Biochemical and Biophysical Research Communications 454 (2014) 167–171

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Ubiquitin E3 ligase dSmurf is essential for Wts protein turnover and Hippo signaling

Lei Cao [1], Ping Wang [1], Yang Gao, Xiaohui Lin, Feng Wang, Shian Wu *

The State Key Laboratory of Medicinal Chemical Biology and College of Life Sciences, Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 8 October 2014
Available online 18 October 2014

Keywords:
dSmurf
Wts
Hippo signaling
Drosophila

ABSTRACT

The Hippo pathway has been implicated in controlling organ size and tumorigenesis and the underlying molecular mechanisms have attracted intensive attentions. In this work, we identified dSmurf as a new regulator of Wts, a core component of the Hippo pathway, in *Drosophila*. Our data revealed that Wts and dSmurf colocalize to cytoplasm and physically form an immunoprecipitated complex in S2 cells. Sufficient knock-down of dSmurf increases the protein abundance of Wts and thus increases phosphorylation level at S168 of Yki, the key downstream target of Wts in the Hippo pathway. Genetic epistasis assays showed that halving dosage of dSmurf dominantly enhances the phenotype caused by overexpression of Wts and restrains Yki activity in *Drosophila* eyes. Our works defines a novel role of dSmurf in animal development through modulating Wts turnover and thereby Hippo signal transduction, implying that targeting dSmurf may be a promising therapeutic strategy to manipulate the Hippo pathway in pathological conditions.

1. Introduction

The Hippo signaling pathway was first discovered in *Drosophila melanogaster*, and then proved to be a highly conserved regulator of organ size control and tumorigenesis in mammals [1–5]. Central to the Hippo pathway is a kinase cascade in which Hippo (Hpo) phosphorylates and activates Warts (Wts) [6–10], and in turn phosphorylates and inactivates the activity of transcriptional co-activator Yorkie (Yki), the key downstream effector of Wts, to transcriptionally regulate target genes involved in cell proliferation and survival [11,12]. Large tumor suppressor 1/2 (Lats1/2), the mammalian homologs of Wts, is often down-regulated or mutated in a variety of human cancers including breast tumor [13], astrocytoma [14] and sarcomas [15]. Given the critical roles in cancer development, studies on Lats/Wts may have important implications in our understanding of tumorigenesis.

Smad ubiquitination regulatory factor 1 and 2 (Smurf1 and 2) are two members of the HECT-type of E3 ubiquitin ligase. Smurf1/2 has been initially implicated to play crucial roles in embryonic development and diverse cellular processes responsive to TGFβ/BMP signaling pathway by promoting ubiquitination and degradation of SMADs [16–18]. In addition to TGFβ/BMP pathway, the substrates of Smurfs are involved in broad biological processes such as cell cycle progression, metastasis and cell apoptosis [19]. Smurf1/2 as well as WWP1/2, NEDDL1/2, NEDD4, NEDD4L and ITCH comprise an E3 ubiquitin ligase group called the NEDD4-like family. The functions of these family members have been implicated in regulation of ubiquitin-mediated trafficking, and lysosome- or proteasome-mediated protein degradation [20,21]. Recent studies have revealed that ITCH, WWP1 or NEDD4 physically associate with Lats1and functionally regulate Lats1 protein stability through their E3 ligase activities in mammalian cells [22–25]. However, the regulation of Lats/Wts turnover in animal development remains largely unknown. Here, we identified that the E3 ligase dSmurf associates with Wts and is essential for Wts turnover and function in *Drosophila*. Our work may provide new insight to understanding Smurfs in tumorigenesis by linking them to the Hippo pathway.

2. Materials and methods

Primary antibodies used in this work: rabbit anti-HSV (1:5000, Abcam) and anti-p-Yki, and mouse anti-Flag (1:5000, Abcam).

2.1. Plasmid construction

For *HSV-wts* construction, full-length of *wts* was amplified from cDNA clone SD26853 (BDGP) by PCR and ligated into the

* Corresponding author. Fax: +86 22 23498210.
E-mail address: wusa@nankai.edu.cn (S. Wu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.10.058
0006-291X/

expression vector of pAc5.1/V5-HisB. The insert was then verified by sequencing. *Flag-dSmurf* and *Flag-dSmurf*^{C1029A} plasmids were gifted by Dr. Dahua Chen [26].

2.2. Cell culture and cell based assays

Drosophila S2 cells were cultured in *Drosophila* cell culture medium (Thermo, SH30278.02) supplemented with 10% fetal bovine serum at 25 °C. X-tremeGENE HP DNA Transfection Reagent (Roche) was used for transient transfection of S2 cells. After 36 h transfection, cells were harvested and lysed in 2× loading buffer (0.25 M Tris–HCl pH 6.8, DTT 78 mg/mL, SDS 100 mg/mL, 50% Glycerine, and 5 mg/mL bromophenol blue) for determining the level of protein expression by Western blotting.

For co-immunoprecipitation, cells were lysed in NP-40 buffer (150 mM NaCl, 1% Triton X-100, 10 mM Tris pH 7.4, 1 mM EDTA pH 8.0, 1 mM EGTA pH 8.0, 0.5% NP-40, and 1 mM PMSF) at 4 °C for 30 min. The supernatants of the samples after centrifugation were incubated with the indicated antibodies at 4 °C for 30 min. Samples were combined with 20 μl Protein A/G agarose (GE, 10080881) for 2 h at 4 °C. Beads were washed three times with NP-40 buffer, followed by Western blotting.

For the luciferase reporter assay, cells were seeded into 24-well plate. Cells were transfected with the indicated plasmids and *pRenilla* as internal control. After 36 h, luciferase activity was assessed using the Dual-Glo Luciferase Assay System (Promega, E2940) according to the manufacturer's protocol.

For immunofluorescence, cells were seeded on coverslips in 24-well plates. 36 h posttransfection, cells were fixed in 4% PBS-buffered formaldehyde, permeabilized with 0.05% Triton X-100 for 20 min at room temperature. Cells were then incubated for 30 min in 3% BSA (Sangon), with primary antibody in 3% BSA for 2 h. Alexa Fluor 488 goat anti-mouse IgG (Invitrogen) and Alexa Fluor cy3 goat anti-Rabbit IgG (Invitrogen) were used to detect primary antibodies. Cells were examined by Zeiss microscopy under 60× magnification.

2.3. Double-stranded RNA synthesis for knock-down of dSmurf in S2 cells

Two independent double-strand RNAs were designed to target different exon regions of *dSmurf* with the primer pairs as follow.

dSmurfi1F: 5′-GAATTAATACGACTCACTATAGGGAGATGTCGCCGGATGACGATGAG-3′
dSmurfi 1R: 5′-GAATTAATACGACTCACTATAGGGAGAGCGTGTTGTGCCGTTGCTG-3′
dSmurfi 2F: 5′-GAATTAATACGACTCACTATAGGGAGAGTACGGACAACGGTCGAG-3′
dSmurfi 2R: 5′-GAATTAATACGACTCACTATAGGGAGATGCAGAGGGTGTCACTGAAG-3′

Then mMESSAGE mMACHINE®Kit (Life technologies, AM1344) was used for reverse transcription according to the technical manual. For knock-down and transfection treatment, S2 cells were seeded into 6-well plate with 8 μg of double strand RNA in the medium. Transient transfection was taken 12 h post seeding. After 36 h transfection, cells were harvested for co-IP or Western blotting.

2.4. Drosophila stocks

Drosophila stocks used in this research were all maintained at 25 °C. *UAS-yki*, *UAS-wts* and *dSmurf*^{15C}/CyO were described previously [6,26–28].

3. Results

3.1. Wts is a dSmurf-associating protein

Recent studies have implicated that the NEDD4-like family members including ITCH, WWP1 and NEDD4 individually regulate Lats1 stability in cultured mammalian cells in a manner dependent on their E3 ligase activity [22–25]. Given the entire family members share a similar structure, we wondered whether Smurf1 and 2 regulate Lats1/2 stability in a similar fashion. To this end, we chose *Drosophila* as the assay system to test this possibility, because of no data to date showing NEDD4-like family members, including dSmurf (the Drosophila homolog of Smurf1/2), regulate the turnover of Wts (the *Drosophila* homologs of Lats1/2). Firstly, we found that Wts is a dSmurf-associating protein in our immunoprecipitation assays when Flag-tagged dSmurf and HSV-tagged Wts were co-expressed in S2 cells (Fig. 1A). We next examined the subcellular localization of dSmurf and Wts, and found that they exclusively co-localized in the cytoplasm of S2 cells (Fig. 1B), suggesting dSmurf and Wts may functionally associate with each other in the cytoplasmic compartment.

3.2. dSmurf is essential for Wts turnover

Previous studies revealed that the NEDD4-like family members ITCH, WWP1 and NEDD4 directly bind to and target Lats1 for ubiquitination-mediated degradation. It is reasonable to expect that dSmurf may also promote Wts degradation in a similar manner. To our surprise, the stability of exogenously expressed HSV-Wts was kept constant when co-expressed with either wild-type of Flag-dSmurf (Fig. 2A) or ligase activity-dead mutant of Flag-dSmurf^{C1029A} (Fig. 2B) in a gradually increasing amount, suggesting overexpression of dSmurf is not sufficient to modulate Wts stability. However, when we knocked down endogenous dSmurf by double-strand RNA mediated RNA interference (RNAi), the accumulation of HSV-Wts was significantly increased (Fig. 2C and D), indicating that dSmurf is an essential factor for Wts turnover in S2 cells.

3.3. Knock-down of dSmurf increases YkiS168 phosphorylation and inhibits Yki activity

As a primary phosphorylation site by Wts, YkiS168 phosphorylation has been generally used to evaluate the activation level of Hippo signaling [27,29–31]. Since knock-down of dSmurf stabilizes Wts abundance, we thus asked whether knock-down of dSmurf modulates the phosphorylation level of YkiS168. To this end, an equal amount of Flag-Yki was transfected into S2 cells in which dSmurf is knocked down or not by double-strand RNAs targeted respectively to dSmurf or GFP control. Cell lysates were immunoprecipitated by anti-Flag antibody for detection of phosphorylated and total Yki by Western blotting. As anticipated, YkiS168 phosphorylation was significantly increased while dSmurf was knocked down (Fig. 3A). As YkiS168 phosphorylation is an inhibitory indicator for Yki transcriptional activity [27,29–31], we then conducted a HRE (Hippo Responsive Element for Sd-binding)-luciferase assay in S2 cells to investigate the effect of dSmurf on Yki-Sd transcriptional activity. As previously shown, a transcription activation of HRE reporter was significantly induced by co-expressing Yki and Sd [11]. Notably, knock-down of dSmurf evidently reduced the transcription activity of the Yki/Sd complex although overexpression of dSmurf had little effect (Fig. 3B). These results suggest that dSmurf is essential for modulating the phosphorylation level of Yki and thereby its activity in transcriptional regulation of S2 cells.

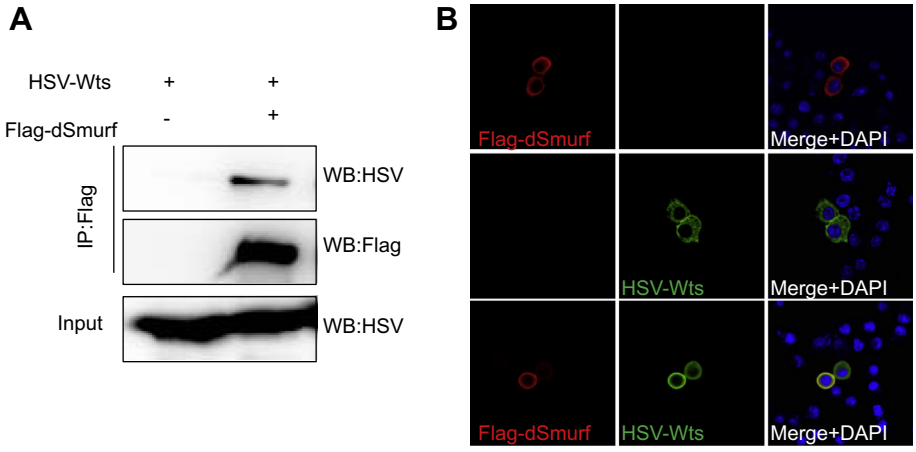

Fig. 1. dSmurf associated and co-localized with Wts in cytoplasm. (A) dSmurf forms a complex with Wts in S2 cells. Cells were co-transfected with *Flag-dSmurf* and *HSV-Wts* plasmids. Cell lysates were immunoprecipitated with anti-Flag antibodies. The immunoprecipitates were analyzed by Western blotting. The total expression of Wts was used to indicate successful transfection. (B) dSmurf co-localizes with Wts in S2 cells. Cells were co-transfected with *Flag-dSmurf* and *HSV-Wts* individually or both. Immunofluorescence was performed to visualize the subcellular localization of dSmurf (Red) and Wts (Green) by confocal microscopy using 60× magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

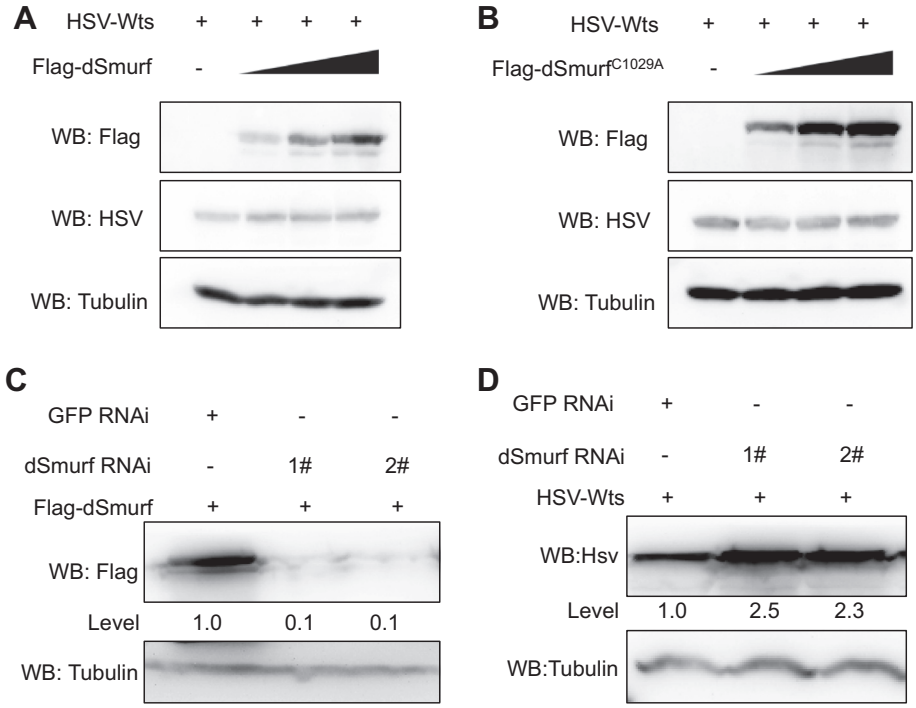

Fig. 2. dSmurf is essential for Wts turnover. (A, B) Ectopically expressed Flag-dSmurf (A) and Flag-dSmurf^{C1029A} (B) did not affect the protein level of Wts. Increasing amounts Flag-dSmurf and Flag-dSmurf^{C1029A} was co-transfected with equal amounts of *HSV-Wts* plasmids. Flag-dSmurf, Flag-dSmurf^{C1029A}, HSV-Wts and Tubulin were detected by Western blotting. (C) Efficient RNA interference of dSmurf. S2 cells were treated double-strand RNAs specifically targeted to dSmurf or GFP control, then transfected with equal amounts of Flag-dSmurf. Flag-dSmurf and Tubulin were detected by Western blotting. The relative level of Flag-dSmurf was determined by densitometry analysis of the Western blotting bands. (D) Knock down of dSmurf accumulates the protein level of Wts. Equal amounts of *HSV-wts* was transfected when dSmurf or GFP were knocked down. HSV-Wts and Tubulin were detected by Western blotting. The relative level of HSV-Wts was determined by densitometry analysis of the Western blotting bands. Tubulin expression was used as a loading control.

3.4. dSmurf modulates Hippo signaling activity in vivo

To assess the functional relevance of dSmurf and the Hippo pathway *in vivo*, we performed genetic epistasis assays in *Drosophila* eyes based on a UAS/Gal4 system. If dSmurf is essential to control the protein turnover of Wts *in vivo*, we expected that down-regulation of dSmurf may stabilize Wts abundance and enhance the eye phenotype caused by overexpression of Wts (GMR > Wts). Consistent with our expectation, one copy removal of *dSmurf* (*dSmurf*^{15C}/+) dominantly enhanced the small and rough eye phenotype resulting from GMR > Wts (Fig. 4A–D and G). More importantly, halving dSmurf further impaired the enlarged eyes caused by overexpression of Yki (GMR > Yki) (Fig. 4E–G), demonstrating that dSmurf steps into the Hippo pathway by modulating Wts turnover and then Yki activity *in vivo*.

4. Discussion

As a tumor suppressor, Lats1/2 plays important roles in organ size control and tumorigenesis. Recent studies have revealed that

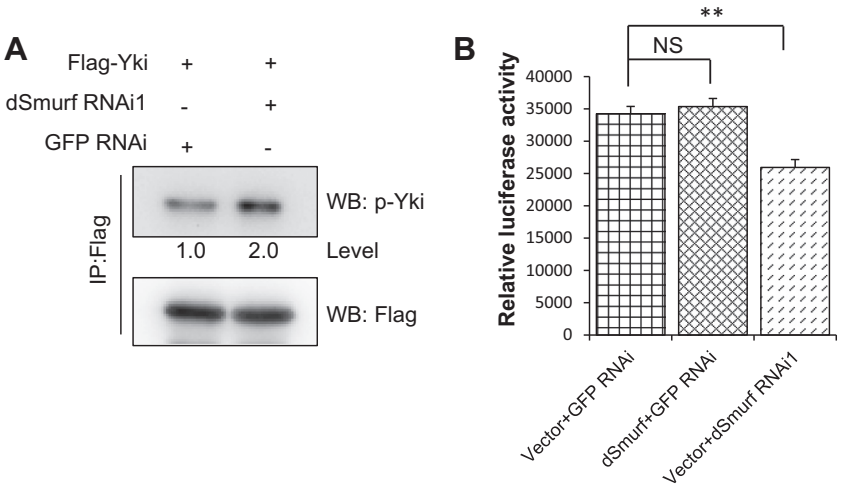

Fig. 3. Knock-down of dSmurf increases YkiS168 phosphorylation and inhibits Yki activity. (A) S2 cells were co-transfected *Flag-Yki* with double-strand RNAs to dSmurf or GFP. The immunoprecipitates by anti-Flag antibody were used to detect phosphorylated (p-Yki) and total Yki (Flag-Yki) via Western blotting. Their relative levels of p-Yki were determined by densitometry analysis of the Western blotting bands. (B) HRE luciferase activity induced by Yki and Sd was evaluated when co-expressing Flag dSmurf or knocking down of dSmurf. Standard error to the mean was shown; unpaired two-tailed student *t*-test was performed showing significance with *p* value.

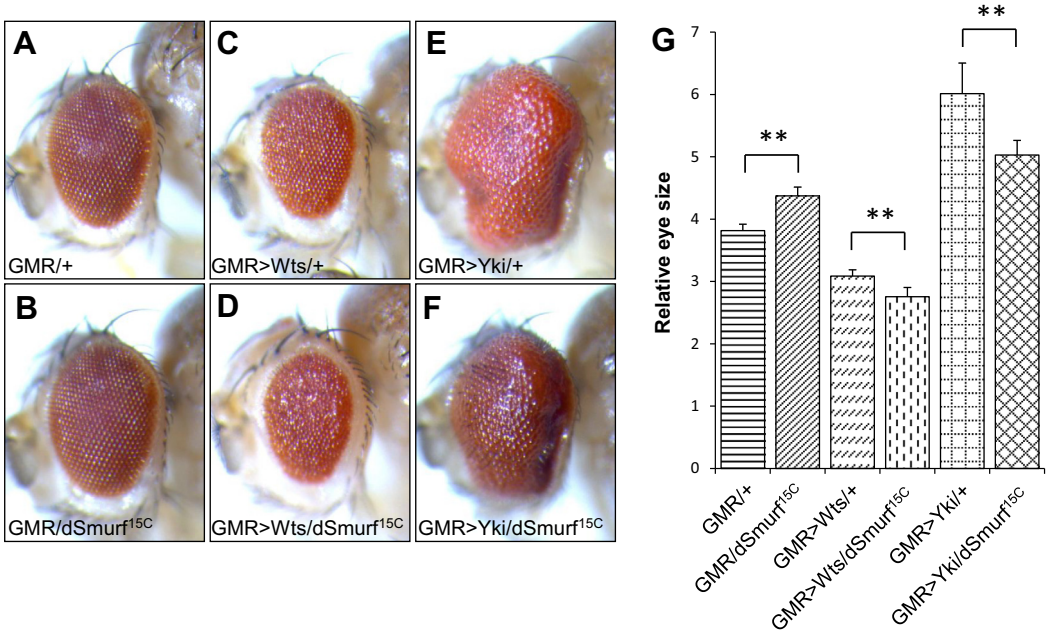

Fig. 4. dSmurf regulates Hippo signaling activity *in vivo*. (A–F) dSmurf was genetic epistasis to Wts and Yki in *Drosophila* eyes. Images of male flies are shown. The genotypes are: (A) *GMR-Gal4/+*, (B) *GMR-Gal4/dSmurf*^{15C}, (C) *GMR-Gal4/+; UAS-Wts*, (D) *GMR-Gal4/dSmurf*^{15C}*; UAS-Wts/+*, (E) *GMR-Gal4, UAS-Yki/+*, (F) *GMR-Gal4, UAS-Yki/dSmurf*^{15C}. (G) Retinal surface areas of the above genotypes were measured. Standard error to the mean was shown; paired two-tailed student *t*-test was performed showing significance with *p* value.

ITCH, WWP1 and NEDD4, the members of the E3 ubiquitin ligase NEDD4-like family, negatively regulate Lats1 stability through ubiquitination- and proteasome-mediated degradation in mammalian cells [22–25]. However, little is known how Lats/Wts stability is regulated in animal models. Based on the similarity of NEDD4-like family members in structure and function, we proposed that Smur1/2 may also regulate Lats1/2 stability in a similar manner. Here, we found that dSmurf (the *Drosophila* homolog of Smurf1/2) interacts with Wts and is essential for Wts protein turnover in *Drosophila* S2 cells. Our data further showed that knock-down of dSmurf regulates Hippo signal transduction evidenced by increasing Yki phosphorylation and thus restraining its transcriptional activity. Importantly, the effect of knocking-down dSmurf on Wts in S2 cells is recaptured in *Drosophila* animal model by halving the dSmurf dosage *in vivo*, supporting a new function of dSmurf in animal development through the important Hippo pathway.

Unlike ITCH, WWP1 and NEDD4, overexpression of dSmurf in S2 cells did not result in obvious alteration of the ubiquitination level and abundance of Wts (data not shown). These observations suggest that dSmurf alone is not sufficient to modulate the Wts turnover through the process of ubiquitination-mediated proteasomal degradation, possibly because of other limiting factors required for this process. However, knocking-down dSmurf significantly promotes Wts protein accumulation, implicating dSmurf may be a *bona fide* regulator and essential for Wts turnover and Hippo signaling. Indeed, the activity of Hippo signaling is regulated in a dosage-dependent manner by dSmurf in *Drosophila* animal development.

Numerous evidence suggests that Smurf1/2 contributes to tumor development and progression by modulating diverse signaling pathways such as TGFβ/BMP, p53 and genomic stability [32–34]. Our study may provide a new way by which Smurfs contributes to cancer development through limiting the accumulation of the Wts/Lats tumor suppressor. Further investigation of the incidence between Smurfs and the Hippo pathway in mammals or clinical samples will provide valuable information for cancer treatment.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We are grateful to Qiang Zhao and Nannan Xiao for technical assistance. This work was supported by grants from the Natural Science Foundation of China (31471319), the National Basic Research Program of China (2011CB943903 and 2010CB912204), and the New Century Excellent Talents Program, the Ministry of Education of China (NCET-08-0294).

References

[1] D. Pan, The hippo signaling pathway in development and cancer, Dev. Cell 19 (2010) 491–505.
[2] B. Zhao, L. Li, Q. Lei, K.L. Guan, The Hippo–YAP pathway in organ size control and tumorigenesis: an updated version, Genes Dev. 24 (2010) 862–874.
[3] F.X. Yu, K.L. Guan, The Hippo pathway: regulators and regulations, Genes Dev. 27 (2013) 355–371.
[4] W. Bossuyt, C.L. Chen, Q. Chen, M. Sudol, H. McNeill, D. Pan, A. Kopp, G. Halder, An evolutionary shift in the regulation of the Hippo pathway between mice and flies, Oncogene (2013).
[5] G. Halder, F.D. Camargo, The hippo tumor suppressor network: from organ size control to stem cells and cancer, Cancer Res. 73 (2013) 6389–6392.
[6] S. Wu, J. Huang, J. Dong, D. Pan, Hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts, Cell 114 (2003) 445–456.
[7] K.F. Harvey, C.M. Pfleger, I.K. Hariharan, The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis, Cell 114 (2003) 457–467.
[8] R.S. Udan, M. Kango-Singh, R. Nolo, C. Tao, G. Halder, Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway, Nat. Cell Biol. 5 (2003) 914–920.
[9] S. Pantalacci, N. Tapon, P. Leopold, The Salvador partner Hippo promotes apoptosis and cell-cycle exit in Drosophila, Nat. Cell Biol. 5 (2003) 921–927.
[10] Z.C. Lai, X. Wei, T. Shimizu, E. Ramos, M. Rohrbaugh, N. Nikolaidis, L.L. Ho, Y. Li, Control of cell proliferation and apoptosis by mob as tumor suppressor, mats, Cell 120 (2005) 675–685.
[11] S. Wu, Y. Liu, Y. Zheng, J. Dong, D. Pan, The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway, Dev. Cell 14 (2008) 388–398.
[12] Y. Goulev, J.D. Fauny, B. Gonzalez-Marti, D. Flagiello, J. Silber, A. Zider, SCALLOPED interacts with YORKIE, the nuclear effector of the hippo tumor-suppressor pathway in Drosophila, Curr. Biol. CB 18 (2008) 435–441.
[13] Y. Takahashi, Y. Miyoshi, C. Takahata, N. Irahara, T. Taguchi, Y. Tamaki, S. Noguchi, Down-regulation of LATS1 and LATS2 mRNA expression by promoter hypermethylation and its association with biologically aggressive phenotype in human breast cancers, Clin. Cancer Res. 11 (2005) 1380–1385.
[14] Z. Jiang, X. Li, J. Hu, W. Zhou, Y. Jiang, G. Li, D. Lu, Promoter hypermethylation-mediated down-regulation of LATS1 and LATS2 in human astrocytoma, Neurosci. Res. 56 (2006) 450–458.
[15] M. Hisaoka, A. Tanaka, H. Hashimoto, Molecular alterations of h-warts/LATS1 tumor suppressor in human soft tissue sarcoma, Lab. Invest. 82 (2002) 1427–1435.
[16] 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.
[17] X. Lin, M. Liang, X.H. Feng, Smurf2 is a ubiquitin E3 ligase mediating proteasome-dependent degradation of Smad2 in transforming growth factor-beta signaling, J. Biol. Chem. 275 (2000) 36818–36822.
[18] P. Kavsak, R.K. Rasmussen, C.G. Causing, S. Bonni, H. Zhu, G.H. Thomsen, J.L. Wrana, Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation, Mol. Cell 6 (2000) 1365–1375.
[19] D. David, S.A. Nair, M.R. Pillai, Smurf E3 ubiquitin ligases at the cross roads of oncogenesis and tumor suppression, Biochim. Biophys. Acta 2013 (1835) 119–128.
[20] C. Chen, L.E. Matesic, The Nedd4-like family of E3 ubiquitin ligases and cancer, Cancer Metastasis Rev. 26 (2007) 587–604.
[21] K.F. Harvey, S. Kumar, Nedd4-like proteins: an emerging family of ubiquitin-protein ligases implicated in diverse cellular functions, Trends Cell Biol. 9 (1999) 166–169.
[22] K.C. Ho, Z. Zhou, Y.M. She, A. Chun, T.D. Cyr, X. Yang, Itch E3 ubiquitin ligase regulates large tumor suppressor 1 stability [corrected], Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 4870–4875.
[23] B. Yeung, K.C. Ho, X. Yang, WWP1 E3 ligase targets LATS1 for ubiquitin-mediated degradation in breast cancer cells, PLoS One 8 (2013) e61027.
[24] Z. Salah, S. Cohen, E. Itzhaki, R.I. Aqeilan, NEDD4 E3 ligase inhibits the activity of the Hippo pathway by targeting LATS1 for degradation, Cell Cycle 12 (2013) 3817–3823.
[25] Z. Salah, G. Melino, R.I. Aqeilan, Negative regulation of the Hippo pathway by E3 ubiquitin ligase ITCH is sufficient to promote tumorigenicity, Cancer Res. 71 (2011) 2010–2020.
[26] S. Huang, Z. Zhang, C. Zhang, X. Lv, X. Zheng, Z. Chen, L. Sun, H. Wang, Y. Zhu, J. Zhang, S. Yang, Y. Lu, Q. Sun, Y. Tao, F. Liu, Y. Zhao, D. Chen, Activation of Smurf E3 ligase promoted by smoothened regulates hedgehog signaling through targeting patched turnover, PLoS Biol. 11 (2013) e1001721.
[27] J. Huang, S. Wu, J. Barrera, K. Matthews, D. Pan, The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP, Cell 122 (2005) 421–434.
[28] S.D. Podos, K.K. Hanson, Y.C. Wang, E.L. Ferguson, The DSmurf ubiquitin-protein ligase restricts BMP signaling spatially and temporally during Drosophila embryogenesis, Dev. Cell 1 (2001) 567–578.
[29] F.A. Grusche, J.L. Degoutin, H.E. Richardson, K.F. Harvey, The Salvador/Warts/Hippo pathway controls regenerative tissue growth in Drosophila melanogaster, Dev. Biol. 350 (2011) 255–266.
[30] L. Zhang, F. Ren, Q. Zhang, Y. Chen, B. Wang, J. Jiang, The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control, Dev. Cell 14 (2008) 377–387.
[31] Y. Chen, Z. Wang, P. Wang, D. Li, J. Zhou, S. Wu, CYLD negatively regulates Hippo signaling by limiting Hpo phosphorylation in Drosophila, Biochem. Biophys. Res. Commun. 452 (2014) 808–812.
[32] Y. Tajima, K. Goto, M. Yoshida, K. Shinomiya, T. Sekimoto, Y. Yoneda, K. Miyazono, T. Imamura, Chromosomal region maintenance 1 (CRM1)-dependent nuclear export of Smad ubiquitin regulatory factor 1 (Smurf1) is essential for negative regulation of transforming growth factor-beta signaling by Smad7, J. Biol. Chem. 278 (2003) 10716–10721.
[33] S.X. Ying, Z.J. Hussain, Y.E. Zhang, Smurf1 facilitates myogenic differentiation and antagonizes the bone morphogenetic protein-2-induced osteoblast conversion by targeting Smad5 for degradation, J. Biol. Chem. 278 (2003) 39029–39036.
[34] J. Nie, P. Xie, L. Liu, G. Xing, Z. Chang, Y. Yin, C. Tian, F. He, L. Zhang, Smad ubiquitylation regulatory factor 1/2 (Smurf1/2) promotes p53 degradation by stabilizing the E3 ligase MDM2, J. Biol. Chem. 285 (2010) 22818–22830.