Regulation mechanism of Fbxw7-related signaling pathways (Review)

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Abstract. F-box and WD repeat domain-containing 7 (Fbxw7), the substrate-recognition component of $SCFF^{bxw7}$ complex, is thought to be a tumor suppressor involved in cell growth, proliferation, differentiation and survival. Although an increasing number of ubiquitin substrates of Fbxw7 have been identified, the best characterized substrates are cyclin E and c-Myc. Fbxw7/cyclin E and Fbxw7/c-Myc pathways are tightly regulated by multiple regulators. Fbxw7 has been identified as a tumor suppressor in hepatocellular carcinoma. This review focused on the regulation of Fbxw7/cyclin E and Fbxw7/c-Myc pathways and discussed findings to gain a better understanding of the role of Fbxw7 in hepatocellular carcinoma.

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Abbreviations: Fbxw7, F-box and WD repeat domain-containing 7; HCC, hepatocellular carcinoma; UPS, ubiquitin-proteasome system; EMT, epithelial-mesenchymal transition; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligases; SKP1, S-phase kinase-associated protein 1; CUL1, cullin 1; RBX1, RING box 1; mTOR, mammalian target of rapamycin; CPD, Cdc4 phosphodegron; GSK3, glycogen synthase kinase 3; NPM, nucleolar protein nucleophosmin; PIK2, Polo-like kinase 2; bHLH, basic helix-loop-helix; bHLH/Zip, basic helix-loop-helix zipper; PP2A, protein phosphatase 2A; BLM, bloom; NEMO, NF-κB essential modulator; CSN, COP9 signalosome

Key words: cancer, tumor suppressor, Fbxw7, cyclin E, c-Myc, hepatocellular carcinoma

1. Introduction

Cancer is considered a terrible disease that leads to a global health threat. Although significant improvements have been made in the management of cancer as well as the comprehension of the molecular mechanisms of neoplasm pathogenesis and progression, cancer remains a common disorder worldwide, accounting for 12.7 million new cancer cases and 7.6 million cancer deaths in 2008, worldwide (1,2). Previous findings have shown that tumorigenesis in humans is a multistep process foundational with mutations which can activate oncogenes and inactivate tumor-suppressor genes. The genetic alterations can result in changes of the proteome, and these changes eventually drive the malignant biological behavior through complex signaling pathways (3-9).

The ubiquitin-proteasome system (UPS), which is responsible for the degradation of >80% of cell proteins, is the main proteolytic mechanisms involved in eukaryocytes (3,4). Most of the proteins involved in cell cycle progression, proliferation and apoptosis are regulated by the UPS (5). Dysregulation of the UPS may contribute to tumor progression, drug resistance and altered immune surveillance (5). Previous studies have focused on the relationship between epithelial-mesenchymal transition (EMT) signal transduction pathways and UPS, and found that many signal transducers and transcription factors involved in EMT are regulated by ubiquitination and the UPS (6). It is well-known that the UPS consists of three classes of enzymes: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and ubiquitin ligases (E3). In most cases, the three enzymes together with 26S proteasome drive target substrate degradation through a series of catalytic processes (5,7). In the human genome there are two E1s, ~30-40 E2s and >600 E3 ligases (8). It should be emphasized that during the ubiquitination, E3 mediates the transfer of ubiquitin from E2 to the substrate protein. Thus, it has a central role in the substrate decision (9).

The SCF complexes belong to the cullin-RING ligase family and are the largest family of E3 ligases comprising four subunits: S-phase kinase-associated protein 1 (SKP1), which is responsible for recruiting the variable F-box protein; cullin 1 (CUL1), which provides a rigid scaffold connecting SKP1 and RBX1 on opposite ends; RING box 1 (RBX1; also known as ROC1 or HRT1), which serves as an interface for E2 ubiquitin-conjugating enzymes; and a member of the F-box

protein family, which functions as a substrate-recognition component (10,11). F-box and WD repeat domain-containing 7 (Fbxw7; also known as Fbw7, hCdc4, hAGO and Sel10) is an evolutionarily conserved protein belonging to the F-box family. It was first identified as Cdc4 in budding yeast in 1973 (12). Sel10 was subsequently identified in *Caenorhabditis elegans* as a negative regulator of Lin-12 (homology of Notch) (13). In parallel, *archipelago*, a gene in *Drosophila melanogaster* encoding a protein containing an F-box domain and seven tandem WD repeats (AGO) domain, was identified (14). From those studies, the human homologue was ascertained and was designated as Fbxw7 or Fbw7.

Fbxw7 is thought to be a tumor suppressor involved in cell growth, proliferation, differentiation and survival (15). Fbxw7 has been found to be inactivated by mutation in various human cancer types (16-18). Fbxw7 has an unusual mutation spectrum whereby biallelic, simple loss-of-function mutations are rare. Instead, most mutations are monoallelic missense changes involving specific arginine residues at β-sheet propellor tips that allow the Fbxw7 protein to recognize its substrates (19). Inactivation of the Fbxw7 protein is associated with the deregulation of several well-known oncoproteins with significant capabilities in pathways that manage cell division and growth, including cyclin E (20), c-Myc (21), c-Jun (22), Notch (23), Mcl-1 (24) and mammalian target of rapamycin (mTOR) (25) (Table I).

Therefore, the dysregulation of Fbxw7-mediated proteasome degradation is likely involved in many signaling pathways which play important roles in human cancers. Among these oncogenic Fbxw7 substrates, cyclin E and c-Myc may be the most clearly investigated oncoproteins that have certain contributions to Fbxw7-associated cancers. In this review, we discuss the two major signaling pathways impacted by Fbxw7 to show how Fbxw7-related signaling pathways are regulated in cancer. In addition, since few studies have focused on the relationship between hepatocellular carcinoma (HCC) and Fbxw7, we assess molecular mechanisms by which Fbxw7 exerts antitumor activity in HCC.

2. Fbxw7 and the mechanism of degradation of its substrates

Fbxw7, which shares the WD40 repeats structure (a substrate interaction domain used to classify F-box proteins), is a well-studied member of the F-box family (54). The human *FBXW7* gene consists of 4 introns and 13 exons and its gene locus maps to chromosome region 4q32, which is commonly deleted in many types of human malignancies (17,55). The *FBXW7* gene encodes the Fbxw7α, Fbxw7β and Fbxw7γ protein isoforms, which have distinct subcellular localizations, with Fbxw7α mainly localizing to nucleoplasm, Fbxw7β to cytoplasm and Fbxw7γ to nucleolus (56). The three isoforms also seem to have tissue specificity with the α-form being found to be extensively expressed in human tissues, while the β-form and/or γ-form are present at lower levels, except in skeletal muscle, brain and to a lesser degree, heart (17).

In addition to the N-terminal region which contains dominant signals for the subcellular localization, each isoform of Fbxw7 shares conserved interaction domains in the C-terminal region: the eight WD40 repeats that determine target specificity; the F-box that recruits the SKP1 of the SCF complex; and the D domain that dimerize the SCF^{Fbxw7}, which allows it to target substrates with low-affinity Cdc4 phosphodegron (CPDs) (15). All these domains are essential for the degradation of its substrates.

Although the three Fbxw7 isoforms share many identical functional domains, recent studies have identified that each isoform has its special function. The α-form is most abundant and accounts for degradation of most tested substrates. Fbxw7β is proved to reside in the endoplasmic reticulum membrane and protects cells from oxidative stress (57). Moreover, Fbxw7α and Fbxw7β are found to play an opposite role in their substrates. For example, PGC-1α, a transcriptional coactivator with broad effects on cellular energy metabolism, is found to have a different fate under different Fbxw7 isoforms (52). Fbw7β reduces cellular PGC-1α via ubiquitin-mediated degradation, whereas Fbw7α increases cellular PGC-1α via ubiquitin-mediated stabilization (52).

Evidence suggests that substrates of Fbxw7 are polyubiquitinated in a GSK3-dependent manner by SCF^{Fbxw7} (22,33,58). Glycogen synthase kinase 3 (GSK3), firstly identified in 1980, is a constitutively active and ubiquitously expressed serine/threonine kinase (59,60). Human cells contain two GSK3 isoforms, known as GSK3α and GSK3β, which are highly similar with respect to sequence (share 97% amino acid sequence within their catalytic domains) and function (61). The activation of GSK3 is dominated by phosphorylation on Ser-21 of GSK3α and Ser-9 of GSK3β (62). It is well established that these phosphorylations are regulated via PI3K/Akt pathway (63). In most cases, inactivation of the PI3K/Akt pathway leads to dephosphorylation of GSK3, which results in the activation of GSK3. The activated GSK3 subsequently phosphorylates the CPDs of the substrates that are primed by phosphorylation at position +4 of the CPD by a yet to-be-identified kinase (in the cyclin E case, which is Cdk2) (33,64). The CPDs of substrates are recognized and interacted with the eight WD40 repeats of Fbxw7 for ubiquitin-mediated proteolytic degradation (65).

CPD is a phosphodegron motif existing in the substrates of Fbxw7. The phosphorylation of CPD by GSK3 is crucial for the interaction between Fbxw7 and its substrate. Mutation of critical residues within the CPD lead to stabilization of substrates, such as c-Myc and has been observed in many human cancers (66). Data gathering from Fbxw7 substrates have ascertained the conserved CDP sequence as ΦXΦΦΦ-T/S-PPX-S/T/E, with Φ standing for a hydrophobic residue and X for any amino acid (65,67). The T/S residue can be phosphorylated by GSK3 and the phosphorylation of S/T/E residue serves as a priming signal for GSK3 phosphorylation. Moreover, studies also found that some substrates have more than one CPD. For example, studies have revealed that cyclin E has two CPDs, with one located in the T380 and the other one being centered at ~T62 (68,69). The two CPDs of cyclin E were essential for efficiency of Fbxw7 binding, in response to different signaling pathways (70).

3. Regulation of Fbxw7-cyclin E/c-Myc signaling pathways

Over the past decade, a number of studies have contributed to the understanding of Fbxw7 molecular mechanisms in human cancers. Several molecules that play an essential role in tumor pathogenesis and progression have been identified to

Table I. Substrates of Fbxw7.

Protein name	Full name	Ref.
Cyclin E	-	20
c-Myc	-	21
c-Jun	-	22
Notch	-	23
Mcl-1	Myeloid cell leukemia 1	24
mTOR	Mammalian target of rapamycin	25
PS1	Presenilin 1	26
N-Myc	-	27
p63	-	28
c-Myb	-	29
HIF-1α	Hypoxia-inducible transcription factor-1α	30
AIB1 (SRC-3)	Amplified in breast cancer 1	31
SREBP1	Sterol regulatory element binding protein-1	32
JunB	-	33
NF-κB2 (p100)	Nuclear factor of κ light polypeptide gene enhancer in B cells 2	34
Aurora A	Aurora kinase B	35
Aurora B	Aurora kinase A	36
CCDC6	Coiled-coil domain containing 6	37
MED13L	Mediator complex subunit 13-like	38
MED13	Mediator complex subunit 13	38
NF1	Neurofibromatosis type 1	39
KLF2	Krüppel-like factor 2	40
KLF5	Krüppel-like factor 5	41
C/EBPα	CCAAT/enhancer binding protein α	42
C/EBPδ	CCAAT/enhancer binding protein δ	43
Nrf1	Nuclear factor E2-related factor 1	44
G-CSFR	Granulocyte colony stimulating factor receptor	45
CREB3L1 (OASIS)	cAMP responsive element binding protein 3-like 1	46
CREB3L2 (BBF2H7)	cAMP responsive element binding protein 3-like 2	46
TopoIIα	Topoisomerase IIα	47
TGIF1	TGFβ-induced factor 1	48
Eya1	Eyes absent 1	49
GATA3	GATA binding protein 3	50
DAB2IP	Disabled homolog 2 interacting protein	51
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator 1α	52
YAP	Yes-associated protein	53

be regulated by Fbxw7, and are now recognized as potential therapeutic targets. In the present review, we focus on the mechanism by which cyclin E and c-Myc are regulated by Fbxw7.

Fbxw7/cyclin E pathway. By binding to its kinase partner Cdk2, cyclin E regulates the cell cycle by promoting the G1-S transition (71). Deregulation of cyclin E causes genomic instability and is thought to directly contribute to cell transformation and tumorigenesis (72). Cyclin E, containing two CPDs that are phosphorylated by GSK3 and autophosphorylated by Cdk2, respectively, is the most well-studied substrate of Fbxw7 (69). Cyclin E has gained much attention as a key mediator for the tumor-suppressor ability of Fbxw7. Specifically, Fbxw7 tightly regulates the abundance of cyclin E through many molecular mechanisms.

p53 has a complicated and incompletely understood interplay between Fbxw7 and cyclin E. Previous observations revealed that the p53-p21 pathway, which is induced by excess cyclin E, suppresses cyclin E kinase activity and this pathway cooperates with SCF^{Fbxw7} to suppress cyclin E-induced genome instability (73,74). Moreover, when using a cDNA-microarray system, p53 protein is found to be a transcriptional activator of *FBXW7b* (75). p53 arrests cell cycle progression at G0-G1 by inducing the Fbxw7β-mediated downregulation of cyclin E expression (75). Similarly, another group provided results showing that, p53 regulates the levels of cyclin E protein to impose a G1-S block through the activation of ago in

Drosophila (76). However, previous findings have also shown that, Fbxw7β is not necessary for the degradation of cyclin E in mammals as mentioned below. Fbxw7 was eventually proven to act as an upstream of p53 by inducing Aurora A degradation (77). Aurora A phosphorylates p53 at S315/215 and thus reduces p53 levels and transcriptional activity (77) (Fig. 1). However, more studies are needed to clarify these paradoxical and complicated relationships.

Some molecules influence the process whereby Fbxw7 modulates cyclin E protein degradation, for instance rictor (78), Artemis (79) and SV40 large T (LT) (80) (Fig. 1). Although rictor is usually identified as a binding partner of mTOR, numerous complexes containing rictor have been recognized and shown to be mTOR-independent (81). Fbxw7α was recently shown to form a complex with rictor. The rictor/Fbxw7 complex functions as an E3 ligase complex, and promotes the degradation of cyclin E in an mTOR-independent manner (78). Artemis, a member of the SNM1 gene family, is a known phosphorylation target of ATM, ATR and DNA-PKcs in response to various types of genotoxic stress (82). In response to UV irradiation, Artemis is phosphorylated at S516 and S645 by the ATR kinase. The phosphorylated Artemis then interacts with Fbxw7α or Fbxw7γ and induces strong ubiquitylation of cyclin E (79). Similarly, the knockdown of rictor by shRNA and inactivation of Artemis by the mutation of S516/645 to alanine can lead to a decreasing ubiquitination of cyclin E (78,79). Contrary to rictor and Artemis, LT negatively regulates the degradation of cyclin E by Fbxw7. LT is a viral oncoprotein producing in cells infected with simian virus 40 (SV40) (83). SV40 LT protein binds to a number of host cell proteins and disrupts their normal functions. Welcker and and Clurman (80) found that LT also has a consensus CPD in its C terminus that can be phosphorylated at T701, through which LT functions as a competitive inhibitor of Fbxw7 and then deregulates ubiquitination of cyclin E. The Ras/MAPK pathway is also involved in the induction of cyclin E stability by altering the physical interaction between Fbxw7 and cyclin E, but not by altering cyclin E phosphorylation on any of its known regulatory sites (84,85) (Fig. 1).

In addition to interacting with Fbxw7, some factors may target cyclin E and disturb the interaction between Fbxw7 and cyclin E. For example, the protein phosphatase PP2A-B55β targets the N- and C-terminal phosphodegrons of cyclin E1 for dephosphorylation, thereby protecting it from degradation mediated by the SCF^{Fbxw7} ubiquitin ligase (86) (Fig. 1). These data suggest the interaction between Fbxw7 and cyclin E is tightly regulated by different regulators. However, as yet unknown regulators remain to be identified in the future.

It has been demonstrated that ubiquitin-mediated degradation of cyclin E in mammals can be divided into two ways: one requires only Fbxw7α and the other requires Fbxw7α and Fbxw7γ, although not Fbxw7β (56,87,88) (Fig. 2). When the expression of cyclin E is low, presumably normal, the inactivation of cyclin E requires Fbxw7α and Fbxw7γ in a two-step manner. Firstly, cyclin E, which is phosphorylated at S384 by Cdk2, interacts with prolyl cis-trans isomerase Pin1 in conjunction with $SCF^{Fbxw7α}$, carries out a noncanonical isomerization of a proline-proline bond in the cyclin E phosphodegron (P382) (56). Then, being mediated by nucleolar protein nucleophosmin (NPM), the complex binds to $SCF^{Fbxw7γ}$ and translocates from the nucleoplasm into the nucleolus where cyclin E is multiubiquitylated, but does not execute proteasomal degradation (87). Notably, Cdk2 kinase was recently demonstrated to interact with Fbxw7γ, result in Fbxw7γ degradation (89). This system serves a novel mechanism for rapid inactivation of cyclin E, through separation of cyclin E from its targets.

Some studies have focused on the upstream of the Fbxw7/cyclin E pathway. Their findings have identified many factors that increase or decrease Fbxw7 expression, thus regulating the Fbxw7/cyclin E pathway (Fig. 1). The PI3K/Akt pathway, which was previously thought to be pivotal for the regulation of the GSK phosphorylation, was recently reported to mediate the phosphorylation of Fbxw7α at S227 (90). This modification stabilizes Fbxw7 and promotes ubiquitylation of the two substrates, cyclin E and c-Myc (90). In addition, Sim *et al* reported a novel mechanism whereby TRIP-Br proteins links to E2F to act as upstream regulators of the Fbxw7/cyclin E pathway in the maintenance of genomic stability (91). This mechanism reveals a function distinct from the conventional function of E2F, which increases cyclin E expression at the transcriptional level. TRIP-Br proteins interact with PHD zinc finger and/or bromodomain proteins such as KRIP-1 and p300/CBP, upregulate the *FBXW7* gene product via the activation of E2F transcriptional activity, and subsequently lead to downregulation of the cyclin E protein (91). Polo-like kinase 2 (PIK2) is also found to directly phosphorylate Fbxw7 at S25, S176 and S349. However, these phosphorylations reduce the stability of Fbxw7, thus stabilizing cyclin E, and contributing to the duplication of centrioles and aneuploidy (92). TAL1, a class II basic helix-loop-helix (bHLH) transcription factor, promotes the malignant phenotype in T-ALL through the repression of Fbxw7 in a miR223-dependent manner. This effect leads to a marked increase of the expression of cyclin E and c-Myc (93).

The most distinctive function of cyclin E is to bind to and activate Cdk2. Activation of the cyclin E/Cdk2 complex subsequently leads to the phosphorylation of its substrates, such as retinoblastoma (81), cdc6, NPM, p21 and p27 (72). Of note, cyclin E/Cdk2 was reported to directly phosphorylate Cdh1, thus inactivating APC^{Cdh1}, an E3 ligase important for genomic stability (94). Furthermore, Lau *et al* showed that the Fbxw7/cyclin E pathway regulates the activation of APC^{Cdh1} through direct phosphorylation of Cdh1 (95). This subsequently leads to the inactivation of APC^{Cdh1} E3 ligase and upregulation of APC^{Cdh1}-specific substrates, which are well-characterized oncoproteins (Fig. 1). These results suggested that Fbxw7 regulates cell cycle through, not only cyclin E itself, but also the downstream substrates of cyclin E/Cdk2 complex.

Taken together, these findings suggest that Fbxw7/cyclin E is regulated by multiple regulators, which partly explains why there are no *FBXW7* gene mutations in some tumors. Moreover, these findings provide insight into the tumor-suppressive function of Fbxw7.

Fbxw7/c-Myc pathway. The c-Myc protein, a basic helix-loop-helix zipper (bHLH/Zip)-type transcription factor, generally combines with its cofactor MAX and activates their target genes transcription by binding E-box motifs (CACGTG), and thus playing a predominant role in cell proliferation and tumor-

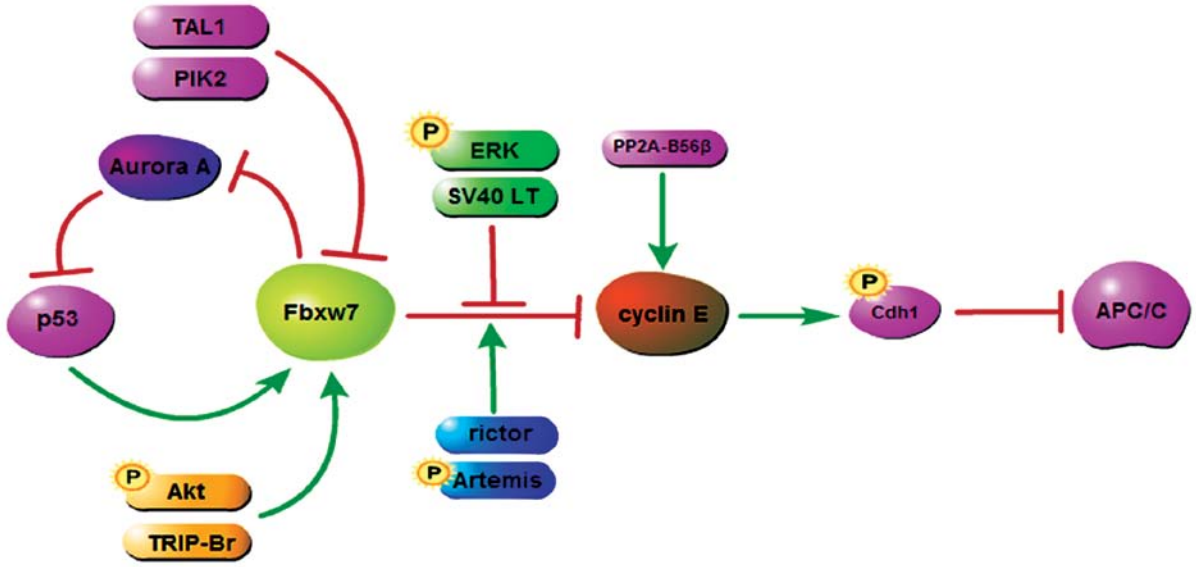

Figure 1. Regulation mechanisms of Fbxw7/cyclin E signaling pathway. Fbxw7/Aurora A/p53 feedback pathway regulates Fbxw7 expression. Other molecular factors such as Akt, TRIP-Br, miR223, PIK2, ERK, SV40 LT, rictor, Artemis, and PP2A-B55β have also been demonstrated to regulate Fbxw7/cyclin E signaling pathway through different mechanisms. Regarding to the downstream of this pathway, APC^{Cdh1} is reported to be an important substrate of the Fbxw7/cyclin E signaling pathway.

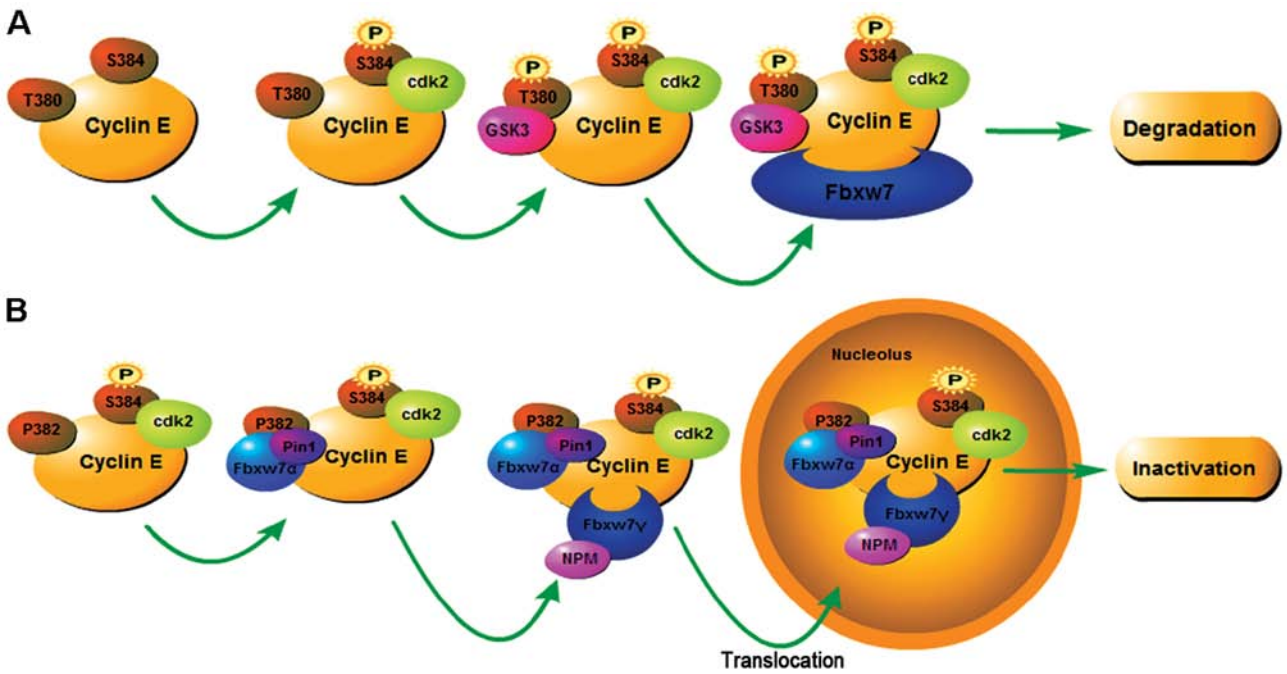

Figure 2. Ubiquitin-dependent proteolysis of cyclin E by Fbxw7 in two situations. (A) When the expression of cyclin E is at a high level, cyclin E is phosphorylated by Cdk2 on S384, and by GSK3 on T380. This hyperphosphorylation on T380 and S384 promotes the contact between Fbxw7α and cyclin E, leading to the ubiquitin-mediated proteolytic degradation of cyclin E. (B) When the expression of cyclin E is at a low level, cyclin E is phosphorylated by Cdk2 on S384. The phosphorylation of cyclin E leads to contact with Pin1 and Fbxw7α on P382. Then, being mediated by NPM, the complex binds to Fbxw7γ and translocates into the nucleolus for inactivation.

igenesis (96). Accumulated evidence has indicated that c-Myc protein turnover is tightly regulated at the post-translational level through ubiquitin-proteasome pathway controlled by the SCF^{Fbxw7} complex (97,98). The oncoprotein c-Myc has only one CPD, containing the sequence PTPPLSP (residues 57-63 in human c-Myc), within which T58 and S62 are the phosphorylation sites (98). However, these two phosphorylation sites exert opposite functions on c-Myc degradation, as the phosphorylation of S62 results in c-Myc stabilization, whereas the T58 phosphorylation by GSK3β contributes to the interaction between Fbxw7 and c-Myc (98,99). The phosphorylation of T58 depends on the prime phosphorylation of Ser-62. This phosphorylation-dependent proteolysis controlled by SCF^{Fbxw7} is involved with complicated feedback mechanisms.

The activation of Ras-dependent phosphorylation pathways is considered to be important for c-Myc stability mainly

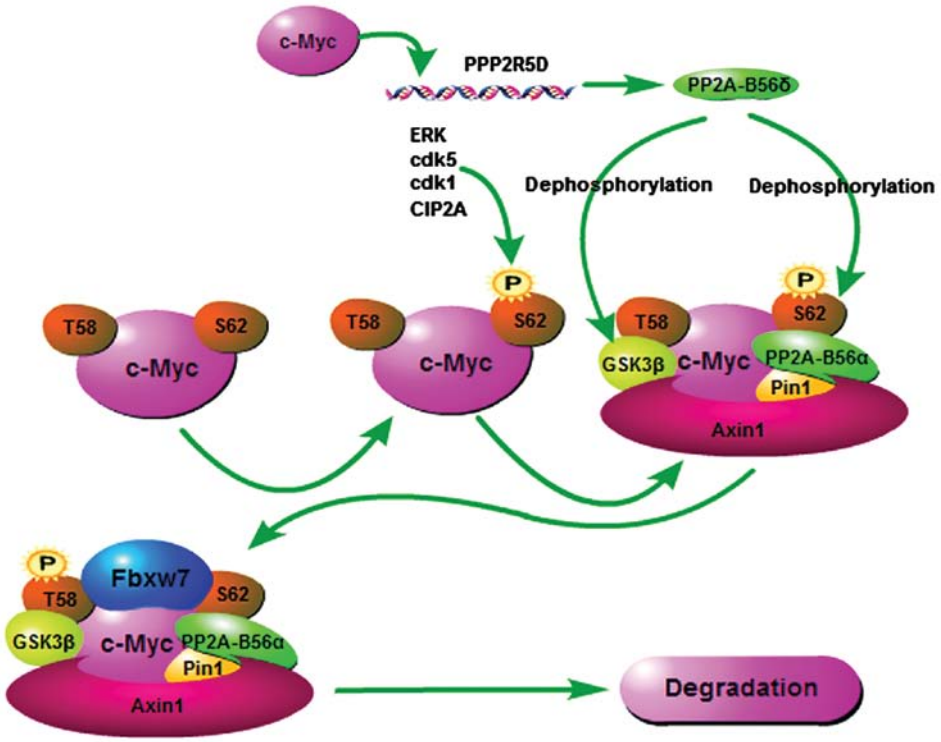

Figure 3. Sequential steps in c-Myc proteolytic degradation by Fbxw7. Multiple factors such as ERK, Cdk5, Cdk1, and CIP2A phosphorylate c-Myc on S62. The initial phosphorylation of S62 allows the connection between Axin1 and c-Myc, forming Axin-Pin1-GSK3β-PP2A/B56α complex to promote c-Myc proteolytic degradation. Moreover, c-Myc can transcriptionally activate the *PPP2R5D* gene that encodes B56δ. PP2A-B56δ can dephosphorylate S62 within c-Myc and S9 within GSK3β, and ultimately facilitate the proteasomal degradation of c-Myc.

through two effective pathways: the Raf/MEK/ERK pathway stabilizes c-Myc by enhancing S62 phosphorylation, and the PI3K/Akt pathway which disrupts the interaction between Fbxw7 and c-Myc by phosphorylating GSK3β at S9, which in turn decreases the phosphorylation of T58 within c-Myc (99). Thus in quiescent cells, where the growth stimulus disappears, Ras activity declines, and the activity of the PI3K/Akt pathway is also downregulated, resulting in the enhancement of T58 phosphorylation and the degradation of c-Myc. It has been shown that the dephosphorylation of S62 is also crucial for the ubiquitin-mediated degradation of c-Myc. T58 phosphorylation is found to facilitate the interaction between c-Myc and Pin1 which catalyzes the isomerization of proline residues in c-Myc to promote S62 dephosphorylation by protein phosphatase 2A (PP2A) (100). Further study showed that Axin1, a multi-domain scaffold protein, regulates this process by facilitating the association of GSK3β, B56α (one of the regulatory subunits of PP2A), and Pin1 with c-Myc, forming an Axin-Pin1-GSK3β-PP2A/B56α complex to promote the ubiquitin-mediated degradation of c-Myc (101). c-Myc has been reported to bind to and transcriptionally activate the *PPP2R5D* gene that encodes B56δ (another regulatory subunit of PP2A) (102). PP2A-B56δ, not only S dephosphorylates S62 within c-Myc in the same manner as PP2A-B56α, but also reverses the GSK3β inhibitory phosphorylation at S9 by PI3K/Akt (102). These studies provide a mechanism that links c-Myc protein degradation controlled by Fbxw7 to a complicated feedback pathway. In addition, Cdk5, Cdk1 and CIP2A increase S62 of c-Myc phosphorylation in a direct or indirect manner, resulting in c-Myc stabilization (103-105) (Fig. 3).

Similar to cyclin E, the Fbxw7/c-Myc pathway appears to be linked to many signal molecules. Previous findings have shown that rictor binds to Fbxw7 and facilitates ubiquitination of c-Myc (78). In addition to rictor, other interaction partners such as stomatin-like protein 1 (SLP-1), NPM, and Bloom (BLM) forms complexes with Fbxw7; in particular, Fbxw7γ, which is specific for the ubiquitination of c-Myc (89,106-108). By performing a two-hybrid screen, Zhang *et al* identified that SLP-1, as a novel interaction partner, can bind to the N-terminus of Fbxw7γ and stabilize Fbxw7γ, leading to an even greater reduction in c-Myc abundance (89). NPM is required by Fbxw7γ for the proper folding, nucleolar localization and stabilization. Mutation of NPM induces delocalization and destabilization of Fbxw7γ and stabilization of c-Myc (106). Recently, BLM, which is a helicase mutated in Bloom syndrome and is conclusively regarded as a sensor of DNA lesion, was reported to have contact with c-Myc and Fbxw7, leading to the degradation of c-Myc and subsequent delay of colorectal tumorigenesis (108,109).

By contrast, many factors serve as binding partners of c-Myc, and block the degradation of c-Myc in a Fbxw7-dependent manner. For example, NF-κB essential modulator (NEMO) induces the upregulation of c-Myc protein through direct interaction with c-Myc protein and inhibits ubiquitination activity of Fbxw7 without interfering with the interaction of Fbxw7 with T58-phoshorylated c-Myc (110).

Although some studies focus on the interaction mechanism between Fbxw7 and c-Myc, other studies provide mechanistic insights for the regulation of the Fbxw7/c-Myc pathway through a reduction of Fbxw7 expression. For example, NF-κB1 (p50),

a ubiquitously expressed subunit of NF-κB, is thought to suppress *FBXW7* gene transcription and upregulate c-Myc protein expression (111). Huang *et al* revealed that Fbxw7 was profoundly upregulated in p50-deficient cells in comparison to that in p50 intact cells, whereas knockdown of Fbxw7 in $p50^{-/-}$ cells restored arsenite-induced c-Myc protein accumulation (111). COP9 signalosome (CSN) has been found to facilitate the autoubiquitination/degradation of Fbxw7, thereby stabilizing c-Myc (112). Similar to protein factors, some miRNAs participate in the regulation of c-Myc by interfering with the expression of Fbxw7. For example, miR-92 mediates the proteolytic degradation of c-Myc by direct repression of Fbxw7 in a Eμ-myc Burkitt's lymphoma model (113). Thus, miR-92 overexpression leads to increase of aberrant c-Myc.

Taken together, the abovementioned studies showed that the Fbxw7/c-Myc pathway is regulated by accurate mechanisms. These mechanisms ensure the cell cycle progression in normal cells and regulate cell proliferation and tumorigenesis in malignant tumors. Moreover, these regulation mechanisms (regulation of the Fbxw7/cylin E and Fbxw7/c-Myc pathways) may also exist in other Fbxw7-specific substrates. Furthermore, the fact that some regulators are involved in the Fbxw7/cyclin E and Fbxw7/c-Myc pathways suggests that the two pathways are important for tumor cell proliferation, and the abnormality of these pathways may be partially regulated by the same mechanisms.

4. Role of Fbxw7 in hepatocellular carcinoma

As a tumor suppressor, mRNA and protein expression levels of Fbxw7 have been shown to be downregulated in various types of human cancer. Fbxw7 has been found to be inactivated by mutation in several malignancies with an overall mutation frequency of ~6% (18). However, no studies have yet reported Fbxw7 mutations in HCC, one of the leading causes of cancer-related mortality worldwide. Notably, Fbxw7 expression is reported to be important in the regulation of lipogenesis and cell proliferation and differentiation in the liver (114). Using liver-specific Fbxw7 null mice, Onoyama *et al* found that the hepatic ablation of Fbxw7 resulted in hepatomegaly and steatohepatitis, and long-term Fbxw7 deficiency resulted in marked proliferation of the biliary system and development of hamartomas (114). Moreover, double heterozygous $p53^{-/-}$ $Fbxw7^{-/-}$ mice have been proven to develop hepatocarcinomas (35). These results suggest that Fbxw7 is critical for the liver development and tumorigenesis.

Previous studies from different groups have identified the role of Fbxw7 as a tumor suppressor in HCC. Chen *et al* were the first group to investigate Fbxw7 in HCC (47). Their findings revealed that Fbxw7 promoted ubiquitin-dependent degradation of TopoIIα in a HADC-dependent manner (47). Liu *et al* found that Fbxw7 was involved in the ubiquitin-dependent degradation of AIB1 in Hbx-related HCC (31). Notably, Tu *et al* were the first group to report that the Fbxw7 mRNA and protein expression in HCC tissues was significantly lower than that in normal tumor-adjacent tissues (115). Subsequently, they also identified that c-Myc, cyclin E, and YAP proteins abundance in HCC was regulated by Fbxw7 (53,116). Consistent with Tu *et al*, Imura *et al* reported a lower Fbxw7 expression in HCC tissues compared with non-tumor liver tissues (117). Coincidentally,

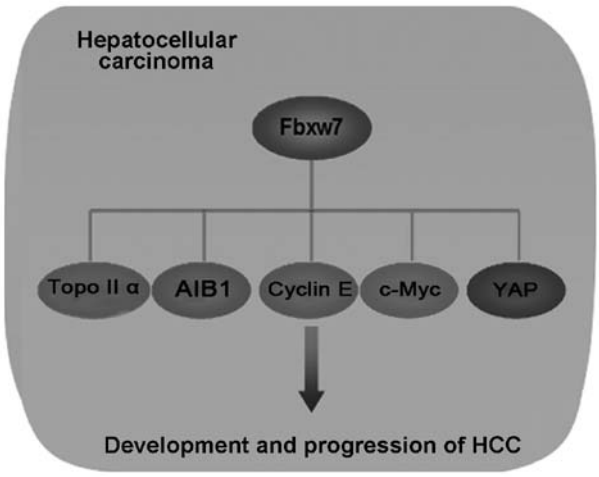

Figure 4. Role of Fbxw7 in HCC. Several proteins such as TopoIIα, AIB1, cyclin E, c-Myc, and YAP are found to be regulated by Fbxw7 in HCC, demonstrating the critical role Fbxw7 plays as a human tumor suppressor.

all these substrates of Fbxw7 in HCC are closely associated with promoting cancer cell proliferation and tumorigenesis (53,71,96,118,119). Given that c-Myc, YAP and AIB1 have transcriptional activity and promote the transcription of many oncogenes with different functions, Fbxw7 may be associated with controlling cell growth and regulating other malignant behavior, such as invasion and metastasis (96,120,121) (Fig. 4). Taken together, these results show that Fbxw7 plays a critical role in HCC. The inactivation of Fbxw7 in HCC is involved in tumorigenesis. However, in-depth investigation is required to determine how Fbxw7 is inactivated in HCC and whether other signal pathways, through which Fbxw7 plays as a tumor suppressor in HCC, exist.

5. Conclusions

Most cell proteins involved in cell cycle progression, proliferation and apoptosis are regulated by the UPS, which consist of three classes of enzymes (E1, E2 and E3). The $SCFF^{bxw7}$ complex is one of the most well-known E3 ligases. Fbxw7, as the substrates-recognition component of the SCF^{Fbxw7} complex, regulates cell proliferation, genetic stability, and tumorigenesis in humans by coordinating the ubiquitin-dependent proteolysis of several key oncoproteins. Several studies (refs?) have identified a growing list of specific substrates of Fbxw7, such as YAP and Eya1. However, cyclin E and c-Myc are the best characterized oncoproteins among the substrates of Fbxw7. Multiple factors tightly regulate the Fbxw7/cyclin E and Fbxw7/c-Myc pathways in different mechanisms. These regulation mechanisms may also exist in other Fbxw7-specific substrates. These mechanisms therefore may be useful in understanding the functions of Fbxw7-related signaling pathways in the regulation of cell proliferation and tumorigenesis in cancer. Furthermore, this understanding reveals Fbxw7-related signaling pathways have potential in developing new targets in cancer therapy. In addition, since many studies have reported that Fbxw7 functions as a tumor suppressor and plays a critical role in regulating several key oncoproteins in HCC, Fbxw7 is a potential therapeutic target in this cancer. However, future

studies should be conducted to invesigate the mechanism of Fbxw7 and how it may serve as a tumor suppressor in HCC.

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