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Unraveling the human protein atlas of metastatic melanoma in the course of ultraviolet radiation-derived photo-therapy

Eumorphia G. Konstantakou[a,1,2], Athanassios D. Velentzas[a,2], Athanasios K. Anagnostopoulos[b,2], Aikaterini F. Giannopoulou[a], Ema Anastasiadou[c], Issidora S. Papassideri[a], Gerassimos E. Voutsinas[d], George Th. Tsangaris[b,*,3], Dimitrios J. Stravopodis[a,*,3]

[a] Section of Cell Biology and Biophysics, Department of Biology, School of Science, National and Kapodistrian University of Athens, Athens, Greece
[b] Proteomics Core Facility, Systems Biology Center, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[c] Basic Research Center, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[d] Laboratory of Environmental Mutagenesis and Carcinogenesis, Institute of Biosciences and Applications, National Center for Scientific Research "Demokritos", Athens, Greece

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ABSTRACT

To explore the photo-therapeutic capacity of UV radiation in solid tumors, we herein employed an nLC-MS/MS technology to profile the proteomic landscape of irradiated WM-266-4 human metastatic-melanoma cells. Obtained data resulted in proteomic catalogues of 5982 and 7280 proteins for UVB- and UVC-radiation conditions, respectively, and indicated the ability of UVB/C-radiation forms to eliminate metastatic-melanoma cells through induction of synergistically operating programs of apoptosis and necroptosis. However, it seems that one or more WM-266-4 cell sub-populations may escape from UV-radiation's photo-damaging activity, acquiring, besides apoptosis tolerance, an EMT phenotype that likely offers them the advantage of developing resistance to certain chemotherapeutic drugs. Low levels of autophagy may also critically contribute to the selective survival and growth of UV-irradiated melanoma-cell escapers. These are the cells that must be systemically targeted with novel therapeutic schemes, like the one of UV radiation and Irinotecan herein suggested to be holding strong promise for the effective treatment of metastatic-melanoma patients. Given the dual nature of UV radiation to serve as both anti-tumorigenic and tumorigenic agent, all individuals being subjected to risk factors for melanoma development have to be appropriately informed and educated, in order to integrate the innovative PPPM concept in their healthcare-sector management.

Significance: This study reports the application of nLC-MS/MS technology to deeply map the proteomic landscape of UV-irradiated human metastatic-melanoma cells. Data bioinformatics processing led to molecular-network reconstructions that unearthed the dual nature of UV radiation to serve as both anti-tumorigenic and tumorigenic factor in metastatic-melanoma cellular environments. Our UV radiation-derived "photo-proteomic" atlas may prove valuable for the identification of new biomarkers and development of novel therapies for the disease. Given that UV radiation represents a major risk factor causing melanoma, a PPPM-based life style and clinical practice must be embraced by all individuals being prone to disease's appearance and expansion.

1. Introduction

Ultraviolet (UV) radiation belongs to the electromagnetic spectrum that ranges between 100 and 400 nm. At the Copenhagen meeting of the 2nd International Congress on Light (1932), UV radiation was conventionally categorized into three spectral regions: 315–400 nm (UVA), 280–315 nm (UVB) and 100–280 nm (UVC). However, the subdivisions are relatively arbitrary and somewhat differ depending on the discipline involved. For example, dermatological and molecular photobiologists normally define the UV radiation-wavelength regions as 320–400 nm for UVA, 290–320 nm for UVB and 200–290 nm for UVC, with the boundary between UVB and UVC radiation being alternatively

* Corresponding authors.
E-mail addresses: gthtsangaris@bioacademy.gr (G.T. Tsangaris), dstravop@biol.uoa.gr (D.J. Stravopodis).
[1] Current Address: Harvard Medical School, Massachusetts General Hospital Cancer Center, Charlestown, Massachusetts, USA.
[2] These authors contributed equally to this work.
[3] These authors also contributed equally to this work.

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specified at 280 nm. Sunlight-derived UV radiation represents a prominent environmental risk factor and a physical carcinogen. Since UVC radiation is filtered out by the ozone layer, only UVA and UVB radiations can reach Earth's surface (90–99% and 1–10%, respectively) and thus are wavelengths of major biological importance [1–5]. However, the increased risk of ozone loss caused by convectively injected water vapor into the stratosphere [6] is foreseen to be associated in the future with a following rise of UV-radiation load onto living organisms. Besides the natural (solar) origin of UV radiation, artificial sources also emit UV radiation of a spectrum of wavelengths frequently used in medicine, industry, research and business, and for cosmetic and domestic purposes, as well. Commercial germicidal lamps that are designed to emit UVC radiation and to disinfect water supplies (and surfaces in laboratories and food-processing industries), indoor tanning salons, and photo-therapy devices represent the most popular and characteristic examples [7,8].

Mechanistically, the most frequent pro-mutagenic DNA lesions induced by UVB and UVC radiations are the *cis-syn* Cyclobutane Pyrimidine Dimers (CPDs) and Pyrimidine (6-4) Pyrimidone Photoproducts ((6-4)PPs) adducts, which if not repaired can promote single C to T or tandem CC to TT transition mutations [1–3]. These UV radiation-specific signature mutations have been previously identified in the tumor-suppressor gene *TP53* of sun-damaged skin suffering by Basal Cell Carcinoma (BCC) or Squamous Cell Carcinoma (SCC) [9,10]. Interestingly, the somatic-mutation catalogue (33,345 substitutions) of COLO-829 human malignant melanoma cell line proved to contain a mutational spectrum of 360 (out of 510) CC > TT/GG > AA dinucleotide substitutions that is reminiscent of the one tightly associated with UV-radiation exposure [11]. From a compilation of 4,938,362 somatic mutations detected in 7042 primary cancers, more than 20 mutational signatures could be extracted, with a UV radiation-derived-damage one dominating the melanoma genomes [12,13]. Taken together, it seems that UV radiation constitutes one of the strongest risk factors for melanoma development [13].

Melanoma arises from malignant transformation of melanocytes, the cells that are committed to melanin production in the skin, eye, mucosal epithelia and meninges. Pigment production in melanocytes is activated by UV radiation-induced DNA damage to adjacent keratinocytes. Stimulated keratinocytes synthesize and secrete α-Melanocyte Stimulating Hormone (αMSH) in a TP53-dependent manner. αMSH binds to Melanocortin 1 Receptor (MC1R), expressed on melanocytes, and through engagement of, among others (e.g. DAG/PKC and NO/PKG), the cAMP/PKA/CREB/MITF signaling axis induces melanin synthesis (a process that is controlled by the MITF target gene *TYR*) and eventual transfer of melanosomes (melanin-containing vesicles) back to keratinocytes [13–20]. Interestingly, certain MC1R variants have been associated with an increased risk for melanoma development [14,17,21–25], while a cAMP-dependent signaling network has proved able to confer resistance of melanoma cells to MAPK-pathway inhibitors [26]. On the other hand, dominant-negative CREB expression can inhibit tumor growth and metastasis of human melanoma cells, and also reduce their resistance to radiation [27,28].

The melanoma incidence in US has dramatically increased from $1/10^5$ (1935) to $23/10^5$ (2012) affected people per year [29]. Melanoma is the most dangerous form that accounts for the majority of skin-cancer deaths. If diagnosed early, it can be treated by surgical resection. However, metastatic melanoma is presented as largely refractory to the applied therapies, with a 5-years survival rate of less than 5% [30]. Via employment of a high-coverage Whole-Genome Sequencing (WGS) approach [31,32], mutated -distinct- landscapes of cutaneous, acral (hands/feet) and mucosal (internal body surfaces) melanoma sub-types have been recently described [33]. Among the mutated genes identified, *BRAF* [34], *TP53* [35], *CDKN2A* [36] and *NRAS* [37] were carried by cutaneous melanoma [33], while *BRAF*, *NRAS* and *NF1* [38] were detected in acral melanoma [33]. Notably, *SF3B1* [39] was characterized as a significantly mutated gene in mucosal melanoma, for the first time [33]. *TERT* [40] promoter mutations were the most frequent of all [33]. Besides distinct sub-types, oncogenic mutations, and/or copy-number increases (amplifications), identified in additional genes, such as *KIT* [41–43] and *MITF* [43–46], critically reflect malignant melanoma's heterogeneity [47]. Cutaneous-melanoma mutation spectra proved to be dominated by three novel UV radiation-exposure signatures, indicating the operation of still unknown mechanisms of UV radiation-mediated damage [33]. Exemplifying the ability of UV radiation to accelerate $BRAF^{V600E}$-driven melanomagenesis by *TP53* targeting [48], all UV radiation-induced signaling networks have to be mechanistically elucidated and pharmaceutically manipulated.

Currently, there are several drugs in the phase of clinical development for melanoma therapeutic management [49,50], with Vemurafenib being the first one approved by FDA (the US Food and Drug Administration) (2011) for treatment of, unresectable or metastatic, $BRAF^{V600E}$-positive melanoma [13,51,52]. Despite its remedial capacity, a number of resistance mechanisms can be developed in mutant-BRAF melanoma, rendering cancer cells refractory to Vemurafenib-based regimens [53]. NRAS upregulation [54], TGFβ-dependent upregulation of EGFR and PDGFRB [55], aberrantly spliced $BRAF^{V600E}$ dimerization [56], tumor stroma-derived HGF secretion [57] and eIF4F translation-complex persistent formation [58] constitute major mechanisms of mutant BRAF-cell tolerance to Vemurafenib (and its related structural analogues). Hence, novel, efficient and systemically safe therapeutic protocols are necessitated to be promptly developed for the successful management of malignant melanoma.

To this direction, and taking as an example the potential of UV radiation to likely serve as a photo-therapeutic tool for the most aggressive primary brain tumor glioblastoma multiforme [59], we have herein attempted to deeply profile the UV radiation-induced human melanoma proteome, in an effort not only to illuminate the UV radiation-driven cytotoxic mechanisms but also to identify novel processes that may orchestrate drug resistance and metastatic progression in response to UV-radiation actions.

2. Materials and methods

2.1. Cell line and culture conditions

WM-266-4 is a hypertriploid human cancer-cell line being characterized by epithelial morphology and adherent pattern of growth. It was originally established from a cutaneous metastasis of malignant melanoma in a 58-years old female patient. WM-266-4 metastatic melanoma-cell line carries wild-type KIT and NRAS, but mutant BRAF (V600D) proteins, while it can produce eumelanin-positive (and -negative) xenograft tumors when injected into SCID (immunocompromised) mice [60]. Cells were obtained from ECACC/Sigma-Aldrich (Munich, Germany), and cultured in 1 × DMEM (supplemented with FBS, L-Glutamine, Sodium Pyruvate, Sodium Bicarbonate, Non-Essential Amino Acids, Penicillin and Streptomycin) at 37 °C and 5% CO_2. For each experimental condition, large-scale cultures of WM-266-4 cells were harvested (via scraping) and subsequently centrifuged at 750*g* for 10 min, while the produced pellet was stored at -20 °C for further processing. All culture media and reagents were purchased from Merck Millipore-Biochrom AG (Merck KGaA, Darmstadt, Germany).

2.2. UV-irradiation protocol

WM-266-4 melanoma-cell mono-layers were washed twice with 1 × PBS and subsequently exposed to UVB (302 nm) or UVC (254 nm) lamp devices (UVP LLC, California, USA). Irradiation time was chosen at 2 or 5 min per UV-radiation type, while distance from UV-light source was set at 15 cm. The surface power density measured for UVB radiation was 5 mW/cm^2 and for UVC radiation was 3 mW/cm^2. Cells were allowed to grow, in 1 × DMEM complete medium, for 24 h post-