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Application of senescence reporter mouse models in panvascular diseases

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Abstract

Vascular ageing accelerates panvascular diseases such as atherosclerosis, age-related arterial stiffening, pulmonary hypertension and cerebral microvascular dysfunction, yet the causal roles of senescent cells remain uncertain. This review aims to clarify those roles by systematically mapping the technological evolution, functional features and disease-specific applications of senescence reporter mouse models. We categorize senescence reporter mouse models into senescence tracing models and senescence eliminating models, and track three successive generations that progressively increased temporal, spatial and functional specificity. First-generation luciferase lines enabled non-invasive whole-body imaging; second-generation fluorescent reporters delivered single cell resolution; third-generation dual recombinase systems achieved lineage tracing and conditional clearance. In parallel, elimination platforms advanced from global ATTAC (apoptosis through targeted activation of Caspase) and trimodality reporter constructs to cell-type-restricted designs. Across atherosclerosis, arterial stiffening, pulmonary vascular remodeling and cerebral microvascular dysfunction, targeted removal or longitudinal tracking of cyclin-dependent kinase inhibitor 2A, CDKN2A ($p16^{Ink4a}$) or cyclin-dependent kinase inhibitor 1A,

CDKN1A ($p21^{Waf1/Cip1}$) positive cells revealed both shared and tissue-specific pathogenic mechanisms, and provided genetic gold standards for benchmarking senolytic drugs. We highlight remaining gaps, particularly the reliance on single biomarkers (p16, p21, p53) that may miss heterogeneous senescent subsets, and propose integrating multi-omics profiling, artificial intelligence-assisted image analysis and next-generation dual recombinase tools to refine mechanistic insight and enable precise anti-senescence interventions in panvascular disease.

Keywords: Vascular ageing, cellular senescence, senescence reporter mice, dual recombinase systems, panvascular diseases

INTRODUCTION

Panvascular disease refers to a group of systemic vascular disorders characterized by atherosclerosis as a common pathological basis, involving large, medium, and micro-vessels and affecting vital organs such as the heart, brain, kidneys, limbs, and aorta. Representative conditions include coronary artery disease, ischemic stroke, peripheral artery disease, aortic aneurysm or dissection, and pulmonary hypertension (PH)[1]. With the acceleration of global population aging, the incidence and mortality rates of panvascular diseases continue to rise, becoming a significant threat to human health. As vascular aging is recognized as a critical component that accelerates panvascular disease progression[2,3], a deeper understanding of its mechanisms is essential for the prevention and treatment of panvascular diseases.

Vascular aging is a complex biological process involving multiple cell types and signaling pathways, with cellular senescence at its core. Cellular senescence is a stable and irreversible cell cycle arrest induced by factors such as DNA damage, oxidative stress, telomere attrition, and chronic inflammation[4]. Senescent cells also secrete various inflammatory cytokines, matrix metalloproteinases (MMPs), and growth factors, collectively termed the senescence-associated secretory phenotype (SASP)[5]. Consequently, several biomarkers, including cell cycle inhibitors [cyclin-dependent kinase inhibitor 2A, CDKN2A ($p16^{Ink4a}$) or cyclin-dependent kinase inhibitor 1A, CDKN1A ($p21^{Waf1/Cip1}$)], senescence-associated β-galactosidase (SA-β-Gal), and autophagy-related markers, are commonly used to detect cellular senescence[6].

Although these senescence markers have illuminated essential features of ageing, they fall short of accurately reflecting the complex microenvironment of vascular tissues *in vivo*. Moreover, the causal relationship between senescent cells and panvascular diseases, whether senescent cells drive pathology or result from disease progression, has remained a longstanding challenge. Consequently, senescence reporter mouse models have emerged, combining senescence marker promoters with various functional elements, enabling real-time and dynamic quantitative detection of senescent cells *in vivo* and providing excellent tools for studying cellular senescence in panvascular diseases.

Senescence reporter mouse technologies are categorized based on their functional characteristics into tracing mouse models and eliminating mouse models. To our knowledge, both categories have undergone three generations of technological evolution, continuously enhancing temporal and spatial specificity, and finally functional specificity aimed at monitoring and manipulating biological processes. This evolution reflects the integration of precision medicine concepts into senescence research, shifting from simple marker detection to systematic analyses of complex biological processes and laying the foundation for studying senescence mechanisms and therapeutic strategies.

Senescence reporter mouse models have demonstrated significant value in studying vascular aging across diverse disease contexts, ranging from atherosclerosis, aortic disease, pulmonary arterial disease to

cerebrovascular dysfunction. However, systematic comparative analyses of their technical features, applicable scopes, and limitations remain lacking. To address this gap, we summarize the development of senescence reporter mouse models, highlight their applications in panvascular disease research, and discuss their advantages and limitations, providing perspectives for future advances in vascular aging studies.

FUNCTIONAL AND MOLECULAR CHARACTERISTICS OF VASCULAR AGING

Vascular aging refers to the age-related remodeling of vascular structure and function. Structural remodeling of aging vessels is characterized by increased vessel wall thickness, decreased elasticity, and lumen dilation[7-9]. Functionally, senescent endothelial and smooth muscle cells alter the vasoconstriction and vasodilation capabilities of blood vessels[10]. Structural and functional changes of aging vessels are interrelated, with vessel diameter negatively correlated with vasodilation function, and alterations in vasoconstriction and vasodilation directly influencing vessel wall diameter and tension.

Senescent vascular cells represent the core pathological mechanism of vascular aging, comprising replicative and stress-induced senescence. Replicative senescence results from telomere shortening due to repeated cell division, whereas stress-induced senescence arises from exposure to inflammatory molecules, oxidative stress, and unhealthy lifestyle factors[11,12]. Cellular senescence triggers DNA damage and is accompanied by upregulation of p53-dependent cyclin-dependent kinase inhibitors, such as $p21^{Wafl/Cip1}$, or $p16^{Ink4a}$, or both[13-15]. Thus, genes such as $p16^{Ink4a}$, $p21^{Wafl/Cip1}$, p53, and galactosidase beta 1 (GLB1, encoding SA-β-Gal) have frequently been utilized as core elements for constructing senescence reporter mouse models[16].

DEVELOPMENT AND CHARACTERISTICS OF SENESCENCE REPORTER MOUSE MODELS

The rapid advancement of *in vivo* imaging systems (IVIS) provides researchers with a novel technological platform for real-time, visual tracking of senescent cells at molecular, cellular, and tissue levels in small animals[17]. Typically, researchers insert reporter genes downstream of senescence marker gene sequences to create senescence reporter mouse models. Upon the onset of cellular senescence, the promoters of these marker genes are activated, driving the expression of reporter genes and generating detectable signals.

Senescence reporter mouse models are primarily classified based on their functions into tracing mouse models and eliminating mouse models. Tracing mouse models are used for real-time monitoring of senescent cells and lineage tracing, while eliminating mouse models investigate the roles of senescent cells in aging and age-related diseases and evaluate the therapeutic benefit of clearing senescent cells.

The first-generation tracing mouse models typically feature single functional elements. Since $p16^{Ink4a}$ is widely recognized as a senescence marker, Yamakoshi *et al.* constructed a humanized $p16^{Luc}$ reporter mouse in 2009, linking the human $p16^{Ink4a}$ promoter and regulatory sequences with a luciferase gene, allowing real-time monitoring of human $p16^{Ink4a}$ expression *in vivo*[18]. Researchers have also explored other senescence-related genes, optimizing expression sites and creating mouse-derived $p16^{Luc}$, mouse-derived $p21^{Luc}$, and p53 response element-driven luciferase reporter ($p53RE^{Luc}$) models[19-21]. Despite pioneering the tracking of senescent cells, luciferase-based systems faced limitations such as unstable luminescence, restricted spatial resolution, and inability to study individual senescent cells, leading to the development of the second-generation fluorescent protein-based models[22]. Liu *et al.* constructed the $p16^{tdTom}$ reporter mouse, dynamically tracking tandem dimer Tomato (tdTomato, tdTom) positive cells across tissues with aging and validating senescent features of tdTom positive macrophages via flow cytometry in inflammation-induced models[23]. Similar strategies were subsequently employed for other senescence markers, optimizing fluorescent protein efficiency and generating models such as p53 response element-

driven enhanced green fluorescent protein reporter (p53RE^{EGFP}) models, mitochondria-targeted Keima (mt-Keima), and Glb1-2A-mCherry (GAC)[24-26]. Secondary senescence arises when senescent cells secrete SASP factors that, via paracrine signaling, drive neighboring cells into a similar arrested state. However, conventional reporter-gene mouse models driven by a single molecular marker cannot reliably distinguish primary from secondary senescent cells in tissue sections or at the whole-organ level[27,28]. To overcome this, Sogabe *et al.* established two complementary inducible mouse systems: oncogene-induced senescence (OIS) and stress-induced senescence (SIS), by selectively activating the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway or the p38-MAPK pathway, respectively, thereby allowing discrete *in vivo* discrimination and analysis of these two senescence programs[29]. According to the inability of p16^{tdTom} to perform lineage tracing, Zhao *et al.* developed the dual recombinase-based Sn-pTracer system, where tdTomato expression is initiated only when both Dre and Cre recombinases are simultaneously expressed, providing a precise new perspective for understanding the heterogeneity of senescence mechanisms[30].

Senescence eliminating mouse models have progressively achieved spatiotemporal and cell-specific regulation, largely due to the mature application of the Cre-LoxP system[31]. Early INK-ATTAC (Ink4a-driven apoptosis through targeted activation of caspase) models first enabled specific targeting and elimination of p16^{Ink4a} positive senescent cells, utilizing FK506-binding protein-fused caspase-8 (FKBP-Caspase-8) fusion proteins and enhanced green fluorescent protein (EGFP) reporter genes under the p16^{Ink4a} promoter and AP20187 (a synthetic FKBP - binding chemical dimerizer, used to induce dimerization and activation of FKBP-fused proteins)-induced apoptosis[32]. Subsequent advancements included p21-ATTAC mice developed by Chandra *et al.*[33]. The p16-3MR (trimodality reporter) system developed by Demaria *et al.*, integrating luciferase (LUC), monomeric red fluorescent protein (mRFP), and herpes simplex virus type 1 thymidine kinase (HSV-TK) to simultaneously identify, isolate, and selectively clear senescent cells via ganciclovir (GCV) sensitivity[34]. Building on the 3MR system, investigators have integrated the cassette under the control of various senescence-associated promoters, such as p21[35]. Nevertheless, these clearance approaches exhibit limited efficacy and specificity in targeting and eliminating senescent cells[36]. Grosse *et al.* further refined control using single recombinase Cre systems driven by the p16^{Ink4a} promoter, enabling permanent labeling and precise manipulation of senescent cells[37]. Zhao *et al.* further enhanced specificity and precision with their innovative dual recombinase Sn-cTracer system, achieving continuous lineage recording and genetic elimination of p16^{Ink4a}-positive cells, marking a pivotal shift from general to precise regulation in senescence-elimination mouse technology[30]. The article summarizes the strain of senescence reporter mouse models in Table 1 and Figure 1.

Tracing models: p16^{Luc} allows whole-body bioluminescent imaging[18]; p16^{tdT} enables single-cell fluorescence detection[23]; and dual-recombinase systems permit tamoxifen-inducible lineage tracing[30]. Elimination models: INK-ATTAC mediates AP20187-induced caspase-8 - dependent apoptosis[32]; p16-3MR enables GCV-driven HSV-TK - based clearance[34]; and the dual-recombinase systems allow diphtheria toxin - induced ablation[30]. All immunofluorescence images in Figure 1 were obtained from a published paper using senescence reporter mice.

Despite the power of next-generation senescence reporter and elimination systems, several technical challenges remain. First, incomplete penetrance is a known limitation, not all senescent cells in a given tissue are labeled or eliminated by the transgene. This limitation often stems from the genomic insertion site and promoter configuration of reporter constructs[46]. For instance, Liu *et al.* generated a p16^{tdTom} knock-in model by replacing one p16^{Ink4a} allele, which resulted in hemizygous expression and partial interference with endogenous gene function[23]. In contrast, Zhao *et al.* inserted the fluorescent cassette into the

Table 1. Development and characteristics of senescence reporter mouse

Model type	Generation	Reporter element	Model name	Construction	Optimal application	Limitation	Authors (year)	References
Tracing mouse models	1st	Luciferase	$p16^{Luc}$	Luciferase reporter under the exogenous $p16^{Ink4a}$ promoter	Real-time imaging of human $p16^{Ink4a}$ promoter activity	May not fully recapitulate mouse endogenous regulation	Yamakoshi *et al.*, 2009	[18]
				Luciferase reporter under the murine $p16^{Ink4a}$ promoter	Endogenous $p16^{Ink4a}$ reporter enabling senescence monitor	Knock-in disrupts endogenous p16 function	Burd *et al.*, 2013	[19]
			$p21^{Luc}$	Luciferase reporter under the exogenous $p21^{Waf1/Cip1}$ promoter	First transgenic p21 reporter enabling *in vivo* visualization of p21 dynamics	Driven by truncated promoter and random-copy insertion	Ohtani *et al.*, 2007	[20]
				Luciferase reporter under the murine $p21^{Waf1/Cip1}$ promoter	Endogenous knock-in reporter allowing physiological p21 promoter activity	Knock-in reduces p21 dosage, potentially altering DNA damage responses	Tinkum *et al.*, 2011	[38]
				LacZ and Fluc integration at the p21 locus	Dual-reporter system combining luciferase for whole-body imaging	Reduced p21 expression from reporter allele	McMahon *et al.*, 2016	[39]
			$p53RE^{Luc}$	Reporter under the p53 responsive P2 element of the MDM2 promoter	Real-time bioluminescent monitoring of p53 oscillatory behavior following ionizing radiation	Restricted to MDM2 regulatory context	Hamstra *et al.*, 2006	[21]
				Reporter under the p53 responsive element of the PUMA promoter	Visualization of chemically induced DNA damage and tissue-specific p53 activation *in vivo*	Reporter driven by a single p53-responsive element	Briat *et al.*, 2008	[40]
	2nd	EGFP	$p53RE^{EGFP}$	EGFP expression under p53 core response elements from the p21 and PUMA promoters	Dual-target design distinguishes different downstream outputs of p53 and allows single-cell visualization	Limited coverage of p53 signaling	Demidenko *et al.*, 2012	[24]
		tdTomato	$p16^{tdTom}$	tdTomato knock-in at exon 1α of the endogenous $p16^{Ink4a}$ locus	Enables single-cell visualization and isolation of $p16^{Ink4a}$ high cells	Knock-in reduces transcript stability	Liu *et al.*, 2019	[23]
		Keima	Mt-Keima	pH-sensitive fluorescent protein Keima targeting of mitochondrial autography	Enables robust *in vivo* quantification of mitophagy across tissues	pH-dependent fluorescence spectra overlap reduces precision	Sun *et al.*, 2017	[25]
		mCherry	Glb1-2A-mCherry (GAC)	mCherry knock-in at the Glb1 stop codon	Converts classical SA-β-Gal activity into an *in vivo* fluorescent reporter	SA-β-Gal/Glb1 as a single marker which is not fully specific for senescence	Sun *et al.*, 2022	[26]
		H2B-GFP	INKBRITE	H2B-GFP expression under the $p16^{Ink4a}$ promoter	Provides the most sensitive and specific detection of low-level p16 activation *in vivo*	Restricted to lung epithelial/mesenchymal contexts	Reyes *et al.*, 2022	[41]
		mCherry	OIS	Constitutively active MEK1 (caMEK1) expression under a doxycycline-inducible Tet-On promoter with IRES-mCherry reporter	The first to discriminate primary and secondary senescence *in vivo*	With insufficient assessment of other tissues, cell types, and temporal changes	Sogabe *et al.*, 2025	[29]
		mCherry	SIS	Constitutively active MKK6 (caMKK6) expression under a doxycycline-inducible Tet-On promoter with IRES-mCherry reporter				
	3rd	Cre-Dre	Sn-pTracer	tdTomato expression activated only in	Lineage trace $p16^{Ink4a}$+ cells fate in a cell-	Need cell type-specific Dre mouse lines	Zhao *et al.*,	[30]

				cells co-expressing Dre and Cre recombinases	type specific manner	and long-term multiple times of Tam treatment	2024	
Eliminating mouse models	1st	ATTAC	INK-ATTAC	FKBP-Caspase-8-EGFP expression under the $p16^{Ink4a}$ promoter with AP20187-inducible dimerization-triggered apoptosis	The first time induces $p16^{Ink4a}$+ senescent cells *in vivo*	Missing p16-independent senescent populations and restricting tissue coverage	Baker *et al.*, 2011	[32]
			p21-ATTAC	FKBP-Caspase-8-EGFP expression under the $p21^{Waf1/Cip1}$ promoter with inducible apoptosis	Selective clearance of $p21^{Waf1/Cip1}$+ senescent cells	Utility in aging or other senescence-associated disorders remains untested	Chandra *et al.*, 2022	[33]
			p16-LOX-ATTAC	INK-ATTAC crossed with cell-type - specific Cre for lineage-restricted senescent cell apoptosis	Provides cell type - specific senolysis	Effects were only partially reproduced by local senolysis	Farr *et al.*, 2023	[42]
	2nd	3MR	p16-3MR	Tricistronic LUC, mRFP and HSV-TK expression under the $p16^{Ink4a}$ promoter with Ganciclovir-induced apoptosis	First model enabling *in vivo* detection and selective elimination of $p16^{Ink4a}$+ senescent cells	With limited organ scope and insufficient temporal analysis of dynamic senescence states	Demaria *et al.*, 2014	[34]
			p21-3MR	Tricistronic LUC, mRFP and HSV-TK expression under the $p21^{Waf1/Cip1}$ promoter with Ganciclovir-induced apoptosis	First model enabling *in vivo* detection and selective elimination of $p21^{Waf1/Cip1}$+ senescent cells	GCV-mediated clearance did not consistently reduce inflammation, and was tested with limited SASP markers	Yi *et al.*, 2023	[35]
		ATD	p21-ATD	AkaLuc, tdTomato and DTR expression under the $p21^{Waf1/Cip1}$ promoter, enabling flow sorting and DTR-mediated apoptosis	Improves upon p21-3MR by allowing simultaneous monitoring, sorting, and selective ablation of $p21^{+}$ senescent cells with higher specificity	Current validation is largely liver-restricted	Chen *et al.*, 2024	[43]
	3rd	Cre	p16-Cre	Cre knock-in at the endogenous p $p16^{Ink4a}$ locus, excising floxed targets in all p16 positive cells	Enables ablation of endogenous p16 high cells	Non-selective clearance disrupts tissue integrity and causes fibrosis	Grosse *et al.*, 2020	[37]
			p16-Cre^{ERT2}-tdTomato	Tamoxifen-induced tdTomato expression in p16+ cells via p16-Cre^{ERT2} × ROSA26-lsl-tdTomato cross	Enables precise lineage tracing without developmental confounding	May cause leakiness or incomplete labeling, limiting efficiency and specificity in long-term studies	Omori *et al.*, 2020	[44]
			p21-Cre	Cre knock-in at the endogenous $p21^{Waf1/Cip1}$ locus, excising floxed targets in p21+ cells	Specifically monitor, trace, and intermittently clear p21 high cells *in vivo*	Targets only a small subset of cells that accumulate with aging	Wang *et al.*, 2021	[45]
		Cre-Dre	Sn-cTracer	DTR expression activated only in cells co-expressing Dre and Cre recombinases	Enables seamless tracing and ablation of p16+ cells in a cell type - specific manner through Cre lines	Cannot perform pulse - chase lineage tracing or precise gene manipulation of p16+ cells in specific lineages	Zhao *et al.*, 2024	[30]

EGFP: enhanced green fluorescent protein; Fluc: firefly luciferase; HSV-TK: herpes simplex virus type 1 thymidine kinase; caMEK1: constitutively active MEK1; caMKK6: constitutively active MKK6; Tet-On: tetracycline-inducible promoter system; IRES: internal ribosome entry site; DTR: diphtheria toxin receptor; lsl: loxP-STOP-loxP; mt-Keima: mitochondria-targeted Keima; tdTomato: tandem dimer Tomato; SA-β-Gal: senescence-associated β-galactosidase; SASP: senescence-associated secretory phenotype; LUC: luciferase; mRFP: monomeric red fluorescent protein; OIS: oncogene-induced senescence; SIS: stress-induced senescence; INK-ATTAC: Ink4a-driven apoptosis through targeted activation of Caspase; PUMA: p53 upregulated modulator of apoptosis; FKBP-Caspase-8: FK506-binding protein - fused caspase-8; ATD: AkaLuc, tdTomato and DTR; LOX: lysyl oxidase; INKBRITE: Ink4a-driven bright reporter of inducible tissue expression.

3'-untranslated region, preserving full p16 transcriptional integrity and achieving more stable and consistent reporter activity across tissues[30]. Even under uniform induction conditions, variation in promoter activity, chromatin accessibility, or epigenetic silencing can further lead to under-representation of senescent cells in the readout[47]. Second, the heterogeneous pharmacokinetics of inducing agents across vascular beds compromises uniform clearance efficiency. Agents such as AP20187 or GCV show organ-specific absorption, metabolism, and clearance rates differences that lead to variable apoptosis efficiency between liver, lung, and heart vasculature[48,49]. Third, reliance on a single biomarker restricts detection scope, since many senescent populations do not synchronously express canonical markers. For example, in a β-cell model using INK-ATTAC mice, residual senescent populations remained after $p16^{Ink4a+}$ cell clearance, indicating incomplete coverage of all senescent subsets[50]. Collectively, these limitations highlight that while current reporter and elimination models have greatly advanced our understanding of vascular ageing, further refinement is required to achieve a comprehensive and accurate depiction of senescent cell dynamics *in vivo*.

APPLICATION OF SENESCENCE REPORTER MOUSE MODELS IN PANVASCULAR DISEASES

Panvascular disease research is grounded in systems biology, emphasizing the systemic and integrative nature of vascular pathologies, covering common features across cardiovascular, cerebrovascular, peripheral vascular systems, and their target organs. By elucidating the interconnections and interactions between diverse vascular pathological processes, it facilitates the establishment of comprehensive strategies for global vascular health regulation[51]. Vascular aging represents a pivotal driving factor in panvascular disease progression; thus, clarifying its underlying mechanisms is critical for intervening in disease development. Senescence reporter mouse models provide powerful technological platforms to unravel the molecular and cellular roles of senescence in panvascular diseases, as we illustrate in Figure 2.

The central illustration depicts senescence reporter mice used for *in vivo* tracing or elimination of senescent cells. Surrounding segments highlight representative disease contexts and mechanistic insights revealed by these models, including atherosclerosis, age-related arterial stiffness and endothelial dysfunction, pulmonary hypertension, and cerebral microvasculopathy. Besides, p16-3MR also serves as a genetic and pharmacologic platform for evaluating senolytics. Together, they demonstrate how reporter mouse models link cellular senescence to functional and pathological alterations across the vascular continuum.

Atherosclerosis

Age is an independent risk factor for the development of atherosclerosis[52]. Recent studies have further demonstrated that senescent cells are not merely a consequence of disease but actively promote plaque formation and progression[53]. Within major arteries such as the coronary arteries, senescent cells predominantly localize to the fibrous caps and margins of the necrotic cores within atherosclerotic lesions. Senescent vascular smooth muscle cells (VSMCs) and foam macrophages in these areas extensively express cell-cycle inhibitors such as $p16^{Ink4a}$, associated with proliferative arrest and secretion of SASP factors[54]. During the early stages of lesion formation, these cells release pro-inflammatory cytokines and chemokines, driving local inflammation. In advanced lesions, they produce MMPs, weakening fibrous cap integrity and increasing the risk of plaque rupture. Moreover, once VSMCs become senescent, their migratory and proliferative capacities are impaired, compromising fibrous cap repair. Notably, Childs *et al.* reported that senescent cells within plaques secrete insulin-like growth factor-binding protein-3 (IGFBP3), antagonizing insulin-like growth factor 1 (IGF-1) signaling, thereby inhibiting the recruitment and extracellular matrix deposition by VSMCs, leading to fibrous cap thinning. Selective clearance of these $p16^{high}$ senescent cells reduced IGFBP3 levels, enhanced VSMC-mediated fibrous cap repair, and significantly improved plaque

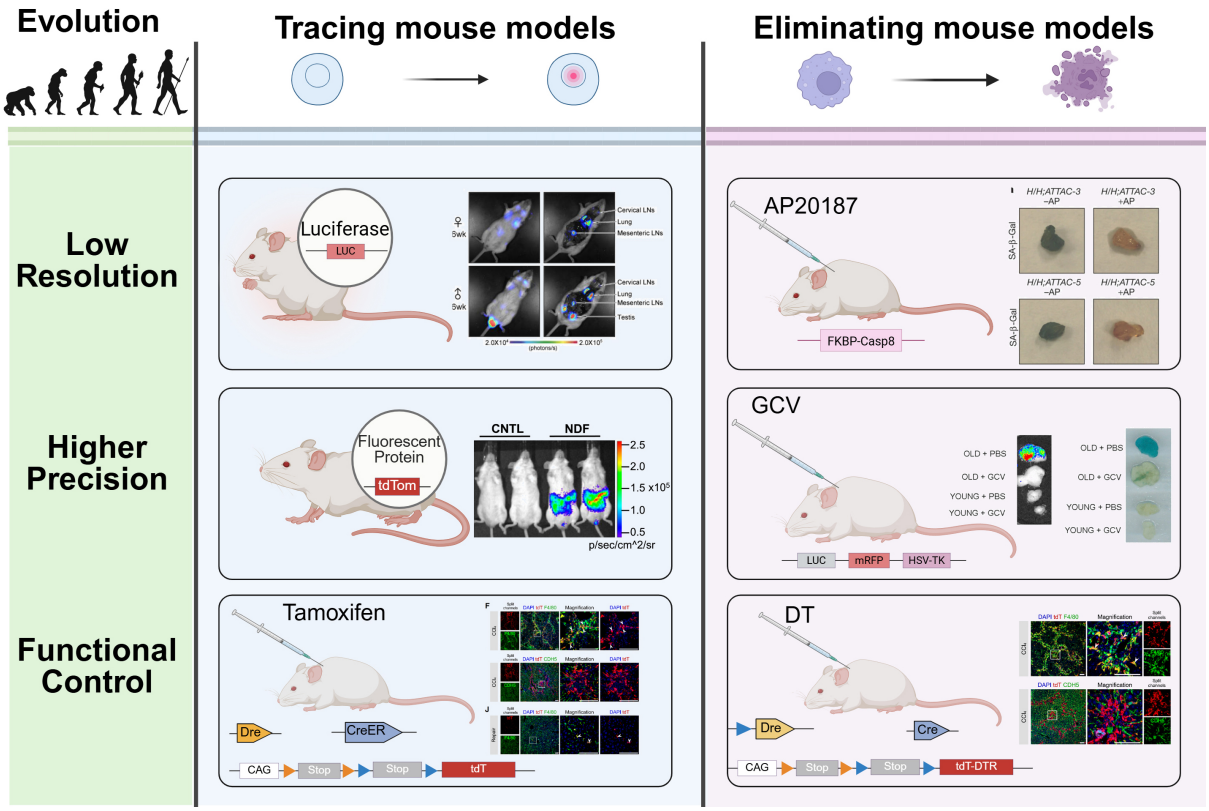

Figure 1. Development and characteristics of senescence reporter mouse models. Created in BioRender. Luccy Carriman (2025) https://BioRender.com/6i1w5nr. LUC: Luciferase; tdTomato, tdTom: tandem dimer Tomato; SA-β-Gal: senescence-associated β-galactosidase; GCV: ganciclovir; mRFP: monomeric red fluorescent protein; HSV-TK: herpes simplex virus type 1 thymidine kinase; DTR: diphtheria toxin receptor; CAG: CAG promoter; CreER: Cre recombinase - estrogen receptor fusion protein; DT: diphtheria toxin; tdT: tandem dimer Tomato (tdTomato); FKBP-Caspase-8: FK506-binding protein - fused Caspase-8; CNTL: control; NDF: neonatal dermal fibroblast; LNs: lymph node(s); DAPI: 4′,6-diamidino-2-phenylindole; PBS: phosphate-buffered saline.

stability[55]. Studies by Roos *et al.* using INK-ATTAC mice, and Sadhu *et al.* employing p16-3MR mouse models to selectively clear hematopoietic-derived p16-positive senescent cells, further support these findings[56,57].

Recent studies have employed senescence reporter mice ($p16t^{dTom}$ and p16-3MR) combined with single-cell and spatial transcriptomics to systematically uncover the senescence heterogeneity of vascular smooth muscle cells, fibroblasts, and T cells within atherosclerotic lesions. This integrated multi-omics approach provides novel insights for the precise diagnosis of cellular senescence and the development of targeted senolytic interventions[58].

Collectively, senescent cells accelerate atherosclerosis progression through dual mechanisms involving inflammatory stimulation and impaired vascular repair.

Age-related arterial dysfunction

Age-related arterial stiffness, characterized by increased aortic pulse wave velocity (PWV), arises primarily from increased intrinsic mechanical stiffness and vascular wall remodeling. This remodeling is typified by collagen deposition, elastin degradation, and cross-linking of structural proteins with advanced glycation end-products (AGEs)[8,59]. Excessive reactive oxygen species (ROS)-mediated oxidative stress, mainly

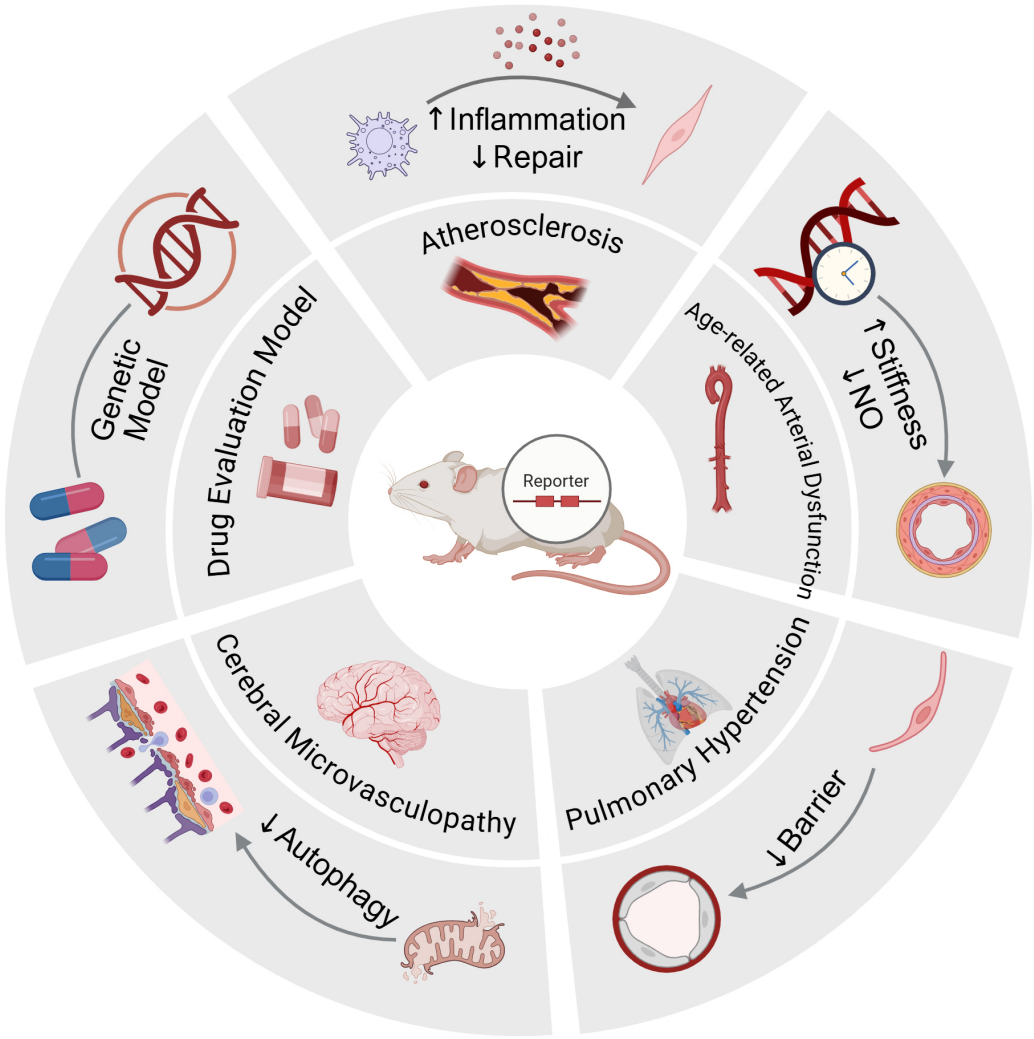

Figure 2. Application of Senescence Reporter Mouse Models in Panvascular Disease. Created in BioRender. Luccy Carriman (2025) https://BioRender.com/m03kj4y.

originating from mitochondrial dysfunction and chronic low-grade inflammation, significantly impairs nitric oxide (NO)-mediated endothelial function, exacerbating arterial stiffness[60,61].

Clayton *et al.* employed the p16-3MR model to selectively clear $p16^{Ink4a}$+ senescent cells in aged mice using GCV[62]. Following senescent cell clearance, aortic PWV significantly decreased, reverting to youthful levels, accompanied by improved endothelium-dependent relaxation. Mechanistic studies revealed enhanced NO bioavailability, reduced superoxide production, and decreased collagen I and AGE content within arterial walls.

Similarly, Mahoney *et al.* also utilized p16-3MR mice to investigate the pathogenic roles of senescent cells in arterial stiffness and assessed the senolytic efficacy of the natural compound 25-hydroxycholesterol (25-HC)[63]. Short-term (five-day) 25-HC treatment markedly decreased aortic PWV in aged mice, achieving improvements comparable to GCV-mediated genetic clearance of p16-positive cells. Importantly, combined 25-HC treatment and GCV-mediated clearance showed no additive effects, further indicating that the

restorative effects of 25-HC on arterial elasticity largely depend on its selective removal of senescent cells.

Pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a rare chronic pulmonary vascular disease characterized by pulmonary vascular remodeling, including abnormal proliferation of pulmonary artery smooth muscle cells and endothelial dysfunction[64]. Similar to atherosclerosis, PH exhibits hyperactive vascular smooth muscle cells, positioning it within the spectrum of panvascular diseases[65]. Emerging evidence suggests that multiple senescent cell types, including endothelial cells, smooth muscle cells, and fibroblasts, are present in PH, exacerbating disease progression[66-68].

Using $p16^{Luc}$ reporter mice, Born *et al.* identified that a significant proportion (approximately 30%) of pulmonary microvascular endothelial cells express $p16^{Ink4a}$ under physiological conditions. Surprisingly, subsequent studies utilizing p16-ATTAC mice and classical senolytic agents for non-selective removal of $p16^{Ink4a}$+ senescent cells resulted in extensive loss of pulmonary capillary endothelial cells, worsened vascular remodeling, enhanced pulmonary artery smooth muscle proliferation, and markedly elevated right ventricular pressures[69].

Senescence also plays a significant role in other PH types, including secondary PH associated with chronic obstructive pulmonary disease (COPD)[70]. Chronic cigarette smoke exposure induces $p16^{Ink4a}$ expression in alveolar epithelial and pulmonary microvascular endothelial cells. SASP factors from these senescent cells activate smooth muscle cells and fibroblasts, accelerating vascular remodeling and resistance increase[71]. Kaur *et al.* demonstrated using p16-3MR transgenic mice that targeted clearance of $p16^{Ink4a}$+ lung senescent cells via GCV significantly reversed lung senescence biomarkers, improved mitochondrial function, and alleviated airspace enlargement within five days. Thus, senolytic strategies targeting p16-positive cells have therapeutic potential to simultaneously mitigate COPD and secondary PH progression[72].

Collectively, these findings suggest that senescence of pulmonary endothelial cells functions as a double-edged sword in pulmonary vascular remodeling disorders: moderate endothelial senescence may act as a brake on pathological proliferation, thereby preventing excessive vascular remodeling, whereas excessive accumulation of senescent cells can injure lung tissue through SASP-mediated inflammation and disruption of barrier function.

Cerebral microvasculopathy

In the cerebral microvasculature, senescent cells predominantly accumulate within the endothelial cells and pericytes of the blood-brain barrier (BBB)[73,74]. As mammals age, the progressive accumulation of mitochondrial DNA (mtDNA) mutations has been identified as a key driver of cellular senescence[75,76]. Concurrently, mitophagy, the selective autophagic clearance of damaged mitochondria, declines in efficiency with advancing age[77]. In various vascular pathologies, impaired mitophagy co-exists with mitochondrial dysfunction and a pro-inflammatory phenotype: uncleared, dysfunctional mitochondria trigger the secretion of cytokines such as interleukin-6 (IL-6), which in turn promotes neuroinflammation and downregulates tight junction proteins, thereby increasing BBB permeability[78,79]. Employing mt-Keima reporter mice, Tyrrell *et al.* demonstrated that aged animals exhibit a markedly elevated senescent burden in cerebral microvascular endothelial cells, accompanied by reduced mitophagic flux and upregulated IL-6 expression, culminating in exacerbated BBB leakage[80]. These results indicate that senescent endothelial cells and pericytes in the brain vasculature compromise barrier integrity and initiate neuroinflammatory cascades, thereby contributing to age-related central nervous system dysfunction.

Growth hormone (GH) secretion and subsequent IGF-1 secretion decline over time, implicating the GH/IGF-1 axis in aging[81]. To investigate the role of IGF-1 signaling in cerebral microvascular senescence, Gulej generated VE-Cadherin-Cre^{ERT2} (VE-Cadherin vascular endothelial cadherin; CreERT2: tamoxifen-inducible Cre recombinase - estrogen receptor T2 fusion protein) / Igf1r$^{fl/fl}$ (insulin-like growth factor 1 receptor floxed allele, a conditional knockout allele in which loxP sites flank the Igf1r gene, allowing Cre-mediated deletion) × p16-3MR mouse models to specifically trace endothelial cell senescence resulting from IGF-1 receptor (IGF-1R) deletion. The study demonstrated that IGF-1R deficiency markedly induced cerebral microvascular endothelial senescence and significantly elevated BBB permeability, emphasizing the protective role of IGF-1 signaling in maintaining vascular integrity[82].

Using p16-3MR senescence reporter mice, multiple studies have shown that unconventional damage factors such as whole-brain irradiation (WBI), paclitaxel (PTX), and cisplatin/methotrexate induce p16+ senescence in cerebral microvascular endothelial and immune cells within months. This leads to increased BBB permeability, capillary rarefaction, and ultimately cognitive impairment. Administration of senolytics three months post-radiation partially restored BBB integrity, promoted capillary regeneration, and improved spatial memory, underscoring the critical value of senescence reporter mice in validating senolytics targeting and mitigating treatment side effects[83-85].

Genetic control models for evaluating senolytic drugs using p16-3MR mice

Senolytics are small molecules capable of selectively eliminating senescent cells, offering novel therapeutic strategies for multiple age-related diseases[86-88]. However, validating their targeted elimination efficacy and specific contributions to tissue function *in vivo* necessitates genetic models that selectively remove senescent cells as controls. The p16-3MR senescence reporter mouse model serves as an ideal functional validation system, providing a clear comparison between conditions with and without senescent cells.

In assessing the therapeutic potential of the natural flavonoid compound fisetin as a senolytic agent, Mahoney *et al.* effectively employed the p16-3MR model[89]. Initially, they compared two groups in *ex vivo* arterial function experiments from aged mice: one group underwent only GCV-mediated clearance of p16+ vascular senescent cells, resulting in significantly improved endothelial relaxation; the other group received fisetin treatment followed by GCV clearance, which did not yield additional improvement beyond the GCV-only group. This comparative approach directly demonstrated that fisetin's vascular protective effects primarily depend on its capacity to clear p16+ senescent cells rather than nonspecific mechanisms, thereby establishing fisetin's effectiveness as a targeted senolytic agent.

With the progressive refinement of senescence biomarker systems and the iterative advancement of reporter mouse technologies, vascular ageing should be regarded as a continuous and multifactorial process rather than a discrete event. In this context, senescence reporter mice represent powerful yet partial tools for *in vivo* evaluation. However, no universally accepted gold standard model has yet been established for studies of panvascular diseases. For instance, in atherosclerosis, evidence indicates that p16-driven systems exhibit inaccuracies in identifying functionally pathogenic senescent cells. Specifically, approaches based on p16, p16 reporters, or p16-linked suicide genes for the detection and/or clearance of senescent cells display notable limitations[90]. Because current models largely rely on a single biomarker, conclusions drawn under highly heterogeneous pathological contexts may be incomplete. To date, only a limited number of studies in vascular aging and related diseases have applied these senescence models. Accordingly, this review does not attempt to define a unified standard, but instead aims to provide illustrative paradigms demonstrating how senescence reporter mouse models have been applied across the spectrum of panvascular diseases.

Endothelial cells represent the most susceptible cell population to senescence across panvascular diseases[91]. The cellular composition and microenvironmental context of distinct vascular beds determine the specific manifestations and consequences of endothelial senescence. Senescence reporter mouse models not only provide powerful tools for precisely targeting endothelial cells *in vivo* but also enable the specific delineation of their senescent features across vascular beds, while endothelial cell-specific clearance models further allow mechanistic dissection of their functional roles in vascular disease development and progression.

DISCUSSION

Targeting senescent cells is widely considered to hold significant therapeutic potential. However, accumulating evidence indicates that simple elimination of senescent cells is not universally beneficial[34,92]. This contradiction primarily arises from the marked heterogeneity of cellular senescence, as senescent cells in different tissues or at different time points may exhibit distinctly different functions. For example, in a liver injury-repair model, Zhao *et al.* demonstrated that removing $p16^{Ink4a}$+ macrophages significantly attenuated liver fibrosis, whereas eliminating $p16^{Ink4a}$+ endothelial cells impaired injury repair and exacerbated fibrosis. This indicates that senescent macrophages primarily exert pro-inflammatory and pro-fibrotic detrimental effects, whereas endothelial cell senescence can have a protective, regenerative-supportive role[30]. Beyond this cellular-level heterogeneity, organ systems themselves may differ in their hierarchical roles during aging. Recent studies have identified the vasculature as a pioneer system in the aging process, functioning as a central senohub that initiates and amplifies systemic aging[93]. Recognizing both the cell-type specificity and the organ-level hub function of vascular aging is therefore critical for designing optimized senescence-targeted interventions.

Senescence reporter mouse models, such as p16-3MR and Sn-cTracer, have significantly advanced vascular aging research. These models use promoters of specific senescence marker genes to drive reporter elements, enabling precise *in vivo* labeling, tracking, and selective manipulation of senescent cells. However, classical senescence markers such as p16, p21, and p53 represent only one aspect of cellular senescence, and not all senescent cells synchronously express these markers. For example, p21 is mainly activated during early senescence, whereas p16 maintains cellular senescence status[94]. The CellAge database (http://genomics.senescence.info/cells/) first compiled 279 core senescence-driving genes and mapped their interactions via protein-interaction and co-expression networks, laying a genomic foundation[95]. Building on this, Saul *et al.*[96] introduced the SenMayo transcriptomic signature to calculate a quantitative "senescence score", and Wang *et al.*[97] developed the human Universal Senescence Index (hUSI) across 34 cell types and 13 senescence stimuli. Together, these multi-gene, network-informed metrics overcome the specificity and sensitivity limits of SA-β-Gal and p16/p21 assays. By uniting curated gene sets with data-driven signatures, they enable scalable, high-resolution frameworks for future multi-omics mapping of cellular aging. Notably, recent studies have demonstrated the feasibility of integrating transgenic reporter models with multi-omic approaches. For example, Mazan *et al.* employed p16-tdTomato reporter mice in combination with single-cell and spatial transcriptomics to systematically analyze senescent cells within atherosclerotic plaques. They identified ten distinct senescent-associated cell clusters, several of which were not captured by traditional markers. By overlaying spatial localization with transcriptional heterogeneity, the study uncovered novel vascular-specific senescence signatures[58]. This example illustrates that the integration of reporter models with multi-omics not only overcomes the limitations of single-marker detection but also enables a cross-scale analytical framework, thereby offering new avenues for precise characterization of vascular senescence heterogeneity.

With the rapid advancement of artificial intelligence (AI) and multimodal imaging, research on vascular ageing is undergoing a methodological transformation. Beyond conventional indices such as PWV and

flow-mediated dilation (FMD), recent studies have shown that AI can detect senescent features at both the cellular and organ levels. For example, deep learning models trained on nuclear morphology have been able to accurately distinguish senescent from proliferative cells across tissues, providing an automated and objective approach for senescence recognition[98]. At the vascular level, AI-based analysis of retinal and vascular imaging has demonstrated the ability to predict vascular ageing status noninvasively, establishing a foundation for image-derived vascular ageing biomarkers[99]. Integrating these AI-driven analytical pipelines with senescence reporter mouse models may ultimately construct a cross-scale framework for mapping senescent cell heterogeneity and advancing precision interventions in vascular ageing.

DECLARATIONS

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Authors' contributions

Drafted the manuscript and prepared all figures: Li H
Conceived the review and performed the literature review: Zhang Y
Supervised the work and provided critical revisions: Tian Z, Zhang S
All authors have read and approved the submitted manuscript.

Availability of data and materials

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Conflicts of interest

Zhang Y serves as a member of the Youth Editorial Board of *Vessel Plus* and as a Guest Editor for the Special Issue Panvascular Aging. Zhang Y was not involved in any steps of the editorial process, notably including reviewer selection, manuscript handling, and decision making. The other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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