The Senescence-Associated Secretory Phenotype: Critical Effector in Skin Cancer and Aging

Kanad Ghosh[1,3] and Brian C. Capell[1,2,3]

Cellular senescence, a state of stable cell cycle arrest in response to cellular stress, is an indispensable mechanism to counter tumorigenesis by halting the proliferation of damaged cells. However, through the secretion of an array of diverse cytokines, chemokines, growth factors, and proteases known as the senescence-associated secretory phenotype (SASP), senescent cells can paradoxically promote carcinogenesis. Consistent with this, removal of senescent cells delays the onset of cancer and prolongs lifespan in vivo, potentially in part through SASP reduction. In this review, we consider the evidence for the SASP and "SASP-like" inflammation in driving skin carcinogenesis, emphasizing how further understanding of both the roles and mechanisms of SASP expression may offer new targets for skin cancer prevention and therapy.

Journal of Investigative Dermatology (2016) ■, ■–■; doi:10.1016/j.jid.2016.06.621

INTRODUCTION

In the 1960s, Leonard Hayflick and Paul Moorhead made a monumental discovery: normal cells have a finite replicative potential in vitro, regardless of culture conditions (Hayflick, 1965). This stable arrest of the cell cycle has been termed cellular senescence, and is believed to be a primary defense against the unlimited cellular proliferation that drives cancer (Mooi and Peeper, 2006). In the decades since, we have learned that senescence is not only caused by this progressive telomere shortening-induced replicative threshold but also by a diverse array of cellular stressors ranging from activated oncogenes and ultraviolet radiation (UVR) to DNA-damaging therapies, oxidative stress, and the depletion of deoxyribonucleotide pools (Mannava, 2013; van Deursen, 2014). For instance, an activated $BRAF^{V600E}$ oncogene can drive the initial proliferation of human melanocytes, which then ultimately growth arrest to form a senescent nevus (Michaloglou et al., 2005). However, senescent cells can escape this cell cycle arrest to form cancers such as melanoma (Figure 1a) (Liu and Sharpless, 2012).

Beyond tumor suppression and cancer, senescent cells have been implicated in critical physiological processes including development, wound healing, and normal aging (Childs et al., 2015). Indeed, the number of senescent cells increases with aging in human tissues, including in both the skin dermis and epidermis (Dimri et al., 1995; Munoz-Espin and Serrano, 2014; Ressler et al., 2006). How senescent cells can have such pleiotropic effects is explained by their ability to secrete an array of inflammatory cytokines, chemokines, growth factors, and proteases known as the senescence-associated secretory phenotype (SASP) (Campisi, 2013; Coppe et al., 2010; Neves et al., 2015). These factors include such well-known cellular mediators as IL1α, IL1β, IL6, IL8, and matrix metalloproteinases (MMP1 and MMP3) (Figure 1b, demonstrating IL6 within a nevus, and Figure 2). Although the role of senescence in aging and wound healing was recently reviewed in the JID (Demaria et al., 2015), here we focus on the SASP, its regulation, and its role in the development and progression of cancer, with particular attention paid to the evidence from melanoma and non-melanoma skin cancers. As in many other fields of research, numerous mechanistic findings in the senescence field have been initially uncovered in primary human fibroblasts. Although keratinocytes and melanocytes have typically displayed similar responses and comparable results when subjected to identical experimental conditions, this caveat must be kept in mind when interpreting results. Further, as cancer-associated fibroblasts are frequently pivotal players in driving carcinogenesis, consideration of cell-type specificity is critical when developing a unified understanding of the role of the SASP in skin carcinogenesis (Gascard and Tlsty, 2016). Thus, in this review, we attempt to note and clarify the cell types employed whenever feasible.

Regulation of the SASP

Although the SASP is associated with senescence, it can be detached from the actual growth arrest of senescence. For instance, in primary human fibroblasts and mammary epithelial cells, overexpression of the cyclin-dependent kinase inhibitor p16INK4a (*CDKN2A*) is sufficient to induce senescence growth arrest (Coppe et al., 2011). However, without any precipitating DNA damage, there is no SASP in

[1]*Epigenetics Program, Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA; and* [2]*Department of Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA*

[3]*These authors contributed equally to this work.*

Correspondence: Brian C. Capell, Smilow Center for Translational Research, University of Pennsylvania, 9-161, Philadelphia, Pennsylvania 19104, USA. E-mail: briancapell@gmail.com

Abbreviations: ATM, ataxia telangiectasia mutated; DDR, DNA-damage response; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; PKD1, protein kinase D1; SASP, senescence-associated secretory phenotype; SCC, squamous cell carcinoma

Received 28 March 2016; revised 1 June 2016; accepted 20 June 2016; corrected proof published online XXX XXXX

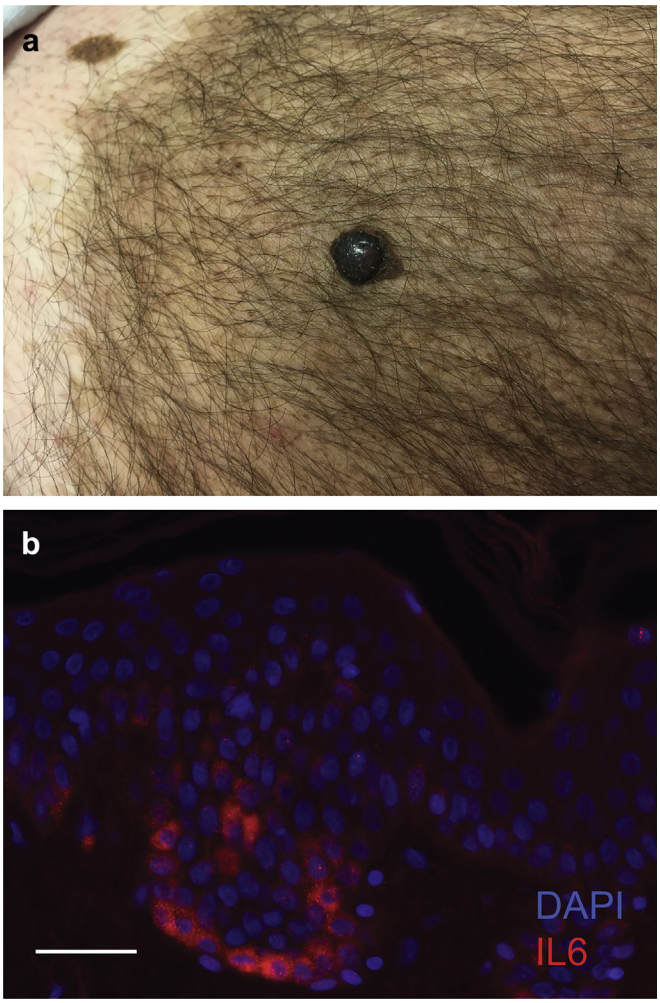

Figure 1. Senescence in the skin. (**a**) A malignant melanoma arising within a longstanding congenital nevus. Congenital nevi typically are driven into OIS by an activated *NRAS* oncogene. (**b**) A human melanocytic nevus displaying positive staining for the SASP cytokine, IL6, around the nevus melanocytes. OIS, oncogene-induced senescence; SASP, senescence-associated secretory phenotype.

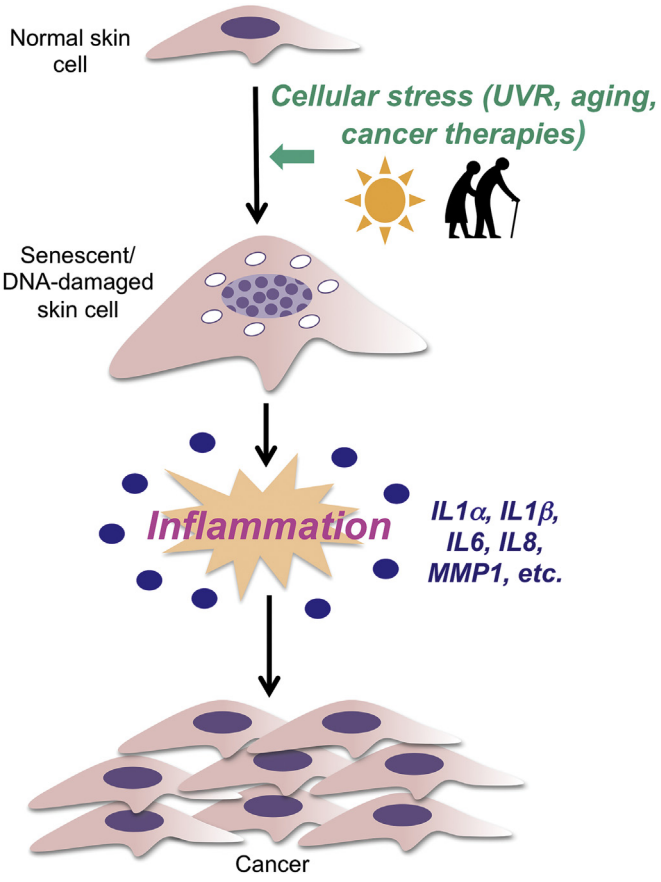

Figure 2. Senescent, damaged cell secretome can drive carcinogenesis. Diverse DNA-damaging stimuli, including UVR, telomere shortening with normal aging, activated oncogenes, and cancer therapies, can drive cells into senescence and lead to the secretion of the array of cytokines, chemokines, growth factors, and proteases known as the SASP. This can create a permissive tissue microenvironment that promotes the initiation, progression, and resistance of cancer cells. MMP1, matrix metalloproteinase 1; SASP, senescence-associated secretory phenotype.

these studies. In contrast, DNA damage leads to a SASP independent of p16INK4a levels (Coppe et al., 2011). These data are in agreement with other studies demonstrating the requirement of an activated ataxia telangiectasia mutated (ATM)-mediated DNA-damage response (DDR) for SASP expression (Rodier et al., 2009) (Figure 3). In a seminal study employing human foreskin fibroblasts and mammary epithelial cells, Di Micco et al. (2006) demonstrated that expression of oncogenic HRasV12 leads to oncogene-induced senescence in a DDR and DNA replication-dependent manner, as cells are unable to induce the DDR when DNA replication is inhibited. Rodier et al. (2009) then later showed that this DDR-SASP link required ATM, but not other DDR regulators, ATR or p53. Mechanistically, ATM is able to couple replication stress to both DDR activation and metabolic reprogramming during senescence (Aird et al., 2015). Despite this, an exception to this DDR-SASP connection was observed during embryonic developmental senescence in mesonephric epithelial cells: although overall displaying a unique gene expression program from damage-induced senescence, embryonic senescence was noted to engage the transforming growth factor-β pathway in the absence of markers of DNA damage (Muñoz-Espín et al., 2013). Collectively, these data demonstrate that although the SASP is typically a response to DNA damage rather than cellular proliferation arrest per se, DNA damage may not be absolutely required in the case of developmentally programmed senescence.

The DDR leads to increased transcription of cytokines such as IL1α, IL1β, IL6, IL8, proteases MMP1 and MMP3, and growth factors including fibroblast growth factor and hepatocyte growth factor among others. In studies performed in both foreskin fibroblasts and mammary epithelial cells, IL1α has been shown to be an upstream regulator of other SASP factors during oncogene-induced senescence, including IL6, IL8, vascular endothelial growth factor, and transforming growth factor-β (Acosta et al., 2013; Orjalo et al., 2009). Critical to the transcription of the SASP is the phosphorylation of the transcription factor NF-κB (Freund et al., 2010) (Figure 3). On senescence or other DNA-damaging stimuli in human embryonic fibroblasts, Chien et al. (2011) demonstrated that the phosphorylated NF-κB p65/RelA subunit