DNA damage responses to low-dose ionizing radiation in normal keratinocytes and diabetes type II keratinocytes

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1. Introduction

Due to the increase in various diagnostic and treatment devices using radiation, the possibility of exposure to low-dose irradiation is increasing. Skin is the outermost part of the body and is known to be easily damaged by direct exposure to radiation. In particular, the skin of diabetic patients is very fragile and is easily damaged by stimulation such as radiation treatment. However, there has been no detailed report on the extent of DNA damage responses in diabetic patients induced by low-dose irradiation. In this study, we compared cell survival, DNA damage and repair-related signaling, and apoptosis in 3D skin organoids using keratinocytes from normal and type II diabetic patients under low-dose irradiation. A decrease in cell viability occurred in both types of cells with increasing irradiation dose. The viability of normal keratinocytes decreased more than that of diabetes type II keratinocytes. The apoptosis observed by the expression of cleaved caspase-3 was further increased in normal keratinocytes with a fast growth rate. Activation of the DNA damage response and activation of repair signals were increased in both cell types with increasing irradiation dose. However, basal expression levels of molecules involved in DNA damage and repair, and radiation-induced activation of these molecules were relatively higher in normal keratinocytes than in diabetes type II keratinocytes. In three-dimensional skin organoids, apoptosis increased as the irradiation dose increased, and the degree of apoptosis increased more significantly in normal keratinocytes. In conclusion, it was confirmed that rapidly proliferating normal keratinocytes responded more strongly to radiation-induced damage and recovery than did diabetes type II keratinocytes. These results suggest standards for skin damage that can occur when normal or diabetic patients are exposed to low-dose radiation during examination or treatment, and can be used as basic data for research to reduce skin damage caused by radiation exposure.

2. Methods

2.1 MTT assay
Normal and diabetes type II keratinocytes were cultured for 7 days after 0, 0.1, 0.5, or 2Gy γ-irradiation. Following the addition of MTT solution, incubation was continued for 4 h. Next, the medium was removed, 200 μl DMSO added, and the optical density of each well measured at 570 nm with a microplate reader.

2.2 Immunoblot analysis
Cells were lysed 1, 24, or 72 h after irradiation. Equal amounts of protein were resolved via SDS-PAGE and transferred to NC membranes. Membranes were incubated with primary antibodies overnight at 4°C. After washing with PBST, blots were incubated with HRP-conjugated secondary antibodies for 1 h at RT. Protein bands were detected with Amersham™ ECL™ Western Blotting Detection Reagents and X-ray films.

2.3 TUNEL assay
Paraffin-embedded 3D skin organoid sample slides were deparaffinized in a xylene-ethanol gradient. After washing with PBS, proteinase K (20 μg/ml) was applied to the slides for 10 min at RT. TUNEL assays were performed using an ApopTag kit (Millipore) as described by the manufacturer. Apoptotic bodies were visualized using the DAB kit.

3. Results

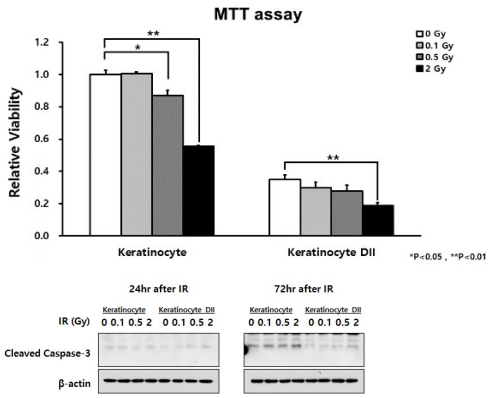

Figure 1. Comparison of cell survival between normal and diabetes type II keratinocytes after ionizing radiation

In the MTT assay, there was no significant difference in cell viability for both normal and diabetes type II keratinocytes when irradiated with 0.1 Gy. On the other hand, as the radiation dose of 0.5 Gy or more increased, the viability of both cells tended to decrease. However, cell survival was further reduced with increasing radiation dose in normal keratinocytes compared to diabetes type II keratinocytes. Apoptosis, seen as expression of cleaved caspase-3, increased on the 3rd day after 2 Gy irradiation, and was weaker in diabetes type II keratinocytes than in normal keratinocytes.

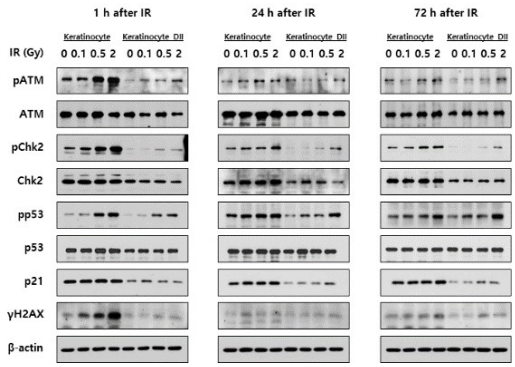

Figure 2. Immunoblot analysis for DNA damage response molecules in normal and diabetes type II keratinocytes after various doses of ionizing irradiation

Next, activation of DNA damage signaling molecules was observed by immunoblot analysis. The active forms of DNA damage response molecules, pATM, pChk2, pp53, and γH2AX, increased with increasing radiation dose, especially at 1-hour post-irradiation in both cell types. However, the expressions of pATM, pChk2, pp53, and γH2AX were weaker in diabetes type II keratinocytes compared to normal keratinocytes. This may be due to low expression of total ATM, Chk2, and p53 in diabetes type II keratinocytes or due to poor response of diabetes type II keratinocytes to irradiation.

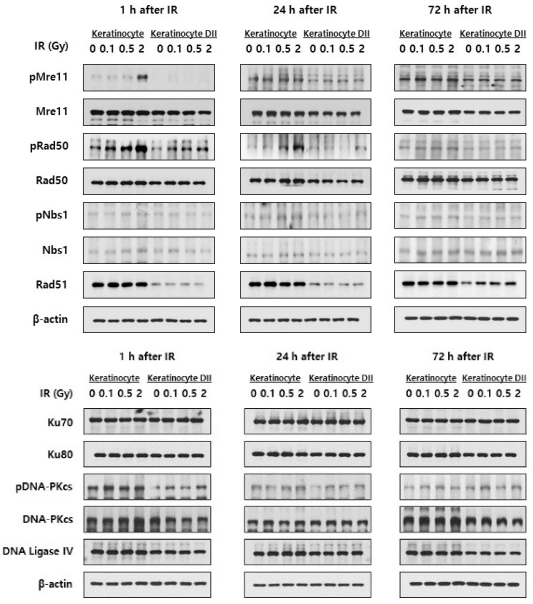

Figure 3. Immunoblot analysis for molecules involved in homologous recombination (HR) and non-homologous end-joining (NHEJ) in normal and diabetes type II keratinocytes after various doses of ionizing irradiation

Activation of DNA repair signaling pathway [Homologous recombination (HR) and Non-homologous End-Joining (NHEJ)] molecules also increased in both cells as radiation dose increased. However, basal expression levels of molecules involved in DNA repair (Mre11, Rad50, Rad51, DNA-PKcs, and DNA ligase IV) and radiation-induced activation of these molecules (pMre11, pRad50, and pDNA-PKcs) were relatively higher in normal keratinocytes than in diabetes type II keratinocytes.

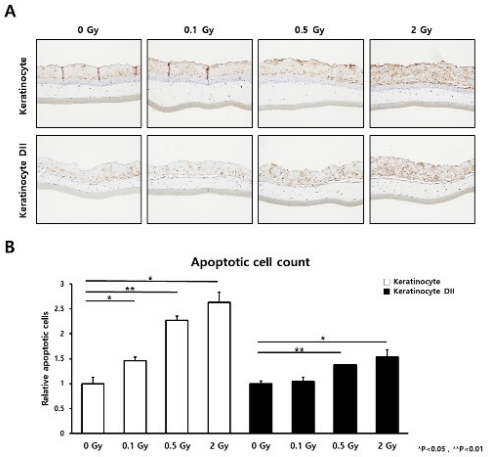

Figure 4. Analysis of apoptosis in 3D skin organoids made with normal or diabetes type II keratinocytes after ionizing radiation

In order to investigate the damage caused by radiation that actually occurs in human skin, we produced 3D skin organoids using normal and diabetes type II keratinocytes. Apoptosis was increased in both 3D skin organoids as the irradiation dose increased but the apoptosis in 3D organoids with normal keratinocytes was significantly increased.

4. Conclusions

In normal or diabetes type II keratinocytes, 0.1 Gy irradiation did not show significant changes in DNA damage and repair responses. On the other hand, when irradiated with more than 0.5Gy, there was a significant change in DNA damage and recovery response. These changes were relatively stronger in normal keratinocytes than in diabetes type II keratinocytes as the irradiation dose increased. This means that diabetes type II keratinocytes have a smaller response to radiation exposure than normal keratinocytes, and suggests that actively proliferating cells can be more sensitive to irradiation even at low doses.

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