Mitogenic factors activate intracellular signaling cascades to induce the expression of D-type cyclins (D1, D2, and D3) that activate CDK4 and CDK6. A well-known target of CDK4/6-cyclin D is the retinoblastoma tumor suppressor protein (RB), which binds some members of the E2F family of DNA-binding transcription factors (E2F) to block their transcriptional activity (Harbour and Dean, 2000). RB phosphorylation promotes its dissociation from E2Fs, releases the E2F transcription factors, and drives the expression of E2F-target genes, including *CCNE*, which encodes cyclin E. In turn, activation of CDK2-cyclin E drives cell-cycle progression from G1 into S phase, during which DNA replication occurs (Duronio and Xiong, 2013; Otto and Sicinski, 2017). Upon successful S-phase entry, cyclin D1 is phosphorylated and degraded by $CRL4^{AMBRA1}$ (Chaikovsky et al., 2021; Simoneschi et al., 2021). CDK2-cyclin E and CDK2-cyclin A become main CDK complexes in S phase, promoting the initiation of DNA and centrosome duplication. The rising of CDK2 activity also requires removal of inhibition of the associated CKIs, such as p21 or p27. $CRL1^{SKP2}$ mediates the ubiquitylation and degradation of p21 and p27, liberating CDK2 from CKI inhibition. Finally, during G2, E2F activity is turned off via the degradation of E2F1/2/3A mediated by FBXO1 (also known as cyclin F) (Burdova et al., 2019; Clijsters et al., 2019; Emanuele et al., 2020).

During S and G2, it is essential for the cell to ensure accurate DNA replication and chromosome integrity. CRL1s are key players in DNA surveillance mechanisms. Upon detection of DNA damage, $CRL1^{\beta TrCP}$ in cooperation with the kinase CHK1 targets the CDK1/2 activating phosphatase CDC25A for degradation (Busino et al., 2003), resulting in an attenuated CDK activity and consequent halt in cell-cycle progression. βTrCP also controls recovery from this checkpoint. Claspin, a checkpoint mediator, is phosphorylated by PLK1 during recovery from genotoxic stress and subsequently degraded in a βTrCP-dependent manner (Mailand et al., 2006; Peschiaroli et al., 2006).

CRLs also monitor the process of centrosome duplication to prevent chromosome aberrations. CP110, a protein essential for centrosome duplication, is targeted by FBXO1 on the centrioles during G2 (D'Angiolella et al., 2010). Thus, FBXO1 ensures that centrosomes are replicated only once during the cell cycle, avoiding centrosome overduplication.

As cells progress through G2 and prepare to enter mitosis, CDK2 gradually steps down from the stage. CDK1 now takes the role to phosphorylate downstream substrates, thereby shaping the mitotic environment. FBXW7 mediates the degradation of cyclin E (Strohmaier et al., 2001), resulting in attenuation of CDK2 activity, whereas βTrCP promotes activation of CDK1 by mediating the degradation of WEE1, a CDK1-inhibitory kinase (Watanabe et al., 2004).

Starting from metaphase, APC/C becomes a key player in promoting mitotic progression and, eventually, mitotic exit (Skaar

and Pagano, 2009). After inactivation of the spindle assembly checkpoint, APC/C^{CDC20} targets securin for degradation, leading to sister chromatid separation. APC/C^{CDC20} also targets cyclin B1 to induce mitotic exit, so this E3 coordinates the segregation of replicated genetic material with the end of mitosis. At the end of anaphase, CDH1 replaces CDC20 within the APC/C complex and promotes further degradation of mitotic proteins, including PLK1, Aurora kinases, and CDC20 itself. βTrCP mediates degradation of the APC/C^{CDH1} inhibitor EMI1, thus contributing to the activation of APC/C (Guardavaccaro et al., 2003). After the exit from mitosis, or during withdrawal from the cell cycle, APC/C^{CDH1} contributes to maintenance of the G1 state or establishment of a stable G0, respectively. APC/C^{CDH1} targets drivers of DNA replication and mitosis (e.g., CDC6, cyclin A, and E2F), as well as SKP2, thus promoting the stabilization of CKIs.

Deregulated ubiquitylation affects cell-cycle progression

Selective degradation of cell-cycle regulators ensures the order and timing of cell-cycle events. The functions of the E3s mentioned above in coordinating cell-cycle progression give them proto-oncogenic or tumor suppressor properties, as supported by their frequent alterations in many tumors.

In addition to cyclin E, $CRL1^{FBXW7}$ targets several well-known oncoproteins that regulate cell proliferation, including c-MYC, c-JUN, and NOTCH (Welcker and Clurman, 2008). FBXW7 is a haploinsufficient tumor suppressor that is frequently subjected to mutations and deletions. Mutations of FBXW7 have been identified in many human cancers, with the highest rate (approximately 30%) in cholangiocarcinoma and T cell acute lymphoblastic leukemias (Welcker and Clurman, 2008). A majority of the FBXW7 mutations are point mutations that occur at key residues that form the substrate-binding interface. Thus, *FBXW7* mutations associated with cancer appear to disrupt substrate recognition. Most of the remaining mutations are nonsense codons that lead to premature termination of FBXW7 translation. Studies leveraging mouse models showed that loss-of-function mutations in FBXW7 promoted hematopoietic or solid organ tumor formation (Mao et al., 2004; Matsuoka et al., 2008), supporting a tumor suppressor role for FBXW7. However, the function of FBXW7 is context dependent, as studies have also shown that FBXW7α functions as a pro-survival gene in multiple myeloma by constitutively targeting the NF-κB inhibitor p100 (Busino et al., 2012), and these tumors do not harbor FBXW7 mutations.

As an important regulator of cell-cycle progression, cyclin D1 is frequently overexpressed in several cancer tissues, including breast cancer, lung cancer, prostate cancer, melanoma, oral squamous cell carcinomas, and colorectal cancer (Alao, 2007; Musgrove et al., 2011). In addition to gene amplification, defects in cyclin D1 degradation account for its overexpression. The literature suggests that several CRL1 SRs and APC/C are the E3s for cyclin D1. However, these studies were not confirmed by genetic studies (Kanie et al., 2012; Qie and Diehl, 2016). Two recent papers also were unable to support a role for CRL1s in cyclin D1 degradation and, instead, they demonstrate that $CRL4^{AMBRA1}$ targets all three D-type cyclins for degradation (Chaikovsky et al., 2021; Simoneschi et al., 2021). *AMBRA1* is frequently mutated in human cancers, resulting in the accumulation of these pro-oncogenic cyclins. Moreover, cancer patients often display somatic mutations in the degron of all three D-type cyclins, also resulting in their accumulation. Finally, mouse models confirm a role of AMBRA1 in the degradation of D-type cyclins, both allowing proper embryogenesis and promoting oncogenesis.

Overexpression of SKP2 levels is pro-oncogenic, due to the enhanced degradation of p27 and the resulting overactivation of CDK1/2. Indeed, amplification of SKP2 is found in multiple human cancers, including lymphomas, prostate cancer, colorectal cancer, melanoma, gastric cancer, pancreatic cancer, and breast cancer (Frescas and Pagano, 2008; Gstaiger et al., 2001).

By virtue of its ability to regulate broad cell-cycle regulators, βTRCP can function as either a tumor suppressor or an oncogenic protein, and may function as an oncoprotein in some tissue types despite having tumor-suppressive effects in others (Frescas and Pagano, 2008; Wang et al., 2014). Overexpression of βTRCP has been reported in colorectal cancer, hepatoblastoma, and breast cancers (Fuchs et al., 2004; Koch et al., 2005; Ougolkov et al., 2004). Somatic mutations in βTRCP that may affect its E3 activity, although rare, were observed in certain human cancers, such as gastric and prostate cancers (Gerstein et al., 2002; Kim et al., 2007a; Saitoh and Katoh, 2001). Moreover, aberrant degradation of βTrCP substrates can be caused by mutations in the substrates themselves or their upstream regulators. Therefore, the roles of βTRCP in cancer need to be evaluated in a tissue-specific or cellular context-dependent manner.

E3s and cell death

Programmed cell death by apoptosis serves as a natural barrier to cancer development. Apoptosis can be mainly initiated through one of two pathways, the intrinsic and the extrinsic (Fulda and Debatin, 2006; Igney and Krammer, 2002). Both pathways are regulated by a number of pro-apoptotic and anti-apoptotic regulators, whose levels need to be carefully controlled, as malregulation can introduce defects in cell-death signaling and confer a survival advantage to cancer cells. E3-mediated ubiquitylation acts at many levels in regulating the apoptotic machinery (Jesenberger and Jentsch, 2002).

Regulation of the extrinsic apoptosis pathway

The extrinsic apoptosis pathway is initiated at the plasma membrane by death receptor ligation. Stimulation of death receptors (e.g., the tumor necrosis factor [TNF] receptor superfamily [CD95/APO-1/Fas] and TNF-related apoptosis-inducing ligand [TRAIL] receptors) by CD95 ligand or TRAIL results in receptor aggregation and recruitment of the death-inducing stimulating complex (DISC) and caspase-8. Upon recruitment, caspase-8 is activated to initiate apoptosis by direct cleavage of downstream effector caspases. The inhibitor of apoptosis (IAP) family of proteins are RING-domain E3s that suppress apoptosis. For example, cIAP1 and cIAP2 induce the ubiquitylation of RIP1, an adaptor subunit of DISC, thus promoting cancer cell survival (Bertrand et al., 2008). Recurrent upregulation of IAP expression has been implicated in cancer development by suppressing apoptosis (Wong, 2011). cIAP2 is highly expressed in pancreatic cancer, and its level correlates to chemotherapy resistance (Lopes et al., 2007). Another IAP, livin, was observed to be highly expressed in human melanoma and other cancers (Vucic et al., 2000).

Regulation of the intrinsic apoptosis pathway

Intrinsic apoptosis signaling is initiated by stress signals through the release of pro-apoptotic factors, such as cytochrome *c* or the

IAP antagonist Smac/DIABLO, from the mitochondrial intermembrane space, an event that ultimately leads to caspase activation and apoptosis. E3s play important roles in stress sensing, signaling, and activation of this apoptotic pathway. For instance, overload of Ca^{2+} into mitochondria can trigger apoptosis (Orrenius et al., 2003). $CRL1^{FBXL2}$ binds the IP3 (inositol 1,4,5-trisphosphate) receptor 3 (IP3R3) and targets it for degradation to limit Ca^{2+} influx into mitochondria (Kuchay et al., 2017). Disruption of FBXL2-mediated regulation of IP3R3 leads to increased cytosolic Ca^{2+} release from the endoplasmic reticulum and sensitizes cells to Ca^{2+}-dependent apoptotic stimuli.

The BCL-2 family of proteins controls cell death largely by regulating mitochondrial outer membrane permeabilization and releasing intermembrane-space pro-apoptotic proteins. The BCL-2 family is divided into anti-apoptotic proteins (such as BCL-2 and BCL-XL) and pro-apoptotic proteins (such as BAX, BAK, BIM, NOXA, and PUMA) (Kale et al., 2018). Hyperactivation of pro-survival signaling (e.g., RAS-ERK and PI3K-AKT) in cancer cells, together with E3s, can disrupt the balance between the pro-apoptotic and the anti-apoptotic proteins and promote cell survival. For example, $CRL1^{\beta TrCP}$ targets BIM for degradation, following its ERK- and RSK-mediated phosphorylation of BIM1 (Dehan et al., 2009). Consequently, knockdown of either βTRCP or RSK sensitizes non-small cell lung cancer (NSCLC) cell lines to gefitinib, a tyrosine kinase inhibitor that induces apoptosis through BIM1.

HCLS1-associated protein X-1 (HAX-1) is a BCL-2-family-related protein that is required to suppress apoptosis in lymphocytes and neurons (Chao et al., 2008). HAX-1 is targeted for degradation by $CRL1^{FBXO25}$ (Baumann et al., 2014). FBXO25 is located at chromosome 8p23.3, a region that is frequently deleted in human mantle cell lymphoma (up to 29%) and other malignancies, such as DLBCL (~8%). In these patients, deletion of FBXO25, and therefore disruption of the FBXO25-mediated HAX-1 degradation, functions as a means for promoting lymphomagenesis.

E3s and DNA-damage response

Living organisms are continuously exposed to several endogenous and exogenous DNA-damaging agents (Jackson and Bartek, 2009). To avoid deleterious mutations, the cell needs to rapidly detect DNA damage, identify the nature of the lesion, and recruit the appropriate repair machinery. Defects in the DNA-damage repair (DDR) mechanisms can cause gross chromosomal abnormalities that may contribute to tumorigenesis. Ubiquitylation of histones and DDR factors plays an important role in recruiting proteins to damaged DNA sites and initiating the repair process.

As an early event of the double-strand break repair pathway, the RING-type RNF8 E3 binds to the damaged sites and assembles Lys-63-linked ubiquitin chains onto histones H1, H2A, and H2AX, in cooperation with the E2 enzyme UBC13 (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; Thorslund et al., 2015; Wang and Elledge, 2007). This recruits a second RING-E3 ligase, RNF168, which recognizes RNF8 ubiquitylation products and catalyzes the monoubiquitylation of histone H2A on K13 and K15 (Mattiroli et al., 2012). RNF168 could also bind the ubiquitylation products of its own activity, thus enabling it to spread the histone ubiquitylation signals at the DNA damage sites. These ubiquitin tags provide a crucial signaling platform for the assembly of the late effector complex, which contains the E3 BRCA1 along with its binding partner BARD1, to form a BRCA1-BARD1 RING heterodimeric E3 (Jackson and Durocher, 2013; Lukas et al., 2011). The RAP80 protein contains tandem ubiquitin-interacting motifs, which bind preferentially to Lys-63-linked ubiquitin chains, and is required for recruitment of the BRCA1 complex and the repair of damaged DNA (Kim et al., 2007b). Mutations in the *BRCA1* gene leading to the expression of a truncated or inactive BRCA1 protein are strongly linked to the development of ovarian and breast cancer (Kuchenbaecker et al., 2017), although more work is needed to clearly define the functions of BRCA1-BARD1 E3 activity.

E3-mediated degradation is also required in DNA-damage detection to enable cells to orchestrate subsequent cell-cycle checkpoints and repair. A classic example is the interplay between MDM2, a RING finger E3, and the tumor suppressor p53. p53, known as the ''guardian of the genome,'' transcriptionally controls a number of genes involved in the regulation of cell cycle, apoptosis, DNA repair, senescence, and angiogenesis (Levine and Oren, 2009; Vousden and Prives, 2009). p53 induces the transcription of MDM2, while MDM2 mediates the ubiquitylation of p53, thus forming a negative feedback loop (Manfredi, 2010; Wade et al., 2010). MDM2, as a homodimer or a heterodimer with the related E3 MDMX/HDMX, binds to and ubiquitylates p53. This results in the proteasomal degradation of p53, keeping p53 levels in check under normal conditions. In response to genotoxic stress, however, various modifications on p53, MDM2, and their regulators inhibit p53 ubiquitylation and degradation, promoting p53 transcriptional activity. The human *MDM2* gene is located on chromosome 12 (12q14-q15) and its amplification is observed in various types of cancers, particularly in sarcomas (Wade et al., 2013). High MDM2 expression levels negatively correlate with p53 protein levels and activity, resulting in poor survival and prognosis (Mccann et al., 1995; Quesnel et al., 1994). Thus, amplification and/or overexpression of MDM2 represents an alternative means of mutation in p53 for escaping growth control in cancer.

The DNA-repair process requires building blocks, the deoxynucleotide triphosphates (dNTPs). $CRL1^{FBXO1}$ participates in genome integrity and DDR by mediating the degradation of RRM2, a subunit of the ribonucleotide reductase that catalyzes the conversion of ribonucleotides to deoxyribonucleotides for both replicative and repair DNA synthesis (D'Angiolella et al., 2012). In response to a variety of genotoxic stimuli, FBXO1 is degraded, allowing the accumulation of RRM2 within the nucleus to enhance dNTP production and facilitate DNA repair. Frequent cancer mutations in *CCNF* (which encodes FBXO1) are thought to contribute to genome instability (D'Angiolella et al., 2013).

E3s and cancer metabolism

To fuel cell growth and proliferation, cancer cells display fundamental changes in energy metabolism to enable increased nutrient acquisition and macromolecular precursor biosynthesis (Pavlova and Thompson, 2016). E3s contribute to the reprogramming of metabolism in cancer cells by regulating components of diverse metabolic processes.

One of the most common metabolic alterations in cancer cells is increased glucose uptake. Through the aerobic glycolysis pathway, cancer cells metabolize glucose at higher rates than normal cells and convert it to lactate, a phenomenon known as the Warburg effect. Some cancer cells also have increased glutamine metabolism, which exceeds the metabolic use of other non-essential amino acids (Wise and Thompson, 2010). Two E3 complexes, APC/C^{CDH1} and $CRL1^{\beta TrCP}$, control the metabolism of glucose and glutamine at specific phases of the cell cycle in cancer cells (Colombo et al., 2011; Duan and Pagano, 2011). APC/C^{CDH1} mediates the degradation of PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3) and GLS1 (glutaminase 1), both of which play key roles in the glycolysis and glutaminolysis pathways, as cells exit mitosis and enter G1. $CRL1^{\beta TrCP}$ specifically targets PFKFB3 during S phase. The oscillation in protein levels of these two enzymes coincides with their respective metabolic activities, in terms of lactic acid generation and glutamine utilization.

To adapt to the high reactive oxygen species (ROS) production due to elevated proliferation and metabolic activity, tumor cells leverage an increased antioxidant program to optimize ROS-driven proliferation and avoid ROS-triggered senescence or apoptosis. The transcriptional factor NRF2 regulates a network of genes with antioxidant functions that mitigate oxidative damage via detoxification of ROS and xenobiotics. NRF2 also regulates metabolic enzymes to rewire cellular metabolism to support antioxidant response (Romero et al., 2017; Wu and Papagiannakopoulos, 2020). Just as NRF2 protects normal cells, studies have shown that aberrant accumulation of NRF2 provides a survival advantage to cancer cells. Indeed, NRF2 overexpression is associated with a poor prognosis in patients with various types of cancer (Jaramillo and Zhang, 2013). At the protein level, NRF2 is regulated by the E3 $CRL3^{KEAP1}$ (Furukawa and Xiong, 2005; Itoh et al., 1999; Zhang et al., 2004). Inactivating mutations in *KEAP1* are found in ~20% of NSCLCs, impairing its ability to target NRF2 for degradation (Singh et al., 2006; Wu and Papagiannakopoulos, 2020). While somatic mutations in KEAP1 occur throughout the protein, NRF2-activating mutations predominantly occur within either the DLG or the ETGE degron, which mediate the interaction between NRF2 and KEAP1 (McMahon et al., 2006; Tong et al., 2006). The high mutation frequency of *KEAP1* and *NRF2* suggests a critical role for this pathway in lung tumor development, as confirmed by numerous mouse models. Genetic alterations in *CUL3* (which encodes the backbone protein of the $CRL3^{KEAP1}$ E3 complex) have also been found to be significantly mutated in human cancers (Armenia et al., 2018; Campbell et al., 2016; Tokheim et al., 2021). Interestingly, at least three other CRL3 SRs (i.e., KLHL6, LZTR1, and SPOP) play a tumor suppressor role (see above); therefore, mutations in *CUL3* are not expected provide a growth advantage by affecting only KEAP1 activity.

E3s and metastasis

Metastasis describes the process through which malignant cells develop the ability to invade tissues beyond their normal boundaries and seed new tumors at secondary sites (Gupta and Massague, 2006; Lambert et al., 2017). For successful colonization, tumor cells need to disseminate from the primary site, enter the circulation, and adapt to foreign tissue microenvironments. Metastasis is a complex process that requires a coordinated network of cellular regulators, including kinases, transcription factors, and E3s. Multiple E3s are reported to either positively or negatively regulate the cellular processes involved in metastasis, and some of these E3s are deregulated in human cancers.

$CRL1^{FBXO22}$ has been shown to suppress metastasis by mediating the degradation of BACH1, a pro-metastatic transcription factor (Lignitto et al., 2019). Heme, the catabolic target of heme oxygenase 1 (HO1) enzyme, enhances the interaction between FBXO22 and BACH1. NRF2 regulates HO1 expression; thus, NSCLCs bearing NRF2-stabilizing mutations (see above) experience higher induction of HO1, leading to prominent catabolic processing of heme. As a result, BACH1 interacts less with FBXO22, accumulates, and promotes metastatic events.

$CRL1^{FBXL7}$ ubiquitylates c-SRC (Moro et al., 2020), a proto-oncoprotein that has been strongly implicated in cancer development and metastasis formation (Summy and Gallick, 2003). In prostate and pancreatic cancer models, downregulation of FBXL7 promotes metastatic progression by increasing cell invasion and migration in a c-SRC-dependent manner. Importantly, the promoter of the gene encoding FBXL7 is hypermethylated in advanced prostate and pancreatic cancers, correlating with decreased FBXL7 mRNA and protein levels (Moro et al., 2020).

E3s and tumor angiogenesis

Cancer cells hijack angiogenesis, the growth of new blood vessels from pre-existing blood vessels, to supply nutrients and oxygen for the rapid expansion of the tumor mass (Potente et al., 2011). In response to hypoxia, tumor tissues produce and release pro-angiogenic factors, such as VEGF (vasculoendothelial growth factor) and fibroblast growth factors, to activate endothelial cells of pre-existing blood vessels (Niu and Chen, 2010). Vessels also need to adjust their morphology and function to meet changing tissue oxygen demands.

A master regulator of hypoxia-induced angiogenesis is the transcription factor HIF (hypoxia-inducible factor) (Semenza, 2012). HIF controls the transcription of multiple genes that promote oxygen delivery to tissues by inducing angiogenesis, including VEGF, erythropoietin, the angiopoietin growth factors, stromal-derived factor 1, and glucose transporter 1 (Koh and Powis, 2012; Krock et al., 2011). HIF contains a labile, oxygen-sensitive α subunit (i.e., HIF1α or HIF2α) and a constitutively expressed β subunit (HIF1β or Aryl). In the presence of high oxygen levels, HIF α subunits are hydroxylated and targeted for degradation by VHL (von Hippel-Lindau), an SR of the CRL2 complex (Kaelin, 2017; Maxwell et al., 1999). Under hypoxic conditions, HIF α subunits escape VHL-mediated degradation and activate hypoxia-inducible genes. Depletion or mutations in *VHL* allows expression of HIF target genes even under normoxic conditions, which supports vascularization and growth of tumors. Inactivation of *VHL* is causative of the von Hippel-Lindau syndrome, a familial predisposition to developing tumors in multiple organs (Lonser et al., 2003). Mutations in the VHL protein are frequently found in familial and sporadic clear cell carcinomas of the kidney, hemangioblastomas of the retina and central nervous system, pancreatic cysts and tumors, and pheochromocytomas, highlighting its tumor suppressor function in the pathogenesis of these tumors (Kaelin, 2017). Neoplasms caused by *VHL*

inactivation are highly vascularized due to constitutive activation of HIF target genes (Kaelin, 2002). HIF activation is an early event in the evolution of neoplastic kidney lesions in VHL patients, and increased expression of certain HIF target genes is associated with disease progression (Mandriota et al., 2002). These observations also suggest the therapeutic strategy of inhibiting HIF or its target genes in VHL-defective carcinogenesis. For example, clear cell renal carcinomas bear high VEGF levels due to loss of pVHL, and are highly sensitive to single agents directed against VEGF (Choueiri and Motzer, 2017). Moreover, an HIF2α inhibitor is showing promise for the treatment of clear cell renal carcinoma (Courtney et al., 2018). These findings demonstrate that targeting the VHL-HIF pathway could be an effective strategy for the treatment of certain angiogenic tumors, notably kidney cancer.

E3s and oncogenic viruses

A few human cancers are triggered by viruses that promote tumorigenesis through activation of oncogenic pathways and/or inactivation of tumor suppressors. The versatility of the ubiquitin system in regulating multiple cellular pathways makes it an attractive target for oncogenic viruses. Viruses may utilize E3s in several ways to promote host cell proliferation: some viral proteins inhibit E3 activities, others hijack host E3s to target new substrate proteins.

Human oncogenic papillomavirus (HPV) infection plays an etiologic role in a subset of cervical and head and neck cancers (Burd, 2003; Tumban, 2019). The major oncoproteins of HPV are encoded by the viral E6 and E7 genes, both of which exploit the ubiquitin system to inactivate cellular tumor suppressor proteins and facilitate HPV-induced carcinogenesis (McLaughlin-Drubin and Munger, 2009; Munger and Howley, 2002; Scheffner and Whitaker, 2003). E6 binds through its N terminus to the E6-AP E3 ligase, the founding member of the HECT family of E3s, thereby promoting binding, ubiquitylation, and consequent degradation of the tumor suppressor p53. Of note, in HPV-negative cells, E6-AP has no role in p53 degradation (BeerRomero et al., 1997; Scheffner, 1998), showing that E6 utilizes a cellular E3 to inactive this tumor suppressor. E6-AP was also shown to target the pro-apoptotic protein BAK for degradation, thus inhibiting apoptosis (Thomas and Banks, 1998). As for E7, an important aspect in its oncogenic activity is the inactivation of the RB tumor suppressor. Analysis of E7-associated proteins uncovered CUL2 as an interactor of E7, and subsequently showed it to promote degradation of RB in an E7-dependent manner (Huh et al., 2007). Similar mechanisms are employed by other viruses. For example, the adenoviral E4orf6 protein hijacks CRL5 to target p53 for degradation (Querido et al., 2001).

In addition to hijacking host E3s to target tumor suppressors, viral proteins may also inhibit E3s to stabilize oncoproteins. The large tumor antigen (LTAg) encoded by simian virus 40 is a powerful oncoprotein that is capable of transforming a variety of cell types (Sullivan and Pipas, 2002). LTAg was found to inhibit FBXW7-driven degradation of proto-oncoproteins such as cyclin E (Welcker and Clurman, 2005). LTAg binds FBXW7 through its phospho epitope within the C terminus that closely mimics the consensus phospho degron for FBXW7. LTAg is not degraded, but instead acts as a decoy, functioning as a competitive inhibitor for FBXW7, thereby reducing the turnover of cellular substrates. LTAg also associates with CUL7 (Ali et al., 2004), and mutants with CUL7 binding deficiency exhibit a decreased ability to support anchorage-independent growth of mouse embryonic fibroblasts (Hartmann et al., 2014). Further studies regarding the link between LTAg-CUL7 interaction and cell transformation will be necessary to better understand the mechanisms by which this viral protein leads to tumorigenesis.

E3s in cancer immunotherapy

The immune system monitors and eradicates the formation and progression of neoplastic tissues in a process called immunosurveillance (Kim et al., 2007c). Often, tumors hijack immune suppression mechanisms to avoid detection by the immune system. In recent years, great achievements have been made in multiple cancers by blocking immune-inhibitory signals, enabling patients to produce an effective anti-tumor response (Mahoney et al., 2015). Immunotherapies that consist of antibodies targeting immune checkpoint proteins, such as PD-1 (programmed cell death protein 1) on T cells, PD-L1 (programmed cell death 1 ligand 1) on antigen-presenting cells and tumor cells, and CTLA-4 (cytotoxic T lymphocyte antigen 4), have revolutionized cancer treatment (Boussiotis, 2016; Dong et al., 2002).

Emerging evidence suggests that E3s contribute to the regulation of tumor immunosurveillance and can be exploited for enhancing anti-tumor immunity (Fujita et al., 2019). Large-scale screens revealed that several E3s, most notably CBL-B, are involved in T cell activation (Shifrut et al., 2018; Zhou et al., 2014). CBL-B negatively regulates T cell activation (Paolino and Penninger, 2010), and *cblb*$^{-/-}$ T cells are less susceptible to PD-1-mediated inhibition (Fujiwara et al., 2016). Therefore, CBL-B has become a focus for understanding the mechanisms of anti-tumor immunity, and its activity could serve as an indicator of responsiveness to PD-1/PD-L1 immunotherapies.

Other studies have suggested a role for E3s in directly regulating the levels of checkpoint molecules. For example, CRL1^{FBXO38} targets PD-1 for proteasomal degradation, thereby promoting T cell anti-tumor immunity (Meng et al., 2018). Moreover, *FBXO38* transcription is downregulated in CD8^{+}PD-1^{+} tumor-infiltrating T cells in colorectal and hepatocellular carcinoma patients, and its levels negatively correlate with cell-surface expression of PD-1. Another study showed that PD-L1 protein abundance is regulated by CRL3^{SPOP} (Zhang et al., 2018). Loss-of-function mutations in *SPOP* compromise PD-L1 degradation, leading to accumulation of PD-L1 levels and reduced numbers of tumor-infiltrating lymphocytes in prostate murine tumors. Further understanding of these biochemical events will provide insight into the mechanisms contributing to the efficacy of tumor immunotherapy and the development of resistance.

E3s as targets in cancer therapy

The critical roles played by E3s in the regulation of fundamental cellular processes and cancer development suggest that they could be key targets for therapeutic strategies. In the following section, we will describe strategies for targeting E3s for cancer therapies (Figure 3).

The success of proteasome inhibitors in cancer treatment is one of the reasons that makes targeting the ubiquitin-proteasome system an attractive therapeutic modality. Despite the

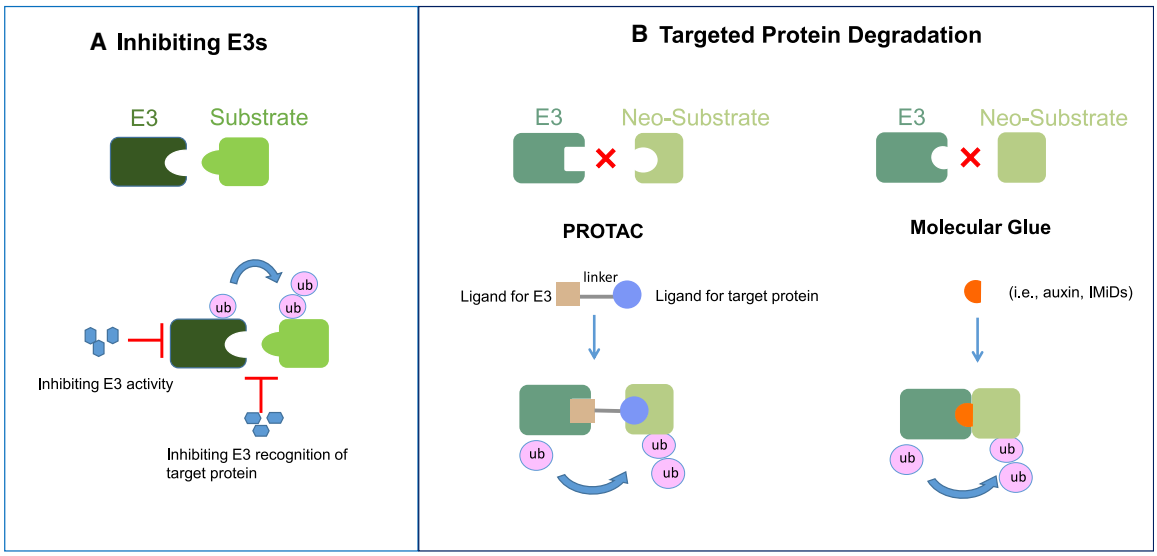

Figure 3. Therapeutic strategies targeting E3s

(A) E3 inhibitors are small molecules that either inhibit the activity of E3 or block its interaction with substrates.

(B) Targeted protein degradation. Proteolysis targeting chimeras (PROTACs) are bifunctional molecules that simultaneously engage a protein of interest and an E3, to form a ternary complex and enable the E3 to mediate the ubiquitylation and subsequent degradation of the neo-substrate. Molecular glues are small compounds that redirect E3s to neo-substrates. They can be either natural compounds (for example, plant hormones) or synthetic compounds (such as IMiDs and indisulam). The proximity induced by the small molecule leads to substrate ubiquitylation by E3s and subsequent proteasomal degradation. Molecular glues do not require high affinity for both the E3 and the neo-substrate, eliminating the need for at least one of the two ligand-binding pockets required for PROTACs.

effectiveness of proteasome inhibitors (e.g., bortezomib, ixazomib, and carfilzomib) in the treatment of certain cancers, especially hematological malignancies, undesirable side effects are observed, presumably due to the non-specific inhibition of the proteasome-dependent degradation of many cellular substrates. Inhibitors targeting specific, oncogenic E3s provide the rationale for the development of more selective drugs with fewer side effects. Specifically, inhibitors may target the E3 levels or their catalytic activity, block its interaction with substrates, or alter its subcellular localization (Nalepa et al., 2006; Skaar et al., 2014).

One obvious target for cancer therapy is MDM2, which targets the tumor suppressor p53 and is overexpressed in several types of human tumors. Small molecules designed to disrupt MDM2-p53 interactions or inhibit MDM2 expression or its activity are expected to activate the p53 pathway, leading to cell-cycle arrest and apoptosis. Intensive research efforts in the past decades resulted in several small-molecule compounds, such as nutlins, that made their way into human clinical trials, demonstrating anti-tumor effects (Tisato et al., 2017; Vassilev et al., 2004; Wang et al., 2017). However, drug-related toxicities and acquired resistance caused by the emergence of p53 mutations are two major concerns arising from these clinical trials. Nevertheless, these discoveries are paving the way for the design of new MDM2 inhibitors with higher potency and selectivity, leading to better pharmacokinetics.

SKP2 is another E3 with oncogenic potentials and is overexpressed in certain human cancers (see above). Different classes of SKP2 inhibitors are designed to suppress the expression of SKP2 (Jiang et al., 2020), to interrupt its interaction with other subunits of the CRL complex (Chan et al., 2013), or to block the interaction with its substrates (Wu et al., 2012). $CRL1^{SKP2}$ and its co-factor CKS1 recognize a phospho degron in the cell-cycle inhibitor p27, and a structure analysis revealed a pocket between SKP2 and CKS1 that is flanked by residues required for p27 binding (Carrano et al., 1999; Hao et al., 2005). Compounds designed to target the binding interface for p27 were shown to selectively inhibit SKP2-mediated p27 degradation, leading to p27 accumulation and cell-cycle arrest. These inhibitors showed some activity in cancer cell lines, as well as in mouse cancer models (Pavlides et al., 2013; Rodriguez et al., 2020; Wu et al., 2012).

Overall, despite huge efforts both in academia and in industry, the strategy of targeting oncogenic E3s did not produce the effects predicted two decades ago (Garber, 2005). The hurdles could come from the fact that E3s are not conventional enzymes, with most of them lacking well-defined activity pockets to be targeted by small molecules. So, inhibition of these enzymes would require blocking protein-protein interactions (e.g., those between the E3 and the substrate or between the SR subunit and the core subunits of the E3), with all the associated challenges.

Targeting E3s with tumor suppressor activity requires different approaches, such as restoring the E3 activity or exploiting the concept of synthetic lethality. For example, as described above, FBXL7 is hypermethylated in advanced prostate and pancreatic cancers, correlating with poor prognosis (Moro et al., 2020). Treatment with decitabine, a DNA-demethylating agent, recovers FBXL7 expression, promoting c-SRC degradation and reducing cell invasion and metastasis formation. The notion of targeting synthetic lethal vulnerabilities in cancer has been validated clinically through the effectiveness of poly(ADP-ribose) polymerase inhibitors in breast and ovarian cancers with defect in BRCA1 and BRCA2 (Ashworth and Lord, 2018). Identifying novel E3 synthetic-lethal interactions will shed light on discovery

of more targetable vulnerabilities in cancers harboring deficiencies or loss-of-function mutations in tumor suppressor E3s.

Targeted protein degradation

In recent years, huge efforts have been made to hijack E3s to promote the degradation of oncogenic substrates by proteolysis-targeting chimaera (PROTAC) technology or compounds that act as molecular glues. Controlled proteostasis provides the opportunity to target the proteome, once considered as ''undruggable'' by the traditional modality of drug discovery.

PROTAC technology utilizes bifunctional molecules to guide an E3 toward the recognition and consequent degradation of oncogenic substrates (Lai and Crews, 2017). Such molecules bind the target protein on one end and an E3 on the other. The PROTAC does not require direct binding to the active site. Compared with catalytic inhibition, degradation could lead to more potent effects on cell proliferation and a robust downstream signaling response. In fact, PROTACs often work at lower concentrations than small-molecule inhibitors, thanks to their catalytic mechanism of action (i.e., the target protein is eliminated and the PROTAC reused to form new E3-PROTAC-substrate trimeric complexes). Our expanding knowledge of E3s and their substrates keeps adding more to the molecular toolbox for the PTOTAC design. A variety of E3s, including cereblon (CRBN), VHL, βTrCP, MDM2, and cIAP, have been successfully exploited by this strategy, and the approach was demonstrated for targets that include hormone receptors (estrogen receptor and AR), kinases, and bromodomain-containing proteins (Cromm and Crews, 2017). ARV-110, which targets the AR, is the first PROTAC that has advanced into the clinic (Mullard, 2019). These trials will provide important insight into the efficacy of these degrader compounds *in vivo*.

However, there are associated challenges for the PROTAC technology, including the requirement of a bifunctional molecule with high affinity on both ends and a molecular weight often above 500 Da, which may limit cell availability. Another emerging strategy for targeted proteolysis utilizes the so-called ''molecular glues,'' which similarly induce a neo-interaction between an E3 and a protein of interest. Two advantages of molecular glues include the use of non-bivalent molecules that are small enough to be drug-like compounds and the lack of requirement for high affinity on both the E3 and the neo-substrate. The latter eliminates the need for at least one of the two ligand-binding pockets required for PROTACs; thus, molecular glues can target not only undruggable substrates but also ''unligandable'' proteins.

The idea of molecular glues in targeted degradation was partly inspired by the finding that auxin, a plant hormone, binds to the plant E3 $CRL1^{TIR1}$, creating a molecular surface that favors substrate binding (Tan et al., 2007). A similar mechanism was found in jasmonate (JA) signaling. JAs are a family of plant hormones that regulate plant growth, development, and response to stress. An amino acid-conjugated form of JA, (3R,7S)-jasmonoyl-L-isoleucine, mediates JA signaling by promoting binding of the F-box protein COI1 to the transcriptional repressor JA ZIM domain proteins, thereby inhibiting transcription of JA-responsive genes (Katsir et al., 2008; Sheard et al., 2010).

Although molecular glues were first characterized in plants, their relevance as a therapeutic modality was first introduced by the discovery of the mechanism of action of immunomodulatory drugs (IMiDs). IMiDs such as thalidomide and its analogs lenalidomide and pomalidomide are used as anti-cancer agents (Bartlett et al., 2004), but the biochemical basis for their antineoplastic activity was not fully understood until recently. IMiDs bind to CRBN, a CRL4 SR (Ito et al., 2010), redirecting $CRL4^{CRBN}$ to neo-substrates, including the lymphoid transcription factors Ikaros and Aiolos (also known as IKZF1 and IKZF3) (Gandhi et al., 2014; Kronke et al., 2014; Lu et al., 2014), casein kinase 1α (CK1α) (Kronke et al., 2015), and the translation termination factor GSPT1 (Matyskiela et al., 2016). Ikaros proteins are essential for the survival of multiple myeloma cells, accounting for the clinical efficacy of IMiDs. CK1α and GSPT1 are the main targets of IMiDs in the treatment of 5q-deletion-associated myelodysplastic syndrome and acute myeloid leukemia, respectively. Structure analysis showed that lenalidomide binds the substrate-binding domain of CRBN, blocking the interaction with (and ubiquitylation of) MEIS2, an endogenous substrate of $CRL4^{CRBN}$ (Fischer et al., 2014). Lenalidomide and CRBN jointly provide the binding interface for neo-substrates (Matyskiela et al., 2016; Schafer et al., 2018). Notably, Ikaros proteins, CK1α, and GSPT1 share no obvious sequence homology; instead, they are recruited to CRBN through a β hairpin containing a glycine residue, suggesting a ''structural degron'' determining CRBN-IMiD selectivity. Moreover, the recruitment specificity of neo-substrates varies among these different thalidomide derivatives. For instance, CK1α is recruited by lenalidomide, but not pomalidomide or thalidomide. Thus, structure and biochemistry insights into the specific interactions between thalidomide analogs and particular substrates will lead to the discovery of more potent compounds. Finally, current efforts are dedicated to the identification of new thalidomide derivatives that may target CRBN to different neo-substrates with additional beneficial effects.

Following these remarkable findings, the aryl-sulfonamide drug indisulam was also shown to redirect $CRL4^{DCAF15}$ to neo-substrates. Indisulam is a potential anti-cancer agent originally discovered by screening sulfonamides for inhibition of cancer cell proliferation (Owa et al., 1999). Structural and biochemical studies revealed that indisulam, and its related compounds E7820 and tasisulum, induces the binding of the RNA splicing factor RBM39 to DCAF15, promoting its degradation and producing anti-proliferative effects (Bussiere et al., 2020; Du et al., 2019; Faust et al., 2020; Han et al., 2017; Uehara et al., 2017).

Continued efforts in the field are leading to the discovery of an increasing number of compounds acting as molecular glues with therapeutic potential (Chamberlain and Hamann, 2019). Three different orthogonal approaches revealed CR8 and analogous compounds as molecular glue degraders (Lv et al., 2020; Mayor-Ruiz et al., 2020; Slabicki et al., 2020a). These molecules direct the CDK12-cyclin K complex to DDB1, a core subunit of the CRL4 complex, thereby inducing cyclin K degradation and exerting toxicity on cancer cells. Another screening looking at compounds that mediate the degradation of the oncogenic transcription factor BCL6 identified BI-3802, which binds to the BTB domain of BCL6 and promotes its protein aggregation and degradation (Kerres et al., 2017; Slabicki et al., 2020b).

This accelerated progress on PROTACs and molecular glues provide new strategies on targeting what otherwise would be considered as undruggable, and even unligandable, protein

targets. It also opens up the possibility of the development of pharmaceutical agents to restore E3-mediated ubiquitylation of substrates that are impaired by genetic alterations on either the E3s or themselves.

CONCLUSIONS

E3s act as molecular switches that control the cellular abundance of key regulatory components of signaling pathways. They are often deregulated in cancer through diverse mechanisms, resulting in altered expression and activity of their target proteins. A better appreciation of the mechanisms underlying deregulation of E3s would help us to understand the initiation and progression of cancer and may provide critical breakthroughs for targeted therapy. In addition to inhibiting E3 activities, targeted protein degradation as a therapeutic modality has attracted substantial interest and investments, and has made great progress in just the last few years. However, the mechanism of action of drugs that function as molecular glues has been identified *a posteriori* in most cases. How to identify *de novo* molecular glues that repurpose E3s for a specific target remains an open, fundamental question in the field of ubiquitin-mediated degradation. Moreover, how to ensure that molecular glues induce productive ubiquitylation, rather than just proximity, remains a major challenge. As the field advances, more rational and systematic approaches are being leveraged, and we anticipate seeing many further clinical successes in this area.

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DECLARATION OF INTERESTS

M.P. is a consultant for and has financial interests in Coho Therapeutics, CullGen, Kymera Therapeutics, and SEED Therapeutics. M.P. is a cofounder of Coho Therapeutics, is on the SAB of CullGen and Kymera Therapeutics, and is a consultant for Santi Therapeutics. S.D. declares no competing interests.

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