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SCF E3 Ubiquitin Ligases as Anticancer Targets

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Abstract

The SCF (Skp1, Cullins, F-box proteins) multisubunit E3 ubiquitin ligase, also known as CRL (Cullin-RING ubiquitin Ligase) is the largest E3 ubiquitin ligase family that promotes the ubiquitination of various regulatory proteins for targeted degradation, thus regulating many biological processes, including cell cycle progression, signal transduction, and DNA replication. The efforts to discover small molecule inhibitors of a SCF-type ligase or its components were expedited by the FDA approval of Bortezomib (also known as Velcade or PS-341), the first (and only) class of general proteasome inhibitor, for the treatment of relapsed/refractory multiple myeloma and mantle cell lymphoma. Although Bortezomib has demonstrated a certain degree of cancer cell selectivity with measurable therapeutic index, the drug is, in general, cytotoxic due to its inhibition of overall protein degradation. An alternative and ideal approach is to target a specific E3 ligase, known to be activated in human cancer, for a high level of specificity and selectivity with less associated toxicity, since such inhibitors would selectively stabilize a specific set of cellular proteins regulated by this E3. Here, we review recent advances in validation of SCF E3 ubiquitin ligase as an attractive anti-cancer target and discuss how MLN4924, a small molecule inhibitor of NEDD8-activating enzyme, can be developed as a novel class of anticancer agents by inhibiting SCF E3 ligase *via* removal of cullin neddylation. Finally, we discuss under future perspective how basic research on SCF biology will direct the drug discovery efforts surrounding this target.

Keywords

Ubiquitin-proteasome system; SCF E3 ubiquitin ligase; anticancer target; drug discovery; neddylation; cullins; F-box proteins; RING ligases

INTRODUCTION

The ubiquitin-proteasome system (UPS) regulates many biological processes through timely degradation of diverse cellular proteins. It, therefore, plays an essential role in maintaining homeostasis and in response to environmental stimuli [1,2]. UPS-targeted protein degradation requires substrate ubiquitination, which is a multi-step enzymatic process catalyzed by a cascade of enzymes, including ubiquitin-activating enzyme E1, ubiquitin-

conjugating enzyme E2, and ubiquitin ligase E3. While E1 and E2 activate and transfer ubiquitin in the reaction, E3 recognizes the substrate and catalyzes the covalent attachment of ubiquitin to the substrate [3]. Multiple runs of this reaction result in polyubiquitination of substrate. The fate of ubiquitinated proteins is determined, however, by the nature of ubiquitin attachment and the type of isopeptide linkage of the polyubiquitin chain. While the K48-linked polyubiquitination predominantly targets protein for degradation after being recognized by the proteasome, the K63-linked polyubiquitination and mono-ubiquitination normally alters protein function, cellular localization, enzyme activity, DNA repair, or interaction with other proteins [4–6]. Fig. (1) illustrates the ubiquitination cascade reactions and three potential fates of ubiquitinated proteins.

The SCF multisubunit E3 ligase complex, consisting of Skp-1, Cullins, F-Box proteins and RBX/ROC RING finger proteins [7,8], is the largest family of ubiquitin ligases that promote the degradation of about 20% of UPS-regulated proteins [9], including cell cycle regulatory proteins, transcription factors, oncoproteins and tumor suppressors among others [10–15] (Table 1). The crystal structure of SCF-RBX complex revealed that Cul-1 acts as a scaffold that binds at its N-terminus the Skp-1 and F-box protein and at its C-terminus the RING protein RBX1 [16]. It is well established that the substrate specificity of SCF complex is determined by the F box proteins that bind to Skp1 and Cullins through its F-box domain and to substrates through its WD40 or leucine rich domains [16], whereas the core SCF E3 ubiquitin ligase is a complex of Cullins-RBX/ROC, in which RBX binds to E2 and facilitates ubiquitin transfer from E2 to substrates [17]. Furthermore, the activity of SCF E3 ubiquitin ligases requires cullin neddylation, which disrupts inhibitory binding of cullin by CAND1 [18–21]. In the human genome, there are 69 F-box proteins, including WD40 domain containing FBXWs, leucine-rich repeats-containing FBXLs and other diverse domains-containing FBXOs, seven cullins (Cul-1, -2, 3, 4A, 4B, -5, and -7) and two RING proteins, RBX1/ROC1 and RBX2/ROC2, also known as SAG (Sensitive to Apoptosis Gene) [22–26]. Cullin-based assembly of SCF E3 ligase subunits can be classified into four categories: Cul1-Skp1-F-box, Cul2/5-Elongins-B/C-VHL/SOCS box, Cul3-BTB, and Cul4A/B-DDB1-DWD [27], making SCF/CRL the largest family of E3 ubiquitin ligases, responsible for the degradation of ~20% of all proteins subjected to proteasomal degradation [9].

SCF COMPLEX E3 UBIQUITIN LIGASES IN CANCER AND AS ANTICANCER TARGETS

The majority of SCF E3 ligase substrates are involved in regulation of cell cycle progression, gene transcription, signal transduction and DNA replication among others [11,12,14]. Through targeted degradation of these substrates, SCF E3 ligases regulate many biological processes. Accumulated evidence strongly suggests that abnormal regulation of SCF E3 ubiquitin ligases contributes to uncontrolled proliferation, genomic instability, and cancer [11]. Among the components of SCF, some are oncogenes (e.g. Skp2) that promote degradation of tumor suppressors and are amplified and/or overexpressed in human cancers, whereas others are tumor-suppressors (e.g. Fbxw7) that target the degradation of oncoproteins and are mutated in human cancers [28–30]. Some deregulated components of SCF E3 ligase in human cancer are listed in Table 2 and discussed below.

RING-Finger Proteins

Two family members of the RING component of SCF E3 ligase in human and mouse are RBX1/ROC1 and RBX2/ROC2/SAG [22–25]. Both members contain a functional RING domain at the carboxyl terminus and are evolutionally conserved with a similar tissue expression pattern [8]. Under overexpressed conditions, both proteins bind to any one of six

members of the cullin family (Cul 1-3, Cul4A, B and Cul-5) [24] and have an *in vitro* E3 ubiquitin ligase activity [31,32]. Either family member can fully rescue yeast death phenotype caused by deletion of *Hrt1*, a yeast orthologue of *Rbx1/Roc1* [24,31,33]. A potential difference between the two members is that RBX1 is constitutively expressed and prefers to bind with Cul2/VHL, whereas RBX2/ROC2/SAG is stress-inducible and preferably binds to Cul-5/SOCS [34,35]. Our recent mouse knockout study revealed that these two members are functionally non-redundant. Under wild type Rbx2 background, Rbx1 deletion caused early embryonic lethality at E7.5 as the result of proliferation defects [36], whereas Sag knockout in the wild type Rbx1 background also caused embryonic lethality at the later stage (E11.5-12.5), associated with cardiovascular defects (manuscript submitted for publication).

RBX1/ROC1—Our recently study showed that compared to normal tissues, RBX1/ROC1 is overexpressed in diverse human primary cancers, particularly in lung cancer. SiRNA silencing of ROC1 triggered the DNA damage response and sequentially induced G2/M cell cycle arrest, senescence and apoptosis in a p53-independent manner, leading to suppression of cancer cell growth [37] (Fig. 2). The underlying mechanism for ROC1 silencing-induced senescence is likely attributable to accumulation of DNA replication licensing proteins (such as Cdt-1 and Orc1), known to be SCF E3 ligase substrates [15,38–41], which trigger the DNA damage response and senescence [42–44]. Thus, RBX1 is a cancer cell survival protein whose inhibition triggers various cell death pathways, eventually leading to cancer cell killing.

RBX2/ROC2/SAG—RBX2/ROC2/SAG is the second member of the RING component of SCF E3 ligases, which was originally cloned as a redox inducible antioxidant protein in our laboratory [22]. As an antioxidant, SAG suppresses apoptosis induced by many stimuli, including redox [22,45], tumor promoter, TPA [34], nitric oxide [46], ischemia/reoxygenation [47], neurotoxins [48], heat-shock [49] and UV-irradiation [50]. When complexed with other components of SCF, SAG exerts E3 ubiquitin ligase activity [31] and promotes the degradation of p27, c-Jun, procaspase-3, I κ B α , HIF-1 α , and Noxa, thus regulating cell proliferation, apoptosis, and skin carcinogenesis [34,51–55]. Significantly, SAG is overexpressed in multiple human tumor tissues, and patients with SAG overexpression have a poor prognosis [55–57]. SAG siRNA silencing selectively inhibited cancer cell proliferation *via* apoptosis induction, suppressed *in vivo* tumor growth and sensitized cancer cells to chemotherapeutic drugs and radiation [52,55], suggesting its potential as an anti-cancer target (Fig. 2).

F-Box Proteins

F-box proteins are the substrate-recognizing subunits of SCF E3 ligase which determine the substrate specificity of SCF. A single F-box protein can recognize and target multiple substrates (e.g. Skp2 targets p27, p21, p57), whereas the same substrate can be recognized and targeted by different F-box proteins (e.g. cyclin E targeted by both Skp2 and Fbxw7). More interestingly, a single F-box protein can target the degradation of several substrates with opposite biological functions (e.g. Skp2 targets p21/p27 as well as cyclin A/D1/E) [15]. Thus, when or whether a particular substrate is targeted for degradation by a given F-box protein will likely be cell context dependent, leading to different biological consequences. Among ~70 F-box proteins in the human genome, only three are well studied: oncogenic Skp2, tumor suppressive Fbxw7, and β -TrCP, which could be tumor suppressive as well as oncogenic in a substrate dependent manner [28,30]. Here we will only focus on Skp2 and β -TrCP as potential cancer targets.

Skp2—Skp2 recognizes and promotes the degradation of several negative cell cycle regulators, including p27, p21, p130 and p57 [11,12,14]. Skp2 is overexpressed in many human cancer types [58] with associated p27 decrease and poor prognosis, seen in gastric cancer [59,60], colon cancer [61,62], and breast cancer [63–65]. Tissue specific expression of Skp2 in mouse prostate gland caused hyperplasia, dysplasia and low-grade carcinoma [66], whereas targeted expression of Skp2 in the T-lymphoid lineage co-operated with activated N-Ras to induce T cell lymphomas with a short latent period and high penetrance [67]. Furthermore, a knock-in mouse model showed a crucial role of Skp2 dependent degradation of p27 for the progress of colon adenomas to carcinoma [68]. Interestingly, in a mouse knockout model, although Skp2 disruption on its own does not induce cellular senescence, Skp2-null environment facilitates tumor-suppressive senescence response upon inactivation of tumor suppressor genes or aberrant proto-oncogenic signals [69]. Consistently, down-regulation of Skp2 using an anti-sense oligonucleotide or siRNA silencing inhibited growth of melanoma [70], oral cancer cells [71], glioblastoma cells [72], and lung cancer cells [73–75]. Thus, pharmacological inhibitors of the Skp2 pathway would be of therapeutic value for cancer treatment.

β -TrCP—The F-box protein, β -TrCP1/2, is a substrate recognizing component of SCF E3 ubiquitin ligases that promote ubiquitination and degradation of various proteins. In some tissues, β -TrCP is characterized as an oncoprotein for targeted degradation of tumor suppressors, such as I κ B [14,76] a negative regulator of NF κ B [77], PDCD4 [78], a protein translation suppressor *via* inhibiting eIF4A [28] and BimEL1 [79], a proapoptotic protein. In a transgenic mouse model, targeted β -TrCP1 expression in mammary gland promotes mammary tumor formation *via* activating NF κ B [80], whereas targeted β -TrCP1 expression in intestine, liver and kidney caused an increased incidence of tumor formation in these organs [81]. Consistently, β -TrCP is overexpressed in human breast cancer cell lines and primary tumors, and β -TrCP inhibition sensitizes breast cancer cells to chemotherapies [82]. Similarly, overexpression of β -TrCP increased NF κ B activity and chemo-resistance, whereas siRNA silencing of β -TrCP reduced NF κ B activation and chemo-resistance in pancreatic cancer cells [83] and sensitized cervical cancer cells to apoptosis induced by etoposide or TRAIL [52]. Thus, disruption of the binding between β -TrCP and its tumor suppressor substrates would be an effective and selective targeting approach.

Cullins

Cullins are scaffold proteins which assembly with other components of SCF into four functionally distinct E3 ubiquitin ligases [27]. So far, seven cullin family members (Cullin 1, 2, 3, 4A, 4B, 5, and 7) have been identified [84]. Among all cullins, Cul-1 is overexpressed in 40% of lung cancers, with active neddylated forms specifically expressed in high-grade neuroendocrine lung tumor tissues [85], whereas Cul-2 frameshift mutations were detected in two out of 41 colon cancers [86]. Cul-5 is a putative tumor suppressor that blocks Src activity [87] and inhibits breast cancer cell growth upon overexpression [88]. Most cullin-related studies focused on Cul-4A, which is overexpressed in a number of human cancers, including breast cancers [89,90] with poor prognosis [91], hepatocellular carcinomas [92], and mesotheliomas [93]. While Cul-4A overexpression was associated with tumor proliferation and cell cycle progression in breast cancer cells [90], knockdown of Cul4A by siRNA caused accumulation of p21 and p27 and G1 cell cycle arrest, leading to growth suppression of mesothelioma cells [93]. In a recent conditional knockout model, skin specific Cul4A disruption dramatically increased resistance to UV-induced skin carcinogenesis [94], which highlights a potential protection from skin cancer caused by UV exposure using pharmacological Cul4A inhibitor. Taken together, these findings indicate that Cul4A amplification and overexpression plays an oncogenic role in carcinogenesis, and that Cul4A could be an attractive target for anticancer therapies. However, since Cul-4A has

many protein substrates [27] that regulate a variety of cellular functions, the challenge will be how to selectively target its oncogenic substrates.

TARGETING SCF E3 UBIQUITIN LIGASES FOR ANTICANCER THERAPY

Bortezomib (also known as Velcade or PS-341) is the first (and only) in class of general proteasome inhibitor, approved by the FDA for the treatment of relapsed/refractory multiple myeloma and mantle cell lymphoma [95,96]. Although it has demonstrated a certain degree of tumor cell selectivity that provides a therapeutic window, the drug is, in general, cytotoxic due to overall inhibition of proteolysis of a wide array of cellular proteins [96,97]. In contrast to general proteasome inhibitors, a specific E3 ligase inhibitor would selectively stabilize a specific set of cellular proteins regulated by this E3, thus avoiding some undesired effects on other cellular proteins. This would, therefore, achieve a high level of specificity with less associated toxicity [29,98,99]. Since the SCF E3 ubiquitin ligase is abnormally activated in many human cancers, contributing to uncontrolled proliferation and genomic instability [11], this E3 ubiquitin ligase or some of its components are being considered and validated as promising anticancer targets [98,99].

In theory, the following approaches can be used to screen for the inhibitors of SCF E3 ubiquitin ligase or its components. The first is to disrupt the interaction between RBX1/2 with E2 ubiquitin conjugation enzyme to prevent ubiquitin transfer to substrates. So far no such inhibitor has been reported. The second approach is to disrupt interaction between SCF components, such as disruption of Cks1-Skp2 interaction [100,101] for p27 accumulation, or to disrupt interaction between F-box proteins and their tumor suppressive substrates (e.g. β -TrCP vs. I κ B) [102]. The third approach, which is not for ligase inhibition per se, but does prevent substrate degradation, is to inhibit a kinase that phosphorylates a particular SCF substrate on a degron/destruction motif to prevent its binding to a corresponding F-box protein. It has been well-established that substrate phosphorylation is a prerequisite for F-box protein binding and subsequent degradation [11,12,15]. This approach, however, is currently being used only as a research option for understanding how the substrate degradation by a particular SCF E3 ligase is regulated. The fourth is to use Protacs (protein-targeting chimeric molecule 1), the artificial chimeric molecules that recruit selected protein substrates to SCF complex for ubiquitination and degradation [103]. A high through-put screen could be set up to identify potential inhibitors. The fifth approach is to screen for small molecule inhibitors of SCF E3 ligase. Although a number of high-throughput screening methods have been established and optimized to screen for small molecule inhibitors of single peptide E3 ligase [104], none of them can be readily converted to screen for inhibitors of multi-component SCF E3 ligases. Recently, a small molecule inhibitor, CpdA, was identified from a biochemical-based screening using *in vitro* transcribed/translated and 35 S-labeled p27, and an *in vitro*-reconstituted system incorporating purified cyclin E/Cdk2, Skp2, Skp1, Cul1, Roc1, and HeLa cell extract. CpdA was found to prevent incorporation of Skp2 into the SCF E3 ligase and to induce G1 arrest as well as p27-dependent cell killing *via* induction of autophagy. Furthermore, CpdA sensitized multiple myeloma to a number of anticancer drugs, including dexamethasone, doxorubicin, melphalan, and bortezomib [105].

TARGETING CULLIN NEDDYLATION AS AN ALTERNATIVE APPROACH FOR INACTIVATION OF SCF E3 LIGASES

Neddylation, a process of addition of ubiquitin-like protein NEDD8 to target proteins, is a novel type of post-translational modifications [106]. Neddylation is mediated by NEDD8 activating enzyme E1 (NAE), NEDD8 conjugating enzyme E2 (Ubc12) and NEDD8-E3 ligase, which consecutively activate and transfer NEDD8 to substrates. The cullin family of

proteins has been well characterized as the major substrates for neddylation [107,108], and activity of SCF E3 ubiquitin ligases requires cullin neddylation, which disrupts inhibitory binding by CAND1 [18–21]. Thus, a clever approach, employed by Soucy *et al.* [9], is to screen for NAE inhibitors with expected inhibitory activity against SCF E3 ubiquitin ligases. Indeed, MLN4924, the first class of such inhibitors was identified *via* a HTS screen and did exactly what was expected: selective inhibition of NAE and SCF E3, leading to accumulation of SCF E3 ligase substrates to trigger cell death [9,109].

Mechanistically, MLN4924 binds to NAE at the active site to create a covalent Nedd8-MLN4924 adduct, which can resemble NEDD8 adenylate, but cannot be further utilized in subsequent intraenzyme reactions. This modification inhibits NAE activity with subsequent abrogation of cullin neddylation [110]. By doing so, MLN4924 inhibits activity of SCF E3 ligases and causes accumulation of a number of SCF E3 substrates, resulting in an abnormal cell cycle profile with accumulation of aneuploid cell populations and induction of apoptosis [9]. *In vivo* xenograft assays also showed that MLN4924 slows tumor growth and was well tolerated in mice at various doses and treatment regimens [9], demonstrating a cancer cell selective killing. Most recently, MLN4924 showed potent activity against acute myeloid leukemia by inducing apoptosis [111,112] and against prostate cancer cell *in vivo* growth by triggering cellular senescence [69]. With all these promises, MLN4924 has advanced to several Phase I clinical trials for solid tumors and hematological malignancies [109].

FUTURE PROSPECTIVES

Compared to the general proteasome inhibitor Bortezomib, which blocks the entire UPS-mediated protein degradation, drugs that target a particular E3 ligase are expected to have better selectivity with less associated toxicity. Indeed, SCF complex E3 ligase inhibitor MLN4924, which blocks ~20% of all cellular proteins subjected to proteasomal degradation, is well-tolerated in mice [9]. Newly released Phase I clinical data showed that patients do develop some symptoms, such as fatigue, nausea, myalgia with increased transaminases¹, which could be associated with an overall inhibition of SCF E3 ligase as well as of other proteins whose function is subjected to neddylation regulation [106]. The following areas of basic research on SCF E3 ligase would further our mechanistic understanding of this multi-functional ligase with the ultimate goal to develop highly selective and specific inhibitors against a subset of pathways regulated by this E3 ligase.

Characterization of F-Box Proteins and their Corresponding Substrates

Among a total of ~70 F-box proteins in the human genome, which serve as the receptors for substrate recognition [26], only few are well characterized [28,30]. A recent global protein stability profiling study has identified over 350 potential SCF E3 substrates which are involved in regulation of cell cycle progression, apoptosis and cell signaling [113,114]. A complete characterization of the F-box proteins and identification of their corresponding substrates with elucidation of associated biological functions will provide an opportunity to screen for drugs that specifically target a given receptor-substrate interaction for potential activation or inactivation of a particular pathway.

Mechanistic Understanding of the Death Pathway Triggered by Inactivation of the SCF E3 Ligase Complex

Our recent work and unpublished data showed that inactivation of SCF E3 ubiquitin ligase complex *via* siRNA silencing of ROC1/RBX1 or treatment with MLN4924 caused

¹Smith, P. G. Inhibition of NEDD8-activating enzyme for cancer treatment: preclinical validation to clinic application of MLN4924. *AACR 101st Annual Meeting 2010*. **2010**, Pg 258.

accumulation of SCF E3 substrates, followed by cancer cell growth arrest and killing *via* apoptosis, senescence, and autophagy [37] (Fig. 2). How these death pathways are mediated, possibly by a subset of accumulated substrates, and whether different types of cell death occur sequentially or in parallel are subjects for future study. Mechanistic understanding of how these death pathways are triggered will help to design screening strategies to target a particular pathway with an aim to reduce normal cell toxicity.

Functional Differentiation of RBX1-SCF or RBX2-SCF E3 Ligase

SCF E3 ubiquitin ligases contain only two RING family members, RBX1/ROC1 or RBX2/ROC2/SAG. Our recent knockout study showed that they are functionally non-redundant. During mouse embryogenesis, Rbx1 controls cell proliferation at the early stage [36], whereas Rbx2/Sag regulates vascular development at the later stage (manuscript submitted). Our cell culture work also showed that siRNA silencing of RBX1 induced cell death *via* apoptosis, senescence, and autophagy [37], whereas siRNA silencing of RBX2/SAG only triggered apoptosis [55]. These studies suggest that RBX1-SCF and RBX2-SCF E3 ligases may selectively target non-overlapping as well as overlapping sets of protein substrates for degradation. Functional characterization of these two types of E3 ligases and their associated substrates would help to design a unique screening strategy for selectively targeting either one of them.

In summary, SCF E3 ubiquitin ligase appears to be a promising anti-cancer target. MLN4924, a small molecule inhibitor of SCF, holds promise for further clinical development as a novel class of anticancer agents. As our understanding of this ligase progresses, more specific drugs targeting a particular subset of SCF controlled pathways will be discovered with expectation of highly cancer cell selectivity with minimal normal cell toxicity.

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ABBREVIATIONS

BTB	Bric-a-brac/Tramtrack/Broad domain
β-TrCP	β-Transducin Repeat-Containing Protein
CAND1	Cullin-Associated and Neddylation-Dissociate-1
Cks1	Cdc kinase subunit 1
CpdA	Compound A
CRL	Cullin-RING ubiquitin Ligases
DDB	Damaged DNA-Binding Protein
Fbxw7	F-box and WD repeat domain-containing 7
HTS	High-Throughput Screen
NAE	Nedd8-Activating Enzyme
NEDD	Neural precursor Cell-expressed Developmentally Down-regulated
RBX1	Ring box protein-1

ROC	Regulator of Cullins
SAG	Sensitive to Apoptosis
SCF	Skp1, Cullins, F-box proteins
siRNA	small interfering RNA
SOCS	Suppressors of cytokine signalling
Skp1	S-phase kinase associated protein 1
Skp2	S-phase kinase associated protein 2
TRAIL	TNF-Related Apoptosis-Inducing Ligand
UPS	Ubiquitin-proteasome system
VHL	Von Hippel-Lindau

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Fig. 1. Ubiquitination cascade in protein degradation and functional regulation

Ubiquitin is first activated by ubiquitin activating enzyme, E1 and transferred to ubiquitin conjugating enzyme E2, and finally transferred to substrates by ubiquitin ligase E3, which recognizes the substrate and catalyzes the ubiquitin transfer. A single run of this ubiquitination reaction results in monoubiquitination of substrate protein with potential functional changes. Multiple runs of such a reaction lead to polyubiquitination of a substrate, which is either recognized by 26S proteasome for targeted degradation, if ubiquitin tag is in a K48 linkage, or subjected to activation, if it is in a K63 linkage.

Fig. 2. Targeting the SCF E3 ubiquitin ligase to trigger multiple cell killing pathways
SCF E3 ubiquitin ligase can be inactivated by targeting its oncogenic components, including RBX1/RBX2, Cul-4A, or Skp2 *via* siRNA silencing approach or by pharmaceutical inhibition of cullin neddylation with a NAE inhibitor, MLN4924. Inactivation of SCF E3 ligase causes the accumulation of its substrates which suppress cancer cell growth by triggering multiple cancer cell killing pathways, including apoptosis, senescence and autophagy with the mechanisms subjected to future investigation.

Table 1**SCF E3 Ubiquitin Ligases and their Substrates**

Name	Substrates	References
1) RBX1/Cullin-1/SKP1/F-Box proteins	Many, for near complete list, see cited references	[11,14,15]
a) Skp2	p21, p27, p57, p130, Cyclins A/D1/E, E2F1, Orc1, Cdt1, c-Myc, B-Myb, Foxo1, Rassf1, Smad4, Brca2, Cdk9	[10,15]
b) β -TrCP	I κ B, β -Catenin, Emi1, Cdc25A/B, Weel, BimEL, Mcl1, p100, p105, p53, p63, Pdcd4, Per1/2, procaspase-3, Rest, Smad3, Snail, Stat-1, Weel, ATF4, Claspin, Cyclin D1, Dlg, H-ras	[10,15,52]
c) Fbxw7	Aurora-A, c-Jun, c-Myb, c-Myc, Cyclin E1/2, mTOR, Notch1/4, Pgc1, Src3, Presenilin, SREBP	[10,15]
2) RBX1/Cullin-2/Elongin BC/VHL	HIF- α , TEL-JAK2	[115,116]
3) RBX1/Cullin-3/BTB-domain proteins	MEI-1, Dishevelled (Dsh), Nrf2, RhoBTB2, topoisomerase I-DNA complex, caspase 8, DAPK	[117–125]
4) RBX1/Cullin-4A/DDB1	Cdt1, p21, Smug, Histone H2A/H3/H4, XPC, DDB2, Hoxa9, Chk1, β -Catenin, p53, TSC2, c-Jun, Merlin, Stat1/2/3, UNG2.	[27,126–134]
5) RBX1/Cullin-5/elongin BC/BC-box proteins/SOCS	Disabled-1 (Dab1)	[135]
6) RBX1/Cullin-7/SKP1/Fbw8	Insulin receptor substrate 1 (IRS-1)	[136]

Table 2

Components of the SCF E3 Ubiquitin Ligase with Oncogenic or Tumor Suppressive Activity

Name	Alterations in cancer	Activity	Major functions	References
ROC1/RBX1	Overexpression	Oncogenic	Cell cycle progression, DNA damage response	[37]
SAG/RBX2	Overexpression	Oncogenic	Anti-apoptosis	[22,55]
Skp2	Amplification, Overexpression	Oncogenic	Cell cycle progression	[28,29]
β -TrCP	Overexpression	Oncogenic	Anti-apoptosis, protein translation	[28,29]
Fbxw7	Mutation, loss of expression	Tumor suppressive	Anti-proliferation, Signaling transduction	[30]
Cul-1	Overexpression	Oncogenic	Cell cycle progression	[85]
Cul4A	Amplification, Overexpression	Oncogenic	DNA repair; Anti-apoptosis	[89,92,93]
Cul5	Loss of expression	Tumor suppressive	Blockage of Src activity, Anti-proliferation	[87,88]