Biochemical and Biophysical Research Communications 458 (2015) 941–945

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

F-box protein Fbxo3 targets Smurf1 ubiquitin ligase for ubiquitination and degradation

Dongnian Li [a, 1], Ping Xie [b, 1], Fei Zhao [c], Jingyi Shu [b], Li Li [a], Yutao Zhan [a, *], Lingqiang Zhang [b, **]

[a] Department of Gastroenterology, Beijing Tongren Hospital, Capital Medical University, No.1 Dongjiaominxiang, Dongcheng District, Beijing 100730, China
[b] State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, No. 27 Taiping Road, Beijing 100850, China
[c] Department of Ultrasound, Peking University Shougang Hospital, No.9 Jinyuanzhuang Road, Shijingshan District, Beijing 100144, China

ARTICLE INFO

Article history:
Received 8 February 2015
Available online 24 February 2015

Keywords:
Protein ubiquitination
Proteasomal degradation
Ubiquitin ligase
Fbxo3
Smurf1

ABSTRACT

It has been demonstrated previously that F-box protein Fbxl15 targets HECT-type E3 Smurf1 and forms a functionally active SCF complex for ubiquitination and proteasomal degradation. Here we show that another F-box protein Fbxo3, belonging to the FBXO type protein family, also interacts with and targets Smurf1 for poly-ubiquitination and proteasomal degradation. Different from Fbxl15, Fbxo3 targets all the Nedd4 family members for their degradation, indicating that Fbxo3 plays an important role in controlling the stability of Nedd4. Taken together, we show that Smurf1 is an endogenous substrate of Fbxo3. Our study gains further insight into the novel role of Fbxo3 in BMP signaling.

1. Introduction

Ubiquitination is the sequential transfer of ubiquitin to an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2) and an E3. E3s are the final effectors of the enzyme cascade controlling ubiquitination, and determine substrate specificity [1]. E3 ubiquitin ligases are RING-finger proteins, and are homologous to the E6AP carboxyl terminus (HECT) domain-containing proteins [2]. Smad ubiquitination regulatory factor 1 (Smurf1) is a member of the HECT-type neural precursor cell expressed and developmentally down-regulated gene 4 (Nedd4) family ligases with a C2-WW-HECT architecture. Smurf1 plays critical roles in the regulation of the bone morphogenetic protein (BMP) signaling pathway [3,4], as well as the Wnt and RhoA pathways [5,6].

Although it has been reported that the stability and activity of Smurf1 is tightly controlled, it remains unclear how the activity of HECT domain E3 is regulated. Previously, we have demonstrated that the PH domain-containing protein casein kinase 2-interacting protein 1 (CKIP-1) functions as an auxiliary factor to enhance Smurf1 activation by binding to the WW domain linker [7]. Neddylation, the covalent attachment of ubiquitin-like protein Nedd8, is critical for the activation of Smurf1 ubiquitin ligase activity [8]. For negative regulation, Smurf2 interacts with Smurf1 to induce the degradation of Smurf1 [9]. A recent study show that an F-box protein Fbxl15 targets Smurf1 and forms a functionally active SCF complex for ubiquitination and proteasomal degradation [10]. F-box proteins usually contain an N-terminal F-box, which mediates Skp1 binding and links the rest of the SCF complex. There are three types of F-box proteins: FBXL, containing an F-box and LRRs; FBXW, containing an F-box and WD repeats; and FBXO, containing either only an F-box or an F-box and another motif [11]. While over 60 F-box proteins are identified, only a few of them are well characterized.

The function and the substrates of the FBXO type proteins are mostly unclear. Recently, we have characterized an FBXO protein, Fbxo3, which forms SCF^{Fbxo3} ubiquitin ligase. Fbxo3 activates pro-inflammatory signaling by mediating proteasomal elimination of Fbxl2, a TRAF protein inhibitor [12]. Fbxo3 promotes the degradation of transcription coactivators HIPK2 and p300 [13]. In addition, Fbxo3 degradates p62 during IFN suppression by RVFV (Rift Valley fever virus) [14]. However, the function and the ubiquitin substrates of Fbxo3 remain unknown. In this study, we found that Fbxo3 interacted with Smurf1 both *in vivo* and *in vitro*, and that it targeted Smurf1 for poly-ubiquitination and proteasomal

* Corresponding author. Fax: +86 10 58268473.
** Corresponding author. Fax: +86 10 66931216.
E-mail addresses: yutaozhan@263.net (Y. Zhan), zhanglq@nic.bmi.ac.cn (L. Zhang).
[1] These authors contributed equally in this study.

http://dx.doi.org/10.1016/j.bbrc.2015.02.089
0006-291X/

degradation. We also demonstrated the positive role of Fbxo3 in BMP signaling. Our results showed that except for Fbxl15, F-box proteins might negatively regulate the stability of Smurf1. Moreover, we established the functional relationship between Fbxo3 and Smurf1-mediated BMP signaling.

2. Materials and methods

2.1. Plasmids, antibodies and reagents

Full-length and truncated forms of Fbxo3 and Smurf1 were amplified by PCR and subcloned into various vectors. Anti-Myc antibody was from Clontech. Anti-Flag M2 monoclonal antibody, the protein synthesis inhibitor cycloheximide (CHX) and the proteasome inhibitor MG132 were from Sigma. Anti-Smurf1 polyclonal antibody (ab-117552) was from Abcam. Anti-HA antibody was from Roche. Anti-GST and Anti-His antibodies were from Tiangen. Anti-Fbxo3 (SC-33158), GAPDH and secondary antibodies were purchased from Santa Cruz Biotechnology.

2.2. Cell culture and transfection

Human embryonic kidney HEK293T cells were cultured in DMEM (Hyclone). Cells were supplemented with 10% fetal bovine serum (FBS; Hyclone), penicillin (50 U/ml) and streptomycin (50 U/ml) (Hyclone). Cells were transfected with Thermo Scientific TurboFect (ThermoScientific) according to the manufacturer's instruction.

2.3. Fluorescent microscopy

At 24 h post-transfection, cells were fixed with 2% paraformaldehyde for 10 min at room temperature, rinsed with PBS, and permeabilized with 3% Triton X-100/PBS for 10 min. The cells were then rinsed with PBS and incubated with monoclonal antibody for 1 h at room temperature, followed by incubation with goat anti-mouse IgG secondary antibody for 1 h at room temperature. The nuclei of the cells were stained with 0.1 g/ml DAPI, and the cells were observed under a fluorescent microscope.

2.4. In vivo ubiquitination assay

Cells were lysed in RIPA lysis buffer [10mMTris-HCl (pH7.5), 150mMNaCl, 5 mM EDTA, 1% (v/v) Nonidet P-40, 1% sodium deoxycholate, 0.025% SDS and protease inhibitors], incubated with the indicated antibody for 3 h at 4 °C and protein A/G-agarose beads (Santa Cruz) for 8 h at4 °C. After three washes, proteins were detected by immunoblotting.

2.5. Immunoprecipitation and immunoblotting

Cells were harvested and lysed in HEPES lysis buffer (20 mM HEPES pH7.2, 50mMNaCl, 0.5%Triton X-100, 1mMNaF and 1 mM dithiothreitol) supplemented with protease inhibitor cocktail (Roche). The lysate was incubated with the indicated antibody for 3 h at 4 °C, followed by incubation with protein A/G-plus agarose and rotated gently for more than 8 h at 4 °C. The immunoprecipitates were washed at least three times in lysis buffer and analyzed by western blotting, followed by detection with a Super Signal chemiluminescencekit (Pierce).

2.6. In vitro GST pull-down assay

Bacteria-expressed GST-Fbxo3 proteins were immobilized on glutathione-Sepharose 4B beads (Amersham) and washed. Then the beads were incubated with His-Smurf1. Beads were washed with GST binding buffer (100 mM NaCl, 50 mM NaF, 2 mM EDTA, 1% Nonidet P40 and protease inhibitor cocktail) and proteins were eluted for western blot analysis [15].

2.7. Luciferase reporter assay

BRE-luciferase reporter assay was carried out as described previously [16]. Cells were lysed with passive lysis buffer (Promega), and luciferase activities in cell extracts were determined with a dual-luciferase assay system (Promega).

2.8. RNA interference

Lentivirus-mediated Fbxo3 shRNAs #1 (5′-CTGTCTAATCACTATCGTT-3′), #2 (5′-GAAGATACATTGACCATTA-3′) and control (5′-TGCGTTGCTAGTACCAAC-3′) were used for the establishment of a stable Fbxo3-depleted 293T cell line. The targeting sequences were cloned into the pGCSIL-PUR vector (Shanghai GeneChem). Depletion efficiency was evaluated by western blot analysis.

3. Results

3.1. Smurf1 interacts with Fbxo3 both in vitro and in vivo

The SCF complex is composed of F-box protein, Skp1, Cullin1 (Cul1) and ROC1. In the SCF complex, F-box protein recognizes specific substrates for ubiquitination. Therefore, different SCF complexes are designated according to their F-box proteins. Fbxo3, whose substrates are few, forms SCF^{Fbxo3} ubiquitin ligase and regulates the degradations of Fbxl2, p62, HIPK2 and p300 through the ubiquitin-proteasome pathway. To confirm the interaction between Smurf1 and Fbxo3, we performed *in vitro* GST pull-down assays with recombinant His-Smurf1 and GST-Fbxo3. There was a specific interaction between Smurf1 and GST-Fbxo3, but not between Smurf1 and GST alone (Fig. 1A). To assess whether Fbxo3 interacted with Smurf1 *in vivo*, we then performed co-immunoprecipitation (Co-IP) assay. The result revealed an association between Flag-Smurf1 and Myc-Fbxo3 in the presence of the proteasome inhibitor MG132 (Fig. 1B). Also, ectopic Fbxo3 proteins were co-immunoprecipitated with endogenous Smurf1 protein (Fig. 1C). To characterize the association between Smurf1 and Fbxo3, we generated several Smurf1 deletion mutants to map the Fbxo3-interacting region. Co-IP assays indicated that the WW and HECT domains, but not the C2 domain of Smurf1, mediated the interaction between Smurf1 and Fbxo3 (Fig. 1D). The interaction between Fbxo3 and Smurf1 in cultured cells suggested that these two types of proteins might localize in the same subcellular compartment. To assess the subcellular localization of Fbxo3 and Smurf1, we co-transfected GFP-Smurf1 and Myc-Fbxo3 into 293T cells in the presence of MG132, and found that Fbxo3 and Smurf1 were co-localized in the cytoplasm (Fig. 1E). Therefore, Smurf1 interacted with Fbxo3 both *in vitro* and *in vivo*.

3.2. Fbxo3 promotes the proteasomal degradation of Smurf1

It has been reported that F-box and LRR domain-containing protein 15 (FBXL15), an F-box protein of the FBXL family, form a Skp1-Cullin1-F-box protein-Roc1 (SCF) FBXL15 ubiquitin ligase complex and target Smurf1 for ubiquitination and proteasomal degradation. We investigated whether Fbxo3 could promote the degradation of Smurf1. The level of Smurf1 was significantly decreased with the presence of Fbxo3 in a dose-dependent manner (Fig. 2A), and the effect was blocked by the proteasome inhibitor MG132 (Fig. 2B). However, overexpressionof Smurf1 had no effect

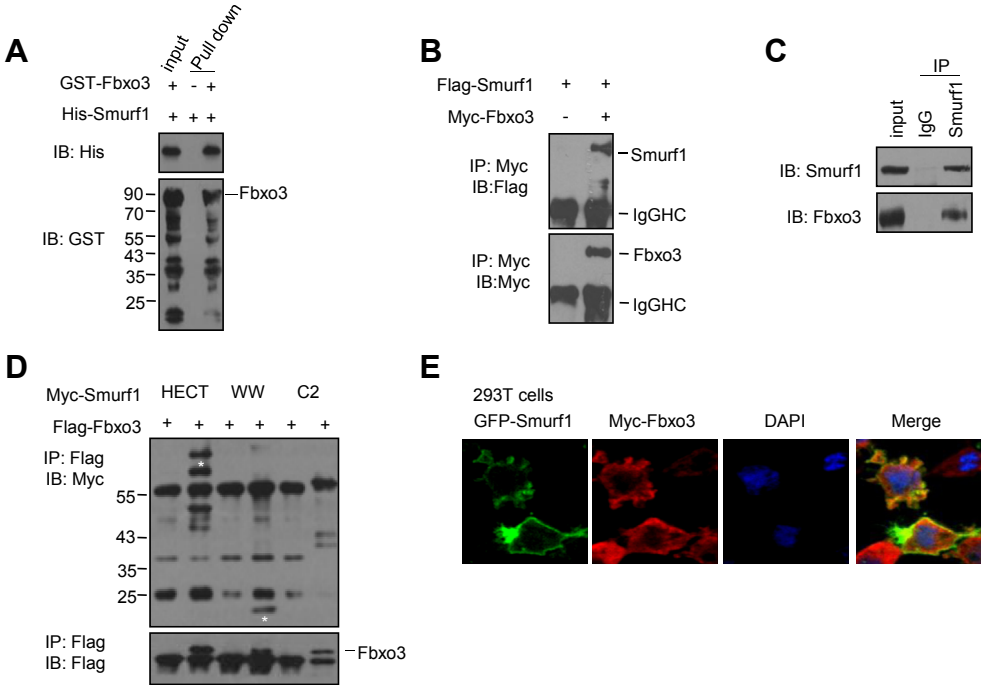

Fig. 1. Smurf1 interacts with Fbxo3 *in vitro* and *in vivo*. (A) The direct interaction between Smurf1 and Fbxo3 is revealed by GST pull-down assays. Input and pull-down samples were both subjected to immunoblotting with anti-GST and anti-His antibodies. Input represents 10% of the proteins being used for pull-down. IB, immunoblotting. (B and C) Coimmunoprecipitation of Smurf1 and Fbxo3. To avoid the degradation of Smurf1, MG132 (20 μM) was added and cells were harvested after 8 h. Cell lysates were immunoprecipitated with relevant antibody and analyzed by immunoblotting. (D) The interaction region of Smurf1 and Fbxo3. Fbxo3 and Smurf1 deletion mutants were transfected into 293T cells. Cell lysates were immunoprecipitated and detected with anti-Myc antibody. (E) Fbxo3 and Smurf1 co-localize in the cytoplasm of 293T cells. Smurf1 proteins were in green and nuclei were stained with DAPI.

on the protein level of Fbxo3 (Fig. 2C), suggesting that Fbxo3 is not a substrate of Smurf1. Next, to examine whether Fbxo3 stabilized Smurf1, we measured the half life of Smurf1. When cells were treated with the protein synthesis inhibitor cycloheximide (CHX), the turnover of Smurf1 was dramatically accelerated when Fbxo3 was co-expressed (Fig. 2D). Because all E3s in the Nedd4 family share similar structure, we asked whether other non-Smurf members could be degraded by Fbxo3. The results showed that the levels of all the Nedd4 family members were significantly decreased in the presence of Fbxo3 (Fig. 2E).

3.3. *Fbxo3 promotes the poly-ubiquitination of Smurf1*

The ubiquitin-proteasome pathway is one of the most important pathways that regulate protein degradation. We next sought to determine whether the Fbxo3-mediated Smurf1 degradation is a

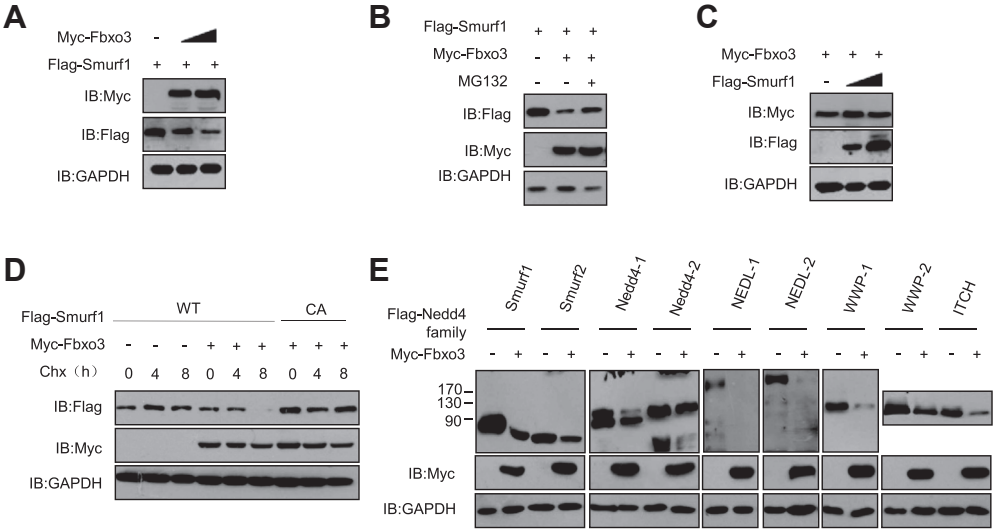

Fig. 2. Fbxo3 destabilizes Smurf1 in 293T cells. (A–C) 293T cells were transfected with Myc-Fbxo3 and Flag-Smurf1, and treated with MG132 before being harvested. (D) 293T cells were transfected with Myc-Fbxo3 and Flag-Smurf1, and treated with the protein synthesis inhibitor cycloheximide (CHX, 10l g/ml) for various time periods before being harvested. (E) Thenine members of the human Nedd4 family were co-transfected with Fbxo3 into 293T cells, respectively. Protein levels were determined.

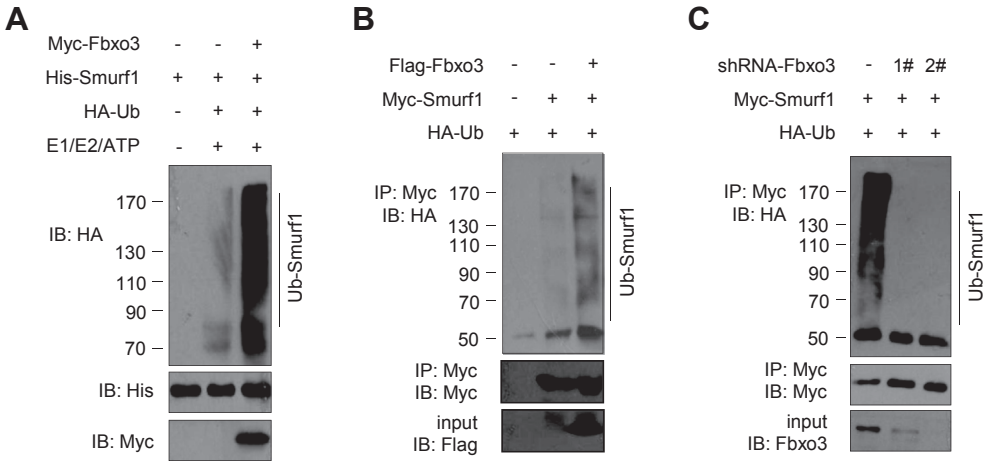

Fig. 3. Fbxo3 promotes the ubiquitination of Smurf1. (A) *In vitro* Smurf1 ubiquitylation assay was performed. Purified HA-ubiquitin, E1, E2 (UbcH5c), purified recombinant Smurf1 and Fbxo3 (overexpressed in mammalian cells, immunoprecipitated by anti-Myc and used as E3) were mixed for *in vitro*ubiquitylation assays and immunoblotted with anti-HA. (B) 293T cells were transfected with HA-Ub, control vector or Myc-Fbxo3, and Flag-Smurf1, and treated with MG132 as indicated. Ubiquitinated Smurf1 was immunoprecipitated (IP) with anti-Flag antibody and detected by immunoblotting with anti-HA antibody. (C) 293T cells were transfected with HA-Ub, control shRNA or Fbxo3 shRNA, and Flag-Smurf1, and treated with MG132 as indicated.

consequence of ubiquitination. To determine whether Fbxo3 could directly catalyze the ubiquitination of Smurf1, we reconstituted an *in vitro* ubiquitination system using purified E1 and E2, bacteria expressed His-Smurf1 and immunoprecipitated Myc-Fbxo3. The E2 used by Fbxo3 remains unknown. Considering UbcH5c could trigger the ubiquitination of FBXL15, we firstly tested the reaction using by UbcH5c. In this system, Fbxo3 efficiently catalyzed the poly-ubiquitination of Smurf1 (Fig. 3A). *In vivo* ubiquitination assay showed that the overexpression of Fbxo3 significantly increased the poly-ubiquitination of Smurf1 in the presence of MG132 (Fig. 3B). Next, Fbxo3 was depleted by the lentivirus-mediated Fbxo3 shRNA. The *in vivo* ubiquitination assay showed that the depletion of Fbxo3 significantly reduced the poly-ubiquitination of Smurf1 (Fig. 3C). Taken together, these results suggest that Fbxo3 functions as a candidate E3 ligase of Smurf1 for ubiquitination and degradation.

3.4. Fbxo3 enhances BMP signaling responsiveness

Smurf1 negatively regulates bone morphogenetic protein (BMP) pathway by ubiquitinating certain signal components for degradation. The results of western blot analysis showed that the overexpression of Fbxo3 significantly decreased the stability of Smurf1, and thus enhanced the protein levels of its substrates Smad1/5 and JunB (Fig. 4A). Next, the effects of selected compounds on BMP signaling responsiveness were delineated by a luciferase reporter assay. BMP-2 treatment remarkably stimulated the BMP-responsive BRE-luc (BMP-2 responsible element) activity. Smurf1 overexpression inhibited BMP-2 mediated elevation of BRE-luc luciferase activity, but the co-expression of Fbxo3 could rescue the reduced responses (Fig. 4B). Importantly, depletion of Fbxo3 resulted in a dramatic enhancement of BRE-luc luciferase activity (Fig. 4C).

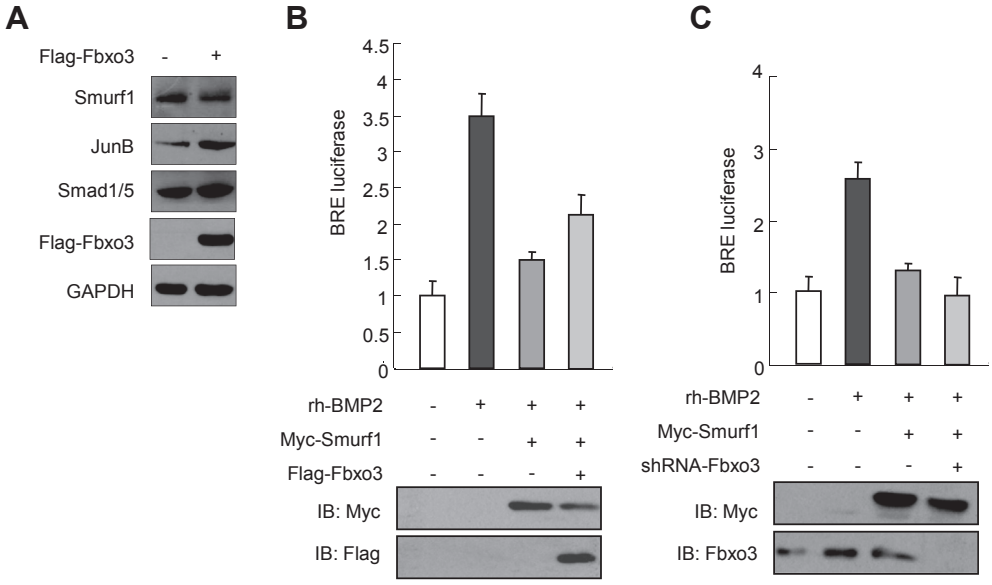

Fig. 4. Fbxo3 enhances BMP-2 signaling responsiveness. (A) Fbxo3 was transfected into 293 cells, and the cells were lysed after 36h and the relevant proteins were analyzed. (B, C) Fbxo3 increased BMP-2 reporter gene activity. 293 cells were co-transfected with Smurf1 and Fbxo3 or mock vector, or with non-targeted control or Fbxo3-specific shRNA. BMP reporter activity was assayed as described in Materials and Methods. Data were determined in triplicate and shown as mean ± SD.

4. Discussion

Previous studies have suggested that there is crosstalk between RING E3 and HECT E3. Cbl is degraded by Nedd4-1 and Itch/AIP4 [17], whereas RNF11 is a target of Smurf2 [18]. However, the regulation of the interactions between HECT-type E3s and F-box E3s remain unclear. Recently, it has been demonstrated that Smurf1 is degraded by the ubiquitin-proteasome pathway through the SCF^{Fbxl15} complex. Our data indicated that the regulation of Smurf1 by Fbxl15 was not specific. In our study, we identified another F-Box protein Fbxo3 and demonstrated that it targeted HECT-type ubiquitin ligase Smurf1 for ubiquitination and degradation in a proteasome-dependent manner. *In vivo* and *in vitro* ubiquitination assays showed that Smurf1 was a new substrate of RING finger-type E3 ligase SCF^{Fbxo3}.

Nedd4 family members contain an N-terminal C2 domain, two to four WW domains and a C-terminal HECT domain. Similar to Fbxl15, Fbxo3 also interacts with Smurf1 both *in vivo* and *in vitro*. Notably, the HECT domain of Smurf1 is both sufficient and necessary for its interactions with Fbxl15 and Fbxo3. Our data suggest that the F-box proteins may bind Smurf1 through a similar mechanism, and that there must be more F-box proteins interacting with Smurf1. Different from Fbxl15, Fbxo3 degrades all the Nedd4 family members, whereas Fbxl15 targets only Smurf1, Smurf2 and WWP2, but not the other six ligases. How the F-box proteins recognize specific Nedd4 family needs to be further investigated.

One of the long-standing central issues in SCF ligase studies is to identify physiological substrates, because the substrates of nearly two-thirds of F-box proteins have not been characterized [19,20]. The known degraded substrates of Fbxo3 are few, and the physiological function of Fbxo3 is unclear. In this regard, our study identified Smurf1 as an endogenous substrate of Fbxo3 and gained further insight into the positive role of Fbxo3 in BMP signaling.

Conflicts of interest

None.

Acknowledgments

This research was supported by the Chinese National Basic Research Programs (2015CB910401) and the Chinese National Natural Science Foundation Projects (31470035, 81041017).

References

[1] A. Hershko, A. Ciechanover, The ubiquitin system, Annu Rev. Biochem. 67 (1998) 425–479.
[2] D. Rotin, S. Kumar, Physiological functions of the HECT family of ubiquitin ligases, Nat. Rev. Mol. Cell. Biol. 10 (2009) 398–409.
[3] H. Zhu, P. Kavsak, S. Abdollah, J.L. Wrana, G.H. Thomsen, A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation, Nature 400 (1999) 687–693.
[4] M. Yamashita, S.X. Ying, G.M. Zhang, C. Li, S.Y. Cheng, C.X. Deng, Y.E. Zhang, Ubiquitin ligase Smurf1 controls osteoblast activity and bone homeostasis by targeting MEKK2 for degradation, Cell 121 (2005) 101–113.
[5] M. Narimatsu, R. Bose, M. Pye, L. Zhang, B. Miller, P. Ching, R. Sakuma, V. Luga, L. Roncari, L. Attisano, J.L. Wrana, Regulation of planar cell polarity by Smurf ubiquitin ligases, Cell 137 (2009) 295–307.
[6] H.R. Wang, Y. Zhang, B. Ozdamar, A.A. Ogunjimi, E. Alexandrova, G.H. Thomsen, J.L. Wrana, Regulation of cell polarity and protrusion formation by targeting RhoA for degradation, Science 302 (2003) 1775–1779.
[7] K. Lu, X. Yin, T. Weng, S. Xi, L. Li, G. Xing, X. Cheng, X. Yang, L. Zhang, F. He, Targeting WW domains linker of HECT-type ubiquitin ligase Smurf1 for activation by CKIP-1, Nat. Cell. Biol. 10 (2008) 994–1002.
[8] P. Xie, M. Zhang, S. He, K. Lu, Y. Chen, G. Xing, Y. Lu, P. Liu, Y. Li, S. Wang, N. Chai, J. Wu, H. Deng, H. Wang, Y. Cao, F. Zhao, Y. Cui, J. Wang, F. He, L. Zhang, Covalent modifier Nedd8 is critical for the activation of Smurf1 ubiquitin ligase in tumorigenesis, Nat. Commun. 13 (2014) 3733.
[9] E. Fukunaga, Y. Inoue, S. Komiya, K. Horiguchi, K. Goto, M. Saitoh, K. Miyazawa, D. Koinuma, A. Hanyu, T. Imamura, Smurf2 induces ubiquitin-dependent degradation of Smurf1 to prevent migration of breast cancer cells, J. Biol. Chem. 283 (2008) 35660–35667.
[10] Y. Cui, S. He, C. Xing, K. Lu, J. Wang, G. Xing, A. Meng, S. Jia, F. He, L. Zhang, SCFFBXL15regulates BMP signalling by directing the degradation of HECT-type ubiquitin ligase Smurf1, EMBO J. 30 (2011) 2675–2689.
[11] J. Jin, T. Cardozo, R.C. Lovering, S.J. Elledge, M. Pagano, J.W. Harper, Systematic analysis and nomenclature of mammalian F-box proteins, Genes. Dev. 18 (2004) 2573–2580.
[12] R.K. Mallampalli, T.A. Coon, J.R. Glasser, C. Wang, S.R. Dunn, N.M. Weathington, J. Zhao, C. Zou, Y. Zhao, B.B. Chen, Targeting F box protein Fbxo3 to control cytokine-driven inflammation, J. Immunol. 191 (2013) 5247–5255.
[13] B.B. Chen, T.A. Coon, J.R. Glasser, B.J. McVerry, J. Zhao, Y. Zhao, C. Zou, B. Ellis, F.C. Sciurba, Y. Zhang, R.K. Mallampalli, A combinatorial F box protein directed pathway controls TRAF adaptor stability to regulate inflammation, Nat. Immunol. 14 (2013) 470–479.
[14] Y. Shima, T. Shima, T. Chiba, T. Irimura, P.P. Pandolfi, I. Kitabayashi, PML activates transcription by protecting HIPK2 and p300 from SCFFbx3-mediated degradation, Mol. Cell. Biol. 28 (2008) 7126–7138.
[15] P. Xie, Y. Tang, S. Shen, Y. Wang, G. Xing, Y. Yin, F. He, L. Zhang, Smurf1 ubiquitin ligase targets Kruppel-like factor KLF2 for ubiquitination and degradation in human lung cancer H1299 cell, Biochem. Biophys. Res. Commun. 407 (2011) 254–259.
[16] Y. Cao, C. Wang, X. Zhang, G. Xing, K. Lu, Y. Gu, F. He, L. Zhang, Selective small molecule compounds increase BMP-2 responsiveness by inhibiting Smurf1-mediated Smad1/5degradation, Sci. Rep. 4 (2014) 4965.
[17] A. Magnifico, S. Ettenberg, C. Yang, J. Mariano, S. Tiwari, S. Fang, S. Lipkowitz, A.M. Weissman, WW domain HECT E3s target Cbl RING finger E3s for proteasomal degradation, J. Biol. Chem. 278 (2003) 43,169–43,177.
[18] V. Subramaniam, H. Li, M. Wong, R. Kitching, L. Attisano, J. Wrana, J. Zubovits, A.M. Burger, A. Seth, The RING-H2 protein RNF11 is overexpressed in breast cancer and is a target of Smurf2 E3 ligase, Br. J. Cancer 89 (2003) 1538–1544.
[19] J.R. Skaar, V. D'Angiolella, J.K. Pagan, M. Pagano, SnapShot: F-box proteins II, Cell 137 (2009) 1358.
[20] J.R. Skaar, J.K. Pagan, M. Pagano, SnapShot: F box proteins I, Cell 137 (2009) 1160.