OUTLOOK

Endothelial cells give a boost to senescence surveillance

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Senescence is a specialized form of cell cycle arrest induced in response to damage and stress. In certain settings, senescent cells can promote their own removal by recruitment of the immune system, a process that is thought to decline in efficiency with age. In this issue of *Genes & Development*, Yin et al. (pp. 533–549) discover a surprising cross-talk where senescent cells instruct endothelial cells to help organize the clearance of the senescent population. This uncovers yet another layer of complexity in senescent cell biology, with implications for cancer treatment and aging.

In cells harboring oncogenic mutations, senescence becomes activated to prevent their uncontrolled proliferation, functioning as a tumor-suppressive mechanism. One hallmark feature of these and other senescent cells is their production of a complex secretome of proteins and extracellular vesicles known as the "senescence-associated secretory phenotype" (SASP), which enables senescent cells to interact with their microenvironment (Muñoz-Espín and Serrano 2014; Rhinn et al. 2019; Birch and Gil 2020). A previous landmark study investigating Ras-induced senescence (RIS) shed light on one key function of the SASP, using an elegant model of transposon-mediated delivery of oncogenic Ras to induce senescence in individual hepatocytes in the liver (Kang et al. 2011). They found that the oncogene-expressing senescent cells promoted their own removal, with the SASP activating the immune system—notably, $CD4^+$ T cells and monocytes/macrophages—to remove the premalignant senescent cells. Critically, if this clearance was blocked, senescent cells persisted and hepatocarcinoma developed. Therefore, the coordinated induction and subsequent removal of senescent cells is a key tumor-suppressive mechanism.

However, it was not fully clear how this process was orchestrated at the cellular and molecular level, and the current study from Yin et al. (2022) advances our understanding, uncovering a previously unknown involvement of endothelial cells (ECs), instructed by the senescent cells, to aid in senescence surveillance.

The investigators previously demonstrated that the SASP can promote lymphocyte adhesion and *trans*-endothelial migration (Hoare et al. 2016), prompting them to explore here how the endothelial cells changed in response to SASP exposure. To address this, they isolated liver sinusoidal endothelial cells (LSECs) from patients and exposed these to the SASP in culture, before performing RNA sequencing. Interestingly, they found that LSECs turned on key inflammatory genes involved in immune cell recruitment and adhesion, such as IL6, IL8, ICAM1, ICOSLG, and others. Transcription factor (TF) motif analysis on the differentially expressed genes implicated the involvement of NF-κB, which they validated at the protein and RNA levels. Importantly, they also demonstrated these SASP-induced changes in other ECs, including human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs).

Interestingly, NF-κB is a master regulator of the SASP in senescent cells (Chien et al. 2011), so the investigators sought to explore whether it now also played a functional role in the ECs. Using genetic or drug inhibitors, they blocked NF-kB signaling in SASP-treated ECs and showed that, indeed, NF-κB mediated the induction of the inflammatory genes, which in culture models was necessary for lymphocyte adhesion and *trans*-endothelial migration.

A critical question was now whether similar cross-talk happens in vivo. To address this, the investigators used the model of transposon-mediated delivery of oncogenic Ras to the liver, validating RIS only in the hepatocytes, and senescent cell clearance after 12 d. In this setting, they then used endothelial-specific Cre-mediated reporter mice to isolate LSECs and confirmed the induction of NF-κB target genes by the SASP in the LSECs in vivo (Fig. 1A).

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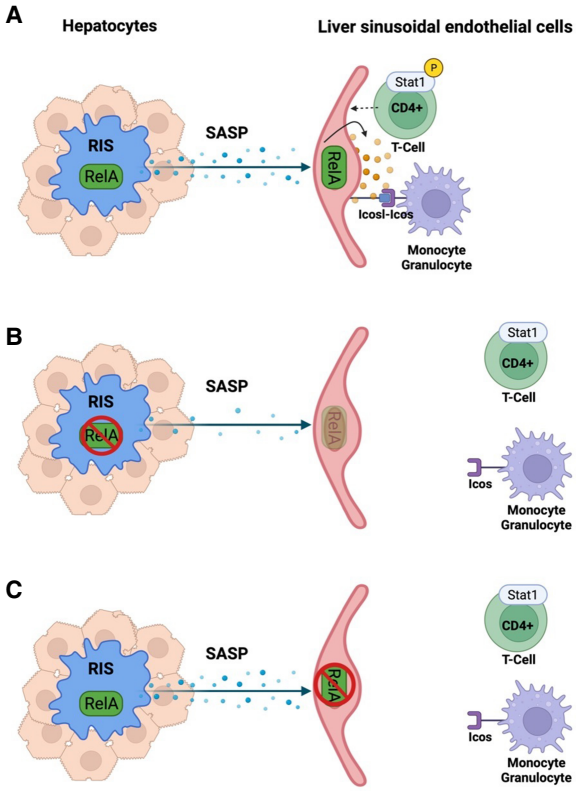

Figure 1. SASP exposure activates LSECs to recruit immune cells for senescence surveillance. (*A*) Oncogene expression in single hepatocytes leads to RAS-induced senescence (RIS) and activation of NF-κB/RelA signaling, which regulates the senescence-associated secretory phenotype (SASP). SASP factors act on adjacent liver sinusoidal endothelial cells (LSECs) and induce NF-κB/RelA. This induces inflammatory gene expression, which activates Stat1 in $CD4^+$ T cells, and up-regulation of Icosl, which leads to recruitment of $Icos^+$ immunocytes. (*B*) Inhibition of *RelA* in RIS decreases the SASP, which now fails to activate RelA in LSECs. (*C*) Inactivation of *RelA* in LSECs decreases recruitment of Stat1 phosphorylated $CD4^+$ cells and Icos-expressing immunocytes, leading to impaired senescence surveillance. Image made with BioRender.

However, what are the functional roles for such signaling? To answer this, they used a systematic combination of cell type-specific Cre-expressing mouse models to disrupt NF-κB either in the hepatocytes or in the LSECs, when RIS was induced only in the hepatocytes. As hypothesized, loss of NF-κB in the senescent cells diminished their own clearance but also caused a decreased paracrine induction of NF-κB target genes in the LSECs (Fig. 1B). However, the key result came when they deleted NF-κB in the LSECs (Fig. 1C). This led to decreased SASP-induced inflammatory gene expression in the LSECs but also impaired clearance of the senescent hepatocytes, conclusively demonstrating that senescent hepatocytes engaged the LSECs to aid in senescence surveillance.

However, it was still not clear whether SASP-primed LSECs were merely favoring immune cell entry or whether they were influencing their functionality. Single-cell sequencing on $CD4^+$ T cells revealed that NF-κB signaling in the endothelium was required for Stat1 signaling in T cells, a key mediator of their polarization and function (Fig. 1). Finally, the investigators returned to the increased ICOSLG expression induced by the SASP in the LSECs. As this is a key immunoregulatory factor in immune cell activation, they investigated its contribution using blocking antibodies to interfere with its ligand *Icos* and mice with diphtheria toxin receptor to ablate $Icos^+$ cells. Ultimately, they found that *Icos–Icosl* signaling was required for granulocyte- and monocyte-mediated clearance of the senescent cells, although the precise role of the LSECs in this remains to be seen.

A major strength of this study is the comprehensive use of mouse models to tease apart the cell type-specific roles of NF-κB, including reporters and deletion and ablation models, coupled with detailed in vivo validation of their specificity. This allowed the investigators to unequivocally show that it is the oncogene-expressing senescent cells that produce the signals to activate the ECs, notably advancing our understanding of how senescent cells communicate and function.

Of course, this also raises additional questions. Previous studies have also shown that the SASP induces functional changes in ECs (Coppé et al. 2006; Ruscetti et al. 2020). It will be interesting to unravel the functions of the other SASP-induced TFs identified here (e.g., Egr1, Jun, and AP1), and how broadly the influence is on other cell types beyond ECs. While the focus of this study is on RIS, senescence also has beneficial roles in development and regeneration, as well as detrimental effects in aging and age-related disease (Rhinn et al. 2019; Di Micco et al. 2021). It will be exciting to explore whether similar communication with ECs is involved in these situations. Do ECs facilitate senescence surveillance only in beneficial settings? How does this change during aging? Interestingly, LSECs are one of the first cells to undergo senescence during aging (Grosse et al. 2020). Might this alter their immune-recruitment functions, ultimately contributing to senescence accumulation during aging? Overall, the study by Yin et al. (2022) provides one more important clue into the complex biology of senescent cells and reinforces the idea that functionally manipulating them—or now their instructed neighbors—could be useful in tumor and aging contexts.

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