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THE ANTICANCER EFFECT OF RAD51-IN-1 IN MDAH-2774 (CVCL_0420) CELLS

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Aim. This study aimed to investigate the effect of pharmacological inhibition of RAD51, via RAD51-IN-1, on the MDAH-2774 cell line

Methods. To achieve these objectives, MDAH-2774 cells were treated with different doses of RAD51-IN-1 to determine the effects on cell viability and the IC50 value of RAD51-IN-1. Intracellular ROS levels of the cells were investigated by DCFDA staining. In the last set of experiments, the effect of RAD51-IN-1 monotherapy application on apoptotic cell death was investigated by acridine orange/ethidium bromide staining.

Results. In the experimental findings obtained, firstly, cells were treated with different doses of RAD51-IN-1 to decrease cell viability and IC50 of RAD51-IN-1 was determined. In addition, it was observed that the amount of intracellular ROS increased after DCFDA staining after RAD51-IN-1 treatment. In the last set of experiments, it was determined that cell death occurred with increasing doses f of RAD51-IN-1.

Conclusion. The RAD51-IN-1 small molecule inhibitor is a promising approach for treating ovarian cancer, but the drug requires further *in vitro* evaluation.

Keywords: Ovarian Cancer, RAD51-IN-1, ROS, Cell Death.

Defects in DNA repair mechanisms are considered one of the fundamental hallmarks of cancer. Cancer cells require DNA repair capacity to repair DNA damage caused by replication stress that occurs during proliferation and exogenous factors such as ionizing radiation and genotoxic antitumor agents. In this context, defects in DNA repair pathways play an important role in tumor formation, progression, and resistance to treatment by leading to genomic instability [1]. For these reasons, the development of inhibitors targeting DNA damage response (DDR) mechanisms at both preclinical and clinical levels has made these pathways attractive targets for therapeutic intervention [2]. Homologous recombination (HR), one of the fundamental components of DDR mechanisms, is a critical pathway for the repair of DNA double-strand breaks (DSBs). This process begins with the recognition of the break site by the MRN complex (MRE11, RAD50, NBS1) and continues with the recruitment and activation of the ATM protein to the site. Activated ATM phosphorylates the Ser139 site of the H2AX protein, resulting in the formation of H2AX; this allows the recruitment of repair proteins such as 53BP1 and RAD50 to the damage site [3]. In addition, other kinases such as ATR and DNA-PK contribute to the DDR response by interacting with the RPA-ATRIP and Ku70/Ku80 complexes, respectively. RAD51 protein, one of the key regulators of homologous recombination, is the homolog of E. coli RecA and recognizes homologous DNA sequences by forming nucleoprotein filaments at the ends of DSBs during the repair process and ensures the repair of the damage through

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rearrangement structures called D-loops [4]. RAD51 filament formation is regulated by BRCA2, RAD52 and RAD51 paralogs. In the literature, it is reported that overexpression of RAD51 is observed in various malignancies such as pancreatic adenocarcinoma, ovarian and breast cancer and this contributes to tumor progression and treatment resistance [5–8].

Inhibition of RAD51 prevents the efficient repair of DNA double-strand breaks, leading to the accumulation of DNA damage in cells. This accumulation increases genomic instability and can result in apoptosis, especially in rapidly proliferating cancer cells that are highly dependent on the homologous recombination repair (HRR) pathway. In contrast, since normal cells can resort to alternative DNA repair mechanisms, RAD51 inhibitors have the potential to cause selective cytotoxicity and minimal damage to healthy tissues [9, 10]. With these properties, RAD51 stands out as a promising molecular target in targeted cancer therapies.RAD51-IN-1, a newly developed homologous recombination RAD51 inhibitor in its class, was shown to exhibit anticancer activity in cell growth in a panel of Triple-negative breast cancer (TNBC) cell lines [11].

In conclusion, DDR and especially the HR pathway have a critical place in cancer biology and treatment approaches. Functional analysis and targeting of proteins in this pathway will enable the development of more effective and personalised oncological treatment strategies in the future.

Aim. This study aims to provide a new avenue for personalized cancer therapy and to elucidate the anticancer effects of the small molecule inhibitor RAD51-IN-1 on ovarian cancer cells (MDAH-2774) for the first time.

Methods. The ovarian cancer (MDAH-2774) cell line was cultured in Dulbecco's Modified Eagle Media (DMEM) medium in a humidified environment containing 5% CO_2 at 37 °C. Cells were collected from flasks with the help of trypsin-EDTA. NutriCulture Cell Viability Determination Kit-8 (CVDK-8) is applied using a highly water-soluble tetrazolium salt. NutriCulture CVDK8 allows sensitive colorimetric assays for determining the number of live cells in cell proliferation and cytotoxicity assays. 2000–5000 cells were seeded per well in 100 ul of medium in a 96-well plate. Cells were incubated in a humidified CO_2 incubator at 37 °C for 24 hours. Various concentrations of substances to be tested were applied to the cells. The cells were incubated for an appropriate time (24, 48h). 10 µl CVDK-8 was added to each well of the plate. The plate was incubated in the incubator for 1–4 hours and the absorbance was measured at 450 nm using a microplate reader (SpectraMax Plus 384 Microplate Reader) [12].

Fluorometric analysis was performed with DCFDA dye as a different method to determine reactive oxygen species. Briefly, 25 µM DCFDA solution was added to the medium of the cells after the treatments and kept in a 37 °C CO_2 incubator for 45 min. At the end of the incubation period, the medium was removed from the wells, and 1X washing solution was added to each well. Then, the absorbance was read at Ex 485 nm/Em 535 nm with a fluorescence spectrophotometer [13].

Cells were plated at a quantity of 10×10^5 in 6-well plates and maintained in DMEM medium with 10% fetal bovine serum (FBS, Sigma, USA; cat. no. F7524) at 37 °C in a 5% CO_2 incubator (ESCO, USA). After overnight treatment, cells were removed with trypsin-EDTA after 48 h, followed by centrifugation, and as a final step, 25 µl of cell suspension (0.5×10^6 to 2.0×10^6 cells/ml) was incubated with 1 µl of AO/EB solution. 10 µl of cell suspension was placed on a microscopic slide, covered with a glass coverslip, and cells were examined under a fluorescence microscope using a fluorescent filter and a 20X objective[14].

Results and Discussion. In this study, firstly, the effect of RAD51-IN-1 on cell viability in the ovarian cancer cell line MDAH-2774 was determined by CVDK-8 assay. The MDAH-2774 cell line was treated for 24 and 48 hours using doses of RAD51-IN-1 (0-100 µM). Under experimental conditions, RAD51-IN-1 inhibited cell viability in the ovarian cancer cell line MDAH-2774 in a dose- and time-dependent manner.

However, a more significant decrease in cell viability was observed in the 48-hour treatment compared to the 24-hour treatment. While the IC50 value of MDAH-2774 was determined as 64.20 µM after 24-hour treatment with RAD51-IN-1, it was defined as 17.51 µM after 48-hour treatment. Therefore, the treatment period of the following experiments was continued with the most effective treatment period of 48 hours. In the study of Ruiz et al., which has similar results to our research, RAD51 was inhibited with siRNA in HeLa cells [15]. As a result of the viability obtained using nm doses of siRAD51, cell viability was reduced in a dose-dependent manner.

In this experiment, we evaluated the effect of RAD51-IN-1 on ROS levels in MDAH-2774 cells. First, MDAH-2774 cells were treated with RAD51-IN-1 (0–0.5–10–20 µM) for 48 hours. After

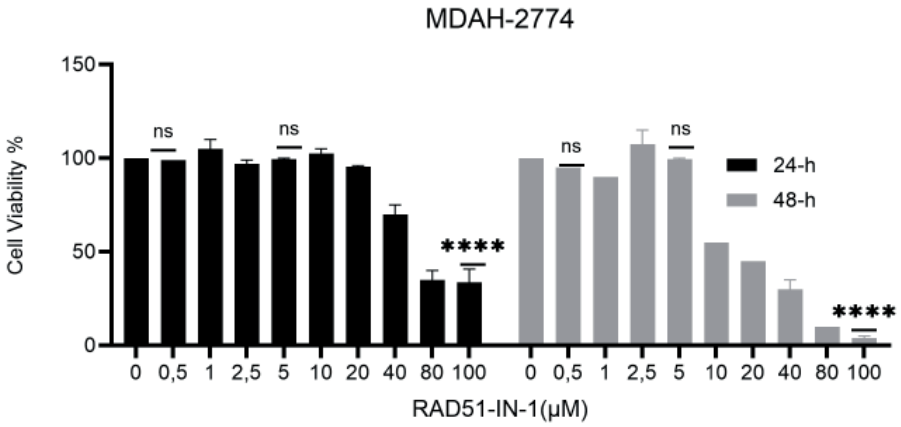

Fig. 1. **The effects of RAD51-IN-1 on cell viability in the MDAH 2774 cell line for 24 and 48 hours of incubation with RAD51-IN-1 at doses of 0, 0.5, 1, 2.5, 5, 10, 20, 40, 80, and 100 μM**
The viability of untreated cells was defined as 100. (****$P < 0.0001$)

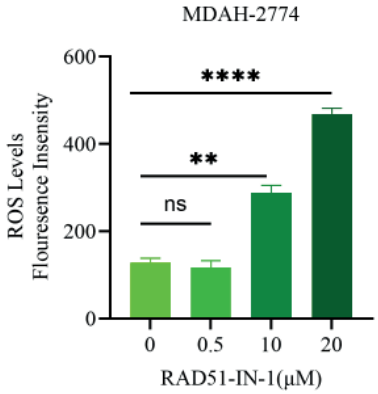

Fig. 2. **ROS levels induced by RAD51-IN-1 treatment. Cellular oxidative stress induced in cells after 48 h of RAD5-IN-1 treatment was measured using a fluorescence spectrophotometer and correlated with the levels of ROS produced. Data represent the mean ± SD of three independent experiments**
(**$P < 0.01$; ****$P < 0.0001$)

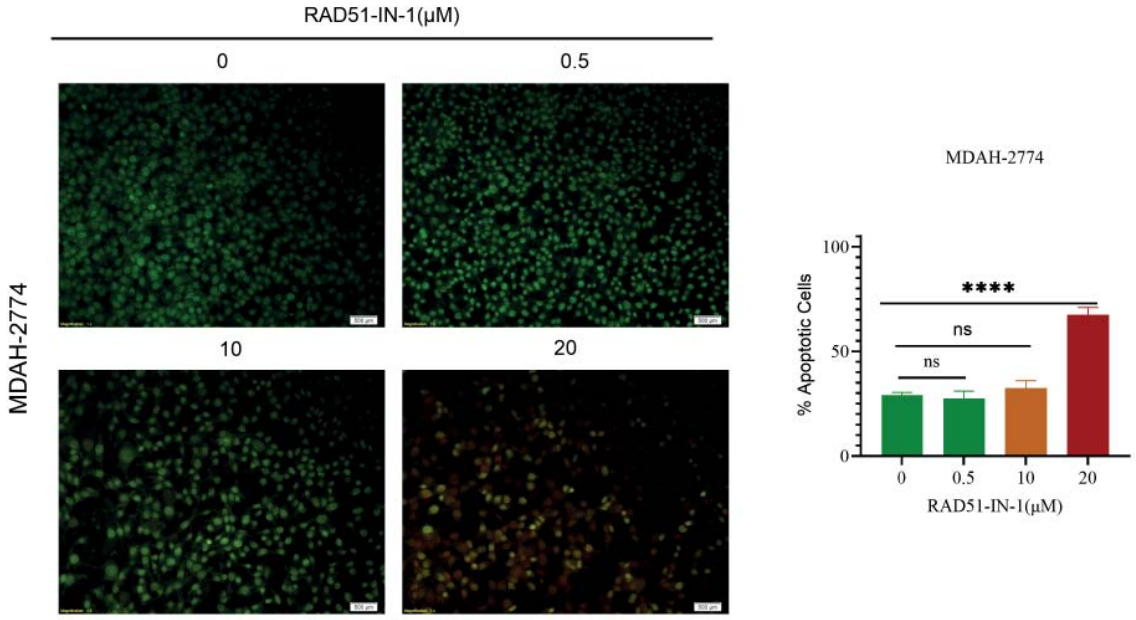

Fig. 3. **Effect of RAD51-IN-1 treatment on apoptosis in MDAH-2774 cells using acridine orange staining and ethidium bromide staining**
Green cells indicate live cells, orange cells indicate early apoptosis, and red cells indicate late apoptosis.
(****$P < 0.0001$)

treatment, cells stained with DCFDA were measured in a fluorescence spectrophotometer. It was observed that RAD51-IN-1 increased the ROS levels of MDAH-2774 cells in a dose-dependent manner and was statistically significant.

To investigate whether RAD51-IN-1 induces apoptotic cell death, an AO/Etbr staining assay was performed. Ovarian cancer cell line MDAH-2774 was treated with RAD51-IN-1 (0-0.5-10-20 μM) for 48 hours. After treatment, cells were stained with AO/Etbr and the images were examined under a microscope. In the obtained microscope images, no significant effect was observed at the dose of 0.5 μM, while a significant effect was observed at the dose of 10-20 μM. As shown statistically, RAD51-IN-1 was shown in MDAH-2774 cells in a dose-dependent manner. In the study by Gu and his team, breast cancer cell lines were treated with RAD-51 (Cpd-1, Cpd-2, Cpd-3, Cpd-4, and Cpd-5). In the apoptotic cell death results obtained, it was statistically determined that it increased cell death, especially in MDA-MB-468 cells [16].

Conclusion. These findings may help develop new treatments for ovarian cancer that exploit the genomic instability of cancer cells.

Authors' contribution

DÖ and CAA provided critical feedback and helped shape the research, analysis and manuscript. DÖ and CAA read and approved the final manuscript.

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