Molecular Targets of Aging Processes

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KEY WORDS: *glycation, AGEs, RAGE receptor, sirtuins, heat shock proteins, clock genes, hyaluronic acid, telomeres, stem cells, mitochondria, oxidative stress, inflammation*

ABSTRACT: *The purpose of this article is to discuss some key target molecules and receptors involved with aging mechanisms. As scientists learn more about the biochemical processes involved with aging, an increasing number of these loci are discovered that could be selected as targets for cosmeceutical ingredients with the final goal of an end-product with good anti-aging benefits.*

The purpose of this article is to discuss some of the key target molecules and receptors involved with aging mechanisms. As scientists learn more about the complex biochemical processes involved with aging, an increasing number of these loci are discovered and may be selected as targets for cosmeceutical ingredients. As the number of potential known targets continues to grow, the problem will be narrowing the field to a few well-chosen sites of action from the plethora of possibilities.

It is impossible to discuss all major aging receptors and target molecules here, thus an effort was made to highlight only those of recent interest; some have no doubt been omitted. Ten to 15 years ago, scientific knowledge was expected to converge on a few mechanisms, or possibly even a single gene that controlled aging.[1] However, although a case could be made for "aging genes" in nematode worms,[2] knowledge of human aging processes seems to be diverging into more complex, intertwined processes with numerous negative and positive feedback loops.

For the past 50 years, the Free Radical Theory of Aging primarily credited to D. Harman, MD, has been discussed and other aging theories often seemed to be subsets of this theory. More recently, however, it has become clear that there are many interrelated aging processes. Free radical damage as a cause of aging is now often referred to as the Oxidative Stress/Inflammation mechanism of aging, and inflammatory processes are being recognized as key in the biochemical and physiologic decline observed in the aged individual—as are the processes of glycation, protein messaging and epigenetics, DNA damage and others. These mechanisms are reviewed here. Note that this article will not suggest specific active ingredients for these sites but rather describe the targets themselves.

Glycation and Glycosylation

Glycation occurs when a sugar moiety becomes attached to a protein. Collagen is the most common protein found in the body and most of it is harbored in the skin, although joints and blood vessels also contain large amounts of collagen. Following the attachment of glucose to collagen, deformability and resilience decrease and the molecule becomes stiff. Individuals subject to increased glycation include diabetics, particularly if "brittle" or under poor control; the obese; and the aged. In those over 40, age is more important than amount of obesity (BMI) in contributing to increased rates of glycation and insulin resistance.

Accumulation of Advanced Glycosylation End-products (AGEs) results from the processes of glycation, which activates the AGE receptor, or RAGE. The RAGE has a wide range of actions that are not only expressed in fibroblasts and keratinocytes of the skin,[3] but also highly expressed in immune cells, neurons, blood vessel walls, bone and tumor cells. RAGE signaling contributes to a microenvironment that is strongly tumorigenic,[4] which occurs via induction of inflammatory cytokines such as IL-1, IL-6 and TNF-alpha. A strong interplay between inflammatory signals and the RAGE creates feed-forward loops that strongly favor the production of malignant cells.

> Inflammatory processes are key in the biochemical and physiologic decline observed with aging.

Cancer could be considered the ultimate result of aging, since animals living long enough will eventually develop cancer. Photoaged skin has a strong malignant potential. Melanoma,[5] solid tumors, chemically induced skin carcinogenesis and squamous cell carcinoma all strongly express the RAGE. Photoaged skin is also subject

to ongoing inflammatory processes, and the RAGE will be found in these environments. Increased RAGE expression drives both acute and chronic inflammation and serves as a bridge between the pro-inflammatory environment and cancer development.

Further, exposure to UV light markedly increases AGE accumulation;[6] the visual appearance of photoaged skin is the ultimate result of both decreased connective tissue functionality and reserves of AGE accumulation, along with ongoing inflammation and its consequences. RAGE activation and inflammatory activation act in concert here. Therefore, AGE receptor antagonists have the potential to ameliorate both ongoing inflammation and its consequences as well as decrease malignant potential.

Sirtuins

Sirtuins were initially described in yeast, and these Silent Information Regulator (Sir) proteins regulate lifespan in yeast as well as other simple organisms. There are seven human sirtuins, SIRT 1–7, and when first discovered, hopes were high that sirtuin modulation might also affect human lifespan. However, due to the complexity and number of processes involved in predicting the human lifespan, human sirtuins have been analytically difficult. Clearly, however, sirtuins have a very key role in the mechanisms involved in lifespan determination.

In every organism tested thus far, calorie restriction has increased life span. Initially it was speculated that this was secondary to decreased metabolic rate, compensating for markedly decreased caloric intake. Instead, the mechanism seems to relate to primary mitochondrial effects. Sirtuins also are heavily involved in life span extension, second to caloric restriction. They clearly are emerging as a theme in anti-aging, as they intimately are involved in the regulation of oxidative stress, inflammation and metabolism.[7]

In humans, SIRT1 is the best-studied sirtuin to date; it plays a role in DNA repair and resistance to oxidative stress. SIRT1 also increases cellular stress resistance, thus increasing SIRT1 expression could impact apoptotic processes, in turn lengthening cellular lifespan and reducing strain on the regenerative capacity of the stem cell population.

With aging, glycation increases and insulin resistance develops. Interestingly, SIRT1 expression has been found to improve insulin sensitivity. Further, there is growing evidence that sirtuins are related to cellular and even overall human lifespan through mitochondrial energy production and mitochondrial functionality. SIRT1 itself is not directly associated with the mitochondria, but it does affect mitochondrial function. SIRT5, on the other hand, is directly linked to mitochondria but less is known about it.[8]

Heat shock proteins, once induced, will protect cells against numerous stressors including UV radiation and inflammation.

Not only do organisms age, but also cells and tissues, such as skin. Organelles including mitochondria also deteriorate functionally and microscopically. Interestingly, although individuals appear more aged with advancing years due to a decline in total physiologic reserve, the rate of aging is highest in younger years, when mitochondrial energy production is most efficient. Mitochondrial organelles produce energy and therefore, via oxidative phosphorylation, also produce the excess free radicals responsible for the oxidative stress state. The workings of skin mitochondria are also tied to apoptotic processes.

There are many small molecules that activate sirtuins and, in particular, SIRT1. Some botanically derived agents are already used in cosmetics to affect sirtuins. Resveratrol, for example, strongly stimulates SIRT1, as do entire groups of polyphenolic plant metabolites including chalcones (butein), flavones (quercitin) and stilbenes (resveratrol).[9]

Heat Shock Proteins

Heat shock proteins (hsp) are found in all cells and in all organisms. They are highly conserved throughout evolution, which indicates their vital importance. There are many of these small proteins, and they are identified with a number which loosely corresponds to their molecular weight. Heat shock proteins hsp72 and hsp27 have been extensively studied; hsp72 is found in all epidermal layers including hair follicles and sweat glands, although not in melanocytes or fibroblasts.

Heat shock proteins improve cellular lifespan by acting as "chaperones" to other proteins. As such and through binding, they protect these other proteins from stress, particularly lower levels of non-lethal stress applied over longer time periods. They encourage other proteins exposed to denaturation stresses to refold and reconfigure, thereby preserving their transport and interaction with other molecules.

Heat shock proteins, once induced by any method, will protect cells against numerous stressors, including UV radiation, increased metabolism, inflammation, and others. UV radiation is a very strong inducer of heat shock proteins, which inhibit the formation of sunburn cells—an apoptotic event mediated by the tumor suppressor protein p53.[10] Improvements in keratinocyte viability, longevity with photoaging, and differentiation can be produced via heat shock proteins.

Notably, organisms living in environmental extremes exhibit more heat shock proteins than those inhabiting gentler environments. However, any organism regardless of its environment will increase transcription of heat shock proteins if stress is applied; for example, human skin under stress has shown increased hsp expression.[11] In contrast to other cell types, keratinocytes show significant basal hsp72 expression at all times and without prior stress. One could speculate this relates to their location in a relatively hostile environment

on the body's surface, which is exposed to UV exposure, cold, dryness and other environmental assaults. Hsp72 definitely confers improved resistance to UVB, although less is known about its effects with UVA exposure. Hsp27 induction reduces tumorigenicity and improves DNA repair.

With aging, the overall ability to produce heat shock proteins is reduced. This is not surprising considering that related to DNA damage, overall synthesis of functional proteins declines with age.[12] However, hsp72 levels in keratinocytes are preserved independent of aging. Hsp27 levels in the skin decline with aging, possibly because they have a higher baseline expression; and since hsp27 is highly related to inhibiting neoplastia, it is not surprising that aged skin is less resistant to dysplasia and tumor development.

The incorporation of heat shock proteins into cosmeceuticals presents a formulating challenge: The stresses that induce heat shock protein synthesis are toxic. These small proteins therefore require direct application or another, yet undiscovered method of induction. However, they remain fascinating molecules with the potential to augment the skin's primary ability, i.e., to protect the body from the environment. Experiments have shown that aging human fibroblasts exhibit improved function post-induction of heat shock proteins.[13]

Clock Genes

"Circadian locomotor cycles kaput" or Clock genes, as their name implies, are involved in circadian rhythm signals of all types extending from the cellular level to overall functioning of the organism. There are numerous functioning oscillators in the skin. The skin, being exposed to day-night cycles and seasonal variations of all sorts, has an active response mechanism to periodic ambient changes and each cell type has a regulatory mechanism that is unique to it. Clock genes have been located in melanocytes, fibroblasts and keratinocytes and in each cell type, the regulatory mechanisms are different but all interact with each other to drive regulatory mechanisms within the skin.[14]

The central protein in the clock mechanism is PERIOD (PER). Levels of PER rise and fall with the time of day. The amount of PER present in the cell directs appropriate function for that time. Two genes, CLOCK and BMAL1, control PER synthesis[15] however, with age, the ability of cells to synthesize PER declines. In response, the larger organism develops sleep disturbances and even chronic illness related to inappropriate immune, surveillance, hormonal and other functions. On the cellular level, these functions decline in the more primary microcosm and normal cycling—including protein synthesis, immune responses, melanin synthesis, appropriate inflammation, cell division, energy metabolism, etc.—become disordered. These factors may relate to the higher incidence of some diseases in shift workers.

Skin repair processes are most active during the night but one could speculate that Clock genes have possible future application by encouraging cell repair during other times, such as highly damaging periods of UV exposure. However, altering the innate biologic clock should be done very

cautiously, considering present knowledge limitations.

Hyaluronan Synthase Genes

The synthesis of hyaluronate or hyaluronic acid (HA) is vitally important for skin health, serving as a key component of the extracellular matrix through which proteins, immune substances and cells must migrate and function. A vast array of cell signals occurs within this milieu. HA has voluminous hydration abilities, a buffering capacity, and acts as a matrix space-filler and support structure. It organizes the extracellular matrix via binding receptors and its interaction with matrix macromolecules. HA also interacts with cell surface receptors and initiates signaling pathways, and is extremely important in wound healing, inflammation, and tumor progression including angiogenesis.

The HA macromolecule is a linear polysaccharide made up of repeating oligosaccharide units. The oligosaccharides have very different signaling properties compared with the larger polysaccharide, including cell proliferation and angiogenesis induction through which tumor progression or wound healing can occur. Three human hyaluronan synthase genes have been identified: HAS1 on Chromosome 19q13.4, HAS2 on Chromosome 16q22.1, and HAS3 on Chromosome 16q22.[16] The function of HA depends on its size, and HAS2 codes for very large molecules while the hyaluronans synthesized from HAS1 and HAS3 are smaller. Hyaluronan also has been shown in vitro to affect keratinocyte differentiation and proliferation. With trauma, mRNA induction of HA occurs, mostly from HAS2 and HAS3, and the healing response begins. This has been shown by tape stripping of murine epidermis.[17]

The induction of HAS genes has the potential to increase hydration, restore the extracellular matrix thus visibly improving skin appearance, induce healing of micro-scars and wrinkles, encourage keratinocyte differentiation, and affect the inflammatory response.

Telomeres

Telomeres are the protective "caps" on the ends of chromosomes. In humans, these consist of the repeating units of DNA base units TTAGGG. With each cell division, the telomere is shortened since DNA polymerase is unable to replicate the final base pairs of the chromosome. Once the telomere reaches a critical shortened length, replication can no longer occur. Hayflick described this as a state of "replicative senescence," in which telomeres in fibroblasts become critically shortened after approximately 50 cell divisions.[18] In this state, the aged cell can no longer enter mitosis.

The tumor suppressor protein p53 is involved in replicative senescence as it is in apoptosis and DNA repair.[19] If the telomeric unit is experimentally disrupted, the cell will also enter either replicative senescence or apoptotic pathways.[20, 21] At present, it appears that once critical telomere shortening occurs, the senescent cell can "choose" apoptosis or mitotic arrest pathways.

Intrinsic aging, i.e., that occurring within cells, involves the accumulation of free radical "hits," which is secondary to their generation via intracellular energy in the mitochondria. Photoaging also involves oxidative damage, in this case occurring from UV radiation. Both intrinsic aging and photoaging cause DNA damage responses and critically shortened telomeric units, with entrance of cellular processes into apoptotic pathways or those of replicative senescence.

Progerias are inherited diseases that have been studied as models of aging. In these, aging occurs at a very accelerated rate. Werner's Syndrome is one progeria in which helicase, an enzyme that unwinds DNA during replication, is defective; and in which there are problems with telomere maintenance.[22] Those challenged by this syndrome develop very aged-looking skin after the teen years and experience the early onset of other diseases of aging. Dyskeratosis congenita, another condition of accelerated aging, is associated with premature graying of hair and early cancer development. Afflicted individuals have very shortened telomeres and are defective in a protein required for normal telomere functioning.[23] Such diseases with impaired telomere function suggest that telomeric manipulation could delay the onset of symptoms and diseases of aging in otherwise healthy individuals.

Telomerase is an enzyme responsible for synthesizing repeat telomere sequences and maintaining telomere length. Thus, with the expression of telomerase, the cell continues to divide and does not enter replicative senescence or apoptosis secondary to critically short telomeres. Some cells are intrinsically "immortal" and express telomerase; these include cells of the germ cell line, i.e., sperm and ova, and the majority of malignant cells.[24] It is a frightening prospect to think that therapeutically protecting telomeres could cause malignant transformation; aging and replicative senescence have been speculated to be evolutionary programs to avoid progression to cancer.[25] However, experiments have demonstrated that the transfection of human fibroblasts with telomerase confers cellular immortality and preservation of normal phenotype without malignant transformation.[26] This evidence suggests there may be room for telomeric maintenance and associated improvement in skin aging while still avoiding malignant transformation and plant actives such as epigallocatechin gallate (EGCG) have been shown to inhibit telomere attrition and the apoptotic pathway.[27]

Both intrinsic aging and photoaging involve accelerated telomere shortening. With critical telomere shortening, the 3′ overhang strand of the telomere becomes exposed. Then DNA damage signals are released via p53 tumor suppressor protein when the 3′ strand exposure is recognized. Although there is much more to learn about the consequences of telomeric manipulation, protection of the telomeric unit exhibits potential for positively impacting the cellular aging processes of skin and other tissues.

Stem Cells

There currently is great public interest in stem cell technology and its potential to increase lifespan and restore youthful functionality and appearance. Stem cells in the skin are located in the hair bulb, sebaceous gland base, and directly above and below the dermal-epidermal junction (DEJ). The stem cell population is drawn upon for replacement as mature cells cease proper functioning for a variety of reasons, i.e., enter apoptosis or replicative senescence, or are injured and become necrotic. The stem cell population serves as the reserve for cell renewal and supplies new, functional skin cells in case of injury.[28]

Less than 1% of cells in the stratum basalis of the epithelium are stem cells, and this vital population itself is also subject to injury and depletion from radical damage, telomere attrition, UV radiation exposure, and genomic instability secondary to severe DNA damage. With time, the number of stem cells declines and their regenerative function becomes impaired. Numerous signaling molecules are involved in the self-renewal ability of stem cells, including the tumor suppressor proteins p16(INK4A), p19(ARF), ATM kinase, p53 and the transcription family of forkhead box O (FOXOs) proteins.[29]

Skin aging results in decreased epidermal turnover time, loss of elasticity and tone, increased susceptibility to injury and infection, decreased immune responsiveness, epidermal dehydration, rhytides, pigmentary alterations, weakness in blood vessels, and increased cancer risk. These processes result in loss of cell functionality and often in cell loss, processes that call upon activation of the skin's adult pluripotent stem cells. With time, the stem cell population also sustains considerable damage and there is loss of the progenitor cell population. In skin obtained from aged individuals, keratinocyte stem cell numbers were lower in photoaged skin having sustained more oxidative damage than in intrinsically aged skin with less total radical damage.[30] Telomere shortening may occur in stem cells with aging, which leads to genomic instability and a decline in stem cell functional capacity.[31]

Bioactive plant compounds have been shown to positively affect stem cell survival in the skin. These include sirtuin-modulators such as resveratrol,[32–34] biopeptides from 1% Kluyveromyces yeast,[35] and others. Mechanisms involved include antioxidant, anti-inflammatory, apoptotic, telomere attrition and senescence. Stem cell compromise is integrally related to skin aging, thus stem cell rescue, protection, and fortification therefore have anti-aging potential.

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Mitochondria, Oxidative Stress and Inflammation

Inflammation has taken its place along with the Free Radical Theory as a "prime mover" in the causes of aging. These two intertwined processes are now sometimes referred to as a combined mechanism of oxidation/inflammation. For somatic cells within the body, about 85% of free radical damage comes from intrinsic cellular metabolism, i.e., from excess high-energy particles produced during oxidative phosphorylation in mitochondria. For skin, the body's guardian against the environment, most radical damage results from photoaging and UV exposure, in addition to intrinsic aging.

Mitochondrial energy creation and intrinsic free radical generation continue to occur in skin and all tissues since all cells require energy for functioning. And as noted, once oxidative damage and inflammation become activated, a continuous feed-forward loop develops in which free radical damage creates more inflammation and inflammatory processes generate more free radicals. These processes are active in every aging target or receptor system discussed herein. Thus, the search for better antioxidants and anti-inflammatory agents continues.

Every organism on the planet has mechanisms for dealing with free radical damage since they all create energy and/or must protect themselves against a hostile external environment of UV radiation and other stresses. However, the strength of these mechanisms differs in individual organisms according to environment and internal metabolism. For plants, there is even variation according to season, portion of the plant harvested, elevation, UV exposure and many other factors.

As well as being a generator of reactive oxygen species (ROS), which act as second messengers, mitochondria are intimately involved in apoptotic processes. Excess ROS imply a death threat for the cell and activate apoptosis, which requires a functioning mitochondrial unit as well as other organelles such as lysosomes. Aged individuals and aged cells contain more nonfunctioning mitochondria, which are less efficient at necessary energy production and protective apoptosis. As lysosomes remove nonviable cellular material, oxidative reactions and inflammatory activation occur with potential for leakage to surrounding structures.

Cytokines mediate inflammatory reactions. They are controlled by feedback mechanisms, they generally act within a short range, and they interact with cell surface receptors before regulating the transcription of a number of cellular genes via second signals. Many of these reactions are incompletely understood. Cytokines may be classified by type, including lymphokines, interleukins, tumor necrosis factors, chemokines, stress proteins (such as heat shock proteins), transforming growth factors, and colony stimulating factors.

Some cytokines, such as IL-1, IL-6 and TNF-alpha, are strongly pro-inflammatory. They are predominantly made by activated macrophages such as skin Langerhans cells. Anti-inflammatory cytokines belong to the T-cell

derived group. There is potential to downregulate excess inflammation by modulating both pro-inflammatory and anti-inflammatory cytokines. Cytokines control the direction and strength of immune responses. They control tissue remodeling after wounding, infection, inflammation and repair; they alter membrane permeability and other properties; and they are involved in further cytokine release, angiogenesis and healing including re-epithelization.[36] Diet, exercise and weight loss have been shown to favorably decrease the degree of pro-inflammatory processes occurring in the body and accompanying cytokine levels. [37]

Antimicrobial peptides (AMPs) are small proteins that are highly evolutionarily conserved throughout species. These molecules are involved in host immune defense and inflammatory responses. Antimicrobial peptides are found on epithelial surfaces. As such, they too are potential targets for therapeutic development. For example, the AMP omiganan is effective in the treatment of acne.[38]

Excessive wound scarring is found with the overproduction of Transforming Growth Factor-beta (TGF-beta) and the suppression of Prostaglandin E2 (PGE2). Notably, nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors inhibit PGE2 production and could potentially contribute to increased scarring,[39] although they are beneficial for pain control.

Cancer development is thought to be the ultimate consequence of aging. Cyclo-oxygenase-2 (COX-2) is over-expressed in premalignant and malignant lesions of skin.[40] Furthermore, COX-2 encourages angiogenesis, which is an important part of tumor progression.[41] Exposure to UV radiation (sunlight) strongly upregulates COX-2 and incites a pro-neoplastic environment.[42] COX-2 inhibitors could have potential as anti-cancer treatments; however, suppression of COX-2 pushes the eicosinoid cascade toward the pro-inflammatory arm, which has negative consequences in some groups such as those predisposed to cardiovascular disease.

Volumes have been written about free radical damage, inflammatory processes and the interaction of their messengers. These reactions and their many mediators remain a fertile field for agents affecting all processes of skin aging, healing, remodeling and tumorigenicity.

Conclusions

Many of the complex receptors herein described are not only important in cosmetics, but also in the pharmaceutical industry. The information explosion has affected all disciplines including cosmeceuticals. Scientific knowledge has grown exponentially even though the regulatory environment seems to be more restrictive in claims allowed. These factors may encourage an interesting future landscape for the cosmetics and personal care market.

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