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AUTHOR(S):
Kondoh, Hiroshi; Hara, Eiji

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Previews in *Cell Metabolism*

**Targeting p21 for diabetes: Another choice of senotherapy**

**Hiroshi Kondoh**$^1*$ and **Eiji Hara**$^{2,3,4}$*

$^1$Geriatric Unit, Graduate School of Medicine, Kyoto University,

Kyoto, 606-8507, Japan

$^2$Research Institute for Microbial Diseases, $^3$Immunology Frontier Research Center,

$^4$Center for Infectious Diseases Education and Research, Osaka University,

Suita, 565-0871 Japan.

*Correspondence: Hiroshi Kondoh (hkondoh@kuhp.kyoto-u.ac.jp) and Eiji Hara (ehara@biken.osaka-u.ac.jp)
Abstract

Senotherapy, elimination of senescent cells, is a cutting-edge treatment for aging-related and lifestyle diseases. In this issue of *Cell Metabolism*, Wang *et al.* report that p21Cip1 highly expressing cells, which represent a senescent cell population, occur in the adipose tissue during obesity. Targeting them genetically or pharmacologically attenuates insulin resistance, suggesting a possible therapeutic approach to treat the metabolic complications of obesity.
Main text

During organismal aging or progression of diseases, normal cells and tissues *in vivo* are subjected to various stresses, causing cellular damage, necessitating repair, adaptation, apoptosis or various defense responses. Cellular senescence is defined as a state in which cells suffer irreversible cell cycle arrest with functional decline due to senescence-inducing stresses (DNA damage, oncogenic stress, oxidative stress, etc) or due to telomere shortening. Senescent cells display several characteristic features, including enlargement with flattened morphology, change in nuclear structure, formation of foci expressing H2Aγ, increased expression of cell cycle inhibitors (p16INK4 and p21Cip1), and impaired proteostasis. Among other phenotypes, senescent cells are also characterized by enhanced secretion of proinflammatory cytokines and chemokines, resulting in what is known as senescence-associated secretory phenotype (SASP), which promotes development of aging-related diseases. Thus, senescent cells accumulate *in vivo* not only in aged tissues, but also in dysfunctional organs resulting from various lifestyle diseases (Wiley and Campisi, 2016). Recent advances in aging research suggest that elimination of senescent cells, known as senolysis, is a promising approach (senotherapy) to reduce chronic inflammation and to treat aging-related diseases.

In this issue of *Cell Metabolism*, Wang *et al.* reported the pathological significance of
senescent cells manifesting elevated expression of $p21^{\text{Cip1}}$ (p21-high) in murine visceral adipose tissues (VAT) (Wang et al. 2021). Their work was initiated by the discovery of p21-high cells in obese mice, using single-cell transcriptomic (SCT) analysis (Wang et al. 2021). These cells comprise pre-adipocytes, endothelial cells and macrophages. They also show that p21-high cells share features of senescence-like $p16^{\text{Ink4}}$-high-expressing cells (p16-high), including an enlarged cell morphology and inducement of chronic inflammation by SASP. However, Wang et al. noted the distinct appearance of tissues and stages between p21-high and p16-high cells (Figure 1). In the VAT of obese mice fed a high-fat diet (HFD), p21-high cells, but not p16-high, accumulate during the early stage of the feeding regimen (2 months), while both are observed later (10 months). Consistently, priming accumulations of $p21^{\text{Cip1}}$ protein are also observed in damaged liver (Sturmlechner et al. 2021). On the other hand, p16-high cells reside predominantly in the aged pancreas.

Earlier work observed that the aging phenotype in progeria mice was partly restored by $p16^{\text{Ink4}}$ deletion, but not by ablation of $p19^{\text{Arf}}$, which results in inactivation of the $p53/p21^{\text{Cip1}}$ pathway, and that genetic removal of p16-high cells has been established as an efficient senotherapy (Childs et al. 2015). However, the beneficial impact of $p21^{\text{Cip1}}$
deletion on aging-related events has been controversial (Cheng, et al. 2000). Even so, recent findings regarding subclasses of senescent cells (Ito et al. 2017) imply that senolytic targets other than p16\textsuperscript{Ink4} could be beneficial. Here, Wang et al. established a genetic approach to eliminate p21-high senescent cells \textit{in vivo} (Figure 1). Elimination of such cells in the VAT suppressed SASP phenotypes and improved both glucose tolerance and insulin sensitivity, although senolysis targeting of p16-high cells to alleviate insulin resistance is less efficient. Notably, inactivation of NF-kB pathway in p21-high senescent cells also results in improvement of glucose tolerance and insulin sensitivity, although p21-high cell number is comparable to control. They showed that the benefits of p21-high cell clearance was observed in short- or long-term induced obesity, female or male obesity and in fat-transplanted mice from human samples. Strikingly, in mice with fat transplanted from patients with obesity, pharmacological-based senotherapy using dasatinib plus quercetin (D+Q) that eliminated both p16-high and p21-high cells exerted effects similar to those resulting from elimination of p21-high cells alone (Figure 1).

Given the identification of ABT263 (an anti-Bcl-2 inhibitor) as a senolytic drug, the targets of senotherpy now include mechanisms involving cell cycle control, anti-apoptosis, autophagy, proteostasis, environmental or metabolism homeostasis and epigenetics (Di
Micco et al. 2021). Now, p21Cip1 can also be added to this list. Exploring p21-specific removal of aging cells might be beneficial in the treatment of some diseased organs, e.g., the VAT in obesity. Thus, p21Cip1 is a potential candidate for senotherapy in human diseases, as p21-high cells are responsible for NF-kB dependent inflammation (Wang et al. 2021). However, precautions should be taken in regard to future application of senotherapy for human diseases. As some senolytic compounds are repurposed anticancer drugs, profound side-effects cannot be overlooked. For example, ABT263 treatment provokes transient thrombocytopenia and neutropenia (Di Micco et al. 2021). Moreover, elimination of p16-high vascular endothelial cells disrupts blood-tissue barriers in mouse liver (Grosse et al. 2020). Notably, unlike p16Ink4, p21Cip1 has significant physiological functions in programmed cell senescence, (Muñoz-Espín et al. 2013). Therefore, careful consideration should be given to when and to which organs p21-high senolysis should be applied in order to minimize these complications.

Moreover, as SASP and senescent cells themselves also serve physiologically important functions in embryonic development, wound healing, tissue repair and tumor-suppression, it is important to distinguish beneficial subpopulations of senescent cells from other subpopulations that negatively impact organismal health. Interestingly, recent findings suggest a significant role of p21Cip1 in SASP, called the p21-activated secretory phenotype.
(PASP) (Sturmlechner et al. 2021). PASP functions as a marker for early immunosurveillance as the macrophage-attracting chemokine, CXCL14, is produced in p21-high cells, not in p16-high cells. Thus, in addition to chemical senolysis (e.g. D + Q), immunosurveillance-coupled senolysis could be a more efficient senotherapy than targeting only p21-high cells. New technology to eliminate senescent cells via immunological tools (e.g., via CAR-T cells) may help to resolve such questions in the future (Amor et al. 2020).

DECLARATION OF INTERESTS

The authors declare no competing interests
References


Figure 1. Distinct and overlapping outcomes of senescent cell clearance in visceral adipose tissues of obesity.

Elimination of p21-high senescent cells by genetic manipulation or chemical therapy is effective to treat diabetes with obesity, as p21-high cells accumulate in adipose tissues at early stages of a high-fat diet. Inactivation of NF-κB pathway in these cells is also beneficial like clearance of senescent cells. Created with BioRender.