Effect of SKN-1 on Stress Responses on Caenorhabditis elegans

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Introduction

Skinhead-1 (SKN-1) is a transcription factor that plays a central role in resisting oxidative stress in *C. elegans.* SKN-1 is related to humans by their ortholog Nrf/CNC protein, which is a regulator of stress response in cells as well as a defense to foreign substances in the body. Furthermore, as evolution has taken place SKN-1 still exhibits important functional conservations with Nrf/CNC. In addition to this *C. elegans* have a variety of similarities to humans such as neurons, skin, and muscles that have been conserved from our common ancestors. Due to this evolutionary conservation of gene functions and physical characteristics, *C. elegans* are advantageous for genetic analysis. *C. elegans* SKN-1 provides a powerful model for investigating how Nrf2 and other Nrf/CNC proteins are regulated, and how they influence the development and functions of normal tissues in vivo (T. Keith,2015).

Methods

Bioproduct

- OMIM was used to identify the Selected SKN-1 gene.
- BLAST and Worms Base was used to identify a Related sequence in *C. elegans*.
- E-rnai web service was used to design primer for PCR amplification of the *C. elegans* gene

Clone gene-specific DNA

- DNA was isolated from *C. elegans.*
- PCR was used to amplify the DNA.
- PCR product was analyzed by gel electrophoresis.
- Recombined PCR product with the vector.

Transformation of E. coli.

- Competent E. coli cells were Transformed using recombine DNA.

Vector analysis

- Single-colony PCR was performed and inoculated overnight culture.
- PCR product was analyzed by gel electrophoresis.

Isolate plasmid DNA

- By performing plasmid miniprep.

Create RNAi feeding strain

- Vector was transformed into the RNAi feeding E. coli strain.
- Beads were used to spread transformed cells on NGM plates.
- Plates were incubated at 37°C for 15-20 h.
- RNAi was induced by feeding.

Assay sensitivity to oxidative stress

- Acute juglone sensitivity assay.
- Five different concentrations.

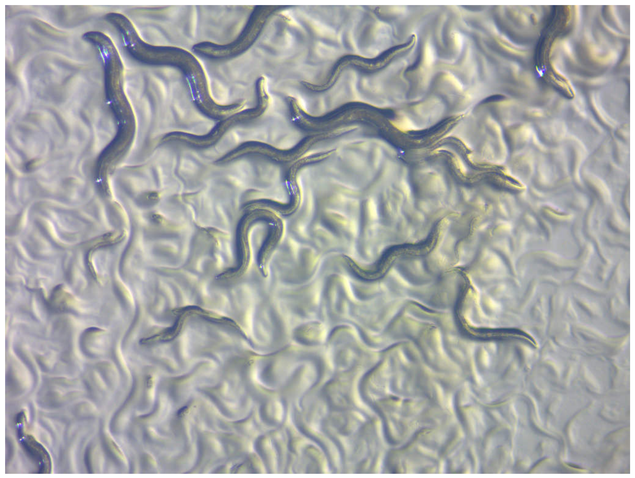
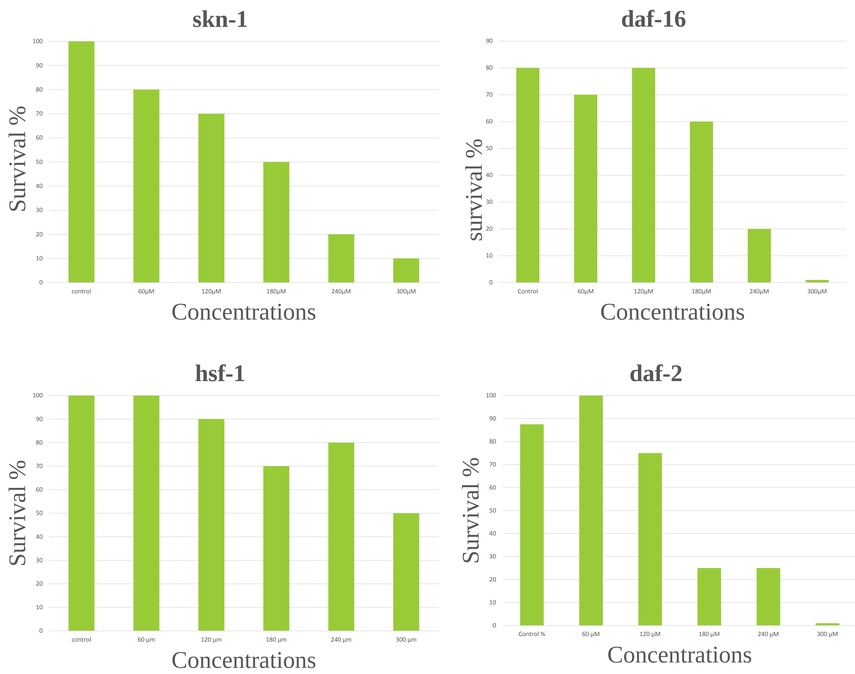


Figure 3. *C. elegans* under the microscope

Figure 2. Survival rate of *C. elegans with v*arious RNAi treatments exposed to different concentrations of juglone



Results

SKN-1 is essential for the increase in oxidative stress resistance. By comparing our control (figure 1) with the other skn-1 silence *C. elegans*, exposed to different concentrations of juglone, we can observe how the loss of function of skn-1 results in shortened lifespan compared to the other RNAi treatments (figure 2). These results show that *C. elegans* oxidative stress response is skn-1-dependent.

Figure 1. Acute juglone sensitivity assay

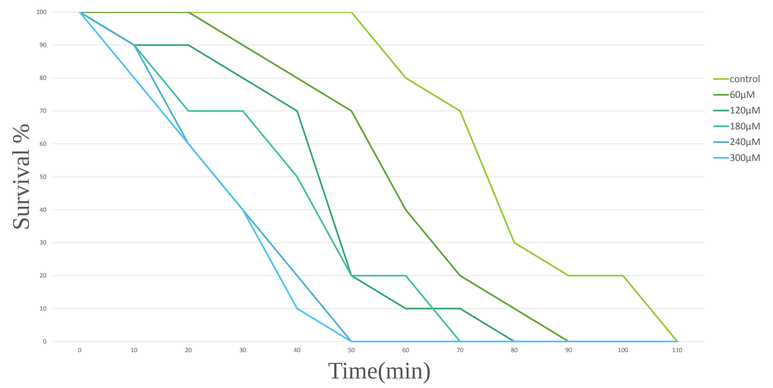


Figure 1.
Survival rate vs juglone concentration. There was a large decrease in survival at higher juglone concentrations

Discussion & Conclusion

Based on the results (figure2), the resistance to oxidative stress was affected by the knockdown of the gene skn-1. The other RNAi treatments didn't have such a significant effect on resistance to oxidative stress indicating that *C. elegans* oxidative stress response is skn-1-dependent. Furthermore, various findings suggest that Nrf/CNC proteins are important for longevity. It is possible that Nrf proteins are directly involved in pathways and mechanisms that promote lifespan, as in the nematode and fruit fly (T. Keith,2015). Therefore, we can assume that Nrf/CNC proteins are likely to be important for longevity assurance in higher organisms such as humans. Overall, these observations demonstrate the role of skn-1 in response to oxidative stress and longevity in *C.elegans*. Further research is hence needed with one or more trials to elucidate the mechanism of SKN-1 in *C. elegans*.

References

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KNOWLEGMENT

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