Testing of a 3D-human skin equivalent for radiation experiments.

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Introduction

Despite the increasing concern about the effect of ionising radiation on living organisms, the majority of radiobiological studies have been conducted *in vitro*, i.e. in 2-dimensional (2D) monocultures. Accumulating evidence suggests that cells behave differently when they are cultivated in a 3D extra-cellular matrix where they also interact with other cells [1-5]. In order to be able to extrapolate the *in vitro* results closer to the *in vivo* situation, it is of great benefit to develop tissue systems suitable for radiobiological assays.

In this project we want to use commercially available 3D-tissue skin equivalents, composed of an epidermal and dermal layer of human keratinocytes and fibroblasts. The extracellular matrix and other cell types resident in skin may have an impact on the occurrence of apoptosis and/or the release of inflammation-related cytokines.

Materials and Methods

To investigate the effect of different irradiation types (UVB; X-rays and carbon ions) a human full-thickness skin equivalent from MatTek was used. Histological stainings and protein extraction methods have been established for morphological and immunological analysis.

After irradiation cultures were fixed in Bouin`s solution, paraffin embedded, sectioned, and stained with hematoxylin and eosin (H & E) to check the morphology. In order to detect differentiation markers, sections were stained with an antibody against cytokeratin 10 (Abcam) and counterstained with hematoxylin.

Lysis with RIPA-buffer was performed after separating mechanically the epidermis from the dermis. The lysates were used for Western Blot analysis to detect E-cadherin, Vimentin (Abcam) and GAPDH (Cell signaling).

Results and Discussion

We could successfully establish methods for fixation, embedding and cutting of the tissue equivalent. Morphological analysis showed a well-organized epiderm with basal, spinous, granular and cornified layers. The dermis is composed of a collagen matrix containing numerous viable normal human fibroblasts (fig.1). The impression of a typical morphology of the skin equivalent could be confirmed by a comparison of tissue slices from normal human skin (courtesy of the dermatological department of the Darmstadt Hospital).

Immunostaining (fig. 1b) shows that cytokeratin 10, a marker for differentiation [6] is expressed in the upper layers of the epiderm (brown staining) but not in the basal layer where the cells remain undifferentiated. A high cell-number in the basal layer is a sign for viable tissue.

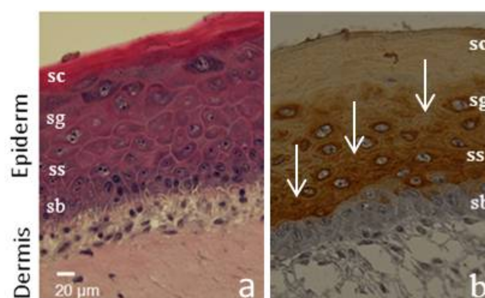

Fig. 1: (a) H&E Staining and (b) immunolocalization of cytokeratin 10 (b) in EpiDermFT400. Cells which express cytokeratin 10 display a brown staining in the cytoplasm (arrows).Stratum basale (sb), stratum spinosum (ss), stratum granulosum (sg) and stratum corneum (sc).

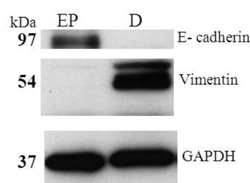

Fig. 2: Western blot analysis of E-cadherin and Vimentin in skin equivalent EFT 400. E-cadherin is detectable in the epiderm (EP) at 97 kDa and Vimentin in the dermis (D) at 37 kDa.GAPDH is used as a loading control; kDa: Kilodalton; EP: epiderm; D: Dermis.

To overcome problems to lyse the tissue samples we tested a combination of mechanical lysis with a douncer homogenizer and ultrasound (bath-type sonicator) which worked very well. EFT-400 offers the possibility to separate the epidermis from the dermis mechanically. Western Blot analysis shows the presence of E-cadherin exclusively in the epiderm and of vimentin in the dermis (fig. 2).

Our study establishes EpiDerm-FT 400 (MatTek) as a suitable model to study the underlying mechanisms of tissue responses to radiation. Analysis of 3D skin equivalents, bridging the gap between in vitro and in vivo models, are important for a better understanding of radiation effects on normal tissues, in particular inflammation and fibrosis during radiotherapy [7].

Reference

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