Killing the old: cell senescence in atherosclerosis

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Atherosclerosis is a disease of aging, and the most common cause of death in the industrialized world. Cell senescence and the therapeutic removal of senescent cells using 'senolytics' are topical areas of science and translational medicine. A new study reports surprising findings on cell senescence and atherosclerosis with important therapeutic implications.

Refers to Childs, B.G. *et al.* Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science* **354**, 472-477 (2016).

Cell senescence is defined by the irreversible loss of proliferative capacity in cells that can normally divide. Cell senescence is driven by exhaustion of replicative potential, for example, by telomere shortening, or as a stress response — so-called 'stress-induced premature senescence'. Both types of senescence are characterized by withdrawal from the cell cycle, and a number of markers, which include upregulation of the cyclin-dependent kinase inhibitor $p16^{ink4a}$, and secretion of a panel of cytokines as part of the 'senescence-associated secretory phenotype' (SASP). In a recent article published in *Science*, Childs and colleagues showed that atherosclerotic plaques from Ldl-receptor null mice contain cells with activity of the pH-sensitive lysosomal enzyme 'senescence-associated-beta galactosidase' (SAβG), and mRNA encoding SASP cytokines[1]. SAβG-positive cells possessed some ultrastructural features of endothelial cells, vascular smooth muscle cells (VSMCs), and macrophages, were present at high concentrations in advanced lesions, but were also found

within only nine days of fat-feeding in areas predisposed to atherosclerosis. The investigators then used a series of transgenic mice containing reporter and/or suicide genes to identify and kill cells based on $p16^{ink4a}$ promoter activity[1]. Removal of cells expressing $p16^{ink4a}$ promoter activity reduced both plaque formation and progression, and promoted some features of stable plaques. These findings suggest that senescence might occur much earlier and be far more frequent than previously thought, that senescence promotes atherosclerosis through SASP proinflammatory cytokines, and that senolytic drugs might have therapeutic potential in atherosclerosis **(Figure 1)**.

Several surprising findings were reported by Childs and colleagues, that are particularly relevant to the specificity of identification and deletion of senescent cells in atherosclerosis. For example, previous studies have shown that advanced human plaques show evidence of senescence, including telomere shortening and SAβG activity[2], and that induction of senescence in human VSMCs induces an IL-1α-dependent SASP[3]. However, SAβG is not specific for senescent cells in mice or humans as it can also mark macrophages[4]. Indeed, most SAβG-positive cells in human plaques seem to be macrophages in the lesion core, with very infrequent intensely-positive VSMCs in the cap that also show other markers such as $p16^{ink4a}$, but no evidence of senescence in the media[2, 3]. Similarly, although cell lineage was not precisely identified nor cell proliferation examined, the SASP components used as senescence markers by Childs and colleagues, such as matrix metalloproteinases, tumour necrosis factor-α, and IL-1α[1], are all highly expressed by macrophages.

Childs and colleagues used a 'suicide' gene approach based on $p16^{ink4a}$ promoter activity to define the role of senescent cells at multiple stages of atherosclerosis. Suicide genes driven from a $p16^{ink4a}$ promoter have been used successfully to define the role of senescent cells in

both disease and normal ageing (reviewed in [5]). However, this technique relies on the transgenic $p16^{ink4a}$ promoter being specific for senescent cells, and not active in other cells (such as plaque macrophages). In contrast, $p16^{ink4a}$ is expressed in resident and inflammatory macrophages, including macrophage-rich areas of human atherosclerotic plaques[6], and is also upregulated when monocytes differentiate into macrophages[7], as occurs in atherosclerosis. $p16^{ink4a}$ can also regulate macrophage polarization, and promote inflammatory signalling in murine macrophages[8].

It is possible that mice show higher levels of cell senescence in atherosclerosis than humans, as aged mice and fat-fed apolipoprotein E $(Apoe)^{-/-}$ mice show an accumulation of telomere-associated DNA damage foci[9], another surrogate marker of senescence. However, these findings also raise the possibility that what the investigators identified as senescent cells might also be inflammatory macrophages, which are present in very early and advanced lesions. Indeed, macrophage ablation alone reduces plaque development and promotes features of plaque stability, particularly in less advanced lesions[10]. Phagocytic cells have also been shown to have SAβG activity in chronologically aged mice, whereas removal of these cells reduces the $p16^{ink4a}$ signal in $p16^{ink4a}$ reporter mice[4]. Together, these observations suggest that macrophages can substantially contribute to SAβG staining, and might be killed via suicide genes expressed from $p16^{ink4a}$ promoters. The precise frequency, lineage, and functional consequences of cell senescence in atherosclerosis thus remains to be determined.

A number of senolytic agents have been developed that selectively kill some types of senescent cells, which can mitigate premature aging of tissues containing those cells[5]. Genetic ablation of senescent cells can also attenuate age-related deterioration of several organs and prolong lifespan[5], raising the prospect that senolytic agents might reduce ageing-

associated diseases such as atherosclerosis. Indeed, the senolytic combination dasatinib plus quercetin improved acute vasomotor function but not arterial compliance in aged mice, and reduced markers of senescence in the media of fat-fed Apoe$^{-/-}$ mice[9]. However, dasatinib plus quercetin did not reduce senescence markers in the intima, and had no effect on plaque size or lipid content[9].

Whatever the specificity of the senescence markers or the identity of the deleted cells, this study has important implications for the use of senolytic agents in atherosclerosis. Removal of cells that have p16^{ink4a} promoter activity reduced plaque development and progression, and promoted features of stability. These effects were seen in multiple vascular beds, in a number of mouse models, and were not due to effects on serum lipids or peripheral blood counts. Local removal of cells that secrete multiple proinflammatory cytokines is an attractive therapeutic goal in atherosclerosis. Removal of p16^{+}/SAβG^{+} macrophages or other cell types that have p16 promoter activity might also be useful therapeutically for a variety of age-associated diseases, or indeed might promote longevity. Ageing has been considered a non-modifiable risk factor for atherosclerosis; however, these new findings by Childs and colleagues suggest that this may be about to change.

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Competing interests

The authors declare no competing interests.

Figure 1 | Effects of cell senescence in atherosclerosis. Senescent cells in atherosclerotic plaques are characterized by lack of proliferation, increased $p16^{ink4a}$ expression, senescence-associated β-galactosidase (SAβG) activity, and establishment of a senescence-associated secretory phenotype (SASP), which leads to secretion of multiple inflammatory cytokines, chemokines, and matrix-degrading proteases. The SASP drives inflammation directly, for example by translocation of IL-1α to the cell surface, and also activates adjacent vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and macrophages, leading to amplification of inflammation and accelerated atherogenesis through secondary pro-inflammatory cytokines. In addition, loss of normal VSMCs and increased protease activity results in reduced structural matrix and weakening of the fibrous cap, leading to plaque instability. Removal of $p16^{ink4a+}$ cells (for example through expression of $p16^{ink4a}$ promoter-driven suicide genes or senolytics) could slow atherogenesis and promote features of plaque stability.

Biographies

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Martin Bennett is a British Heart Foundation Professor of Cardiovascular Sciences and Consultant Cardiologist at Cambridge, UK. He undertook his PhD at ICRF In

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Murray Clarke is a British Heart Foundation Senior Fellow at the University of Cambridge, UK. He undertook his PhD in Edinburgh examining cell death in platelets, and then postdoctoral research in Cambridge. He is interested in the downstream consequences of cell death, and how this drives inflammation and its effects on vascular diseases, particularly through the IL-1 family.

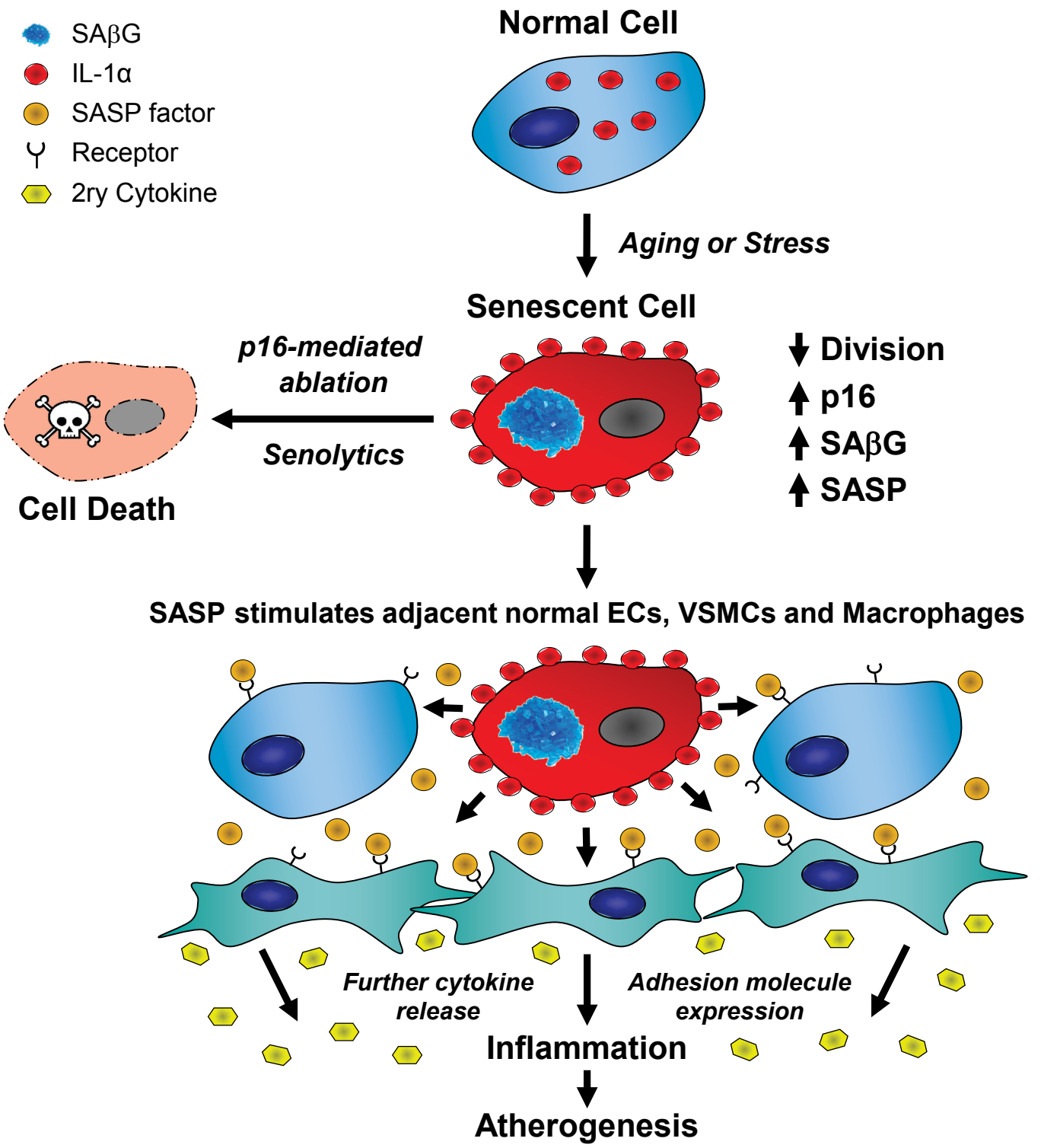

Figure 1: Effects of Cell Senescence in Atherosclerosis.
Senescent cells in atherosclerotic plaques are characterised by lack of proliferation, increased $p16^{ink4a}$ expression, senescence-associated β-galactosidase (SAβG) activity and establishment of a senescence-associated secretory phenotype (SASP), which leads to secretion of multiple inflammatory cytokines, chemokines and matrix-degrading proteases. The SASP drives inflammation directly, for example by translocation IL1α to the cell surface, and also activates adjacent vascular smooth muscle cells (VSMCs), endothelial cells (ECs) and macrophages, leading to amplification of inflammation and accelerated atherogenesis through secondary cytokines. In addition, loss of normal VSMCs and increased protease activity results in reduced structural matrix and weakening of the fibrous cap, leading to plaque instability. Removal of $p16^{ink4a+}$ cells, for example through expression of $p16^{ink4a}$ promoter-driven suicide genes or the use of senolytics, could slow atherogenesis and promote features of plaque stability.