Niraparib enhances radiosensitivity in glioblastoma with redistribution of DDX21

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Abstract

Objective

To explore the feasibility and mechanism of PARP inhibitor niraparib as a radiosensitizer for Glioblastoma (GBM).

Methods

Bioinformatics analysis was used to reveal the mechanism of PARP inhibitor in glioma and its correlation with radiotherapy. CCK8 determined the optimal concentration and time of Niraparib in GBM cell lines (A172, U251 and U87). Clonogenic assay was used to detect the radiosensitivity of niraparib in GBM cells. The radiosensitization mechanism of niraparib in GBM cells was studied by flow cytometry, western blot, immunofluorescence and CCK8.

Results

Pathways relevant to ribosome biosynthesis and functions such as eukaryotic translation initiation, rRNA processing was found to be responsible for cytotoxicity of niraparib in 519 tumor cell lines. Moreover, mRNA expression of PARP1/2, genes participated in ribosome biosynthesis and homologous recombination (HR) were all significantly negatively associated with SF2 in 44 NCI-60 cell lines. The IC50 of niraparib in A172 and U87 cell lines were 10.77 ± 3.31 and 32.37 ± 2.84 uM respectively. The DEF37 was established as 1.99 at 348 nM, 2.17 at 1044 nM for A172 cell line and 1.10 at 1056 nM, 1.44 at 3169 nM for U87 cell line, respectively. The treatment of 1056nM niraparib combination with radiation (4Gy) resulted in significant increase in fraction of G2 phase in A172 and U87 cells. The protein expression of DDX21 was only slightly decreased in treated with irradiation alone or niraparib combined with irradiation. Niraparib combined with irradiation could promote the redistribution of DDX21 from the nucleolus to the nucleoplasm in U87 cells. Knockdown of DDX21 significantly reduced proliferation in U87 cells. Niraparib combined with radiotherapy further decreased clonogenic number in U87 cells compared with those treated with niraparib alone or irradiation alone, likewise the U87 cells after knockdown of DDX21. Interestingly, knockdown of DDX21 resulted in significant increasing in clonogenic number of U87 cells.

Conclusion

Niraparib affected ribosome biosynthesis via redistribution of DDX21 from the nucleolus to the nucleoplasm, caused G2/M phase arrest, thus increased the radiosensitivity of U87 cells. Niraparib may be a good radiosensitizer for GBM.
Introduction

Glioblastoma (GBM) is the most common primary intracranial tumor, although treatment of Stupp significantly prolonged progression-free survival (PFS) and overall Survival (OS) of GBM, the 5-year Survival rate is still below 10%. Poor prognosis of GBM is closely related to resistance to radiotherapy and chemotherapy (Tan et al. 2020). DNA repair defects, special biological behavior of stem cells, and microRNA-mediated expression regulation all directly affect the sensitivity of GBM to radiotherapy and chemotherapy (Oldrini et al. 2020; Sharanek et al. 2020; Wang et al. 2017).

Poly (ADP ribose) polymerase (PARP) is a protein superfamily responsible for transferring ADP ribose groups to target proteins. It has been found that 17 members of the family, which play a role in maintaining genomic stability and telomerase length. PARP1, as the earliest target of tumor therapy in PARP family, is involved in base excision repair which is the main repair mechanism of DNA single-strand damage in cells. Inhibition of PARP1 activity can hinder DNA single-strand damage repair and induce DNA double-strand damage in cells. Cells with homologous recombination deficiency (HRD), such as those carrying BRCA1/2 mutations, are unable to carry out effective repair of DNA double-strand damage. PARP inhibitors can block cellular DNA single-strand damage repair and induce death through the "synthetic lethal" effect in HRD tumor cells. PARP inhibitors have been successfully used in the treatment of ovarian and breast cancer based on this effect (Aberle et al. 2020; Liu et al. 2017; Miller et al. 2020; Sun et al. 2021; Tung et al. 2021; Yan et al. 2021).

In addition, studies have shown that PARP inhibitors can inhibit cell proliferation through ribosome biosynthesis via DDX21 in breast cancer cells with intact homologous recombination (HR) repair capacity (Keung et al. 2020). DDX21 is one of the important members of DEAD box RNA helicase, which can affect ribosome biosynthesis by participating in regulating rDNA transcription, rRNA processing and modification (Calo et al. 2015). Thus, PARP inhibitors can also affect PAR-dependent ribosomal protein transcriptional activation and reduce ribosome biosynthesis via DDX21 (Kim et al. 2019). In general, the GBM tumor cells retain the complete homologous recombination repair function (Zhang et al. 2021). On the other hand, Verhagen's study had suggested that PARP inhibition is effective in cell lines with intact homologous recombination repair as homologous recombination deficiency cell lines (Verhagen et al. 2015). Therefore, this study was aimed to explore the effect of the PARP inhibitor niraparib on the radiosensitivity of GBM tumor cell lines with intact HR and reveal the possible role of DDX21 in the radiosensitizing effect of niraparib.

Materials And Methods

Data acquisition and preprocessing

The 50% inhibition concentration (IC\textsubscript{50}) of Olaparib on 946 Cell lines in the Cancer Cell Line Encyclopedia (CCLE) and the IC\textsubscript{50} of Talazoparib on 910 Cell lines in CCLE were downloaded from the genomic f drug
susceptibility in cancer database (GDSC, https://www.cancerrxgene.org). All IC\textsubscript{50} values are converted by natural logarithm and used for statistical calculation.

Forty-one genes of HSA03440 homologous recombination repair pathway were obtained from KEGG database, and mutation data of 41 HR genes in CCLE cell lines were extracted from cBioPortal database. 45 breast cancer cells, 30 ovarian cancer cells and 33 glioma cell lines, both with IC\textsubscript{50} data of Olaparib and Talazoparib and 41 HR gene mutation status were included in the analysis.

The raw CEL files ([HG-U219] Affymetrix Human Genome U219 Array) of CCLE cell lines were obtained from https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-3610/files/raw/. The raw CEL files and RMA function in “limma” R package were used to perform background correction, normalization and probe summarization to obtain the expression matrix at probe cluster level. When there were multiple probes corresponding to a gene, the probes with the largest interquartile range were taken to represent the gene expression value. A total of 18464 genes from 108 CCLE cell lines were analyzed. The survival fraction at 2 Gy (SF2) of NCI60 cell lines were obtained from reference (Eschrich et al. 2009). The raw CEL files of 48 cell lines were obtained from the ArrayExpress (www.ebi.ac.uk/biostudies/arrayexpress/studies/ E-MTAB-3610). The SF2 values were statistically analyzed after natural logarithm transformation. The expression profiles data of 529 GBM and 10 normal brain tissue were obtained from TCGA database, and the clinical characteristics and progression-free interval (PFI), disease-specific survival (DSS) and overall survival (OS) were obtained from UCSC (https://xenabrowser.NET/datapages/).

**Calculation Of Parp Sensitivity Signature And Identification Of Genes Modifying Prediction Of Parp Sensitivity Signature**

PARP sensitivity signature is originally identified according to the sensitivity of CCLE cell lines to PARP inhibitors by comparing resistant and sensitive cells (McGrail et al. 2017). The PARP sensitivity signature was calculated through Pearson correlation coefficient of expression of genes belonging to signature and Log2FC values published by the original article (McGrail et al. 2017).

To investigate the active mechanism of PARP inhibitors in the different cell lines (breast cancer, ovarian cancer, glioma), the method similar to that used to identify genes reflecting T cell dysfunction in the development of tumor immune dysfunction and exclusion (TIDE) was used (Jiang et al. 2018). Multiple linear regression was performed with PARP inhibitor IC\textsubscript{50} as dependent variable and PARP sensitivity signature, gene expression value and their interaction as covariates. The interaction t-values of 18464 genes were extracted. The enrichment analysis of the Reactome gene set was perfomed with t-values as the test variable by using the clusterProfiler package gsePathway function (Yu et al. 2012). FDR $P<0.15$ was considered as a significant enrichment pathway.

**Calculation Of Radiosensitivity Zeta Value**
There were 10127 genes available shared between TCGA GBM expression matrix and signatures of ribosome biosynthesis and HR. 48 NCI60 cell lines was used to develop RADzeta to evaluate radiosensitivity of any given tumor sample. Differentially expressed genes were firstly identified through lmFit in the “limma” R package between 12 cell lines with highest SF2 versus 12 cell lines with lowest SF2. Vector projection was used to calculate RADzeta (Bao et al. 2006). The centroids of 12 cell lines with highest SF2 and 12 cell lines with lowest SF2 was shown in Supplementary Table 1. The RADzeta indicated the degree of resistance to radiotherapy. The gene list of HR and GO termed with “GO_RIBOSOME GO_RIBOSOME_BIOGENESIS”, “GO_RIBOSOME_ASSEMBLY” were extracted from GO gene sets. The average expression of 10 genes belonging to HR was used to determine the measurement of HR capacity. For ribosome biosynthesis, 45 genes of ribosome biosynthesis signature were identified through overlapping genes belonging to GO_RIBOSOME GO_RIBOSOME_BIOGENESIS, GO_RIBOSOME_ASSEMBLY and significantly up-regulated genes in tumor tissue samples of GBM versus normal tissues. The average expression of 45 genes of ribosome biosynthesis signature was used to evaluate the capacity of ribosome biosynthesis.

**Main Materials**

Niraparib (ZL-2306) was donated by ZaiLab(Shanghai) Co. Cell Cycle Analysis Kit was purchased from Beyotime Institute of Biotechnology; DDX21 antibody was purchased from Proteintech; DDX21 interfering lentiviral vector was purchased from Shanghai Genechem Co.,Ltd; the sequences of shDDX21 was follow: F:5’-CGCTCCTTGATCAACTCAAAT-3’, R:5’-GGAGACACTGCGAAAGCAAAC-3’.

**Cell Culture**

Human Glioblastoma cell lines A172, U251, U87 were purchased from National Collection of Authenticated Cell Cultures (Shanghai,China). A172 has wild-type p53 and PTEN, U251 has mutant p53 and wild-type PTEN, U87 has wild-type p53 and mutant PTEN. The mutation status of BRAC in three cells is unknown. All cells were cultured in DMEM/F12 medium containing 10% fetal bovine serum, 100 u/ml penicillin and 0.1 mg/ml streptomycin at 37°C incubator with 5% CO₂.

**Niraparib Configuration**

Niraparib dry powder was dissolved in DMSO at 200 mM to obtain storage solution (Meng et al. 2021). This high concentration solution was stored in -20°C refrigerator after through 22 um sterilizing filter to remove bacteria and impurities.

**Growth Inhibition Assay**
Growth inhibition was evaluated by the cell counting kit-8 assay (CCK8). Cells (3000 cells/well) were seeded into 96-well plate and treated with niraparib at the final concentration 0, 3.125, 6.25, 12.5, 25, 50, 100, 200, 300 μM for 24h and 48h. Culture medium containing drug were removed. 100 ul 10% CCK-8 solution was added in each well and incubated at 37°C for 2h. Cell viability was measured using the colorimetric readings at 450 nm. Drug inhibition rate=(Acon-Aexp)/Acon×100%, the half-maximal inhibitory concentration values (IC₅₀) were calculated from dose-response curves by utilizing Prism (Version 8.01).

**Clonogenic Assay**

The effects of drug and radiation on colony formation ability were assessed with clonogenic assay. A172, U251 cells (1000/well) and U87 cells (2000/well) were seeded into 60 mm culture dishes. Cells were treated with niraparib at 1/30, 1/50, 1/100 IC₅₀ for 30min before irradiated with 8MV X-rays using a linear accelerator (Synergy, Elekta) with a dose of 600 Gy/min at different dose (0, 2, 4, 6, 8 Gy) (Moreno-Villanueva et al. 2019). All cells were further cultured at 37°C for 2 weeks. All cells were fix with 4% paraformaldehyde at room temperature for 10 min, washed with PBS for three times, and stained with 1% crystal violet at room temperature for 10 min. The cells were counted under the microscope, and cell clusters with more than 50 cells were counted as one colony, and then calculated plating efficiency (PE, PE = colonies/ inoculated cells) and surviving fraction (SF, SF = PE_exp/PE_con).

**Cell Cycle Assay**

Cells were seeded into 6-well plate, one group of cells treated with niraparib (1/50 IC₅₀), another group of cells without treatment of niraparib. The cells were irradiated with 4 Gy X-ray, were further cultured at 37°C. The cells were fixed with ice 70% ethanol at -20°C, incubated with propidium iodide (PI) solution and examinated with flow cytometer.

**Western Blot**

Cells were lysed by RIPA buffer. The extracted proteins (5ug) were separated by 10% SDS-PAGE gel, and transfered to polyvinylidene fluoride membrane (PVDF). The membranes were blocked with 10% skim milk at room temperature for 2 hours. The primary antibodies solutions were incubated for overnight at 4°C. The antibody/antigen complexes were revealed by the enhanced chemiluminescence kit, the membranes was scanned and analyzed by chemiluminescent gel imaging system and Image Lab software (Version 6.1).

**Immunofluorescence Studies**
Cells were fixed in 4% paraformaldehyde (Beyotime, shanghai, China) for 10min at room temperature. Then treated with 0.5% Triton X-100 (Beyotime, shanghai, China) in PBS for 20min room temperature. The cells were incubated with primary antibodies solutions for overnight at 4°C, and then washed with PBS three times. Cells were incubated with FITC Green goat anti-rabbit (ZSGB- BIO, beijing, China) for 1h at 37°C. Nucleus were stained with DAPI ((Beyotime, shanghai, China). The images were captured with the fluorescence microscope (Olympus, Japan).

**Statistical analysis**

Images were analyzed using Image j software, statistical analysis was performed using graphpad prism software (version 8.1. 1). Hill equation was used to fit dose-response curves to calculate IC\(_{50}\). Kruskal-Wallis test was used to compare IC\(_{50}\) among cell lines of different cancer types with correction for multiple comparisons. \(P<0.05\) was considered statistically significant.

**Results**

**Mechanism of PAPR inhibitor activity in CCLE cell lines**

In breast cancer (BRCA), ovarian cancer (OV), and glioma (GBM), the median IC\(_{50}\) and interquartile range of the two PARP inhibitors were shown in Supplementary Table 2. The IC\(_{50}\) value of PARP inhibitor was higher in breast cancer than in ovarian cancer and glioma, especially for talazoparib. The IC\(_{50}\) values of the two PARP inhibitors were not significantly different between the cell lines harboring any truncated mutant HR genes and the cell lines with wild-type HR genes in the three tumor types (Kruskal-Wallis test: olaparib \(P=0.769\), talazoparib, \(P=0.239\)). In contrast, PARP sensitivity signature was strongly associated with the IC\(_{50}\). In ovarian cancer cell lines, PARP sensitivity signature manifested the strongest correlation with IC\(_{50}\) of olaparib (\(P=0.001\)). In breast cancer cell lines, PARP sensitivity signature was the strongest predictor for IC\(_{50}\) of talazoparib (\(P=0.001\)) (Supplementary Table 2). These results demonstrated that PARP sensitivity signature was able to faithfully represent intrinsic sensitivity of different cell lines to PARP inhibitors.

Linear regression with interaction term was used separately in BRCA, OV and GBM cell lines. The results of GSEA analysis showed that ribosome biosynthesis was the main mechanism affecting olaparib sensitivity in BRCA cell lines (Fig. 1, Supplementary Table 3). However, the genes affecting olaparib sensitivity in GBM cell lines were mainly involved in ribosome biosynthesis and mitosis (Supplementary Table 3). These results preliminarily suggested that ribosome biosynthetic pathway was shared between GBM and BRCA in terms of olaparib sensitivity.

**Effect Of Ribosome Biosynthesis And Hr On Radiosensitivity Of Gbm**
RADzeta is highly positively correlated with SF2 in 48 NCI60 cell lines \( (r = 0.728, P < 0.001) \). In 44 solid tumor cell lines, HR repair ability was highly positively correlated with ribosome biosynthesis \( (r = 0.632, P = 4.182 \times 10^{-6}) \). However, HR repair ability \( (r = -0.432, P = 0.003) \), ribosome biosynthesis, cyclins expression level (CCNE1 and CCNB2) were all significantly negatively correlated with RADzeta (Fig. 2A and 2B). These results were also observed in the 529 primary tumor tissues with GBM (Fig. 2). These results suggested that the high HR repair ability could be associated with high ribosome biosynthesis and high sensitivity to irradiation in GBM.

**Inhibited Proliferation Of Gbm Cells By Niraparib**

As shown in Fig. 3, niraparib can effectively inhibited the cell viability of A172, U251 and U87 cells at different concentrations. The IC\(_{50}\) value of niraparib were established as 10.77 ± 3.31 uM, 18.91 ± 2.95 uM, 32.37 ± 2.84 uM for A172, U251 and U87 cell line respectively. Because the main purpose of our study is to explore the radiosensitization of niraparib on GBM cells, three lower concentrations \( (1/10, 1/30, 1/100 \text{IC}_50) \) were used for further radiosensitization experiments in A172 and U-87 cell lines (Zheng et al.2013; Zhou et al. 2017).

**Increased Radiosensitivity Of A172 And U87 Cells By Niraparib At Low Concentrations**

As shown in Fig. 4, the surviving colonies decreased significantly with the increase of drug concentration in the three GBM cell lines before irradiation. The surviving colonies further decreased significantly with the increase of irradiation dose in A172 and U87 cells. The radiation dose enhancement factor at 37% survival fraction (DEF\(_{37}\)) were established as 1.99 at 348 nM, 2.17 at 1044 nM for A172 cell line and 1.10 at 1056 nM, 1.44 at 3169 nM for U87 cell line, respectively. However, the surviving colonies had no significant difference between U251 cells with or without irradiation. Therefore, we only selected A172 and U87 cells, and the treatment concentration of niraparib was 1/30 IC50, and the irradiation with 4Gy X-ray for the further study.

**G2/m Phase Arrest In A172 And U87 Cells By Niraparib**

As shown in Fig. 5, there were synergistic cross-effects between irradiation and niraparib. The A172 and U87 cells treated with niraparib \( (1/30 \text{IC}_50) \) and irradiation \( (4\text{Gy}) \) revealed a significant increase in G2/M phase, which was within the radiosensitive phases of cell cycle. This result suggested niraparib increases radiosensitivity through G2/M phase arrest in A172 and U87 cells.

**Niraparib Combined With Irradiation Promoted The Redistribution Of Ddx21 From The Nucleolus To The Nucleoplasm**
The expression of DDX21 protein in U87 cells was not changed after treated with 1056nM niraparib alone for 48h. However, the protein expression of DDX21 was only slightly decreased in treated with irradiation (4Gy) alone or niraparib combined with irradiation (Fig. 6A and 6B).

As shown in Fig. 6C, niraparib combined with irradiation could affect DDX21 localization in the nucleus, promoted the redistribution of DDX21 from the nucleolus to the nucleoplasm. This phenomenon was not observed in the niraparib alone or irradiation alone.

**Increased Radiosensitivity Of Ddx21 Knockdown U87 Cells By Niraparib**

The expression of DDX21 in A172, U251 and U87 cells were detected by western blot. As shown in Fig. 7A, DDX21 was not expressed in A172 cells, but highly expressed in U251 and U87 cells. Because of niraparib couldn’t increase radiosensitivity in U251 cells, and DDX21 was faintly expressed in A172 cells, studies of the mechanism of radiosensitization by niraparib was performed only in U87 cells and knockdown of DDX21 expression in U87 cells was carried out by delivering small interfering lentiviral vector (Fig. 7B).

As shown in Fig. 7C and 7D, in the U87 cells or the U87 cells after knockdown of DDX21, the surviving colonies of cells treated with niraparib combined with irradiation significantly lower than cells treated with niraparib or radiation alone. However, in U87 cell after knockdown of DDX21, the colony formation ability was strongly increased in all groups.

**Discussion**

Through bioinformatics analysis, we found that the mechanism of olaparib activity in GBM was mainly involved in the ribosome biosynthesis and protein translation pathway (Fig.1, Supplementary Table 3). Our study found that HR repair ability was positively correlated with ribosome biosynthesis ability in GBM, suggested the HR repair mechanism might participate in ribosome biosynthesis pathway as observed in breast cancer. However, HR repair ability, ribosome biosynthesis, cyclins mRNA expression (CCNE1 and CCNB2) were all significantly negatively correlated with RADzeta (Fig.2A and 2B). These results implied that GBM has high HR repair ability and ribosome biosynthesis ability, which is more likely to cause delayed DNA damage repair after radiation and in turn more sensitive to radiotherapy. The above results suggested that the factors affecting radiosensitivity in GBM may not only be DNA damage repair, but also be related to ribosome biosynthesis mediated by HR repair. In fact, the genes down-regulated in response to irradiation in GBM cell lines U251 and U343 treated with 25Gy X-ray for 24h were mainly involved in small nucleolar RNA (snoRNA), ribosome biosynthesis and ribosome RNA metabolism pathways (Choudhary et al. 2020). Therefore, we speculated that HR repair ability and ribosome biogenesis were closely related to the radiosensitivity of GBM.
It has been confirmed that ribosomal proteins are involved in the nucleolar stress of colorectal cancer cells, DNA replication stress in GBM cells to induce cell cycle arrest and cell apoptosis (Awah et al. 2020; Pecoraro et al. 2019). The identification and functional study of this ribosomal protein can provide further clues for the study of GBM radiosensitivity mechanism. Some research reported PARP1 could participate in ribosome biosynthesis by enriching in nucleoli and affecting ADP ribosylation (ADP Rylation) of nucleolar proteins (Boamah et al. 2012). It has been reported that in breast cancer cells with proficient HR repair ability, the PARP inhibitor niraparib inhibited tumor cell proliferation by inhibiting PARP1 activity and reduced DDX21 ADP ribosylation and ribosome biosynthesis (Kim et al. 2019). Therefore, we speculated that ribosome proteins such as DDX21, S11 and uL3 that are regulated by ADP ribosylation may enhance radiosensitivity treated with PARP inhibitor as a radiosensitizer in GBM.

In our study, we used A172, U251 and U87 cells to study the radiosensitization of PARP inhibitor niraparib and the mechanism of DDX21 involved in radiosensitization, the early results of our study had been reported in AACR Annual Meeting 2022 as wall report (Luo et al. 2022). Firstly, we found that niraparib had radiosensitization in A172 and U87 cells of p53 wild-type at the concentration far below IC$_{50}$ (Fig.4). Niraparib increased radiosensitivity through cell G2/M phase arrest in A172 and U87 cells (Fig.5). In contrast, niraparib had no effect on radiosensitization in U251 cells which harbored p53 mutation. The results suggested that niraparib as a radiosensitizer might be associated with the p53 mutation status of GBM cells. It was previously reported that DDX21 achieved cell cycle arrest by affecting ribosome biosynthesis and regulated p53 function (Koltowska et al. 2021), this may be the reason why niraparib had no radiosensitizing effect in U251 cells. Secondly, the results of bioinformatics analysis showed that PARP inhibitors could also participate in PAR-dependent ribosomal protein transcriptional activation and promote ribosome biosynthesis. Recent studies have shown that when DDX21 localized to nucleolus, activation of PARP1 ADP-ribosylates DDX21 and promoted rDNA transcription, redistribution of DDX21 from nucleolus to nucleoplasm will affect cell ribosomal biosynthesis (Calo et al. 2015; Kim et al. 2019). We further clarify the role of DDX21 which affects ribosome biogenesis in radiosensitivity of niraparib. We found that niraparib combined with irradiation had no significant effect on the expression of DDX21 protein by western blot (Fig.6A and 6B). We evaluated the effect of niraparib combined with irradiation on DDX21 localization by immunofluorescence. We found that niraparib combined with irradiation could promoted the redistribution of DDX21 from the nucleolus to the nucleoplasm (Fig.6C). Therefore, we speculated that niraparib, as a radiosensitizer in U87 cells, affected ribosomal biosynthesis by promoting the redistribution of DDX21 from the nucleolus to the nucleoplasm, resulting in G2/M phase arrest. Thirdly, we knocked down DDX21 in U87 cells (Fig.7B) and found that knockdown of DDX21 in U87 cells with high expression of DDX21 resulted in radiotherapy resistance in all treatment groups (Fig.7C and 7D), which suggested that DDX21 might play a key role in GBM radiotherapy resistance. However, the precise mechanism by which the DDX21 participates in radiosensitivity needs further investigation in more details. Thirdly, niraparib has radiosensitization effect on A172 cells with low expression of DDX21 and U87 cells with knockdown of DDX21 (Fig.4, Fig.7C), the phenomena may imply that niraparib render radiosensitizaion in GBM cells by other proteins that are commonly regulated by ADP ribosylation.
There were also shortcomings in our research. We observed no change in DDX21 expression in U87 cells treated with and without niraparib, and increased DDX21 protein expression in U87 cells treated with niraparib plus irradiation. In combination with bioinformatics analysis results, we tentatively speculated that DDX21 might be involved in radiosensitizing through cell cycle arrest by promoted ribosome biosynthesis in niraparib, this conclusion needs to be confirmed by knockdown of Ribosomal protein in later studies. At the same time, the mechanism of niraparib had a radiosensitivity effect in GBM radiotherapy resistance induced by DDX21 knockdown need to be further studied.

**Conclusion**

Our study proposed for the first time that niraparib had radiosensitization on HR repair intact GBM cells (A172 and U87 cells). Niraparib affected ribosome biosynthesis via redistribution of DDX21 from the nucleolus to the nucleoplasm, caused G2/M phase arrest, thus increased the radiosensitivity of U87 cells. Radiotherapy resistance is an important cause of GBM treatment failure. Our study provided a new therapeutic strategy to address GBM radiotherapy resistance in clinical, it has important clinical significance.

**Declarations**

**Funding**

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**Data availability statement**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Conflict of Interest** None.

**Ethical approval** The protocol of this study was approved by the ethics committee of Daping Hospital of Army Medical University. Ethics approval number is 2022(No. 270).

**References**


Figures
Figure 1

Reactome analysis showing enrichment of rRNA processing. A BRCA B GBM.
Figure 2

Correlation between cyclins, ribosome signature and SF2 in 44 NCI60 cell lines (A) and 529 GBM primary tumor tissues (B). Dotplot showing stepwise correlation among all signatures including RADzeta in 44 NCI60 cell lines (C) and 529 GBM primary tumor tissues (D).
Figure 3

Inhibition rate of A172, U251, and U87 cells by drug concentration of niraparib. A: 24h, B: 48h.
Figure 4

Survival rate of A172, U251 and U87 cells at different doses. A and B: A172 cells, C and D: U251 cells, E and F: U87 cells.
Figure 5

Figure 6

A and B: Niraparib combined with radiotherapy effect the expression of DDX21 in U87 cells by western blot. C Niraparib combined with radiotherapy effect the redistribution of DDX21 from the nucleolus to the nucleoplasm.
Figure 7

A: Expression of DDX21 in A172, U251 and U87 cells. B: DDX21 was knocked down in U87 cells. C and D: Niraparib combined with radiotherapy effect the survival rate of U87 after knockdown of DDX21

Supplementary Files

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- SupplementaryTable.pdf