Apoptotic response to ionizing radiation in human skin cells.

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Numerous studies indicate that radiation exposure leads to inflammatory responses in skin cells and tissue [1, 2]. On the other hand, low doses of irradiation with α-particles emitted from a radon source exert anti-inflammatory effects in patients suffering from chronic inflammation. This indicates a complex dependence of the regulation of inflammation on radiation quality, dose and the interaction of irradiated cells with the tissue environment. In general the two major types of cell death, apoptosis and necrosis, are involved in processes related to inflammation [3]. In contrast to necrosis, apoptosis is a controlled process which leads to the elimination of the cells without triggering inflammation. In this project we want to investigate changes induced by ionizing radiation that are potentially related to induction or inhibition of inflammation in skin, i.e. the occurrence of apoptosis and the release of cytokines and other relevant factors.

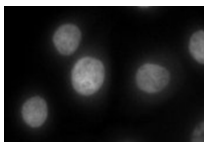
Methods

To investigate the effect of different irradiation types, primary keratinocytes (NHEK; Lonza) and a human full-thickness skin equivalent (MatTek; Ashland) were irradiated with X-ray and carbon ions (186keV/µm).The effects were compared with previous results obtained in immortalized HaCaT cells. For each irradiation type a low dose and a high dose was used. Doses have been chosen to obtain nearly equivalent survival levels. 24 hours and 3 days after irradiation, cells were stained with DAPI to investigate changes in the morphology or fixed with 4 % of PFA. The skin equivalent was used for protein extraction with RIPA-Buffer or was fixed with Bouin's solution, dehydrogenated and embedded in paraffin. Serial sections (5 µm) of irradiated and unirradiated skin equivalents were stained with hematoxylin and eosin (H & E) according to commonly used procedures. In order to detect apoptotic cells, sections were stained with an antibody against cleaved caspase3 (Cell signaling). All sections were counterstained with hematoxylin. Protein extracts of monolayer cell culture and full thickness skin equivalents were used for Western Blot analysis.

Results and Discussion

In NHEK, no morphological changes indicating primary apoptosis could be detected in DAPI stainings 24 hours after irradiation with high doses of X-ray (fig.1). HaCaT cells respond similarly at this early time point, but 3 days after irradiation a significant amount of the cleaved form of caspase 3 was detected, indicating late apoptosis [4], whereas no cleaved caspase 3 could be detected in NHEK cells, also not after carbon ion exposure (data not shown). Growth kinetics analysis performed with NHEK suggests that the cells stop to proliferate after irradiation but do not undergo primary and late apoptosis (data not shown).

Fig. 1: DAPI stained cell nuclei of NHEK cells 3 days after X-ray irradiation (10 Gy).



To compare the effects of ionizing irradiation on monolayer and 3-D cultures we irradiated a human full-thickness skin equivalent with different doses of X-rays and carbon ions. In our first experiments the cells of the human skin equivalent turned out to be very resistant to ionizing irradiation. After irradiation with a high dose of carbon ions or X-rays cleaved caspase 3 could not be detected in antibody stainings (fig.2) and Western Blot analysis (fig.3).

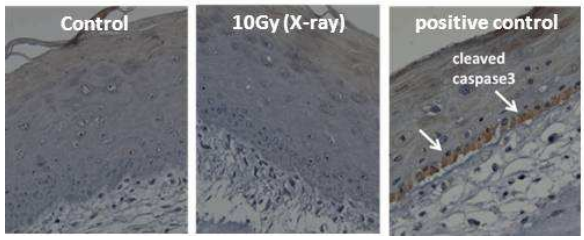


Fig. 2: Immunolocalization of cleaved caspase3 in EpiDermFT-400 3 days post irradiation with X-rays. Cells which express cleaved caspase 3 are indicated with a brown staining (arrows).

Fig. 3: Western blot analysis of Caspase-3 in skin equivalents 24hours and 3 days after irradiation with carbon-ions. PC: positive Control (Lysats of HaCaT cells 3 days after irradiation with 10 Gy of X-ray); kDa: Kilodalton; fl:full-length; cl: cleaved length.

Taken together, primary keratinocytes do not respond with early or late apoptosis to ionizing irradiation. This is in contrast to HaCaT cells that undergo secondary apoptosis after continued proliferation, probably due to mutated p53 in HaCat cells [5]. Epidermal keratinocytes of skin equivalents do not undergo apoptosis irrespective of dose and radiation quality.

We conclude that apoptosis of keratinocytes is not a direct trigger for pro- or anti-inflammatory responses in skin after exposure to ionizing radiation.

References

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