DNA ligase IV mutations confer shorter lifespan and increased sensitivity to nutrient stress in Drosophila melanogaster

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Abstract
The nonhomologous end-joining pathway is a primary DNA double-strand break repair pathway in eukaryotes. DNA ligase IV (Lig4) catalyzes the final step of DNA end ligation in this pathway. Partial loss of Lig4 in mammals causes Lig4 syndrome, while complete loss is embryonically lethal. DNA ligase 4 (DNAlig4) null Drosophila melanogaster is viable, but sensitive to ionizing radiation during early development. We proposed to explore if DNAlig4 loss induced other long-term sensitivities and defects in D. melanogaster. We demonstrated that DNAlig4 mutant strains had decreased lifespan and lower resistance to nutrient deprivation, indicating Lig4 is required for maintaining health and longevity in D. melanogaster.

Keywords Drosophila melanogaster · DNA ligase IV · Lifespan · Starvation

Introduction
DNA damage is a major hallmark of cancer (Hanahan and Weinberg (2011)), and aberrations in pathways maintaining genomic fidelity are associated with multiple cancers (Brown 2017). In mammals, DNA double-strand breaks (DSB) are primarily repaired by homologous recombination (HR) and classical nonhomologous end-joining (cNHEJ); if cNHEJ is compromised, cells may use alternative NHEJ (altNHEJ). Double-strand breaks are recognized by the Ku70-Ku80 dimer, which recruits DNA-PKcs, Artemis, and DNA ligase IV (Lig4) with XRCC4 (Lieber 2010). Lig4 is an ATP-dependent ligase that catalyzes the phosphodiester bond formation in cNHEJ-mediated DSB repair (Lieber 2010), and, in contrast to DNA ligases I and III which facilitate homeostatic DNA metabolism, the activity of Lig4 is restricted to cNHEJ (Lieber 2010).

Drosophila melanogaster has been extensively used as a model to study DNA repair. The DSB repair in D. melanogaster, like in mammals, is via the two primary pathways: the template-dependent homologous recombination and template-independent end-joining (EJ) pathway as well as the alternative end-joining (altEJ) pathway (Gorski 2003; Mota 2019). These pathways and corresponding components in D. melanogaster are similar to the mammalian system, yet there are some key differences. In the D. melanogaster EJ pathway, DSB are recognized by orthologs of the Ku70/80 heterodimer: Irbp and Ku80. The D. melanogaster ligation complex is composed of D. melanogaster DNAlig4 and orthologs of XRCC4 (CG3448) and XLF (CG12728 and CG32756). One striking deviation of the D. melanogaster EJ is the absence of the key protein, DNA-PKcs, a critical component of the mammalian NHEJ pathway (Mota 2019). D. melanogaster EJ pathway also lacks polymerases μ and λ and the nuclease Artemis (recently reviewed (Sekelsky 2017)).

Lig4 hypomorphic mutations in humans cause the Lig4 syndrome (recently reviewed (Altmann and Gennery 2016)), and Lig4−/− mice are inviable, as mutations cause p53-mediated neuronal apoptosis resulting in embryonic lethality (Frank 2000). In contrast, D. melanogaster males and females lacking DNAlig4 function are viable and fertile; however, these mutants are hypersensitive to ionizing radiation (IR)—induced DNA damage during early development (Gorski 2003; McVey et al. 2004). An accumulation of DNA damage is commonly observed as a result of aging, and metabolic alterations resulting from nutrient deprivation...
can induce oxidative stress which in turn induces DNA damage (Filomeni et al. 2015). We wanted to investigate whether mutations in \textit{DNAlig4} modulates \textit{D. melanogaster} longevity and response to nutrient deprivation.

\textbf{Results and discussion}

We obtained three strains of \textit{D. melanogaster} from the Bloomington Drosophila Stock Center: \textit{w}^{1118} (wild-type \textit{DNAlig4}) and two \textit{DNAlig4} mutants: \textit{DNAlig4}^{57} and \textit{DNAlig4}^{57} (Gorski 2003). The \textit{D. melanogaster} stocks were maintained at 25 °C on normal yeast-cornmeal food (ingredients: 337.5 g yeast, 195 g soy flour, 1425 g cornmeal, 95 g \textit{Drosophila} food-grade Agar type II, 900 g malt extract, 1.5 g molasses, 100 mL propionic acid, 250 mL 10% Tegosept, and 25 L tap water) unless mentioned otherwise. Flies were harvested from two groups of 10 flies each of mixed sexes.

\section*{B. Lifespan and response to nutrient deprivation.}

Median survival (expressed as hours into starvation) is listed in Table 1 (\textit{p}=0.001 for \textit{w}^{1118} compared to \textit{DNAlig4}^{57}, \textit{p}<0.0001 for \textit{w}^{1118} compared to \textit{DNAlig4}^{57}, \textit{n}=40 flies per strain). Survival under starvation conditions was assessed as mentioned in the Results and discussion section. Median survival (expressed as hours into starvation) is listed in Table 1 (\textit{p}<0.001 for \textit{w}^{1118} compared to \textit{DNAlig4}^{57}, \textit{n}=100 flies per strain).

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\caption{\textit{DNAlig4} mutants have a reduced lifespan and are more sensitive to starvation compared to wild-type \textit{D. melanogaster}. A Transcript levels of \textit{DNAlig4} were analyzed by qPCR using primers spanning the first two exons which were deleted in the mutant strains. RNA expression was assayed from two groups of 10 flies each of mixed sexes. The RNA expression for each of the \textit{DNAlig4} mutants was each compared to the wild-type (\textit{w}^{1118}) flies. **\textit{p}<0.01, \textit{n}=2. B Lifespan was assessed in wild-type (\textit{w}^{1118}) and \textit{DNAlig4} flies. Median lifespan (expressed as days post emergence) is listed in Table 1 (\textit{p}=0.001 for \textit{w}^{1118} compared to \textit{DNAlig4}^{57}, \textit{p}<0.0001 for \textit{w}^{1118} compared to \textit{DNAlig4}^{57}, \textit{n}=40 flies per strain). C Survival under starvation conditions was assessed as mentioned in the Results and discussion section. Median survival (expressed as hours into starvation) is listed in Table 1 (\textit{p}<0.001 for \textit{w}^{1118} compared to \textit{DNAlig4}^{57}, \textit{p}<0.001 for \textit{w}^{1118} compared to and \textit{DNAlig4}^{57}, \textit{n}=100 flies per strain).}
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\end{figure}
flies was 53 days and 40.5 days for the DNAlig45 and DNAlig457 strains, respectively, and both DNAlig4 mutant fly strains were statistically similar to one another (Fig. 1B and Table 1). Our results are in agreement with a previous study where a different DNAlig4 mutant, the DNAlig4169a, was shown to have reduced lifespan compared to the wild-type strain (Garcia 2011).

We investigated the effect of nutrient deprivation on the survival of DNAlig4 mutants. Briefly, age-matched flies were mated as above, and freshly emerged male and female virgin flies were collected and cultured in groups of 10 into vials containing approximately 10 mL of standard fly food for 48 h. Flies were then transferred to vials with 1% agar with water. Dead flies were scored twice a day (in a cycle of 16 h and 8 h intervals) until all the flies died; 100 flies per strain were assessed. Survival curves were created for survivorship and statistically analyzed as above. DNAlig45 flies were significantly more resistant to nutrient deprivation than either of the DNAlig4 mutant strains, which, again, were statistically similar to one another (Fig. 1C and Table 1).

The reduced lifespan we observed in DNAlig4 mutants is likely a function of accumulation of unresolved DNA damage. We attribute the diminished capacity to withstand nutrient stress to induction of oxidative DNA damage that is resolved with less efficiency in DNAlig4 mutant strains. Overall, we can conclude that while loss of DNAlig4 does not hinder viability of progeny from homozygous mutant parents or manifest any obvious phenotypic defects in D. melanogaster, it does negatively impact the lifespan of adult flies and sensitizes them to nutrient deprivation. We conclude that DNA Lig4 is required for maintaining health and longevity in D. melanogaster; the role of DNA Lig4 in supporting health and lifespan of other organisms is currently unknown.

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Author contribution AKA, JC, and RJ designed the experiments. RJ and SB conducted the experiments, and RJ, SB, and AKA analyzed the data under the supervision of JC and AKA. RJ, SB, JC, and AKA wrote the manuscript.

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Declarations

Ethical approval This article does not contain any studies with human participants or vertebrate animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

References

Altmann T, Gennery AR (2016) DNA ligase IV syndrome; a review. Orphanet J Rare Dis 11(1):137
Brown JS et al (2017) Targeting DNA repair in cancer: beyond PARP inhibitors. Cancer Discov 7(1):20–37
Filomeni G, De Zio D, Cecconi F (2015) Oxidative stress and autophagy: the clash between damage and metabolic needs. Cell Death Differ 22(3):377–388
Frank KM et al (2000) DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the p53 pathway. Mol Cell 5(6):993–1002
Garcia AM et al (2011) Loss of the bloom syndrome helicase increases DNA ligase 4-independent genome rearrangements and tumorigenesis in aging Drosophila. Genome Biol 12(12):R121
Gorski MM et al (2003) The Drosophila melanogaster DNA ligase IV gene plays a crucial role in the repair of radiation-induced DNA double-strand breaks and acts synergistically with Rad54. Genetcs 165(4):1929–1941
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674
Joshi RR, Ali SI, Ashley AK (2019) DNA ligase IV prevents replication fork stalling and promotes cellular proliferation in triple negative breast cancer. J Nucleic Acids 2019:9170341. https://doi.org/10.1155/2019/9170341
Lieber MR (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem 79:181–211
Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4):402–408

McVey M, Radut D, Sekelsky JJ (2004) End-joining repair of double-strand breaks in Drosophila melanogaster is largely DNA ligase IV independent. Genetics 168(4):2067–2076

Mota MBS et al (2019) DNA damage response and repair in perspective: Aedes aegypti, Drosophila melanogaster and Homo sapiens. Parasit Vectors 12(1):533

Sekelsky J (2017) DNA repair in Drosophila: mutagens, models, and missing genes. Genetics 205(2):471–490

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