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Review

Physiological functions of FBW7 in cancer and metabolism

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ABSTRACT

FBW7 is one of the most well characterized F-box proteins that serve as substrate recognition subunits of SCF (Skp1-Cullin 1-F-box proteins) E3 ubiquitin ligase complexes. SCF^{FBW7} plays key roles in regulating cell cycle progression, differentiation, and stem cell maintenance largely through targeting a broad range of oncogenic substrates for proteasome-dependent degradation. The identification of an increasing number of FBW7 substrates for ubiquitination, and intensive *in vitro* and *in vivo* studies have revealed a network of signaling components controlled by FBW7 that contributes to metabolic regulation as well as its tumor suppressor role. Here we mainly focus on recent findings that highlight a critical role for FBW7 in cancer and metabolism.

1. Introduction

1.1. Ubiquitin-proteasome system (UPS)

Protein degradation is often essential for a rapid response to signal transduction and the recycling of amino acids as part of protein turnover. The vast majority of protein degradation is processed by the ubiquitin-proteasome system (UPS) [1]. Ubiquitin is an evolutionally conserved protein of 76 amino acids and covalently linked to target proteins in a multi-step process involving three key enzymes; an ubiquitin-activating enzyme (E1); an ubiquitin-conjugating enzyme (E2) and an ubiquitin ligase (E3). The E1 enzyme activates ubiquitin in an ATP-dependent manner resulting in a thioester bond between the ubiquitin protein and the E1. Sequentially, the C-terminus of ubiquitin binds with the Cys residue in the active site of an E2 enzyme, and then is covalently attached to the ε-amino group of the Lys residue on target molecules by the E3 ubiquitin ligase. Ubiquitin E3 ligases are classified into two major groups; the homologous to the E6AP carboxyl terminus (HECT) domain containing E3s and Really Interesting New Gene (RING) domain containing E3s. In contrast to HECT type E3s that form a thioester bond with ubiquitin, RING-type E3s directly conjugate ubiquitin from E2s to substrates. Although there are only two E1s and thirty-seven E2s, the human genome encodes over six hundred E3s, suggesting that E3 ligases functionally determine the substrate specificity for ubiquitination [2].

Ubiquitin can be added sequentially to form a polyubiquitination chain on the substrate protein. Since ubiquitin has seven Lys residues, Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63, various types of polyubiquitination linkages are formed that can result in different physiological outcomes [3]. For instance, Lys48 linked polyubiquitinated proteins are recognized by the 26S proteasome, which is composed of a 20S core sub-complex, the 19S regulatory sub-complex, and the 11S sub-complex. Polyubiquitin chains bind to the 19S core particle and are then cleaved off from substrate proteins. Subsequently, the target protein is unfolded and degraded by peptidase in the 20S core subunit. Recent studies began to reveal that polyubiquitin chains composed of apical chain linkages *via* the other six Lys residues within ubiquitin are not only involved in protein degradation, but also play important roles in various cellular events including DNA repair response, endocytosis, and signal transduction [4].

1.2. SCF (Skp1-Cullin1-F-box protein) type of E3 ligase complexes

Cullin-RING ubiquitin ligases (CRLs) are one of the RING type E3 ligases, and are composed of a Cullin, RING finger protein, a variable substrate-recognition subunit, and an adaptor subunit. All Cullins contain a conserved domain in their C-terminal regions and binds to either RING-box protein 1 (Rbx1) or Rbx2, which recruits the E2 enzyme to transfer ubiquitin molecules to the target protein. In eukaryotes, eight types of Cullin (Cullins 1, 2, 3, 4A, 4B, 5, 7, and 9) have been identified and each Cullin forms a functionally distinct CRL complex. Since substrate recognition domains specifically recruit target molecules, they determine the substrate selectivity and are the largest contributor to the diversity of cellular functions of CRLs.

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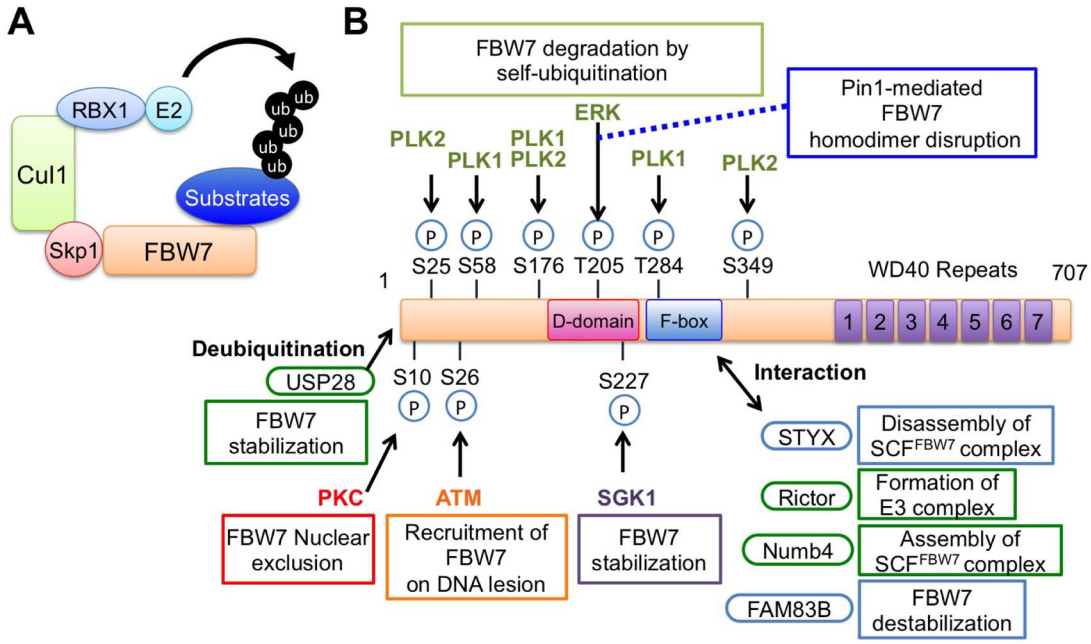

Fig. 1. (A) A schematic illustration of SCF^{FBW7} E3 ubiquitin ligase complex. (B) Upstream FBW7 signaling that modulates FBW7 function, stability, and subcellular localization. FBW7 E3 ligase activity is coordinately controlled by phosphorylation, ubiquitination, and interaction with key regulatory proteins.

CRL1, which is also denoted as the Skp1-Cullin 1-F-box protein (SCF) E3 ligase complex, is the best-characterized member among all CRLs. The SCF complex contains the invariant components S-phase kinase-associated protein 1 (Skp1), Rbx1, and Cullin 1, as well as a variable substrate recognition subunit F-box protein (Fig. 1A). So far, 69 F-box proteins have been identified in the human genome, and according to the substrate recognition domains, they are grouped into three major sub-classes; FBXW (WD40 repeat domain), FBXL (leucine-rich repeat domain), and FBXO (other various domains). Importantly, substrate recognition by F-box proteins often involves post-translational modifications of the target proteins such as phosphorylation or glycosylation [5]. SCF complexes often target key molecules involved in cell cycle progression and are thus considered one of the master regulators of the cell cycle machinery [5].

1.3. The F-box protein FBW7

The F-box protein FBW7, also known as FBXW7 and Cdc4, is one of the most well-studied components of the SCF type of E3 ubiquitin ligases (Fig. 1A). *FBXW7* encodes three splicing variants, FBW7α, β and γ [6]. Each isoform differs in their N-terminal sequence but shares three conserved interaction domains; a D domain for promoting FBW7 dimerization, an F-box domain for recruitment of the SCF complex through Skp1, and a C-terminal WD40 repeat domain for substrate recognition. Thus, all FBW7 isoforms are considered to be functionally identical in principal. However, these isoforms show different subcellular localizations; FBW7α, β and γ localize in the nucleoplasm, cytoplasm and nucleolus, respectively [7]. In addition, tissue distribution also varies among these three isoforms. FBW7α is ubiquitously expressed in mice, whereas FBW7β is exclusively expressed in brain and testis, and FBW7γ is expressed in skeletal muscle and heart [8], which is consistent with the results of human multi-tissue Northern blot analysis [6].

FBW7 substrates typically contain a conserved Cdc4 phosphodegron (CPD) motif (L)-X-pT/pS-P-P-X-pS/pT/E/D (X represents any amino acid) [9,10]. FBW7 recognizes and ubiquitinates its substrates in response to phosphorylation of this motif. In many cases, GSK3 phosphorylates the CPD motif of FBW7 substrates in concert with priming kinases, which in turn triggers SCF^{FBW7}-directed substrate ubiquitination [11–13].

FBW7 is a well-established tumor suppressor that promotes the degradation of various oncogenic proteins such as cyclin E [14–16], c-Myc [17,18], c-Jun [11,19], and MCL1 [20,21]. *FBW7* is located within chromosomal region 4q32 that is frequently lost in cancers [6]. A comprehensive screening of over 1500 human cancers reveal that approximately 6% of all human cancers harbor *FBW7* mutations [22]. Notably, mutations were frequently detected in cholangiocarcinomas (35%) and T cell acute lymphoblastic leukemia (T-ALL; 31%), and mutation frequencies in the range 6–9% were found in colon, endometrium, and stomach tumors. In human cancers, the most common missense mutations of *FBW7* occurs at R465, R479, and R505 [22,23], critical residues in the WD40 domain involved in substrate binding (Supplementary Fig. 1), which strongly indicates that FBW7 dysfunction leads to tumorigenesis. A mammalian genetic screen for p53-dependent genes involved in tumorigenesis further revealed that $Fbw7^{+/-}$ mice were susceptible to radiation-induced tumorigenesis, and $Fbw7^{+/-}$ $Tp53^{+/-}$ mice have increased susceptibility, suggesting that FBW7 is likely a haploinsufficient tumor suppressor [24].

2. Roles of FBW7 in cancer

2.1. FBW7 downstream substrates

FBW7 targets multiple oncoproteins and oncogenic transcription factors for ubiquitination-mediated proteolysis (Supplementary Table 1). As such, dysregulation of FBW7-dependent proteolysis of these oncogenic proteins contribute to development of various cancers. Given the crucial function of FBW7 as a tumor suppressor, the list of FBW7 substrates still continue to grow (Supplementary Table 1), revealing roles for FBW7 in controlling multiple biological processes such as metastasis, stress responses, and immune functions.

SOX9 is a transcription factor that is involved in cell fate control and is frequently upregulated in various human cancers. In medulloblastoma, missense and nonsense *FBW7* mutations are frequent events, and the deficiency of functional FBW7 leads to SOX9 stabilization, which in turn enhances metastatic potential and chemo-resistance [25]. Another independent study indicates that FBW7 is involved in DNA damage-induced SOX9 destabilization, further suggesting that