A role for Shugoshin in human cilia?

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Abstract

We have recently described a novel role for the conserved centromeric/kinetochore protein and cohesin protector, Shugoshin, in cilia of C. elegans. Worms are unusual in that the sole Shugoshin protein (SGO-1) is dispensable for chromosome segregation but required for cilia function in fully differentiated sensory neurons. Depletion of sgo-1 leads to an array of sensory defects observed in other cilia mutants with a compromised diffusion barrier. Accordingly, SGO-1 loads to the base of cilia in sensory neurons and can be observed occupying the transition zone, the critical ciliary domain that regulates trafficking in and out of ciliary compartments. Here we start to address a potential conserved role in cilia for vertebrate Shugoshin by asking whether human Shugoshin can: (1) localize to cilia and (2) rescue defects due to Shugoshin depletion in C. elegans. Our preliminary results suggest that human Shugoshin is detectable in the cilia base but show limited functional conservation when expressed in C. elegans sensory neurons.

Figure 1. Human Shugoshin is observed in the base of cilia in human neuroblastoma cells and localizes to distal dendrites and cilia proper when ectopically expressed in C. elegans sensory neurons.
Description

Originally identified in flies and yeast based on their essential role in ensuring timely chromosome separation in anaphase, Shugoshins show poor sequence homology across species (Watanabe, 2005; Gutiérrez-Caballero et al., 2012). Most Shugoshin proteins have been identified bioinformatically based on the presence of a coiled coil domain in the N-terminus and an arginine-rich, basic domain in the C-terminus. (Kitajima et al., 2004; Rabitsch et al., 2004; Figure 1A). Along with weak amino acid conservation, Shugoshins show several species-specific protein partners and specialized functions in different cell division contexts, arguing against a direct orthology, and complicating the understanding of the evolution of this protein family (Gutiérrez-Caballero et al., 2012; Grishaeva et al., 2016). Unifying among these functions, however, are the sites of action of Shugoshins: the kinetochore, spindle, centromosome and primary cilium, all of which are microtubule-based cellular structures.

The *C. elegans* genome encodes a single Shugoshin homolog, SGO-1, that localizes to chromosomes in mitotic and meiotic cells, and to cilia in the adult sensory organs (de Carvalho 2008; Bohr 2016, Reed et al., 2023). Surprisingly, SGO-1-depleted worms are viable and fertile but show behavioural defects consistent with cilia dysfunction. We asked whether the cilia function of SGO-1 in worms might be a conserved feature in humans, in which two Shugoshin paralogs exist, Sgo1 (Sgo11) and Sgo2 (Sgo2). We chose to concentrate on Sgo1 as the candidate for a potential function in cilia for three reasons. First, Sgo1 has likely evolved different extra-nuclear activities. Of note, sSgo1 (292aa) lacks 268 residues substitution in the N-terminal domain of Sgo1 (K23E) disrupts its ability to directly interact with the pacemaker ion channel HCN4 in the membrane of cardiomyocytes of patients with Chronic Atrial and Intestinal Dysrhythmia Syndrome (CAID) (Liu et al., 2021). Thus, Sgo1 has likely evolved different extra-nuclear activities. Of note, sSgo1 (292aa) lacks 268 residues encoded by exon 6, but retains both conserved Shugoshin domains and is of similar size as *C.33H5.15* (Sgo1) and *C.33H5.15a* (Sgo1). For this analysis we used human SH-SY5Y neuronal-like cells that were induced to enter ciliogenesis by serum starvation. The presence of cilia was confirmed with an anti-polyglutamylation antibody that marks the centromeres (*Figure 1B*). Immunostaining of SH-SY5Y cells in anaphase showed the expected foci-like staining attributed to centromeres (*Figure 1B*). In interphasic cells, we did not detect positive anti-Sgo1 staining on chromosomes, but rather detected a dispersed signal within the cytoplasm. A similar signal distribution for Sgo1 has been described elsewhere in mouse brain sections using monoclonal anti-mouse Sgo1 antibodies (Song et al., 2017a). We found that among SH-SY5Y cells with anti-Tubulin-positive cilia, around 65% showed strong anti-Sgo1 signal co-localizing with anti-Tubulin (Figure 1C, D). The Sgo1 signal was strongest at the base of the cilium, in a region consistent with the basal body, but also extended distally into the ciliary shaft. In the remaining anti-Tubulin positive cells, no detectable anti-Sgo1 signal was observed in or around cilia.
The localization of Sgo1 in SH-SY5Y cells is reminiscent of SGO-1 localization in worms, which is observed primarily in the cilia base and transition zone of sensory cilia (Reed et al., 2023). Definitive proof of a cilia localization for Sgo1 will require, however, the investigation of sgo1-depleted cells.

Considering its low homology with C. elegans SGO-1, the presence of Sgo1 in cilia of neuroblastoma cells was surprising and hinted at potential functional conservation in this organelle. To gain insight into a possible shared ciliary function between human and C. elegans Shugoshin, we expressed sSgo1::GFP in C. elegans sensory neurons using a pan-cilia promoter (Posm-5) and asked whether the human protein could localize to cilia in worms. In these heterologous overexpression lines, we detected sSgo1::GFP on sensory organs in the head of hermaphrodites (Figure 1C, D). While we cannot rule out the contribution of overexpression in the dendritic and periciliary sSgo1::GFP signal, we noticed that, as observed already with SGO-1, sSgo1 is able to cross into the highly impermeable transition zone region, suggesting that the cilia localization of sSgo1::GFP is the product of active transport. It remains possible, however, that transport of Sgo1 into cilia is not biologically relevant in worms (Reed et al., 2023, inset panels in Figure 1E). We reasoned that if Sgo1 has a conserved function in the cilia proper, it may be able to rescue sensory defects in sgo-1 mutant worms. To assess if Sgo1 is functionally redundant with SGO-1 in cilia, we expressed sSgo1::GFP in sensory neurons of sgo-1 (tm2443) worms and evaluated whether the human protein could rescue the sensory defects in these animals carrying a truncated protein product (Reed et al., 2023). We found no evidence of this: while sgo-1 (tm2443) worms can be partially rescued by a SGO-1::GFP transgene, mutant worms expressing sSgo1::GFP display similar levels of dauer formation and glycerol avoidance defects as sgo-1 (tm2443) mutants (Figure 1G, H). The failure of a human Sgo-1 to rescue sgo-1 sensory defects in worms is not unexpected, in view of how Shugoshin proteins have diverged both sequence-wise and functionally in different species. However, these results do not rule out a potential ciliary function of Sgo1 in human cells, a possibility that was not investigated here.

Several integral kinetochore components of the Knl1/Mis12/Ndc80 (KMN) network and transient kinetochore proteins such as Aurora kinases that mediate microtubule attachment and chromosome segregation have been recently implicated in the development of cilia-containing sensory organs during C. elegans embryonic development (Cheerambathur et al., 2019; Bai et al., 2020). Together with the extra-nuclear localization of Shugoshin proteins in neuronal cells, these findings reinforce the perception that protein modules controlling the build up and disassembly of transitory microtubule-based structures during cell division and ciliogenesis have been extensively co-opted during evolution.

In conclusion, our preliminary results raised the intriguing possibility that vertebrate Shugoshin may access and perhaps regulate primary cilia function. Further experiments looking at expression of sgo1 isoforms in human cells and assessing cilia defects upon sgo1 depletion will be necessary to confirm these findings and probe into any functional implication. Nevertheless, a conserved role of human Shugoshin in cilia will invite a revision of the current focus in understanding pathologies recently linked to Shugoshin mutations in fully differentiated cells, such as CAID and Alzheimer’s disease, from exclusively cohesinopathic in origin to a possible ciliopathic perspective (Rao et al., 2018).

**Methods**

**Culture and immunostaining of SH-SY5Y cells**

2x10⁵ SH-SY5Y cells were seeded on poly-D-lysine coated coverslips and allowed to establish in DMEM/F12 (1:1) (HEPES) + 10% FBS for 24 hours. 48 hours of serum deprivation (0.5% FBS) was used to induce ciliogenesis. Cells were fixed in 4% formaldehyde solution at room temperature for 20 minutes followed by incubation in 0.1 M glycine in PBS for 5 minutes. After three washes in PBS, cells were permeabilized with 0.5% Triton X-100/PBS for 30 minutes at room temperature, washed once in PBS for 5 minutes and blocked under gentle agitation with a 5% Normal Donkey Serum + 1% BSA solution. Primary antibody incubation was performed in 1% Normal Donkey Serum + 1% BSA overnight at 4°C. After washing three times with 0.05% Triton X-100 / PBS solution, cells were exposed to the secondary antibody for 1 hour at room temperature before mounting using ProLong™ Glass Antifade Mountant (Invitrogen; P36980). Primary antibodies used were: 1:500 rabbit anti-Sgo1 polyclonal antibody (Invitrogen; #PA5-30869; from now on referred as anti-Sgo1) and 1:1000 mouse anti-polyglutamylation modification monoclonal antibody (AdipoGen Life Sciences; AG-20B-0020-C100; anti-Tubglut). Secondary antibodies used were: anti-rabbit IgG Alexa Fluor Plus 594 (1:2,000; Invitrogen) and anti-mouse IgG Alexa Fluor Plus 488 (1:2,000; Invitrogen). Two rounds of staining were performed. As control for staining, mitotic figures with expected anti-Sgo1 signal on centromeres were confirmed on both experiments. Cells with anti-Tubglut signal were scored for presence or absence of anti-Sgo1 signal in cilia to calculate the prevalence of Sgo1 in cilia.

**C. elegans strains maintenance**

*C. elegans* strains were cultured on Normal Growth Medium (NGM) plates at 20°C in accordance with standard conditions (Brenner, 1974). Strains used in this study are listed in Table 1.
Microscopy

Cell and worm images were taken using a Delta Vision deconvolution system (GE). 3D projections of image stacks (0.2 μm optical sections) are shown. When necessary, image deconvolution was accomplished using Softworxs (GE). For imaging C. elegans, two-day old adult worms were used. Animals were mounted on 2% agarose pads with 25 mM of sodium azide in M9 buffer and imaged immediately.

Glycerol avoidance

The drop test method was used to quantify avoidance behavior to 3M glycerol (Hilliard et al., 2004). In this test, a drop of glycerol solution is placed on the tail of a forward moving worm tracking on an agar surface. As the solution reaches the sensory organs in the head by capillarity, an immediate backward movement response follows. Failure to back within 4 seconds of entering in contact with the solution is scored as lack of avoidance. Tests were repeated for the same worms 10 times. 30 worms per genotype were tested for a total of 300 avoidance responses recorded. Avoidance indexes (AI) for each worm (the number of positive avoidance movements divided by the number of tests and AI averages) were calculated. Only worms that completed all 10 tests were considered for calculation.

Dauer formation

Resistance to SDS treatment was used to quantify dauer formation (Cassada et al., 1975). Mixed worm populations derived from four L4 hermaphrodites per plate were grown on standard size (1 cm²) OP50 lawns and monitored for starvation. Five days after food depletion, worms were collected and washed in M9, resuspended in 1 ml of 1% SDS and incubated with gentle shaking for 30 min. All surviving dauers were identified on petri dishes thrashing under a Nikon 745 stereoscope. Three experiments were carried out, each composed of 12 independent plates. Plates were scored for the present or absence of dauers. The % average of plates containing dauers in the three experiments is presented.

Statistics

Significance (p<0.05) was calculated using two-tailed t-tests with equal variance (MS Excel).

Cloning of Posm-5::sSgo1::GFP and microinjection into gonads

A C. elegans codon-optimized human sSgo1 cDNA (876bp, see sequence below) was cloned downstream of the ciliated neuron specific promoter Posm-5 (329bp) in pUC57 to make pCEC62. Subsequently the Posm-5::sSgo1 insert was subcloned upstream to and in frame with the GFP cassette in pPD95.75 using SpII / KpII, to make pCEC65 (Posm-5::sSgo1::GFP::unc-54 UTR). All plasmids were confirmed by sequencing. Microinjection was performed using a Nikon inverted microscope equipped with a Narishige Micromanipulator. pCEC65 and Parl-13::mks-5::mCherry::unc-54 UTR plasmid were injected into N2 gonads to make CEC279 worms. sgo-1(tm2443); Posm-5::sSgo1::GFP::unc-54 UTR rescue lines (CEC290 and CEC291) were made by injecting pCEC65 into sgo-1(tm2443) worms. ccGFP (pCFJ68) was used as co-injection marker to generate these strains. A list of plasmids is provided in Table 2.

Reagents

Table 1. C. elegans strains
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<th>genotype</th>
<th>source</th>
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<td><em>Caenorhabditis elegans</em> wild type isolate</td>
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<td>CV138</td>
<td><em>sgo-1(tm2443)</em></td>
<td>M. Colaiacovo</td>
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<td>CEC279</td>
<td><em>sasEx75</em> [Posm-5::sSgo1::GFP::<em>unc-54</em> UTR; Parl-13::mks-5::mCherry::<em>unc-54</em> UTR; ccGFP]</td>
<td>This work</td>
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<td>CEC290</td>
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<td>This work</td>
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<tr>
<td>CEC291</td>
<td>*sgo-1(tm2344)*IV ; sasEx84 [Posm-5::sSgo1::GFP::<em>unc-54</em> UTR; ccGFP] line 2</td>
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<tr>
<td>CEC239</td>
<td>*sgo-1(tm2443)*IV; sasEx52 [Posm-5::sgo-1::GFP; Podr-1::DsRed]</td>
<td>Reed et al., 2023</td>
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**Table 2. Plasmids**

<table>
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<td>pCFJ68</td>
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<td>E. Jorgensen</td>
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<td>-</td>
<td>Parl-13::mks-5::mCherry::<em>unc-54</em> UTR</td>
<td>marker for TZ</td>
<td>Wei et al., 2013</td>
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<td>pCEC62</td>
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<td>human sSgo1 - codon-optimized for C. elegans</td>
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<tr>
<td>pCEC65</td>
<td>Posm-5::sSgo-1cDNA::GFP::<em>unc-54</em> UTR</td>
<td>Cilia-driven sSgo-1::GFP translation reporter</td>
<td>This work</td>
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**References**


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