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# Targeting Cell Senescence to Improve Cardiac Regeneration

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## Background

"Cell senescence is a detrimental cell state triggered by stressful insults and certain physiological processes" (ie, oxidative stress, reactive oxygen species), whereby damaged cells exit the cell cycle permanently but remain metabolically active.[1] A key feature of senescent cells is that they produce and secrete proinflammatory factors, termed the *senescence-associated secretory phenotype* (SASP). Senescent cells accumulate in all tissues, including the heart, with aging and at etiological sites in multiple chronic diseases. Senescent cells also accumulate in the heart due to anthracycline-induced cardiotoxicity. In preclinical animal models, long-term persistence of senescent cells and their SASP contribute to pathophysiology and organ deterioration, including in the heart and cardiovascular system, and have been causally linked to decreased lifespan and health span.[2-4]

Through exploiting senescent cells' dependence on specific prosurvival pathways, a new class of agents called *senolytics* have emerged. Senolytics specifically kill senescent cells without affecting proliferating or quiescent differentiated cells.[5] Senolytic elimination of senescent cells has been shown to improve cardiovascular and physical function, to reduce frailty, and to increase lifespan and health span.[6-8] Moreover, initiating senolytics in later life can increase rates of survival.[8]

## Current Opportunities for Improvement

Senescence-associated pathways are important in several normal physiological processes, including wound healing, tissue regeneration, and development or morphogenesis. The effectiveness of senolytics on the heart, which has limited regenerative capacity, needs to be determined.

## Recent Developments

Aging impairs the heart's ability to repair and regenerate. As people age, their cardiac stem (ie, cardiac progenitor cells [CPCs]) become senescent, upregulating key markers of senescence, such as p16Ink4a and senescence-associated β-galactosidase, and markers of DNA damage, such phosphorylated histone 2AX. Senescent CPCs also possess critically short telomeres and an SASP.[9] Indeed, by the time a person is 75 years old, approximately 50% of their CPCs are senescent. Cardiac progenitor cells from older patients are dysfunctional, showing impaired growth, clonogenicity, and cardiomyogenic differentiation potential.[9] When senescent human CPCs were transplanted into a

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myocardial-infarcted mouse heart, there was decreased cardiac regeneration and cardiac function compared with when nonsenescent, cycling-competent CPCs were transplanted.[9]

Next, the effects of senolytics and global senescent cell removal on the aged heart were determined. In this experiment, 24- to 32-month-old wild-type mice were randomly assigned to vehicle or senolytic dasatinib and quercetin (D&Q) treatment, administered in 4 cycles at 3 consecutive days per cycle, with the cycles occurring 12 days apart. P16Ink4a messenger RNA expression decreased ($P < .05$) in the heart following D&Q treatment in older mice.[9] Morphometric analysis of heart sections showed that D&Q-treated mice had decreased fibrosis and hypertrophy and that senolytic treatment had induced compensatory cardiomyocyte renewal and replacement.[9] An increased number of smaller ventricular 5-ethynyl-2′-deoxyuridine–positive or Ki-67–positive cardiomyocytes were found, suggesting that these mice had cardiomyocytes that were immature and newly formed compared with vehicle-treated mice, which exhibited only rare, small 5-ethynyl-2′-deoxyuridine–positive or Ki-67–positive cardiomyocytes but a greater proportion of hypertrophied myocytes.[9] Finally, D&Q treatment rejuvenated the heart's regenerative potential, activating and increasing the number of CPCs.[9]

To test the effects of senescence and D&Q senolytics on different cell types of the human heart in vitro, a transwell insert, co-culture, stress-induced premature senescence human cell model system was developed. It showed that the co-culture of human senescent cells with human CPCs, induced pluripotent stem cell–derived cardiomyocytes, and endothelial cells leads to decreased survival and cell cycle activity.[9,10] Moreover, endothelial cells show impaired tube formation and migration.[10] By eliminating senescent cells, D&Q senolytics improved human CPC and induced-pluripotent stem cell–derived cardiomyocyte survival as well as DNA synthesis (Fig. 1).[9,10] Similarly, D&Q senolytics improved endothelial cell survival, migration, and tube formation (Fig. 2).[10] The mechanism of action supports the secretion of an SASP by senescent cells—especially plasminogen activator inhibitor 1, interleukin-6, and interleukin-8—which was abrogated with D&Q treatment.[9,10]

### Abbreviations and Acronyms

| | |
|---|---|
| CPC | cardiac stem/progenitor cells |
| D&Q | dasatinib and quercetin |
| SASP | senescence-associated secretory phenotype |

## Future Directions

The present findings support targeting senescence using senolytics to prevent, delay, and treat multiple age-related heart disorders as well as the toxic and senescence-inducing effects of cancer chemotherapy on the heart.

Clinical trials on senolytics are already underway. The Translational Geroscience Network in the United States is conducting 15 clinical studies on senolytics for age-related conditions. They have developed assays for measuring biomarkers in the blood and tissues that can be used to test the efficacy of senolytics in the proposed trials and to identify people who are most likely to benefit from senolytic therapy. Research into understanding how senolytics act on the human heart, in clearing senescent cells, or whether they have any off-target side effects is greatly needed.

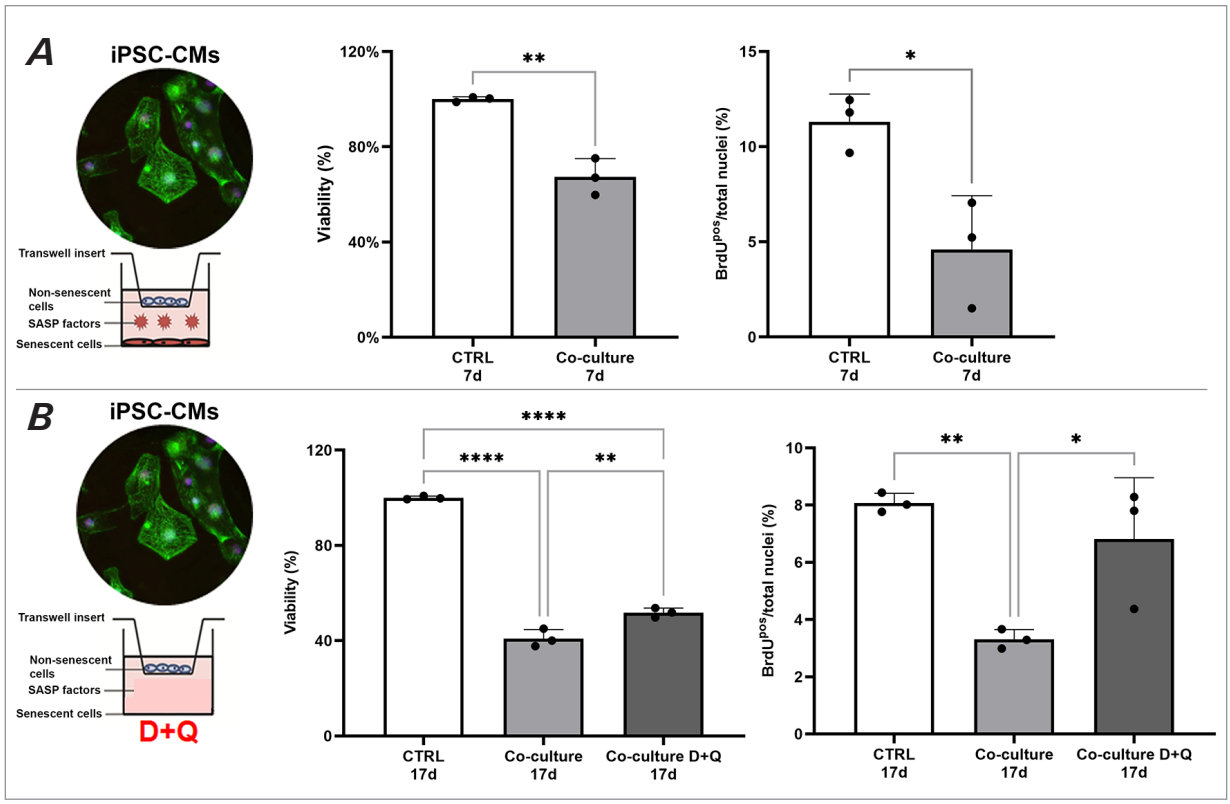


**Fig. 1** Senolytic treatment improves survival and DNA synthesis of human iPSC-derived cardiomyocytes. **A**) iPSC-CMs were co-cultured with senescent CPCs. Graphs show iPSC-CM crystal violet staining (viability) and BrdU-positive staining when cultured alone (CTRL 7d) or co-cultured with senescent CPCs (co-culture 7d) for 7 days. Data are mean (SD). Significance was determined by t test, *$P$ < .05, **$P$ < .01. Individual data points represent independent replicates/wells. **B**) iPSC-CMs were co-cultured with senescent CPCs and treated with D&Q senolytics to remove senescent CPCs. Graphs show the percentage of crystal violet–stained iPSC-CMs (viability) and BrdU-positive iPSC-CMs when cultured alone (CTRL 17d), co-cultured with senescent CPCs (co-culture 17d), or co-cultured with senescent CPCs and treated with D&Q (co-culture D&Q 17d) for 17 days. Data are mean (SD). Significance was determined by ANOVA, followed by Tukey post hoc test to identify the differences. *$P$ < .05, **$P$ < .01, ****$P$ < .0001. Individual data points represent independent replicates/wells.

ANOVA, analysis of variance; BrdU, bromodeoxyuridine; CPC, cardiac progenitor cell; D&Q, dasatinib and quercetin; iPSC-CM, induced pluripotent stem cell–derived cardiomyocyte; SASP, senescence-associated secretory phenotype.

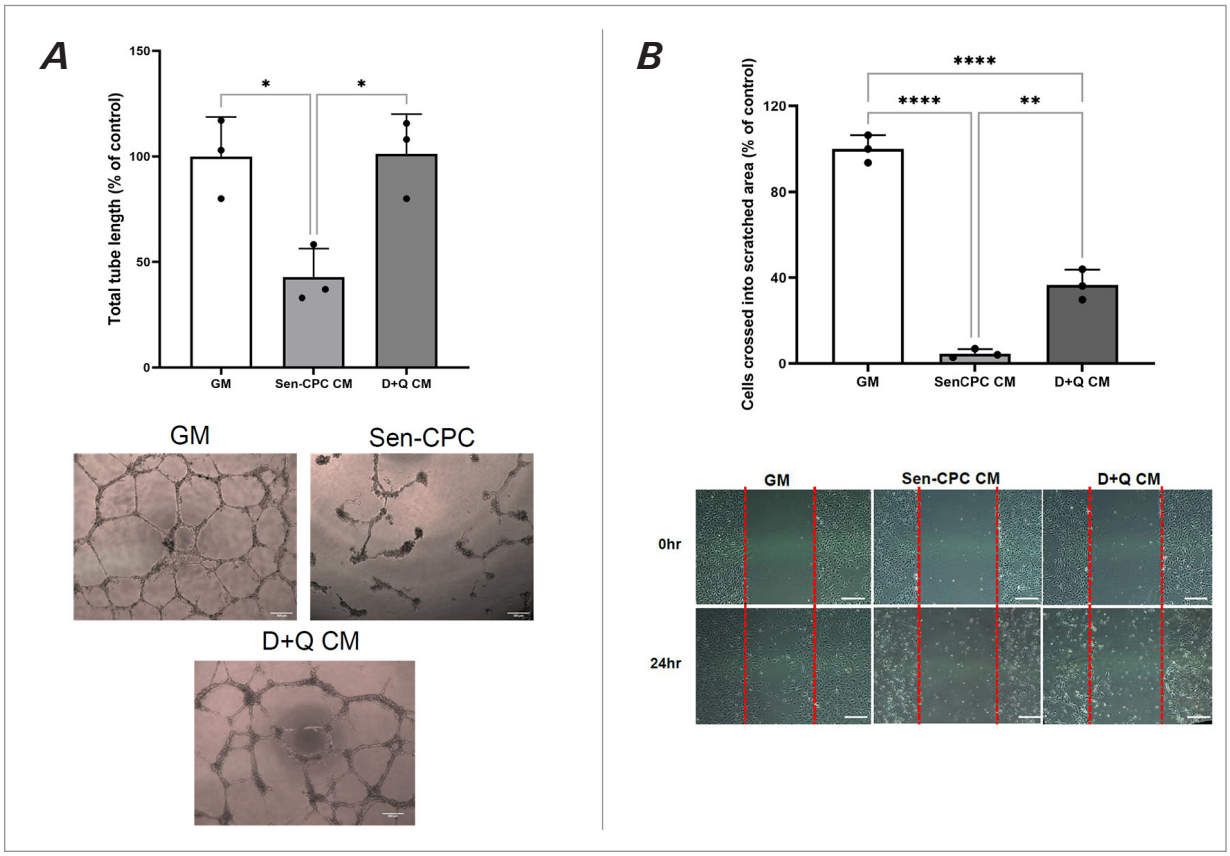


**Fig. 2** Senolytic treatment improves angiogenesis. **A**) Total tube length formed by HUVECs when cultured in normal GM, Sen-CPC CM, and D&Q CM. Data are a mean (SD) percentage of the control group (GM). Significance was determined by ANOVA, followed by Tukey post hoc test to identify the differences. *$P < .05$. Individual data points represent independent replicates/wells. Representative field of view micrograph images of HUVEC tube formation. Scale bar = 200 μm. **B**) HUVEC migration measured by the scratch assay when supplemented with normal GM, Sen-CPC CM, and D&Q CM for 24 hours. Data are a mean (SD) percentage of the control group (GM). Significance was determined by ANOVA, followed by Tukey post hoc test to identify the differences. ****$P < .0001$, **$P < .01$. Representative field of view micrograph images of the scratch (red dotted lines) in the different media conditions.

ANOVA, analysis of variance; D&Q CM, dasatinib and quercetin–conditioned media; GM, growth media; HUVEC, human umbilical vein endothelial cell; Sen-CPC CM, senescent cardiac progenitor cells–conditioned media.

## Article Information

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