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Specific inhibition of Wee1 kinase and Rad51 recombinase: A strategy to enhance the sensitivity of leukemic T-cells to ionizing radiation-induced DNA double-strand breaks

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ABSTRACT

Present-day oncology sees at least two-thirds of cancer patients receiving radiation therapy as a part of their anticancer treatment. The objectives of the current study were to investigate the effects of the small molecule inhibitors of Wee1 kinase II (681641) and Rad51 (RI-1) on cell cycle progression, DNA double-strand breaks repair and apoptosis following ionizing radiation exposure in human leukemic T-cells Jurkat and MOLT-4. Pre-treatment with the Wee1 681641 or Rad51 RI-1 inhibitor alone increased the sensitivity of Jurkat cells to irradiation, however combining both inhibitors together resulted in a further enhancement of apoptosis. Jurkat cells pre-treated with inhibitors were positive for γH2AX foci 24 h upon irradiation. MOLT-4 cells were less affected by inhibitors application prior to ionizing radiation exposure. Pre-treatment with Rad51 RI-1 had no effect on apoptosis induction; however Wee1 681641 increased ionizing radiation-induced cell death in MOLT-4 cells.

1. Introduction

Exposure to ionizing radiation (IR) damages cells directly by interacting with DNA and other critical cellular targets or indirectly by generating reactive oxygen species. Among various types of DNA damage, the DNA double-strand breaks (DSBs) are assumed to be the most important for the therapeutic effect mediated by IR [1]. Unfortunately, the resistance of cancer cells to IR is currently considered one of the main limiting factors restricting treatment efficacy.

One of the described biological factors discriminating the sensitivity of cells to anticancer therapy is p53, a well-established and frequently mutated tumour suppressor in human cancers [2]. The loss of the p53 function is generally recognized as an adverse prognostic factor in human cancers. Whereas p53 wild-type cells repair damaged DNA preferentially during G_1 and/or G_2-arrest, cells lacking a functional p53 would not be anticipated to demonstrate a G_1 checkpoint and would preferentially depend on the G_2 checkpoint to permit DNA repair prior to undergoing mitosis [3].

Cdk1-cyclin B (cyclin dependent kinase 1) is a member of cyclin-dependent kinases implicated in cell cycle control. Cdk1-cyclin B acts as a serine/threonine protein kinase composed of the catalytic subunit Cdk1 and its positive regulatory subunit cyclin B. At the G_2/M boundary, Wee1 kinase acts as a negative regulator of Cdk1 kinase activity and as a major kinase phosphorylating Cdk1 on tyrosine 15. The action of the Wee1 kinase is opposed by dephosphorylation mediated by Cdc25 phosphatases in a late G_2 phase before mitosis [4].

To evade cell death following anticancer treatments, cell cycle progression of cancer cells must be delayed to provide enough time for DNA repair, which prevents damaged cells from entering mitosis. It is well known that DSBs caused by IR or other chemotherapy drugs are repaired by non-homologous end-joining (NHEJ), which occurs predominantly in G_1 and pre-S, and by homologous recombination repair (HR), which occurs prior to mitosis in late S and G_2 where DNA templates with perfect homology are available. Eukaryotic recombinase Rad51 seems to be a crucial protein of HR, which forms helical nucleoprotein filaments on the 3′-tail of ssDNA to coordinate homology search and DNA strand invasion. In response to DNA damage, Rad51 is translocated to the nucleus where it sequesters into foci together with other proteins involved in HR [5].

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Since Wee1 kinase delays the cell cycle at the G_2 boundary by adding a negative phosphorylation on Cdk1 and Rad51 recombinase acts as a central catalyst of HR for premitotic DNA repair, its inhibition could represent an excellent target for the sensibilization to anticancer treatments. The aim of the study was to assess the cytotoxic and apoptosis-inducing effects of ionizing radiation, the specific inhibitor of Wee1 kinase 681641, the novel inhibitor of Rad51 recombinase RI-1 and its combination on Jurkat (p53 deficient) and MOLT-4 (p53 wild-type) leukemic cell lines.

2. Materials and methods

2.1. Cell cultures and culture conditions

The experiments were carried out with the Jurkat (p53 mutant E6.1) and MOLT-4 (p53 wild-type) cell lines from the European Collection of Cell Cultures (ECACC, Salisbury, UK). Jurkat cells were propagated in RPMI 1640 medium supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 1 mM pyruvate, 10 mM HEPES, MEM Non-Essential Amino Acids 10 μL/mL, 50 μg/mL penicillin and 50 μg/mL streptomycin (all reagents from Life Technologies, Grand Island, NY, USA). MOLT-4 cells were cultured in RPMI 1640 medium supplemented with 20% foetal calf serum, 2 mM L-glutamine, 1 mM pyruvate, 10 mM HEPES, MEM Non-Essential Amino Acids 10 μL/mL, 50 μg/mL penicillin and 50 μg/mL streptomycin (all reagents from Life Technologies, Grand Island, NY, USA). Jurkat and MOLT-4 cells in the maximum range of 20 passages and in an exponential growth phase were used for this study.

2.2. Wee1 kinase and Rad51 recombinase inhibitor treatment

The specific Wee1 kinase inhibitor II 681641 and Rad51 inhibitor II RI-1 (both from Merck, Darmstadt, Germany) were dissolved in dimethyl sulfoxide – DMSO (Sigma–Aldrich, St. Louis, MO, USA). For the experiments, the stock solutions were diluted using a complete culture medium creating final concentrations of 5 μM for Wee1 kinase inhibitor II 681641 and 15 μM for Rad51 inhibitor II RI-1. Inhibition experiments were performed as previously described for Wee1 kinase inhibitor II 681641 [6] and Rad51 inhibitor II RI-1 [7]. Both inhibitors were applied 60 min before irradiation, making sure the concentration of DMSO is <0.1% to avoid the toxic effects on examined cells. Control cells were treated with a DMSO vehicle only (0.1%; Control).

2.3. Gamma-irradiation

Aliquots of cell suspension in concentration of 1×10^5/mL were plated into 25 cm^2 flasks (TPP, Trasadingen, Switzerland) and were irradiated at room temperature using a ^{60}Co γ-ray source (Chisotron Chirana, Prague, Czech Republic) at a distance of 1 m from the source with a photon dose-rate of 1 Gy/min. Dosimetry was performed using an ionization chamber (Dosemeter PTW Unidos 1001, Serial No. 11057, with Ionization Chamber PTW TM 313, Serial No. 0012; RPD Inc., USA); the set was validated by the Czech Metrology Institute (CMI) – Inspectorate for Ionizing Radiation (IIR), Protocol No. 9011-OL-U4124/2005). After irradiation by 2 Gy and 4 Gy (Jurkat) or 1 Gy and 2 Gy (MOLT-4), flasks were incubated in 5% CO_2 atmosphere at 37 °C. Non irradiated control cells were handled in the same way, except that irradiation was omitted.

2.4. Cell viability

Cell viability was tested using the colorimetric XTT II cell proliferation and viability assay kit (Roche, Basel, Switzerland). For each condition, 100,000 cells were seeded in a 96-well plate at 100 μl per well. The labelling reagent was added to the electron-coupling reagent in a 1:50 ratio. Fifty millilitres of the XTT reagent were then added to each well in a 96-well plate. The plates were than incubated at 37 °C for 2 h. Following incubation, the plates were spectrophotometrically analysed using microplate reader Tecan Infinite M200 at a wavelength of 480 nm (Tecan Group Ltd., Männedorf, Switzerland). Cell viability experiments were performed on three separate occasions. Viability was calculated as described in the paper by Havelek and colleagues using the following formula: (%) viability = (A480sample − A480blank)/(A480control − A480blank) × 100, where A480 is the absorbance of utilized XTT formazan measured at 480 nm [8].

2.5. Analysis of apoptosis

Apoptosis was determined by flow cytometry using an APOPTEST™-FITC kit (Dako, Glostrup, Denmark) according to the manufacturer's instructions. Measurement was performed immediately using a CyAn™ ADP (Beckman Coulter, Miami, FL, USA) flow cytometer. Listmode data were analysed using Summit v4.3 software (Beckman Coulter, Miami, FL, USA).

2.6. Cell cycle analysis

For the analysis of cell cycle distribution, the cells were washed with ice cold PBS and fixed with 70% ethanol. For detection of low molecular-weight fragments of DNA, the cells were incubated for 5 min at room temperature in a buffer (192 mL 0.2 M Na_2HPO_4 + 8 mL of 0.1 M citric acid, pH 7.8) and then labelled with propidium iodide in Vindelov's solution for 1 h at 37 °C. The DNA content was determined using the flow cytometer Dako CyAn (Beckman Coulter, Miami, FL, USA) with an excitation wave length of 488 nm. The data were analysed using Multicycle AV software (Phoenix Flow Systems, San Diego, CA, USA).

2.7. Immunofluorescence staining, epi-fluorescence and confocal microscopy

For each condition, 100,000 cells were loaded on poly-L-lysine-coated glass slides and fixed with 4% freshly prepared paraformaldehyde for 10 min at room temperature, washed with PBS, permeabilized in 0.2% Triton X-100/PBS for 15 min at room temperature, and washed with PBS (reagents from Sigma–Aldrich, St. Louis, MO, USA). Before incubation with primary antibodies (overnight at 4 °C), the cells were incubated with 7% inactivated foetal calf serum + 2% bovine serum albumin in PBS for 30 min at room temperature. Rabbit monoclonal anti-phospho-histone H2AX Ser139 (Cell Signaling, Danvers, MA, USA) and mouse anti-53BP1 antibody (Millipore, Billerica, MA, USA) were used for γH2AX and 53BP1detection. For the secondary antibody, the affinity pure donkey anti-rabbit-FITC-conjugated and affinity pure donkey anti-mouse-tetramethylrhodamine (TRITC)-conjugated antibody was purchased from the Jackson ImmunoResearch Laboratories (West Grove, PA, USA). The secondary antibody was applied to each slide (after their pre-incubation with 5.5% donkey serum in PBS for 30 min at room temperature), incubated for 1 h in the dark and washed (3 × 5 min) with PBS. The slides were mounted with an antifading polyvinyl alcohol mounting medium with DABCO® (Sigma–Aldrich, St. Louis, MO, USA). Images of all of the examined slides were obtained by a Nikon Eclipse 80i fluorescence microscope; the exposure time and dynamic range of the camera in all the channels were adjusted to the same values for all the slides to portray quantitatively comparable images. High-resolution imaging of single isolated cell nuclei was performed by means of the Nikon $C2^+$ confocal microscope system.

Images were further processed and merged using NIS-Elements Advanced Research 4.13 (all instruments and software from Nikon, Tokyo, Japan).

2.8. Western blot analysis

Whole-cell lysates (Cell Lysis Buffer, Cell Signaling Technology, Danvers, MA, USA) were prepared 24 h following irradiation of Jurkat and MOLT-4 cells treated with or without inhibitors, and quantification of the protein content was performed using the BCA assay (Sigma–Aldrich, St. Louis, MO, USA). The lysates (20 μg purified protein) were loaded into each lane of a polyacrylamide gel. After electrophoretic separation, the proteins were transferred to a PVDF membrane (Bio-Rad, Hercules, CA, USA). Non-specific binding of the membranes was blocked for 1 h in a Tris-buffered saline containing 0.05% Tween 20% and 5% non-fat dry milk. The membranes were washed in TBS/T. Incubation with a primary antibody against specific antigens (p53 – Exbio, Prague, Czech Republic; β-actin, $p21^{WAF1/Cip1}$, $p16^{INK4A}$ – Sigma–Aldrich, St. Louis, MO, USA; Chk1, Chk1_serine 345, Chk2, Chk2_threonine 68 – Cell Signaling, Danvers, MA, USA) was performed at 4 °C overnight. The following day the membranes were washed 5-times with TBST, each time for 5 min, and once with TBS, for 10 min, and then incubated with an appropriate secondary antibody (DakoCytomation, Glostrup, Denmark) for 1 h at room temperature. Band detection was performed using a chemiluminescence detection kit (Roche, Basel, Switzerland). To ensure equal protein loading, each membrane was reprobed and β-actin was detected.

2.9. Statistical analysis

The descriptive statistics of the results were calculated and the charts were made in Microsoft Office Excel 2003 (Microsoft, Redmond, WA, USA) or GraphPad Prism 5 biostatistics (GraphPad Software, La Jolla, CA, USA). In this study, all the values were expressed as arithmetic means with S.D. of triplicates unless otherwise noted. The significant differences between the groups were analysed using the Student's *t*-test and *P* values $\leqslant 0.05$ were considered statistically significant.

3. Results

3.1. Wee1 681641 and Rad51 RI-1 inhibitors reduced Jurkat and MOLT-4 cell survival following irradiation

Using cell viability assay we investigated a dose and time-depended decrease in Jurkat cell viability in response to IR as measured 24 h and 72 h after irradiation. However, in the later examined interval of 144 h Jurkat cells irradiated by 2 Gy recovered and their viability started to increase (Supplementary Fig. 1). Similarly, the viability of MOLT-4 cells decreased with increasing time (24 and 72 h) after irradiation excepting dose of 1 Gy. At 144 h after irradiation MOLT-4 cells exposed to both examined doses of radiation recovered and started to increase in viability (Supplementary Fig. 2). Combining Wee1 681641 and Rad51 RI-1 with IR significantly reduced cell survival for both Jurkat and MOLT-4 cells. Application of the Wee1 681641 inhibitor followed by exposure to IR decreased survival in both cell lines more intensively than Rad51 RI-1. At 144 h MOLT-4 cells pre-treated in combination with Wee1 681641, Rad51 RI-1 and IR by 1 Gy started to increase in viability, whereas cells irradiated by 2 Gy not. Contrary thereto, Jurkat cells irradiated in the presence of Wee1 681641 and Rad51 RI-1were 144 h later completely eradicated from the culture either of irradiating with 2 or 4 Gy.

3.2. Wee1 681641 and Rad51 RI-1 inhibitors sensitizes human Jurkat and MOLT-4 leukemic T-cells to ionizing radiation

Ionizing radiation exposure stand-alone induced a statistically significant dose dependent increase in the numbers of early and late apoptotic cells, which was obviously more pronounced in MOLT-4 cells in contrast to Jurkat cells. Although Jurkat cells displayed radioresistance compared to MOLT-4, Jurkat pre-treatment with Wee1 inhibitor II 681641 and irradiated significantly decreased the survival rate ($P < 0.05$) compared to IR alone. Application of the RI-1 inhibitor caused lower radiosensitization of Jurkat cells in contrast to 681441 in parallel experiments. Combining both inhibitors statistically significantly ($P < 0.05$) increased sensitivity to IR in terms of the cell death of Jurkat leukemic cells, except the dose of 4 Gy 24 h after irradiation when compared with inhibitors as monotherapy agents (Figs. 1A–D). Having demonstrated the effect of both inhibitors on Jurkat cells, we then investigated the response MOLT-4 cells. Unexpectedly, combining Wee1 inhibitor II 681641 with IR significantly ($P < 0.05$) enhanced the apoptosis of MOLT-4 cells during both analysed intervals. Although simultaneous treatment with the Wee1 inhibitor and Rad51 inhibitor followed by irradiation caused the highest apoptosis between the studied groups, the effect of Rad51 inhibitor RI-1 was moreover additive in comparison to the effect of Wee1 inhibitor II 681641 in monotherapy. MOLT-4 cells were not affected by Rad51 RI-1 treatment in contrast to cells irradiated alone (Figs. 2A–D).

3.3. Wee1 681641 and Rad51 RI-1 inhibitors pre-treatment lead to G_2/M cell cycle arrest abrogation in irradiated Jurkat cells

Irradiation of Jurkat itself caused statistically significant G_2 cell cycle arrest, which was dose-dependent. In parallel experiments both inhibitors reduced the proportion of cells in the G_2 phase of the cell cycle and increased the proportion of cells in the G_1 phase relative to irradiated cells. Furthermore, the simultaneous combination of both inhibitors in pre-treatment almost totally abrogates the G_2/M checkpoint induced upon exposure to 2 Gy of gamma radiation (Fig. 3A and Supplementary Fig. 3A). To the best of our knowledge, this is the first report describing the capability of RI-1 to abrogate IR-induced cell cycle arrest in the G2 phase of p53-deficient cells.

3.4. Wee1 681641 inhibitor pre-treatment increases the proportion of MOLT-4 cells in the G_2/M phase of the cell cycle in comparison to cells irradiated alone

IR itself caused significantly lower increase in G_2-phase MOLT-4 cells compared to substantial a dose-dependent increase in G_2 accumulation in Jurkat cells. In irradiated MOLT-4 cells, application of the Wee1 inhibitor resulted in an increase in the percentage of cells in the G_2 phase of the cell cycle compared to irradiated cells. Exposure to Rad51inhibitor RI-1or Wee1 and RI-I in combination followed by irradiation had a less dramatic effect on the cell cycle profile of MOLT-4 cells compared to IR alone (Fig. 3B and Supplementary Fig. 3B).

3.5. Wee1 681641 and Rad51 RI-1 inhibitors cause persistence of DSBs in Jurkat but not in MOLT-4 leukemic cells after irradiation

Epi-fluorescence microscopy showed a retention of γH2AX 24 h post-irradiation in the Jurkat cells that were pre-treated with Wee1, Rad51 or both inhibitors in combination (Supplementary Fig. 4) Jurkat cells pre-treated with the Wee1 inhibitor and exposed to 4 Gy of IR had a diffuse occurrence of γH2AX foci through the

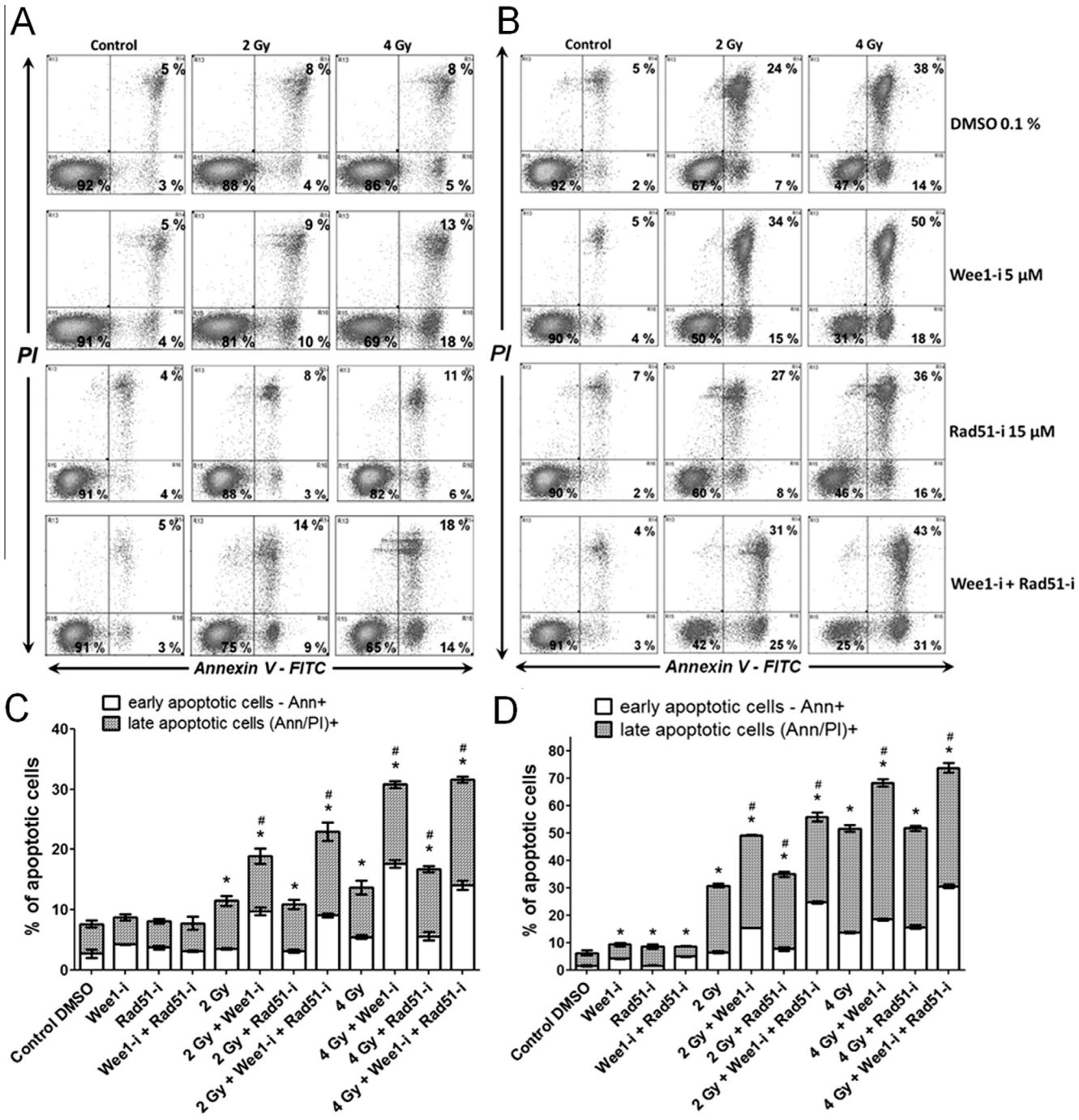

Fig. 1. The effect of inhibitors and ionizing radiation on Jurkat leukemic cells apoptosis. Histograms of Annexin V/propidium iodide double staining (A) 24 h and (B) 48 h after irradiation. The bar graph represents the percentage of early and late apoptotic cells (C) 24 h and (D) 48 h following irradiation. The results are shown as the mean ± SD from three experiments. * – significantly different to control ($P < 0.05$), # – significantly different from irradiation alone ($P < 0.05$).

entire cell nucleus. Contrary thereto, Jurkat cells treated with the Rad51 inhibitor and irradiated with the same dose had a more discrete pattern of γH2AX foci colocalized with the 53BP1. Cells pre-treated in combination had a brighter γH2AX foci immunofluorescence indicating a higher amount of unrepaired DSBs in contrast to Wee1-, or Rad51-inhibitor-treated cells (Fig. 4A). MOLT-4 cells treated with inhibitors together with IR, or irradiated alone by 2 Gy had a comparable amount of γH2AX foci positive cells with corresponding controls (Supplementary Fig. 5).

3.6. Wee1 681641 inhibitor pre-treatment potentiates ionizing radiation-induced Chk1 and Chk2 activation in Jurkat cells

Proteins synthesized in order to decelerate the cell cycle, inhibitors of cyclin-dependent kinases $p21^{Cip1/Waf1}$ and $p16^{INK4a}$ were not expressed in Jurkat cells after irradiation or treatment with Wee1 681641 and Rad51 RI-1. Similarly we did not detect p53 expression in Jurkat cells after irradiation (data not shown). We observed an increase in the amount of Chk1 phosphorylated at Ser345 in irradiated, Wee1 inhibitor-, Wee1 and Rad51

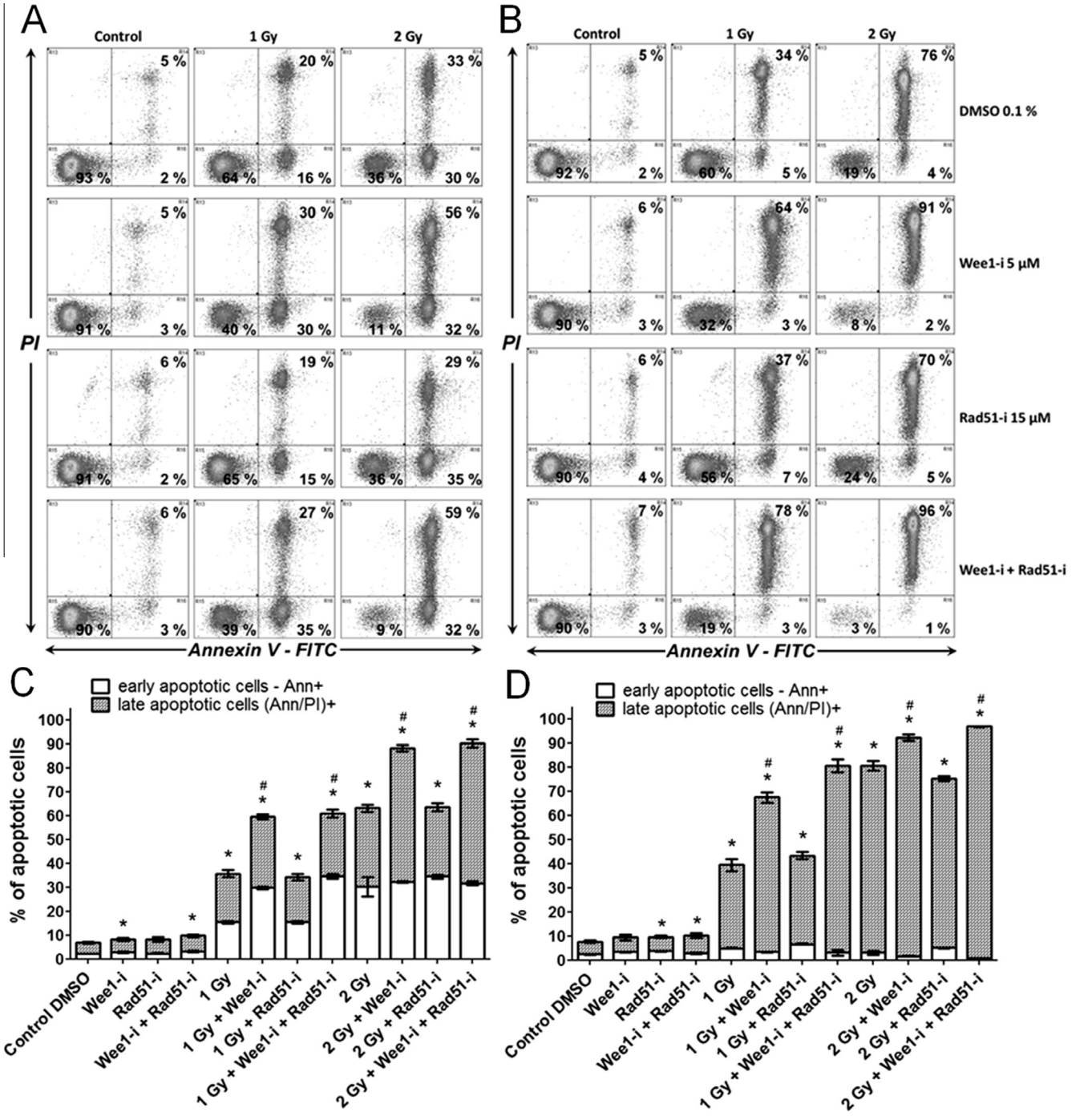

Fig. 2. The effect of inhibitors and ionizing radiation on MOLT-4 leukemic cells apoptosis. Histograms of Annexin V/propidium iodide double staining (A) 24 h and (B) 48 h after irradiation. The bar graph represents the percentage of early and late apoptotic cells (C) 24 h and (D) 48 h following irradiation. The results are shown as the mean ± SD from three experiments. * – significantly different to control ($P < 0.05$), # – significantly different from irradiation alone ($P < 0.05$).

inhibitor-pre-treated cells followed by irradiation. Although the amount of Chk1 phosphorylated at Ser345 increased after irradiation despite inhibitor pre-treatment, it was even more increased in cells irradiated after application of the Wee1 inhibitor. Phosphorylation of Chk2 at Thr68 had the same tendency as phosphorylation of Chk1 (Fig. 4B).

Irradiation of MOLT-4 cells stand alone with 1 Gy of gamma radiation resulted in an increased accumulation of p53. The expression of both p53 and $p21^{Cip1/Waf1}$ induced by IR was not changed by pre-treatment with both examined inhibitors. Both Wee1 681641 and Rad51 RI-1 inhibitors slightly increased the amount of Chk1 phosphorylated at Ser345, whereas the overall amount decreased. IR-induced phosphorylation of Chk2 at Thr68 was not significantly affected by the studied inhibitors (Fig. 4C).

4. Discussion

In this study, we focused on comparing the effects of the small molecule inhibitors of Wee1 kinase and Rad51 recombinase with the classical DNA-damaging agent gamma radiation stand alone, or in combination on two different representatives of leukemic

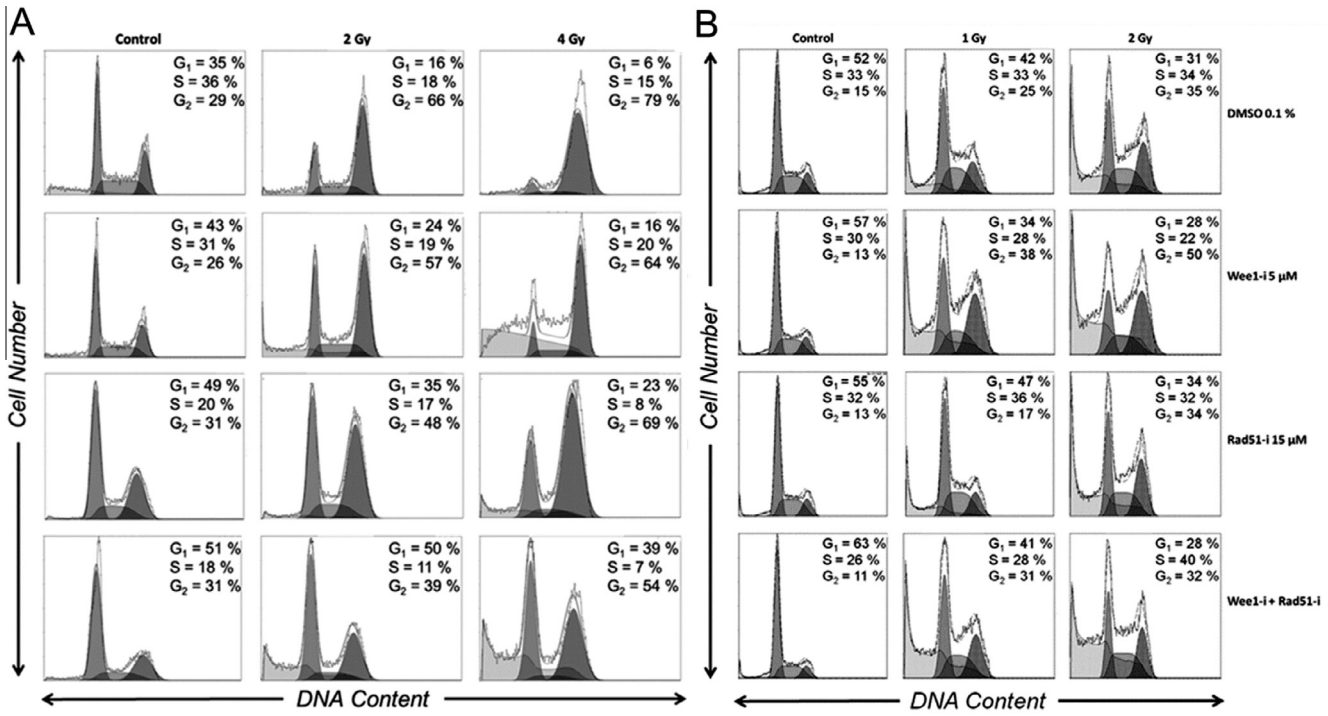

Fig. 3. The effect of inhibitors and ionizing radiation on the cell cycle in Jurkat and MOLT-4 leukemic cells. The numbers in the histograms represent the percentage of (A) Jurkat and (B) MOLT-4 cells cycling through phases G_1, S and G_2/M of the cycle. The pooled results 1 of 3 independent experiments are shown.

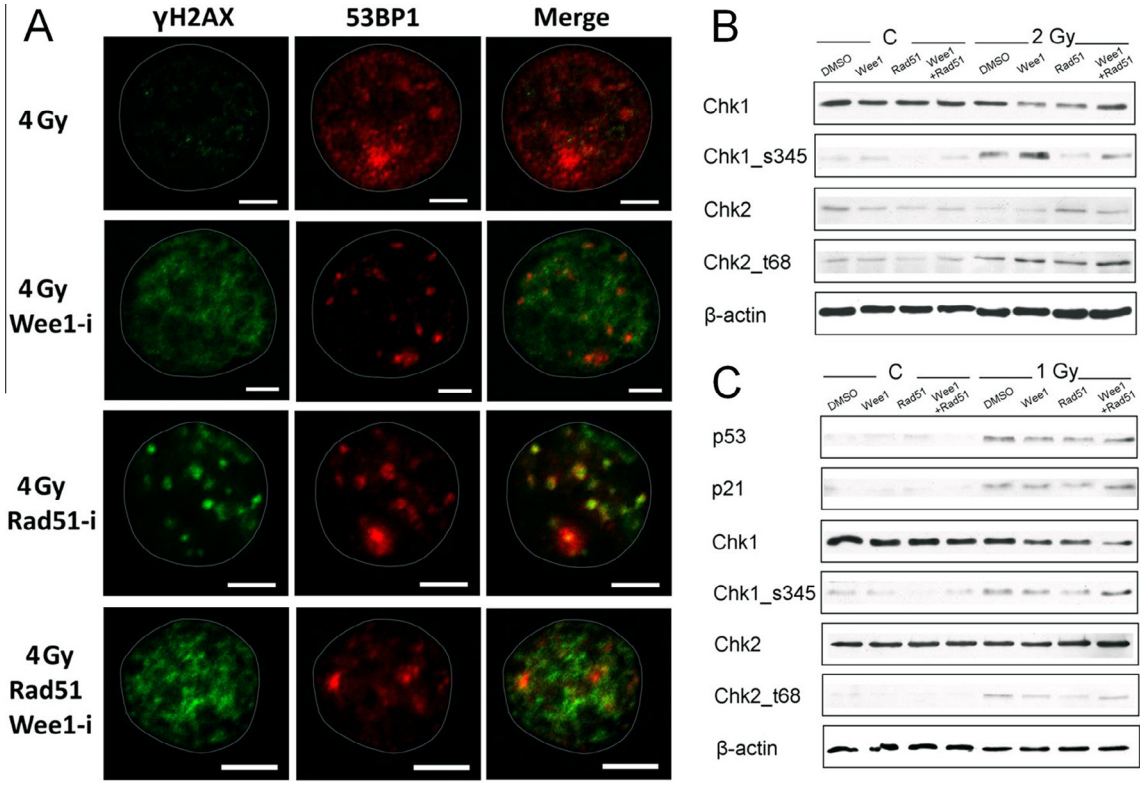

Fig. 4. Induction and activation of proteins in Jurkat and MOLT-4 leukemic cells treated with inhibitors and ionizing radiation. (A) The confocal microscopic images of single isolated cell nuclei of Jurkat leukemic cells. The surrounding yellow frame outlines the location of the cellular nucleus (scale bar: 2 µm). Experiments were performed in triplicate and photographs from representative chambers are shown. Using Western blotting we evaluated changes in proteins that regulate cell cycle in (B) Jurkat and (C) MOLT-4 cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

T-cell lymphoblasts. The major characteristic of the Jurkat cells, which lack the p53 function and which thus lead to an increased accumulation in the G_2/M phase after gamma radiation exposure, was diminished using both inhibitors. It is interesting that the treatment of p53 wild-type MOLT-4 cells with the Wee1 inhibitor lead to an increase in the amount of cells in G_2 in comparison to cells irradiated without the inhibitor. In conformity with our data, Wee1 inhibitor MK-1775 abrogated the radiation-induced G_2 block in p53-defective cells derived from lung cancers (H1299 and Calu-6) but not in p53 wild-type lines (A549 and H460) [9].

Combining the Wee1 inhibitor with IR resulted in a statistically significant increase in the sensitivity of Jurkat cells to the cytotoxic effect of IR. This concurs with reported literature where targeting Wee1 using the ATP-competitive inhibitor MK-1775 revealed increased sensitivity to IR in human lung, breast, and prostate cancer cells [9]. A growing body of evidence in conformity with our results showing that the effect of the Wee1 inhibitor is independent of p53 functionality was reported in Van Linden's study. In the acute myelogenous leukemic cell lines tested, regardless of p53 functionality, Wee1 inhibitor MK-1775 abrogated the S-phase checkpoint and augmented apoptosis induced by cytarabine [10].

Jurkat cells irradiated with 4 Gy of gamma radiation and stained by PI 24 h later were about 79% arrested in the G_2 phase of the cell cycle. At the same time, cells pre-treated with the examined inhibitors showed persistent γH2AX foci, which were obliviously more intensive using a combination of both inhibitors and rarely present in irradiated cells with 0.1% DMSO vehicle. Our results are in conformity with the conclusions published by Leonardo Bee and his team. According to their experimental work on human neonatal lung fibroblasts, G_2-phase CCD-34Lu cells treated with the HR inhibitor Rad51 RI-1 at 10 μM concentrations exhibited a higher level of unrepaired DSBs 6 h after irradiation as assayed through γH2AX foci [11]. The observations that the inhibition of Wee1 kinase interferes with normal DNA damage response are in conformity with the conclusions reported by Maryla Krajewska and colleagues. When they analysed DNA damage responses in cells treated with MK-1775, they observed a marked reduction of 53BP1 at sites of DNA damage along with an increase in γH2AX staining after irradiation [12].

In Jurkat cells pre-treatment with Wee1 681641 caused an increase in the amount of Chk1 phosphorylated at Ser345 whereas the overall amount of Chk1went down. The increased accumulation of Chk1 phosphorylated at Ser345 observed in Jurkat cells after Wee1681641 pre-treatment and irradiation probably acts as a compensatory mechanism to diminished Wee1 kinase activity maintaining G_2 cell cycle arrest. In the study published by Guertin and his team the pairing of Wee1 MK-1775 and Chk1 MK-8776 led to the rapid phosphorylation of Chk1at Ser345 in A2058, HT-29 and LoVo cell lines [13].

In conclusion, our results have demonstrated for the first time that targeting G_2/M checkpoint signalling and homologous recombination using Wee1 kinase and Rad51 recombinase inhibitors could be a potential strategy to improve the therapeutic outcome in cancer cells. The radiosensitizing effect of combination therapy with Wee1 681641 and Rad51 RI-1 inhibitors was associated with the impairment of DNA damage repair in Jurkat cells, the loss of cell survival, enhanced apoptosis and G_2 cell cycle arrest abrogation. In MOLT-4 cells the combination of Wee1 681641 and Rad51 RI-1 inhibitors augmented IR-induced cell death and decreased cell survival without a higher amount of residual γH2AX foci observed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.123.

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