

Fbxw7 Tumor Suppressor: A Vital Regulator Contributes to Human Tumorigenesis

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Abstract: Rapidly accumulating data indicate that F-box/WD repeat-containing protein 7 (Fbxw7) is one of the most frequently mutated genes in human cancers and regulates a network of crucial oncoproteins. These studies have generated important new insights into tumorigenesis and may soon enable therapies targeting the Fbxw7 pathway.

We searched PubMed, Embase, and ISI Web of Science databases (1973–2015, especially recent 5 years) for articles published in the English language using the key words “Fbxw7,” “Fbw7,” “hCDC4,” and “Sel-10,” and we reviewed recent developments in the search for Fbxw7.

Fbxw7 coordinates the ubiquitin-dependent proteolysis of several critical cellular regulators, thereby controlling essential processes, such as cell cycle, differentiation, and apoptosis. Fbxw7 contains 3 isoforms (Fbxw7 α , Fbxw7 β , and Fbxw7 γ), and they are differently regulated in substrate recognition. Besides those, Fbxw7 activity is controlled at different levels, resulting in specific and tunable regulation of the abundance and activity of its substrates in a variety of human solid tumor types, including glioma malignancy, nasopharyngeal carcinoma, osteosarcoma, melanoma as well as colorectal, lung, breast, gastric, liver, pancreatic, renal, prostate, endometrial, and esophageal cancers.

Fbxw7 is strongly associated with tumorigenesis, and the mechanisms and consequences of Fbxw7 deregulation in cancers may soon enable the development of novel therapeutic approaches.

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Abbreviations: AIB1 = amplified in breast cancer 1, Aurora-A = Aurora kinase A, CPD = Cdc4 phospho-degron, EGFR = epidermal growth factor receptor, EMT = epithelial-to-mesenchymal transition, ENO1 = Enolase 1, ER = estrogen receptor, FAM83D = family with sequence similarity 83, member D, Fbxw7 = F-box/WD repeat-containing protein 7, GC = gastric cancer, G-CSFR = granulocyte colony stimulating factor receptor, GSK-3 = glycogen

synthase kinase 3, HCC = hepatocellular carcinoma, HIF-1 α = hypoxia inducible factor-1 α , KLF5 = Kruppel-like factor 5, Mcl-1 = Myeloid cell leukemia-1, miRNAs = microRNAs, mRNA = messenger RNA, mTOR = mammalian target of rapamycin, NF- κ B2 = Nuclear factor- κ B2, NSCLC = nonsmall cell lung cancer, OS = osteosarcoma, Pin1 = prolyl isomerase 1, RCC = renal cell cancer, RITA = RBP-J-interacting and tubulin-associated, SCF = SKP1-CUL1-F-box, SCNC = small cell neuroendocrine carcinoma, SREBP = sterol regulatory element binding protein, YAP = Yes-associated protein.

INTRODUCTION

Ubiquitin proteasome system can regulate many important cellular processes by degrading short-term proteins. The target proteins are ubiquitinated and degraded through a series of actions of ubiquitin ligase. The SKP1-CUL1-F-box (SCF) ubiquitin ligases mainly comprised of F-box protein, Skp1, Cullin 1, and Roc1/Rbx1/Hrt1. F-box/WD repeat-containing protein 7 (Fbxw7) is a member of the F-box protein family, which function as the substrate recognition component of the SCF E3 ubiquitin ligase. The Fbxw7 (also known as hCDC4, Sel-10) was first identified in 1973 by Hartwell¹ at Washington University in his research for genetic control of the cell division cycle in yeast. Since this discovery in yeast, a wealth of experimental evidence, suggests that Fbxw7 is a tumor suppressor through negative regulation of many oncogenic proteins. It is also the substrate-recognition component of the SCF ubiquitin ligases that can bring together protein substrates and the catalytic core of the ubiquitin machinery. However, compared with the research of its substrates, little is known about the regulation of Fbxw7 itself, and how different Fbxw7 isoforms play in substrate recognition and ubiquitylation. Therefore, in the following sections, we will mainly discuss the Fbxw7 isoforms, the regulation of Fbxw7, and its role in different human solid tumors and its mechanisms for inactivation.

ETHICAL REVIEW

The study presented here is a literature review and it does not need any ethical approval. This study does not involve interaction with any human subjects and does not collect any identifiable private information.

METHODS

Relevant literature focusing on the field of Fbxw7 in human tumorigenesis was identified through searching in PubMed, Embase, and ISI Web of Science databases by key-words “Fbxw7,” “Fbw7,” “hCDC4,” and “Sel-10,” from 1973 to 2015, especially recent 5 years. There were no limitations imposed on language and study types. The references cited by the articles searched were also analyzed.

Three independent investigators (contributing authors), CJ, GMH, and LZQ, conducted the searching process and the

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writing manuscript. Relevant literature was chosen according to the objective of this review and the availability of full text.

Fbxw7 Structure and Isoforms

The Fbxw7 gene locus maps to chromosome region 4q32, which is frequently deleted in a broad spectrum of human tumor types, and is composed of 4 untranslated and 13 coding exons spanning approximately 210kb of the human genome. The Fbxw7 structure consists of a WD40-repeat domain, an F-box domain, a 5 residue tail, and an α -helical linker domain. The WD40 domain forms a canonical 8-bladed β -propeller structure, which consists of 4 antiparallel β strands and resembles a cylinder with narrow and wide ends and a solvent-filled central channel, and it can be combined with the specific substrate.

Mammalian cells contain 3 Fbxw7 isoforms (Fbxw7 α , Fbxw7 β , and Fbxw7 γ), and they are derived from the same locus on chromosome 4. All isoforms contain a D domain, an F box domain, and a WD40-repeat domain. They share 10 C-terminal exons, which encode for the F-box and substrate-recognition motifs, and then direct a variety of substrates to degradation. Each isoform can identify substrate that contains a phosphorylated consensus Fbxw7 phospho-degron motif. However, other factors affect the interactions between a substrate and a specific isoform: the subcellular localization and the amount of Fbxw7 isoform at a specific setting. And their subcellular localization is determined by a unique N-terminal exon specific for each isoform. Fbxw7 α , the most abundant isoform in proliferating cells, mainly localizes to the nucleoplasm, whereas Fbxw7 β localizes to cytoplasm and Fbxw7 γ localizes to nucleolus. Fbxw7 α is thought to perform most Fbxw7 function. Yang et al² reported that in breast cancers, the association of phosphatase and tensin homolog gene with both amplified in breast cancer 1 (AIB1) and Fbxw7 α could lead to the downregulation of AIB1 transcriptional activity, which resulted in regulating the oncogenic function of AIB1. Furthermore, Fbxw7 α regulated positively epidermal growth factor receptor (EGFR) by influencing a proteasome-dependent ubiquitination step essential for constitutive degradation and stability of EGFR. Strohmaier et al³ found that Fbxw7 α was the predominant form in most human tissues including cancers, while Fbxw7 β was mostly expressed as the form only in nonproliferating tissues like brain and skeletal muscle, and tended to be repressed in some cancers. These observations suggest that Fbxw7 α may control of the cell cycle in proliferating cells, and Fbxw7 β may be needed for the permanent cell-cycle arrest that leads to senescence. What is more, van Drogen et al⁴ indicated that the combined activity of Fbxw7 α and Fbxw7 γ were essential for the degradation of cyclin E. In the study of Sionov et al,⁵ they showed that Fbxw7 β was induced by all the stress stimuli tested, including vinblastine, cisplatin, and etoposide treatments, in a p53-dependent manner. But the expression of Fbxw7 α and Fbxw7 γ was p53-independent and their response to most stress stimuli was limited. What is more, under certain conditions, the same genotoxic agent stimulated induction of Fbxw7 β and repression of Fbxw7 α . The expression of 3 isoforms is controlled by different promoters and upstream regulation elements, but the regulation mechanisms controlling the expression of 3 isoforms are still obscure. Above all, we can see that the 3 isoforms of Fbxw7 are differentially regulated and cannot be categorized as one protein.

Biochemical Function as Ubiquitin Ligases to Degrade Target Proteins

The ubiquitin-proteasome pathway plays a vital role in many cellular functions by determining the abundance of

cellular proteins, including short-lived, regulatory, and misfolded/denatured proteins. Dysregulation of the proteolytic system would result in uncontrolled proliferation, genomic instability, and leading to tumorigenesis. Ubiquitylation needs the concerted action of ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s). The E2s have been emerged as key mediators of chain assembly, and the E3s mainly decide the substrate specificity. The cell cycle, in particular, is primarily controlled by 2 ubiquitin ligases, SCF and anaphase promoting complex/cyclo-some, and perturbation of their function can result in tumorigenesis. SCF ubiquitin ligases compose of an invariable core complex of Cul1, Skp1, and Rbx1, along with the F-box protein family that play as substrate recognition components (Figure 1). Fbxw7 is a member of the F-box protein family, which is the component of an SCF E3 ubiquitin ligase. It contributes to the ubiquitin-mediated degradation of c-Myb,⁶ Mediator 13,⁷ Kruppel-like factor 2,⁸ Kruppel-like factor 5 (KLF5),⁹ granulocyte colony stimulating factor receptor,¹⁰ eya1,¹¹ BCL-3,¹² neurofibromatosis type 1,¹³ nuclear factor E2-related factor 1,¹⁴ p100/nuclear factor- κ B2 (NF- κ B2),^{15,16} GATA3,¹⁷ JunB,¹⁸ Myeloid cell leukemia-1 (Mcl-1),^{19,20} c-Myc,^{21–27} CyclinE,^{21–27} CDK2,¹⁶ Hes-1,¹⁶ CyclinD1,¹⁶ sterol regulatory element binding protein (SREBP),²⁸ c-Jun,^{23,29} Hypoxia inducible factor-1 α (HIF-1 α),³⁰ Notch1,^{23,31,32} DEK,²³ Enolase 1 (ENO1),³³ Yes-associated protein (YAP),³⁴ mammalian target of rapamycin (mTOR),^{35,36} Ki-67,^{24,37} TOP2A,²⁰ coiled-coil-domain containing 6,³⁸ Aurora kinase A (Aurora-A),^{26,37,39} Notch4,³⁷ proliferation cell nuclear antigen, and³⁷ MYCN.⁴⁰ They all function as cell-cycle promoters or oncogenic regulators of proliferation, growth, and apoptosis. The loss of Fbxw7 results in accumulation of its substrates, which leads to oncogenesis. However, little is known about which accumulating substrate is most related to human tumorigenesis in Fbxw7 deficiency cancer cells.

The 3rd, 4th repeat domain of the WD40 of Fbxw7 contains a highly conserved arginine residues (R465, R479, and R505), which bind with high affinity to Cdc4 phospho-degron (CPD), a consensus phosphopeptide motif⁴¹ (Figure 1). CPD is the common phosphorylation motif of most Fbxw7 substrates. When glutamate or phosphorylation offers a negative charge, the serine or threonine in the “+4” position of CPD can be phosphorylated. The phosphorylation of CPD is very important for recognition and subsequent degradation by Fbxw7. In some cases, the substrates of Fbxw7, including c-Myc, Cyclin E, c-Jun, SREBP, Notch, and c-Myb, have mutations within their CPDs, resulting in escaping from Fbxw7-mediated degradation.⁴²

Fbxw7 also contains a conserved dimerization motif called the D domain (Figure 1), whose protomer consists of 3 α helices which self-associate in a right-handed superhelical manner to form a parallel dimer. The D domain mediates dimerization in a homotypic fashion, and the dimerization of the SCF complex facilitates ubiquitin conjugation but not substrate recognition. The D domain may accommodate considerable interaction specificity.⁴¹ For example, Sic 1, the target protein of Fbxw7 with weak affinity in yeast, has no CPD with high affinity, only carries several degradation determinants of low affinity, whose combination with Fbxw7 requires the help of Fbxw7 dimerization.⁴⁴ Other substrates, which do not provide a negatively charged amino acid in the +4 position, or which cannot accommodate an extra phosphate in their phospho-degrons, may be absolutely dependent on Fbxw7 dimerization for their turnover.⁴⁵

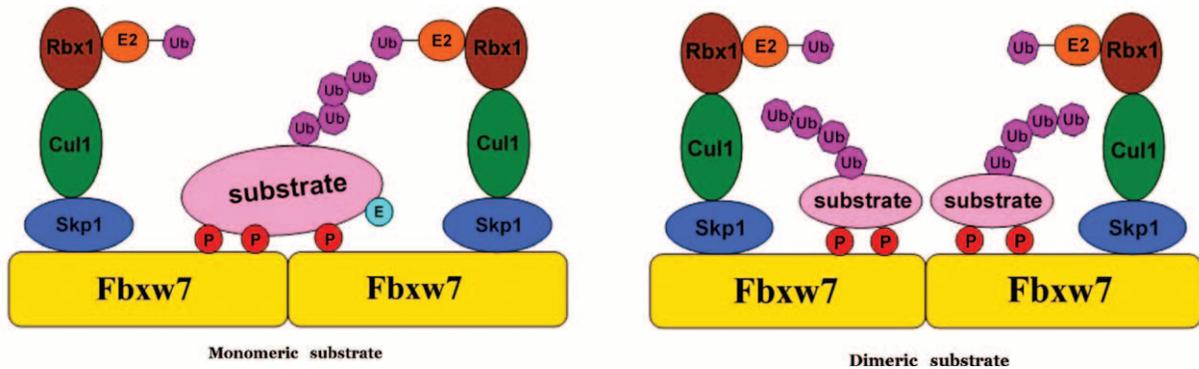


FIGURE 1. Schematic illustration of the SCF-type of E3 ubiquitin ligase complex (modified from ref.).⁴³ The SCF (Skp1-Cullin 1-F-box) complex consists of 4 components: Skp1, Cul-1, Rbx1, along with the F-box protein family that play as substrate recognition components. Fbxw7 is a member of the F-box protein family, which recognizes the targeted substrates. High-affinity substrates have a consensus phosphopeptide motif termed CPDs that contain 2 phosphorylated residues (red “P”); lower-affinity CPDs contain a negatively charged amino acid (blue “E”) in place of the second phosphate. Fbxw7 also contains a conserved dimerization motif called the D domain, mediates Fbxw7 dimerization. CPD = Cdc4 phospho-degron, SCF = SKP1-CUL1-F-box.

Glycogen synthase kinase 3 (GSK-3) is also the coregulator for turnover of some Fbxw7 substrates, which has the conserved phospho-epitope as the CPDs. It phosphorylates the central threonine or serine of the CPD in each substrate. GSK-3 has been found to phosphorylate many proteins playing crucial roles in a variety of cellular processes, such as cell proliferation, differentiation, and apoptosis. Study from Flugel et al⁴⁶ indicated that phosphorylation of HIF-1 α by GSK-3 β recruited Fbxw7, which then promoted ubiquitylation of the HIF-1 α protein. Pérez-Benavente and Farras¹⁸ demonstrated that GSK3 β -mediated phosphorylation of JunB on a vital consensus phosphodegron that induced Fbxw7 E3-ligase recruitment and its degradation in late G2. Dysregulation of GSK3 β -FBXW7-JUNB axis may be relevant in cancer.

Polo-like kinases perform important functions during mitosis, cytokinesis, and centriole duplication. Plk2 is involved in the reproduction of centrosomes and is activated in early G1 phase. However, little is known about the mechanisms underlying Plk2-induced centriole duplication. Cizmecioglu et al⁴⁷ showed that Plk2 phosphorylated Fbxw7 on serine 176 and the 2 proteins (Polo-like kinases and Fbxw7) form a complex in vitro and in vivo, ultimately decreased the Fbxw7 protein stability, resulting in accumulation of cyclin E and increased potential for centriole reproduction. As we know, substrate phosphorylation is the main mechanism that ensures timely destruction of Fbxw7 substrates. Schulein et al⁴⁸ showed that PI3K-dependent phosphorylation of Fbxw7 stimulated its ability to ubiquitinate and degrade its substrates, which controlled the balance between turnover of Fbxw7 and its substrates to fine-tune their activity. Another study showed that the novel PI3 kinase inhibitor NVP-BKM120 decreased Mcl-1 levels through facilitating its degradation in a GSK3/FBXW7-dependent mechanism, that contributed to induction of apoptosis and enhancement of trail-induced apoptosis in human lung cancer cells.¹⁹ In a study from Isobe et al,⁴⁹ they showed that early region 1A, an oncogene product derived from adenovirus, interacted with Fbxw7 and attenuated the ubiquitylation of its target proteins in vivo. It may be helpful to explain the mechanism whereby adenovirus infection induced unregulated proliferation.

Regulation of Fbxw7 activity

Regulation of kinase activity is important to maintain homeostasis as inappropriate activation or inhibition can always

lead to cancer development. Fbxw7 is a tumor suppressor that controls the protein levels of many oncogenes. However, little is known about the regulation of Fbxw7 itself. There are some established mechanisms of regulation that can be applied to the regulation of Fbxw7: regulation of expression is at the transcription and protein level, and posttranslational modifications such as phosphorylation. But sometimes, the ways of regulation are different in 3 Fbxw7 isoforms.

p53

Fbxw7 is a p53-dependent tumor suppressor and its activation by p53 results in ubiquitination-mediated suppression of several oncoproteins. For example, Mao et al⁵⁰ reported that Fbxw7 expression was upregulated when p53 expression was induced by radiation, and the baseline expression of Fbxw7 was suppressed in p53^{-/-} mice. Furthermore, p53-dependent loss of Fbxw7 led to genetic instability by mechanisms that involved the activation of Aurora-A, c-Jun, and Notch4, and they also found a p53-binding site that was consisted in a promoter region of the Fbxw7. In glioblastoma, Kimura et al⁴¹ found that Fbxw7 β expression, but not the alternative isoform of this gene, Fbxw7a or Fbxw7 γ , was activated in a p53-dependent manner in response to genotoxic stresses, such as UV irradiation and adriamycin treatment, suggesting that each isoform had a different functional role. In gastric cancers (GCs), the deletion of 1 copy of Fbxw7 and TP53 were discovered in 45.5% and 21.2% of gastric tumors, respectively, both the Fbxw7 and TP53 messenger RNA (mRNA) expressions were lower in tumors than in paired nonneoplastic specimens. And it was associated with a more invasive phenotype in GC cell lines.⁵¹ Previous studies had shown that, compared with inactivation of p53 or Fbxw7 alone, the simultaneous disruption of p53 and Fbxw7, two cell cycle checkpoint genes, led to poorer prognosis in clinical GC. This report showed that the status of Fbxw7 and p53 was vital for prognosis determination of GC patients.⁵² In human hepatocellular carcinoma (HCC), Fbxw7 protein expression was negatively correlated with mutant p53²¹ and could be activated by adenoviral delivery of p53. Perez-Losada et al⁵³ showed that Fbxw7 was a p53-transcriptional target that controlled genomic instability and tumor development in epithelial tumors. Grim et al⁵⁴ initially found that Fbxw7 and p53 cooperatively suppressed advanced and chromosomally unstable intestinal cancer. Li et al⁵⁵ found that Fbxw7-mutated

colorectal cancer cells exhibited aberrant expression of phosphorylated-p53 at Serine-15. Li et al³⁹ discovered that p53 mutation led to increased expression of miR-25 and down-regulation of Fbxw7, resulting in elevated levels of Aurora-A, which is critically important for the rapid proliferation and aggressive behavior of prostatic small cell neuroendocrine carcinoma. These reports strongly suggest that transcription of Fbxw7 is regulated by p53 activity and identifying additional transcriptional regulators that modulate Fbxw7 expression will be helpful in understanding the role of Fbxw7 in tumorigenesis.

MicroRNAs (miRNAs) Including miR-223, miR-25, miR-27a, miR-182, miR-503, miR-129-5p, and miR-92a

Recently, miRNAs have increasingly become recognized as one of the regulatory genes which can bind mRNA through sequence complementarities and cause inhibition of protein translation and/or degradation of mRNA. The miRNA complexes can act as oncogenes or tumor suppressors in the development of cancers, finally affect the progression of human tumors and the prognosis of the patients. Lately, multiple studies have identified several miRNAs which can regulate Fbxw7 expression. MiR-223 had been shown to have a significant adverse impact on the survival of oesophageal squamous cell carcinoma patients through repression of the function of Fbxw7.⁵⁶ In addition, miR-223 played as an oncogene by inhibiting the expression of Fbxw7 in human GC⁵⁷ and T cell acute lymphoblastic leukemia.⁵⁸ Eto et al⁵⁹ also revealed that the miR-223/Fbxw7 pathway regulated the sensitivity of a human epidermal growth factor receptor 2-positive GC cell line to trastuzumab through the modulation of apoptosis. Zhou et al⁶⁰ discovered that miR-223 promoted the cisplatin resistance of GC cell via regulating cell cycle by targeting Fbxw7. Lu et al⁶¹ demonstrated that miRNA-25 had important roles in reprogramming mouse fibroblast cells to induced pluripotent stem cells by regulating some candidate gene targets including Fbxw7. Xiang et al⁶² found that miRNA-25 was significantly upregulated in nonsmall cell lung cancer (NSCLC) and promoted NSCLC cells proliferation and motility partially by targeting Fbxw7. Gong et al⁶³ showed that miRNA-25 was overexpressed in primary tumor tissues of GC patients and promoted GC progression by directly downregulating Fbxw7 expression. In a study from Li et al,⁶⁴ they showed that a sequential expression of miR-503 and miR-182 in benign adenoma cooperatively regulated Fbxw7, contributing to the malignant transformation of colon adenoma to adenocarcinoma. Lerner et al⁶⁵ demonstrated that attenuation of Fbxw7 by miR-27a overexpression led to inappropriate cell cycle progression and DNA replication stress, in accordance with the dysregulation of cyclin E expression. Recently, over-expression of miR-129-5p was identified to upregulate Fbxw7 expression. However, the underlying mechanism is unclear.⁶⁶ Zhou et al⁶⁷ indicated that miR-92a was upregulated in cervical cancer and promoted cell proliferation and invasion by suppressing the expression level of Fbxw7. Therefore, the expression of Fbxw7 appears to be tightly regulated by several different miRNAs. However, so far, no studies have identified whether the regulation of miRNAs has much difference in Fbxw7 α , Fbxw7 β , and Fbxw7 γ .

RBP-J-Interacting and Tubulin-Associated (RITA)

RITA is a novel RBP-J-interacting protein that down-regulates Notch-mediated transcription. Recently, Wang

et al⁶⁸ initially explored its molecular mechanism in HCC, they demonstrated that noncancerous liver tissues exhibited increased RITA expression compared to HCC tissues. Moreover, RITA overexpression upregulated p53 and reduced cyclin E levels, and RITA levels were associated with cell proliferation and apoptosis. More recently, Wang et al¹⁶ found that RITA overexpression increased protein expression of Fbxw7 and p53 and downregulated the expression of cyclin E, cyclin D1, CDK2, Hes-1, and NF- κ B p65. It indicated that RITA exerted tumor-suppressive effects in hepatocarcinogenesis.

SREBP2

The SREBP family of transcription factors controls lipid and cholesterol metabolism. These proteins are rapidly degraded by the ubiquitin-proteasome pathway. However, the signals and factors required for this are unknown. Some researches indicated that the phosphorylation-dependent degradation of the SREBP family of transcription factors was regulated by Fbxw7.²⁸ But another study showed that SREBP2 regulated miR-182 by targeting Fbxw7, resulting in a feedback pathway to regulate SREBP transcriptional activity.^{69,70}

NF- κ B1

The NF- κ B family proteins are well-known transcription factors in regulation of multiple gene transcription and cellular processes, including the control of cell survival, tumor invasion, stress response, and drug resistance.⁷¹ It has been well documented that the transcription factor NF- κ B2 (p100/p52) is one of the candidate Fbxw7 substrates, and Fbxw7 promotes degradation of p100 in a GSK3 β phosphorylation-dependent manner. It indicates that Fbxw7 may exert its tumor-suppressor function by regulating NF- κ B activity.⁷² But recently, studies have shown that NF- κ B signaling pathways regulate miR-223/FBXW7 axis in T-cell acute lymphoblastic leukemia.⁷³ NF- κ B1 inhibited Fbxw7 expression and then suppressed its target c-Myc protein degradation,⁷⁴ indicating that NF- κ B1 may be an upstream regulator of Fbxw7. The biological function of NF- κ B1 and NF- κ B2 are different, therefore, more studies and increased research efforts are needed to investigate the underlying mechanisms.

Regulation of Fbxw7 Activity by Other Factors

Emerging evidence has suggested that Fbxw7 could be regulated by some other factors such as prolyl isomerase 1 (Pin1), family with sequence similarity 83, and member D (FAM83D). For example, Min et al⁷⁵ indicated that Pin1 might be an upstream regulator of Fbxw7. Indeed, Pin1 directly bound to Fbxw7 and disrupted Fbxw7 dimerization in a phosphorylation-dependent manner. Depletion of Pin1 upregulated the expression of Fbxw7 protein, subsequently decreased Mcl-1 abundance, leading to the inhibition of tumor cell proliferation and transformation, also enhancing Taxol sensitivity in cancer cells. Wang et al²⁹ discovered that FAM83D downregulated Fbxw7 and upregulated Fbxw7 targets, such as c-Myc, c-Jun, and mTOR, then promoting cell proliferation and migration as well as invasion in breast cancer cells. Balamurugan et al³⁰ reported that CCAAT/enhancer-binding protein- δ , an inflammatory response gene and candidate tumor suppressor, directly inhibited the expression of Fbxw7 and upregulated the substrates of Fbxw7, like mTOR and HIF-1 α , enhancing the mTOR/Akt/S6K1 signaling and promoting breast tumor metastasis. Rocher-Ros et al⁷⁶ indicated that presenilin played a novel role on epidermal growth and transformation by reciprocally

regulating the Notch and EGFR signaling pathways through the ubiquitin ligase Fbxw7. Recently, Welcker et al⁷⁷ found that the Epstein–Barr nuclear antigen 1-binding protein 2 (EBP2) behaved mostly like the Fbxw7 pseudo-substrate that directly bound to Fbxw7 and regulated Fbxw7's nucleolar localization. Study from Sancho et al⁷⁸ demonstrated that the downstream Notch signaling effector Hes-5 directly repressed transcription of Fbxw7 β . Moreover, they revealed that the NICD/Hes-5/FBXW7 β positive feedback loop underlied Fbxw7 haploinsufficiency. Numb was found to be required for cell fate determination during the neuroblast division. Recently, Numb4, one of the predominant Numb isoform, has been shown to promote Fbxw7 ubiquitin ligase assembly and activation, leading to increased Notch degradation.⁷⁹ Fbxw7-dependent substrate ubiquitination was antagonized by the Usp28 deubiquitinase. Schulein-Volk et al⁸⁰ discovered that dual regulation of Fbxw7 activity by Usp28 (which was equivalently disrupted by loss or overexpression of Usp28) was a safeguard mechanism for maintaining physiological levels of proto-oncogenic Fbxw7 substrates. Mo et al⁸¹ suggested that activated serum-and glucocorticoid-inducible protein kinase1 inhibited the Notch1 signaling pathway via phosphorylation of Fbxw7 at serine 227.

Role of Fbxw7 as Tumor Suppressor in Human Solid Tumors and Its Mechanisms for Inactivation

Tumor suppressors are defined as those in which loss of function leads to tumor formation. Fbxw7 is a tumor suppressor gene that is responsible for the degradation of several proto-oncogenes and its functional inactivation can dysregulate the cell division process, and potentially lead to tumorigenesis. Since then, an explosion of studies explicitly addressed the role of Fbxw7 in human tumors. Fbxw7 has been implicated in astrocytoma, and in cancers of the lung, breast, gastric, liver, pancreatic, cervix, and esophagus. Several mechanisms have been reported for the inactivation of Fbxw7 in the progress of human cancer including mutation, deletion, and hypermethylation, of which the Fbxw7 mutation is most common. A lot of effort has been concentrated on finding Fbxw7 mutation in various types of human cancers, which has shown that the overall point mutation frequency is 6% to 35% in human cancers with tissue specificity.⁸²

Fbxw7 in Gastric Cancer

Lee et al⁸³ reported that the Fbxw7 mutation rate in GC tissues was from 3.7% to 6% and did not differ in early or advanced GC, which might play a role in the prognosis of GC. Yokobori et al⁸² pointed out that Fbxw7 mRNA expression level in cancerous tissues was lower than that in noncancerous tissues. And patients with low Fbxw7 expression had a remarkable poorer prognosis than those with high Fbxw7 expression. Fbxw7 expression was associated with the progressive tumor size, lymph node metastasis, peritoneal dissemination, venous invasion, and clinical stage. Experiments had also shown that loss of heterozygosity of Fbxw7 occurred in 32% of early-onset gastric cancers, and the loss of Fbxw7 expression had a significant correlation with the upregulation of c-Myc.²² Furthermore, Li et al⁸⁴ found that Fbxw7 induced tumor apoptosis and growth arrest and inhibited the epithelial-to-mesenchymal transition (EMT) in part by downregulating the RhoA signaling pathway in GC.

Fbxw7 in Colorectal Cancer

In the research of Rajagopalan et al,⁸⁵ somatic mutations in the Fbxw7 gene were found in 22 of the 190 colorectal tumors

patients. Most mutations occurred in exon 7th to 10th, and caused truncation of the protein at a position amino-terminal to the 4th WD40 domain, which interrupted the binding of Fbxw7 to its substrates. The inactivation of Fbxw7 would lead to an increase of cyclin E and cell morphological abnormalities. Babaei-Jadidi et al²³ discovered that the expression level of Fbxw7 in colorectal cancer tissue was lower compared with the normal tissue, and the low expression of Fbxw7 was associated with the poor prognosis. Fbxw7 mutation enhanced expression of c-Myc and cyclin E proteins and upregulated cell proliferation. Fbxw7 mutation also resulted in accumulation of multiple substrates and occurred in impaired degradation of Notch, Jun, and DEK, which cooperatively led to carcinogenesis. Miyaki et al⁸⁶ discovered that Fbxw7 mutations were 9% in hereditary nonpolyposis colorectal cancer, 9% in familial adenomatous polyposis carcinomas, and 10% in sporadic carcinomas. Frame-shift mutations were observed in hereditary nonpolyposis colorectal cancer tumors, while single-base substitutions predominantly happened in familial adenomatous polyposis and sporadic tumors. Loss of heterozygosity at the chromosome 4q region in Fbxw7 gene was seldom detected in tumors. Almost 25% of patients with colorectal cancer had a reduced copy number of Fbxw7, and the incidence of the genetic alteration was concordantly increased with the progression of disease stage. Multivariate analysis revealed that Fbxw7 expression in colorectal cancer was an independent prognostic factor for 5 year survival following surgery. Fbxw7 may be a useful prognostic indicator in colorectal cancer. Zhan et al³³ showed that Fbxw7 physically binds to ENO1 and targets ENO1 for ubiquitin-mediated degradation, and suppressed the ENO1-induced gene expression, lactate production, cell proliferation, and migration in colorectal cancer.

Fbxw7 in Hepatocellular Carcinoma

Since then, an explosion of studies explicitly addressed the role of Fbxw7 in HCC. Tu et al³⁴ demonstrated that Fbxw7 expression was impaired in HCC tissues and loss of Fbxw7 expression was related to poor clinicopathological features including venous infiltration, large tumor size, high pathological grading and, advanced tumor node metastasis stage. Additionally, some researchers found that the low expression of Fbxw7 correlates with the tumor recurrence after hepatectomy in patients with HCC.⁸⁷ Tu et al²¹ discovered that p53 contributed to hepatocarcinogenesis partially through the down-regulation of Fbxw7 activity and the accumulation of c-Myc and cyclin E. Furthermore, Tu et al³⁴ indicated that Fbxw7 was inversely associated with YAP protein expression, and it regulated YAP protein abundance by targeting YAP for ubiquitination and proteasomal degradation in HCC tissues. Yu et al⁸⁸ reported that Fbxw7 increased chemosensitivity in HCC cell lines through suppression of EMT. These results indicate that Fbxw7 may serve as a prognostic marker and that c-Myc, cyclin E, YAP may be potential targets of Fbxw7 in HCC. Yang et al⁸⁹ reported a crucial role of Fbxw7 in cholangiocarcinoma metastasis by regulating EMT.

Fbxw7 in Breast Cancer

In breast cancer, the somatic mutation rate of the Fbxw7 gene was found to be less than 1%, which was based on the catalogue of somatic mutations in cancer database. But deletions of chromosome 4q31 in which Fbxw7 located were found to be more than 30% of breast cancer cell lines and primary cancers.⁹⁰ In addition, a study of breast cancer⁹¹

demonstrated another mechanism for inactivation of Fbxw7, namely promoter specific methylation. Methylation of Fbxw7 was related to favorable prognosis despite its association with poorly differentiated tumors. Wei et al⁹² found that the Fbxw7 expression level was significantly reduced in breast cancer compared to the normal breast tissues, and the lower level of Fbxw7 expression was associated with shorter disease-free survival, especially in patients with estrogen receptor-negative and basal subtype tumors. Silencing Fbxw7 enhanced expression of mTOR,³⁵ c-Myc,²⁴ Ki-67,²⁴ cyclin E,²⁴ and KLF5⁹³ proteins and upregulated both cell proliferation and G1-S transition.

Fbxw7 in Lung Cancer

Villaruz et al³⁶ firstly described the role of Fbxw7 mutation in NSCLC. They found the mTOR inhibitor temsirinolimus was still effective in a patient with adenocarcinoma of the lung, who had previously progressed on multiple lines of systemic therapy. Furthermore, Fbxw7 upregulation significantly increased cisplatin cytotoxicity in NSCLC.⁹⁴ Recently, Yokobori et al²⁰ revealed that low Fbxw7 expression presented with more progressive cancer and shorter survival time than patients with high Fbxw7 expression. What is more, silenced Fbxw7 revealed enhanced MS-275 (a class I-specific histone deacetylase [HDAC] inhibitor) sensitivity and taxol resistance. Silencing Fbxw7 enhanced expression of Mcl-1^{19,20} and TOP2A.²⁰ Zhao et al³⁸ also showed that Fbxw7 interacted with and targeted coiled-coil-domain containing 6 for ubiquitin-mediated proteasomal degradation, but it could be impaired by Ataxia Telangiectasia Mutated during DNA damage response in lung cancer cells.

Fbxw7 in Glioma Malignancy

Glioma malignancy is the most common type of primary malignant brain tumor and may arise from a cell with neural stem-like properties. Until now, the silencing mechanisms for the Fbxw7 in gliomas were unknown. Gu et al⁹⁵ suggested that mutation and methylation was not the major cause of the suppression of Fbxw7 in gliomas. Matsumoto et al⁹⁶ implicated that Fbxw7 played an important role in the degradation of Notch family members, thus regarded as a pivotal regulator of “stemness” and neuronal-gial differentiation in neural stem cells. In the research of Kim et al,²⁵ they proposed that p53 mutations led to gliomagenesis by both allowing the upregulation of c-Myc through downregulation of Fbxw7 and by protecting against c-Myc-induced apoptosis. Hagedorn et al³⁷ found the expression level of Fbxw7 was significantly reduced in more than 80% grade IV glioma. Furthermore, in grade IV glioma biopsies, 2 targets of Fbxw7, Aurora-A and Notch4 were preferentially immunodetected. In human glioma cell lines, overexpression of Fbxw7 lost the expression of proliferation cell nuclear antigen,³⁷ Ki-67,³⁷ Cyclin E1,²⁶ MYC,²⁶ and AURKA.²⁶ These observations suppose that Fbxw7 is a tumor suppressor in glioma malignancy, and interfering with Fbxw7 or its downstream targets would be a new therapeutic strategy.

Fbxw7 in Renal Malignancy

Williams et al⁴⁰ identified Fbxw7 as a novel Wilms' tumor gene, mutated or deleted in approximately 4% of tumors they examined, and they also found MYCN copy number gain in 9 of 104 (8.7%) cases, which was a target of Fbxw7-mediated ubiquitination and degradation. By using a rapid breakpoint cloning procedure in a case of renal cell cancer (RCC), Kuiper

et al⁹⁷ discovered that the Fbxw7 gene was disrupted by a constitutional t(3;4)(q21;q31). The analysis of the tumor tissue revealed the presence of some anomalies, concluding loss of the derivative chromosome 3. Therefore, disruption of the Fbxw7 gene might play a critical role in the development of human RCC. Fu et al⁹⁸ found that the expression level of Fbxw7 in RCC tissues was highly related to its clinical pathologic grade and tumor node metastasis phase and was highly lower than in paracancerous normal tissues, and Fbxw7 overexpression suppressed RCC cell proliferation and induces apoptosis. These data suggested that Fbxw7 is a significant tumor suppressor gene in RCC.

Fbxw7 in Other Solid Tumors

In esophageal squamous cell carcinoma, cases with a loss of Fbxw7 copy number usually had low Fbxw7 expression and a poorer prognosis than those with no loss of copy number, and silencing Fbxw7 upregulated the expression of c-Myc, which played an important role in cell cycle regulation.²⁷ In pancreatic cancer, Calhoun et al⁹⁹ discovered that 6% of pancreatic adenocarcinomas overexpressed cyclin E, which was accompanied by a novel somatic homozygous mutation in Fbxw7. In addition, nuclear retention of Fbxw7 by specific inhibitors of nuclear export led to Notch1 degradation in pancreatic cancer.³¹ Furthermore, Ji et al¹⁰⁰ found that Fbxw7 could be phosphorylated and destabilized by KRAS mutation in pancreatic cancer. Aydin et al³² firstly provided evidence on Fbxw7 as a vital tumor suppressor mutated and inactivated in melanoma that resulted in sustained Notch1 activation and made Notch signaling inhibition as a promising therapeutic strategy in melanoma. Garcia-Dios et al¹⁰¹ found that mutations in Fbxw7 correlated with high endometrial cancer type, tumor grade, and lymph node status, which prompted that Fbxw7 played as a suppressor gene in endometrial carcinoma. In human nasopharyngeal carcinoma, Song¹⁰² discovered that Fbxw7 increased drug sensitivity to cisplatin by downregulating the expression of multidrug resistance-associated protein. Li et al¹⁰³ found that the expression of Fbxw7 in osteosarcoma (OS) cases were significantly lower than those in normal bone tissues, and the low expression of Fbxw7 was correlated with advanced clinical stage, high T classification, poor histological differentiation, and a worse 5-year survival of OS patients. Multivariate Cox regression analysis also indicated that Fbxw7 was an independent prognostic marker in OS.

CONCLUSION

This review has shed light on the magnitude of Fbxw7 within the human tumorigenesis and suggested that Fbxw7 is a vital tumor suppressor gene, which can be inactivated in the progress of human tumorigenesis through mutation, deletion, and hypermethylation, leading to an increase in several oncoproteins, including c-Jun, c-Myc, cyclin E, Notch1, mTOR, SREBP, c-Myb, Aurora-A, KLF5, Mcl-1, Neurofibromatosis type 1, Nuclear factor E2-related factor 1, ENO1, HIF-1 α , and so on, most of which possess strong oncogenic roles. Furthermore, in different human solid tumor types, the downstream targets of Fbxw7 are also different (Figure 2). Fbxw7 contains 3 isoforms (Fbxw7 α , Fbxw7 β , and Fbxw7 γ), and they are differently regulated in subtract recognition. Besides those, recently, accumulating evidence has shown that several molecules such as p53, miRNAs including miR-223, miR-25, miR-27a, miR-182, miR-503, and miR-129-5p, RITA, and FAM83D, as well as Pin1, CCAAT/enhancer-binding

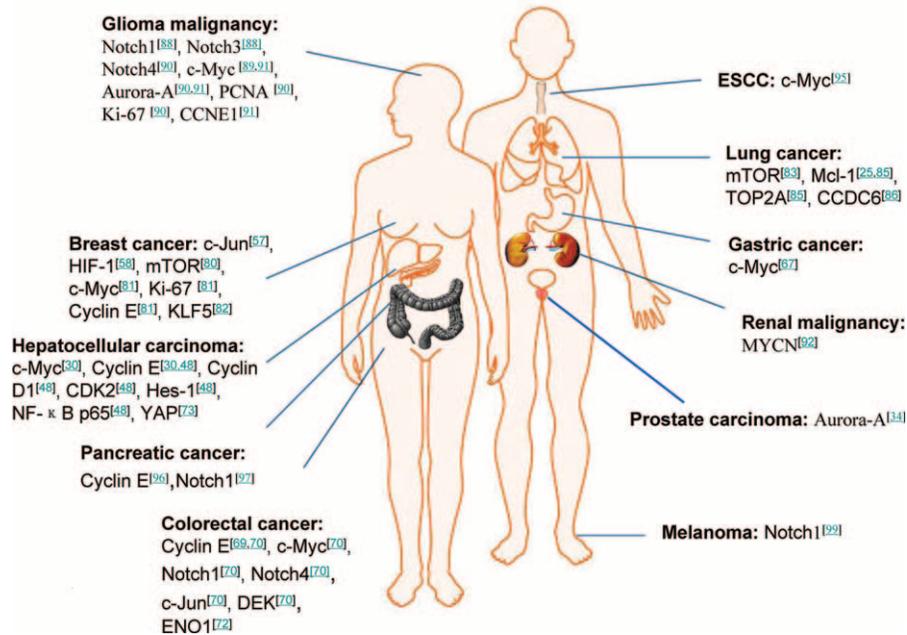


FIGURE 2. The downstream targets of F-box/WD repeat-containing protein 7 (Fbxw7) in different human solid tumor types. Fbxw7 coordinates the ubiquitin-dependent proteolysis of many key oncoproteins, and identifying critical Fbxw7 substrates are important for understanding tumorigenesis and discovering therapeutic targets. As shown above, in different human solid tumor types, the downstream targets of Fbxw7 are also different, which largely provide a new clue for the development of therapeutic targets in our fight against different cancer.

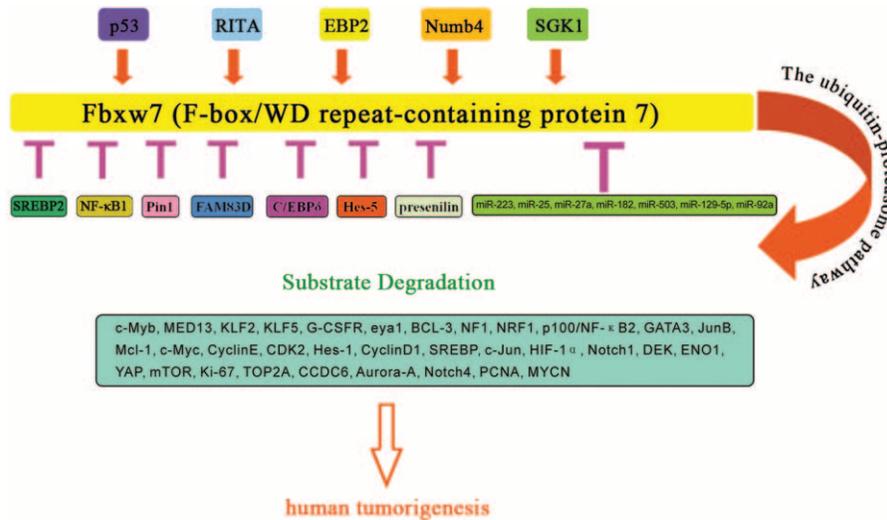


FIGURE 3. The upstream regulators of Fbxw7 and its major downstream targets that contributes to human tumorigenesis. Fbxw7 coordinates the ubiquitin-dependent proteolysis of several key oncoproteins, such as c-Myb,⁶ MED13,⁷ KLF2,⁸ KLF5,⁹ G-CSFR,¹⁰ eya1,¹¹ BCL-3,¹² NF1,¹³ NRF1,¹⁴ p100/NF-κB2,^{15,16} GATA3,¹⁶ JunB,¹⁸ Mcl-1,^{19,20} c-Myc,²¹⁻²⁷ CyclinE,²¹⁻²⁷ CDK2,¹⁶ Hes-1,¹⁶ CyclinD1,¹⁶ SREBP,²⁸ c-Jun,^{23,29} HIF-1 α ,³⁰ Notch1,^{23,31,32} DEK,²³ ENO1,³³ YAP,³⁴ mTOR,^{35,36} Ki-67,^{24,37} TOP2A,²⁰ CCDC6,³⁸ Aurora-A,^{26,37,39} Notch4,³⁷ PCNA,³⁷ MYCN,⁴⁰ and their function linked to defects in cell proliferation, differentiation, genetic instability, and ultimately tumorigenesis. What is more, several proteins such as p53, RITA, EBP2, Numb4, SGK1, SREBP2, NF- κ B1, Pin1, FAM83D, C/EBP δ , Hes-5, presenilin, miR-223, miR-25, miR-27a, miR-182, miR-503, miR-129-5p, and miR-92a are found to regulate the expression of Fbxw7. Aurora-A=Aurora kinase A, CCDC6=coiled-coil-domain containing 6, ENO1=Enolase 1, Fbxw7=F-box/WD repeat-containing protein 7, G-CSFR=Granulocyte colony stimulating factor receptor, HIF-1 α =Hypoxia inducible factor-1 α , KLF2=Kruppel-like factor 2, KLF5=Kruppel-like factor 5, Mcl-1=Myeloid cell leukemia-1, MED13=Mediator 13, mTOR=mammalian target of rapamycin, NF1=Neurofibromatosis type 1, NF- κ B2=p100/Nuclear factor- κ B2, NRF1=Nuclear factor E2-related factor 1, PCNA=proliferation cell nuclear antigen, SREBP=sterol regulatory element binding protein, YAP=Yes-associated proteins.

protein- δ , presenilin, SREBP2, NF- κ B1, EBP2, Numb4 and serum-and glucocorticoid-inducible protein kinase1 could regulate Fbxw7 (Figure 3). Mutations of Fbxw7 are closely related to tumor progression and prognosis, and the detection of Fbxw7 mutations has potential clinical applications. In-depth study of Fbxw7 mutations will not only contribute to the diagnosis of cancer using Fbxw7 mutation as biomarkers, but also help in the development of targeted therapeutics for these mutations, which has positive implications for the prevention of cancer and individualized treatment.

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