

## Regulation of UV-induced Apoptosis in Human Melanocytes

CECILIA BIVIK

Department of Clinical and Experimental Medicine, Division of Dermatology, Faculty of Health Sciences, Linköping, Sweden. E-mail: [cecilia.bivik@liu.se](mailto:cecilia.bivik@liu.se)

Cecilia Bivik from the Department of Clinical and Experimental Medicine, Division of Dermatology, Faculty of Health Sciences, Linköping, Sweden, defended her doctoral thesis on 25 May 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=1&pid=diva2:23441>.



Malignant melanoma arises from the pigment producing melanocytes in epidermis and is the most aggressive type of skin cancer. The incidence of malignant melanoma is increasing faster than any other type of cancer in the white population worldwide, with a doubling rate every 10–20 years. So far, the only identified external risk factor for malignant melanoma is sun exposure. Elimination of photodamaged cells by apoptosis (programmed cell death) is essential to prevent tumour formation. Melanocytes are considered relatively resistant to apoptosis; however, the mechanism of regulation of apoptosis in these cells is still unknown. The aim of this thesis was to investigate the apoptotic process following ultraviolet (UV) irradiation in primary cultures of human melanocytes. The focus of this study was on the regulation of mitochondrial stability by Bcl-2 family proteins and the possible participation of lysosomal proteases, cathepsins.

The findings demonstrate that UV irradiation activates the mitochondrial pathway of apoptosis in melanocytes, leading to cytochrome c release, caspase activation and nuclear fragmentation. No change in protein expression of Bax and Bcl-2 was observed in response to UV. Instead, translocation of the Bcl-2 family proteins from cytosol to mitochondria was important in the regulation of survival and death of melanocytes. The pro-apoptotic Bcl-2 family proteins Bax and Bid were translocated to the mitochondria in apoptotic melanocytes with fragmented nucleus, whereas in the surviving population, the anti-apoptotic proteins Bcl-2 and Bcl-XL were redistributed to the mitochondria, counteracting apoptosis.

The lysosomal compartment is the major site of intracellular protein degradation and, until recently, the function of lysosomal proteases, cathepsins, was presumed to be limited to

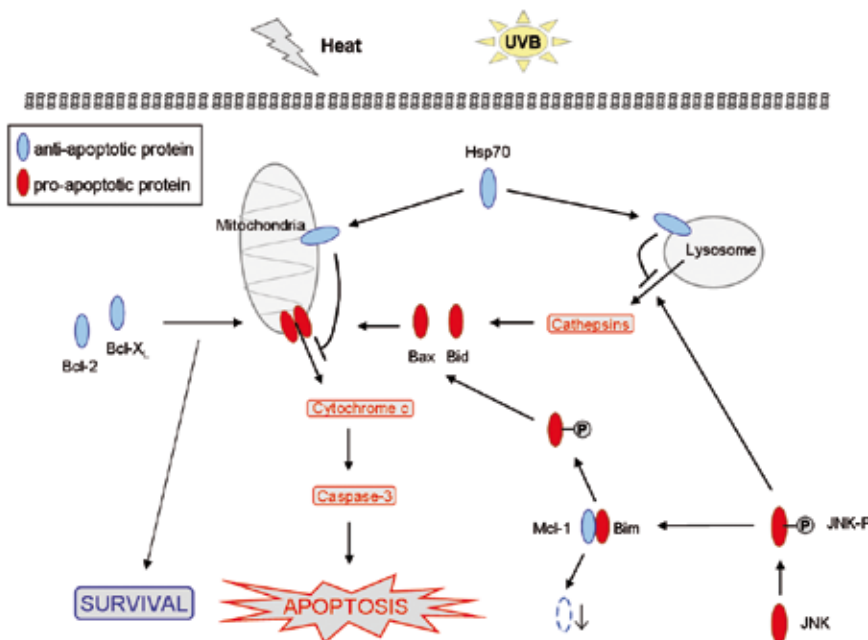


Fig. 1. Proposed model of apoptotic signaling following heat and ultraviolet (UV) irradiation in human melanocytes based on the results of this thesis.

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 trigger 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

PETRA WÄSTER

*Department of Clinical and Experimental Medicine, Division of Dermatology, Faculty of Health Sciences, Linköping, Sweden. E-mail: [petra.waster@liu.se](mailto:petra.waster@liu.se)*

**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