

# Function and regulation of F-box/WD repeat-containing protein 7 (Review)

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**Abstract.** The ubiquitin-proteasome system is an important post-translational modification system involved in numerous biological processes, such as cell cycle regulation, gene transcription, signal transduction, apoptosis, differentiation and development. F-box/WD repeat-containing protein 7 (FBXW7) is one of the most studied F-box (FBX) proteins, serving as substrate recognition component of S phase kinase-associated protein 1-Cullin 1-FBX protein complexes. As a tumor suppressor, FBXW7 recognizes numerous proto-oncoproteins and promotes their ubiquitination and subsequent proteasomal degradation. FBXW7 is regulated at different levels, leading to tunable and specific control of the activity and abundance of its substrates. Therefore, genetic mutations or decreases in its expression serve an important biological role in tumor development. In-depth studies and identification of additional substrates targeted by FBXW7 have suggested a signaling network regulated by FBXW7, including its tumor-inhibitory role. The present review focused on the role of FBXW7 in tumor suppression and its application in cancer therapy.

3. Post-translational modifications of FBXW7
4. Genetic alterations of FBXW7 in cancers
5. Substrates and mechanisms of FBXW7 involved in tumor suppression
6. Therapeutic exploitation of FBXW7 signaling
7. Conclusions

## 1. Introduction

Numerous cell signaling pathways are regulated by the ubiquitin-proteasome system. Ubiquitination of the target protein generally requires the joint action of three enzymes. First, E1 activates the ubiquitin molecule; E2 then binds the ubiquitin molecule; and finally, an E3 ubiquitin ligase binds to a specific substrate and E2 covalently transfers the ubiquitin protein to one or more lysine residues of the target protein (1-3). Thousands of ubiquitination processes are ongoing in a cell at any moment (4). The high specificity of this degradation mechanism targeting specific proteins is determined mainly by the E3 ubiquitin ligase (5). Therefore, the E3 ligase plays a key role in degradation of the target protein (6). E3 ubiquitin ligases with a RING domain comprise a large group of E3 ubiquitin ligases that is responsible for ~20% of ubiquitin-mediated protein degradation (6); additionally, degradation events regulated by Cullin RING ligases (CRLs), which are composed of Cullin (Cul) proteins, account for a large proportion (7). Previous studies have shown that CRLs not only are the primary factors in protein degradation but also are involved in the initiation and progression of some cancers including colorectal cancer, cholangiocarcinoma, etc (8,9).

The Cul protein family was first reported in 1996 to form an active complex that can regulate the cell cycle (10). The human genome contains 7 Cul proteins, including, Cul1, Cul2, Cul3, Cul4A, Cul4B, Cul5, Cul7 and Cul9 (11). All six have conserved, homologous Cul domains, which function to bind the subunit RING domain proteins RING-box protein 1 (Rbx)1 or Rbx 2 in the whole complex. In addition to Cul7 and Cul9, five other members have three serial N-terminal Cul repeats, which are used to recognize subunits in the E3 ubiquitin ligase complex. Since it was discovered and defined

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nearly 20 years ago, subsequent studies have revealed that the multipart E3 ligase complex consisting of this family of proteins has an intricate structure and serves important functions in the cell cycle, signal transduction, cell development and other physiological processes (12,13).

Among the CRLs, CRL1 [also known as S phase kinase-associated protein 1-Cul1-F-box (SCF)] has been extensively studied. The SCF complex is composed of Cul1, Rbx1, the linker protein Skp1 and variable F-box (FBX) proteins. Within the SCF structure, differences in the FBX proteins, determines the specificity of substrate binding to accomplish the degradation of different substrates. To date, 69 FBX proteins have been found and identified in the human genome, and most can form the CRL1 complex (14). FBX proteins can be roughly divided into three types according to the other domains present: i) FBXW, which contain a tryptophan-aspartic acid 40 (WD40) repeat domain; ii) FBXL, which contain a leucine-rich repeat domain; and iii) FBXO, which contain other domain motifs. Accumulating evidence has indicated that dysregulation of FBX proteins is involved in the development, angiogenesis, proliferation and metastasis of a number of malignancies (9). F-box/WD repeat-containing protein 7 (FBXW7) is also called AGO, hCDC4 and SEL-10; SEL-10 was first identified from yeast, AGO was first found in *Drosophila*, and CDC4 is a yeast gene (15). The SCF E3 ubiquitin ligase complex contains FBXW7 which targets several important oncoproteins including c-Jun, c-Myc and Notch1 etc. for ubiquitylation (Fig. 1A). The human FBXW7 gene is located on chromosome 4 (4q31.3) and is mainly expressed in the brain, heart and testes, but at lower levels in the liver, lungs and kidneys (16).

The FBXW7 gene produces three mRNA transcripts (FBXW7 $\alpha$ , FBXW7 $\beta$  and FBXW7 $\gamma$ ) through selective splicing following transcription. The tissue expression and subcellular localization differ among the three subtypes; FBXW7 $\alpha$  mRNA is expressed in almost all tissues, FBXW7 $\beta$  mRNA can be detected only in the brain and testes, and FBXW7 $\gamma$  mRNA mainly exists in the heart and skeletal muscle (17). FBXW7 $\alpha$  and FBXW7 $\beta$  are localized in the cytoplasm, whereas FBXW7 $\gamma$  is in the nucleolus (18). FBXW7 $\alpha$  plays the most important role among the three subtypes and can occasionally replace the other two (19). However, the  $\beta$  and  $\gamma$  subtypes may also play unique roles under specific conditions, as their abnormal localization may cause cell functional defects (20). The human FBXW7 $\alpha$  contains 707 amino acid residues. The N-terminus of FBXW7 $\alpha$  contains an FBX motif composed of ~40 amino acids, which mainly contributes to ubiquitin ligase activity (21). In addition, FBXW7 $\alpha$  has a seven-tandem repeat WD40 domain that is necessary for substrate recognition, and a dimerization domain that is associated with the binding of FBXW7 to the substrate (Fig. 1B). FBXW7 recognizes and binds substrates with the specific phosphorylated amino acid sequence, (L)-X-pT/pS-P-(P)-X-pS/pT/E/D (where X can be any amino acid residue) (22). In numerous cases, the conserved Cdc4 phosphodegron (CPD) motif of FBXW7 substrates is phosphorylated by glycogen synthase kinase 3 (GSK3), which initiates FBXW7-mediated substrate ubiquitination (23,24).

FBXW7 is generally recognized as a cancer suppressor that regulates the ubiquitination and degradation of various proliferation- and survival-related proteins, including cyclin E (25), Notch (15), c-Myc (26) and mammalian target of

rapamycin (mTOR) (27). The mutation rate of FBXW7 in colorectal cancer (28), breast cancer (29) and other malignant tumors is ~10%, whereas in some types of leukemia, the mutation rate is as high as 60% (30). Moreover, the mutational status of FBXW7 is directly related to the therapeutic effect of chemotherapeutic drugs (31). In addition, FBXW7 plays a role in embryonic development, as mice with FBXW7 knockout mutation die on day 11, the middle stage of embryonic development, mainly because of a developmental deformity of the digestive organs (32). FBXW7 also serves an important role in the proliferation and maturation of stem cell populations (33).

## 2. Transcriptional and translational regulation of FBXW7 expression

**Transcriptional regulation.** On the one hand, FBXW7 affects the ubiquitination state and protein content of various substrates; on the other hand, this process is tightly regulated from DNA to protein expression (Fig. 1B) (21). FBXW7 expression can be regulated at a number of levels, including at the transcriptional level through the regulation of multiple transcription factors. FBXW7 $\alpha$  is negatively regulated by CCAAT/enhancer-binding protein  $\delta$  (CEBP $\delta$ ), a transcription factor involved in adipocyte differentiation as well as inflammation reactions (34). In mammary tumors, CEBP $\delta$  expression is induced under a hypoxic microenvironment, which can lead to tumor metastasis by directly binding to the promoter of FBXW7 to inhibit its expression (35,36). Additionally, presenilin, a regulator of Notch processing and  $\beta$ -catenin signaling, can also indirectly downregulate FBXW7 $\alpha$  mRNA expression (37). FBXW7 $\beta$  and FBXW7 $\gamma$  are upregulated in a p53-dependent manner, and their upregulation is required for the apoptotic response and tumor suppression induced by p53 (38).

**Translational regulation.** A number of previous studies have indicated that the direct binding of multiple non-coding microRNAs (miRNAs) to the 3' untranslated region of the mRNA can prevent protein translation of FBXW7 (39,40). Overexpression of miRNA (miR)-223 in T cell acute lymphoblastic leukemia (T-ALL), colorectal cancer and gastric cancer has been reported to downregulate FBXW7 (39,40). In T-ALL, high levels of miR-223 promote the proliferation of tumor cells, and its inhibition increases the sensitivity to  $\gamma$ -secretase inhibitor drugs (39). FBXW7 is also repressed by miR-27a overexpression in an adenomatous polyposis coli protein (APC) mutation-mediated murine model of colorectal adenocarcinoma and in human high-grade colorectal adenocarcinomas associated with preinvasive adenomas (41,42). Inhibition of miR-27a increases FBXW7 expression and downregulates FBXW7 substrates, which delays tumor formation in model systems and inhibits the proliferation of colorectal cancer cells (43). miR-92a is reported to decrease the expression levels of FBXW7 mRNA and protein, and to increase c-Myc expression, which facilitates Myc-mediated apoptosis and proliferation in a model of B-cell lymphoma (44). Knockdown of miR-92a suppresses cancer cell invasion and proliferation through the upregulation of FBXW7 (45).

Additional miRNAs, including miR-548, miR-544a, miR-367, miR-182, miR-503, miR-155-3p and miR-32, have also been demonstrated to modulate FBXW7 activity via

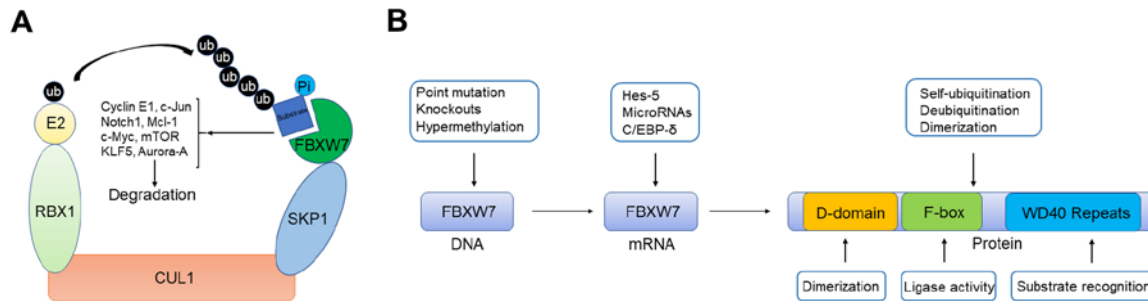


Figure 1. Schematic illustration presenting the functional model and regulation of FBXW7. (A) Functional model of FBXW7. Schematic illustration showed that SCF E3 ubiquitin ligase complex containing FBXW7 can target several important oncoproteins including c-Jun, c-Myc, and Notch1 *et al* for ubiquitylation. (B) Regulation of FBXW7 from gene to protein. FBXW7 contains a D-domain for dimer formation and two key domains of F-box protein family including WD repeat and F-box for substrate binding and enzymatic activity. Regulatory mechanisms and related proteins that induce FBXW7 dysfunction at different levels. FBXW7, F-box/WD repeat-containing protein 7; RBX1, RING-box protein; KLF5, Krüppel-like factor 5; E2, enzyme 2; ub, ubiquitin; Pi, phosphate; SKP1, S phase kinase-associated protein 1; CUL1, cullin 1; Hes-5, hairy and enhancer of split 5; C/EBP- $\delta$ , CCAAT enhancer binding protein  $\delta$ .

various mechanisms (46-51). Although individual miRNAs are weakly related to FBXW7 expression in patients with cancer, suggesting that more than one miRNA is involved in the regulation of FBXW7, miRNA-mediated suppression of FBXW7 synchronously targets the three FBXW7 isoforms, which may influence additional FBXW7 substrates (52).

**Epigenetic regulation.** The transcription and translation of FBXW7 are regulated by epigenetics; specifically, histone modifications have been reported to regulate FBXW7. For example, the histone methyltransferase enhancer of zeste homolog 2 (EZH2), is associated with epigenetic inactivation of genes, including FBXW7; EZH2 promotes trimethylation of histone H3 Lys27 residue of FBXW7, which results in inactivation of FBXW7 gene function (53). Notably, FBXW7 has been reported to target EZH2 for ubiquitination and degradation in pancreatic cancer cells and to be negatively associated with the expression of EZH2 in human pancreatic cancer samples (54). In addition to histone modifications, DNA modifications are reported to regulate FBXW7 expression. In contrast to FBXW7 $\alpha$ , which exhibits ubiquitous expression in cell lines and a broad tissue distribution, FBXW7 $\beta$  is expressed in specific cell lines and tissues (55). Histone and DNA modifications have been reported to epigenetically regulate the FBXW7 $\beta$  promoter, which is methylated in 51% of primary breast cancer tumors and 43% of 60 human cancer cell lines originating from brain, breast, kidney, prostate, cervix, blood, skin, lung, thyroid and bone (56). The expression of the FBXW7 $\beta$  gene is negatively associated with its methylation level (56). Patients with lymph node-positive breast cancer with higher FBXW7 methylation levels have longer overall survival times, although methylation of FBXW7 correlates with high-grade tumors (56). In addition, patients with ovarian cancer with p53 mutations have been reported to exhibit lower FBXW7 expression compared with those with wild-type p53, and hypermethylation of the FBXW7 promoter associated with mutations in p53 leads to decreased FBXW7 expression (57).

### 3. Post-translational modifications of FBXW7

**Autoubiquitination.** Post-translational modifications of FBXW7 are involved in its autoubiquitination, deubiquitination,

dimerization and localization. In addition to targeting substrates for ubiquitination and degradation, FBXW7 can also be regulated by autoubiquitination. Peptidyl-prolyl *cis-trans* isomerase NIMA-interacting 1 (Pin1) has been reported to destabilize and downregulate FBXW7 by mediating a decrease in dimerization and promoting FBXW7 self-ubiquitination and degradation (58). In addition, phosphorylation at Thr205 by extracellular signal-regulated kinase and at Ser176 by polo-like kinase 2 leads to destabilization and degradation (59,60). By contrast, phosphorylation mediated by serum- and glucocorticoid-inducible kinase 1 (SGK1) and phosphoinositide 3-kinase at Ser227 stabilizes FBXW7 but increases ubiquitination of cyclin E, Notch and Myc (53,54). FBXW7 stability can also be controlled by SCF-dependent mechanisms. For instance, COP9 signalosome complex subunit 6, a member of the COP9 signalosome complex, increases FBXW7 autoubiquitination and proteasome-mediated degradation by regulating Cull1 neddylation (61).

**Deubiquitination.** Autoubiquitination and degradation of FBXW7 can be reversed by the deubiquitinating enzyme 28 (USP28). Overexpression of USP28 not only allows the degradation of several FBXW7 substrates, inhibits progenitor cell proliferation and delays tumor formation (62), but also represses autocatalytic ubiquitination of FBXW7 (63). Genetic ablation of USP28 negatively regulates FBXW7 and its substrates in the pancreas, liver and lungs. In addition, abrogation of USP28 facilitates the transformation of mouse fibroblasts due to FBXW7 destabilization (63).

**Dimerization.** FBXW7 is characterized by its ability to form dimers through a conserved D domain. Previous studies of endogenous FBXW7 with mutations preventing dimer formation have revealed that dimerization facilitates ubiquitination of FBXW7 substrates with low-affinity degrons, but it is unimportant to substrates with high-affinity degrons, including cyclin E and Myc (64,65). Dimer-deficient mutants of CDC4 in yeast variant show increased autocatalytic ubiquitination and instability *in vivo* (66). In addition, isomerization mediated by Pin1 and phosphorylation of human FBXW7 at Ser205 repress its dimerization and enhance its autoubiquitination (58).

**Location.** Abnormal cellular distribution of FBXW7 impairs its interaction with substrates. For example, nucleophosmin is necessary for the nucleolar localization of FBXW7 and is often mutated in acute myelogenous leukemia, leading to FBXW7 instability and an increase in c-Myc expression (67). Additionally, phosphorylation of FBXW7 $\alpha$  at Ser10 inhibits one of its nuclear localization signals (68).

#### 4. Genetic alterations of FBXW7 in cancers

The important role of FBXW7 in tumor suppression has been confirmed by different genetic alterations of FBXW7 in various human cancers. Functional silencing of FBXW7 by deletion, mutation and hypermethylation ultimately leads to tumorigenesis and cancer progression (69). Although rare, FBXW7 mutations occur often in breast, pancreatic, gastric and cervical cancers but rarer promoter hypermethylation and genetic deletion of FBXW7 occurs in bladder, cervical and breast cancers (65). However, missense point mutations are the most common type of genetic alterations in FBXW7 and are observed on three arginine residues (R465, R479 and R505) at the  $\beta$  propeller (65). FBXW7 is usually expressed as a functional wild-type protein because the second wild-type allele is retained. Consistent with this observation, mouse models with monoallelic deletion of FBXW7 show a milder tumor phenotype compared with biallelic gene deletions (70). Therefore, biallelic FBXW7 mutations are assumed to silence the function of the wild-type protein as dominant negative alleles (65).

In the intestine and the hematopoietic system, knock-in mice with a heterozygous FBXW7 mutation show accelerated tumorigenesis compared to mice with heterozygous wild-type FBXW7 (FBXW7<sup>+/+</sup>) (70,71). The hematopoietic stem cells in FBXW7<sup>Mut/+</sup> mice show a significant increase in Myc but do not exhibit the characteristic hyperproliferative phenotype of those in FBXW7<sup>-/-</sup> animals (70). Mice with T-cell leukemias induced by an activated Notch allele show accumulation of sterol regulatory element-binding protein 1 and Myc (70). Moreover, additional depletion of p53 does not promote the onset of disease, indicating the functional difference between complete loss and mutation of FBXW7 (70). By contrast, the observation that only Krüppel-like factor 5 (KLF5) and homeobox protein TGIF1 (TGIF1), instead of most tested FBXW7 substrates, are substantially affected by mutated FBXW7, reveals that the influence of FBXW7 mutations on substrate regulation is strongly context dependent (71). Although FBXW7 mutations enhance intestinal tumorigenesis driven by the multiple intestinal neoplasia, a mutant allele of APC, the levels of Notch, Jun and Myc remain normal in these mice with developing adenomas (71). Therefore, heterozygous mutations in FBXW7 may promote tumorigenesis by the regulation of non-canonical substrates such as TGIF1 and KLF5.

#### 5. Substrates and mechanisms of FBXW7 involved in tumor suppression

Ubiquitin ligases play a biological role by affecting the expression levels of specific target proteins. The most common mechanism by which ubiquitin ligases participate in cancer processes is through regulating the content of cell

cycle-related factors. FBXW7 can effectively recognize a variety of substrates, such as cyclin E, KLF5, mTOR, Aurora A, c-Myc, c-Jun and induced myeloid leukemia cell differentiation protein Mcl-1 (MCL-1). Most of these protein substrates are involved in the regulation of cell cycle processes or homeostasis. Additionally, they are the expressed products of oncogenes or potential oncogenes (Fig. 2; Table I).

**Cyclin E.** Cyclin E binds and activates the cell cycle-dependent protein kinase cyclin-dependent kinase 2 (CDK2) to promote transition of the cell cycle from G<sub>1</sub> to S phase (72). Increased expression of CDK2 can cause chromosomal instability and accelerate the occurrence of cancer (73). Cyclin E is expressed mainly in tissues exhibiting vigorous cell division, in direct contrast with FBXW7, which is expressed mainly in non-proliferative tissues (74). Cyclin E has a typical CPD, in which phosphorylation of Thr380 and Ser384 is necessary for degradation (32). FBXW7 can catalyze the degradation of cyclin E2 in addition to E1, the most common cyclin E subtype. Cyclin E2 contains two typical CPDs, that are specifically recognized by FBXW7, and the double-site phosphorylation of Thr392 and Ser396 initiates ubiquitination and proteasomal degradation (74), explaining why the decrease in FBXW7 expression and the increase in cyclin E in primary invasive breast cancer lead to the appearance of a large number of cells with chromosomal polyploidy (75).

**Aurora A.** Aurora A (also known as serine/threonine protein kinase 15) serves a role in mitosis and meiosis by regulating the phosphorylation of specific substrates, and its activity is the highest during G<sub>2</sub>/S phase transition (76). Aurora A is overexpressed in a variety of tumors, resulting in abnormal centrosome expansion, increased chromosomal instability and eventual carcinogenic transformation (76,77). FBXW7-deficient HCT116 and HeLa cells exposed to vincristine, paclitaxel and spindle toxin exhibit extensive mitotic delay and nuclear duplication, which are important causes of polyploidy. Loss of FBXW7 can increase the content of cyclin E and Aurora A, but the increase of single cyclin E or Aurora A could not cause drug-induced polyploidy. This observation shows that the increase in cyclin E and Aurora A is a cause of polyploidy (78).

**Notch.** Notch is a highly conserved signaling system in multicellular organisms, playing an important role in cell proliferation, differentiation and apoptosis (79). In mammals there are four isoforms of Notch receptors, all of which are single-pass transmembrane receptors in which the N-terminus is located outside the cell and accounts for most of the structure and only a small part (C-terminus) is inside the cell (80). When a ligand binds to the extracellular domain of the Notch receptor, it can induce a proteolysis reaction and release the intracellular domain (81). The intracellular domain of the Notch receptor acts as a transcription factor to regulate the expression of specific genes (81). Overactivation of the Notch signaling pathway can cause abnormal cell proliferation and cancer (82). Ubiquitination of the intracellular domain of Notch 1 and Notch 4 by FBXW7 significantly weakens Notch signal transduction, whereas inhibition of FBXW7 enhances Notch-mediated activation of downstream signaling (83).

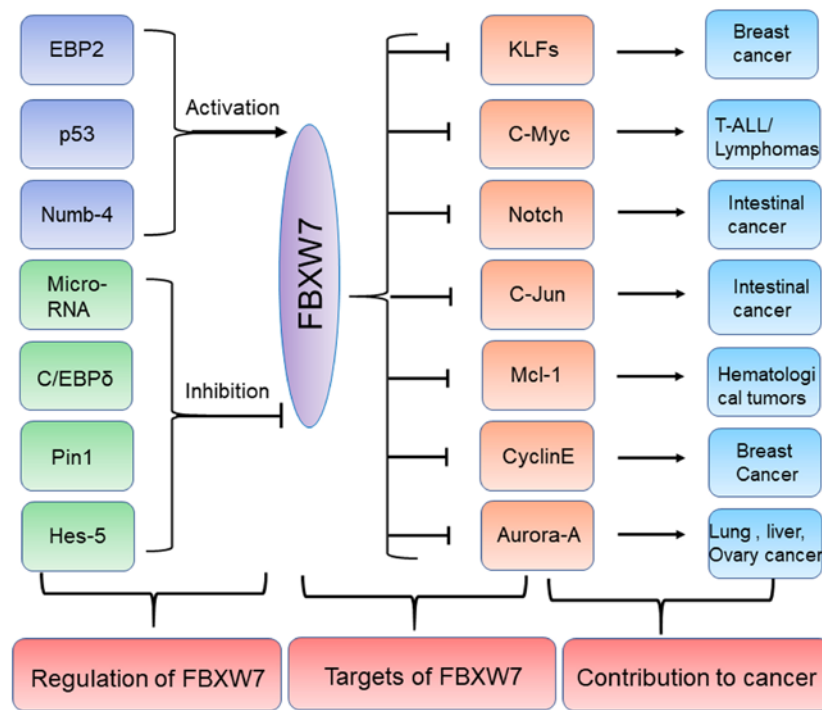


Figure 2. Regulators and substrates of FBXW7 in human cancers. Several upstream proteins including EBP2, p53 and Numb-4 can positively regulate FBXW7 while other upstream regulators including Pin1, Hes-5 and C/EBPδ etc. can negatively regulate FBXW7. The specific substrates of FBXW7 including c-Myc, c-Jun and Mcl-1 can promote development of some tumors including lymphomas, intestinal cancer and hematological tumors. FBXW7, F-box/WD repeat-containing protein 7; EBP2, eIF4E-binding protein 2; C/EBP-δ, CCAAT enhancer binding protein δ; Pin1, peptidyl-prolyl *cis-trans* isomerase NIMA-interacting 1; Hes-5, hairy and enhancer of split 5; KLFs, Krüppel-like factor; T-ALL, T cell acute lymphoblastic leukemia; Mcl-1, induced myeloid leukemia cell differentiation protein Mcl-1. Numb-4, NUMB endocytic adaptor protein.

FBXW7 gene knockout results in the abolishment of Notch 4 signal transmission, which can lead to abnormal development of blood vessels in mouse embryos and may result in death at ~11 days (84). Similarly, mice with brain-specific FBXW7 knockout succumb after birth owing to the accumulation of Notch 1 and Notch 3 proteins in the brain, which results in increased expression of target genes and abnormal differentiation of neural stem cells, causing abnormal brain development and morphology in these mice (85).

**MCL-1.** MCL1 is an antiapoptotic protein of the BCL2 family that can promote cancer development by reducing apoptosis (86). A number of hematological tumors, such as B-cell lymphoma and chronic myeloid leukemia, exhibit an abnormal increase in MCL1 expression, which is considered an important cause of chemotherapeutic resistance. In normal cells, the half-life of the MCL1 protein is short, and it is easily degraded by ubiquitination modification. However, in tumor cells, the MCL1 protein content is increased, although a detailed understanding of this mechanism is lacking. MCL1 can be phosphorylated by GSK3, which initiates ubiquitination and degradation of MCL1 (86). Loss of FBXW7 in a human T-ALL cell line is accompanied by an increase in MCL1 content. This cell line is sensitive to a variety of kinase inhibitors, such as sorafenib but is resistant to ABT-737, an inhibitor of BCL2 (22). When FBXW7 function is restored or MCL1 is lost, sensitivity to ABT-737 can be restored. Therefore, these findings confirm that the increase in the MCL1 protein content caused by FBXW7 deficiency is a mechanism underlying tumor chemoresistance (22). In addition, paclitaxel treatment

induces phosphorylation modification of MCL1, which can be recognized by FBXW7, leading to MCL1 ubiquitination and degradation, consequently increasing apoptosis (31). By contrast, when FBXW7 is inactivated or expression of FBXW7 is decreased, the protein stability of MCL1 is increased. Correspondingly, resistance to microtubule-targeted drugs, such as paclitaxel, is increased in tumor patients, and the its chemotherapeutic effect is significantly reduced (31).

**c-Myc.** c-Myc is an important oncogenic protein that can regulate cell growth and division, serving a number of roles in human cancer. In lymphoid tumor cell lines, mutation of the Thr58 site in c-Myc is the most frequent mutation and results in the failure of FBXW7 to regulate c-Myc protein content, accumulation of c-Myc protein and eventual tumor development (87). In addition to GSK3, NEMO-like kinase (NLK) also regulates the c-Myc protein content. NLK can directly bind to c-Myc and catalyze the phosphorylation of multiple C-terminal sites. This modification promotes ubiquitination and proteasomal degradation of FBXW7. Mutation of these sites can abrogate the c-Myc-FBXW7 interaction and protein ubiquitination (88).

Abnormal localization of c-Myc proteins can cause its accumulation and may lead to tumorigenesis (63). USP28 can bind to FBXW7 and inhibit ubiquitination modification of c-Myc by the latter, thus increasing the protein stability of c-Myc (89). DNA damage caused by ultraviolet radiation can reduce c-Myc protein content due to dissociation of USP28 and FBXW7, thus increasing FBXW7-mediated ubiquitination and degradation of c-Myc protein. Because both cyclin E

Table I. Substrates targeted by F-box/WD repeat-containing protein 7.

Author, year	Substrate	Genomic location	Function/signalling pathways	Molecular size (amino acids)	Phospho-degron (phosphorylation sites)	Putative modifying kinase	(Refs.)
Finkin <i>et al</i> , 2008	Aurora-A	20q13	Protein kinase; cell cycle	403	LGTVYREL	GSK3	(78)
Klotz <i>et al</i> , 2009	Cyclin E1	19q12	Cyclin, cell cycle	410	LLTPPQSG	GSK3	(74)
Tsunematsu <i>et al</i> , 2004	Notch1	9q34	Transcription factor; Notch signalling pathway	2,555	FLTPSPES	Cdk8	(84)
Yang-Yen <i>et al</i> , 2006	Mcl-1	1q23	BCL2-family protein; cell survival	350	IMSPEEL, DGSLPSTP	GSK3	(86)
Welker <i>et al</i> , 2004	c-Myc	8q24	Transcription factor; cell proliferation	439	LPTPLSP	GSK3	(87)
Mao <i>et al</i> , 2008	mTOR	1p36	Protein kinase; PI3K signalling pathway	2,549	LLTPSIHL	-	(27)
Liu <i>et al</i> , 2010	KLF5	13q22	Transcription factor; adipocyte differentiation and lipid metabolism	457	PPSPPSSE, LNTPDLDL, NLTPPPSY	GSK3	(92)
Wei <i>et al</i> , 2005	c-Jun	1p32	Transcription factor; cell proliferation	331	GETPPLSP	GSK3	(23)

KLF5, Krüppel-like factor 5; GSK3, glycogen synthase kinase 3; Mcl-1, induced myeloid leukemia cell differentiation protein Mcl-1.

and c-Myc are positive regulators of the cell cycle, decreases in their levels can cause cell cycle exit. However, FBXW7 deficiency increases the protein levels of these two factors and promotes cell cycle re-entry and G<sub>1</sub>/S phase transition, which is conducive to cell division (90). This mechanism is an important reason for cancer driven by FBXW7 mutations.

**mTOR.** mTOR is a protein kinase that promotes cell growth and division by regulating protein synthesis and cell autotrophism. The increase in mTOR content and activity is a common feature of tumorigenesis, and mTOR is widely used as a drug target in tumor therapy (27). In human breast cancer cell lines and patients with primary breast cancer, FBXW7 can contain a gene deletion or a functional inactivation mutation, which leads to an increase in the mTOR protein content and activation of its downstream signaling pathway (27). The sensitivity of breast cancer cells harboring wild-type FBXW7 to rapamycin is significantly increased, which suggests that loss of FBXW7 function is a mechanism underlying the resistance of tumor patients to mTOR pathway inhibitors (27).

**KLF5.** KLF5 is a development-related transcription factor that can promote the expression of multiple development-related genes and may serve a role in cell proliferation, cell cycle, apoptosis and cell migration and differentiation (73). KLF5 is overexpressed in various cancers and can promote proliferation and deterioration of breast cells (91). KLF5 is a short-lived protein that can be rapidly degraded by ubiquitination. KLF5 contains three CPDs recognized by FBXW7; simultaneous mutation of these sites can inhibit the interaction and ubiquitination of FBXW7 and KLF5, whereas the point mutations in FBXW7 can significantly delay degradation of the KLF5 protein, leading to accumulation of KLF5 in cells (92). The binding and ubiquitination of KLF5 and FBXW7 are dependent on phosphorylation of KLF5 at Ser303 by GSK3 $\beta$  (93). In tumor cells, mutation or abnormal activation of FBXW7 results in a decrease in the ubiquitination and the protein degradation of KLF5. In turn, excessive accumulation of KLF5 promotes the occurrence of cancer by increasing the expression of target genes.

## 6. Therapeutic exploitation of FBXW7 signaling

Given the substantial tumor control exhibited by FBXW7 in mouse models, therapeutic exploitation of FBXW7 and its related pathways for several types of cancer has attracted intense interest. Various oncogenic transcription factors and oncoproteins are degraded through FBXW7-mediated ubiquitination. Most substrates with increased levels due to FBXW7 silencing mutations are oncoproteins. Such substrates are likely to alter regulation of the cell cycle, perturb stress response and rewire metabolic pathways (28). Previous studies have reported that the accumulation of c-Myc and MCL1 induce synthetic lethal interactions between non-functional FBXW7 and tumor necrosis factor-like death ligands or mitotic inhibitors in tumor cells (31,94). Similarly, FBXW7 mutant cells with increased c-Myc are more sensitive to suppression of additional enzymes, such as CDK1, and energy-sensing enzymes, such as AMP-activated protein kinase (95,96). Alternatively, post-translational modifications are important for FBXW7 E3 ligase activity, suggesting that aberrant alteration of upstream signaling



might impair the antitumor effect of FBXW7. One possibility is to target deubiquitinases, considering that USP36 and USP28 can antagonize FBXW7 activity. Growth of established tumors can be inhibited by acute decrease of USP28, showing that the development of tumors depends on USP28 (56), and that certain types of tumor could be effectively treated using inhibition of USP28 with small molecules. USP7 inhibitors successfully stabilize p53 and promote apoptosis in myeloma cells that are not sensitive to available therapies (97). Additionally, several studies have demonstrated that loss of p53, or mutation or deletion of FBXW7 promotes tumorigenesis (10,98). Since frequent mutant FBXW7 alleles result in impaired substrate recognition, recovering substrate binding by FBXW7 may effectively inhibit tumor development. This may be potentially achieved in a manner analogous to p53, in which mutant forms of the protein can be reactivated by small molecules (99). Moreover, since mutant FBXW7 has the wild-type allele in most human tumors, several enzymes, such as Pin1 and SGK1, which modulate the activity of FBXW7, may provide a reasonable approach for tumor suppression by increasing FBXW7 activity (53,100). Therefore, restoring the antitumor function of FBXW7 by blocking oncogenic upstream mediators may be an effective therapeutic strategy.

## 7. Conclusions

Ubiquitination of specific proteins plays an important role in tumor initiation, development, metastasis and chemoresistance. Currently, the drugs designed to target ubiquitin-modified molecules exhibit good potential in cancer treatment. FBXW7 has been suggested to have tumor-suppressive effects in various tumors. On the one hand, mutation of FBXW7 or a reduction in its activity promotes the occurrence and progression of tumors; on the other hand, it also increases the chemoresistance of tumors. These developments are important to improving our understanding of tumor occurrence mechanisms, the development of diagnostic reagents, and the optimization and design of therapeutic drugs. In the study of FBXW7, a series of important problems remain to be solved. For example, are there still unidentified FBXW7 substrates? Is there a network mechanism underlying the regulation of multiple substrates by FBXW7? What are the precise mechanisms of FBXW7 regulation? In conclusion, the present review examined the crucial role and molecular mechanism of FBXW7 in tumor inhibition, and may offer a putative therapeutic approach for multiple cancers.

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## Availability of data and materials

Not applicable.

## Authors' contributions

XY, ZZ, XX, SJ and WX were involved in the conception of the review. ZZ, QH, WL and ML were involved in the writing of the article. QS, GF, ZY, and YQ were involved in critically revising and proofreading the manuscript. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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