Abstract

Investigation of DNA repair mechanisms in human oral squamous cell carcinoma cells HSC2 and radiation-resistant cells HSC2-R using DNA-PKcs inhibitor NU7441 and Rad51 inhibitor IBR2

2023
Graduate School of Dentistry,
Health Sciences University of Hokkaido

Kentaro　OHUCHI

Introduction

Owing to recent improvements in physical accuracy, radiotherapy has achieved non-invasive local control over normal tissues and now plays an important role in cancer treatment. However, local recurrence and metastasis may occur. One of the reasons for this is that radioresistant cells remain during fractionated X-ray irradiation, and these radioresistant cells are thought to cause recurrence and metastasis.

The main cell-killing effect of ionizing radiation is thought to involve DNA damage, particularly DNA double-strand breaks. Mechanisms that can repair this DNA damage do exist, two types of the main ones being non-homologous end joining and homologous recombination. Homologous recombination is considered to be the repair method associated with fewer errors, but error-prone non-homologous end joining can induce genomic instability and cause the expansion of radioresistant clones. However, the details of the biological mechanism underlying the acquisition of radioresistance in cancer cells are unknown. Therefore, to clarify the mechanism by which radioresistant cells develop, we investigated the mechanism of action of DNA damage repair proteins. Specific inhibitors were used to study the response to DNA damage of radioresistant cell lines established by fractionated X-ray irradiation.

Method

The oral squamous cell carcinoma cell line HSC2 was used as the parental cell line in this study. It was subjected to X-ray irradiation at 2 Gy per day 30 times (total dose 60 Gy) to establish the surviving radiation-resistant oral squamous cell carcinoma cell line HSC2-R. HSC2 cells and HSC-2 cells were provided by the Cell Resource Center for Biomedical Research's Cell Bank at the Institute of Development, Aging and Cancer, Tohoku University. We investigated the DNA damage repair mechanism by adding NU7441, a DNA-PKcs inhibitor, and IBR2, a Rad51 inhibitor, to HSC2 cells and HSC2-R cells and then irradiating them with X-rays. DNA-PKcs is a protein involved in non-homologous end joining, and Rad51 is a protein that plays a central role in homologous recombination.

First, we confirmed by colony formation assay that HSC2-R cells are resistant to radiation compared with HSC2

cells. Next, we added NU7441 or IBR2 to the culture medium of HSC-2 cells and HSC-2R cells, and performed colony formation assay. The toxicity of these inhibitors was evaluated by determining the cell viability. Specifically, NU7441 or IBR2 adjusted to a non-toxic concentration was added, and 1 h later, X-rays were irradiated at 6 Gy to compare the cell viability. The percentages of apoptotic cells 24 and 48 h after irradiation were then analyzed by flow cytometry using FITC-labeled AnnexinV and propidium iodide (PI). In addition, cell cycle distribution and DNA damage repair dynamics were analyzed by flow cytometry using FITC-γH2AX and PI. The statistical significance of differences was tested using the Mann–Whitney U test in the case of two groups, while the Steel–Dwass test was used in the case of three or more groups, with $p<0.05$ being considered statistically significant.

Results and Discussion

The survival rate of HSC2-R cells treated with X-ray irradiation alone was significantly higher than that of HSC2 cells, confirming that HSC2-R cells are more resistant to X-rays than HSC2. When an inhibitor was added to the culture medium and the cell survival rate was measured, no decrease in the survival rate was observed at 5 μM for NU7441 and 10 μM for IBR2. Therefore, these inhibitors were used at these concentrations and X-ray irradiation was also performed. The results revealed there were no colonies for HSC2 cells and HSC2-R cells after NU7441-added X-ray irradiation. This shows that NU7441, a DNA-Pkcs inhibitor, has no effect on HSC2-R cells. It is thought that this decreases the repair of radiation and reduces radiation resistance.

FITC-Annexin V and PI staining was used to measure the percentage of apoptotic cells 24 and 48 h after treatment. The rate of apoptosis of HSC2-R cells treated with X-ray irradiation alone was significantly lower than that of HSC2 cells. When X-ray irradiation was performed after adding an inhibitor (NU7441 or IBR2), the apoptosis of HSC2-R increased.

γH2AX, an index of DNA damage, was also measured over time. Upon treatment with X-ray irradiation alone, the γH2AX-positive fraction of HSC2 cells and HSC2-R cells increased 1 h after irradiation, but rapidly decreased 6 h after irradiation. It is thus thought that intracellular DNA damage peaked approximately 1 h after X-ray irradiation, and then the γH2AX-positive fraction decreased due to DNA repair. In the case of NU7411-added X-

ray irradiation, The γH2AX-positive fraction of cells and HSC2-R cells similarly increased 1 h after irradiation. Furthermore, in HSC2 cells, the γH2AX-positive fraction remained high even 6 h after irradiation, but decreased 24 h after irradiation. Meanwhile, in HSC2-R cells, the γH2AX-positive fraction remained high until 48 h after irradiation. With IBR2-added X-ray irradiation, the γH2AX-positive fraction of HSC2 cells and HSC2-R cells was almost the same as with X-ray irradiation alone. These results suggest that NU7411 inhibited DNA repair in HSC2-R cells and did not reduce the γH2AX-positive fraction.

At the same time as γH2AX, we measured the cell cycle distribution. In HSC2 cells, after treatment with X-ray irradiation alone, the cell cycle distribution did not change until 6 h later, but after 24 h, the proportion of cells in the G2/M phase increased. The percentages of cells in G0/G1 phase and S phase decreased. G2/M phase arrest was confirmed, while, after 48 h, this arrest tended to be released. In contrast, for HSC2-R, the number of cells in G2/M phase did not increase significantly, while the number of cells in G0/G1 phase did not decrease significantly either. Upon irradiation with X-rays containing NU7441, the number of cells in G2/M phase increased over time for both cell types. The number of cells in the G0/G1 phase decreased continuously 1 h until 48 h after treatment. This suggests that, when NU7441 is added, DNA repair does not proceed in HSC2-R cells with the cell cycle being arrested at the G2/M checkpoint, leading to an increase in polyploid cells. There was no significant difference in the tendency for both cells in the case of IBR2 administration in combination with X-ray irradiation compared with the findings upon X-ray irradiation alone.

Conclusion

This study suggests that the radioresistance of HSC-R cells is dependent on non-homologous end joining involving DNA-PKcs.