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review

Emerging roles of the FBW7 tumour suppressor in stem cell differentiation

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FBW7 is a ubiquitin E3 ligase substrate adaptor that targets many important oncoproteins—such as Notch, c-Myc, cyclin E and c-Jun—for ubiquitin-dependent proteolysis. By doing so, it plays crucial roles in many cellular processes, including cell cycle progression, cell growth, cellular metabolism, differentiation and apoptosis. Loss of FBW7 has been observed in many types of human cancer, and its role as a tumour suppressor was confirmed by genetic ablation of FBW7 in mice, which leads to the induction of tumorigenesis. How FBW7 exerts its tumour suppression function, and whether loss of FBW7 leads to de-differentiation or acquisition of stemness—a process frequently seen in human carcinomas—remains unclear. Emerging evidence shows that FBW7 controls stem cell self-renewal, differentiation, survival and multipotency in various stem cells, including those of the haematopoietic and nervous systems, liver and intestine. Here, we focus on the function of FBW7 in stem cell differentiation, and its potential relevance to human disease and therapeutics.

Keywords: stem cell; FBW7; differentiation; Notch; c-Jun

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See Glossary for abbreviations used in this article.

What is FBW7?

Ubiquitination is a post-translational modification that has many regulatory roles in the cell. An important one is the promotion of protein degradation by the 26S proteasome, a multi-subunit protease complex. This pathway controls diverse cellular processes, including cell proliferation, cell cycle progression, transcription, immune response, DNA damage repair and apoptotic cell death (Nalepa *et al*, 2006). Ubiquitination is mediated through a cascade of three enzymatic reactions catalysed by the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and the ubiquitin ligases (E3s), which can physically bind to and add ubiquitin chains to the target protein, often resulting in its recognition and degradation in an ATP-dependent manner by the proteasome (Nalepa *et al*, 2006).

On the basis of common structural motifs, E3s are grouped into several main classes: RING-finger, HECT, U-box, PHD-finger and RBR (Eisenhaber *et al*, 2007; Rotin & Kumar, 2009). RING-finger E3 ligases are grouped into subfamilies, one of which consists of the Cullin-based E3 ligases, including the Skp1–Cullin 1–F-box (SCF) complex (Frescas & Pagano, 2008). The core of the SCF complex consists of Skp1, Rbx1 and Cullin 1; it additionally contains variable F-box proteins (Frescas & Pagano, 2008). Each F-box protein has two main functional domains: the F-box, which interacts with the SCF core complex through binding to Skp1; and the carboxy-terminal domains—such as WD40 or the LRR domain—which bind to specific substrates (Nakayama & Nakayama, 2006). More than 70 putative F-box proteins have been found in the human genome (Winston *et al*, 1999), and their function and physiological substrates are mostly unknown. Among them, FBW7—also known as Fbxw7, Ago, CDC4 and Sel10—has been reported to target various oncogenic proteins for degradation (Welcker & Clurman, 2008). There are three mammalian FBW7 isoforms —FBW7α, FBW7β and FBW7γ—derived from alternative splicing, which encode unique amino-termini (Welcker & Clurman, 2008). This leads to differences in cellular localization and tissue distribution: FBW7α and FBW7γ localize in the nucleus and nucleolus, respectively, whereas FBW7β is mainly expressed in the cytoplasm (Welcker & Clurman, 2008). The exact physiological role of each isoform remains largely unknown.

Only a handful of FBW7-specific substrates have been described, such as Aurora (Finkin *et al*, 2008), cyclin E (Koepp *et al*, 2001; Moberg *et al*, 2001; Strohmaier *et al*, 2001), c-Jun (Nateri *et al*, 2004; Wei *et al*, 2005), c-Myc (Welcker *et al*, 2004; Yada *et al*, 2004), c-Myb (Kanei-Ishii *et al*, 2008), KLF5 (Zhao *et al*, 2010), Mcl-1 (Inuzuka *et al*, 2011; Wertz *et al*, 2011), mTOR (Mao *et al*, 2008), Notch (Gupta-Rossi *et al*, 2001; Hubbard *et al*, 1997; Oberg *et al*, 2001; Wu *et al*, 2001), presenilin (Rocher-Ros *et al*, 2010) and SREBP (Sundqvist *et al*, 2005; Fig 1). All are oncogenic proteins often overexpressed in human cancers that have essential roles in signalling pathways involved in cell growth, division, differentiation and apoptosis. Therefore, FBW7 is believed to function as a tumour suppressor

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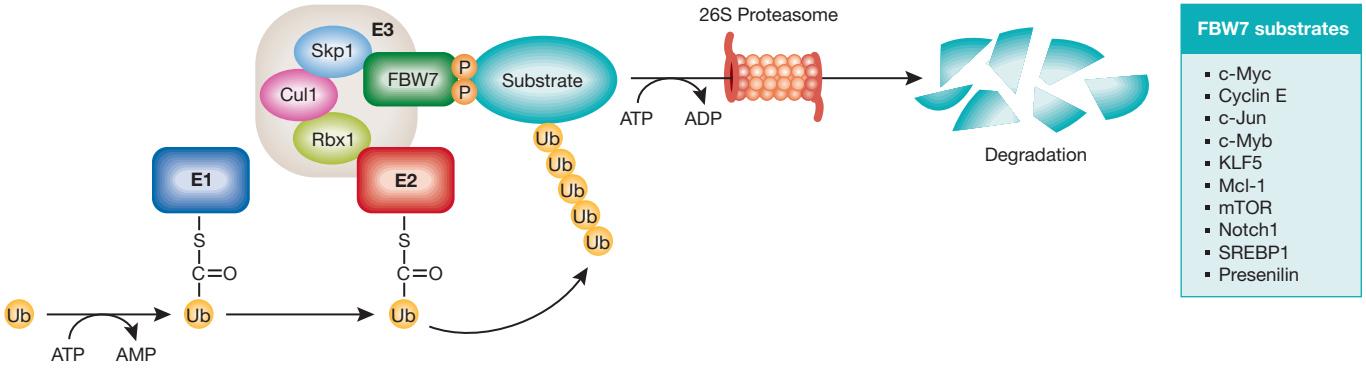

Fig 1 | Pathway of FBW7-mediated degradation. Proteins are targeted for degradation by the ubiquitin proteasome system through an enzymatic cascade involving three enzymes: the ubiquitin-activating E1, the ubiquitin-conjugating E2 and a ubiquitin ligase, E3. The initial step is ATP-dependent and involves the linkage of ubiquitin to E1. Ubiquitin is then activated and transferred to E2. The ubiquitin-charged E2 then interacts with a specific E3 partner such as SCF(FBW7), which transfers the ubiquitin molecule to the substrate, resulting in its recognition and degradation in an ATP-dependent manner by the 26S proteasome. FBW7, F-box and WD-repeat-domain-containing 7; Ub, ubiquitin.

due to the negative regulation of these oncogenic proteins. Indeed, FBW7 is frequently inactivated by deletion, mutation or promoter hypermethylation in cancer (Crusio *et al*, 2010). FBW7 mutations occur in approximately 30% of T-cell acute lymphoblastic leukaemia (T-ALL), 16% of primary endometrial cancer and 11% of colorectal cancer. Overall, approximately 6% of all human primary tumours have mutations in FBW7 (Perry & Li, 2008).

How does FBW7 exert its anti-tumoral activity? The exact molecular mechanisms by which FBW7 suppresses cancer development and progression are still not fully understood (Sidebar A). However, several studies have begun to shed some light on this question. For example, FBW7 deletion leads to activation of p53 through c-Myc accumulation in mature T lymphocytes (Onoyama *et al*, 2007). Although FBW7 deletion alone is not sufficient for tumorigenesis, the combination of FBW7 and p53 inactivation efficiently promotes lymphomagenesis (Onoyama *et al*, 2007). Furthermore, loss of FBW7 induces gut adenomas mainly due to overexpression of Notch and c-Jun (Babaei-Jadidi *et al*, 2011), and inhibition of FBW7 induces the development of thymic lymphoma through upregulation of c-Myc (Onoyama *et al*, 2007). Additionally, depletion of FBW7 delays c-Myb turnover and increases its abundance in a GSK3-dependent manner in myeloid leukaemia cells (Kitagawa *et al*, 2009). FBW7 targets mTOR for ubiquitination and degradation, and loss of FBW7 leads to activation of mTOR in human breast cancer (Mao *et al*, 2008). More recently, FBW7 was described to target Mcl-1 for ubiquitination and destruction (Inuzuka *et al*, 2011; Wertz *et al*, 2011). As a result, elevated expression of the pro-survival factor Mcl-1 in FBW7-deficient cells results in resistance to anti-tubulin chemotherapeutic agents and accelerated tumorigenesis (Wertz *et al*, 2011). Similarly, FBW7-deficient T-ALL cells with higher expression of Mcl-1 are more sensitive to the Mcl-1 antagonist sorafenib, while acquiring resistance to the Bcl-2 family inhibitor ABT-737 (Inuzuka *et al*, 2011). Although inhibition of FBW7 substrates might contribute to suppress tumour growth, many questions remain before we will fully understand the complex roles of FBW7 as a tumour suppressor (Sidebar A).

Sidebar A | In need of answers

(i) How does loss of FBW7 result in cancer?
(ii) How does FBW7 regulate abnormal cell division, resulting in cancer?
(iii) Why are numerous oncoproteins regulated by one ubiquitin E3 ligase, SCF(FBW7)?
(iv) How does FBW7 control stem cell self-renewal and differentiation?
(v) Does loss of the FBW7 tumour suppressor lead to de-differentiation or acquisition of the cancer stem cell phenotype, thus contributing to tumour development?
(vi) Can one specific signalling pathway control stem cell differentiation to a specific cell type?
(vii) Does targeting FBW7 regulate stem cell differentiation? Can this be harnessed to design novel therapeutic strategies for human disease?

Stem cells at a glance

In addition to its known role in tumour suppression, FBW7 has recently been implicated in the control of stem cell biology, which is the focus of this review. Stem cells are classically defined as cells with unlimited capacity for self-renewal and a remarkable potential to differentiate into a full spectrum of cells, allowing the formation of tissues and/or full organisms (Reya *et al*, 2001). There are three main types of stem cell: embryonic, germinal and somatic. Embryonic stem cells (ESCs) are derived from the blastocyst—a 3–5-day-old embryo—and have the capacity to generate all the cell types of a mature organism, as well as the ability to replicate unlimitedly (Young, 2011). Germinal stem cells from the germinal layer of the embryo eventually undergo differentiation to generate either eggs or sperms (Barroca *et al*, 2009). Somatic stem cells (also known as adult stem cells, ASCs) differentiate into many characteristic cell types within a specific organ or tissue (Wagers & Weissman, 2004). Recently, a new type of stem cell, termed induced pluripotent stem cells (iPSCs), were generated by transduction of four defined transcription factors—OCT4, SOX2, KLF4 and c-Myc—thus allowing some specialized adult cells to be genetically reprogrammed into a stem-cell-like state (Takahashi & Yamanaka, 2006; Wu & Hochedlinger, 2011). However, further studies revealed that iPSCs differ significantly from ESCs in many aspects (Pappas & Yang, 2008). The concept of cancer stem cells (CSCs)—also known as cancer-stem-like cells—was introduced a few years ago and remains controversial (Clevers, 2011). CSCs with the capacity to self-renew and differentiate have been identified and isolated from a wide variety of human cancers including haematopoietic tumours,

Glossary

BLBP	brain lipid-binding protein
DAPT	N-[N-(3,5-difluorophenacetyl)- l-alanyl]-S-phenylglycine t-butyl
FBW7	F-box and WD-repeat-domain-containing 7
GSK-3	glycogen synthase kinase 3
HECT	homologous to E6-associated protein carboxyl terminus
Hes	hairy and enhancer of split 1
Hey	hairy/enhancer-of-split related with YRPW motif protein 1
KLF	Kruppel-like factor
mTOR	mammalian target of rapamycin
RBR	ring between ring fingers
SOX	SRY-box
SREBP	sterol regulatory element-binding protein
Wnt	wingless-type MMTV integration site family

breast, lung, prostate, colon, brain, head and neck, and pancreatic cancer (Clevers, 2011). However, it remains unclear how CSCs contribute to tumorigenesis *in vivo*.

Stem cells are found in particular locations or microenvironments within tissues, which are known as stem cell niches (Lin, 2002). The niche consists of neighbouring proliferating and differentiating cells that provide the appropriate environment so that the stem cells can remain undifferentiated (Lin, 2002). In tissues such as the gut and bone marrow, stem cells function to repair damaged cells and/or replace those that were lost due to normal wear, tear or injury (Yen & Wright, 2006). However, in other organs—including the heart and pancreas—stem cells only divide under special conditions, such as in response to injury (Laflamme & Murry, 2011; Zaret & Grompe, 2008). Interestingly, unlike somatic cell division, which yields two identical daughter cells, stem cell division is asymmetrical. Each daughter cell has the potential to either remain a stem cell or give rise to a specialized cell, such as a red blood, muscle or brain cell (Cohen & Melton, 2011).

Although research on stem cells has generated several important discoveries over the past decades, we still do not understand the signals that trigger the process of stem cell differentiation and have only a limited picture of what maintains 'stemness'. Therefore, research into these questions is urgently needed. Several factors and signals, such as components of Notch, Wnt and Sonic hedgehog, have been shown to have pivotal roles in stem cell differentiation (Reya *et al*, 2001) and have been comprehensively reviewed elsewhere (Takebe *et al*, 2011). In addition, a body of recent literature has shown that FBW7 is also involved in stem cell differentiation (Hoeck *et al*, 2010; Iriuchishima *et al*, 2011; Matsumoto *et al*, 2011; Matsuoka *et al*, 2008; Perry & Li, 2008; Reavie *et al*, 2010; Thompson *et al*, 2008). The following sections discuss the potential roles of FBW7 in stem cell differentiation.

FBW7 in stem cell differentiation

It is important to note that ESC-specific depletion of FBW7 does not induce phenotypic changes or alter the expression of germline markers, suggesting that FBW7 is dispensable for the self-renewal of ESCs (Reavie *et al*, 2010). Nevertheless, several studies have shown that FBW7 regulates several transcription factors—such as c-Myc, Notch and c-Jun—involved in the quiescence and self-renewal of haematopoietic stem cells (HSCs; Iriuchishima *et al*, 2011; Matsuoka *et al*, 2008; Thompson *et al*, 2008). Furthermore, two independent groups have shown that FBW7 is a key regulator of neural stem cell (NSC) maintenance and differentiation in the brain (Hoeck *et al*, 2010; Matsumoto *et al*, 2011). FBW7 also regulates the maintenance of intestinal progenitors, as progenitor cells accumulate in the crypts of mice lacking FBW7 in the intestine and their differentiation into goblet cells is impaired (Sancho *et al*, 2010). However, the expression of stem cell markers remained unchanged in the intestines of FBW7-deficient mice, arguing against an expansion of the stem cell compartment (Sancho *et al*, 2010). More recently, FBW7 deficiency has also been shown to lead to a shift in the differentiation of liver stem cells from the hepatocyte lineage to cholangiocytes, as well as to an increase in cell proliferation (Onoyama *et al*, 2011). In the following subsections, we discuss the contributions of FBW7 to the maintenance and differentiation of some stem cell types.

Neural stem cells. The adult brain contains NSCs, which are able to generate three main cell types: neurons, astrocytes and oligodendrocytes (Sahni & Kessler, 2010). NSCs are known to change their competency during the development process. They initially give rise to neurons and later to other cell types that are also known as later-born cell types and include astrocytes, oligodendrocytes and ependymal cells. Both the cell number and the later-born cell types are decreased if NSCs are prematurely depleted, suggesting that NSC maintenance is necessary to generate the proper cell quantity and full cellular diversity before the final stage of development (Hatakeyama *et al*, 2004). In fact, the size and shape of the nervous system primarily depends on the number of times an NSC periodically re-enters the cell cycle (Ohnuma & Harris, 2003). In support of this idea, mutation of cyclin-dependent kinase inhibitor p27 blocks cell cycle progression and delays cell differentiation, leading to an enlarged brain (Nakayama *et al*, 1996). Furthermore, cell differentiation delayed by expression of stabilized β-catenin in turn increased the size of NSCs and neurons, also resulting in an enlarged brain (Chenn & Walsh, 2002). In addition, the inactivation of the Notch targets Hes1, Hes3 and Hes5 accelerates cell differentiation and causes a wide range of brain malformations (Hatakeyama *et al*, 2004).

FBW7 has been recently shown to have a crucial role in NSCs to regulate the abundance of Notch proteins (Matsumoto *et al*, 2011). Mice with conditional ablation of FBW7 in the brain die shortly after birth and lack suckling behaviour (Matsumoto *et al*, 2011). In these mice, the differentiation of neural progenitor cells was skewed towards astrocytes rather than neurons. This phenotype is consistent with the observed accumulation of Notch 1 and Notch 3, as well as overexpression of Notch downstream target genes, including *Hes1*, *Hes5*, *Hey1*, *Hey2* and *BLBP*, most of which are associated with the maintenance of NSCs (Matsumoto *et al*, 2011). Inhibition of the Notch pathway by the pharmacological inhibitor DAPT increased the number of neurons and reduced the number of astrocytes (Matsumoto *et al*, 2011), indicating that excessive and persistent Notch signalling impairs the differentiation of NSCs to neurons, favouring astrocytes instead.

In agreement with an important role of FBW7 in NSCs, another study identified that FBW7 is a key regulator of neural progenitor viability and NSC differentiation (Hoeck *et al*, 2010). This group also used conditional knockout mice to inactivate FBW7 in the nervous system and found that mice lacking FBW7 died during the perinatal period. The absence of FBW7 resulted in decreased neurogenesis and an accumulation of cells expressing radial glia markers in cultured neurospheres, indicating that FBW7 inactivation

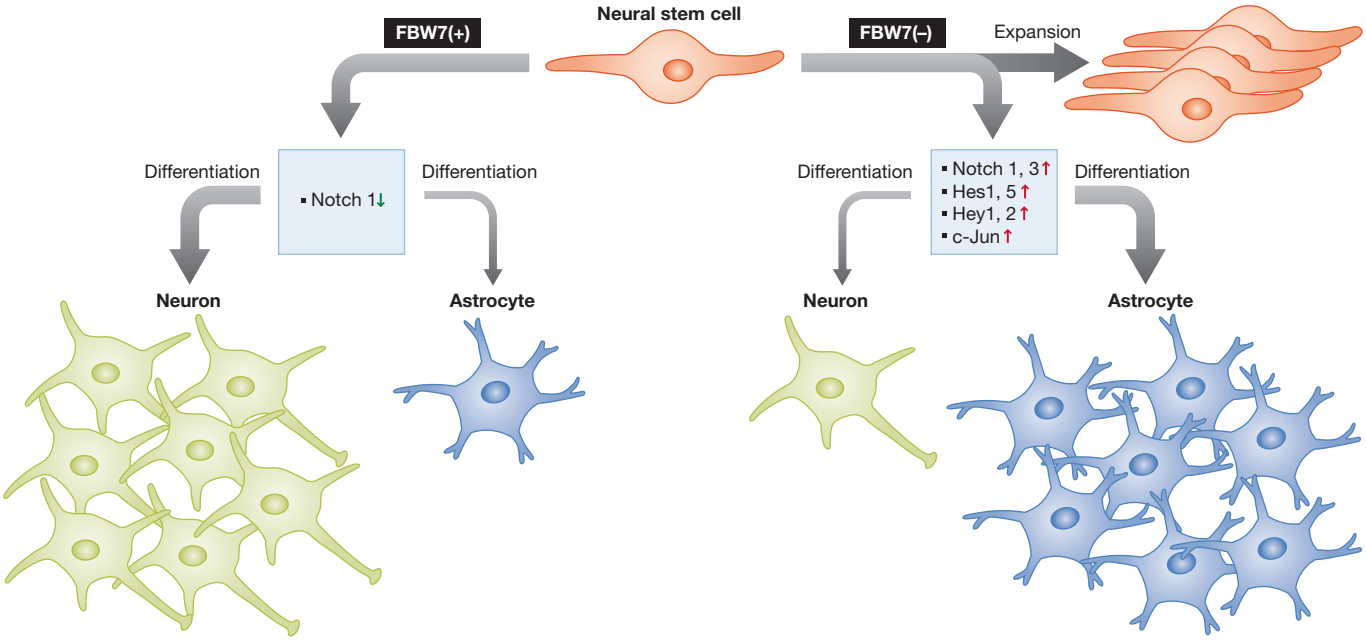

Fig 2 | FBW7 is required for neural stem cell differentiation. FBW7 controls NSC differentiation through downregulation of its ubiquitin substrates, such as Notch 1. Loss of FBW7 leads to impaired NSC differentiation due to the upregulation of c-Jun, Notch 1, Notch 3 and the Notch target genes *Hes1*, *Hes5*, *Hey1* and *Hey2*. After deletion of FBW7, differentiation of NSCs is skewed towards astrocytes at the expense of neurons, suggesting that excessive and persistent Notch signalling impairs the differentiation of NSCs to neurons. FBW7, F-box and WD-repeat-domain-containing 7; Hes, hairy and enhancer of split 1; Hey, hairy/enhancer-of-split related with YRPW motif protein 1; NSC, neural stem cell.

might lead to increased generation of radial glia neural stem cells (Hoeck *et al*, 2010). In addition, the loss of FBW7 led to impaired NSC differentiation and markedly increased apoptotic death of neural progenitors, due to the upregulation of Notch 1 and c-Jun, respectively. Inhibition of the Notch pathway with DAPT alleviated the blocking of stem cell differentiation (Hoeck *et al*, 2010). Together, these two independent studies demonstrate that FBW7 might be required for NSC differentiation (Fig 2; Hoeck *et al*, 2010; Matsumoto *et al*, 2011).

Haematopoietic stem cells. These cells reside in the bone marrow and give rise to all mature blood cell types: red blood cells, B and T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, macrophages and platelets (Schroeder, 2010). HSCs remain quiescent or dormant during homeostasis, although they are the adult stem cell with the highest potential to generate a large number of progenitor cells (Schroeder, 2010). To maintain homeostasis and respond rapidly to haematopoietic stresses—such as bleeding, toxic insults and chemotherapeutic agents—HSCs self-renew and differentiate to produce new blood cells (Schroeder, 2010). Several signalling pathways and molecules have been found to control the fate of HSCs, including Notch (Clements *et al*, 2011; Loeffler *et al*, 2011), Sonic hedgehog (Trowbridge *et al*, 2006), Smad (Blank *et al*, 2008; Larsson & Karlsson, 2005), Wnt (Duncan *et al*, 2005) and c-Myc (Hoffman *et al*, 2002; Laurenti *et al*, 2008), indicating that the self-renewal and quiescence of HSCs are controlled by a highly orchestrated integration of intrinsic and extrinsic signals. Several independent groups have recently shown that FBW7 regulates HSC quiescence and differentiation (Matsuoka *et al*, 2008; Reavie *et al*, 2010; Thompson *et al*, 2008).

FBW7-deficient mice die at embryonic day 10.5 due to defects in haematopoiesis and vascular development (Tetzlaff *et al*, 2004; Tsunematsu *et al*, 2004). The inactivation of FBW7 in bone marrow HSCs causes premature HSC death through p53-dependent apoptosis (Matsuoka *et al*, 2008). Furthermore, FBW7-deficient HSCs upregulate c-Myc and Notch 1 expression, and downregulate Mdm2 expression, which suppresses p53 function (Matsuoka *et al*, 2008). Interestingly, loss of FBW7 confers a selective advantage to cells in which p53 function is inhibited, resulting in the development of T-ALL; this suggests that FBW7 acts as a fail-safe mechanism against both premature HSC loss and leukaemia (Matsuoka *et al*, 2008). In addition, FBW7 controls HSC quiescence and self-renewal, as its deletion leads to defective stem cell quiescence, which results in impaired self-renewal and loss of repopulating capacity (Thompson *et al*, 2008). Deletion of FBW7, which is highly expressed in non-cycling HSCs, specifically affects the expression of several important regulators of cell cycle entry, such as p57 and E2F2, inducing exit from quiescence and entry into the cell cycle (Thompson *et al*, 2008). As in the previous study, deletion of FBW7 in bone marrow stem cells and progenitor cells also induced c-Myc stabilization, suggesting that c-Myc overexpression could be an important regulator of cell cycle entry in HSCs (Thompson *et al*, 2008). Slight changes in the stability and abundance of c-Myc—which is controlled by FBW7—can profoundly modify the transcriptional programme of HSC and therefore trigger their quiescence or self-renewal (Reavie *et al*, 2010).

FBW7α is the FBW7 isoform preferentially expressed in primitive HSCs (Iriuchishima *et al*, 2011). Overexpression of FBW7α causes cell-cycle dormancy, mainly through downregulation of FBW7 substrates such as c-Myc, Notch 1 and the mTOR target

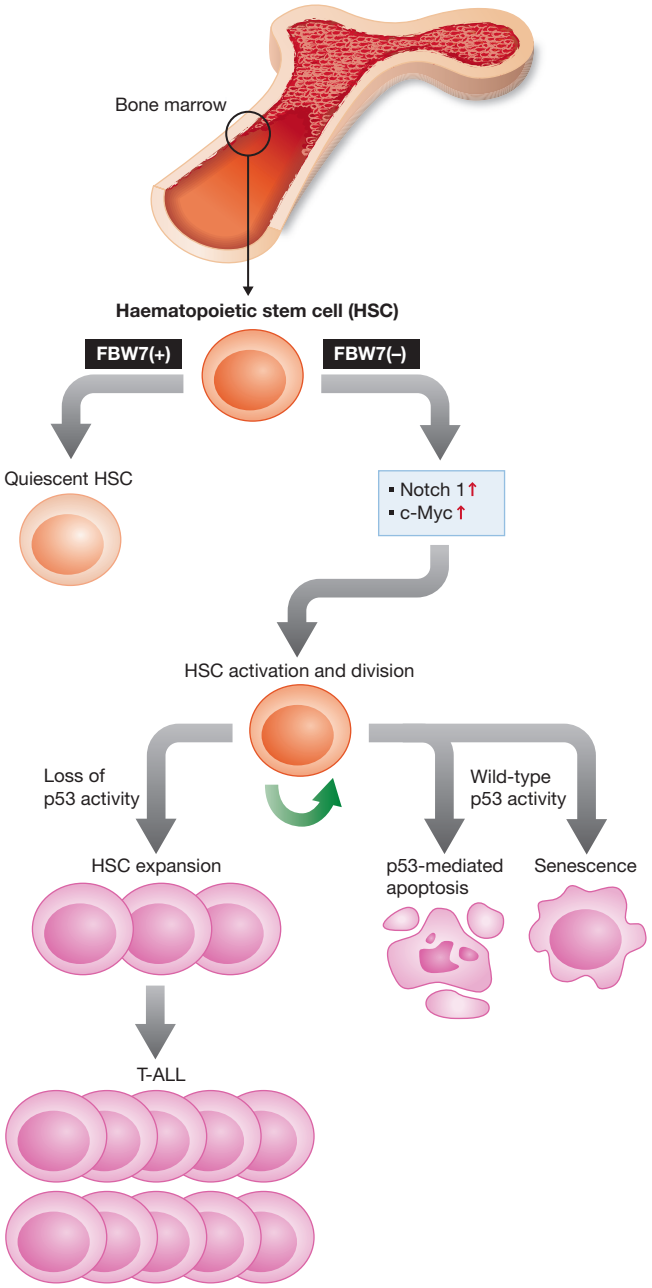

Fig 3 | Hypothesis of FBW7 function in haematopoietic stem cells. FBW7 is required for maintenance of HSC quiescence. Loss of FBW7 results in the accumulation of Notch 1 and c-Myc, leading to an exit from quiescence and entry into the cell cycle, which results in HSC expansion. HSC expansion is inhibited by either p53-dependent apoptosis or senescence. Loss of p53 activity leads to uncontrolled HSC expansion and eventually results in leukaemia. FBW7, F-box and WD-repeat-domain-containing 7.

S6 (Iriuchishima *et al*, 2011). In addition, c-Myc expression is inhibited in an FBW7α-dependent manner during hypoxia, which is an essential factor for HSC quiescence (Iriuchishima *et al*, 2011). Thus, FBW7α sustains HSC quiescence through the inhibition of the c-Myc, Notch 1 and mTOR pathways. Interestingly, only attenuation of c-Myc rescues the proliferative abnormality of FBW7-null $CD4^+CD8^+$ cells (Onoyama *et al*, 2007; Welcker & Clurman, 2008). All of these studies point to c-Myc as a crucial FBW7 substrate in the context of HSC self-renewal and maintenance of the haematopoietic system. On the basis of these data, we propose a pathway through which inactivation of FBW7 leads to exhaustion of quiescent HSCs, mediated in part through activation of the c-Myc and Notch signalling pathways (Fig 3).

Cancer stem cells. Although the existence of CSCs was first proposed more than a century ago, they have recently regained considerable attention due to advances in stem cell research (Visvader & Lindeman, 2008). Nevertheless, the concept of CSCs remains controversial and the role they play in tumour biology probably depends on the type of tumour. They are defined as a population of cells that have the capacity to self-renew and, thus, maintain tumours. Specific markers that identify CSCs in a variety of human cancers have been described (Visvader & Lindeman, 2008). Emerging evidence suggests that cancers could arise from CSCs, as they can self-renew, differentiate and regenerate the phenotypic cells of the original tumour when implanted into severely combined immunodeficient mice (Visvader & Lindeman, 2008). The concept of CSCs will undoubtedly help us to understand tumour biology and hopefully design novel therapeutic strategies for the complete eradication of tumour growth by targeting CSCs.

Notch—an FBW7 substrate—is deregulated in CSCs, leading to their uncontrolled self-renewal and a predisposition to tumour development, indicating that FBW7 could have a role in CSC biology through the Notch pathway (Ranganathan *et al*, 2011). Indeed, the Notch signalling network is frequently overexpressed in human malignancies including cervical, lung, colon, head and neck, and pancreatic cancers, renal carcinoma, acute myeloid leukaemia, and Hodgkin and large-cell lymphomas (Wang *et al*, 2010). The fate of CSCs has been shown recently to be controlled in part by the Notch pathway. For example, breast CSCs upregulate Notch, and its inhibition decreases the number of stem cells and their self-renewal capacity (Harrison *et al*, 2010; Kondratyev *et al*, 2011). Furthermore, breast CSCs contribute to the development of brain metastases due in part to increased Notch activity (Harrison *et al*, 2010; McGowan *et al*, 2011). Therefore, inhibition of Notch 1 reduces the number of CSCs and the formation of brain metastases from breast cancer (McGowan *et al*, 2011). Similar trends were observed in other CSCs: inhibition of Notch 1 decreased glioma CSC proliferation and glioma growth (Wang *et al*, 2011), as well as the growth of hepatocellular carcinoma (Nishina *et al*, 2011) and leukaemia (Alcalay *et al*, 2003). Notably, FBW7 deletion leads to stem cell activation and leukaemogenesis through the Notch target c-Myc (Matsuoka *et al*, 2008). Taken together, FBW7 targets Notch for degradation, which is a key regulatory process that controls CSC self-renewal. Furthermore, c-Myc is one of the crucial factors in the generation of iPSCs (Takahashi & Yamanaka, 2006). It is therefore possible that the accumulation of c-Myc (Takahashi & Yamanaka, 2006) or KLF5 (Liu *et al*, 2010; Zhao *et al*, 2010) contributes to the partial induction of stem-cell-like phenotypes in $FBW7^{-/-}$ cancers.

Clinical implications for therapy

Given the potential of stem cells to undergo self-renewal and differentiation, their therapeutic use in the context of transplantation, regenerative medicine and cancer treatment is under intense investigation. In fact, adult bone-marrow HSCs have been used

in transplants for more than 40 years (Blade *et al*, 2010). As stem cells exist in many organs, including the brain and heart, these cells could become the basis of transplantation-based therapies if we can harness the conditions to control stem cell differentiation. Stem cell research also opens a new therapeutic window for regenerative or reparative medicine for diseases such as diabetes and heart disease (Wagers & Weissman, 2004). For example, it is now possible to regenerate bone by using cells derived from bone marrow stroma, to repair damaged heart muscle with cardiac muscle cells and to develop insulin-producing cells for the possible treatment of diabetes (Wagers & Weissman, 2004). To reach these goals, we need to be able to generate large quantities of adult stem cells and to differentiate them into specific, fully functional cell types.

As mentioned above, FBW7 plays a pivotal role in stem cell self-renewal and differentiation. It is also required for the maintenance of HSC quiescence; consequently, the loss of FBW7 promotes transient HSC proliferation, eventually leading to their exhaustion (Matsuoka *et al*, 2008; Thompson *et al*, 2008). On the other hand, loss of FBW7 leads to impaired NSC differentiation and increased apoptotic death of neural progenitors (Hoeck *et al*, 2010). Therefore, regulation of FBW7 could theoretically be used to control stem cell differentiation for potential clinical therapies. In this regard, NSC manipulation could potentially be used in the treatment of neurological diseases such as stroke, multiple sclerosis and Parkinson disease. Furthermore, due to the role of FBW7 in CSCs, it might be possible to target and eliminate them through upregulation of FBW7 activity, which would consitute a novel strategy for cancer treatment. Overexpression of FBW7, or induction of FBW7 expression, is possible by manipulating its upstream regulators, including p53 (Yokobori *et al*, 2009) and microRNAs (Lerner *et al*, 2011; Mavrakis *et al*, 2011). This would subsequently reduce Notch activity, eventually inhibiting the ability of CSCs to repopulate the cells forming the tumour mass. For example, gliomas—which are the most common types of tumour of the central nervous system—have inactivation of FBW7 (Hagedorn *et al*, 2007) and higher expression of Notch (Stockhausen *et al*, 2010), and might arise from neural CSCs. Given its ability to promote degradation of Notch and subsequently enhance NSC differentiation, FBW7 is expected to be a novel target to treat gliomas. Interestingly, several types of CSC were shown to share many properties—including self-renewal, pluripotency and quiescence—with normal HSCs (Clevers, 2011; Rasheed *et al*, 2011), in which FBW7 is required for survival by suppression of c-Myc activity. Therefore, inhibition of FBW7 might also be effective for eradication of CSCs. However, it should be noted that considerable additional research is required to understand fully how to use stem cells for cell-based or tissue-based therapies to treat human diseases.

Conclusions and future perspectives

We have discussed recent studies that identify FBW7 as a key player in controlling the balance between stem cell dormancy and self-renewal. It is noteworthy that the function of FBW7 in stem cells is dependent on the organ system of interest. For example, FBW7 is required for neural differentiation, while loss of FBW7 in HSCs leads to defective maintenance of quiescence and premature depletion of HSCs (Hoeck *et al*, 2010; Thompson *et al*, 2008). These observations suggest that the lack of FBW7 leads to opposite functional consequences in HSCs and NSCs. Given its ability to control stem cell differentiation, regulation of FBW7 by novel approaches is likely to have a significant impact in designing new disease-specific therapies, including cancer treatment.

However, at the current stage, it is imperative to elucidate further our molecular understanding of how FBW7 controls the self-renewal and survival capacity of stem cells. Although some progress has been made in this regard, we have only touched the tip of the iceberg. Future studies should address many important outstanding questions (Sidebar A). For example, how are stem cells maintained in the adult tissues? In addition to the well-characterized lateral inhibition by the Notch signalling pathway, what are other molecular mechanisms that allow stem cells to remain undifferentiated after the cells around them have undergone differentiation? Can one specific signalling pathway control stem cell differentiation to a specific cell type? How does FBW7 regulate abnormal cell division, resulting in cancer? The answers to these questions will help us to understand how cancer cells are regulated, and the involvement of FBW7 in this process. Furthermore, addressing these questions might enable us to control stem cell differentiation and thereby grow cells or tissues for medical purposes, such as cell-based therapies, as well as develop novel strategies to target cancer and other complex diseases.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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