Cell Autonomous and Non-Autonomous Effects of Senescent Cells in the Skin

Marco Demaria[1], Pierre Yves Desprez[1,2], Judith Campisi[1,3] and Michael C. Velarde[1]

Human and mouse skin accumulate senescent cells in both the epidermis and dermis during aging. When chronically present, senescent cells are thought to enhance the age-dependent deterioration of the skin during extrinsic and intrinsic aging. However, when transiently present, senescent cells promote optimal wound healing. Here, we review recent studies on how senescent cells and the senescence-associated secretory phenotype contribute to different physiological and pathophysiological conditions in the skin with a focus on some of the cell autonomous and non-autonomous functions of senescent cells in the context of skin aging and wound healing.

Journal of Investigative Dermatology advance online publication, 9 April 2015; doi:10.1038/jid.2015.108

INTRODUCTION

Cellular senescence is a complex stress response that renders cells incapable of cell division, even in the presence of growth stimuli (Campisi, 2013). Senescent cells are distinct from quiescent cells, which retain the ability to proliferate in response to appropriate stimuli. Senescent cells are also distinct from post-mitotic cells and terminally differentiated cells, which generally lose the ability to divide as a consequence of developmental, as opposed to stress-activated, programs.

A senescence response is typically induced by cellular damage (often nuclear DNA damage or mitochondrial dysfunction; von Zglinicki *et al.*, 2005; Ziegler *et al.*, 2015). As part of the senescence response, senescent cells express a number of non-exclusive markers, including the cell cycle inhibitor $p16^{INK4A}$ and elevated levels of a lysosomal enzyme, termed as senescence-associated β-galactosidase (Rodier and Campisi, 2011). Many senescent cells also secrete several cytokines, growth factors, and matrix metalloproteinases (MMPs), collectively termed as the senescence-associated secretory phenotype (SASP; Coppe *et al.*, 2008), which differ from those secreted by non-senescent quiescent, post-mitotic, and/or differentiated cells.

Senescent cells can alter tissue homeostasis and promote age-related diseases, including degenerative pathologies and cancers (Campisi, 2013; van Deursen, 2014). The inability of senescent cells to proliferate can impair tissue regeneration after injury, causing prolonged or permanent tissue damage with age. In addition, the SASP factors that are secreted by senescent cells can alter tissue microenvironments through their paracrine effects and promote age-related phenotypes (Coppe *et al.*, 2008). Indeed, removal of senescent cells in a premature aging mouse model reduced selected age-related pathologies, such as sarcopenia, cataracts, and loss of subdermal adipose tissue (Baker *et al.*, 2011).

Although cellular senescence is often viewed as a negative contributor to tissue function during the aging process, senescent cells, and particularly the SASP, can also have beneficial effects, such as the promotion of proper wound healing. This aspect of senescent cells could explain why cellular senescence evolved and has been preserved during evolution, even though it contributes to age-related phenotypes later in life. Here, we discuss how cellular senescence can be both beneficial and detrimental during skin aging and wound healing, and how the contrasting cell autonomous and non-cell autonomous effects of senescent cells can depend on the physiological context. On the one hand, senescent cells can accelerate aging phenotypes through the loss of tissue homeostasis by promoting chronic inflammation, persistent degradation of the extracellular matrix, and stem cell exhaustion. On the other hand, senescent cells can also have essential roles during wound healing by limiting excessive proliferation and fibrosis and promoting the formation of granulation tissue.

CELLULAR SENESCENCE AND SKIN AGING

Skin aging is caused by both intrinsic and extrinsic factors. Intrinsic aging, sometimes termed as chronologic aging, refers mainly to sun-protected areas of the skin. Intrinsic aging is associated with morphological changes primarily in the epidermal layer, manifest as marked thinning and loss of undulation (flattening of the dermo–epidermal junction; Makrantonaki and Zouboulis, 2007). Intrinsic aging also reduces subcutaneous fat and dermal thickness with an accompanying loss of cellularity and vascularity (Farage *et al.*, 2013). In contrast, extrinsic aging, particularly photoaging (sun exposure), markedly affects both the epidermal and dermal layers, with the latter showing a striking loss of collagen and extracellular matrix (Quan *et al.*, 2004, 2010).

[1]*Campisi Laboratory, Buck Institute for Research on Aging, Novato, California, USA;* [2]*California Pacific Medical Center Research Institute, San Francisco, California, USA and* [3]*Lawrence Berkley National Laboratory, Life Sciences Division, Berkeley, California, USA*

Correspondence: Michael C. Velarde, Campisi Laboratory, Buck Institute for Research on Aging, Campisi Laboratory, 8001 Redwood Boulevard, Novato, California 94945, USA. E-mail: mvelarde@buckinstitute.org

Abbreviations: MMP, matrix metalloproteinase; SASP, senescence-associated secretory phenotype

Received 7 January 2015; revised 26 February 2015; accepted 7 March 2015

There is also accumulation of abnormal elastic tissues (Bernstein *et al.*, 1994; Mitchell, 1967), which are due to formation of structurally different elastic fibers (Watson *et al.*, 2001).

Senescent cells increase with age in both the epidermis and dermis, as determined by elevated levels of senescence-associated β-galactosidase activity and $p16^{INK4A}$ expression (Dimri *et al.*, 1995; Krishnamurthy *et al.*, 2004; Ressler *et al.*, 2006; Waaijer *et al.*, 2012). Because senescent cells cannot proliferate, their presence in aged skin can potentially impair tissue homeostasis, regeneration, and youthful tissue structure/function (Campisi, 2013; Signer and Morrison, 2013). For example, in a three-dimensional organotypic culture model using neonatal dermal fibroblasts and epidermal keratinocytes from human donors of varying ages, increasing the expression of $p16^{INK4A}$ in keratinocytes isolated from young (30–40 years) donors yielded a thin epidermal layer similar to that formed by keratinocytes from elderly (53–66 years) donors; decreasing the expression of $p16^{INK4A}$ in keratinocytes from elderly donors transformed the aged skin phenotype of a thin epidermal layer into a thicker epidermis, similar to that formed by keratinocytes from young donors (Adamus *et al.*, 2014).

Aside from the cell autonomous effects of non-proliferating senescent cells on skin homeostasis, SASP factors secreted by senescent cells are also thought to contribute to skin aging phenotypes in a cell non-autonomous manner. SASP factors, especially the MMPs, become elevated with age and can alter the tissue microenvironment and accelerate skin aging phenotypes (Table 1). MMPs can degrade collagens, including type I collagen, which is the most abundant protein in the dermal extracellular matrix. Loss of collagen is associated with several clinical manifestations of aging skin, including wrinkles, sagging, and laxity (Jariashvili *et al.*, 2012; Quan *et al.*, 2010; Shuster *et al.*, 1975). Hence, increased expression and activity of MMPs during aging can decrease the amount of collagen in the skin (Varani *et al.*, 2004), diminish fibroblast-collagen interactions, and reduce mechanical tension, explaining the wrinkling phenotype observed in aged skin (Varani *et al.*, 2006). Another hypothesis for facial wrinkling is the enhanced elastase activity upon UVB stimuli, which is associated with a reduction in the elastic properties of the skin (Imokawa, 2009). The role of cellular senescence in promoting changes in the elastic tissue has not been addressed yet but represents an important avenue for future clinical approaches.

Although it is still unclear which specific stimuli are responsible for inducing cellular senescence during aging, both intrinsic and extrinsic aging have been linked to the age-related increase in the number of senescent cells in the skin. For example, the hereditary disorders, the Werner syndrome, xeroderma pigmentosum, and the Hutchinson–Gilford progeria syndrome, which are due to defects in DNA damage repair or nuclear organization, are associated with increased cellular senescence and accelerated age-related phenotypes in the skin (Davis *et al.*, 2007; Harada *et al.*, 1999; Liu *et al.*, 2006). Extrinsic factors, such as X-rays, UV light, and cigarette smoke, also can induce cellular senescence, as well as age-related phenotypes in the skin (Shin *et al.*, 2012; Velarde *et al.*, 2012; Yang *et al.*, 2013).

UV light can induce photoaging via direct damage to extracellular matrix components, such as collagen and fibrillin fibers (Jariashvili *et al.*, 2012; Menter *et al.*, 2001; Sherratt *et al.*, 2010), or indirect damage through mitochondrial dysfunction. Indeed, mitochondrial dysfunction is suggested to have a role in both intrinsic and extrinsic aging and may potentially serve as a common link between the two (Krutmann and Schroeder, 2009). UV radiation–induced photoaging of human skin is associated with large-scale deletions in mitochondrial genomes (mitochondrial DNA (mtDNA); Berneburg *et al.*, 1997; Birch-Machin *et al.*, 1998). Intra-individual studies have revealed that the frequency of a 4,977 bp deletion, also defined as "common deletion", is increased up to 10-fold in photoaged skin compared with sun-protected skin (Berneburg *et al.*, 1997). The majority of these deletions are detectable in the dermis of human skin exposed to physiological doses of UVA (Berneburg *et al.*, 2005). UV radiation also induces this common deletion in cultured skin fibroblasts and decreases mitochondrial function (Berneburg *et al.*, 2005). Because mitochondrial damage and dysfunction induces cellular senescence in culture and *in vivo* (Passos *et al.*, 2006; Velarde *et al.*, 2012), and UV light also promotes mitochondrial damage and cellular senescence, it would be interesting to test whether the UV-induced common deletion contributes to skin aging through mitochondrial dysfunction–associated senescence.

Table 1. List of MMPs involved in skin aging, cellular senescence, and wound healing

	MMP expression	References
Skin aging	Elevated expression of MMP1, MMP3, and MMP9	Quan *et al.*, 2009
Cellular senescence	Elevated expression of MMP1, MMP3, MMP8, MMP10, MMP12, and MMP13	Coppe *et al.*, 2010; Freund *et al.*, 2011
Wound healing	Temporal upregulation of MMP1, MMP2, MMP9, MMP3, MMP10, MMP14, MMP8, MMP12, MMP13, MMP19, MMP26, MMP28	Martins *et al.*, 2013

Abbreviation: MMP, matrix metalloproteinase.

CELLULAR SENESCENCE AND WOUND HEALING

Wound healing is a complex process by which the skin repairs itself after injury. This process is classically divided into four distinct but overlapping phases (Singer and Clark, 1999): (i) hemostasis, (ii) inflammation, (iii) proliferation, and (iv) remodeling. During the first two phases, platelets promote coagulation and begin an inflammatory cascade by secreting a variety of cytokines and chemokines to attract macrophages and neutrophils (Fuhrman *et al.*, 1991; Kim *et al.*, 2008; Shallo *et al.*, 2003). Before the inflammatory phase ends, fibroblasts are recruited to the wound site and endothelial cells mature from progenitor cells to reestablish vascularization (Chen *et al.*, 2008; Postlethwaite *et al.*, 1987;