Inflammation-related response to irradiation in different human skin culture systems*

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Introduction

Chronic inflammatory skin diseases (eczema, psoriasis) can be treated with low doses of irradiation [1]. Exposure to UV, photons and radon can alleviate the symptoms suggesting an anti-inflammatory effect. Apoptosis can be related to this. The absence of an inflammatory response during apoptosis is not only due to lack of pro-inflammatory signals, but apoptotic cells can actively suppress an inflammation by the release of anti-inflammatory cytokines like TGF-β or IL-10 [2]. Changes induced by irradiation that are potentially related to inflammation in skin will be investigated.

Materials and Methods

Model systems with different complexity-levels have been used: monolayer cultures of human primary keratinocytes (NHEK; Lonza), co-cultures of primary keratinocytes and fibroblasts (NHDF; Lonza) and human full-thickness skin equivalents (EFT400; MatTek; Ashland). Samples were irradiated comparing low versus high X-ray and C-ion (186 keV/μm) doses or UV-B intensities, fixed and processed 12 hours, 24 hours and 3 days after irradiation for microscopic analysis and protein extraction. Hematoxylin & Eosin (H&E) staining and immunostaining against Caspase 3 and PARP have been performed. Cytokine release was quantified by ELISA.

Results and Discussion

No apoptosis was observed after X-ray and C-ion irradiation in all model systems [3]. Cleaved caspase 3 and cleaved PARP could only be detected in monolayer cultures of NHEK after UV-B intensities of 40 and 60 mJ/cm^2 (Fig. 1A); the same intensities could not induce apoptosis in the more complex systems (data not shown). These suggest that apoptosis is not a trigger for the release of anti-inflammatory cytokines. C-ion irradiation induced morphological changes after exposure to a high dose; but earlier compared to UV-B (not shown). 3 days after UV-B irradiation (100 mJ/cm^2), cobblestoned morphology of the basal layer and a reduced cohesion of the stratum corneum occur (Fig. 1B), indicating a radiation induced impairment of the differentiation process. The inflammatory response was assessed by detection of TGF-β and Il-1α release in monolayer- and co-cultures. In unirradiated co-cultures the release of TGF-β and Il-1α is suppressed by intercellular communication between NHEK and NHDF (data not shown) which is in agreement with the literature [4]. The enhancement of the pro-inflammatory cytokine IL1α in NHEK monocultures (data not shown) and co-cultures (fig.2) after exposure to moderate doses/intensities shows that the cytokine-balance is changed due to irradiation. Taken together, an induction of apoptosis was only observed for UV-B exposure, not for ionizing irradiation. Modifications of the keratinocyte differentiation and release of inflammatory factors was only observed for moderate and high doses of UV-B and ionizing radiation, but not for low doses. In future experiments the influence of immune cells on the radiation response will be considered.

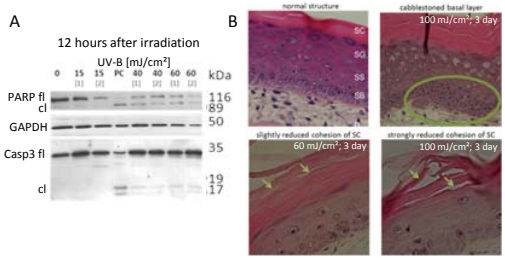

Figure 1: (A) Western Blot analysis of NHEK cells after UV-B irradiation. PC: positive control (HaCaT cells 5 days; 10 Gy X-ray); kDa: kilodalton; fl: full-length; cl: cleaved (B) H&E staining of tissue sections. circle: cobblestoned morphology in the stratum basale (sb); arrows: reduced cohesion of stratum corneum (sc)

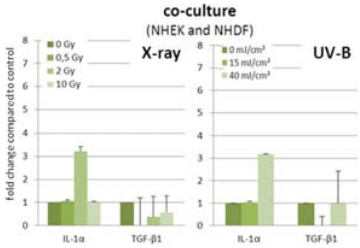

Figure 2: IL1α and TGF-β release of co-cultures after irradiation with x-ray and UV-B

References

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