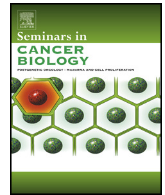
Seminars in Cancer Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer



F-box proteins: Keeping the epithelial-to-mesenchymal transition (EMT) in check

Víctor M. Díaz [a,b,c,*], Antonio García de Herreros [a,b,c,*]

[a] Programa de Recerca en Càncer, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain
[b] Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain
[c] Parc de Recerca Biomèdica de Barcelona, Doctor Aiguader, 88, E-08003 Barcelona, Spain

ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form 1 October 2015
Accepted 17 October 2015

Keywords:
EMT
Snail
Twist
Zeb
F-box
Ubiquitination
Proteasome
Cancer

ABSTRACT

F-box proteins are the key recognition subunit of multimeric E3 ubiquitin ligase complexes that participate in the proteasome degradation of specific substrates. In the last years, a discrete number of F-box proteins have been shown to regulate the epithelial-to-mesenchymal transition (EMT), a process defined by a rapid change of cell phenotype, the loss of epithelial characteristics and the acquisition of a more invasive phenotype. Specific EMT transcription factors (EMT-TFs), such as Snail, Slug, Twist and Zeb, control EMT induction both during development and in cancer. These EMT-TFs are short-lived proteins that are targeted to the proteasome system by specific F-box proteins, keeping them at low levels. F-box proteins also indirectly regulate the EMT process by controlling EMT inducers, such as Notch, c-Myc or mTOR. Here we summarize the role that these F-box proteins (Fbxw1, Fbxw7, Fbxl14, Fbxl5, Fbxo11 and Fbxo45) play in controlling EMT during development and cancer progression, a process dependent on post-translational modifications that govern their interaction with target proteins.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Ubiquitination is a post-translational modification that strongly impacts protein turnover. Further, mono- and polyubiquitination, with its multiple variants, also affect protein localization and regulate cell cycle progression, apoptosis and transcription. In the last years, scientists have put much effort into deciphering the ubiquitin-modified proteome (ubiquitinome) [1,2]; however, for most proteins, it is still unknown which ubiquitin ligases catalyse the reaction. Many oncogenes and tumor suppressors are short-lived proteins targeted by the ubiquitin proteasome system, and their deregulated proteolysis is involved in various diseases, including cancer. In this review, we focus on the regulation of protein stability of factors that control the epithelial-to-mesenchymal transition (EMT), a cellular conversion that takes place not only during development but also in tumour progression. EMT is governed by several short-lived transcriptional factors, the stability of which is controlled by several F-box ubiquitin ligases.

* Corresponding authors at: Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Programa de Recerca en Càncer, C/ Doctor Aiguader 88, Barcelona, Spain. Tel.: +34 933160433.
E-mail addresses: victor.diaz@upf.edu (V.M. Díaz), agarcia@imim.es (A.G. de Herreros).

http://dx.doi.org/10.1016/j.semcancer.2015.10.003
1044-579X/© 2015 Elsevier Ltd. All rights reserved.

2. The SCF subfamily of E3 ubiquitin ligases

Ubiquitination consists of the conjugation of the 76-amino acid protein ubiquitin to a target protein and requires the sequential action of three enzymes that act in a concerted manner: E1 (ubiquitin-activating enzyme) activates the ubiquitin molecule in an ATP-dependent manner, E2 (ubiquitin-conjugating enzyme) transfers the activated ubiquitin directly onto the substrate or to the third enzyme, the E3 ubiquitin ligase [3,4]. This enzyme is responsible for substrate binding and specificity, coordinating the interactions and successive ubiquitination (polyubiquitination) of specific residues in a protein. The process is reversible because polyubiquitin chains can be disassembled by a family of about 95 deubiquitinating enzymes (deubiquitinases, or DUBs) [5]. E1 and E2 have been only minimally linked to cancer development, while the loss or gain of the E3 function is a key factor in cancer initiation and progression.

The way in which ubiquitin is loaded onto its substrate depends on the specificity of about 600 existing E3s that belong to two large subfamilies, which contain either a RING/RING-like domain (RING, "really interesting new gene") or a HECT ("homologous to E6-AP C-terminus")-type domain. The largest family of E3s belong to the multi-subunit RING-type and associate to one of the seven Cullin (Cul) proteins, giving them the name Cullin-RING ubiquitin ligases (CRLs). Cul proteins act as molecular scaffolds to connect the

Please cite this article in press as: Díaz VM, de Herreros AG. F-box proteins: Keeping the epithelial-to-mesenchymal transition (EMT) in check. Semin Cancer Biol (2015), http://dx.doi.org/10.1016/j.semcancer.2015.10.003

E2 enzyme with the substrate via a multi-subunit complex. Thus, the Cul C-terminus binds to a RING finger protein (Rbx1/Roc1 or Rbx2/Roc2) that directly links with the E2 enzyme (for examples, see Figs. 1–5). On its N-terminal side, Cul interacts with several adaptor proteins, depending on the Cul variant: Cul2 and Cul5 interact with the adaptor Elongin BC; Cul3, with the BTB (Broad complex, Tramtrack and Bric-a-brac) proteins; Cul4A and Cul4B, with DDB1 (DNA damage-binding protein 1); and Cul1 and Cul7, with the adaptor Skp1 (S-phase-kinase-associated protein-1) [6]. Skp1 interacts specifically with F-box proteins through their F-box domain [7]. The assembled E3 multimeric complex of Skp1–Cullin1–F-box gives the name to the SCF subfamily that contains 69 F-box proteins [8]. Moreover, an active SCF complex requires the neddylation of Cul1 (that is, the incorporation of the ubiquitin-like polypeptide Nedd8 to Cul1 by a Nedd8 E3 ligase). Neddylation blocks the association of Cul with the negative regulator CAND1 (cullin-associated and neddylation-dissociated 1) [9]. The reverse process of Nedd8 removal, or deneddylation, requires the isopeptidase activity of the COP9 signalosome [10,11].

As stated above, the F-box protein is the substrate-recognition subunit of the SCF complex and contains an F-box domain, a conserved, N-terminal 50 amino acid motif that binds Skp1 [6,12,13]. Depending on the presence of particular domains in their C-terminal part, the 69 F-box proteins are grouped into three classes: 11 Fbxw proteins, characterized by WD40 repeat domains; 21 Fbxl proteins, with leucine-rich repeats (LRRs); and 37 Fbxo proteins that contain other domains [14]. In general, F-box proteins recognize a degradation signal within a protein or degron [6] that, as we describe below, often corresponds to a phosphorylated sequence.

3. EMT is governed by short-lived factors: The Snail, Twist and Zeb proteins

EMT is a reversible process that induces epithelial cells to acquire mesenchymal traits and that was first identified during development [15]. EMT is essential for gastrulation and neural crest delamination [16–19] but also takes place during tumor invasion, correlating with enhanced invasiveness [20,21]. EMT is observed at the tumor edge in close contact with peritumoral extracellular matrix (ECM) [22]. Lineage tracing experiments with pancreas tumors have demonstrated the existence of EMT in murine cancer models [23]. Phenotypically, tumor cells undergoing EMT become spindle-shaped and motile and acquire new biological characteristics, such as the capability to activate stromal cells. This crosstalk between tumor and stromal cells facilitate their invasive behaviour, generating a reciprocal influence between the tumor and its microenvironment [22,24,25]. EMT induction promotes the generation of cancer stem cells (CSCs) as well as of cancer-associated fibroblasts (CAFs) [26]. CAFs synthesize and release collagens, laminin and fibronectin, increase the stiffness of the ECM and promote anisotropic fiber orientation that sustains oriented tumor cell migration and invasiveness [27]. Another important property of mesenchymal cells resulting from EMT is their increased resistance to cell death and to chemo- and immunotherapies [16,28–30].

The molecular hallmark of EMT is the down-regulation of the epithelial marker E-cadherin due to transcriptional inhibition [31,32] that precedes the activation of mesenchymal genes, such as fibronectin [33]. The members of the Snail family Snail1 and Snail2 (formerly Snail and Slug), the Zeb family (Zeb1 and Zeb2) and Twist1 have been described to bind and repress the E-cadherin (CDH1) promoter [21,31]. Snail1/2 contains a N-terminal SNAG box that recruits several co-repressors and is required for transcriptional repression [34–38]. The C-terminus of Snail1/2 contains several zinc finger (ZnF) domains that bind E-boxes with a core 5′-CACCTG-3′ sequence, and, in the case of Snail1, also contains a nuclear localization signal (NLS) [39,40]. Although structurally unrelated, members of the ZEB family of transcription factors, ZEB1 (δEF1) and ZEB2 (SIP1), are also potent inhibitors of CDH1 expression, likewise functioning by binding to co-repressors [41–45]. As indicated above, another family of E-cadherin repressors involved in EMT contains the class II basic helix–loop–helix (bHLH) Twist proteins (Twist1 and Twist2) [21,46–48], the class I bHLH, TCF3 (E12 and E47 isoforms) [49] and the TCF4 (E2-2A and E2-2B isoforms) proteins [50,51]. Finally, an emerging family of zinc finger transcription factors involved in EMT are the Krüppel-like factors (KLFs) [52] such as KLF8 [53].

Snail, Zeb and Twist are activated by common extracellular cues that induce EMT, such as TGF-β, Wnt and growth factors [17,54–58]. Among these, TGF-β is the best studied and is known to up-regulate Snail1, Zeb1 and Twist1 to promote EMT [59]. EMT is also triggered by cell conditions that induce stress [60], such as hypoxia [56,61], reactive oxygen species (ROS) [62–65] and genotoxic stress caused by DNA damage [66]. EMT stimulated by stress conditions facilitates the acquisition of chemo- and radio-resistance, enhances invasion and metastasis of tumor cells and provides CSC-like properties [58,67–72].

Snail1 expression in TGF-β-induced EMT is transient yet required for a full cellular conversion [73]. In general, most of the EMT-TFs are short-lived proteins expressed at very low levels in epithelial cells [74]. Accordingly, half-lives of Snail1, Snail2, Zeb1 and Twist are approximately 25 min [75], 30 min [76], less than 1 h [55] and about 1.2 h [77], respectively. In the last years, EMT induced by cytokines or stress signalling has been associated to EMT-TF protein stabilization [55,78–80]. In the next sections, we summarize the mechanisms that control protein degradation and stabilization during EMT and discuss how this depends on the concerted action of several F-box proteins.

4. F-box proteins regulating EMT

Several F-box proteins (β-TrCP1/Fbxw1, Fbxw7, Ppa/Fbxl14, Fbxl5, Fbxo11 and Fbxo45) have been described to be relevant in EMT by participating in the degradation of EMT-TFs. These F-box proteins present different subcellular localization, substrate specificity and physio/pathological roles, as summarized in Table 1.

4.1. β-TrCP1/Fbxw1

Fbxw1, also named "β-transducin repeat-containing protein" (β-TrCP1), contains seven C-terminal WD40 repeats as well as an N-terminal F-box domain [14] (Fig. 1). The first substrates identified for this F-box protein were phosphorylated proteins, such as the NFκB inhibitor IκBα and β-catenin [81,82]. Since then, more than 35 substrates for β-TrCP1 and the highly related β-TrCP2 (Fbxw11) have been described [83]. Surprisingly, despite this high number of substrates, β-TrCP1(−/−) mice develop normally, showing only moderate impairment of spermatogenesis and reduced fertility for males [84] resulting from the stabilization of the β-TrCP1 substrates cyclin A and Emi1 in the testes [84,85]. Other substrates, such as β-catenin, IκBα and Snail1 are present at normal levels due to the redundant role of β-TrCP2 [84,85]. Mice with both isoforms depleted have a severe testicular phenotype [85].

Snail1 is one of the best-characterized targets for β-TrCP1 (Fig. 1A). Snail1 has a phospho-serine rich domain (amino acids 90–120) with a phosphodegron DpS^{96}GxxpS^{100} sequence similar to that found in β-catenin [86]. Snail1 phosphorylation and degradation requires GSK-3β activity in two consecutive steps: first, the phosphorylation on residues S104 and S107 promotes Snail1 localization to the cytoplasm [75,87] by uncovering a nuclear export sequence (NES) (amino acids 132–143) [88]; later, the

Please cite this article in press as: Díaz VM, de Herreros AG. F-box proteins: Keeping the epithelial-to-mesenchymal transition (EMT) in check. Semin Cancer Biol (2015), http://dx.doi.org/10.1016/j.semcancer.2015.10.003

Download English Version:

https://daneshyari.com/en/article/8362083

Download Persian Version:

https://daneshyari.com/article/8362083

Daneshyari.com