SHORT TAKE

Neuronal protein with tau-like repeats (PTL-1) regulates intestinal SKN-1 nuclear accumulation in response to oxidative stress

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Summary

Oxidative stress is a central pathomechanism in Alzheimer’s disease (AD) and other diseases with tau pathology. The Nrf2 transcription factor induces detoxification enzymes and improves tau pathology and cognition. Its homologue in C. elegans is SKN-1. We previously showed that the worm tau homologue, PTL-1, regulates neuronal aging and lifespan. Here, we tested PTL-1’s involvement in the stress response. ptl-1 mutant animals are hypersensitive to oxidative stress and are defective in stress-mediated nuclear accumulation of SKN-1. This defect can be rescued by PTL-1 re-expression under the control of the ptl-1 promoter. Given the close relationship between aging and stress tolerance, we tested lifespan and found that PTL-1 and SKN-1 regulate longevity via similar processes. Our data also suggest that PTL-1 functions via neurons to modulate SKN-1, clarifying the role of this protein in the stress response and longevity.

Key words: C. elegans; lifespan; neurons; oxidative stress; PTL-1; SKN-1.

Introduction, results and discussion

The most common form of dementia, AD, is characterised by Aβ-containing plaques and neurofibrillary tangles composed of hyperphosphorylated tau (Ittner et al., 2011). Protein with tau-like repeats-1 (PTL-1) is the sole Caenorhabditis elegans homologue of the mammalian tau/MAP2/MAP4 family (Goedert et al., 1996). PTL-1 has a predominantly neuronal expression pattern and functions in the nervous system to mediate kinesin-based transport (Tien et al., 2011).

Activation of Nrf2, a mediator of the oxidative stress response, reduces tau hyperphosphorylation and aggregation (Jo et al., 2014; Stack et al., 2014). Its C. elegans homologue, SKN-1, similarly regulates an oxidative stress response (An et al., 2003). SKN-1 exists in 3 isoforms (Bishop et al., 2008). Most studies have focused on isoforms a/c in the oxidative stress response (An et al., 2005).

Loss of ptf-1 causes neuronal and organisal aging defects (Chew et al., 2013, 2014). As aging and stress pathways are intimately linked, we tested whether ptf-1 mutant animals are stress-sensitive. ptf-1(ok621) and ptf-1(tm543) mutant worms showed decreased survival after exposure to H₂O₂ (Fig 1A). We next tested whether SKN-1 was affected by defective PTL-1. Wild-type animals carrying the lds7[SKN-1::GFP] transgene show reporter expression in the cytoplasm of intestinal cells that rapidly accumulates in the nucleus in response to stress. In contrast, in ASI neurons, SKN-1 is constitutively localised to nuclei (An et al., 2003)(Fig 1B). In the following assays, we used azide stress as this was shown to effectively induce SKN-1 nuclear accumulation (An et al., 2003). In nonstress conditions, no SKN-1 nuclear accumulation was observed in any of the tested strains (data not shown). Both ptf-1 mutant strains displayed a defect in SKN-1 nuclear accumulation in intestinal nuclei in response to azide (Fig 1Bii), which could be rescued by re-expression of PTL-1 under control of the ptf-1 promoter (Fig 1Bii). We next tested whether GCS-1, a detoxification enzyme that is induced by SKN-1, is affected by mutations in ptf-1. lds3[Pgcs-1::gfp] expression is low under normal conditions (Fig S1) but is induced in the intestine under stress conditions (Fig 1Ci) (An et al., 2003). Pgcs-1::gfp induction in response to stress was defective in ptf-1 mutants, and this defect was rescued by PTL-1 re-expression (Fig 1Ci). We also found that the induction of two other SKN-1 targets, gst-4 (Park et al., 2009) and hsp-4 (Glover-Cutter et al., 2013), following azide treatment was compromised in ptf-1 mutants and could be rescued by PTL-1 re-expression (Fig S2). We previously showed that ptf-1 mutants are short-lived (Chew et al., 2013, 2014). Others reported that skn-1(zu67), which affects SKN-1a and c, also confers a short-lived phenotype (An et al., 2003). Interestingly, ptf-1;skn-1 double-mutant animals did not have a significantly different lifespan compared to skn-1 or ptf-1 single-mutant animals (Fig 1D, Table S1), suggesting that SKN-1 and PTL-1 regulate lifespan via the same pathway.

Using the pan-neuronal aex-3 promoter, we re-expressed PTL-1 to test whether neuronal PTL-1 regulates SKN-1. This was sufficient to rescue the defect in sensitivity to H₂O₂ (Fig S3), SKN-1 nuclear accumulation (Fig 2Ai), Pgcs-1::gfp induction (Fig 2Aii) and induction of gst-4 and hsp-4 (Fig S2) in ptf-1 null mutants in response to stress. These data suggest a role for neuronal PTL-1 in regulating intestinal SKN-1. However, as aex-3 is also reported to function in the intestine (Mahoney et al., 2008), a contribution from non-neuronal tissues to the observed rescue of ptf-1 mutant phenotypes cannot be excluded. We therefore also performed RNAi knockdown of ptf-1 and found that SKN-1 nuclear accumulation in response to stress is only compromised when the nervous system is sensitised to RNAi, supporting a role for neuronal PTL-1 in intestinal SKN-1 regulation (Fig S4). Given that SKN-1b is expressed in ASI neurons (An et al., 2003; Bishop et al., 2007), we tested whether re-expressing PTL-1 in ASI neurons alone, using a gpa-4
promoter, affected intestinal SKN-1. However, ASI-specific PTL-1 re-expression neither rescued the defect in SKN-1 nuclear accumulation nor enabled Pgcs-1::gfp induction (Fig 2B). These findings suggest that PTL-1 in the nervous system, but not ASI neurons alone, modulates SKN-1 accumulation in the intestinal nuclei in response to stress. PTL-1 may be required for communication between neurons and the intestine, via synaptic vesicle (SV) transport. We found that unc-13(e450) mutants that are defective in SV exocytosis (Richmond et al., 1999) are also

**Fig. 1** PTL-1 regulates the stress response and longevity in the same pathway as SKN-1. A) *ptl-1* mutants are hypersensitive to H$_2$O$_2$ stress. B) Intestinal SKN-1::GFP nuclear accumulation in response to sodium azide stress is indicated by arrows pointing to intestinal nuclei. Arrowheads indicate ASI neurons. Bii) *ptl-1* mutants are defective in SKN-1 nuclear accumulation in response to azide, which can be rescued by PTL-1 re-expression. SKN-1 nuclear accumulation was scored as positive if GFP was localised to ≥1 intestinal nucleus. n = 15 per assay for 2 replicates. C) Azide induces Pgcs-1::gfp expression. Scoring was conducted as in (Wang et al., 2010). Cii) *ptl-1* mutants show defective Pgcs-1::gfp induction in response to azide, which can be rescued by PTL-1 re-expression. n = 40 per replicate for 3 replicates. D) Survival curves at 25 °C. n = 120 per assay for 2 replicates (one shown). For graphs in Bii) and Cii), error bars indicate mean ± SEM. p-value: *<0.05, ns=not significant. For details of statistical analysis see Experimental Procedures.

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Fig. 2 Neuronal PTL-1 regulation of intestinal SKN-1 may involve UNC-13. A) Pan-neuronal re-expression rescues the defect in i) intestinal SKN-1 nuclear accumulation and ii) PgcS-1::GFP induction in response to azide. B) ASI neuron-specific re-expression fails to rescue the defect in i) intestinal SKN-1 nuclear accumulation and ii) PgcS-1::GFP induction in response to azide. C) unc-13 mutant animals are defective in i) intestinal SKN-1 nuclear accumulation and ii) PgcS-1::GFP induction in response to azide. iii) intestinal SKN-1 nuclear accumulation for unc-13;ptl-1 double-mutant animals treated with azide. Scoring was conducted as in (Tullet et al., 2008; Wang et al., 2010). For SKN-1::GFP, n = 15 per assay for 3 replicates; for PgcS-1::GFP, n = 40 per assay for 3 replicates. Error bars indicate mean±SEM. p-value: *<0.05, ns=not significant. For details of statistical analysis, see Experimental Procedures.
defective in SKN-1 nuclear accumulation and Pgc-1p::gfp induction in response to azide stress (Fig 2C). When we generated a unc-13;ptl-1 (ok621) double-mutant strain, we did not observe differences in SKN-1 localisation between azide-treated unc-13 and unc-13;ptl-1 strains (Fig. 2Ciii), suggesting that PTL-1 and UNC-13 act in the same pathway to regulate SKN-1.

We have shown that the tau-like protein PTL-1 is involved in regulating the response to oxidative stress and in regulating aging, likely in the same pathway as SKN-1. In addition to DAF-2 (Tullet et al., 2008) and p38 MAPK (Inoue et al., 2005), we propose PTL-1 as an additional factor required for nuclear localisation of intestinal SKN-1. We did not find a role for insulin signalling in PTL-1-mediated SKN-1 regulation (Fig SS). As PTL-1 regulates kinesin-based transport (Tien et al., 2011), neuronal PTL-1 may regulate intestinal SKN-1 via signalling molecules carried by SVs. In support, we showed that SKN-1 nuclear accumulation requires UNC-13. Interestingly, unc-13 expression may be regulated by SKN-1 (Staab et al., 2014).

Our data contribute to an emerging picture of a complex communication network between the nervous system and the intestine in C. elegans. Related to this is work on the SKN-1 negative regulator WDR-23, which is widely expressed and targets SKN-1 for proteasomal degradation (Choe et al., 2009). Intestinal WDR-23 expression is sufficient to rescue the neuromuscular defect in wdr-23 mutant animals (Staab et al., 2013), implying that intestinal SKN-1 regulates neuronal function. We previously showed that PTL-1 regulates neuronal and organisinal aging (Chew et al., 2013, 2014), and now show that it modulates the stress response via SKN-1. Given that tau pathology has also been linked to oxidative stress, our findings provide an interesting avenue for a further investigation into the role of a tau-like protein in stress tolerance and longevity.

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Author contributions

YLC, JG and HRN designed experiments, analysed data, wrote the manuscript. YLC performed experiments, wrote the manuscript. YLC, JG and HRN designed experiments, analysed data, reviewed the manuscript. The authors contributed equally to the manuscript. YLC performed experiments, wrote the manuscript.

Conflict of interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Supplementary text including Supplementary Results and Discussion, Experimental Procedures and Supplementary Figure legends.

Fig. 51 Non-stressed animals display a low basal induction of Pgc-1p::gfp.

Fig. 52 Induction of SKN-1 targets gst-4 and hsp-4 is impaired in ptl-1 mutant animals and can be rescued by PTL-1 re-expression.

Fig. 53 Re-expression of PTL-1 under the control of the ptl-1 promoter or the aex-3 promoter restores resistance to hydrogen peroxide treatment.

Fig. 54 Knockdown of ptl-1 results in defective SKN-1 nuclear accumulation when neurons are sensitised to RNAi.

Fig. 55 PTL-1 does not regulate DAF-16::GFP nuclear localisation, and SKN-1 nuclear accumulation is not affected by mutations in daf-2.

Table 51 Summary of data obtained in two independent lifespan assays for ptl-1(ok621), skn-1(zu67) and ptl-1(ok621);skn-1(zu67) strains together with a wild-type control.