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Brief Report

Transplanted Senescent Cells Induce an Osteoarthritis-Like Condition in Mice

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Abstract

Osteoarthritis (OA) is the leading form of arthritis in the elderly, causing pain, disability, and immobility. OA has been associated with accumulation of senescent cells in or near joints. However, evidence for a causal link between OA and cellular senescence is lacking. Here, we present a novel senescent cell transplantation model involving injection of small numbers of senescent or nonsenescent cells from the ear cartilage of luciferase-expressing mice into the knee joint area of wild-type mice. By using bioluminescence and 18FDG PET imaging, we could track the injected cells in vivo for more than 10 days. Transplanting senescent cells into the knee region caused leg pain, impaired mobility, and radiographic and histological changes suggestive of OA. Transplanting nonsenescent cells had less of these effects. Thus, senescent cells can induce an OA-like state and targeting senescent cells could be a promising strategy for treating OA.

Keywords: Senolytics—Cell transplantation—Bioluminescence imaging—Fluorodeoxyglucose—Mechanical allodynia

Osteoarthritis (OA) is one of the leading causes of pain and disability worldwide (1). It can greatly increase health care costs and reduce quality of life. The key characteristics of age-related OA in humans include damage of articular cartilage with joint space narrowing, osteophyte formation, subchondral bone alteration, and degeneration of soft tissues (2). Age is the leading predictor for developing OA. Other risk factors include increased body weight, female sex, diet, genetics, race, and injuries (3). Injury-induced OA can be modeled in mice by damaging joints surgically (2). However, modeling age- or senescence-associated OA, which may be distinct from injury-related OA (2), in mice has been challenging. So far, no disease-modifying drug has been approved to treat OA other than pain reducers, partly because etiological mechanisms of age-related OA have been poorly understood to date (2). Potential cellular mechanisms contributing to the development of OA include low-grade inflammation (4), chondrocyte alteration (5), mitochondrial dysfunction (6), loss of glycosaminoglycans (7), and dysregulated energy metabolism (8,9). In addition, a potential contribution by senescent cells has been suggested (10,11).

Cellular senescence refers to a state of stable arrest of cell proliferation in replication-competent but apoptosis-resistant cells (12,13). Senescent cells accumulate with aging in various tissues (14), including the articular cartilage (15). A variety of stimuli and stresses can cause senescence, including telomere shortening, genomic DNA damage, oncogenic insults, metabolic stresses, epigenetic changes, and mitochondrial dysfunction (12,16). One key feature of senescent cells is secretion of an array of pro-inflammatory cytokines, chemokines, and growth factors, termed the senescence-associated secretory phenotype (SASP) (17,18). The SASP is observed across a number of senescent cell types, including fibroblasts and mesenchymal stem cells (17,19). Although mounting evidence suggests that cellular senescence is associated with OA (10,11,20–22), whether this link is causal remains to be determined.
We transplanted senescent cells into the knee joint region of mice and found that these cells induced a phenotype with features resembling OA, including articular cartilage erosion, increased pain, and impaired function. To our knowledge, this is the first evidence suggesting that cellular senescence can actually cause OA. Our findings also imply that targeting senescent cells is a promising approach for preventing or treating OA.

Materials and Methods
Details about methods are included in Supplementary Information.

Results
Establishing a Senescent Cell Transplantation Model
To test if senescent cells cause an OA-like arthropathy, we injected either senescent or control nonsenescent fibroblasts into the knee joint region of mice. We transplanted seven mice with control cells and seven with senescent cells. We isolated mouse fibroblasts from ear clips of CAG-luc mice and induced cellular senescence with 10 Gy radiation. Twenty days after radiation, more than 95% of fibroblasts were senescence-associated β-galactosidase (SABG) positive, whereas less than 5% of nonradiated parallel control fibroblasts were SABG positive (Figure 1A). p16Ink4a and p21Cip1 transcript levels, two key cellular senescence markers, were significantly increased in the radiated fibroblasts (Figure 1B). To assess the SASP, we collected conditioned medium from radiated and control fibroblasts for 24 hours. Twenty-three cytokine and chemokine proteins were measured in conditioned medium using an addressable laser bead-based multiplex assay. We found that the secreted protein levels of 19 SASP components were significantly increased in conditioned medium collected from radiated fibroblasts compared with nonradiated control cells (Supplementary Table 1). The most highly induced SASP components were: granulocyte-colony stimulating factor...
Some damage to the nonsenescent cell-injected knees (Figure 2B) aged menisci, hallmarks of OA (Figure 2C, arrows). We did observe phyte formation, subchondral bone structure alteration, and damage to the growth plate (histology score = 16.8 ± 3.3); however, this damage was significantly less than in the senescent cell-transplanted knees (Figure 2E). These data indicate that injection of senescent cells in and around the joint space of the knee causes pain.

We next tested whether senescent cell transplantation into knees impaired locomotor function. We found that Rotarod performance was significantly decreased in the mice injected with senescent cells compared with animals injected with control nonsenescent cells or those that were not injected (Figure 2F). In addition, we found that mice injected with senescent cells moved less and traveled shorter distances than mice injected with control nonsenescent cells (Figure 2F and G). Thus, senescent cell transplantation causes reduced locomotor function in mice.

Discussion

OA is a major cause of pain, immobility, and disability in the elderly (1). No disease-modifying interventions are now in clinical use, with current treatments focused on pain control, mobility aides such as canes, braces, or walkers, or surgical joint replacement (25). Impeding progress in developing disease-modifying treatments is a lack of understanding of the initial pathogenesis of age-related OA and a lack of mouse models of this condition, as opposed to the surgical damage-induced OA in mice that models injury-associated OA. Here, we provide evidence that accumulation of senescent cells in and around previously healthy joints can actually cause an OA-like arthropathy in mice. This both provides a new model of OA and implies that clearing senescent cells with senolytics or interfering with their pro-inflammatory SASP could be a disease-modifying therapeutic option. A next step will be to test such interventions in our senescent cell-transplanted model.

One of the potential mechanisms by which senescent cells could induce an OA-like phenotype is through the SASP. OA is linked to inflammation (4) and immune cells have been found in early stage OA (26). IL-6, one of the key SASP components, is highly associated with OA progression (27,28). We found that the senescent cells we transplanted secreted 20 times more IL-6 than nonsenescent cells (Figure 1C and Supplementary Table 1). We previously reported that senescent cells increase immune cell chemotraction (19). Furthermore, immune cell chemokines, such as the macrophage chemottractant, MCP-1, were secreted by the senescent cells we transplanted (Supplementary Table 1). Thus, senescent cell-provoked immune cell infiltration and the inflammation that they, in turn, induce might contribute to the OA-like phenotype we observed. In addition, senescent cells can directly impair progenitor function through the SASP (29) and spread senescence to nearby cells (30,31), both of which might contribute to dysfunction of chondrocytes and therefore to OA. Moreover, transplanted senescent cells might persist longer than nonsenescent cells in vivo due to the fact that they are resistant to apoptosis (13), which could allow them more time to damage the knee.

In our model, we transplanted 2 × 10^6 ear fibroblasts into the knee joint region. It will be important to ascertain the minimum number of injected senescent cells sufficient to induce the OA-like phenotype. In addition, extensive studies comparing effects of different transplanted senescent cell types (such as chondrocytes or mesenchymal stem cells), dose responses, effects of gender, effects of recipient age, use of other species such as rats, persistence of different types of transplanted cells, use of different drugs such as senolytics and SASP inhibitors after transplantation, additive effects of stressing the joints...
around which senescent cells have been transplanted, time course of histological changes to the knee following senescent cell transplantation, and many other analyses remain to be done to fully characterize this model.

One of the limitations of our model is that the injection itself might cause minor damage to the joint, which we did observe in some of the nonsenescent cell-injected joints (Figure 2B). We speculate that some of the damage to the control nonsenescent cell-injected joints resulted from the injection itself or the fact that some of nonsenescent cells possibly become senescent in vivo after injection. However, the damage to the senescent cell-injected joints was significantly more severe (Figure 2B). Additionally, only senescent cell-injected knees exhibited mechanical allodynia (Figure 2E), indicating that senescent cells contribute to the pain that is a consequence of OA. Advantages of our model are that it is relatively simple and it does not require breeding of genetically modified mice. Also, animals can be followed in real time in vivo during development of OA or before and after administering interventions by functional and pain testing and luciferase and PET imaging. Specifically, senescent cell abundance can be followed. Moreover, our model is clinically relevant because senescent cells accumulate within the joints in both OA patients (22) and aged individuals (15).

The finding that cellular senescence can drive development of an OA-like state is consistent with the geroscience hypothesis—

![Figure 2](image-url)

**Figure 2.** Senescent cell transplantation induces osteoarthritis-like phenotypes and impairs function. Seven-month-old C57BL/6 female mice were subjected to control nonsenescent (CON) or senescent (SEN) primary ear fibroblast transplantation in the knee region. (A) Safranin O/Fast Green staining was performed 3 months after transplantation. Representative images (5x magnification of the knee joint) are shown. (B) Histology scores for the knee joints are shown as mean ± SEM (N = 5). (C) Representative radiographs are shown. (D and E) The von Frey filament assay was performed 3 months after transplantation. Paw withdrawal frequencies using both 0.16g (D) and 0.4g filaments (E) are shown as mean ± SEM (N = 7). (F) Rotarod assay was performed before and 1 month after transplantation. Results are shown as percent change in time to falling relative to baseline. Results (N = 7) are expressed as mean ± SEM. (G and H) Locomotor activity during 20 minutes of evaluation was monitored 3 months after transplantation. (G) Stationary time (ST) and active time (AT) are shown as mean ± SEM (N = 7). (H) Distance traveled is shown as mean ± SEM (N = 7).
fundamental aging mechanisms, of which cellular senescence is one, predispose to age-related disabilities and chronic diseases, such as OA. If correct, this would imply that senescent cell accumulation may not only predispose to OA, but to multiple other age-related conditions, as is increasingly appearing to be the case (19,29,32–34). The fact that we were able to detect senescent cells in vivo using \textsuperscript{18}FDG PET imaging without any genetic modification (Figure 1E) might open a potential avenue to assess senescent cell burden in humans with a variety of conditions involving local accumulation of senescent cells, such as in idiopathic pulmonary fibrosis or at sites subjected to therapeutic radiation.

A variety of anti-inflammatory agents have been used for OA, mainly for pain control. They have not proven to be effective in modifying disease progression (2). Furthermore, these agents have side effects and toxicity due to continuous administration (35). Therefore, a novel therapeutic strategy is needed. Our findings increase the likelihood that senolytic agents or SASP inhibitors will be disease modifying. Based on a hypothesis-driven drug discovery approach, we recently identified agents that eliminate senescent cells selectively—senolytic drugs (33,36). We predict that senolytics or SASP inhibitors such as ruxolitinib, which decreases IL-6 secretion and effects by senescent cells and also alleviates the senescent cell-induced stem cell dysfunction caused by TGFβ-related SASP factors (19,29), will delay, prevent, or alleviate OA. Consistent with this possibility, we found that senolytics attenuate age-related loss of glycosaminoglycans, a contributor to developing OA (7), from the intervertebral discs of progeroid mice (33). Moreover, senolytics are effective when administered periodically (33), likely because senescent cells do not of course divide and may be slow to re-accumulate once cleared in the absence of a strong continuing insult. We predict that senolytics may have fewer side effects than the anti-inflammatory agents currently used for controlling pain.

In summary, our study indicates that senescent cells can directly contribute to the pathogenesis of OA. We also present a novel model of OA, which is clinically relevant and allows us and others to test whether clearing senescent cells or interfering with the SASP alleviates or delays OA. Thus, our model could be useful for the OA research field in conjunction with current surgical injury-based models. Targeting senescent cells might be a promising way to delay, prevent, or treat OA.

Supplementary Material

Please visit the article online at http://gerontology.oxfordjournals.org to view supplementary material.

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Conflict of Interest

JK, TT, TP and Mayo Clinic have a financial interest related to this research. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and is being conducted in compliance with Mayo Clinic conflict of interest policies.

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