The Dangerous Sun – Implications of UV Irradiation for Initiation and Invasion of Malignant Melanoma

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During the last years, our research group has characterized signalling pathways for UVA and UVB in skin cells as well as in melanoma cells. UV irradiation is both mutagenic and mitogenic in skin cells, and considered as a complete carcinogen. The exact mechanism of damage is however not fully elucidated. UVB irradiation is thought to be associated with direct DNA damage, whereas the damaging effect of UVA is mediated by oxidative stress-induced damage and is less well characterized. Since UVA is the dominant wavelength of sunlight, it is of critical importance to characterize its negative effects on the cells. We have demonstrated that the cell damaging effect of UVB is mediated via DNA-damage and p53 activation, while UVA generates reactive oxygen species in the cytosol (1). Further, we have characterized the signalling pathways of UVA- and UVB-induced apoptosis in human melanocytes (2, 3) and found release of lysosomal proteases to the cytosol which is preceded by stress signalling by AP-1 and NF-KB (4, 5). Moreover, we have presented evidence that melanocyte apoptosis is reduced if co-cultured with UVB-irradiated keratinocytes (6).

Recently, we made the unique finding that during UVA-induced plasma membrane damage in keratinocytes and lysosomes, the cellular degrading unit are translocated to the plasma membrane donating their own membranes to repair the wound (7). Noteworthy, such damage was not detected after UVB, indicating that we have identified a specific lysosome-related mechanism induced by UVA irradiation. Furthermore, the lysosomal exocytosis results in release of lysosomal content outside the damaged cells into the extracellular space. To further characterize the phenomenon and show that this damage is not selective to keratinocytes, we have performed experiments in melanocytes and melanoma cells, which all show that UVA causes plasma membrane damage followed by lysosomal exocytosis (8). Note that none of the cell lines showed exocytosis following UVB-irradiation.

Moreover, the release of lysosomal proteases from melanoma cells facilitates invasion through adjacent tissues (9). The key players in this process are different proteases including lysosomal cathepsins released from the tumour cells (Fig. 1). Important knowledge is that we recently found that extracellular cathepsins exerted mitogenic stimulation on neighbouring cells (8). Thus, lysosomal function is often altered in cancer cells and their tumour promoting action could be an important target for future therapy.

To gain a deeper understanding of how lysosomal exocytosis affects cell signalling and proliferation, we use several models including co-culture in transwell systems and 3D models of reconstituted skin. From human skin, we isolate and grow melanocytes, keratinocytes and fibroblasts from the same donor and produce 3D models of reconstructed skin (Fig. 2). The morphology of the reconstructed skin is very similar to skin *in situ* and we are able to use relatively high UV intensities to imitate severe sunburn that would be unethical to use on human volunteers or laboratory animals. Moreover, Zebrafish embryo (*Danio rerio*) models are used to study the invasion process.

Our research provide important knowledge of the interplay of keratinocytes and melanocytes during UV irradiation and depict signalling pathways regulating UV-induced lysosomal

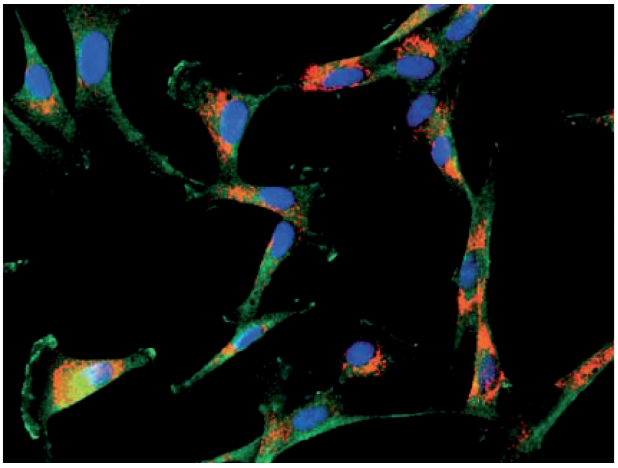

Fig. 1. Spontaneous secretion of cathepsins from melanoma cells facilitates invasion and migration. Immunocytochemical fluorescence in melanoma cells. Staining of cathepsin K (green), LAMP-2 (red) and nuclei (blue).

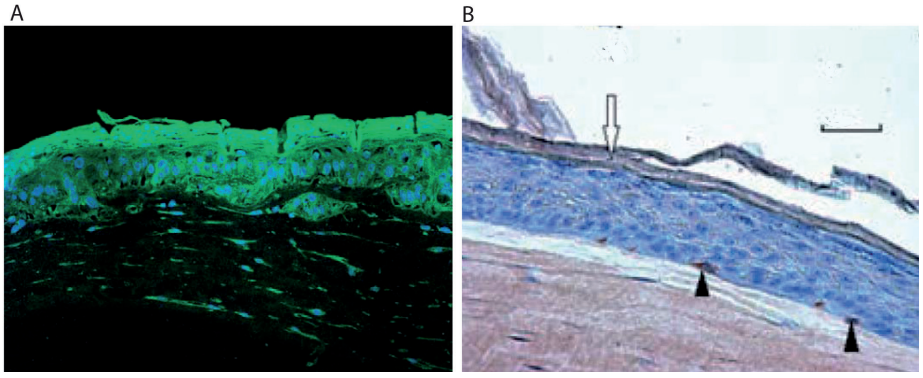

Fig. 2. Artificial skin constructs. A. Immunocytochemical fluorescent staining of LAMP-2 (green) and nuclei (blue) of both epidermal melanocytes and keratinocytes as well as dermal fibroblasts. B. Immunocytochemical staining were the black arrows indicate Melan A staining (brown) and the white arrow points at stratum corneum (×200, bar = 50 µm).

exocytosis and cell proliferation (7, 10). Importantly, we evaluate the impact of UVA-induced lysosomal exocytosis for the invasion of melanoma cells. Lysosomal exocytosis is a hitherto unrecognized process and a thorough characterization of its consequences is mandated. We will continue to elucidate the effect of lysosomal exocytosis on signalling events and put the finding into physiological settings.

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