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Ubiquitin E3 ligase SCF$^{\beta\text{-TRCP}}$ regulates TRIB2 stability in liver cancer cells

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ABSTRACT

Tribbles homolog 2 (TRIB2) is functionally important for liver cancer cell survival and transformation. Our previous study demonstrates TRIB2 is stable in liver cancer cells due to the impaired ubiquitination by Smurf1. However, overexpression of Smurf1 alone cannot completely abolish TRIB2 protein expression, whether other potential factors involved in the degradation of TRIB2 still remains unclear. In the present study, we reveal that the stability and ubiquitination of TRIB2 can also be controlled by ubiquitin E3 ligase SCF$^{\beta\text{-TRCP}}$. Depletion of either Cullin1 or β-TRCP up-regulates TRIB2 protein expression. Moreover, knockdown of β-TRCP extends the half-life, whereas reduces ubiquitylation of TRIB2. Similar to Smurf1, β-TRCP exerts its role through the TRIB2 Degradation Domain (TDD) at the N-terminus of the TRIB2 protein. Hence, we add TRIB2 to the substrate list of SCF$^{\beta\text{-TRCP}}$ and the finding may be helpful in the treatment of TRIB2 dependent liver cancer.

1. Introduction

TRIB2 is a member of the tribbles family pseudokinase proteins originally identified by their roles in Drosophila morphogenesis [1]. Emerging evidence suggests a potential role of tribbles proteins in both solid and non-solid malignancies [2–5]. By using a combination of genomic and cellular approaches, we recently identified TRIB2 as a critical downstream effecter of Wnt signaling in liver cancer cells [6]. Moreover, we found TRIB2 is also functionally important for liver cancer cell survival and transformation, and acts as a crucial signaling nexus to couple the Hippo/YAP and C/EBPα pathways to Wnt-induced liver tumorigenesis [6]. Given that TRIB2 modulates many cellular functions, it can be hypothesized that aberrant TRIB2 expression may be responsible for the development of human cancer and could represent a novel therapeutic target.

Our and other studies suggest that TRIB2 functions as a protein that interacts with ubiquitin E3 ligases, and thereby modulates the protein stability of downstream effecters [3–7]. However, the stability control of TRIB2 per se is still largely unclear. Protein ubiquitination mediated by the ubiquitin proteasome system (UPS) plays an essential regulatory role in critical cellular processes. In this system, E3 ubiquitin ligases play an indispensable role by recognizing specific substrates, and facilitating or directly catalyzing ubiquitin transfer to the respective molecular targets [8]. The relevance of the E3s in several biological processes is emphasized in vivo and often accompanied by the occurrence of cancer [9,10]. Most recently, we described ubiquitin E3 ligase Smurf1 is involved in the ubiquitination and proteasomal degradation of TRIB2. Phosphorylation of TRIB2 by P70S6K was also found to promote Smurf1 induced degradation. Impaired ubiquitination and phosphorylation by Smurf1 and P70S6K in liver cancer cells contributes to the relative higher expression and more stability of TRIB2 compared to that in the normal hepatic cells [11]. However, overexpression of either Smurf1 or P70S6K cannot completely abolish TRIB2 protein expression in liver cancer cells. Therefore it is important to reveal other potential related factors involved in the degradation of TRIB2.

The SCF is a four-subunit RING-type E3 ligase consisting of the RING domain protein Rbx1, two scaffold proteins (Culin1 and SKP1), and one of the many F-box proteins [12]. The F-box proteins determine substrate specificity [12]. β-Transducin Repeat-Containing Proteins (β-TRCP) is a member of the F-box protein family. β-TRCP was reported that plays redundant roles in the ubiquitination of various of substrates, including β-catenin [13], IkB [14], and YAP [24]. In our previous study, we demonstrated that TRIB2

Abbreviations: TRIB2, tribbles homolog 2; HCC, hepatocellular carcinoma; IF, immunofluorescence; CHX, cycloheximide; β-TRCP, beta-transducin repeat containing E3 ubiquitin protein ligase; SCF complex, SKP1–cullin–F-box complex; TDD domain, TRIB2 degradation domain.

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interacts with β-TRCP at C-terminus [6], however; there is no direct evidence showing that β-TRCP regulates TRIB2 stability.

In this study, we reveal that the stability and ubiquitination of TRIB2 can be also controlled by Ubiquitin E3 ligase $SCF^{\beta\text{-}TRCP}$. Similar to Smurf1, β-TRCP exerts its role through the TRIB2 Degradation Domain (TDD) at the N-terminus of the TRIB2 protein. Hence, we add TRIB2 to the substrate list of $SCF^{\beta\text{-}TRCP}$ and the finding may be helpful in the treatment of TRIB2 dependent liver cancer.

2. Materials and methods

2.1. Cell culture and vectors

Liver cancer cell lines Bel-7402 and SMMC-7721 cells were cultured in DMEM. Cells were treated by 50 μg/ml protein synthesis inhibitor, cycloheximide (CHX, Sigma), or 25 μM proteasome inhibitor, MG132 (Cayman) 5 h before harvest. Constructions of the expression plasmids for β-TRCP, Cullin1 and TRIB2 as well as the lentiviral ShRNA against β-TRCP were described in our previous study [6,9,11]. Lentiviral shRNA against Cullin1 (TRCN0000003392) was purchased from Open Biosystem. Dominant negative forms of Cullin2, 3, 4A, 4B, and 5 were constructed according to the previous study [15].

2.2. Immunofluorescence (IF), and western blotting (WB)

For IF, cells were fixed by 4% paraformaldehyde for 15 min, washed with PBS and blocking buffer (3% FBS + 1% HISS + 0.1% Triton X-100), and then incubated overnight at 4 °C in primary antibodies against TRIB2 (Abnova, #H00028951-M04) and Cullin1 (Epitomics, #2436). Alexa-Fluor-488 or -555 fluorescent conjugated secondary antibodies (life technologies) were used for detection.

For WB, proteins were resolved on SDS–PAGE gels followed by standard WB. Primary antibodies used were: β-TRCP (Cell Signaling Technology (CST), #4394), HA (CST, #3724 or #2367), Flag (Sigma, #F3165 or CST, #2368), Myc (CST, #2278 or #2276), Ub (CST, #3933), TRIB2 (Abcam, ab84683), GFP (Epitomics, #1533) and GAPDH (CST, #5174).

2.3. Quantitative RT-PCR

Quantitative RT-PCR was performed as previously described [6,9,11]. qPCR primers used for detection TRIB2 were listed as follows: forward: GGTGTGCAAGGTGTTTGATATCAG, and reward: GAAGGAATGCATGTCCCCATAG.

2.4. Immunoprecipitation

Cells were washed with PBS and subsequently lysed in Western/IP lysis buffer (Beyotime). Protein lysates were centrifugated at 14,000×*g* for 10 min to pellet debris. After preclearing for 1 h with 50 μl of protein A/G-Sepharose (Novex), the supernatants were incubated at 4 °C overnight with 3 μg antibodies as indicated crosslinked to protein A/G-Sepharose beads. Beads were washed five times with lysis buffer, resuspended in SDS loading buffer, and analyzed by WB analysis with antibodies as indicated.

2.5. In vivo and in vitro ubiquitination Assays

For the in vivo ubiquitination assay, cells were treated with MG132 (25 μM) for 5 h before harvest to avoid the proteasome-mediated degradation. The cell lysate was prepared in HEPES lysis buffer supplemented with protease inhibitors (CST), and proteins were immunoprecipitated with the indicated antibody and detected by WB with antibody as indicated.

For the in vitro ubiquitination assay, active E1 and E2 (Boston Biochemistry), HA-Ub (a gift from Jiabin Sun from Shanghai Jiaotong University), $SCF^{\beta\text{-}TRCP}$ complex (GST-BTRC, GST-Cullin1, GST-Skp1 and GST-Rbx1) (Abnova), and TRIB2-Flag (expressed in HEK293T cells and purified by immunoprecipitation with an anti-Flag antibody) were incubated at 30 °C for 2 h, and the assay was terminated with protein loading buffer as described by Guo, et al. [16].

3. Results

3.1. Cullin1 interacts and controls TRIB2 expression

As multisubunit Cullin–Ring complexes comprise the largest known family of E3 ligases [17], we first tested whether a specific Cullin–Ring complex was involved in regulating TRIB2 degradation. Because both TRIB2-Flag and Cullin1-Myc were cloned into the pcDNA3 vector, and the expression of the two proteins were all driven by the CMV promoter, limited cellular context needed for the activation of the CMV promoter may be shared by these two plasmids when they co-transfected. Thus, increasing concentration of pcDNA-Cullin1-Myc may reduce the CMV promoter efficiency from the co-transfected constant amount of pcDNA-TRIB2-Flag plasmid, and inevitably leading to the does dependent down-regulation of the TRIB2-Flag without demonstrating whether Cullin1-Myc protein has a genuine negative effect on TRIB2-Flag protein expression. To avoid this, increasing concentration of pcDNA-TRIB2-Flag (0.25 μg for lane 1, 0.5 μg for lane 2, 0.75 μg for lane 3, and 1 μg for lane 4) expression plasmid was co-transfected with increasing concentration of pcDNA-Cullin-Myc expression plasmid (0 μg for lane 1, 0.1 μg for lane 2, 0.15 μg for lane 3, and 0.25 μg for lane 4) into Bel-7402 cells, and we found that even increasing expression of TRIB2-Flag could not prevent its down-regulation by overexpression of Cullin1-Myc in a doses dependent manner (Fig. 1A). Furthermore, ectopic expression of other dominant-negative forms of Cullins had no notably effect on TRIB2 expression in Bel-7402 cells (Fig. 1B), indicating a specific role of Cullin1 on TRIB2 stability.

To further support the role of Cullin1 on TRIB2 expression, we infected Bel-7402 cells with shRNA against Cullin1, and found endogenous TRIB2 could be up-regulated by knockdown of Cullin1 (Fig. 1C). Moreover, overexpression of Cullin1 was sufficient to reverse Cullin1 shRNA induced TRIB2 expression (Fig. 1D), suggesting the specificity of the Cullin1 shRNA.

To confirm the interaction between TRIB2 and Cullin1, we performed microscopy analysis and observed co-localization of endogenous TRIB2 and Cullin1 in both Bel-7402 and SMMC-7721 cells (Fig. 1E). By co-imunoprecipitation (co-IP) experiments, we also found that TRIB2-HA could be readily pulled down by Cullin1-Myc (Fig. 1F), further support a physiological role for Cullin1 in the control of TRIB2 stability. Take together; the above data strongly suggests the involvement of the SCF type of E3 ligase complex in the regulation of TRIB2 stability.

3.2. β-TRCP is the specific F-box protein that controls TRIB2 expression

Although Cullin1 controls TRIB2 expression has been described in the Section 3.1, the identity of the specific F-box protein that binds with Cullin1 to form a functional SCF type of E3 ligase complex remains unknown. In our previous study, we reported an F-box factor, β-TRCP has a direct interaction with TRIB2 [6]. However, whether β-TRCP acts as a regulator to TRIB2 stability is still unclear. In support of β-TRCP capable of regulating TRIB2

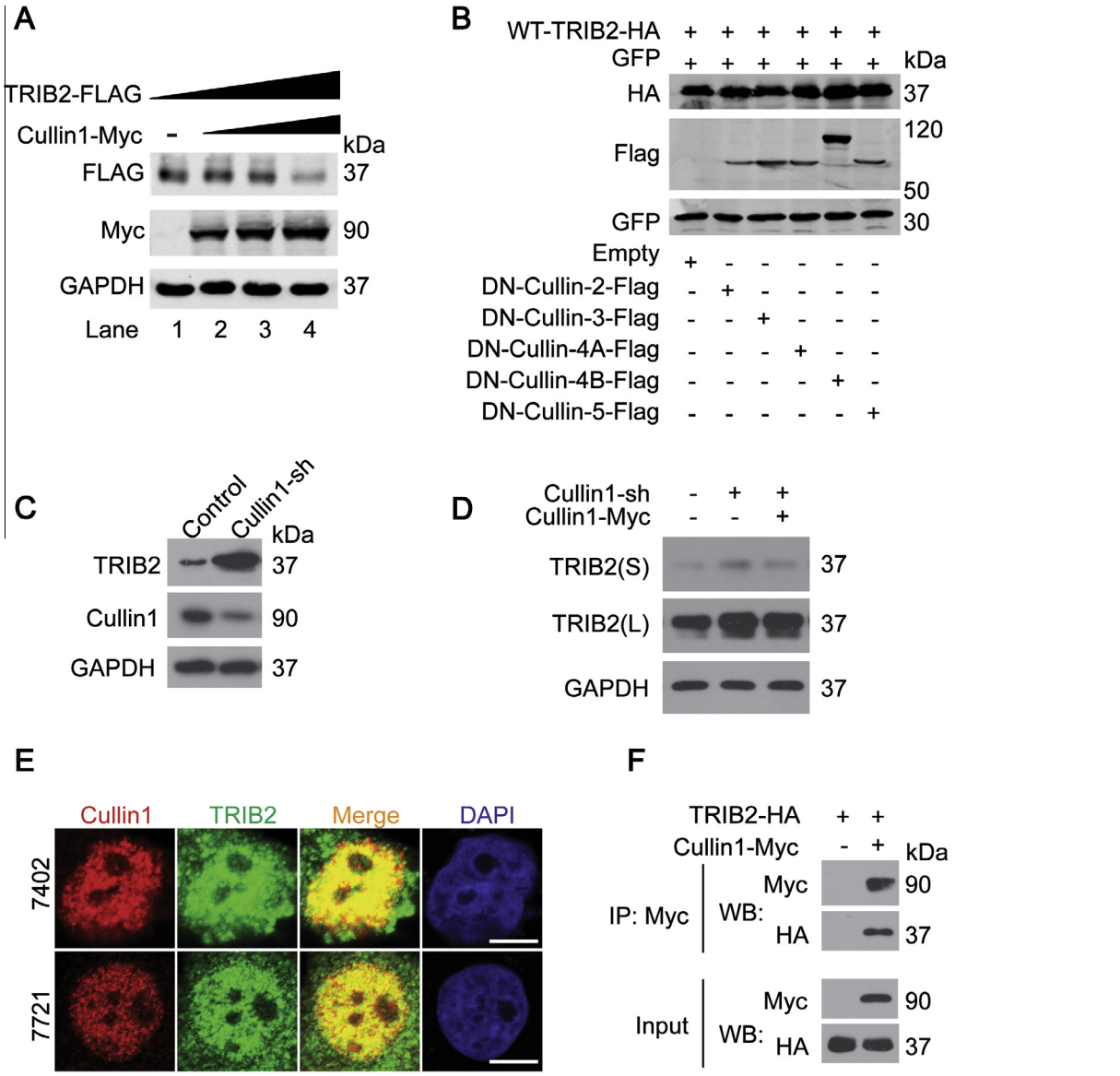

Fig. 1. The Cullin-1 of SCF complex interacts and decreases TRIB2S protein. (A) Overexpression of Cullin1 decreases TRIB2 protein level. Bel-7402 cells were transfected with the plasmids as indicated. Exogenous TRIB2-Flag protein was detected by WB analysis. (B) TRIB2-Flag was co-transfected into Bel-7402 cells with different dominant negative (DN) forms of cullins as indicated. The steady level of TRIB2 was determined by WB analysis. (C) Silencing of Cullin1 up-regulates TRIB2. Western blots of endogenous TRIB2 and Cullin1 in control (infected with shRNA against GFP) and Bel-7402 cell with Cullin1 knocked down. (D) Overexpression of Cullin1 rescues knockdown of Cullin1 induced TRIB2 expression. Western blots of TRIB2 in Bel-7402 cells under different treatment as indicated. (E) Co-localization of Cullin1 and TRIB2. Co-localization of endogenous TRIB2 with Cullin1 in Bel-7402 and SMMC-7721 cells as measured by IF assay. Scale bar, 15 μM. (F) Interaction between Cullin1 and TRIB2. TRIB2-HA was co-transfected with Cullin1-Myc into Bel-7402 cells. TRIB2 and Cullin1 associations were examined by co-IP assay as indicated.

degradation, we detected an up-regulation of TRIB2 in Bel-7402 cells with β-TRCP knocked down compared to the control cells (Fig. 2A). Furthermore, we ruled out the possibility that β-TRCP regulates TRIB2 expression at transcriptional level, because qPCR data revealed that TRIB2 mRNA level remained almost unchanged before and after knockdown of β-TRCP (Fig. 2B).

We reported previously that TRIB2 is an unstable protein with a half-life time of approximately 5 h in liver cancer cells [11], thereby we tested whether β-TRCP affects TRIB2 stability. Depletion of β-TRCP extended the half-life of TRIB2 (Fig. 2C), confirming the importance of β-TRCP in regulating TRIB2 turnover. Consistently, ubiquitination of TRIB2 was markedly reduced after knockdown of β-TRCP in both Bel-7402 and SMMC-7721 cells (Fig. 2D), suggesting that β-TRCP is critical for stability and ubiquitination of TRIB2. In addition, co-IP experiments demonstrated that TRIB2-HA could be readily pulled down by β-TRCP-Flag in Bel-7402 cells (Fig. 2E).

Because the TRIB2 Degradation Domain (TDD, the most proximal five amino acids MNIHR at the N-terminus of the TRIB2 protein) is capable of inducing a significant enhanced degradation of TRIB2 with E3 ligase Sumrf1 [11], we tested if β-TRCP mediated depredation of TRIB2 is also through this domain. As shown in Fig. 2F, we found that only wild type (WT)-TRIB2 was up-regulated compared to the mutant-TRIB2 without TDD domain (FW-a) after depletion of β-TRCP, suggesting TDD is also important for β-TRCP induced regulation of TRIB2.

3.3. Ubiquitination of TRIB2 by $SCF^{\beta\text{-}TRCP}$ in vivo and in vitro

We next asked whether $SCF^{\beta\text{-}TRCP}$ complex functions as an E3 ligase to promote the ubiquitination of TRIB2. Overexpressed β-TRCP enhanced the ubiquitination of TRIB2 in cultured Bel-7402 cells (Fig. 3A). In addition, purified $SCF^{\beta\text{-}TRCP}$ complex promoted the ubiq-

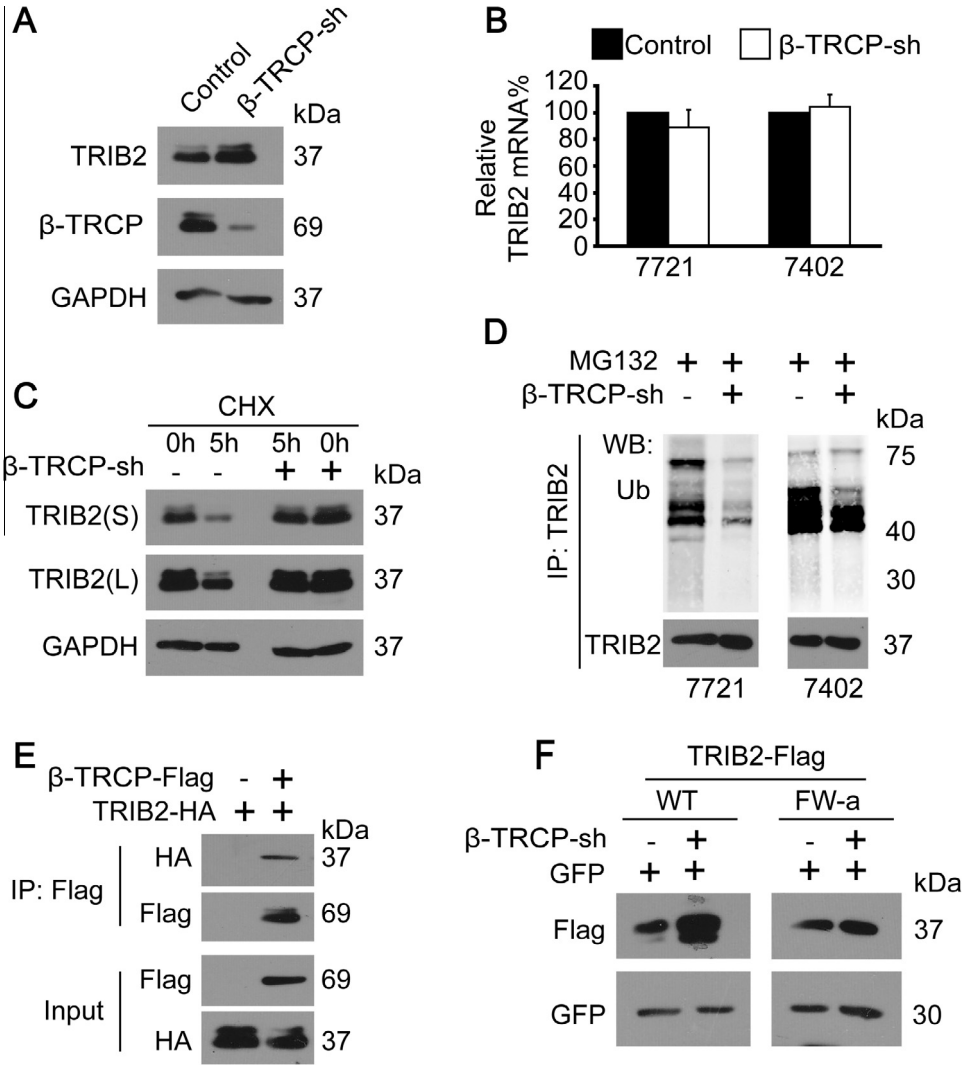

Fig. 2. β-TRCP interacts and negatively regulates TRIB2 expression and stability. (A) Knockdown of β-TRCP induces TRIB2. Western blots of TRIB2 and β-TRCP in control (infected with shRNA against GFP) and Bel-7402 cells with β-TRCP knocked down. (B) β-TRCP does not affect TRIB2 transcription. mRNA levels of TRIB2 were detected in Bel-7402 and SMMC-7721 cells under same treatment as described in (A). GAPDH mRNA was regarded as a loading control. (C) Knockdown of β-TRCP protects TRIB2 from degradation. Endogenous TRIB2 was measured by WB analysis in control (infected with shRNA against GFP) and Bel-7402 cells with β-TRCP knocked down. Protein synthesis was blocked by treatment of CHX (50 μg/ml) for the indicated time. (D) Ubiquitination of TRIB2 is reduced after knockdown of β-TRCP. Bel-7402 cells were treated with MG132 (25 μM) for 5 h before harvest. Endogenous TRIB2 was immunoprecipitated and WB analysis was done using anti-TRIB2 or anti-Ub antibody. (E) Interaction between exogenous TRIB2-HA and β-TRCP-Flag. TRIB2-HA was co-transfected with β-TRCP-Flag into Bel-7402 cells. TRIB2 and BTRC associations were examined by co-IP assay. (F) β-TRCP regulates TRIB2 through TDD domain. Plasmids expressing WT (Wild type)-TRIB2 or FW-a-TRIB2 (without TDD domain) were transfected into control (infected with shRNA against GFP) or Bel-7402 cells with β-TRCP knocked down. Cells were then harvested and protein lysates were subjected into WB analysis.

uitination of TRIB2 directly in vitro (Fig. 3B). Taken together, these data indicate that $SCF^{\beta\text{-}TRCP}$ acts as a biologically relevant E3 ligase for TRIB2 (Fig. 4).

4. Discussion

The F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1–cullin–F-box), which function in phosphorylation-dependent ubiquitination [18]. $SCF^{\beta\text{-}TRCP}$ recognizes a DSGXXS motif in which the serine residues are phosphorylated, and therefore this motif is called phosphodegron [19]. Phosphorylation of phosphodegron is the major mechanism regulating $SCF^{\beta\text{-}TRCP}$ substrate binding [19]. Additional bona fide substrates of $SCF^{\beta\text{-}TRCP}$, such as PERIOD, BIM, and DEPTOR proteins contain phosphodegrons that diverge significantly from the canonical motif, with a phosphoserine or phosphothreonine replacing the aspartic acid in the canonical motif [20]. Like PERIOD, there is a TSGSYS (aa. 226–231) motif within the TRIB2 protein, which may recruit $SCF^{\beta\text{-}TRCP}$. However, the functional role of this motif needs to be further investigated. We reported previously that TRIB2 can be phosphorylated by P70 S6 kinase (P70S6K) at Serine 83, a amino acid residue conserved among human, mouse, cattle and frog, and Smurf1-mediated ubiquitination requires phosphorylation of S83 [11]. However, whether phosphorylation of S83 leads to subsequent phosphorylation of TSGSYS needs to be investigated. More importantly, both Smurf1 and $SCF^{\beta\text{-}TRCP}$ regulates TRIB2 expression through TDD domain (Fig. 2F), leading us to re-considerate the relationship between Smurf1 and $SCF^{\beta\text{-}TRCP}$, and is it a competition or cooperation? There is accumulating evidence showing that β-TRCP is overexpressed in multiple cancers, including liver cancer [21–23]. By contrast, we detected weak IHC signaling represents Smurf1 in most of 617 liver cancer samples tested in our previous study [11]. These findings suggest Smurf1 and β-TRCP may antagonize with each other, and which E3 ligase plays dominant role on controlling TRIB2 stability needs to be explored further.

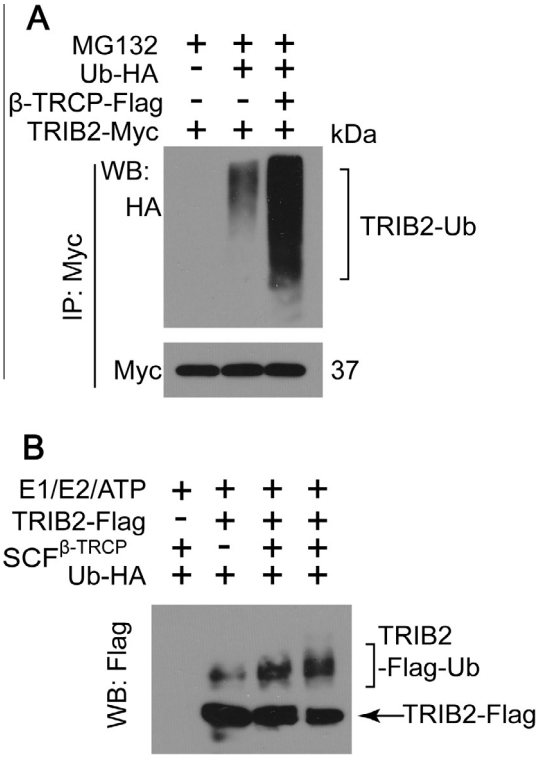

Fig. 3. Ubiquitination of TRIB2 by $SCF^{\beta\text{-}TRCP}$ in vivo and in vitro. (A) β-TRCP promotes TRIB2 ubiquitination in vivo. Bel-7402 cells were transfected with expression plasmids as indicated. Five hours before cell harvest, the cells were treated with the proteasome inhibitor MG132 (25 μM) to avoid the proteasome-mediated degradation. Cell lysates were then prepared and immunoprecipitated with an anti-Myc antibody. The immunoprecipitates (IP) were analyzed by WB with the anti-HA antibody to indicate the ubiquitinated TRIB2. (B) $SCF^{\beta\text{-}TRCP}$ enhances TRIB2 ubiquitination in vitro. E1, E2, HA-Ub, $SCF^{\beta\text{-}TRCP}$ Complex (GST-β-TRCP, GST-Skp1, GST-Cullin1, and GST-Rbx1), and TRIB2-Flag were incubated at 30 °C for 2 h in ubiquitination buffer. Ubiquitinated TRIB2 was visualized by WB with an anti-Flag antibody.

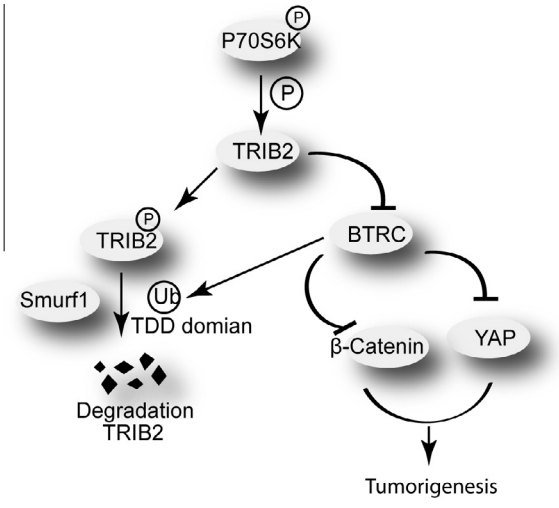

Fig. 4. Possible mechanism underlying the regulation network of TRIB2 in liver cancer cells.

$SCF^{\beta\text{-}TRCP}$ is involved in YAP proteasomal degradation [24], and we described that TRIB2 protects YAP from degradation through inhibition to β-TrCP [6]. Simultaneous knockdown of β-TrCP successfully rescues TRIB2 shRNA-induced YAP down-regulation [6]. In this study, we describe $SCF^{\beta\text{-}TRCP}$ plays a negative role on TRIB2 stability. To our knowledge, we reveal a novel dual-inhibitory auto-regulatory feedback between TRIB2 and $SCF^{\beta\text{-}TRCP}$.

Conflict of interest

None.

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References

[1] J. Mata, S. Curado, A. Ephrussi, P. Rorth, Tribbles coordinates mitosis and morphogenesis in *Drosophila* by regulating string/CDC25 proteolysis, Cell 101 (2000) 511–522.
[2] F. Zanella, O. Renner, B. García, et al., Human TRIB2 is a repressor of FOXO that contributes to the malignant phenotype of melanoma cells, Oncogene 29 (2010) 2973–2982.
[3] K.B. Grandinetti, T.A. Stevens, S. Ha, et al., Overexpression of TRIB2 in human lung cancers contributes to tumorigenesis through downregulation of C/EBPα, Oncogene 30 (2011) 3328–3335.
[4] K. Keeshan, Y. He, B.J. Wouters, et al., Tribbles homolog 2 inactivates C/EBPalpha and causes acute myelogenous leukemia, Cancer Cell 10 (2006) 401–411.
[5] M.M. Hannon, F. Lohan, Y. Erbilgin, et al., Elevated TRIB2 with NOTCH1 activation in paediatric/adult T-ALL, Br. J. Haematol. 158 (2012) 626–634.
[6] J. Wang, J.S. Park, Y. Wei, et al., TRIB2 acts downstream of Wnt/TCF in liver cancer cells to regulate YAP and C/EBPα interaction, Mol. Cell 51 (2013) 211–225.
[7] K. Keeshan, W. Bailis, P.H. Dedhia, et al., Transformation by Tribbles homolog 2 (Trib2) requires both the Trib2 kinase domain and COP1 binding, Blood 116 (2010) 4948–4957.
[8] Z.M. Eletr, D.T. Huang, D.M. Duda, et al., E2 conjugating enzymes must disengage from their E1 enzymes before E3-dependent ubiquitin and ubiquitin-like transfer, Nat. Struct. Mol. Biol. 12 (2005) 933–934.
[9] J. Wang, L. Ma, W. Weng, et al., Mutual interaction between YAP and CREB promotes tumorigenesis in liver cancer, Hepatology 58 (2013) 1011–1020.
[10] Y.L. Chen, R.H. Yuan, W.C. Yang, et al., The stem cell E3-ligase Lin-41 promotes liver cancer progression through inhibition of microRNA-mediated gene silencing, J. Pathol. 229 (2013) 486–496.
[11] J. Wang, Y. Zhang, W. Weng, et al., Impaired phosphorylation and ubiquitination by P70 S6 kinase (P70S6K) and Smad ubiquitination regulatory factor 1 (Smurf1) promotes Tribbles homolog 2 (TRIB2) stability and carcinogenic property in liver cancer, J. Biol. Chem. (2013), http://dx.doi.org/10.1074/jbc.M113.503292 (in press).
[12] R.J. Deshaies, SCF and Cullin/Ring H2-based ubiquitin ligases, Annu. Rev. Cell Dev. Biol. 15 (1999) 435–467.
[13] M. Hart, J.P. Concordet, I. Lassot, et al., The F-box protein b-TrCP associates with phosphorylated b-catenin and regulates its activity in the cell, Curr. Biol. 9 (1999) 207–210.
[14] Q. Li, I.M. Verma, NF-kB regulation in the immune system, Nat. Rev. Immunol. 2 (2002) 725–734.
[15] J. Jin, X.L. Ang, T. Shirogane, et al., Identification of substrates for F-box proteins, Methods Enzymol. 399 (2005) 287–309.
[16] X. Guo, S. Shen, S. Song, et al., The E3 ligase Smurf1 regulates Wolfram syndrome protein stability at the endoplasmic reticulum, J. Biol. Chem. 286 (2011) 18037–18047.
[17] M.D. Petroski, R.J. Deshaies, Function and regulation of cullin–RING ubiquitin ligases, Nat. Rev. Mol. Cell Biol. 6 (2005) 9–20.
[18] D. Frescas, M. Pagano, Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer, Nat. Rev. Cancer 6 (2008) 438–449.
[19] S.Y. Fuchs, V.S. Spiegelman, K.G. Kumar, The many faces of b-TrCP E3 ubiquitin ligases: reflections in the magic mirror of cancer, Oncogene 23 (2004) 2028–2036.
[20] D. Gao, H. Inuzuka, M.K. Tan, mTOR drives its own activation via SCF(βTrCP)-dependent degradation of the mTOR inhibitor DEPTOR, Mol. Cell 44 (2011) 290–303.
[21] A. Ougolkov, B. Zhang, K. Yamashita, et al., Associations among beta-TrCP, an E3 ubiquitin ligase receptor, beta-catenin, and NF-kappaB in colorectal cancer, J. Natl. Cancer Inst. 96 (2004) 1161–1170.
[22] Y. Kudo, D. Guardavaccaro, P.G. Santamaria, et al., Role of F-box protein betaTrcp1 in mammary gland development and tumorigenesis, Mol. Cell. Biol. 24 (2004) 8184–8194.
[23] A. Koch, A. Waha, W. Hartmann, et al., Elevated expression of Wnt antagonists is a common event in hepatoblastomas, Clin. Cancer Res. 12 (2005) 4295–4304.
[24] B. Zhao, L. Li, K. Tumaneng, et al., A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP), Genes Dev. 24 (2010) 72–85.