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Ce-wts-1 plays important roles in *Caenorhabditis elegans* development

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ABSTRACT

The Hippo–Warts pathway defines a novel signaling cascade involved in organ size control and tumor suppression. However, the developmental function of this pathway is less understood. Here we report that the *Caenorhabditis elegans* homolog of Warts, *Ce-wts-1*, plays important roles during worm development. The null allele of *Ce-wts-1* causes L1 lethality. Partial loss of *Ce-wts-1* function by RNAi reveals that *Ce-wts-1* is involved in many developmental processes such as larval development, growth rate regulation, gut granule formation, pharynx development, dauer formation, lifespan and body length control. Genetic analyses show that *Ce-wts-1* functions synergistically with the TGF-β Sma/Mab pathway to regulate body length. In addition, CE-WTS-1::GFP is enriched near the inner cell membrane, implying its possible membrane-related function.

1. Introduction

Cell proliferation, differentiation and programmed cell death are essential processes during animal development. Any aberration in these processes may lead to tumorigenesis. Mutations of two classes of genes, protooncogenes and tumor suppressor genes, can lead to cancer development [1].

Recently, the Salvador–Hippo–Warts cascade was identified as a new tumor suppressor network [2]. The signal from extracellular milieu is received by transmembrane proteins, such as Fat. Then it is relayed by cytoplasmic proteins, including Expanded, Merlin, Hippo, Salvador, Warts (Wts; also called Lats in mammals), and Mats. Finally, it leads to the phosphorylation and inhibition of Yorkie, a transcriptional coactivator that positively regulates cell proliferation and survival. A lack of these tumor suppressors leads to overgrowth in a variety of tissues in *Drosophila melanogaster*, while a gain-of-function leads to reduced proliferation and ectopic apoptosis [3]. The importance of this pathway is emphasized by its simultaneous and unisonous control of cell proliferation and apoptosis, and its evolutionary conservation. The increasing evidence also indicates that the deregulation of this pathway occurs in human tumors.

To understand how inactivation of tumor suppressors leads to tumorigenesis, it is common to decipher the pathways in animal developmental processes. The developmental roles of the Hippo–Warts pathway are poorly documented, especially in *C. elegans* [2]. In this report, we explored the function of Warts, a core component of the Hippo/Warts pathway, in worm development.

We identified the *C. elegans* homolog of *warts*, *Ce-wts-1*. Null allele of *Ce-wts-1* leads to L1 lethality. Partial depletion of *Ce-wts-1* exhibits many defects during development. Genetic analyses show that *Ce-wts-1* genetically interacts with the TGF-β pathway and other *small* mutations. The expression pattern of Ce-WTS-1::GFP reveals that Ce-WTS-1 is mainly expressed intracellularly near the membrane.

2. Materials and methods

2.1. Strains

The nematode *C. elegans* was maintained as described by Brenner [4]. Worms were grown at 20 °C unless otherwise noted. The following alleles were used in this work: LGI: *rnt-1(ok351)*, *mef-2(gv2)*, *Ce-wts-1(ok753)*; LGII: *sma-6(wk7)*, *eat-2(ad465)*, *eat-3(ad426)*; LGIII: *sma-2(e502)*, *sma-3(e491)*, *daf-7(e1372)*; LGIV: *sma-4(e729)*, *tax-6(p675)*, *eat-1(ad427)*, *pha-3(ad607)*; LGV: *dbl-1(wk70)*, *sma-1(ru18)*; LGX: *kin-29(oy38)*, *pha-2(ad472)*, *sma-5(n678)* [5]. The transgenic markers are: *juIs76 (Punc-25::gfp)* for D-type neurons, *wIs51 (scm-1::gfp)*, *jcIs1 (ajm-1::gfp)* for seam cells, *fwEX1(pRF4, Ce-wts-1::gfp)*.

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2.2. Construction of Ce-wts-1::gfp reporter

The *Ce-wts-1::gfp* reporter contains the promoter, coding sequence and 3'UTR (T20F10, nt 17 884–27 380). *gfp* was inserted at the C-terminus of *Ce-wts-1*. The reporter DNA was coinjected with pRF4 (*rol-6*) and at least two transgenic lines were analyzed.

2.3. RNAi and microinjection

Single-stranded RNA was transcribed from the T7 and SP6-flanked PCR templates. The PCR template used for synthesizing RNA is: *Ce-wts-1* (cDNA, nt 1645–2620). The single-stranded RNAs were annealed and injected into N2 and different mutants. Eggs laid between 24 and 48 h after microinjection were collected for further analyses.

2.4. Body length measurement

L4 hermaphrodites grown at 20 °C were transferred to fresh NGM plates. One day later, animals were mounted on 5% agarose pad and photographed under 10× or 20× objectives with Zeiss AxioCam. Only animals with split vulva, mature oocytes and few embryos ($\leqslant$2) were used for body length measurement by Image J [5].

2.5. Measurement of seam cell size

The *ajm-1::gfp* that specifically marked the seam cell membrane was used to measure the seam cell area [6,7]. Late L3 larvae were picked and seam cells were photographed under 100× objective. Three consecutive cells ideal for measurement were selected per worm and measured by Zeiss AxioVision.

3. Results

3.1. Ce-wts-1 encodes the warts homolog in C. elegans

Searching *C. elegans* database revealed a worm homolog of *warts*, *T20F10.1*, which we named *Ce-wts-1*. Similar to Warts in fly and mammals, *Ce-wts-1* encodes a kinase of the Nuclear Dbf-2-related (NDR) family [8,9]. The predicted Ce-WTS-1 product contains 908 amino acids, with a characteristic Serine/threonine kinase domain between aa502 and aa807. The Ce-WTS-1 kinase domain is 54% identical to those of fly Warts and human LATS kinase domains. *ok753* allele of *Ce-wts-1* (provided by *C. elegans* Gene Knockout Consortium), contains a frame-shift deletion from aa544 to aa657, and causes L1 lethality (data not shown). We injected a 10 kb genomic DNA which contains 2 kb promoter, 6 kb coding region and 2 kb 3′UTR of *Ce-wts-1* into heterozygous *ok753* worms, and found that this *Ce-wts-1* genomic DNA could completely rescue $ok753^{-/-}$ lethality, indicating that the L1 lethal arrest of $ok753^{-/-}$ is due to *Ce-wts-1* mutation (data not shown). Since *ok753* removes most of the kinase domain, which has been demonstrated to be crucial for Warts function [10], we believe that *ok753* may be a null allele of *Ce-wts-1*. We also noted that *Ce-wts-1* genomic DNA lacking the first 1 kb intron failed to rescue $ok753^{-/-}$, suggesting that the first intron may contain positive gene regulatory elements.

3.2. Loss of Ce-wts-1 function has pleiotropic effects in C. elegans development

Because $ok753^{-/-}$ animals arrest at L1 stage, we could not explore the developmental role of *Ce-wts-1* beyond L1 stage. Alternatively, we used RNAi to create the partial loss of *Ce-wts-1* function. We synthesized *Ce-wts-1* double-stranded RNA (dsRNA) and microinjected it into worms. We checked the specificity of *Ce-wts-1(RNAi)* by injecting it into *fwEX1(pRF4, Ce-wts-1::gfp)* worms. The results showed that the expression of *Ce-wts-1::gfp* was largely eliminated by *Ce-wts-1(RNAi)*, confirming that the dsRNA is indeed targeting *Ce-wts-1* gene (Fig. 1I and J). We found *Ce-wts-1(RNAi)* caused many developmental defects, including larval lethality, constitutive dauer and longer lifespan, growth retardation, less gut granules, distorted pharynx, and small body size.

3.2.1. Larval lethality

As shown in Fig. 1A, 47% (n = 241) of RNAi-treated worms die at different larval stages, and 53% of them can grow to the adult stage.

3.2.2. Growth retardation

Decrease of *Ce-wts-1* activity leads to slow growth (Fig. 1B). It took nearly 95 h for severely affected worms to grow to the young adult stage at 20 °C. The rest worms took about 73 h to enter the young adult stage, while N2 worms needed about 55 h. Interestingly, $Lats1^{-/-}$ knockout mice also display decreased growth rate [11], suggesting the function of *Ce-wts-1* in regulating animal growth may be conserved.

3.2.3. Constitutive dauer formation and long lifespan

Data from the Ruvkun lab showed that inactivation of *Ce-wts-1* by RNAi feeding could make worms live longer [12]. Our experiments confirmed this observation (data not shown). Study from the Kenyon lab showed that a weak allele of *daf-2* lives longer, while a strong allele becomes dauer worm at L2 stage [13]. We thus examined the role of *Ce-wts-1* in dauer formation. In *C. elegans*, the TGF-β dauer pathway inhibits entry into dauer stage in wild-type worms, and DAF-7 is its ligand. *daf-7(e1372)* is a temperature-sensitive mutation. At 15 °C, only a small fraction of *daf-7* mutants get into dauer stage. At 25 °C, 100% become dauer worms. When *Ce-wts-1* dsRNA was injected into *daf-7* worms at 15 °C, the percentage of dauer worm increased from 13% (n = 50) to 79% (n = 153) (Fig. 1A), suggesting a genetic interaction between the Hippo–Warts pathway and the TGF-β dauer pathway.

3.2.4. Less gut granules

Gut granules can be visualized by its autofluorescence under microscopic DAPI channel. We found that autofluorescence from gut granules drastically diminished in *Ce-wts-1(RNAi)* worms (Fig. 1C and D).

3.2.5. Distorted pharynx

We found that in the slower-growing RNAi worms, the pharynxes were distorted to some extent (Fig. 1E and F). This phenotype is also found in other *small* mutants.

3.2.6. Small body size

In *D. melanogaster*, loss of *warts* function leads to tissue overgrowth [9]. However, $Lats1^{-/-}$ knockout mice exhibit small size/decreased weight, although there are hyperplastic changes in the pituitary [11]. In *C. elegans*, knockdown of *Ce-wts-1* also displayed the small body size phenotype (Fig. 1G and H). At young stage, the body length of RNAi-treated worms was 29% shorter than that of wild-type animals (n = 37) (Table 1). We checked whether the small body size phenotype was the consequence of less cells. We injected *Ce-wts-1* dsRNA into *juIs76* and *wIs51*, which specifically marked D-type neurons and hypodermal seam cells [14]. We found in *Ce-wts-1(RNAi)* worms, numbers of D-type neurons and hypodermal seam cells (19.8 ± 0.8, 16.0 ± 0.8, n = 15) were similar to those of wild-type worms (18.9 ± 0.3, 16.0 ± 0.4, n = 15). We then measured seam cell size using *jcIs1(ajm-1::gfp)* that labels the seam cell membrane [6,7]. In late L3 *Ce-wts-1(RNAi)*

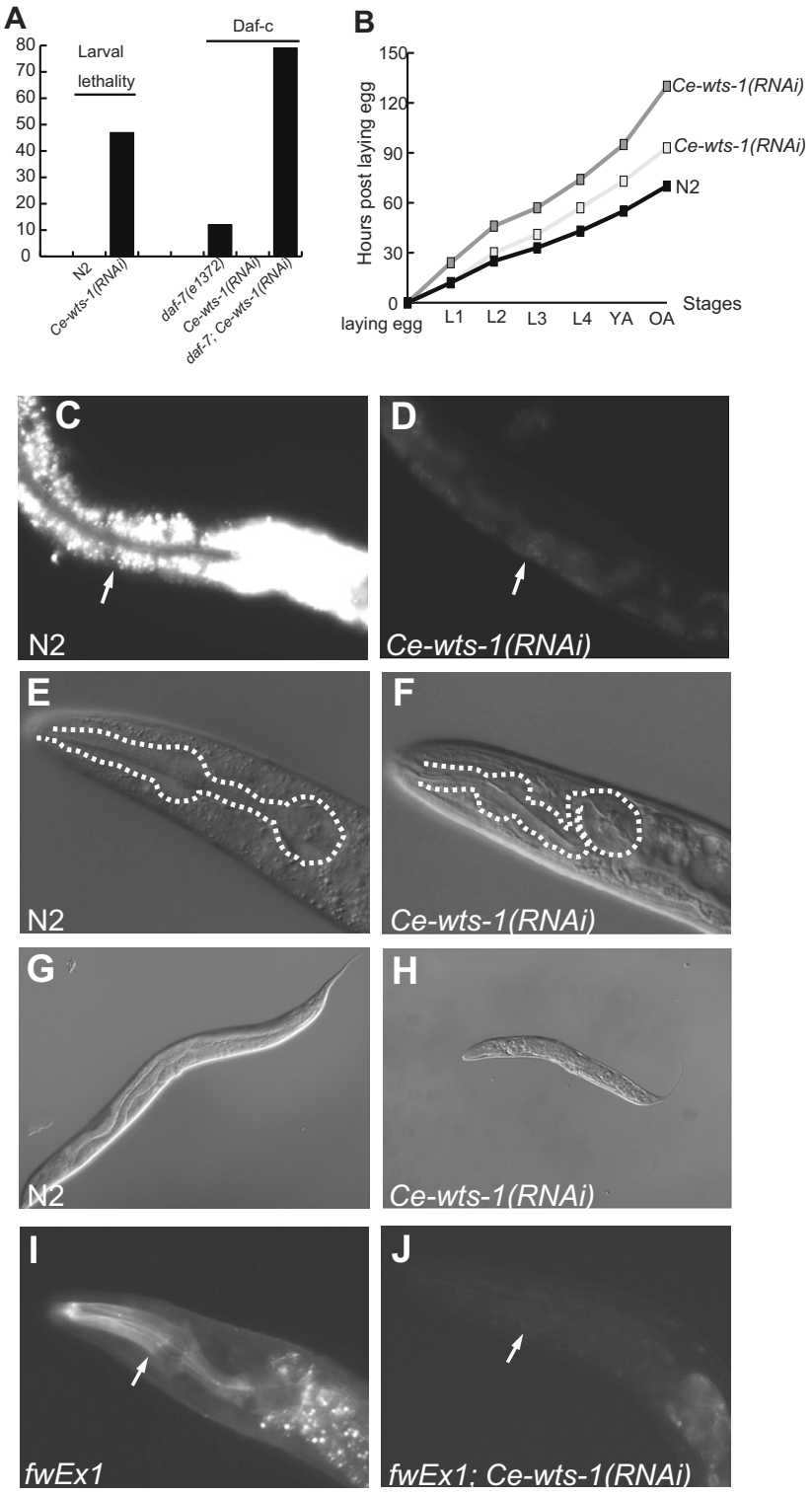

Fig. 1. *Ce-wts-1(RNAi)* causes pleiotropic defects in worm development. (A) Depletion of *Ce-wts-1* activity lead to larval lethality and increased constitutive dauer formation (Daf-c). (B) *Ce-wts-1(RNAi)* worms grew much slower than N2. (C and D) Knockdown of *Ce-wts-1* caused decreased gut granules (arrows, pictures were taken at the same exposure time). (E and F) Pharynxes were distorted in *Ce-wts-1(RNAi)* worms (broken line). (G and H) *Ce-wts-1(RNAi)* worms were smaller than wild-type. (I and J) Pharynx GFP was largely eliminated by *Ce-wts-1(RNAi)* (J), compared with worms before RNAi (I).

worms, the average area of seam cells was $83.4 \pm 17.6\ \mu m^2$ ($n = 52$), significantly smaller than that of wild-type worms ($117.4 \pm 11.3\ \mu m^2$, $n = 39$; $P < 0.001$). These results imply that smaller cell size may be responsible for the small size phenotype in *Ce-wts-1(RNAi)* worms.

3.3. Ce-wts-1 synergistically interacts with small genes

To explore how *Ce-wts-1* interacts with signaling pathways during development, we choose the *small* phenotype of *Ce-wts-1(RNAi)* worms to do genetic analyses. In *C. elegans*, four pathways have

Table 1
Genetic interactions between *Ce-wts-1* and other *Small* mutations.

Genotype	Body length (mm)[a] (means ± S.D.)	Relative value of the mean body length	Protein	References
N2	0.96 ± 0.04	100		
Ce-wts-l(RNAi)	0.68 ± 0.07	71[b]	Serine/threonine kinase	
dbl-1(wk70)	0.59 ± 0.04	62	Ligand of TGF-β signaling	[15]
dbl-1; Ce-wts-l(RNAi)	0.43 ± 0.04	45[b]		
sma-2(e502)	0.54 ± 0.04	57	R-Smad	[16,18]
sma-2; Ce-wts-l(RNAi)	0.43 ± 0.04	44[b]		
sma-3(e491)	0.60 ± 0.05	62	R-Smad	[16,18]
sma-3; Ce-wts-1(RNAi)	0.39 ± 0.02	41[b]		
sma-4(e729)	0.52 ± 0.02	61	Co-Smad	[16,18]
sma-4; Ce-wts-1(RNAi)	0.48 ± 0.01	50[b]		
sma-6(wk7)	0.59 ± 0.03	61	Type I receptor of TGF-β signaling	[17]
sma-6; Ce-wts-1(RNAi)	0.44 ± 0.03	46[b]		
lon-1(e185)	1.03 ± 0.04	107	With homology to CRISP protein	[29]
lon-1; Ce-wts-1(RNAi)	0.96 ± 0.07	100		
lon-2(e678)	1.01 ± 0.05	105	Glypican protein of HSPG	[30]
lon-2; Ce-wts-1(RNAi)	0.75 ± 0.03	78[b]		
sma-1(ru18)	0.67 ± 0.06	70	βH-spectrin	[19]
sma-1; Ce-wts-1(RNAi)		Embryo lethal		
sma-5(n678)	0.50 ± 0.03	52	Serine/threonine kinase	[27]
sma-5; Ce-wts-1(RNAi)		L1 lethal		
kin-29(oy38)	0.62 ± 0.05	65	Serine/threonine kinase	[28]
kin-29; Ce-wts-1(RNAi)		L1 lethal		
eat-1(ad427)	0.80 ± 0.04	83	α-Actinin associated LIM protein	[5]
eat-1; Ce-wts-1(RNAi)		L1 lethal		
eat-3(ad426)	0.76 ± 0.06	79	Dynamin-like GTP binding protein	[25]
eat-3; Ce-wts-1(RNAi)		L1 lethal		
pha-2(ad472)	0.73 ± 0.05	76	Homeodomain transcription factor	[26]
pha-2; Ce-wts-1(RNAi)		L1 lethal		

[a] Body length was only measured for young adult worms with split vulva, mature oocytes and ⩽2 embryos. At least 15 worms were measured per genotype.
[b] These body length data differ significantly from those of wild-type and the parental single mutants in unpaired *t*-test ($P < 0.005$).

been reported in regulating body length [5]. These pathways are: a TGF-β Sma/Mab pathway including *dbl-1*, *sma-2*, *sma-3*, *sma-4* and *sma-6* [15–18]; a spectrin pathway including *sma-1*, *spc-1* and *unc-70* [19–21]; a calcineurin pathway including *tax-6* and *cnb-1* [22,23]; a feeding defective pathway including *eat-1*, *eat-2*, *eat-3*, *pha-2*, *pha-3*, etc. [5,24–26]. In addition, there are still some *small* genes that have not been assigned to these pathways, like *sma-5*, *kin-29*, etc. [27,28]. To explore whether *Ce-wts-1* also genetically interacts with these mutations, we injected *Ce-wts-1* dsRNA into *dbl-1(wk79)*, *sma-2(e502)*, *sma-3(e491)*, *sma-4(e729)*, *sma-6(wk7)*; *sma-1(ru18)*; *tax-6(p675)*; *eat-1(ad427)*, *eat-2(ad465)*, *eat-3(ad426)*, *pha-2(ad472)*, *pha-3(ad607)*; *sma-5(n678)*, *rnt-1(ok351)*, *kin-29(oy38)*, *tph-1(mg280)*.

Inactivation of TGF-β Sma/Mab pathway leads to worms with 60–70% of body length of wild-type (Table 1). However, when *Ce-wts-1* dsRNA was injected into TGF-β Sma/Mab pathway mutants, the double mutants' body length was only 41–50% of wild-type (Table 1). In addition, double mutants grow much slower, usually taking 6–7 days to enter adulthood. The brood size of double mutants also drastically decreased (data not shown). According to previous studies [15], *wk70* is a null allele of *dbl-1*. Double mutations of *dbl-1* and *sma-2/sma-3/sma-4* did not enhance the Small phenotype of single mutants. Therefore, there is strong redundancy between *Ce-wts-1* and TGF-β Sma/Mab pathway in regulating body length. Additionally, *lon-1*, which was negatively regulated by TGF-β Sma/Mab pathway [29], could suppress *Ce-wts-1* Small phenotype, which indicated *lon-1* was downstream of *Ce-wts-1* (Table 1). Moreover, the Long phenotype of *lon-2*, an upstream regulator of TGF-β Sma/Mab pathway [30], was suppressed by *Ce-wts-1(RNAi)* (Table 1). Based on these data, we conclude that *Ce-wts-1* functions redundantly with TGF-β Sma/Mab pathway, sharing the same upstream regulator and downstream target.

We also found that *Ce-wts-1; sma-1* was totally embryonically lethal; *Ce-wts-1; sma-5*, *Ce-wts-1; kin-29*, *Ce-wts-1; eat-1*, *Ce-wts-1; eat-3*, *Ce-wts-1; pha-2* were 100% L1 lethal, while other double mutants did not exhibit obvious synthetic phenotypes (Table 1). The synthetic larval lethality of these double mutants implies *Ce-wts-1* synergistically interacts with *sma-5*, *kin-29*, *sma-1*, *eat-1*, *eat-3*, *pha-2* to regulate worm early development.

3.4. Ce-WTS-1 is expressed near the cell membrane

To determine the temporal and spatial expression patterns of *Ce-wts-1*, we constructed *Ce-wts-1::gfp* fusion gene and microinjected it into wild-type animals. The fusion gene contains a full length genomic *Ce-wts-1* DNA, with *gfp* ligated in frame to its C-terminus. This fusion reporter could fully rescue *ok753*$^{-/-}$ lethal phenotype, suggesting it may reveal the endogenous expression pattern of Ce-WTS-1.

The expression of *Ce-wts-1* begins at the comma stage (Fig. 2A and B) and proceeds during the entire larval and adult stages. Ce-WTS-1::GFP was detectable in many tissues, including pharynx, gut, vulval, spermathecal, and seam cells (Fig. 2C–I). The subcellular localization of Ce-WTS-1 appears to be close to the membrane or membrane-associated, i.e., in gut apical membrane, vulval cell membrane, spermathecal cell membrane and seam cell membrane, by comparing Ce-WTS-1 expression patterns with NHX-2::GFP expression in gut [31], AJM-1::GFP expression in vulval, spermathecal and seam cells [32]. However, the 'DAS' transmembrane domain prediction program predicted no transmembrane domain in CE-WTS-1 (data not shown). In addition, neither fly Warts or human LATS1 contains transmembrane domains [33]. Therefore, Ce-WTS-1 is likely accumulated intracellularly near the cell membrane and may interact with membrane or membrane associated proteins.

4. Discussion

In this report, we have identified a *C. elegans* homologue of the tumor suppressor *warts*. Ce-WTS-1 has the characteristic Serine/

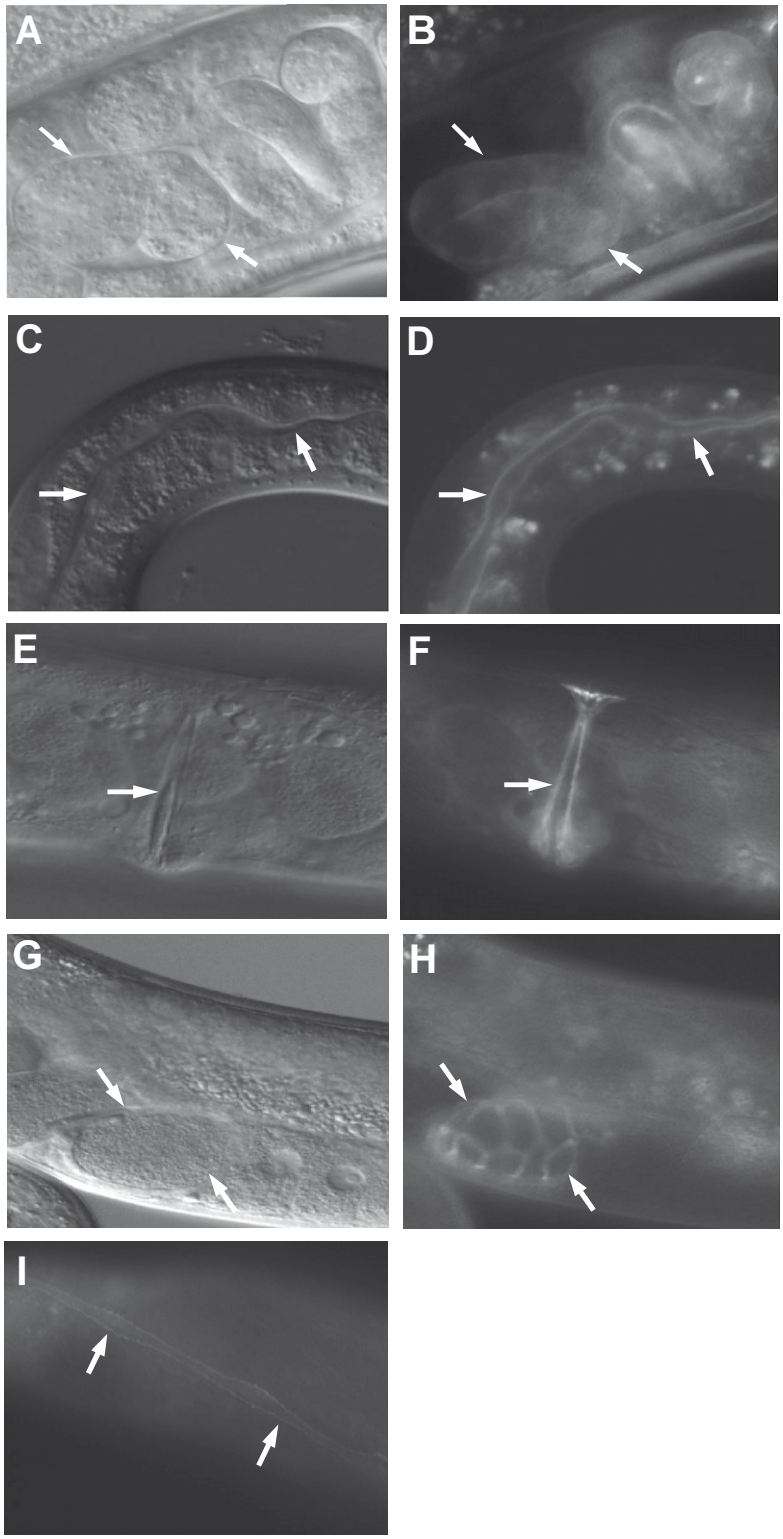

Fig. 2. The expression of *Ce-wts-1* was enriched beneath the cell membrane. (A and B) The onset of *Ce-wts-1* expression was detected at comma stage. (C–I) Obvious membrane localization of *Ce-wts-1* was evident at apical membrane of intestine cells (arrows, D), vulval cell membrane (arrows, F), cell membrane of inflated spermatheca (arrows, H), and hypodermal seam cell membrane (arrows, I). The corresponding DIC pictures were on the left (C, E and G).

threonine kinase domain of the Nuclear Dbf-2-related (NDR) family. Like human Lats1 [11], loss of *Ce-wts-1* function leads to larval lethality, slow growth rate and small animal size/decreased weight. However, we have not observed obvious tissue overgrowth or hyperplastic cells in *Ce-wts-1(RNAi)* worms, which is different from *warts*' role in inhibiting tissue growth in *D. melanogaster*. Further efforts are required to verify whether *Ce-wts-1* inhibits tissue over-growth.

It is known that cytoplasmic Warts inhibits Yorkie translocation into nucleus by phosphorylating Yorkie. Recently, several studies

in human cultured cells indicated that plasma membrane anchoring is important for Lats1 kinase activity [34,35]. They showed that when LATS1 was co-expressed with membrane-bound hMOB1, an interacting protein of LATS1, its kinase activity greatly increased, compared with non-membrane-bound hMOB1. However, it is still unclear whether Warts is physically membrane-attached. In our study, we constructed a *Ce-wts-1::gfp* fusion gene and found Ce-WTS-1 is mainly localized intracellularly near the cell membrane in multiple tissues of worm. Interestingly, the subcellular location of *T10H10.1*, the *C. elegans* homologue of Warts interacting protein, Salvador, is also expressed at the membrane region of spermathecal cells (unpublished data). Therefore, the expression pattern revealed by Ce-WTS-1::GFP may provide an in vivo evidence for membrane anchoring property of Warts and further investigation is required to address how membrane association is related to the physiological functions of Warts.

In *C. elegans*, there are at least two TGF-β-like signaling pathways: the TGF-β dauer pathway and the TGF-β Sma/Mab pathway. Through double mutant analyses, we found that *Ce-wts-1* genetically interacted with both TGF-β pathways in *C. elegans*. *Ce-wts-1* synergistically interacted with *daf-7* to inhibit worms entering into dauer stage and with the Sma/Mab pathway components to regulate worm body length. In cancer biology, the TGF-β pathway is a well-known tumor suppressor pathway and the Hippo–Warts pathway is a novel tumor suppressor pathway. Considering the evolutionary conservation of these pathways, it is tempting to speculate that both the Hippo–Warts pathway and the TGF-β pathway may coordinate to inhibit the tumorigenesis.

Body size is determined by cell number and cell size. In *Ce-wts-1(RNAi)* worms, we did not observe obvious changes of cell number in D-type neurons and hypodermal seam cells. However, the size of seam cells in *Ce-wts-1(RNAi)* worms is obviously smaller than that of wild-type, implying that the small body size of *Ce-wts-1(RNAi)* animals may be due to decreased cell size. Studies on cell size control in mouse and fly revealed that insulin/IGF-mTOR-S6K/eIF4E phosphorylation cascade positively regulates protein synthesis and cell size [36]. The Xu T. lab found that there was a 25% decrease of growth hormone level in *Lats1*$^{-/-}$ knockout mice [11]. Therefore, it is likely that *Ce-wts-1* might use similar insulin/IGF-mTOR-S6K/eIF4E pathway to regulate protein synthesis and body size. Indeed, the Ohshima Y. lab recently showed there was a drastic decrease of total protein contents in *sma-1*, *sma-2*, *sma-4*, *sma-5* and *sma-6* [37]. Thus, total protein contents and insulin signaling should be measured in *Ce-wts-1(RNAi)* worms in future experiments to explore how *Ce-wts-1* regulates body size.

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References

[1] Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. Cell 100, 57–70.
[2] Pan, D. (2007) Hippo signaling in organ size control. Genes Dev. 21, 886–897.
[3] Harvey, K. and Tapon, N. (2007) The Salvador–Warts–Hippo pathway – an emerging tumour-suppressor network. Nat. Rev. Cancer 7, 182–191.
[4] Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.
[5] Morck, C. and Pilon, M. (2006) *C. elegans* feeding defective mutants have shorter body lengths and increased autophagy. BMC Dev. Biol. 6, 39.
[6] Mohler, W.A., Simske, J.S., Williams-Masson, E.M., Hardin, J.D. and White, J.G. (1998) Dynamics and ultrastructure of developmental cell fusions in the *Caenorhabditis elegans* hypodermis. Curr. Biol. 8, 1087–1090.
[7] Wang, J., Tokarz, R. and Savage-Dunn, C. (2002) The expression of TGFbeta signal transducers in the hypodermis regulates body size in *C. elegans*. Development 129, 4989–4998.
[8] Justice, R.W., Zilian, O., Woods, D.F., Noll, M. and Bryant, P.J. (1995) The Drosophila tumor suppressor gene *warts* encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. Genes Dev. 9, 534–546.
[9] Xu, T., Wang, W., Zhang, S., Stewart, R.A. and Yu, W. (1995) Identifying tumor suppressors in genetic mosaics: the Drosophila *lats* gene encodes a putative protein kinase. Development 121, 1053–1063.
[10] Huang, J., Wu, S., Barrera, J., Matthews, K. and Pan, D. (2005) The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell 122, 421–434.
[11] St. John, M.A. et al. (1999) Mice deficient of Lats1 develop soft-tissue sarcomas, ovarian tumours and pituitary dysfunction. Nat. Genet. 21, 182–186.
[12] Curran, S.P. and Ruvkun, G. (2007) Lifespan regulation by evolutionarily conserved genes essential for viability. PLoS Genet. 3, e56.
[13] Apfeld, J. and Kenyon, C. (1998) Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. Cell 95, 199–210.
[14] Eastman, C., Horvitz, H.R. and Jin, Y. (1999) Coordinated transcriptional regulation of the *unc-25* glutamic acid decarboxylase and the *unc-47* GABA vesicular transporter by the *Caenorhabditis elegans* UNC-30 homeodomain protein. J. Neurosci. 19, 6225–6234.
[15] Suzuki, Y., Yandell, M.D., Roy, P.J., Krishna, S., Savage-Dunn, C., Ross, R.M., Padgett, R.W. and Wood, W.B. (1999) A BMP homolog acts as a dose-dependent regulator of body size and male tail patterning in *Caenorhabditis elegans*. Development 126, 241–250.
[16] Savage, C., Das, P., Finelli, A.L., Townsend, S.R., Sun, C.Y., Baird, S.E. and Padgett, R.W. (1996) *Caenorhabditis elegans* genes *sma-2*, *sma-3*, and *sma-4* define a conserved family of transforming growth factor beta pathway components. Proc. Natl. Acad. Sci. USA 93, 790–794.
[17] Yoshida, S., Morita, K., Mochii, M. and Ueno, N. (2001) Hypodermal expression of *Caenorhabditis elegans* TGF-beta type I receptor SMA-6 is essential for the growth and maintenance of body length. Dev. Biol. 240, 32–45.
[18] Savage-Dunn, C. (2005) TGF-beta signaling. WormBook, 1–12.
[19] Hammarlund, M., Davis, W.S. and Jorgensen, E.M. (2000) Mutations in beta-spectrin disrupt axon outgrowth and sarcomere structure. J. Cell Biol. 149, 931–942.
[20] McKeown, C., Praitis, V. and Austin, J. (1998) *Sma-1* encodes a betaH-spectrin homolog required for *Caenorhabditis elegans* morphogenesis. Development 125, 2087–2098.
[21] Norman, K.R. and Moerman, D.G. (2002) Alpha spectrin is essential for morphogenesis and body wall muscle formation in *Caenorhabditis elegans*. J. Cell Biol. 157, 665–677.
[22] Bandyopadhyay, J. et al. (2002) Calcineurin, a calcium/calmodulin-dependent protein phosphatase, is involved in movement, fertility, egg laying, and growth in *Caenorhabditis elegans*. Mol. Biol. Cell 13, 3281–3293.
[23] Singaravelu, G., Song, H.O., Ji, Y.J., Jee, C., Park, B.J. and Ahnn, J. (2007) Calcineurin interacts with KIN-29, a Ser/Thr kinase, in *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. 352, 29–35.
[24] McKay, J.P., Raizen, D.M., Gottschalk, A., Schafer, W.R. and Avery, L. (2004) *Eat-2* and *eat-18* are required for nicotinic neurotransmission in the *Caenorhabditis elegans* pharynx. Genetics 166, 161–169.
[25] Kanazawa, T. et al. (2008) The *C. elegans* Opa1 homologue EAT-3 is essential for resistance to free radicals. PLoS Genet. 4, e1000022.
[26] Morck, C., Rauthan, M., Wagberg, F. and Pilon, M. (2004) *Pha-2* encodes the *C. elegans* ortholog of the homeodomain protein HEX and is required for the formation of the pharyngeal isthmus. Dev. Biol. 272, 403–418.
[27] Watanabe, N., Nagamatsu, Y., Gengyo-Ando, K., Mitani, S. and Ohshima, Y. (2005) Control of body size by SMA-5, a homolog of MAP kinase BMK1/ERK5, in *C. elegans*. Development 132, 3175–3184.
[28] Maduzia, L.L. et al. (2005) *C. elegans* serine-threonine kinase KIN-29 modulates TGFbeta signaling and regulates body size formation. BMC Dev. Biol. 5, 8.
[29] Maduzia, L.L., Gumienny, T.L., Zimmerman, C.M., Wang, H., Shetgiri, P., Krishna, S., Roberts, A.F. and Padgett, R.W. (2002) *Lon-1* regulates *Caenorhabditis elegans* body size downstream of the *dbl-1* TGF beta signaling pathway. Dev. Biol. 246, 418–428.
[30] Gumienny, T.L., MacNeil, L.T., Wang, H., de Bono, M., Wrana, J.L. and Padgett, R.W. (2007) Glypican LON-2 is a conserved negative regulator of BMP-like signaling in *Caenorhabditis elegans*. Curr. Biol. 17, 159–164.
[31] Nehrke, K. and Melvin, J.E. (2002) The NHX family of Na^+–H^+ exchangers in *Caenorhabditis elegans*. J. Biol. Chem. 277, 29036–29044.
[32] Koppen, M., Simske, J.S., Sims, P.A., Firestein, B.L., Hall, D.H., Radice, A.D., Rongo, C. and Hardin, J.D. (2001) Cooperative regulation of AJM-1 controls junctional integrity in *Caenorhabditis elegans* epithelia. Nat. Cell Biol. 3, 983–991.
[33] Cserzo, M., Wallin, E., Simon, I., von Heijne, G. and Elofsson, A. (1997) Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. Protein Eng. 10, 673–676.

[34] Stegert, M.R., Hergovich, A., Tamaskovic, R., Bichsel, S.J. and Hemmings, B.A. (2005) Regulation of NDR protein kinase by hydrophobic motif phosphorylation mediated by the mammalian Ste20-like kinase MST3. Mol. Cell. Biol. 25, 11019–11029.
[35] Hergovich, A., Schmitz, D. and Hemmings, B.A. (2006) The human tumour suppressor LATS1 is activated by human MOB1 at the membrane. Biochem. Biophys. Res. Commun. 345, 50–58.
[36] Ruvinsky, I. and Meyuhas, O. (2006) Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. Trends Biochem. Sci. 31, 342–348.
[37] Nagamatsu, Y. and Ohshima, Y. (2004) Mechanisms for the control of body size by a G-kinase and a downstream TGFbeta signal pathway in *Caenorhabditis elegans*. Gene Cell 9, 39–47.