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53bp1 mutation enhances *brca1* and *bard1* embryonic lethality in *C. elegans*

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Abstract

In mice, mutation of *brca1* results in embryonic lethality, which is partially suppressed by *53bp1* mutation. In contrast, mutation of the *C. elegans* BRCA1 ortholog, *brc-1*, or its binding partner, *brd-1*, lead to only mild embryonic lethality. We show that in *C. elegans*, *brc-1* and *brd-1* embryonic lethality is enhanced when *53bp1* ortholog, *hsr-9*, is also mutated. This is not a consequence of activating *polq-1*-dependent microhomology-mediated end joining, as *polq-1* mutation does not suppress embryonic lethality of *hsr-9*; *brc-1* mutants. Together, these results suggest that BRC-1-BRD-1 and HSR-9 function in parallel pathways and do not act antagonistically as in mammals.

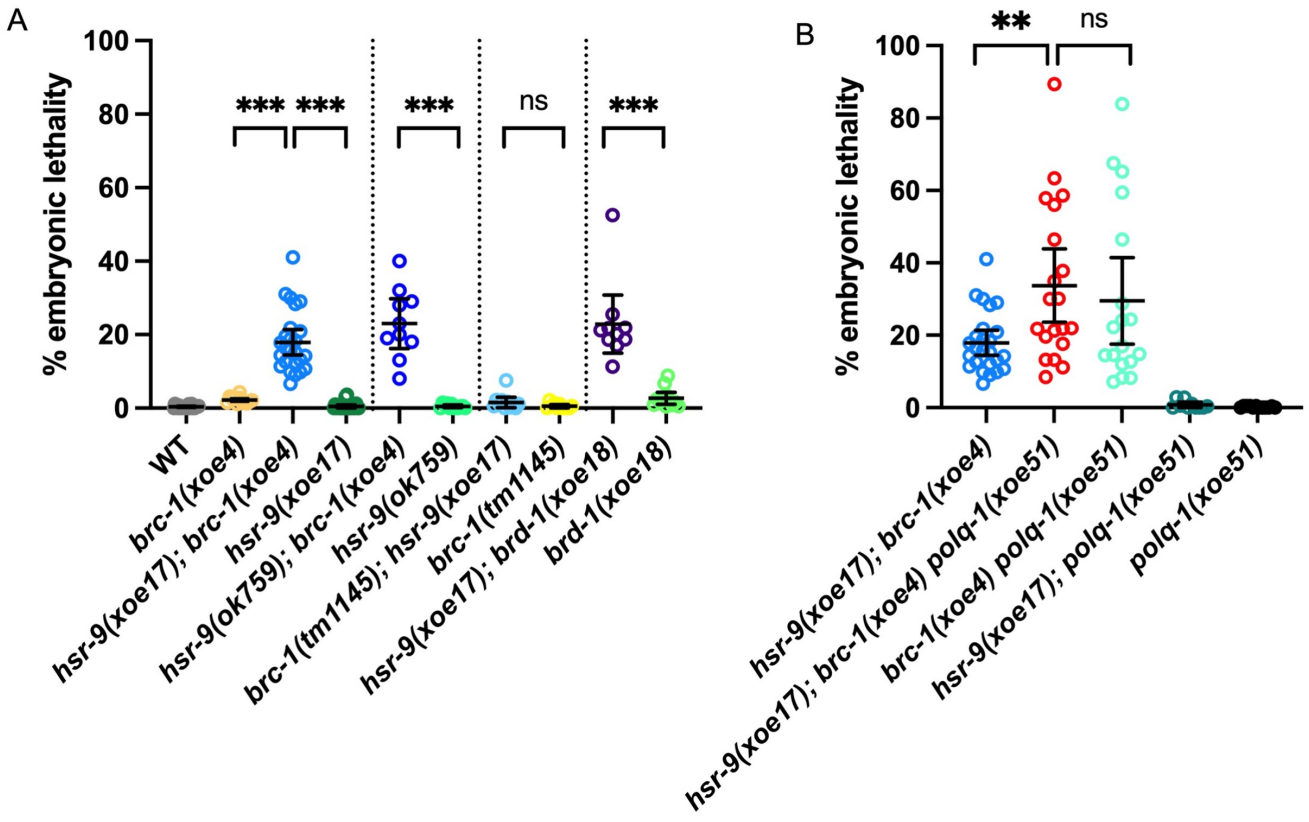

Figure 1. Embryonic lethality in different allele combinations of *brc-1, brd-1, hsr-9,* and *polq-1*:

A) Embryonic lethality in wild type (26), *brc-1(xoe4)* (12), *hsr-9(xoe17); brc-1(xoe4)* (25), *hsr-9(xoe17)* (23), *hsr-9(ok759); brc-1(xoe4)* (12), *hsr-9(ok759)* (12), *hsr-9(xoe17); brc-1(tm1145)* (11), *brc-1(tm1145)* (11), *hsr-9(xoe17); brd-1(xoe18)* (10), and *brd-1(xoe18)* (12) animals. Number of animals examined are in paratheses. B) Embryonic lethality in *hsr-9(xoe17); brc-1(xoe4)* (25), *hsr-9(xoe17); brc-1(xoe4) polq-1(xoe51)* (20), *brc-1(xoe4) polq-1(xoe51)* (18), *hsr-9(xoe17); polq-1(xoe45)* (10) and *polq-1(xoe51)* (12). Mean and 95% Confidence Interval shown; *** $p < 0.001$; ** $p < 0.01$; ns = not significant by Mann-Whitney.

Description

BRCA1-BARD1 is an essential E3 ubiquitin ligase that functions as a tumor suppressor through promoting double strand break (DSB) repair by homologous recombination (Brzovic et al. 2003; Hashizume et al. 2001; Tarsounas and Sung 2020). Several groups have shown that the early embryonic lethality of *brca1* mutant mice can be partially suppressed by mutation of the tumor suppressor *53bp1*, which promotes the error prone non-homologous end joining (NHEJ) pathway (Cao et al. 2009; Chen et al. 2020; Li et al. 2016). In *C. elegans*, orthologs of BRCA1 and BARD1, (*brc-1* and *brd-1*, respectively), also play roles in DSB repair but have only mild embryonic lethal phenotypes (Boulton et al. 2004; Janisiw et al. 2018; Li et al. 2018). Additionally, analysis of the *53bp1* ortholog, *hsr-9*, did not reveal an obvious role in NHEJ (Ryu et al. 2013). These results suggest that the function of *hsr-9* and relationship between *brc-1*-*brd-1* and *hsr-9* may be different in this metazoan than in mammals.

We constructed *hsr-9; brc-1* and *hsr-9; brd-1* double mutants and analyzed embryonic lethality to examine the genetic interaction between these genes in *C. elegans*. In contrast to what has been reported in mice, we observed elevated embryonic lethality in *brc-1* and *brd-1* null alleles [*brc-1(xoe4)*, *brd-1(xoe18)* (Li et al. 2023; Li et al. 2018)], in combination with either *hsr-9(ok759)* (Ryu et al. 2013) or a new putative null allele *hsr-9(xoe17)* (Figure 1A). On the other hand, a hypomorphic *brc-1* allele, *brc-1(tm1145)* (Li et al. 2018), in combination with *hsr-9(xoe17)* did not result in elevated embryonic lethality (Figure 1A). To determine whether the elevated embryonic lethality was due to *polq-1*-dependent microhomology-mediated end joining (MMEJ), which is mutagenic and activated in the absence of *brc-1* (Kamp et al. 2020), we also constructed a new putative null allele of *polq-1* [*polq-1(xoe51)*]. We found that the *hsr-9; brc-1 polq-1* triple mutant had levels of embryonic lethality similar to *brc-1 polq-1* but higher than *hsr-9; brc-1*, suggesting that the elevated embryonic lethality of *hsr-9; brc-1* is not a consequence of activation of MMEJ (Figure 1B). Therefore, our results are consistent with a model where BRC-1-BRD-1 and HSR-9 function in parallel pathways to promote viable progeny, most likely through DSB repair, and do not appear to be antagonist as in mammals.

Methods

CRISPR-mediated genome editing: *hsr-9(xoe17)* and *polq-1(xoe51)* alleles were engineered by incorporating the stop-in cassette (Wang et al. 2018) early in the coding region of each gene and were generated using the co-CRISPR method (Paix et al. 2015). The *hsr-9* repair template (gattttgcctcttaaataaaatttcagCAAAAAACCGAGGGGAGACTTGCAATAGGGAAG TTTGTCCAGAGCAGAGGTGACTAAGTGATAAGCTAGCTCTCGGATCATCTTGCAAACATGCTTATTGCTGgtaggtattg caacc) and guide RNA (AGGGGAGACTTGCAATATCT) were injection into N2 and the resulting progeny were analyzed by PCR using TGAAATTAAGGTGGTCACTCGAAG and GTTGTTGTGGGGAGGCTGAA. The *polq-1* repair template (AGAGAATTCTCTGAAGATCCATTAATATTGCTTACCGAAGGGGAAGTTTGTCCAGAGCAG AGGTGACTAAGTGATAAGCTAGCAGAGTTTTCGCCGCAATTCTCAGACTTTGGTAATGATTTC) and guide RNA (ATTGCGGCGAAAACTCTCTT) were injected into N2 and the resulting progeny were analyzed by PCR using ATAGGCAAATGGCTGGACGG and TCAAAGCAGTCTTCTCGGCA. Worms were outcrossed a minimum of three times.

Embryonic lethality: L4 hermaphrodites of indicated genotypes were picked onto individual plates and transferred to new plates every 24hr for 3 days. Embryonic lethality was determined by counting eggs and hatched larvae 24hr after removing the hermaphrodite and calculating percent as eggs/(eggs + larvae).

Reagents

Strains:

Strain	Genotype	Available from
N2	*Caenorhabditis elegans*	CGC
JEL730	*brc-1(xoe4)*	JE lab, deposited in CGC
JEL1000	*hsr-9(xoe17)*	JE lab, will be deposited in CGC
WB240	*hsr-9(ok759)*	CGC

JEL1162	*brd-1(xoe18)*	JE lab, will be deposited in CGC
JEL1016	*hsr-9(xoe17); brc-1(xoe4)*	JE lab, will be deposited in CGC
JEL838	*hsr-9(ok759); brc-1(xoe4)*	JE lab
JEL1166	*hsr-9(xoe17); brc-1(tm1145)*	JE lab
JEL1319	*hsr-9(xoe17); brd-1(xoe18)*	JE lab, will be deposited in CGC
JEL1134	*polq-1(xoe51)*	JE lab, will be deposited in CGC
JEL1142	*hsr-9(xoe17); brc-1(xoe4) polq-1(xoe51)*	JE lab, will be deposited in CGC
JEL1104	*brc-1(xoe4) polq-1(xoe51)*	JE lab
JEL1199	*hsr-9(xoe17); polq-1(xoe51)*	JE lab

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