

# UV-Radiation, Apoptosis and Skin

Nives Pustišek<sup>1</sup> and Mirna Šitum<sup>2</sup>

<sup>1</sup> Children's Hospital Klaićeva, »Sestre milosrdnice« University Hospital Center, Zagreb, Croatia

<sup>2</sup> Department of Dermatology and Venerology, »Sestre milosrdnice« University Hospital Center, Zagreb, Croatia

## ABSTRACT

*Apoptosis or programmed cell death is a key function in regulating skin development, homeostasis and tumorigenesis. The epidermis is exposed to various external stimuli and one of the most important is UV radiation. The UVA and UVB spectra differ in their biological effects and in their depth of penetration through the skin layers. UVB rays are absorbed directly by DNA which results in its damage. UVA can also cause DNA damage but primarily by the generation of reactive oxygen species. By eliminating photodamaged cells, apoptosis has an important function in the prevention of epidermal carcinogenesis. UV-induced apoptosis is a complex event involving different pathways. These include: 1. activation of the tumour suppressor gene p53; 2. triggering of cell death receptors directly by UV or by autocrine release of death ligands; 3. mitochondrial damage and cytochrome C release. The extrinsic pathway through death receptors such as fibroblast-associated, tumour necrosis factor receptor and TNF related apoptosis inducing ligand receptor activate caspase cascade. The intrinsic or mitochondrial pathway of apoptosis is regulated by the Bcl-2 family of proteins, anti-apoptotic (Bcl-2, Bcl-xl, Bcl-w) and the pro-apoptotic (Bax, Bak, Bid). The balance between the pro-apoptotic and anti-apoptotic proteins determines cell survival or death. We discuss recent findings in the molecular mechanisms of UV induced apoptosis.*

**Key words:** apoptosis, UV radiation, UV skin damage

## Introduction

Apoptosis or programmed cell death is a key regulator of physiological growth control and tissue homeostasis. The process of apoptosis in the skin has an important role in the maintenance of normal cellular proliferation and normal skin thickness as well as formation of the corneum<sup>1</sup>. In the epidermis, diffusely and under the control of complex genetic programmes, proliferation, differentiation and apoptosis take place interchangeably. Alternating between these processes is necessary for the normal structure and homeostasis of the skin<sup>1,2</sup>. Proliferation is more pronounced in the upper layers of the epidermis and differentiation in the lower layers. In contrast to these two processes, apoptotic gene programme is present diffusely in all skin layers<sup>1,2</sup>. Differences in the expression of specific genes and proteins that play key roles in apoptosis in different epidermal layers are manifest. For instance, basal layer of the epidermis is protected from apoptosis by increased expression of Bcl-2, which cannot be found in suprabasal layers. The expression of Bak protein increases from the basal layers upwards towards the surface of the epidermis and in this way, apoptosis is stimulated<sup>1,3,4</sup>.

Apoptosis is also an important defence mechanism against skin tumours. Namely, when skin cells accumulate excess mutations or genetic damages, apoptotic pathways are activated and cause cell death. The ability to induce apoptosis is essential in order to avoid clonal expansion of damaged cells. Resistance to apoptosis is an important hallmark for malignant transformation<sup>5</sup>. Epidermis is exposed to a variety of factors that may induce apoptosis and probably the most important factor is sun ultraviolet (UV) radiation. UV radiation is capable of activating several apoptotic pathways by inducing DNA damage with activation of tumour suppressor gene p53<sup>6</sup>, by activation of death receptors in the plasma membrane and by activation the mitochondrial pathway<sup>7</sup>.

## UV-Radiation and Apoptosis

The mechanisms of the carcinogenic effect of UV rays are multiple. The most oncogenic portion of the solar spectrum is the UVB rays portion. Namely, DNA absorbs UV rays of the wavelength between 245 to 290 nm<sup>8</sup>. This

is, for the most part, UVB radiation (280–300 nm), as UVC rays (200–280 nm) do not reach the earth surface. At the molecular level, the absorbed UV radiation causes damage to the cellular DNA and induces the formation (6–4) of photoproducts (between two pyrimidines) and cyclobutane dimers (between thymine and cytosine)<sup>8</sup>. These damages are usually repaired by DNA repair mechanisms<sup>8</sup> as normal cell has the ability to repair and remove DNA damages<sup>8–11</sup>. The role of UVA radiation in the process of carcinogenesis is not as well documented as the role of UVB radiation. UVA radiation causes damage indirectly, by forming reactive oxygen free radicals<sup>8,11,12</sup>. Another important component of UV radiation in the process of carcinogenesis is the suppression of the immunological response, which indirectly promotes carcinogenesis<sup>8,11,12</sup>.

The key element of the DNA repair processes is the tumour suppression gene p53, an anti-oncogene located on chromosome 17p. Tumour suppressor p53 is activated under the influence of acute UV radiation and it stops the cell cycle in G1 phase, thus enabling the repair of damaged DNA prior to its replication in S1 phase of the cell cycle. While the cell cycle is on standby, cyclobutane dimers and 6–4 photoproducts are removed using a complex process of excision repair (nucleotide excision repair – NER)<sup>10,11</sup>. Should the repair fail, the death or apoptosis of the affected cell will succeed. Apoptosis is regulated by tumour suppressor p53 as well, and this is why p53 is often called »the guardian of the genome«. Mutations in p53 gene cause the loss of UV light-induced apoptosis in keratinocytes. Later exposure to UV light selectively promotes clonal expansion of cells carrying mutated p53 gene<sup>10,11</sup>. The emergence of malignant tumours is the consequence of the cell's inability to repair damaged DNA<sup>11,12</sup>. Damage to the DNA is the first step in oncogenesis. If the damage is strong or repeated, the protective mechanisms of the cell are no longer able to repair all the damage and that leads to the emergence of carcinoma<sup>11–13</sup>.

DNA damage appears to be the predominant factor determining whether a cell undergoes apoptosis, but it is definitely not the only factor. Death receptors on the cell membrane are involved in UV-induced apoptosis too<sup>7</sup>. They can be activated either directly by UV or by the respective ligands whose release is induced by UV in an autocrine or paracrine manner. This is also called extrinsic apoptotic pathway<sup>7</sup>. Death receptors belong to the tumour necrosis factor (TNF) receptor gen superfamily, which is defined by similar, cysteine-rich extracellular domains and a homologous cytoplasmic sequence termed »death domain«<sup>14,15</sup>. Put simply, activation of the death receptor on the cell surface activates caspase cascade. Caspases are proteases with cysteine tail, which cleave proteins behind the aspartic acid end, leading to termination of apoptosis<sup>15</sup>. So far twelve caspases have been identified, among which there are initiator and effective caspases. Initiator caspases (e.g. 8 and 9) cleave inactive pro-forms of effector caspases, thereby activating them, while effector caspases (e.g. 3, 6 and 7) subsequently

cleave various nucleoproteins, followed by DNA fragmentation and apoptotic cell death<sup>15</sup>.

The intrinsic or mitochondrial pathway is activated through mitochondrial membrane permeabilization. It is not yet clear whether these changes are direct consequences of UV exposure or are related to the membrane and DNA changes<sup>16–18</sup>. The mitochondrial pathway of apoptosis is regulated by the Bcl-2 family of proteins which consists of antiapoptotic (e.g. Bcl-2, Bcl-XL, Bcl-w) and proapoptotic (e.g. Bax, Bak, Bid) proteins<sup>16–18</sup>. Balance between antiapoptotic and proapoptotic proteins determines whether apoptosis is induced or prevented<sup>16–18</sup>. The Bcl-2 proteins are involved in the control of mitochondrial permeability by forming pores in the outer membrane or by regulating the opening and closing of the permeability pores. In response to an apoptotic stimulus, such as UV radiation, proapoptotic proteins are activated and this causes mitochondrial release of cytochrome c and caspase activation<sup>16–18</sup>.

### Clinical Significance of UV Induced Apoptosis

Melanocytes are generally considered to be more resistant to apoptosis than keratinocytes<sup>19</sup>. This can be attributed to several factors, including less extend DNA damage because of the photoprotective effect of melanin, more efficient DNA repair, and/or higher activity of anti-apoptotic and survival pathways in melanocytes<sup>20</sup>. It is well documented that high levels of Bcl-2 are constitutively expressed in melanocytes<sup>21</sup>. Florelli et al. have shown very low rates of apoptosis in nevi<sup>22</sup>. Survivin, an inhibitor of apoptosis, is expressed in melanocytic nevi and melanoma but not in normal melanocytes. Alanko et al. have shown nevomelanocytes to be more resistant to apoptosis than normal melanocytes, and increased resistance to apoptosis in nevi is likely mediated by expression of apoptotic inhibitors<sup>23</sup>.

Although the role of apoptosis in the maintenance of epidermal melanocytes population is not clear, this in-born resistance of melanocytes to apoptosis may have a role in the development of malignant melanoma. Although sun exposure probably plays an important role in melanoma development<sup>24,25</sup>, aetiology of these tumours is variable and multifactorial. Molecular events that regulate cell survival, growth arrest, apoptosis and cell differentiation are important in malignant cell growth<sup>15</sup>. It is well known that malignant tumour cells have defective mechanisms of regulation and apoptosis leading to clonal expansion of malignant cells. Patients with advanced melanoma respond poorly to conventional therapies, largely due to acquired apoptosis resistance in tumour cells<sup>26</sup>. Multiple mechanisms of apoptosis resistance in melanoma have been identified<sup>27</sup> such as dysfunctional death receptor signalling, transcriptional repression of proapoptotic regulators and up regulation of apoptotic inhibitors and so on<sup>27</sup>. The role of apoptosis inhibition in melanoma development is not well understood<sup>27</sup> and it is

one big area of research due to the discovery of new treatment opinions.

Finally, we may conclude that new knowledge on the regulation and mechanisms of apoptosis on the molecular level provides novel insights about the role of apoptosis in tumour pathogenesis and opens up new therapeutic opportunities. Unfortunately, malignant melanoma remains a tumour resistant to therapy. Early diagnosis – meaning good prevention and screening – notwithstanding, the success rates of other therapeutic options remain

low. Understanding of the process and the role of apoptosis in melanocytes, nevomelanocytes and melanoma will open up new opportunities. For instance, the possibility of activation and induction of apoptosis in nevi, especially dysplastic nevi, would be an excellent method of preventing malignant melanoma. Until that becomes a reality, we are left with correct and continual sun protection as the chief protective method against the key etiological factor in skin tumorigenesis.

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N. Pustišek

Children's Hospital Klaićeva, »Sestre milosrdnice« University Hospital Center, Klaićeva 16, 10 000 Zagreb, Croatia  
e-mail: nives.pustisek@kbcsm.hr

## UV ZRAČENJE, APOPTOZA I KOŽA

### SAŽETAK

Apoptoza ili programirana stanična smrt ima ključnu ulogu u procesu razvoja i održavanja homeostaze stanica kože, te u procesu tumorogeneze. Epidermis je izložen cijelom nizu vanjskih čimbenika i jedan od najvažnijih je sunčevo UV zračenje. UVA i UVB zračenje se razlikuje po biološkom efektu i dubini prodora kroz slojeve kože. UVB zračenje se absorbira direktno u molekulu DNA što dovodi do njenog oštećenja. UVA zračenje također uzrokuje DNA oštećenje, ali ne direktno već preko formiranja slobodnih kisikovih radikala. Uklanjanje fotooštećene stanice, apoptoza ima važnu ulogu u sprječavanju epidermalne karcinogeneze. UV inducirana apoptoza je kompleksni proces koji uključuje različite putove. To su: 1. aktivacija tumor supresor gena p 53; 2. aktivacija receptora smrti na površini stanice direktno ili preko liganda; 3. mitohondrijalno oštećenje i oslobađanje citokroma C. Ekstrinzični put aktivacije preko receptora smrti kao *fibroblast-associated, tumour necrosis faktor receptora* i *TNF related apoptosis inducing ligand receptora* uzrokuje aktivaciju kaspaza. Unutarnji ili mitohondrijalni put aktivacije apoptoze je reguliran s Bcl-2 obitelji proteina, antiapoptotičnim (*Bcl-2, Bcl-xl, Bcl-w*) i proapoptotičnim (*Bax, Bak, Bid*) proteinima. Ravnoteža između antiapoptotičnih i proapoptotičnih proteina određuje da li će stanica živjeti ili umrijeti. U radu smo prikazali najnovije spoznaje o molekularnim mehanizmima UV inducirane apoptoze.