Montagna Symposium 2013—Light and Skin: How Light Sustains, Damages, Treats, Images and Modifies Skin Biology

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The 62nd Annual Montagna Symposium on the Biology of Skin, ''Light and Skin: How Light Sustains, Damages, Treats, Images and Modifies Skin Biology,'' was held from October 10 to 14, 2013, in Stevenson, Washington. Life and skin evolved under sunlight; dermatology will forever be entwined with photomedicine. We routinely use microscopy, optical diagnostics, phototherapy, photodynamic therapy, and lasers while dealing with melanoma, non-melanoma skin cancer, photosensitivity disorders, and photoaging. Moreover, there are misunderstandings, recent surprises, mysteries, and challenges. How do the protean cutaneous and systemic effects of light really happen? Exactly what is healthy and unhealthy about light? Can these effects be separated? What is the ideal sunscreen? What can we ''see'' inside live skin? Which technologies are pushing the limits for therapy and diagnosis? How to ''translate'' promising research all the way to practical impact? The Symposium was organized by Molly Kulesz-Martin and chaired by Rox Anderson with Session Chairs David Fisher, Barbara A. Gilchrest, and Steven Jacques.

The meeting started (and ended) with provocative talks by British dermatologist-scientists. Antony Young delivered the keynote address, ''Impact of Climate Change on Skin,'' describing the first large prospective database regarding human behavior, sun exposure, sunscreen use, and biomarkers for UV exposure. He reported large prospective, collaborative studies measuring solar UV exposure, hydroxy-vitamin D3 levels, and biomarkers of DNA damage and repair in ''free-ranging'' Europeans at home and on holiday. The subjects kept detailed logs of their activities and sunscreen use, wore a watch-like monitor of solar UV exposure, and provided blood and urine samples. Sun exposure was highly correlated with thymidine dimer products in the urine and significantly correlated with circulating 25-hydroxy-vitamin D3 levels, and both were decreased with sunscreen use. Surprisingly, the slope of the solar exposure–vitamin D3 dose-response curve was independent of skin pigmentation, suggesting that substantial vitamin D3 photochemistry occurs in the upper epidermis above the heavily pigmented basal cell layer.

Session Chair David Fisher presented the molecular, cell, and skin biology underlying pigmentation and melanoma. The UV-induced melanogenesis cascade includes keratinocyte synthesis of pro-opiomelanocortin macropeptide, which is cleaved to produce both MSH (melanocyte-stimulating hormones) and endorphins. Mice (much like Young's Europeans) become addicted to UV exposure and actively choose it, a preference that was suppressed when endorphin receptors were blocked by naloxone. Why has a systemic system for sun-seeking behavior evolved, with skin at its center? Melanocortin receptor (MC1R) sequence polymorphisms that ablate function lead to red hair and increased susceptibility to UV-induced skin cancers. In mice, forskolin, a cAMP agonist, bypasses the MC1R defect and leads to the production of eumelanin. Potentially, phosphodiesterase PDE4D3 inhibitors that increase cAMP levels in humans could reduce melanoma risk in redheads. But how does MC1R deficiency increase skin cancer risk? Mice lacking pheomelanin (deficient in both MC1R and tyrosinase) are less susceptible, strongly suggesting that pheomelanin *per se* enhances UV-independent and UV-induced skin cancers. Furthermore, increased DNA oxidation products were found without any UV exposure in the red-haired mice. These results from the Fisher lab suggest that pheomelanin elevates production of reactive oxygen species, which can drive UV-induced mutations and skin cancer, and that antioxidant protection strategies may work best in redheads.

Emi Nishimura reported that targeting c-kit produces temporary graying of hair in mice, without loss of follicular melanocytes. In contrast, aging decreases and eventually depletes the melanocyte stem cells (MSCs) in the bulge region of hair follicles, after which the hair remains permanently white. Mutation and/or genetic instability appears to have a central role in this. Aging and X-ray exposure are associated with increased expression of γH2AX, a DNA damage marker, in MSCs of the hair follicle bulge. Nishimura hypothesized that follicular MSCs may have unusual susceptibility to ionizing radiation damage, and subsequently found that activated MSCs are less sensitive to X-ray than nonactivated MSCs. The mechanisms involved may shed light on hair graying, including its prevention and reversal.

Andrew Borkowski reported mechanisms by which damage-associated molecular

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pathways (DAMPs), in particular small noncoding RNAs, are important for skin-barrier maintenance. Toll-like receptor 3 (TRL3) is a target for DAMPs. Inhibition of TLR3 caused transient profound decrease of skin-barrier response genes. In particular, UVB exposure damages the skin barrier and stimulates a robust epidermal barrier repair response. Richard Gallo further discussed the role of DAMPs in mediating UV-induced immunosuppression and apoptosis. Keratinocytes not exposed to UV but to media from UV-exposed cells exhibit a bystander injury response, which can be blocked by addition of RNAse or TLR3 inhibitors. Gallo noted that the skin-commensal bacterium *S. epidermidis* partially inhibits some TLR3-mediated responses, presumably conferring tolerance and perhaps some abrogation of UV responses. Could TLR3 inhibitors treat certain photosensitivity diseases—such as lupus erythematosus—with DAMP-mediated pathophysiology?

UV- and light-induced genotoxicity occurs from multiple mechanisms, including primary DNA photochemistry, oxidative photoproducts, delayed damage from endogenous response pathways, and faulty repair. Sergiy Kyryachenko introduced the topic of keratinocyte-derived factors, such as alpha-melanocyte-stimulating hormone-sand endothelin, in promoting melanoma, and Arup Indra discussed RXR retinoid receptors in modulating innate immunity and cell survival after UV exposure. Sancy Leachman discussed the complex mechanisms and action spectrum for UV-induced genotoxicity, with emphasis on oxidative damage. Nrf-2 is part of a MC1R-mediated pathway that regulates antioxidant genes. In MC1R-deficient mice, Nrf-2 is inactive and antioxidant gene expression is reduced. Leachman reported a series of studies showing that sulforaphane, an antioxidant contained in broccoli sprouts, restores Nrf-2 activity through interaction with a protein called Keap-1.

Session Chair Barbara Gilchrest spoke about the presumptive role of telomeres in UV responses. She noted that the disruption of the telomere loop experimentally can initiate DNA damage–like signaling, resulting in cell senescence (permanent inability to divide) or apoptosis depending on the cell type. Because telomeres in all mammalian species are tandem repeats of TTAGGG and its complement, the telomere is an excellent target for DNA damage: thymidine dinucleotides, the substrate for perhaps 70% of UVB damage; and guanine, the target for oxidative DNA damage and major target for UVA irradiation. The extensive damage introduced during UV irradiation or the influx of repair proteins might therefore disrupt the telomere loop, leading to DNA damage signaling that, if sufficiently severe, would result in cell senescence and apoptosis in the skin, consistent with photoaging, or if less severe to p53-mediated protective responses such as the well-documented enhanced melanogenesis (tanning) and the upregulation of DNA repair capacity. As further support for a central role of telomeres in cellular response to UV, identical responses can be induced by treatment of cells or intact skin with telomere homolog oligonucleotides that interact with telomeric DNA to activate the identical signaling pathway.

Craig Elmets discussed the role of cyclooxygenase (COX) genes in mediating UV-induced skin cancer. UV induces COX-2 and downstream prostaglandin E2, a major inflammatory cytokine. In mice, knocking out COX-2 or administering the selective inhibitor celecoxib significantly reduces UV-induced skin cancer. In a randomized controlled clinical trial of celecoxib in >200 skin cancer–prone subjects, there was no significant reduction in actinic keratosis, but non-melanoma skin cancers were significantly reduced by more than 50%. Topical COX-2 inhibitors could possibly be developed for skin cancer reduction in organ transplant recipients.

Which proteases have a role in photoaging? Thomas Ruenger reported that solar elastosis involves cathepsin K, a lysosomal enzyme that is debatably the most potent of human collagenases. Cathepsin K knockout mice exhibit hypertrophic scarring. Cathepsin K also processes elastin, which accumulates in photoaged dermis. In fibroblasts from young individuals, but not old individuals, cathepsin K was induced by UVA (not UVB). Other disorders exist in which abnormal extracellular proteins accumulate during aging due to the failure of lysosome functioning as chaperone protein-mediated autophagy decreases. In the Hutchinson Gilford progeria syndrome, a protein called progerin accumulates. Runger reported that UVA (again, not UVB) induced accumulation of progerin in normal skin fibroblasts, along with cell nuclear changes similar to Hutchinson Gilford progeria syndrome. Preventive strategies may emerge from this research.

A promising new tool for evaluating the pathogenesis of various types of skin inflammation was presented by Phillip Tong. He described a cutaneous ''immune atlas'' created by using intravital multiphoton microscopy (MPM) to visualize fluorescently tagged immune populations in reporter mice. He showed three-dimensional imaging of immune cells infiltrating living mouse skin, and quantified macrophages, dermal dendritic cells, mast cells, and T cells in defined locations in the skin at multiple body sites.

Session Chair Steven Jacques led a tour through imaging technologies introducing a superb session on optical imaging, microscopy, and spectroscopy. He explained the energy source and wavelength-dependent ability to use light in order to probe tissue structure on the nano-, micro-, and meso-scales. In particular, second-harmonic-generation imaging nanoscale sensing (1–10 nm) and its dependence on asymmetrical structure was explained. Light scattering as a contrast mechanism, offering macroscale sensing (50–10 μm), was discussed with examples of monitoring collagen fibrils transition to larger collagen fiber bundles.

The intrinsic optical absorption and scattering properties of skin can be determined from external measurements. Anthony Durkin is using spatial light modulation over a range of wavelengths to noninvasively map the oxygen saturation of cutaneous blood. He found that the detection of cutaneous burn injury depth was successful in a swine model, even for burns deeper than the eye could see.

In vivo microscopy is painless and harmless, and yields images immediately without artifacts, but lacks the advantage of specific tissue staining. Haishan Zeng presented impressive images of skin by coregistering reflectance confocal microscopy (RCM), MPM, and Raman