Solar radiation is an important risk factor for skin cancer, the incidence of which is increasing, especially in the fair-skinned populations of the world. While the ultraviolet (UV)B component has direct DNA damaging ability, UVA-induced effects are currently mainly attributed to the production of reactive oxygen species. In our recent study, we compared the effects of UVA and UVB radiation on human keratinocytes and found that UVA-induced plasma membrane damage was rapidly repaired by lysosomal exocytosis, which was detected based on the expression of lysosomal membrane associated protein-1 (LAMP-1) on the plasma membrane of non-permeabilized cells. Later, the keratinocytes died through caspase-8 mediated apoptosis. In contrast, the plasma membranes of keratinocytes exposed to UVB showed no LAMP-1 expression, and, although the cells died by apoptosis, no initial caspase-8 activity was detected. We have also demonstrated the occurrence of UVA-induced lysosomal exocytosis in reconstructed skin and shown the relocation of lysosomes from the center of cells to the vicinity of the plasma membrane. Thus, we suggest that lysosomal exocytosis also occurs in keratinocytes covered by the stratum corneum following exposure to UVA. Our findings provide new insight into the mechanism of UVA-induced skin damage.

**UVA-Induced Cell Damage**

Well-established epidemiological evidence has shown that UV irradiation is a major environmental skin carcinogen, but the underlying mechanisms are still not fully elucidated. UV is classified into three types depending on the wavelength. Short-wave UVC (200–280 nm) irradiation is filtered by atmospheric ozone and is not involved in skin damage. The mid-wave UVB (280–320 nm) is partially filtered by the ozone layer, but approximately 5% reaches the earth. UVB is biologically active and penetrates the superficial layers of skin, down to the basal layer of the epidermis, where it generates harmful reactive oxygen species (ROS). Chromophores, such as melanin, and the bases of the DNA molecule are able to absorb the light. Such damage to the DNA molecule can contribute to mutagenic lesions, and the ROS produced in the course of energy release after light absorption is responsible for inflammation of the skin and sunburn. Less energetic than UVB, but present in larger amounts, UVA radiation (320–400 nm) penetrates deeper into the skin, reaching the dermis. Several chromophores absorb UVA energy, including melanin and the riboflavin-containing FAD and FMN. Compared with UVB, UVA causes less obvious damage and was therefore, until recently, considered rather harmless. However, UVA is more penetrating because it reaches the stem cell compartment of the skin; and, because the generated ROS alter proteins, lipids and DNA, UVA increases the risk of cancer development. Indeed, UVA- and UVB-induced adduct formation and signature mutations have been detected in human skin squamous cell carcinomas and solar keratosis. However, UVA fingerprint mutations were mostly localized in the...
basal germinative layer, while UVB fingerprint mutations had a predominantly suprabasal localization, indicating that longer, more penetrating UV wavelengths are important risk factors in cancer development.\(^5\) In addition, UVA significantly contributes to premature skin aging and wrinkle formation. UVB and UVA alter the immune response, either alone or in combination; however, given that UVA is 20-times more abundant in sunlight, the latter is generally considered the main culprit in solar-induced immunosuppression.\(^6\) Taken together, recent data points out UVA as a critical component in skin cancer development. More detailed studies of the mechanisms behind UVA-induced cell damage are, however, needed to elucidate its mechanism of action.

**Exocytosis of Lysosomes**

Lysosomes are acidic cytoplasmic organelles that are responsible for the degradation and recycling of old and damaged macromolecules and organelles.\(^7\) They appear morphologically heterogeneous due to variations in the digested material. However, research conducted over the last several decades have proven lysosomes to be advanced organelles that are crucial regulators of cell homeostasis and are involved in many cellular processes, such as plasma membrane repair, cholesterol homeostasis, and cell death.\(^8,9\) The roles of exocytosis of secretory lysosomes in the immune response, bone resorption, cell signaling, and plasma membrane repair have been studied.\(^10,11\)

After damage, restoration of plasma membrane integrity is essential for the survival of the cell, and conventional lysosomes have been shown to be exocytosed during repair of the plasma membrane. Plasma membrane injury is considered a frequent event in mammalian cells, especially in those that operate under conditions of mechanical stress, such as muscle and skin cells.\(^12\) Moreover, pore-forming toxins such as streptolysin O can induce lysosomal exocytosis within minutes.\(^13\) Lysosomal translocation is controlled by microtubule-mediated long-range transport and results in the formation of a lysosomal patch that eventually fuses with the plasma membrane and restores plasma membrane integrity.\(^14\) Damage to the plasma membrane results in calcium

![Figure 1. Model of lysosomal exocytosis for the repair of UVA-induced plasma membrane damage in human keratinocytes. UVA irradiation induces free radical-mediated plasma membrane damage, which triggers lysosomes to fuse with the plasma membrane in a synaptotagmin VII and calcium-dependent manner. Lysosomal exocytosis is detected by the appearance of lysosome-associated membrane protein (LAMP)-1 at the plasma membrane and extracellular release of the lysosomal content. Acid sphingomyelinase (aSMase) generates ceramide, thereby inducing the formation of lipid rafts, which are important signaling platforms. Exocytosis could be reduced by the pre-treatment of keratinocytes with the antioxidant α-tocopherol (α-Toc), addition of anti-synaptotagmin VII antibodies (Syt-VII-ab), addition of vacuolin-1 or disruption of the lysosomal pH using NH\(_4\)Cl.](image-url)
influx, which causes a conformational change in synaptotagmin VII (Syt VII) at the lysosomal membrane and thus facilitates the formation of a four-helix bundle between t-and v-SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors). This interaction is essential for fusion of the lysosomal and plasma membranes.\textsuperscript{15-17}

Exocytosis can be monitored by the appearance of lysosome-associated membrane proteins (LAMP-1 and LAMP-2) on the plasma membrane of non-permeabilized cells and by the extracellular release of lysosomal enzymes, such as β-hexosaminidase, acid sphingomyelinase and N-acetyl-β-D-glucosaminidase.\textsuperscript{15,18}

**UVA-Induced Lysosomal Exocytosis in Keratinocytes**

We recently conducted a study on the damage done by UVA and UVB radiation to the human keratinocyte plasma membrane, and we found that lysosomal exocytosis is involved in the repair of UVA irradiation-induced plasma membrane damage.\textsuperscript{19} First, we compared the plasma membrane damage induced by UVA and UVB irradiation and found that UVA, but not UVB, induced plasma membrane damage followed by Ca\textsuperscript{2+}-dependent lysosomal exocytosis and caspase-8-mediated apoptosis. Lysosomal exocytosis resulted in the extracellular release of the lysosomal enzymes cathepsin D and acid sphingomyelinase and the appearance of LAMP-1 on the plasma membrane of non-permeabilized cells, as detected by antibodies directed to the luminal portion of LAMP-1. We also prevented exocytosis by adding vacuolin-1\textsuperscript{20}

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**Figure 2.** UVA-induced translocation of lysosomes to the plasma membrane. Human reconstructed skin was exposed to UVA (60 J/cm\textsuperscript{2}) or UVB (500 mJ/cm\textsuperscript{2}) and immediately fixed and processed for immunohistochemistry. A) Representative images of control and UVA-irradiated reconstructed skin stained for lysosomal associated membrane protein-2 (anti-LAMP-2) and nuclei (DAPI) and a merged image with an inset at higher magnification. An image captured using transmitted light (T-PMT) shows the gross morphology, with the stratum corneum marked (*). Note the redistribution of lysosomes from the perinuclear region in control keratinocytes (arrow) to the vicinity of the plasma membrane (arrowhead) in UVA-exposed reconstructed skin. B) Quantification of cells with LAMP-2 located at the plasma membrane (PM) in 5 randomly selected areas in epidermis below stratum corneum (≥ 500 cells) directly after irradiation. *P ≤ 0.05 compared with the control (reconstructed skin from three individuals).
and anti-synaptotagmin VII antibodies, as shown by the significant reduction in LAMP-1 translocation to the plasma membrane after UVA exposure. When lysosomal pH was increased by the addition of NH4Cl, exocytosis was reduced, indicating that lysosomal acidification facilitated exocytosis (Fig. 1). Following exocytosis we identified endocytosis and in the formed vacuolar structures active caspase-8 was detected and did colocalize with cathepsin D. Keratinocyte cultures in monolayers lack keratinized cells from the stratum corneum in the skin; however, it could be argued that this model is not completely relevant from a physiological perspective. To test whether the protective layer of the stratum corneum could protect keratinocytes from UVA-induced plasma membrane damage, we extended our model to reconstructed skin produced using cells derived from human foreskin. Fibroblasts were incorporated into a collagen matrix, on top of which keratinocytes were seeded and allowed to proliferate and differentiate, as described previously. After exposing reconstructed skin to UVA followed by histological preparation and staining of lysosomes with LAMP-2, we found that relocation of the lysosomes from the center of the cells to the vicinity of the plasma membrane did take place in the skin model (Fig. 2A). In accordance with the findings from keratinocytes in mono-layer cultures, UVB-radiation did not cause relocation of lysosomes to the plasma membrane (Fig. 2B). Thus, our findings suggest that lysosomal exocytosis might occur in keratinocytes covered by the stratum corneum following exposure of the skin to UVA.

**New Challenges**

These findings raise several important questions regarding the physiological function of lysosomal exocytosis in the skin. Release of lysosomal content into the extracellular space must be elucidated, and the function must be identified. As studied previously, the release of acid sphingomyelinase catalyzes the production of ceramide, which is important for the formation of lipid rafts and signaling platforms in the plasma membrane. We found that cathepsin D was released into the extracellular space in keratinocyte monolayers, which has been reported to also occur in keratinocytes exposed to ionophores. Interestingly, pro-cathepsin D has been shown to have a mitogenic effect on fibroblasts when released extracellularly. Cathepsins also promote tumorigenesis by stimulating tumor growth, which enhances angiogenesis and facilitates invasion. Thus, it is possible that, following lysosomal exocytosis, the cathepsins located outside of keratinocytes might participate in UVA-induced mitogen stimulation and contribute to skin cancer progression. Furthermore, UVA-induced immunosuppression is mediated by several cytokines, including interleukin-4 (IL-4). IL-4 was recently shown to induce cathepsin activity in macrophages, which promoted pancreatic tumor growth and invasiveness in culture, indicating that UV might promote cathepsin expression.

It will be of great interest to determine whether melanocytes exposed to UVA will also present plasma membrane damage and lysosomal exocytosis. UV-irradiation stimulates the production of melanin, but no consensus model for the delivery of melanosomes to keratinocytes has been presented. The filopodial-phagocytosis and shedding-vesicle models have been presented, but convincing experimental proof for either model is still lacking. Therefore, it would be of interest to study melanosome transfer and compare its mechanism with those of lysosomal exocytosis.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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