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Deregulated proteolysis by the F-box proteins SKP2 and β-TrCP: tipping the scales of cancer

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Abstract

The maintenance and preservation of distinct phases during the cell cycle is a highly complex and coordinated process. It is regulated by phosphorylation — through the activity of cyclin-dependent kinases (CDKs) — and protein degradation, which occurs through ubiquitin ligases such as SCF (SKP1–CUL1–F-box protein) complexes and APC/C (anaphase-promoting complex/cyclosome). Here, we explore the functionality and biology of the F-box proteins, SKP2 (S-phase kinase-associated protein 2) and β-TrCP (β-transducin repeat-containing protein), which are emerging as important players in cancer biogenesis owing to the deregulated proteolysis of their substrates.

Cellular transformation results from aberrant responses to otherwise normal cues that regulate processes involved in proliferation, differentiation and apoptosis. These processes are regulated by transcription, translation, post-translational modifications and degradation of key regulatory proteins, and, as such, the ubiquitin–proteasome system (UPS) has a crucial role in maintaining and regulating cellular homeostasis. In order for target proteins to be recognized and degraded by the proteasome, the small protein ubiquitin is transferred and covalently attached to substrates by the sequential action of three enzymes, namely E1 (ubiquitin-activating enzyme), one of many E2s (ubiquitin-conjugating enzymes (UBCs)) and one of many E3s (ubiquitin ligases). The specifics of how these three enzymes work in concert are reviewed elsewhere[1]. Important for this Review, however, is the understanding that the specificity of ubiquitylation is provided by ubiquitin ligases, which physically interact with target substrates. With the number of ubiquitin ligases extending to several hundred, these complex molecular machines provide specificity and adaptability to regulated protein degradation.

Ubiquitin ligases are classified into two main classes on the basis of structural similarities: the RING-finger proteins and the HECT-domain proteins. Many multi-subunit, RING-finger type ubiquitin ligases contain a cullin (Cul) protein subunit, a name derived from the ability of cullin RING ubiquitin ligases (CRLs) to 'cull' or sort substrates for degradation[2]. In mammals there are six different CRLs, including the SKP1–CUL1–F-box protein (SCF) complex. In addition, there are CRL-like ligases such as the anaphase-promoting complex/cyclosome (APC/C).

The SCF ubiquitin ligases are the most characterized mammalian CRLs[3]. The SCF complex, together with the UBC component, forms a 'super-enzyme' (BOX 1). Most SCF substrates are recognized and bound by the F-box protein subunit only when they are post-translationally modified, usually through phosphorylation at specific sites. This is in contrast to other ligases,

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such as APC/C, which are only activated when needed and recognize substrates on the basis of a degradation motif (degron) in the primary sequence of their targets.

Sixty-nine F-box proteins have been identified in humans[4–6], and they have been classified into three categories: those with WD40 domains (FBXWs), those with leucine-rich repeats (FBXLs) and those with other diverse domains (FBXOs). Notably, only nine of the sixty-nine SCF ubiquitin ligases have well-established or proposed substrates; these include $SCF^{\beta\text{-TrCP1}}$, $SCF^{\beta\text{-TrCP2}}$, SCF^{SKP2}, SCF^{FBXL3}, SCF^{FBXL20}, SCF^{FBXO4}, SCF^{FBXW7}, SCF^{FBXO7} and SCF^{FBXW8}. Substrates of these SCF complexes can be sub-divided into two main groups: direct regulators of cyclin-dependent kinases (CDKs) and regulators of gene transcription (or both). Numerous studies have described the roles of SCF ubiquitin ligases in controlling cell size, proliferation and survival, and, given the diverse and important roles of SCF ligases, their deregulation has been implicated in aberrant cellular growth and tumorigenesis. Such is the case for FBXW7, which has a central role in cell cycle progression, cellular growth and differentiation by targeting oncogenic proteins for degradation, and several cancer-associated mutations have been shown to exist in *FBXW7* (REF. 7). In this Review, we will focus on SKP2 (S-phase kinase-associated protein 2, also known as FBXL1) and β-TrCP (β-transducin repeat-containing protein), the two other prototypical and best characterized mammalian F-box proteins. Notably, mammals express two distinct paralogues of β-TrCP with biochemical properties that are indistinguishable: β-TrCP1 (also known as FBXW1, FBW1A and FWD1) and β-TrCP2 (also known as FBXW11, FBW11, FBXW1B, FBX1B and HOS). We will therefore use the term β-TrCP to refer to both, unless specified. SKP2 and β-TrCP are key players in regulating many cellular processes that are related to cancer through targeted degradation of substrates. We will highlight their respective substrates and discuss the role of these molecules in cancer biogenesis and tumour progression.

SKP2 and β-TrCP control the activity of CDKs

Tight regulation of the cell division cycle through proper activation and inactivation of CDKs is crucial to preventing cellular transformation[8]. In addition to the catalytic subunit of the CDK, each CDK complex contains one of many activating subunits, called cyclins, the levels of which oscillate during the cell cycle. Although CDK1 (also known as cell division cycle 2) is the only CDK in mammals that is essential for viability, several distinct cyclin–CDK complexes finely regulate progression through distinct phases of the cell cycle. CKIs (CDK inhibitors), such as p21 and p27, function by inhibiting CDK activity and promoting cell cycle arrest and/or delaying the response to anti-mitogenic stimuli. CDK1 and CDK2 are active from late G1 to anaphase, and for this reason, this temporal interval is defined as CDK phase (or C phase)[9]. Whereas CDK2 activity peaks in S and G2 phases, CDK1 activity is relatively low in S, increases in G2, and peaks in early mitosis, only to disappear abruptly during anaphase. SCF complexes and APC/C provide specific, rapid and timely proteolysis of cell cycle regulators (which ultimately control CDKs), thereby finely modulating their activities during cell cycle progression. Whereas SKP2 activates CDK1 and CDK2 by directing the degradation of p21 and p27, β-TrCP has a dual role in controlling CDK1 activity: turning it off by inducing the degradation of CDC25A (cell division cycle 25A) and EMI1 (also known as F-box protein 5), in S phase and mitosis, respectively, and turning it on by inducing the degradation of claspin and WEE1 at G2–M (FIG. 1).

Notably, β-TrCP has also emerged as a key player in the S and G2 DNA-damage response checkpoint[10], the main function of which is to mediate cell cycle arrest to allow time to repair DNA lesions. This is mainly accomplished by limiting the activity of CDKs to prevent premature hyperactivation of CDK1 and entry into mitosis before the completion of DNA repair. Upon DNA damage, activation of CHK1 (checkpoint kinase-1) and CHK2 through phosphorylation by ATR (ataxia-telangiectasia mutated and RAD3-related) and ATM (ataxia-

telangiectasia mutated), respectively, results in hyperphosphorylation of CDC25A, leading to increased proteolysis of CDC25A (mediated by β-TrCP) and attenuation of CDK1 activity (FIG. 1). Importantly, upon recovery from DNA damage, β-TrCP additionally functions to restore CDK1 activity by targeting claspin and WEE1 for degradation. Therefore, given the crucial function of the cell cycle machinery in regulating cell cycle progression, the altered proteolysis of cell cycle regulators is clearly a contributing factor in the unrestrained proliferation that is typical in cancer cells.

At a glance

- Targeted protein proteolysis of key regulatory proteins by the ubiquitin–proteasome system (UPS) has a central role in maintaining and regulating growth. As such, components of the UPS can promote or prevent cellular transformation, which results from an aberrant response to otherwise normal cues that regulate processes involved in proliferation, differentiation and apoptosis.
- The SCF (SKP1–CUL1–F-box protein) ubiquitin ligases are the best characterized mammalian cullin RING ubiquitin ligases, and the F-box protein provides the substrate targeting specificity of the complex.
- Out of sixty-nine F-box proteins that have been identified in humans, only nine have been matched with their respective substrates. The F-box proteins SKP2 (S-phase kinase-associated protein 2) and β-TrCP (β-transducin repeat-containing protein) have emerged as key regulatory molecules with roles in cellular processes that are intimately related to tumorigenesis.
- SKP2 is an oncogenic protein that targets tumour suppressor proteins for degradation. As a positive regulator of cell cycle progression, a major target of SKP2 is the cyclin-dependent kinase (CDK) inhibitor p27, as has been shown *in vivo* and *in vitro*. Increased levels of SKP2 and reduced levels of p27 are observed in many types of cancer, and these levels are in several cases used as independent prognostic markers.
- Whereas β-TrCP has been previously suggested to possess both oncogenic and tumour suppressive characteristics — mainly owing to the diversity in β-TrCP substrates — recent evidence indicates β-TrCP is mainly oncogenic.
- Previous attempts at targeting components of the degradation machinery have been successful for laboratory and clinical use, as observed in the effectiveness of the proteasome inhibitor bortezomib (Velcade) in multiple myeloma. The development of pharmaceutical compounds targeting specific SCF ubiquitin ligases is timely and is complemented by structural and basic biochemical studies that have identified substrates for important cellular regulators such as SKP2 and β-TrCP.

SKP2 targets tumour suppressor proteins

SKP2 was first identified as a component of the cyclin-A– CDK2 complex and was subsequently shown to promote entry into S phase[11]. Since then, SKP2 has been extensively characterized as an SCF ubiquitin ligase that is crucial for cell cycle progression (see above and FIG. 1) and cell proliferation.

Biochemical studies identified SKP2 as the rate-limiting component of the SCF machinery that ubiquitylates the tumour suppressor p27 *in vitro* and *in vivo*[12–14]. In particular, SKP2 recognizes p27 only when p27 is phosphorylated on Thr187 by one of many CDK complexes.

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Moreover, the ubiquitylation of p27 requires CKS1 (CDK subunit 1), which binds to SKP2 and increases the affinity of phosphorylated p27 for SKP2 (REFS 15,[16]). Importantly, this result was the first demonstration of a requirement of an accessory protein for SCF function.

Mouse models of p27 function have provided genetic evidence that the loss of *Cdkn1b* (which encodes p27) promotes cell proliferation, which leads to endocrine dysfunction and the development of cancer[17]. Mice lacking p27 have been shown to be larger than wild-type, control littermates and exhibit generalized organomegaly. Similar to mice lacking *Rb1* (retinoblastoma 1), *p27*$^{-/-}$ mice spontaneously develop pituitary tumours, confirming that p27 and RB1 function in similar regulatory pathways. Interestingly, all *p27*-null 129/Sv mice develop pituitary adenomas by the age of ten weeks and die with massively enlarged pituitary tumours that result in compression of the brain. Thymic enlargement in *p27*$^{-/-}$ mice was associated with an increase in T-lymphocyte proliferation, and, in the spleen, the absence of p27 enhanced proliferation of haematopoietic progenitor cells.

Box 1 | SCF complexes are super-enzymes

The SCF (SKP1–CUL1–F-box protein) ubiquitin ligase complex is the best characterized mammalian cullin RING ubiquitin ligase (CRL). The cullin subunit CUL1 functions as a molecular scaffold that interacts at the amino terminus with the adaptor subunit SKP1 (S-phase kinase-associated protein 1) and at the carboxyl terminus with a RING-finger protein RBX1 (also known as ROC1), RBX2 (also known as ROC2) or Ro52 and a specific E2 enzyme or ubiquitin-conjugating enzyme (UBC), such as UBC3, UBC4 or UBC5 (REF. 3). The F-box protein functions as the variable component that binds SKP1, through the F-box domain, and the substrate, through different protein–protein interaction motifs, which in most cases are localized C-terminally of the F-box. There is a debate as to whether SCF ligases are true enzymes. In many ways, the molecular composition and functionality of SCF ligases, together with the UBC component, can be considered a super-enzyme. When compared to classical enzymes, such as kinases, many fundamental characteristics support this view. In the classical enzymatic reaction, the kinase transfers a small chemical group (that is, a phosphate) by way of an active catalytic site to targeted substrates. These substrates are selected on the basis of their ability to bind the specific kinase through a substrate-binding domain. Lastly, the orientation of the substrate and its positioning towards the active site is determined within a single protein chain. In comparable mechanical fashion, SCF ubiquitin ligases transfer a small protein (that is, the ubiquitin moiety) by way of an activated UBC component to specific substrates that are selected through a particular substrate-binding protein (that is, the F-box protein). Here, the cullin and F-box protein dictate the orientation of the substrate and its presentation to the RING-finger protein–UBC pair. Finally, SCF complexes have been shown to contain intrinsic ubiquitin ligase activity *in vitro*, as purified, recombinant CUL1–RBX1 complexes (in the absence of SKP1 and the F-box protein subunits) can catalyse UBC-dependent, substrate-independent ubiquitylation through the formation of free ubiquitin chains. Therefore, based on the action, specificity, and multi-subunit composition of the SCF, the notion of the super-enzyme can be used to characterize these ubiquitin ligases.

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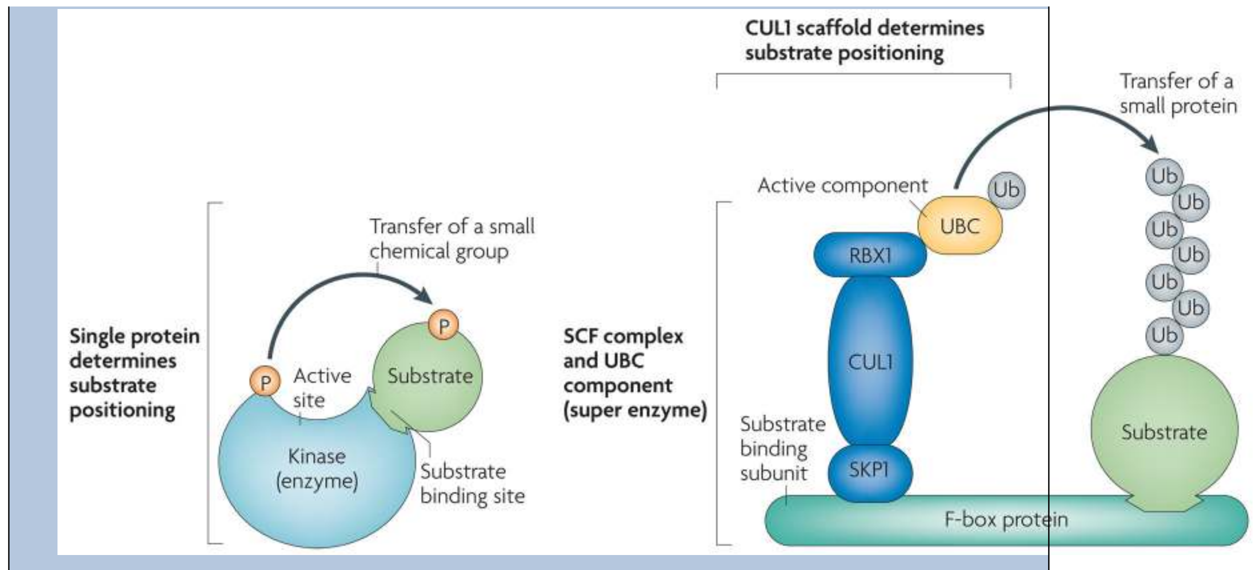

In agreement with biochemical data acquired in cultured cells, the absence of SKP2 in mice results in the accumulation of p27, and *Skp2*$^{-/-}$ mice were shown to be smaller than wild-type littermates[18]. *Skp2*$^{-/-}$ cellular phenotypes included nuclear enlargement and polyploidy in cells of the liver, lung, kidney and testis, and an increased number of centrosomes in mouse embryonic fibroblasts (MEFs)[18]. Importantly, all these phenotypes disappear in *Skp2*$^{-/-}$; *p27*$^{-/-}$ double-mutant mice, indicating that p27 is a key target of SKP2 (REFs 19,20). *Cks1*$^{-/-}$ mice are also smaller than wild-type animals, and cells derived from these mutant mice were shown to proliferate poorly, probably owing to elevated levels of p27 (REF. 15).

Other SKP2 substrates are also tumour suppressor proteins, such as the CKIs, p21 (REFs 21, 22) and p57 (encoded by *CDKN1C*)[23]; TOB1 (transducer of ERBB2)[24]; RASSF1 (Ras association domain family 1)[25]; and RBL2 (retinoblastoma-like 2; also known as p130)[26]. FOXO1, a member of the forkhead box-containing transcription factors that are involved in various cellular processes including cell cycle regulation, differentiation, stress responses and apoptosis, is also targeted for degradation by SKP2 as a consequence of phosphorylation by Akt, which is a pro-survival kinase[27].

Several other proteins have been reported to be targeted by SKP2 (TABLE 1). Importantly, SKP2 recognizes substrates for ubiquitylation through phosphorylation of consensus sequence (s) rather than recognizing a degron in the primary sequence. Therefore, although some of these substrates, such as USP18 (ubiquitin-specific peptidase 18)[28] and cyclin D1 (REF. 18), have been observed to accumulate in *Skp2*$^{-/-}$ MEFs, they have never been shown to be ubiquitylated *in vitro* via SKP2, implying that they might be indirectly upregulated in the absence of SKP2. Another group of proposed substrates (such as CDK9, which is the catalytic subunit of the positive-transcription elongation factor B[29,30]) have not been validated by follow-up studies or cannot be confirmed by other groups. As *Skp2*$^{-/-}$; *p27*$^{-/-}$ mice and MEFs overcome phenotypes that are associated with SKP2 deficiency, it is possible that p27 is the crucial substrate of SKP2 *in vivo*. Alternatively, SKP2 substrates other than p27 might be efficiently targeted by additional ubiquitin ligases, reducing the role of SKP2 in their regulation. For example, human CDT1 (chromatin licensing and DNA replication factor 1) is also degraded through CRL4; p21 is also degraded through APC/C^{CDC20}; and cyclin E is also degraded through SCF^{FBXW7}. Future studies will be required to validate all SKP2 substrates, identify the biological relevance of their degradation and characterize their potential roles in promoting or preventing cancer.

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SKP2 as an oncogene

The transformation potential of SKP2 was initially observed in tissue culture systems, in which forced expression of SKP2 in immortalized cells was found to induce degradation of p27, promote entry into S phase and induce growth in the absence of adhesion to the extracellular matrix[31]. The introduction of a dominant-negative SKP2 mutant into breast cancer cells was shown to result in fewer colonies in soft agar, and accordingly, enforced expression of SKP2 in hormone-dependent breast cancer cells conferred resistance to a G1 arrest mediated by anti-oestrogens and oestrogen deprivation[32]. Expression of SKP2 was observed to increase with androgen addition in human prostate cells, leading to an increase in p27 degradation and increased proliferation[33,34]. Moreover, overexpression of SKP2 overcomes cell cycle arrest by androgens[33]. Together, these results indicate that overexpression of SKP2 (and the subsequent low levels of p27) serves as a growth advantage for cancer cells.

Several mouse models confirm the function of the SKP2–p27 axis in tumorigenesis. For example, in mammary epithelium, p27 deficiency cooperates with the *ErbB2* oncogene in breast tumour development. Similarly, overexpression of SKP2 in T cells cooperates with activated NRAS in lymphomagenesis[35], and lymphomas arising in mice that are deficient for the transcriptional activator CBP (cAMP-response-element-binding protein (CREB)-binding protein) exhibit reduced levels of p27 and increased levels of SKP2 (REF. 36). Introduction of a *p27*-null allele into $Cbp^{-/-}$ mice accelerates lymphomagenesis and obviates the need for SKP2 upregulation. In addition, constitutive expression of SKP2 in mouse prostate promoted marked overproliferation, resulting in hyperplasia, dysplasia and low-grade carcinoma of the prostate gland, and, consistent with its crucial role in p27 proteolysis, resulted in a significant downregulation of p27 (REF. 37). Similarly, xenografts of breast cancer cell lines expressing SKP2 grow faster than those expressing lower levels of SKP2 (REF. 38). Analyses of a p27-T187A knock-in mouse, which generates a p27 mutant that cannot be bound by SKP2 owing to the loss of T187 phosphorylation, showed that the SKP2-dependent degradation of p27 is crucial for the progression of colon adenomas to colon carcinomas[39]. Finally, increased expression of CKS1 in MYC-induced mouse lymphomas correlates with low levels of p27 (REF. 40), in agreement with the function of CKS1 in promoting SKP2-mediated degradation of p27. Importantly, loss of CKS1 in *E*μ-*Myc* B cells impairs the ability of MYC to induce cell proliferation, lymphomagenesis and metastasis.

Numerous studies in patients have shown low levels of p27 rather than complete loss of p27 expression in tumours, and this is in agreement with the notion of p27 as a haploinsufficient tumour suppressor[41]. The inactivation of p27 in human tumours is rarely due to the loss of one allele or inactivating mutations, and loss of heterozygosity has never been reported. Instead, the low levels of p27 are attributed to a decrease in protein stability[17]. As such, increases in SKP2 expression (mRNA and protein) are associated with downregulation of p27 in a wide range of human tumours and cell lines. The mechanisms that lead to the upregulation of SKP2 in human cancers have been identified for a small set of tumours (TABLE 2). Notably, in all studied cases of cancer, high levels of SKP2 correlate with poor overall survival. Elevated expression of CKS1 (mRNA and protein) is also observed in some tumours, and, importantly, CKS1 was found to be an independent prognostic marker for breast, colorectal and gastric cancers[42–46] (TABLE 2).

Presumably, SKP2 overexpression or improper temporal expression (such as expression in G1 when it is normally low) confers a growth advantage by enhancing the degradation of p27. However, there are certain cancers in which elevated levels of SKP2 do not correlate with low levels of p27. It is possible that in these tumours p27 is degraded via alternative ubiquitin ligases — through KPC[47] or PIRH2[48], for example — and it is important to investigate whether in these same tumours there is a positive selective pressure to enhance the degradation

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of different SKP2 substrates (such as FOXO1, TOB1, p130 or RASSF1). Experiments that were conducted in prostate carcinoma cells show that SKP2 overexpression eliminates the pro-apoptotic effect of FOXO1, and the levels of FOXO1 and SKP2 are inversely correlated in mouse lymphoma models, suggesting that elevated SKP2 is a major factor responsible for the degradation of FOXO1 in lymphomagenesis. Furthermore, FOXO1 and SKP2 levels were also found to be inversely correlated in endometrial cancer cells[49]. Notably, FOXO1 regulates cell cycle arrest by transcriptional activation of negative cell cycle regulators, such as p27, p21 and p130 (all of which are also targeted by SKP2). Therefore, characterizing the expression of FOXO1 in human tumours, its correlation with levels of SKP2 and p27, levels of Akt activity and the resulting clinical outcomes will be crucial to fully understanding the role of this tumour suppressor. Finally, a role for RBL2 loss has been suggested for many tumours[50–56], and future studies will be needed to determine whether the levels of RBL2 are affected in tumours overexpressing SKP2.

β-TrCP functions in diverse pathways

As mentioned above, mammals express two distinct paralogues of β-TrCP with biochemical properties that are indistinguishable. Work by several groups has demonstrated the versatility of β-TrCP in regulating various cellular processes through mediating the degradation of a variety of targets (TABLE 3). β-TrCP recognizes a DSGXXS destruction motif or its variants (for example, DSG/DDG/EEG/SSGXXS/E/D motifs) in which the serine residues are phosphorylated by specific kinases to allow binding to β-TrCP (TABLE 3). Targets of β-TrCP can be divided into two main groups: cell cycle regulators and pro-apoptotic regulators. Accordingly, inhibition by RNA interference or forced expression of a dominant-negative β-TrCP mutant induces apoptosis in human malignant melanoma and breast cancer cells, and augments the cytotoxic effects of anticancer drugs and ionizing radiation[57–59]. This effect is probably due to pro-apoptotic factors (for example, IκB (inhibitor of nuclear factor κB), PDCD4 (programmed cell death 4) and others) and CDC25A (which promotes mitotic catastrophe) that accumulate when β-TrCP is inhibited (see below).

β-Trcp1$^{-/-}$ mice have been shown to have impairment in spermatogenesis and reduced fertility without signs of gross tissue abnormalities[60,61]. Overall viability of these animals is also unaffected. This result is probably due to redundancy with β-TrCP2, which presumably continues to target β-TrCP substrates for degradation. However, MEFs isolated from *β-Trcp1*$^{-/-}$ animals display centrosome overduplication that is associated with the presence of multipolar metaphase spindles, misaligned chromosomes and a lengthened G2–M transition. The stabilization of claspin[62,63] and WEE1 (REF. 64) is probably the main reason that *β-Trcp1*$^{-/-}$ MEFs progress slower than wild-type cells through the G2–M transition and mitosis.

β-TrCP is an oncoprotein (in some tissues)

Owing to the diversity in its substrates, β-TrCP might be expected both to be oncogenic and display tumour suppressor activity (TABLE 3). However, overwhelming evidence indicates that β-TrCP possesses mainly oncogenic characteristics. Indeed, overexpression of β-TrCP has been reported in many cases. One study, examining colorectal cancer tissues, showed that 56% of the tissues tested had increased β-TrCP1 mRNA and protein levels, and that this increase was associated with decreased apoptosis and poor prognosis[65]. Furthermore, chemoresistant pancreatic cancer cell lines also display markedly elevated levels of β-TrCP1 and constitutive activation of NFκB (nuclear factor κB; see below)[66]. β-TrCP1 is also overexpressed in hepatoblastomas[67], whereas β-TrCP2 overexpression is observed in some breast cancers[68]. Similarly, mammary epithelia of female mice exogenously expressing high levels of β-TrCP1 proliferate faster, with 38% of these mice developing carcinomas[69]. Interestingly, targeted expression of β-TrCP in lymphoid organs produces no phenotype, suggesting that β-TrCP-

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dependent tumorigenesis is tissue specific[69]. Together, these observations advocate a role for β-TrCP in promoting tumour development in certain tissues.

β-TrCP targets tumour suppressor proteins for UPS-mediated degradation

NFκB is an inducible, dimeric transcription factor complex composed of members of the Rel family of DNA-binding proteins that activate a large number of genes in response to infection, inflammation and other stresses[70]. NFκB targets include genes that encode various regulatory cytokines (for example, interleukin and TNFα (tumour necrosis factor-α)), transcription factors, survival factors (for example, BCL-xL), growth factors (for example, interleukin 6 and GMCSF (granulocyte macrophage colony-stimulating factor)), adhesion molecules (for example, MMP9 (matrix metalloproteinase 9)), cell surface receptors and immune modulators. Under normal conditions, inactive NFκB is sequestered in the cytoplasm and a family of inhibitory proteins, the IκBs, actively bind NFκB to mask its nuclear localization signal, thereby preventing nuclear import of NFκB and consequently inhibiting its activity[70]. The exposure of cells to a variety of stresses leads to the rapid phosphorylation and ubiquitylation of IκB via β-TrCP[71–78]. The subsequent proteolytic degradation of IκB frees NFκB to translocate to the nucleus, where it can regulate gene transcription. Together, this activation of NFκB through degradation of IκB is considered the classical NFκB signalling pathway.

Constitutive activation of NFκB is observed in many inflammation-associated human cancers, where it contributes to tumorigenesis[70]. Being a negative regulator of NFκB, IκB functions as a tumour suppressor, and the aberrant activation of NFκB due to defective IκB activity has been demonstrated in several malignancies. For example, NFκB activation is often observed in human hepatocellular carcinomas (HCCs)[79]. Similarly, MDR2 (multidrug resistance 2; also known as ABCB4)-knock-out mice develop HCC owing to chronic inflammation caused by the accumulation of bile acids. However, the removal of NFκB signalling, following induction of a liver-specific, non-degradable IκB transgene (*ΔN-IκB*), leads to a dramatic decrease in tumour progression, similar to that observed in NFκB-deficient animals[80]. Together, these data suggest that aberrant loss of IκB, which might occur in the context of β-TrCP overexpression, might lead to the development of HCC. In fact, this process also appears to occur in melanomas: increased survival of melanoma cells is thought to result primarily from constitutive NFκB activation associated with high levels of β-TrCP and constitutive IκB kinase activity[81,82].

Whereas the molecular deregulation of many tumour suppressor genes and/or oncogenes occurs at the transcriptional level, increasing evidence suggests that cellular transformation can originate from altered translational regulation of tumour suppressors and oncogenes. Several eukaryotic translation initiation factors, including eIF4F (eukaryotic translation initiation factor 4F), participate in the regulation of protein translation. Within this complex, the RNA helicase eIF4A catalyses the unwinding and the consequent cap-dependent translation of mRNAs with structured 5′ UTRs (untranslated regions), including mRNAs of proteins that positively regulate cell growth and survival.

PDCD4 is a tumour suppressor that binds to and inhibits *eIF4A*, subsequently inhibiting translation[83]. Degradation of PDCD4 through β-TrCP was identified as a key factor in the branch of the mTOR (mammalian target of rapamycin) pathway that controls translation[84]. In this pathway, S6K1 was shown to phosphorylate PDCD4, marking the protein for β-TrCP-dependent destruction. As PDCD4 blocks translation, suppresses cell growth and promotes apoptosis, the loss of PDCD4 function is thought to contribute to cell transformation. In fact, decreased expression of PDCD4 is observed in advanced carcinomas of the breast and prostate[85–87]. Similarly, the protein levels of PDCD4 were observed to be downregulated in certain HCCs compared with normal liver[88]. Recently, the loss of PDCD4 was shown to be an independent risk factor in colorectal cancer and analysis of PDCD4 levels showed that its

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loss was associated with poor disease-specific survival in some patients and poor overall survival in all patients[89]. In the lung, loss of PDCD4 expression was widely observed in primary carcinomas of all subtypes, and this expression correlated with higher grade and disease stage, suggesting that the loss of PDCD4 expression is a prognostic factor in lung cancer[90]. Future studies will be needed to determine whether the levels of PDCD4 are affected in tumours overexpressing β-TrCP and/or displaying overactivation of the PI3K (phosphoinositide 3-kinase)–Akt–mTOR–S6K1 pathway.

A third β-TrCP substrate with oncogenic relevance is REST (repressor element 1 (RE1)-silencing transcription factor), which was originally discovered as a transcriptional repressor of a large number of neuronal differentiation genes in non-neuronal cells and neural stem/progenitor cells[91]. REST has a dual role as a tumour suppressor (in epithelial cells) and as an oncogenic protein (in neuronal cells; see below)[91]. Supporting a role for REST in tumour suppression, knockdown of *REST* using RNA interference has been shown to promote transformation of breast epithelial cells, and deletions encompassing the *REST* locus have been found in many aggressive human carcinomas[92]. Exogenous β-TrCP expression transforms human mammary epithelial cells, and reconstitution of REST function by the introduction of a non-degradable mutant of REST abrogates oncogenic transformation by β-TrCP[93]. The proposed tumour suppressor function of REST seems to lie in its ability to inhibit the PI3K pathway[92]. If REST function is lost or mutated in cancer cells, activation of PI3K is thought to contribute to cell transformation. Therefore, it will be important to investigate levels of REST and the PI3K pathway in cancers overexpressing β-TrCP.

Defective substrate targeting by β-TrCP in cancer

Although the oncogenic potential of β-TrCP is potentially related to the reduced stability of antiproliferative and pro-apoptotic substrates, defective substrate targeting by β-TrCP might also have a role in oncogenesis. Owing to the redundancy of β-TrCP paralogues, the complete loss of both genes by inactivating mutations and/or deletions is unlikely, and therefore, β-TrCP might have a greater role as an oncogenic protein than as a tumour suppressor. However, rare aberrations that could potentially impede the substrate targeting capabilities of β-TrCP have been found (TABLE 4). A screen for genetic alterations of *FBXW11* (which encodes β-TrCP2) in gastric cancer cell lines and primary gastric cancer tissues identified a nucleotide substitution in its seventh WD-repeat domain[94]. Complementing this study, an analysis of somatic mutations in 95 gastric cancer specimens found five missense mutations in *FBXW11*, and in these particular tissues, β-catenin levels were higher than controls[95]. Finally, two mutations in *BTRC* (encoding β-TrCP1) were found in prostate tissues, and an in-frame insertion in *FBXW11* has been identified in one breast tumour[96,97].

Mutations in β-TrCP might lead to the stabilization of oncogenic substrates such as β-catenin, which has been implicated in tumorigenesis[98]. Indeed, high levels of β-catenin in many cancers, such as colorectal cancers, hepatocellular carcinomas and malignant melanomas, are often due to its stabilization, but stabilization, rather than being due to β-TrCP alterations, is frequently due to mutations in the genes encoding APC (adenomatous polyposis coli) and axin, which are crucial to the phosphorylation of β-catenin and its consequent degradation via β-TrCP[76,99–104]. The stabilization of β-catenin leads to the accumulation of the protein, nuclear translocation and transcription of a number of pro-proliferative genes such as *MYC* and cyclin D1. Another way for β-catenin to potentially escape degradation is through mutations in the N-terminal phospho-degron. Similarly, some human breast cancers impede the phosphorylation of prolactin receptor — phosphorylation is required for its β-TrCP-dependent degradation, increasing the mitogenic effects of prolactin in mammary tissues[105].

Another substrate that evades proteolysis via β-TrCP is REST. As mentioned above, REST behaves as an oncoprotein in neuronal cells. REST is ubiquitylated and targeted for degradation

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by β-TrCP during G2 to allow the transcriptional derepression of MAD2 (mitotic arrest deficient 2)[106], an essential component of the spindle assembly checkpoint. Expression of a stable REST mutant inhibits MAD2 expression in G2 and results in a phenotype that is consistent with faulty activation of the spindle checkpoint. Importantly, an indistinguishable phenotype was observed by expressing REST-FS, an oncogenic frameshift mutant identified in colorectal cancer[92] that lacks the β-TrCP-binding domain. Moreover, another study suggests that β-TrCP-mediated degradation of REST is required for proper neural differentiation and that non-degradable REST mutants attenuate differentiation[93]. Together, these two studies indicate that levels of REST must be accurately controlled to avoid putting neuronal tissues at risk of cancer. Indeed, increased levels of REST due to overproduction[107–109] and/or C-terminal truncations (D. Guardavaccaro and M.P., unpublished observations), as observed in human medulloblastomas and neuroblastomas, would inhibit differentiation and generate chromosomal instability, two fundamental mechanisms that contribute to tumorigenesis. Interestingly, REST variants (which lack the β-TrCP-binding motif) are also observed in non-neuronal tumours, but the implication of these findings is not yet understood. It is possible that such truncated variants contribute to cell transformation by promoting aneuploidy and genetic instability in both neuronal and non-neuronal tissues.

Other substrates that are expected to provide an oncogenic gain-of-function when they accumulate in cells harbouring β-TrCP mutations are the Cdc25 dual-specificity phosphatases CDC25A and CDC25B[58,110], and EMI1. High levels of Cdc25 have been found in many different human cancers[111], including breast, ovarian and colorectal cancer, in which overexpression of CDC25A and CDC25B correlates with clinical outcome[112,113]. In addition, CDC25A and CDC25B are overexpressed in some non-Hodgkin lymphomas, and these high levels correlate with the aggressiveness of high-grade lymphomas[114–116]. Although oesophageal, gastric, lung, thyroid, and head and neck cancers also show overexpression of CDC25A and CDC25B, the relationships between β-TrCP expression or mutations and these substrates are unknown, although in some cell lines, CDC25A levels increase because of its stabilization[117]. The levels of EMI1 transcript and protein have also been found to be upregulated in several human tumour cell lines[118] and human malignant cancers[119,120]. Again, it is not known whether accumulation of EMI1 depends on defective targeting by β-TrCP (either by mutational inactivation of β-TrCP or mutation or truncation of EMI1).

SKP2 and β–TrCP as targets for therapy

The development of pharmaceutical compounds targeting specific SCF ubiquitin ligases is timely and is complemented by basic biochemical studies that have identified substrates for important cellular regulators such as SKP2 and β-TrCP. Many structural biology studies have provided crystallographic, three-dimensional maps of SCF components[3]. Together with established biochemical data, these resources are ideal for the discovery of small molecules that inhibit SCF ligases either allosterically or by blocking the interaction between F-box proteins and substrate(s).

Previous attempts at targeting components of the degradation machinery have been successful for laboratory and clinical use, as observed with the effectiveness of the proteasome inhibitor bortezomib (velcade) in multiple myeloma[121]. Proteasome inhibition by bortezomib is achieved by directly binding to the β-subunit of the proteasome, presumably resulting in increased protein stability of factors that promote apoptosis, with resultant increases in sensitivity to chemotherapeutic treatments and radiation therapy. One of the major molecules involved in mediating the effect of proteasome inhibition by bortezomib is NFκB, and as discussed previously, activation of NFκB is dependent on the degradation of IκB following ubiquitylation by β-TrCP. However, whereas drugs such as bortezomib act to stabilize large,

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nonspecific pools of proteins that are regulated by the proteasome, small molecules designed to prevent degradation of specific proteins by particular ubiquitin ligases, such as SKP2 and β-TrCP, are in development and could prove more efficacious and have fewer side effects. Despite moderate efforts in the industry to develop and utilize such inhibitors, the use of small molecule inhibitors to block the action of SKP2 and β-TrCP has not been reported.

Conclusions and perspectives

The control of regulatory proteins by the UPS is crucial for maintaining the integrity of basic cellular processes such as those governing cell growth, proliferation and survival. This precision is accomplished, in part, through the specific recognition of substrates by the F-box component of SCF ubiquitin ligases. Deregulation of the mechanisms that control protein stability, such as those discussed in this Review, have been shown to contribute to aberrant cellular growth and tumorigenesis. The involvement of the F-box proteins SKP2 and β-TrCP in promoting tumorigenesis is extensive and continues to be elucidated in ongoing studies. The development of small molecule inhibitors against F-box proteins, in particular SKP2 and β-TrCP, is fundamental for future cancer therapy approaches. Additionally, with the majority of the 69 human F-box proteins remaining orphans (that is, unmatched to their substrates), the contribution of SKP2 and β-TrCP in cancer might only be a small indication of a larger trend for this family of proteins. Indeed, this trend is observed for FBXW7, which is a prototypical tumour suppressor gene that is mutated in various human cancers[7]. Several substrates have been identified for FBXW7, including the oncogenic proteins MYC, cyclin E, NOTCH1, NOTCH4, AURKA (aurora kinase A) and JUN. Moreover, the recent identification of FBXL3 as a component of the circadian clock machinery that targets members of the cryptochrome family for degradation[122], together with the increasing links between nightshift work and the incidences of breast and endometrial cancers, suggests that this F-box protein might be involved in processes contributing to cancer[123]. Another example is FBXL10, a tumour suppressor protein that is resident in the nucleolus in human cells and represses the transcription of ribosomal RNA genes to control cell growth[124]. Finally, there are F-box-like proteins such as NIPA (nuclear interaction partner of ALK), which is involved in the oncogenic signalling of NPM (nucleophosmin)–ALK (anaplastic lymphoma kinase) and other oncogenic fusions of ALK [125]. Future studies identifying the substrates of orphan F-box proteins are poised to advance the field of protein degradation and cancer biology.

DATABASES

Enterz Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

APC | ATM | ATR | AURKA | axin | β-TrCP | BCL-ξL | CBP | CDC25A | CDC25B | CDK1 | CDK2 | CDK9 | Cdkn1b | CDKN1C | CDT1 | CHK1 | CHK2 | CKS1 | claspin | CUL1 | cyclin D1 | eIF4F | EMI1 | ErbB2 | FBXL10 | FBXW7 | FOXO1 | GMCSF | interleukin 6 | JUN | KPC | MAD2 | MMP9 | MYC | NIPA | NOTCH1 | NOTCH4 | NRAS | PDCD4 | PIRH2 | RASSF1 | Rb1 | RBL2 | REST | S6K1 | SKP1 | SKP2 | TNFα | TOB1 | USP18 | WEE1

National Cancer Institute: http://www.cancer.gov breast cancer | colorectal cancer | endometrial cancer | gastric cancer | head and neck cancer | lung cancer | lymphoma | melanoma | oesophageal cancer | pancreatic cancer | thyroid cancer

FURTHER INFORMATION

M. Pagano's homepage: http://pathology.med.nyu.edu/Pagano

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Glossary

Ubiquitin, A small, 7.5-kDa protein that is ubiquitously expressed in all eukaryotes. Chains of ubiquitin moieties (connected by Lys48) target proteins for proteasomal degradation. Monoubiquitylation or polyubiquitylation through different lysine residues controls the function (not the proteolysis) of various proteins.
Proteasome, A large multisubunit protein complex (approximately 2.5 MDa) that is found in all eukaryotes and archaea, the main function of which is to degrade excessive, unneeded or damaged proteins by proteolysis using a chemical reaction that breaks peptide bonds in an ATP-dependent manner.
Ubiquitin-activating enzyme (E1), An enzyme that activates ubiquitin in a process that requires ATP as an energy source.
Ubiquitin-conjugating enzyme (E2), An enzyme that accepts the transfer of ubiquitin from the ubiquitin-activating enzyme (E1) and transfers it to substrates.
Ubiquitin ligase (E3), An enzyme that functions as the substrate recognition component of the ubiquitylation machinery. E3 enzymes are capable of interacting with E2 enzymes and substrates to facilitate the transfer of ubiquitin to the selected substrate.
RING-finger proteins, Proteins that interact with E2 ubiquitin enzymes to serve as an E3 enzyme. They are subdivided structurally into multi-subunit and single-subunit types, including those containing RING-like folds such as the U-box.
HECT-domain proteins, Proteins that are characterized by the presence of a C-terminal HECT domain, which is a domain of approximately 350 amino acids that is catalytically involved in the attachment of ubiquitin to substrates.
F-box domain, Originally identified in cyclin F as a stretch of approximately 40 amino acids linking F-box proteins to sKP1 to form the core of the sCF complex.
Degron, specific sequence of amino acids in a protein substrate typically conserved through evolution that directs the recognition of an E3 ubiquitin ligase.
Paralogues, Homologous genes that have resulted from a gene duplication event within a single genome. This is in contrast to othologous genes, which are separated by a speciation event.
C phase, The mammalian cell cycle is divided into four distinct phases called G1, s, G2 and mitosis. C phase is defined as the temporal interval between the G1–s transition and the end of mitosis when CDK activity is present.
Organomegaly, The abnormal enlargement of organs.
Mitotic catastrophe, A death resulting from failure of a cell to arrest before mitosis following DNA damage, resulting in severe aberrancies in chromosomal structure and segregation. It might share downstream events with apoptosis.

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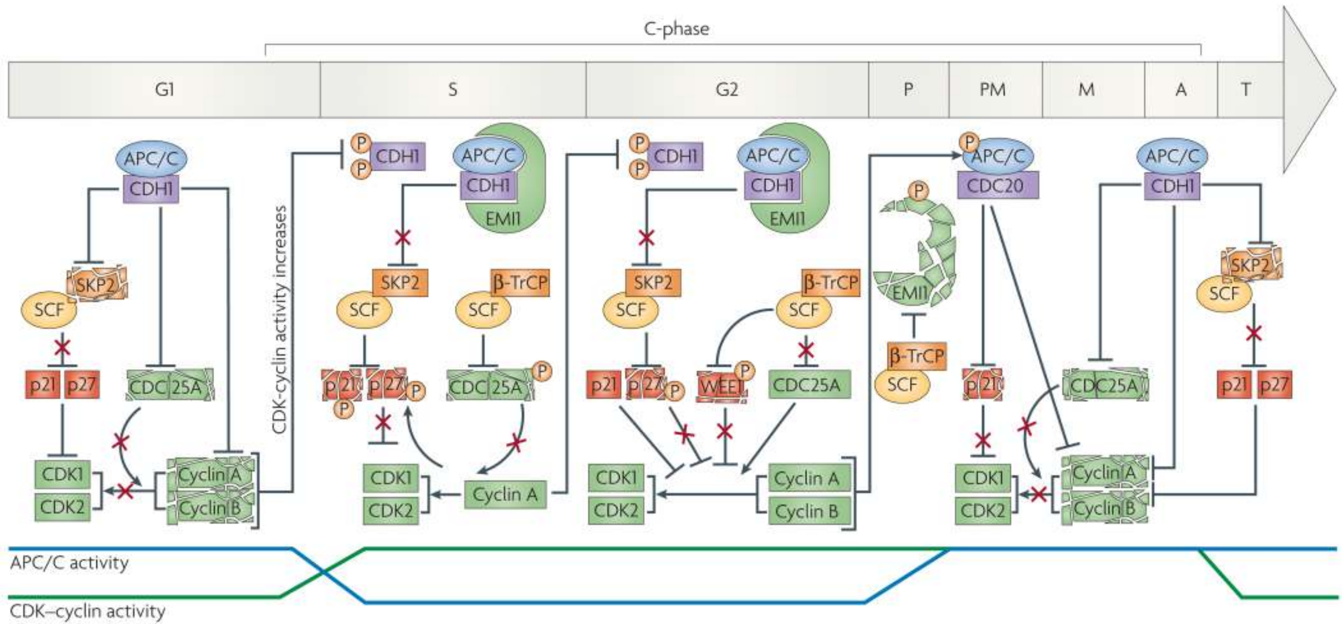

Figure 1. The UPS controls the cell cycle

The cell division cycle is regulated primarily by the activity of cyclin-dependent kinases (CDKs) and protein degradation by the ubiquitin–proteasome system (UPS). Each CDK complex contains one of many activating subunits, termed cyclins, the levels of which oscillate during the cell cycle. CKIs (CDK inhibitors), such as p27 and p21, inhibit CDK activity and promote cell cycle arrest and/or delay. SCF complexes and the APC/C (anaphase-promoting complex/cyclosome) provide the specific, rapid and timely proteolysis of cell cycle regulators, which ultimately controls CDK1 and CDK2 to finely modulate their activities during cell cycle progression. The best characterized cell cycle ubiquitin ligases are SCF^{SKP2}, SCF^{FBXW7} (not shown), $SCF^{\beta\text{-TrCP}}$, APC/C^{CDH1} and APC/C^{CDC20}. SCF^{SKP2} is a positive regulator of cell cycle progression (by promoting the degradation of p21 and p27), whereas $SCF^{\beta\text{-TrCP}}$ is both a positive and negative regulator of the cell cycle (by targeting CDC25A (cell division cycle 25A), claspin, WEE1 and EMI1 (also known as F-box protein 5)). APC/C^{CDH1} and APC/C^{CDC20} always attenuate CDK1 activity (by directing the degradation of cyclins A and B), except in early mitosis, when APC/C^{CDC20} targets p21 for degradation. Finally, SCF^{FBXW7} attenuates CDK1 and CDK2 by inducing the degradation of cyclin E. SCF complexes and the APC/C control each other, with SKP2 being ubiquitylated by APC/C^{CDH1} in G1 and $SCF^{\beta\text{-TrCP}}$ targeting EMI1, which is an inhibitor of APC/C^{CDH1}, for proteolysis in early mitosis. Additionally, SCF complexes and the APC/C share common substrates that are targeted by their respective ubiquitin ligase(s) only at particular times during the cell cycle. For example, SCF^{SKP2} targets p21 for degradation at G1–S, whereas APC/C^{CDC20} targets p21 during prometaphase. This scenario is also true for the targeted degradation of CDC25A by APC/C^{CDH1} in G1 phase, which is followed by $SCF^{\beta\text{-TrCP}}$-mediated degradation during S phase. Moreover, phosphorylation by CDKs modulates the activity of SCF complexes and the APC/C. CDK activity inhibits binding of CDH1 to the APC/C while promoting the activation of APC/C^{CDC20}, and phosphorylation of certain SCF substrates by CDKs allows recognition by the F-box protein subunit. β-TrCP, β-transducin repeat-containing protein; CDH1, also known as FZR1 (fizzy/cell division cycle 20 related 1); FBXW7, F-box protein with WD domain 7; SKP2, S-phase kinase-associated protein 2.

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Table 1

Reported substrates of SKP2

Reported substrates	Function	Upregulated in *Skp2*$^{-/-}$ MeFs	Ubiquitylation reconstituted *in vitro*	Refs
p27*	Cell cycle control	Yes	Yes	12, 13, 14
p21*	Cell cycle control	Yes	Yes	21, 22, 126
p57	Cell cycle control	Yes	Yes	23
Cyclin A	Cell cycle control	Yes	No	18
Cyclin E‡	Cell cycle control	Yes	Yes	18
Cyclin D1	Cell cycle control	Yes	No	22
CDT1	DNA replication	No	Yes	127
ORC1	DNA replication	ND	No	128
BRCA2	DNA repair	ND	No	129
RAG2	DNA repair	ND	Yes	130
TOB1	Gene transcription	Yes	Yes	24
RBL2*	Gene transcription	ND	Yes	26
FOXO1	Gene transcription	ND	Yes	27
MEF/ETS	Gene transcription	ND	Yes	131
MLL	Gene transcription	ND	Yes	132
MYB	Gene transcription	ND	No	133
MYC§	Gene transcription	ND	No	134–135
E2F1	Gene transcription	No	No	136
HPV-E7	Viral oncogenesis	Yes	Yes	137
USP18	Interferon signaling	Yes	No	28
MKP1	ERK signalling	ND	Yes	138
SMAD4	Signal transduction	ND	Yes	139
CDK9	Transcriptional elongation	Yes	No	29–30
E2A	B- and T-cell development	ND	No	140–141
TAL1	Erythroid differentiation	ND	No	142
RASSF1	Microtubule stabilizer	Yes	Yes	25

*Requires CKS1 (CDK subunit 1) for binding to SKP2.

‡Only free (CDK-unbound) cyclin E.

§Ubiquitylation seems to promote transcriptional activity. BRCA2, breast cancer associated 2; CDK9, cyclin-dependent kinase 9; CDT1, chromatin licensing and DNA replication factor 1; FOXO1, Forkhead box-containing, O subfamily 1; HPV-E7, human papillomavirus E7 protein; MEFs, mouse embryonic fibroblasts; MEF/ETS, myeloid ELF1-like factor; MKP1, mitogen-activated protein kinase (MAPK) phosphatase 1; MLL, myeloid/lymphoid leukaemia; ND, not determined; ORC1, origin recognition complex 1; RAG2, recombination activating gene 2; RASSF1, Ras association domain family 1; RBL2, retinoblastoma-like 2 (also known as p130); SKP2, S-phase kinase-associated 2; TAL1, T-cell acute lymphocytic leukaemia 1 (also known as SCL); TOB1, transducer of ERBB2; USP18, ubiquitin-specific peptidase 18 (also known as UBP43).

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Table 2

Cancers associated with SKP2 deregulation

Human cancer	Correlation with poor prognosis	Correlation with low p27 levels	Refs
Biliary tract cancer	Yes	No	143
Breast cancer[*]	Yes	Yes	32, 42, 144–145
Cervical cancer	Yes	No	146
Colon cancer[*]	Yes	Yes	39, 43, 147–148
Endometrial cancer	Yes	ND	149–150
Gastric cancer[*]	Yes	Yes	45, 151–152
Glioma/glioblastoma[‡]	Yes	Yes	153–154
Kaposi sarcoma	Yes	No	155
Lung cancer (NSLC) [*‡]	Yes	Yes	156–158
Lung cancer (SLC)[*]	Yes	Yes	157–159
Lymphoma and leukaemia	Yes	No	40
Multiple myeloma	Yes	Yes	160–161
Melanoma	Yes	Yes	162–165
Oral cancers	Yes	Yes	147, 166–169
Ovarian cancer	Yes	Yes	170–171
Prostate cancer	Yes	Yes	34, 172–173

[*] CKS1 (CDK subunit 1) also upregulated.

[‡] Gene amplification. ND, not determined; NSLC, non-small-cell lung cancer; SKP2, S-phase kinase-associated protein 2; SLC, small-cell lung cancer.

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Table 3

Reported substrates of β-TrCP reported substrates

Reported substrates	Function	Degron in humans	Refs
IκBα	Inhibitor of NFκB	DSGLDS	71–78
IκBβ	Inhibitor of NFκB	DSGLGS	72
IκBε	Inhibitor of NFκB	DGSIES	72
p100	NFκB signalling	DSAYGS	174–175
p105	NFκB signalling	DSGVETS	176–177
WEE1	CDK1 inhibitory kinase	DSAFQE/EEGFGS	64
CDC25A	Phosphatase, CDK1 activator	STDSG	58, 110
CDC25B	Phosphatase, CDK1 activator	DDGFVD/DSGFCLDS	110
β-catenin	Wnt signalling	DSGIHS	76, 99–104
PDCD4	Protein synthesis	DSGRGDS	84
Claspin	DNA replication and damage stress Transcriptional repressor	DSGQGS	62, 63
REST	Transcriptional repressor	DEGIHS/STDSG	93, 106
ATF4	Transcription factor	DSGICMS	178
PRL-R	Growth hormone signalling	DSGRGS	179
CD4 (HIV VPU)*	Viral replication	ND	180
IFNR	Cytokine signalling	DSGNYS	181
DLG	Cell contact and polarity	DSGLPS	182
EMI1‡	CDH1 inhibitor	DSGYSS	60
Snail	Animal patterning	DSGKGS	75
PER1	Circadian clock transcription	TSGCSS	183
PER2	Circadian clock transcription factor	SSGYGS	184–185
PC2§	Calcium signalling	ND	186
MCL1	Pro-apoptotic protein	DGSLPS	187
Pro-caspase 3	Pro-apoptotic protein	ND	188
p63	Epithelial differentiation and apoptosis	ND	189
GHR	Growth hormone signalling	ND	190
Bora	PLK1 activator	DSGYNT	191
STAT1	Transcription factor	ND	192

*CD4 has no DSG motif; VPU (viral protein U) targets CD4 to β-TrCP.

‡Upregulated in *β-Trcp*$^{-/-}$ mouse embryonic fibroblasts.

§PC2 has no DSG motif; tafazzin targets PC2 to β-TrCP. ATF4, activating transcription factor 4; β-TrCP, β-transducin repeat-containing protein; CDC25A, cell division cycle 25A; CDH1, also known as fizzy/cell division cycle 20 related 1 (FZR1); CDK1, cyclin-dependent kinase 1; DLG, discs large tumour suppressor; EMI1, also known as F-box protein 5; GHR, growth hormone receptor; IκB, inhibitor of NFκB; IFNR, interferon receptor; MCL1, myeloid cell leukaemia 1; ND, not determined; NFκB, nuclear factor κB; p100, NFκB2 protein precursor; p105, NFκB1 protein precursor; PC2, protein polycystin 2; PDCD4, programmed cell death 4; PER1, period homologue 1; PLK1, polo-like kinase 1; PRL-R, protein tyrosine phosphatase 4A3; REST, repressor element 1 (RE1)-silencing transcription factor; STAT1, signal transducer and activator of transcription 1.

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Table 4

Cancers associated with β-TrCP deregulation

Human cancer	Level of upregulation, observed alterations and correlations	Refs
Cancers displaying high β-TrCP levels		
Breast cancer	Upregulated mRNA levels in primary tumors; mRNA and protein levels in several cell lines	68
Colon cancer	Upregulated mRNA and protein levels; high NFκB levels; correlation with poor prognosis	65
Hepatoblastoma	Upregulated mRNA levels; activation of Wnt signalling	67
Pancreatic cancer	Overexpression in one cell culture line; correlation with NFκB activity and chemoresistance	66
Melanoma	Upregulated mRNA and protein levels; high NFκB levels; increased survival of cells	81, 82
Cancers displaying β-TrCP alterations		
Breast cancer	In-frame insertion in *FBXW11*	97
Gastric cancer	Mutation of *FBXW11* in one cell culture line and 5 mutations in the 95 gastric cancer tissues tested	95
Prostate cancer	2 mutations in *BTRC* found in the 22 tissues tested	96

β-TrCP, β- transducin repeat-containing protein; FBXW11, F-box and WD repeat domain containing 11 (which encodes β-TrCP2); NFκB, nuclear factor κB.