Effects of Ultraviolet Radiation on Skin Cell Proteome

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Abstract

Ultraviolet (UV) radiation is known to cause both positive and negative health effects for humans. The synthesis of vitamin D is one of the rare beneficial effects of UV. The negative effects, such as sunburn and premature photoaging of the skin, increase the risk of skin cancer, which is the most detrimental health consequence of UV radiation. Although proteomics has been extensively applied in various areas of the biomedical field, this technique has not been commonly used in the cutaneous biology. Proteome maps of human keratinocytes and of murine skin have been established to characterize the cutaneous responses and the age-related differences. There are very few publications, in which proteomic techniques have been utilized in photobiology and hence there is no systematic research data available of the UV effects on the skin proteome. The proteomic studies have mainly focused on the UV-induced photoaging, which is the consequence of the long-term chronic UV exposure. Since the use of proteomics has been very narrow in the photobiology, there is room for new studies. Proteomics would offer a cost-effective way to large-scale screen the possible target molecules involved in the UV-derived photodamage, especially what the large-scale effects are after the acute and chronic exposure on the different skin cell populations.

Keywords

Proteome • Skin • Non-ionizing radiation • Ultraviolet radiation • Photo aging • Skin cancer • Melanoma

9.1 The Effects of UV on the Skin

Sunlight is the most prominent source of ultraviolet (UV) radiation. Ultraviolet radiation spans a wavelength of 100–400 nanometers (nm), being both non-ionizing and non-visible. The terrestrial

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spectrum of solar UV radiation consists, depending on latitude and season of the year, of 1–5% ultraviolet B radiation (280–320 nm), whereas the majority of the radiation reaching the Earth's surface belongs in the ultraviolet A (320–400 nm) region. Several artificial UV sources, mainly the UV lamps, have been developed for the various purposes, such as for the cosmetic tanning of the skin, for the therapeutic use in the phototherapy, and for the sanitation and germicidal use.

The acute signs of UV exposure are pigmentation (tanning), erythema (sunburn) and the synthesis of vitamin D, which is one of the rare beneficial health effects of UV radiation. Chronic exposure to UV radiation causes premature skin aging and increases the risk of skin cancer. The carcinogenic potential of UV is associated with its ability to suppress the cell-mediated immune responses. Primarily this phenomenon was supposed to prevent the development of excessive inflammation. However, UV-induced immunosuppression may comprise a major risk factor for the development of skin cancer by allowing cancer cells to escape from the immunosurveillance [1].

Skin consists of two major layers. The outermost layer, epidermis, is made up of the stratified squamous epithelium providing the protective barrier against environmental stress. It is formed mainly of keratinocytes, Langerhans cells, and melanocytes. Beneath the epidermis lies a dermis layer, composed of fibroblasts and the connective tissue. Dermis offers skin the strength and elasticity and it also contains the blood capillary system that provides nutrients, and aids in the body's temperature regulation and in metabolic waste product removal.

UVA and UVB radiation have different biological effects on skin. UVB wavelengths are estimated to contribute 80% of the harmful effects of exposure to the sun [2]. Photon energy grows along with the shorter wavelength and thus, UVB wavelengths are more potent in initiating skin carcinogenesis through DNA damage than UVA. UVB mutagenesis is characterized by a high frequency of CC to TT transitions in a DNA strand. These CC to TT tandem mutations are considered as the UVB signature mutations, i.e. so called pyrimidine hot-spots [3, 4]. The genotoxicity of UVA occurs mainly through an indirect photosensitization process via the generation of reactive oxygen species (ROS) that are capable of inducing oxidative DNA damage and mutations [5, 6].

UV radiation is considered as a complete carcinogen of non-melanoma skin cancers (NMSC) by initiating and promoting the carcinogenesis of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). A direct correlation between UVB-induced pyrimidine hot spot mutations in the tumor suppressor protein p53 and the onset of SCC and BCC provides direct evidence for the mutagenic role of UVB in skin carcinogenesis [7–9]. In the melanoma development the main etiological risk factor is the UV radiation, although hereditary reasons also play a notable role in the progression of malignant melanoma. As with the NMSC, individuals sensitive to the sun, who do not tan and burn easily, are at the greatest risk.

Photoaging, i.e. premature aging of the photodamaged skin, is a result of chronic exposure to UV radiation. The clinical signs of photoaging are dryness, roughness, deep wrinkles, irregular pigmentation, elastosis and telangiectasia, which appear in areas heavily exposed to the sun, such as the face, neck and the upper extremities. Photoaging predisposes to the formation for solar keratosis, which is considered a precursor of squamous cell carcinoma. Although UVB radiation is mainly responsible for DNA damage and eventually skin cancer formation, UVA is considered a major factor in the process of skin photoaging. UVA-derived reactive oxygen species lead to the accumulation of disorganized elastic fibers in the dermal compartment causing also loss of interstitial collagen, the main component of the dermal connective tissue [10]. Indeed, the histological hallmark of photoaging, called as solar elastosis, is the massive accumulation of atypical elastotic material in the upper and middle dermis.

9.2 Proteome Analysis of the Skin

9.2.1 Mapping the Human Skin Cells

Proteomics provides nowadays an effective tool to analyze simultaneously the expression profiles of the several proteins. The high throughput protocols, e.g. two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), fluorescent two dimensional difference in-gel electrophoresis (2D-DIGE), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and various chromatography techniques, like liquid chromatography (LC), or tandem mass spectrometry (MS/MS) allow the large-scale analysis of the whole proteome and enable the profiling of different protein isoforms and post-translational modifications, such as phosphorylation and glycosylation.

Although proteomics has been extensively applied in various areas of the biomedical field, this technique has not been commonly used in the cutaneous biology [11, 12]. In 1990s, Celis et al. gathered novel information from the epidermal keratinocytes of the biopsied human skin using 2D-PAGE, microsequencing, and mass spectrometry [13–16]. They established a keratinocyte 2D-PAGE database, in which over 1,000 keratinocyte-derived proteins were identified. This database has been used in profiling bladder squamous cell carcinoma, which resembles closely skin keratinocytes both in morphology and protein expression patterns [17]. Quantitative analysis of protein expression profiles of human epidermis, biopsied from the elderly people, was also generated by Celis' group [18]. Skin proteome was identified by matching the gels with the master 2D gel image of human keratinocyte reference map described above, and selected proteins were confirmed by immunoblotting and/or mass spectrometry. Quantitative analysis of 172 proteins showed that the majority of them (148) remained unaffected by the aging process, but the 22 deregulated proteins, like manganese-superoxide dismutase (Mn-SOD) and heat shock protein 60 (HSP60), support the notion that aging is linked with increased oxidative stress, and the concomitant apoptosis. This is in accordance with the notion that aging is related with the increased oxidative stress, exacerbated by increased production of reactive oxygen species (ROS).

Other proteome maps of skin structures have also been generated. For example a proteome map of a human fibroblast cell line has been established to determine collagen and collagen-related proteins, and to screen the metabolic disorders of the skin [19]. In addition, human epidermal plasma membrane has been characterized by 2D-LC and MS/MS [20]. In that study 57.3% of the identified proteins were assigned as integral membrane or membrane-associated proteins, such as intercellular adhesion proteins and gap junction proteins.

There are also few publications about proteome profiling of special skin organelles. Mitochondrial proteins are essential in metabolics and in regulating apoptosis, and number of disorders is known to be related to mitochondrial dysfunction. Mitochondria from human fibroblasts were characterized by nano-LC-MS/MS analysis of iTRAQ-labeled peptides to study the relative amounts of mitochondrial protein levels, and to identify the metabolic imbalance and cellular stress [21]. Melanosomes [22] and lamellar bodies of the epidermis [23] were identified by 2D-MS/MS, or by nano-LC-MS/MS, respectively, in a scope to study further the biogenesis of lysosome-related organelles. These organelles are responsible for many critical functions in the skin cells and proteome analysis will help and improve the understanding of their biological function.

9.2.2 Mapping the Murine Skin

To characterize the epidermal and dermal responses for the environmental changes, a reference proteome map from BALB/c

abdominal area was established by 2-DE and mass spectrometry [24]. Out of identified 34 proteins, 25 proteins were found to be expressed in the epidermal compartment, whereas 9 proteins were expressed more predominantly in the dermis. Proteins were involved for example in the stress response, apoptosis, growth inhibition, energy metabolism, cholesterol transport and scavenging the free radicals. In the future, this map might help to identify the protein targets for the prediction and prevention of environmentally induced skin conditions, such as skin allergy, carcinogenesis and microbial infections [12, 25].

Scott et al. studied age-related differences in the mouse model. They identified 179 differentially expressed proteins in neonatal BALB/c mice skin as compared to the adult tissue [26]. One such protein was Stefin A, which was abundant in the neonatal skin, but its expression decreased with age, suggesting a functional change during development. Since Stefin A is normally up-regulated in the proliferative diseases, like psoriasis, authors postulated that it is likely that this protein could provide a useful target for diseases of abnormal proliferative conditions, including cancer [26, 27].

9.3 Proteome Analysis of Skin Cells After UV Exposure

Proteomics is not widely used method in the photobiology. There are very few publications, in which proteomic techniques have been utilized in analyzing the UV-derived effects, and hence there is no systematic research data available of the UV effects on the skin proteome.

There is only one study so far, where the *acute* photodamage has been studied among the benign skin cells. Photodamage was induced by a single UVB exposure (100 mJ/cm^2) in the dermal fibroblasts by 2D-PAGE/MS, and obtained protein spots were confirmed by qPCR and Western blot analysis [28]. In the UVB-treated cells, 18 differentially expressed proteins were identified, from which receptor-interacting protein (RIP) and vimentin were significantly up-regulated. RIP plays a role in triggering apoptosis, whereas vimentin is a vital cytoskeletal protein in fibroblasts, protecting them from physical injury and DNA damage. Overexpression of vimentin can also delay a cell death. Authors speculated that RIP may represent a key regulatory step in triggering cell death after UVB exposure, whereas vimentin may contribute to the resistance of cells to UVB-induced damage [28].

The proteomic studies have mainly focused on the UV-induced photoaging, which is the consequence of the long-term *chronic* UV exposure. Bertrand-Vallery et al. studied the effect of the multiple UVB radiation doses (8×300 mJ/cm^2) on the keratinocytes by 2D-DIGE [29]. Sixty-nine differentially expressed proteins, which were involved in the keratinocyte differentiation and survival, were identified by LC-MS/MS. Out of the identified proteins the most interesting candidate for further analysis was TRIparite Motif Protein 29 (TRIM29), validated by Western blot, immunochemistry, and RT-PCR. It was found to be expressed very abundant in keratinocytes and reconstructed epidermis. Originally TRIM29 was discovered from the cells of Ataxia Telangienctasia patients, and it is known to partially suppress the sensitivity to ionizing radiation [30]. Bertrand-Vallery et al. suggested that TRIM29 participates in the survival of differentiating keratinocytes and may allow them to enter a protective alternative differentiation process, rather than die massively after UV-induced stress. This finding is a novel mechanism that allows the survival of keratinocytes as they migrate and differentiate in the skin [29].

When the chronic UVA exposure-induced alterations were studied by 2D-DIGE/MS in the dermal fibroblast cell culture, the most pronounced protein undergoing UVA-induced down-regulation was cathepsin B, verified by Western blot analysis [31]. The used UVA regimen was given as a chronic exposure for 3 weeks and the total UVA dose was 59.4 J/cm^2. Cathepsin B is a lysosomal cysteine-protease, which is known to be inactivated by oxidative-stress related conditions in the cells. Indeed, Lamore et al. showed, that UVA-induced loss of cathepsin B enzymatic activity in fibroblasts was

suppressed by antioxidant intervention, and that UVA-irradiation of fibroblasts led to the massive lipofuscin accumulation in lysosomes, most likely due to the impaired cathepsin B activity. Authors discussed the possibility that cathepsin B may be a crucial target of UVA-induced photo-oxidative stress causatively involved in dermal photodamage through the impairment of lysosomal removal of lipofuscin [31].

In addition to the cell culture studies, the albino hairless HR-1 mouse model has also been utilized when studying the photoaging [32]. Mice were irradiated 5 days per week for 7 weeks with the total UVB dose of 2.31 J/cm^2. Proteomic analysis revealed 17 differently expressed proteins in the photoaged skin, for example superoxide dismutase (SOD) and malonialdehyde (MDA). Additionally in this set-up, the antioxidant properties of Korean deciduous tree extract, *Machilus thunbergii Sieb et Zucc* (*M. thunbergii*), widely used in the traditional medicine, was examined. The dorsal skin of mice was treated topically with *M. thunbergii* for 2 h prior to UV irradiation and proteomes from the skin of each study group were analyzed. *M. thunbergii* treatment altered the expression of several proteins. Interestingly, among 15 proteins upregulated by UV-irradiation, 13 proteins were downregulated after treatment with *M. thunbergii*. Also the thickness of the dorsal skin was significantly decreased in the group that was UV-exposed and treated with *M. thunbergii* extract. This result possibly indicates that *M. thunbergii* might have had antiphotoaging effects [32].

So far, there is only one proteomic study performed with human excised native skin after the chronic UV exposure. When skin samples were irradiated with solar simulated UV radiation for periods of 3 or 4 h (the total cumulative UV dose of 11.25 J/cm^2 and 15 J/cm^2, respectively) and analyzed by 2D-PAGE/MS, oxidative stress markers were seen expressed differentially in the UV irradiated samples and the non-exposed controls [33]. Apart from the proteins known as the UV-related oxidative stress markers, such as HSP27 and MnSOD, authors identified also two novel proteins that were down-regulated after UV exposure. Further analysis revealed that these proteins were the de-phosphorylated forms of cofilin-1 and destrin, which are known to be actin-cytoskeleton modulators [33].

9.4 Conclusions

As a summary, so far the UV-induced alterations found by the proteomic approach have mainly been involved in the oxidative stress-related changes. Since the use of proteomics has been very narrow in the photobiology, there is room for new studies. Proteomics would also offer a cost-effective way to large-scale screen the possible target molecules involved in the UV-derived photodamage. Both in vitro and especially in vivo experimental set-ups are required to better answer the question what the large-scale effects are after the acute and chronic exposure on the different skin cell populations, and what novel target molecules can be found on the skin proteome after exposing the skin cells with the different wavelengths of UV radiation.

Finally, since the skin cancer formation is the most detrimental health hazard deriving from the UV radiation, proteomics might offer a useful way to study photocarcinogenesis. Malignant melanoma is associated with poor prognosis, especially if the tumor has progressed into the metastatic phase. Thus, early detection would improve substantially patient survival. Proteomics also appears to be an ideal choice for the discovery of new melanoma biomarkers in humans [34]. Proteins specifically secreted by tumor cells, for example proteins shed into the blood, may serve as early cancer biomarkers. Several proteomic approaches have been utilized in the search for clinically relevant biomarkers in melanoma, but so far the results have been relatively limited. Human melanoma proteomic studies have been reviewed in a detailed manner in the excellent review article written by Sabel et al. [35].

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