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Review

# Fbw7 and its counteracting forces in stem cells and cancer: Oncoproteins in the balance

Catherine A. Cremona[a], Rocio Sancho[a,*], Markus E. Diefenbacher[a,**,1], Axel Behrens[a,b,***]

[a] The Francis Crick Institute, Lincoln's Inn Fields Laboratory, 44 Lincoln's Inn Fields, London WC2A 3LY, UK
[b] School of Medicine, King's College London, Guy's Campus, London SE1 1UL, UK

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ABSTRACT

Fbw7 is well characterised as a stem cell regulator and tumour suppressor, powerfully positioned to control proliferation, differentiation and apoptosis by targeting key transcription factors for ubiquitination and destruction. Evidence in support of these roles continues to accumulate from in vitro studies, mouse models and human patient data. Here we summarise the latest of these findings, highlighting the tumour-suppressive role of Fbw7 in multiple tissues, and the rare circumstances where Fbw7 activity can be oncogenic. We discuss mechanisms that regulate ubiquitination by Fbw7, including ubiquitin-specific proteases such as USP28 that counteract Fbw7 activity and thereby stabilise oncoproteins. Deubiquitination of key Fbw7 substrates to prevent their destruction is beginning to be appreciated as an important pro-tumourigenic mechanism. As the ubiquitin-proteasome system represents a largely untapped field for drug development, the interplay between Fbw7 and its counterpart deubiquitinating enzymes in tumours is likely to attract increasing interest and influence future treatment strategies.


## 1. Introduction

Fbw7 (also known as Fbxw7, Ago, Cdc4, Sel10) is the substrate recognition component of a Skp1-Cul1-F-box (SCF)-type E3 ubiquitin ligase that targets several oncoproteins for K48-linked ubiquitination and proteasomal degradation [1]. The *Fbw7* gene locus encodes three alternatively spliced forms, Fbw7α, β and γ, which are localised to the nucleoplasm, cytoplasm and nucleolus respectively. The role of all three Fbw7 isoforms is to function as an assembly platform for the different proteins of the SCF complex, via the F-box domain, and as a bridging factor with the substrate protein to enable ubiquitination. Like other F-box proteins of the Fbw class, Fbw7 contains tandem WD40 domains that mediate its interaction with specific substrates. In most cases, Fbw7 recognises a phosphodegron motif (CPD, Cdc4 phosphodegron) on its target protein, often a GSK3β phosphorylation site separated by 3 amino acids from a second phospho-serine or phospho-threonine residue. The arginine residues on Fbw7 responsible for binding this motif are frequently mutated in cancer, underlining the importance of this interaction for proper cell function. Prominent Fbw7 substrates include proteins important for cell division, growth, survival and differentiation. Fbw7 directly controls the levels of crucial oncoproteins such as c-Myc, cyclin E, Notch intracellular domain (NICD), and c-Jun [2–4], as well as Aurora [5], c-Myb [6], KLF5 [7], Mcl-1 [8], mTOR [9], Presenilin [10], SREBP [11], HIF-1α [12] and a host of other substrates (Fig. 1). Its role in tumour suppression and tissue homeostasis is therefore not surprising. The substrate recognition properties of Fbw7 and their role in tumourigenesis have recently been reviewed in detail [13].

The importance of ubiquitin-mediated degradation for protein stability is well established, but targeting this system as an anti-cancer strategy has only just begun to be exploited [14,15]. To date, the only clinically approved drugs that influence the ubiquitin-proteasome system are proteasome inhibitors such as bortezomib, which are designed to induce general toxicity in cells that are very active in protein synthesis. While these have shown some efficacy in multiple myeloma [16], the action of these inhibitors at a universal step of the pathway means that their activity can result in unpredictable outcomes including side effects. Targeting

* Corresponding author.
** Corresponding author.
*** Corresponding author at: The Francis Crick Institute, Lincoln's Inn Fields Laboratory, 44 Lincoln's Inn Fields, London WC2A 3LY, UK.
*E-mail addresses:* Rocio.Sancho@crick.ac.uk (R. Sancho), markus.diefenbacher@uni-wuerzburg.de (M.E. Diefenbacher), Axel.Behrens@crick.ac.uk (A. Behrens).
[1] Present address: Theodor Boveri Institute, Biocenter, University of Würzburg, Würzburg, Germany. Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany.

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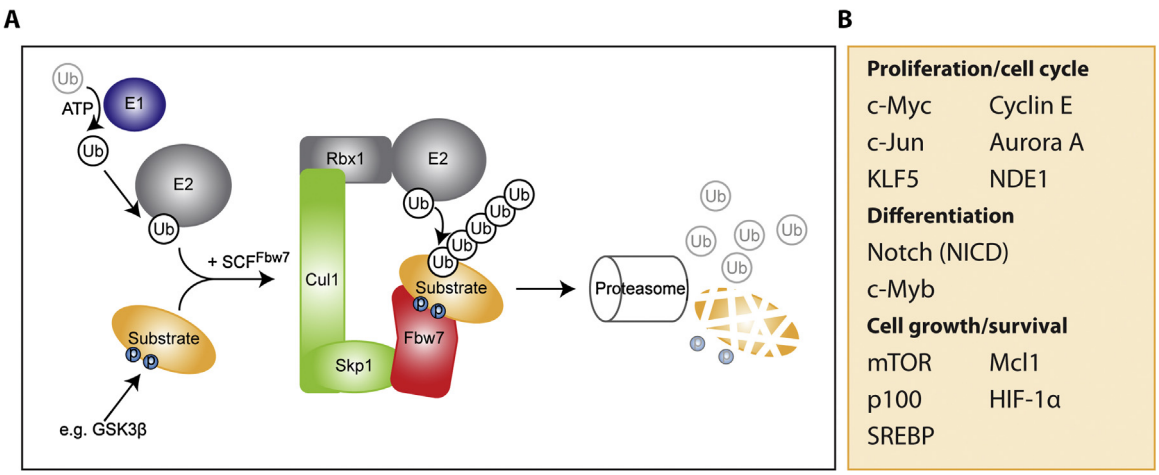


**Fig. 1.** Fbw7 enzymatic function and substrates. (A) Fbw7 destabilises protein targets by functioning as the substrate recognition component of an SCF-type E3 ubiquitin ligase. In order to ubiquitinate a substrate, the cleaved ubiquitin moiety is first activated by an E1 enzyme and passed to the E2 ubiquitin-conjugating enzyme. The E3 multisubunit SCF complex (Skp1, Cul1, Fbw7, plus Rbx1) brings the E2-ubiquitin conjugate into close proximity with the substrate to allow ubiquitin ligation to occur. Fbw7 (also known as Cdc4) typically recognises and binds a phosphorylated degron motif (CPD) on the substrate. Polyubiquitination using lysine 48-linked chains targets the substrate for proteasomal degradation. (B) Some examples of Fbw7 substrates particularly relevant to cancer. Functional categorisations are indicative, and proteins may belong to more than one category.

the system at the level of substrate recognition or modification would enable a more restricted intervention that would be more specific to particular sets of proteins [17,18]. The set of Fbw7 substrates includes many proteins with oncogenic activity, and Fbw7 function is frequently lost in tumours, resulting in stabilisation of one or more of these proteins. Studies in model systems have confirmed that Fbw7 loss of function contributes to tumourigenesis and tumour progression. In order to exploit this knowledge to be able to restore or boost the turnover of Fbw7 substrates in tumours, a detailed understanding of Fbw7 activity and interactions in different tissues is required. In this review we will summarise the latest findings relevant to Fbw7's role in cancer (recently reviewed elsewhere: [13,19]), and then focus on the regulatory mechanisms surrounding Fbw7 and its activity, particularly its relationship with its counterpart deubiquitinating enzymes (DUBs).

## 2. Fbw7 controls stem cell differentiation, proliferation and apoptosis

In addition to its role as a tumour suppressor, Fbw7 functions in the control of stem cell biology (recently reviewed by Wei and colleagues [20]). Somatic stem cells are classically defined as adult tissue cells with unlimited capacity for self-renewal and the potential to differentiate into mature tissue cells, allowing maintenance and proper tissue homeostasis. In addition, it is well accepted that tissue stem cells can serve as the 'cells of origin' of tumours when challenged with specific oncogenic mutations. The best example of tissue stem cells as the cells of origin of tumours was provided by elegant lineage tracing experiments performed in the mouse intestine [21]. Conditional overactivation of the Wnt signalling pathway specifically in intestinal stem cells (using Lgr5-Cre) led to the development of tumours, while the same trigger in more differentiated cells was not efficient in generating tumours [21]. These data provided evidence that the mechanisms that operate in the regulation of tissue stem cells and tissue homeostasis are crucial factors in tumourigenesis.

Despite the many advances in stem cell research over the years, knowledge of the factors that maintain the stem cell pool and control cell differentiation in different tissues is still incomplete. Fbw7 has been described to play a key role in regulating stem cell biology and tissue homeostasis in the nervous system, intestinal tissue, haematopoietic stem cells and liver [22–28], and we summarise these findings below (Fig. 2) [20].

### 2.1. Fbw7 in the nervous system

Fbw7 has a crucial role in neural stem cells (NSCs) to regulate the abundance of the transcriptionally active form of Notch (NICD). Mice lacking Fbw7 die perinatally, and the absence of Fbw7 results in decreased neurogenesis and accumulation of radial glial neural stem cells. In addition, the loss of Fbw7 leads to impaired NSC differentiation and increased apoptosis of neural progenitors, due to upregulation of NICD and c-Jun respectively [22,28].

### 2.2. Fbw7 in the intestine

Consistent with its role in regulating NICD/c-Jun and the importance of Notch/c-Jun signalling in intestinal homeostasis, conditional loss of Fbw7 in the intestinal tissue leads to accumulation of intestinal progenitors in the intestinal crypts and impaired differentiation of secretory cells [24]. The effect of Fbw7 loss in the intestine was also observed in Fbw7 heterozygous mice, due to the existence of a positive regulatory loop between Notch and Fbw7, thus reinforcing the previously observed haploinsufficiency for Fbw7 observed in human intestinal tumours [29].

### 2.3. Fbw7 in haematopoietic stem cells

Several independent groups have shown that Fbw7 regulates HSC quiescence and differentiation [25,26,30,31]. Inactivation of Fbw7 in the bone marrow causes premature HSC death through p53-dependent apoptosis. Fbw7 null HSCs upregulate c-Myc and Notch1, and downregulate Mdm2 expression, which suppresses p53 [26]. In addition, Fbw7 controls HSC quiescence and self-renewal by stabilising c-Myc, suggesting that c-Myc is a crucial regulator of cell cycle entry in HSCs [25]. Conversely, overexpression of Fbw7α causes cell cycle dormancy in HSCs through downregulation of c-Myc and Notch [30]. These data indicate that inactivation of Fbw7 in HSCs leads to exhaustion of quiescent HSCs, mediated through activation of the c-Myc and Notch signalling pathways.

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