Oxaliplatin Compromised CDK1 Activity Sensitizes BRCA-Proficient Cancers to PARP Inhibition in Oxaliplatin Resistance Gastric Cancer

Huafu Li
SAHSYSU: The Seventh Affiliated Hospital Sun Yat-sen University

Chunming Wang
The Seventh Affiliated Hospital Sun Yat-sen University

Linxiang Lan
The Francis Crick Institute

Wenhui Wu
The Seventh Affiliated Hospital Sun Yat-sen University

Ian Evans
Francis Crick Institute

E Josue Ruiz
Francis Crick Institute

Leping Yan
The Seventh Affiliated Hospital Sun Yat-sen University

Haiyong Zhang
The Seventh Affiliated Hospital Sun Yat-sen University

Zhijun Zhou
Oklahoma State University Center for Health Sciences

Zhenran Hu
The Seventh Affiliated Hospital Sun Yat-sen University

Wei Chen
The Seventh Affiliated Hospital Sun Yat-sen University

Axel Behrens
Francis Crick Institute

Yulong He
The Seventh Affiliated Hospital Sun Yat-sen University

Changhua Zhang (zhchanghsysuedu@163.com)
Sun Yat-Sen University

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Abstract

**Background:** Oxaliplatin resistance is one of the most important problems in the treatment of cancer. The successful culture of tumor organoid in gastric cancer can help us to study oxaliplatin resistance and its mechanism. Thus, it is convenient for us to successfully solve oxaliplatin resistance and improve the prognosis of patients.

**Methods:** Two oxaliplatin resistant patients and two oxaliplatin sensitive patients were enrolled through our Gastric Cancer Center of Sun Yat-sen University. Core genes of oxaliplatin resistant and non-resistant patients were analyzed by sequencing. The overexpression and knockdown of core genes were carried out by organoid in vivo, combined with oxaliplatin-resistant cell lines AGS, MKN74 and SNU719 for cell viability, WB and immunofluorescence, etc., to verify the role of core genes in oxaliplatin resistance. Again, in vivo experiments were verified by subcutaneous tumor formation in vitro.

**Results:** Through sequencing, we found that PARP1 is an important core gene leading to oxaliplatin resistance. In vivo organoids, oxaliplatin resistant cell lines and subcutaneous tumor formation in vivo. We found that PARP1 was an important cause of oxaliplatin resistance. Oxaliplatin can inhibit CDK1 activity and make cancer with normal BRCA1 function sensitive to PARP inhibition. Through the combination of oxaliplatin and PARP1 inhibitor olaparib, we can effectively kill tumor cells. Through the patients' follow-up data, we found that the expression level of PARP1 was significantly correlated with oxaliplatin resistance.

**Conclusion:** Our results indicate that PARP1 is an important core gene leading to oxaliplatin resistance. Combined oxaliplatin and PARP1 inhibitor olaparib can effectively kill tumor cells. Oxaliplatin can inhibit CDK1 activity and make cancers with normal BRCA1 function more sensitive to PARP inhibitors.

**Background**

The standard treatment for gastric cancer is surgical resection, but in most cases, this is not possible because most of them are diagnosed at an advanced stage, thus losing the chance of surgery (1). Alternative therapies such as radiotherapy and chemotherapy are very ineffective. Available chemotherapy, based on cisplatin and 5-fluorouracil (5-FU) or their combined derivatives, such as oxaliplatin and capecitabine, fail in 95% of non-surgical gastric tumors, respectively (2). Therefore, there is an urgent need to advance our understanding of the mechanisms of chemotherapy resistance. This is necessary to more accurately select the best treatment for each patient and to develop new strategies to overcome chemotherapy resistance. Early results suggest that patient-derived cell lines or xenografts may facilitate the discovery of new therapies because they are more closely related to primary characteristics that do not change, allowing them to be used to guide chemotherapy selection (3). Tumor organoid are the research means and methods for us to be more authentic and close to primary tumors. Yan et al. found that gastric cancer organoid are a better and more effective method for clinical drug screening for tumors (4). By analyzing the clinical samples of patients in the Gastric Cancer Center of Sun Yat-sen
University and conducting drug sensitivity experiments with gastric cancer organoid, we included the clinical specimens of four gastric cancer patients for organ-like culture. In vivo and in vitro studies showed that 2 patients had significantly higher tolerance to oxaliplatin than the other 2 patients. Sequencing showed that PARP1 was an important factor affecting oxaliplatin resistance, and oxaliplatin combined with PARP1 inhibitor olaparib could effectively kill oxaliplatin resistant cells. This improves the prognosis.

**Methods**

**Cell culture**

GC cell lines AGS (ATCC® CRL-1739™) and MKN74 (ABC-TC0689) were ordered from The Francis Crick Institute Cell Services, SNU719 were provided by Nanjing Kegen Biotechnology Co., Ltd. They were then expanded, and fluorescence-based mycoplasma detection were performed, and subsequently confirmed with agar culture. A large number of primary cells were frozen. The cells were passaged twice a week for 20 passages. All GC cell lines are grown in complete medium containing 10% FCS and RPMI.

In order to cultivate a stable Oxaliplatin-resistant GC cell line (No. S1224, Selleckchem) for long term, AGS, SNU719 and MKN74 cells were exposed to RPMI with an initial Oxaliplatin concentration of 1 \( \mu \text{mol/L} \) and 10% fetal bovine serum. The surviving cell population was grown to a concentration of 80% and passaged twice within 9 days to ensure survival. The above process was repeated for the surviving cells with higher Oxaliplatin concentration of 10 \( \mu \text{mol/L} \) (15 days), 20 \( \mu \text{mol/L} \) (30 days), 50 \( \mu \text{mol/L} \) (60 days), 100 \( \mu \text{mol/L} \) (90 days), and 200 \( \mu \text{mol/L} \) (120 days), finally reaching the relevant Oxaliplatin concentration of 200\( \mu \text{mol/L} \). After a successful culture, resistance analysis was carried out by IC50 and colony forming test (see Figure S1A-C).

**Human tissue and organoids**

Human GC tissues were taken from patients who underwent gastric cancer surgery in the First Affiliated Hospital of Sun Yat-sen University. They agreed and signed a donation and research consent form. This was approved by the clinical research and animal experiment ethics committee of the First Affiliated Hospital of Sun Yat-sen University (Ethical Review [2017] No. 208). This research complied with all the ethics of human participation in research. the organoids of our gastric cancer diagnosis and treatment center was screened through the Scientific Research Center of the Seventh Affiliated Hospital of Sun Yatsen University and organoid strains of 4 patients were finally selected to be included in the experiment. The patient strains of these organoids were named GC1, GC2, GCR1, GCR2.

After surgery, the GC sample was placed in 50 U/ml penicillin-streptomycin (Thermo Fisher) frozen G solution. The tissue was minced on ice and incubated in DMEM containing 1 mg/ml collagenase V (Sigma-Aldrich) for 1 h at 37°C. Iced PBS was added to stop the digestion, the mixture was then centrifuged at 4°C (300 G, 5 min). The samples were further digested with TrypLE (Thermo Fisher) at 37°C
for 5 minutes, and then stopped with a large quantity of PBS. The suspension was filtered through 40 nylon meshes, centrifuged, and the cells were fixed in the medium. It was passaged with TrypLE every 2 weeks. The medium for establishing and culturing human GC organoids was as described in the literature (5).

**lentivirus production and infection of organoids**

Control and shRNA_PARP1-expressing pLKO vectors were purchased from Sigma. PARP1 overexpression vectors were designed to generate the lentivirus by Shanghai Genechem Co., Ltd. All lentiviral particles were produced in HEK293T cells by standard procedures, concentrated by ultracentrifugation at 100,000g for 2h and resuspended in sterile PBS. Organoids were extracted from Matrigel using TrypLE Express (Thermo Fisher), resuspended in OptiMEM with 10 µg/ml Polybrene, and then mixed with the virus solution in an incubator for 6h. Cells were plated back into Matrigel and split 3 to 7 days later when antibiotic selection was started.

**Quantitative real-time PCR**

When the number of cells were few, MagMAX-96 Total RNA Isolation Kit (Ambion) or RNeasy Mini Kit (Qiagen) was used to extract RNA according to the manufacturer's instructions. Random hexamer primers (Invitrogen) was used to SuperScript III First-Strand cDNA synthesis kit or use iScript cDNA synthesis kit (BioRad) according to the manufacturer's instructions to generate cDNA. 5 times dilution was performed on the cDNA with distilled water, diluted 2 mol/L to use each RT-qPCR reaction, and the measurement was performed on the Express SYBR GreenER (Thermo Fisher) ABI7500 (Applied Biosystems). The primers were designed using the Universal Probabilistic Analysis and Design Center (Roche) to ensure that they span the exon-exon junction. The family gene transcription level (actin) was used for normalization. The RT-qPCR primers were listed in Supplementary Table 2.

**Western blotting**

The total protein of the extracted cells was lysed in frozen cell lysis buffer (NEB) containing 1 mM PMSF and 1:100 protease inhibitor cocktail (Sigma). The lysate was pre-cleared with 15×l protein A Sepharose 4B beads (Sigma) at 4°C for 30 minutes. NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (thermo Fisher, 78833) were used to extract nucleoprotein from cells. The pre-determining BCA (Pierce, Rockford, IL) and Western Blot procedures were as described above (6). Antibodies include Anti-beta Actin antibody (1:50000, Abcam, ab49900), Anti-gamma H2A.X (phospho S139) antibody (1:1000, Abcam, ab2893), PARP-1 antibody (F-2) (1: 500, Santa Cruz, sc-8007), Cdc2 p34 antibody (17) (1:500, Santa Cruz, sc-54), BRCA1 antibody (D-9) (1:500, Santa Cruz, sc-6954), Phospho-cdc2 (Tyr15) Antibody (1:1000, cellsignal, #9111), Phospho-BRCA1 (Ser1497) Polyclonal Antibody (1:1000, thermofisher, # PA5-64621), Rad51 Antibody (G-5) (1:500, Santa Cruz, sc-133089). XRCC1 (1:1000, Abcam, ab44830).
**Flow cytometry**

Annexin V-PI apoptosis assay were performed using Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich) according to the manufacturer's protocol. FlowJo 10 software was used to analyze the data.

**Colony Formation Assay and Cell Viability**

In a 6-well plate with a total of 500 cells per well, the following solution was added: control (DMSO), Olaparib (No.S1060, Selleckchem) (25μM/ml), Oxaliplatin (10μM/ml), cis Platinum (5μM/ml) and CDK1 inhibitor (AG-024322, BIOQUOTR, 837364-57-5) (0.12μM/ml). After two weeks, the formation of colonies or colosphere was evidently visible, the cell colonies were then fixed, stained with 0.1% crystal violet in 20% methanol solution, and counted. This process was repeated three times per solution type.

In a 96-well transparent bottom blackboard, 3000 cells were planted in each well (organoids were planted in Matrigel). The drug was then added to each well according to a 10-fold concentration gradient. Adenosine triphosphate (ATP) levels (Promega, Madison, WI) were determined by CellTiter-Glo using a luminometer (PerkinElmer Life and Analytical Sciences, Boston, MA) 48 hours later.

**Immunofluorescent staining**

Immunofluorescence staining of organoids and cell lines: the body was placed in a glass bottom tissue culture plate (ibidi, lot:191218/2), fixed with 5% NBF for 10 minutes, and blocked with PBS containing 10% FCS, 1% BSA (Sigma) and 0.2% Triton-X. The primary antibody was incubated in blocking buffer at 4°C for 16h. The fluorescent secondary antibody was incubated with 3 DAPI in blocking buffer at 20°C for 1-6h. Fluorescence staining was imaged on a Zeiss LSM 780 confocal microscope. Tissues were prepared as above and the same antigen retrieval procedure was applied. Secondary antibodies were fluorophoreconjugated and incubated with 3µM DAPI in the dark. Before mounting, slides were incubated in 0.1% (w/v) Sudan black B (Sigma) in 70% ethanol to reduce background signal.

**The PDOX mouse model**

*In vivo* experiments had been performed in accordance with the Institutional Animal Care and Use Committee (IACUC) regulations. It was approved by the clinical research and animal experiment ethics committee of the First Affiliated Hospital of Sun Yat-sen University (Ethical Review [2017] No. 208). The experiment was performed by the staff of the Animal Center of the First Affiliated Hospital of Sun Yat-sen University. In order to study the tumorigenesis ability of oxaliplatin resistance, we inoculated 100,000 cells selected into BALB/C NUDE mice with Matrigel (BD, 354230). After 25 days, 6 mice with organoids transplantation tumors received a treatment of Oxaliplatin (Selleckchem, s1224) at a dose of 5 mg/kg twice a week for 4 weeks.
For the other 6 mice, PBS were injected intraperitoneally. The cancer-bearing BALB/C NUDE mice were sacrificed 4 weeks later, and tumors were harvested for measuring and weighing. In order to study the drug resistance of PARP1 expression, we inoculated 100,000 cells of plko and PARP1-sh1 (GCR1 and GCR2) into BALB/C NUDE mice with Matrigel (BD, 354230). After 25 days, 6 mice with organoids transplantation tumors received a treatment of Oxaliplatin (Selleckchem, s1224) at a dose of 5 mg/kg twice a week for 4 weeks. The cancer-bearing BALB/C NUDE mice were sacrificed 4 weeks later, and tumors were harvested for measuring and weighing. We inoculated 200,000 cells of control and PARP1 overexpression (GC1 and GCR2) into BALB/C NUDE mice with Matrigel (BD, 354230). After 25 days, 6 mice with organoids transplantation tumors received a treatment of Oxaliplatin (Selleckchem, s1224) at a dose of 5 mg/kg twice a week for 4 weeks. The cancer-bearing BALB/C NUDE mice were sacrificed 4 weeks later, and tumors were harvested for measuring and weighing.

The organoids of GCR1 and GCR2 were digested into single cells by TrypLE (glibco, 12604-013) and then counted, and 100,000 cells were placed on Matrigel (BD, 354230) and inoculated subcutaneously into BALB/C NUDE mice (6 per group). After 25 days, the organoids transplanted BALB/C NUDE mice received intraperitoneal injection of either Oxaliplatin (Selleckchem, s1224) + Olaparib (Selleckchem, AZD2281, s1060), Oxaliplatin, Olaparib, or PBS. Oxaliplatin dose was 5 mg/kg, Olaparib dose was 50 mg/kg, combined group dose was 5 mg/kg of Oxaliplatin and 25 mg/kg of Olaparib twice per week, each treatment lasting for 4 weeks. The tumor size and body mass were measured every three days. The mice were sacrificed one month later, and tumor tissues were prepared for histological examination. Tumor volume (mm$^3$) = 0.5 x width$^2$ x length. All animal experiments were carried out in accordance with health guidelines, and the protocol was set by the Sun Yat-sen University Animal Protection and Use Committee. When the mice reached the end point, the tumor was photographed and the tumor was weighed.

**Immunohistochemical staining.**

The tissues were collected, fixed with 10% neutral buffered formalin (NBF, Sigma) for 16 hours, dehydrated with 70% ethanol, and embedded in 4×m paraffin sections. H&E staining was performed by the experimental histopathology laboratory according to standard procedures. After heat-mediated antigen extraction in 10 mM sodium citrate buffer (pH 6.2), the endogenous peroxidase was blocked with 1.6% hydrogen peroxide, and PARP-1 (Proteintech, 13371-1-AP), Ki67 (Abcam, ab15580), Caspase 3 (Proteintech, 19677-1-AP), BRCA1 (Affinity Biosciences, AF6289), Phospho-BRCA1-Ser1497 (Affinity Biosciences, AF8204), CDK1 (Abcam, ab133327), Phospho-CDK1-Y15 (Abclonal, AP0016) were stained with DAB according to manufacturer's manual. Positive cells were counted in 5 random field of view per slide.

**RNA isolation and microarray**

Total RNA was extracted from tissue samples, and Nanodrop 2000 was used to detect the concentration and purity of the RNA. Agarose gel electrophoresis was used to detect RNA integrity, and Agilent 2100
was used to determine the RIN value. A single library construction required that the total amount of RNA was no less than 5μg, the concentration $\geq 200\text{ng/μL}$, and the OD260/280 between 1.8 and 2.2. The mRNA capture and library preparation were completed by the advanced sequencing equipment of Shanghai Origin-gene Biomedical Technology Co., Ltd. using KAPA mRNA HyperPrep kit (Roche). The biological triplicate libraries were sequenced on the Illumina Truseq TM RNA sample prep Kit platform of the facility, and each sample produced an average of 25 million single-ended reads of 75 bp. Align the post quality control, high-quality sequence with the designated reference genome. The PDOX sample was first compared with mice reference genome. After removing mice-related data, it was then compared with human reference genome. The human reference genome was obtained from Ensembl database, genome version GRCh38, gene annotation information was Ensemble 92. Before alignment, cutadapt (version 1.9.1) is used for quality control and adaptor trimming of the original reading. Use the annotation release 86 to sequence the Reads of the human genome GRCh38 using RSEM 1.3.0 and STAR 2.5.2, and count the subsequent gene levels. In version 3.6.1 of R package, the DESeq2 package (version 1.24.0) was used for normalization and differential expression analysis of raw count data. Regularized logarithmic transformation was performed on the rlog function.

**Clinical GC patient samples**

From May 2010 to February 2020, the progressive GC tissue samples before the start of Oxaliplatin treatment were collected from the First Affiliated Hospital of Sun Yat-sen University (n = 100) through surgical specimens or biopsy, and the patients’ research consent form were signed and documented. This study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (Ethical Review [2018] No. 087). The Oxaliplatin group received at least 6 cycles of Oxaliplatin treatment. The detailed clinical characteristics of the patients were shown in Table 1. The tumor response to chemotherapy was evaluated by the three-dimensional volume reduction rate or tumor response rate (radiological evaluation), and evaluated in accordance with the response evaluation criteria in the solid tumor (RECIST) guidelines (7). In the validation phase, patients with worsening symptoms, new lesions, or radiologically assessed tumor regeneration $\geq 25\%$ were assigned to the progressive disease (PD) group (n = 45) and the remaining non-PD group (n = 55). PFS is defined as the duration from tumor resection to PD. Follow-up was performed every 3 months (for the initial 0-2 years), 6 months (subsequent 2-4 years), and once a year until death or February 2020. The follow-up study included abdominal computed tomography and postoperative physical examination.
|                      | Non-Progress(55) | Progress(45) | P     |
|----------------------|------------------|--------------|-------|
| **Gender**           |                  |              |       |
| male                 | 29               | 24           | 0.952 |
| female               | 26               | 21           |       |
| **Age**              |                  |              |       |
| ≤65                  | 15               | 20           | 0.060 |
| >65                  | 40               | 24           |       |
| **M staging**        |                  |              |       |
| M0                   | 55               | 45           | -     |
| M1                   | 0                | 0            |       |
| **T staging**        |                  |              |       |
| T1                   | 1                | 3            | 0.370 |
| T2                   | 19               | 2            |       |
| T3                   | 31               | 31           |       |
| T4                   | 4                | 9            |       |
| **N staging**        |                  |              |       |
| N0                   | 27               | 10           | 0.343 |
| N1                   | 20               | 15           |       |
| N2                   | 5                | 9            |       |
| N3                   | 3                | 11           |       |
| **Differentiation**  |                  |              |       |
| High                 | 31               | 31           | 0.130 |
| Moderately           | 18               | 11           |       |
| Poorly               | 6                | 3            |       |
| Undifferentiation    | 0                | 0            |       |
| **PARP1**            |                  |              |       |
| High                 | 43               | 26           | 0.032 |
Patient information in public databases

The transcriptome data of patients with gastric adenocarcinoma confirmed by pathology was downloaded from the TCGA website (https://portal.gdc.cancer.gov/) in June 2020, including data from 416 patients with gastric adenocarcinoma and general information of the corresponding cases. Data that did not list survival time were excluded, leaving 416 cases of gastric cancer and 33 cases of adjacent tissues. Inclusion criteria: (a) Diagnosis age ≥ 8 years old; (b) Tumor site: stomach; (3) Cases with clear pathology. The exclusion criteria are as followed: (a) multiple tumor; (b) carcinoma in situ; (c) incomplete follow-up data; (d) deaths within 30 days. Proteomics data of patients with gastric adenocarcinoma were downloaded from the CPTAC website (https://cptac-data-portal.georgetown.edu/study-summary/S025) in June 2020, including data and corresponding general information of 130 gastric adenocarcinoma patients.

Gene Set Enrichment Analysis (GSEA)

GSEA was performed using software (GSEA V4.0.3) developed by the Broad Institute of MIT and Harvard University (https://www.gsea msigdb.org/gsea/index.jsp). For the cancer and para-cancer RNA-seq datasets of Oxaliplatin resistance patients, normalized RNA read counts were used for analysis, and the following settings were applied: permutation number = 1000, permutation type = gene set, enrichment statistics = weighting, a measure of gene ranking = signal noise. For the TCGA gastric cancer dataset, the samples were grouped according to their expression above or below the median value. The normalized RSEM read count was used for analysis, and the following settings were applied: number of permutations = 1000, permutation type = phenotype, enrichment statistics = weighting, measurement of gene ranking = signal 2 noise. Recognized marker gene set 40, KEGG pathway or gene ontology (GO) terms, and false discovery rate (FDR q) <0.05 were considered significant enrichment.

Screening of differentially expressed genes (DEGs)

The expectation-maximization method RNA-Seq was used to normalize the 3-level transcriptome data of the data set, and the logarithmic transformation of all gene expression values was performed. Approximate data were normally distributed after normalization by quantiles (8). In this study, the R package limma program v3.28.14 was used to analyze the differential genes of gene expression data, and its mRNA satisfied P<0.01, false discovery rate (FDR)<0.01 and |log2 fold change (FC)|>1.5, where P
<0.05 indicated that the hypothesis test was statistically significant. FDR is a control indicator for the error rate of the hypothesis test. As an evaluation index of the selected differential genes, the number of false rejections was proportional to the number of rejected invalid hypotheses. FC was usually used to describe the degree of change from the initial value to the final value. In this study, the ratio of tumor tissue gene expression value to normal tissue gene expression value was used, also known as the fold change. The heatmap and volcano map of the differential genes were constructed in R language for visual comparison.

WGCNA Co-expression Network Construction

Gene expression data (mRNA-seq data) was downloaded from the TCGA database. A total of 24,991 genes were identified in each sample. Analysis of variance was performed and then sorted from largest to smallest. The SD value of each gene was calculated and sorted from largest to smallest, and then the top 5000 genes were selected for WGCNA. WGCNA package in R software was used to construct a gene co-expression network from the expression data map of these 5000 genes (9). Using the adjacency function in WGCNA, an adjacency matrix was constructed by calculating the Pearson correlation between all pairs of genes in the selected sample. In this study, \( \beta = 7 \) (scale-free R2 = 0.9) was used as the soft threshold parameter to ensure a scale-free network. In order to further identify the functional modules in the co-expression network of these 5000 genes, the adjacency matrix was used to calculate the Topological Overlap Measure (TOM), which represents the overlap in the shared neighborhood. We identify related modules by calculating the correlation between MEs and PARP1 expression levels. Then the log10 transformation of the p value (GS=\(\log_2P\)) in the linear regression of gene expression and clinical PARP1 expression level information was defined as gene significance (GS). In addition, module significance (MS) is defined as the average GS of all genes in a module. In general, among all the selected modules, the module with the highest absolute value of MS was considered to be the module related to the level of PARP1 expression.

PPI network construction of key module gene

The Hub gene, which is highly interconnected with the nodes in the module, is considered to have important functions. We selected the top 30 Hub genes in the module network as candidate genes for further analysis and verification. The STRING data set is an online biological resource that can decode the interaction between proteins and proteins to obtain the actual precise functions of proteins (10). The candidate gene was submitted to the protein interaction of STRING, and the binding confidence interval of the cutoff value was set to 0.4. In the plugin, Molecular Complex Detection (MCODE), the significant models with strong protein-protein connection were calculated and selected with the default parameters (degree cut \(\geq 2\), node score cut \(\geq 2\), K-core \(\geq 2\), maximum depth = 100). P<0.05 was considered statistically significant.
**Statistical analysis**

The images and graphs shown represent several experiments repeated on different individuals at different times. Each experiment was repeated independently. All statistics were performed using SPSS and R software. The statistical test was explained in the figure legend. All results were statistically different based on the mean ± SD, P < 0.05.

**Results**

**PARP1 is an Important Core Gene Leading to Oxaliplatin Resistance**

In order to find the cause of chemotherapy resistance, we used four PDOs (GC1, GC2, GCR1, GCR2), of which GCR1 and GCR2 were from patients whose GC recurred after postoperative chemotherapy, while GC1 and GC2 were patients without recurrence after postoperative chemotherapy. In a viability assay, GCR1 (IC50 = 19.95μm/L) and GCR2 (IC50 = 63.09μm/L) were found to be more resistant to Oxaliplatin than GC1 (IC50 = 0.93μm/L) and PT4 (IC50 = 3.03μm/L) (see Figure S1A). In order to explore the differences between oxaliplatin resistant and non-resistant patients in organoids and the regulation of core genes. We performed mRNA sequencing on organoids of oxaliplatin-resistant and non-resistant patients. Figure 1A shows their significantly different genes. Compared with non-drug-resistant patients, the main enrichment pathways for drug-resistant patients include Homologous Recombination, DNA Replication, Base Excision Repair, Cell Cycle (Fig. 1B). Finally, we searched for the core gene through String and found that PARP1 was the core gene affecting drug resistance (Fig. 1C, D).

**PARP1 is Upregulated in Gastric Cancer Oxaliplatin Resistance Organoid**

Through our experiments on oxaliplatin resistance of GCR1, GCR2, GC1 and GC2 in vitro and in vivo, it was found that the tolerance of GCR1 and GCR2 to oxaliplatin was significantly higher than that of GC1 and GC2 (Figure S1D, Fig. 2A-F). Moreover, it was found that the expression level of PARP1 in GCR1 and GCR2 of oxaliplatin-resistant organoid was significantly higher than that in GC1 and GC2 of oxaliplatin-sensitive organoid. To illustrate the important role of PARP1 in oxaliplatin resistance.

**PARP1 plays an important role in maintaining oxaliplatin resistance**

Our previous study found that PARP1 expression was significantly elevated in oxaliplatin resistance. To study the role of PARP1 in oxaliplatin resistance. In vitro, through PARP1 overexpression in oxaliplatin-sensitive organoid, it was found that oxaliplatin-sensitive organoid showed significantly increased tolerance to oxaliplatin, and after PARP1 knockdown in oxaliplatin-resistant organoid, it was found that oxaliplatin-resistant organoid showed decreased tolerance to oxaliplatin (Fig. 3A-C, Figure S3A). In vivo,
we also further verified that the subcutaneous tumor-forming experiment was conducted after the overexpression of PARP1 in oxaliplatin-sensitive organoid, and it was found that the tolerance of subcutaneous tumors to oxaliplatin was significantly increased after intraperitoneal injection of oxaliplatin. Moreover, PARP1 knockdown was performed on oxaliplatin resistant organoid, and it was found that the tolerance of tumors to oxaliplatin in vivo was reduced (Fig. 3D-F). These results indicate that PARP1 plays a very important role in oxaliplatin resistance in vitro and in vivo.

**PARP1 inhibition by Olaparib sensitizes gastric cancer to Oxaliplatin**

Since PARP1 might be an important gene for Oxaliplatin resistance we had verified. To verify whether PARP1 inhibitor combined with oxaliplatin can effectively inhibit oxaliplatin resistance. We will carry out further in vitro and in vivo experiments. First of all, by using both the PARP1 inhibitor, Olaparib, and Oxaliplatin, the drug combination was found to effectively inhibit the viability, size, cell count, and proliferation of the organoids of oxaliplatin resistant gastric cancer (ORGC, GCR1 and GCR2) (Fig. 4A-C). The drug combination could significantly inhibit the activity and proliferation of Oxaliplatin resistance gastric cancer cell lines (Figure S2A, B). BALB/C NUDE mice in vivo tumorigenesis experiments have also confirmed that these drugs when used together could effectively inhibit tumor growth when compared with their use individually (Fig. 4D-F), and can induce cell apoptosis and affect proliferation of tumor cells (Fig. 4G-L). By comparing Olaparib + Oxaliplatin versus Oxaliplatin, it was found that the Olaparib + Oxaliplatin group was mainly enriched in oxidative phosphorylation and PPAR signaling pathway (see Figure S2C, D). These two pathways are primarily important enrichment pathways for tumor apoptosis after chemotherapy-induced DNA damage (11, 12). The main pathways enriched in the Oxaliplatin group were JAK-STAT, MAPK, NOTCH and WNT signaling pathways (see Figure S2E-H). In fact, these pathways are not only related to drug resistance in tumor, but also closely related to proliferation. PARP1 is an important factor affecting oxaliplatin resistance, and inhibition of PARP1 by olaparib can significantly change oxaliplatin tolerance. Thus for the clinical combination of drug use to bring convenience.

**Combined Oxaliplatin with Olaparib inhibits BER and HR repair pathways via blocking both CDK1-BRCA1 and PARP1-related activities**

Through the study above, the increase in PARP1 expression was found to be an important mediating factor for Oxaliplatin resistance. Now we will explore the role of PARP1 in Oxaliplatin resistance. PARP1 is usually used to repair single base breaks in DNA. Single base break is a type of commonly occurring DNA damage, and unrepaired single base breaks are not harmful to cells. However, when these broken bases are transcribed or replicated, they will destroy and cause damage to the new DNA copies. The activation of PARP1 can promote DNA base excision repair (BER) and inhibit the binding of transcription factors to single-stranded DNA, thus inhibiting the transcription of damaged DNA and DNA repair (13). Meanwhile, the main role of Oxaliplatin is to disrupt the DNA synthesis of cells, thereby affecting the cell cycle and
leading to DNA damage. The sensitivity and resistance of cells to platinum-based chemotherapy are largely determined by the activity of the DNA damage response (14). PARP1 is highly likely to mediate Oxaliplatin resistance through its regulation of DNA repair mechanisms. First, the effect of Oxaliplatin on DNA damage in Oxaliplatin resistant cells and sensitive cells was studied and results showed that the resistant cells were able to effectively repair DNA after the damage (Fig. 5A). Next, the effect of PARP1 activity on the repair of DNA damage caused by Oxaliplatin was studied and PARP1 inhibition was found to significantly inhibit the repair process (Fig. 5B, C). BER is an important pathway used in platinum-based drug. The role of BER in cancer drug resistance had been proposed by many studies (15–18), and PARP1 plays an important role in the BER pathway (19). To this end, the effect of PARP1 inhibitor combined with Oxaliplatin on the BER pathway marker, XRCC1 was studied, and Olaparib + Oxaliplatin was found to significantly inhibit the BER pathway when compared to Oxaliplatin alone (Fig. 5A, D, E). However, the transcription levels of XRCC1 in Oxaliplatin resistance patients and non-resistant patients, and XRCC1 of Oxaliplatin resistance and non-resistant strains did not change significantly (Figure S3 B, C). It showed that PARP1 can cause Oxaliplatin resistance by affecting the BER signaling pathway. Oxaliplatin will cause single-stranded damage after acting on tumor cells. At this point, if PARP1 had sufficient function, it will lead to BER pathway activation and thus DNA repair, which will eventually lead to drug resistance in tumor cells (20).

The role of PARP1 is to bind to DNA damage sites (mostly single-stranded DNA breaks) and catalyze the synthesis of poly ADP ribose chains on protein substrates (21). In order to study the core target of PARP1 interaction, weighted gene co-expression network analysis (WGCNA) method was used to find the core gene that interacted with PARP1 and it was found that CDK1 played a very key role in the high expression of PARP1 (Figure S4, Table S1). Cyclin-dependent kinase (CDK) 1 is the core component of the cell cycle mechanism, forming a complex with cyclin A and B to promote the progression of S phase, G2 phase and M phase. Recently, CDK1 and its other family members had been shown to be involved in the upstream of the DNA damage response pathway (22). Studies had found that CDK1 can inhibit homologous recombination by inhibiting the phosphorylation of BRCA1 (23, 24). Thus, we verified whether Oxaliplatin can directly act on BRCA1 or CDK1 to inhibit BRCA1 and cause homologous recombination failure.

To do this, we investigated the interaction of Oxaliplatin with BRCA1 and CDK1. First, Olaparib + Oxaliplatin drug combination was compared with single drug Oxaliplatin. Oxaliplatin was seen to significantly inhibit the phosphorylation of BRCA1 and CDK1(Fig. 6A, B), but Olaparib had no significant effect (Fig. 6A, 7A-C, 8A-F). In addition to affecting the functions of BRCA1 and CDK1, Oxaliplatin can also decrease the expression level of RAD51 (Fig. 6A, Fig. 7D, E). RAD51 is an important marker in homologous recombination. Oxaliplatin may be able to inhibit homologous recombination by affecting the function of BRCA1, which in turn leads to a decrease in RAD51 and ultimately aggravating DNA damage (such as increased expression of H2AX). But whether Oxaliplatin indirectly inhibited BRCA1 function by inhibiting CDK1 or directly inhibiting BRCA1 function remains unclear. So their relationship was compared by inhibiting the effect of CDK1. Figure 6A showed that CDK1 inhibitors significantly inhibited the phosphorylation of BRCA1, and the effect was similar to that of Oxaliplatin. In order to examine whether Oxaliplatin can bypass CDK1 and directly inhibit BRCA1, the functional effects of
cisplatin, which is also a platinum-based drug, was used on CDK1 and BRCA1 and compared to that of Oxaliplatin and found that cisplatin cannot inhibit the functions of CDK1 and BRCA1 (Fig. 6A). Moreover, it was shown through proliferation and colony formation assay that the effect of cisplatin combined with PARP1 and CDK1 inhibitors was not significantly different from the effect of Oxaliplatin combined with PARP1 inhibition (Fig. 6C, D). It showed that CDK1 plays an important role in killing tumor cells in platinum-based chemotherapy drugs. In fact, although the principle of action of Cisplatin and Oxaliplatin is basically the same, but the effect of cisplatin is worse than that of Oxaliplatin (25). CDK1 may be the main reason.

**PARP1 expression predicts the relapse of human gastric cancer after surgery**

It showed that PARP1 play an important role in Oxaliplatin resistance in gastric cancer. In order to clinically verify the importance of PARP1 in the recurrence of gastric cancer after curative surgery and adjuvant chemotherapy, we enrolled gastric cancer patients undergoing adjuvant chemotherapy in Sun Yat-sen University's Gastric Cancer Research Center. Through immunohistochemistry and recurrence status of patients after adjuvant chemotherapy, we found that PARP1 were highly expressed in the specimens of patients who relapsed after adjuvant chemotherapy (Figure S5 A, B, C). Moreover, the recurrence time of patients with high expression of PARP1 was significantly shorter than that of patients with low expression (Figure S5D, E). It showed that PARP1 can be used as important indicators to clinically predict recurrence in postoperative adjuvant chemotherapy patients, and it also showed that PARP1 play an important role in chemotherapy resistance.

**Discussion**

While trying to define and understand the biological characteristics of Oxaliplatin resistance cell subsets in GC, we found that PARP1 was significantly increased in relapsed patient after postoperative chemotherapy through the use of the sequencing results of patients who relapsed after Oxaliplatin chemotherapy and the patients with good chemotherapy response. We then verified that PARP1 have strong characteristics and Oxaliplatin resistance through *in vivo* organoids and Oxaliplatin resistant cell lines and *in vivo* BALB/C NUDE mice tumorigenesis. Then we found that PARP1 is the core gene of their common differences by looking for the difference genes between Oxaliplatin resistance patients and non-resistant patients. In order to explore the relationship between PARP1 and Oxaliplatin resistance, we inhibited PARP1 to significantly enhance the ability of Oxaliplatin to kill cancer and Oxaliplatin resistance cells. The combined use of PARP1 inhibitors can significantly inhibit activity of GC organoids, which affects their tumor initiation ability. *In vivo* experiments have also shown that inhibiting PARP1 can significantly overcome the resistance to Oxaliplatin. Subsequently, we found that PARP1 mediates the DNA repair ability of Oxaliplatin resistance cells by regulating the DNA repair pathway BER, and after the combination of PARP1 inhibitor, Olaparib, the joint effect allowed the drugs to effectively cause homologous recombination failure through CDK1 and BRCA1 functions, eventually leading to tumor cell apoptosis.
PARP1 is a multifunctional protein post-translational modifier found in most eukaryotic cells. It is activated by recognizing fragments of DNA that are structurally damaged and is thought to be a DNA damage receptor. It also performs polyadenosine diphosphate ribosylation of many nucleoproteins. Proteins modified by it include histones, RNA polymerase, DNA polymerase, DNA ligase, etc., and through the ADP-ribosylation of histones, histones are detached, which is helpful to repair the binding of proteins and repair DNA damage (26). PARP1 inhibitors can enhance the efficacy of radiotherapy, alkylating agents and platinum-based chemotherapy by inhibiting DNA damage repair and promoting apoptosis of tumor cells (27). It wasn't until 2014 that olaparib, the world's first PARP (polyADP ribosome polymerase) inhibitor, was approved for the treatment of ovarian cancer, followed by the Pani Family of lucaparib, niraparib and tarazoparib (28). PARP and BRCA are both masters of DNA repair, and PARP is responsible for single-strand repair. When PARP is controlled by inhibitors, the single-strand breaks of cells continue to increase and gradually develop into double-strand breaks. At this time, BRCA is required to take on the responsibility of high-Fi precision repair (homologous recombination) of double-strand breaks to prevent cell death caused by DNA instability (29). Although most studies have shown that PARP1 inhibitors can effectively enhance the efficacy of chemotherapy drugs, the mutation rate of BRCA1 mutations in most gastrointestinal tumors is actually not high (30).

### Conclusion

Our study found that PARP1 inhibitors in combination with oxaliplatin have a powerful anti-tumor effect in gastric cancer patients without BRCA1 mutations. The mechanism is currently unexplained. However, our study found that oxaliplatin itself can affect BRCA1 by inhibiting the function of CDK1. Causing BRCA1 dysfunction, allowing PARP1 inhibitors to function effectively.

### Abbreviations

GC1: Gastric cancer 1; GC1: Gastric cancer 2; GCR1: Gastric cancer resistance 1; GCR2: Gastric cancer resistance 2; ATP: Adenosine triphosphate; IACUC: Institutional Animal Care and Use Committee; OXA: Oxaliplatin; OLP: Olaparib; CON: control group; AGSR: AGS Oxaliplatin resistance; SNU719R: SNU719 Oxaliplatin resistance; MKN74R: MKN74 Oxaliplatin resistance; CISP: cisplatin; PCDK1: CDK1 phosphorylation antibody; PBRCA1: BRCA1 phosphorylation antibody; TOM: Topological overlap measure; GS: Gene significance; MS: Module significance; MCODE: Molecular Complex Detection; DAVID: Database for Annotation, Visualization and Integrated Discovery; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

### Declarations

#### Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (Ethical Review [2018] No. 087). And the clinical research and animal experiment ethics committee of the
First Affiliated Hospital of Sun Yat-sen University (Ethical Review [2017] No. 208).

Consent for publication

Not applicable.

Availability of data and material

The tumor data of BALB/C NUDE mice have been deposited in the NCBI BioProject database (www.ncbi.nlm.nih.gov/bioproject) under BioProject accession no. PRJNA669415. The RNA-Seq data in gastric cancer in the TCGA database was downloaded from https://cptac-data-portal.georgetown.edu/study-summary/S025. Proteomics data of gastric cancer was downloaded from the CPTAC website (https://cptac-data-portal.georgetown.edu/study-summary/S025). Obtain single-cell sequencing data of early gastric cancer tissue from the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE134520). All relevant data could be obtained from the corresponding author.

Competing interests

No potential conflicts of interest were disclosed.

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Authors' contributions

All authors contributed to writing the manuscript. AB, YH, CZ, HL and CW designed the study. HL, CW and LL Development of methodology. HL, CW, LL, IE, and ZZ acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.). HL, CW, LL, ER analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis). HL, CW, WC, ZH and LY Cultivation and development organoid.

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References

1. Yuan, L., Xu, Z.Y., Ruan, S.M., Mo, S., Qin, J.J. and Cheng, X.D. (2020) Long non-coding RNAs towards precision medicine in gastric cancer: early diagnosis, treatment, and drug resistance. *Molecular cancer*, 19, 96.

2. Wei, L., Sun, J., Zhang, N., Zheng, Y., Wang, X., Lv, L., Liu, J., Xu, Y., Shen, Y. and Yang, M. (2020) Noncoding RNAs in gastric cancer: implications for drug resistance. *Molecular cancer*, 19, 62.

3. Remy, C., Borniard, J. and Perez, J. (2020) Analysis of Unscheduled Telephone Calls Received by a Specialized Cancer Pain Nurse. *Pain management nursing : official journal of the American Society of Pain Management Nurses*, 21, 255-258.

4. Yan, H.H.N., Siu, H.C., Law, S., Ho, S.L., Yue, S.S.K., Tsui, W.Y., Chan, D., Chan, A.S., Ma, S., Lam, K.O. et al. (2018) A Comprehensive Human Gastric Cancer Organoid Biobank Captures Tumor Subtype Heterogeneity and Enables Therapeutic Screening. *Cell stem cell*, 23, 882-897.e811.

5. Seidlitz, T., Merker, S.R., Rothe, A., Zakrzewski, F., von Neubeck, C., Grützmann, K., Sommer, U., Schweitzer, C., Schölch, S., Uhlemann, H. et al. (2019) Human gastric cancer modelling using organoids. *Gut*, 68, 207-217.

6. Ruiz, E.J., Diefenbacher, M.E., Nelson, J.K., Sancho, R., Pucci, F., Chakraborty, A., Moreno, P., Annibaldi, A., Liccardi, G., Encheva, V. et al. (2019) LUBAC determines chemotherapy resistance in squamous cell lung cancer. *The Journal of experimental medicine*, 216, 450-465.

7. Eisenhauer, E.A., Therasse, P., Bogaerts, J., Schwartz, L.H., Sargent, D., Ford, R., Dancey, J., Arbuck, S., Gwyther, S., Mooney, M. et al. (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer (Oxford, England : 1990)*, 45, 228-247.

8. Li, B. and Dewey, C.N. (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics*, 12, 323.

9. Luo, Y., Coskun, V., Liang, A., Yu, J., Cheng, L., Ge, W., Shi, Z., Zhang, K., Li, C., Cui, Y. et al. (2015) Single-cell transcriptome analyses reveal signals to activate dormant neural stem cells. *Cell*, 161, 1175-1186.

10. Snel, B., Lehmann, G., Bork, P. and Huynen, M.A. (2000) STRING: a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene. *Nucleic acids research*, 28, 3442-3444.

11. Yadav, N., Kumar, S., Marlowe, T., Chaudhary, A.K., Kumar, R., Wang, J., O’Malley, J., Boland, P.M., Jayanthi, S., Kumar, T.K. et al. (2015) Oxidative phosphorylation-dependent regulation of cancer cell apoptosis in response to anticancer agents. *Cell death & disease*, 6, e1969.

12. Yang, W.L. and Frucht, H. (2001) Activation of the PPAR pathway induces apoptosis and COX-2 inhibition in HT-29 human colon cancer cells. *Carcinogenesis*, 22, 1379-1383.
13. Ray Chaudhuri, A. and Nussenzweig, A. (2017) The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nature reviews. Molecular cell biology, 18*, 610-621.

14. Slyskova, J., Sabatella, M., Ribeiro-Silva, C., Stok, C., Theil, A.F., Vermeulen, W. and Lans, H. (2018) Base and nucleotide excision repair facilitate resolution of platinum drugs-induced transcription blockage. *Nucleic acids research, 46*, 9537-9549.

15. Horton, J.K., Srivastava, D.K., Zmudzka, B.Z. and Wilson, S.H. (1995) Strategic down-regulation of DNA polymerase beta by antisense RNA sensitizes mammalian cells to specific DNA damaging agents. *Nucleic acids research, 23*, 3810-3815.

16. Sawant, A., Floyd, A.M., Dangeti, M., Lei, W., Sobol, R.W. and Patrick, S.M. (2017) Differential role of base excision repair proteins in mediating cisplatin cytotoxicity. *DNA repair, 51*, 46-59.

17. Yang, J., Parsons, J., Nicolay, N.H., Caporali, S., Harrington, C.F., Singh, R., Finch, D., D'Atri, S., Farmer, P.B., Johnston, P.G. *et al.* (2010) Cells deficient in the base excision repair protein, DNA polymerase beta, are hypersensitive to oxaliplatin chemotherapy. *Oncogene, 29*, 463-468.

18. Preston, T.J., Henderson, J.T., McCallum, G.P. and Wells, P.G. (2009) Base excision repair of reactive oxygen species-initiated 7,8-dihydro-8-oxo-2'-deoxyguanosine inhibits the cytotoxicity of platinum anticancer drugs. *Molecular cancer therapeutics, 8*, 2015-2026.

19. Ronson, G.E., Piberger, A.L., Higgs, M.R., Olsen, A.L., Stewart, G.S., McHugh, P.J., Petermann, E. and Lakin, N.D. (2018) PARP1 and PARP2 stabilise replication forks at base excision repair intermediates through Fbh1-dependent Rad51 regulation. *Nature communications, 9*, 746.

20. Faivre, S., Chan, D., Salinas, R., Woynarowska, B. and Woynarowski, J.M. (2003) DNA strand breaks and apoptosis induced by oxaliplatin in cancer cells. *Biochemical pharmacology, 66*, 225-237.

21. Mateo, J., Lord, C.J., Serra, V., Tutt, A., Balmaña, J., Castroviejo-Bermejo, M., Cruz, C., Oaknin, A., Kaye, S.B. and de Bono, J.S. (2019) A decade of clinical development of PARP inhibitors in perspective. *Annals of oncology : official journal of the European Society for Medical Oncology, 30*, 1437-1447.

22. Myers, J.S., Zhao, R., Xu, X., Ham, A.J. and Cortez, D. (2007) Cyclin-dependent kinase 2 dependent phosphorylation of ATRIP regulates the G2-M checkpoint response to DNA damage. *Cancer research, 67*, 6685-6690.

23. Johnson, N., Cai, D., Kennedy, R.D., Pathania, S., Arora, M., Li, Y.C., D'Andrea, A.D., Parvin, J.D. and Shapiro, G.I. (2009) Cdk1 participates in BRCA1-dependent S phase checkpoint control in response to DNA damage. *Molecular cell, 35*, 327-339.

24. Johnson, N., Li, Y.C., Walton, Z.E., Cheng, K.A., Li, D., Rodig, S.J., Moreau, L.A., Unitt, C., Bronson, R.T., Thomas, H.D. *et al.* (2011) Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. *Nature medicine, 17*, 875-882.

25. Liu, Q., Zeng, X., Wang, W., Huang, R.L., Huang, Y.J., Liu, S., Huang, Y.H., Wang, Y.X., Fang, Q.H., He, G. *et al.* (2019) Awareness of risk factors and warning symptoms and attitude towards gastric cancer screening among the general public in China: a cross-sectional study. *BMJ open, 9*, e029638.

26. Alemasova, E.E. and Lavrik, O.I. (2019) Poly(ADP-ribosyl)ation by PARP1: reaction mechanism and regulatory proteins. *Nucleic acids research, 47*, 3811-3827.
27. Wang, Q., Xiong, J., Qiu, D., Zhao, X., Yan, D., Xu, W., Wang, Z., Chen, Q., Panday, S., Li, A. et al. (2017) Inhibition of PARP1 activity enhances chemotherapeutic efficiency in cisplatin-resistant gastric cancer cells. *The international journal of biochemistry & cell biology, 92*, 164-172.

28. Wang, S., Han, L., Han, J., Li, P., Ding, Q., Zhang, Q.J., Liu, Z.P., Chen, C. and Yu, Y. (2019) Uncoupling of PARP1 trapping and inhibition using selective PARP1 degradation. *Nature chemical biology, 15*, 1223-1231.

29. D'Andrea, A.D. (2018) Mechanisms of PARP inhibitor sensitivity and resistance. *DNA repair, 71*, 172-176.

30. Narod, S.A. and Foulkes, W.D. (2004) BRCA1 and BRCA2: 1994 and beyond. *Nature reviews. Cancer, 4*, 665-676.

**Figures**
Figure 1

PARP1 is the central gene of Oxaliplatin resistance in gastric cancer. A, Heatmap of mRNA differential expression of GC1 and GC2 tumors against GCR1 and GCR2 tumors. The abscissa represents the gene name. Red represents High, blue represents Low. B, Analysis of enrichment of mRNA differential expression of GC1 and GC2 tumors against GCR1 and GCR2 tumors. C, STING database protein interaction network diagram of mRNA differential expression in GC1 and GC2 tumors compared to GCR1.
and GCR2 tumors. Edges represent protein-protein associations. Cambridge blue: from curated databases. Violet: experimentally determined. Green: gene neighbourhood. Red: gene fusions. Blue: gene co-occurrence. Reseda: text mining. Black: co-expression. Lilac: protein homology.

Comparison of NODE string number of two gene sets in core genes of C.

Figure 2
PARP1 is upregulated in oxaliplatin resistance gastric cancer. A, B, C, GC1, GC2, GCR1, GCR2 organoids were treated with Oxaliplatin respectively before checking for a representative image (A) number of organoid (B), size of organoid (C). D, Representative images of tumorigenesis in BALB/C NUDE mice with Oxaliplatin. The ruler represents 1cm. E, Tumor growth curves of PDOX BALB/C NUDE mice of GCOR. The curve shows the average tumor volume. Error bars represent mean-standard deviation. F, Mass of tumors of PDOX in BALB/C NUDE mice. The curve shows the average tumor mass. G, Representative images of PARP1 levels stained by immunofluorescence in organoid and tumors. The scale represents of tumor is 20um. The scale represents of organoid is 200um. H, proportion of PARP1+ cells in organoid in G. I, proportion of PARP1+ cells in tumor in G. The Student’s t test was used for statistical analysis. Error bars indicate mean ± standard deviation. * p<0.05, ** p<0.01, *** p<0.001.
Figure 3

PARP1 is required for Oxaliplatin resistance development. A, B, C, GC1, GC2 and GC1 PARP1 overexpression, GC2 overexpression, GCR1, GCR2 and GCR1 PARP1 knock-down, GCR2 knock-down organoids were treated with Oxaliplatin respectively before checking for a representative image (A) number of organoid (B, C). D, Representative images of tumorigenesis in BALB/C NUDE mice with Oxaliplatin. The ruler represents 1cm. E, Tumor growth curves of PDOX BALB/C NUDE mice of GC1, GC2...
and GC1 PARP1 overexpression, GC2 overexpression. The curve shows the average tumor volume. Error bars represent mean-standard deviation. F, Tumor growth curves of PDOX BALB/C NUDE mice of GCR1, GCR2 and GCR1 PARP1 knock-down, GCR2 knock-down. The curve shows the average tumor volume. Error bars represent mean-standard deviation.
PARP1 inhibition by Olaparib sensitizes gastric cancer to Oxaliplatin. A, B, C ORGC organoids were treated with Olaparib+Oxaliplatin, Oxaliplatin, and Olaparib respectively before checking for a representative image (A) number of organoid(B), size of organoid (C). The scale represents 500um. Different drug concentrations were used to act on cells and before cell viability tests (A, E). D, Representative images of tumorigenesis in BALB/C NUDE mice with Olaparib+Oxaliplatin, Oxaliplatin, Olaparib and a blank control group. The ruler represents 1cm. E, Tumor growth curves of PDOX BALB/C NUDE mice of GCOR. The curve shows the average tumor volume. Error bars represent mean-standard deviation. F, Mass of tumors of PDOX in BALB/C NUDE mice. The curve shows the average tumor mass. G, The effect of different medication groups on the apoptosis of ORGC organoids. H, Comparison of the proportion of apoptosis in different groups in G. I, Representative images of KI67 stained by IF staining of ORGC organoids treated with olaparib+oxaliplatin, oxaliplatin, olaparib and a blank control group. The red stains indicate KI67 positive. The scale represents 2um. J, Representative images of KI67 and Caspase3 stained by IHC staining after tumorigenesis of BALB/C NUDE mice treated with Olaparib+Oxaliplatin, Oxaliplatin, Olaparib and a blank control group. The brown stains indicate KI67 and Caspase3 positive. The scale represents 200um. K, Statistical analysis of KI67+ cells in I. L, Comparison of the percentage of positive cells stained with KI67 and Caspase3. Student’s t test was used for statistical analysis. Error bars indicate mean± standard deviation. OXA, Oxaliplatin. OLP, Olaparib. CON, control group. ORGC, oxaliplatin resistance gastric cancer. * p<0.05, ** p<0.01, *** p<0.001.
The inhibition of PARP1 can significantly enhance the DNA damage and inhibit BER of Oxaliplatin-resistant GC. A, comparison of γH2AX expression in Olaparib+Oxaliplatin, Oxaliplatin, Olaparib, and the blank control group and the MKN74, SNU719, AGS resistant strains and their corresponding wild-type cell lines at different times. Comparison of XRCC1 expression in Olaparib+Oxaliplatin, Oxaliplatin, Olaparib, and the blank control group in MKN74, SNU719, and AGS Oxaliplatin resistance strains. B,
immunofluorescent comparison of γH2AX expression in Olaparib+Oxaliplatin, Oxaliplatin, Olaparib and the blank control group and the MKN74, SNU719, AGS resistant strains and their corresponding wild-type cell lines at different times. C, ratio of γH2AX+ cells in MKN74, SNU719, AGS Oxaliplatin resistance strains and their corresponding wild-type cell line in B. D, immunofluorescent comparison of XRCC1 expression in Olaparib+Oxaliplatin, Oxaliplatin, Olaparib, and the blank control group in MKN74, SNU719, and AGS Oxaliplatin resistance strains. The scale represents 2um. E, ratio of XRCC1 cells in MKN74, SNU719, AGS Oxaliplatin resistance strain and their respective wild-type cell lines in D respectively. The Student’s t test was used for statistical analysis. Error bars indicate mean ± standard deviation. OXA, Oxaliplatin. OLP, Olaparib. CON, control group. AGSR, AGS Oxaliplatin resistance. SNU719R, SNU719 Oxaliplatin resistance. MKN74R, MKN74 Oxaliplatin resistance. * 0.05, ** 0.01, *** 0.001.
Figure 6

Treatment of Oxaliplatin inhibits HR repair pathways via blocking CDK1-BRCA1 activities in Oxaliplatin resistance cell line. A, Verification by WB on the effects of Olaparib+Oxaliplatin, Oxaliplatin, Olaparib, AG-02432 and cisplatin on CDK1 expression and its phosphorylation, BRCA1 expression and its phosphorylation, RAD51 expression in SNU719, MKN74, and AGS Oxaliplatin resistance strains. Drug action time was 36h. B, Histochemical results of protein phosphorylation in gastric cancer patients. C, D,
the effects of Olaparib+Oxaliplatin and cisplatin combined with CDK1 inhibitor Olaparib on colony formation of overexpressed RARP1 and normally expressed RARP1 cell lines in SNU719, MKN74, and AGS Oxaliplatin resistance strains. Colonies were stained with crystal violet. The Student’s t test was used for statistical analysis. Error bars indicate mean ± standard deviation. OXA, Oxaliplatin. OLP, Olaparib. CON, control group. CISP, cisplatin. PCDK1, CDK1 phosphorylation antibody. PBRCA1, BRCA1 phosphorylation antibody. AGSR, AGS Oxaliplatin resistance. SNU719R, SNU719 Oxaliplatin resistance. MKN74R, MKN74 Oxaliplatin resistance. * ≥ 0.05, ** < 0.01, *** < 0.001.
Figure 7

Oxaliplatin inhibits HR repair pathways via blocking both CDK1-BRCA1. A, The representative images of immunofluorescent CDK1 phosphorylation and BRCA1 phosphorylation staining of Olaparib+oxaliplatin, Oxaliplatin, Olaparib, and blank control group in MKN74, SNU719, AGS Oxaliplatin resistance cell lines. The scale represents 20um. B, proportion of CDK1 phosphorylation and BRCA1 phosphorylation positive cells in A respectively. C, representative images of immunofluorescent phosphorylation staining comparisons of Olaparib+Oxaliplatin, Oxaliplatin, Olaparib and blank control group and immunofluorescent phosphorylation staining of RAD51 and BRCA1 of MKN74, SNU719, AGS resistant strains RAD51 and BRCA1 at different time. The scale represents 2um. D, Statistical analysis of RAD51+ cells in C. The Student’s t test was used for statistical analysis. Error bars indicate mean ± standard deviation. OXA, Oxaliplatin. OLP, Olaparib. CON, control group. PCDK1, CDK1 phosphorylation antibody. PBRCA1, BRCA1 phosphorylation antibody. AGSRE, AGS Oxaliplatin resistance. SNU719RE, SNU719 Oxaliplatin resistance. MKN74RE, MKN74 Oxaliplatin resistance. * \( < 0.05 \), ** \( < 0.01 \), *** \( < 0.001 \).
Figure 8

Treatment of Oxaliplatin inhibits HR repair pathways via blocking CDK1-BRCA1 activities in ORGC Organoid and PDOX. A, representative images of immunofluorescent staining comparison of CDK1 phosphorylation and BRCA1 phosphorylation in ORGC organoids under the effects of Olaparib+Oxaliplatin, Oxaliplatin, Olaparib and the blank control group. The scale represents 20μm. B, C, proportion of CDK1 phosphorylation and BRCA1 phosphorylation positive cells in A respectively. D,
Representative images of comparison of IHC staining of CDK1 and its phosphorylation and BRCA1 and its phosphorylation in BALB/C NUDE mice after tumorigenesis under the effects of Olaparib+Oxaliplatin, Oxaliplatin, Olaparib and blank control group. The scale represents 200um. E, F, proportion of CDK1 phosphorylation and BRCA1 phosphorylation positive cells in P respectively. The Student’s t test was used for statistical analysis. Error bars indicate mean ± standard deviation. OXA, Oxaliplatin. OLP, Olaparib. CON, control group. CISP, cisplatin. PCDK1, CDK1 phosphorylation antibody. PBRCA1, BRCA1 phosphorylation antibody. ORGC, Oxaliplatin resistance gastric cancer (GCR1 and GCR2). * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001.

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