Animal Models

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Abstract

Skin aging is a complex process that is categorized into either intrinsic or extrinsic actinic aging. Extrinsic actinic aging results from environmental factors, namely ultraviolet (UV) radiation, which is commonly referred to as photoaging, and is characterized by wrinkles and dryness. In contrast to extrinsic aging, intrinsic aging is gene dependent and occurs over time due to variety of physiological factors (ex/ hormonal changes). Numerous studies have been performed in an attempt to understand how the architecture of the skin changes with age and to uncover the mechanism by which this occurs [1]. There is also a large body of studies focused on determining how to prevent and reverse the effects of photoaging. As such, providing an in-depth review of the literature would not be possible in a single chapter. Therefore, the aim of this chapter is to lay a general framework of the various studies that have been performed in animal models regarding skin aging and to highlight what conclusions can be drawn from them and what has yet to be uncovered.

Introduction

Aging is influenced by a combination of biological, physiological, and environmental factors. Skin aging in particular can be divided into actinic aging and intrinsic aging. Actinic aging refers to

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ultraviolet (UV) radiation-induced changes, whereas intrinsic aging refers to changes that occur independent of the environment and are influenced by biologic and physiologic factors. In an attempt to understand age-related changes that occur within the skin, numerous in vivo studies using model organisms have been performed. Since human and mouse genomes are ninety-nine percent conserved, rodents are great animal models to study human disease. Moreover, the ability to genetically engineer rodents enables us to study the relationship between genetics and the pathophysiology of various diseases. Here we present a basic framework of the leading in vivo studies that have been performed which have shaped what we currently know about aging.

Actinic Aging

In an attempt to understand the relationship between long-term UV exposure and the development of elastosis, studies were initially performed using the Dublin Imprinting Control Region (ICR) Albino Random Bred mouse, which contains a mutation in the gene encoding tyrosinase, an enzyme needed to produce melanin. In 1964, Sams et al. reported that exposing the shave back of these mice to long-term UV radiation resulted in solar elastosis [2]. While the study recapitulated the effects of long-term exposure to UV radiation, the dose applied greatly exceeded normal levels of UV radiation. Moreover, the mice developed tumors that were thought to be different from those produced in humans; thus, scientists were in search of a more appropriate mouse model to study the effects UV radiation has on humans.

The hairless mouse became the model of choice after Winkelmann et al. observed that an engineered hairless mouse possessing mutant alleles of the hairless gene (hr gene) produced tumors in response to UV radiation that were similar to human tumors [3, 4]. Since then, the hairless mouse has been used to study various photobiologic phenomena including phototoxicity, photoimmune effects, carcinogenesis, and UV-induced DNA damage [5]. Subsequent investigation reveled that the UV-induced connective tissue damage in these mice is analogous to that in humans and that the acute edema that develops in response to UV radiation is comparable to the erythema humans develop when they get a sunburn [6, 7]. Although pigmented and albino hairless mice can be engineered, the most commonly used hairless mouse model to study photoaging is the albino Skh hairless mouse [8].

Elastosis has been the most common measure for assessing the long-term effects of UV exposure. In 1980, Berger et al. used the naked (Ng/-) albino strain to produce elastosis in a hairless mouse [9]. Since then, numerous studies have demonstrated ultraviolet-induced elastosis. Johnston et al. demonstrated that the levels of cross-linked elastin in the skin of mice treated with UVA or UVB were decreased, while the levels of collagen remained unchanged. Biological assays reveled that collagen synthesis via prolyl hydroxylase (PH) was impaired with UVA exposure and thus may lead to decreases in collagen synthesis and subsequent dermal atrophy over time [10]. Further studies using immunohistochemistry, electron microscopy, and biochemical experiments showed UV radiation-induced elastosis [7, 11].

While the former studies emphasized the impact of UV light on elasticity, a dermal component, other studies have demonstrated the effect of UV light on the viscosity of the skin, an epidermal component. In order to assess the influence 1,25-dihydroxyvitamin D_3 has on photo-wrinkling, Fujimura et al. treated female HR/ICR hairless mice topically with 1,25-dihydroxyvitamin D_3, which in humans is produced in the kidneys [12]. The mice were treated once daily, 5 days per week with 1.00 μg, 0.20 μg, or 0.05 μg of 1,25-dihydroxyvitamin D_3. Skin sagging was assessed using a scale described by Bisset et al. [13]. Four grades were used, with the fourth being the most severe. After 6 weeks of treatment with 1.0 μg/day of 1,25-dihydroxyvitamin D_3, the skin on the backs of these mice developed coarse, deep wrinkles. While this suggested that 1,25-dihydroxyvitamin D_3 may promote degeneration of skins architecture, it was unclear if it was due to changes in the epidermis or dermis. This led to further experiments investigating the effect of topical 1,25-dihydroxyvitamin D_3 on the

immediate distension (U_e) and the delayed distension (U_v), which are parameters of skin elasticity and viscosity, respectively. While topical application of high levels of 1,25-dihydroxyvitamin D_3 did not influence U_e, it did lead to a decrease in U_v. Since U_e is largely a measure of the dermal component and U_v an epidermal component, this study suggests that changes in the mechanical properties of the skin after topical 1,25-dihydroxyvitamin D_3 are due to physical changes in the epidermis [12].

Fujimura et al. also compared immediate retraction (U_r) and final distension (U_f) before and after topical application of 1,25-dihydroxyvitamin D_3 and found that the ratio of U_r/U_f decreased after application of 1,25-dihydroxyvitamin D_3, as is expected with normal aging. Interestingly, the decrease that resulted from application of 1,25-dihydroxyvitamin D_3 was a result of a decrease in U_r, instead of an increase in U_f which usually occurs with age-related skin changes. Further studies are needed to elucidate the reason for this difference. Together these experiments by Fujimara et al. suggest that although vitamin D is recommended to prevent osteoporosis, vitamin D may have damaging effects on other organs. Therefore, it may be worthwhile to investigate if vitamin D causes accelerated degradation of organs other than the skin.

The degradation of collagen and other components of the dermal extracellular matrix in part is due to upregulation of matrix metalloproteinases (MMPs). Matrix metalloproteinases are a family of endopeptidases that cleave the constituents of the extracellular matrix in connective tissues [14]. MMPs have been implicated in the pathogenesis of various conditions including atherosclerosis and emphysema. Their association with photoaging has been revealed in studies showing the upregulation of MMPs in irradiated cultured fibroblasts [15]. UVB induces expression of MMP-1, MMP-3, and MMP-9, whereas UVA induces expression of MMP-1, MMP-2, and MMP-3 [16, 17]. Further experiments revealed that in addition to MMPS, expression of matrix metalloelastase changed in response to UV exposure. Immunohistochemistry of samples of skin from of hairless mice exposed to UV radiation for increasing periods of time reveled that the longer the mice were exposed to radiation, the higher the level of expression of MME in the dermis. This suggests that photoaging may be attributed to UV-induced degeneration of the dermal extracellular matrix by both MMPs and MMEs.

Since UVB rays induce expression of MMP-1, MMP-3, MMP-9, via Cathepsin G, a serine protease, Son et al. hypothesized that inhibition of Cathepsin G may prevent UVB-induced photoaging [18]. Comparison of the skin of hairless mice before and after exposure to UVB radiation revealed that the mice treated with topical Cathepsin G had less of a decrease in collagen and an attenuated upregulation of MMP compared to mice that did not have Cathepsin applied to their skin. Thus, topical application of Cathepsin G inhibitors may be useful for the prevention of UVB-induced photoaging in humans by attenuating upregulation of MMP, thereby minimizing damage to the extracellular matrix.

Since UV radiation is oxidizing, Hwang et al. postulated that topical application of Gallic acid (GA), a substance that possesses antioxidant and anti-inflammatory properties, would prevent UVB-induced photoaging [19]. To test this hypothesis, hairless mice were randomly divided into three groups of six mice that received varying amounts of GA following UVB irradiation and two control groups, one that received no UVB exposure and no GA and the other that received UVB exposure without topical GA. For 3 weeks, the mice received 1 h of UVB radiation followed by topical application of GA. Comparison of skin from mice exposed to UVB with and without GA showed that GA-treated mice had less prominent wrinkles, less dryness, decreased skin thickness, and lower levels of MMP-1. Thus, topical application of GA may be useful in preventing UVB-induced photoaging by negatively modulating levels of MMP-1.

Since UV-induced photoaging is mediated by reactive oxygen species that in turn generate lipid peroxidation carbonyl-containing products, such as acrolein (4HNE), Larroque-Cardoso et al. hypothesized that 4HNE mediates photoaging through formation of adducts with elastin and therefore topical application of a carbonyl

scavenger like carnosine should reverse UV-induced photoaging [20]. Using immunofluorescence, Hwang et al. showed that the dermis of hairless mice exposed to UVA daily exhibited an increase in 4HNE and 4HNE-elastin adducts and demonstrated that daily application of carnosine completely reversed the development of photoaging alternations including 4-HNE-adduct formation on elastin. In summary, this study highlights the role of 4HNE-elastin adducts in photoaging and suggests that application of topical carbonyl scavengers has a protective effect against photoaging.

All-*trans*-retinoic acid (RA) is another topical treatment for repairing UV-induced skin damage that has been well studied. Klingman et al. for example used the Skh *hairless*-1 albino mouse model to determine if topical all-*trans*-retinoic acid (RA) could reverse UV-induced skin damage using the Skh *hairless*-1 albino mouse model. The mice were irradiated three times a week for 10 weeks to produce UV-induced changes in the skin, and RA was subsequently applied in varying concentrations. Histology and electron microscopy were used to assess structural changes in the skin before and after the use of RA. The study revealed that RA could repair UV-induced dermal damage in mice by hyperactivating fibroblasts, which in turn leads to increased collagen synthesis [21]. Soon Park et al. performed another study regarding reversing photoaging. Since prior wound repair studies demonstrated that adipose-derived stem cells (ADSCs) could stimulate fibroblast migration and collagen synthesis [22], Soon Park et al. hypothesized that ADSCs could be used as a cosmetic treatment to reverse collagen degradation and deceleration of collagen synthesis resulting from photodamage. To test this hypothesis, ADSCs were injected intradermally into the backs of three micropigs twice over a 14-day interval. Histologic and western blot analysis revealed that there was an increase in collagen in skin 1 month after injection of ADSCs, suggesting that ADSCs may be a potential treatment to reverse skin aging.

Apart from UV radiation, there are many other environmental oxidizing agents that are damaging to the skin. Tobacco smoke, for example, has been shown in in vitro studies to have particularly deleterious effects on the extracellular matrix of the skin [23]. There is evidence from in vivo studies that tobacco smoke induces premature skin aging [24]. In 2007, Tanaka et al. was the first to examine the effects of cigarette smoke on the connective tissue matrix of hairless mice. An aqueous smoke solution prepared by dissolving cigarette smoke in phosphate buffered saline was either applied topically or administered intracutaneously to the backs of the hairless mice three times a week over a 6-month period. Immunohistochemistry revealed that there was a loss of discernable collagen bundles in the dermis of the mice treated with the aqueous smoke solution as compared to the control. This was the first of many studies to show that tobacco smoke directly induces premature aging of the skin using an in vivo model [24]. Subsequently, many groups studied the influence cigarette smoke has on blood flow to the skin. According to *Leow and Maibach's* review of the literature, all studies showed a consistent decrease in blood flow during the first two minutes of smoking cigarettes [25]. A later study by Manfrecola et al. reported a 38.1 % reduction in cutaneous blood flow in smokers and 28.1 % reduction in nonsmokers, with smokers having a shorter recovery time than for smokers [26].

Intrinsic Aging

Our understanding of intrinsic aging is limited in comparison with that of actinic aging. Since UV radiation is a quantitative external variable, the consequences of actinic damage can be observed more readily. Intrinsic aging however occurs over many years due to various biologic or physiologic influences, independent of any environmental factors. It is therefore more difficult to experimentally design studies to investigate intrinsic aging. Scientists have yet to understand why different individuals age at different rates. The experiments reviewed below are just the beginning of our attempt to elucidate the various factors responsible for intrinsic aging.

Hiromi Kimoto-Nira et al. used senescence-accelerated mice (SAM) to observe various physical changes associated with aging, including those in the skin. As their name implies, SAK develop normally, but show an early onset of aging. It is therefore a great mouse model to study how age alone effects various organs in the body. Hiromi Kimoto-Nira et al. examined how probiotics influence aging by orally administering *Lactococcus lactic* subsp. *cremoris* H61(strain H61), a probiotic to SAM for 5–9 months [27]. Using a grading system developed by Hosokawa et al. in 1984, Hiromi Kimoto-Nira et al. observed that the mice receiving probiotics had a lower incidence of skin ulcers and diminished rate of hair loss [27, 28], suggesting that probiotics may be able to slow the progression of intrinsic aging.

In 1975, Murai et al. attempted to understand how collagen changes on a molecular level with age, by examining the levels of hydroxylysine-linked carbohydrates units in collagen molecules at various ages. Since glycosylation renders molecules to be more insoluble, Murai et al. examined how the ratio of soluble to insoluble fractions obtained from skin of rats changed with age. Examination of the degree of glycosylation in the insoluble fraction revealed an absolute decrease in levels of glycosylated hydroxylysine in collagen with age in addition to a gradual increase in ratio insoluble : soluble collagen with age [27]. While the role of glycosylation in formation of collagen had yet to be understood at the time this experiment was performed, this was among the first experiments to use an animal model to examine molecular changes in collagen with age.

Another model that has been employed to study changes in skin attributed to intrinsic aging is the Ishibashi (IS) rat. Interestingly, their skin develops wrinkles and furrows at 12 weeks of age independent of UV radiation; thus, Sakuraoka et al. compared how the skin composition of elastin and collagen of IS rats changed over time compared to control Sprague–Dawley (SD) rats [28, 29]. Using the method described by Prockop et al., collagen content was determined by measuring levels of hydoxyproline, while high performance liquid chromatography was used to estimate elastin content by measuring isodesmosine, a lysine derivative in elastin molecules [30]. No significant difference in the amount of collagen was observed between aged skin of IS and SD rats and their younger counterparts. This suggests that the intrinsic aging observed in the skin of IS rats is unlikely related to collagen. Subsequent comparison of levels of isodesmosine between aged and young counterparts of IS and SD rats revealed that while aged IS rats had a notable reduction in isodesmosine content, aged SD rats did not. This decrease in isodesmosine content in the skin of IS rats coupled with the lack of changes in isodesmosine levels in skin of SD rats suggests that intrinsic aging of skin in IS rats may be related to changes in elastin content. Thus, the IS rat may be a good model to understand intrinsic aging in humans.

Although a majority of in vivo studies related to skin aging have been performed in rodents, there are few studies that have been performed using dogs. In one study, Mexican hairless dogs with spotty pigmentation were used to study the effects of kinetin (KN) on reversing hyperpigmentation. After 50 days of topical application of one side of the body, the KN-treated sites showed normalization of pigmentation and a more rejuvenated appearance of the skin. Thus, the authors proposed that kinetin may be a safe treatment in humans to reverse age-associated hyperpigmentation [31].

Fgf23 and Klotho as Future Models

Fibroblast growth factor 23 (FGF-23) null mice and *klotho* mice are two transgenic strains that have a premature aging phenotype characterized by early onset arteriosclerosis, osteopenia, ectopic calcifications, pulmonary emphysema, diminished hearing, and senile atrophy of the skin [32]. Thus, these mice may be good models to understand the various factors influencing intrinsic aging. The similar phenotypes of these mice are likely a result of both strains of mice possessing mutations in genes encoding proteins linked via a common pathway [33].

Preliminary studies with these mice reveal that *Klotho* and *FGF-23* null mice have high increased

vitamin D activity in their serum, suggesting that there may be a correlation between vitamin D and premature aging in various organs including the skin, as suggested previously by Fujimara et al. More recent data from Yamashita et al. showing that klotho mice exhibit significant phenotypic similarities with aged skin such as atrophy and delayed wound healing suggest that the Klotho mouse may be a good model to investigate wound healing in the elderly [34].

Conclusion

The examples discussed here are just a few of the studies investigating changes associated with skin aging. Furthermore, this chapter only reviews studies using animal models and does not include any examples of studies performed in humans or in xenografts. It is our hope that this text allows one to familiarize themselves with the various models available and the methods employed to study skin aging. As our understanding of the aging mechanism becomes more intricate, new models more closely resembling processes in humans will be needed to further our understanding of aging.

While the number of studies investigating actinic aging greatly outweigh those on intrinsic aging, the *Klotho and FGF-23* mice are two models provide us with a means to better characterize how systemic processes influence the structure, function, and appearance of skin over time. In addition to broadening our understanding of how the skin is influenced by systemic factors, it is our hope that future studies regarding skin aging will also able to shed light on how various organs change with age.

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