HELZ-BRCA2 Complex Resolves R-loops to Drive Co-Transcriptional Homologous Recombination

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Introduction

Mutations in BRCA2 predispose individuals to various cancers. The BRCA2 protein, with its partner DSS1, is crucial for genomic integrity, mainly through homologous recombination (HR) repair of double-strand breaks (DSBs) and replication fork protection (RFP). It helps load RAD51 recombinase onto single-stranded DNA (ssDNA) or stalled replication forks. Recent studies show BRCA2-DSS1 also helps resolve R-loops, which are DNA-RNA hybrids that can cause genome instability if unresolved. Key questions remain about how R-loops are formed and resolved during HR and how BRCA2-DSS1 regulates them. This study used proximity labeling and mass spectrometry to find new BRCA2-DSS1 partners under DNA damage conditions, identifying HELZ, an RNA helicase, as a novel partner. HELZ's role in DNA repair and genome maintenance was previously unknown.

Materials and Methods

Full-length HELZ, miniBRCA2, and truncated BRCA2 mutants were purified from E. coli or insect cells for various in vitro biochemistry assays, including EMSA binding assay, R-loop unwinding, AFM imaging, and affinity pull-down. Stable mammalian cell lines (HeLa wild-type or helicase-dead mutant of HELZ) were established for multiple analyses, such as proximity-dependent biotinylation (PDB) assays, mass spectrometry analysis, DNA repair reporter assay, foci imaging, DART assay with immunofluorescence staining, laser-induced micro-irradiation, RNA/DNA hybrid slot blot, and clonogenic survival assays with various DNA damage agents and irradiation.

Results

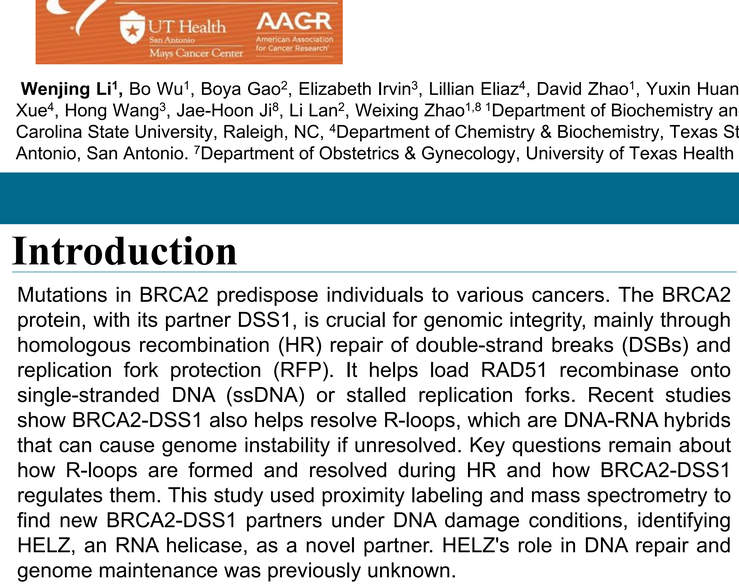


Figure 1 HELZ is a novel BRCA2-DSS1 interacting partner that directly binds to BRCA2 CTRB domain. **a.** Volcano plots indicates HELZ as a high-ranking partner of DSS1 by BioID-LC-MS/MS. Red dots represent significantly upregulated proteins (p<0.05 and log2FC>1). Blue dots represent significantly downregulated proteins (p<0.05 and log2FC<-1). Black dots represent non-significant proteins. Dashed lines illustrate the cutoffs. X axis, fold change at log2 scale; y axis, minus p value at log10 scale. **b**. Immunoprecipitation (IP) of HELZ from HeLa cells showing that HELZ interacts with BRCA2, but not BRCA1 in the presence of RNase/DNase. **c**. Illustration of BRCA2 and various truncations such as miBRCA2 and CTRB. Abbreviations: NTD, N-terminal DNA binding domain; BRC repeat, breast cancer repeat; DBD, DNA binding domain; HD, helical domain; OB, oligonucleotide/oligosaccharide binding; and CTRB, C-terminal RAD51-binding. **d**. SDS-PAGE analysis of insect purified HELZ and BRCA2 with Coomassie blue staining. **e**. IP showing direct interaction of recombinant HELZ and BRCA2. **f.** GST and Flag beads pull down indicate that BRCA2-CTRB provides the major interface for HELZ binding. Samples were analyzed by SDS-PAGE for Coomassie blue staining and western blot with anti-HELZ antibody.

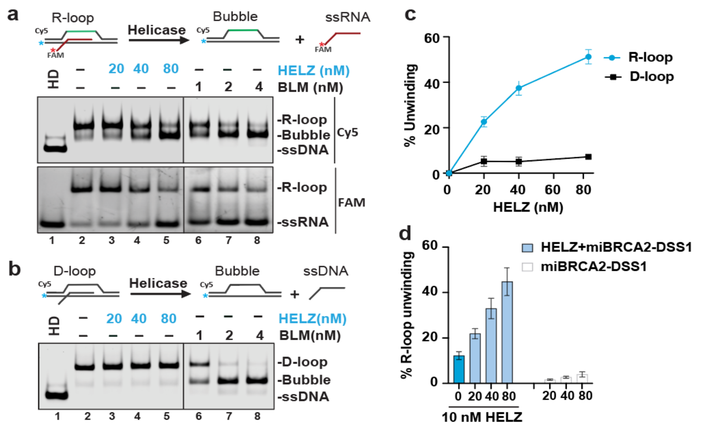


Figure 2 HELZ has specific RNA helicase activity. a. Schematic of the R-loop unwinding assay with ssRNA labeled with FAM and bubble DNA labeled with Cy5 (top). PAGE gel showing representative R-loop unwinding by HELZ (20 nM, 40 nM, and 80 nM; lanes 3-5) and BLM (1 nM, 2 nM, and 4 nM; lanes 6-8) (bottom). **b**. Schematic of the D-loop unwinding assay with bubble DNA labeled with Cy5 (top). PAGE gel showing representative D-loop unwinding by HELZ (20 nM, 40 nM, and 80 nM; lanes 3-5) and BLM (1 nM, 2 nM, and 4 nM; lanes 6-8) (bottom). c. Quantification of the R-loop and D-loop unwinding experiments by HELZ in **a** and **b**. The data represent the mean ± SD of at least three independent experiments. **d.** Quantification of the stimulation of miBRCA2-DSS1 on R-loop unwinding by HELZ. The data represent the mean ± SD of at least three independent experiments.

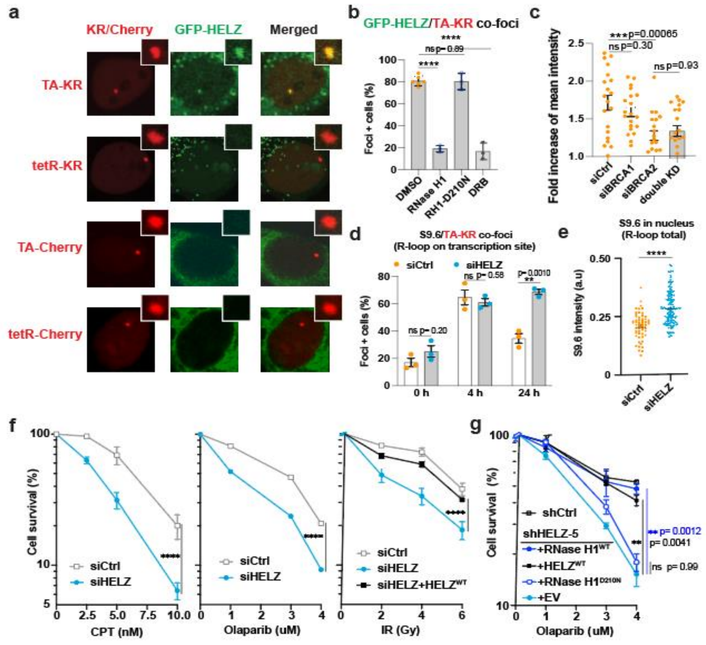


Figure 3 The recruitment of HELZ to R-loop is required for cell survival. a. U2OS-TRE cells transfected with pBROAD3 TetR-KR/Cherry or pBROAD3 TA-KR/Cherry and the expression vector of GFP-HELZ were light-activated and recovered 20 min before fixation. Representative images of GFP foci recruitment at sites of TA-KR, TetR-KR, TA-Cherry or TetR-Cherry were shown. **b.** Foci-positive cells with HELZ colocalization with TA-KR upon treatment with DRB and overexpression of RNase H1 or RNase H1D210N were quantified. ns, not significant; ****P ≤ 0.0001(student t test). **c.** Foci-positive cells with HELZ colocalization with TA-KR upon treatment with siBRCA1 or siBRCA2 or the combination were quantified. **d**. Foci-positive cells with S9.6 colocalization with TA-KR upon treatment with siHELZ were quantified. ns, not significant (student t test). **e**. Quantification (mean ± SEM) of enrichment of R-loop (detected by S9.6 antibody) in the nuclei of HeLa with HELZ siRNAs. au arbitrary unit. ****P ≤ 0.0001(student t test). **f**. Clonogenic survival of HeLa cells depleted with HELZ upon treatment with CPT, Olaparib, IR. Symbol: EV, empty vector; HELZ^{WT}, with ectopic expression of wild type HA-HELZ. ****P ≤ 0.0001(two-way ANOVA). **g**. Clonogenic survival of HeLa cells depleted with shHELZ-5 and ectopic expression of HELZ or RNase H1 upon treatment with Olaparib. Symbol: EV, empty vector. P values indicated in the figure (two-way ANOVA).

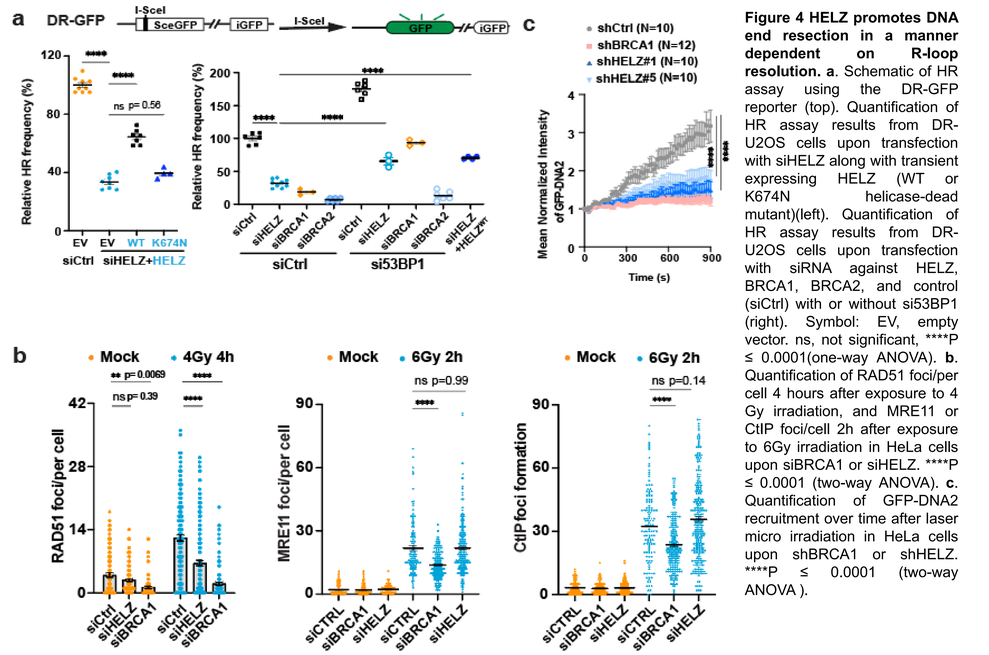


Figure 4 HELZ promotes DNA end resection in a manner dependent on R-loop resolution. a. Schematic of HR assay using the DR-GFP reporter (top). Quantification of HR assay results from DR-U2OS cells upon transfection with siHELZ along with transient expressing HELZ (WT or K674N helicase-dead mutant)(left). Quantification of HR assay results from DR-U2OS cells upon transfection with siRNA against HELZ, BRCA1, BRCA2, and control (siCtrl) with or without si53BP1 (right). Symbol: EV, empty vector. ns, not significant, ****P ≤ 0.0001(one-way ANOVA). **b.** Quantification of RAD51 foci/per cell 4 hours after exposure to 4 Gy irradiation, and MRE11 or CtIP foci/cell 2h after exposure to 6Gy irradiation in HeLa cells upon siBRCA1 or siHELZ. ****P ≤ 0.0001 (two-way ANOVA). **c.** Quantification of GFP-DNA2 recruitment over time after laser micro irradiation in HeLa cells upon shBRCA1 or shHELZ. ****P ≤ 0.0001 (two-way ANOVA).

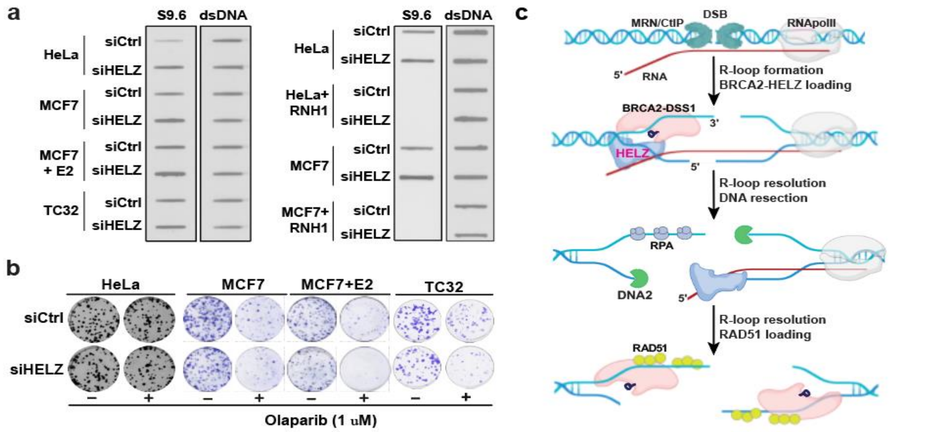


Figure 5 The implication of HELZ in R-loop driven cancers. a. R-loop level (detected by S9.6 antibody) was tested in genomic DNA of HeLa, TC32 and MCF7 with/without estrogen hormone 17β-estradiol (E2) treatment. **b**. Representative images of clonogenic survival of HeLa, TC32 and MCF7 cells depleted with HELZ upon treatment with Olaparib. **c**. Model for HELZ functions in R-loop resolution and DNA end resection during HR at transcription active sites. HELZ-BRCA2 is recruited to R-loops formed at transcription active sites near DSBs and resolves R-loops to promote long-range DNA resection, partially through the recruitment of DNA2 or other factors.

Conclusion

Our study identifies HELZ as a novel RNA helicase and BRCA2 partner essential for resolving R-loops and promoting homologous recombination (HR) repair. BRCA2 recruits HELZ to R-loops, where HELZ facilitates DNA end resection, aiding their resolution and preserving genome stability. HELZ is particularly critical in estrogen receptor-positive (ER+) breast cancer cells and other R-loop-driven tumors such as Ewing sarcoma. Loss of HELZ in these contexts heightens genomic instability and markedly increases sensitivity to PARP inhibitors. These findings establish HELZ as a key genome maintenance factor and highlight its potential as a context-dependent therapeutic target to enhance PARP inhibitor efficacy in ER+ breast cancers and Ewing sarcoma, offering new opportunities for precision medicine in R-loop-driven cancers.

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