

**REVIEW**

Metformin as a Geroprotective Agent: Multifaceted Mechanisms Targeting Cellular Senescence and Aging Hallmarks

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ABSTRACT: Metformin is widely recognized for its pleiotropic effects on aging hallmarks, yet the evidence remains descriptive and mechanistically fragmented. This is a mechanism-focused narrative review; the evidence is illustrative rather than being systematically retrieved. We critically evaluate the proposed geroprotective mechanisms of metformin, including AMP-activated protein kinase activation (AMPK), mitochondrial complex I inhibition, senescence-associated secretory phenotype (SASP) suppression, and epigenetic modulation. While ample data support these pathways in preclinical models, resolving the relative contribution of these pathways requires: (i) locus-specific analysis of context-dependent H3K27me3 regulation (reported as both increased and decreased), and (ii) direct testing of AMPK-dependent versus AMPK-independent pathways in aged organisms using tissue-specific AMPK-knockout mice and chromatin profiling at SASP gene promoters. We provide specific, falsifiable predictions and experimental designs for each of these requirements in the main text. Existing studies have overgeneralized the senolytic capacity of metformin because they lack a comprehensive assessment of context-dependent effects. Further validation through rigorous human clinical trials (e.g., the ongoing Targeting Aging with Metformin study) is required, along with a clear dissection of AMPK-dependent versus AMPK-independent mechanisms, which is necessary to guide rational repurposing of metformin for aging intervention.

KEYWORDS: Metformin; cellular senescence; aging hallmarks; AMP-activated protein kinase (AMPK); epigenetics; senescence-associated secretory phenotype (SASP)

1 Introduction

1.1 Overview of Cellular Senescence

According to Hayflick and Moorhead's original definition [1], cellular senescence describes the limited replicative capacity of human fibroblasts in culture, characterized by an essentially irreversible cell-cycle arrest [2]. This stable arrest serves as an alarm response to various stressors—including genotoxic agents, nutrient deprivation, hypoxia, mitochondrial dysfunction, and oncogene activation [3]—and distinguishes senescence from quiescence (a reversible arrest) and terminal differentiation (a permanent exit via distinct pathways) [4]. Although typically irreversible, senescence arrest can be bypassed under specific conditions, particularly in cancer cells [5–7].

1.2 The Dual Role of Cellular Senescence

Cellular senescence acts as a “double-edged sword” in health and disease. It is essential for tumor suppression and tissue repair [8]; however, persistent accumulation of senescent cells and their senescence-associated

secretory phenotype (SASP) drives chronic inflammation, tissue dysfunction, and aging [3]. For a comprehensive overview of senescence biomarkers and experimental considerations, readers may refer to the review by Lucas et al. [9].

A key mechanism underlying this duality involves reactive oxygen species (ROS). Under physiological conditions, ROS function as essential signaling molecules for homeostasis, immunity, and mitochondrial function [10]. Conversely, excessive ROS generation induces oxidative damage, accelerates senescence, and promotes age-related pathologies [10]. Thus, the functional outcome of senescence critically depends on redox balance: while moderate ROS are indispensable, their overabundance exacerbates SASP and tissue damage. Mechanistically, ROS homeostasis in senescence is orchestrated by three interconnected systems: the nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant defense pathway, NADPH oxidases (NOX) as major ROS sources, and the SOD/GPx antioxidant enzyme axis. Nrf2—the master transcriptional regulator of antioxidative stress—counteracts senescence by inducing phase-II detoxifying enzymes and restoring redox balance; conversely, Nrf2 dysfunction exacerbates SASP and age-related inflammation [11]. NOX family enzymes (notably NOX1 and NOX4) drive ROS generation that fuels DNA damage and a NOX1–IL-6 proinflammatory loop, thereby perpetuating SASP and tissue dysfunction [12]. The balance between superoxide dismutase (SOD) and glutathione peroxidase (GPx) further determines cellular H₂O₂ levels: elevation of the SOD/GPx activity ratio leads to H₂O₂ accumulation and premature senescence [13]. Importantly, the functional duality of the SASP depends on the senescence stage. “Early” (non-terminal) senescent cells secrete a beneficial SASP that promotes tissue repair and tumor suppression. However, as cells transition into a “late” senescence stage, their SASP shifts to become enriched with pro-inflammatory factors (e.g., IL-6, IL-8) [14,15], driving chronic inflammation and age-related pathologies. Therefore, SASP-modulating therapies must consider not only suppression but also stage-specific compositional changes. This observation leads us to propose the hypothesis that precisely titrating intracellular ROS levels—neither eliminating nor overwhelming them—could confer therapeutic benefits in aging and senescence-associated pathologies [16]. This hypothesis requires direct testing in future studies. Reactive oxygen species (ROS) have dual roles: essential for homeostasis [10] but excessive ROS drive senescence and SASP [11–13]. We propose a testable hypothesis: precisely titrating intracellular ROS levels—neither eliminating nor overwhelming them—confers therapeutic benefits. This hypothesis predicts that moderate ROS reduction (e.g., 30–50%) suppresses SASP without impairing basal signaling, whereas complete elimination abolishes beneficial repair functions. A direct test would use graded doses of mitochondrial-targeted antioxidants or controlled Nrf2 activation in senescent cell models, measuring SASP factors and tissue repair capacity. If this prediction fails, ROS modulation is not the primary mechanism; alternative pathways (e.g., direct NF-κB inhibition) are prioritized.

1.3 Background of Metformin

Metformin, a traditional biguanide, has been a first-line therapy for type 2 diabetes for over six decades due to its efficacy, safety, and low cost [17]. Beyond glucose-lowering, accumulating evidence reveals pleiotropic effects across age-related conditions, including cardiovascular protection, cancer risk reduction, and metabolic modulation [18,19]. Interest has grown in repurposing metformin as a geroprotective agent, driven by preclinical studies reporting extended longevity in some animal models [20]. This transition from antidiabetic to potential anti-aging therapy is exemplified by the Targeting Aging with Metformin (TAME) trial, which targets the biology of aging per se [21]. These developments underscore that metformin’s actions extend far beyond glycemic control, highlighting its broader role in modulating fundamental aging mechanisms.

Key experimental evidence supporting these mechanisms across model systems is highlighted in Table 1 (selected examples). The multifaceted anti-aging mechanisms of metformin—integrating effects on energy sensing, oxidative stress, inflammation, and epigenetics—are summarized in Fig. 1. We propose that metformin’s geroprotective actions emerge from a tri-partite network: (1) a core energy-stress module (mitochondrial complex I → AMPK → mTOR/autophagy); (2) an inflammatory-control module (NF-κB → SASP); and (3) an epigenetic-remodeling module (SAM → H3K4me3/H3K27me3 → gene expression). These modules are not linear but interact via feedback loops (e.g., ROS ↔ NF-κB, AMPK ↔ TET2). The following Perspectives are structured around this integrative framework.

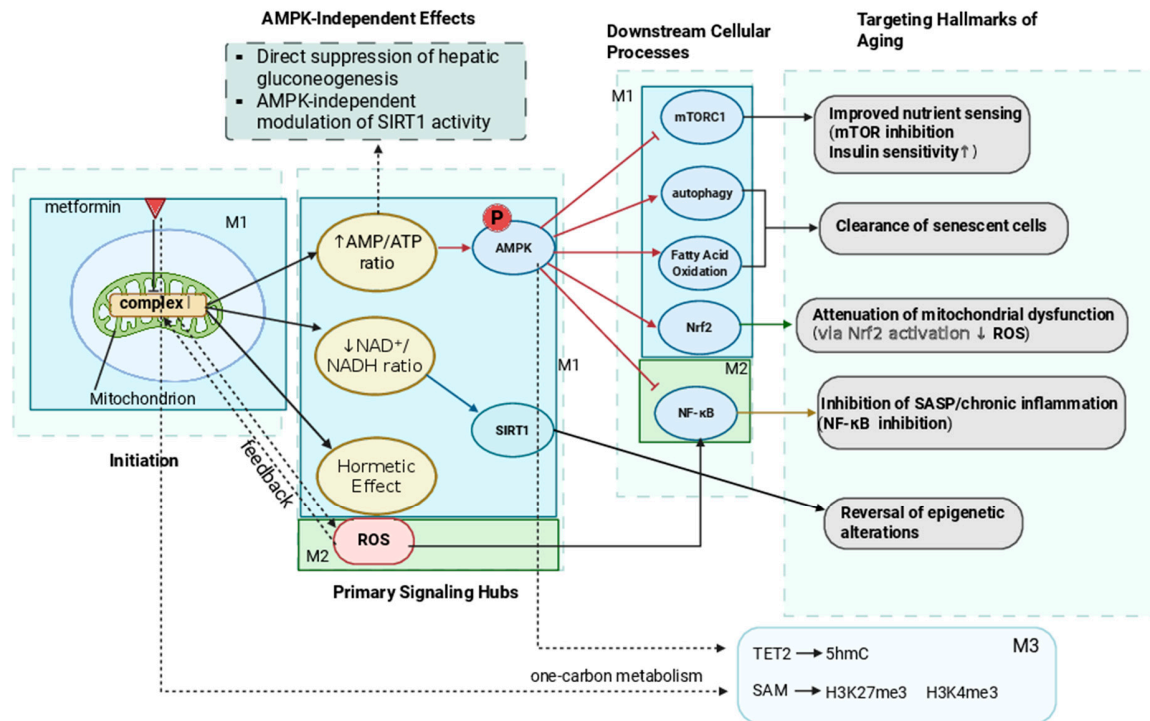


Figure 1: An integrated tri-partite network of metformin’s geroprotective mechanisms. Metformin acts through three interconnected modules: M1 (energy stress)—inhibition of mitochondrial complex I increases AMP/ATP ratio, activates AMPK and SIRT1, suppresses mTORC1, and enhances autophagy; M2 (inflammation control)—mild ROS production (hormetic effect) is kept below the threshold that would otherwise activate NF-κB and the SASP; dashed feedback arrow (ROS ↔ complex I) illustrates self-limiting hormesis; M3 (epigenetic remodeling)—metformin modulates histone methylation (via one-carbon/SAM) and DNA hydroxymethylation (via AMPK → TET2 → 5hmC). The four aging hallmarks shown on the right side are targets of the integrated M1–M3 network. Solid arrows indicate activation or progression; dashed arrows represent cross-module feedback and crosstalk. “↑” and “↓” denote increase or decrease. Abbreviations: AMPK, AMP-activated protein kinase; SIRT1, sirtuin 1; AMP, adenosine monophosphate; ATP, adenosine triphosphate; NAD⁺, nicotinamide adenine dinucleotide; NADH, reduced NAD⁺; ROS, reactive oxygen species; mTORC1, mechanistic target of rapamycin complex 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; SASP, senescence-associated secretory phenotype; Nrf2, nuclear factor erythroid 2-related factor 2; TET2, ten-eleven translocation 2; 5hmC, 5-hydroxymethylcytosine; SAM, S-adenosylmethionine; H3K4me3, trimethylation of histone H3 lysine 4; H3K27me3, trimethylation of histone H3 lysine 27. Created with BioRender under a publication license (Jan 2026).

2 Perspective 1: Core of Energy Metabolism and Nutrient Sensing—The Central Role of AMPK

Establishes AMPK as the central hub integrating metformin-induced complex I inhibition to coordinate catabolic restoration and anabolic suppression.

Mitochondria are indispensable organelles for ATP production and the regulation of redox balance, energy homeostasis, and cell survival. Understanding metformin's effects on mitochondrial function is therefore crucial for elucidating its anti-senescence mechanisms.

2.1 Mechanism

A foundational action of metformin is the direct, reversible, and concentration-dependent inhibition of mitochondrial respiratory chain complex I (NADH: ubiquinone oxidoreductase) [22,23]. By impairing electron transport, this inhibition compromises oxidative phosphorylation efficiency, leading to a decrease in adenosine triphosphate (ATP) synthesis and a concomitant rise in intracellular adenosine monophosphate (AMP) levels, thereby significantly elevating the AMP/ATP ratio [22,24,25]. This disruption also perturbs the cellular redox equilibrium, often manifesting as a reduced nicotinamide adenine dinucleotide (NAD⁺) to its reduced form (NADH) ratio [26].

Importantly, this initiating metabolic stress is AMPK-independent. Key evidence comes from studies using hepatocytes isolated from liver-specific Ampk α 1/ α 2 double-knockout mice, where metformin retained its ability to selectively inhibit oxygen consumption driven by complex I substrates and to increase the AMP/ATP ratio [24,25]. This conclusively positions complex I inhibition as the primary, upstream event.

The resultant energy deficit (elevated AMP/ATP ratio) is a potent signal for the activation of AMP-activated protein kinase (AMPK), primarily through phosphorylation at its Thr172 residue [25,27]. In parallel, metformin can upregulate sirtuin 1 (SIRT1) protein expression and activity via AMPK-independent pathways. For instance, in podocyte models, metformin-induced SIRT1 upregulation persisted even after small interfering RNA (siRNA)-mediated knockdown of AMPK α 1 or AMPK α 2 [26]. The co-activation of AMPK and SIRT1 orchestrates a comprehensive metabolic adaptation. A prime example is the stimulation of fatty acid β -oxidation: AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC), leading to a drop in malonyl-CoA levels. As a potent allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT-1), reduced malonyl-CoA relieves the brake on mitochondrial fatty acid import, facilitating their oxidation as an alternate energy source [27,28].

Notably, metformin's cardinal effect of suppressing hepatic gluconeogenesis—and thereby lowering blood glucose—does not strictly require AMPK. The drug-induced energy stress itself can directly constrain this ATP-demanding process, as demonstrated in the aforementioned AMPK-knockout models. While species-specific differences in AMPK isoform expression exist (e.g., human hepatocytes predominantly utilize AMPK α 1) [25], the core mechanism of complex I inhibition is evolutionarily conserved.

In summary, metformin instigates a mitochondria-orchestrated energy and redox crisis. This pivotal event engages a dual response: (1) the direct curtailment of energy-intensive anabolic pathways, and (2) the activation of a sophisticated energy-sensor network (centered on AMPK and SIRT1) to promote catabolic restoration of homeostasis. This integrated pharmacological response forms the mechanistic cornerstone of its metabolic and potential geroprotective actions [24,26].

2.2 Downstream Anti-Aging Effects

Before detailing specific downstream effects, we first address a critical knowledge gap regarding the AMPK dependency of these effects.

A critical caveat: While AMPK-independent initiation of energy stress is established in young or metabolically healthy livers [24,25], most mechanistic studies have been conducted in young or cancer models. AMPK activity declines with age [29], yet AMPK-independent pathways (direct SIRT1 activation [26], direct ROS scavenging [30]) have been demonstrated only in young or healthy models. We therefore propose the following hypothesis: in aged individuals with diminished AMPK function, AMPK-independent routes may assume a dominant role in metformin's geroprotective effects.

Research roadmap for AMPK dependency in aged organisms:

In aged, tissue-specific AMPK α 1/ α 2 double-knockout mice treated with metformin, measure (i) healthspan and senescence markers, and (ii) SIRT1 activity and mitochondrial ROS. Prediction: Metformin benefits knockouts, with persistent SIRT1 activation and ROS reduction $\rightarrow$ AMPK-independent pathways are sufficient. If not (no benefit or no SIRT1/ROS change) $\rightarrow$ AMPK required; alternative geroprotectors may be needed.

Evidence for AMPK-independent mechanisms.

Although mitochondrial complex I inhibition occurs independently of AMPK [24], most of metformin's downstream effects (mTORC1 suppression, autophagy, anti-inflammation) have long been attributed primarily to AMPK activation. However, accumulating evidence supports AMPK-independent pathways: metformin can directly activate SIRT1, inhibit mTORC1 via Rag-GTPases, suppress NF- κ B-mediated inflammation [31], exert direct ROS scavenging [30], and modulate PKD/MAPKAPK2 [32] (see also reviews [33–35]). Thus, attributing all downstream anti-aging effects solely to AMPK is an oversimplification.

The compensatory capacity of AMPK-independent pathways in aged organisms is directly testable with the knockout model described above. In summary, metformin's anti-aging action reflects complex synergy and compensation between AMPK-dependent and AMPK-independent pathways [33,35]. When extrapolating to clinical translation (e.g., the TAME trial), this multi-mechanistic nature should guide the interpretation of trial outcomes.

2.2.1 Inhibition of mTORC1

The mTORC1 is a master regulator of cell growth and anabolism [36], integrating nutrient and energy cues to promote processes like protein synthesis via phosphorylation of downstream effectors such as p70 S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). Under the energy stress induced by metformin, activated AMPK exerts potent suppressive effects on mTORC1 signaling through two well-established, complementary mechanisms: (i) phosphorylating and activating the tuberous sclerosis complex 1 and 2 (TSC1/TSC2) complex, which inhibits the mTORC1 activator Ras homolog enriched in brain (Rheb) [37], and (ii) directly phosphorylating the scaffold subunit regulatory associated protein of mTOR (Raptor), thereby impairing mTORC1 substrate recruitment and activity [38]. This concerted inhibition of a central anabolic hub represents a critical link through which metformin may counteract the hyperactive growth signaling often associated with cellular senescence. Unlike rapamycin's direct, nanomolar-affinity inhibition of mTORC1, metformin acts indirectly via AMPK, resulting in milder, context-dependent suppression [39]. Whether this AMPK-dependent mTORC1 suppression operates similarly in aged organisms is directly testable using the AMPK-knockout mouse model described in the roadmap above.

2.2.2 Improvement of Insulin Sensitivity

Correcting the aging-associated hallmark of “deregulated nutrient sensing” is central to metformin’s therapeutic action. Its ability to enhance systemic and cellular insulin sensitivity is largely mediated through AMPK activation [28]. In models such as renal podocytes, metformin triggers a signaling cascade involving ecto-ATPase inhibition and P2 receptor activation [40], leading to robust AMPK phosphorylation (increases of 54–74%) even under hyperglycemic conditions, thereby restoring insulin-stimulated glucose uptake. This process involves both AMPK and SIRT1, as optimal insulin response requires both sensors [26]. Importantly, metformin demonstrates therapeutic adaptability, retaining the capacity to enhance basal glucose uptake through compensatory mechanisms when the canonical SIRT1-AMPK axis is impaired [26]. This multi-targeted efficacy is particularly relevant given that SIRT1 expression and activity are frequently downregulated in states of insulin resistance and metabolic syndrome [41], highlighting its role as a key node in metabolic aging.

2.2.3 Promotion of Autophagy

Autophagy is an essential cellular quality-control and recycling process, crucial for maintaining proteostasis and organellar integrity [42]. AMPK serves as a direct and potent activator of autophagy initiation under conditions of energy stress. It phosphorylates the key autophagy-initiating kinase, unc-51 like autophagy activating kinase 1 (ULK1) at specific residues (e.g., Ser317 and Ser777), triggering the formation of autophagosomes [43,44]. Therefore, metformin-induced AMPK activation provides a direct mechanistic conduit to enhance autophagic flux [44]. The upregulation of this cellular “cleansing” pathway is vital for the timely removal of damaged mitochondria (mitophagy) and aggregated proteins, thereby alleviating fundamental drivers of cellular decline and aging.

3 Perspective 2: Targeting Mitochondrial Dysfunction and Oxidative Stress

3.1 Mechanism: Mild Inhibition of the Mitochondrial Respiratory Chain by Metformin (“Hormetic Effect”)

Addresses metformin’s mild, hormetic complex I inhibition that lowers ROS production and reinforces antioxidant defense, breaking the ROS-driven feedforward loop of senescence and tissue damage.

Metformin, a first-line medication for type 2 diabetes, has long been a focus of research regarding its mechanism of action. Recent studies suggest that metformin can regulate energy metabolism and improve insulin resistance and excessive hepatic glucose output through mild inhibition of mitochondrial respiratory chain complex I [23]. This effect involves not only direct mitochondrial inhibition but may also be achieved via indirect signaling pathways, thereby maintaining cellular homeostasis while avoiding excessive oxidative stress.

Two landmark studies established the mechanistic foundation for metformin’s mitochondrial action, albeit through complementary experimental approaches. Owen et al. demonstrated that metformin directly and time-dependently inhibits complex I in intact hepatocytes, an effect associated with reduced cellular energy status (decreased ATP/ADP ratio) and characteristic metabolic shifts consistent with suppressed gluconeogenesis [23]. In contrast, El-Mir et al. revealed that metformin’s inhibitory effect requires cellular integrity, suggesting an indirect signaling pathway that leads to persistent and specific complex I suppression without directly affecting other respiratory complexes in isolated mitochondria [22]. Collectively, these findings support a model in which metformin achieves mild, self-limiting inhibition of complex I—either

through gradual mitochondrial accumulation or via an unidentified signaling cascade—thereby restraining hepatic gluconeogenesis while avoiding a catastrophic energy crisis.

In summary, metformin achieves mild, self-limiting inhibition of respiratory chain complex I either through direct accumulation within mitochondria or by activating specific cellular signaling pathways. This inhibition not only restricts hepatic gluconeogenesis and promotes peripheral glucose utilization but may also reduce excessive reactive oxygen species production by moderating electron transport chain efficiency, thereby alleviating oxidative stress. This mechanism provides crucial insight into the pleiotropic effects of metformin in metabolic diseases.

3.2 Downstream Anti-Aging Effects

3.2.1 Reduction of ROS Production

Using extracellular flux analysis and ROS assays, the study by Ohguro et al. revealed that metformin (Met) modulates the metabolic phenotype of high glucose-exposed mouse Schwann cells by shifting energy production from mitochondrial respiration towards glycolysis, an effect associated with a significant reduction in intracellular ROS levels [45]. This observation underscores a cytoprotective mechanism wherein metformin, by dampening mitochondrial respiratory flux, effectively diminishes electron overflow from the over-reduced electron transport chain (ETC) under high glucose stress. Consequently, this attenuation of ETC efficiency leads to a marked decrease in the generation of superoxide and other downstream reactive oxygen species (ROS). The alleviation of oxidative stress directly mitigates the ensuing damage to critical macromolecules—including lipids, proteins, and DNA—thereby preserving cellular integrity and function.

3.2.2 Enhancement of Antioxidant Defense

It is noteworthy that the regulatory effects of metformin on mitochondria extend beyond merely alleviating oxidative stress to directly promoting functional cellular regeneration. In the nervous system, research has found that metformin upregulates the expression of myelin proteins and/or myelin sheath structures across multiple culture systems—including human monocultures, organoids, and chimeric models—even when the human cells retain fetal-like characteristics [46]. In chimeric models, metformin treatment not only increased the mitochondrial area within transplanted human cells but also promoted morphological changes in the mitochondria of host mouse axons; concurrently, the upregulation of transcripts related to mitochondrial function and metabolism suggests that metformin facilitates myelination through a non-cell-specific mechanism, mediated by the regulation of mitochondrial morphology and function [46]. This finding directly links the mitochondrial effects of metformin to the crucial process of remyelination.

Metformin has demonstrated potential in alleviating oxidative stress and inflammation in bovine mastitis models via modulating the AMPK/Nrf2/nuclear factor kappa B (NF- κ B) signaling pathway. In a study by Arbab et al. (2021), using an inflammatory model of primary bovine mammary epithelial cells (PBMECs) induced by lipoteichoic acid (LTA), pretreatment with metformin (3 mM, 12 h) effectively activated the AMPK signaling pathway, as evidenced by significantly increased levels of phosphorylated AMPK (P-AMPK) and its downstream target ACC. This activation of AMPK further promoted the nuclear translocation and stabilization of the key antioxidant transcription factor Nrf2, leading to the upregulation of downstream antioxidant enzyme genes such as heme oxygenase-1 (HO-1) and glutathione peroxidase 1 (Gpx1). This cascade of molecular events enhanced the cellular antioxidant defense system, effectively countering LTA-induced oxidative damage. Experimental evidence from Western blotting, qRT-PCR, and

immunofluorescence collectively supported the role of the AMPK/Nrf2 axis in mediating the antioxidant effects of metformin [47].

The detailed molecular and metabolic consequences of metformin's mild inhibition of mitochondrial complex I are depicted in Fig. 2.

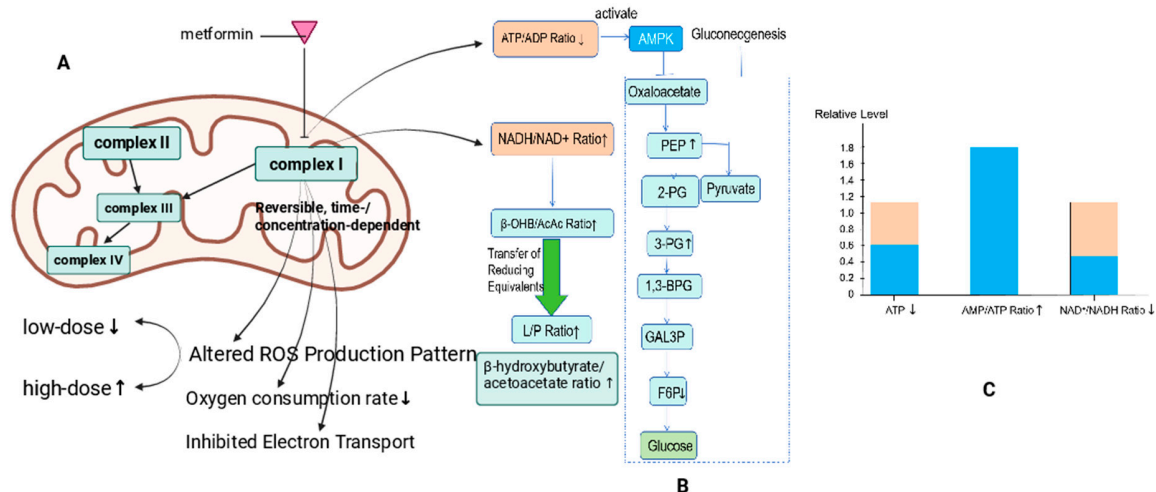


Figure 2: Metformin-induced mitochondrial modulation and its metabolic consequences. (A) Inhibition of mitochondrial complex I and its direct consequences. The schematic depicts the electron transport chain (ETC) embedded in the inner mitochondrial membrane (IMM). Metformin reversibly and in a time- and concentration-dependent manner inhibits complex I, impairing electron flow from NADH. This leads to reduced proton pumping, a decreased proton motive force (Δp), diminished oxygen consumption rate (OCR), and ultimately, lower ATP synthesis by complex V (ATP synthase). This primary inhibition directly increases the matrix AMP/ATP ratio and decreases the NAD⁺/NADH ratio. Inhibition of Complex I also biphasically modulates reactive oxygen species (ROS) production: mild inhibition may decrease superoxide ($O_2^{\cdot-}$) generation by preventing over-reduction of downstream sites, while severe inhibition causes upstream electron accumulation and increased ROS from Complex I itself or other sites. (B) Impact on metabolic pathway intermediates. Metformin treatment alters the levels of key metabolites. In gluconeogenesis, precursors like phosphoenolpyruvate (PEP) and glycerate-3-phosphate accumulate, while downstream hexose phosphates decrease, reflecting inhibition of the pathway. The cellular redox state is shifted, as indicated by increased lactate/pyruvate and β -hydroxybutyrate/acetoacetate ratios, consistent with a more reduced cytosolic and mitochondrial NADH pool, respectively. (C) Changes in cellular energy and redox parameters. The bar graphs summarize the net cellular outcomes resulting from the mechanisms shown in (A,B): a decrease in total ATP levels, a significant increase in the AMP/ATP ratio (activating AMPK), and a decrease in the NAD⁺/NADH ratio (influencing sirtuin activity). IMS, intermembrane space; Q, ubiquinone; Cyt c, cytochrome c; Δp , proton motive force. In this figure, the upward arrow (↑) denotes an increase; the downward arrow (↓) denotes a decrease. Annotations 'low-dose ↓' and 'high-dose ↑' indicate that low-dose metformin decreases ROS production, whereas high-dose metformin increases ROS production (biphasic modulation). Abbreviations: ETC, electron transport chain; IMM, inner mitochondrial membrane; OCR, oxygen consumption rate; Δp , proton motive force; ROS, reactive oxygen species; AMP, adenosine monophosphate; ATP, adenosine triphosphate; NAD⁺, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide. Created with BioRender under a publication license (Jan 2026).

4 Perspective 3: Inhibition of the Senescence-Associated Secretory Phenotype (SASP)

Focuses on metformin's suppression of the pro-inflammatory SASP via NF- κ B, counteracting inflammaging downstream of metabolic and oxidative stress modules.

The senescence-associated secretory phenotype (SASP), a hallmark of senescent cells, exerts context-dependent effects ranging from tumor suppression and tissue repair to the promotion of chronic inflammation and cancer progression [48,49]. As noted in Section 1.2, the SASP transitions from a beneficial (early senescence) to a pro-inflammatory (late senescence) profile, marking a critical therapeutic window [14,15]. Pharmacological modulation of the SASP has thus emerged as a promising therapeutic strategy. Among the identified SASP inhibitors, the anti-diabetic drug metformin has been demonstrated to effectively suppress the pro-inflammatory arm of the SASP [50,51]. Mechanistically, metformin primarily targets the NF- κ B signaling pathway, a master regulator of SASP gene expression [52,53]. Evidence indicates that metformin interferes with IKK/NF- κ B activation, thereby selectively repressing the transcription of key SASP factors such as interleukin-6 (IL-6) and interleukin-8 (IL-8) without affecting the senescence-associated growth arrest [52]. This NF- κ B-dependent SASP (N-SASP) is potently inhibited by metformin, an effect that is linked to the drug's action on mitochondrial function [52,54]. While the transcription factor C/EBP β is another critical regulator of SASP component expression, such as IL-6 and GRO α [55,56], a direct mechanistic link between metformin and C/EBP β inhibition remains less clearly established. Therefore, the current understanding positions the suppression of the NF- κ B pathway as the central mechanism by which metformin curtails the SASP [52] (see Table 1, SASP Inhibition), highlighting its potential for mitigating senescence-associated inflammation in age-related diseases and cancer.

Table 1: Selected examples of key experimental evidence supporting the geroprotective mechanisms of metformin.

Mechanism Category	Model System	Key Findings	Reference
Complex I Inhibition	Hepatoma cells, isolated mitochondria	Time- and concentration-dependent inhibition of respiration supported by glutamate/malate; no effect on succinate oxidation.	[23]
AMPK-Dependent Effects	Primary human hepatocytes	Increased AMP/ATP ratio and activation of AMPK (Thr172 phosphorylation).	[25]
AMPK-Independent Effects	Hepatocytes from liver-specific Ampk α 1/ α 2 KO mice	Persistent inhibition of complex I-supported respiration, elevated AMP/ATP ratio, and suppression of gluconeogenesis in the absence of AMPK.	[24]
SIRT1 Activation	Podocytes	Increased SIRT1 protein levels and improved insulin resistance even after knockdown of AMPK α 1/ α 2.	[26]
mTORC1 Inhibition/Autophagy Promotion	Various cell lines	AMPK phosphorylates Raptor/TSC2 to inhibit mTORC1; it phosphorylates ULK1 to initiate autophagy.	[42]
ROS Reduction/Antioxidant Enhancement	Mouse Schwann cells	Shifted energy metabolism from oxidative phosphorylation to glycolysis, leading to decreased intracellular ROS levels.	[45]
SASP Inhibition	Review	Metformin suppresses the pro-inflammatory arm of SASP, in part through NF- κ B pathway modulation. The TAME trial is proposed to validate its geroprotective effects.	[21]
Epigenetic Regulation	C. elegans, Lung cancer cells	Reduced H3K4me3 via SAM restriction; downregulated MLL2 to decrease H3K4me3 at promoters of cell-cycle genes.	[57,58]

Table 1: *Cont.*

Mechanism Category	Model System	Key Findings	Reference
Pro-apoptotic (In cancer cells) and Senomorphic Effects	Endothelial cells, Breast cancer cells	Induced apoptosis ($\uparrow$ Bax, $\downarrow$ Bcl-2); inhibited SASP secretion ($\downarrow$ IL-6, TNF- α).	[51,59]

Note: Abbreviations: AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; NADH, nicotinamide adenine dinucleotide; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; interleukin-6 (IL-6); interleukin-8 (IL-8); tumor necrosis factor alpha (TNF- α), tumor necrosis factor alpha; SIRT1, sirtuin 1; mTORC1, mechanistic target of rapamycin complex 1; ULK1, unc-51 like autophagy activating kinase 1; TSC2, tuberous sclerosis complex 2; SAM, S-adenosylmethionine; MLL2, mixed-lineage leukemia 2; H3K4me3, trimethylation of histone H3 lysine 4. Gene names (e.g., Bax, Bcl-2) are not expanded. Arrows indicate direction of change: $\uparrow$ denotes increase/upregulation; $\downarrow$ denotes decrease/downregulation. This table provides illustrative examples of representative studies and is not the result of a systematic review. The mechanisms shown are selected to highlight major pathways discussed in the text.

4.1 Downstream Anti-Aging Effects

The anti-aging properties of metformin are closely associated with the activation of its core target, AMPK, with the suppression of NF- κ B-driven chronic inflammation being a pivotal mechanism. Research in an isoprenaline-induced *in vivo* injury model demonstrated that metformin treatment potently enhanced and modulated AMPK activity. Crucially, this AMPK activation effectively inhibited the NF- κ B signaling pathway, evidenced by reduced NF- κ B protein expression or nuclear translocation, which subsequently led to a significant decrease in the synthesis and release of key downstream pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor alpha (TNF- α) [60]. This well-defined “AMPK-NF- κ B” axis elucidates a core molecular mechanism through which metformin alleviates chronic inflammation. Given that chronic, low-grade inflammation (“inflammaging”) is a hallmark of cellular senescence and tissue functional decline [61], metformin’s ability to suppress NF- κ B signaling via AMPK activation provides a compelling mechanistic explanation for its potential to delay cellular aging.

Beyond NF- κ B, the mammalian target of rapamycin (mTOR) pathway represents another critical node in SASP regulation that is modulated by metformin. In head and neck squamous cell carcinoma models, metformin treatment inhibited mTOR pathway activation induced by CDK4/6 inhibitors, consequently suppressing the expression of tumor-promoting SASP factors, including IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) [62]. This effect is mechanistically distinct from AMPK-mediated NF- κ B inhibition, as metformin can suppress mTOR signaling both via AMPK-dependent (TSC2/Raptor phosphorylation) and AMPK-independent mechanisms, collectively attenuating the pro-inflammatory arm of the SASP.

p38 mitogen-activated protein kinase (p38 MAPK) functions as an upstream activator of NF- κ B and directly enhances the DNA damage-driven transcriptional activity of NF- κ B on SASP gene promoters, including IL6, IL8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [63]. Although direct evidence for metformin suppressing p38 MAPK is less extensive than for NF- κ B, metformin’s well-established ability to alleviate oxidative stress—a potent activator of p38 MAPK—likely contributes to reducing p38 MAPK phosphorylation in senescent cells, thereby dampening the downstream inflammatory cascade.

CCAAT/enhancer-binding protein β (C/EBP β) constitutes a third transcription factor governing SASP gene expression. C/EBP β directly binds to the promoters of SASP components such as “IL-6” and GRO α , cooperating with RB: E2F complexes to implement oncogene-induced senescence [55]. A comprehensive review on SASP regulation explicitly identifies metformin as a drug that “inhibits the

inflammatory components of the SASP” while noting NF- κ B, C/EBP, and p53 as the principal transcription factors controlling this phenotype [64]. While direct evidence linking metformin to C/EBP β inhibition remains less established, the drug’s modulation of upstream signaling pathways (AMPK, mTOR) that intersect with C/EBP β activity suggests an indirect regulatory potential.

4.2 Epigenetic Regulation of SASP

Accumulating evidence indicates that metformin is a pleiotropic epigenetic regulator capable of shaping the chromatin landscape through multiple mechanisms. Its ability to activate the AMPK pathway can subsequently modulate the activities of histone acetyltransferases and deacetylases, leading to a global increase in histone acetylation and chromatin opening [65,66]. More importantly, metformin exerts significant regulatory effects on histone methylation and has been shown to specifically reduce repressive marks such as H3K9me2 and H3K27me3, which are crucial in neuroprotection and metabolic improvement [58]. Given the central role of repressive marks like H3K27me3 in maintaining gene silencing, several studies have examined metformin’s effects on this modification. Notably, metformin has been reported to increase global H3K27me3 levels in liver tissue, breast cancer xenografts, and serum of treated patients [67]. We propose a testable hypothesis: metformin enriches H3K27me3 specifically at the promoters of IL-6 and IL-8 in senescent cells. This hypothesis predicts that (a) H3K27me3 occupancy at these loci increases upon metformin treatment, and (b) CRISPR-mediated removal of H3K27me3 at these sites abrogates metformin’s suppression of IL-6/IL-8. A direct test would be ChIP-seq for H3K27me3 in metformin-treated versus untreated senescent fibroblasts, coupled with locus-specific demethylation (e.g., dCas9-KDM6B). Selective enrichment at these promoters would support an epigenetic mechanism of SASP suppression; its absence would instead point to NF- κ B-mediated pathways as the dominant explanation.

4.3 Knowledge Gaps in Metformin’s Modulation of SASP Composition

We propose a testable hypothesis: metformin selectively suppresses late-stage pro-inflammatory SASP factors (IL-6, IL-8, MCP-1) while preserving early-stage repair factors (EGF, PDGF, VEGF). Test: SASP proteomics (early versus late senescent fibroblasts $\pm$ metformin) and scRNA-seq of aged mouse adipose tissue. Predictions: (i) Late-stage factors $\downarrow \geq 50\%$, early-stage factors $\downarrow < 20\%$; (ii) SASP score $\downarrow$ without change in p16^{Ink4a+} cell frequency. Refutation: Global suppression of both early and late factors, or decreased p16^{Ink4a+} cells (senolytic shift).

5 Perspective 4: Epigenetic Regulation and Gene Expression

Describes how metformin remodels the epigenetic landscape—through SAM restriction and modulation of histone methyltransferases/demethylases—to silence pro-aging gene programs, acting in parallel with metabolic and inflammatory pathways.

Emerging evidence suggests that the antitumor and antineoplastic effects of metformin are closely linked to its central role in modulating one-carbon metabolism, particularly its impact on intracellular S-adenosylmethionine (SAM) levels. Pioneering metabolomic fingerprinting by Corominas-Faja et al. revealed that metformin treatment in breast cancer cells induces a significant accumulation of the folate cycle intermediate 5-formimino-tetrahydrofolate, concurrently impairing *de novo* nucleotide synthesis and glutathione metabolism. This metabolic reprogramming mimics the action of multi-targeted antifolates like pemetrexed, implying that metformin disrupts folate-mediated one-carbon flux, thereby indirectly influencing the methionine cycle and SAM production [68]. This mechanism has been further elucidated in an invertebrate model. Zhang et al. demonstrated that in the rotifer *Brachionus asplanchnoidis*,

metformin, akin to dietary restriction (DR), reduces the N6-methyladenosine (m6A) modification on mRNA, leading to the downregulation of methionine synthase (MTR). The decreased MTR expression limits the remethylation of homocysteine to methionine, ultimately resulting in a significant reduction of intracellular SAM [69]. This study established that the m6A-MTR axis-mediated decline in SAM is a crucial mechanism for metformin-induced lifespan extension, an effect that can be reversed by exogenous SAM or methionine supplementation. Although the study by Kang et al. primarily focused on FGF21, its use of metformin as a positive control highlights metformin's broader metabolic influence, potentially including AMPK-mediated effects on methyl donor balance [70]. Collectively, these findings indicate that metformin, through interference with the folate and methionine cycles—partly via epitranscriptomic regulation of m6A—effectively lowers the level of SAM, a key methyl donor, providing a critical metabolic perspective for understanding its mechanisms in delaying aging and inhibiting cancer.

5.1 Downstream Anti-Aging Effects

Metformin exerts its anti-aging effects partly through epigenetic regulation, particularly by modulating key histone methylation marks such as H3K27me3 and H3K4me3, which are critically involved in aging and age-related diseases.

5.1.1 Regulation of H3K4me3

In lung cancer cells, metformin significantly reduces H3K4me3 levels at the promoters of positive cell cycle regulatory genes (e.g., CCNB2, CDK1, CDK6, E2F8), leading to downregulation of their mRNA expression. This effect is mediated through the downregulation of H3K4 methyltransferase MLL2, and knockdown of MLL2 mimics metformin's suppressive effects on cell proliferation. Moreover, MLL2 overexpression is associated with poor recurrence-free survival in lung adenocarcinoma, highlighting its potential as a therapeutic target in aging-related cancers [58].

In *Caenorhabditis elegans*, metformin extends healthspan by reducing H3K4me3 levels via S-adenosylmethionine (SAM) restriction, which depends on H3K4me3 modifiers such as set-2 and rbr-2. This epigenetic modulation downregulates mTOR/S6K signaling, a key pathway in aging [57].

5.1.2 Regulation of H3K27me3

Metformin has been shown to decrease H3K27me3 levels in ovarian cancer cells by suppressing the polycomb repressive complex 2 (PRC2) components, including EZH2, SUZ12, and EED. This reduction is mediated through AMPK activation and contributes to the inhibition of tumor proliferation and migration. Overexpression of EZH2 abrogates metformin's anti-tumor effects, confirming the central role of H3K27me3 in its mechanism of action [71].

Conversely, in non-malignant or metabolic contexts (e.g., hepatocytes, breast cancer xenografts, and serum from breast cancer patients), metformin can elevate global H3K27me3 levels by directly targeting the H3K27me3 demethylase UTX/KDM6A [67].

Context-Dependent Regulation of H3K27me3 by Metformin

The above two sets of findings—H3K27me3 reduction in ovarian cancer [71] and H3K27me3 elevation in other contexts [67]—collectively indicate that metformin does not exert a unidirectional effect on this mark. Instead, its impact is highly context-dependent, varying with cell type, disease state, and experimental conditions.

Importantly, global H3K27me3 levels do not necessarily reflect changes at specific genomic loci. The seemingly opposing reports on H3K27me3 (decreased in ovarian cancer [71] vs. increased in hepatocytes

and breast cancer xenografts [67]) are not errors but likely reflect context-dependent biology (cancer vs. non-malignant, cell-type specific, and locus-specific effects). Integrated analysis suggests that metformin's net effect on H3K27me3 is not unidirectional. Metformin globally increases H3K27me3 in some contexts [67] but decreases it in others [71]. Extrapolating to senescent cells, we propose a testable hypothesis: metformin selectively enriches H3K27me3 at the promoters of pro-inflammatory SASP genes (IL-6, IL-8) but not at housekeeping genes. This hypothesis predicts that (a) H3K27me3 ChIP-seq signal increases specifically at these loci upon metformin treatment, and (b) pharmacological inhibition of EZH2 reverses metformin's suppression of IL-6/IL-8. A direct test would combine ChIP-seq with EZH2 inhibitor co-treatment in primary senescent fibroblasts.

Research roadmap: Perform H3K27me3 ChIP-seq in paired cancer versus non-cancerous cells treated with metformin. Prediction: opposite directional changes at cell-cycle versus inflammatory gene promoters. Refutation: same directional change in both cell types, implicating basal metabolic state (e.g., Warburg effect) as the modifier.

5.2 Metformin and DNA Methylation: Potential Influence on Aging-Related Gene Expression

Dysregulation of epigenetic modifications is a hallmark of aging, with alterations in DNA methylation patterns being particularly important. Beyond its effects on histone modifications, emerging evidence suggests that metformin can also modulate the DNA methylation landscape. Its action may be mediated through Ten-eleven translocation 2 (TET2), a key enzyme in active DNA demethylation. Research indicates that metformin upregulates TET2 expression and activity via AMPK activation [72]. The functionally active TET2 enzyme oxidizes 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), a crucial intermediate in the DNA demethylation pathway. This mechanism is supported by a sepsis-induced liver injury model, where metformin treatment reversed global DNA hypermethylation and significantly increased 5hmC levels [73]. Metformin upregulates TET2 and increases global 5hmC in sepsis-induced liver injury [72,73]. We hypothesize that metformin increases locus-specific 5hmC at promoters of aging-related inflammation genes (e.g., IL-6, Tnf) via the AMPK-TET2 axis. This can be tested by 5hmC profiling in aged tissues with TET2 knockdown: If promoter-restricted 5hmC increases are lost upon TET2 depletion, the hypothesis is supported; if not, TET2 is not a major effector in aging tissues, and metformin's epigenetic effects in this context may be limited to histone modifications.

However, its impact is context-dependent. For instance, in lung cancer cells, genome-wide DNA methylation analysis revealed that while metformin significantly affected H3K4me3, it did not markedly alter the DNA methylation status at the promoter regions of cell cycle regulatory genes [58]. Thus, metformin robustly modifies histone methylation, but its effects on DNA methylation are context-dependent and not universal, at least in cancer cell models. Two recent studies provide the strongest evidence to date for metformin-mediated DNA methylation modulation. In aged non-human primates, metformin reversed tissue-specific DNA methylation age (e.g., frontal lobe −6.1 years) [74]. In older adults, metformin reduced monocyte epigenetic age without affecting CD8⁺ T cells, highlighting cell-type specificity [75]. These findings support a genuine, albeit context-dependent, influence of metformin on the aging methylome.

5.3 Summary of Epigenetic Regulation

Metformin regulates key epigenetic marks associated with aging—including H3K4me3, H3K27me3, and DNA hydroxymethylation (5hmC)—by modulating methyltransferases and SAM metabolism. These changes help reverse age-related epigenetic dysfunction and inhibit pro-aging signaling pathways such as mTOR. While its direct impact on DNA methylation may be limited in specific models, its regulation of

the active demethylation process via the AMPK-TET2 axis suggests a broader epigenetic influence. This can be tested directly: does metformin increase locus-specific 5hmC at the promoters of aging-related inflammatory genes (e.g., IL-6, Tnf) in an AMPK- and TET2-dependent manner? A negative result would indicate that this pathway is not the primary mediator.

A summary of metformin's epigenetic landscape remodeling through SAM metabolism, histone methylation, and DNA hydroxymethylation is presented in Fig. 3.

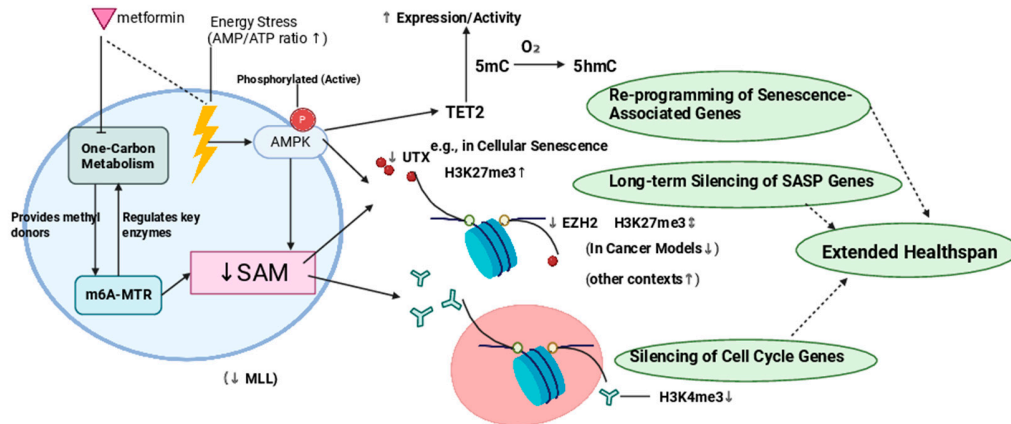


Figure 3: Epigenetic landscaping by metformin in the context of aging artistic representation of how metformin reshapes the epigenetic landscape during aging. The model highlights two upstream drivers: reduced SAM levels (via one-carbon metabolism/m6A-MTR axis) and AMPK activation. Downstream effects include decreased H3K4me3 (via MLL inhibition and SAM limitation), context-dependent modulation of H3K27me3 (through EZH2 suppression or UTX inhibition), and enhanced DNA demethylation via AMPK-mediated TET2 activation. These epigenetic changes contribute to the silencing of cell-cycle and SASP genes, reprogramming of senescence-associated gene expression, and ultimately, extension of healthspan. Arrows: upward (↑) indicates increase/activation/upregulation; downward (↓) indicates decrease/inhibition/downregulation. The proposed causal relationships in this figure are partly based on hypotheses requiring experimental validation (see Sections 4.2 and 5.2); notably, H3K27me3 enrichment at SASP promoters requires experimental testing (e.g., ChIP-seq). Abbreviations: SAM, S-adenosylmethionine; AMPK, AMP-activated protein kinase; H3K4me3, trimethylation of histone H3 lysine 4; H3K27me3, trimethylation of histone H3 lysine 27; TET2, Ten-eleven translocation 2; m6A, N6-methyladenosine; MTR, methionine synthase; UTX/KDM6A, Ubiquitously Transcribed Tetratricopeptide Repeat on X chromosome/Lysine Demethylase 6A; PRC2, polycomb repressive complex 2. Created with BioRender under a publication license (Jan 2026).

These opposing effects are not contradictions but reflect context-dependent biology: cell type and dose determine whether metformin increases or decreases H3K27me3 or ROS. Thus, any unidirectional model of metformin action is inadequate (Table 2).

Table 2: Context-dependent effects of metformin on key aging-related mechanisms.

Mechanism	Context (Cell Type/Condition)	Reported Effect	Proposed Explanation for Context Dependence
H3K27me3	Ovarian cancer cells	↓ (decrease)	PRC2 suppression via AMPK [71]
H3K27me3	Hepatocytes, breast cancer xenografts	↑ (increase)	UTX/KDM6A inhibition [67]
ROS production	Low-dose metformin	↓ (decrease)	Mild complex I inhibition reduces electron leak (Fig. 2)

Table 2: Cont.

Mechanism	Context (Cell Type/Condition)	Reported Effect	Proposed Explanation for Context Dependence
ROS production	High-dose metformin	↑ (increase)	Severe complex I blockade causes upstream electron accumulation (Fig. 2)

↑: increase; ↓: decrease.

6 Perspective 5: Direct and Indirect Clearance of Senescent Cells

Evaluates metformin’s dual capacity as a senomorphic agent (suppressing SASP) and potential senolytic agent (inducing apoptosis), while also promoting tissue regeneration as an effector module downstream of AMPK and NF-κB signaling.

6.1 Pro-Apoptotic in Cancer Cells: an Unconfirmed Senolytic Mechanism

6.1.1 Senolytic (Pro-Apoptotic) Effects

Metformin induces apoptosis in cancer cells via Bax/caspase-3 upregulation and Bcl-2 downregulation [59,76]. To test whether metformin acts as a direct senolytic in normal cells, apoptosis should be compared in senescent versus proliferating primary fibroblasts after metformin treatment. Selective apoptosis in senescent cells would support a senolytic effect; conversely, absence of selective apoptosis would indicate that metformin’s geroprotective actions are senomorphic (SASP suppression) rather than senolytic.

6.1.2 Senomorphic (SASP-Inhibitory) Effects

In addition to its direct pro-apoptotic effects, metformin is more frequently characterized as a “senomorphic” agent. Rather than directly killing senescent cells, it suppresses their deleterious senescence-associated secretory phenotype (SASP), thereby mitigating their negative impact on the tissue microenvironment. In a model of endothelial cell senescence induced by doxorubicin, metformin significantly reduced the secretion of numerous SASP factors, including IL-6, TNF-α, MCP-3, and MMP-3, and suppressed the expression of adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and endothelial selectin (E-selectin) [51]. Mechanistically, this study revealed that metformin acts by inhibiting the c-Jun N-terminal kinase (JNK) and NF-κB signaling pathways, which are central regulators of the SASP network. Moreover, metformin effectively ameliorated the “hyper-inflammatory response” of senescent endothelial cells upon lipopolysaccharide (LPS) stimulation, further confirming its role in modulating the senescent cell phenotype. These findings suggest metformin has senomorphic potential, blunting senescent cell paracrine toxicity and potentially delaying tissue dysfunction and chronic inflammation.

6.2 Promoting Cellular Regeneration and Functional Recovery

The anti-aging benefits of metformin extend beyond the clearance or suppression of senescent cells to include the direct promotion of functional tissue regeneration and repair. Research in the nervous system demonstrates that metformin treatment enhances the differentiation capacity of aged rat oligodendrocyte progenitor cells (OPCs), restoring their responsiveness to pro-differentiation cues. This pro-regenerative effect is also conserved in human OPCs derived from embryonic stem cells, where metformin significantly promotes differentiation into oligodendrocytes even when the cells retain a fetal-like state [46]. These findings reveal a “pro-regenerative” potential of metformin that surpasses mere “anti-aging”, providing a novel theoretical basis for its application in fields such as neurodegenerative diseases.

The dual strategies of metformin in targeting senescent cells—senolytic and senomorphic actions—along with its pro-regenerative potential, are illustrated in Fig. 4. Metformin can both directly induce apoptosis in senescent cells and suppress their harmful secretory phenotype, exhibiting senolytic and senomorphic properties, respectively (Table 1).

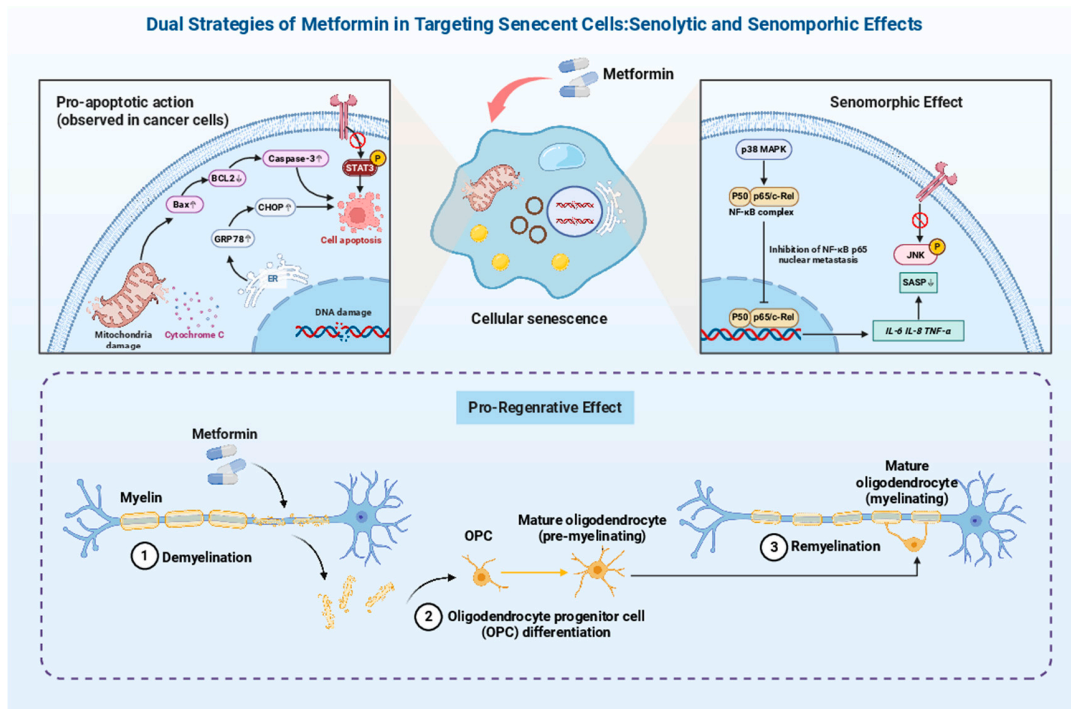


Figure 4: Dual strategies of metformin in targeting senescent cells: senolytic and senomorphic effects. This cartoon-style illustration compares the two distinct strategies by which metformin targets senescent cells. Left: Pro-apoptotic action (observed in cancer cells)—metformin promotes apoptosis of senescent cells through mitochondrial (Bax/Caspase-3 upregulation, Bcl-2 downregulation) and ER stress (CHOP/GRP78 upregulation) pathways, along with Stat3 inhibition. Right: Senomorphic action—metformin suppresses SASP secretion by inhibiting NF-κB and JNK signaling, thereby “taming” the senescence-associated secretory phenotype. The bottom panel additionally depicts the pro-regenerative effect of metformin, shown as enhanced differentiation of oligodendrocyte precursor cells into mature myelinating oligodendrocytes. Arrows: ↑ indicates increase/upregulation/activation; ↓ indicates decrease/downregulation/inhibition. Abbreviations: Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CHOP, C/EBP homologous protein; GRP78, glucose-regulated protein 78; Stat3, signal transducer and activator of transcription 3; NF-κB, nuclear factor kappa B; JNK, c-Jun N-terminal kinase; SASP, senescence-associated secretory phenotype. Created with BioRender under a publication license (Jan 2026).

7 Conclusion and Future Perspectives

Metformin’s effects on AMPK, ROS, H3K27me3, and SASP are context-dependent, varying with cell type, dose, and senescence stage—reflecting a network modulator, not a unidirectional linear pathway. Metformin demonstrates broad geroprotective potential by targeting multiple pillars of aging—cellular senescence, metabolic dysregulation, oxidative stress, and chronic inflammation. Its ability to fine-tune energy homeostasis via AMPK and SIRT1, dampen mitochondrial ROS production, suppress SASP through NF-κB inhibition, and remodel epigenetic marks positions it as a multifunctional aging-modulatory agent.

Notably, metformin not only attenuates senescence-associated damage but also promotes regenerative processes, as evidenced in neural and mammary models.

However, several key questions remain. Two specific experiments are prioritized: (1) ChIP-seq for H3K27me3 in metformin-treated vs. untreated senescent fibroblasts to test whether H3K27me3 occupancy increases specifically at IL6/IL8 promoters [67,71]; (2) lifespan and healthspan assessment in aged, tissue-specific AMPK α 1/ α 2 double-knockout mice treated with metformin to determine whether AMPK-independent pathways [24–26] suffice for geroprotection. The context-dependent effects of metformin on epigenetic marks (e.g., H3K27me3) and its precise impact on DNA methylation in aging tissues warrant further clarification, although initial evidence in diabetic patients suggests a potential to slow epigenetic aging in peripheral blood [77]. While preclinical studies, including recent work in non-human primates demonstrating decelerated multi-omics aging clocks [74], underscore its senomorphic and pro-regenerative capacities, translational gaps persist regarding optimal dosing, long-term safety, and efficacy in non-diabetic aging populations. Future research should prioritize human trials—such as the TAME (Targeting Aging with Metformin) initiative, which was proposed precisely to validate metformin’s geroprotective benefits in clinical settings [21]. Additionally, combining metformin with other senolytics or lifestyle interventions may synergistically enhance healthspan. Elucidating tissue-specific mechanisms and identifying biomarkers of metformin response will be crucial for personalized aging interventions.

Limitations and safety considerations

While the NF- κ B-dependent suppression of the SASP represents a core mechanism of metformin’s anti-inflammatory and geroprotective actions, it is critical to recognize that NF- κ B also plays essential roles in host immune defense and tissue repair. Sustained, systemic inhibition of NF- κ B—if it occurs with long-term metformin use—could theoretically increase susceptibility to infections or impair wound healing, particularly in the elderly population, where immune function is already compromised. Currently, however, the clinical evidence does not support a clear increased risk of common infections with metformin in older adults. In fact, retrospective studies and small-scale trials have shown that metformin use is associated with improved influenza vaccine responses—including enhanced serum antibody titers and reduced T-cell exhaustion markers—in both diabetic and non-diabetic elderly individuals [78,79]. Moreover, several observational studies have reported lower risks of pneumonia and improved COVID-19 outcomes in metformin users [80,81]. These findings suggest that metformin’s anti-inflammatory effects may predominantly target pathological, chronic inflammation rather than compromising beneficial acute immune responses.

Nevertheless, important caveats remain. First, most clinical safety data come from diabetic populations; the long-term immunological effects of metformin in non-diabetic, healthy aging individuals are less well characterized. Second, the theoretical risk of NF- κ B inhibition—namely, impaired immune surveillance and potential acceleration of immunosenescence—cannot be dismissed entirely based on existing evidence. Animal studies have shown that sustained NF- κ B inhibition can be detrimental to muscle maintenance and other physiological functions [82], underscoring that the balance between benefit and harm is context-dependent. Third, A testable question for future trials (e.g., TAME) is: does metformin achieve a 20–50% suppression of NF- κ B target gene expression in primary immune cells, and does this vary with age or frailty? Observational studies suggest improved vaccine responses [78,79] and lower pneumonia risk [80,81] in metformin users, implying that pathological inflammation is reduced without compromising host defense. Direct quantification can be obtained by measuring NF- κ B-dependent transcripts (e.g., IL6, TNF, IL1B) in PBMCs from metformin-treated vs. placebo older adults, stratified by baseline inflammatory status.

Therefore, although metformin appears safe and well-tolerated based on decades of clinical use, the possibility of adverse immunological consequences with very long-term use in aging populations should be vigilantly monitored in future trials, including the ongoing TAME study. Pragmatic outcomes such as infection rates, vaccine responsiveness, and wound healing should be incorporated as secondary endpoints in upcoming geroprotective clinical trials. Within the framework of geroscience, the goal is not to completely silence NF- κ B, but to tune down its pathologically overactive state while preserving its indispensable homeostatic functions [83]. Future mechanistic studies employing single-cell approaches and tissue-specific NF- κ B reporters are warranted to dissect the immune-cell-type-specific actions of metformin and to identify biomarkers that predict individuals who may benefit most—or potentially be at risk—from long-term NF- κ B modulation.

It is important to acknowledge that not all studies support a universal geroprotective effect of metformin. Null or even detrimental outcomes have been reported depending on model system, genetic background, timing of intervention, and specific healthspan endpoints. For example, in certain *Caenorhabditis* species, metformin failed to extend lifespan or even shortened it [84]. Moreover, a recent randomized controlled trial in older adults with sarcopenia (MET-PREVENT) found no improvement in muscle strength or physical function, with poor tolerability at low dose [85]. These findings do not invalidate the proposed mechanisms but highlight context dependence. Two key experiments would clarify major uncertainties. (i) H3K27me3 ChIP-seq in metformin-treated senescent fibroblasts: no enrichment at IL-6/IL-8 promoters would argue against epigenetic SASP suppression. (ii) Metformin in aged AMPK α 1/ α 2 double-knockout mice: no healthspan improvement would indicate that AMPK-independent pathways alone are insufficient.

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Conflicts of Interest: The authors declare no conflicts of interest.

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