Supporting Information

for Adv. Sci., DOI: 10.1002/advs.201903616

BRCA1 Deficiency Impairs Mitophagy and Promotes Inflammasome Activation and Mammary Tumor Metastasis

Qiang Chen,* Josh Haipeng Lei, Jiaolin Bao, Haitao Wang, Wenhui Hao, Licen Li, Cheng Peng, Takaaki Masuda, Kai Miao, Jun Xu, Xiaoling Xu, and Chu-Xia Deng*
Figure S1. Loss of BRCA1 impairs stress-induced mitophagy. A) Immunoblot analysis of BRCA1 in Hela-mCherryParkin with control shRNA or different BRCA1 shRNA. B) Analysis of mitophagy activities in shCtrl and shBRCA1 Hela-mCherryParkin under CCCP treatment by clearance of ATP5B. Scale bar, 20 µm. C) Quantification for ATP5B levels after CCCP treatment (more than 30 cells were counted per group). D) The mRNA levels of BRCA1 in Brca1^flox/flox MEFs infected with AdGFP or AdCre. E) Immunoblot analysis of COXII, COXIV and TOM20 in Brca1^flox/flox MEFs infected with AdGFP or AdCre treated with CCCP. F) Quantification of COXII, COXIV and TOM20 levels in (D), which normalized by β-actin level (n = 3 per group). G) The mRNA levels of BRCA1 in Brca1^flox/flox;Tam-Cre MEFs with or without 4-HT treatment. H, I) Mitophagy activity indicated by mt-mKeima in Brca1^flox/flox;Tam-Cre MEFs. (H) Dual-excitation imaging (488/561 nm) of mt-mKeima. Scale bar, 50 µm. (I) Quantification of fluorescence intensity from (H) (more than 50 cells were counted per group). A high ratio (561/488) of puncta indicates a low pH area. The cells were treated with or not CCCP (30 µM). Data represent the mean ± SEM and are representative of three independent experiments. Significant differences were determined by one-way (C) or two-way (F, I) ANOVA with Tukey multiple comparison testing or unpaired two-tailed t test (D and G). *p < 0.05, ***p < 0.001, ****p < 0.0001.
Figure S2. BRCA1 deficiency has no effect on CCCP induced PINK1/Parkin pathway activation. A) Immunoblot analysis of PINK1 in shCtrl and shBRCA1 Hela cells after CCCP (10 µM) treatment for indicated time points. B) shCtrl and shBRCA1 Hela-mCherryParkin were treated by CCCP for 1 h, and then stained by MitoTracker Green for labeling mitochondria. Hoechst33258, DNA-binding dye. Scale bar, 10 µm. C) The ubiquitin level on mitochondria in shCtrl and shBRCA1 Hela-HA-Parkin with or without CCCP (10 µM) treatment for 3 h, as measured by immunostaining for ubiquitin (Ub). Mitochondria were labeled by TOM20 immunostaining. Scale bar, 10 µm. D) Immunoblot analysis of MFN1 and MFN2 levels in Hela-HA-Parkin shCtrl and shBRCA1 cells under CCCP treatment for indicated time points. Data are representative of at least two independent experiments.
Figure S3. BRCA1 is required for mitophagy, but not for general autophagy. A) Immunoblot analysis of p62 and LC3 in Brca1^{floxed} MEFs infected with AdGFP or AdCre under starvation (HBSS treatment) for indicated times. B) The co-localization of lysosomes with mitochondria in Hela-HA-Parkin shCtrl and shBRCA1 cells under CCCP (10 µM) treatment for 6 h. Lysosomes and mitochondria were marked with LAMP1-RFP and MitoTracker Green, respectively. Scale bar, 5 µm. C) Pearson’s coefficient is shown as the quantification of lysosomes co-localized with mitochondria per cell in (B) (10 fields were counted per group). Data represent the mean ± SEM and are representative of at least two independent experiments. Significant differences were determined by unpaired two-tailed t test (C) ***p < 0.001.
Figure S4. BRCA1 regulates MFN1/2 mediated-mitochondrial fusion. A) The mRNA levels of BRCA1, Mfn1 and Mfn2 in Brca1floxflox, Tam-Cre MEFs with or without 4-HT (5 µM) treatment, as measured by real-time PCR (n = 3 per group). B) Immunoblot analysis of BRCA1, MFN1 and MFN2 in HCC1937 cells stably transfected with empty vector (VEC) or BRCA1 expressing vector (BRCA1). Asterisk (*) indicates MFN2 band. C) The mRNA levels of Mfn1 and Mfn2 in VEC and BRCA1 HCC1937 cells measured by real-time PCR (n = 3 per group). D) Mitochondrial morphology of VEC and BRCA1 HCC1937 cells stained by MitoTracker Green. Scale bar, 10 µm. E) The mRNA levels of Mfn1 and Mfn2 in breast cancer PDX models (TM00096, BRCA1 WT; TM00089, BRCA1 MT) cells measured by real-time PCR (n = 3 per group). F) Representative images of immunostained MFN1 in breast cancer PDX models. Scale bar, 20 µm. Data represent the mean ± SEM and are representative of at least two independent experiments. Significant differences were determined by unpaired two-tailed t test. **p < 0.01, ***p < 0.001, ****p < 0.0001.
Figure S5. BRCA1 is required for stress-induced mitochondrial fission via mediating AMPK activation. A) Immunoblot analysis of Flag-BRCA1 in Flag-tagged BRCA1 (Flag-BRCA1) MEFs compared with wildtype (WT) MEFs. B) Immunoblot analysis of BRCA1 level on mitochondria in Flag-BRCA1 MEFs under CCCP (30 μM) treatment. TOM20 and Lamin A are loading control for mitochondrial and nuclear protein, respectively. C) Representative images of BRCA1 in Flag-BRCA1 MEFs stained with Flag antibody. The cells were treated with or not CCCP. Scale bar, 50 μm. D) BRCA1 is required for Oligomycin-induced AMPK activation in 293T cells. shCtrl and shBRCA1 293T cells were treated by Oligomycin (10 μM) for indicated time points. E) BRCA1 has no effect on AICAR-induced AMPK activation. Brca1flax/flax MEFs infected with AdGFP or AdCre were treated with AICAR (2.5 mM) for 1 h. F, G) BRCA1 deficiency impairs CCCP-induced mitochondrial fission. (F) The BRCA1 levels in shCtrl and shBRCA1 Hela-HA-Parkin; (G) Mitochondrial morphology of shCtrl and shBRCA1 Hela-HA-Parkin with or without CCCP treatment for indicated time points. Mitochondria were marked by MitoDsRed. Arrows indicate mitochondrial morphology. H) Loss of DRP1 blocks CCCP-induced mitophagy. shCtrl and shDRP1 Hela-mCherryParkin were treated with CCCP for indicated time points. Data are representative of at least two independent experiments.
Figure S6. CCCP-induced AMPK activation is independent on LKB1 or CaMMKβ. A) LKB1 KD has no effect on CCCP-induced AMPK activation. shCtrl and shLKB1 293T cells were treated by CCCP for indicated time points. B) CaMMKβ KD has no effect on CCCP-induced AMPK activation. shCtrl and shCaMMKβ 293T cells were treated by CCCP for indicated time points. C) Immunoblot analysis of AMPK activation in shCtrl and shBRCA1 Hela cells that lack of LKB1. Data are representative of at least two independent experiments.
Figure S7. Inflammatory response significantly increases in *Brca1* mutant mammary gland. GSEA plot of enrichment in “inflammatory response” gene set upregulated in *Brca1* MT mammary glands.
Figure S8. The expression of inflammasome associated genes in different cell lines. A) NLRP3 mRNA levels. B) PYCARD mRNA levels. C) CASP1 mRNA levels. D) TLR4 mRNA levels.
NLRP3 Inflammasome Inhibitor: Glibenclamide (GLI) (5 mg/kg)

Brca1\text{floxed} \text{MMTV-Cre}

A

Inhibitor injection at one day before removing tumor

Inhibitor injection at every three days after removing tumor for five times

Remove primary tumor

Check tumor relapse and metastasis

B

P2: CD8\textsuperscript{+} cells

P3: Activated CD8\textsuperscript{+} cells

Control

CD8

CD3

CD44

GLI

P2: CD8\textsuperscript{+} cells

P3: Activated CD8\textsuperscript{+} cells

C

Control

GLI

D

MDSC

Brca1 mut tumor

Enrichment score

NES = -0.599

pvalue = 0.945

Position in the Ranked List of Genes

E

Macrophages

Brca1 mut tumor

Enrichment score

NES = -0.843

pvalue = 0.772

Position in the Ranked List of Genes
Figure S9. Inhibition of inflammasome activation attenuates Brca1 mutant mammary tumor recurrence and metastasis. A) The flowchart of animal experiments. Brca1\textsubscript{flox/flox} MMTV-Cre mice developing mammary tumors (diameter around 1 cm) were i.p. injected with PBS or NLRP3 inflammasome inhibitor Glibenclamide (GLI, 5 mg/kg) one day before primary tumor removal. Then, the mice were administrated with PBS or GLI by i.p. continuous injection every three days for five times. B) Flow cytometric gating strategy for CD8\textsuperscript{+} T cell analysis. C) Representative images of lung metastasis of tumors after primary tumor removal. Arrows indicate metastatic tumors on lung. D) GSEA plot of enrichment in “MDSC” gene set has no significant difference in B.T versus Trp53.T. E) GSEA plot of enrichment in “Macrophages” gene set has no significant difference in B.T versus Trp53.T.