MONTAGNA SYMPOSIUM 2013 Light and Skin: How Light Sustains, Damages, Treats, Images and Modifies Skin Biology

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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 October 10–14, 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 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 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 activity 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, 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 (POMC) macropeptide, which is cleaved to produce both MSH (melanocyte-stimulating hormones) and endorphins. Mice (much like Young's

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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 anti-oxidant 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 appear to play a central role. Aging and X-ray exposure are associated with increased expression of γH2AX, a DNA damage marker, in MSCs of the hair follicle bulge. She hypothesized that follicular MSCs may have unusual susceptibility to ionizing radiation damage, and subsequently found that activated melanocyte stem cells are *less* sensitive to X-ray than non-activated MSCs. The mechanisms involved may shed light on hair graying, including prevention and reversal.

Andrew Borkowski reported mechanisms by which damage-associated molecular pathways (DAMPs), in particular small non-coding 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 TLR-3 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-MSH and 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. She 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 disruption of the telomere loop experimentally can initiate DNA damage-like signaling, resulting in cell senescence (permanent inability to divide) or apoptosis, depending on 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 (TT), the substrate for perhaps 70% of UVB damage, and guanine (G), 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 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 cyclo-oxygenase (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 giving 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 play a role in photoaging? **Thomas Runger** reported that solar elastosis involves cathepsin K, a lysosomal enzyme that is debatably the most potent of human collagenases. Cathepsin K knock-out 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 failure of lysosome functioning as chaperone protein-mediated autophagy decreases. In the Hutchinson Gilford progeria syndrome (HGPS), a protein called progerin accumulates. He reported that UVA (again, not UVB) induced accumulation of progerin in normal skin fibroblasts, along with cell nuclear changes similar to HGPS. Preventive strategies may emerge from this research.

A promising a new tool for evaluating the pathogenesis of various types of skin inflammation was presented by **Phillip Tong.** He described a cutaneous “immune atlas” created 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 to probe tissue structure on the nano-, micro- and meso-scales. In particular, second-harmonic-generation imaging (SHG) nano-scale sensing (1–10 nm) and its dependence on asymmetrical structure was explained. Light scattering as a contrast mechanism, offering macro-scale sensing (50nm-10um), 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 non-invasively map the oxygen saturation of cutaneous blood. He found that 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 co-registering reflectance confocal microscopy (RCM), MPM and Raman spectroscopy (RS). RCM has been in steady use for live human skin imaging for more than a decade. RS interrogates molecular structure, e.g. of small metabolites, lipids, nucleic acids and proteins. Zeng discussed microscopes that combine RCM for structural imaging with MPM for identification of elastin, NADH, collagen and other molecules, and with RS for point determination of chemical structures.

Impressive progress of RCM toward clinical applications was presented by **Milind Rajadhyaksha**. Tumor cell nuclei are easily seen when aluminum chloride or acetic acid is applied as contrast agent for about 1 minute. For basal cell carcinoma, RCM has sensitivity of 92–100% and specificity of 88–97%. Rajadhyaksha proposed a “real-time” analogue of Mohs surgery, using RCM to guide an erbium (Er) laser for tissue removal. Imaging was possible through the thin layer of thermal injury left by erbium laser, and a false color rendition of RCM images was able to mimic the familiar appearance of H&E. **Daniel Gareau** presented computer vision algorithms to differentiate histologically identified melanomas from benign nevi. Using a novel polar representation of dermoscopy image metrics, neural network training was performed on a set of 88 lesions, which yielded ten metrics significantly correlated with melanoma. Next, in a prospective set of 60 melanomas and 60 nevi, the neural network achieved 100% sensitivity and 30% specificity, which is much better than expected for a commercially available mole-imaging device.

Optical coherence tomography (OCT) is an optical analogue to ultrasound imaging, detecting back-scattered light as a function of depth in tissue. Blood flow modulates the back-scattered light, and **Ruikang Wang** showed remarkable images of skin microvasculature obtained by Doppler OCT. Another strategy for label-free imaging of perfusion is speckle modulation. Speckle is an interference pattern of back-scattered light

from coherent (laser) sources, which moves when the light-scattering objects move, e.g. with blood flow. Overall this session showed that huge strides have been made in the imaging field, and these are heading for clinical applications.

At the Saturday banquet, **John Parrish** presented via video (now available at montagnasymposium.org) an entertaining and substantial personal history of the birth of sunscreens, PUVA, narrow-band UVB and dermatological lasers, including rich stories that sparked (literally) reminiscences of early lasers in the clinic.

The final day focused on emerging optical therapies. **Rox Anderson** discussed target-selective optical treatments, including using 1720 nm laser pulses preferentially absorbed by sebum lipids as a potential treatment for acne. Another strategy uses light-absorbing gold nanoshells forced into the glands by a topical route, followed by laser exposure. In collaborative studies, the nanoshells were driven into sebaceous glands by mechanical vibration then exposed to a near infrared laser resulting in local thermal damage to sebaceous glands, with clinical improvement. **Conor Evans** presented outstanding 3-D images using coherent anti-stokes Raman scattering (CARS), of lipids in live mouse sebaceous glands obtained *in situ* without stains. After exposing mouse ears to cold temperatures, Evans and colleagues showed that sebocytes *in vivo* suffer selective injury as a result of cytoplasmic lipid crystallization.

The potential therapeutic role of “ultrashort” lasers was considered. While there is but one picosecond laser now in dermatological use (for tattoo removal), there are no femtosecond (fs) lasers in dermatology practice (for anything). Focused femtosecond lasers are capable of very precise dermal cutting without thermal damage. Tracy (Hequn) Wang discussed the potential of fs laser pulses for skin treatment, showing local tissue effects near a laser focus within intact skin. A system that simultaneously provides skin imaging by MPM and precise cutting of the imaged tissue appears to be feasible.

Highly effective treatment of microvascular skin malformations, especially portwine stains (PWS), remains an elusive goal. Evidence is growing that hypoxia after pulsed dye laser (PDL) treatment may induce unwanted angiogenesis. PDL treatment of neonates less than about 3 months old is much more effective than treatment of older children or adults with PWS. A novel suggestion was made during discussion that fetal hemoglobin, which confers greater tolerance to hypoxia, may be responsible. Studies at the University of California, Irvine by **Kristen Kelly, Wesley Moy** and others have shown better response when angiogenesis inhibitors (rapamycin, imiquimod) are administered before and after PDL treatment. The addition of photosensitizing drugs also enhances efficacy. Wesley Moy reported that intravenous administration of the photodynamic photosensitizer NPe6 produces significantly greater vessel clearance after PDL in animal studies, while Dr. Kelly discussed new clinical evidence. Several photodynamic therapy drugs have been shown to be effective for PWS after intravenous administration and light exposure. Laser speckle modulation imaging was also used to monitor local microvascular blood flow during PDL treatment of PWS. A laser fluence-dependent, immediate decrease in perfusion was observed, representing microvascular injury. However, loss of perfusion was patchy, with reperfusion

noted in some areas that could be reduced by multiple “passes” of laser treatment. At present it remains unknown whether local perfusion is a reliable guide during PWS treatment.

Fractional laser treatments use a narrow laser beam to create an array of small zones of thermal damage that heal rapidly without scarring, stimulating a tissue remodeling process. Ablative fractional CO_2 or Er lasers make an array of vertical microchannels, extending to controllable depth in the skin. **Merete Haedersdal** is using these microchannels as conduits for topical drug delivery. In *ex vivo* human skin, there was a very large increase in the transport of small and large hydrophilic molecules. A study was performed comparing clinical cure rates of actinic keratosis lesions by PDT using topical methyl-aminolevulinic acid and red LED light sources, with vs. without fractional Er laser to assist the drug uptake. There was significantly greater clearance, with significantly reduced recurrence, after fractional laser-assisted drug delivery. Considering that there is little data available about fractional laser-assisted uptake for the host of topical medications available in dermatology, caution was advised.

A critical update on fractional laser treatments was presented by **Dieter Manstein**. Photoaging, scars, tattoos, drug-induced pigmentation and some pigmentary disorders respond. Manstein suggested a new strategy, in which the microchannels created by laser ablation are used to deliver a different laser wavelength that would otherwise not penetrate well into skin. He reported that a large volume of deep dermal or even subcutaneous tissue can be affected this way. Manstein is exploring fractional laser treatment in other organs, i.e. fibrosis in heart and liver.

The meeting ended with something controversial and something hopeful. Properly revealed, changes in appearance motivate people to actually use sunscreen. **Trevor Jones** summarized a pilot study in which 112 adults attending a SnowSports convention were briefly shown UV camera images of themselves that highlighted photodamage. More than ¾ of the 41% who replied two months later reported greater sun avoidance and sunscreen use.

Richard Weller delivered a surprising and controversial talk. High blood pressure, the leading Disability Adjusted Life Years risk factor for death and disability, is correlated with latitude ($r^2 = 0.26$) and season. Evidence is mounting that UVA exposure of skin at solar levels decreases blood pressure, probably through nitric oxide (NO) pathways. Weller and colleagues previously reported in this journal that UV could release NO stores in skin, independent of NO synthetases (Mowbray et al). An NO-activated fluorescent imaging probe revealed that UVA releases a dose- and time-dependent pool of NO, primarily in the upper epidermis. UVA exposure to subjects’ forearms produces a local increase in cutaneous blood flow, but decrease in peripheral vascular resistance may not be the dominant mechanism for UVA-induced decrease in blood pressure. In 24 adults exposed to UVA, blood pressure was lowered, along with concomitant decreases in circulating nitrate and increases in circulating nitrite.

A final brainstorming session was stimulated by **William Ju**’s presentation on how to foster and support innovative, impactful treatments for patients with skin conditions. There is a jagged continuum of steps including problem definition, scientific discovery, invention,

investment, product development, regulatory approval, commercialization, access (including affordability), and finally, patient use. Our skin research community has outstanding diversity, scientific substrate, creativity and resourcefulness but is challenged to navigate this jagged pathway.

All told, the 2013 Montagna Symposium brought together a group of skin scientists as diverse, interactive and provocative as photobiology itself. This field is poised to make major contributions and fits John Parrish's definition of dermatology – "the skin and everything in it." Unprecedented optical diagnostics are on clinical dermatology's threshold, and so are novel light therapies. I sincerely thank the organizers, co-chairs, speakers, poster authors, sponsoring organizations, and all who attended, especially students and residents.

Society for Investigative Dermatology Eugene M. Farber Travel Awards for Young Investigators

Andrew W. Borkowski, University of California, San Diego; Marcus Calkins, Ph.D., Oregon Health & Science University; Daniel Gareau, Ph.D., The Rockefeller University; Jodi L. Johnson, Ph.D., Northwestern University; Kivanc Kose, Ph.D., Memorial Sloan-Kettering Cancer Center; Rajan Kulkarni, M.D., Ph.D., University of California, Los Angeles; Wesley Moy, M.S., University of California, Irvine; Philip L. Tong, M.D., Centenary Institute, Royal Prince Albert Hospital/University of Sydney; Tracy (Hequn) Wang, Ph.D., University of Washington

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