degradation of long-lived proteins. Recently, however, it has been shown that release of cathepsins to the cytosol following permeabilization of the lysosomal membrane is involved in the apoptotic process in several different cell types. This work provides, for the first time, evidence that UV irradiation induces lysosomal permeabilization and that the subsequent cathepsin release is a crucial event in triggering apoptosis in melanocytes. These findings further demonstrate that lysosomal permeabilization occurs early in the apoptotic process and that both aspartic cathepsin D and the cysteine cathepsins B and L are potent pro-apoptotic mediators triggering apoptosis upstream of Bax translocation and mitochondrial membrane permeabilization.

Sunlight includes both UV irradiation, which stimulates melanocytes to divide, and infrared irradiation, which generates heat. In response to both heat and UV irradiation, this thesis demonstrates a marked increase in expression of the stress-induced heat shock protein 70 (Hsp70), which was shown to inhibit apoptosis by binding lysosomal and mitochondrial membranes and to counteract the release of cathepsins and cytochrome c. The infrared irradiation from the sun has so far not been considered an aetiological factor for malignant melanoma. However, the anti-apoptotic action of Hsp70 may result in survival of melanocytes containing UV-induced DNA damage, which might constitute potential tumour precursors.

Furthermore, these studies show that UV irradiation activates c-jun N-terminal kinase (JNK). This kinase was found to triggered apoptosis by operating upstream of lysosomal membrane permeabilization and cathepsins release. The pro-apoptotic Bcl-2 family protein Bim, normally sequestered by the anti-apoptotic Mcl-1 protein, was demonstrated to be phosphorylated in a JNK-dependent manner in response to UV irradiation. In addition, a significant decrease in Mcl-1 protein level was observed.

The thesis illustrates that permeabilization of mitochondria and lysosomes and release of their constituents to the cytosol participates in UV-induced apoptosis signalling in human melanocytes in vitro (Fig. 1). The process is regulated by a complex network of pro- and anti-apoptotic proteins, exerting their effect by translocation to new intracellular locations. Increased knowledge of the apoptotic process in melanocytes might lead to better understanding of the development of malignant melanoma and might in the future contribute to new strategies for the prevention and therapy of melanoma.

UVA/B-induced Redox Alterations and Apoptosis in Human Melanocytes

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Petra Wäster, from the Department of Clinical and Experimental Medicine, Division of Dermatology, Faculty of Health Sciences, Linköping, Sweden, defended her doctoral thesis on 5 June 2007 at the University of Linköping. The thesis publication is available at Linköping University Electronic Press: http://www.ep.liu.se/smash/record.jsf?searchId=2&pid=diva2:23600.

Malignant melanoma is one of the most rapidly increasing cancers and accounts for approximately three-quarters of all skin cancer deaths worldwide. Despite compelling evidence that ultraviolet (UV) irradiation causes melanoma, there is limited knowledge of how various wavelength spectra affect the balance between proliferation and apoptosis controlling the homeostasis of the melanocyte population. The aim of this thesis was to elucidate the regulation of UVA/B-induced apoptotic signalling in human epidermal melanocytes *in vitro* in relation to redox alterations and antioxidant photoprotection.

UVB radiation has been regarded as the main cause of malignant melanoma, ascribed to the fact that these wavelengths are absorbed within nucleic acids and are also effective in inducing painful erythema, associated with high risk for malignant melanoma. Recently, however, attention has focused on the role of UVA in the aetiology of melanoma. This thesis demonstrates an altered redox balance, destabilization of plasma membrane integrity, decreased cell proliferation and increased apoptosis in human epidermal melanocytes immediately after irradiation with UVA. In comparison, melanocytes were

found to be markedly resistant to UVB irradiation, although apoptosis was triggered.

Furthermore, we investigated possible protective effects of α-tocopherol and β-carotene in UVA/B-irradiated human melanocytes. α-Tocopherol provided photoprotection through several modes of action affecting redox alterations and signalling, stabilizing the plasma membrane, and decreased proliferation and apoptosis rate, while β-carotene did not show the same protective capacity. Altogether, α-tocopherol might be a useful substance in protecting melanocytes from UV-induced damage.

The ability of tumour cells to evade apoptosis is a central hallmark of the complex multistep process leading to tumour development. Melanocytes have not been described to undergo apoptosis in the epidermis. Melanocytes express constitutively high levels of the anti-apoptotic protein Bcl-2, suggesting resistance to apoptosis. Inability to undergo apoptosis might lead to survival of cells with DNA damage and an increased risk of tumour development. This thesis provides evidence that UVA/B induces apoptosis in melanocytes, which is regulated by the intrinsic mitochondrial pathway. The apoptotic activity of mitochondria depends on the balance between positive and negative apoptotic regulators, such as members of the Bcl-2 protein family, and also on lysosomal cathepsins released to the cytosol. In cells with fragmented nuclei, we report translocation of the pro-apoptotic proteins Bax, Bid, Puma and Noxa from the cytosol to the mitochondria in both UVA- and UVB-exposed melanocytes. The pro-apoptotic process is counteracted by anti-apoptotic proteins, such as Bcl-2 and Bcl-x_L. Both of these anti-apoptotic proteins were found to translocate from the cytosol to the mitochondria in melanocytes having normal-shaped nuclei and thus identified as surviving cells. The Bcl-2 protein is generally considered to be attached strictly to membranes. For the first time, these findings describe cytosolic localization of Bcl-2, which, after UVA/B irradiation, was translocated to the mitochondria. The lysosomal compartment and the function of lysosomal cathepsins were earlier presumed to be limited to degradation of long-lived proteins. However, lysosomal membrane permeabilization and concomitant release of cathepsins to the cytosol have been assigned specific functions during apoptosis initiation. In human melanocytes, we report for the first time UV irradiation to induce lysosomal membrane permeabilization with concomitant release of cathepsins to the cytosol. Pre-treatment with the cathepsin inhibitors prevented apoptosis induction, suggesting pro-apoptotic properties of the cathepsins in human melanocytes.

The p53 protein is an important transcription factor, regulating cellular response following intracellular alterations. UVB irradiation induces direct DNA damage, which is one of the most potent signals leading to p53 activation and, consequently, p53 accumulates in the nucleus in melanocytes. UVA irradiation, instead, gives rise to reactive oxygen species, which induces translocation of p53 to the mitochondria. In addition to the transcriptional role of p53, there is accumulating evidence for transcription-independent p53-mediated apoptotic pathways. The oxidative intracellular environment induced by UVA might lead to oxidation of critical cysteines in p53, resulting in deactivated p53 inducing direct apoptosis at the level of the mitochondria (Fig. 1). Thus, this study shows that, depending on the UV wavelength, p53 mediates apoptosis in melanocytes by transcriptional-dependent or -independent activity. These results emphasize p53 as an important pro-apoptotic component in the regulation of apoptosis.

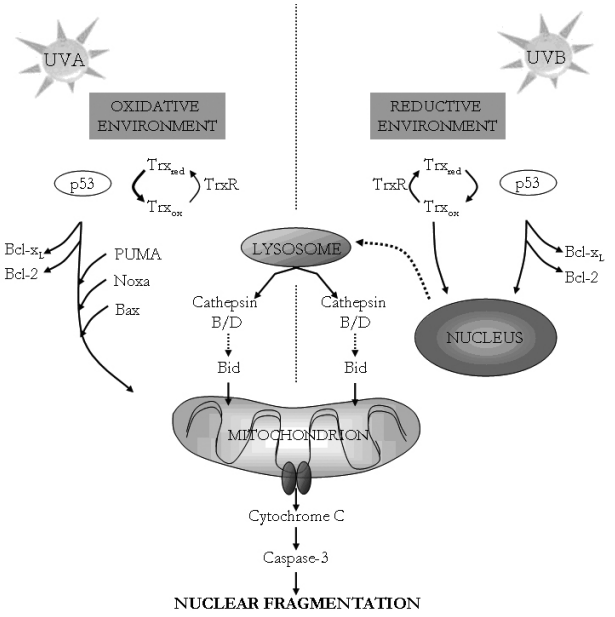

Fig. 1. Model based on the findings of p53-mediated pro-apoptotic events after UVA/B exposure of human melanocytes.

In summary, this thesis provides new insight into the variety of cellular damage and the impact on human melanocytes of different wavelengths within the UV spectrum. The research projects present novel knowledge of UV-mediated apoptosis signalling through the lysosome and the intrinsic mitochondrial pathway in melanocytes. Future investigations will address the initiation process of this pathway upstream of lysosomes, which might lead to the identification of new targets for melanoma therapy. Improved knowledge of the apoptosis regulatory systems in melanocytes might lead to a better understanding of the formation of pigment naevi and malignant melanoma and, in the future, might provide better strategies to prevent and eliminate tumour development and progression.