Biological Response of Tissue Engineered Mucosa to Ionizing Radiation: A Time Study

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Introduction

Tissue Engineered Mucosa (TEM) was originally designed to reconstruct large oral defects remaining in the oral cavity after oncological resection. TEM consists of human keratinocytes and fibroblasts seeded onto an a-cellular dermal scaffold. Previous study in our laboratory showed that TEM could also be used as a tool to study the biological response to ionizing radiation (IR), as IR is a major part of the treatment of patients after oncological resection. This study focused on the biological response of TEM in time after exposure to a single dose of IR. We hypothesize that TEM is capable to detect DNA double strand breaks (DSBs) caused by IR, and that TEM can repair this damage using the main DNA repair pathways, non-homologous end-joining (NHEJ) and homologous recombination (HR).

Materials and Methods

Human primary keratinocytes and fibroblasts were isolated from buccal biopsies, and cultured. TEM was created by seeding fibroblasts and keratinocytes onto an a-cellular dermal scaffold. After two weeks of culturing the TEM at the Air/Liquid interface, TEM was irradiated with a single dose of 16.5Gy, 0Gy was used as control. TEM was harvested 1, 6, 24, 48 and 72h after exposure to IR.

DNA damage and repair, cell proliferation, apoptosis and extracellular matrix components were quantified using immunohistochemistry. Statistical significance was determined using the Tukey-Kramer Multiple Comparisons test.

Results

1h and 6h after exposure to IR the number of 53BP1, a DNA DSB marker, positive cells was significantly increased when TEM was irradiated with 16.5Gy (Fig. 1). Also the number of cells positive for the NHEJ (Ku70/80) and HR (Rad51) repair pathways were significantly increased. IR with 16.5Gy resulted in a significant increase of the number of apoptotic cells 24 and 48h after irradiation (Fig. 2). Changes in cell proliferation (Ki67) were not detected by irradiation with a single dose of 16.5Gy.

Discussion and Conclusions

Our results indicate that TEM is capable to detect and repair DNA DSBs using both NHEJ and HR. This study provides new insights in the mechanism underlying irradiation damage in TEM. Future research will include the use of radioprotective agents to improve the quality of TEM after IR.

References


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