Mutant *RB1* enhances therapeutic efficacy of PARPis in lung adenocarcinoma by triggering the cGAS/STING pathway

Qi Dong, …, Yang Hui, Yunyan Gu

*JCI Insight.* 2023;8(21):e165268. [https://doi.org/10.1172/jci.insight.165268](https://doi.org/10.1172/jci.insight.165268).

Graphical abstract

---

**Find the latest version:**

[https://jci.me/165268/pdf](https://jci.me/165268/pdf)
Mutant RB1 enhances therapeutic efficacy of PARPis in lung adenocarcinoma by triggering the cGAS/STING pathway

Qi Dong,1,2 Tong Yu,3,4 Bo Chen,1 Mingyue Liu,1 Xiang Sun,3 Huiying Cao,3 Kaidong Liu,1 Huanhuan Xu,1 Yuquan Wang,3 Shuping Zhuang,3 Zixin Jin,1 Haihai Liang,3 Yang Hui,2 and Yunyan Gu1

1Department of Systems Biology, College of Bioinformatics Science and Technology, and 2Department of Biochemistry and Molecular Biology, Harbin Medical University, Harbin, China. 3Department of Pharmacology (State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education), College of Pharmacy, Harbin Medical University, Harbin, China. 4Shanghai Frontiers Science Research Center for Druggability of Cardiovascular noncoding RNA, Institute for Frontier Medical Technology, Shanghai University of Engineering Science, Shanghai, China.

Introduction
Lung adenocarcinoma (LUAD) is the major histological subtype of non–small cell lung carcinoma (NSCLC); the majority of diagnosed LUAD patients are in the middle and late stages (1). Despite promising new therapies that have been proposed, the 5-year survival rate of LUAD patients is 15% due to the typically late stage of diagnosis and innate or acquired resistance (2). Effective treatment strategies are needed for LUAD patients. Several clinical trials demonstrated that poly (ADP-ribose) polymerase inhibitors (PARPis) had the potential to improve the prognosis for NSCLC patients (3, 4). PARPis may therefore be useful treatments for LUAD patients.

PARPis are clinically approved drugs according to their synthetic lethal interactions, and clinical trials have been applied in non–small cell lung cancer. However, the therapeutic efficacy of PARPis in lung adenocarcinoma (LUAD) is still unknown. We explored the effect of a mutated retinoblastoma gene (RB1) on PARPi sensitivity in LUAD. Bioinformatic screening was performed to identify PARPi-sensitive biomarkers. Here, we showed that viability of LUAD cell lines with mutated RB1 was significantly decreased by PARPis (niraparib, rucaparib, and olaparib). RB1 deficiency induced genomic instability, prompted cytosolic double-stranded DNA (dsDNA) formation, activated the cGAS/STING pathway, and upregulated downstream chemokines CCL5 and CXCL10, triggering immune cell infiltration. Xenograft experiments indicated that PARPi treatment reduced tumorigenesis in RB1-KO mice. Additionally, single-cell RNA sequencing analysis showed that malignant cells with downregulated expression of RB1 had more communications with other cell types, exhibiting activation of specific signaling such as GAS, IFN response, and antigen-presenting and cytokine activities. Our findings suggest that RB1 mutation mediates the sensitivity to PARPis through a synthetic lethal effect by triggering the cGAS/STING pathway and upregulation of immune infiltration in LUAD, which may be a potential therapeutic strategy.
Genetic interactions refer to the combination of genetic variants of 2 genes contributing to a phenotype that is different from the expectation based on their individual variants, including SL and synthetic viability (10). Genetic interactions participate in the drug response of cancer cells. Hu et al. developed a quantitative chemical-genetic interaction mapping strategy in human mammary epithelial cells, charting the effect of knocking down 625 DNA repair- and cancer-relevant genes on the sensitivity or resistance to 29 drugs (11). Martins et al. generated a systematic chemical-genetic interaction map to predict 90 drug responses for triple-negative breast cancer (TNBC) cell lines with cancer gene aberrance (12). SL interactions contribute to the identification of new drug targets or drug response biomarkers.

Thus, we systematically explored candidate responsive biomarkers for PARPis based on genetic interactions by utilizing bioinformatics screening, and revealed that mutation of retinoblastoma tumor suppressor gene (RB1) is a candidate PARPi-sensitive biomarker for lung cancer (13). RB1 is a negative regulator of the cell cycle. It has been shown that 8.2% of NSCLC patients carried mutations in RB1, and 4% of LUAD patients had mutations in RB1 in The Cancer Genome Atlas (TCGA) (14). NSCLC patients with RB1 mutation are associated with nonresponse to immunotherapy (15). Therefore, investigation of the role of RB1 mutation in response to PARPis could be of great benefit for clinical treatment of NSCLC. Furthermore, the therapeutic response to PARPis is significantly influenced by the tumor microenvironment (TME) (16). Exploring and understanding the mechanism of the TME’s regulation of the response to PARPis is necessary for improving the effectiveness of treatment.

In the current study, we systematically explored the mechanism of RB1 mutation involved in the response to PARPis (niraparib, rucaparib, and olaparib). Then, we investigated the effect of RB1 mutation on the transcriptome and proteome. We found that RB1 mutation increases genomic instability and upregulates the immune response by activating cGAS/STING signaling in LUAD. In vivo, rucaparib treatment significantly reduced tumor volume in RB1-knockout (RB1-KO) xenograft mice. Together, our results reveal that mutant RB1 mediates sensitivity to PARPis in LUAD cells by SL interaction.

**Results**

**RB1 mutation enhances sensitivity to PARPis in LUAD.** Based on the systematic screening of genomic variations that have SL interactions with PARPis, we found that RB1 mutation may mediate sensitivity to PARPis in lung cancer cells (Figure 1A) (13). RB1-mutant lung cancer cells tended to be sensitive to olaparib, niraparib, and talazoparib in the Genomics of Drug Sensitivity in Cancer (GDSC) and the Cancer Therapeutics Response Portal (CTRP) data sets (Figure 1, B–J). Moreover, RB1 mutation had SL interaction with PARP3 knockdown in the RNA interference screening data set (Cancer Dependency Map [DepMap] Portal, Achilles) (Figure 1K). In RB1-mutant LUAD patients, high expression of PARP1 showed poorer overall survival than patients with low expression of PARP1 (Figure 1L).

Then, we investigated the relationship between RB1 mutation and PARP1/2/3 expression. The expression of PARP1 and PARP2 in RB1-mutant LUAD samples was significantly higher than RB1 wild-type (WT) samples (Figure 1, M and N). Furthermore, the expression of PARP1, PARP2, and PARP3 was significantly negatively correlated with RB1 expression (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/jci.insight.165268DS1).

H2228 and H1781 are RB1-mutant cell lines, whereas A549, H1650, and H1975 are RB1-WT cell lines (Figure 2A). CCK8 analysis showed that RB1-mutant cell lines were sensitive to PARPis (Figure 2, B–D). Consistent with these results, the IC50 values of the LUAD cells were significantly lower in cell lines with RB1 mutation compared with WT cell lines (Figure 2, B–D). Furthermore, H2228 formed fewer colonies than A549 when exposed to the same concentration of niraparib and rucaparib for the same time (Figure 2, E and F). TUNEL assay showed that RB1-mutant cell lines H2228 and H1781 had more apoptotic cells compared with WT cell lines A549 and H1975 treated with niraparib. Cells treated with DMSO served as the negative control (Figure 2, G and H). The results indicate that RB1 mutation increases the sensitivity to PARPis in LUAD cells.

**Mutated RB1 shows low expression at the level of transcriptome and proteome and mediates sensitivity to PARPis.** LUAD samples, lung squamous cell carcinoma (LUSC) samples, and lung cancer cell lines with RB1 mutations showed significantly lower expression of RB1 and encoded proteins than cell lines with WT RB1 (Figure 3, A–E). In addition, RB1 expression in RB1-mutant cell lines (H2228 and H1781) was lower compared with RB1-WT cell lines (A549, H1650, and H1975) (Figure 2A). RB1 expression analysis was carried out on the LUAD cell lines H2228, H1781, A549, H1650, and H1975 using quantitative
real-time PCR (qRT-PCR) and Western blot assay. The results showed that mRNA and protein expression levels of RB1 were downregulated in RB1-mutant samples compared with RB1-WT samples (Figure 3, F and G). The above analysis indicates that RB1 mutations are loss-of-function mutations.

We explored the hypothesis that RB1 expression mediates PARPi sensitivity. Lung cancer cell lines with low RB1 expression (RB1-L) were sensitive to olaparib according to the pharmacogenomic data in the GDSC data set (Figure 3, H and I). We found that RB1-mutant breast cancer cell lines were significantly sensitive to rucaparib and olaparib in the GDSC data set (Figure 3, J and K). Then, TNBC patients treated with veliparib were grouped according to RB1 expression. There were more patients with pathological...
Figure 2. LUAD cell lines with RB1 mutations reduce viability promoted by PARPi.

(A) Expression and mutations of RB1 in 5 LUAD cells in the DepMap and COSMIC databases.

(B–D) The viability of LUAD cell lines with mutated RB1 or WT RB1 treated with PARPi olaparib, niraparib, and rucaparib were analyzed by CCK8 assay (n = 4). Representative images of colony formation assay of A549 and H2228 cell lines treated with 1 μM niraparib, rucaparib, and DMSO for 72 hours followed by 14-day recovery. Statistical analysis of colony formation assay (n = 4). **P < 0.01 vs. DMSO by unpaired, 2-tailed Student’s t test and Dunnett’s test. NS, no significance.

(G) TUNEL assay of apoptotic cells among A549, H1975, H2228, and H1781 treated with 1 μM DMSO or niraparib. Scale bars: 50 μm. (H) Statistical analysis of TUNEL assay (n = 6). *P < 0.05, **P < 0.01 vs. DMSO by unpaired, 2-tailed Student’s t test.
complete response (pCR) in the RB1-L group than in the high-RB1-expression (RB1-H) group (Figure 3L). In addition, the expression of RB1 in pCR patients was significantly lower than that in residual disease (RD) patients (Figure 3M). Altogether, our results show that RB1 mutation is associated with RB1-L and mediates sensitivity to PARPis in lung cancer and breast cancer.

**RB1 deficiency facilitates the effect of PARPis on DNA damage.** Some evidence demonstrated that RB1 mutation increases DNA damage and instability in cancer cells (17). Retinoblastoma localizes to DNA double-strand breaks (DSBs) and promotes DSB repair through HR (18). Next, we explored the relevance between the DNA damage response (DDR) and RB1 in the TCGA LUAD cohort. RB1-mutant samples exhibited significantly higher telomeric allelic imbalances (NtAI), loss of heterozygosity (LOH), and homologous recombination damage (HRD) scores in comparison with WT RB1 (Figure 4A). Moreover, NtAI, large-scale transition (LST), and HRD scores in RB1-L samples were significantly greater than in RB1-H samples (Figure 4B). Then, we assessed the disparity of chromosomal instability using the weighted genome integrity index (wGII) and the frequency of LOH (FLOH). The wGII and FLOH scores in RB1-mutant samples were significantly higher than RB1-WT samples (Figure 4C), and the wGII scores in RB1-L samples were significantly higher compared with RB1-H samples (Figure 4D). Furthermore, RB1 mutation was related to increases in “fraction altered,” “aneuploidy,” “silent mutation rate,” and “nonsilent mutation rate” scores (Figure 4, E–H). These results revealed that RB1 deficiency enhances DDR and induces genomic instability.

As the functions of PARPis are to interfere with DNA repair and induce cell death in cancer cells, we posited that RB1 might affect cancer cell responses to PARPis by disrupting DNA damage repair. To test this hypothesis, we generated an RB1-KO cell line from RB1-WT A549 lung cancer cells using the CRISPR/Cas9 system. RB1 KO was confirmed with qRT-PCR and Western blotting (Figure 4I). When the same concentration of niraparib and rucaparib was added to cell lines, A549-RB1-KO cell viability was significantly reduced (Figure 4J). We also observed a similar result in cell colony formation assays where the number of cell clones was reduced in A549-RB1-KO cells (Figure 4K). We then examined cytosolic double-stranded DNA (dsDNA) in A549-RB1-WT and A549-RB1-KO cells treated with niraparib and rucaparib using immunofluorescence. As shown in Figure 4L, niraparib and rucaparib promoted the accumulation of cytosolic dsDNA in A549-RB1-KO cells compared with the cells treated with DMSO. More importantly, PARPis did not affect nuclear DNA DSBs in A549-RB1-WT cells, supporting the idea that the presence of RB1 may partially repair the DNA damage induced by PARPis. Consistently, immunofluorescence analysis of RAD51 (a key marker of DNA damage) in A549 cells exposed to the same concentration of niraparib indicated that PARPis aggravated the DNA damage response in A549-RB1-KO cells, but not in A549-RB1-WT cells (Figure 4, M and N).

To further examine PARPis’ SL interactions with RB1 in DNA damage repair, we constructed an RB1 overexpression plasmid and transfected it into RB1-mutant cell line H2228 (H2228-RB1). The transfection efficiency was verified by qRT-PCR and Western blotting (Figure 5A). Cell viability assays indicated that overexpression of RB1 partially restored the proliferation of H2228 cells when given the same dose of niraparib and rucaparib (Figure 5B). We obtained similar results using cell colony formation experiments (Figure 5, C and D). Moreover, we found that niraparib and rucaparib treatments were able to induce the formation of cytosolic dsDNA in H2228, while reexpression of RB1 protected against DNA damage and reduced the amount of cytosolic dsDNA (Figure 5E). Consistent with this finding, RB1 reexpression increased the fluorescence intensity of RAD51 and decreased γ-H2AX expression compared with the solvent control group treated with DMSO (Figure 5, F and G). Taken together, our results show that PARPi-mediated activation of DDR was associated with RB1 gene stabilization in LUAD cells.

**PARPis induce the secretion of chemokines CCL5 and CXCL10 via the cGAS/STING pathway in an RB1-dependent manner.** PARPi treatment is known to produce cytosolic dsDNA that is subsequently recognized by cyclic GMP-AMP synthase (cGAS), which leads to the activation of innate immune signaling. In our experiments, RB1-mutant samples showed significantly increased expression of several cGAS/STING-related genes in comparison with RB1-WT samples in the TCGA LUAD cohort (Figure 6, A–E); IFNA6, IFNA2, IFNA14, and IFNA18 showed the same trend (Supplemental Figure 2, A–D). In the LUAD cohort of Gillette et al. (19), the expression of CCL5, CXCL10, and IFNB1 in RB1-mutant samples was significantly higher than in RB1-WT samples (Figure 6F). Thus, RB1 mutation stimulated cGAS/STING signaling.
We further investigated the above findings at single-cell resolution. Cells from 11 early-stage LUAD patients (tLung) in the NCBI Gene Expression Omnibus (GEO) database (GSE131907) were integrated. Cells were annotated into 8 cell types (B lymphocytes, endothelial cells [ECs], epithelial cells, fibroblasts, mast cells, myeloid cells, natural killer [NK] cells, and T lymphocytes) and further divided into cell subtypes. The copy number karyotyping of aneuploid tumors (CopyKAT) algorithm was used to identify malignant cells in the epithelial cell cluster, and further classified by RB1 expression (see Methods). We applied the CellChat algorithm to infer the communications in GAS signaling among high-RB1-expressing malignant epithelial cells (ME-RB1-H), low-RB1-expressing malignant epithelial cells (ME-RB1-L), and other cell subtypes. ME-RB1-L cells communicated with multiple cell subtypes via the GAS signaling pathway, including “activated DCs” (dendritic cells) and several subtypes of ECs and fibroblasts (Figure 6G), whereas ME-RB1-H cells did not have communications related to GAS signaling with other cells. The communicational probability of GAS signaling of ME-RB1-L cells with other cells was higher than that of ME-RB1-H cells (Figure 6H).

Then, we investigated whether PARPi-induced cytosolic dsDNA could activate innate immune signaling through the cGAS/STING pathway in RB1-mutant LUAD cells. Therefore, we assessed the colocalization of cGAS and dsDNA. Immunofluorescent staining of cGAS and staining with PicoGreen probe in A549-RB1-WT and A549-RB1-KO cell lines exposed to the same concentrations of niraparib and rucaparib revealed a marked increase in the number of cytoplasmic cGAS foci and colocalization with cytosolic dsDNA in A549-RB1-KO cells, but not in A549-RB1-WT cells (Figure 6I). We examined whether cytosolic dsDNA and cGAS could activate STING signaling. Immunoblotting analysis of cGAS, STING, phosphorylation of TBK1 (pSer172 TBK1) and IRF3 (pSer386 IRF3), critical components in the cGAS/STING pathway, were detected in A549 cell lines exposed to PARPis (20). As shown in Figure 6J, niraparib and rucaparib treatment increased cGAS and STING expression in A549-RB1-KO cells, but not A549-RB1-WT cells. Indeed, niraparib and rucaparib treatment also elevated phosphorylation levels of both TBK1 and IRF3 in A549-RB1-KO cells. We then evaluated the mRNA expression level of CCL5 and CXCL10, key downstream targets of STING activation (21). We found that RB1-KO significantly upregulated the mRNA levels of CCL5 and CXCL10 induced by PARPis (Figure 6K). Consistent with these changes in mRNA levels, PARPis substantially increased the production of proinflammatory cytokines CCL5 and CXCL10 in A549 cell culture supernatant, as detected by ELISA (Figure 6L).

To investigate whether reexpression of RB1 restored PARPi-induced DNA DSBs, we overexpressed RB1 in H2228 cells following PARPi treatment. Analysis of cGAS staining intensity within dsDNA indicated a marked decrease in response to PARPis niraparib and rucaparib in H2228 cells transfected with RB1 (Figure 6M). Furthermore, we found that overexpression of RB1 led to the inactivation of components of the STING pathway, including cGAS, STING, p-TBK1, and p-IRF3 in H2228 cells treated with PARPis (Figure 6N). The mRNA levels of CCL5 and CXCL10 were downregulated in H2228 cells transfected with RB1 (Figure 6O). Consistent with these observations, the concentration of CCL5 and CXCL10 in supernatants of H2228-RB1 cells revealed a similar decrease after exposure to PARPis (Figure 6P).

**PARPi suppressed tumorigenesis in RB1-KO mice.** The above experiments in vitro indicated that RB1 mutation increased the therapeutic efficacy of PARPis in LUAD cells. Next, to validate the suppressive effect of a PARPi on LUAD in vivo, we established xenograft models in human peripheral blood mononuclear cell–engrafted mice (huPBMC-NOG) to generate human mature T cells. The volume and weight of tumors in mice with A549-RB1-KO cells were significantly reduced compared with A549-WT cells after concurrent treatment with rucaparib (Figure 7, A and B). Immunohistochemical staining showed that the expression of caspase-3 was obviously increased in A549-RB1-KO cells treated with rucaparib. A Ki67 proliferation assay also showed that knockdown of RB1 obviously increased the antitumor effect of rucaparib (Figure 7C). Western blot analysis revealed that A549-RB1-KO mice treated with rucaparib...
**Figure 4. RB1 deficiency enhances genomic instability.** (A) Distribution of genomic scar scores between RB1-mutant and RB1-WT samples. (B) Distribution of genomic scar scores between RB1-H and RB1-L samples. (C) Comparison of chromosomal instability in RB1-mutant and RB1-WT samples. (D) Comparison of chromosomal instability in RB1-H and RB1-L samples. (E–H) Comparison of “fraction altered,” “aneuploidy,” “silent mutation rate,” and “nonsilent mutation rate” values between RB1-mutant and RB1-WT samples. (I) Protein and mRNA expression of RB1 in A549-RB1-WT cells and A549-RB1-KO cells assessed by Western blotting and qRT-PCR assays (n = 6). **P < 0.01 vs. A549-RB1-WT. (J) CCK8 analysis of cell viability (n = 10). **P < 0.01 vs. A549-RB1-WT. (K) Colony formation assay of A549-RB1-WT and A549-RB1-KO cells treated with 1 μM niraparib, rucaparib, and DMSO for 72 hours followed by 14-day recovery (n = 4). **P < 0.01 vs. A549-RB1-WT. (L) Representative immunofluorescence images of A549-RB1-WT and A549-RB1-KO cells treated with niraparib, rucaparib, and DMSO for 72 hours. Release of dsDNA into the cytoplasm detected by PicoGreen assay. Green, PicoGreen; Blue, DAPI. Scale bars: 5 μm. (M) Immunofluorescence of RAD51 and γ-H2AX in A549-RB1-WT and A549-RB1-KO cells. Scale bars: 20 μm. (N) Western blot analysis for RAD51 and γ-H2AX in A549-RB1-WT and A549-RB1-KO cells. P values were calculated by 1-sided Wilcoxon’s rank-sum test (A–H) and unpaired, 2-tailed Student’s t-test (I–K).
could activate the cGAS/STING signaling pathway (Figure 7D). qRT-PCR experiments further showed that knockdown of RB1 increased mRNA expression of the inflammatory factors CCL5 and CXCL10, illustrating the consequent activation of a T cell immune response (Figure 7, E and F).

**RB1 deficiency activates immune infiltration.** Compared with RB1-WT samples, fractions of “macrophages M1" ($P = 0.039$) and “macrophages M0” ($P = 0.046$) were significantly higher in RB1-mutant samples (Figure 8, A and B). Additionally, “Th2 cells” (T helper 2 cells) ($P = 0.0012$) and “IFN-γ response” ($P = 0.0020$) scores in RB1-mutant samples were significantly higher than RB1-WT LUAD samples (Figure 8, C and D). Next, we further determined the infiltration distinction between RB1-L and RB1-H samples. Infiltration of “T cells follicular helper” ($P = 2.6 \times 10^{-3}$), “T cells regulatory (Tregs)” ($P = 2.4 \times 10^{-4}$), “NK cells activated” ($P = 2.6 \times 10^{-4}$), and “macrophages M0” ($P = 0.050$) in RB1-L samples was higher than in RB1-H samples (Figure 8, E–H). The “proliferating NK/T cells in lung” gene set was significantly enriched in RB1-mutant samples (Figure 8I).

To gain insight into the discrepancy in the immune microenvironment between RB1-L and RB1-H samples, we performed cell-cell communication analysis at the single-cell level. After eliminating the batch effect by using the R package “harmony,” cells collected from 9 LUAD samples in GSE171145 were merged and clustered, in which samples showed uniform distribution (Supplemental Figure 3 and Supplemental Figure 4A). We annotated clusters by the singleR method and expression of canonical marker genes (Supplemental Figure 4, B and C, and Supplemental Figure 5A). A total of 10 distinct cell clusters were identified, including T cells, epithelial cells, B cells, macrophages, NK cells, fibroblasts, neutrophils, mast cells, ECs, and basal cells (Figure 8J).
In the GSE131907 (tLung) and GSE171145 data sets, the number and strength of interactions between ME-RB1-L cells and other cell types were higher than those between ME-RB1-H cells and other cell types, both in sender and receiver cells (Figure 8K, Supplemental Figure 5B, and Supplemental Figure 6A), especially tissue stem cells and macrophages in GSE171145, fibroblasts and myeloid cells in GSE131907 (tLung), ECs, T cells, and NK cells in both data sets. Similarly, subtype cells frequently communicated with ME-RB1-L cells compared with ME-RB1-H in both early-stage and advanced-stage LUAD patients in GSE131907 (Figure 8L), and the level of communication strengthened (Supplemental Figure 6B). In addition, we investigated specific communications in ME-RB1-L cells. In the GSE171145 and GSE131907 (tLung) data sets, interactions of secreted phosphoprotein 1 (SPP1), laminin, intercellular adhesion molecule (ICAM), and major histocompatibility complex class II (MHC-II) signaling pathway were specifically present in ME-RB1-L cells, indicating immune upregulation in these cells (Supplemental Figure 7, A and B).

**Discussion**

PARPis target *PARP* genes by reducing DNA repair function and increasing replication fork errors in cancer cells. *RB1* is located in the nucleoplasm (Supplemental Figure 8) and performs DDR functions. We revealed that loss-of-function mutations of *RB1* induce DNA damage and promote dsDNA accumulation in the cytoplasm. DSBs are subsequently recognized by the cGAS/STING pathway, followed by triggering of innate immune signaling. Immune cells (e.g., macrophages, T cells, NK cells, DCs, and B cells) are recruited to the surface of lung cancer cells and destroy tumor cells (Supplemental Figure 8J–M). Moreover, our study indicated that the immunomodulatory capacity of PARPis could improve PARPi’s efficacy in *RB1*-mutant LUAD patients.

Some studies showed that increasing *PARP1* expression dictated the sensitivity to PARPis (22). Our study revealed that the expression of *PARP1* and *PARP2* was significantly upregulated in *RB1*-mutant samples. Moreover, we found a negative correlation between *PARP1/2* expression and *RB1* expression, which also confirmed our assumption that *RB1* deficiency mediates sensitivity to PARPis based on SL interactions for LUAD.

PARPis have the potential to treat multiple cancers. In 2023, the US Food and Drug Administration approved talazoparib with enzalutamide for metastatic castration-resistant prostate cancer patients with mutations of an HR repair gene (*ATM, ATR, BRCA1, BRCA2, CDK12, CHEK2, FANCA, MLH1, MRE11A, NBN, PALB2, or RAD51C*), and approved niraparib for advanced ovarian, fallopian tube, or primary peritoneal cancer patients with HRD-positive status in 2019 (23, 24). Moreover, in phase Ib and II trials of avelumab plus talazoparib treatment in NSCLC patients, the overall response rate of DDR-positive patients was higher than DDR-WT patients (25). In this study, our results suggest that LUAD patients with *RB1* mutation may benefit from PARPis due to the increase in DNA damage and immune infiltration. Currently, there is a lack of publicly available PARPi-response data for LUAD patients with corresponding transcriptome or genome sequencing. *RB1* is an important regulator of the cell cycle, and functional inactivation of *RB1* has been recognized to occur in multiple human cancers, including TNBC (26). TNBC and LUAD have common oncogenic mechanisms (27, 28); therefore, PARPi-response data for TNBC was used to verify the therapeutic effect of *RB1* on PARPis (Figure 3, J–M).

Moreover, *RB1* mutation has been verified to induce PARPi hypersensitivity in osteosarcoma.
tumor cells (29). Hence, development of basket clinical trials to access the efficacy and safety of PARPis in RB1-mutant LUAD patients is a priority in the near future.

In this study, we overexpressed RB1 using a constructed plasmid (pcDNA3.1 vector, 0.2 μg) and found increased fluorescence intensity of RAD51 and decreased γ-H2AX expression, indicating that overexpressed RB1 may increase the capacity of DNA damage repair (Figure 5, F and G). Tang et al. transfected a gastric cancer cell line with 2 concentrations of pcDNA3.1 vector (0.2 μg and 0.5 μg) to overexpress RB1 and derived consistent results (30). In this study, whether the effect of RB1 is dose dependent in lung cancer cells warrants future detailed research.

Figure 7. RB1 KO enhanced the therapeutic efficacy of PARPi in LUAD xenograft. (A) Tumor volumes of 4 treatment groups with A549-RB1-WT (100 μL PBS), A549-RB1-KO (100 μL PBS), A549-RB1-WT + rucaparib (60 μL DMSO, 240 μL PEG 300, 30 μL Tween 80, 270 μL normal saline, 1.08 mg rucaparib every 2 days) and A549-RB1-KO + rucaparib (60 μL DMSO, 240 μL PEG 300, 30 μL Tween 80, 270 μL normal saline, 1.08 mg rucaparib every 2 days). (B) The tumor growth curves of 4 treatment groups for 30 days. **P < 0.01. (C) Representative images of IHC staining in LUAD xenograft samples. Scale bars: 50 μm. (D) Western blot analysis of proteins in the STING pathway in LUAD xenograft samples (n = 3). (E and F) qRT-PCR analysis of CCL5 and CXCL10 mRNAs in A549-RB1-WT and A549-RB1-KO cells after treatment with PARPis in mice (n = 5). *P < 0.05 vs. A549-RB1-WT + rucaparib. P values in D–F were calculated by unpaired, 2-tailed Student’s t test.
Previous studies have reported that PARPis induce innate immunity via PARP1 trapping–induced DNA damage and activation of cGAS/STING signaling (31). Our study indicated a synergistic effect of \( \text{RB1} \) mutation and treatment with PARPis on the activation of cGAS/STING. Our findings indicated that \( \text{RB1} \) deficiency significantly upregulated the mRNA and protein levels of CCL5 and CXCL10 induced by PARPis. According to CellChat analysis, CCL signaling specifically communicated in ME-RB1-L cells, and CCL5 sends communications to atypical chemokine receptor 1 (ACKR1) and C-C chemokine receptor type 1 (CCR1) (Supplemental Figure 7A). CCL5 and its receptors are critical for the recruitment of effector immune cells to the site of inflammation. CXCL10 and CXCL5 overexpression is associated with the presence of CD8+ T cells in lung cancer (21). Taken together, the results indicate the crucial role of CCL5 and CXCL10 in mediating T cell recruitment in \( \text{RB1} \)-deficient cancer cells.

We revealed that type I IFNs were significantly upregulated in \( \text{RB1} \)-mutant LUAD samples (Figure 6, B and C, and Supplemental Figure 2). The type II IFN (IFN-II), IFN-\( \gamma \), plays an important role in both innate and adaptive immunity, which was upregulated in \( \text{RB1} \)-mutant samples (32). IFN-II and MHC-II signaling pathways specifically communicate in ME-RB1-L cells (Supplemental Figure 7, A and B). IFN-II is primarily secreted by adaptive immune cells, and upregulates MHC-II expression on antigen-presenting cells. Previous studies revealed that PARPis upregulate MHC-II on DCs, enhancing antigen presentation and T cell interactions (33). In addition, numerous regulators of SPP1 and ICAM appeared to have specific interactions in ME-RB1-L cells (Supplemental Figure 7, A and B). SPP1 is a cytokine that upregulates the expression of IFN-\( \gamma \), and is related to cytokine activity and extracellular matrix binding. ICAM-1 is a cell adhesion molecule that plays a key role in cell-cell interactions leading to the immune response. Our analysis demonstrated the upregulation of cytokine activity and cell adhesion–activated immune response in \( \text{RB1} \)-deficient LUAD samples, and an increase in adaptive and innate immunities. Moreover, the intercellular communications were inferred by CellChat based on single-cell RNA-seq (scRNA-seq) data; the accuracy of the interactions among cells need further experimental validation.

Infiltration of immune cells was evaluated in LUAD bulk tissue. M0/M1 macrophages, T cells, and activated NK cells were upregulated in \( \text{RB1} \)-deficient cells. Furthermore, the communications between ME-RB1-L cells and immune cells was higher compared with ME-RB1-H cells at single-cell resolution. Taken together, these results prompted us to conjecture that the immune cells (e.g., macrophages, T cells, NK cells, DCs, and B cells) are recruited to the surface of \( \text{RB1} \)-mutant lung cancer cells (Supplemental Figure 7C). A deeper understanding of the change in the TME after PARPi treatment in \( \text{RB1} \)-mutant LUAD patients warrants further investigation.

In summary, our study demonstrated the remarkable efficacy of PARPis in treating \( \text{RB1} \)-mutant LUAD cells, which provides a rationale for the therapy in clinical settings and a new treatment strategy for LUAD.

**Methods**

Multiomics data of lung cancer cell lines and bulk LUAD samples. We downloaded gene mutation and expression data of 182 lung cancer cell lines from the Catalogue of Somatic Mutations in Cancer (COSMIC v87, https://cancer.sanger.ac.uk/cosmic; accessed December, 2018), and obtained somatic mutation, gene expression, and protein expression data for 244 lung cancer cell lines from the DepMap portal v19Q1 (https://depmap.org/portal/; accessed March, 2019). RNA interference (RNAi) screen data were downloaded from the DepMap Portal, Achilles v2.20.2.

Somatic mutation, gene expression, and protein expression data of LUAD and LUSC patients from TCGA were downloaded from the Genomic Data Commons (https://portal.gdc.cancer.gov/; accessed March, 2019). Gene expression and \( \text{RB1} \)-mutant information of 110 LUAD patients was obtained from
the study of Gillette et al. (19). Silent mutations and mutations in 5′-flanks, introns, intergenic regions, 5′-UTR, and 3′-UTR were excluded (13).

Pharmacological data of PARPis. Pharmacological screening data of PARPis in lung cancer cell lines were obtained from the GDSC v17Jul19 (https://www.cancerrxgene.org/; accessed September, 2019) and the CTRP v2.1 (https://portals.broadinstitute.org/ctrp/v2.1/; accessed January, 2018). We obtained ln(IC50) (natural log of the fitted half-maximal inhibitory concentration) values and AUC (area under the drug inhibition curve) values of PARPis. Cell line information for GDSC was referenced from COSMIC, and cell line information for CTRP was available from the DepMap portal.

Drug response and gene expression data of TNBC patients from a randomized phase III trial, BrighTNess, were downloaded from the GEO (GSE164458). We focused on 237 TNBC patients who were treated with veliparib plus DNA-damaging agents (carboplatin and paclitaxel), 127 TNBC patients were labeled as pCR, and 110 TNBC patients achieved RD. Expression values were log2 normalized.

scRNA-seq data processing and analysis. The scRNA-seq data of 9 LUAD samples were obtained from GSE171145. We used R package “harmony” (https://cran.r-project.org/web/packages/harmony/index.html) to integrate cells across individuals. scRNA-seq data were clustered and annotated according to the singleR package (https://bioconductor.org/packages/release/bioc/html/SingleR.html) and canonical marker genes. scRNA-seq data of 11 early-stage LUAD patients (tLung) and 4 advanced stage LUAD patients (tL/B) in the study of Kim et al. were obtained from GSE131907 (34). scRNA-seq data were annotated according to canonical marker genes in Kim et al. (Supplemental Table 2).

We performed the CopyKAT algorithm (https://github.com/navinlabcode/copykat) to estimate genomic copy number profiles. Epithelial cells labeled as “aneuploid” were considered as malignant cancer cells. RB1-expressing malignant epithelial cells were grouped by median of RB1 expression into ME-RB1-H and ME-RB1-L. The cell-cell communications were analyzed using the R package “CellChat.” See Supplemental Methods for details.

Evaluation of chromosomal and genomic instability. Integrated previously published measures of genomic scar and chromosomal instability of LUAD samples in TCGA were obtained from Marquard et al. (35). Genomic scar signatures include the NtAI, LST, and LOH. The sum of the 3 scores is titled the HRD score. Chromosomal instability measures contain the wGHI and the FLOH.

The “aneuploidy” score reports the total number of arm-level amplifications and deletions in cancer samples. The “fraction altered” means somatic copy number alterations divided by copy number variation. The 2 measures of LUAD samples in TCGA were obtained from Knijnenburg et al. (36). In addition, the measures of “nonsilent mutation rate” and “silent mutation rate” for LUAD samples in TCGA were acquired from Thorsson et al. (37).

Components in cGAS/STING pathway. A total of 31 genes in the cGAS/STING pathway were collected from the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/kegg/) database, and studies by Chen et al. (38), Motwani et al. (39), Chabanon et al. (40), and Loo et al. (41) (Supplemental Table 1).

Evaluation of immune cell infiltration in bulk LUAD samples. Immune cell fractions of TCGA LUAD samples were estimated by the Cell type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) algorithm (https://cibersortx.stanford.edu/cshome.php). The fraction of “Th2 cells” in TCGA LUAD samples was obtained from Thorsson et al. (37). Estimation of “IFN-γ response” in TCGA LUAD samples was acquired from the study of Wolf et al. (42).

Cell type signature gene sets curated from cluster markers identified in scRNA-seq studies of human tissues were downloaded from the Molecular Signatures Database (MSigDB: https://www.gsea-msigdb.org/gsea/msigdb; accessed December, 2021). The R package “limma” was used to identify differentially expressed genes between RB1-mutant and RB1-WT LUAD samples in TCGA, and Gene Set Enrichment Analysis (GSEA) was used to explore the cell type that significantly differentially infiltrated RB1-mutant samples by R package “clusterProfiler.”


Cell culture. The human LUAD cancer cell lines A549, H1650, H1975, H2228, and H1781 in this study were purchased from the Chinese Academy of Sciences. H1650, H1975, H1781, and H2228 were cultured in RPMI 1640 medium (VivaCell) with 10% fetal bovine serum (FBS, VivaCell), 100 U/mL penicillin, and 100 μg/mL streptomycin. A549 were maintained with an F-12K Nutrient Mixture (Gibco). All cells were maintained at 37°C with 5% CO2 in a humidified incubator. All cell lines were short tandem repeat (STR) typed to confirm identity before the study.
CRISPR/Cas9-mediated RB1-KO cell line. For RB1-KO cells, single guide RNAs (sgRNAs) were designed using the online CRISPR design tool (Red Cotton, https://en.rc-crispr.com/). See Supplemental Methods for details.

Chemical inhibitors. The PARPi olaparib (AZD2281) was purchased from Selleck Chemicals. Rubcarparib (AG014699) and niraparib (MK4827) were purchased from MedChemExpress. Inhibitor stock solutions were prepared in DMSO and stored in aliquots at –80°C. According to DrugBank (https://go.drugbank.com/), the targets of olaparib and rucarparib are PARP1, PARP2, and PARP3, and the targets of niraparib are PARP1 and PARP2.

Cell viability measurement. CCK8 assay was used to assess the cell growth. Approximately 1 × 10^5 cells/well were plated in a 96-well plate. After treatment, the cells were incubated with CCK8 (TransGen Bio-tech) solution at 37°C for 2 hours. The absorbance was measured at 450 nm using an Infinite 200PRO microplate spectrophotometer (Tecan).

Colony formation. The cells were seeded in a 6-well culture plate. Following drug incubation for 72 hours, the medium was aspirated, and cells were incubated with no-drug media for 14 days. Next, the cells were fixed with cold methanol for 20 minutes, stained with 0.1% crystal violet for 15 minutes, washed with PBS, and imaged. Colonies were counted manually using a stereomicroscope and quantitated using Image-Pro Plus software (Media Cybernetics).

Xenograft model. The huPBMC-NOG-dKO models were used to generate human mature T cells. Six- to 8-week old huPBMC-NOG-dKO female mice were purchased from Beijing Vital River Laboratory Animal Technology. Cells (5 × 10^6) containing A549-RB1-KO or A549-WT were suspended in 100 μL PBS, and A549-WT was transplanted subcutaneously into the mouse’s left armpit and A549-RB1-KO was subcutaneously transplanted into the mouse’s right armpit. The dosing group was injected with 10 mg/kg rucarparib intraperitoneally every 2 days (1.08 mg rucarparib, 60 μL DMSO, 240 μL PEG 300, 30 μL Tween 80, and 270 μL normal saline; 100 μL/time each). Tumor volume was measured every 4 days with vernier calipers. Animals were randomly assigned to experimental groups. During the experiment, the researchers did not know the population distribution when evaluating the results. The sample size for each condition was 6. All mice were sacrificed after 4 weeks.

IHC analysis. Samples collected from huPBMC-NOG-dKO female mice tumor tissue sections were dewaxed in xylene and rehydrated with gradient ethanol. After washing with double-distilled water, tissue sections were incubated with 3% hydrogen peroxide (Solarbio) for 10 minutes and antigen retrieval was performed with sodium citrate antigen recovery solution (Solarbio). Then, sections were blocked with 50% goat serum for 1 hour, incubated overnight with anti-caspase-3 (Wanleibio; catalog WL01927; 1:200) and anti-Ki67 (Affinity; catalog AF0198; 1:100) primary antibodies at 4°C, and then secondary antibody conjugated with horseradish peroxidase (HRP) (Zhongshan Science and Technology) staining solution, and then reverse stained with hematoxylin (LABEST) for 10 seconds. Photographs were obtained using a Zeiss microscope.

H&E staining. Tissues were fixed in 4% paraformaldehyde for 24 hours, embedded in paraffin, and cut into 5-μm-thick sections. The sections were then stained with H&E (Solarbio) according to the manufacturer’s instructions and observed under a Zeiss microscope.

TUNEL staining. TUNEL assay was performed to detect apoptotic cells using an in situ cell death detection kit (fluorescein, Roche Applied Science) according to the manufacturer’s instructions. See Supplemental Methods for details.

Western blotting. See Supplemental Methods for details.

qRT-PCR. See Supplemental Methods for details.

Immunofluorescent staining. See Supplemental Methods for details.

PicoGreen staining. PicoGreen staining was performed using Quant-iT Pico-Green dsDNA reagent kits from Thermo Fisher Scientific. See Supplemental Methods for details.

ELISA. Detection of cytokines CCL5 and CXCL10 in cell supernatants was measured by ELISA kits (Proteintech, KE00093 and KE00128, respectively). See Supplemental Methods for details.

Statistics. One-sided Wilcoxon’s rank-sum test was used to assess the discrepancy between RB1-mutant and -WT samples, or to test the difference between RB1-L and RB1-H samples, which were separated by the median expression value of RB1. The correlations between PARP1/2/3 expression and RB1 expression were evaluated by Pearson’s correlation analysis. Data are presented as mean ± SD, and the
unpaired, 2-tailed Student’s *t* test was used to compare the mean values of independent samples unless otherwise noted. A *P* value of less than 0.05 was considered statistically significant. Statistical analyses were performed using R software v4.1.2 (https://www.r-project.org) and GraphPad Prism software v8.0 (https://www.graphpad.com/scientific-software/prism/).

**Study approval.** All animal procedures were approved by the Animal Experiment Ethics Committee of the Harbin Medical University (no. IRB3022823), Harbin, China.

**Data availability.** The public data used in this study can be downloaded from public databases or retrieved from associated files of papers. All data generated or analyzed during this study are included in the article and its Supporting Data Values file. The underlying data generated as part of this study are available from the corresponding author upon request.

**Author contributions**

YG and YH conceptualized the study. QD and TY developed the methodology. QD, TY, BC, ML, XS, HC, and ZJ carried out the investigation. QD, TY, BC, and ML analyzed the data. QD, TY, XS, and HC generated figures. QD, TY, KL, and HX validated the data. QD and TY wrote the original draft of the manuscript. YG, YH, HL, KL, HX, YW, and SZ reviewed and edited the manuscript. The order of co–first authors was assigned according to discussions and votes by all the authors.

**Acknowledgments**

The authors acknowledge the efforts of all of the researchers who have contributed the data to the public databases of TCGA, DepMap, CTRP, GDSC, COSMIC, and MSigDB. The interpretation and reporting of these data are the sole responsibility of the authors. This work was supported by the National Natural Science Foundation of China (no. 32270710 to YG; no. 82271208 to YH), the Outstanding Youth Foundation of Heilongjiang Province of China (no. YQ2021H005 to YG), and the HMU Marshal Initiative Funding (no. HMUMIF-21023 to YG and HL).

Address correspondence to: Yunyan Gu, Department of Systems Biology, College of Bioinformatics Science and Technology, Harbin Medical University, No. 157 Baojian Road, Nangang District, Harbin, Heilongjiang, 150081, China. Phone: 86.0451.86699584; Email: guyunyan@ems.hrbmu.edu.cn. Or to: Yang Hui, Department of Biochemistry and Molecular Biology, Harbin Medical University, No. 157 Baojian Road, Nangang District, Harbin, Heilongjiang, 150100, China. Phone: 86.0451.86671684; Email: huiyang79@126.com.