HMGB2 holds the key to the senescence-associated secretory phenotype

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The senescence-associated secretory phenotype (SASP) is a hallmark of senescence with an important physiological impact, but how it is established is unclear. In this issue, Aird et al. (2016. *J. Cell Biol.* https://doi.org/10.1083/jcb.201608026) describe how chromatin-bound HMGB2 fine tunes SASP expression by avoiding heterochromatin spreading.

Cellular senescence was first identified as a type of irreversible cell cycle arrest that occurs when cells reach the end of their replicative potential (Hayflick and Moorhead, 1961). Many other stresses, such as irradiation, oxidative stress, DNA damage, or oncogenic activation, also trigger premature senescence (Kuilman et al., 2010). We are just starting to understand the broad relevance of senescence and its many pathophysiological implications. Senescence is generally considered to be a protective response: it is tumor suppressive and it limits the extent of fibrotic responses. However, the accumulation of senescent cells can have detrimental consequences, such as in age-related pathologies. Therefore, understanding how senescence is implemented and defining the impact of the senescence program on cells and their environment is of great importance.

The defining characteristic of senescence is a highly stable cell cycle arrest, triggered by the up-regulation of cyclin-dependent kinase inhibitors such as $p16^{INK4a}$ and $p21^{CIP1a}$. Senescent cells also undergo dramatic changes in their morphology and in the organization and architecture of their cellular compartments. The most notorious changes happen in the nuclei, with widespread chromatin changes triggered by depletion of lamin B1 (Shah et al., 2013) and a dramatic reorganization of heterochromatin, termed senescence-associated heterochromatin foci (SAHF; Narita et al., 2003). Genes necessary for cell cycle progression, such as E2F-dependent genes, are incorporated into the SAHF and are thereby silenced, contributing to the stability of the growth arrest. Although senescent cells repress proliferation-promoting genes, they also induce the gene program necessary for the implementation of senescence. Some genes encoding secreted factors such as proinflammatory cytokines, growth factors, and matrix metalloproteinases are up-regulated during senescence; this secretory program is a hallmark of senescence and is referred to as the senescence-associated secretory phenotype (SASP; Coppé et al., 2010). The SASP is thought to mediate many of the physiological functions associated with senescent cells. For example, the SASP of preneoplastic cells undergoing oncogene-induced senescence (OIS) initiates an immune surveillance response that behaves as a cell-extrinsic tumor suppressor mechanism (Kang et al., 2011). The SASP can reinforce or induce senescence, which might contribute to limit tumor progression (Acosta et al., 2008). However, because of its proinflammatory nature, the SASP can also promote tumor progression and present other detrimental long-term effects, especially during aging (Coppé et al., 2010). Therefore, one current challenge is to resolve this paradox so as to preserve the beneficial effects of senescence (associated with stable growth arrest) while minimizing the detrimental effects caused by chronic inflammation. To achieve this, we need to gain a better understanding of how senescence is implemented. Senescence is associated with a deep chromatin reorganization that is essential for fine-tuning gene expression, and understanding the epigenetic mechanisms controlling senescence is necessary to delineate how the different aspects of the senescence program and specifically of the SASP are regulated. In this issue, Aird et al. (2016) identify a key role for high mobility group box (HMGB) 2 in shaping the chromatin landscape associated with the SASP, bringing to light an important component of the machinery underlying the SASP.

Aird et al. (2016) endeavored to identify factors regulating chromatin reorganization during senescence. By performing a new analysis of previously published gene expression datasets, they identified a decrease of HMGB2 expression as one of the most significant changes associated with senescence. HMGB proteins are nonhistone molecules that bind to DNA and affect the chromatin state, allowing transcription factors to access gene promoters. HMGB1 has been previously linked to senescence (Davalos et al., 2013), and loss of nuclear HMGB1 is considered a marker of senescent cells. HMGB1 also functions as an alarmin, a protein secreted upon stress to elicit an innate immune response (Davalos et al., 2013). The role of HMGB1 in senescence has been linked to this alarmin function; extracellular HMGB1 stimulates cytokine secretion through TLR-4 and NF-κB signaling and precedes SASP induction. HMGB2 has not been described to behave as an alarmin, so this intriguing hit stimulated the authors to investigate how it could regulate senescence.

Aird et al. (2016) confirmed the down-regulation of HMGB2 mRNA and protein levels in the human lung fibroblast IMR90 cell line either undergoing OIS or replicative

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The Rockefeller University Press $30.00
J. Cell Biol. Vol. 215 No. 3 297–299
https://doi.org/10.1083/jcb.201610044

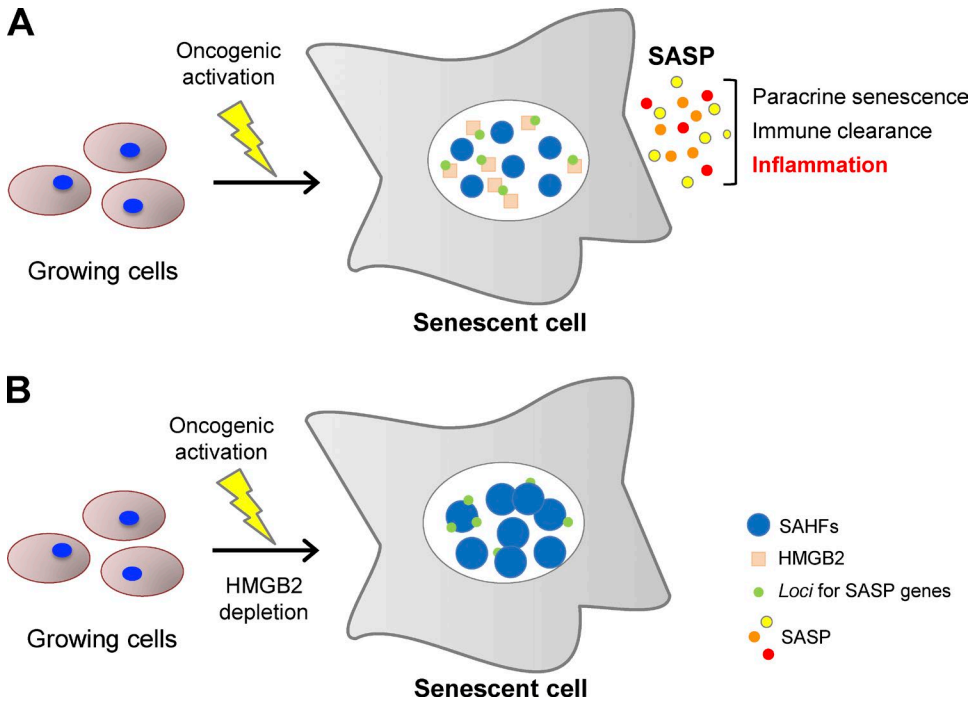

Figure 1. **HMGB2 binds to SASP genes to induce their transcription.** (A) Upon oncogenic expression, cells reorganize their heterochromatin in repressive SAHF. HMGB2 limits their spread and allows the transcription of SASP genes. (B) HMGB2 depletion results in repression of the expression of SASP genes due to their heterochromatinization.

senescence. The authors additionally validated their finding in senescent cells isolated from a published mouse model of accelerated aging. Either knockdown or knockout of HMGB2 triggered senescence in IMR90 cells. It is important to note that, although the overall HMGB2 levels decreased in senescent cells, the investigators could still detect a significant amount of HMGB2 bound to chromatin during senescence. As HMGB2 typically promotes gene transcription, to investigate the role that chromatin-bound HMGB2 plays during senescence, Aird et al. (2016) performed HMGB2 chromatin immunoprecipitation sequencing (ChIP-Seq) and combined the data with published gene expression profiles. Among the genes with increased HMGB2 association that were upregulated during senescence, the authors found many SASP genes, including *IL-8* and *IL-6*. The researchers further investigated whether the association of HMGB2 with SASP genes depends on NF-κB or C/EBP-β, known transcriptional regulators of the SASP. Knockdown of the NF-κB subunit RELA had no effect on the binding of HMBG2 to SASP gene loci. However, the authors found that C/EBP-β is itself a target of HMGB2 in senescent cells based on ChIP-Seq data, and HMGB2 depletion led to decreased C/EBP-β levels. Given the broad role of C/EBP-β in SASP regulation, it will be important to clarify the relationship between HMGB2 and C/EBP-β in future work.

Remarkably, Aird et al. (2016) observed that the decrease in chromatin-bound HMGB2 correlated with reduced SASP gene expression but did not affect cell cycle arrest. Moreover, they show that loss of HMGB2 resulted in the spread of heterochromatin marks into SASP gene loci. In other words, upon HMGB2 depletion, SASP gene loci are incorporated into the SAHF and become silenced (Fig. 1). Additional experiments will be needed to assess whether HMGB2 also excludes SASP loci from the SAHF in other cell types and conditions of senescence induction, as SAHF are not as prominent in cells other than fibroblasts or when senescence is induced by stresses other than oncogenes. However, these results suggest that one strategy to ablate the SASP without affecting the senescence growth arrest could be HMGB2 inhibition.

Two recent studies also investigated the epigenetic mechanisms controlling the SASP. Capell et al. (2016) focused on MLL1, an oncoprotein and transcriptional activator required for the expression of pro-proliferative cell cycle genes. Expression of MLL1 correlates with a DNA damage response (DDR). DDR activation can result in SASP activation, and it was shown that inhibition of MLL1 attenuates SASP expression without affecting the stability of the growth arrest. However, the impact of MLL1 on SASP expression is indirect. Another study demonstrated that there is a global remodeling of the enhancer landscape during OIS (Tasdemir et al., 2016). In particular, superenhancers are enriched in the proximity to key SASP genes. The bromo and extra-terminal domain protein BRD4 was identified as an essential cofactor for the transcription of superenhancer-associated genes, and its inhibition blunted SASP expression without affecting the senescent growth arrest. Collectively, these recent studies and the study by Aird et al. (2016) reinforce the idea that it is possible to blunt SASP induction without affecting the senescent growth arrest. Using epigenetic mechanisms to target the SASP could serve to uncouple the long-term deleterious effects of the SASP from other beneficial effects such as those connected to the senescence growth arrest. An effective pharmacological agent targeting HMGB2 may be highly valuable in a therapeutic context to reduce the SASP and target chronic inflammation in age-related pathologies. Given the role of not only HMGB2 but also HMGB1 in controlling inflammatory

responses, it would also be interesting to dissect their respective contributions in inflammation and investigate whether their combined targeting has an additive or synergistic effect. In this regard, the small molecule inflachromene directly binds and inhibits HMGB proteins. It was also shown to effectively inhibit microglia-mediated neuroinflammation and displayed both antiinflammatory and neuroprotective properties (Lee et al., 2014).

Targeting proteins controlling the senescent state has many therapeutic implications. Senescent cells accumulate with age and are detrimental for tissue structure and function, and aging is a risk factor in many diseases. The SASP is a likely contributor to the negative effects of senescent cells on tissue homeostasis. A seminal study found that clearance of *p16^{INK4}* senescent cells delays aging and improves progression of already established age-related disorders (Baker et al., 2016), thereby opening a race to identify strategies to overcome the side effects of senescence. Senolytic compounds, drugs that specifically kill senescent cells, are a new and exciting avenue. These drugs rely on specific vulnerabilities of senescent cells as compared with normal growing cells. For example, the senolytic compounds ABT-263 and ABT-737 target Bcl-2–family antiapoptotic proteins, which are up-regulated during senescence (Chang et al., 2016; Yosef et al., 2016). Although very promising, the senolytic drugs identified so far might be too toxic for clinical applications. An alternative approach to prevent the negative effects of senescence involves suppressing the SASP to reduce inflammation. Antiinflammatory drugs, although not devoid of side effects, are relatively safe and able to reduce the incidence of certain cancer types. By identifying HMGB2 as a factor governing heterochromatin spreading and SASP gene expression, the study by Aird et al. (2016) enhances our understanding of the epigenomic regulation of the SASP and sets the stage for the development of new therapies aimed at suppressing the inflammatory component of senescence.

Acknowledgments

We apologize to those whose papers have not been cited here due to space restrictions.

Research in J. Gil's laboratory is funded by core support from the Medical Research Council (grants MC-A652-5PZ00 and MC_U120085810).

The authors declare no competing financial interests.

Submitted: 13 October 2016
Accepted: 17 October 2016

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