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Synergistic effect of RAD50 downregulation on combination of rucaparib and doxorubicin

RAD50'nin downregülasyonunun rucaparib ve doksorubisin kombinasyonuna sinerjik etkisi

Ramazan Gundogdu[1] Mehmet Kadir Erdoğan[2] Aydın Sever[2] Yusuf Toy[2]

[1] Vocational School of Health Services, Department of Pharmacy Services, Bingol, Türkiye

[2] Bingol University, Faculty of Arts and Sciences, Department of Biology, Bingol, Türkiye

ABSTRACT

Aim: The MRN (MRE11-RAD50-NBS1) protein complex functions as a DNA damage sensor and plays essential roles to coordinate the repair of DNA double-strand breaks. Although dysfunctional MRN activity has been shown to sensitize cancer cells to certain DNA-damaging agents or PARP inhibitors, the functional significance of RAD50 upon rucaparib and doxorubicin treatments has yet to be studied. The aim of this research was to investigate the response of RAD50-defective cancer cells toward the combination of rucaparib and doxorubicin.

Materials and Methods: Human bone osteosarcoma epithelial cells (U2OS) were used in this study to assess the therapeutic potential of RAD50 expression levels. The RNA interference technology was applied to silence the expression of the RAD50 mRNA activity. The qRT-PCR technique was used to investigate the mRNA expression levels of the relevant genes. Western blotting analysis was conducted to assess the relevant protein expression levels. Clonogenic survival assay was performed to dissect the effect of RAD50-loss on the rucaparib and doxorubicin combination treatment.

Results: RAD50 knockdown resulted in a significant decrease in MRE11 and NBS1 protein levels, whereas it did not affect p53 and p21 expressions at mRNA and protein levels. Furthermore, the cells with RAD50-loss had impaired DNA damage response activation against acute doxorubicin treatment. We finally showed that RAD50 depletion increased the cytotoxicity of doxorubicin when combined with the PARP inhibitor rucaparib.

Conclusion: Taken together, our preclinical findings suggest that RAD50 expression levels can be explored as a predictive biomarker in the evaluation for precision cancer treatments involving PARP inhibitors.

Keywords: RAD50, MRN complex, rucaparib, doxorubicin.

ÖZ

Amaç: *MRN (MRE11-RAD50-NBS1) protein kompleksi bir DNA hasar sensörü olarak işlev görür ve homolog rekombinasyon onarım mekanizması ile DNA çift sarmal kopmalarının onarımının koordine edilmesinde önemli roller oynar. Fonksiyonel olmayan MRN aktivitesinin kanser hücrelerini DNA'ya zarar veren ajanlara veya PARP inhibitörlerine karşı duyarlı hale getirdiği gösterilmiş olsa da, RAD50'nin rucaparib ve doksorubisin tedavileri üzerindeki fonksiyonel önemi henüz araştırılmamıştır. Bu araştırmanın amacı, RAD50 defektif kanser hücrelerinin rucaparib ve doksorubisin kombinasyonuna yanıtının araştırılmasıdır.*

Corresponding author: Ramazan Gundogdu
Vocational School of Health Services, Department of Pharmacy Services, Bingol, Türkiye
E-mail: *rgundogdu@bingol.edu.tr*

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Gereç ve Yöntem: *Bu çalışmada RAD50 ekspresyon seviyelerinin terapötik potansiyelini değerlendirmek için insan kemiği osteosarkoma epitel hücreleri (U2OS) kullanıldı. RAD50 mRNA aktivitesinin ifadesini susturmak için RNA interferans teknolojisi uygulandı. İlgili genlerin mRNA ekspresyon seviyelerini araştırmak için qRT-PCR tekniği kullanıldı. İlgili protein ekspresyon seviyelerini değerlendirmek için Western blot analizi yapıldı. RAD50 kaybının rucaparib ve doksorubisin kombinasyon tedavisi üzerindeki etkisini incelemek için klonojenik sağkalım analizi gerçekleştirildi.*

Bulgular: *Azalan RAD50 ifadesinin, MRE11 ve NBS1 protein seviyelerinde önemli bir düşüşe neden olduğu gözlenirken, p53 ve p21'in mRNA ve protein seviyelerini etkilemediği görüldü. Ayrıca, RAD50 kaybı olan hücrelerin akut doksorubisin tedavisi ile DNA hasar yanıt aktivasyonunu kaybettiği belirlendi. Son olarak, RAD50 susturulmasının PARP inhibitörü olan rucaparib ile birleştirildiğinde doksorubisinin sitotoksisitesini arttırdığı gözlendi.*

Sonuç: *Tüm bu sonuçlar birlikte ele alındığında, klinik öncesi bulgularımız, PARP inhibitörlerinin kanser tedavisinde kullanılmasında RAD50 ekspresyon seviyelerinin prediktif bir biyobelirteç olarak araştırılabileceğini göstermektedir.*

Anahtar Sözcükler: *RAD50, MRN kompleksi, rucaparib, doksorubisin.*

INTRODUCTION

Human cells constantly receive endogenous and/or exogenous genotoxic insults, which potentially cause various structural DNA alterations, including the sugar-phosphate backbone breaks of DNA (1, 2). DNA double-strand breaks (DSBs) are among the most catastrophic lesions, and cells utilize several multi-step molecular pathways to detect and repair these complex damages (2–4). DSBs are primarily repaired by homologous recombination (HR) and non-homologous end-joining (NHEJ) repair pathways (4, 5). Unrepaired DSBs eventually induce tremendous cellular consequences, including cell death or genome instability which is defined as a hallmark of cancer (6–8). Although dysfunctional DSB repair may consequently lead to cellular transformation, novel cancer treatment strategies aim to harness the inefficient DSB repair capacity of cancer cells to offer more specific and potent treatment regimens for cancer patients (9). Therefore, it is of clinical importance to study the underlying molecular mechanisms of dysfunctional DSB repair activities.

The MRN (MRE11-RAD50-NBS1) protein complex functions as a DNA damage sensor. Following DSB formation, MRN is recruited to the vicinity of damaged DNA, where it is mainly responsible for processing the broken DNA ends and activating the ATM (ataxia telangiectasia mutated) kinase (10). The primary function of RAD50 is to tether the two broken DNA ends in close proximity to allow MRE11-driven DNA end resection and subsequent DSB repair through the HR mechanism (11, 12). Among others, the products of *BRCA1* and *BRCA2* genes have significant roles in the HR-dependent DSB repair, and therefore, loss-of-functional mutations in either of these two genes impair the integrity of the HR mechanism (2, 7, 13).

The PARP enzymes have been reported to participate in many cellular processes, including DNA single-strand break (SSB) repair pathways. As members of ADP-ribosyltransferases (ARTs), PARPs are capable of transferring ADP-ribose molecules from NAD+ (nicotinamide adenine dinucleotide) to acceptor proteins in order to recruit the relevant DNA repair proteins towards damaged chromatin by constituting PAR units (polymers of ADP- ribose, a process termed PARylation) (14). Genetic or chemical inactivation of PARP activity leads to the accumulation of spontaneous SSBs, which are mostly converted to complex DSBs during DNA replication (15). Thus, cancer cells with HR deficiency are hypersensitive to PARP inhibition due to intolerable DSB accumulation (16–18). Cancer cells with *BRCA1/2*-deficiency become vulnerable to PARP inhibitors, including rucaparib (19–21). Although clinically-approved PARP inhibitors have been successfully used to treat cancer patients with *BRCA1/2* mutations, research have revealed that PARP inhibition may also be effective against *BRCA1/2*-efficient conditions, which underlines the importance of defining alternative predictive biomarkers for better patient stratifications (18, 22, 23). Considering the PARP inhibitor resistance reported in the clinic (24, 25), research on the characterization of further predictive biomarkers and combination treatments with conventional

drugs will strengthen the anti-cancer effects of PARP inhibitors (26–29).

In this report, we have investigated the functional significance of RAD50 loss and PARP inhibition in combination with chemotherapeutic agent doxorubicin. The research herein demonstrates that the MRN complex is indeed required for a fully functional DDR response upon DNA damage induction. We discovered that U2OS cancer cells with RAD50 knockdown have reduced MRE11 and NBS1 protein levels, and inefficient activation of DDR, as judged by diminished CHK2 and p53 phosphorylations. We further revealed that RAD50 loss sensitizes cancer cells to doxorubicin treatment, which becomes more synergistic when combined with small molecule PARP inhibitor rucaparib. These findings have significant preclinical implications regarding RAD50-dependent DDR response upon combination treatment of rucaparib and doxorubicin.

MATERIALS and METHODS

Chemicals and antibodies

Dimethyl sulfoxide (DMSO) and crystal violet were purchased from Bioshop Canada and Sigma Aldrich, respectively. The primary antibodies for anti-RAD50, anti-p53 and β-Actin were bought from Santa Cruz (#sc-56209, #sc-126 and #sc-1616, respectively); anti-MRE11 and anti-NBS1 were obtained from BD Biosciences (#611366 and #611870, respectively); anti-p21, anti-phospho-p53 (ser15), anti-CHK2 and anti-phospho-CHK2 (thr68) were bought from Cell Signaling Technology (#2947, #9284, #2662 and #2661, respectively). The HRP(horseradish peroxidase)-linked secondary antibodies against anti-rabbit, anti-rat and anti-mouse were purchased from GE Healthcare (#GENA934, #GENA935 and #GENA931, respectively), and anti-goat were obtained from Santa Cruz (#sc2056). Rucaparib phosphate (AG-014699) and doxorubicin hydrochloride were purchased from Selleckchem (#S1098) and Sigma (#D1616), respectively.

Cell culture

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Trypsin-EDTA (0.25%) and Dulbecco's phosphate buffered saline (dPBS) were purchased from Gibco (Thermo Fisher Scientific). Penicillin and streptomycin were purchased from Sigma Aldrich and Bioshop Canada, respectively. U2OS osteosarcoma cancer cell line was gently provided by Dr Alexander Hergovich (UCL, London, UK) and maintained in DMEM supplemented with 10% fetal bovine serum with 1% antibiotic (final concentration of 64 µg/mL penicillin and 100 µg/mL Streptomycin). Cells were grown in humidity-saturated cell culture incubators at 37°C with 5% CO_2. Stock solutions of rucaparib and doxorubicin were prepared in DMSO and stored at -80°C. Drug/inhibitor treatments were performed as indicated in the corresponding figure legends.

Cell transfection

Lipofectamine RNAiMAX transfection reagent was bought from Thermo Fisher Scientific (#13778150). Reduced serum medium (OPTI-MEM) was purchased from Gibco (Thermo Fisher Scientific). All siRNAs were from Qiagen (FlexiTube GeneSolution GS10111 for RAD50) and sequences are available upon request. Briefly, U2OS cells plated at a density of 20,000 cells/cm^2 in the antibiotic-free medium were transfected with final concentration of 5 nM siRNA using Lipofectamine RNAiMAX. All the experiments were performed 48h after siRNA transfection unless otherwise stated. The efficacy of all siRNA transfections was confirmed by Western blot analysis.

qRT-PCR

Total RNA isolation was performed at room temperature using TRIzol® Reagent (Invitrogen) as instructed by the manufacturer. Briefly, TRIzol (for 6-cm plates) was applied to cells, which were collected into a microcentrifuge tube by using a cell scraper. Chloroform was then added into collected samples to separate RNA from other cellular contents. After the resulting mixture had been centrifuged at 10,000 rpm for 15 minutes at 4°C, the upper aqueous phase was transferred into a microcentrifuge tube, which was mixed with isopropanol to precipitate RNA. The samples were incubated for 10 minutes at room temperature and then centrifuged at 10,000 rpm for 10 minutes at 4°C. Consequently, the pellets were washed with ethanol at room temperature and centrifuged again at 8,000 rpm for 5 minutes at 4°C. The RNA pellet was dissolved in nuclease-free water and incubated at 55-60°C for 10 minutes. cDNA synthesis was performed using iScript One-Step RT-PCR Kit (Bio-Rad) according to the manufacturer's protocol. qPCR was performed using verified qPCR primers (Qiagen) and the QuantiTect SYBR Green PCR

Kit (Bio-Rad) using the Mastercycler system (Eppendorf, Germany). 18S rRNA was employed as internal control for standardization.

Western blotting

The cell pellets were incubated in standard lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 1% NP-40, 5 mM EDTA, 0.5 mM EGTA, 50 mM NaF, 20 mM beta-glycerophosphate, 1 mM Na_3VO_4, 1 mM DTT, 1 mM benzamidine, 0.5 mM PMSF, 1 mM leupeptin, pH 8.0) on ice for 90 minutes. After centrifugation at 10,000 rpm for 20 minutes at 4°C, the soluble protein fractions were transferred into microcentrifuge tubes. Laemmli SDS sample buffer (1 mM Tris-HCl, 0.05% SDS, 5% beta-mercaptoethanol, 10% bromophenol blue) was added to the total cell lysates and the mixtures were heated at 95°C for 5 minutes. Proteins were resolved by 12% SDS-PAGE before being transferred to a PVDF membrane. Membranes were blocked with 5% skim milk prepared in TBS-T (50 mM Tris, 150 mM NaCl, 0.5% Tween-20, pH 7.5) and then incubated with the corresponding antibody overnight. The protein-antibody complex was then probed by secondary antibodies conjugated with HRP and finally subjected to ECL (Amersham) substrates for chemiluminescent detection. Densitometry analysis of Western blots was conducted using the NIH ImageJ program.

Colony survival assays

Clonogenic survival assays were performed as described in (30, 31). Briefly, 500 of exponential phase cells were seeded in 6-well plates and allowed to adhere for 24 hours, before being treated with rucaparib with/out doxorubicin for 72 hours. Cell medium was refreshed every 3-4 days until each colony has more than 50 cells (7-10 days). Colonies were first fixed with methanol and acidic acid solution (3:1), then stained with 0.5% crystal violet dissolved in methanol. All survival experiments were conducted in triplicate with three independent experiments.

Statistical Analysis

Graphics and statistical analyses were acquired by GraphPad Prism software (GraphPad, CA, USA) and results were plotted displaying the means ± SEM. The significance of differences between the means was determined using one-tailed unpaired Student's t-test. Differences were considered statistically significant when p- values were below 0.05 (*), 0.01 (**), 0.001 (***) or 0.0001 (****) for all experiments.

RESULTS

RAD50 knockdown by RNA interference

In our experiments we used U2OS osteosarcoma cancer cells since they have been widely used as a suitable cell model for identification of molecular and biological significance of related DDR proteins (32). The efficacy of four different siRNA constructs targeting RAD50 mRNA in U2OS cells was evaluated by transient transfection. Cells were harvested at 24h, 48h and 72h after transfection and subjected to Western blot analysis (Figure-1A). Densitometric analyses of immunodetected bands revealed that all siRNA oligonucleotides but siRAD50#1 effectively silenced the RAD50 mRNA at 48h after transfection (Figure-1B). We selected siRAD50#2 for our further siRNA-based gene knockdown experiments.

We next aimed to investigate whether RAD50 loss would have any effect on the protein levels of MRE11 and NBS1, other two components of the MRN complex. In line with the previous studies (33), we showed that RAD50 depletion causes reduced MRE11 and NBS1 protein levels in U2OS cells (Figure-1C,D), suggesting MRN deficiency. In other words, siRNA-based RAD50 targeting is likely to impair the conformation and stability of the MRN complex since RAD50 is large structural component of the heterotrimeric complex. p53 is a tumour suppressor protein that is activated upon DNA damage induction and further activates a set of genes at the transcriptional and protein levels to determine the cell faith (34). As a transcriptional target of p53, p21 protein plays a key role in cell cycle arrest in response to endogenous and exogenous DNA damages (35, 36). Therefore, we also assessed whether RAD50 loss would have any effect on the p53 and p21 mRNA and protein levels. RAD50 depletion did not affect p53 and p21 protein levels (Figure-1C,D) and our qRT-PCR experiments revealed that RAD50 targeting (Figure-1E) did not alter transcriptional profile of both genes (Figure-1F,G).

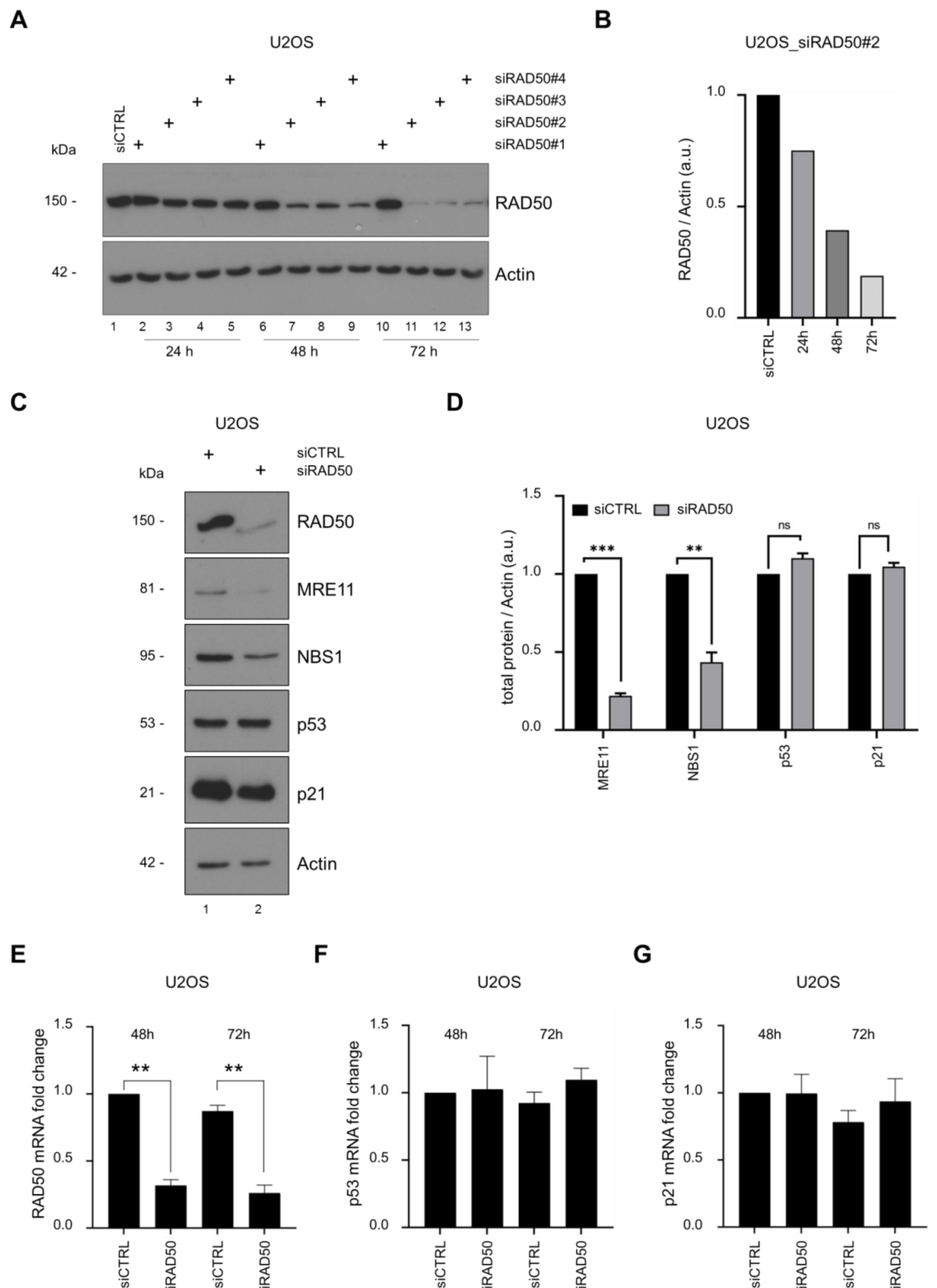

Figure-1. RAD50 knockdown impairs the MRN stability by inhibiting MRE11 and NBS1 expressions. (A) Western blotting with indicated antibodies of U2OS cell lysates from cells transfected with indicated siRNAs in order to investigate the silencing efficacy of four different siRNA oligonucleotides against RAD50 mRNA. (B) histogram showing the time-dependent silencing efficacy of the siRAD50#2 construct, obtained by densitometric quantification of Western blots represented in A. Arbitrary units were normalized to the expression of the corresponding β-actin (n=1). (C) Western blotting with indicated antibodies of U2OS cell lysates from cells transfected with indicated siRNA. (D) histogram showing the expression profile of indicated proteins, obtained by densitometric quantification of Western blots represented in D. Arbitrary units were normalized to the expression of the corresponding β-actin (n=3). (E,F,G) qRT-PCR analysis of the RAD50, p53 and p21 genes in U2OS cells with/out siRAD50#2 at indicated time points (n=3).

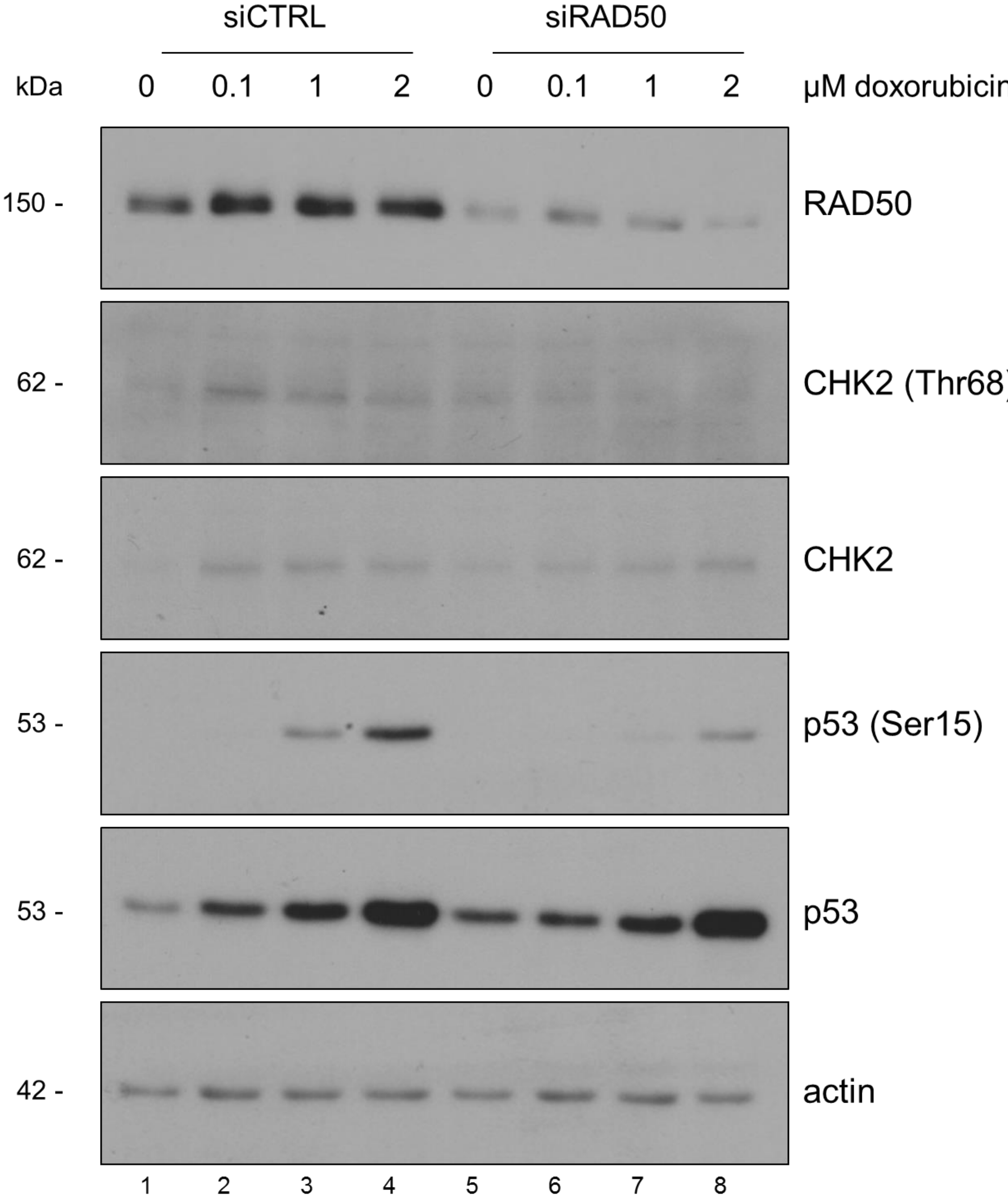

Figure-2. RAD50 supports DDR activation upon exogenous DNA damage exposure. (A) Western blotting with indicated antibodies of U2OS cell lysates from cells transfected with indicated siRNAs and treated with/out doxorubicin. 48h after siRNA transfection cells were treated either with DMSO or doxorubicin (0 - 0.1 - 1 - 2 μM) for 1h, then collected and subjected to Western blot analysis. (B,C) histograms showing the expression profile of indicated proteins, obtained by densitometric quantification of Western blots represented in D. Arbitrary units were normalized to the expression of the corresponding total protein (n=3).

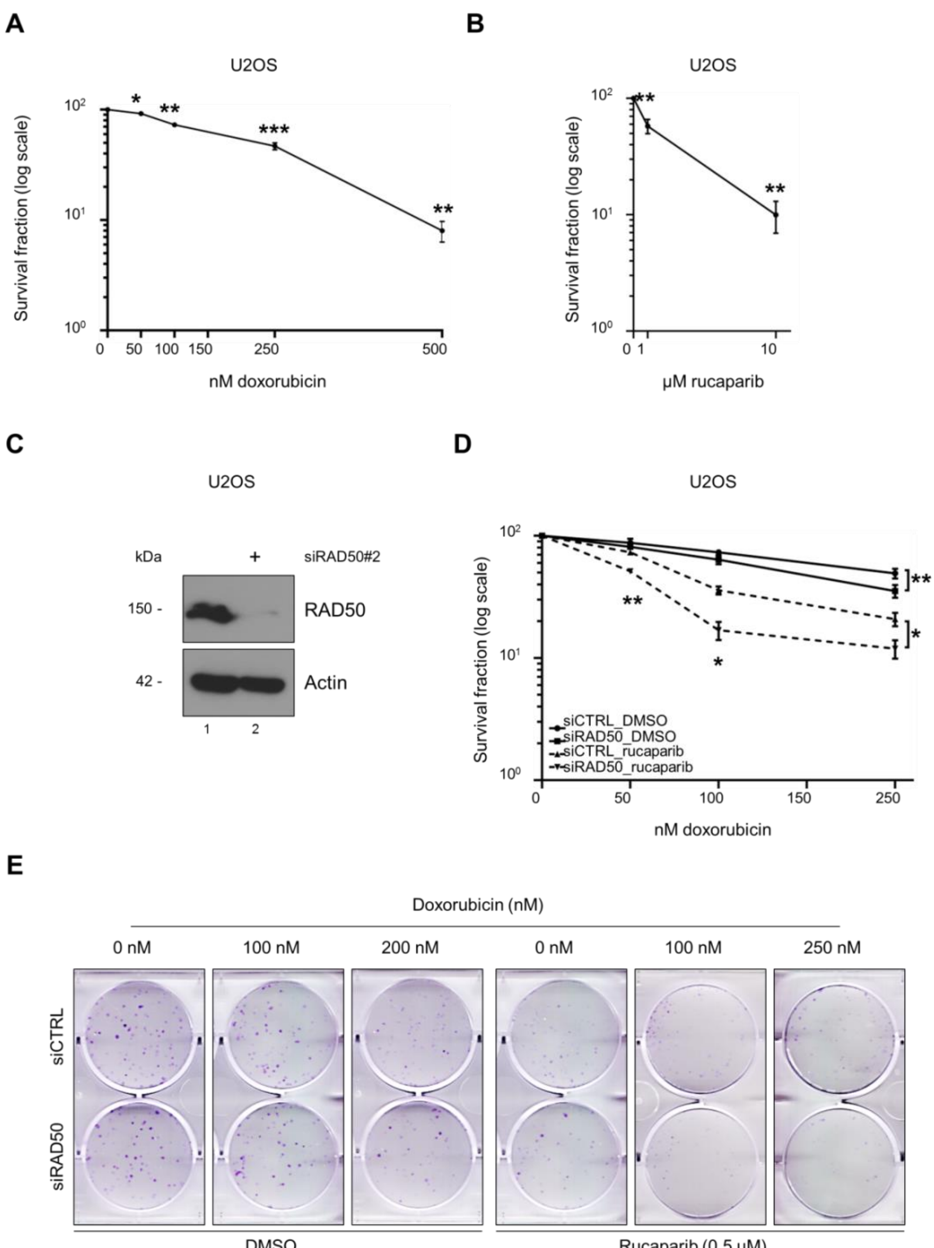

Figure-3. RAD50 loss sensitizes cancer cells to the rucaparib-doxorubicin combination treatment. (A,B) Clonogenic survival of U2OS cells in response to doxorubicin treatments (0 - 50 - 100 - 250 - 500 nM) or rucaparib treatments (0 - 1 - 10 μM) for 3 days. Quantifications are displayed as percentage (in log scale) of colonies formed after treatment with indicated doses (n=3). Results were corrected according to plating efficiencies of the corresponding untreated control. (C) Western blotting with indicated antibodies of U2OS cell lysates from cells transfected with indicated siRNAs. 24h after siRNA transfection cells were harvested and re-plated for either clonogenic survival assays or Western blot analysis. 24h after plating cells were collected and subjected to Western blot analysis to confirm the successful RAD50 silencing. (D) Clonogenic 5 survival of U2OS cells upon RAD50 knockdown (siRAD50#2) compared with controls in response to the increasing doses of doxorubicin alone (0 - 50 - 100 - 250 nM) or combined with rucaparib (0.5 μM). Quantifications are displayed as percentage of colonies formed after treatment with indicated doses (n=3). Results were corrected according to plating efficiencies of the corresponding untreated controls. (E) Representative images of the clonogenic survival assays shown in D.

RAD50 loss impairs the DDR activation

The DDR signaling pathway becomes activated in the case of a DSB induction in the chromatin. The heterotrimeric MRN complex detects the damage and controls the management of the proper repair. The inactive ATM kinase dimers are activated by an auto-phosphorylation and becomes active monomers, which then target and phosphorylate CHK2 kinases at Thr68. ATM kinases directly or through CHK2 kinases stimulate the activation of downstream effector proteins including p53 phosphorylation at Ser15 (2, 37, 38). Since these events are indispensable for a proper DDR signaling upon DNA damage, we next analyzed the DDR integrity in RAD50-defective cancer cells in response to acute doxorubicin treatment. 48h after transfection with siRAD50 there was no change in expressions of p53 and p21 (Figure-1C and D), therefore, 48h after transfection U2OS cells were treated with increasing doses of doxorubicin (0 - 0.1 - 1 - 2 µM) for 1h and collected for Western blot analysis. As shown in Figure-2, siRNA-based targeting successfully silenced the RAD50 protein expression. Remarkably, compared to the relevant total protein expressions, Western blot assays using phospho-specific antibodies revealed a significant decrease in the phosphorylation of CHK2 and p53 proteins (Figure-2), suggesting that cells with RAD50 knockdown have a defective DDR signaling upon exogenous DNA damage.

RAD50 loss sensitizes U2OS cancer cells to rucaparib combined with doxorubicin

To investigate the biological significance of our previous results we performed *in vitro* clonogenic survival assays with U2OS cancer cells. Doxorubicin is an anthracycline antibiotic widely used as an anti-neoplastic agent to treat patients in the clinic (39). The small molecule PARP inhibitor rucaparib is a clinically-approved novel anti-cancer drug for the treatment of cancer patients with *BRCA*-mutations (21). Recently published reports demonstrated that rucaparib treatment augmented anti-cancer effects of conventional chemotherapeutics, including doxorubicin (21). Since RAD50 knockdown impairs the MRN complex stability by affecting MRE11 and NBS1 expressions, and probably, therefore, disrupts the functionality of DDR signaling in response to exogenous damage induction, we hypothesized that RAD50 deficiency would render cancer cells vulnerable to doxorubicin and rucaparib combination. First, we determined the individual cytotoxic effects of doxorubicin and rucaparib treatments in U2OS cells, revealing that the 72h-treatment with 250 nM doxorubicin or 1 µM rucaparib killed nearly half of the cells (Figure-3A and B, respectively). Therefore, 48h after transfection (Figure-3C) cells were exposed to increasing doses of doxorubicin (0 - 50 - 100 - 250 nM) with/out rucaparib (0.5 µM) for 72 hours. RAD50-depleted cancer cells became significantly sensitive to high dose of doxorubicin (250 nM) whereas rucaparib combination synergistically potentiated the cytotoxic activity of doxorubicin in the cells (Figure-3D and E). Taken together, our results suggest that cancer cells with decreased RAD50 levels may respond better to the combination treatment of PARP inhibitors with conventional anti-cancer agents.

DISCUSSION

The common mechanism of action that chemotherapeutics employ is introducing genotoxic stress and activating programmed cell death mechanisms to eliminate rapidly dividing neoplastic cells from the proliferation pool (40). Although these conventional anti-cancer agents have been successfully used to treat cancer patients in the clinic, their lack of specificity impedes their clinical success. As standard chemotherapeutics target both rapidly proliferating normal and cancer cells, they cause adverse side effects frequently observed in the patients. Another success-limiting factor of cancer treatments is acquired chemotherapy resistance, mostly appearing as a result of altered DDR capacity (41, 42). Therefore, identifying the biological significance of cellular proteins especially involved in the DDR and repair pathways, including ATR, ATM, CHK1, CHK2, DNA-PK and WEE1 has attracted great attention in recent years (9,26,40,43,44). The status of regulator DDR proteins may enable us to predict the treatment response since neoplastic cells can be addicted to their altered activity for survival. In addition, pharmacological inhibition of their augmented activity may render neoplastic cells sensitive to standard anti-cancer therapies (26, 27, 44). The synthetic lethality defined between PARP and BRCA proteins stands as a milestone achievement among the DDR-targeted anti-cancer treatment strategies (45). Inhibition of PARP activity causes

accumulation of SSBs, which become DSBs during replication. PARP inhibition is specifically cytotoxic to cancer cells with dysfunctional or inefficient HR activity since they are unable to resolve these replication-associated DSBs. Therefore synthetic lethality concept offers selective cancer cell killing without harming healthy ones (45–47).

The MRN DNA damage sensor complex instantly detects DSB formations and activates the corresponding DDR pathways (48). *MRE11* and *NBS1* genes are mostly mutated in human cancers, leading to heritable genomic instability disorders ataxia-telangiectasia-like disorder (ATLD) and Nijmegen-breakage syndrome (NBS), respectively. Functional inactivation of both genes may lead to cellular hypersensitivity to ionizing radiation (IR) and transformation (49,50). RAD50 is a member of the SMC (structural maintenance of chromosome) protein family, which have been identified to function in supporting sister-chromatid cohesion and chromosome condensation in cells (10). The ATPase domain of RAD50 holds an N-terminal Walker A and a C-terminal Walker B motifs, both of which are required for its activities to bind and partially unwind DNA double helix strands upon DSB induction (51,52). Koppensteiner and her colleagues identified MRE11 expression as a candidate biomarker of PARP inhibitor treatment (53). A recent whole-exome deep sequencing study demonstrated that the *RAD50* gene copy number deletion could be used as a potential biomarker to predict PARP inhibitor response of ovarian cancer patients (54). Flores-Pérez et al. revealed that RAD50 supports DDR and breast cancer cell survival against cisplatin treatment (55). Furthermore, RAD50 targeting was shown to sensitize cancer cells to platinum drugs (56, 57). However, to our knowledge, there is no direct demonstration of whether RAD50 loss renders cancer cells vulnerable to rucaparib combined with DNA-damaging agents.

We define herein the functional consequence of RAD50 loss in survival of cancer cells encountered with exogenous therapeutic stress. Our immunoblotting analyses showed that RAD50 knockdown precludes the integrity of the MRN complex since the MRE11 and NBS1 protein levels were significantly decreased in RAD50-defective U2OS cancer cells, suggesting an inefficient HR activation upon RAD50 loss. Given that MRN activation and recruitment towards damaged chromatin is important for the activation of ATM kinase and corresponding signaling axis, compromised CHK2 and p53 activation upon RAD50 knockdown would consolidate our findings that RAD50 loss reduces MRN activity therefore, negatively affects the ATM-CHK2-p53 pathway activation. Our clonogenic survival assays with U2OS cells revealed that RAD50 depletion renders cancer cells sensitive to the doxorubicin and rucaparib combination treatment. Doxorubicin inhibits the topoisomerase II activity, thus mostly inducing DSBs (58). Although RAD50 depletion sensitizes cancer cells to doxorubicin treatment alone, rucaparib combination potentiates the chemotoxicity of doxorubicin in RAD50-defective cells. Considering the synthetic lethal interaction between the BRCA-dependent HR and the PARP-dependent SSB repair mechanisms (19, 20), one may argue that RAD50 knockdown results in impaired MRN functionality in HR, which in turn weakens the cellular response of PARP-inhibited cancer cells towards chemotherapeutics. A recent report revealed that nearly 18% of ovarian cancer patients with wild-type *BRCA* exhibit RAD50 deletion (54). Together with several recent preclinical studies showing that RAD50 silencing sensitized various tumour cells to anti-cancer treatments (55–57,59,60), our results suggest RAD50 for further evaluation as a potential biomarker of PARP inhibitor treatments.

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Declaration of interest statement

The author declares no conflict of interest.

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