Montagna Symposium 2014—Skin Aging: Molecular Mechanisms and Tissue Consequences

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The 63rd annual Montagna Symposium on the Biology of Skin, “Skin Aging: Molecular Mechanisms and Tissue Consequences,” was held from 9–13 October 2014, in Gleneden Beach, Oregon. The meeting brought together basic gerontologists, dermatologists, and skin biologists working on mechanisms and problems of skin aging, industry scientists attempting to create products to address unmet needs in the field, and trainees wishing to acquire a better understanding of the aging process and its consequences in the skin. The many recent advances in both basic and applied aspects of cutaneous aging led to productive exchanges among these participants, broadened everyone’s horizons, and stimulated several new collaborations. The Symposium was chaired by Barbara A. Gilchrest with Session Chairs Judith Campisi, Howard Chang, and Gary Fisher.

The meeting opened with a Keynote presentation by Jan Vijg. Dr Vijg began by pointing out the great progress that has been made over the last two centuries in increasing both life expectancy and health span—i.e., the average time span an individual can expect to remain functional. However, further progress depends entirely on gaining a deeper understanding of the intrinsic mechanisms that underlie the aging process and predisposition to disease. Among the many theories of how we age, one of the oldest is based on the accumulation of errors in genome and epigenome. Although conceptually fairly simple, this theory proved extremely difficult to test because mutations and epimutations occur at low frequency, turning each tissue into genome mosaics. Dr Vijg presented data from his group on their single-cell approach to the study of somatic DNA mutations and epimutations in aging tissues. Making use of the most recent next-generation sequencing methods, their data indicated that the frequency of somatic mutations is much higher than previously thought, with many mutations inactivating gene function. Dr Vijg discussed these results in the context of some broader philosophical and ethical questions posed by the study of aging. For example, in view of a gradual erosion of genome and epigenome integrity, is it feasible to develop interventions to delay, halt, or even reverse aging, and are there ethical costs? This discussion provided participants with an engaging, big-picture introduction to the meeting’s theme.

Session Chair Howard Chang drew the connection between aging and studies of epigenetics—the mechanisms of gene memory over time—and then introduced recent findings that the human genome encodes thousands of gene memory over time—and then introduced recent findings that the human genome encodes thousands of long noncoding RNAs, many of which have functions in chromatin regulation. Chang reported that the stress-responsive transcription factor NF-κB is increasingly active with age in the skin and other tissues and that interruption of NF-κB activity in aged skin can restore many features of young skin. He then described long noncoding RNAs that are regulated by NF-κB, focusing on Lethe, which is induced by NF-κB and in turn dampens the NF-κB response, helping cells forget that they were stressed in the past. Chang concluded by describing a new technology that can map chromatin changes in just a few thousand cells, finding that many age-associated changes are only evident in the long-lived stem cell compartment of tissue.

Ruby Ghadially discussed functional studies of human epithelial stem cells. Although human skin stem cells can be enriched by one or more cell surface markers, their functional characterization had previously depended on in vitro colony-forming assays. Dr Ghadially showed that intradermal injection of skin stem cells can recapitulate some aspects of wound healing and lead to the formation of epidermal inclusion cysts where cells undergo keratinization. This assay system also allowed comparison of the proliferative capacity of epithelial stem cells in vivo, albeit in a xenograft setting, and can be used to compare stem cells in different settings, including aging.

If seeing is believing, Valentina Greco continued the session with an exciting presentation on in vivo imaging of stem cell niche. Dr Greco developed cutting-edge two-photon microscopy methods and transgenic animals with fluorescent nuclei in skin cells in order to make movies of how individual cells in hair follicles move and develop. Direct visualization of cell dynamics

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overturned several dogmas in the skin stem cell field, including the idea that stem cells are somehow uniquely specified. In fact, if existing hair follicle stem cells are destroyed, other cells can take their place, influenced by the unique environment of the niche. During hair follicle regression in catagen, Dr Greco showed that only the outer root sheath cells die, whereas the inner root sheath cells move up the follicle. The signal for outer root sheath cells to die emanates from mesenchymal cells in the dermal papilla. These results raised the concept that the mesenchymal cells in the dermal papilla may be the intrinsic clock for hair follicle cycling over time.

Ray Monnat followed with studies of the Werner Syndrome, a disease with many features of premature aging arising from loss of function mutations in the WRN helicase gene. A consequence of this progeroid syndrome is the increased incidence of cancers, including melanoma, with an over 50-fold increase in Werner Syndrome patients. Dr Monnat identified several aspects of DNA metabolism that are altered in Werner Syndrome cells. He found that the rate of the DNA replication fork progression is altered, and many genes exhibit altered expression levels. The changes in gene expression apparently had large effects on genes encoding tRNA synthetase pathways; thus, unexpectedly, a defect in DNA metabolism or replication may indirectly impact protein translation.

Danica Chen concluded the session with a presentation on aging and sirtuins. Although sirtuins were first recognized for their roles in chromatin regulation in the nucleus, Chen reported the novel roles of the mitochondrial resident Sirt3 protein in aging. She showed that Sirt3 is important to deacetylate superoxide dismutase 2, a key enzyme to detoxify reactive oxygen species, and that Sirt3 is necessary to prevent the build-up of reactive oxygen species in aged hematopoietic stem cells. Another sirtuin, Sirt7, responds to stress signals in the endoplasmic reticulum and signals the translational machinery to slow down accordingly. Sirt7-deficient animals exhibit fatty liver and altered hematopoietic stem cell function, which can be reversed by pharmacologic alleviation of endoplasmic reticulum stress.

Session Chair Judith Campisi introduced the multifaceted stress response termed cellular senescence. Senescent cells are characterized by an essentially irreversible arrest of cell proliferation and a complex senescence-associated secretory phenotype composed of numerous growth factors, inflammatory cytokines, and proteases. The senescence growth arrest is known to be an important tumor suppressive mechanism. The senescence-associated secretory phenotype, however, is a double-edged sword. Using a mouse model that allows the visualization, sorting, and selective elimination of senescent cells, Campisi presented evidence that skin wounding is accompanied by a rapid but transient appearance of senescent cells, primarily fibroblasts and endothelial cells that secreted platelet-derived growth factor AA, a newly identified senescence-associated secretory phenotype factor that was essential for optimal wound closure. By contrast, the chronic presence of senescent cells, which can occur in response to whole-body radiation, systemic genotoxic chemotherapy, or oxidative stress, is deleterious and can promote tumor metastasis and retard skin wound healing.

Anne Chang followed with a presentation on the marked individual variation in the ability to maintain skin youthfulness during aging, a phenotype that thus far has been poorly characterized. At least some of the ability to maintain healthy, youthful-appearing skin into advanced age appears to have a genetic basis. Chang suggested that a shift in focus from skin aging to skin youthfulness can uncover new key pathways that regulate skin health and potential interventions to restore skin youthfulness pathways in aged individuals.

Barbara A. Gilchrest discussed the promise of T-oligos, oligonucleotides that are homologous to the vertebrate telomere DNA repeat sequence (TTAGGG). Human telomeres shorten progressively with repeated cell division owing to the biochemistry of DNA replication and because most human cells only minimally express telomerase, the enzyme that adds telomeric sequences de novo. Long-term exposure to T-oligos mimics the effects of short, dysfunctional telomeres, but short-term exposure to T-oligos elevates DNA repair capacity, increases telomerase activity, and elongates telomeres. These studies indicate another doubled-edged sword. At low levels, DNA damage signaling, such as that caused by short-term T-oligo exposure, is protective, increasing DNA repair systems, telomerase expression, and telomere length. At higher levels, such as that caused by long-term T-oligo and excessive sun exposures, the protective effects are overwhelmed and DNA damage signaling then drives apoptosis and senescence.

Steven Artandi closed the session by elucidating how telomerase expression and assembly are controlled. In both mice and humans, the identification of telomerase-positive cells has been elusive owing to the very low expression of the holoenzyme components. The Artandi laboratory recently overcame this limitation by generating a knock-in reporter mouse model in which the open reading frame of TERT, the reverse transcriptase subunit of the enzyme, is replaced by fluorescent proteins, allowing tracking of telomerase-positive stem and transit amplifying cells and determining their role in telomere length maintenance. New evidence suggests that telomerase is assembled in subnuclear domains such as Cajal bodies, an important step in the optimal expression and localization of the enzyme. These findings promise deeper insights into telomerase regulation during carcinogenesis and natural aging.

Session Chair Gary Fisher presented three-dimensional videos of second harmonic generation signals and atomic force microscopy nanoscale images that illustrated deterioration of collagen fibrils with aging. Multiphoton fluorescence microscopy images further revealed that attachment of fibroblasts to collagen fibrils is disrupted by fragmentation, leading to reduced fibroblast spreading. Fibroblasts respond to loss of attachment by upregulating production of collagen-degrading matrix metalloproteinases and downregulating production of collagen, leading to further collagen deficit, with attendant decline of skin health. Human intervention
studies demonstrated that fibroblasts in aged skin could be “rejuvenated” by enhancing structural support and mechanical force within the dermis, by injection of cross-linked hyaluronic acid (HA) dermal filler. Thus, functional decline of dermal fibroblasts may be largely a reflection of their tissue environment and not irreversible.

The theme of the reversibility of some features of skin aging was further advanced by Sewon Kang, who presented an overview of retinoids and their ability to improve the appearance of aged human skin. Although the mechanisms by which retinoids improve appearance are not precisely known, the induction of collagen appears to be a critical factor. Retinoic acid stimulated production of type VII collagen anchoring fibrils and type I collagen. Retinoid dermatitis, a side effect due to the actions of retinoids on the epidermis, could be partially mitigated by combination with inhibitors of EGFR, potentially improving tolerability and therapeutic benefit.

Vera Gorbunova presented fascinating connections between longevity, essentially complete resistance to all types of cancers, and the unique properties of hyaluronic acid, a long chain polysaccharide composed of repeating units of the D-glucuronic acid and N-acetylglucosamine, in the naked mole rat. This mammal has a life expectancy exceeding 30 years. High viscosity of culture media from naked mole rat fibroblasts was traced to secretion of large amounts of very long chains (molecular weights 6–12 times greater than in mice or humans) of HA. Increased length of HA chains was found to be due to two amino acid substitutions within the active site of the major HA-synthesizing enzyme HAS2. Both increased expression of HAS2 and reduced expression of HA-degrading activities were found to be responsible for high HA content in the naked mole rat. Interestingly, inhibition of high molecular weight HA production conferred oncogenic transformation on normally resistant naked mole rat fibroblasts. These data reveal that long chain HA has unique anti-cancer properties; however, the precise mechanism of this activity remains to be determined. We eagerly await the results of ongoing studies to determine whether transgenic expression of naked mole rat HAS2 will extend the life span of mice.

Yong Li presented the role of prostatoglandins in loss of skin collagen during aging, a novel counterpart to the activity of these well-known lipid mediators of immunity and inflammation in the skin. cDNA microarray analysis of the skin from persons of varying ages revealed that the gene responsible for prostat glandin E2 synthesis, PTGES1, strongly positively correlated with age. Compelling data showed that PTGES1 expression is highly sensitive to fibroblast spreading. The potential for clinical translation of these findings is high. Nonsteroidal anti-inflammatory drugs, which are well-known to inhibit prostaglandin E2 production, provided proof of concept, with one such drug raising collagen levels in aged human skin.

Richard Clark described his recent studies on fibronectin-derived peptide P12. Fibronectin provides a substrate for dermal cell attachment, migration, and growth during wound healing. P12 peptide displays strong binding to platelet-derived growth factor BB, and this binding enhances its growth promoting activity. Aged pre-senescence fibroblasts lack sufficient traction force to expose P12 and therefore respond poorly to platelet-derived growth factor BB. Responsiveness to platelet-derived growth factor BB could be markedly improved by addition of exogenous P12. This raises the intriguing possibility that P12 may be therapeutic to improve wound healing in the elderly.

Vincent Monnier wrapped up the session by discussing glycation of extracellular matrix molecules, such as collagen and elastin, resulting in accumulation of advanced glycation end products during aging. This accumulation alters the structure and mechanical properties of dermal collagen and elastin, deleterious to both the physical properties and functions of dermal cells. Unfortunately, to date, strategies to remove advanced glycation end products in vivo have been unsuccessful; however, understanding of the chemistry of glycation has led to identification of several molecules that retard glycation formation. Thus, using such agents to extend skin health during aging remains a possibility.

To open Barbara A. Gilchrest’s session, Vladimir Botchkarev discussed epigenetic regulation of skin stem cell activity in skin development, aging, and cancer. Specifically, his group looked at how p63 and epigenetic regulators interact to promote, establish, and maintain epidermal differentiation program in keratinocytes and how these epigenetic mechanisms provide novel approaches for the treatment of age-associated skin disorders.

Calvin B. Harley outlined methods for measuring telomerase activity and telomere length and the potential of these methods and measurements to improve clinical practice in dermatology and beyond. Beginning with an overview of telomere biology, Dr Harley went on to present telomere measurement techniques such as quantitative reverse transcriptase in real time and quantitative fluorescent in situ hybridization and their applications in cancer care and the wellness market, as well as a potential role for these methodologies in such areas as improved skin biopsies and telomere vaccines.

J. Silvio Gutkind presented his group’s multi-institutional clinical trial exploring the anti-tumor effects of rapamycin on newly diagnosed head and neck squamous cell carcinoma, which also yielded data about the role of mTOR in skin aging, specifically, that the inhibition of mTOR may prevent premature aging of the skin without increasing cancer incidence.

Maria Eriksson presented her group’s work on the Hutchinson-Gilford progeria syndrome (HGPS) as a model for normal aging, including recent studies on the expression of HGPS mutation in basal cells of the interferollicular epidermis resulting in the upregulation of lamin B receptor in the suprabasal cell layer, and on the development of transgenic mouse models with expression of this mutation only in certain cell types, shedding light on systemic responses underlying aging phenotypes.

Dr Eriksson’s talk was followed by three short talks. Rosemarie Osborne described her group’s efforts to define molecular pathways involved in
intrinsic skin aging and photoaging, using transcriptional profiling tools that elucidate the expression of genes associated with changes in cellular energy production. Abbas Raza presented his lab’s current work on acyclothymidine dinucleosides as structural mimetics of DNA, with data showing that topical application of acyclothymidine dinucleosides resulted in DNA photoprotection in both mouse and human skin exposed to UVB. Abdoelwaheb el Ghalbzouri described his group’s work on reticular and papillary fibroblasts in human skin equivalents.

The meeting’s final session began with five short talks on current trends in industry research. Raaj Khusial presented work on a novel role for autophagy in epidermal differentiation. Michael Milane outlined a phase 2 clinical study evaluating the safety and efficacy of aminolevulinic acid photodynamic therapy for reducing the incidence of facial actinic keratoses in high-risk patients. Florence Nadal-Wollbold presented data from an industry-academic collaboration on the role of carbonyl stress in actinic elastosis and photoaging. Tracy Shafizadeh gave an overview of the metabolome and how it may be useful in the development of therapeutics and skin care products. Betty Yu presented data showing a platform that employs topically applied invisible cross-linked polymer films to enhance hydration, provide support, and otherwise improve compromised skin.

Barbara A. Gilchrest and Sewon Kang moderated the culminating Future Directions panel. Panelists Chris de Bryune, John Doux, and Serge Lichtsteiner led a discussion on current approaches and opportunities/barriers to new dermatologic products, drugs, and cosmeceuticals and implications for translation from bench to bedside. This lively and candid exchange shared experiences, shared perspectives of obstacles, and set forth new mechanisms for synergy between industry, academia, and government researchers, bringing the meeting to a noteworthy conclusion.

Society for Investigative Dermatology
Eugene M. Farber Travel Awards for Young Investigators: Nicole K. Brogden, PharmD, Ph.D., University of Iowa; Marco Demaria, Ph.D., Buck Institute for Research on Aging; Vyacheslav Labunskyy, Ph.D., Boston University School of Medicine; George Man, University of California, Berkeley; Michael C. Velarde, Ph.D., Buck Institute for Research on Aging. Japanese Society for Investigative Dermatology Travel Award for Young Investigators: Hideo Kudo, MD, Ph.D., Kumamoto University/Pharmaceuticals Medical Devices Agency, Japan.

CONFLICT OF INTEREST
H.Y.C. receives grant support from the Life Extension Foundation and has financial interests in Epinomics, Inc., and RaNA Therapeutics. The other authors state no conflict of interest.

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Montagna Symposium 2015
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