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Roles of F-box proteins in cancer

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Abstract

F-box proteins, which are the substrate-recognition subunits of SKP1–cullin 1–F-box protein (SCF) E3 ligase complexes, have pivotal roles in multiple cellular processes through ubiquitylation and subsequent degradation of target proteins. Dysregulation of F-box protein-mediated proteolysis leads to human malignancies. Notably, inhibitors that target F-box proteins have shown promising therapeutic potential, urging us to review the current understanding of how F-box proteins contribute to tumorigenesis. As the physiological functions for many of the 69 putative F-box proteins remain elusive, additional genetic and mechanistic studies will help to define the role of each F-box protein in tumorigenesis, thereby paving the road for the rational design of F-box protein-targeted anticancer therapies.

Ubiquitylation by the ubiquitin proteasome system (UPS) is a post-translational modification that governs diverse cellular processes such as cell proliferation, cell cycle progression, transcription and apoptosis. The UPS exerts its biological functions through a cascade of three enzymatic reactions, which are catalysed by the ubiquitinactivating E1 enzyme, the ubiquitin-conjugating E2 enzyme and the ubiquitin-protein E3 ligase. Crucially, E3 ligases determine the substrate specificity for ubiquitylation and subsequent degradation.

Among more than 600 putative E3 ubiquitin ligases that are coded in the human genome[1], the largest family is the cullin–RING E3 ligase (CRL) complex family, which contains eight members: namely, CRL1, CRL2, CRL3, CRL4A, CRL4B, CRL5, CRL7 and CRL9 (REFS 2,3). Generally, CRL E3s consist of a cullin scaffold protein, an adaptor protein, a substrate receptor protein and/or a RING protein that recruits the E2 enzyme. Within the eight CRLs, CRL1 is so far the best-characterized family member, which is also designated as the SKP1–cullin 1–F-box protein (SCF) E3 ligase complex[4,5].

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The SCF complex is composed of the invariant components S-phase kinase-associated protein 1 (SKP1), the E3 ligase RBX1 (also known as ROC1) and cullin 1, as well as variable F-box proteins that confer substrate selectivity by targeting a distinct subset of substrates for ubiquitylation[4,5]. Each F-box protein consists of at least two major functional domains: various carboxy-terminal domains that bind to specific substrates, and the F-box motif, which is a protein–protein interaction domain that was first identified in F-box only 1 (FBXO1; also known as cyclin F)[5] and that recruits F-box proteins into the SCF complex via direct binding with the adaptor protein SKP1 (REF. 6).

Besides SCF, another multi-component E3 ligase, APC/C (anaphase promoting complex/cyclosome), has also been well established as a crucial regulator of multiple cellular processes, including cell cycle progression, such as S phase entry and G2/M phase exit[4,6]. Specifically, the SCF complex primarily regulates entry into S phase by degrading G1 cyclin-dependent kinase inhibitors (CKIs) and G1 cyclins[4], and β-transducin repeat-containing protein 1 (β-TRCP1; also known as F-box/WD repeat-containing protein 1A (FBXW1A))-dependent degradation of WEE1 is required for the initiation of M phase[7]. APC/C governs timely cell cycle progression in both M and G1 phases[6]. Interestingly, although it is composed of approximately 14 subunits, APC/C shares structural similarity with SCF by containing a cullin-like scaffolding protein, APC2 (also known as ANAPC2), and a substrate recognition subunit, CDH1 (also known as FZR1) or CDC20, both of which are WD40 repeat-containing proteins that are analogous to F-box proteins in SCF[8,9].

The F-box protein families

F-box proteins can be organized into three subclasses according to the presence of specific substrate recognition domains. The FBXW subclass, which contains WD40 repeat domains, comprises ten proteins, including the well-studied β-TRCP1, FBXW7 (also known as FBW7 and CDC4) and β-TRCP2 (also known as FBXW11). There are 22 F-box and leucine-rich repeat protein (FBXL) family members, including SKP2 (also known as FBXL1), all of which contain leucine-rich repeat domains. The remaining 37 F-box proteins are designated as FBXO proteins that contain various domains that are not fully characterized. However, recent studies have begun to reveal some interesting biological functions that are attributed to otherwise uncharacterized functional domains in several FBXO proteins[10–13].

How do F-box proteins recognize their substrates? In most cases, they target specific degrons, which are short, defined motifs within their substrates. Moreover, proper post-translational modifications of the substrates are often required for their interaction with respective F-box proteins[14]. For example, FBXW7 substrates typically contain the conserved CDC4 phosphodegron (CPD) sequence (Leu)-X-pThr (or pSer)-Pro-Pro-X-pSer (or pThr, Glu or Asp) (X represents any amino acid)[15,16], and phosphorylation of this motif is required for FBXW7 to recognize and ubiquitylate its substrates. In addition to phosphorylation, F-box proteins can also recognize degrons that are modified by glycosylation or the addition of mannose oligosaccharides. For instance, FBXO6 binds to a glycosylated degron in T cell receptor α-chain[17], and FBXO2 can ubiquitylate proteins with *N*-linked high-mannose oligosaccharides, such as precursor β1 integrin[18]. Notably, recent studies showed that FBXO1 recognizes centrosomal protein of 110 kDa (CP110)[10] and

ribonucleoside-diphosphate reductase subunit M2 (RRM2)[19] with an unmodified degron (Arg-X-Leu) for degradation, whereas phosphorylation unexpectedly inhibits the degradation of FBXL2 (REF 20) and FBXO11 (REFS 21,22) substrates. These results indicate that other mechanisms, including restriction of degron access or regulation of F-box protein localization or stability, might also be involved in controlling F-box protein-mediated degradation[14]. Hence, further in-depth investigations are warranted to explore whether other degron-recognition mechanisms, such as lysine methylation[23], lysine acetylation or tyrosine phosphorylation[20], contribute to F-box protein-mediated ubiquitylation.

Emerging experimental and clinical data indicate that misregulated destruction of cell cycle regulators, many of which have either tumour suppressive or oncogenic functions, is tightly linked to cancer initiation and progression[4]. To this end, the crucial role of F-box proteins in tumorigenesis began to be appreciated owing to the pivotal and indispensable roles of these proteins in cell cycle regulation[24]. Over the past 17 years, research efforts have been devoted to identifying the substrates that are ubiquitylated by a given F-box protein and thereby reveal its physiological and cellular functions. In addition, there are ongoing studies that aim to establish mouse models to decipher the physiological role of F-box proteins in tumorigenesis, or that aim to link genetic alterations of F-box genes to pathological contributions of F-box proteins in human cancers. In this Review, we provide a comprehensive summary of the known and potential roles of the F-box proteins in tumorigenesis on the basis of the available data from three major categories: physiological evidence (mouse models), pathological evidence (human cancer relevance) and biochemical evidence (identified downstream ubiquitin substrates) (BOX 1; TABLE 1). On the basis of these three standards, we have grouped the 69 putative human F-box proteins into one of four categories: tumour suppressive, oncogenic, context-dependent or undetermined functions in cancer (FIG. 1).

Tumour-suppressive F-box proteins

Multiple F-box proteins function as tumour suppressors, or show emerging roles in suppressing tumorigenesis (TABLE 2).

FBXW7 is a tumour suppressor

FBXW7 is a well-established tumour suppressor that targets various oncogenic proteins for degradation[25,26]. *Fbxw7*$^{-/-}$ mice showed impaired vascular development that led to lethality at embryonic day 10.5, which might be caused by the accumulation of the FBXW7 substrates NOTCH1 and NOTCH4 (REFS 27,28). Notably, extensive screenings for *FBXW7* mutations in human cancers have shown that approximately 6% of all primary human cancers harbour *FBXW7* mutations[29]. The most frequent *FBXW7* mutations were identified in T cell acute lymphoblastic leukaemia (T-ALL; 30%) and cholangiocarcinomas (35%). The most common missense mutations of *FBXW7* occurred at R465, R479 and R505 in human cancers[25]. To further understand the physiological functions of FBXW7 in tumorigenesis, mouse models with tissue-specific ablation or knock-in of *Fbxw7* have been developed and analysed, especially in bone marrow-specific and intestine-specific models. In particular, mice with conditional ablation of *Fbxw7* in the T cell lineage developed

thymic lymphoma, partly owing to excessive accumulation of MYC[30]. Bone marrow-specific *Fbxw7*-knockout mice showed extremely severe pancytopenia by 12 weeks and developed T-ALL within 16 weeks. This was partly due to exhaustion of haematopoietic stem cells (HSCs), which resulted from accumulated MYC and NOTCH1 (REFS 31,32), echoing the observation of frequent *FBXW7* mutations or deletions in patients with T-ALL[33].

Consistently, HSCs with a heterozygous mutation of *Fbxw7* in the substrate recognition pocket ($Fbxw7^{R465C/+}$) kept normal HSC function, but FBXW7-R465C-mediated MYC accumulation in collaboration with Notch 1 mutation increased leukaemia-initiating potential[34]. Although mice with intestine-specific *Fbxw7* ablation did not develop intestinal tumours, mice with mutation of adenomatous polyposis coli ($Apc^{Min/+}$ mice) and intestine-specific *Fbxw7* ablation substantially increased intestinal tumorigenesis, in part via accumulation of NOTCH1 and JUN[35]. A more recent study showed that mice with the heterozygous missense mutation R482Q ($Fbxw7^{R482Q/+}$; the equivalent of R479Q in humans) in an *Apc*-mutant background ($Apc^{1322T/+}$) develop tumours faster than *Fbxw7*-heterozygous mice in the same $Apc^{1322T/+}$ background, in part because $Fbxw7^{R482Q/+}$ $Apc^{1322T/+}$ mice have elevated levels of Kruppel-like factor 5 (KLF5) and homeobox protein TGIF1 (REF. 36). As FBXW7 can form dimers through its amino-terminal dimerization domain[37,38], the stronger effects of $Fbxw7^{R482Q/+}$ on tumorigenesis are thought to stem from dominant-negative effects owing to heterodimer formation between wildtype and R482Q-mutant proteins, which dilutes the wild-type FBXW7 homodimers and their tumoursuppressor function. Furthermore, *Fbxw7* deficiency in the liver caused development of hamartomas, partly owing to the accumulation of sterol regulatory element-binding protein (SREBP; also known as SREBF1) and NOTCH1 (REF. 39). Notably, FBXW7 was further shown to function as a haploinsufficient tumour suppressor, as $Fbxw7^{+/-}$ mice were susceptible to radiation-induced tumorigenesis[40], and irradiated $Fbxw7^{+/-}$ $Tp53^{+/-}$ mice developed multiple tumours in the lung, liver and ovary (susceptibility: wild-type < $Fbxw7^{+/-}$ < $Tp53^{+/-}$ < $Fbxw7^{+/-}$ $Tp53^{+/-}$ << $Tp53^{-/-}$ < $Fbxw7^{+/-}$ $Tp53^{-/-}$)[40]. Taken together, the FBXW7 tumour suppressor might function in a haploinsufficient manner.

Besides T-ALL and cholangiocarcinomas, *FBXW7* is mutated in 9% of primary endometrial cancer, 9% of colorectal cancer and 6% of stomach cancer[29]. Human FBXW7 has three isoforms (FBXW7α, FBXW7β and FBXW7γ) as a result of alternative splicing. *FBXW7* promoter hypermethylation has been shown to contribute to the inactivation of FBXW7β in breast cancer and is correlated with poorly differentiated tumours[41]. However, it remains unclear why the rate of cancer development in mice that lacked FBXW7β was not increased[42]. Notably, lower expression of FBXW7 contributed to more malignant phenotypes, such as lymph node metastasis, larger tumour size and poor prognosis in gastric cancer[43]. Similarly, FBXW7 expression was inversely associated with advanced stage and recurrence in prostate cancer[44]. Taken together, these pathological data further confirm the notion that FBXW7 mainly functions as a tumour suppressor in various types of human cancers.

Further biochemical evidence suggests that FBXW7 exerts its tumour-suppressor role by promoting the degradation of various oncoproteins[26]. The growing list of FBXW7-specific

substrates, many of which have oncogenic functions (see Supplementary information S1 (table)), includes cyclin E, MYC, JUN, NOTCH, myeloid cell leukaemia 1 (MCL1), SREBP, mTOR, KLFs, CCAAT/enhancer-binding proteins (C/EBPs) and the mediator complex components MED13 and MED13L.

The emerging tumour-suppressor roles of other F-box proteins

It has been recently reported that FBXO11 targets the BCL-6 oncoprotein for ubiquitylation and subsequent degradation. Biologically, the overexpression of FBXO11 inhibited cell growth and induced cell death through promoting BCL-6 degradation in diffuse large B cell lymphoma (DLBCL) cells[11]. *In vivo* xenograft studies further indicated that FBXO11 contributes to lymphomagenesis by governing BCL-6 stabilization. Specifically, deletion or mutation of *FBXO11* was observed in patients with primary DLBCL[11], and it was significantly associated with the poor survival of patients with pancreatic cancer[45]. Additionally, it has been shown that FBXO11 interacts with CDT2 (also known as DTL), which forms part of the CRL4 complex, and promotes its proteasomal degradation. This leads to regulation of the timing of cell cycle exit[22], in part through the stabilization of p21 (encoded by *CDKN1A*) and the N-lysine methyltransferase SETD8, and also leads to increased cell migration[21]. Genetically, homozygous *Fbxo11* mutation was shown to cause severe middle ear infections, which indicates its crucial role in regulating inflammation[46]. However, further mouse modelling studies would help to elucidate the *in vivo* function of FBXO11 in tumorigenesis.

FBXW8 was found to assemble with cullin 7 to form a functional E3 ligase complex[47]. Notably, more than 60% of *Fbxw8*$^{-/-}$ embryos die *in utero* owing to retardation of intrauterine growth and abnormal placenta development[47,48], and this has restricted further studies to appreciate its physiological role in tumorigenesis. However, mTOR complex 2 (mTORC2) was recently found to phosphorylate and subsequently stabilize FBXW8, leading to accelerated turnover of insulin receptor substrate 1 (IRS1)[49]. Moreover, it has been reported that FBXW8 negatively regulated cancer cell proliferation through proteolysis of cyclin D1 (REFS 50,51) (see Supplementary information S2 (table)). Nonetheless, tissue-specific knockout mouse models and identification of additional FBXW8 substrates are necessary to determine whether FBXW8 has a crucial role in suppressing tumorigenesis.

In 2007, three groups independently identified FBXL3 as a regulator of the circadian clock by degrading cryptochrome (CRY) proteins through different genetic and biochemical approaches[52–54]. Indeed, *Fbxl3*$^{-/-}$ mice showed a longer circadian period[55]. Interestingly, prolonged circadian rhythm in *Fbxl3*$^{-/-}$ mice could be partially reversed by simultaneously knocking out *Fbxl21* (REF. 55), because FBXL21 could protect CRY from FBXL3-mediated degradation in the nucleus and promote CRY degradation within the cytoplasm[55]. The crystal structure of the CRY2 and FBXL3–SKP1 complex was recently solved, and it showed a novel F-box protein–substrate bipartite interaction mechanism[56]. As mice with mutations in CRYs are arrhythmic (they lack a circadian rhythm) with a faster rate of growth of implanted tumours[57], and as disrupted circadian rhythms might lead to aberrant cell division and subsequent malignant cell growth[58], FBXL3 might be involved in tumorigenesis through promoting CRY degradation. Despite biochemical evidence that

pinpoints its crucial role in the mammalian circadian clock, the physiological and the pathological roles of FBXL3 in human cancer development remain to be further explored.

FBXO1 contains a cyclin-box domain and hence is also known as cyclin F, although little is known about its role in cell cycle regulation. Mouse modelling studies have shown that *Fbxo1* heterozygous mice are normal and fertile, whereas *Fbxo1*$^{-/-}$ mice die around embryonic day 10.5 owing to failures in yolk sac and chorioallantoic placentation[59]. However, the depletion of *Fbxo1* in mouse embryonic fibroblasts (MEFs) caused cell cycle defects by an unclear mechanism[59]. Notably, FBXO1 is downregulated in hepatocellular carcinoma, and low expression levels of FBXO1 are associated with larger tumour size, advanced clinical stage, tumour multiplicity and poorer survival, which suggests that FBXO1 could be a promising prognostic marker for hepatocellular carcinoma[60].

Mechanistically, FBXO1 has been reported to govern centrosome duplications by mediating the degradation of CP110, which is essential for centrosome duplication[10], as well as to participate in genome integrity and DNA repair control by degrading RRM2, which catalyses the conversion of ribonucleotides to deoxyribonucleotides for both replicative and repair DNA synthesis[19] (see Supplementary information S3 (table)). These findings revealed an emerging tumour-suppressive role for FBXO1, although further genetic mouse models and biochemical studies are required to clearly define its physiological contribution to tumorigenesis.

Fbxo4$^{-/-}$ mice were developed to evaluate the role of FBXO4 in tumorigenesis. However, different phenotypes were observed by two independent groups. Vaites *et al*.[61] reported that *Fbxo4*$^{-/-}$ mice developed lymphomas, as well as histiocytic, mammary and hepatocellular carcinomas, whereas Kanie *et al*.[62] observed no abnormality in *Fbxo4*$^{-/-}$ mice. These discrepancies may have stemmed from the different mouse genetic backgrounds used in each study, which warrants further in-depth evaluation. Nonetheless, under pathological conditions, FBXO4 levels are reduced in tumour cell lines and in a subset of primary human cancers with a correspondingly increased accumulation of cyclin D1, which is a forkhead box protein O4 (FOXO4) substrate[63]. A further study showed that cancer-associated mutations in *FBXO4* inhibited the dimerization-mediated activation of SCF complexes containing FBXO4, which led to the accumulation of nuclear cyclin D1 and oncogenic transformation[64]. To this end, αβ-crystallin was identified as a necessary chaperone for the recognition of cyclin D1 by FBXO4 (REF. 63), whereas impairment of the FBXO4–αβ-crystallin interaction caused an accumulation of cyclin D1, which led to faster cell cycle progression[63] (see Supplementary information S4 (table)). Overall, it seems that the most likely antitumorigenic function of FBXO4 is to degrade cyclin D1, although further studies are required to solve the discrepancies in the existing mouse models.

FBXO10 binds to the anti-apoptotic BCL-2 oncoprotein to promote its degradation, which indicates an antitumour activity for FBXO10 (REF. 65). In support of this notion, there is pathological evidence indicating that low expression levels of FBXO10 were tightly associated with breast cancer risk[66–68]. Similarly, *FBXO18* is often deleted or mutated in melanoma cells, and the depletion of FBXO18 enhances ultraviolet (UV)-mediated transformation of human melanocytes, which suggests a tumoursuppressive role of FBXO18

(REF. 69). Multiple studies have shown that FBXO31 is downregulated in human breast cancer and hepatocellular carcinoma cell lines[70,71]. Conversely, the overexpression of FBXO31 suppressed cell proliferation and colony formation, partly through the degradation of cyclin D1 (REFS 70,71). However, although cyclin D1 was identified as a novel FBXO31 substrate[13] (see Supplementary information S3 (table)), depletion of FBXO31 in MEFs did not lead to the accumulation of cyclin D1 (REF. 62). Nevertheless, DNA-damaging agents and γ-irradiation caused increased FBXO31 levels through ataxia telangiectasia mutated (ATM)-mediated phosphorylation of FBXO31, which suggests that FBXO31 has a role in DNA damage-induced growth arrest[13]. Establishing additional mouse models and identifying additional FBXO31 substrates will be required to further characterize the role of FBXO31 in cancer.

Oncogenic F-box proteins

In contrast to the tumour-suppressive F-box proteins described above, mounting evidence shows that many other F-box proteins have oncogenic functions.

SKP2 is an oncoprotein

The F-box protein SKP2 was first identified as a key cell cycle regulator because it mediates ubiquitylation and subsequent degradation of various cell cycle regulators, such as p27 (encoded by *CDKN1B*) and p21 (REFS 72–75) (see Supplementary information S4 (table)). A growing body of literature strongly implies that SKP2 has oncogenic roles in human cancers[4] (TABLE 3). The oncogenic potential of SKP2 was further shown in transgenic mice, as overexpression of *Skp2* in the mouse prostate gland induced hyperplasia, dysplasia and lowgrade carcinoma[76]. Moreover, transgenic mice expressing *Skp2* in the T-lymphoid lineage and co-expressing *Nras* developed T cell lymphomas with shorter latency and showed decreased survival[77]. Transgenic mice that expressed the SKP2 splice variant SKP2B under the control of the `mouse mammary tumour virus promoter` (MMTV promoter) develop mammary tumours, partly through the downregulation of REA (also known as prohibitin 2), which is an inhibitor of the oestrogen receptor[78]. Consistent with a crucial oncogenic role for SKP2, *Skp2*-knockout mice are resistant to the development of sarcomas and lymphomas in an $Arf^{-/-}$ background, and are resistant to adrenal and prostate cancers when the tumour suppressor gene *Pten* is lost[79]. *Skp2* deficiency also delayed breast cancer development and prolonged the survival of *MMTV-Neu* mice[80]. Deleting retinoblastoma 1 (*Rb1*) using Pro-opiomelanocortin (*Pomc*)-Cre was sufficient to induce melanotroph tumorigenesis in the pituitary gland[81]. Notably, SKP2 deficiency abolished pituitary tumour formation in *Pomc*-Cre $Rb1^{lox/lox}$ $Trp53^{lox/lox}$ mice but not in *POMC*-Cre $Rb1^{lox/lox}$ $Cdkn1b^{-/-}$ mice[81]. Furthermore, the inactivation of *Skp2* completely prevents spontaneous pituitary tumorigenesis in $Rb1^{+/-}$ mice[82], and *Skp2* deficiency inhibits skin tumorigenesis induced in the `DMBA–TPA model`[83]. Taken together, these findings in mice provide evidence to support a role for SKP2 as a proto-oncogene.

In further support of an oncogenic role, SKP2 has been reported to be overexpressed in various types of human tumour samples (TABLE 3), including lymphoma[84], prostate cancer[85], colorectal cancer[86], melanoma[87], nasopharyngeal carcinoma[88], pancreatic cancer[89] and breast carcinoma[90,91]. Moreover, SKP2 might function as a prognostic marker

for various human cancers. For instance, upregulation of SKP2 was associated with increased histological grade and tumour size in human hepatocellular carcinoma[92]. Consistently, patients with a higher level of SKP2 expression have poorer prognosis in breast cancer[80], gastric cancer[93] and melanoma[87], which suggests the potential for the use of SKP2 as a biomarker for poor prognosis in various types of human cancers.

Recent studies support the notion that SKP2 exerts its oncogenic functions through degradation of its substrates[4], most of which are bona fide tumour suppressors. Well-characterized substrates of SKP2 include p21, p27, p57 (encoded by *CDKN1C*), retinoblastoma-like protein 2 (RBL2; also known as p130), FOXO1 and others (see Supplementary information S4 (table)), which suggests that SKP2 has a crucial role in governing many key cellular processes. For example, p21, p27 and p57 suppress cell cycle progression, RBL2 prevents cell division and FOXO1 is a transcription factor that positively regulates apoptosis. SKP2 deletion was recently shown to retard tumorigenesis through p27 accumulation when RB1 and p53 are inactivated[81]. However, further biochemical studies to identify additional SKP2 substrates and the generation of additional compound mouse models are required to pinpoint the major downstream signalling pathways by which aberrant SKP2 activation promotes tumorigenesis in different tissue settings or under different pathological conditions.

The emerging oncogenic roles of other F-box proteins

FBXO5 (also known as EMI1 and FBX5) functions as an endogenous inhibitor of APC/C. *Fbxo5*$^{-/-}$ mice are embryonic lethal owing to defects in pre-implantation development[94] (TABLE 3). Pathologically, FBXO5 has been suggested to have a possible oncogenic role in human cancers. For instance, the overexpression of FBXO5 leads to increased proliferation, tetraploidy and genomic instability of p53-deficient cells, which suggests that p53 loss might function together with FBXO5 to promote tumorigenesis[95]. In further support of its oncogenic role, FBXO5 was shown to be strongly expressed in malignant tumours compared with benign tumours[96]. Consistent with this notion, significant overexpression of FBXO5 was detected in ovarian tumour tissues[97]. Overexpression of FBXO5 was associated with a high histological grade and worse survival in ovarian cancer[98], and it was positively correlated with stage and poor outcome in hepatocellular carcinoma[99]. Biochemically, FBXO5 has been shown to govern cell cycle progression to S phase and mitosis via stabilizing APC/C ubiquitin substrates that have oncogenic activity, such as cyclin A, cyclin B or securin, by binding to APC/C co-activator proteins to inhibit APC/C activation[100]. In keeping with a possible oncogenic role for FBXO5, the BCR–ABL fusion oncoprotein increases FBXO5 stability and thereby blocks SKP2 degradation via APC/C-induced ubiquitylation, leading to enhanced cell proliferation in chronic myeloid leukaemia cells[101]. In summary, targeting the FBXO5 oncoprotein might be a novel anticancer therapeutic approach, but its oncogenic role warrants further studies that use tissue-specific transgenic mouse modelling.

Furthermore, the potential oncogenic role of FBXO9 was supported by recent findings that FBXO9 promotes survival in multiple myeloma cell lines through degrading telomere maintenance 2 (TELO2) and TELO2-interacting protein 1 (TTI1) — integral components of

mTORC1 and mTORC2, which control the cellular abundance of mammalian PI3K-related kinases (PIKKs)[102] (see Supplementary information S3 (table)). Specifically, FBXO9 only targets mTORC1-associated TELO2 and TTI1 for degradation, thereby suppressing mTORC1 to restrain cell growth but meanwhile releasing the negative feedback regulation on the PI3K–mTORC2–AKT pathway to sustain survival[102]. Although *FBXO9* mutation has been identified in patients with juvenile myoclonic epilepsy[103], further mouse modelling studies are required to assess an oncogenic role for FBXO9.

Context-dependent functions

β-TRCP1 and β-TRCP2

It is noteworthy that some F-box proteins, such as β-TRCP1 and β-TRCP2, have context-dependent roles in governing tumorigenesis. β-TRCP1 and β-TRCP2 exert their oncogenic or tumour-suppressive functions depending on the specific tumour type or cellular context. Notably, β-TRCP1 and β-TRCP2 are reported to have indistinguishable biochemical functions in recognizing and degrading their substrates[104]. The growing list of identified β-TRCP1 and β-TRCP2 ubiquitin substrates includes β-catenin, CDC25A, FBXO5, vascular endothelial growth factor receptor 2 (VEGFR2), inhibitor of nuclear factor-κB (IκB), programmed cell death protein 4 (PDCD4) and DEP domain-containing mTOR-interacting protein (DEPTOR) (see Supplementary information S5 (table)). Although some β-TRCP1 and β-TRCP2 substrates show oncogenic properties, many have possible tumour-suppressive functions.

Btrcp1$^{-/-}$ mice did not have an increased cancer incidence[105,106] (TABLE 4). Notably, mammary glands of *Btrcp1*$^{-/-}$ female mice showed a hypoplastic phenotype. However, 38% of transgenic mice that expressed β-TRCP1 that was targeted to the mammary gland and other epithelial tissues under the control of the MMTV promoter developed tumours such as mammary, ovarian and uterine carcinomas, which indicates that β-TRCP1 could facilitate epithelial tumorigenesis *in vivo*[107]. Moreover, transgenic mice with inducible, selective expression of dominant-negative β-TRCP2 in the epidermis had decreased UVB-induced oedema, hyperplasia and inflammatory response in the skin, which suggests a possible oncogenic role for β-TRCP2 in the skin cancer setting[108].

In addition, analysis of clinical samples suggests that β-TRCP1 could be an oncoprotein in certain tissues. For example, 56% of colorectal cancer tissues showed upregulation of β-TRCP1 at both the mRNA and protein levels that correlated with poor clinical prognosis[109]. Moreover, β-TRCP1 is highly expressed in pancreatic cancer[110] and hepatoblastoma biopsy samples[111]. Similarly, elevated levels of β-TRCP2 have also been observed in various human tumour tissue samples, such as those of prostate, breast and gastric cancers[104]. These findings suggest that the overexpression of β-TRCP1 or β-TRCP2 could be a common trend in human cancers. However, somatic mutations in *BTRCP1* and *BTRCP2* that prevent E3 ligase activity have also been identified in human gastric cancer; this correlated with stabilization of β-catenin in these tissues and could explain the development of these tumours[112,113]. This highlights the tumour-suppressive role of β-TRCP1 and β-TRCP2, at least in the gastric cancer setting. Collectively, β-TRCP1 and β-TRCP2 might exert either an oncogenic or a tumour-suppressive activity in a tissue-specific or cellular context-dependent

manner; therefore, further in-depth investigations are required to explore the exact mechanisms by which these proteins exert their biological functions in cancer.

FBXL10

FBXL10 (also known as KDM2B) is an F-box protein that contains a Jumonji C domain that carries out demethylase activity (TABLE 1). Consistent with its molecular structure, FBXL10 has been shown to have dual functions: histone H3 lysine 36 demethylation and histone H2A lysine 119 ubiquitylation[114], both of which might be crucial to negatively regulate embryonic stem cell pluripotency. A recent study has also shown direct evidence for dysregulation of FBXL10 in cancer. FBXL10 overexpression was observed in human pancreatic cancer tissue samples, and higher expression levels of FBXL10 correlated with disease grade and stage, as well as with metastasis[115]. More importantly, downregulation of FBXL10 abrogated the tumorigenicity of pancreatic cancer cell lines, whereas overexpression of FBXL10 cooperated with the *KRAS*^{G12D} mutation to induce pancreatic tumorigenesis in mice[115]. In line with this notion, depletion of FBXL10 caused upregulation of ARF in MEFs, which suggests that FBXL10 might suppress cell death by inhibiting the ARF tumour-suppressor pathway[116]. In addition, overexpression of FBXL10 promoted cell proliferation and inhibited cellular senescence, in part by suppressing the expression of the CDK4 inhibitor p15 (also known as INK4B)[117]. However, contrary to the oncogenic properties of FBXL10, tumour suppression by FBXL10 has been reported, in which retroviral insertion-mediated suppression of FBXL10 expression led to the development of an aggressive brain tumour in mice[118]. Hence, further studies are required to solidify the possible context-dependent role of FBXL10 in tumorigenesis.

Undetermined but probable functions in cancer

Unfortunately, owing to a lack of compelling physiological, pathological and biochemical evidence, the potential roles of many F-box proteins in cancer remain mostly undetermined (TABLE 4). For the F-box proteins discussed in this section, either initial mouse models have been attempted but have no cancerous phenotypes, or there is a lack of supporting mouse-modelling studies. Thus, additional whole-body, tissue-specific or compound mouse-modelling studies that are coupled with additional pathological and biochemical evidence are required to decipher the potential roles of these F-box proteins in cancer.

The FBXW family

FBXW4 was found to be mutated in the dactylaplasia mouse, the phenotype of which resembles the human autosomal dominant `split hand/foot malformation disease` (SHFM disease); however, no cancer incidence was observed. Further pathological studies showed that FBXW4 is mutated, lost or underexpressed in multiple human cancer cell lines and clinical tumour samples[119]. Moreover, reduced FBXW4 expression correlates with decreased survival in patients with non-small-cell lung cancer[119]. Although inactivation of FBXW4 has been reported in human cancers[119], further studies using knockout or knock-in mouse models, as well as identification of the ubiquitin substrates of FBXW4, are required to assess its role in cancer.

FBXW5 was originally identified as a negative regulator of TGFβ-activated kinase 1 (TAK1; also known as MAP3K7) in the interleukin-1β (IL-1β) signalling pathway[120], which suggested its tumour-suppressive potential; this was supported by the ability of FBXW5 to promote the degradation of the MYB oncoprotein[121]. However, FBXW5 was recently reported to control the stability of deleted in liver cancer 1 (DLC1; also known as RHO GTPase-activating protein 7)[122] and tuberous sclerosis 2 (TSC2)[123] tumour suppressors (see Supplementary information S2 (table)), which suggests a possible oncogenic role for FBXW5, but this requires knockout or knock-in mouse models for further validation.

FBXW15-selective expression has been reported in mouse oocytes[124] and recently implicated as a possible tumour suppressor by promoting the degradation of histone acetyltransferase binding to ORC1 (HBO1; also known as KAT7) to inhibit replication licensing and cell proliferation[125] (see Supplementary information S2 (table)). However, establishing engineered mouse models will facilitate further characterization of a potential role for FBXW15 in cancer.

The FBXL family

FBXL2 was recently authenticated as an ubiquitin E3 ligase component that exerts antitumour activity. Specifically, FBXL2 targets cyclin D2 for degradation in B lymphocytes and leukaemic cells, which leads to G0 arrest and apoptosis[126] (see Supplementary information S6 (table)). Similarly, FBXL2 could suppress lung cancer cell proliferation through the degradation of cyclin D3 (REF. 127). FBXL2 was also reported to inhibit tumorigenesis by promoting the degradation of Aurora B[128], whereas the FBXL2-mediated degradation of p85β (a PI3K regulatory subunit) suggests a possible oncogenic role[20].

FBXL5 was found to regulate the turnover of p150, which is an activator of microtubule motor cytoplasmic dynein that is crucial for vesicular transport and mitotic spindle organization[129]. However, its role in tumorigenesis remains to be defined. *Fbxl5*$^{-/-}$ mice die during embryogenesis as a result of excessive iron accumulation[130,131] owing to the loss of iron regulatory protein 2 (IRP2; also known as IREBP2) degradation[130]. This restricts further use of these mice to understand the role of FBXL5 in tumorigenesis and warrants the development of tissue-specific knockout mice.

Notably, FBXL20 might have a possible oncogenic function, as overexpression was recently observed in human colorectal adenocarcinoma[132]. Interestingly, FBXL20 was validated as a direct target of oncogenic miR-3151 in cytogenetically normal acute myeloid leukaemia, which suggests a possible tumoursuppressor function in leukaemia[133]. These findings indicate that, analogous to β-TRCP1 and β-TRCP2, FBXL20 might also govern tumorigenesis in a context-dependent manner.

Although FBXL21 has been shown to target CRY proteins for degradation and *Fbxl21*$^{-/-}$ mice showed compromised daily activity[134], its connection with human cancer remains mostly undefined and warrants further in-depth investigation.

The FBXO family

Fbxo7$^{-/-}$ mice show increased pro-B cell and pro-erythroblast populations[135], but no information has been obtained regarding the role of FBXO7 in tumorigenesis. Biochemically, FBXO7 catalyses the ubiquitylation of hepatoma upregulated protein (HURP; also known as DAP5)[136]; *HURP* is a putative oncogene, as its overexpression has been observed in human hepatocellular carcinoma, colon cancer, breast cancer and transitional cell carcinoma[136]. FBXO7 also catalyses the ubiquitylation of inhibitor of apoptosis protein 2 (IAP2; also known as BIRC2)[137], which is another putative oncoprotein that inhibits apoptosis and facilitates cell cycle transition. However, FBXO7 directly binds to the CDK6–cyclin D complex, and depletion of FBXO7 led to a reduced association of cyclin D with CDK6 (REF. 138). Moreover, overexpression of FBXO7 transformed immortalized fibroblasts in a CDK6-dependent manner[138]. In further support of the oncogenic potential of FBXO7, the expression of FBXO7 in haematopoietic progenitor cells cooperates with p53 loss to promote T cell lymphomagenesis[139]. Consistent with this finding, a high expression of FBXO7 was observed in epithelial tumours but not in normal tissues[138]. As opposite roles of FBXO7 in tumorigenesis have been suggested by different biochemical analyses, the generation of tissue-specific or compound knockout or knock-in mouse models for *Fbxo7*, as well as the identification of additional ubiquitin substrates of FBXO7, is required to determine the role of FBXO7 in human cancer.

Pluripotent stem cells can be induced from mouse fibroblasts through the overexpression of FBXO15 (REF. 140), and this suggests a potential oncogenic role for FBXO15, given the connection between cellular transformation and differentiation[141]. However, *Fbxo15*$^{-/-}$ mice are viable, with no noticeable tumourassociated phenotypes[142], which suggests that further transgenic mouse models should be generated to assess the possible oncogenic role of FBXO15.

FBXO28 might have an oncogenic role by stimulating transcription through non-proteolytic ubiquitylation of MYC[143]. Consistent with a putative oncogenic role, high expression levels of FBXO28 correlate with poor outcome in breast cancer[143]. Moreover, FBXO32 was identified as a muscle-specific F-box protein[144], and studies of *Fbxo32*-overexpressing and *Fbxo32*$^{-/-}$ mice showed that FBXO32 has a crucial function in muscle homeostasis[145]. FBXO32 has been reported as an apoptosis regulator and a tumour suppressor[146]. In support of this notion, *FBXO32* promoter methylation correlated with poor prognosis in human ovarian cancer, and reintroducing FBXO32 markedly inhibited cell proliferation[147]. However, further studies are warranted to define the role of FBXO32 in tumorigenesis.

Overexpression of FBXO39 was observed in various cancer cell lines, such as breast, colon, hepatoma, renal, thyroid anaplastic, ovary, sarcoma and lung[148]. Moreover, FBXO39 was characterized as a new `cancer testis antigen` from patients with colon cancer, which implies that FBXO39 could be useful for diagnosis and immunotherapy, but its oncogenic role remains undefined[148]. Interestingly, FBXO44 was recently shown to target BRCA1 for ubiquitylation and destruction; and consistent with a possible oncogenic role, overexpression of FBXO44 was observed in sporadic breast cancer with low BRCA1 levels[149]. Given that the loss of BRCA1 activity facilitates breast cancer development, it is imperative to

determine whether induced expression of FBXO44 in mouse mammary glands is sufficient to drive breast tumorigenesis. FBXO45 was reported to promote the degradation of p73, which is a member of the p53 tumour-suppressor family[150]. Depletion of FBXO45 stabilized p73 and induced cell death, which suggests that FBXO45 could have an oncogenic role[150]. However, *Fbxo45*$^{-/-}$ mice are embryonic lethal[151]. Thus, further tissue-specific knockout or knock-in mouse models, as well as identification of additional FBXO45 substrates, are required to define the physiological role of this protein in cancer.

F-box proteins as therapeutic targets

Given that a substantial number of F-box proteins have tumour-suppressive or oncogenic roles, F-box proteins could function as therapeutic targets for the prevention and/or treatment of human cancers (FIG. 2). For example, oncogenic SKP2 is a promising target, and using highthroughput screening, several small-molecule inhibitors have been developed, including Compound A and Compound #25 (also known as SZL-P1-41), to block SKP2 E3 ligase activity[152,153]. Compound A induces apoptosis and cell cycle arrest in multiple myeloma cells[153]. Compound #25 selectively suppressed SKP2 E3 ligase activity but not the activity of other SCF complexes, and it inhibited AKT-mediated glycolysis and activated cellular senescence[152]. Recently, an *in silico* screen also identified small-molecule inhibitors, called ‘skpins’, that block SKP2-mediated p27 degradation, thereby leading to p27 accumulation and subsequently causing cell cycle arrest at G1 or G2/M in cancer cells[154]. With a similar mechanism, SKP2 E3 ligase inhibitors (SKP2E3LIs), which are another group of inhibitors that block the interaction of SKP2 with p27, efficiently hindered the oestrogen-induced stimulation of cell growth and the degradation of nuclear p27 (REF. 155).

In addition, two recent studies showed that loss of FBXW7 in cancer cells promotes resistance to taxol and ABT-737 (REFS 156,157). Thus, upregulation of FBXW7 could reverse drug resistance, and the development of inhibitors for upstream regulatory proteins to induce FBXW7 activity might also be a viable therapeutic approach. To this end, peptidyl-prolyl *cis-trans* isomerase NIMA-interacting 1 (PIN1) negatively regulated the stability and tumour-suppressor function of FBXW7, which suggests that enzymatic inhibition of PIN1 that leads to elevated FBXW7 activity could be a promising approach for anticancer therapy[158]. As many F-box proteins have context-dependent functions in different cancer types, any anticancer treatments might require the use of tissue-specific targeting of the responsible F-box protein.

Conclusions and perspectives

In conclusion, our current knowledge demonstrates that F-box proteins could have essential roles in tumorigenesis, mainly through the regulation of substrate turnover. Interestingly, it has been shown that some F-box proteins could also exert their cellular functions via non-proteolytic regulation, which can occur in an E3 ligase activity-dependent or activity-independent manner. For example, SKP2 exerts its oncogenic function in part through the K63-linked ubiquitylation and activation of AKT[80] and NBS1 (also known as nibrin)[159]. There are also examples of F-box proteins having E3 activity-independent functions, such as

FBXO5 functioning as an endogenous and SCF-independent suppressor of APC/C[160] (more of these examples are reviewed in REF. 161).

Among the 69 F-box proteins that have been identified, extensive studies have been heavily focused on only four F-box proteins — FBXW7, SKP2, β-TRCP1 and β-TRCP2 — whereas only limited attention has been devoted to the remaining 65 members. Notably, as inhibitors of SKP2 are beginning to show therapeutic potential, this is encouraging the search for other F-box proteins that might also have oncogenic roles, as well as for compounds that target them. Functional characterization of each F-box protein would help us to understand cellular functions of individual F-box proteins and whether they are potential anticancer targets. To this end, there are still many important remaining questions. For example, does a given F-box protein exert its tumour-suppressive or oncogenic role in certain tissues or in certain pathological settings? In this regard, two recent studies indicated that FBXW7 could also function as an oncoprotein in chronic myeloid leukaemia. These studies showed that ablation of *Fbxw7* resulted in the eradication of leukaemia-initiating cells by allowing them to exit quiescence, thereby leading to remission of leukaemia during combination therapy with imatinib in mice[162,163]. However, it remains unclear whether FBXW7, which mostly functions as a tumour suppressor, could have oncogenic activity in other tissue settings. More importantly, it remains unaddressed whether the E3 ligase activity per se of any SCF E3 ligase complex is required for its tumorigenesis-related functions. What is the upstream signalling pathway (or pathways) that governs the activation or inactivation of each given SCF type of E3 ligase? Is there crosstalk between individual F-box proteins? What are the physiological substrates for many orphan F-box proteins? How can we establish more systematic and novel approaches to screen for additional substrates for each F-box protein, and how can we validate the physiological function for each F-box protein? To address these questions, we will need to develop whole-body or tissue-specific knockout or knock-in mice. We also need to design novel methods to identify additional ubiquitin substrates, and thereby define the mechanistic roles of F-box proteins in cancer development. For F-box proteins that have different roles in different tumour types, more studies should be focused on the identification of context-specific substrates that bear a causal relationship with the function of the F-box protein in tumorigenesis, which could be confirmed by using reverse genetic methods. In conclusion, addressing these questions might help to facilitate the development of novel strategies or therapeutic combinations to target F-box proteins in human cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Pancytopenia	A disease that is characterized by a reduction in the number of all formed elements in the blood, such as red cells, white cells and platelets.
***Apc*$^{Min/+}$ mice**	Mice with the multiple intestinal neoplasia (Min) mutation are predisposed to intestinal adenoma formation, which is a widely used mouse model for exploring the role of adenomatous polyposis coli (APC) in intestinal tumorigenesis.
Hamartomas	Benign tumour-like malformations composed of tissue elements that are normally found at a given site but that grow in a disorganized mass.
αβ-crystallin	A water-soluble structural protein that is found in the lens and the cornea of the eye, accounting for the transparency of the structure. It has also been identified in other tissues, such as the heart, and in aggressive breast tumours. Recent studies showed that it is a cofactor for SKP1–cullin 1–F-box protein (SCF)-F-box only 4 (FBXO4)-mediated ubiquitylation of cyclin D1.
Mouse mammary tumour virus promoter (MMTV promoter)	The MMTV promoter is hormonally regulated and its expression is found primarily in the mammary gland and other tissues, such as the lung, kidney, salivary gland, testes and the prostate. The MMTV promoter is often used in model systems of breast cancer to elucidate the genetics and biology of breast cancer.
DMBA–TPA model	A two-stage chemical skin carcinogenesis model using a single dose of the genotoxic carcinogen DMBA, followed by multiple doses of a non-genotoxic tumour-promoter, TPA.
Split hand/foot malformation disease (SHFM disease)	A congenital limb defect that predominantly affects the central rays of the autopod.
Cancer testis antigen	A member of a group of tumour antigens with higher expression in normal testis but not in adult somatic tissues. Overexpression of cancer testis antigens has been observed in several types of human cancers, suggesting that they could be used as biomarkers and are attractive drug targets for cancer treatment.

References

1. Li W, et al. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. PLoS ONE. 2008; 3:e1487. [PubMed: 18213395]

2. Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. Annu. Rev. Biochem. 2009; 78:399–434. [PubMed: 19489725]

3. Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. Nature Rev. Mol. Cell Biol. 2005; 6:9–20. [PubMed: 15688063] References 2 and 3 are two review articles that provide a comprehensive overview of the cullin ring type of E3 ligases.

4. Frescas D, Pagano M. Deregulated proteolysis by the F-box proteins SKP2 and β-TrCP: tipping the scales of cancer. Nature Rev. Cancer. 2008; 8:438–449. [PubMed: 18500245] This is an excellent review of the roles of SKP2, β-TRCP1 and β-TRCP2 in cancer.

5. Bai C, et al. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell. 1996; 86:263–274. [PubMed: 8706131] This ground-breaking work identified the F-box motif in FBXO1 and was the pioneering study that opened the SCF research field.

6. Nakayama KI, Nakayama K. Ubiquitin ligases: cell-cycle control and cancer. Nature Rev. Cancer. 2006; 6:369–381. [PubMed: 16633365] This is an excellent review about the role of ubiquitin ligases in controlling cell cycle progression and tumorigenesis.

7. Watanabe N, et al. M-phase kinases induce phospho-dependent ubiquitination of somatic Wee1 by SCFβ-TrCP. Proc. Natl Acad. Sci. USA. 2004; 101:4419–4424. [PubMed: 15070733]

8. Peters JM. The anaphase promoting complex/cyclosome: a machine designed to destroy. Nature Rev. Mol. Cell Biol. 2006; 7:644–656. [PubMed: 16896351] This article provided a comprehensive summary of APC/C E3 ligases.

9. Harper JW, Burton JL, Solomon MJ. The anaphase-promoting complex: it's not just for mitosis any more. Genes Dev. 2002; 16:2179–2206. [PubMed: 12208841]

10. D'Angiolella V, et al. SCF(Cyclin F) controls centrosome homeostasis and mitotic fidelity through CP110 degradation. Nature. 2010; 466:138–142. [PubMed: 20596027]

11. Duan S, et al. FBXO11 targets BCL6 for degradation and is inactivated in diffuse large B-cell lymphomas. Nature. 2012; 481:90–93. [PubMed: 22113614] This paper identified BCL-6 as a novel substrate of FBXO11 and demonstrated that FBXO11-mediated BCL-6 degradation is crucial for diffuse large B cell lymphoma development and that FBXO11 is a haploinsufficient tumour suppressor.

12. Rye MS, et al. FBXO11, a regulator of the TGFβ pathway, is associated with severe otitis media in Western Australian children. Genes Immun. 2011; 12:352–359. [PubMed: 21293382]

13. Santra MK, Wajapeyee N, Green MR. F-box protein FBXO31 mediates cyclin D1 degradation to induce G1 arrest after DNA damage. Nature. 2009; 459:722–725. [PubMed: 19412162]

14. Skaar JR, Pagan JK, Pagano M. Mechanisms and function of substrate recruitment by F-box proteins. Nature Rev. Mol. Cell Biol. 2013; 14:369–381. [PubMed: 23657496]

15. Nash P, et al. Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication. Nature. 2001; 414:514–521. [PubMed: 11734846] This is a pioneering study that identified the central phosphorylation domain within most of FBXW7 substrates.

16. Orlicky S, Tang X, Willems A, Tyers M, Sicheri F. Structural basis for phosphodependent substrate selection and orientation by the SCFCdc4 ubiquitin ligase. Cell. 2003; 112:243–256. [PubMed: 12553912]

17. Yoshida Y, et al. Fbs2 is a new member of the E3 ubiquitin ligase family that recognizes sugar chains. J. Biol. Chem. 2003; 278:43877–43884. [PubMed: 12939278]

18. Yoshida Y, et al. E3 ubiquitin ligase that recognizes sugar chains. Nature. 2002; 418:438–442. [PubMed: 12140560] This is the first report of an F-box protein interacting with modifications other than phosphorylation.

19. D'Angiolella V, et al. Cyclin F-mediated degradation of ribonucleotide reductase M2 controls genome integrity and DNA repair. Cell. 2012; 149:1023–1034. [PubMed: 22632967]

20. Kuchay S, et al. FBXL2- and PTPL1-mediated degradation of p110-free p85β regulatory subunit controls the PI(3)K signalling cascade. Nature Cell Biol. 2013; 15:472–480. [PubMed: 23604317]

21. Abbas T, et al. CRL1-FBXO11 promotes Cdt2 ubiquitylation and degradation and regulates Pr-Set7/Set8-mediated cellular migration. Mol. Cell. 2013; 49:1147–1158. [PubMed: 23478445]

22. Rossi M, et al. Regulation of the $CRL4^{Cdt2}$ ubiquitin ligase and cell-cycle exit by the SCF^{Fbxo11} ubiquitin ligase. Mol. Cell. 2013; 49:1159–1166. [PubMed: 23478441]

23. Lee JM, et al. EZH2 generates a methyl degron that is recognized by the DCAF1/DDB1/CUL4 E3 ubiquitin ligase complex. Mol. Cell. 2012; 48:572–586. [PubMed: 23063525] This paper showed for the first time that lysine-methylation could be a recognization signal for CRL.
24. Nakayama KI, Nakayama K. Regulation of the cell cycle by SCF-type ubiquitin ligases. Semin. Cell Dev. Biol. 2005; 16:323–333. [PubMed: 15840441]
25. Crusio KM, King B, Reavie LB, Aifantis I. The ubiquitous nature of cancer: the role of the SCF^{Fbw7} complex in development and transformation. Oncogene. 2010; 29:4865–4873. [PubMed: 20543859]
26. Welcker M, Clurman BE. FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. Nature Rev. Cancer. 2008; 8:83–93. [PubMed: 18094723] This is an excellent review, which updates the tumour-suppressor role of FBXW7.
27. Tsunematsu R, et al. Mouse Fbw7/Sel-10/Cdc4 is required for notch degradation during vascular development. J. Biol. Chem. 2004; 279:9417–9423. [PubMed: 14672936]
28. Tetzlaff MT, et al. Defective cardiovascular development and elevated cyclin E and Notch proteins in mice lacking the Fbw7 F-box protein. Proc. Natl Acad. Sci. USA. 2004; 101:3338–3345. [PubMed: 14766969]
29. Akhoondi S, et al. FBXW7/hCDC4 is a general tumor suppressor in human cancer. Cancer Res. 2007; 67:9006–9012. [PubMed: 17909001]
30. Onoyama I, et al. Conditional inactivation of Fbxw7 impairs cell-cycle exit during T cell differentiation and results in lymphomatogenesis. J. Exp. Med. 2007; 204:2875–2888. [PubMed: 17984302]
31. Matsuoka S, et al. Fbxw7 acts as a critical fail-safe against premature loss of hematopoietic stem cells and development of T-ALL. Genes Dev. 2008; 22:986–991. [PubMed: 18367647]
32. Thompson BJ, et al. Control of hematopoietic stem cell quiescence by the E3 ubiquitin ligase Fbw7. J. Exp. Med. 2008; 205:1395–1408. [PubMed: 18474632]
33. Maser RS, et al. Chromosomally unstable mouse tumours have genomic alterations similar to diverse human cancers. Nature. 2007; 447:966–971. [PubMed: 17515920] This paper identified FBXW7 as being frequently inactivated or deleted in T-ALL.
34. King B, et al. The ubiquitin ligase FBXW7 modulates leukemia-initiating cell activity by regulating MYC stability. Cell. 2013; 153:1552–1566. [PubMed: 23791182]
35. Sancho R, et al. F-box and WD repeat domain-containing 7 regulates intestinal cell lineage commitment and is a haploinsufficient tumor suppressor. Gastroenterology. 2010; 139:929–941. [PubMed: 20638938]
36. Davis H, Lewis A, Behrens A, Tomlinson I. Investigation of the atypical *FBXW7* mutation spectrum in human tumours by conditional expression of a heterozygous propellor tip missense allele in the mouse intestines. Gut. 2013 http://dx.doi.org/10.1136/gutjnl-2013-304719. References 34 and 36 established mouse models that recapitulated the loss of tumour-suppressor function of human FBXW7 via β-propeller point mutations.
37. Welcker M, Clurman BE. Fbw7/hCDC4 dimerization regulates its substrate interactions. Cell Div. 2007; 2:7. [PubMed: 17298674]
38. Tang X, et al. Suprafacial orientation of the SCFCdc4 dimer accommodates multiple geometries for substrate ubiquitination. Cell. 2007; 129:1165–1176. [PubMed: 17574027]
39. Onoyama I, et al. Fbxw7 regulates lipid metabolism and cell fate decisions in the mouse liver. J. Clin. Invest. 2011; 121:342–354. [PubMed: 21123947]
40. Mao JH, et al. *Fbxw7/Cdc4* is a p53-dependent, haploinsufficient tumour suppressor gene. Nature. 2004; 432:775–779. [PubMed: 15592418]
41. Akhoondi S, et al. Inactivation of FBXW7/hCDC4-β expression by promoter hypermethylation is associated with favorable prognosis in primary breast cancer. Breast Cancer Res. 2010; 12:R105. [PubMed: 21122106]
42. Matsumoto A, et al. Fbxw7β resides in the endoplasmic reticulum membrane and protects cells from oxidative stress. Cancer Sci. 2011; 102:749–755. [PubMed: 21205095]
43. Yokobori T, et al. p53-Altered FBXW7 expression determines poor prognosis in gastric cancer cases. Cancer Res. 2009; 69:3788–3794. [PubMed: 19366810]

44. Koh MS, Ittmann M, Kadmon D, Thompson TC, Leach FS. *CDC4* gene expression as potential biomarker for targeted therapy in prostate cancer. Cancer Biol. Ther. 2006; 5:78–83. [PubMed: 16357515]
45. Mann KM, et al. Sleeping Beauty mutagenesis reveals cooperating mutations and pathways in pancreatic adenocarcinoma. Proc. Natl Acad. Sci. USA. 2012; 109:5934–5941. [PubMed: 22421440]
46. Hardisty-Hughes RE, et al. A mutation in the F-box gene, *Fbxo11*, causes otitis media in the Jeff mouse. Hum. Mol. Genet. 2006; 15:3273–3279. [PubMed: 17035249]
47. Tsunematsu R, et al. Fbxw8 is essential for Cul1–Cul7 complex formation and for placental development. Mol. Cell. Biol. 2006; 26:6157–6169. [PubMed: 16880526]
48. Tsutsumi T, Kuwabara H, Arai T, Xiao Y, Decaprio JA. Disruption of the *Fbxw8* gene results in pre- and postnatal growth retardation in mice. Mol. Cell. Biol. 2008; 28:743–751. [PubMed: 17998335]
49. Kim SJ, et al. mTOR complex 2 regulates proper turnover of insulin receptor substrate-1 via the ubiquitin ligase subunit Fbw8. Mol. Cell. 2012; 48:875–887. [PubMed: 23142081]
50. Okabe H, et al. A critical role for FBXW8 and MAPK in cyclin D1 degradation and cancer cell proliferation. PLoS ONE. 2006; 1:e128. [PubMed: 17205132]
51. Lin P, et al. Fbxw8 is involved in the proliferation of human choriocarcinoma JEG-3 cells. Mol. Biol. Rep. 2011; 38:1741–1747. [PubMed: 20878477]
52. Siepka SM, et al. Circadian mutant Overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. Cell. 2007; 129:1011–1023. [PubMed: 17462724]
53. Godinho SI, et al. The after-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period. Science. 2007; 316:897–900. [PubMed: 17463252]
54. Busino L, et al. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science. 2007; 316:900–904. [PubMed: 17463251]
55. Yoo SH, et al. Competing E3 ubiquitin ligases govern circadian periodicity by degradation of CRY in nucleus and cytoplasm. Cell. 2013; 152:1091–1105. [PubMed: 23452855]
56. Xing W, et al. SCF^{FBXL3} ubiquitin ligase targets cryptochromes at their cofactor pocket. Nature. 2013; 496:64–68. [PubMed: 23503662]
57. Kelleher FC, Rao A, Maguire A. Circadian molecular clocks and cancer. Cancer Lett. 2014; 342:9–18. [PubMed: 24099911]
58. Lahti T, Merikanto I, Partonen T. Circadian clock disruptions and the risk of cancer. Ann. Med. 2012; 44:847–853. [PubMed: 23072403]
59. Tetzlaff MT, et al. Cyclin F disruption compromises placental development and affects normal cell cycle execution. Mol. Cell. Biol. 2004; 24:2487–2498. [PubMed: 14993286]
60. Fu J, et al. Low cyclin F expression in hepatocellular carcinoma associates with poor differentiation and unfavorable prognosis. Cancer Sci. 2013; 104:508–515. [PubMed: 23305207]
61. Vaites LP, et al. The Fbx4 tumor suppressor regulates cyclin D1 accumulation and prevents neoplastic transformation. Mol. Cell. Biol. 2011; 31:4513–4523. [PubMed: 21911473]
62. Kanie T, et al. Genetic reevaluation of the role of F-box proteins in cyclin D1 degradation. Mol. Cell. Biol. 2012; 32:590–605. [PubMed: 22124152]
63. Lin DI, et al. Phosphorylation-dependent ubiquitination of cyclin D1 by the $SCF^{FBX4\text{-}B\ crystallin}$ complex. Mol. Cell. 2006; 24:355–366. [PubMed: 17081987] This paper identified αβ-crystallin as a cofactor in promoting FBXO4-mediated degradation of cyclin D1.
64. Barbash O, et al. Mutations in *Fbx4* inhibit dimerization of the SCF^{Fbx4} ligase and contribute to cyclin D1 overexpression in human cancer. Cancer Cell. 2008; 14:68–78. [PubMed: 18598945]
65. Chiorazzi M, et al. Related F-box proteins control cell death in *Caenorhabditis elegans* and human lymphoma. Proc. Natl Acad. Sci. USA. 2013; 110:3943–3948. [PubMed: 23431138]
66. Samuelson DJ, et al. Rat Mcs5a is a compound quantitative trait locus with orthologous human loci that associate with breast cancer risk. Proc. Natl Acad. Sci. USA. 2007; 104:6299–6304. [PubMed: 17404222]

67. Smits BM, et al. An insulator loop resides between the synthetically interacting elements of the human/rat conserved breast cancer susceptibility locus MCS5A/Mcs5a. Nucleic Acids Res. 2012; 40:132–147. [PubMed: 21914726]
68. Xu X, et al. Human *MCS5A1* candidate breast cancer susceptibility gene *FBXO10* is induced by cellular stress and correlated with lens epithelium-derived growth factor (LEDGF). Mol. Carcinog. 2012 http://dx.doi.org/10.1002/mc.21977.
69. Jeong YT, Cermak L, Guijarro MV, Hernando E, Pagano M. FBH1 protects melanocytes from transformation and is deregulated in melanomas. Cell Cycle. 2013; 12:1128–1132. [PubMed: 23466708]
70. Kumar R, et al. *FBXO31* is the chromosome 16q24.3 senescence gene, a candidate breast tumor suppressor, and a component of an SCF complex. Cancer Res. 2005; 65:11304–11313. [PubMed: 16357137]
71. Huang HL, Zheng WL, Zhao R, Zhang B, Ma WL. FBXO31 is down-regulated and may function as a tumor suppressor in hepatocellular carcinoma. Oncol. Rep. 2010; 24:715–720. [PubMed: 20664978]
72. Carrano AC, Eytan E, Hershko A, Pagano M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nature Cell Biol. 1999; 1:193–199. [PubMed: 10559916]
73. Sutterluty H, et al. p45SKP2 promotes p27Kip1 degradation and induces S phase in quiescent cells. Nature Cell Biol. 1999; 1:207–214. [PubMed: 10559918] References 72 and 73 established p27 as a bona fide substrate for SKP2.
74. Tsvetkov LM, Yeh KH, Lee SJ, Sun H, Zhang H. $p27^{Kip1}$ ubiquitination and degradation is regulated by the SCF^{Skp2} complex through phosphorylated Thr187 in p27. Curr. Biol. 1999; 9:661–664. [PubMed: 10375532]
75. Yu ZK, Gervais JL, Zhang H. Human CUL-1 associates with the SKP1/SKP2 complex and regulates p21(CIP1/WAF1) and cyclin D proteins. Proc. Natl Acad. Sci. USA. 1998; 95:11324–11329. [PubMed: 9736735]
76. Shim EH, et al. Expression of the F-box protein SKP2 induces hyperplasia, dysplasia, and low-grade carcinoma in the mouse prostate. Cancer Res. 2003; 63:1583–1588. [PubMed: 12670908]
77. Latres E, et al. Role of the F-box protein Skp2 in lymphomagenesis. Proc. Natl Acad. Sci. USA. 2001; 98:2515–2520. [PubMed: 11226270]
78. Umanskaya K, et al. Skp2B stimulates mammary gland development by inhibiting REA, the repressor of the estrogen receptor. Mol. Cell. Biol. 2007; 27:7615–7622. [PubMed: 17785450]
79. Lin HK, et al. Skp2 targeting suppresses tumorigenesis by Arf-p53-independent cellular senescence. Nature. 2010; 464:374–379. [PubMed: 20237562] This paper showed that inactivation of SKP2 inhibited tumorigenesis through ARF-p53-independent cellular senescence, which suggests that SKP2 might function as an oncoprotein.
80. Chan CH, et al. The Skp2-SCF E3 ligase regulates Akt ubiquitination, glycolysis, herceptin sensitivity, and tumorigenesis. Cell. 2012; 149:1098–1111. [PubMed: 22632973]
81. Zhao H, et al. Skp2 deletion unmasks a p27 safeguard that blocks tumorigenesis in the absence of pRb and p53 tumor suppressors. Cancer Cell. 2013; 24:645–659. [PubMed: 24229711]
82. Wang H, et al. Skp2 is required for survival of aberrantly proliferating *Rb1*-deficient cells and for tumorigenesis in $Rb1^{+/-}$ mice. Nature Genet. 2010; 42:83–88. [PubMed: 19966802]
83. Sistrunk C, et al. Skp2 deficiency inhibits chemical skin tumorigenesis independent of $p27^{Kip1}$ accumulation. Am. J. Pathol. 2013; 182:1854–1864. [PubMed: 23474082]
84. Seki R, et al. Prognostic significance of S-phase kinase-associated protein 2 and p27kip1 in patients with diffuse large B-cell lymphoma: effects of rituximab. Ann. Oncol. 2010; 21:833–841. [PubMed: 19889619]
85. Wang Z, et al. Skp2: a novel potential therapeutic target for prostate cancer. Biochim. Biophys. Acta. 2012; 1825:11–17. [PubMed: 21963805]
86. Li JQ, et al. Correlation of Skp2 with carcinogenesis, invasion, metastasis, and prognosis in colorectal tumors. Int. J. Oncol. 2004; 25:87–95. [PubMed: 15201993]
87. Rose AE, et al. Clinical relevance of SKP2 alterations in metastatic melanoma. Pigment Cell. Melanoma Res. 2011; 24:197–206. [PubMed: 20883453]

88. Fang FM, et al. Effect of S-phase kinase-associated protein 2 expression on distant metastasis and survival in nasopharyngeal carcinoma patients. Int. J. Radiat. Oncol. Biol. Phys. 2009; 73:202–207. [PubMed: 18538504]

89. Schuler S, et al. SKP2 confers resistance of pancreatic cancer cells towards TRAIL-induced apoptosis. Int. J. Oncol. 2011; 38:219–225. [PubMed: 21109943]

90. Radke S, Pirkmaier A, Germain D. Differential expression of the F-box proteins Skp2 and Skp2B in breast cancer. Oncogene. 2005; 24:3448–3458. [PubMed: 15782142]

91. Zheng WQ, Zheng JM, Ma R, Meng FF, Ni CR. Relationship between levels of Skp2 and P27 in breast carcinomas and possible role of Skp2 as targeted therapy. Steroids. 2005; 70:770–774. [PubMed: 16024059]

92. Lu M, et al. The expression and prognosis of FOXO3a and Skp2 in human hepatocellular carcinoma. Pathol. Oncol. Res. 2009; 15:679–687. [PubMed: 19404778]

93. Ma XM, Liu Y, Guo JW, Liu JH, Zuo LF. Relation of overexpression of S phase kinase-associated protein 2 with reduced expression of p27 and PTEN in human gastric carcinoma. World J. Gastroenterol. 2005; 11:6716–6721. [PubMed: 16425372]

94. Lee H, et al. Mouse emi1 has an essential function in mitotic progression during early embryogenesis. Mol. Cell. Biol. 2006; 26:5373–5381. [PubMed: 16809773]

95. Lehman NL, Verschuren EW, Hsu JY, Cherry AM, Jackson PK. Overexpression of the anaphase promoting complex/cyclosome inhibitor Emi1 leads to tetraploidy and genomic instability of p53-deficient cells. Cell Cycle. 2006; 5:1569–1573. [PubMed: 16861914]

96. Lehman NL, et al. Oncogenic regulators and substrates of the anaphase promoting complex/cyclosome are frequently overexpressed in malignant tumors. Am. J. Pathol. 2007; 170:1793–1805. [PubMed: 17456782]

97. Gutgemann I, Lehman NL, Jackson PK, Longacre TA. Emi1 protein accumulation implicates misregulation of the anaphase promoting complex/cyclosome pathway in ovarian clear cell carcinoma. Mod. Pathol. 2008; 21:445–454. [PubMed: 18204430]

98. Min KW, et al. Clear cell carcinomas of the ovary: a multi-institutional study of 129 cases in Korea with prognostic significance of Emi1 and Galectin-3. Int. J. Gynecol. Pathol. 2013; 32:3–14. [PubMed: 23202783]

99. Zhao Y, et al. Early mitotic inhibitor-1, an anaphase-promoting complex/cyclosome inhibitor, can control tumor cell proliferation in hepatocellular carcinoma: correlation with Skp2 stability and degradation of $p27^{Kip1}$. Hum. Pathol. 2013; 44:365–373. [PubMed: 22995332]

100. Hsu JY, Reimann JD, Sorensen CS, Lukas J, Jackson PK. E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC^{Cdh1}. Nature Cell Biol. 2002; 4:358–366. [PubMed: 11988738]

101. Chen JY, Wang MC, Hung WC. Bcr-Abl-induced tyrosine phosphorylation of Emi1 to stabilize Skp2 protein via inhibition of ubiquitination in chronic myeloid leukemia cells. J. Cell. Physiol. 2011; 226:407–413. [PubMed: 20717963]

102. Fernandez-Saiz V, et al. SCFFbxo9 and CK2 direct the cellular response to growth factor withdrawal via Tel2/Tti1 degradation and promote survival in multiple myeloma. Nature Cell Biol. 2013; 15:72–81. [PubMed: 23263282]

103. Suzuki T, et al. Mutation analyses of genes on 6p12-p11 in patients with juvenile myoclonic epilepsy. Neurosci. Lett. 2006; 405:126–131. [PubMed: 16876319]

104. Fuchs SY, Spiegelman VS, Kumar KG. The many faces of β-TrCP E3 ubiquitin ligases: reflections in the magic mirror of cancer. Oncogene. 2004; 23:2028–2036. [PubMed: 15021890]

105. Nakayama K, et al. Impaired degradation of inhibitory subunit of NF-κ B (I κ B) and β-catenin as a result of targeted disruption of the β-TrCP1 gene. Proc. Natl Acad. Sci. USA. 2003; 100:8752–8757. [PubMed: 12843402]

106. Guardavaccaro D, et al. Control of meiotic and mitotic progression by the F box protein β-Trcp1 *in vivo*. Dev. Cell. 2003; 4:799–812. [PubMed: 12791266]

107. Kudo Y, et al. Role of F-box protein βTrcp1 in mammary gland development and tumorigenesis. Mol. Cell. Biol. 2004; 24:8184–8194. [PubMed: 15340078]

108. Bhatia N, Demmer TA, Sharma AK, Elcheva I, Spiegelman VS. Role of β-TrCP ubiquitin ligase receptor in UVB mediated responses in skin. Arch. Biochem. Biophys. 2011; 508:178–184. [PubMed: 21187057]
109. Ougolkov A, et al. Associations among β-TrCP, an E3 ubiquitin ligase receptor, β-catenin, and NF-κB in colorectal cancer. J. Natl Cancer Institute. 2004; 96:1161–1170.
110. Muerkoster S, et al. Increased expression of the E3-ubiquitin ligase receptor subunit βTRCP1 relates to constitutive nuclear factor-κB activation and chemoresistance in pancreatic carcinoma cells. Cancer Res. 2005; 65:1316–1324. [PubMed: 15735017]
111. Koch A, et al. Elevated expression of Wnt antagonists is a common event in hepatoblastomas. Clin. Cancer Res. 2005; 11:4295–4304. [PubMed: 15958610]
112. Kim CJ, et al. Somatic mutations of the β-TrCP gene in gastric cancer. APMIS. 2007; 115:127–133. [PubMed: 17295679]
113. Saitoh T, Katoh M. Expression profiles of βTRCP1 and βTRCP2, and mutation analysis of βTRCP2 in gastric cancer. Int. J. Oncol. 2001; 18:959–964. [PubMed: 11295041]
114. Wu X, Johansen JV, Helin K. Fbxl10/Kdm2b recruits polycomb repressive complex 1 to CpG islands and regulates H2A ubiquitylation. Mol. Cell. 2013; 49:1134–1146. [PubMed: 23395003]
115. Tzatsos A, et al. KDM2B promotes pancreatic cancer via Polycomb-dependent and -independent transcriptional programs. J. Clin. Invest. 2013; 123:727–739. [PubMed: 23321669]
116. Fukuda T, Tokunaga A, Sakamoto R, Yoshida N. Fbxl10/Kdm2b deficiency accelerates neural progenitor cell death and leads to exencephaly. Mol. Cell. Neurosci. 2011; 46:614–624. [PubMed: 21220025]
117. He J, Kallin EM, Tsukada Y, Zhang Y. The H3K36 demethylase Jhdm1b/Kdm2b regulates cell proliferation and senescence through p15(Ink4b). Nature Struct. Mol. Biol. 2008; 15:1169–1175. [PubMed: 18836456]
118. Frescas D, Guardavaccaro D, Bassermann F, Koyama-Nasu R, Pagano M. JHDM1B/FBXL10 is a nucleolar protein that represses transcription of ribosomal RNA genes. Nature. 2007; 450:309–313. [PubMed: 17994099]
119. Lockwood WW, Chandel SK, Stewart GL, Erdjument-Bromage H, Beverly LJ. The novel ubiquitin ligase complex, SCF^{Fbxw4}, interacts with the COP9 signalosome in an F-box dependent manner, is mutated, lost and under-expressed in human cancers. PLoS ONE. 2013; 8:e63610. [PubMed: 23658844]
120. Minoda Y, Sakurai H, Kobayashi T, Yoshimura A, Takaesu G. An F-box protein, FBXW5, negatively regulates TAK1 MAP3K in the IL-1β signaling pathway. Biochem. Biophys. Res. Commun. 2009; 381:412–417. [PubMed: 19232515]
121. Kanei-Ishii C, Nomura T, Egoh A, Ishii S. Fbxw5 suppresses nuclear c-Myb activity via DDB1-Cul4-Rbx1 ligase-mediated sumoylation. Biochem. Biophys. Res. Commun. 2012; 426:59–64. [PubMed: 22910413]
122. Kim TY, et al. CRL4A-FBXW5-mediated degradation of DLC1 Rho GTPase-activating protein tumor suppressor promotes non-small cell lung cancer cell growth. Proc. Natl Acad. Sci. USA. 2013; 110:16868–16873. [PubMed: 24082123]
123. Hu J, et al. WD40 protein FBW5 promotes ubiquitination of tumor suppressor TSC2 by DDB1-CUL4-ROC1 ligase. Genes Dev. 2008; 22:866–871. [PubMed: 18381890]
124. De La Chesnaye E, et al. Fbxw15/Fbxo12J is an F-box protein-encoding gene selectively expressed in oocytes of the mouse ovary. Biol. Reprod. 2008; 78:714–725. [PubMed: 18094359]
125. Zou C, et al. SCF^{Fbxw15} mediates histone acetyltransferase binding to origin recognition complex (HBO1) ubiquitin-proteasomal degradation to regulate cell proliferation. J. Biol. Chem. 2013; 288:6306–6316. [PubMed: 23319590]
126. Chen BB, et al. F-box protein FBXL2 targets cyclin D2 for ubiquitination and degradation to inhibit leukemic cell proliferation. Blood. 2012; 119:3132–3141. [PubMed: 22323446]
127. Chen BB, Glasser JR, Coon TA, Mallampalli RK. F-box protein FBXL2 exerts human lung tumor suppressor-like activity by ubiquitin-mediated degradation of cyclin D3 resulting in cell cycle arrest. Oncogene. 2012; 31:2566–2579. [PubMed: 22020328]

128. Chen BB, Glasser JR, Coon TA, Mallampalli RK. Skp-cullin-F box E3 ligase component FBXL2 ubiquitinates Aurora B to inhibit tumorigenesis. Cell Death Dis. 2013; 4:e759. [PubMed: 23928698]

129. Zhang N, et al. FBXL5 interacts with p150Glued and regulates its ubiquitination. Biochem. Biophys. Res. Commun. 2007; 359:34–39. [PubMed: 17532294]

130. Ruiz JC, Walker SD, Anderson SA, Eisenstein RS, Bruick RK. F-box and leucine-rich repeat protein 5 (FBXL5) is required for maintenance of cellular and systemic iron homeostasis. J. Biol. Chem. 2013; 288:552–560. [PubMed: 23135277]

131. Moroishi T, Nishiyama M, Takeda Y, Iwai K, Nakayama KI. The FBXL5-IRP2 axis is integral to control of iron metabolism *in vivo*. Cell. Metab. 2011; 14:339–351. [PubMed: 21907140]

132. Zhu J, Li K, Dong L, Chen Y. Role of FBXL20 in human colorectal adenocarcinoma. Oncol. Rep. 2012; 28:2290–2298. [PubMed: 23023584]

133. Eisfeld AK, et al. miR-3151 interplays with its host gene BAALC and independently affects outcome of patients with cytogenetically normal acute myeloid leukemia. Blood. 2012; 120:249–258. [PubMed: 22529287]

134. Hirano A, et al. FBXL21 regulates oscillation of the circadian clock through ubiquitination and stabilization of cryptochromes. Cell. 2013; 152:1106–1118. [PubMed: 23452856]

135. Meziane el K, Randle SJ, Nelson DE, Lomonosov M, Laman H. Knockdown of Fbxo7 reveals its regulatory role in proliferation and differentiation of haematopoietic precursor cells. J. Cell Sci. 2011; 124:2175–2186. [PubMed: 21652635]

136. Hsu JM, Lee YC, Yu CT, Huang CY. Fbx7 functions in the SCF complex regulating Cdk1-cyclin B-phosphorylated hepatoma up-regulated protein (HURP) proteolysis by a proline-rich region. J. Biol. Chem. 2004; 279:32592–32602. [PubMed: 15145941]

137. Chang YF, Cheng CM, Chang LK, Jong YJ, Yuo CY. The F-box protein Fbxo7 interacts with human inhibitor of apoptosis protein cIAP1 and promotes cIAP1 ubiquitination. Biochem. Biophys. Res. Commun. 2006; 342:1022–1026. [PubMed: 16510124]

138. Laman H, et al. Transforming activity of Fbxo7 is mediated specifically through regulation of cyclin D/cdk6. EMBO J. 2005; 24:3104–3116. [PubMed: 16096642]

139. Lomonosov M, et al. Expression of Fbxo7 in haematopoietic progenitor cells cooperates with p53 loss to promote lymphomagenesis. PLoS ONE. 2011; 6:e21165. [PubMed: 21695055]

140. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007; 448:313–317. [PubMed: 17554338]

141. Valent P, et al. Cancer stem cell definitions and terminology: the devil is in the details. Nature Rev. Cancer. 2012; 12:767–775. [PubMed: 23051844]

142. Tokuzawa Y, et al. Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development. Mol. Cell. Biol. 2003; 23:2699–2708. [PubMed: 12665572]

143. Cepeda D, et al. CDK-mediated activation of the SCF^{FBXO28} ubiquitin ligase promotes MYC-driven transcription and tumourigenesis and predicts poor survival in breast cancer. EMBO Mol. Med. 2013; 5:999–1018. [PubMed: 23776131]

144. Bodine SC, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science. 2001; 294:1704–1708. [PubMed: 11679633]

145. Li HH, et al. Atrogin-1 inhibits Akt-dependent cardiac hypertrophy in mice via ubiquitin-dependent coactivation of Forkhead proteins. J. Clin. Invest. 2007; 117:3211–3223. [PubMed: 17965779]

146. Tan J, et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev. 2007; 21:1050–1063. [PubMed: 17437993]

147. Chou JL, et al. Promoter hypermethylation of *FBXO32*, a novel TGF-β/SMAD4 target gene and tumor suppressor, is associated with poor prognosis in human ovarian cancer. Lab. Invest. 2010; 90:414–425. [PubMed: 20065949]

148. Song MH, Ha JC, Lee SM, Park YM, Lee SY. Identification of BCP-20 (FBXO39) as a cancer/testis antigen from colon cancer patients by SEREX. Biochem. Biophys. Res. Commun. 2011; 408:195–201. [PubMed: 21338577]

149. Lu Y, et al. The F-box protein FBXO44 mediates BRCA1 ubiquitination and degradation. J. Biol. Chem. 2012; 287:41014–41022. [PubMed: 23086937]

150. Peschiaroli A, Scialpi F, Bernassola F, Pagano M, Melino G. The F-box protein FBXO45 promotes the proteasome-dependent degradation of p73. Oncogene. 2009; 28:3157–3166. [PubMed: 19581926]

151. Saiga T, et al. Fbxo45 forms a novel ubiquitin ligase complex and is required for neuronal development. Mol. Cell. Biol. 2009; 29:3529–3543. [PubMed: 19398581]

152. Chan CH, et al. Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression. Cell. 2013; 154:556–568. [PubMed: 23911321] This paper showed that Compound #25 selectively inhibited SKP2 activity and retarded tumour growth through restricting cancer stem cell traits.

153. Chen Q, et al. Targeting the p27 E3 ligase SCF^{Skp2} results in p27- and Skp2-mediated cell-cycle arrest and activation of autophagy. Blood. 2008; 111:4690–4699. [PubMed: 18305219]

154. Wu L, et al. Specific small molecule inhibitors of Skp2-mediated p27 degradation. Chem. Biol. 2012; 19:1515–1524. [PubMed: 23261596]

155. Pavlides SC, et al. Inhibitors of SCF-Skp2/Cks1 E3 ligase block estrogen-induced growth stimulation and degradation of nuclear p27kip1: therapeutic potential for endometrial cancer. Endocrinology. 2013; 154:4030–4045. [PubMed: 24035998]

156. Inuzuka H, et al. SCF^{FBW7} regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. Nature. 2011; 471:104–109. [PubMed: 21368833]

157. Wertz IE, et al. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature. 2011; 471:110–114. [PubMed: 21368834] References 156 and 157 showed that MCL1 is a novel substrate of FBXW7 and is involved in conferring chemoresistance.

158. Min SH, et al. Negative regulation of the stability and tumor suppressor function of Fbw7 by the Pin1 prolyl isomerase. Mol. Cell. 2012; 46:771–783. [PubMed: 22608923]

159. Wu J, et al. Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1. Mol. Cell. 2012; 46:351–361. [PubMed: 22464731]

160. Miller JJ, et al. Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. Genes Dev. 2006; 20:2410–2420. [PubMed: 16921029]

161. Nelson DE, Randle SJ, Laman H. Beyond ubiquitination: the atypical functions of Fbxo7 and other F-box proteins. Open Biol. 2013; 3:130131. [PubMed: 24107298]

162. Reavie L, et al. Regulation of c-Myc ubiquitination controls chronic myelogenous leukemia initiation and progression. Cancer Cell. 2013; 23:362–375. [PubMed: 23518350]

163. Takeishi S, et al. Ablation of Fbxw7 eliminates leukemia-initiating cells by preventing quiescence. Cancer Cell. 2013; 23:347–361. [PubMed: 23518349]

164. Agrawal N, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in *NOTCH1*. Science. 2011; 333:1154–1157. [PubMed: 21798897]

165. Le Gallo M, et al. Exome sequencing of serous endometrial tumors identifies recurrent somatic mutations in chromatin-remodeling and ubiquitin ligase complex genes. Nature Genet. 2012; 44:1310–1315. [PubMed: 23104009]

166. Huber C, et al. Identification of mutations in *CUL7* in 3-M syndrome. Nature Genet. 2005; 37:1119–1124. [PubMed: 16142236]

167. Chiaur DS, et al. Five human genes encoding F-box proteins: chromosome mapping and analysis in human tumors. Cytogenet. Cell Genet. 2000; 88:255–258. [PubMed: 10828603]

168. Laulier C, Cheng A, Huang N, Stark JM. Mammalian Fbh1 is important to restore normal mitotic progression following decatenation stress. DNA Repair (Amst.). 2010; 9:708–717. [PubMed: 20457012]

169. Signoretti S, et al. Oncogenic role of the ubiquitin ligase subunit Skp2 in human breast cancer. J. Clin. Invest. 2002; 110:633–641. [PubMed: 12208864]

170. Gstaiger M, et al. Skp2 is oncogenic and overexpressed in human cancers. Proc. Natl Acad. Sci. USA. 2001; 98:5043–5048. [PubMed: 11309491]

171. Yokoi S, et al. Amplification and overexpression of SKP2 are associated with metastasis of non-small-cell lung cancers to lymph nodes. Am. J. Pathol. 2004; 165:175–180. [PubMed: 15215173]

172. Kanarek N, et al. Spermatogenesis rescue in a mouse deficient for the ubiquitin ligase SCFβ-TrCP by single substrate depletion. Genes Dev. 2010; 24:470–477. [PubMed: 20194439]

173. Chen S, et al. An insertion/deletion polymorphism in the 3' untranslated region of beta-transducin repeat-containing protein (βTrCP) is associated with susceptibility for hepatocellular carcinoma in Chinese. Biochem. Biophys. Res. Commun. 2010; 391:552–556. [PubMed: 19931512]

174. Scott DK, et al. Identification and analysis of tumor suppressor loci at chromosome 10q23.3–10q25.3 in medulloblastoma. Cell Cycle. 2006; 5:2381–2389. [PubMed: 17102621]

175. Watanabe T, Hikichi Y, Willuweit A, Shintani Y, Horiguchi T. FBL2 regulates amyloid precursor protein (APP) metabolism by promoting ubiquitination-dependent APP degradation and inhibition of APP endocytosis. J. Neurosci. 2012; 32:3352–3365. [PubMed: 22399757]

176. Malard V, et al. Global gene expression profiling in human lung cells exposed to cobalt. BMC Genomics. 2007; 8:147. [PubMed: 17553155]

177. Takagi H, Setou M, Ito S, Yao I. SCRAPPER regulates the thresholds of long-term potentiation/depression, the bidirectional synaptic plasticity in hippocampal CA3-CA1 synapses. Neural Plast. 2012; 2012:352829. [PubMed: 23316391]

178. Nelson RF, et al. Selective cochlear degeneration in mice lacking the F-box protein, Fbx2, a glycoprotein-specific ubiquitin ligase subunit. J. Neurosci. 2007; 27:5163–5171. [PubMed: 17494702]

179. Babaei-Jadidi R, et al. FBXW7 influences murine intestinal homeostasis and cancer, targeting Notch, Jun, and DEK for degradation. J. Exp. Med. 2011; 208:295–312. [PubMed: 21282377]

180. Davis H, et al. *FBXW7* mutations typically found in human cancers are distinct from null alleles and disrupt lung development. J. Pathol. 2011; 224:180–189. [PubMed: 21503901]

181. Jandke A, et al. The F-box protein Fbw7 is required for cerebellar development. Dev. Biol. 2011; 358:201–212. [PubMed: 21827743]

182. Grim JE, et al. Fbw7 and p53 cooperatively suppress advanced and chromosomally unstable intestinal cancer. Mol. Cell. Biol. 2012; 32:2160–2167. [PubMed: 22473991]

183. Matsumoto A, et al. Fbxw7-dependent degradation of Notch is required for control of "stemness" and neuronal-glial differentiation in neural stem cells. J. Biol. Chem. 2011; 286:13754–13764. [PubMed: 21349854]

184. Hoeck JD, et al. Fbw7 controls neural stem cell differentiation and progenitor apoptosis via Notch and c-Jun. Nature Neurosci. 2010; 13:1365–1372. [PubMed: 20935640]

185. Sancho R, et al. Fbw7 repression by hes5 creates a feedback loop that modulates notch-mediated intestinal and neural stem cell fate decisions. PLoS Biol. 2013; 11:e1001586. [PubMed: 23776410]

186. Kwon YW, et al. Pten regulates Aurora-A and cooperates with Fbxw7 in modulating radiation-induced tumor development. Mol. Cancer Res. 2012; 10:834–844. [PubMed: 22513362]

187. Nakayama K, et al. Targeted disruption of Skp2 results in accumulation of cyclin E and p27(Kip1), polyploidy and centrosome overduplication. EMBO J. 2000; 19:2069–2081. [PubMed: 10790373]

188. Zhong L, Georgia S, Tschen SI, Nakayama K, Bhushan A. Essential role of Skp2-mediated p27 degradation in growth and adaptive expansion of pancreatic β cells. J. Clin. Invest. 2007; 117:2869–2876. [PubMed: 17823659]

189. Sakai T, et al. Skp2 controls adipocyte proliferation during the development of obesity. J. Biol. Chem. 2007; 282:2038–2046. [PubMed: 17082193]

190. Fotovati A, Nakayama K, Nakayama KI. Impaired germ cell development due to compromised cell cycle progression in Skp2-deficient mice. Cell Div. 2006; 1:4. [PubMed: 16759351]

191. Minamishima YA, Nakayama K. Recovery of liver mass without proliferation of hepatocytes after partial hepatectomy in Skp2-deficient mice. Cancer Res. 2002; 62:995–999. [PubMed: 11861371]

192. Agarwal A, et al. Absence of SKP2 expression attenuates BCR-ABL-induced myeloproliferative disease. Blood. 2008; 112:1960–1970. [PubMed: 18559973]

193. Kratzat S, et al. Cks1 is required for tumor cell proliferation but not sufficient to induce hematopoietic malignancies. PLoS ONE. 2012; 7:e37433. [PubMed: 22624029]

194. Kossatz U, et al. Skp2-dependent degradation of p27kip1 is essential for cell cycle progression. Genes Dev. 2004; 18:2602–2607. [PubMed: 15520280]

195. Sistrunk C, Macias E, Nakayama K, Kim Y, Rodriguez-Puebla ML. Skp2 is necessary for Myc-induced keratinocyte proliferation but dispensable for Myc oncogenic activity in the oral epithelium. Am. J. Pathol. 2011; 178:2470–2477. [PubMed: 21641375]

196. Suzuki S, et al. The amelioration of renal damage in Skp2-deficient mice canceled by p27 Kip1 deficiency in $Skp2^{-/-}$ $p27^{-/-}$ mice. PLoS ONE. 2012; 7:e36249. [PubMed: 22558406]

197. Wang J, et al. The role of Skp2 in hematopoietic stem cell quiescence, pool size, and self-renewal. Blood. 2011; 118:5429–5438. [PubMed: 21931116]

198. Baguma-Nibasheka M, Kablar B. Abnormal retinal development in the Btrc null mouse. Dev. Dyn. 2009; 238:2680–2687. [PubMed: 19705444]

Key points

- F-box proteins have pivotal roles in the development and progression of human malignancies through governing the turnover of key factors that are involved in multiple cellular processes, including cell proliferation, apoptosis, invasion, angiogenesis and metastasis.
- In order to determine the possible role for each of the 69 identified mammalian F-box proteins in tumorigenesis, we focused on summarizing experimental evidence derived from physiological mouse models, pathological gene alterations in cancer patients and biochemical substrates identified for respective F-box proteins.
- F-box/WD repeat-containing protein 7 (*FBXW7*) exerts its tumour-suppressor role mostly by promoting the degradation of various oncoproteins that regulate important cellular processes including cell cycle progression, cellular metabolism, differentiation and apoptosis. Moreover, FBXW7 somatic mutations have been observed in various human cancers, and genetic ablation of *Fbxw7* in different tissue settings predisposes mice to cancer.
- S-phase kinase-associated protein 2 (SKP2) promotes tumorigenesis mostly by promoting the ubiquitylation and subsequent degradation of a cohort of tumour-suppressor proteins, including p27, p21, p130 and forkhead box protein O1 (FOXO1). *Skp2* transgenic mouse models, and overexpression of SKP2 in various types of human cancers further support a role for SKP2 as a proto-oncoprotein.
- β-transducin repeat-containing protein 1 (β-TRCP1) and β-TRCP2 exert their oncogenic or tumour-suppressive function in a tissue-specific, or cellular context-dependent manner.
- For a subset of F-box proteins with emerging oncogenic or tumour-suppressive roles, genetic mouse models have been established to shed light on the potential role of these proteins in tumorigenesis. However, further additional compound or tissue-specific mouse models, as well as identification of downstream substrates, are necessary to clearly define the roles of these proteins in cancer.
- F-box proteins with undetermined roles in tumorigenesis are defined for those members without studies from cancerous mouse models, although pathological genetic alterations or characterized tumour-associated substrates might have provided minimal support for their possible involvement in tumorigenesis.
- Targeting F-box proteins, or F-box protein signalling pathways, could be an effective strategy for prevention or treatment of human cancers.

Box 1 | Definitions of physiological, pathological and biochemical evidence as used in this Review

Three major standards are used in this Review to determine the role of a given F-box protein in tumorigenesis. 'Physiological evidence' refers to results that are derived from mouse modelling studies, including knockout and transgenic mouse models, either at the whole-body level or in a tissue-specific manner. Physiological evidence is considered to be the most reliable evidence for examining the physiological role of an F-box protein in tumorigenesis. 'Pathological evidence' shows the correlation between the expression or function of a given F-box protein and various cancer-related pathological conditions. These pathological changes include gene-level alterations such as gene amplification, gene mutation and gene deletion, as well as protein-level changes such as protein overexpression and downregulation in various types of human cancers. These relationships are not causal, and they are not reliable or sufficient to define the role of an F-box protein in cancer. However, pathological evidence is considered to be strong supportive data that indicate the involvement of a given F-box protein in tumorigenesis. 'Biochemical evidence' mainly refers to the identified ubiquitin substrates for each F-box protein. Typically, if an E3 ligase degrades an oncogene, it might be considered to be a tumour suppressor, or if it degrades a tumour suppressor, it might be considered to be an oncogene. However, biochemical evidence is only considered to be supporting evidence, and a thorough evaluation of the role of each F-box protein in tumorigenesis should be a comprehensive evaluation of evidence that has been derived from all three standards, with physiological evidence weighing the most.

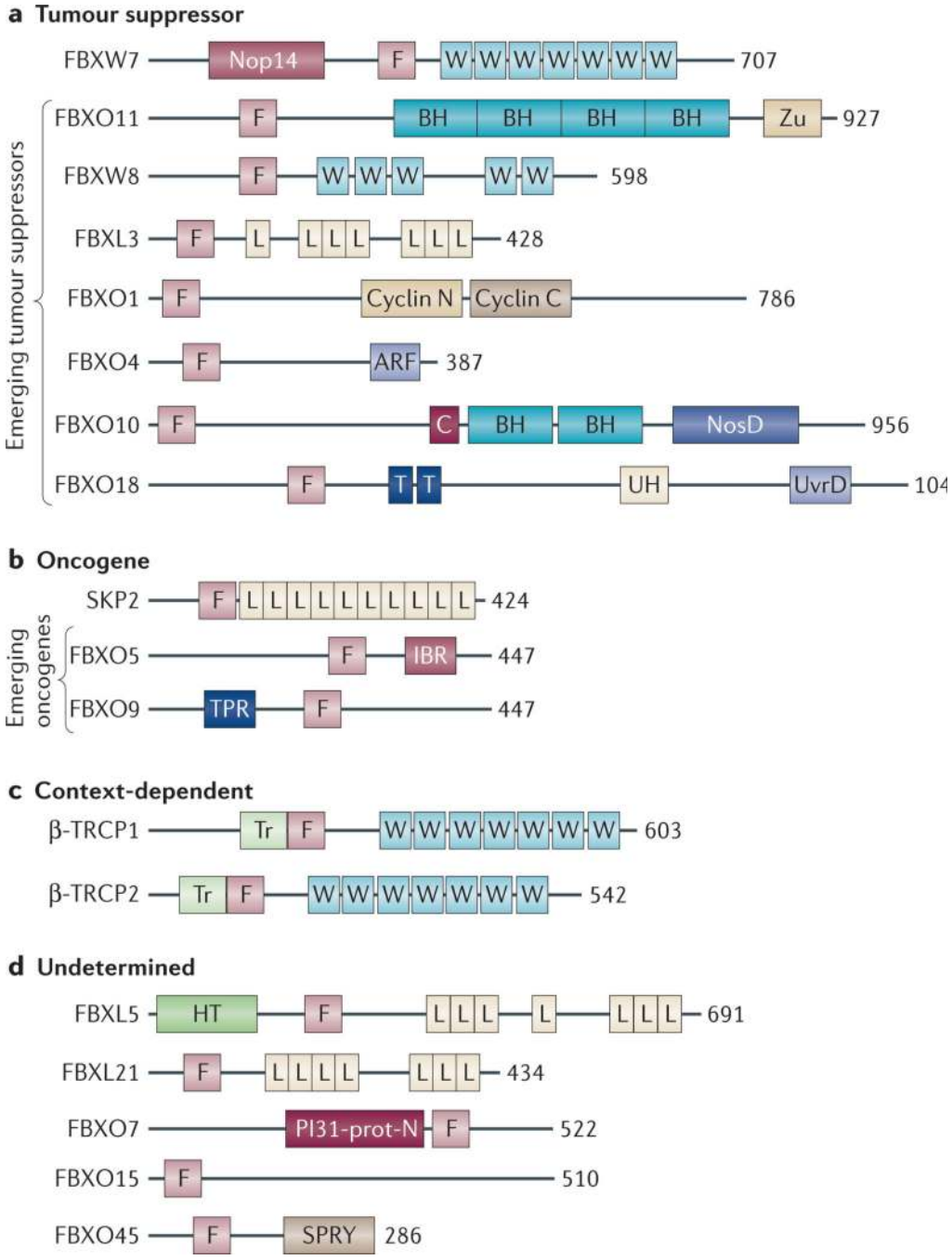

Figure 1. Illustration of functional domains in the highlighted F-Box proteins, grouped by their potential functions in cancer that have been shown by available mouse models

On the basis of data from mouse genetics, pathological profiles and biochemical substrates identified for each F-box protein we group F-box proteins into four categories to indicate their roles in tumorigenesis: tumour suppressor (part **a**), oncogene (part **b**), context-dependent (part **c**) and undetermined (part **d**). β-TRCP, β-transducin repeat-containing protein; BH, β-helix; F, F-box motif; FBXL, F-box and leucine-rich repeat protein; FBXO, F-box only; FBXW, F-box/WD repeat-containing protein; HT, hemerythrin domain; IBR, in

between ring fingers domain; L, leucine-rich repeat; Nop14, NOP14-like family domain; NosD, periplasmic copper-binding protein; PI31-prot-N, PI31 proteasome regulator amino-terminal domain; SKP2, S-phase kinase-associated protein 2; SPRY, SPLA and the ryanodine receptor domain; T, transmembrane region; Tr, D domain of β-TRCP; TRP, tetratricopeptide repeat; UH, UvrD/REP helicase N-terminal domain; UvrD, UvrD-like helicase C-terminal domain; W, WD40 repeat; Zu, putative zinc finger in N-recognin.

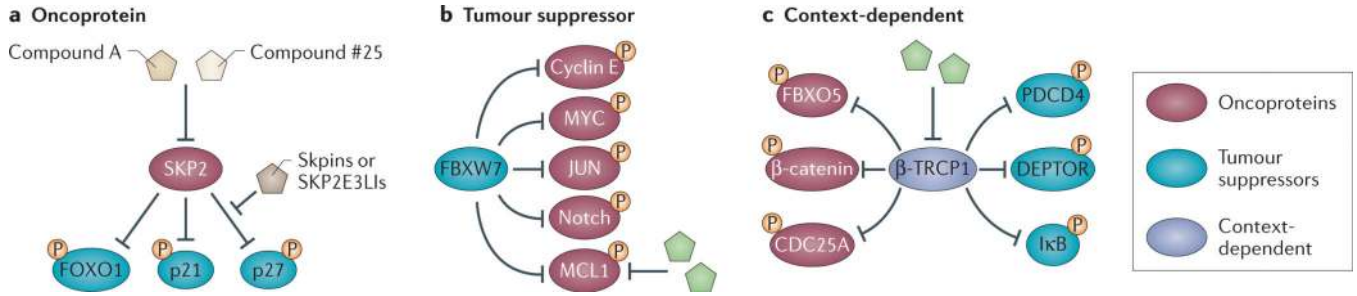

Figure 2. Different strategies for targeted therapy that aims to inhibit F-box E3 ligases on the basis of their roles in tumorigenesis

a | As indicated, for oncogenic F-box proteins, inhibitors that directly block SKP1–cullin 1–F-box protein (SCF) E3 complex formation or inhibit E3 interaction with substrates could be used as anticancer drugs. To this end, Compound #25 (also known as SZL-P1-41) has been characterized to impair the incorporation of S-phase kinase-associated protein 2 (SKP2) into a functional SCF complex, thereby terminating the oncogenic activity of SKP2. Similarly, 'skpins' and SKP2 E3 ligase inhibitors (SKP2E3LIs) were identified to specifically disrupt the interaction of SKP2 with p27, thereby leading to the accumulation of p27 and subsequent cell cycle arrest. **b** | For tumour-suppressive F-box proteins such as F-box/WD repeat-containing protein 7 (FBXW7), as genetic ablation or depletion of FBXW7 was frequently observed in cancer patients, a rational targeting of its downstream oncogenic pathway (or pathways) could be used as an efficient anticancer approach. **c** | For F-box proteins with context-dependent or undetermined roles in cancer, such as β-transducin repeat-containing protein 1 (β-TRCP1), disease-specific analysis would be necessary to further guide the design of personalized therapies. CDC25A, cell division cycle 25 homologue A; DEPTOR, DEP domain-containing mTOR-interacting protein; FBXO5, F-box only 5; FOXO1, forkhead box protein O1; IκB, inhibitor of nuclear factor-κB; MCL1, myeloid cell leukaemia 1; P, phosphorylation; PDCD4, programmed cell death protein 4.

Table 1

Physiological, pathological and biochemical evidence for potential roles of highlighted F-box proteins in cancer

Evidence	F-box protein	Physiological evidence (mouse models for cancerous phenotypes)	Pathological evidence	Biochemical evidence (major substrates)
Tumour suppressors				
Physiological, pathological and biochemical	FBXW7	• Knockout[31,40] (tumorigenesis) • Transgenic[34,36] (tumorigenesis upon expressing FBXW7 mutants)	Mutation[33,164,165]	Cyclin E, MYC, JUN and MCL1
Physiological, pathological and biochemical	FBXO4	Knockout[62,61] (tumour or normal)	Mutation[64]	Cyclin D1 and TRF1
Emerging tumour suppressors				
Biochemical	FBXW8	Knockout[47,48] (embryonic lethal)‡	*CUL7* mutations in 3-M syndrome‡ (REF. 166)	IRS1, TBC1D3 and cyclin D1
Pathological and biochemical	FBXL3	Knockout‡ (REF. 134)	Mutation[167]	CRY
Pathological and biochemical	FBXO1	Knockout[59] (embryonic lethal)‡	Reduced expression[60]	RRM2 and CP110
Pathological and biochemical	FBXO10	None	Associated with breast cancer risk[66–68]	BCL-2
Physiological, pathological and biochemical	FBXO11	Jeff mouse (deaf and otitis media)[46]	Associated with severe otitis media[46] and inactivated in diffuse large-B cell lymphoma[11]	BCL-6 and CDT2
Physiological, pathological and biochemical	FBXO18	*Fbxo18*$^{f/f}$ and *Fbxo18*$^{-/-}$ (impaired mitotic progression following decatenation stress)[168]	Deleted or mutated in melanoma cells[69]	ATF1
Biochemical	FBXO31	Knockout[62] (normal)‡	None	Cyclin D1
Oncogene				
Physiological, pathological and biochemical	SKP2	• Knockout[79,81–83] (tumour resistant) • Transgenic[76–78] (tumorigenesis)	Overexpression[169–171]	p27, p21, RBL2 and FOXO1
Emerging oncogenes				
Pathological and biochemical	FBXO5	Knockout[94] (embryonic lethal)‡	Overexpression[96,97,101]	Inhibitor of APC^{CDH1} and APC^{CDC20}
Pathological and biochemical	FBXO9	None	Mutation[103]	TELO2 and TTI1
Context dependent				
Physiological, pathological and biochemical	β-TRCP1 and β-TRCP2	• Knockout[106,172] (no tumour) • Transgenic[107] (tumorigenesis in certain tissues)	• Overexpression[110,173] (suggests oncogenic role) • Mutation[112,113,174] (suggests tumour-suppressive role)	β-catenin, CDC25A, IκB and DEPTOR
Pathological and biochemical	FBXL10	Knockout‡ (REF. 116)	Overexpression[115]	H2A
Undetermined				

Evidence	F-box protein	Physiological evidence (mouse models for cancerous phenotypes)	Pathological evidence	Biochemical evidence (major substrates)
Pathological	FBXL2	Transgenic‡ (REF. 175)	Overexpression[176]	p85β and APP ‡
Biochemical	FBXL5	Knockout[130,131] (embryonic lethal)‡	None	$p150^{Glued}$ and IRP2
Pathological	FBXL20	Knockout‡ (REF. 177)	Overexpression[132]	Not applicable
Biochemical	FBXL21	Knockout‡ (REF. 134)	None	CRY
Biochemical	FBXO2	Knockout[178] (hearing loss)‡	None	Pre-integrin β1, SHPS1 and NR1
Biochemical	FBXO7	Knockout‡ (REF. 135)	None	CD43
Biochemical	FBXO15	Knockout[142] (normal)‡	None	MDR1
Biochemical and pathological	FBXO32	• Knockout[145] • Transgenic‡ (REF. 145)	Reduced expression[147]	MYOD1 and EIF3F
Biochemical	FBXO45	Knockout[151] (embryonic lethal)‡	None	p73 and UNC13A

APC, anaphase promoting complex; APP, amyloid-β A4 protein; ATF1, activating transcription factor 1; β-TRCP, β-transducin repeat-containing protein; BCL, B cell lymphoma; CP110, centrosomal protein of 110 kDa; CRY, cryptochrome; *CUL7*, cullin 7; DEPTOR, DEP domain-containing mTOR-interacting protein; EIF3F, eukaryotic translation initiation factor 3 subunit F; FBXL, F-box and leucine-rich repeat protein; FBXO, F-box only; FBXW, F-box/WD repeat-containing protein; FOXO1, forkhead box protein O1; H2A, histone H2A; IκB, inhibitor of nuclear factor-κB; IRP2, iron regulatory protein 2; IRS1, insulin receptor substrate 1; MCL1, myeloid cell leukaemia 1; MDR1, multidrug resistance protein 1; MYOD1, myoblast determination protein 1; NR1, N-methyl-d-aspartate receptor subunit NR1 (also known as NMDAR1); RBL2, retinoblastoma-like protein 2; RRM2, ribonucleotide reductase small subunit; SHPS1, SHP substrate 1; SKP2, S-phase kinase-associated protein 2; TBC1D3, TBC1 domain family member 3; TELO2, telomere maintenance 2; TRF1, telomeric repeat-binding factor 1; TTI1, TELO2-interacting protein 1; UNC13A, protein UNC-13 homologue A.

*Indicates emerging tumour suppressors or oncoproteins.

‡Although data are shown in these cells, they are not supportive of a role in cancerous phenotypes.

Table 2

Summary of mouse models for F-box proteins with characterized or emerging tumour-suppressive functions

F-box protein	Knockout mouse models		Transgenic mouse models
	Whole body	Tissue specific	Tissue specific
Characterized tumour suppressor			
FBXW7	• *Fbxw7*$^{+/-}$; radiation-induced tumorigenesis[40] • *Fbxw7*$^{-/-}$ *Trp53*$^{-/-}$; colorectal cancer[182] • *Fbxw7*$^{+/-}$ *Apc*$^{+/-}$; impaired intestinal and neural stem cell differentiation[185] • *Fbxw7*$^{+/-}$ *Trp53*$^{+/-}$; tumours in epithelial tissues[40] • *Fbxw7*$^{+/-}$ *Pten*$^{-/-}$; accelerated tumour formation[186]	• Gut; intestinal tumour[179] • Cerebellar anlage; decreased cerebellar size and defects in axonal arborization[181] • Brain; die after birth[183,184] • Liver; hepatomegaly and steatohepatitis[39] • Intestine; impaired progenitor cell differentiation[35] • Haematopoietic stem cells; defective maintenance of quiescence[32] • Haematopoietic tissue; T-ALL[31] • T cell; thymic lymphoma[30] • Leukaemia-initiating cells; abrogation of quiescence[163] or tumour inhibition[162]	• Whole body; *Fbxw7*^{R482Q}; die perinatally[180] • Whole body; *Fbxw7*^{R468C}; T-ALL[34] • Intestine; *Fbxw7*$^{fl(R482Q)/+}$; intestinal tumour[36]
Emerging tumour suppressors			
FBXO11	*Fbxo11*^{mut}; reduced weight, deafness and otitis media in Jeff mouse[46]	None available	None available
FBXW8	*Fbxw8*$^{-/-}$; smaller littermates[48]; abnormal placenta[47]	None available	None available
FBXL3	*Fbxl3*$^{-/-}$; longer period[134]	None available	None available
FBXO1	*Fbxo1*$^{-/-}$; embryonic lethal[59]	Various tissues; normal[59]	None available
FBXO4	• *Fbxo4*$^{+/-}$ or *Fbxo4*$^{-/-}$; lymphomas, histiocytic sarcomas, mammary and hepatocellular carcinomas[61] • *Fbxo4*$^{-/-}$; viable and normal[62]	None available	None available
FBXO18	*Fbxo18*$^{f/f}$ and *Fbxo18*$^{-/-}$; impaired mitotic progression following decatenation stress[168]	None available	None available

Apc, adenomatous polyposis coli; FBXL, F-box and leucine-rich repeat protein; FBXO, F-box only; *Fbxw*, F-box/WD repeat-containing protein; T-ALL, T cell acute lymphoblastic leukaemia.

Table 3

Summary of mouse models for F-box proteins with characterized or emerging oncogenic functions

Knockout mouse models	Transgenic mouse models	
Whole body	**Whole-body expression**	**Tissue-specific expression**
Characterized oncogene: SKP2		
• *Skp2*$^{-/-}$; smaller littermates[187]; inhibited skin tumorigenesis[83]; hypoinsulinaemia, and glucose intolerance[188]; protected mice from the development of obesity[189]; massive apoptosis[187] in spermatogenic cells[190]; hepatocytes increased in size[191] • *Pten*$^{+/-}$ *Skp2*$^{-/-}$ and *Arf*$^{-/-}$ *Skp2*$^{-/-}$; senescence* (REF. 79) • *Rb1*$^{+/-}$ *Skp2*$^{-/-}$; no tumour* (REF. 82) • *Skp2*$^{-/-}$ *Cdkn1b*$^{-/-}$; skin tumour* (REF. 83); nephropathy* (REF. 196) • *Pten*$^{+/-}$ *Skp2*$^{-/-}$; normal HSC quiescence* (REF. 197) • *Skp2*$^{-/-}$ *Pomc*-Cre *Rb1*$^{lox/lox}$ *Trp53*$^{lox/lox}$; no tumour* (REF. 81) • *Skp2*$^{-/-}$ *Pomc*-Cre *Rb1*$^{lox/lox}$ *Cdkn1b*$^{-/-}$; Pituitary tumour* (REF. 81) • *Skp2*$^{-/-}$ *MMTV-Neu*; delayed breast tumour formation* (REF. 80)	• BCR–ABL-infected *Skp2*$^{-/-}$ marrow; myeloproliferative syndrome[192] • *Cdkn1b*^{T187A} *Skp2*$^{-/-}$; short period in G1[194] • K5-*Myc Skp2*$^{-/-}$; tumorigenesis[195]	• B cells; *Skp2*; no tumour[193] • Mammary glands; *MMTV-Skp2b*; breast tumour[78] • Prostate; *Skp2*; prostate cancer[76] • T lymphoid; *Skp2* and/or *Nras*; T cell lymphomas[77]
Emerging oncogene: FBXO5		
Fbxo5$^{-/-}$; embryonic lethal[94]; role in tumorigenesis is not determined	None available	None available

Cdkn1b, cyclin-dependent kinase inhibitor 1B, which encodes p27; FBXO, F-box only; HSC, haematopoietic stem cell; K5, keratin 5 promoter; *MMTV*, mouse mammary tumour virus promoter; *Pomc*, pro-opiomelanocortin; *Rb1*, retinoblastoma 1; *Skp2*, S-phase kinase-associated protein 2.

* Compound mouse models.

Table 4

Summary of mouse models for F-box proteins with context-dependent or undetermined roles in cancer

F-box protein	Whole-body knockout mouse models	Tissue-specific transgenic mouse models
Context dependent		
β-TRCP1	*Btrcp1*$^{-/-}$; viable and fertile (male)[105]; reduced fertility (male)[106]; abnormal retinal development[198]	Mammary glands; human *BTRCP1*; hypoplastic phenotype[107]
β-TRCP2	Inducible β-TRCP2 shRNA in *Btrcp1*$^{-/-}$ mice; severe testicular defect[172]	Epidermis; dominant-negative *Btrcp2*; decreased UVB-induced oedema and hyperplasia[108]
Undetermined		
FBXL5	• *Fbxl5*$^{-/-}$; die *in utero*[130,131] • *Fbxl5*$^{+/-}$; normal[130]	None available
FBXL21	*Fbxl21*$^{-/-}$; compromised daily activities[134]	None available
FBXO7	*Fbxo7*$^{-/-}$; increased pro-B cell and pro-erythroblast populations[135]	None available
FBXO15	*Fbxo15*$^{-/-}$; normal[142]	None available
FBXO45	*Fbxo45*$^{-/-}$; embryonic lethal[151]	None available

β-TRCP1, β-transducin repeat-containing protein 1; FBXL, F-box and leucine-rich repeat protein; FBXO, F-box only; shRNA, short hairpin RNA; UVB, ultraviolet B.