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Chapter

Genetic and Epigenetic Influences on Cutaneous Cellular Senescence

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Abstract

Skin is the largest human organ system, and its protective function is critical to survival. The epithelial, dermal, and subcutaneous compartments are heterogeneous mixtures of cell types, yet they all display age-related skin dysfunction through the accumulation of an altered phenotypic cellular state called senescence. Cellular senescence is triggered by complex and dynamic genetic and epigenetic processes. A senescence steady state is achieved in different cell types under various and overlapping conditions of chronological age, toxic injury, oxidative stress, replicative exhaustion, DNA damage, metabolic dysfunction, and chromosomal structural changes. These inputs lead to outputs of cell-cycle withdrawal and the appearance of a senescence-associated secretory phenotype, both of which accumulate as tissue pathology observed clinically in aged skin. This review details the influence of genetic and epigenetic factors that converge on normal cutaneous cellular processes to create the senescent state, thereby dictating the response of the skin to the forces of both intrinsic and extrinsic aging. From this work, it is clear that no single biomarker or process leads to senescence, but that it is a convergence of factors resulting in an overt aging phenotype.

Keywords: skin, DNA damage, telomeres, epigenetics, immunosenescence, inflammaging

1. Introduction

A consensus agreement on the definition of cellular senescence may be stated as a viable but non-proliferative condition distinct from the G0 quiescent phase of the cell cycle and postmitotic terminal differentiation [1, 2]. While aged living organisms accumulate senescent cells, aging and senescence are not synonymous terms—the cellular and molecular pathways that eventuate in the senescent state can be activated by diverse mechanisms, not necessarily chronologic aging nor the limit of replicative cell division. It was the latter phenomenon, in fact, that led early investigators to the original concept of cellular senescence as an *in vitro* observation; that replicating fetal "skin tissue cells" stop dividing at a certain passage number, the so-called "Hayflick Limit" [3]. This review focuses on our current understanding of how cellular senescence occurs in the skin, its irreversible (and possibly reversible) characteristics, description of known trigger points involving genetic and epigenetic factors and their clinical implications in health and disease.

Cellular senescence is characterized by cell cycle arrest [4], the expression of senescence associated secretory phenotype (SASP) [5, 6], damage to DNA [7–9], deregulated metabolic profile [2, 10], changes to the epigenome [11] and transcriptome [12], resistance to apoptosis [13, 14], and altered immune surveillance [15, 16]. It can be triggered by multiple factors [2], the mechanisms of which appear to categorize the 'type' of senescence into two main groups; so-called replicative senescence (RS) due to shortened telomeric DNA resulting from excessive cell division cycles [17–20]; and a state generally termed 'premature senescence' (PS), in which both oncogene-induced senescence (OIS), triggered by activation of oncogenes such as *ras* [21], and several other 'molecular stresses' [4] also eventuates in the senescent phenotype.

There are a variety of biomarkers for cellular senescence but not all senescent cells express the same biomarkers due to these differential molecular induction pathways. Several senescent biomarkers have been identified in the skin [22]; however, it is currently unclear how the multitude of cell types that comprise this tissue respond to senescence-inducing triggers and how this correlates with skin aging, other than the fact that senescent cells accumulate in all skin compartments with age, just like other organ systems. What is becoming clear, however, is that cellular senescence plays critical roles in the pathobiology of skin aging and disease [23].

2. Aging of the skin

Both intrinsic (time, genetic and hormonal) and extrinsic (environmental) factors contribute to skin aging. Old skin not only appears clinically different from young skin but has altered physiology due to a combination of molecular, cellular, and biochemical processes, and tracing the pathogenic origin of the 'skin aging phenotype' remains a work in progress. From a clinical perspective however, the skin of most people older than 6–7 decades of life, particularly in photo-exposed areas, is thinner, looser, less tethered to underlying tissue, more wrinkled, more translucent with more visible capillary vessels, more discolored, drier, and less padded by the subcutaneous layer [24, 25]. Scalp skin also ages, commonly observed as pigment loss (graying), and most people experience hair loss as another inevitable esthetic problem.

Anatomically, the structure of human integument tissue we call 'skin' is composed of ectodermal-derived epithelial cells layered as stratified squamous epithelium on top of mesenchymal-derived dermis separated by a specialized basement membrane zone (BMZ) called the dermal-epidermal junction (DEJ). Directly below the dermis is the fatty hypodermis (or subcutaneous layer) separating fascia and muscle from the skin. Epithelial-mesenchymal interactions that occur during embryogenesis (and wound repair) contribute to the formation of glandular structures buried within these compartments (called adnexa) which are comprised of eccrine, apocrine, sebaceous and hair follicle structures. Peripheral nerves and blood vessels traverse the subcutaneous and dermal layers and together with all other structures of the integument, serve the functions of barrier protection, retention of heat and water, sensation, contractility, and lubrication [26].

This multi-compartmental system is the largest organ of the body and is composed of about 20 different cell types responsible for skin function and its stratification [27], all of which also change with age to contribute to the overall 'skin aging phenotype'. At the microscopic level, skin tissues of older individuals exhibit common characteristics regardless of whether sun protected or chronically exposed to ultraviolet (UV) light [28]. The most obvious and well documented structural changes include epidermal thinning, loss of rete ridges and flattening of dermal papillae [29, 30], keratinocyte

and melanocyte architectural changes [31, 32], BMZ/DEJ alterations [33], less dense and altered reticular dermal collagen structure [34], accumulation of altered elastin and elastic fiber structural abnormalities [35], altered shape and loss of papillary dermal capillary loops [36–38], and size and structural alterations in glandular structures of the eccrine, apocrine and sebaceous units [39]. Concomitant with morphologic changes observed in aged skin, senescent cell populations increase in all skin compartments.

2.1 Effects of aging on epidermal structure and function

The epidermis, consisting of 5 different layers of keratinocytes, continuously renews itself on an approximate 27-day cycle by a differentiation program involving basal cells which are maintained and replenished by stem cells residing in the bulge region of the hair follicle and the interfollicular epidermis [40]. Of particular importance to aging of the epidermal compartment is the general concept that the cellular microenvironment (or niche) of stem cell populations plays a critical role in homeostatic resupply of transient amplifying basal cells [41]. The epidermis maintains a dynamic equilibrium by proliferating in the basal layer that is attached to the DEJ, then cell division ceases and basal keratinocytes undergo terminal differentiation while spatially migrating towards the top of the epidermis. During this transition, keratinocytes acquire specialized cytoskeletal components and create an intercellular diffusion barrier, eventually forming the outermost epidermal layer called the stratum corneum (SC). The SC is a specialized acidic, hydrophobic, protein-lipid-carbohydrate flexible 'shell' resistant to wear and tear, water loss, and invasion of microbes [42]. The "barrier function" of the skin is derived from the SC.

The epidermal compartment appears to deal with the ravages of extrinsic aging in a fundamentally different way than the dermal compartment because terminally differentiated (cell cycle arrested) keratinocytes are continuously shed, thus removing accumulated DNA and other macromolecular damage that otherwise trigger the senescent phenotype. But since the epidermis is continuously replenished by stem cells arising from the interfollicular niche, its alteration can affect epidermal biology in profound ways. In fact, epidermal stem cell niche can be affected during aging by both basal keratinocytes [43] and dermal fibroblasts [44]. Niche microenvironments can be altered by intrinsic and extrinsic aging at cell-cell, cell-matrix and paracrine signaling levels, leading to stem cell depletion and the 'atrophic epidermal phenotype' observed in intrinsic aged skin [45].

Many other cell types localize to the epidermis, including pigment-producing melanocytes found in the basal layer that protect against UV radiation. Pigment is synthesized within the melanocyte but transferred to neighboring basal keratinocytes (and specialized hair follicle-associated keratinocytes) via a complex melanosomal exo/phagocytosis mechanism localizing at the dendritic tips of melanocytes which interdigitate with up to 20 keratinocytes [46]. Melanocyte dysfunction associated with extrinsic aging (mostly photoaging) manifests clinically as abnormally dispersed and/or diminished melanin pigment (i.e., dyschromia, lentigines, and in the scalp, canities). Senescence of the melanocyte has been observed both *in vitro* and *in vivo* and the molecular pathways involved identified [47]. In fact, based on biomarker (e.g., P16) expression, senescent melanocytes appear to represent most senescent cells in aged epidermis [48] and their contributions to development of the epidermal atrophic phenotype via autocrine and paracrine (i.e., SASP) mechanisms have been identified [49].

The epidermal immune system is a network of resident antigen-presenting dendritic Langerhans cells (LC) thought to function as immune sentinels [50] together with trafficking lymphoid immune cells including resident memory T cells as CD8+

and CD4+ cells [51]. Of interest, a specialized CD4 + T cell (Treg) residing near the hair follicle bulge areas (located in the dermis) has been shown to play a role in hair growth cycling [52]. Skin aging is associated with variable deterioration of both adaptive and innate immune function, generally referred to as cutaneous 'immunosenescence'. This term has become controversial in the literature [16] because immune cell senescence is, in part, a physiologic adaptive response to survival and fitness of the organism. Its use to describe altered skin immune responses with age appears appropriate in the context of inflammaging [53], since the concept of immunosenescence encompasses both systemic chronic, low-grade inflammation [i.e., elevated serum levels of interleukins IL-6 and IL-8 and increased tumor necrosis factor alpha (TNF-α), etc.] and the presence of dysfunctional immune responses in various skin compartments apparently related to both tissue level RS and PS. Currently unknown, however, is whether cutaneous inflammaging is a cause or an effect of dysfunctional innate immune responses observed in the elderly and whether cellular senescence is responsible.

Examples of cutaneous aged-related immune dysfunction include reported reductions in the number and functionality of LC in aged skin and this correlates with both age related defective epidermal barrier function and inflammaging [54]. Likewise, defective physiologic immune clearance of senescent cells that contribute to aged skin pathologies have been demonstrated in dermal fibroblasts by the observation that these cells express a nonclassical major histocompatibility antigen (HLA-E). Its increased expression appears to block activation of natural killer (NK) cells and CD8+ cells responsible for clearing damaged cells, suggesting that evasion of dermal immunosurveillance leads to persistence of senescent dermal fibroblasts [55].

Aging is a clinical comorbidity in many skin diseases pathogenically linked to defective cutaneous innate and adaptive immune responses [53]. The incidence and prevalence of autoimmune blistering disorders such as bullous pemphigoid (BP), pemphigus vulgaris, and epidermolysis bullosa acquisita are all increased in older populations, BP being the most common example [56]. Likewise, aging is a comorbidity in the development of skin cancers, and the loss of immunosurveillance due to dysfunctional LCs is thought to contribute to progression of both non-melanoma skin cancer (NMSC) [57] and melanoma [58].

Another unique cell type scattered along the DEJ, considered part of the epidermal compartment, and possessing mixed neuronal, endocrine, and immunologic functions (as well as embryonic origin) is the Merkel cell (MC) [59]. Its involvement in the skin's somatosensory system is key to the sense of fine touch discrimination, which is decreased in the elderly [60]. In glabrous skin MC form complexes with intraepidermal sensory neurites found at the DEJ termed 'touch domes' or Merkel's discs. Digital skin of aged humans contains less of these complexes, lower density of MC and decreased expression of the stretch-activated ion-channel component Piezo2 [61]. Occurring mainly in aged humans, a rare but very aggressive skin cancer, Merkel cell carcinoma (MCC) has attracted recent attention due to its mysterious etiopathogenesis. 80% of MCC is associated with integration of a newly identified polyoma virus (MCPyV) [62], whereas 20% appear linked to accumulation of UV light-induced somatic mutations [63]. As detailed in the next section on epigenetics, it is of interest that the majority of MCC display expected chronologic age but DNA methylation patterns of epigenetically youthful cells [64].

2.2 Aging and the dermis

The dermal compartment is divided into superficial, reticular, and deep dermis with unique cellular, vascular, extracellular matrix (ECM), and adnexal components

that define each space. Much of the 'business-end' of the dermis is localized to the superficial dermal compartment and the DEJ is central to its structure and functionality. It is considered part of both the epidermis and the superficial dermis because cellular components of each layer contribute to its synthesis, maintenance, and renewal. Serving as an adhesive scaffolding for basal keratinocytes, a shear-resistant Velcro-like surface securing the dermis to the epidermis, a complex paracrine factor-sequestering and mechano-transducer signaling layer, the DEJ modulates a remarkable number of cutaneous cellular processes involved in skin structure, function, regeneration, and resistance to trauma [33]. Comprised mainly of Type IV collagen and laminin, like other BMZs, DEJ complexity has been dissected at the molecular level to reveal a complex network of other collagens (VII, XVII and XVIII), 4 different isoforms of laminin (511, 521, 311 and 332), perlecan, nidogens, SPARC, fibrulins-1 and -2, dystroglycans, and integrins a3b1 and a6b4. All of these DEJ components are altered during aging and these changes correlate with age dependent increases in both DEJ thickness and stiffness [65].

Immediately beneath the DEJ, forming nipple-like structures projecting into the epidermal compartment and containing unique ECM, microvasculature, specialized fibroblasts, and dermal mesenchymal stem cells is the papillary dermis (PD). Here, undulating dermal protrusions interdigitate with epidermal rete ridges (pegs) to increase surface area for nutrient transfer, trafficking of immune cells, and increased tensile strength. The transition of superficial to reticular dermis is static and defined mostly by changes in ECM structure but dynamic during repair and disease. The majority of space in reticular dermis is occupied by thick bundles of interstitial collagens I and III, elastin and fibrillin fibers, and amorphous 'ground substance' comprised of hyaluronic acid, proteoglycans and glycoproteins. Like the DEJ, most if not all these dermal ECM components are altered and/or dysfunctional due to aging [35]. Both intrinsic and extrinsic aging correlate with the loss of rete ridges and flattening of dermal papillae [29, 30]. Compared to young, non-exposed and old photo-protected skin, the PD of chronically photodamaged skin displays marked structural changes, the most dramatic feature of which is the presence of "solar elastosis" in the superficial dermis. Solar elastosis consists of pathologically altered elastin fibrils [66] that present as dense accumulations of amorphous material best visualized with trichrome staining.

The cellular composition of these dermal sub-compartments is a complex mix of fibroblasts, endothelial cells, myofibroblasts, macrophages, mast cells, trafficking immune cells, adipocytes, various stem cells, sensory neurites, and the differentiated cellular components of dermal adnexa (including the hair follicle). An example of such cellular complexity, the significance of which continues to evolve, is the apparent post-natal plasticity of the dermal fibroblast. Single cell RNA sequencing has revealed at least four different subpopulations of human dermal fibroblasts [67, 68], and skin aging has been demonstrated to have a strong effect on both dermal 'fibroblast' phenotype and functionality. For example, young papillary dermal fibroblasts can direct reformation of youthful DEJ and epidermal structure and function, whereas old papillary and/or reticular dermal fibroblast populations cannot [69]. Furthermore, it is the senescent PD fibroblast and CD271+, laminin 332-expressing interfollicular stem cells that contribute to age-associated pathologic remodeling of the DEJ [33, 70]. Recent attention has focused on specific dermal fibroblast subpopulations and their involvement in wound healing, fibrosis, and loss of epidermal stem cell 'stemness' due to niche signaling dysfunction. The homeobox gene engrailed-1 (*EN-1*) expression appears to distinguish two types of fibroblasts; those cells expressing *EN-1* are associated with fibrotic healing phenotype whereas *EN-1* negative fibroblasts promote physiologic remodeling [71, 72]. Epigenetic modulation of the fibrotic phenotype is reviewed in the next section.

2.3 Subcutaneous layer involvement in skin aging dysfunction

The subcutaneous compartment (hypodermis) is composed mainly of cellular lipid storage units (adipocytes) separated by thin weblike networks of specialized ECM stroma containing microvasculature, adipose derived mesenchymal stem cells (ADSC) and immune cells. It functions as a thermoregulatory and shock-resistant barrier, as well as a reservoir of bioactive factors involved in systemic lipid metabolism, energy balance, and endocrine function [73]. Subcutaneous fat also undergoes age-related changes that are generally like the epidermal and dermal compartments where an 'atrophic' phenotype becomes clinically evident. With aging, subcutaneous fat deposits in various body locations disappear and/or are redistributed to visceral locations elsewhere in the body, causing esthetic concerns; this redistribution is associated with a variety of systemic age-related disease states, including insulin resistance, metabolic syndrome, cardiovascular disease, and obesity [74, 75]. Of note, senescent cells have been shown to accumulate in aged adipose tissue [76], contributing to systemic inflammaging. Experimental clearance of senescent cells can dramatically affect the redistribution of fat from the visceral to the subcutaneous compartment and decrease SASP expression [77]. The mechanism(s) of adipose cell senescence has not been clearly defined; however, ADSC exhaustion, oxidative stress by reactive oxygen species (ROS), and niche disruption appear to play important roles [78].

The influence of adipogenic hormones in skin aging and senescence has received recent attention with the discovery that UVB-light induced PS in human keratinocytes can be rescued by adiponectin via its suppression of inflammatory signaling pathways and human beta defensin-2 (*hBD2*) expression [79]. Human dermal fibroblasts express adipokine receptors and both leptin and adiponectin have been shown to stimulate expression of the ECM components hyaluronic acid and interstitial collagen [80]. These adipogenic hormones secreted by subcutaneous fat cells thus appear to represent paracrine cutaneous anti-aging factors for both the epidermal and dermal compartments.

'Fat grafting' has become a popular procedure in esthetic medicine and a variety of other clinical indications [81, 82] with special attention focused on ADSC. These cells can be isolated from subcutaneous fat removed during liposuction procedures after the stromal vascular fraction (SVF) is either mechanically sorted or enzymatically digested with bacterial collagenase, decanted (or centrifuged), washed, and grafted [83]. SVF is composed of cellular components (pre-adipocytes, adipocytes, histiocytes, endothelial cell progenitor cells and ADSCs) and is a rich source of growth factors (i.e., bFGF, IGF-1, VEGFs, PDGF-BB), matrikines, and other paracrine cellular factors. The ADSC secretome has been well characterized, consisting of soluble protein factors and lipid membrane particles (exosomes and ectosomes) that are used internationally in multiple therapeutic clinical trials for a vast array of indications, including dermatologic conditions (esthetics, wound healing, fibrotic diseases, dermatoporosis, etc). It is the loss of ADSC stemness, decreased proliferative potential, and dysfunctional secretome expression accompanying skin aging that continues to draw intense interest [82].

3. Genetic influences on cutaneous cellular senescence

The two major molecular pathways resulting in RS and PS have been observed in the skin [23]. These are reviewed in the following section by examining first the genetic aspects of cutaneous cellular senescence, followed by epigenetic influences. It should be noted here that acquisition of these cellular senescence phenotypes plays a critical role in both normal

organismal and tissue level physiology by, for example, dampening fibrotic responses during the remodeling phase of wound repair or suppressing tumor formation [84]. However, it also appears to be a major pathologic driver in age-related disease states [85].

3.1 DNA damage related to telomere biology

The senescent phenotype can be activated by DNA damage at the ends of all eukaryotic chromosomes, called telomeres, which consist of DNA loops containing noncoding repeats of guanine-rich sequences complexed with protective oligomeric proteins (Shelterins). Discovery that chromosomal replicative machinery responsible for somatic cell division cannot synthesize exact duplicates of these structures led to the concept of the 'end-replication problem' during serial passaging [20]; thus, telomeric DNA is subjected to attrition because DNA polymerase fails to replicate the 3′ lagging strands.

Telomeric DNA are shortened by approximately 50–200 bp per cell division and thus a molecular clock is achieved, reflecting the replicative history of primary cells [86]. A specialized DNA polymerase (telomerase) is responsible for fixing the 'end replication problem', maintaining telomeric length, but its expression and function are restricted to immortal postnatal cells; *in vivo,* comprising stem, progenitor, and cancer cells. When cells reach their 'Hayflick Limit' telomeres lose enough DNA [87] to trigger a genomic instability signal and chromosomes become 'uncapped' by loss of Shelterin. This genomic instability signal is a specialized DNA damage response (DDR) and generates telomere dysfunction-induced foci (TIFs). Approximately half of all persistent DNA damage foci are localized to telomeres, and these can trigger RS. But senescent cells can harbor many other forms of persistent chromosomal DNA damage foci, called DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence) [9]. These dynamic structures can also trigger cell cycle arrest and SASP induction.

Independent of telomere length or uncapping by loss of Shelterins, guanine-rich telomeric DNA repeats can become damaged by ROS, generating DDR telomere-associated foci (TAFs), which are associated with triggering the senescence phenotype [19]. This observation has particular relevance to the state of chronic inflammation, SASP expression, and tissue aging (Inflammaging) in skin and other tissues [15, 88, 89], as discussed in Section 3.2.

While epidermal, dermal, and subcutaneous cellular compartments all harbor evidence of RS in aged skin tissue, direct evidence that telomeric DNA associated RS is involved in skin aging is supported by experiments involving ectopic expression of human telomerase (*hTERT*). We reported that neonatal human dermal microvascular endothelial cells (HDMEC) undergo RS *in vitro* but can become immortalized with viral transfer of the catalytic subunit of *hTERT* [90]. Furthermore, these telomerized HDMEC formed fully functional microvessels *in vivo* (perfused with murine blood) that exhibited superior durability with time after xenografting in immunodeficient mice *versus* vessels created with *in vitro*-aged primary HDMEC [91]. As previously reviewed, cutaneous microvasculature of aged papillary dermis is markedly reduced and abnormally structured versus young dermis [38], presenting clinically as telangiectasia and senile purpura/dermatoporosis. The roles of RS, OIS, and other senescent pathways on skin vasculature remain to be determined.

3.2 Genotoxic and exposome insults

As noted in the introduction, cellular senescence can be induced in the absence of any telomeric damage or loss and this premature senescence (PS) has similar

deleterious effects on aged tissues, including the skin. The triggers for the PS program generally fall into (a) accumulation of subcytotoxic, unrepairable, non-telomeric DNA damage, including mitochondrial DNA (mtDNA), (b) macromolecular insults to cytosolic and secreted proteins and lipids, and (c) metabolic dysfunction involving an altered mitochondrial-lysosomal axis [92]. All of these PS triggers have been demonstrated in skin cells *in vitro* and *in vivo* [93].

The molecular and cellular effects of chronic UV light exposure (photoaging) have also been well-documented and, in many ways, more extensively than intrinsically aged human skin. Both UVA (320–400 nm, less energy) and UVB (280–320 nm, more energy) light cause photoaging but UVB is mostly absorbed by the epidermis, where it causes sunburns. UVA penetrates the superficial and reticular dermal compartments and is considered a major factor in photoaging. While both UVA and UVB wavelengths generate reactive oxygen species (ROS), indirectly damaging DNA, UVB is also directly mutagenic, causing DNA defects called cyclobutene pyrimidine dimers and 6–4 photoproducts [94]. Remodeling of dermal ECM favoring an atrophic phenotype is triggered in unwounded skin by UV exposure via the activation of mitogen-activated protein kinase (*MAPK*) and activator protein 1 (*AP-1*) signaling pathways which causes downstream expression of matrix metalloproteinases (MMPs) in both the epidermal and dermal compartments [95]. These same pathways block transforming growth factor beta (TGF-β)/SMAD signaling via TGF-b type II receptor down regulation causing decreased collagen synthesis [96, 97]. Dissection of the molecular effects of chronic UV exposure on the DEJ and PD have been recently reviewed [33].

Our understanding of the role senescent cells play in cutaneous aging pathologies continues to evolve. In the past, senescent cells observed in the skin with biomarkers *in vivo* were believed to be passive, unresponsive bystanders recognized morphologically by their enlarged, seemingly flattened, abnormal shapes and senescence-associated (SA) β-galactosidase staining. But characterization of SASP expression in senescent cells (and their paracrine effects) provided compelling evidence that senescent cells are anything but passive.

It is now widely accepted that senescent cells remarkably influence surrounding non-senescent neighbors and ECM networks via secretion of inflammatory cytokines, chemokines, matrikines, MMPs, tissue inhibitors of metalloproteinases (TIMPs), and other proteinase-inhibitor systems that comprise the tissue 'proteinase web' [98]. One such example is the role played by plasminogen activator inhibitor-1 (*PAI-1*) in modulating senescence. *PAI-1* is a soluble and matrix bound serine protease inhibitor with multiple matricellular functions and can be found at increased levels in both dermal fibroblasts from aged donors and premature aging syndrome patients [99–101]. Ectopic expression of *PAI-1* in fibroblasts induces the senescent phenotype and is both necessary and sufficient for RS downstream of p53 [102]. Many other examples of SA ECM alterations have been reviewed [33].

The quintessential example of extrinsic aging involves the postmitotic dermal fibroblast population which responds to 'expososomal' damage [103] by activating DDR pathways, triggering PS and subsequent expression of macromolecular damage profiles involving mtDNA damage. One mechanism of mtDNA damage appears to involve UV light-induced deletion of a significant length of mtDNA, termed the 'common deletion' (CD) [104]. This 49 kb mtDNA fragment contains codons for electron transport chain (ETC) protein complexes I, IV and V which together express 72 ETC subunits, the loss of which cripples physiologic functions of mitochondrial energy metabolism, ROS protective mechanisms, and calcium homeostasis. Tracking the mtDNA CD in human skin revealed that both intrinsic (photo-protected) and extrinsic (chronic UV-damaged)

skin contain this marker [105], and that dermal fibroblasts appear to be the culprit for subsequent age-related tissue damage of ECM [106, 107].

3.3 Genetic skin diseases associated with DNA repair pathway defects

Analysis of progeroid syndromes have provided insights into molecular mechanisms of intrinsic and extrinsic skin aging. Common skin phenotypic signs and symptoms shared by both these premature aging disorders and skin aging in the general population include skin atrophy, alopecia, fibrosis, telangiectasia, poikiloderma, canities and both NMSC and melanoma. Rare autosomal recessive patterns of different mutations in DNA repair genes group these heritable disorders into those involving; (1) multiple defects in nucleotide excision repair (NER) genes [e.g., DNA polymerase eta (*POLH*) among six others] coding for repair proteins in xeroderma pigmentosum (XP) [108], (2) transcription and transcription-coupled NER genes in Cockayne syndrome (CS) and (3) mutations in the gene family of RecQ helicases involved in DNA double strand break repair in Werner syndrome, Bloom syndrome, and Rothman-Thomson syndrome [109]. In the latter three disorders, mitochondrial defects have been well documented and correlate with cellular senescence phenotypes [110, 111]. In XP-V null mouse models, loss of *POLH* leads to obesity and marked adipose tissue senescent phenotype expression [112].

3.4 SNPs and transcriptomics

Several genome-wide association studies (GWAS) and meta-analyses performed on young and old populations have identified single nucleotide polymorphisms (SNPs) in genes thought to be correlated with skin aging [113–118] or 'perceived' facial age' [119]. These large cohort-based studies suggest specific allelic variants of pigmentation gene (*MC1R*), aryl hydrocarbon receptor gene (*AHR*), basonuclin 2 gene (*BCN2*), type-1 collagen alpha-2 gene (*Col1A2*) or SNPs within or near the DIAPH2, KCND2 and EDEM1 loci all appear to correlate with both intrinsic and extrinsic skin aging phenotypes and/or youthful skin appearance.

Of all these identified genes and their allelic variants, the biology of *MC1R* gene has received perhaps the most recent attention due to its central role in modulating human (and murine) skin pigmentation systems, the clinical influence of which led to the categorization of Fitzpatrick Skin Phototypes. *MC1R* signaling is associated with both skin cancer and skin aging via its mixed role in UV induced PS in melanocytes and promotion of efficient DNA damage repair [120]. Genetic variants of *MC1R* (coding for G protein-coupled transmembrane melanocortin receptor-1 on melanocytes) are strongly linked to increased risk of both NMSC and melanoma in both red and brown Caucasian phototype cohorts [121]. Meta-analysis of several GWAS studies demonstrated SNPs in or near *MC1R* (and SLC45A2 and IRF4) correlated with different skin aging phenotypes using a skin surface topographic scoring system of solar elastosis [116] and the *MC1R* gene may also affect inflammaging via generation of ROS independent of its function in melanin production [122].

Gene expression studies of the skin aging phenotype have revealed several important observations about the complexities of distinguishing intrinsic from extrinsic mechanisms, as they appear to overlap in many important ways. In human skin, gene profiling and transcriptomic analyses [115, 123–128] have identified thousands of upregulated and downregulated genes in old vs. young and intrinsically aged vs. extrinsically aged (photoaged) skin. One transcriptomic study showed genes associated

with mitigating oxidative stress, control of lipid synthesis, and epidermal differentiation were all downregulated in both exposed and photo-protected skin, whereas, elastin expression was increased in exposed skin (consistent with formation of solar elastosis), and interstitial collagen expression decreased in sun protected skin (consistent with intrinsic aging) [127]. Similarly, confirming histologic studies, expression profiling of human aging that spanned subjects between the ages of 24–70 years demonstrated younger-appearing skin upregulated expression of the *LAMA5* gene (DEJ component) and epidermal cell-cell adhesion complex (desmosomal) genes *DSC3* and *CDH1* [126].

Race, sex, and skin tone of subjects also all play a role in the genetic correlates of skin aging. The expression of some aging related genes was found to be sex-dependent in a Caucasian sample [129], and studies in a Han Chinese sample showed distinct genetic variants and phenotypes from that in a Caucasian population [118]. These discrete expression patterns further highlight the complexity of cataloging aging mechanisms in the skin and suggest that much more information would be required from a wider diversity of samples to understand any potential global age-related changes.

Altogether, genetic factors, including telomere DNA loss, genotoxic accumulation of mutations in both genomic and mtDNA, DDR signaling, DNA repair dysfunction, and allelic variations in key cutaneous protective genes controlling pigmentation, inflammation, dermal, epidermal, and subcutaneous physiology all converge on our emerging understanding of the central role cellular senescence plays in skin aging. What follows is a review of how epigenetic factors also influence cellular senescence in cutaneous biology and the aging phenotype.

4. Epigenetic influences on cutaneous cellular senescence

Though the evolution to senescence is usually characterized as a genomically driven phenotype, its manifestation can be largely characterized as an epigenetically entrenched state. The baseline definition of a senescent cell is an otherwise mitotic cell that has entered permanent cell cycle arrest, but this also begets a broader shift in cell behavior and protein production. For these changes to be permanent they must be encoded in long-term gene expression tendencies, i.e., in the cellular epigenome. The epigenome is the composite architecture consisting of chemical and physical modifications to the DNA that do not alter the underlying coding and noncoding sequences but instead modify its oligomeric structure and transcription. These modifications span multiple layers from the local control of specific gene promoters to large scale regulation of entire domains of genes.

Canonically, the epigenome is most strongly associated with cell identity, as it makes accessible the portions of genomic DNA needed for the cell's functional role while segregating and silencing irrelevant regions. Thus, the most dramatic epigenetic shifts are observed when cells differentiate from stem or progenitor states. In this full and dramatic state transition, the function of the cell is redefined, affecting everything from its morphology to its protein production and factor secretion [130]. For skin this can mean, for instance, a transiently amplifying cell in the basal epidermis fully differentiating to a corneocyte through natural turnover or endothelial progenitor cells differentiating into new endothelium in response to an angiogenic signal. Conversely, cells undergo constant but more minor epigenetic events as they are exposed to regular stimuli from the environment, which can lead to upregulation or downregulation of certain behaviors [131]. For instance, methylation sequencing of the same cell type across patients (e.g., epidermal keratinocytes) will show a distribution with perturbation on the mean

population value based on internal and local external stimuli [132]. This heterogeneity evolves with different stressors, and with aging itself becomes more prominent. Eventually the stressors can lead to enough diversity to characterize pseudostates and pseudostate transitions that may by-and-large retain the cell's identity but with a different grade of functionality across multiple genes. We will discuss a few additional examples of this in the following sections, like fibrotic versions of connective tissue cells and pro-/anti-inflammatory versions of macrophages; however, the focus of this section that could also be considered an epigenetic pseudostate is senescence. It meets the criteria in that it still retains core cell identity but also dramatically affects a multitude of genes to alter protein production and secretion profile, and thus requires a core epigenetic component. In this section we will review the multitude of epigenetic changes that accompanies this pseudostate evolution in skin cells, what role they play in establishing the senescent phenotype and how they may potentially be engaged therapeutically.

4.1 Sequence specific modulation

One modality of epigenetic is sequence specific meaning it targets specific regions of the genetic code—either DNA or RNA. DNA base pair methylation is a well-known example of this modality. Cytosine is the most commonly methylated base in eukaryotic cells and when methylated, often serves to block the activity of RNA polymerase, as in the context of CpG islands. Found in the promoter region of many genes, CpG islands are clusters of methylated cytosine followed by guanine, wherein the methylation inhibits (silences) the transcription of that gene [132]. Widespread hypomethylation has been documented in aging and senescent fibroblasts, and in some cases, impairs cell cycling pathways through the suppression of cyclin pathways. Specifically, a lack of methylated sites leads to the upregulation of *p16INK4a* which inhibits cyclin D/CDK4 to suppress G1 phase progression, while upregulation of *p14ARF* leads to activation of p53/p21 and inhibits cyclin E/CDK4 to prevent S phase progression [7]. The global methylation status of fibroblasts is directly and strongly correlated with donor chronological age through regression algorithms known as 'epigenetic clocks.' These algorithms calculate a weighted linear combination of the beta coefficients (the percent signal from the methylated out of the total unmethylated and methylated alleles) [133]. When dermal fibroblasts were passaged towards replicative senescence (RS) these epigenetic clocks show aggregated methylomic evolution. The cell cycle was reengaged by overexpressing the telomerase gene *hTERT*, causing cells to progress to further doubling. However, the epigenetic clock did not reverse and the cells continued to age, bypassing RS, further hinting that a broader epigenetic change was occurring through the progression to senescence rather than just the suppression of a few mitotic arrest genes [134].

Conversely, sequence specific epigenetic regulation on the level of transcribed RNA is accomplished through feedback mechanisms by families of non-coding RNA-including microRNAs, siRNAs, long and short non-coding RNAs, and others. These non-coding RNAs will interact with other DNA, RNA, and proteins to regulate their expression, further enhancing the complexity of the transcriptome over the more rigid landscape of the methylome [135]. Thus, non-coding species are often used to not just reinforce but also propagate the senescence response. The particular influence of miRNAs, short sequences that complement and bind to specific regions of mRNAs to limit their stability and thus their translation likelihood, has been explored in the context of cutaneous cell senescence [136]. For instance, UV-induced senescent fibroblasts are known to produce miR-34 which targets a number of transcripts within these cells for cell cycle regulatory genes like *MYC* and *BCL2* as well as genes for other epigenetic factors such as E2H and SIRT1 [137].

Meanwhile, in wounding-induced senescence the extracellular secretion of miR-21 as part of the SASP phenotype triggers the activation of resident macrophages to drive the local inflammatory response [138], but these represent only a few of a handful of drivers. Senescent keratinocytes, for instance, have displayed upregulation of over a hundred different microRNAs correlated with expression of the senescence biomarkers p16, p53, and senescence-associated β-galactosidase (SA-β-Gal) [136]. Together, these mechanisms represent the precise regulation of specific genomic targets and interfering with transcription machinery as one mode of enforcing the senescent epigenetic state.

4.2 Compaction

For regulation across gene domains (~150 base pairs or greater), the epigenome uses methods of physical compaction to close off regions of the genome from transcription. The negative charge of the DNA attracts it to wrap around the positively charged protein octamer spools called histones, which segregate the sequences away from transcription machinery. Chemical modifications like methylation, acetylation, and ubiquitination of the amino acid residues on the tails of these histone proteins alter the charge interaction with DNA and with other histones influencing oligomeric structure [139]. Senescence engages in this mechanism by modulating the enzymatic activity that regulates these histone tails. For example, the activity of methyltransferases like EZH2, which adds trimethylation to the lysine residue 27 of histone 3 (H2K27me3), is reduced in senescent cells. This reduction in the resulting H3K27me3, especially at the *INK4a/ARF* locus mentioned previously, reinforces the discontinuation of the cell cycle [8]. Other forms of histone tail modification include acetylation, which tends to promote more transcription. One of the most well-studied classes of deacetylation enzymes is the sirtuin family of proteins. In both fibroblasts and keratinocytes, Sirt1 and Sirt6 directly respond to DNA damage and inflammation, but their expression is diminished in senescent cells [140]. Interestingly, both Sirt1 and Sirt6 also play an active role in regulating collagen balance, thus their downregulation could be conceptually likened to senescence of the dermal ECM and its turnover, just like that of cellular turnover.

Histones can also be modified through changes within the core octamer proteins themselves and a hallmark example of this phenomenon is the variant species of the H2A protein known as H2A.J. This modified protein is prevalent in a lot of senescent skin cell types where it weakens the binding of another histone in the complex, H1, triggering a signaling cascade that preempts the interferon response and contributes to initiation of SASP expression [141]. In senescent epidermal keratinocytes in particular, the increase in H2A.J variants is correlated with arrested cell cycle and maturation of the basal cells into mature corneocytes, thus it may play a direct role in the morphologic phenomena of epidermal thinning seen with age [142]. The broader contribution of these histone changes, along with local DNA methylation shifts, is the transition to wide-reaching genome compaction in senescent cells, for example the condensation of senescence associated heterochromatin foci, as in H3K9me3 rich regions of nuclease resistant compact facultative heterochromatin [11]. These foci are seen across skin cell types like fibroblasts and keratinocytes and are thought to entrench the senescent state by long term segregation and silencing of mitotic genes [143]. However, the evolution of these foci seems to be specific to the type of senescence induction, most prominent in OIS, suggesting that senescence itself may even be a family of pseudo-states rather than a distinct, singular manifestation [144]. Nevertheless, in general, these forms of epigenetic modification which bias entire regions of genes from active to passive and vice versa truly embody a cell state/pseudostate.

4.3 Alternative epigenetic pseudostates

The natural and prevalent engagement of senescence, even in young tissues, reflects its role as a form of stress response. In fact, a major function of senescence is to prevent the evolution of alternate, more detrimental states of the cells and tissue under these conditions. One such competing epigenetic pseudostate is fibrosis. The fibrotic transition is a common feature in the pathological evolution of many tissues, i.e., hypertrophic scarring and keloids in the skin, idiopathic pulmonary fibrosis in the lung, cirrhosis in the liver [145]. A key component of fibrosis is the differentiation of various cell types including fibroblasts, adipocytes, epithelial cells, and endothelial cells into a population known as myofibroblasts [146]. As mentioned, differentiation is canonically an epigenetic event as cells convert and specify their functional gene regions while silencing other unused regions. It involves the same modalities of control—methylation, histone tags, chromatin structure, etc.—often with more dramatic and permanent modifications. These activated myofibroblasts are critical for the repair response in that they secrete superfluous extracellular matrix (ECM) components (Collagen 1, alpha-smooth muscle actin (α-SMA), fibronectin, etc) that accumulate in the connective tissue [147]. At the same time, these cells diminish the process of anabolic degradation of ECM through reduction of MMPs [148]. Unbridled overgrowth of these myofibroblasts, as evidenced by the overactivation of growth factors like connective tissue growth factor (CTGF), leads to the buildup and disorganization of the connective tissue [149]. Senescence in this context is thought to be a responsive, secondary epigenetic evolution that is engaged to shut down this population and stop the overgrowth [150]. These processes—from the epigenetic cell identity shift (e.g., epithelial-to-mesenchymal or fibroblast-to-myofibroblast transitions, depending on the starting cell types) to the epigenetic proliferation-suppressed state (induction of senescence)—represent relatively fast epigenetic turnovers. As such, a key mediator of this rapid transition is thought to be the slew of non-coding RNAs, like let-7 g to engage TGFbeta driven myoblast conversion and miR-127-3p to induce p53/p21 drivers of senescence [151, 152].

Another alternative pseudostate that competes with senescence is of course cancer and more particularly for skin, melanoma. Like senescence, cancer is a state transition that involves bypassing apoptotic pathways, yet these aberrant cells also bypass the suppression of their cell cycle gene networks [14]. It is thought that melanoma cells are able to undo the senescence epigenetics and re-engage the cell cycle due to the deleterious recruitment of epigenetic enzymes, like histone demethylases and Jumonji proteins [153]. This means a host of pathways whose methylation would otherwise lead to cell cycle suppression, like the p15INK4B or the p27Kip1 pathways, are methylated without cell cycle arrest in melanoma [154–156]. The use of inhibitors to target these epigenetic enzymes seems to be a promising methodology to restore the cell cycle arrest and control the cancerous growth [157].

An interesting intersection of epigenetic and oncogenic pseudostates is highlighted in Merkel cell carcinoma (MCC). This aggressive, non-melanoma skin cancer is rare but occurs primarily in the elderly and immunosuppressed. Interestingly, methylation clock analysis of MCC cells shows their epigenetic age as significantly younger than the chronologic age of the patients from which they were derived—a stark contrast from the continually progressing epigenetic age of senescent cells. Further analysis of these MCC cells did not indicate any signs of pluripotency [64]. The mechanism by which MCCs reverse their epigenetic age is still unknown, however, it may be related to other epigenetic alterations recently discovered in this cell type, including decreased H3K27me3 expression [158, 159] and overactivity of the

lysine-specific histone demethylase 1A [160, 161]. These are just some examples of this fundamental need to tightly control and disengage mitotic networks and why senescence requires a complex regulatory architecture like the epigenome.

4.4 Enablers of senescence

The phenomenon of senescence is promoted by the epigenetics of not just the arrested cells in question, but also that of the other resident cells that enable this transition. Though senescence is thought to be a permanent state, the persistence of senescence in the tissue is only meant to be transient. This is because the key function of this state is to respond to stressors by retaining cells, despite their damage, to maintain the tissue temporarily while preventing them going down the more detrimental alternate routes mentioned, all the while signaling the immune system and other repair mechanisms. When the immune system is young and efficient its cells are recruited to the skin and other tissues to clear out the senescent cells [162]. With aging, however, the number and lifetime of these senescent populations increases due to the altered epigenetic pseudostates of the senescent clearing cells as well, contributing to innate immunosurveillance dysfunction of the skin. One example of this is in the dominance of the pro-inflammatory M1 macrophage pseudostate over the anti-inflammatory M2 macrophage pseudostate [163]. There are a number of histone methylation and acetylation modifiers that play a role in pseudostate fate decision, for instance histone deacetylase 3 promoting M1 macrophages or the SYMD family of methyltransferases promoting M2 macrophages [164]. With the accumulation of stressors over a lifetime, the more pro-inflammatory epigenetic pseudo-states are favored in skin and other tissues, especially in response to factors like SASP or inflammaging [165]. In addition, in some disease states like type 2 diabetes, the wound healing response and inflammation tends to exaggerate the M1 state response with focal DNA methylation components at sites like peroxisome proliferator activated receptor gamma (PPARγ) or and elevation of miR-125b [164]. This epigenetic shift in the balance of macrophage cells then ties back to senescence as the M1 macrophage predominantly engages in more phagocytic clearance of foreign pathogens, while the M2 macrophages carry out more phagocytic clearance of damaged host cells (efferocytosis) [166]. This, coupled with the fact that senescent cells develop ways to better evade apoptosis, means that they are more likely to accumulate [167] in aged tissue. There are additional immune cell types that are similarly driven by the pro-inflammatory transition, yet become impaired at senescent cell clearance, including NK cells and neutrophils [167]. Altogether, this epigenetic evolution of the regulator cells, part of inflammaging, proves just as critical to the manifestation of a sustained senescence pressure in cutaneous tissue as epigenetic changes engaged in the non-dividing cells themselves.

4.4.1 Distinction from temporary cell cycle arrest

Though sometimes associated with senescence, somatic stem cells (as opposed to differentiated cells) are typically associated with another form of cell cycle arrest, known as quiescence. Because their role is to remain as a niched tissue reserve, they often enter periods of temporary cell cycle arrest with a prolonged G0, instead of a permanent one, until they are called to activate, to proliferate and differentiate, by a stressor [168]. One major epigenetic distinction that enables this temporary quiescence vs. permanent senescence is the utilization of bivalent domains. These are regions of genes that are regulated by both a repressive histone tag as well as an activating one, that allows the region to rapidly switch from one state to another depending on stimulus [169]. A prime example of the use of this

is in the coinciding utilization of repressive H3K27me3 and activating H3K4me3, which maintains a tenuous baseline suppression of the gene region. This pair forming a bivalent domain is widely used throughout the embryonic stem cell genome, establishing its broad potency as a cell type with the potential to express a lot of different proteins [170]. But when the same domains were searched for in dermal hair follicle stem cells (HFSC), they were found to be substantially restricted to lineage-specific factors like *Sox9* and *Nfatc1* and growth factor *FGF18* [171]. Then, when these HFSC were stimulated to activate, many of the genes with H3K4me3 activating markers, which are located primarily near the gene promoters, were further reinforced by additional H3K79 dimethylation in the gene body, to tip the scale from suppression to activation [171]. These genes included many cell cycle regulators which, when combined with the cell lineage factors, properly executed differentiation. Thus, this mechanism of readily switchable suppression to expression establishes a major distinction in epigenetic regulation from cell cycle in quiescence from that of senescence where the cell cycle genes are more permanently, epigenetically suppressed.

4.4.2 Manipulability of senescent epigenetics

Earlier, we mentioned how drugs targeting epigenetic enzymes represent one methodology for modulating some of the epigenetic changes that drive senescence, such as senolytic therapies. However, a broader and more dramatic approach of epigenetic evolution is through the process of cellular reprogramming. This technology was inspired by the core epigenetic reset that occurs during the process of reproduction in which sperm and egg, two cells with very precise roles and epigenetic identities, are reprogrammed to make embryonic cells—epigenetically plastic cells that can differentiate into any cell in the body. The isolation and recapitulation of this process in any desired cell type was achieved through the discovery of core transcription factors [172]. When overexpressed in cells, this set of core transcription factors would drive a full epigenetic remodeling to produce embryonic-like cells with all their differentiation potential. This process is called induced pluripotent stem cell reprogramming (iPSC) and has been utilized in a variety of different cell types with dermal fibroblasts being the gold standard for many studies [173]. Even fully senescent fibroblast populations established from 51 population doublings and maintained for two months in culture, successfully showed iPSC reprogramming, as evidenced by revived proliferation, reduced p16 and p21, and re-differentiation after reaching the pluripotent state [174]. Crucially, the re-differentiated progeny were once again able to be passaged into senescence, thus suggesting that malignant transformation was not induced during the entire process. Furthermore, one of the key reprogramming factors *Oct4*, has been shown to independently re-engage senescent hair follicle mesenchymal stem cells back into cycling by engaging a host of DNA methyltransferase to inhibit the p21 pathway [175]. More recently researchers have shown that the prevalence of senescence in a population can be reduced with even a transient application of the reprogramming factors [176–178]. Though whether this means a re-engagement of senescent cells in the cell cycle or simply competitive growth advantage of healthy cells remains to be seen. This represents an enticing new possibility in that epigenetic manipulation may possibly counter the accumulation of senescent cells in many aged and diseased tissues, including the skin.

5. Conclusion

The skin represents an excellent organ system in which the effects of cellular senescence manifest as observed clinical changes in organismal health and disease.

A myriad of processes drives the genomic erosion that instigates the transition to senescence. Some of these processes are more stereotyped, engineered into the cell by design, and are observed in chronologically aged skin, while others are stochastic and driven by environmental conditions, exemplified by exposomal damage. Either way, the result is an evolution of the entire state of the cell. This means more than just the direct arrestation of the cell cycle, but also entails changes through the cellular transcriptome, proteome, and secretome as encoded by alterations to the core cellular epigenome. This also involves a myriad of changes to the many layers of architecture that encode a cell's function and identity. The global process is critical for the skin's ability to retain functional integrity upon stress and insult as the first line of defense for the body, and in many ways, senescence represents the least of multiple evils.

This review gives a glimpse of how and why intrinsic and extrinsic factors trigger cutaneous cellular senescent phenotypes, leaving several important questions unanswered. For example, which genetic and epigenetic factors determine the dominant decision pathways favoring senescence vs. apoptosis or any other disease states for different skin compartments? How are the various types of senescence manifestations comparable in terms of evolution and manipulability? What are the molecular and cellular consequences of therapeutic re-engagement of senescent cells into the cell cycle? As the focus on aging grows as an ever more prominent factor in clinical and investigative dermatology, insights on these questions into the nature of senescence become a critical step towards both dermatologic therapeutic advancement specifically and translational medicine in general.

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Conflict of interest

The authors declare no conflict of interest.

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