An Overview of the Histology of Aging Skin in Laboratory Models

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Contents

Abstract

This article is an overview of histomorphological changes documented during various stages of life in many laboratory models utilized in skin aging studies. A bewildering variety in cutaneous aging response has been observed in different strains. The commonly used laboratory models like the rat and the mouse show dissimilar aging changes in skin. There are a few mouse models which seem to resemble the general trend of skin attrition related to advancing age observed in some human studies. Caloric restriction has been observed to modulate skin aging changes in the rat and the mouse. Despite the wide variation in observational studies related to aging changes, some rodent models are useful to aging research and experimental response of aging skin.

Introduction

Human populations worldwide are living longer, and skin from older people becomes more susceptible to diseases and malformations apart from being ravaged by environmental trauma like ultraviolet radiation. The science of clinical dermatology documents numerous diseases of human skin, but scant information has been paid to basic research in the microanatomy of aging skin. This is a subject that invokes biological and evolutionary interest about age-induced involution of the

"What lies beneath this aging skin? The untold destruction stealthy creeping, The bones the organs the nerves, the brain, I am breathing, I am functioning, Am I living?"
Julie A. Crippin, 2008

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integumentary system. It is logical to introspect about the phenomenon of chronological aging of animal skin from other mammalian species. Despite many descriptive studies published in early literature, there are still many gaps in our knowledge about the patterns of intrinsic aging in various laboratory models. Although animal models may not fully corroborate biochemical data of aging skin and may show inconsistent results, they are still useful tools to interpolate the fundamental etiologies of skin aging and antiaging effects [1].

One reason for the lack of documentation in this area of research is scant availability of animal models of skin aging with clear documentation regarding age or the stages of the life cycle. Animal husbandry for long-term maintenance of aging animals in a disease-free colony is a costly project and is usually beyond the reach of an average laboratory. The aging human skin with increased roughness and wrinkling is sagging, and inelastic, and shows many signs of atrophy, such as epidermal thinning or abnormal collagen and elastic fibers. It is logical to ask if similar or different conditions are observed when a comparative anatomical analysis is extended to different models from the mammalian kingdom. This review explores whether intrinsic aging also affects skin histology in laboratory models in a comparable manner. The data are very limited and were acquired through painstaking investigations by researchers dating back to several decades using classical histological techniques; the reported quantitative data were often uncorroborated by statistical methodology.

The Human Scenario: Skin Aging Histology

Morphological changes of the human integument due to intrinsic aging caused by time-induced physiological changes are understandable, but can be altered due to personal and environmental factors, can vary in different anatomical sites, and are also linked to endocrine factors [2]. Despite these problems, several accounts of intrinsic aging of human skin have been published over the last few decades. Skin atrophy is marked only after the fifth decade of human life and shows a plethora of morphological changes including epidermal thinning, flattening of the dermal-epidermal junction, loss of melanocytes, and immunocompetent Langerhans cells; some physiological functions, such as surface lipid production, and thermoregulation are also affected by the aging process (review in Rittie and Fisher [3]).

There are also dermal changes such as reduced fibroblast population and sebaceous glands. These histopathologic events have been reviewed [4]. Morphometric measurement of collagen fibers from stained human skin biopsies further showed that collagen fiber density started decreasing from around 30–40 years, with thinner and more spaced fibers [5]. Other investigators found no difference in epidermal or dermal thickness in a study of wound healing comparing skin from young and elderly volunteers [6]. The biochemical profile of collagen metabolism of human skin, however, changes with age. A steady decline in synthesis of hydroxyproline in human skin up to the fourth decade has been described [7].

Reports of dermal elastic fiber changes with age including abnormalities and disintegration have been published by some authors. As per the study of Vitellaro-Zuccarello et al. [5], elastic fiber distribution has a different aging pattern in men and women. While the density of elastic fibers in the papillary dermis is not modified as a function of age, in the reticular dermis of both sexes, the fiber density increases in the first decade, followed by a drop only in the female. Biochemically, elastin biosynthesis is stable up to approximately the fourth decade of life [7]. Although reports by different investigators vary in detail due to different kinds of sampling and histological methods employed, a general trend of attrition of the dermal connective tissue fibers is noted as a correlate of the aging process. Histopathologic changes in the epidermis and dermis of human skin in sun-exposed and sun-protected areas have been reviewed by Monteiro-Riviere [8].

Aging in Rodent Models

Some accounts of histological and cellular kinetic changes throughout the life span or representative stages of rodent life span are available. Most of the studies were conducted on the hairless mouse, other strains of mice (CBA, C57131/6NNia, Balb/c), and rats (Wistar rat, Fischer 344). It is difficult to make a meaningful comparison between older studies related to skin aging histopathology, as different authors have reported data on both sexes of various mouse models, which were often based upon limited sample numbers. Some of these important articles lack statistical evaluation. However, some noteworthy findings from early literature on different rodent models are summarized here.

The hairless mouse owes its hairlessness to a homozygous recessive genetic condition, and structural changes of skin accompanying development of hairlessness in this animal were described in the older literature. Age-related modifications in epidermal cell kinetics in this species were described by Iverson and Schjoelberg [9]. Using autoradiography, it was shown that epidermal cell proliferation increased from birth to approximately 20 weeks of age and remained steady. This detailed study could not confirm whether epidermal thickness or cell proliferation rate decreases systematically with increasing age. On the other hand, Haratake et al. [10] presented data showing that the thickness of the epidermis in hairless mice decreases with intrinsic aging. There was also less incorporation of tritiated thymidine in the epidermis in older mice.

The tritiated thymidine technique was used in mice up to 19 months of age, and the data revealed an age-dependent decline in the cell proliferation rate [11]. The data reported from Balb/c mice [12] seemed to indicate epidermal atrophy with age. The epidermis was thinner, with smaller nuclei, in 20-month-old animals compared to 2-month-old animals, although there was no decrease in mitotic activity and DNA labeling index. The loss of epidermal mass was related to a decrease in protein and RNA content of the epidermis. However, similar epidermal changes were not observed in a detailed study in young and old C57B1/6NNia mice [13]. In fact, the epidermis from the ear and footpad showed a statistically significant increase in thickness and augmented cell size. The index of labeling with tritiated thymidine showed no difference between young and old mice. Moreover, in C57BL/6 N mice, the number of epidermal cell layers and the epidermal thickness remained constant from 1 to 22 months of age [14].

Most of the reported studies on mouse skin were mainly concerned with the aging effect on the epidermal cell size or cell kinetics, and scarce attention has been devoted to the morphological changes of the dermal constituents in aging animals. CBA mouse skin was investigated in three age groups (1, 6, and 27 months) from animals procured from NIH colonies [15]. As the rate of skin aging differs in different areas of the body [13], samples were studied from the dorsal, ventral, and pinna skin and the footpad of these young, young adult, and old animals. A negative linear effect of age on epidermal depth with a significant reduction in cell count (cell/mm) and pilosebaceous unit profiles in dorsal skin samples and footpad was observed. The sebaceous glands appeared atrophied with pyknotic nuclei (Figs. 1 and 2). No consistent change in depth of the dermis or area fraction of collagen as determined by histomorphometry was noticed. The dermal elastic fibers in the dorsal skin and footpad showed proliferation in higher age groups in this mouse model. This can be compared with a study in the C57/B16 mice from NIH colonies [16] where decreased dermal cellularity and thickness and decreased epidermal proliferation were reported.

Further investigation of the CBA mice from NIH colonies as described in the previous paragraph [15] was made to compare epidermal morphometry and the index of proliferating cell nuclear antigen (PCNA-I) by immunohistochemistry in respect to the dorsal and pinna skin, ventral skin, and the footpad from other areas of aging experimental animals (Bhattacharyya, unpublished observations). There was an attrition of epidermal thickness in dorsal and ventral skin and the footpad in relation to aging. PCNA-I showed a reduction

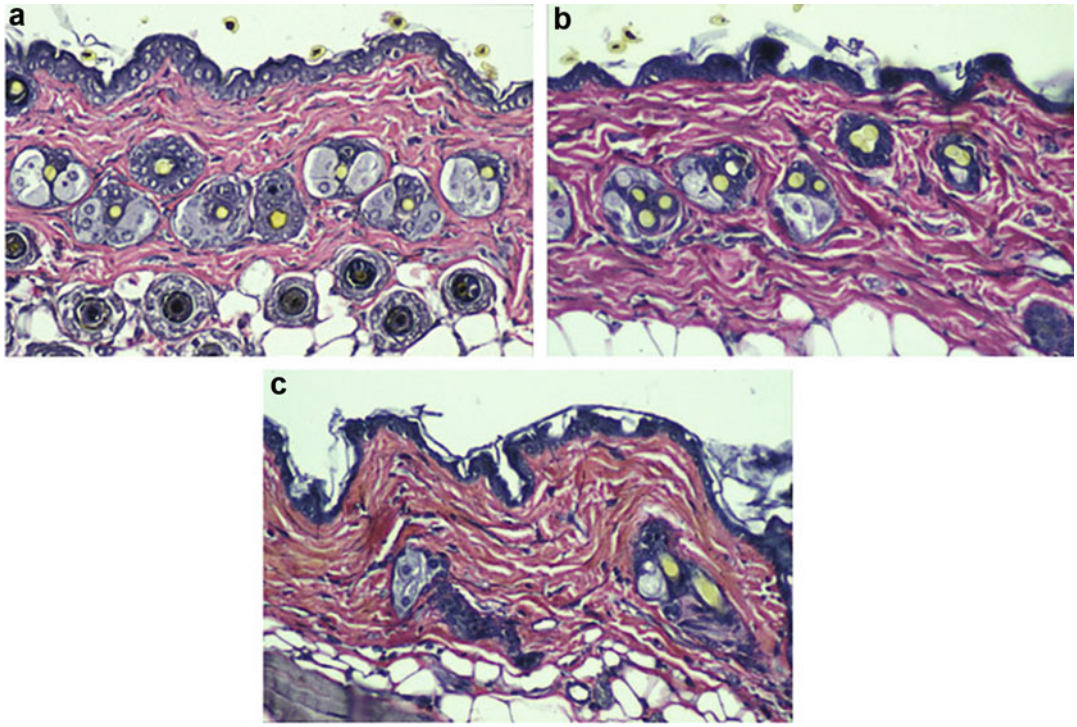

Fig. 1 Histological preparation of the dorsal skin in aging CBA mice in young (**a**), young adult (**b**), and old (**c**) animals, showing increasing atrophy of the epidermis and shrinkage of sebaceous follicles. Dermal elastic fibers can be seen in **c**. Verhoeff-van Giesen stain

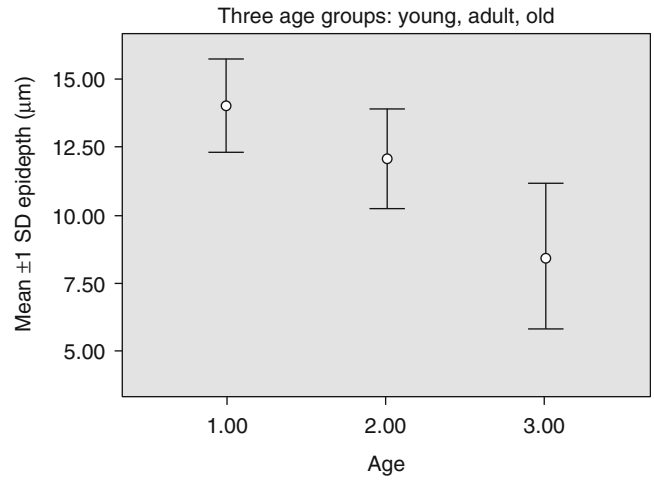

Fig. 2 Error bar chart of epidermal width measurements in three groups of CBA mice

across the ages only in the pinna skin and dorsal surface (Table 1; Figs. 3a–c).

The aging skin in the rat shows nonuniform patterns in different strains when it comes to epidermal and dermal thickness, and the age-associated changes seem to differ from the trend noted in murine species. In an early study of Wistar Institute rats, no significant age-related alteration was noted although the author described many qualitative differences in epidermal layers [17]. In Sprague

Table 1 PCNA-I and epidermal thickness data from aging CBA mice

	PCNA-I	*EPI width (*μm)
DS		
Y	31.9 + 5.0	16.3 + 0.8
AD	32.6 + 5.4	14.1 + 1.0
O	24.1 + 4.3	12.3 + 0.7
	*F 5.46, P 0.01	* F 32.43, P 0.0005
PS		
Y	37.0 + 7.2	15.6 + 1.5
AD	26.8 + 5.3	14.1 + 1.3
O	31.2 + 5.9	13.3 + 3.2
	*F 4.01, P 0.04	
FP		
Y	40.8 + 12.2	88.7 + 11.8
AD	32.6 + 6.5	72.9 + 8.1
O	40.9 + 6.1	55.3 + 8.7
		*F 17.28 P 0.0001
VS		
Y	35.0 + 4.8	14.6 + 0.6
AD	34.8 + 2.7	12.1 + 0.9
O	32.1 + 3.1	9.8 + 0.9
		* F 54.91 P 0.0005

DS dorsal skin, *PS* pinna skin, *FP* footplate, *VS* ventral skin, *Y* young, *AD* adult, *O* old

Dawley rats, the foot epidermis was explored to determine age-related changes in cell kinetics using single-pulse [3H]-thymidine labeling and the percent labeled mitosis technique [18] and led to the conclusion that there is a progressive decline in the rates of cell proliferation associated with age. However, these data were presented from rats only up to the age of 52 weeks. The authors of this chapter referred to five reports published earlier, which showed that rodent epidermal cell proliferation decreased in middle age and then remained constant or increased in senile animals. Skin from aging Wistar rats up to the age of 34 months was studied using histomorphometric analysis by Voros and Robert [19]. Average epidermal and dermal thickness did not show appreciable change with senility in this species. In aging Fischer 344 rats, epidermal thickness remained constant from 3 months of age onward [14]. However, increasing values in epidermal depth and nuclear population were noted in the ventral and dorsal skin and footplate skin samples from young, 1-year-old, and 2-year-old Fischer 344 rats [20, 21]. Earlier, Lapiere [22] commented that instead of becoming atrophic, rat skin increases in size, with more collagen, although the rate of increase is greatly diminished with aging. This is due to reduced collagen biosynthesis and increased degradation of macromolecules, but the balance between synthesis and degradation remains positive in rats.

Aging changes in cells other than epidermal keratinocytes, such as melanocytes or cells of Langerhans, have also been documented in some studies. Ultraviolet radiation has important health consequences on the Langerhans cells of human skin. The numerical density of Langerhans cells in aging inbred mice was studied from epidermal sheets and showed reduction when compared to that in young animals, although cutaneous immunoreactivity was not compromised [23]. Age-related neurodegenerative changes in peripheral nerves are a widespread phenomenon of clinical importance, and rat studies have attested to this inhibitory pattern. Age-associated loss in size of Meissner's corpuscles, with smaller and disorganized axonal processes, was reported in the digital pads of mice aged to the maximum life expectancy [24].

Interpretation of morphological changes of aging skin has many limitations, as discreet biochemical changes underlying such alterations cannot be visualized. In contrast to sparsely available accounts of dermal histochemical or morphological transformations in relation to life history in laboratory models, biochemical studies showing quantitative changes in dermal glycosaminoglycans, hydroxyproline concentration, acid mucopolysaccharides, and skin collagen and elastin changes in aging mice, rats, rabbits, and hamsters have been published. Only a few papers are cited here [25, 26]. Oxidative damage to the lipids and DNA increases with age in Fischer 344 rats, which was studied by measuring antioxidant enzyme activity [27]. In the hairless mouse, however, skin aging was not accelerated due to decreased antioxidant capacity [28]. Such molecular changes of the skin in intrinsically aged laboratory animals can only be revealed by immunohistochemistry as more suitable antibodies become available for research.

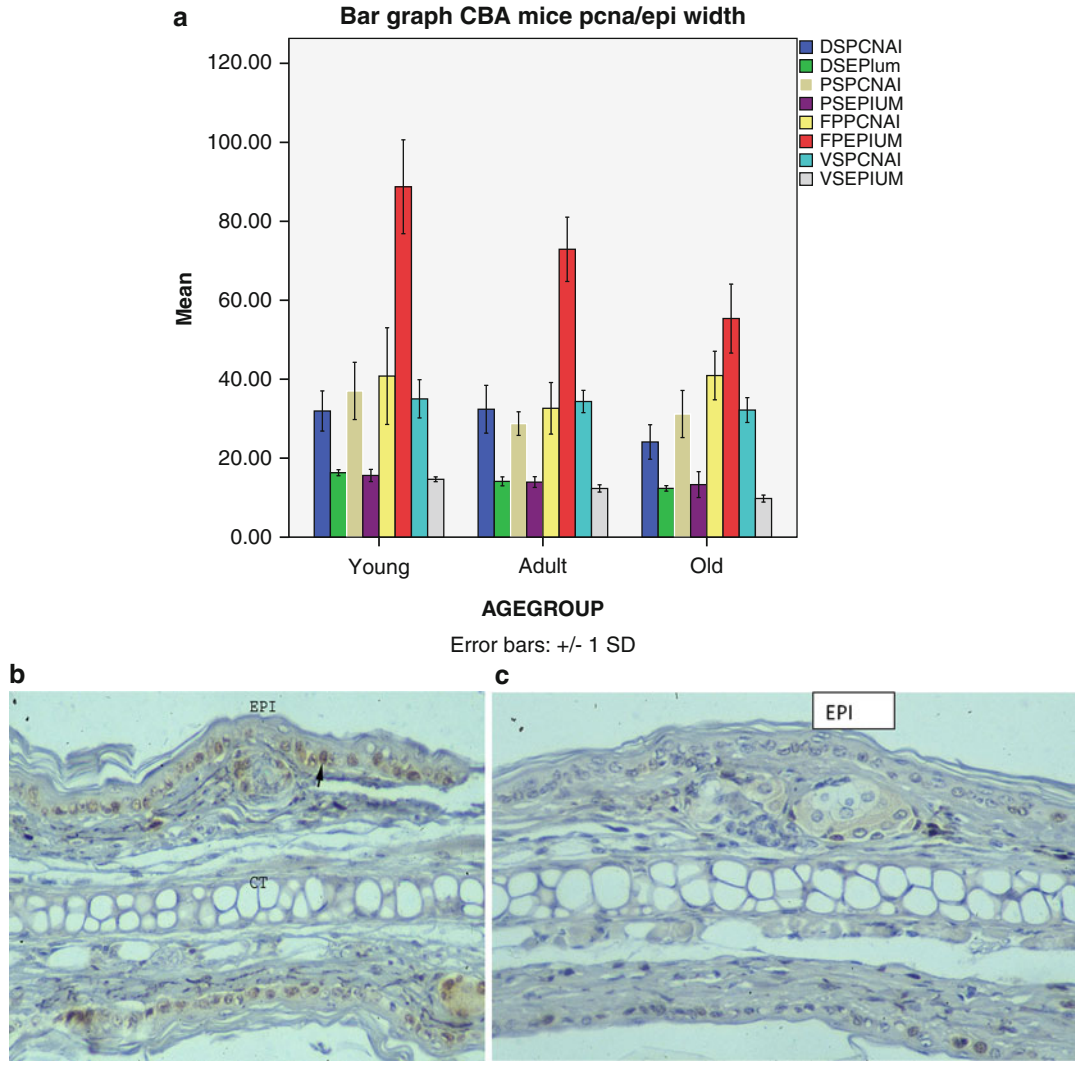

Fig. 3 (**a**) A composite bar diagram showing epidermal width and PCNA-I (PCNA index) in skin samples from dorsal and ventral areas, footpad, and the pinna in three age groups. (**b**). Pinna skin section from a CBA young specimen shows PCNA staining in epidermis (*EPI, arrow*). CT cartilage. (**c**) Pinna skin immunostained for PCNA in an old specimen shows absence of reactive nuclei in a thinner epidermis (EPI)

Morphological Changes with Aging in Other Species

Some sporadic accounts have also been published relating to mammals other than commonly available laboratory rodent models. Age-induced reduction in sebaceous glands was described in sheep by Warren et al. [29]. Epidermal flattening, fewer hair follicles and sebaceous glands, and a decrease in melanocytes were age-related changes in the hairless dog [30]. Veterinary textbooks describe aging changes in skin of domesticated dogs and cats. Senile changes in the skin of old cats and dogs include alopecia, callus formation over pressure points, orthokeratotic hyperkeratosis of the epidermis, and atrophied hair follicles [31]. Due to certain structural similarities with human skin, the pig skin model has been used in many experiments for studying responses to

surgical and physiological manipulations [32], but no account is available on aging morphological changes in this species.

Calorie Restriction (CR) and Skin Aging

Calorie restriction (CR) can reverse age-associated alterations in many organs like the heart, the liver, and the brain [33] and has been shown to exert beneficial effects on many skin disorders, although its morphological effect on the aging skin has not been thoroughly explored. Whether CR can modify age-related histomorphological features of the skin in colony-raised rats was evaluated with morphometric procedures in Fischer 344 rats. [20, 21, 34, 35]. Three age groups (young, adult, old) of this strain belonging to ad libitum and CR feeding regimens were obtained from NIH colonies, and skin samples from the dorsum, footpad, and abdominal skin were analyzed with morphometric procedures to evaluate various skin compartments (thickness of stratum corneum, epidermis, PCNA index, dermis, fat layer, percentage fraction of dermal collagen, elastic fibers, fibroblast density, capillary profiles, and staining intensity of dermal glycosaminoglycans). The Fischer 344 rat showed many age-related skin changes, and these were prevented or delayed by CR, presumably due to metabolic alterations imposed by the dietary regimen (Fig. 4).

CR reduces cell proliferation in some tissues, with inhibited pace of DNA replication, and this makes those tissues less susceptible to DNA damage by carcinogens. Epidermal cell proliferation as quantified by immunohistochemistry was also correlated to age-related changes in epidermal thickness in these colony-raised Fischer 344 rats. Just as CR somewhat inhibited the trend of increasing epidermal width in aging rats, the keratinocyte proliferation rate as measured by staining of proliferating cell nuclear antigen (PCNA) was correspondingly lower in aging CR rats. This trend was observed in the epithelium of the dorsal skin as well as the footplate (Figs. 5 and 6) [34]. In C57BL/6 J mice, epidermal cell proliferation was reduced by CR and alternate-day fasting regimens [36]. A study in SENCAR mice also shows that dietary calorie restriction

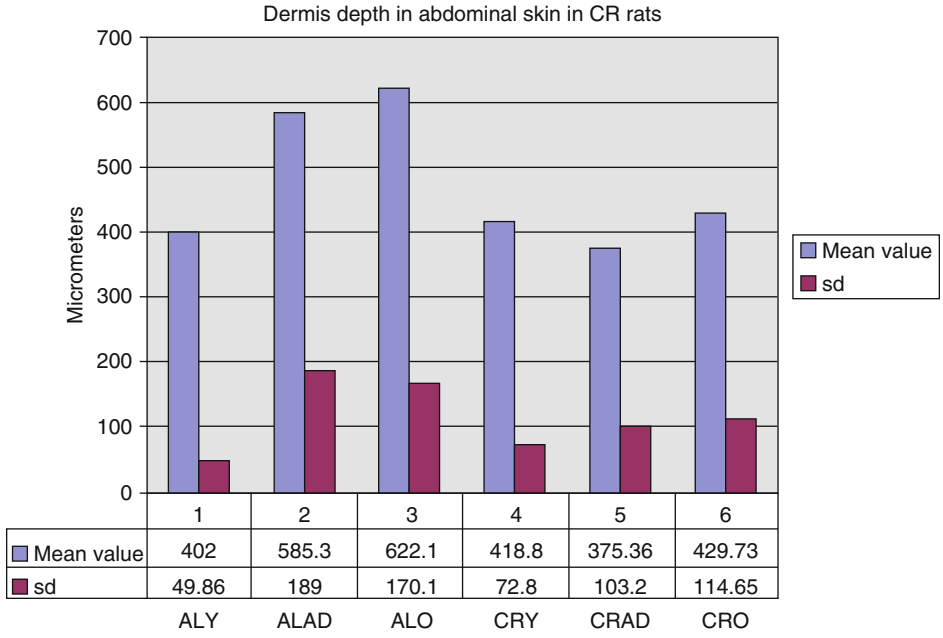

Fig. 4 Bar graph representation of the dermis depth from Fischer 344 rats in CR study. Three age groups of ad libitum (*AL*) and calorie-restricted (*CR*) animals are represented

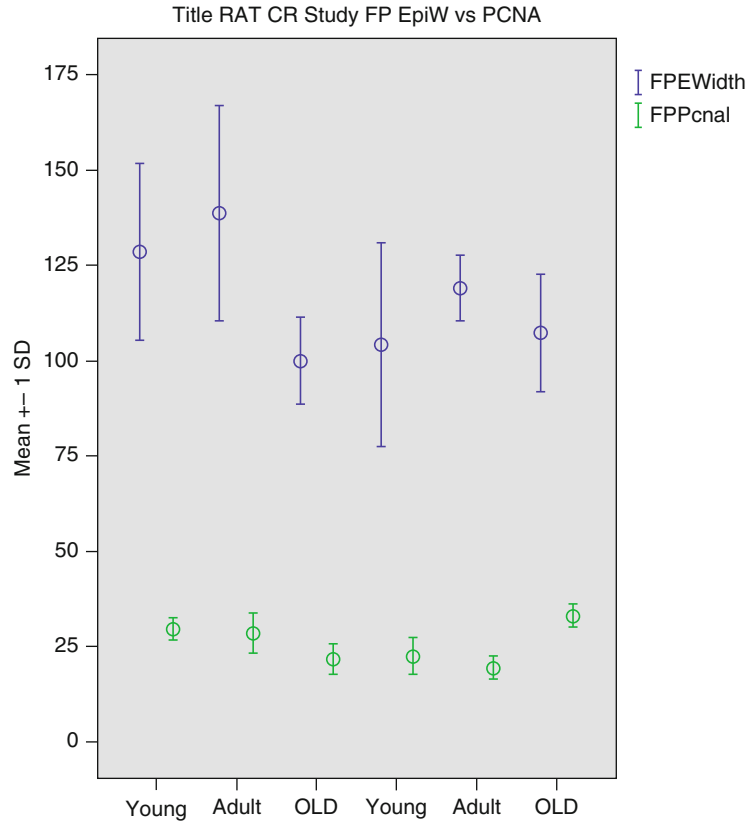

Fig. 5 Representation of epidermal width and PCNA index from footplate (*FP*) in rats from CR study. Three age groups from AL and CR animals are represented

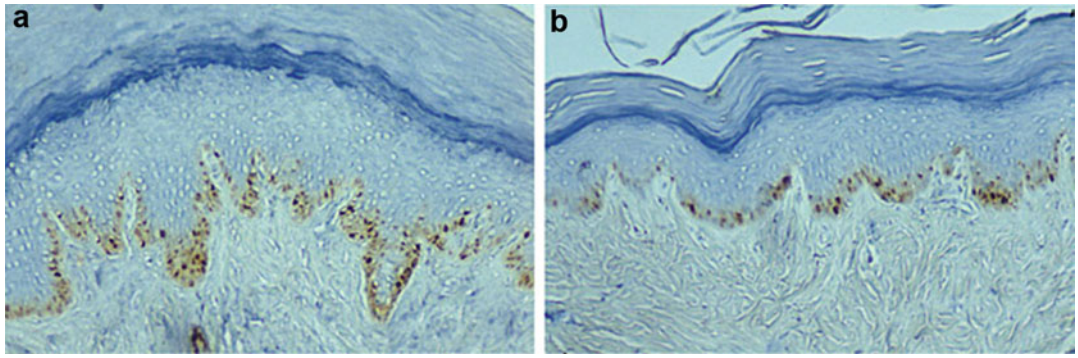

Fig. 6 PCNA staining in footplate epidermis from adult (**a**) and old (**b**) CR rats. (**a**) CR rat, adult group, footplate section. A broad epidermis with a thick cornified layer and brown stained PCNA reactive cells in the basal layer can be observed. (**b**) CR rat, old group. The footplate section shows regression of the epidermis and fewer PCNA positive cells

may inhibit gene expression in skin tissues relevant to cancer risks [37]. CR has been reported to reduce the level of free radicals and prevent accumulation of advanced glycation products and thus may be beneficial to aged skin. On the other hand, loss of subdermal adipose tissue stores, with restricted feeding, may accentuate fine wrinkles in human facial skin. CR can diminish subdermal adipose tissue [21]; therefore, its aesthetic effect on the profile of the aging human face remains to be seen.

Conclusion

Due to the ease of studying skin aging phenomena and age-associated progressive changes in short-lived mammals, published studies were mostly confined to laboratory rodent models. Unfortunately, many earlier descriptions of the histopathologic changes in aging skin in such models did not consider old or senile specimens and therefore lack a proper population sampling. Thus, the story is somewhat incomplete when compared to gerontologic cutaneous changes studied from skin biopsy samples of 90-year-old human patients! It should be noted that rodent life span is also highly variable in different strains. Early literature has recorded that among inbred mice strains used for aging research, a mean life span can vary from a low of 276 days in the AKR/J strain to a maximum of 799 days in the LP/J strain [38]. The lack of availability of suitably aged rodent models is a handicap to such research. This review, however, shows that the mouse model may be more suitable for documenting age-related histological changes that can be compared to human data, despite the caveat that comparing skin from laboratory-grown rodents with a short life span with the human skin assaulted by years of disease and environmental challenge is a rather risky endeavor. Histological deficits in intrinsically aged human skin affect the epidermis, dermal thickness, cellularity, and elastic fiber system. Efforts should be made to search for analogous chronological changes in animal models that can be utilized for studies aimed at rejuvenation of the aged skin. Similarities between human and murine skin have been reported in many studies, making mouse skin a suitable material for studying inflammatory skin diseases. Apart from certain similarities in basic molecular and physiological processes between the mouse and the human [39], the mouse can be maintained under proper husbandry conditions and may be a more suitable animal for studying the process of senescence of the integumentary system. Virtually no data are available on histological changes from intrinsic aging in other animals like the dog, the domestic pig, or the rabbit, although such species are extensively used for biomedical and physiological studies.

Wrinkle formation is a vexing problem of aging human facial skin, and there is little information about skin wrinkles in any animal model at the senile state. Suitable models for studying the histophysiology of wrinkles or their reversal have not been reported, except for one study. Cross-breeding between the Wistar and the wild rat was reported to generate a rat model (the Ishibashi rat) in which skin aging, with wrinkles and furrows, appeared at 12 weeks. Wrinkle formation in this new model was due to a reduction in elastin and collagen contents of the aging skin [40]. This kind of experimental model would prove useful to define the underlying anatomical correlates of skin wrinkle formation, to study its profilometry, and to test the effect of topical anti-wrinkle products for its amelioration [41].

In recent years, stem cells in skin have attracted a lot of scientific introspection due to their potential clinical application in wound healing, burns, and alopecia [42], and these cells can go through self-renewal and terminal differentiation and may regulate tissue aging [43]. It remains to be determined whether stem cells in aging mouse skin can show temporal regression. In vitro studies have shown aged mouse epidermal keratinocytes can function comparably as those cells from young mice [44]. Keratin 15 (K15) promoter activity, which is specific for stem cells, was shown to be active in the hair follicle bulge in murine skin, and the basal epidermal expression of K15 gradually decreased with age [42]. On the other hand, in C57/BI6 mice from NIH colonies, despite a loss in dermal cellularity and thickness in the dorsal skin associated with skin aging, epidermal stem cells were maintained at normal levels throughout life [16]. Further research will elaborate how stem cells can respond to intrinsic aging in different mouse models and whether they can be pharmacologically stimulated to reverse the process of cutaneous aging.

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