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# Skp2 in the ubiquitin-proteasome system: A comprehensive review

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## Abstract

The ubiquitin-proteasome system (UPS) is a complex process that regulates protein stability and activity by the sequential actions of E1, E2 and E3 enzymes to influence diverse aspects of eukaryotic cells. However, due to the diversity of proteins in cells, substrate selection is a highly critical part of the process. As a key player in UPS, E3 ubiquitin ligases recruit substrates for ubiquitination specifically. Among them, RING E3 ubiquitin ligases which are the most abundant E3 ubiquitin ligases contribute to diverse cellular processes. The multisubunit cullin-RING ligases (CRLs) are the largest family of RING E3 ubiquitin ligases with tremendous plasticity in substrate specificity and regulate a vast array of cellular functions. The F-box protein Skp2 is a component of CRL1 (the prototype of CRLs) which is expressed in many tissues and participates in multiple cellular functions such as cell proliferation, metabolism, and tumorigenesis by contributing to the ubiquitination and subsequent degradation of several specific tumor suppressors. Most importantly, Skp2 plays a pivotal role in a plethora of cancer-associated signaling pathways. It enhances cell growth, accelerates cell cycle progression, promotes migration and invasion, and inhibits cell apoptosis among others. Hence, targeting Skp2 may represent a novel and attractive strategy for the treatment of different human cancers overexpressing this oncogene. In this review

  

article, we summarized the known roles of Skp2 both in health and disease states in relation to the UPS.

**KEYWORDS**
$p27^{KIP1}$, RING E3 ubiquitin ligase, $SCF^{Skp2}$, Skp2

## 1 | THE UBIQUITIN-PROTEASOME SYSTEM

The ubiquitin-proteasome system (UPS) mainly regulates the stability and activity of proteins which subsequently influences numerous cellular functions such as cell proliferation, cell cycle progression, transcription, and apoptosis.[1-3] The ubiquitination process, a sophisticated posttranslational modification cascade, involves the activity of three enzymes (ubiquitin-activating E1, ubiquitin-conjugating E2, and ubiquitin-protein E3 ligase enzymes) that work sequentially to attach ubiquitin (Ub) to substrate proteins, which ultimately results in altered protein function in a variety of ways or proteasomal degradation (Figure 1).[4-12] Crucially, the specificity of substrate recognition is conferred largely by E3 ubiquitin ligases for ubiquitination and subsequent degradation.[2,6,7,12]

### 1.1 | The ubiquitin molecule

Ub is a small protein (8.6 kDa) distributed in nucleus, cytoplasm, and on the cell membranes of eukaryotic cells. It can be covalently conjugated to either another ubiquitin molecule or other proteins through three main reaction sequences: activation, conjugation, and ligation that are catalyzed by the corresponding ubiquitin enzymes in an ATP dependent manner.[16,17] The structure of ubiquitin, a polypeptide chain of 76-amino acid residues, is divided into two important parts: a globular domain which comprises a four-stranded mixed β-sheet and a single α-helix, and a flexible C-terminal tail ending with the simplest amino acid glycine. The C-terminus is at the center of ubiquitin function by participating in a highly ordered sequence of covalent interactions that terminates in ubiquitin attachment to the target protein.[4,17]

### 1.2 | Ubiquitination site

Protein ubiquitination requires the formation of covalent conjugates between the ubiquitin molecule and the target via amide linkages.[17] More specifically, ubiquitination typically occurs on lysine (or less frequently on methionine) residues of either a substrate protein or another ubiquitin molecule creating stable isopeptide (or peptide) linkages with the C-terminus of ubiquitin.[13] Hence, two distinct types of ubiquitin-protein conjugates exist. The first type of ubiquitination involves an isopeptide linkage between ubiquitin and the ε-amino groups of target protein's lysine residues. The second type of conjugate, the less common form of ubiquitination, on the other hand is formed by ubiquitination of the free N-terminal α-amino group of methionine residue of the acceptor protein through a peptide bond.[17,18] Besides, ubiquitin has also been found thioester-bound to cysteine residues in certain target proteins.[19]

### 1.3 | Consequences of ubiquitination

Protein ubiquitination involves either a single ubiquitin (monoubiquitin) or polyubiquitin chains. Polyubiquitination is formed by when multiple ubiquitin molecules are linked to each other in such a way that the C-terminus of

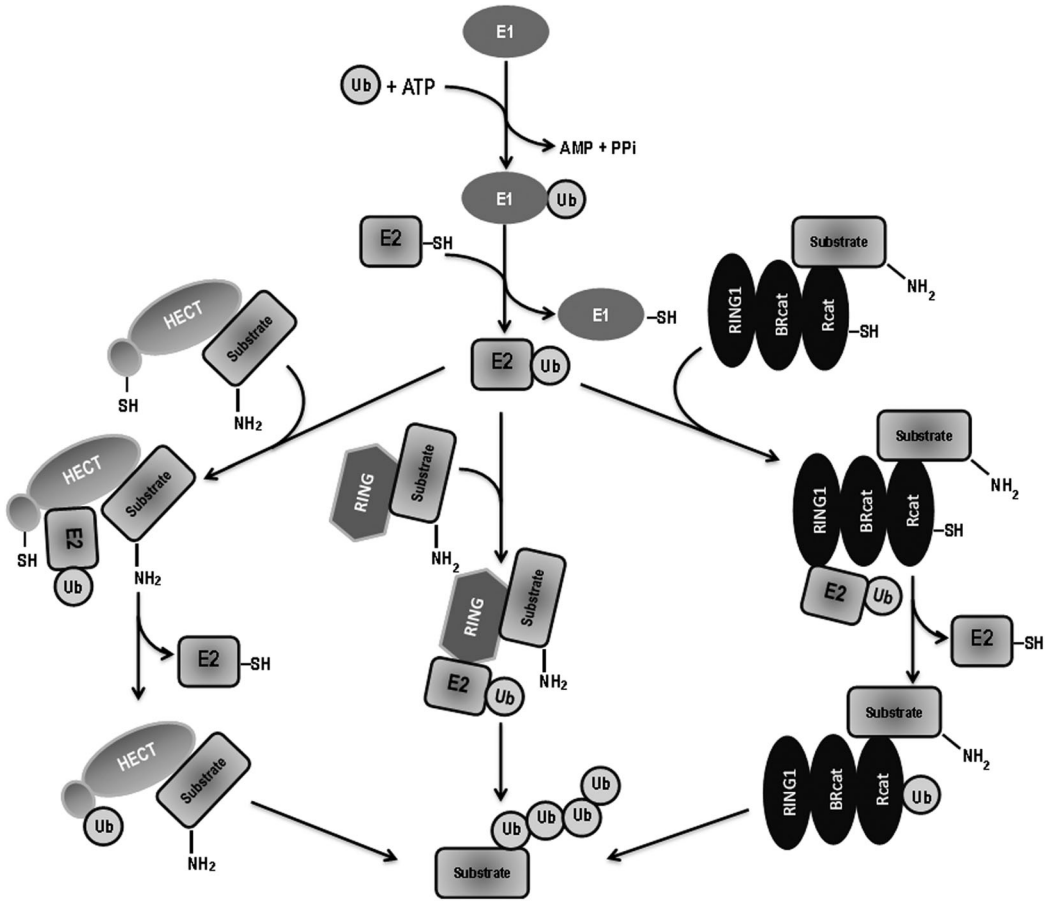


**FIGURE 1** The ubiquitination pathway. Protein ubiquitination by the ubiqutin proteasome system is carried out by the concerted action of three enzymes that act sequentially. The process starts with the activation of a ubiquitin (Ub) molecule in which its C-terminus is linked to the active site Cys of E1 by a thioester bond to form E1~Ub complex. This reaction is catalyzed by the ubiquitin-activating enzyme (E1) and is dependent on ATP. In a transthiolation reaction, the ubiquitin is then transthiolated to the conserved catalytic Cys residue of one of ~40 different (in mammals) ubiquitin-conjugating enzymes (E2), generating an E2~Ub thioester. Ubiquitin protein ligases (E3s) interact with both E2~Ub and the substrate to which ubiquitin is to be transferred, thus providing much of the specificity in the ubiquitin system. However, different E3 ubiquitin ligases exhibit different mode of action. RING E3 ubiquitin ligases function as scaffolds by engaging the E2~Ub complex to their RING domain to optimally orient it with respect to the substrate protein for efficient ubiquitin transfer directly from E2~Ub to the substrate. The HECT E3 ubiquitin ligases bind the E2~Ub complex via their N-terminal lobe. Unlike RING E3 ubiquitin ligases, HECT E3 ubiquitin ligases form a covalent intermediate between the C-terminus of ubiquitin and the conserved catalytic Cys residue in their C-terminal lobe (transthiolation reaction). This HECT~ubiquitin intermediate is then poised for the subsequent transfer of ubiquitin to a substrate protein. In RBR E3 ubiquitin ligases, the RING1 engages with the E2~Ub complex in a similar manner to the RING E3 ubiquitin ligases, whereas the Rcat acts in a similar fashion to the C-terminal lobe of the HECT E3 ubiquitin ligases by performing a transthiolation reaction to form a thiolester bond between the C-terminus of ubiquitin and the catalytic Cys of their Rcat domain.[13,14] Hence, the RBR E3 ubiquitin ligases use a combination of the RING and HECT mechanisms.[15] Once ubiquitinated the protein may be destined for either degradation by the proteasome (main fate of the ubiquitinated proteins) or directed for other pathways (not shown). BRcat, benign-catalytic domain; HECT, homologous to E6-associated protein C-terminus E3 ubiquitin ligases; RBR, RING1-BRcat-Rcat E3 ubiquitin ligases; Rcat, required-for-catalysis domain

one ubiquitin is covalently bonded to the amino group of any of the seven lysine's side chain in the previous ubiquitin molecule in the sequence. Hence, a single target can be modified by diverse polyubiquitin chains simultaneously.[20,21] To a large extent, target protein specificity selected for ubiquitination, the attachment sites on the substrate protein, ubiquitin chain length as well as the lysines involved (ie, $K^{11}$, $K^{48}$, and $K^{63}$) depends on the specific combination of E2 and E3 enzymes.[7]

It is inevitable for most proteins to be ubiquitinated at some point in their cellular lifespan[22] which leads to multiple cellular changes.[23] The ultimate fate and functional consequence of the target protein ubiquitinated largely depends on the nature of ubiquitination. Mono-ubiquitination play a key role in certain cellular functions including DNA repair, protein trafficking and transcription, and usually occurs when the E2 loses the capability of forming ubiquitin chain and this activity is negatively regulated by cofactors and deubiquitinating enzymes.[24] Moreover, multi mono-ubiquitination and chains of four or more ubiquitin molecules linked through $K^{48}$ can lead to proteasomal degradation of target proteins.[17,25-27] Likewise, the anaphase-promoting complex/cyclosome (APC/C) marks its substrates for proteasomal degradation through $K^{11}$-linked ubiquitin chain formation[28] and chains linked through other lysines may also have the same outcome.[29,30] However, in NF-κB signaling pathway, $K^{63}$-linked and linear ubiquitin chains are associated with nonproteasomal outcomes.[31,32] In addition, DNA repair functions and lysosomal degradation of cell surface and endocytic proteins also involve $K^{63}$-linked chains.[33-40]

### 1.4 | Ubiquitin modifications

Once attached to a substrate, ubiquitin is subjected to further modifications to generate diversified chains and hence to create a host of distinct signals with varied cellular outcomes.[22] The chains are assembled through isopeptide bond formation between the C-terminal glycine and any of the seven internal lysines ($K^6$, $K^{11}$, $K^{27}$, $K^{29}$, $K^{33}$, $K^{48}$, or $K^{63}$) or the free N-terminal methionine of ubiquitin molecules that are already substrate-bound. This produces multi- or poly-ubiquitin chains that can encompass complex topologies.[17] These lysine residues can either alternatively or simultaneously undergo modification by ubiquitin-like molecules (such as SUMO or NEDD8). Moreover, further modifications of ubiquitin such as acetylation of lysine residue or phosphorylation of serine, threonine, and tyrosine residues carry a huge potential to drastically alter the signaling outcome.[22] Several deubiquitinase (deubiquitylating or deubiquitinating) enzymes oppose the function of E3 ubiquitin ligases and break ubiquitin chains to halt the ubiquitin signal.[10,41]

## 2 | THE E3 UBIQUITIN LIGASES

E3 ubiquitin ligases are diverse groups of proteins characterized by the presence of several defining motifs.[10] Owing to their multifaceted properties and interactions, E3 ubiquitin ligases provide a powerful and specific mechanism of protein elimination.[42] The human genome encodes more than 700 E3 ubiquitin ligases.[10] Based on the presence of specific domains, the E3 ubiquitin ligases that have been identified to date fall into one of the three main classes: homologous to E6-associated protein C-terminus (HECT) E3 ubiquitin ligases, Really Interesting New Gene (RING) E3 ubiquitin ligase which also include the RING-like E3 ubiquitin ligases, and RBR (RING1-BRcat-Rcat/RING-in-between-RING/RING-between RING-RING) E3 ubiquitin ligases.[14,15,42,43] Each of these E3 ubiquitin ligases contains its own specific domain: HECT, RING and RBR domains, respectively. Moreover, individual E3 ubiquitin ligases under each class can vary widely from each other by possessing additional protein interaction domains.[42]

The role of E3 ubiquitin ligases in ubiquitin conjugation is to mediate ubiquitin transfer from E2 ubiquitin-conjugating enzyme to target protein. However, it should be noted that due to the overlapping binding sites used by E1 and E3 on E2 enzymes, the last step of the ubiquitination cascade is uncoupled from the first two steps which involve an ATP-dependent ubiquitin activation catalyzed by E1 followed by its conjugation with E2 enzymes.[10]

The RING and U-box E3 ubiquitin ligases function as scaffolds that orient E2~ubiquitin thioester complex close to the target protein so that ubiquitin transfer takes place efficiently. In other words, the RING E3 ubiquitin ligases facilitate the direct transfer of the ubiquitin from the E2 cysteine to the target substrate.[7,10,13] By contrast, HECT E3 ubiquitin ligases are directly involved in catalysis, akin to E1 and E2 during ubiquitination.[42] They form a thioester-intermediate between their C-lobe catalytic cysteine residue and the C-terminus of ubiquitin (ie, they serve as catalytic intermediates) before transferring the cargo ubiquitin onto its target.[44-46] The RBR E3 ubiquitin ligases on their part use both RING and HECT-like mechanisms.[15]

The main function of E3 ubiquitin ligases is to regulate target protein polyubiquitination mainly for subsequent degradation. Furthermore, E3 ubiquitin ligases regulate several cellular processes and are implicated in multiple disease conditions due to loss of function or inappropriate targeting.[42]

## 2.1 | HECT E3 ubiquitin ligases

In human beings, HECT E3 ligases comprise nearly 30 different enzymes that directly catalyze protein ubiquitination and have been found to noncovalently interact with ubiquitin.[11,44] HECT E3 ubiquitin ligases have direct role in protein ubiquitination. First, a catalytic intermediate thioester is formed between the C-lobe cysteine residue (an active-site cysteine within the HECT domain) of HECT3 E3 ligase and the C-terminus of ubiquitin (Figure 1).[44-47] Ubiquitin is then transferred to target proteins that are bound to the substrate recognition determinants of the E3 ubiquitin ligase.[48] Structurally, HECT E3 ubiquitin ligases have a common C-terminal HECT domain which consists of approximately 350 amino acids divided into two lobes. The larger N-lobe is a binding site for ubiquitin-charged E2 and the smaller C-lobe is responsible for catalysis by retaining a conserved catalytic cysteine residue.[48,49] The lobes are tethered by a short flexible hinge, and conformational flexibility about this linker was found to be important for juxtaposing the active-site cysteines of the E2. Moreover, the N-lobe of $HECT^{Nedd4}$ has large and small subdomains.[44]

## 2.2 | RBR E3 ubiquitin ligases

The RBR E3 ubiquitin ligases are a unique family of E3 ubiquitin ligases that comprise 12 complex multidomain enzymes such as Parkin, HOIP, HOIL-1, HHARI, and TRIAD1 E3 ubiquitin ligases.[10,14] They are characterized by a highly conserved catalytic unit composed of a RING1, an in-between RING (IBR), and a RING2 domain. The C-terminal RING2 and the central IBR domains are renamed as required-for-catalysis (Rcat) and benign-catalytic (BRcat) domains, respectively, to reflect their structure and function accurately. Rcat is essential for the catalytic activity of RBR E3 ubiquitin ligases. BRcat has the same folding pattern as Rcat but does not have ubiquitination activity due to the lack of a catalytic cysteine residue. Like the other E3 ubiquitin ligase types, the RBR E3 ubiquitin ligases also contain other domain structures that are specific to each member. Moreover, RBR E3 ubiquitin ligases share common features both with the RING and HECT E3 ubiquitin ligases. They directly catalyze ubiquitin transfer from an intrinsic catalytic cysteine found in the Rcat like HECT E3 ubiquitin ligases and recruit thioester-bound E2 enzymes via a RING domain like the RING E3 ubiquitin ligases (Figure 1).[14,15]

## 2.3 | RING E3 ubiquitin ligases

RING-type E3 ubiquitin ligases (also known as RING finger, RING motif, or RING domain E3 ubiquitin ligases) and RING finger-like E3 ubiquitin ligases such as U-box proteins constitute the vast majority of the E3 ubiquitin ligases in mammalian cells. These enzymes function together with the E2 ubiquitin-conjugating enzymes to mediate the ubiquitination process.[13] Mechanistically, these E3 ubiquitin ligases act as adapter-like molecules to bring E2

loaded with ubiquitin and substrate protein into sufficiently close proximity to facilitate target protein ubiquitination (Figure 1).[42] All RING E3 ubiquitin ligases create a conserved E2 binding platform by coordinating two $Zn^{2+}$ ions via eight cysteine and histidine residues in a cross-brace arrangement[13,50] to facilitate ubiquitin transfer from their cognate E2~ubiquitin to target protein.[51-54] The RING finger-like U-box family of E3 ubiquitin ligases which contain a modified RING motif works in a similar manner but without employing $Zn^{2+}$ coordination.[13]

RING-type domains exist in different structural contexts including monomeric, dimeric, and multimeric forms. Many RING E3 ubiquitin ligases such as MDM2 and c-Cbl exist as single-chain (monomeric) enzymes. But, a notable feature of RING-type E3 ubiquitin ligases is their tendency to form dimers: homodimers (cIAP, RNF4, BIRC7, IDOL, and CHIP and Prp19 [U-box proteins])[51,55-60] or heterodimers (BRCA1-BARD1, Mdm2-MdmX [or HdmX/Hdm4 in humans], and RING1B-Bmi1). In the case of homodimeric RING E3 ubiquitin ligases, the intrinsic capacity of both RINGs to functionally interact with E2s is retained. This, however, is not the case for some heterodimeric RINGs.[61-64]

## 2.4 | Complex RING E3 ubiquitin ligases

Cullin-RING ligase (CRL) superfamily are a striking example of multimeric RING-type E3 ubiquitin ligases.[65] CRLs display enormous flexibility in substrate specificity and are composed of a cullin protein, a small RING protein, and either an adapter protein(s) that binds interchangeable substrate recognition elements or, in the case of CRL3, proteins that bind both the cullin protein (CUL3) and substrate.[66] Even though the CRL superfamily overwhelmingly exhibits the greatest range of substrate recognition, other multisubunit E3 ubiquitin ligases such as the highly complex APC/C E3 ubiquitin ligase with more than a dozen of core subunits displays even greater structural complexity and plays a critical role in different phases of cell cycle.[67] The Fanconi Anemia (FANC) E3 ubiquitin ligase,[68-70] and mindbomb E3 ubiquitin ligase which contains three RINGs in its C-terminal region are also multisubunit RING E3 ubiquitin ligases.[71]

### 2.4.1 | Cullin proteins

Cullin (CUL) proteins are molecular scaffolds that associate with RING proteins and E3 ubiquitin ligases. These proteins are widely expressed both in nucleus and cytoplasm, and play a huge role in the ubiquitination of diverse proteins. There are eight members of the mammalian cullin protein family: CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5, and the atypical cullins CUL7 and the p53-associated parkin-like cytoplasmic protein (PARC) which is also known as CUL9. They contain a highly conserved structural feature called the cullin homology (CH) domain at their C-terminus. The CH domain contains about 200 amino acid residues and is essential for binding the RING-finger proteins. Moreover, CUL1 to CUL5 have a long stalk-like amino-terminal domain which consists of three cullin repeats (CR1 to CR3) whereas CUL7 and CUL9 are larger in size and contain additional homology domains.[66,72,73] The cullin proteins are components of CRL complexes, the major family of E3 ubiquitin ligases with about 300 members, tethering both a substrate-targeting unit, often through an adapter protein, and the RING finger component: regulator of cullins 1 (ROC1, also called RING box protein [Rbx]1) or ROC2 (Rbx2).[66,72]

### 2.4.2 | Cullin1-RING E3 ubiquitin ligases

CRLs are the largest E3 superfamily in mammals and regulate a dazzling array of cellular and organismal processes.[65] CRLs (CRL1, CRL2, CRL3, CRL4A, CRL4B, CRL5, CRL7, and CRL9) comprise interchangeable substrate receptors (such as F-box proteins) dedicated to distinct cullin-RING catalytic cores.[65,74] The N-terminal domain of a cullin protein is essential to recruit a substrate receptor either directly or indirectly via an adapter protein. Each cullin has a large family of distinct substrate receptors that recognize a specific “degron” motif in a substrate protein. The C-terminal domain of

cullin proteins is used to bind an Rbx RING protein which then recruits an E2~ubiquitin intermediate. This positions a target protein bound to the substrate receptor in close proximity to the RING-bound E2 ubiquitin conjugating enzyme and facilitate efficient transfer of ubiquitin directly to the substrate protein.[75]

CRL1, alternatively called as S-phase kinase-associated protein 1 (Skp1)-Cullin1-F-box (SCF) E3 ubiquitin ligase, is the prototype and most intensively studied of all CRLs.[65] SCF E3 ligases comprise several dozen modular enzymes, most of which remain uncharacterized, that have diverse roles in biological regulation.[76,77] The SCF E3 ubiquitin ligases share two common structural features: a catalytic core (CUL1-Rbx1) and a variable substrate recognition module (F-box protein [FBP]-Skp1). The variable F-box proteins which could form a variety of different SCF type E3 ubiquitin ligase complexes contain substrate receptor domain and therefore confer substrate selectivity to the SCF complex by specifically targeting a distinct set of substrates for ubiquitination and subsequent proteasomal degradation.[78-82] Owing to their substrate specificity, SCF E3 ubiquitin ligases can be exploited as promising therapeutic targets.[83-85] Dysregulated degradation of oncoproteins or tumor suppressors due to gain or loss of function of specific SCF E3 ubiquitin ligases could lead to tumorigenesis and other diseases. However, the oncogenic and tumor suppressor function of F-box proteins depends on the biological function of their ubiquitin substrate.[86-88] $SCF^{Skp2}$, $SCF^{FBW7}$, and $SCF^{\beta\text{-}TRCP}$ are the three highly characterized E3 ubiquitin ligases in human cancers.[89,90]

### 2.4.3 | F-box proteins

As the substrate-recognizing subunits of the SCF E3 ubiquitin ligase complexes, F-box proteins play pivotal roles in the regulation of multiple cellular processes such as cell proliferation, migration and invasion, metabolism, angiogenesis, cell death and DNA damage response through recruiting target proteins for ubiquitination and subsequent degradation.[12,91-93] Nonetheless, the physiological roles of many F-box proteins remain largely unknown. According to the structural characteristics, the F-box proteins are categorized into three classes: FBWs, FBLs, and FBXs that contain WD-40 domains, leucine-rich repeats, and different protein-protein interaction modules or no recognizable motifs, respectively (Table 1). Each F-box protein consists of various protein-protein interaction motifs at the C-terminal that bind target substrates, and an N-terminal F-box motif, a stretch of 40-amino acid domain that binds Skp1, an adapter protein in the SCF E3 ubiquitin ligase complex.[86,94]

### 2.4.4 | Substrate recognition by F-box proteins

The C-terminal domains of F-box proteins are used to recruit a unique set of proteins harboring specific "degron" motifs. These short, defined motifs may require additional posttranslational modifications such as phosphorylation, glycosylation and addition of mannose oligosaccharides for their interaction with respective F-box proteins.[73,95-98] F-box proteins can also recognize target proteins with unmodified degron motifs.[99,100] Moreover, phosphorylation of degron motifs is reported to block the degradation of substrates of some F-box protein.[101-103] Thus, F-box protein-mediated protein degradation might be controlled by additional mechanisms including degron access restriction and regulating F-box protein localization or stability.[73]

## 3 | Skp2: STRUCTURE AND REGULATION

### 3.1 | Structure of Skp2

S-phase kinase-associated protein 2 (Skp2, also known as FBXL1 or p45), member of the FBXL subclass of F-box proteins, is a well-characterized cancer-related protein discovered in 1995. This 45 kDa oncoprotein is localized in

**TABLE 1** Classification of F-box proteins[6]

| F-box proteins | Alternative names | Substrate recognition domain | Number of proteins (confirmed to date) | Functions | Examples |
|---|---|---|---|---|---|
| FBXW subclass | FBXW-WD40 (WD) repeats | WD40 repeat domain | 10 | Mainly target substrates involved in cell cycle regulation and tumorigenesis | FBXW-1$^{\beta\text{-TRCP1}}$, FBXW-2, FBXW-4, FBXW-5, FBXW-7, FBXW-8, FBXW-9, FBXW-10, FBXW-11$^{\beta\text{-TRCP2}}$, FBXW-12 |
| Leucine-rich repeat proteins | FBXL proteins | Leucine-rich repeat (LRR) domain | 22 | Tumor suppressors or oncoproteins | FBXL1 (Skp2), FBXL2, FBXL3A, FBXL3B, FBXL4-7, FBXL9 and FBXL11 |
| FBXO proteins | … | Uncharacterized domains | 37 | Miscellaneous | FBXO1 (cyclin F), FBXO2, FBXO3 - FBXO12, FBXO20 - FBXO26, FBXO29 and Elongin A |

nucleus and cytoplasm mainly.[104-106] The structure of Skp2 consists of 424 amino acid residues divided into four distinct domains: destruction domain (D-box) which controls its stability, nuclear localization signal (NLS) and F-box domain situated at its N-terminus, and 10 consecutive C-terminal leucine-rich repeats (LRR) domain that recognize different substrates or other elements (Figure 2).[106-108] One of the LRR has a partially disordered loop instead of the helix characteristic of LRRs.[109] The C-terminus stabilizes the interaction of Skp2 with Skp1 as it folds back near the F-box.[108] Moreover, Skp2 has a second isoform called Skp2B or FBXL2 that lacks exon 10 and include exon 11 at its C-terminus domain.[110] Consequently, the two isoforms differ in their substrate specificities. Hence, Skp2B does not exhibit a significant effect on Skp2 substrates, and most importantly, it does not affect the level of p27.[108,110]

## 3.2 | Regulation of Skp2

### 3.2.1 | Regulation of Skp2 gene expression

*Regulation of Skp2 expression by transcription activators*

Mammalian Skp2 protein is degraded by the ubiquitin-proteasome pathway but its expression is mostly regulated at the transcriptional level during the cell cycle (Table 3). Several transcription factors such as E2F1,[112,113] NF-κB,[114,115] SP1,[116] CBF1,[117] GABP,[118] FOXM1,[119] and MYCN[120] are found to be associated in the promoter region of Skp2 gene. In addition, Skp2 expression can be regulated by many signaling pathways. For example, notch1 signaling not only induces Skp2 gene expression by associating with CBF1 but also triggers Skp2-dependent

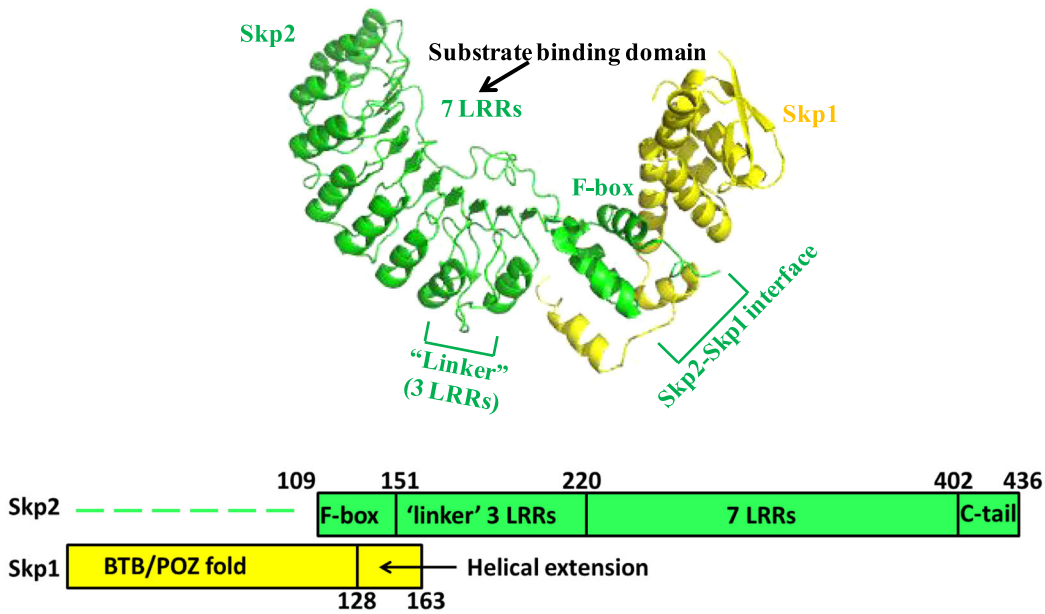


**FIGURE 2** The structure of Skp1-Skp2 Complex. The Skp1 (yellow) that binds to Skp2 (green) through the N-terminal F-box domain of the latter is an adapter protein to recruit Skp2 into the $SCF^{Skp2}$ complex; The α helices at the C-terminal represent the defining domain (LRRs) of Skp2 that recognize substrates.[111] The location of different domains and regions of interaction between Skp1 and Skp2 are shown below the structure. The 100-amino acid residues at the N terminus of Skp2 are missed and indicated with dashed lines.[109] The D-box and NLS of Skp2 in the N terminus is not shown here. BTB/POZ: Broad Complex, Tramtrack, Bric-a-brac/Pox virus and Zinc finger [Color figure can be viewed at wileyonlinelibrary.com]

p21 and p27 degradation and cell cycle progression.[117] Similarly, other oncoproteins such as HBXIP are found to stimulate the promoter of Skp2 and upregulates Skp2 expression via activating E2F1.[121] A recent study by Katona *et al* also showed that a nuclear scaffolding protein called menin promoted Skp2 expression by binding to the promoter region of its gene in colorectal cancer.[122]

*The PI3K/Akt pathway regulates Skp2 expression*

Ablation of the PI3K/Akt signaling pathways either by inhibition of the PI3K activity or by depletion of Akt1 leads to the downregulation of Skp2 expression at the transcription level.[123-125] Mechanistically, the reduction of Skp2 mRNA levels following Akt inactivation is due to the reduced protein levels of E2F1 and its binding to the promotor of Skp2 gene, and impaired NF-κB signaling which is subjected to regulation by Akt1.[114,125] In addition, several oncogenic signals such as Bcr-Abl and Her2/Neu that are overexpressed in human cancers induced Skp2 gene expression through the PI3K/Akt signaling pathway.[126,127]

*The IL-6/JAK2/STAT3 pathway regulates Skp2 expression*

Skp2 is found to be a direct transcriptional target of STAT3 signaling.[128] The IL-6/JAK2/STAT3 pathway positively regulated the expression of Skp2. Consistently, STAT3 depletion leads to suppression of Skp2 expression, and thereby elevating expression of Skp2 substrates p27 and p21.[129,130] Moreover, constitutive activation of STAT3 signaling restored high-level Skp2 expression and lowered p27 expression to promote cell cycle progression through G0/G1.[130]

*MicroRNAs inhibit Skp2 expression*

MicroRNAs (miRNAs or miRs) have a critical role in gene expression regulation.[131] Several miRNAs are found to directly target the Skp2 gene and decrease its expression in several human cancers. For example, miR-186,[132] miR-30a-5p,[133] miR-339,[134] miR-340,[135] and miR-508-5p[136] downregulate the expression of Skp2 in pituitary tumor cells and esophageal squamous cell carcinoma, ovarian cancer, lung cancer, hepatocellular carcinoma cells, and gastric cancer, respectively (Table 2). The consequence of Skp2 expression inhibition by miRNAs is decreased cell viability, proliferation, migration and invasion, cell cycle arrest at the G0/G1 phase and $p27^{Kip1}$-mediated induction of apoptosis.[132-136]

*Regulation of Skp2 expression by transcription inhibitors*

Unlike the transcriptional activators, repressors for Skp2 expression are less clear. One such transcriptional repressor of Skp2 expression is the forkhead box O3 (FOXO3a). It inhibits Skp2 protein expression through direct binding to the promoter of Skp2 gene.[137,138] In line with this, FOXO3a also induced $p27^{KIP1}$ transcription.[139,140] Two additional studies in mice revealed that another tumor suppressor called the forkhead box P3 (FOXP3) also binds to the promoter region of Skp2 and suppresses its expression to induce cell cycle arrest.[141,142]

### 3.2.2 | Regulation of Skp2 stability and localization

*The UPS regulates Skp2 stability*

Skp2 is a short-lived protein and its stability is regulated by the UPS during the cell cycle. The multisubunit E3 ubiquitin ligase $APC^{Cdh1}$ complex is found to trigger Skp2 ubiquitination and causes its subsequent degradation early in the G1 phase (Table 3).[143,144] Consistent with this, the level of Skp2 expression is found to increase and promoted S-phase transition upon Cdh1 gene silencing. This is in accord with the low level of Cdh1 protein observed in G1/S transition and the corresponding rise in Skp2 expression level.[143,145] Cdh1 binds to the N-terminal D-box motif of Skp2, and unsurprisingly, removal of this domain spares Skp2 from $APC^{Cdh1}$-mediated ubiquitination and subsequent degradation.[143,144] In contrast, ubiquitin-specific peptidase 10 (USP10) is reported as a novel deubiquitinase of Skp2. USP10 mediates the deubiquitination and stabilization of Skp2 which leads to

**TABLE 2** MicroRNAs interfering with Skp2 expression

| Micro RNAs | | Human tumor cells | | | | | | miRNAs effect on Skp2 | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | Pituitary | Esophageal squamous cell | Ovarian | Lung | Hepatocellular | Gastric | | |
| miRNAs | 186 | √ | √ | | | | | ↓ Skp2 gene expression | He et al[132] |
| | 30a-5p | | | √ | | | | | Wang et al[133] |
| | 339 | | | | √ | | | | Ren et al[134] |
| | 340 | | | | | √ | | | Wang et al[135] |
| | 508-5p | | | | | | √ | | Duan et al[136] |

increased activation of the tyrosine kinase Bcr-Abl through $K^{63}$ ubiquitination in chronic myeloid leukemia. Furthermore, loss of USP10 decreased Skp2 expression as well as the subsequent Bcr-Abl activation and its downstream signals.[146]

*Phosphorylation prevents Skp2 destruction*

The serine/threonine-protein kinase Akt directly controls Skp2 stability by a mechanism that involves degradation by the $APC^{Cdh1}$ ubiquitin ligase complex (Table 3).[123] Skp2 undergoes phosphorylation at two critical serine residues, $Ser^{64}$ and $Ser^{72}$, by Akt and Cdk2.[123,147] Owing to their proximity to the D-box motif of Skp2, these phosphorylation sites play a critical role in regulating its stability. The phosphorylation of Skp2 on these residues effectively halts Cdh1 binding to Skp2 and thereby attenuating $APC^{Cdh1}$-mediated Skp2 ubiquitination and degradation. It is apparent that the activity of both Akt and Cdk2 kinase is stringently regulated by the cell cycle.[104]

**TABLE 3** Summary of Skp2 regulatory mechanisms

| Regulatory molecules | Regulatory mechanism | Effects |
|---|---|---|
| Transcription activators | Stimulate the promoter of Skp2 gene | Promote Skp2 gene expression |
| MiRNAs | Silence Skp2 gene | Inhibit Skp2 gene expression |
| NEDD8 | Neddylation of Cullin1 | Promote $SCF^{Skp2}$ complex formation |
| Akt | Phosphorylation | Enhance activity (Nucleus) |
| | | Promote $SCF^{Skp2}$ complex formation |
| | | Increase Skp2 cytosolic localization (14-3-3 binding) |
| | | Deter Skp2 ubiquitination (increase stability) |
| p300 | Acetylation | Increase Skp2 cytosolic localization (14-3-3 binding) |
| | | Deter Skp2 ubiquitination (increase stability) |
| $APC^{Cdh1}$ | Ubiquitination | Promote Skp2 degradation |
| FOXO3a | Transcription Inhibitor | Repress Skp2 gene expression |
| | | Disrupt $SCF^{Skp2}$ complex formation |
| STAT3 | Transcription activator | Increase Skp2 expression |

Similarly, mTORC1 stabilizes Skp2 and regulate its oncogenic function in gastric cancer. This kinase directly phosphorylates Skp2 at $Ser^{64}$, and which in turn protects its elimination by UPS.[148]

*Akt promotes cytoplasmic localization and activity of Skp2*

Sustained hyperactivation of the PI3K/Akt pathway is considered as the hallmark of many cancers. Activation of this pathway has been shown to enhance p27 destruction, probably through influencing Skp2 activity (Table 3).[124,126,149] To further strengthen this notion, it has been shown that loss of Cdh1 which mediates Skp2 degradation by the mechanism discussed above is not a frequent event in human cancer compared with the frequency of Skp2 overexpression.[123] This puzzle cannot be solved unless there is a cause to the increased activity of Skp2 in several types of cancers while Cdh1 activity is yet not fully compromised. To this end, stimulation of the PI3K/Akt pathway causes the phosphorylation of Skp2 at $Ser^{72}$ within the putative nuclear localization sequence and leads to the cytoplasmic translocation of Skp2[123] through promoting its interaction with 14-3-3 scaffold protein and preventing the interaction of Skp2 with importin α5 and α7 that mediate Skp2 nuclear import. Moreover, $Ser^{72}$ phosphorylation also enhances the $SCF^{Skp2}$ complex formation and its E3 ubiquitin ligase activity which in turn promotes cell cycle progression and tumorigenesis.[104] Moreover, Akt can also strengthen the role of Skp2 in tumor cells through an indirect mechanism. Activation of the PI3K/Akt pathway inhibits apoptosis by promoting the phosphorylation and subsequent cytoplasmic localization of downstream proapoptotic protein targets including Bad,[150] FOXO1,[151] and FOXO3a.[152] Akt upregulation can also promote cell growth by inactivating p21[153] and p27.[154-156]

*Acetylation enhances Skp2 stability and cytosolic localization*

Skp2 is subjected to acetylation on two lysine residues, $K^{68}$ and $K^{71}$, within the nuclear localization signal (NLS) by a p53 activator called p300. The functional consequence of this modification is to enhance Skp2 stability and mediate its cytoplasmic retention (Table 3).[157,158] Once in the cytoplasm, Skp2 plays a crucial role in the progression of human cancers.[108,157,159-161] Moreover, Inuzuka et al[157] have also reported the importance of Skp2 acetylation in regulating its substrates, such as p21, p27, and FOXO1.

*Regulation of $SCF^{Skp2}$ complex formation and its activity*

The formation and structural integrity of $SCF^{Skp2}$ complex are obviously critical for the enzymatic activity of the $SCF^{Skp2}$ E3 ubiquitin ligase. Interestingly, $SCF^{Skp2}$ complex formation and its activity can be regulated by different factors. One such mechanism is by the neddylation and deneddylation cycles of its component protein (CUL1) with a small ubiquitin-like modifier called Nedd8 (Table 3). Neddylation of CUL1 stabilizes the $SCF^{Skp2}$ complex by preventing the binding of CUL1 to CAND1 which is a negative regulator of the $SCF^{Skp2}$ complex formation. The Skp2-Skp1 complex promotes the neddylation of CUL1 by dissociating CUL1 from CAND1. In contrast, the COP9 signalosome (CSN) complex, an eight-subunit protein complex, mediates deconjugation of Nedd8 from the cullin subunits of SCF and other CRLs. Thus favoring CUL1 interaction with CAND1 and preventing its binding to Skp1 and Skp2.[104,162] This process is essential to maintain the proper activity of CRLs in cells.[162] Therefore, the exchange of F-box proteins within the SCF scaffold takes place through a complex cycle involving neddylation and deneddylation.[74] Furthermore, the transcription factor FOXO3a has also been reported to disrupt $SCF^{Skp2}$ complex formation in a transcription-independent manner.[137]

## 4 | Skp2 FUNCTIONS

### 4.1 | Skp2 tissue expression

Skp2 is broadly expressed in several tissue types including the placenta, lymph node, adrenal, appendix, bone marrow, brain, colon, duodenum, endometrium, esophagus, fat, gall bladder, heart, kidney, liver, lung, ovary,

pancreas, prostate, salivary gland, skin, small intestine, spleen, stomach, testis, thyroid, and urinary bladder (https://www.ncbi.nlm.nih.gov/gene/6502#gene-expression). The expression of both Skp2 mRNA and Skp2 protein is regulated by the cell cycle. Under physiological conditions, Skp2 accumulates late in the G1 phase and its expression peaks appear during S and G2 phases of the cell cycle, rather than the G1 phase. Skp2, therefore, primarily mediates p27 degradation at the G2, but not G0/G1 phases. The level of Skp2 declines as cells progresses through the M phase. Therefore, Skp2 is indispensable for progression into mitosis during cell cycle.[106,163-168]

## 4.2 | Skp2 substrates

Skp2 targets several protein substrates involved in cell cycle progression, signal transduction, and transcription for ubiquitination most of which end up with degradation in the proteasome. The substrates of Skp2 include but not limited to p21,[169] p27,[170] p57,[171] p130,[172] UBP43,[173] PDCD4,[174] RASSF1A,[175] Tob1,[176] RAG-2,[177] Cdt1,[178] TAL1,[179] hOrc1p,[180] Cdk9,[181] FOXO1,[182] cyclin E,[183] E2F1, TRUSS E3 ubiquitin ligase,[184] and c-Myc[175,185,186] which are involved in different cell functions. Owing to its broader tissue distribution and the wide range of substrates it recognizes, Skp2 is very crucial in a multitude of cellular processes such as cell cycle regulation, cell proliferation, apoptosis, differentiation, and survival. Interestingly, these processes which involve $SCF^{Skp2}$ E3 ubiquitin ligase-mediated ubiquitination significantly influence tumorigenesis.[89]

### 4.2.1 | $p27^{KIP1}$: The main Skp2 substrate

The tumor suppressor $p27^{KIP1}$ which belongs to the kinase inhibitor protein (KIP) family of Cdk inhibitory proteins (CKIs) is the key target of Skp2. It is a 198-amino acid protein that controls cell cycle progression at the G1 phase.[187,188] This CKI inhibits a broad range of cyclin-dependent kinases (Cdks) including Cdk2–cyclin E and Cdk2–cyclin A complexes, the activities of which are greatly required for the G1 phase to S-phase transition and to trigger DNA replication.[77,189] The Cdk-inhibitory domain of $p27^{KIP1}$ resides in its N-terminal portion and is sufficient to arrest cells at G0/G1. The nuclear localization signal of $p27^{KIP1}$ is found in the less conserved C-terminal portion of the molecule.[105]

Several transcription factors such as menin, Myc and Pim modulate the expression of $p27^{KIP1}$.[105] However, it is widely accepted that genetic or epigenetic changes in the $p27^{KIP1}$ gene are rare events in carcinomas.[190] The rise and fall of $p27^{KIP1}$ is regulated predominantly at the posttranscriptional and posttranslational levels that modulate its cellular localization and extent of E3 ubiquitin ligase-mediated degradation.[105] The major E3 ubiquitin ligase that clears nuclear $p27^{KIP1}$ during the G1/S-phase transition is the $SCF^{Skp2}$.[190] The phosphorylation of $p27^{KIP1}$ at $Thr^{188}$ by either Cdk2/cyclin E or Cdk2/cyclin A complex is a primary prerequisite that marks it for recognition by Skp2.[191] Moreover, $SCF^{Skp2}$-mediated $p27^{KIP1}$ ubiquitination requires an accessory protein called cyclin-dependent kinase subunit 1 (Cks1).[192] Cks1 serves as an adapter protein that binds Cdk/cyclin complex, phosphorylated $p27^{KIP1}$and Skp2. In doing so, Cks1 greatly increases the binding affinity of $Thr^{188}$-phosphorylated $p27^{KIP1}$ to Skp2.[190] For this reason, the phospho-p27/Cks1/Cdk2/cyclin E complex is regarded as $SCF^{Skp2}$ substrate.[193]

## 4.3 | Skp2 in cell cycle progression

Eukaryotic cell proliferation requires sequential activation of the catalytic units of cell division cycle called Cdks.[189] The enzymatic activity of Cdks is regulated by transcriptional, translational, and posttranslational mechanisms such as association with cyclins (positive regulatory subunits), phosphorylation and dephosphorylation, and interaction with CKIs.[77,189,194] The CKIs are classified into two families based on their structure and Cdk targets.[189] The INK4

(inhibitors of Cdk4) family specifically inhibit the catalytic subunits of Cdk4 and Cdk6 by blocking the association of Cdks with cyclin D. Members of this family include $p16^{INK4a}$,[195] $p15^{INK4b}$,[196] $p18^{INK4c}$,[197,198] and $p19^{INK4d}$.[199] The CIP/KIP family, on the other hand, comprises $p21^{Cip1}$,[200-205] $p27^{KIP1}$,[206,207] and $p57^{KIP2}$.[208,209] They are broadly acting inhibitors (Cf. the INK family) affecting the activities of cyclin D-, E-, and A-dependent kinases. They bind to preassembled Cdk/cyclin complexes and inhibit their activities.[210-214]

The levels of cyclins, CKIs, and many other cell cycle regulators oscillate during the cell cycle as a result of periodic proteolysis.[215] The APC/C and the SCF complexes are the major ubiquitin ligases that regulate cell cycle progression through proteolysis of short-lived cell cycle regulatory proteins and thereby controlling their intracellular concentrations.[215-218] As a critical component of the F-box family of substrate-recognizing subunits of SCF ubiquitin-protein ligase complexes, Skp2 recognizes and ubiquitinates several protein targets to exert its impact in cell cycle progression and other cellular processes.[89,219] The ability of Skp2 to influence cell proliferation, metabolism and tumorigenesis is mediated through promoting ubiquitination and degradation of CKIs.[112,137,183,220,221] In a normal cell cycle, the level of $p27^{KIP1}$ is elevated in the G0/G1 phase.[222] As cells enter the cell cycle (G1 phase) and S-phase, $p27^{KIP1}$ levels must be decreased to allow Cdk activation. Skp2, as an oncoprotein, plays a pivotal and indispensable role in cell cycle progression by triggering $K^{48}$ ubiquitination and destruction of $p27^{KIP1}$.[84]

## 5 | Skp2 IN CANCER MODULATION

### 5.1 | Oncogenic role of skp2

Dysregulated destruction of cell cycle regulators, many of which have either tumor-suppressive or oncogenic functions, is tightly linked to neoplastic transformation and cancer progression.[89] Skp2 plays a critical role in tumorigenesis of different human cancers owing to its vital role in the cell cycle.[168] The $SCF^{Skp2}$ E3 ubiquitin ligase complex regulates the degradation of several cell cycle regulators.[223] Since most of its substrates, notably the CKIs, ubiquitinated and degraded by proteasome are tumor suppressors Skp2 is regarded as an oncoprotein. The oncogenic function of Skp2 is well established in a variety of human cancers.[107,224,225] Deregulated Skp2 function promotes neoplastic transformation and this is consistent with the observation that Skp2 is overexpressed in many human cancers.[123,226]

Skp2 is activated and mediates tumorigenesis in many types of human cancers like lymphomas, non–small cell lung cancer, prostate cancer, melanoma, nasopharyngeal carcinoma, pancreatic cancer, breast carcinomas, cervical cancer, osteosarcoma, uveal melanoma, multiple myeloma, and gastric cancer.[107,225,227-235] Consistent with this notion, the downregulation of $p27^{KIP1}$, particularly nuclear-expressed $p27^{KIP1}$, is implicated both in cancer progression and poor prognosis in a variety of cancers.[236] Absence or reduced levels of $p27^{KIP1}$ due to excessive degradation is a frequent event in several types of aggressive human tumors[222,229,237-240] and low levels of $p27^{KIP1}$ correlate with aggressive tumor biology, high-grade tumors, and poor prognosis.[226,229,241] One main mechanism responsible for the decreased level of $p27^{KIP1}$ protein in carcinomas is the increased expression of $SCF^{Skp2}$, especially Skp2.[86,190,226] Depletion of Skp2 which spares the $p27^{KIP1}$ levels decreases cell growth and metastasis and increases apoptosis in certain tumors.[224,230-232,242,243-245] In addition to $p27^{KIP1}$ degradation, Skp2 activates cancer critical growth and survival-signaling pathways.[124,126,246-248] Moreover, a recent study by Krishnan et al[249] showed Skp2 as a potential driver for Cdk1 expression. Skp2 increased the expression of Cdk1 through phosphorylation of the transcription factor FOXM1.

### 5.2 | Skp2 inhibits apoptosis in cancer cells

Skp2 amplification and overexpression impedes p53 transactivation function and represses apoptosis mediated by DNA damage or p53 stabilization. This function of Skp2 is independent of ubiquitination. Mechanistically, Skp2

binds to the CH1 and CH3 domains, p53 binding sites, of p300 to hinder its interaction with p53. Skp2, therefore, suppresses p300-mediated acetylation of p53 and its transactivation ability. In addition to inhibiting the interaction between p53 and p300, Skp2 alters p53 activity by promoting p300 degradation. Therefore, Skp2 attenuates p53 function by affecting the activity and the level of the acetyltransferase p300.[224,250] Moreover, overexpression of Skp2 and Skp2B has been reported to induce cell proliferation and inhibit apoptosis in breast cancer through a mechanism involving the p53-inducible gene 3 (PIG3). Mechanistically, Skp2 and Skp2B decreased the expression of PIG3 mediated by p53 and prohibitin (PHB), respectively.[229,251]

## 5.3 | Skp2 promotes cancer cell proliferation and metastasis

Skp2 promotes cancer cell progression through either influencing certain cellular processes or making a cross-talk with other signaling pathways activated in cancer cells. Cell growth, cell cycle progression, invasion, and metastatic abilities of tumor cells are positively regulated by Skp2. It also inhibits apoptosis and induces drug resistance.[219,224,252,253] For instance, Skp2 overexpression promoted cell growth and inhibited apoptosis in osteosarcoma (OS) cells. Moreover, Skp2 upregulation accelerated cell cycle progression and enhanced migration and invasion ability in these cells. These effects were due to decreased E-cadherin, FOXO1, p21, and p57, and increased matrix metalloproteinase (MMP)-9 protein levels mediated by Skp2.[107]

Further assessment of the role of Skp2 in cancer revealed that the abrogation of Skp2 expression in Skp2 overexpressing MGC-803 cells led to marked inhibition of cell viability, proliferation, colony formation, and migration and invasion while promoting apoptosis. The ability of these cells to form a tumor and to metastasize, and the growth of established tumors was also suppressed in vivo due to inhibition of cell proliferation and enhanced apoptosis. Interestingly, the impact of Skp2 shRNA was minimal in BGC-823 cells with respect to the above processes. These cells express a low level of Skp2 and when induced to overexpress Skp2 tumorigenesis is promoted in mice.[254]

Studies on uveal melanoma (UM), and U266 and RPMI 8226 multiple myeloma cell lines with SKP2 inhibitor C1 (SKPin C1) further support the critical roles of Skp2 in cancer cells. Skp2 overexpression promoted cell cycle progression in these cell lines through p27 degradation.[231,232] SKPin C1, a highly selective Skp2 inhibitor, prevents Skp2-mediated p27 degradation. The increased p27 protein level then restrained the cell cycle, inhibited cell proliferation, and induced apoptosis.[231,232] Skp2 depletion caused upregulation of p27, p21, and p57 and downregulation of cyclin E and Cdk2 which leads to induction of cell cycle arrest in the G1/S-phase. Moreover, caspase-3 activity and expression and activity of MMP-2 and MMP-9 were also increased upon Skp2 depletion.[254]

## 5.4 | Skp2 promotes glycolysis and tumorigenesis in cancer cells

Akt kinase (protein kinase B (PKB)) is a key protein that transmits extracellular growth factor signals to the inside of the cell in response to survival signals. It coordinates signal pathways that regulate cell proliferation, cell survival, metabolism, and tumorigenesis.[255-258] Moreover, dysregulation of the PI3K/Akt kinase signaling pathway is a frequent event in human cancers.[259] E3 ubiquitin ligases are found to play a role in Akt kinase activation. For example, the TRAF6 (TNF receptor-associated factor 6) E3 ubiquitin ligase mediates Akt ubiquitination and activation following IGF-1 signaling.[260,261] Moreover, activation of ErbB family receptors such as the epidermal growth factor (EGF) receptors also induced Akt ubiquitination. But, it is the $SCF^{Skp2}$ E3 ligase complex, not the TRAF6, which orchestrates Akt ubiquitination, and its recruitment to the cell membrane and eventual activation in response to EGF receptors activation by the EGF. This Skp2-mediated $K^{63}$ Akt ubiquitination and activation induces Glut1 expression to increase glucose uptake and glycolysis and promotes tumorigenesis.[220]

### 5.5 | Skp2 regulates cancer stemness

F-box proteins are reported to regulate the differentiation of cancer stem cells (CSCs) which may be responsible for tumor metastasis and recurrence.[91] A growing body of evidence showed that Skp2 deficiency suppressed stem cell features of different human cancers. Skp2 depletion not only halted CSC self-renewal but also decreased tumor growth and metastasis.[85,233,242,262] Resistance to certain anticancer agents was also improved upon genetic depletion or pharmacologic inhibition of Skp2, at least in part. As described by Ruan et al,[242] the ability of Skp2 to maintain CSCs population and augment their self-renewal is mediated through nondegradative ubiquitination and stabilization of Twist protein.

### 5.6 | Skp2 causes drug resistance

Increased expression of Skp2 poses a challenge in cancer treatment by mediating resistance against the clinically used anticancer agents or other small molecule inhibitors that are under development. Overexpression of Skp2 is identified to be closely related to resistance to chemotherapeutic agent doxorubicin in breast cancer.[263] The emergence of resistance to small molecule inhibitors of the PI3K pathway which leads in rebound Akt activation has also been correlated with the E3 ligase Skp2 in breast cancer cells.[259] Moreover, Skp2 silencing or pharmacological inhibition is also found to sensitize cancer cells to traditional anticancer agents including paclitaxel and doxorubicin, cisplatin and other target-derived antitumor agents like herceptin, rapamycin, and bortezomib.[220,264-267] It is, therefore, plausible to conclude that targeting Skp2 can be exploited not only to abolish its oncogenic functions but also to improve the sensitivity of cancer cells to other chemotherapeutic agents. The different roles of Skp2 in cancer discussed so far are summarized in Figure 3.

### 5.7 | Skp2 in tumor microenviroment

The initiation, progression, and metastasis of tumors are complex processes that depend on a constant cross-talk between the tumor cells and the tumor stroma. The stromal microenvironment (also known as tumor microenvironment, TME) that surrounds tumor cells and a small portion of slowly dividing tumor stem cells includes the vasculature, fibroblasts, immune cells and the extracellular matrix.[268-270] TME not only plays a key role in tumorigenesis but also represents a crucial target for cancer treatment in addition to targeting tumor cells.[269,271-273] The $SCF^{Skp2}$ E3 ubiquitin ligase is reported to be involved in modulating TME in a context-dependent manner. For example, androgen deprivation therapy is implicated in the development of androgen-independent prostate cancer through modulation of the prostate tumor microenvironment by secretory senescent cells.[274] Interestingly, the induced cellular senescence is mediated in part by Skp2 downregulation caused by the depletion of androgen activity.[274,275] On the other hand, TME is found to mediate Skp2 downregulation through Cdh1 upregulation in mantle cell lymphoma and other non-Hodgkin lymphoma cells which led to a G1 arrest, though reversible, due to elevated p27 and p21 protein levels.[268] Despite these initial findings, further studies are required to fully understand the exact role of Skp2 in TME.

## 6 | Skp2 IN CANCER THERAPY

### 6.1 | Skp2 is a potential drug target

Skp2 is now confirmed to be involved in pertinent signaling pathways including survival and apoptotic pathways of many different cancers. High aggressiveness and poor prognosis of various malignant tumors were confirmed to be

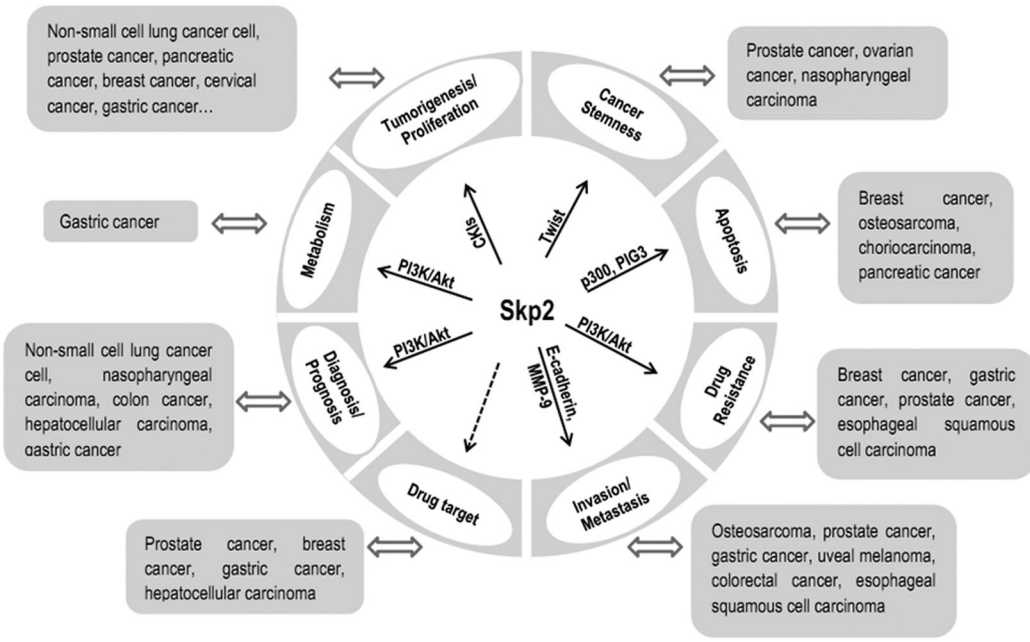


**FIGURE 3** Summary of Skp2 functions in cancer

associated with reduced expression of p27.[176,228,236,276] Suppression of p53 function by Skp2 also decreased apoptosis.[251] Moreover, there is mounting evidence supporting the notion that activation of survival pathways by Skp2 could lead to tumorigenesis. It is therefore plausible to look for different approaches that could impair the function of Skp2 at various levels.[277,278] Indeed, different strategies that decrease expression level and activity of Skp2, and interfere with its stability and localization have been found to halt cell cycle entry and progression,[105,228] inhibit cell proliferation, and profoundly restrict cancer metastasis.[254]

Skp2 has also been found to limit the therapeutic efficacy of different anticancer agents[265] by conferring resistance through multiple mechanisms.[259] Interestingly, inhibition of Skp2 by small molecule inhibitors improved the sensitivity of cancer cells to anticancer drugs.[264,279,280] Moreover, Skp2 could act as both a diagnostic and prognostic marker in cancer.[228,229,281,282] Taken together, it can be concluded that targeting Skp2 at different levels may represent a novel and a promising approach for the treatment of different human cancers with Skp2 overexpression.[107,226,228,229,243,254,283]

## 6.2 | Small molecule inhibitors of Skp2

The $SCF^{Skp2}$ E3 ubiquitin ligase and its accessory protein, Cks1, promote cell proliferation mainly by inducing the degradation of its key substrate, $p27^{Kip1}$. Skp2 and Cks1 are frequently overexpressed in many cancer types and cause unrestrained proliferation mainly by promoting $p27^{KIP1}$ degradation. For this reason, disruption of the interaction of Skp2 with its cofactor Cks1 by small molecules to spare Skp2-mediated $p27^{KIP1}$ degradation would represent an ideal target for pharmacological intervention.[193] In addition, compounds that prevent Skp2-SCF complex formation would also be invaluable tools in the coming age of cancer therapy.[284] SCF-Skp2-specific small molecule inhibitors may also be exploited as novel strategies to treat human cancers that depend on the Skp2-$p27^{KIP1}$ axis.[285] Moreover, small molecules inhibitors that inhibit Skp2 expression or promote its degradation would be of great importance in cancer treatment.[286-288] It should, however, be noted that all the strategies

ultimately have the potential to mainly restore $p27^{KIP1}$ levels in human cancers to halt tumorigenesis through the mechanisms discussed above.

### 6.2.1 | Inhibitors of Skp2 expression

Decreasing Skp2 expression through small molecule inhibitors may be a useful approach for treatment of cancers with Skp2 overexpression. A small molecule inhibitor of p27 depletion called SMIP004 (N-(4-butyl-2-methyl-phenyl) acetamide) was reported to stabilize p27 and p21 protein levels in a proteasome-independent manner through downregulating Skp2 expression in LNCaP prostate cancer cells leading to cell cycle arrest in the G1 phase.[286,287] However, regulation of Skp2 expression by SMIP004 is secondary to the decreased E2F transcription factor activity caused by SMIP004-mediated cyclin D1/Cdk4 activity inhibition.[112,286,287] In a recent study, Zhou et al[288] reported a new way of targeting Skp2 protein by increasing its turnover using dioscin. This natural product is found to increase Skp2 recruitment into the $APC^{Cdh1}$ E3 ubiquitin ligase complex by promoting its interaction with Cdh1 resulting in $K^{48}$ ubiquitination and degradation of Skp2 in colorectal cancer. Other natural products such as the neddylation inhibitors Gartanin[289] and flavokawain A,[290] and curcumin[230] are also found to decrease Skp2 expression in prostate and pancreatic cancers, respectively.

### 6.2.2 | Skp1-Skp2 complex formation inhibitors

As stated elsewhere in this review, Skp2 is an integral component of the $SCF^{Skp2}$ E3 ubiquitin ligase which helps the ligase by specifically targeting the proteins destined for proteasomal degradation. Compounds that disturb this structural integrity are under development and are found to be effective in halting the Skp2-mediated tumorigenesis. For example, a small molecule inhibitor named Compound #25 (also known as SZL-P1-41), which was identified by high-throughput virtual screening approaches based on two potential pockets, is found to restrain growth in multiple differentiated cancer cells by attenuating p27 ubiquitination and subsequent cell cycle progression. This small molecule prevented Skp2 and Skp1 association by binding to the F-box domain of Skp2 and hence, block $SCF^{Skp2}$ complex formation. The functional consequences of this inhibition are augmentation of p27-mediated apoptosis or senescence and impairment of Akt-driven glycolysis.[84,220] Chen et al also reported another small molecule called Compound A which prevented Skp2 incorporation into the $SCF^{Skp2}$ ligase complex. This blockade similarly interfered with the function of $SCF^{Skp2}$ ligase and spared Skp2 substrates from ubiquitination mediated proteasomal degradation in vitro.[284] Moreover, Malek et al[266] identified a new $SCF^{Skp2}$ inhibitor called DT204 through chemical library screening which reduced Skp2 binding to CUL1 and Commd1, a CUL1-binding protein that enhances $SCF^{Skp2}$ activity, in bortezomib (BTZ)-resistant multiple myeloma.

### 6.2.3 | Skp2-p27 binding inhibitors

Wu *et al* identified a set of small molecule inhibitors collectively called as SKPins (Compounds C1, C2, C16, and C20) specific to SCF-Skp2 activity using in silico screens targeted to the binding interface for p27 (Figure 4). These selective inhibitors of Skp2-mediated p27 degradation fit into a molecular surface pocket at the Skp2-Cks1 interface and block p27 ubiquitination in vitro. They, however, do not block the non-Skp2-p27 interfaces of the active SCF. Moreover, the compounds neither impaired the function of E1, E2 and other E3 ubiquitin ligase enzymes nor interfered with the stability of other Skp2 substrates. Functionally, SKPins increased p27 protein level in Skp2-dependent manner and promoted cell cycle arrest in the G1 or G2/M phases in cell-type-specific manner in cancer cells.[285,291] In addition,

Ooi et al[292] identified linichlorin A and gentian violet as inhibitors of the interaction between Skp2-Cks1 complex and p27, though they failed to provide the detailed binding mechanism.

### 6.2.4 | Skp2-Cks1 protein-protein interaction inhibitors

By now, it is clear that $p27^{KIP1}$ ubiquitination by the $SCF^{Skp2}$ E3 ubiquitin ligase requires Cks1 protein. This protein is crucial for facilitating the interaction between Skp2 and $p27^{KIP1}$.[192] As a result, a direct protein-protein interaction between Skp2 and Cks1 is required for $p27^{KIP1}$ degradation. Two compounds named NSC689857 and NSC681152 ($IC_{50}$ = 76 μM) have been found to inhibit the $SCF^{Skp2}$ E3 ubiquitin ligase-mediated $p27^{KIP1}$ degradation by perturbing Skp2-Cks1 protein-protein interaction based on a high-throughput AlphaScreen (amplified luminescent proximity homogeneous assay screen) assay (Figure 4). This strategy may potentially be a useful approach for the control of excessive $p27^{KIP1}$ degradation in cancer cells.[193] Through a ELISA based high-throughput screen system, Singh et al[293] synthesized a series of quinoline inhibitors that disrupt the protein-protein interaction between Skp2 and Cks1, in which compound 22d exhibited the highest activity ($IC_{50}$ = 0.17 μM).

### 6.2.5 | Skp2/p300 interaction inhibitors

In addition to promoting the proteasomal degradation of tumor suppressor, notably the CKIs, Skp2 also inhibit p53-mediated apoptosis in a nonproteolytic manner by antagonizing p300-mediated p53 activation.[224] Oh et al[250] identified a Skp2 inhibitor called M1 that targets the p300 binding site of Skp2 to block its interaction with p300

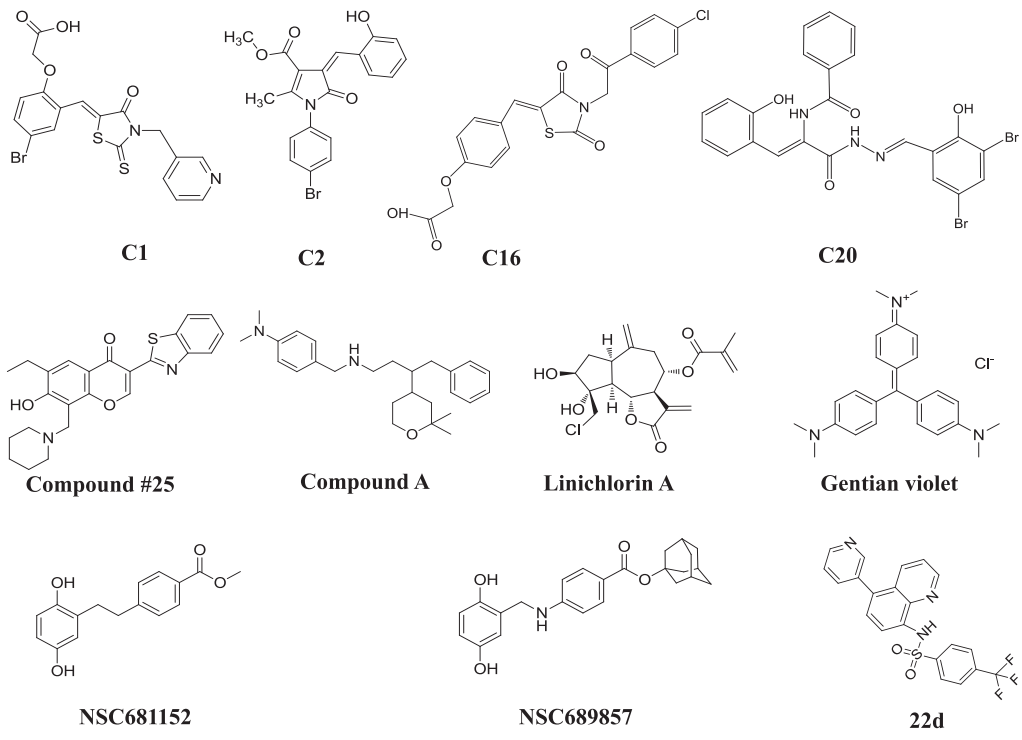


**FIGURE 4** Structures of Skp2 inhibitors

using AlphaScreen assay. Interestingly, this inhibitor does not interfere with the substrate binding site of Skp2, and hence the proteolytic functions of Skp2 remain unaffected by M1 inhibition as evidenced by unaltered p27 levels. The level of Skp2 bound to p300 and cellular Skp2 protein level is decreased by M1 treatment. On the other hand, p300-mediated p53 acetylation and stabilization is increased which induced p53-mediated apoptosis and cell death in cancer cells.[250]

## 7 | FUTURE PERSPECTIVES

Skp2 is a defined oncogene in multiple human cancers. This clearly paves the way to study the role of Skp2 in multiple aspects of different cancers. However, not all of its substrates and downstream signaling molecules are fully explored. As a result, Skp2 could have other unexplored roles in specific cancer. In addition, the role of $SCF^{Skp2}$ E3 ubiquitin ligase complex in modulating TME is not studied very well. Therefore, additional functional and mechanistic studies are required to further understand the role of Skp2 in cancer. Moreover, in spite of the initial efforts made so far, the quest for other potential molecular targets and small molecules that can bind them in Skp2-mediated signaling pathways remains wide open and needs further investigation. Small molecules that inhibit p300 or Akt-mediated Skp2 activation and compounds that disrupt Skp2 stabilization and localization among others could be exploited as further investigation areas.

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