Faces of cellular senescence in skin aging

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ABSTRACT

The skin is comprised of diverse cell types with different proliferative capacities. Skin aging occurs with chronological age and upon exposure to extrinsic factors such as photodamage. During aging, senescent cells accumulate in different compartments of the human skin, leading to impaired skin physiology. Diverse skin cell types may respond differently to senescence-inducing stimuli and it is not clear how this results in aging-associated skin phenotypes and pathologies. This review aims to examine and provide an overview of current evidence of cellular senescence in the skin. We will focus on cellular characteristics and behaviour of different skin cell types undergoing senescence in the epidermis and dermis, with a particular focus on the complex interplay between mitochondrial dysfunction, autophagy and DNA damage pathways. We will also examine how the dermis and epidermis cope with the accumulation of DNA damage during aging.

1. Skin aging and senescence

The skin is comprised of as many as 20 different cell types with different proliferative capacities. During aging, irreversibly growth arrested, senescent cells accumulate in different compartments of the human skin, leading to impaired skin physiology. Currently, we do not fully understand how different skin cell types respond to senescence-inducing stimuli, and how this results in the various aging-associated skin phenotypes and pathologies. Here, we summarise the potential role(s) of cellular senescence during skin aging, with a focus on the factors that trigger senescence and the cellular characteristics of different skin cell types undergoing senescence in the epidermis and dermis.

2. Skin aging

The skin is part of the integumentary system; it plays major roles in thermoregulation and provides the first barrier against environmental insults and pathogens. It is the largest organ in the human body, consisting of multiple compartments and approximately 20 cell types that contribute to skin function and stratification (Blanpain and Fuchs, 2006). The epidermis is the outermost layer of the skin and consists of 5 sublayers, namely stratum basale, spinosum, granulosum, lucidum and corneum (Sotiropoulou and Blanpain, 2012). Keratinocyte stem cells residing in the basal layer undergo self-renewal and differentiate upwards to form the upper suprabasal layers, ending in the stratum corneum, where anucleated, dead cells are constantly being sloughed off. Melanocytes are found in the basal layer of the epidermis; they rarely proliferate but produce melanin in specialized melanosomes, which are transferred to their neighbouring keratinocytes (Hearing, 2005). The basement membrane separates the epidermis from its underlying dermis. The dermis is supplied by a network of blood vessels, nerve endings and is home to appendages such as sebaceous glands, sweat glands, sensory nerves and hair follicles. Fibroblasts constitute the main cell type within the dermis and are responsible for the production of extracellular matrix (ECM) components that confer structural integrity, resilience and elasticity to the skin.

Aging of the skin is characterised by slower regeneration and eventual erosion of skin structure and functionality. Skin aging is associated with a compromised protective role – specifically impaired wound healing and barrier function, increased inflammation, impaired water and thermal homeostasis for the organism, and susceptibility to various skin disorders including cancer (Velarde and Demaria, 2016).

Skin aging is a multifactorial process. It can be attributed to intrinsic (genetically determined and age-related) factors and extrinsic causes. Intrinsic aging is most prominent in sun-protected skin, manifesting clinically as fine lines, dry skin, altered pigmentation and loss of elasticity. Biologically, slower regeneration of the epidermis due to reduced proliferative capacity of keratinocyte stem cells and depletion of epidermal stem cell reservoirs contribute to chronological skin thinning. Women undergoing menopause may experience accelerated aging of the skin due to declining estrogen levels, which greatly influence skin...
appearance (Farage et al., 2013). Flattening of the epidermal/dermal interface renders the epidermis more fragile to shear forces and reduces nutrient flux between the epidermis and dermis which in turn may negatively impact keratinocyte proliferation (Lavker et al., 1989). Melanocyte number and/or activity are altered, giving rise to hypo- and hyperpigmented lesions in aged skin (Ortonne, 1990a). The dermis experiences a loss of volume and thickness in the elderly. Decreased collagen production and altered assembly of the elastin network result in loss of elasticity and favours wrinkle formation in the aged skin (Fig. 1) (Farage et al., 2013; Lavker et al., 1989).

The skin is constantly subjected to environmental insults and pollutants, with ultraviolet radiation (UVR) from the sun being the most damaging. As such, the effects of extrinsic aging are often compounded upon those resulting from intrinsic factors. Chronic exposure to UVR induces accelerated skin aging and results in photoaging (dermaheliosis) and increased photocarcinogenesis (Panich et al., 2016). Photoaged skin appears rough and dry with deep wrinkles and is characterised by the appearance of visible blood vessels (telangiectasia), mottled pigmentation (actinic lentigines / age spots, ephelides / freckles) and decreased skin elasticity (Chung et al., 2003; Fitsiou et al., 2020; Gosain and DiPietro, 2004; Ortonne, 1990b; Rittie and Fisher, 2015). Photodamage is characterised by disorganization of the dermal ECM, in terms of hypocollagenesis, the extent of which correlates with the severity of photodamage; and the accumulation of amorphous elastin fibers (Quan and Fisher, 2015). As a result of these intrinsic and extrinsic factors, a number of skin conditions such as dry skin, several forms of dermatitis and fungal infections are prevalent especially in the elderly. These conditions render the aging skin more prone to development of more severe issues such as chronic or slow healing wounds and skin infections among others (Kottner et al., 2015).

3. Cellular senescence

3.1. Characteristics of cellular senescence

Aging can be defined as an age-related functional decline of healthspan and physiological functions (Lopez-Otin et al., 2013). On an organisinal level, aging is the major risk factor for the development of chronic illnesses, including cardiovascular disease, diabetes and cancer. On a cellular level, Leonard Hayflick (Hayflick, 1965) first described this phenomenon in human diploid lung fibroblasts in culture. He observed that, after a finite number of population doublings, cells entered a stable growth arrest termed cellular senescence. Initially thought to be a cell culture artefact, the advent of early biomarkers to detect senescent cells in vitro and in vivo resulted in enormous progress towards our understanding of the role of cellular senescence during aging. Senescent cells exhibit several classical morphological and physiological alterations including a flattened and enlarged appearance, senescence-associated-β-galactosidase (SA-β-gal) activity (Dimri et al., 1995), chromatin and epigenetic modifications (Rodier and Campisi, 2011; Zhang et al., 2007), and resistance to mitogenic (Mathon and Lloyd, 2001) or apoptotic signals (Wang, 1995). In addition, senescent cells secrete a number of long-range acting molecules, including chemokines (GRO-α), inflammatory cytokines (IL-1, IL-6, IL-8), matrix metalloproteinases (MMP-3, MMP-1, MMP-9) and growth factors (HGF, IGFBPs), collectively referred to as the senescence-associated secretory phenotype (SASP) (Acosta et al., 2013; Coppe et al., 2008; Freund et al., 2010).
Senescence can be beneficial during normal development and physiology where it is necessary for embryonic growth and patterning (Munoz-Espin et al., 2013; Storer et al., 2013) and wound healing (Demaria et al., 2014; Jun and Lau, 2010). Importantly, senescence is an intrinsic tumor-suppressive mechanism that limits proliferation of preneoplastic cells with irreparable DNA damage (Collado and Serrano, 2010). However, senescence is a double-edged sword. Far from being silent bystanders, senescent cells alter tissue homeostasis, remain metabolically active, secrete a SASP and may induce senescence in their neighbouring cells in vitro and in vivo (da Silva et al., 2019). This process can occur via diverse mechanisms, including SASP factors, transfer of microRNAs via extracellular vesicles such as exosomes, or by intercellular protein transfer using cytoplasmic bridges (Biran et al., 2015; Terlecki-Zaniewicz et al., 2018). Accumulation of dysfunctional, senescent cells therefore has deleterious effects in aging tissues due to their decreased ability to contribute to tissue repair and regeneration, resulting in compromised tissue homeostasis, integrity and function. A chronic state of tissue inflammation or inflammaging resulting from elevated inflammatory signalling enabled by the SASP of senescent cells may contribute to increased susceptibility to various cancers and increased likelihood of metastases to distal locations (Franceschi and Campisi, 2014).

### 3.2. Biomarkers of cellular senescence

Detection of SA-β-gal activity remains the gold standard to identify senescent cells. However, it poses a number of challenges and technical hurdles such as the limited ability to detect enzymatic SA-β-gal activity in fixed samples and non-specific activity in certain cell types and tissue compartments (Dimri et al., 1995; Krishna et al., 1999; Lee et al., 2006). A host of other senescence biomarkers have emerged in recent years, for example activation of the p53/p21 and p16/pRb pathways, decreased levels of the non-histone nuclear protein HMGB1 and nuclear envelope protein lamin B1, and chromatin rearrangements (Wang and Dreesen, 2018). Despite intense research efforts, the detection of senescent cells in vivo remains challenging and the search for biomarkers that unambiguously identify senescent cells is an ongoing quest.

Senescent skin cells have been reported with age and in various age-related skin pathologies such as diabetic and chronic wounds (Mendez et al., 1998; Wilkinson et al., 2019), hyperpigmented lesions (Yoon et al., 2018) and psoriasis (Chaturvedi et al., 2003; Qin et al., 2002). A variety of extraneous stress signals including photodamage and oncogene expression can activate the senescence program in skin. Based on biomarkers such as p16 expression, loss of lamin B1, HMGB1 translocation, nuclear and mtDNA alterations, senescence has been observed in diverse cell types in the dermal and epidermal compartments both in vitro and in vivo (Wang and Dreesen, 2018). Dermal fibroblasts undergoing replicative senescence or upon UVR exposure display elevated SA-β-gal staining, p16 or p21 expression and loss of lamin B1 (Campisi, 1996; Chen et al., 2008; Freund et al., 2012; Munro et al., 2001; Shimi et al., 2011; Wang et al., 2017). Keratinocytes with loss of lamin B1 expression have been observed in chronologically aged skin and in murine keratinocytes upon UVR exposure (Dreesen et al., 2013; Wang et al., 2017). Increased p16 (and p21) staining has been shown in the basal layer of human epidermis (Hickson et al., 2019; Ressler et al., 2006; Vicorelli et al., 2019) and in ‘biologically aged’ skin (Waijler et al., 2012). HMGB1 translocation into the cytoplasm has also been previously reported in human and mouse epidermis (Johnson et al., 2013; Vicorelli et al., 2019). In addition, melanocytes with activating mutations in N-RAS or its downstream target BRAF undergo oncogene-induced senescence (OIS) as judged by lamin B1 and p16 staining (Gray-Schopfer et al., 2006; Ivanov et al., 2013; Michaloglou et al., 2005).

One caveat with most existing biomarkers is their non-exclusive nature to cellular senescence. For example, not all forms of senescence are dependent on p53/p21 and p16 activity (Mirzayans et al., 2012; Prieur et al., 2011). Induction of p53 and p21 also occurs upon DNA damage in cells undergoing transient cell cycle arrest (Karimian et al., 2016; Lakin and Jacob, 1999). Similarly, p16 can be expressed in non-senescent cells, for example during differentiation of melanocytes and in cancer cells with pRb inactivation or p16 mutations (Al Dhaybi et al., 2011; Romagosa et al., 2011; Shapiro et al., 1995). HMGB1 is actively exported from nuclei of senescent cells (Davalos et al., 2013), however it is also secreted during tissue inflammation (Bertheloot and Lutz, 2017). Therefore, it is prudent to use a combination of these markers for a more accurate interpretation of the biological context(s). Fundamentally, these challenges highlight the need to identify senescence markers that can be detected non-invasively. The utility of Raman spectroscopy in skin aging for example, is a promising avenue although more efforts are needed to distinguish the various cell types within different skin compartments, and to ensure applicability in a clinical setting (Liendi et al., 2020).

The murine model has been an invaluable resource in biological research. However, there are physiological differences between human and mouse, as such, they do not completely mirror the pathogenesis of certain human conditions including wound healing and skin repair (Zomer and Trentin, 2018). Human and mouse skin differ in organization and histological features (Fig. 2). While having similar layers of cells as mouse skin, human skin is considerably thicker (100 μm), firmer and adheres to the underlying tissue structure. Gender-specific differences in mouse skin are also present (Zomer and Trentin, 2018). Moreover, in contrast to human, mouse interfollicular melanocytes disappear shortly after birth, whilst a pool of melanocytes is retained within the hair follicle (Chou et al., 2013; Hirobe, 1984). These differences need to be taken into consideration when interpreting and translating data between human and mice. Likewise, careful interpretation is needed regarding cellular senescence in studies using murine cells as a model of human senescence, as there are fundamental differences between human and mouse, particularly in terms of telomere-dependent replicative senescence and oxygen sensitivity (Coppe et al., 2010; Itahana et al., 2004).

Despite their limitations, senescence biomarkers paved the way to detect senescent cells in vivo, and more importantly, demonstrate that they accumulate in aging tissues, preneoplastic lesions and in chronic diseases, including atherosclerosis, diabetes etc., highlighting an association between senescence, chronological aging and age-related pathologies (Lopez-Otin et al., 2013). Studies of human premature aging syndromes and transgenic aging mouse models have been instrumental in allowing the systematic identification and characterisation of senescent cells in many age-associated diseases (Carrero et al., 2016; Kudlow et al., 2007). Together with the development of genetic strategies and drug-based approaches to selectively eliminate senescent cells, these advances established causal links between cellular senescence, organisinal aging and the development of age-related pathologies (Baker et al., 2016, 2011; Chang et al., 2016; Childs et al., 2015; Ovadya and Krizhanovsky, 2018; Yosef et al., 2016).

### 4. Cellular senescence in the skin

The skin houses diverse cell types with different functions and proliferative capacities (Blanpain and Fuchs, 2006). Senescent cells accumulate with age in the skin, leading to a decline in various aspect of skin function (Wang and Dreesen, 2018). Currently, there is a gap in our understanding of how the diverse skin cell types respond to senescence-inducing stimuli, and how this, in turn results in age-associated skin changes. Despite significant advances in the understanding of senescence and its roles in normal and pathological skin conditions, it remains unclear whether senescent skin cell types display cell-type (or cell state) specific differences in the transcriptome, and particularly in their proteome. This has important implications on how senescence in a particular cell type affects another, and how this contributes to, or causes the various age-related skin phenotypes.
The following section will examine skin aging in detail, particularly summarising differences in skin cell senescence in the epidermis and the underlying dermis, as well as how different skin cell types respond to senescence-inducing stimuli such oxidative or genotoxic stress during photoaging.

4.1. Dermis

Dermal fibroblasts are the most abundant cell type in the dermis and are responsible for organizing the extracellular matrix. Fibroblast senescence has been extensively studied as this has been the classic cell type used in seminal in vitro senescence studies. Most of these studies induced senescence via replicative or stress/oncogene-induced protocols using the classic molecular senescence markers such as an enlarged and flattened morphology, permanent cell cycle arrest, and acquisition of a classical SASP (Coppe et al., 2008). Chromatin modifications including ‘DNA segments with chromatin alterations reinforcing senescence’ or DNA-SCARs which are persistent nuclear foci with PML/53BP1 have been described in senescent human cells and mouse tissues and are thought to play a role in maintaining the SASP (Rodier et al., 2011).

Recently, an alternate form of senescence driven by loss of mitochondrial function has been described in human fibroblasts (Wiley et al., 2016). Specifically, the depletion of mitochondrial sirtuins led to the mitochondrial-dysfunction-associated senescence (miDAS) phenotype characterised by lower NAD+/NADH ratios and a distinct SASP that was independent of the IL-1 pathway. Additionally, the miDAS SASP has been shown to promote keratinocyte differentiation as conditioned media from fibroblasts undergoing miDAS enhanced differentiation based on transglutaminase (TGM1) and loricrin (LOR) expression (Wiley et al., 2016). In addition to the classical SASP components, Narzt et al. have also identified oxidized bioactive lipids as novel SASP factors in dermal fibroblasts induced to senesce via replicative or stress protocols (Narzt et al., 2020). These elevated lysophospholipid species are pro-inflammatory and modulate TLR2/CD36 signalling and phagocytic activity in macrophages, suggesting that alterations in the epilipidome may play a role in facilitating immune evasion and contributing to age-associated skin inflammaging (Narzt et al., 2020). These pleiotropic characteristics possibly reflect diverse molecular pathways involved in triggering or maintaining different facets of cellular senescence arising from different stress signals. It remains to be elucidated whether changes, including altered NAD+/NADH ratios or levels of oxidized lipids are molecular features induced by in vitro protocols or represent a universal characteristic of senescent cell types in situ.

Having said that, there are noteworthy differences between senescent fibroblasts in vitro versus in vivo. For instance, Waldera Lupa et al. reported that intrinsically (or in vivo) aged dermal fibroblasts exhibit an age-specific Skin Aging Associated Secretion Proteome, or SAASP, that is distinct from the originally characterized SASP (Waldera Lupa et al., 2015). Formation of nuclear foci reminiscent of DNA-SCAR was observed in intrinsically aged fibroblasts. However these foci did not correlate with increased DNA double strand breaks or telomere attrition, but displayed an association with SAASP development. Analysis of biological pathways between the SAASP and the canonical SASP further revealed common processes such as matrix degradation and proinflammatory pathways. This analysis also shed light on processes unique to the SAASP including ECM remodelling, adherens junction interactions, metabolism of carbohydrates and sphingolipids/glycosphingolipids, among others (Waldera Lupa et al., 2015). An earlier study by the same group also revealed that age-associated protein abundance changes are not correlated to the transcriptome in primary dermal fibroblasts across different age groups, suggesting that post-transcriptional mechanisms contribute to age-related changes in protein levels (Waldera-Lupa et al., 2014).

4.2. Epidermis

The epidermis forms the first barrier of defense against environmental insults, pathogens as well as mutagens including UVR. Keratinocytes are the most abundant cell type in the human epidermis and are responsible for regeneration and stratification of the epidermis. The widely accepted model of epidermal homeostasis is that epidermal stem cells in the basal layer undergo cell division and produce transient amplifying progenitor cells that detach from the basement membrane. In
the suprabasal layer, these differentiating cells migrate towards the epidermal surface, where they terminally differentiate, become anucleated cornocytes, and form the stratum corneum. This well-defined life cycle of keratinocytes in the human skin takes place over approximately 20–28 days. Epidermal turnover over this limited time span has led the view that there is little room for the development of a lasting pool of senescent keratinocytes in the skin. Moreover, innate mechanisms such as immune clearance may limit the deleterious effects of senescent cells (Kale et al., 2020). Recently, Notch signalling has been implicated in clearance of senescent cells from the basal layer (Yoshikawa et al., 2021). Indeed, the incidence of senescent skin cells is low even when the epidermis is constantly exposed to damaging stimuli (Idda et al., 2020). Nonetheless, turnover of the epidermis slows down in aged individuals, giving rise to a thinner epidermis and overall more fragile skin in the elderly (Farage et al., 2013). At the same time, gradual decline of innate mechanisms such as the immune system provide partial explanations as to why senescent cells may accumulate in aged skin (Montecino-Rodriguez et al., 2013). While there is arguably limited evidence, we have shown that based on lamin B1 staining, senescent cells may accumulate in aged human epidermis (Dreesen et al., 2013). Consistent with our data, Victorelli et al. demonstrated loss of HMGB1 throughout the epidermis in aged skin (Victorelli et al., 2019). However, our analysis was limited to aged (above 60) and very young skin (1 year old); thus, further analysis of additional age groups is essential to provide a more comprehensive picture regarding the accumulation of lamin B1/ HMGB1-negative cells within the epidermis and dermis (Dreesen et al., 2013). In addition, it has been shown that p16- and p21-positive senescent cells accumulate in the skin of the elderly compared to that of young subjects, although the accumulation of senescent (or p16-) cells appears to be largely restricted to the basal layer and resemble melanocytes (Ressler et al., 2006; Waaijer et al., 2012). Lastly, by assessing lamin B1 levels at single cell resolution, we quantified the accumulation and clearance of senescent cells within the basal and suprabasal layer of mouse epidermis upon a chronic low exposure to UV, as well as upon skin regeneration, respectively. Due to the low dose used in these experiments, the dermis remained unaffected (Wang et al., 2017). These and other experiments highlight the utility of lamin B1 to detect senescent cells in specific cell types or tissue compartments, such as the skin or kidneys (Baar et al., 2017; Wang et al., 2017).

It should be appreciated that the epidermis is comprised of keratinocyte subpopulations with distinct differentiation status and functions (Matsui and Amagai, 2015). However, our current understanding of the characteristics and behaviours of the different keratinocyte subpopulations is still in its infancy. In order to fully characterise these distinct cell states, complementary approaches to transcriptomic profiling such as detailed analyses of the proteome, secretome and metabolome of these keratinocyte subpopulations are necessary. Importantly, their response(s) to environmental cues, and to aging may differ significantly. A multitude of models have been proposed to explain how keratinocytes are committed to differentiation including transcription factor mediated changes, differential expression of master cell cycle regulators, as well as differences in Ca²⁺ concentrations within the suprabasal layers (Quek et al., 2018; Tu and Bikle, 2013). However, what triggers this switch between proliferating basal cells and their non-proliferating, differentiating progeny, and how this process changes during aging, remains unclear.

Terminal differentiation involves permanent growth arrest and exit from the cell cycle, where the line with a senescent state is blurred. These observations raise a debatable and intriguing question whether differentiated keratinocytes share certain hallmarks of cellular senescence, or simply represent a façade of cellular senescence. Do basal keratinocytes embark on a path towards senescence as the transient amplifying progenitor cells initiate epidermal differentiation? Counter-arguments for this hypothesis are the lack of certain features of cellular senescence in differentiated cells (Gorgoulis et al., 2019). Clearly, a prerequisite to answering this question is our ability to distinguish between cellular differentiation and senescence. Data from our group showed that even in young skin, terminally differentiated cells displayed a loss of lamin B1, whilst in aged skin, these cells were present throughout the epidermis (Dreesen et al., 2013), raising the possibility that terminally differentiated cells may resemble senescent cells. In addition, it has been proposed that lamin B1 is functionally involved in triggering senescence. However, it is important to note that the absence of lamin B1 and B2 did not affect development or cell proliferation in mouse skin (Yang et al., 2011). In agreement with these findings, we demonstrated that downregulation of lamin B1 did not directly lead to senescence unless additional stressors were present, indicating that loss of lamin B1 is a consequence of senescence and other mechanism(s) are necessary to initiate senescence (Dreesen et al., 2013). Nonetheless, it is possible that late epidermal differentiation markers may overlap with senescence markers, and a better understanding of these markers and their cellular context(s) is required for us to tease apart differences or similarities between these two cell states. Taken together, a thorough characterization of the different senescence biomarkers and the identified senescent cell types within human and mouse epidermis (and dermis) is imperative to determine to what extent these markers overlap with differentiation markers, and how senescent cells contribute to overall skin aging.

4.3. Functional interaction between dermal fibroblasts and epidermis is altered during aging

Paracrine signalling between dermal fibroblasts and keratinocytes is essential for tissue homeostasis in physiological conditions and dermatological disorders (Russo et al., 2020). During aging, crosstalk between these cell populations is altered. For example, junctional structures that anchor the epidermal basal keratinocytes are lost, reducing the interaction between keratinocytes and fibroblasts, thereby impairing the proliferation of basal stem cells and rendering aged skin more fragile (Langton et al., 2016). Consistent with these changes, senescent fibroblasts do not support proper epidermal stratification of keratinocytes in 3D organotypic cell culture models (Weimullner et al., 2020).

IGF-1 secreted by dermal fibroblasts is a major regulator of keratinocyte proliferation; the effects of which are balanced by keratinocytes’ expression of several IGF binding proteins (IGFBPs) and IGF-1R (Fig. 1). The IGF-1/IGF-R1 axis is altered in geriatric skin. Specifically, lower expression of IGF-1 from senescent fibroblasts led to failure to sustain appropriate IGF-1R in keratinocytes. In response to UVB exposure, activated IGF-1R protects keratinocytes from apoptosis by invoking cellular senescence through generation of ROS and p21-dependent pathways (Lewis et al., 2008). This ensures that the skin barrier function is maintained while limiting proliferation of UV-damaged cells. Altered IGF-1/IGF-1R impairs the keratinocyte response to UVB exposure and renders keratinocytes more sensitive to UV-induced apoptosis. However, surviving keratinocytes can evade senescence and proliferate in the presence of DNA damage, contributing to the development of cellular transformation and malignancies. Importantly, ectopic supplementation with IGF-1 was able to reverse this defective response to UVB (Lewis et al., 2010).

Communication between fibroblasts and keratinocytes can be mediated not only by soluble factors. Recently, CD63-positive extracellular vesicles carrying miRNAs have also been implicated as paracrine messengers between human dermal fibroblasts and keratinocytes in 2D cell culture and 3D skin models (Takasugi, 2018; Terlecki-Zaniewicz et al., 2019). Of note, miR-23a-3-p is a mediator of senescence (Rock et al., 2015), whose expression correlates positively with age and is secreted by senescent fibroblasts (Terlecki-Zaniewicz et al., 2018). In addition, miR-23a has been shown to regulate lamin B1 mRNA stability in senescent fibroblasts (Dreesen et al., 2013).

The interaction between keratinocytes and fibroblasts also plays a key role in wound healing (Werner et al., 2007), which occurs in three overlapping phases, namely inflammation, proliferation and
remodelling and depends on a dynamic interplay between several cell types. Following hemostasis to stop the bleeding, immune cells are attracted to the wound in the early inflammatory phase by various cytokines, chemokines and growth factors secreted by endothelial cells, and cells at the wound edge. As neovascularization occurs, fibroblasts proliferate and form granulation tissue while epithelial cells migrate to close the wound. The process of re-epithelialization is induced by growth factors such as EGF, bFGF and PDGF while keratinocyte growth factor (KGF) stimulates keratinocyte proliferation (Kiritsy et al., 1993). A subset of fibroblasts transition into myofibroblasts, which play an essential role in depositing ECM, mediate wound contraction and eventually remodel the new ECM (Rodrigues et al., 2019).

Fibroblast senescence was found to be increased in venous ulcers and the incidence of which decreased upon healing of the lesions (Mendez et al., 1998; Vande Berg et al., 2005). Exposure to wound fluid induced dermal fibroblasts to switch to a synthetic mode where they deposit ECM to a matrix-degrading, senescent phenotype, exhibiting SA-β-gal activity and elevated p21 levels (Mendez et al., 1999; Telgenhoff and Shroot, 2005). However, it remains unclear, which soluble factors may mediate this switch or whether additional factors influence fibroblast senescence in the wound. As wound closure and healing is completed, it has been proposed that senescent fibroblasts are cleared by natural killer cells and macrophages (Kale et al., 2020). This parsimonious mechanism limits tissue fibrosis, in part through expression of CCN1/CYR61 and CCN2/CTGF, and promotes skin regeneration via a PDGFA-enriched SASP (Demaria et al., 2014; Jun and Lau, 2010). Consistent with this data, Demaria and colleagues observed excessive fibrosis along with delayed wound closure in mice in which p16-positive senescent cells were ablated (Demaria et al., 2014). The benefits of cellular senescence in wound healing relies on rapid clearance of senescent cells upon wound closure. With advancing age, and possibly due to reduced immune clearance, senescent fibroblasts persist within the dermis, resulting in chronic inflammation that may impede wound healing in aged skin (Sgonc and Gruber, 2013; Swift et al., 2001).

5. Drivers of cellular senescence

Cellular senescence is a complex, dynamic and highly pleotropic process (van Deursen, 2014). Fundamentally however, it is a cellular stress response gone awry. The stressors are diverse and include telomeric shortening as a result of the end replication problem, aberrant expression of genes involved in extracellular matrix synthesis and degradation and inflammation contribute to the appearance of photo-aged skin (Krutmann and Schroeder, 2009). Recently, a novel mitochondria-to-nucleus signalling that mediates shedding of cytoplasmic chromatin fragments (CCFs), which can lead to transcription of proinflammatory genes via a CGAS-STING-NFkB pathway, have been elucidated in primary human cells and in mice (Vizioli et al., 2020). Senescent cells often display mitochondrial dysfunction resulting in decreased efficiency of oxidative phosphorylation, decreased energy production and increased levels of ROS which in turn drive damage in DNA or other macromolecules. The Passos group showed that signals from the DNA damage response (DDR) can be integrated to trigger PGC-1α-dependent mitochondrial biogenesis via an ATM/AKT/mTORC1 cascade. This in turn facilitates ROS-mediated activation of the DDR and cell cycle arrest in multiple cellular models from human and mice. Importantly, mitochondrial depletion by inhibition of mTORC1 or PGC-1α reduced the pro-inflammatory and pro-oxidant phenotype of senescent cells while sustaining their cell cycle arrest in aging mouse liver. These results highlight mitochondria as candidates for anti-aging interventions (Correia-Melo et al., 2016). For instance, NAD+ supplementation in primary human keratinocytes by addition of the precursor nicotinamide (NAM) reduced expression of epidermal differentiation markers and increased their proliferative capacity. Consistent with this, NAM administration to 3D skin models inhibited differentiation of the upper epidermal layers and maintained the proliferative abilities of cells in the basal layer (Tan et al., 2019). It has been proposed that NAD+ might regulate aging via modulation of DNA repair and prevention of stem cell senescence, and the latter effect may involve positive modulation of mitochondrial oxidative phosphorylation (Tan et al., 2019; Zhang et al., 2016).

Autophagy allows unwanted organelles and dysfunctional cellular components to be degraded and recycled through a lysosome-mediated pathway (Kroemer, 2015). Damaged mitochondria are removed by a specialised autophagic process called mitophagy (Chen et al., 2020). Studies in premature aging syndromes showed decreased mitophagy and accumulation of damaged mitochondria, suggesting an association between DNA damage, cellular bioenergetics and redox balance (Fang et al., 2014; Pallardo et al., 2010; Scheibye-Knudsen et al., 2012; Valentin-Vega et al., 2012). Indeed, increased oxidative stress and mitochondrial damage are common features in age-associated diseases (Buchan et al., 2020; Wallace, 2012). The efficiency of autophagy decreases with age and may be caused by mitochondrial dysfunction and increased inability to maintain redox homeostasis due to mitochondrial defects in aged systems (Kroemer, 2015). Coupled with the observation that autophagy deficient systems often show hallmarks of aging and cellular senescence, it has been suggested that autophagy has an anti-aging function. However, caution is warranted as restoration of autophagy in aged mice led to rejuvenation of some tissues, but also an increased

Broad skin phenotypes have been reported in human patients with diseases caused by mitochondrial dysfunction (Bodemer et al., 1999; Feichtinger et al., 2014). In addition, primary or secondary mitochondrial pathologies as well as mutations in nuclear genes encoding mitochondrial proteins or defects in mtDNA have been observed in several common and rare skin disorders (Feichtinger et al., 2014). Some human skin phenotypes have been recapitulated in mouse models. For example, POLG knockout mice which harbour a mutation in the mtDNA polymerase PolG, and epidermal-specific superoxide dismutase 2 (SOD2)-deficient mice present an aged skin phenotype with accumulation of senescent keratinocytes within the epidermis as determined by SA-β-gal staining and loss of HMG1 (Velarde et al., 2015; Wiley et al., 2016).

Mitochondria play a critical role in establishing the pro-oxidant and pro-inflammatory phenotype during senescence. Damaged mitochondria accumulate with age in skin cells and also in response to UVR exposure (Mellem et al., 2017; Panich et al., 2016). Mitochondrial perturbations lead to retrograde mitochondrial signalling that has been linked to various diseases including photoaging, where the altered expression of genes involved in extracellular matrix synthesis and degradation and inflammation contribute to the appearance of photo-aged skin
incidence in spontaneous tumors, highlighting the biphasic role of autophagy in aging and cancer (Cassidy et al., 2020).

Involvement of autophagy and mitophagy in fibroblast senescence has been demonstrated by several lines of evidence (Chen et al., 2020; Sreedhar et al., 2020). However, it is unknown whether the same mechanisms apply to keratinocyte senescence. In contrast to a highly connected mitochondrial network in keratinocytes in young human skin, aged keratinocytes had fewer mitochondrial clusters and a fragmented mitochondrial network, which suggests mitophagy defects (Mellem et al., 2017). Deletion of autophagy related 7 (Atg7), which is essential for autophagy, led to increased sensitivity to oxidative stress in mouse keratinocytes. These autophagy-deficient keratinocytes became senescent with exacerbated DNA damage and inflammatory signalling, along with an altered lipid metabolism associated with mitochondrial damage (Song et al., 2017). During epidermal differentiation, keratinocytes undergo a series of changes which culminate in nuclear degradation before becoming corneocytes that form the cornified epidermal layer (Fischer et al., 2011). The precise mechanisms that regulate these tightly orchestrated patterns of nuclear changes remain poorly understood, although autophagy has been suggested to play a role in keratinocyte differentiation and subsequent nuclear degradation (Aymard et al., 2011; Rogerson et al., 2018). Consistent with this notion, defects in autophagy result in nuclear retention (parakeratosis) in psoriatic skin (Akinduro et al., 2016). Interestingly, keratinocytes in psoriatic lesions have been described to exhibit features of senescence (Chaturvedi et al., 2003; Qin et al., 2002; Wrone-Smith et al., 1997).

The emerging connection between skin senescence, mitochondrial defects and autophagy raises fascinating questions. It remains debated whether mitochondrial dysfunction is a cause or consequence of cellular senescence – the answer to this question is likely context dependent. Furthermore, it is unknown whether senescent skin cells are present in mitochondrial disorders presenting with skin phenotypes. It also remains to be elucidated how alterations in autophagy impact DNA repair pathways. These changes may have implications on both cell behaviour as well as the paracrine cross talk between the epidermal and dermal compartments, contributing to the complex overall aging phenotype.

5.2. Persistent DNA damage

Skin aging and age-related diseases are exacerbated by sun exposure and intrinsically linked to the accumulation of DNA damage and an impaired ability to repair damaged DNA (Lopez-Otin et al., 2013). Both nuclear and mtDNA accumulate DNA damage lesions during chronological aging and as a result of photoaging (Lombard et al., 2012). The DNA damage response (DDR) is a conserved network of proteins involved in sensing, signalling and repair of DNA damage (Jackson and Bartek, 2009). The five main repair pathways are mismatch repair (MMR), nucleotide excision repair (NER), base excision repair (BER), homologous recombination (HR) and non-homologous end joining (NHEJ). MMR, BER and NER resolve lesions requiring single-strand base excisions, whereas HR and NHEJ are responsible for DSB repair (Jackson and Bartek, 2009). These mechanisms are altered in aged skin cells, impairing their repair capability, leaving the genome vulnerable to UVR exposure and promoting skin aging and/or tumorigenesis (Chung et al., 2003). While nuclear DNA repair is well-characterised, we lack a clear understanding of the mechanisms governing mtDNA repair, although it has been established that BER plays a central role in maintaining mitochondrial genome integrity (Kazak et al., 2012). Interestingly, mutations in key DNA repair genes have been linked to mitochondrial dysfunction through direct or indirect mechanisms manifesting in various skin disorders.

Most of the photolesions and oxidative DNA damage induced by UVR are repaired by NER which can be classified into Transcription-Coupled Repair (TCR) and Global Genome Repair (GGR). Xeroderma Pigmentosum C (XPC) is a p53-regulated and UVR-inducible protein essential for GGR-based DNA repair (Amundson et al., 2002). Deletion or functional inactivation of XPC, results in Xeroderma pigmentosum (XP), a disease characterised by accelerated photoaging and a predisposition to skin cancers. Likewise, XPC-deficient mice display premature skin aging, linked to increased oxidative stress resulting from NAPDH Oxidase 1 (NOX1) overactivation (Hosseini et al., 2013). In addition, mitochondrial function of young XPC-deficient mice was found to be reminiscent of that in aged wild-type mice, consistent with exacerbated oxidative stress (Hosseini et al., 2015). Mutations in another NER pathway gene, CSA causes Cockayne Syndrome (CS) where patients display premature aging and cachexia, along with neurological dysfunction and skin photosensitivity. Together in a complex with DDB1, cullin4A and Roc1, CSA appears to mediate the TCR branch of NER (Saijo, 2013). Interestingly, TCR-deficient but GGR-competent CS patient cells did not show increased mutations following UV exposure, which may explain the lack of carcinogenesis in CS patients (Reid-Baylis et al., 2016). Overexpression of CSA in primary keratinocytes from CS patients rescued UV sensitivity and protected the cells from premature senescence by restoring DNA repair, reducing oxidative damage and maintaining redox balance (Cordisco et al., 2019). Interestingly, normal human keratinocytes use the more efficient GGR, as opposed to fibroblasts which mainly adopt the TCR pathway (D’Errico et al., 2005, 2003; Otto et al., 1999). It is possible that the functions of TCR and GGR may complement each other to repair UV-induced damage in keratinocytes, providing a robust protective mechanism for genomic stability in the epidermis.

The RecQ helicas are highly conserved proteins that play critical roles in DNA replication and repair processes. There are 5 RecQ helicas in human, namely RECQ1L, BLM, WRN/RECQL2, RECQL4 and RECQL5. Mutations in these helicas cause inherited disorders with skin abnormalities and mitochondrial dysfunction. For example, mutations in WRN/RECQL2 ligase cause Werner Syndrome, an adult accelerated aging syndrome, characterized by short stature, a sclerodermalike skin phenotype and premature hair greying. Expression of helicase-deficient WRN mutants or Wrrn deletion results in impaired telomere replication, telomeric DNA damage and premature senescence in vitro and in vivo (Chang et al., 2004; Crabbé et al., 2007, 2004). In addition, knockdown of WRN by siRNA in human fibroblasts resulted in mitochondrial ROS generation through stabilization of HIF1a (Llabé et al., 2012). Loss of RECQL4 results in Rothmund-Thomson Syndrome, a rare disease in which patients develop erythema, skin atrophy and abnormal pigmentation. RECQL4 is localized to the mitochondria and RECQL4 deficiency in human WI-38 and BJ cells is associated with mtDNA damage and increased mtDNA copy number, which has been proposed as a compensatory mechanism to mtDNA damage (Groteau et al., 2012). In vitro, human primary fibroblasts depleted of BLN, WRN or RECQL4 display increased senescence based on elevated SA-β-Gal, p16 and p21 staining along with persistent foci of DNA damage (Lu et al., 2014).

The ataxia telangiectasia mutated (ATM) gene is a master regulator of the DDR to dsDNA breaks. Loss of ATM leads to Ataxia Telangiectasia, a neurodegenerative disorder with some patients showing abnormal skin pigmentation, inflammation and premature hair greying. Depletion of ATM in patient-derived cells and tissues obtained from ATM-deficient mice have significant nuclear and mtDNA instability resulting in impaired mitochondrial function (Sharma et al., 2014). Much of our understanding of the DDR comes from studies of dermal fibroblasts. But it has become increasingly clear that different cell types in various skin compartments may respond differently to DNA damage. Indeed, the epidermis is home to keratinocyte subpopulations with differing proliferative capabilities. Proliferating keratinocyte stem cells residing in the basal layer produce transient amplifying progenitor cells that differentiate upward into the suprabasal layer where they exit the cell cycle, become terminally differentiated, and eventually form the stratum corneum. There is evidence suggesting that keratinocytes in the
epidermis appear to be more resistant to photodamage than dermal fibroblasts (D’Errico et al., 2005; Otto et al., 1999). Moreover, within the epidermis, keratinocyte stem cells in the basal layer appear to be more resistant to UV damage than their differentiated progeny in the suprabasal layer (Metral et al., 2018; Qin et al., 2002). This may be partially attributed to an evolutionary adaptation of the DDR in keratinocytes, and especially in keratinocyte stem cells, in order to efficiently and rapidly resolve photodamage-induced DNA lesions. But how do keratinocytes in different epidermal layers respond to DNA damage and does their differentiation status influence how they respond to genotoxic insults? Conversely, does the outcome of DDR signalling differ at various stages during the keratinocyte life cycle?

5.2.2. DDR during the keratinocyte life cycle

When normal cells encounter DNA damage, they undergo a transient cell cycle arrest to repair damaged DNA; upon completion of which, the cell cycle is resumed. A persistent activation of DNA damage checkpoints, due to excessive damage, unreparable lesions or delayed repair triggers cellular programs such as senescence or apoptosis. p53 plays a pivotal role in these cell fate decisions by activating cell cycle checkpoints to safeguard genome integrity (Giono and Manfredi, 2006). Activity of cyclins and cyclin-dependent kinases (CDKs) drive cell cycle progression. Cyclins D and E, in conjunction with CDK4/6, and CDK2, respectively, allow for G1 progression and entry to S phase through phosphorylation of pRB and activation of E2F target genes (Vermeulen et al., 2003). Upon detection of DNA damage, p53 activates the cyclin-dependent kinase inhibitor p21, which in turn inhibits cyclin/CDK activity and arrests the cell cycle at the G1/S boundary (el-Deiry et al., 1993). Keratinocytes, unlike dermal fibroblasts, lack an efficient G1/S arrest when they encounter UV-induced damage (D’Errico et al., 2003). The lack of G1/S arrest has been hypothesized to increase the incidence of unrepaired lesions during DNA replication, thus resulting in a persistent activation of the G2/M checkpoint, followed by initiation of cellular senescence or apoptosis (D’Errico et al., 2005).

The role of mitotic checkpoints in determining epidermal cell fate has been explored by several groups (de Pedro et al., 2018; Quek et al., 2018; Sanz-Gomez et al., 2020; Zanet et al., 2010). Substrate-specific co-activators of the anaphase promoting complex (APC), CDC20 and Cdh1, have been reported to regulate the balance between stemness and differentiation, respectively in keratinocytes (Quek et al., 2018). While it remains controversial whether keratinocytes undergo mitotic slippage after prolonged mitotic arrest, and existing data regarding the presence of polyploid keratinocytes remains conflicting, evidence seems to suggest that DNA damage may trigger keratinocyte differentiation through activation of mitotic checkpoints (de Pedro et al., 2018; Quek et al., 2018). For example, activation of G2/M arrest by nocodazole correlates with onset of human keratinocyte differentiation (Gandarillas et al., 2005; Quek et al., 2018). Overexpression of the proto-oncogene Myc or the cell cycle regulator cyclin E led to replication stress and DNA damage in primary keratinocytes, triggering a mitotic block via p53/21 activation and terminal differentiation (Freije et al., 2012; Gandarillas et al., 2005) although in some cases, p21 can be induced independently of p53 (Dexter et al., 1995). In human skin, the Cdk1 inhibitor Wee1 is proposed to be required for the mitotic-differentiation checkpoint as inactivation of Wee1 abrogates the checkpoint function and cells undergo apoptosis (de Pedro et al., 2018). Indeed, it has been argued that the mitosis-differentiation checkpoint serves as a protective mechanism against apoptosis to avoid epithelial cell loss, and at the same time suppress pre-cancerous cells via shedding of keratinocytes in the event of irreparable DNA damage (de Pedro et al., 2018).

A provocative hypothesis is that activation of DDR may provide a first signal to initiate differentiation (Molinuevo et al., 2020; Sherman et al., 2011). Through manipulation of DDR pathways using chemical modulators and gene silencing studies, Molinuevo et al. showed that DDR signalling in the epidermis controls keratinocyte differentiation independent of DNA damage (Molinuevo et al., 2020). Further to this, while high doses of UV induce apoptosis of keratinocytes, induction of DNA damage through exposure to sublethal doses of UV increased squamous differentiation and production of the cornified layer in various systems (Del Bino et al., 2004; Matsui et al., 1996). However, it is not known whether senescence is also ensued in these cells. Interestingly, in response to UVB exposure, we and others have reported that primary keratinocytes become prematurely senescent and lamin B1-negative senescent cells accumulate in mouse epidermis (Dreesen et al., 2013; Wang et al., 2017). Immortalised human keratinocytes deficient for p16 provide a model where differentiation and premature senescence are uncoupled from one another (Bertrand-Vallery et al., 2010). Subjected to repeated UVB exposure, p16-deficient keratinocytes showed accumulation of DNA damage, underwent prolonged cell cycle arrest and increased expression of involucrin and filaggrin but did not become senescent (based on SA-beta-gal activity). The lack of proper demarcation between terminal differentiation and senescence, coupled with the non-uniformity in the experimental dosage of UVR in these studies and the diversity of biomarkers selected to identify senescent cells are among plausible explanations for the observed discrepancies and represent issues that are crucial to resolve.

5.2.3. DDR during terminal differentiation and senescence

To date, a caveat in the field has been that most DDR studies utilized proliferating and often transformed cells. It is unclear whether, or to which extent, non-proliferating or terminally differentiated cells within the 3D context of the skin respond to the same DNA damage, and whether they activate similar response pathways. As such, the DDR in terminally differentiated cells is poorly characterized (Fortini and Dogliotti, 2010; Nouspikel and Hanawalt, 2002). The general notion is that terminally differentiated cells have a dampened DNA damage response, possibly attributed to different physiological requirements of differentiated cells which no longer replicate their DNA, and whose DNA repair machinery is mainly associated with maintenance of the active transcriptome. For example, and in contrast to neural stem cells, astrocytes lack functional DDR signalling and are radioresistant (Schneider et al., 2012). Similarly, there is limited evidence that the DDR may differ significantly between senescent and non-senescent keratinocytes and fibroblasts. While keratinocytes predominantly accumulate SSBs, aged fibroblasts accumulate both DSBs and SSBs (Nassour et al., 2016). Lastly, these senescent keratinocytes display defective activation of ATM signalling which may be attributed to reduced ATM transcription and suppression of key DDR genes (Collin et al., 2018). Given the tremendous interest in the development of anti-aging interventions, it is imperative that we better understand how the different resident skin cell types respond to different genotoxic stressors.

Senescent cells are associated with widespread chromatin rearrangement and epigenetic alterations that promote metabolic rewiring and give rise to the characteristic secretory phenotype of senescent cells (Lee and Schmitt, 2019; Nakao et al., 2020). Senescent fibroblasts display subnuclear heterochromatin domains called senescence-associated heterochromatin foci (SAHF) which are thought to contain silenced chromatin domains required for cell cycle progression such as E2F target genes (Narita et al., 2003). SAHF formation also coincides with recruitment of heterochromatin proteins, which may facilitate chromatin remodelling in order to lock cells in a senescent state (Narita et al., 2003). That said, SAHF formation is not exclusive to cellular senescence, and also depends on the senescence trigger, species and cell type (Aird and Zhang, 2013). Altered chromatin states can also have implications on the DNA repair process. For example, DNA-SCARS are nuclear structures in senescent cells that are formed on persistent DNA lesions (Rodier et al., 2011). These distinct nuclear markers lack
characteristics of transient DNA damage foci such as ssDNA and DNA repair proteins RPA and RAD53, but show activated ATM/ATR, CHK2 and p53 signalling (Rodier et al., 2011). In UVR-damaged cells, the p53 target gene GADD45 can interact with core histones that have been altered by UVR and may modulate DNA accessibility to repair factors (Carrier et al., 1999). The significance of the different nature of DDR observed in senescent keratinocytes and different skin cell types remains somewhat unclear and warrants further investigation. In addition, the consequences of this differential response on skin homeostasis will need to be further elucidated.

6. Conclusions and perspectives

Although the main drivers of intrinsic and extrinsic skin aging and cell senescence have been well defined, further characterization of existing senescence and differentiation biomarkers in human skin cells is essential to shed light onto where, and at what frequency, these cells occur, and how they may contribute to skin aging. Moreover, it is unclear how diverse resident skin cell types in the epidermis and dermis cope with DNA damage accumulation during chronological aging or photoaging, and how this in turn, affects skin function. This question is complicated by the complex interplay between these heterogeneous subpopulations of epidermal keratinocytes with different proliferative capabilities. A multitude of factors including changes in mitochondrial metabolism, calcium homeostasis and chromatin modifications have been reported to play roles in the epidermal differentiation switch. Studies of cell cycle checkpoints and clues from human genetic disorders arising from defects in DNA damage response pathways point towards DDR signalling as an important driver of epidermal differentiation. Additional stressors, including DNA damage encountered by differentiated populations could lead to activation of senescence pathways as these cells undergo terminal differentiation. As we have summarised in this review, the current literature depicts a tightly interwoven relationship between DNA damage, mitochondrial dysfunction and oxidative stress in skin cell senescence and/or differentiation that requires further investigation. In terms of DDR regulation, more work needs to be done to understand the role of mitochondrial energy metabolism and regulation of autophagy in modulating the DDR in proliferating versus terminally differentiated and senescent cell types. Also noteworthy is the need to understand whether (and to what extent) altered chromatin configuration in differentiating and senescent keratinocytes affects the DDR (Botchkarev et al., 2012). In this regard, a detailed characterization of the fundamental differences between chromatin organization in differentiating versus senescent populations of keratinocytes will provide mechanistic insights about the DDR in the skin, and perhaps shed new light to distinguish these two cell populations. The skin offers a veritable model system to study tissue aging and presents unique opportunities to translate in vitro findings towards a clinical setting. It is a well-defined and well-characterized organ that is accessible for imaging and “minimally invasive sampling” techniques. These, in conjunction with the development of an arsenal of excellent in vitro and in vivo tools, including 3D organotypic culture models, 3D bioprinted skin tissues, transdermal delivery tools and excellent animal models brought in a golden era for skin research. In addition, incorporation of patient-derived skin cells into 3D skin constructs provide a valuable resource for generating skin disease models towards the goal of personalized medicine (Randall et al., 2018). Recent development of aged skin biomimetics or ‘seno-skins’ offers promising avenues for studying intercellular interaction during differentiation and aging, as well as efficacy testing and to validate anti-aging therapies (Weinmüller et al., 2020). However, challenges remain in the manufacturing of physiological skins that recreate physiologically relevant microenvironment and encompass all three main skin cell types (keratinocytes, fibroblasts and melanocytes), as well as incorporate a perfused vasculature, appendages such as glandular structures and hair follicles, or components of the immune system (Randall et al., 2018).

Last but not least, there is an immense interest in strategies to eliminate senescent cells, particularly in the discovery of drug candidates that can induce cell death in senescent cells, termed senolytics. To date, there are few studies that investigated the effects of senolytics on skin. Hickson et al. reported in a pilot study that oral administration of the senolytic combination of dasatinib and quercetin reduced p21 and p16 positive cells in the epidermis (Hickson et al., 2019). Challenges remain for the development of senolytics as critical issues such as drug efficacy and safety need to be considered to limit adverse effects to the tissue. An important question is whether the tissue in question will repopulate with younger, healthier cells after senescent cells have been eliminated. In this regard, novel tools such as ‘seno-skins’ and animal models of skin aging are ideal experimental systems to test senolytics in skin. Collaborative and interdisciplinary approaches that bring together biologists, engineers and clinicians will be highly beneficial to accelerate progress in this exciting field.

Declaration of Competing Interest

The authors report no declarations of interest.

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