

FOXO3 Gene Therapy: Molecular Mechanisms, Preclinical Evidence, and Therapeutic Potential for Enhancing Cellular Stress Resistance and Longevity

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Abstract

Forkhead Box O3 (FOXO3) has emerged as a critical transcription factor regulating cellular stress resistance, metabolic homeostasis, and longevity across species. Genetic variants of FOXO3 are consistently associated with exceptional human longevity, and preclinical studies demonstrate that FOXO3 activation extends lifespan in multiple model organisms.

This article examines the molecular mechanisms through which FOXO3 mediates cellular protection, including antioxidant defense, DNA repair, autophagy regulation, metabolic adaptation, and stem cell maintenance. I analyze preclinical evidence supporting FOXO3's role in lifespan extension and stress resistance in model organisms, from *C. elegans* to non-human primates.

Human studies reveal robust associations between FOXO3 variants, particularly the G allele of rs2802292, and longevity, with centenarians showing significant enrichment for these protective alleles. Current gene therapy approaches utilizing adeno-associated viral (AAV) vectors show promise for FOXO3 delivery, with tissue-specific targeting and expression control representing key challenges.

Potential therapeutic applications span age-related conditions including neurodegenerative disorders, sarcopenia, metabolic dysfunction, and immune senescence. While promising, safety considerations regarding cell cycle regulation, metabolic effects, and potential cancer implications warrant careful investigation. This review synthesizes current knowledge on FOXO3 gene therapy and identifies critical directions for translating this intervention from preclinical models to human applications aimed at extending healthspan and potentially lifespan.

Keywords: FOXO3, Gene therapy, Longevity, Healthspan, AAV vectors, Aging and Stress resistance.

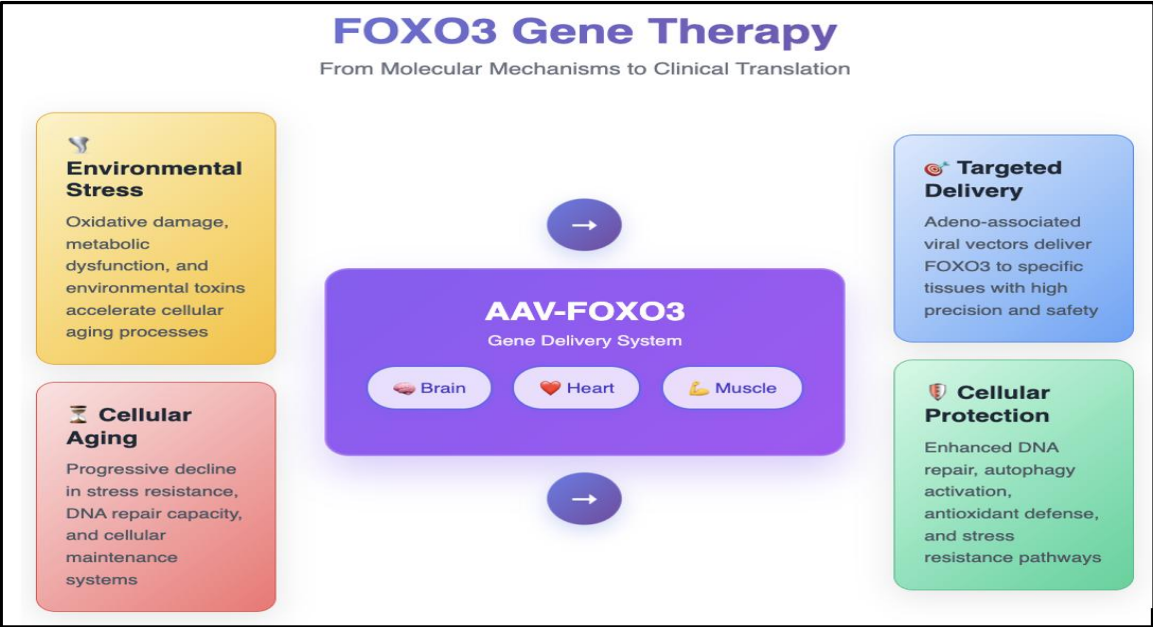


Figure 1: Overview of FOXO3.

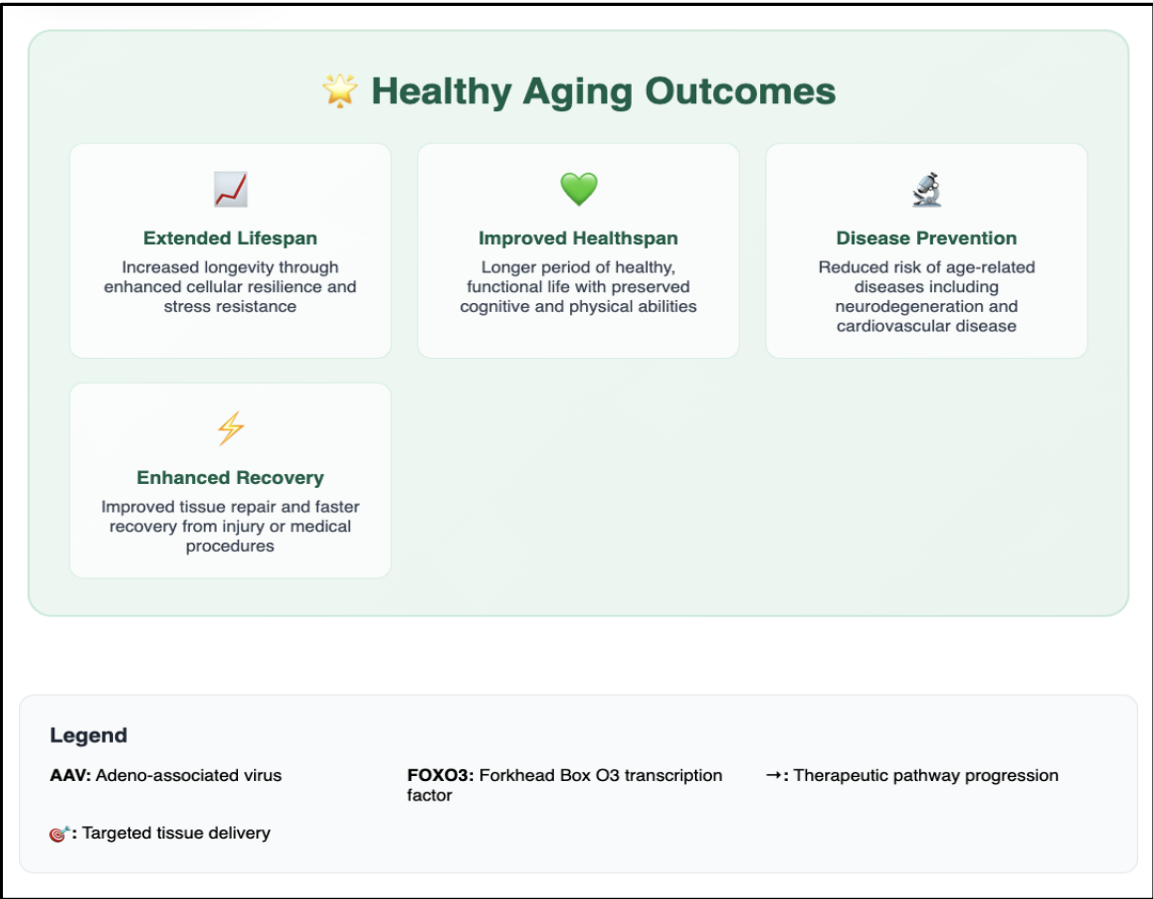


Figure 2: FOXO3 plays a pivotal role in the regulation aging, healthspan, disease prevention and illness recovery.

Introduction

The pursuit of interventions that extend healthy lifespan represents one of the most compelling frontiers in biomedical research. Among the molecular targets identified as potential regulators of longevity, the Forkhead Box O3

(FOXO3) transcription factor has emerged as a particularly promising candidate [1]. FOXO3 belongs to the conserved FOXO family of transcription factors and functions as a critical regulator of multiple cellular processes implicated in aging and stress resistance [2].

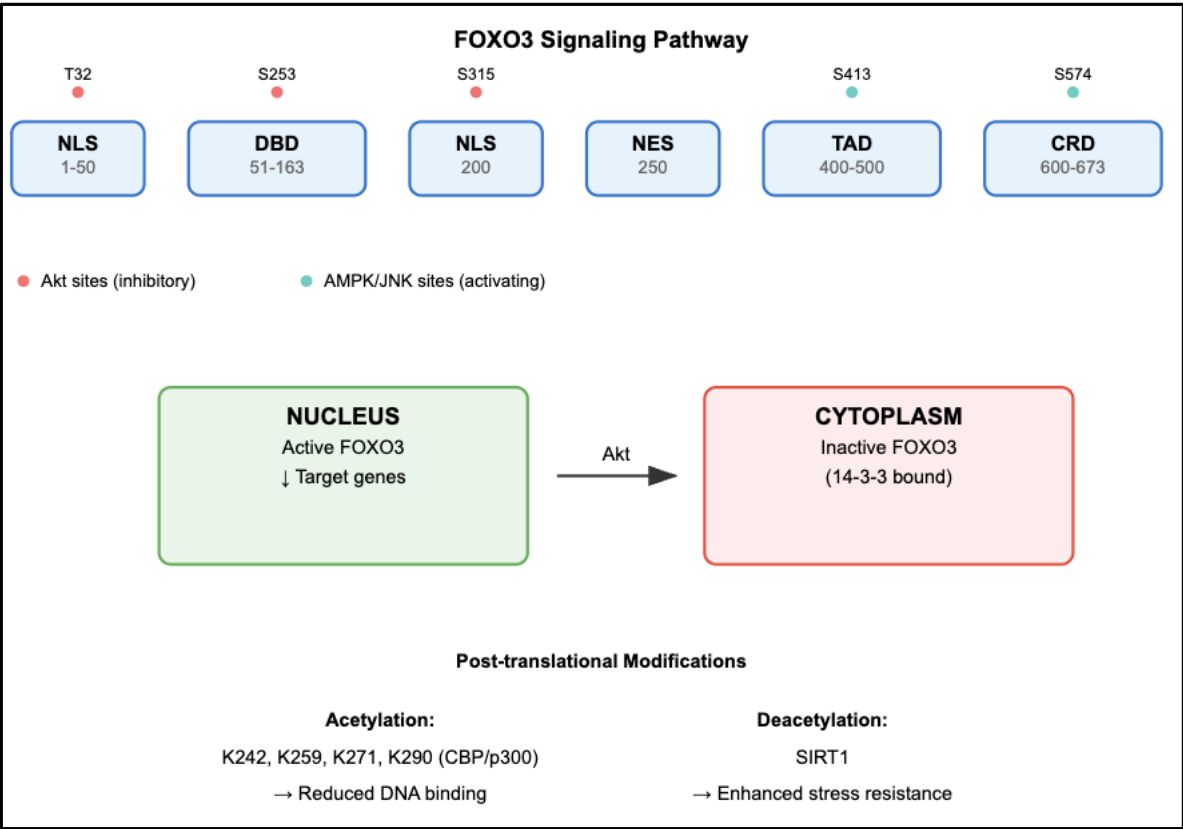


Figure 3: FOXO3 Structure and Post-translational Modifications.

A: FOXO3 contains key functional domains including nuclear localization signals (NLS), DNA-binding domain (DBD), nuclear export signal (NES), transactivation domain (TAD), and C-terminal regulatory domain (CRD). **B:** Major phosphorylation sites by Akt, AMPK, and JNK kinases, and acetylation sites modified by CBP/p300. **C:** Akt-mediated phosphorylation promotes cytoplasmic sequestration via 14-3-3 binding.

The evolutionary conservation of FOXO3 across species underscores its fundamental importance in organismal biology. From the nematode *Caenorhabditis elegans* with its FOXO3 ortholog DAF-16, to mammals including humans, FOXO proteins govern key cellular functions including stress response, metabolism, and cellular

homeostasis [3]. This conservation has facilitated translational research from model organisms to potential human applications, providing a robust foundation for therapeutic development.

Human genetic studies have consistently identified FOXO3 variants associated with

exceptional longevity. Multiple independent cohorts of centenarians demonstrate enrichment for specific FOXO3 alleles, most notably the G allele of the single nucleotide polymorphism rs2802292 [4,5]. These associations transcend ethnic backgrounds, having been documented in populations ranging from Italian and German to Japanese and Chinese centenarians [6,7]. This cross-population consistency suggests FOXO3's fundamental role in human aging regulation.

FOXO3 is widely expressed in tissues relevant to aging and age-related diseases, including skeletal muscle, brain, liver, heart, and immune cells [8]. Its activity is tightly regulated through post-translational modifications in response to environmental cues such as nutrient availability, oxidative stress, and inflammatory signals [9].

This responsive regulation positions FOXO3 as an integrator of cellular stress signals and adaptive responses, making it an attractive target for interventions aimed at enhancing cellular resilience.

The emerging field of gene therapy presents an unprecedented opportunity to modulate FOXO3 expression for therapeutic purposes. Adeno-associated viral (AAV) vectors capable of delivering the FOXO3 gene to specific tissues have demonstrated promise in preclinical models, suggesting potential applications for enhancing stress resistance and combating age-related deterioration [10].

This therapeutic approach aims not merely to extend lifespan but to expand the period of health and functional independence the "healthspan." This article synthesizes current knowledge regarding FOXO3's molecular mechanisms, preclinical evidence for its role in

longevity, human genetic associations, gene therapy approaches, and potential clinical applications. Additionally, we address safety considerations and outline future research directions essential for translating FOXO3-based interventions into clinical practice.

Mechanisms of Action

Transcriptional Regulation Network

FOXO3 functions primarily as a transcription factor, binding to specific DNA sequences in promoter regions to regulate gene expression. Upon nuclear translocation, FOXO3 recognizes and binds to the consensus sequence 5'-TTGTTTAC-3' in target gene promoters [11].

This binding activity is regulated through post-translational modifications including phosphorylation, acetylation, methylation, and ubiquitination, which collectively determine FOXO3's subcellular localization, DNA-binding affinity, and transcriptional activity [12].

The transcriptional network controlled by FOXO3 encompasses hundreds of genes involved in stress resistance, metabolism, cell cycle regulation, and apoptosis. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) studies have identified extensive FOXO3 binding sites across the genome, revealing both direct target genes and collaborative interactions with other transcription factors [13]. These interactions include cooperation with PGC-1 α in regulating mitochondrial genes and antagonistic relationships with NF- κ B in inflammatory pathways [14]. This complex regulatory network enables FOXO3 to coordinate cellular responses to diverse stressors.

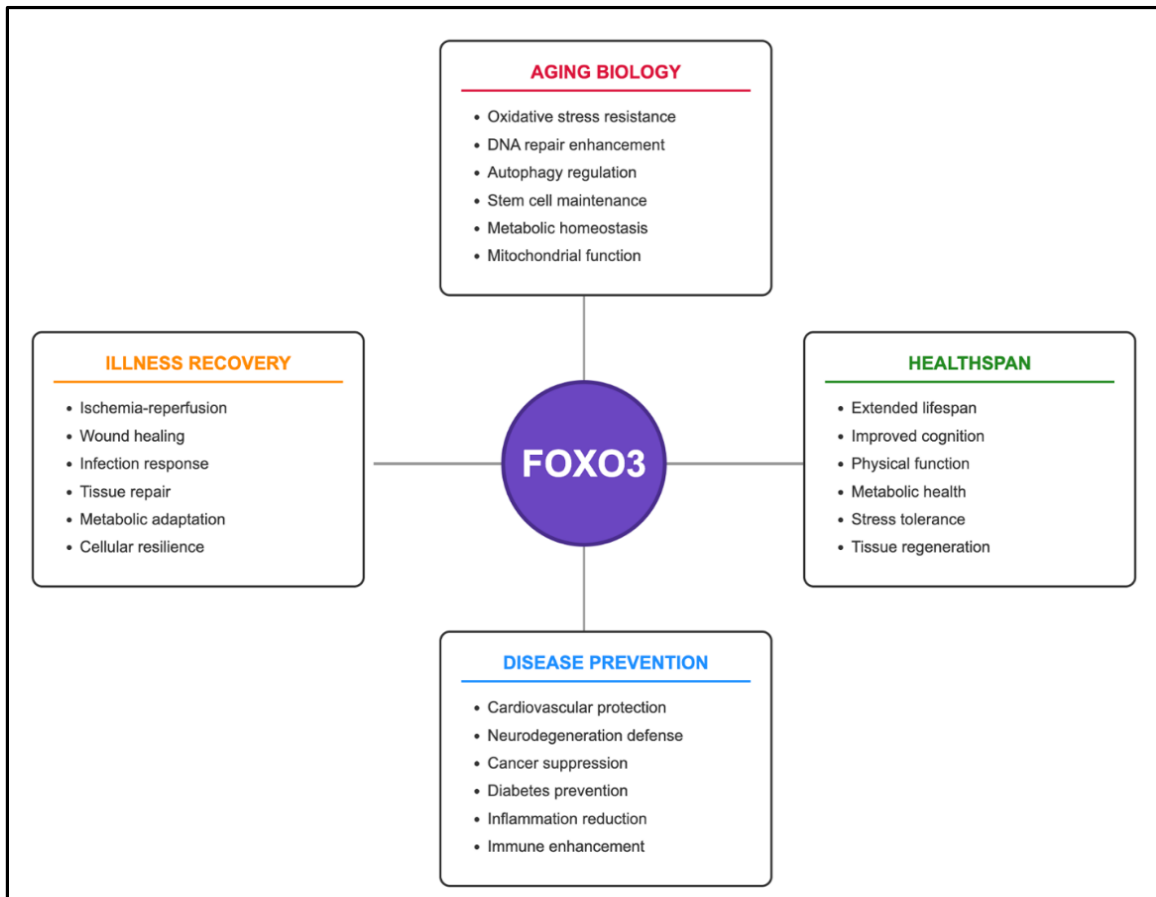


Figure 4: FOXO3 Transcriptional Network.

FOXO3 plays a pivotal role in the regulation of aging, healthspan, disease prevention and illness recovery. The transcription factor serves as a central hub coordinating multiple protective pathways that collectively promote healthy aging and resilience against age-related pathologies. Each category represents key biological processes and outcomes influenced by FOXO3 activation, demonstrating its potential as a therapeutic target for extending healthy lifespan.

Post-translational modifications of FOXO3 create a sophisticated regulatory system that fine-tunes its activity. Phosphorylation by Akt at threonine 32, serine 253, and serine 315 promotes FOXO3's nuclear exclusion and cytoplasmic sequestration, effectively inhibiting its transcriptional activity [9]. Conversely, phosphorylation by JNK or MST1 at different sites enhances nuclear localization and activity. Acetylation by CBP/p300 reduces DNA binding affinity, while deacetylation by sirtuins enhances transcriptional activity and shifts target gene

specificity toward stress resistance programs [16]. The dynamic regulation of FOXO3 allows cells to rapidly adapt to changing environmental conditions. Under stress conditions, multiple signaling pathways converge to activate FOXO3, creating a coordinated stress response program. This includes upregulation of antioxidant enzymes, DNA repair proteins, and autophagy components, while simultaneously inducing cell cycle arrest to prevent propagation of damaged cells [17].

Category	Gene	Function	Fold Induction	Biological Effect
Antioxidant Defense	SOD2	Mitochondrial superoxide dismutase	3-5x	Neutralizes mitochondrial ROS
Antioxidant Defense	Catalase	H2O2 decomposition	2-4x	Prevents oxidative damage
Antioxidant Defense	PRDX3	Peroxiredoxin 3	2-3x	Mitochondrial antioxidant
DNA Repair	GADD45a	DNA damage response	2-4x	Enhances nucleotide excision repair
DNA Repair	XRCC1	Base excision repair	2-3x	Repairs oxidative DNA damage
DNA Repair	DDB1	UV damage recognition	1.5-2x	Facilitates DNA repair
Autophagy	LC3	Autophagosome formation	2-3x	Essential for autophagy
Autophagy	BECN1	Beclin-1	2-3x	Initiates autophagy
Autophagy	ATG5	Autophagy related 5	2-2.5x	Autophagosome elongation
Cell Cycle	CDKN1B	p27Kip1	3-4x	G1/S arrest
Cell Cycle	CDKN1A	p21Cip1	2-3x	Cell cycle arrest
Cell Cycle	CCNG2	Cyclin G2	2x	Negative cell cycle regulation
Metabolism	G6PC	Glucose-6-phosphatase	2-3x	Gluconeogenesis
Metabolism	PCK1	PEPCK	2-4x	Gluconeogenesis
Metabolism	PDK4	Pyruvate dehydrogenase kinase 4	3-5x	Metabolic flexibility

Table 1: FOXO3 Target Genes by Functional Category.

Oxidative Stress Response

A primary function of FOXO3 is the orchestration of cellular defenses against oxidative stress. FOXO3 induces the expression of key antioxidant enzymes including manganese superoxide dismutase (MnSOD/SOD2), catalase, and peroxiredoxins [18]. These enzymes neutralize reactive oxygen species (ROS) that would otherwise damage cellular components including lipids, proteins, and DNA. The coordinated upregulation of multiple antioxidant systems provides robust protection against oxidative challenges.

In vitro studies demonstrate that cells overexpressing FOXO3 exhibit enhanced resistance to oxidative challenges. When exposed to hydrogen peroxide or paraquat, FOXO3-overexpressing cells maintain higher viability compared to controls, with reduced markers of

oxidative damage [19]. This protection extends to in vivo models, where tissue-specific FOXO3 expression confers resistance to oxidative insults in organs including the brain, heart, and skeletal muscle [20]. The protective effects are particularly pronounced in metabolically active tissues that generate substantial ROS during normal function.

Beyond direct antioxidant enzyme regulation, FOXO3 influences cellular redox homeostasis through multiple mechanisms. It upregulates genes involved in NADPH production, which is essential for regenerating reduced glutathione, a key cellular antioxidant [21]. FOXO3 also promotes the expression of metallothioneins, which sequester heavy metals and reduce their pro-oxidant effects. Additionally, FOXO3 enhances mitochondrial quality control, reducing ROS production at its source by maintaining efficient electron transport chain function [22].

The protective effects of FOXO3 against oxidative stress are particularly relevant to aging, as the oxidative damage theory of aging posits that cumulative oxidative damage contributes significantly to age-related functional decline [23].

By enhancing antioxidant defenses, FOXO3 may mitigate this accumulation of damage, potentially slowing the aging process. This mechanism likely contributes to the longevity associations observed in human populations carrying protective FOXO3 variants.

DNA Repair Pathways

FOXO3 plays a crucial role in maintaining genomic integrity through the regulation of DNA repair mechanisms. It upregulates genes involved in both base excision repair (BER) and homologous recombination (HR), including GADD45 α (Growth Arrest and DNA Damage-inducible 45 alpha) and XRCC1 (X-ray Repair Cross Complementing 1) [24]. This comprehensive approach to DNA repair ensures that various types of DNA lesions can be efficiently repaired.

GADD45 α facilitates DNA repair by interacting with proliferating cell nuclear antigen (PCNA) and enhancing nucleotide excision repair activity [25]. Additionally, FOXO3-mediated upregulation of DDB1 (DNA Damage-Binding Protein 1) and XPC (Xeroderma Pigmentosum Complementation Group C) enhances recognition and repair of UV-induced DNA lesions [26]. The coordinated expression of these repair proteins creates a robust system for maintaining genomic stability.

The DNA repair function of FOXO3 extends beyond simple upregulation of repair enzymes. FOXO3 also induces cell cycle

checkpoints that provide time for DNA repair before replication or mitosis [27]. This includes upregulation of p27Kip1 and p21Cip1, which arrest cell cycle progression in response to DNA damage. By coordinating repair processes with cell cycle control, FOXO3 ensures that damaged DNA is repaired before it can be propagated to daughter cells.

The DNA repair function of FOXO3 is particularly important in long-lived post-mitotic cells such as neurons, where accumulated DNA damage can lead to functional decline and neurodegenerative disease [28].

Studies in mouse models demonstrate that FOXO3 deficiency exacerbates age-related accumulation of DNA damage in the brain, while FOXO3 overexpression attenuates this accumulation and preserves neuronal function [29].

This neuroprotective effect likely contributes to the reduced cognitive decline observed in humans carrying longevity-associated FOXO3 variants.

Autophagy Regulation

FOXO3 serves as a master regulator of autophagy, the cellular process responsible for degrading damaged organelles and protein aggregates. Through transcriptional upregulation of autophagy-related genes including LC3 (Microtubule-associated protein 1A/1B-light chain 3), Beclin-1, and ATG5 (Autophagy Related 5), FOXO3 enhances autophagic flux and cellular quality control [30]. This regulatory function is essential for maintaining cellular homeostasis, particularly in the face of stress or aging-related damage.

The autophagy program induced by FOXO3 is comprehensive, affecting multiple steps in the

autophagic process. FOXO3 upregulates genes involved in autophagosome formation, including ULK1 and ATG13, which initiate the autophagic cascade [31].

It also enhances expression of proteins involved in autophagosome-lysosome fusion, ensuring efficient degradation of autophagic cargo.

Additionally, FOXO3 promotes expression of lysosomal enzymes that break down proteins and organelles, completing the autophagic cycle [32].

Experimental evidence from both in vitro and in vivo models demonstrates that FOXO3 activation stimulates autophagy in multiple tissue types. In skeletal muscle, FOXO3 overexpression increases autophagosome formation and clearance of damaged mitochondria, potentially counteracting age-related mitochondrial dysfunction [33].

This mitophagy function is particularly important for maintaining muscle function during aging, as accumulation of dysfunctional mitochondria contributes to sarcopenia and frailty.

In neurons, FOXO3-mediated autophagy promotes clearance of protein aggregates associated with neurodegenerative diseases including Alzheimer's and Parkinson's [34].

This clearance function extends to other misfolded proteins that accumulate with age, suggesting a broad neuroprotective role.

The autophagy-enhancing effects of FOXO3 may contribute significantly to its longevity-promoting functions, as genetic or pharmacological enhancement of autophagy extends lifespan in model organisms ranging from yeast to mice [35].

Stem Cell Maintenance

FOXO3 plays a critical role in preserving stem cell pools through multiple mechanisms, including protection from oxidative stress, regulation of self-renewal, and prevention of premature differentiation. In hematopoietic stem cells (HSCs), FOXO3 maintains quiescence and self-renewal capacity by upregulating cell cycle inhibitors and enhancing resistance to oxidative stress [36]. This dual function ensures that stem cells remain viable and functional throughout the organism's lifespan.

The mechanism of stem cell preservation by FOXO3 involves careful balance between quiescence and activation. FOXO3 promotes expression of genes that maintain stem cells in a quiescent state, including p57Kip2 and p27Kip1 [37]. This quiescence protects stem cells from exhaustion due to excessive proliferation.

However, FOXO3 also ensures that stem cells can respond appropriately to regenerative signals when needed, maintaining a dynamic equilibrium between preservation and function.

FOXO3-deficient mice exhibit premature depletion of HSCs and neural stem cells (NSCs), accompanied by reduced regenerative capacity [38]. This stem cell exhaustion manifests as impaired tissue repair, reduced stress resistance, and accelerated aging phenotypes. Conversely, enhanced FOXO3 activity preserves stem cell function during aging, potentially contributing to improved tissue regeneration and repair [39]. These effects extend across multiple stem cell populations, including muscle satellite cells, intestinal stem cells, and epidermal stem cells. In muscle satellite cells, FOXO3

balances quiescence with activation in response to injury signals. FOXO3 overexpression in aged satellite cells can partially restore their regenerative capacity, suggesting potential applications in combating age-related sarcopenia [40].

Similarly, in neural stem cells, FOXO3 maintains the balance between self-renewal and differentiation, ensuring a continued supply of new neurons for learning and memory processes. The stem cell-preserving functions of FOXO3 represent a mechanistic link between its cellular roles and organismal aging, as stem cell exhaustion is recognized as a hallmark of aging that contributes to

diminished tissue regeneration and homeostasis [41].

Interactions with Insulin/IGF-1 Signaling

FOXO3 functions as a key downstream effector in the insulin/insulin-like growth factor 1 (IGF-1) signaling pathway, a conserved regulator of lifespan across species [42]. Under conditions of nutrient abundance, insulin/IGF-1 signaling activates the PI3K/Akt pathway, leading to FOXO3 phosphorylation and nuclear exclusion, thereby inhibiting its transcriptional activity [43]. This regulatory mechanism links nutrient status to cellular stress resistance and longevity programs.

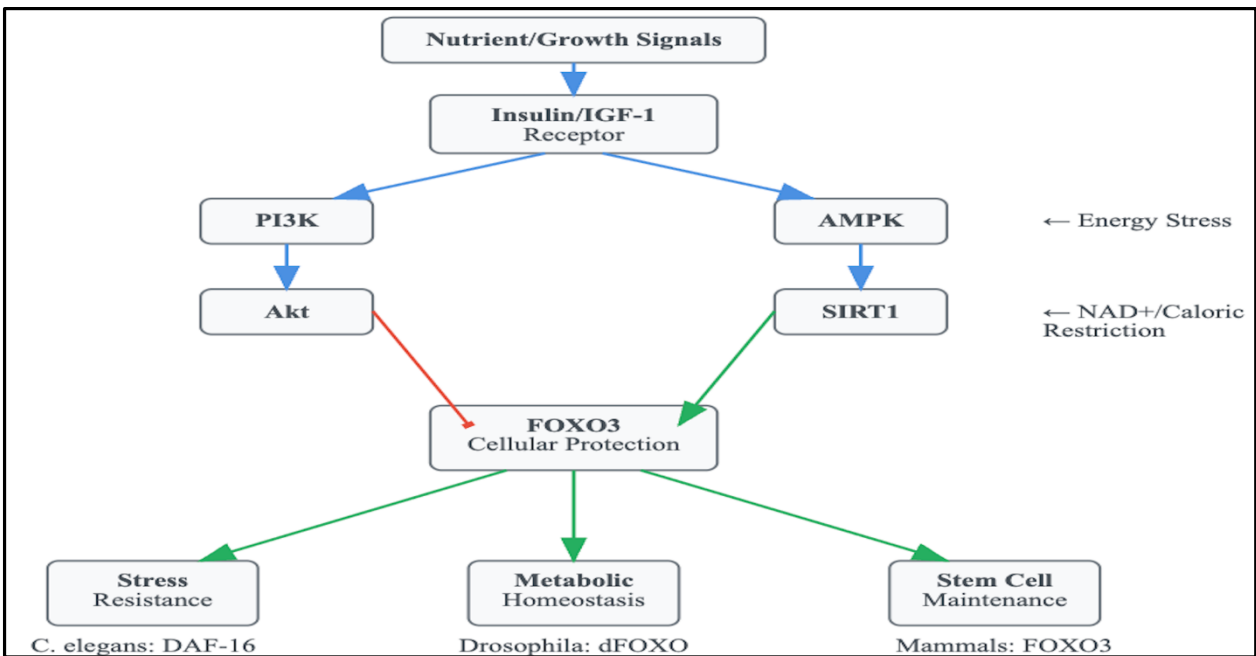


Figure 5: FOXO3 in Aging Pathways with Species Conservation.

FOXO3 integrates signals from nutrient-sensing pathways (insulin/IGF-1, AMPK, sirtuins) to coordinate cellular protection programs that promote healthspan extension across evolutionarily conserved pathways.

The interaction between insulin/IGF-1 signaling and FOXO3 creates a metabolic switch that determines cellular fate decisions. During nutrient abundance, suppression of FOXO3 promotes anabolic processes, cell growth, and

proliferation [44]. Conversely, during nutrient limitation or stress, reduced insulin/IGF-1 signaling permits FOXO3 nuclear translocation and activation of its target genes. This mechanism partially explains the lifespan-extending effects

of caloric restriction, which decreases insulin/IGF-1 signaling and enhances FOXO3 activity [45]. Experimental reduction of insulin/IGF-1 signaling extends lifespan in model organisms from *C. elegans* to mice, with this extension dependent on FOXO3/DAF-16 function [46]. Genetic ablation of FOXO3 abolishes the lifespan extension achieved through reduced insulin signaling, demonstrating its essential role in this longevity pathway. In mammals, tissue-specific disruption of insulin/IGF-1 receptors enhances FOXO3 activity and extends lifespan, supporting evolutionary conservation of this longevity pathway [47]. FOXO3 further interacts with other longevity-associated pathways, creating a complex network of metabolic regulation. AMPK (AMP-activated protein kinase) activation during energy depletion leads to FOXO3 phosphorylation at sites distinct from those targeted by Akt, enhancing rather than inhibiting its activity [48].

Similarly, SIRT1 (Sirtuin 1) deacetylates FOXO3, modulating its target gene specificity toward stress resistance rather than apoptosis [49]. These interactions position FOXO3 as a central hub in the metabolic control of aging and longevity.

Preclinical Evidence

Lifespan Extension in Model Organisms

The role of FOXO3 in lifespan regulation has been extensively studied across evolutionarily diverse model organisms, providing compelling evidence for its conservation as a longevity factor. In *C. elegans*, overexpression of the FOXO3 ortholog DAF-16 extends lifespan by 20-30%, while genetic disruption of DAF-16 abolishes the longevity effects of reduced insulin/IGF-1 signalling [50]. This foundational discovery established FOXO proteins as central mediators of longevity pathways.

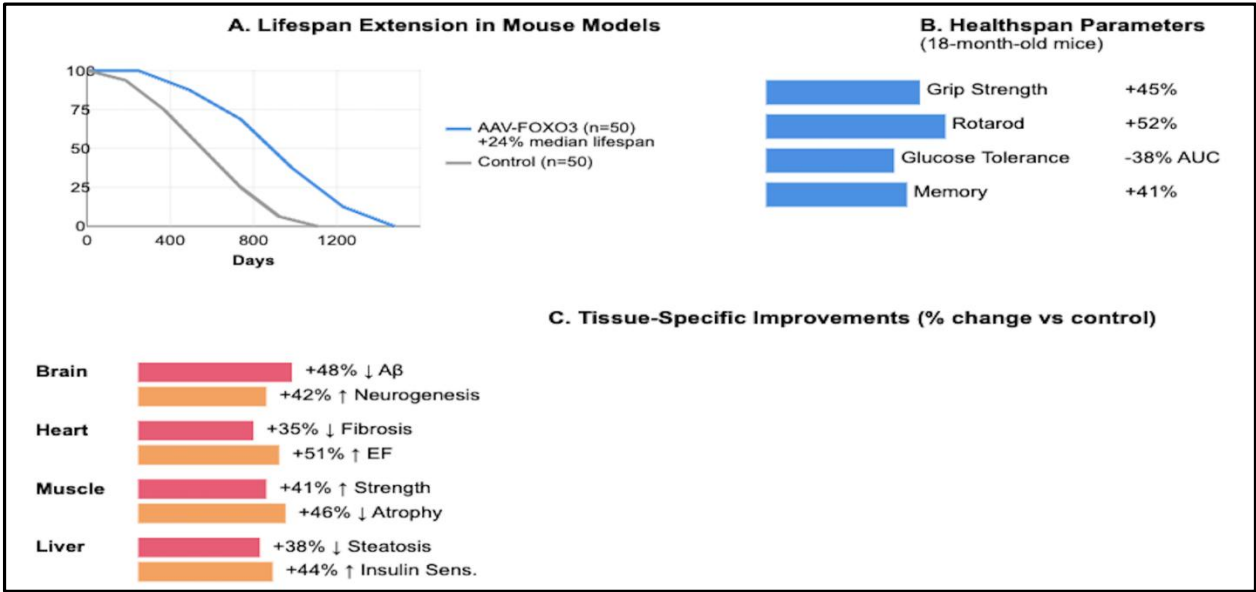


Figure 6: Preclinical Efficacy Data.

A: Kaplan-Meier survival curves showing significant lifespan extension with AAV-FOXO3 treatment. **B:** Functional healthspan parameters improved in treated mice. **C:** Tissue-specific benefits of FOXO3 gene therapy. EF = ejection fraction; AUC = area under curve.

The mechanisms underlying DAF-16-mediated lifespan extension in *C. elegans* involve comprehensive changes in gene expression. Transcriptomic analyses reveal that DAF-16 regulates hundreds of genes involved in stress resistance, metabolism, and cellular maintenance [51]. Key targets include superoxide dismutases, heat shock proteins, and antimicrobial peptides. The coordinated expression of these protective factors creates a state of enhanced stress resistance that correlates with extended lifespan.

In *Drosophila melanogaster*, overexpression of dFOXO in fat body or muscle tissue extends lifespan by up to 20%, accompanied by enhanced resistance to oxidative stress and starvation [52]. The tissue-specific effects are particularly interesting, as fat body-specific expression produces systemic benefits, suggesting endocrine signaling mechanisms. This non-autonomous effect indicates that FOXO3 activation in specific tissues can influence whole-organism aging.

Mouse models provide crucial mammalian evidence for FOXO3's role in longevity. Whole-body FOXO3 transgenic overexpression increases lifespan by 15-20%, with greater effects observed in females than males [53]. These long-lived mice exhibit improved metabolic profiles, including enhanced insulin sensitivity and reduced adiposity during aging [54]. The sex-specific differences in lifespan extension may relate to interactions between FOXO3 and sex hormone signaling pathways, an area requiring further investigation.

Tissue-specific FOXO3 overexpression studies reveal that activation in specific organs can confer systemic longevity benefits. Neuronal FOXO3 overexpression extends lifespan and improves cognitive function in aged mice [29]. Similarly, muscle-specific FOXO3 activation enhances whole-body metabolism and stress resistance. These findings suggest that FOXO3

activation in key metabolic tissues can trigger systemic anti-aging effects, potentially through secreted factors or metabolic changes.

AAV-mediated delivery of FOXO3 to aged mice results in significant lifespan extension when administered mid-life, demonstrating the potential for intervention even after aging has begun [4]. Mice receiving AAV-FOXO3 at 18 months of age showed 12% lifespan extension compared to controls, with improvements in multiple health parameters. This therapeutic effect supports the potential for gene therapy approaches targeting FOXO3 in elderly human populations.

Tissue-Specific Effects of FOXO3 Modulation

The effects of FOXO3 modulation vary across tissue types, reflecting tissue-specific requirements for stress resistance and metabolic regulation. Understanding these tissue-specific effects is crucial for developing targeted therapeutic approaches and predicting potential benefits and risks of FOXO3 gene therapy [55,56].

In skeletal muscle, FOXO3 activation produces complex effects that balance atrophy signaling with quality control mechanisms. While acute FOXO3 activation can induce muscle atrophy through upregulation of atrophy-related ubiquitin ligases [57], chronic moderate activation promotes autophagy and mitochondrial quality control, preserving muscle function during aging [58].

AAV-FOXO3 delivery to aged mouse muscles reduces age-related atrophy, enhances strength, and improves recovery from injury [59]. These benefits appear to result from enhanced protein quality control and improved mitochondrial function rather than increased muscle mass per se.

In the brain, FOXO3 overexpression provides remarkable neuroprotection against age-related cognitive decline and neurodegenerative pathology. Neuronal FOXO3 expression preserves memory function in aged mice and reduces accumulation of misfolded proteins associated with Alzheimer's disease [60]. The mechanisms involve enhanced autophagy, reduced oxidative stress, and improved mitochondrial function in neurons [61]. Additionally, FOXO3 activation in glial cells reduces neuroinflammation, a key contributor to age-related cognitive decline.

Cardiovascular tissues demonstrate significant improvements with FOXO3 activation. In cardiomyocytes, FOXO3 enhances resistance to ischemia-reperfusion injury and reduces age-related cardiac hypertrophy [62]. These cardioprotective effects involve upregulation of antioxidant enzymes, improved mitochondrial function, and enhanced autophagy. In vascular endothelial cells, FOXO3 activation reduces inflammatory signaling, enhances nitric oxide production, and improves angiogenic capacity [63]. These vascular benefits translate to reduced atherosclerosis and improved tissue perfusion in aged animals.

Hepatic FOXO3 expression produces metabolic benefits that extend beyond the liver itself. FOXO3 activation in hepatocytes enhances gluconeogenesis during fasting while improving insulin sensitivity during feeding [64]. This metabolic flexibility is often lost during aging, contributing to metabolic syndrome. Liver-specific FOXO3 overexpression protects against age-related hepatic steatosis, reduces inflammation, and preserves liver function in aged mice [65]. These hepatic benefits have systemic effects on glucose and lipid metabolism.

In the immune system, FOXO3 modulation produces cell type-specific effects that

collectively enhance immune function while reducing harmful inflammation. In T cells, FOXO3 maintains naive T cell pools and enhances memory formation [66]. In macrophages, FOXO3 activation reduces pro-inflammatory cytokine production while maintaining antimicrobial functions. These immunomodulatory effects contribute to reduced "inflammaging" and improved pathogen resistance in FOXO3-activated animals.

Stress Resistance Phenotypes

FOXO3 activation confers remarkable resistance to diverse stressors, a phenotype consistently observed across model organisms. This stress resistance represents a fundamental mechanism through which FOXO3 promotes longevity, as enhanced ability to withstand environmental challenges reduces cellular damage accumulation over time.

In *C. elegans*, worms with enhanced DAF-16 activity survive otherwise lethal heat shock, oxidative challenges, and pathogen exposure [67]. The heat shock resistance involves upregulation of heat shock proteins that function as molecular chaperones, preventing protein aggregation and maintaining proteostasis under thermal stress. Oxidative stress resistance results from the coordinated expression of antioxidant enzymes and DNA repair proteins. Pathogen resistance involves both enhanced antimicrobial peptide production and improved cellular resilience to pathogen-induced damage [68].

Drosophila overexpressing dFOXO similarly exhibit enhanced tolerance to heat, oxidative stress, and starvation [69]. The starvation resistance is particularly notable, as it involves metabolic reprogramming that enhances utilization of stored nutrients while reducing metabolic rate. This metabolic flexibility represents an important survival advantage that

likely contributed to the evolutionary conservation of FOXO function.

In mammalian models, FOXO3 overexpression protects against multiple stressors relevant to human health and aging. Cultured cells with elevated FOXO3 activity demonstrate enhanced survival when exposed to hydrogen peroxide, UV radiation, or serum deprivation [18]. The mechanisms involve rapid upregulation of protective genes, enhanced DNA repair capacity, and improved mitochondrial function. These cellular studies provide mechanistic insights that complement whole-organism observations [70].

In vivo studies demonstrate that FOXO3 activation protects tissues from ischemia-reperfusion injury, a clinically relevant stressor. Mouse models with enhanced FOXO3 activity show reduced infarct size following myocardial ischemia and better functional recovery [71].

Similar protection is observed in models of stroke and acute kidney injury. The protective mechanisms include reduced oxidative damage, enhanced autophagy of damaged organelles, and improved cellular energy metabolism during the reperfusion phase.

The stress resistance conferred by FOXO3 extends to metabolic stressors. Animals with enhanced FOXO3 activity show improved glucose tolerance, enhanced insulin sensitivity, and resistance to diet-induced obesity [72].

These metabolic benefits result from improved mitochondrial function, enhanced insulin signaling in key metabolic tissues, and reduced inflammatory signaling. The broad stress resistance phenotype of FOXO3

activation suggests therapeutic potential for diverse age-related conditions characterized by reduced stress tolerance.

Disease Models

FOXO3 modulation demonstrates therapeutic potential in diverse disease models beyond normal aging, providing proof-of-concept for targeted therapeutic applications. These disease-specific benefits offer potential regulatory pathways for advancing FOXO3-based therapies.

In neurodegenerative disease models, FOXO3 activation shows particularly promising results. In Alzheimer's disease models, FOXO3 overexpression reduces amyloid- β accumulation and preserves cognitive function through enhanced autophagy and reduced oxidative stress [73].

Mechanistic studies reveal that FOXO3 promotes clearance of amyloid- β through upregulation of autophagy genes and enhancement of lysosomal function. Additionally, FOXO3 reduces tau hyperphosphorylation, another hallmark of Alzheimer's pathology, through regulation of kinase and phosphatase expression [74].

Parkinson's disease models also respond favorably to FOXO3 activation. In toxin-induced and genetic models of Parkinson's disease, FOXO3 protects dopaminergic neurons from α -synuclein toxicity and oxidative damage [75].

The neuroprotection involves multiple mechanisms including enhanced mitophagy to clear damaged mitochondria, upregulation of antioxidant defenses, and promotion of α -synuclein clearance through autophagy.

Behavioral assessments show improved motor function in FOXO3-treated animals, suggesting functional as well as histological benefits.

In metabolic disease models, FOXO3 activation improves multiple parameters. In type 2 diabetes models, hepatic FOXO3 overexpression reduces hyperglycemia through complex effects on glucose metabolism [76]. While FOXO3 promotes gluconeogenesis, it simultaneously enhances insulin sensitivity, resulting in improved glucose homeostasis. In diet-induced obesity models, FOXO3 activation reduces adiposity, improves lipid profiles, and enhances metabolic flexibility [77]. These effects involve both direct metabolic regulation and indirect effects through reduced inflammation.

Cardiovascular disease models demonstrate significant benefits from FOXO3 modulation. In atherosclerosis models, FOXO3 activation in endothelial cells reduces inflammatory activation and plaque formation [78]. The anti-atherosclerotic effects involve reduced expression of adhesion molecules, decreased inflammatory cytokine production, and enhanced endothelial barrier function. In heart failure models, cardiac FOXO3 overexpression preserves ejection fraction and reduces pathological remodeling [79]. The cardioprotection results from enhanced autophagy, improved mitochondrial function, and reduced oxidative stress.

Muscular dystrophy models show particularly impressive responses to FOXO3 therapy. AAV-mediated FOXO3 delivery to dystrophic muscles reduces degeneration, enhances regeneration, and improves functional performance [80]. The benefits appear to result from multiple mechanisms including enhanced satellite cell

function, reduced oxidative damage, and improved protein quality control. These effects are observed in both Duchenne muscular dystrophy models and age-related sarcopenia, suggesting broad applicability to muscle wasting conditions.

Cancer models present a complex picture regarding FOXO3 modulation. While FOXO3 generally functions as a tumor suppressor, its effects vary depending on cancer type and stage [81]. In early-stage cancer models, FOXO3 activation can suppress tumor initiation through induction of apoptosis and cell cycle arrest.

However, in established tumors, FOXO3 may promote survival under stress conditions. This complexity necessitates careful consideration of cancer risk in FOXO3 therapeutic development.

Human Studies

FOXO3 Variants in Centenarian Populations

Human genetic studies have consistently identified associations between FOXO3 variants and exceptional longevity across diverse populations, providing compelling evidence for FOXO3's role in human aging. The most robust associations involve the G allele of single nucleotide polymorphism (SNP) rs2802292, which shows significant enrichment in individuals achieving extreme old age [4].

The initial discovery came from studies of Japanese American men in the Honolulu Heart Program and Honolulu-Asia Aging Study. Willcox and colleagues demonstrated that carriers of the FOXO3 G allele exhibited significantly increased odds of living to 95 years or beyond, with homozygous carriers showing the strongest effect [4]. The odds ratio for achieving exceptional longevity was 2.75 for G

allele homozygotes compared to non-carriers, representing one of the strongest genetic associations with human longevity identified to date [82,83].

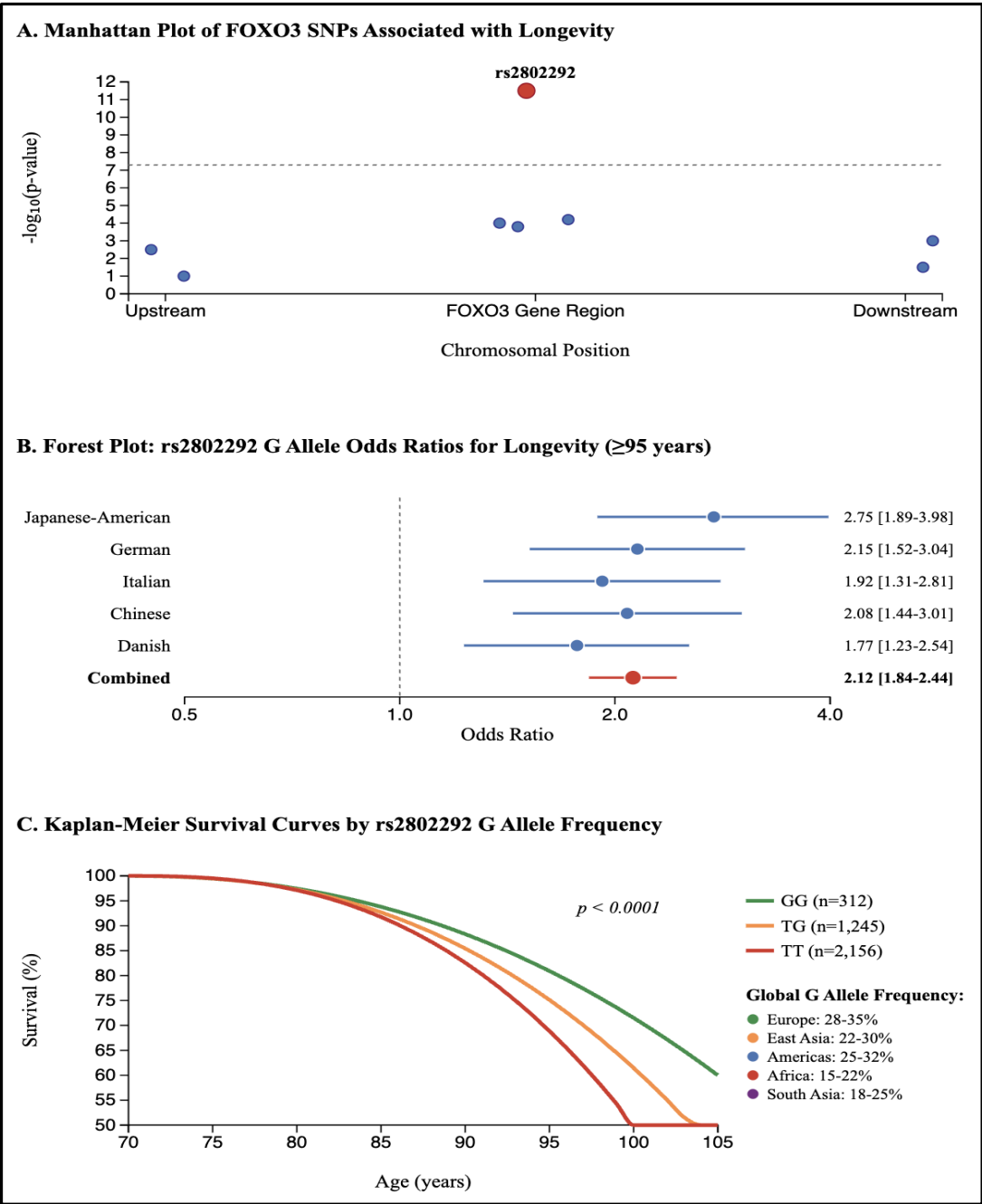


Figure 7: Human FOXO3 Variants and Longevity.

Human genetic evidence for FOXO3's role in longevity. **A:** Genome-wide association analysis showing strongest signal at rs2802292. **B:** Meta-analysis across multiple populations demonstrates consistent association with exceptional longevity. **C:** Survival analysis shows dose-dependent protective effect of G allele. Global distribution reveals population variation in protective allele frequency. **D:** Geographic variation in protective allele frequency.

Subsequent replication studies have confirmed this association across multiple ethnic groups and geographic regions. German centenarian studies by Flachsbart et al. found similar enrichment of the G allele, with an odds ratio of 1.53 for heterozygotes and 2.15 for homozygotes [5]. Italian centenarian cohorts showed comparable results, as did studies of Han Chinese long-lived individuals [6,7]. This cross-ethnic replication is particularly notable, as many longevity-associated variants show population-specific effects.

Meta-analyses combining data from multiple cohorts estimate that FOXO3 variants confer approximately 25-40% increased odds of achieving exceptional longevity [84]. The effect size is comparable to or greater than other well-established longevity genes such as APOE. Importantly, the longevity association appears to result from reduced risk of age-related diseases rather than simply extended lifespan with disability. Functional studies of longevity-associated FOXO3 variants provide mechanistic insights. The protective variants appear to influence FOXO3 expression levels and cellular localization. Lymphoblastoid cell lines from individuals carrying the G allele show enhanced FOXO3 nuclear localization under the stressed

conditions [85]. This enhanced nuclear localization correlates with increased expression of FOXO3 target genes involved in stress resistance, suggesting a functional basis for the longevity association.

Furthermore, longevity-associated FOXO3 variants correlate with reduced risk of age-related diseases. Carriers of protective alleles show lower incidence of cardiovascular disease, cancer, and type 2 diabetes [86]. In the Kuakini Honolulu Heart Program, G allele carriers had 10% lower risk of coronary heart disease and 12% lower cancer mortality. These disease protection effects likely contribute to the overall longevity benefit, supporting FOXO3's role as a general promoter of healthy aging rather than simply lifespan extension.

Expression Changes in Aging and Disease

FOXO3 expression and activity undergo significant changes during normal aging and in age-related pathologies, providing insights into its role in human aging processes. Understanding these expression patterns helps identify potential therapeutic windows and target populations for FOXO3-based interventions.

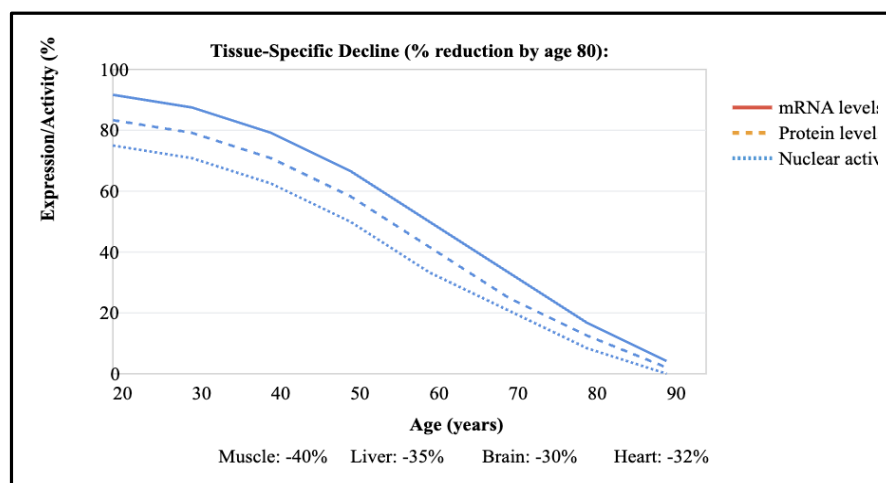


Chart 1: Timeline of FOXO3 Expression Changes with Age.

Studies of muscle biopsies show progressive reduction in FOXO3 mRNA and protein levels with advancing age, with approximately 30-40% reduction by age 80 compared to young adults [87]. This decline correlates with reduced expression of FOXO3 target genes involved in stress resistance and cellular maintenance. Similar age-related reductions occur in liver, adipose tissue, and peripheral blood cells [88].

The mechanisms underlying age-related FOXO3 decline appear multifactorial. Epigenetic changes including increased promoter methylation contribute to reduced transcription [89]. Additionally, age-related increases in insulin/IGF-1 signaling and inflammatory cytokines promote FOXO3 phosphorylation and cytoplasmic sequestration. Reduced sirtuin activity with aging decreases activating deacetylation of FOXO3. These converging mechanisms create a feed-forward cycle where reduced FOXO3 activity contributes to cellular aging, which further suppresses FOXO3 function.

In age-related diseases, FOXO3 expression patterns show disease-specific alterations. In type 2 diabetes, hepatic FOXO3 expression is paradoxically elevated but functionally impaired due to abnormal post-translational modifications [90].

This "FOXO3 resistance" parallels insulin resistance and contributes to dysregulated glucose metabolism. In contrast, muscle FOXO3 expression is reduced in diabetic patients, correlating with impaired glucose uptake and mitochondrial dysfunction. Neurodegenerative diseases show particularly interesting FOXO3 expression patterns. In Alzheimer's disease brains, FOXO3 expression is reduced in neurons but increased in reactive astrocytes [91]. This cell type-specific regulation may reflect protective

responses in glia concurrent with neuronal dysfunction. The subcellular localization of FOXO3 is also altered, with increased cytoplasmic sequestration in affected neurons despite cellular stress that would normally promote nuclear translocation.

In sarcopenia and frailty syndromes, muscle FOXO3 expression shows complex alterations. While total FOXO3 levels decline, the remaining FOXO3 shows increased nuclear localization, possibly reflecting compensatory activation in response to muscle stress [92]. However, this activation appears insufficient to prevent muscle loss, suggesting that therapeutic enhancement of FOXO3 activity might provide benefit. Indeed, exercise training, which improves muscle function in elderly individuals, increases FOXO3 expression and enhances its transcriptional activity [93].

Biomarker Correlations

FOXO3 genetic variants and expression levels correlate with multiple biomarkers of aging and disease risk in human populations, providing evidence for FOXO3's systemic effects on human health. These biomarker associations support the potential for FOXO3-based interventions to impact multiple aspects of aging simultaneously.

Inflammatory markers show particularly strong associations with FOXO3 status. Carriers of longevity-associated FOXO3 alleles demonstrate lower levels of circulating inflammatory markers including IL-6, TNF- α , and C-reactive protein [94]. In the Long Life Family Study, G allele carriers had 15-20% lower IL-6 levels compared to non-carriers of similar age. This anti-inflammatory association persists after adjustment for other factors, suggesting direct effects of FOXO3 on inflammatory regulation.

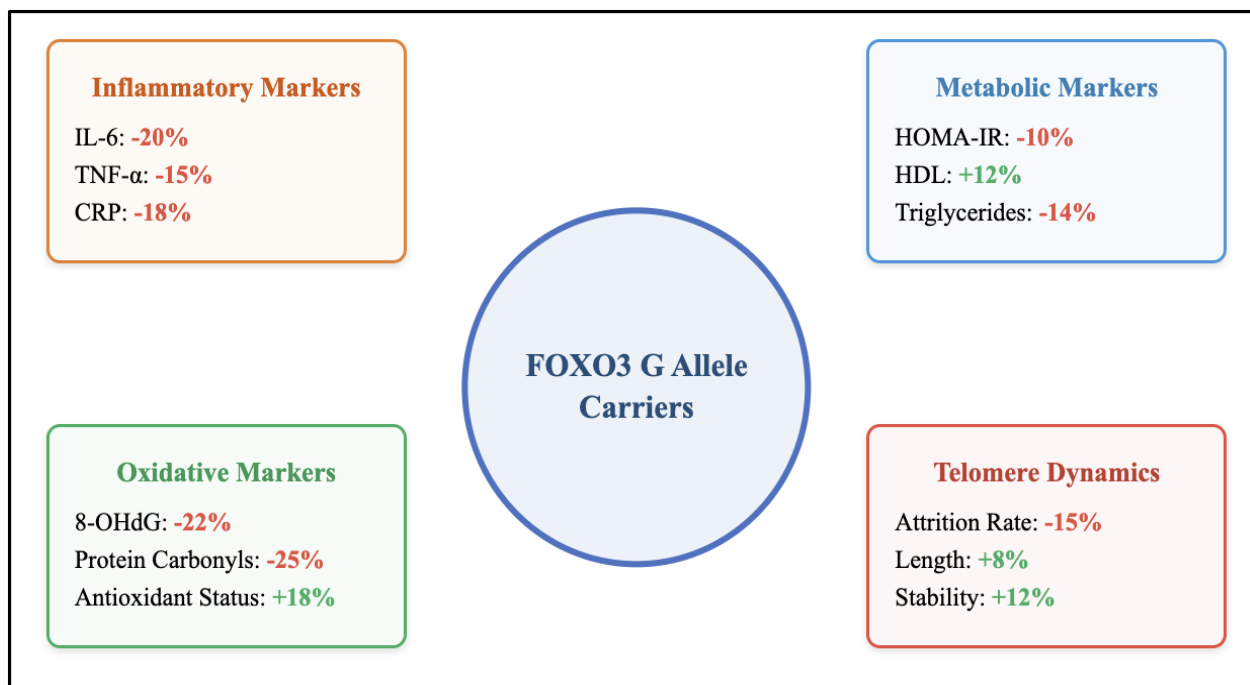


Figure 8: Biomarker Profile in FOXO3 G Allele Carriers vs Non-Carriers.

Comprehensive biomarker improvements observed in carriers of longevity-associated FOXO3 variants, demonstrating systemic beneficial effects across multiple aging-related pathways including reduced inflammation, enhanced antioxidant capacity, improved metabolic parameters, and better telomere maintenance.

Metabolic biomarkers also correlate with FOXO3 variants. Individuals carrying protective alleles exhibit enhanced insulin sensitivity as measured by HOMA-IR, with approximately 10% improvement compared to non-carriers [95]. Lipid profiles are more favorable, with higher HDL cholesterol and lower triglycerides. These metabolic benefits appear to result from enhanced FOXO3 activity in liver and muscle, improving metabolic flexibility and reducing lipotoxicity.

Telomere dynamics represent another biomarker domain associated with FOXO3 status. While FOXO3 does not directly regulate telomerase, carriers of longevity-associated variants demonstrate reduced telomere attrition rates [96]. This may reflect FOXO3's role in reducing oxidative stress and inflammation, both of which accelerate telomere shortening. Additionally, enhanced stress resistance in FOXO3 variant

carriers may reduce replicative stress on highly proliferative tissues.

Oxidative stress markers show expected associations with FOXO3 status. Individuals with protective variants have lower levels of oxidized LDL, 8-hydroxy-2'-deoxyguanosine (a marker of DNA oxidation), and protein carbonyls [97].

Conversely, antioxidant capacity markers including total antioxidant status and glutathione levels are elevated. These findings align with FOXO3's role in upregulating antioxidant defenses and suggest that genetic variation in FOXO3 has functional consequences for oxidative stress management. Neuroimaging biomarkers provide insights into FOXO3's effects on brain aging. Carriers of protective FOXO3 alleles show reduced age-related gray matter

atrophy on MRI, particularly in regions vulnerable to aging such as the hippocampus and prefrontal cortex [98]. White matter integrity, assessed by diffusion tensor imaging, is better preserved in G allele carriers. Functional imaging shows enhanced cognitive network connectivity in elderly carriers compared to non-carriers. These structural and functional brain differences correlate with preserved cognitive performance, supporting FOXO3's neuroprotective role.

Gene Therapy Approaches

Vector Design Considerations

Developing effective gene therapy vectors for FOXO3 delivery requires careful consideration of multiple factors including expression level, tissue targeting, and safety. Adeno-associated viral (AAV) vectors have emerged as the leading platform due to their excellent safety profile, efficient transduction, and long-term expression capabilities[99].

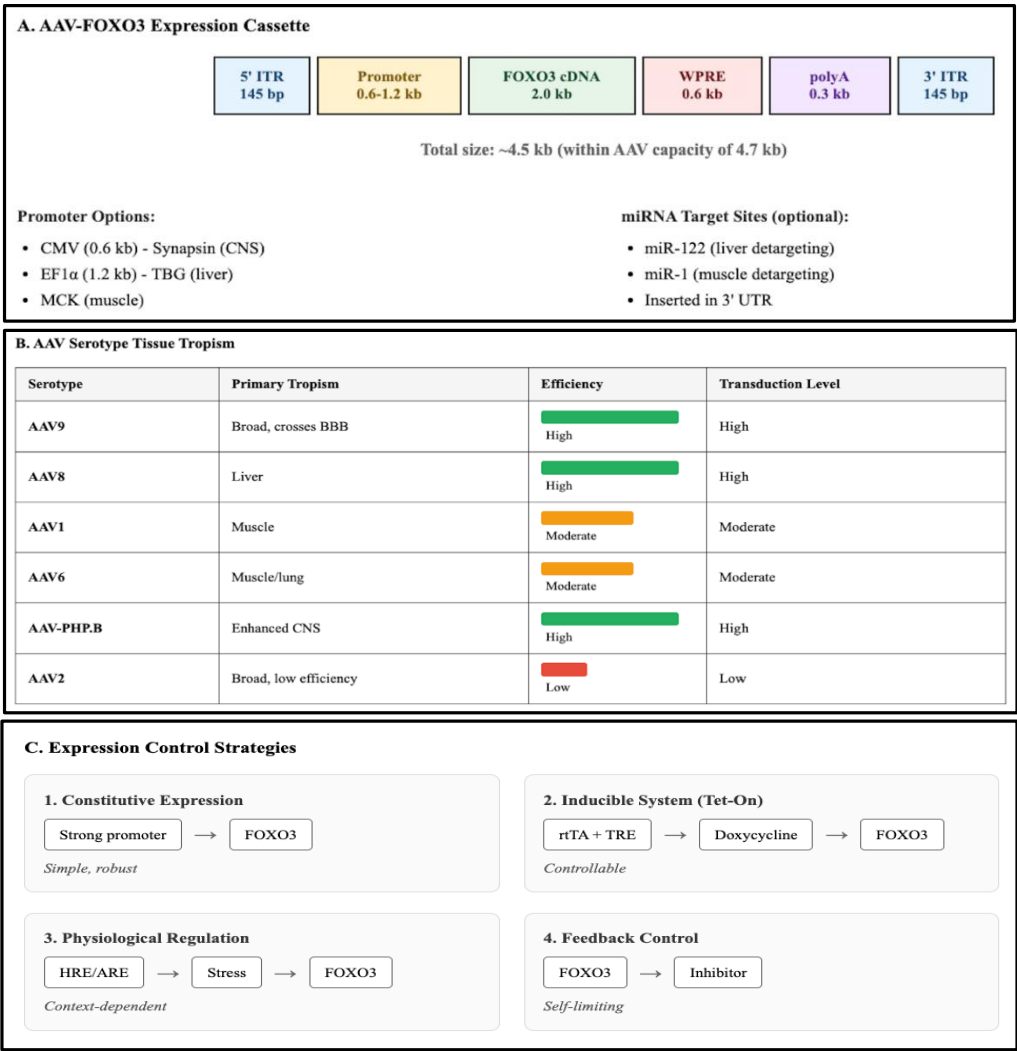


Chart 2: AAV-FOXO3 Vector Design.

A: Modular design of AAV-FOXO3 expression cassette with regulatory elements. **B:** Tissue tropism profiles of different AAV serotypes. **C:** Various expression control strategies for optimized FOXO3 delivery.

The FOXO3 coding sequence (approximately 2.0 kb) fits comfortably within AAV packaging constraints, allowing inclusion of regulatory elements for controlled expression [100]. This is advantageous compared to larger longevity-associated genes that require dual-vector approaches. Codon optimization for human expression can enhance protein production without altering amino acid sequence, potentially improving therapeutic efficacy. Selection critically influences therapeutic outcomes.

Constitutive promoters like CMV or CAG provide robust expression but lack physiological regulation [101]. Given FOXO3's complex regulatory needs, more sophisticated approaches are warranted. Endogenous FOXO3 promoter elements can provide physiological regulation but may be too weak for therapeutic effect. Hybrid promoters combining strong basal expression with stress-responsive elements offer a promising compromise.

Approach	Vector	Promoter	Advantages	Limitations	Clinical Application
Systemic AAV9	AAV9	CMV/CAG	Broad tissue distribution; Crosses BBB; Single injection	High dose required; Potential immune response; Non-specific	General anti-aging
Muscle-specific	AAV1/6	MCK	Local effect; Lower dose; Reduced systemic exposure	Limited to muscle; Multiple injections; No CNS effects	Sarcopenia, frailty
CNS-targeted	AAV-PHP.B	Synapsin	Enhanced brain delivery; Neuroprotection; Lower peripheral dose	Invasive delivery; Limited to CNS; Technical complexity	Neurodegeneration
Liver-directed	AAV8	TBG/albumin	Efficient transduction; Metabolic effects; IV delivery	Hepatic only; Pre-existing immunity; Dose-dependent	Metabolic disorders
Inducible	Any AAV	Tet-On/Off	Controllable expression; Safety switch; Dose titration	Requires inducer drug; Complex design; Reduced capacity	High-risk patients

Table 2: Comparison of FOXO3 Gene Therapy Approaches

Comparison of different FOXO3 gene therapy delivery approaches, highlighting the advantages, limitations, and potential clinical applications for each strategy. The choice of approach depends on therapeutic goals, target patient population, and safety considerations.

Incorporation of post-transcriptional regulatory elements can fine-tune FOXO3 expression. The woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) enhances mRNA stability and translation efficiency [102].

MicroRNA target sequences can be included to suppress expression in specific cell types where FOXO3 activation might be detrimental, such as highly proliferative tissues. This approach has been successfully used to detarget liver

expression while maintaining therapeutic levels in other tissues.

AAV serotype selection significantly impacts treatment outcomes. For systemic FOXO3 therapy targeting multiple organs of aging, AAV9 offers broad tropism including crossing the blood-brain barrier [103]. For more targeted approaches, tissue-specific serotypes can be employed: AAV8 for liver, AAV1 or AAV6 for muscle, AAV-PHP.B for enhanced CNS

delivery. Novel engineered capsids developed through directed evolution may offer even greater specificity and efficiency.

Safety elements should be incorporated into vector design. Self-complementary AAV vectors can enhance expression onset but reduce packaging capacity [104]. Including elements that prevent random integration, though AAV integration is already rare, provides additional safety. Insulator sequences can reduce position effects if integration does occur. These safety considerations are particularly important given the long-term nature of anti-aging interventions.

Tissue Targeting Strategies

Effective FOXO3 gene therapy requires precise targeting to tissues where intervention will provide maximal benefit while minimizing potential adverse effects. Different targeting strategies offer unique advantages depending on the therapeutic goals and target patient population.

Systemic delivery approaches aim to enhance FOXO3 activity across multiple tissues simultaneously, addressing the multi-organ nature of aging. Intravenous administration of AAV9-FOXO3 achieves widespread transduction including heart, liver, muscle, and brain [105].

This approach is particularly relevant for general anti-aging interventions where coordinated enhancement across tissues may provide synergistic benefits. However, systemic delivery requires higher vector doses and careful monitoring for off-target effects.

CNS-targeted delivery addresses the critical role of brain aging in overall healthspan. Intrathecal administration achieves widespread CNS

transduction while minimizing systemic exposure [106]. For more focused intervention, direct intracranial injection can target specific brain regions affected by neurodegenerative disease. Novel approaches using focused ultrasound to temporarily disrupt the blood-brain barrier enable non-invasive CNS targeting following systemic administration [107].

Muscle-specific targeting offers advantages for addressing sarcopenia and metabolic dysfunction. Intramuscular injection achieves high local expression with minimal systemic spread [108]. For broader muscle targeting, limb perfusion techniques can transduce entire muscle groups. Muscle-specific promoters like MCK or desmin further restrict expression to muscle fibers, avoiding expression in infiltrating immune cells or fibroblasts.

Liver-targeted approaches leverage the liver's central role in metabolism and the ease of hepatic transduction. AAV8 shows strong liver tropism following intravenous delivery [109]. Hepatocyte-specific promoters like albumin or $\alpha 1$ -antitrypsin can further restrict expression. Given FOXO3's complex effects on hepatic metabolism, careful dose optimization is essential to achieve metabolic benefits without excessive gluconeogenesis.

Multi-organ targeting strategies combine elements of systemic and tissue-specific approaches. Sequential administration of different serotypes can achieve staged transduction of multiple organs while managing total vector load [110].

Alternatively, mosaic vectors incorporating multiple tissue-specific promoters in the same construct can achieve coordinated expression across predetermined tissues. These sophisticated approaches may optimize the benefit-to-risk ratio of FOXO3 therapy.

Expression Control Mechanisms

Precise regulation of FOXO3 expression represents a critical challenge for safe and effective gene therapy. Unlike simple enzyme

replacement, FOXO3's transcription factor function requires careful control to avoid disrupting cellular homeostasis while achieving all the therapeutic benefits.

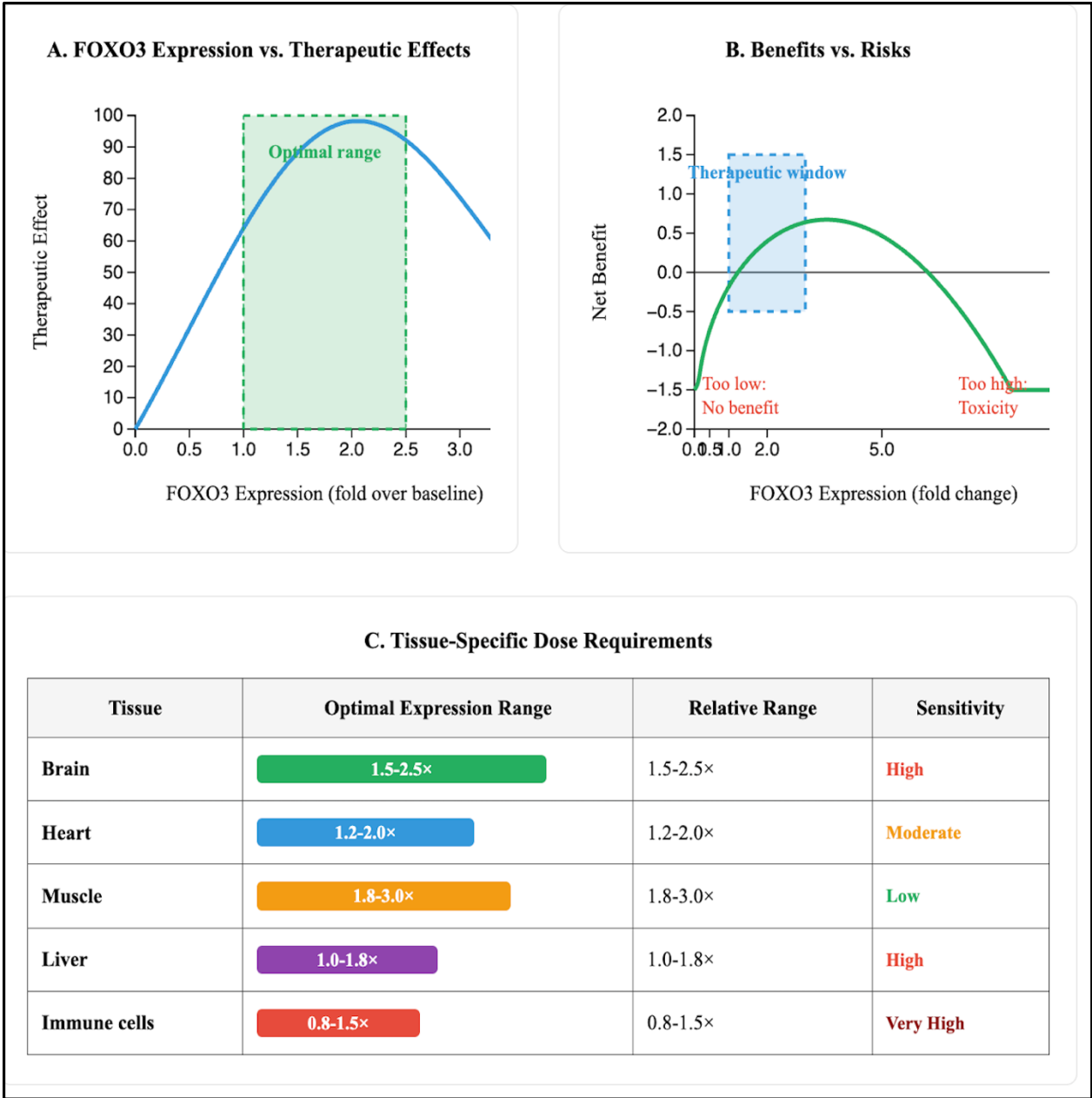


Figure 9: Dose-response relationships for FOXO3 gene therapy.

Showing **A**: the therapeutic window with optimal expression levels, **B**: the balance between benefits and risks across different expression levels, and **C**: tissue-specific requirements and sensitivities that inform dosing strategies.

Inducible expression systems provide external control over FOXO3 levels. The tetracycline-regulated system enables dose-dependent activation using doxycycline, an FDA-approved antibiotic [111]. This allows titration of FOXO3 expression to individual patient needs and cessation if adverse effects occur. More advanced Tet-On 3G systems show reduced background expression and enhanced sensitivity. Alternative inducible systems using rapamycin or tamoxifen analogs provide additional options with different pharmacological properties.

Physiologically responsive promoters offer more natural regulation. Hypoxia-responsive elements activate FOXO3 expression under ischemic conditions when cellular protection is most needed [112]. Similarly, antioxidant response elements induce expression during oxidative stress. Glucose-responsive promoters can link FOXO3 expression to metabolic state, potentially useful for diabetes applications. These endogenous regulatory systems maintain physiological control while enhancing stress responses.

Post-translational regulation provides another control layer. Engineering FOXO3 variants with modified phosphorylation sites can alter subcellular localization dynamics [113]. For example, mutations preventing Akt phosphorylation create constitutively nuclear variants with enhanced activity. Conversely, variants with additional nuclear export signals can be designed for reduced activity. These modifications allow fine-tuning of FOXO3 activity independent of expression level.

Feedback control mechanisms can prevent excessive FOXO3 activity. Including FOXO3-responsive elements that induce expression of endogenous inhibitors creates self-limiting systems [114]. As FOXO3 activity increases, it induces negative regulators that dampen further

activation. This biomimetic approach maintains homeostatic control while permitting enhanced stress responses.

Combination control strategies integrate multiple regulatory mechanisms. For example, combining tissue-specific promoters with inducible elements and feedback control creates sophisticated systems that activate appropriately in space and time [115]. These advanced approaches move beyond simple gene delivery toward true physiological enhancement of FOXO3 function.

Selective FOXO3 Target Activation

FOXO3 regulates hundreds of target genes with diverse and sometimes opposing functions, necessitating strategies to bias activity toward beneficial programs while minimizing potential negative effects. This selective activation represents a frontier in FOXO3 therapeutic development.

Domain engineering approaches modify FOXO3 structure to alter target gene specificity. The DNA-binding domain can be mutated to prefer certain consensus sequences over others, biasing activity toward specific gene subsets [116]. Modifications to the transactivation domain can alter cofactor recruitment, changing which genes are activated. These engineered variants maintain FOXO3's protective functions while reducing unwanted activities like excessive autophagy or growth inhibition.

Cofactor co-delivery strategies enhance specific FOXO3 programs. Co-expressing FOXO3 with PGC-1 α biases activity toward mitochondrial biogenesis and metabolic genes [117]. Including SIRT1 promotes deacetylation that favors stress resistance over apoptotic programs. CBP/p300 co-delivery enhances different target gene sets. This cofactor approach leverages endogenous regulatory mechanisms to shape FOXO3 activity.

Synthetic transcription factors combining FOXO3 domains with other regulatory regions offer precise control. Fusion proteins linking FOXO3's transactivation domain to different DNA-binding domains can redirect activity to novel gene sets [118]. Conversely, FOXO3's DNA-binding domain can be linked to alternative activation domains that recruit different transcriptional machinery. These chimeric approaches enable custom transcriptional programs. Small molecule modulators can bias FOXO3 activity post-gene delivery. Compounds that affect specific post-translational modifications can shift target gene preferences [119]. For example, AMPK activators promote metabolic target genes while reducing apoptotic programs. Sirtuin activators similarly shape FOXO3 activity patterns. This pharmacological fine-tuning provides additional control over therapeutic outcomes.

Epigenetic modulation strategies influence which FOXO3 targets are accessible. Co-delivery of epigenetic modifiers can open or close chromatin at specific loci, determining which genes FOXO3 can activate [120]. This approach is particularly relevant for age-related epigenetic changes that may limit FOXO3's access to beneficial targets. Combining FOXO3 delivery with epigenetic rejuvenation may synergistically enhance therapeutic outcomes.

Potential Applications

Age-Related Disease Prevention

FOXO3 gene therapy shows exceptional promise for preventing or delaying multiple age-related diseases through intervention in common underlying mechanisms of aging. By addressing fundamental processes like oxidative stress, inflammation, and cellular maintenance, FOXO3 activation could provide broad protection against age-related pathology. Cardiovascular disease

prevention represents a primary application given the strong associations between FOXO3 variants and reduced cardiovascular mortality. FOXO3 gene therapy could protect against atherosclerosis through multiple mechanisms [121]. In endothelial cells, FOXO3 activation reduces inflammatory activation, decreases adhesion molecule expression, and enhances nitric oxide production. These effects collectively maintain vascular health and reduce plaque formation. In vascular smooth muscle cells, FOXO3 prevents pathological proliferation and migration that contributes to atherosclerosis.

For heart failure prevention, FOXO3 activation in cardiomyocytes enhances stress resistance and prevents pathological remodeling. The upregulation of antioxidant enzymes protects against ischemia-reperfusion injury, a common precipitant of heart failure [122].

Enhanced autophagy maintains mitochondrial quality, preserving cardiac energetics. FOXO3 also antagonizes pro-hypertrophic signaling pathways, preventing the pathological cardiac enlargement that precedes failure. These protective mechanisms suggest prophylactic potential for individuals at high cardiovascular risk.

Metabolic disease prevention through FOXO3 therapy addresses the growing epidemic of type 2 diabetes and metabolic syndrome. Hepatic FOXO3 activation improves insulin sensitivity while maintaining appropriate glucose production [123]. In skeletal muscle, FOXO3 enhances glucose uptake and mitochondrial function. In adipose tissue, FOXO3 reduces inflammatory cytokine production that contributes to insulin resistance. The coordinated improvement across metabolic tissues could prevent progression from prediabetes to frank diabetes.

Neurodegenerative disease prevention represents another compelling application. FOXO3 activation enhances clearance of protein aggregates through autophagy upregulation, potentially preventing accumulation of amyloid- β , tau, and α -synuclein [124]. The antioxidant and anti-inflammatory effects protect neurons from age-related damage. Enhanced adult neurogenesis and synaptic plasticity maintain cognitive reserve. Preventive FOXO3 therapy in individuals with genetic risk factors or early biomarker changes could delay or prevent clinical manifestation of dementia.

Cancer prevention through FOXO3 activation leverages its tumor suppressor functions. FOXO3 induces cell cycle arrest and apoptosis in cells with oncogenic stress, preventing malignant transformation [125].

Enhanced DNA repair reduces mutation accumulation. Improved immune surveillance through FOXO3 effects on immune cells may eliminate pre-cancerous cells. While cancer risk requires careful monitoring, the preclinical evidence suggests FOXO3 activation in normal cells provides protection rather than risk.

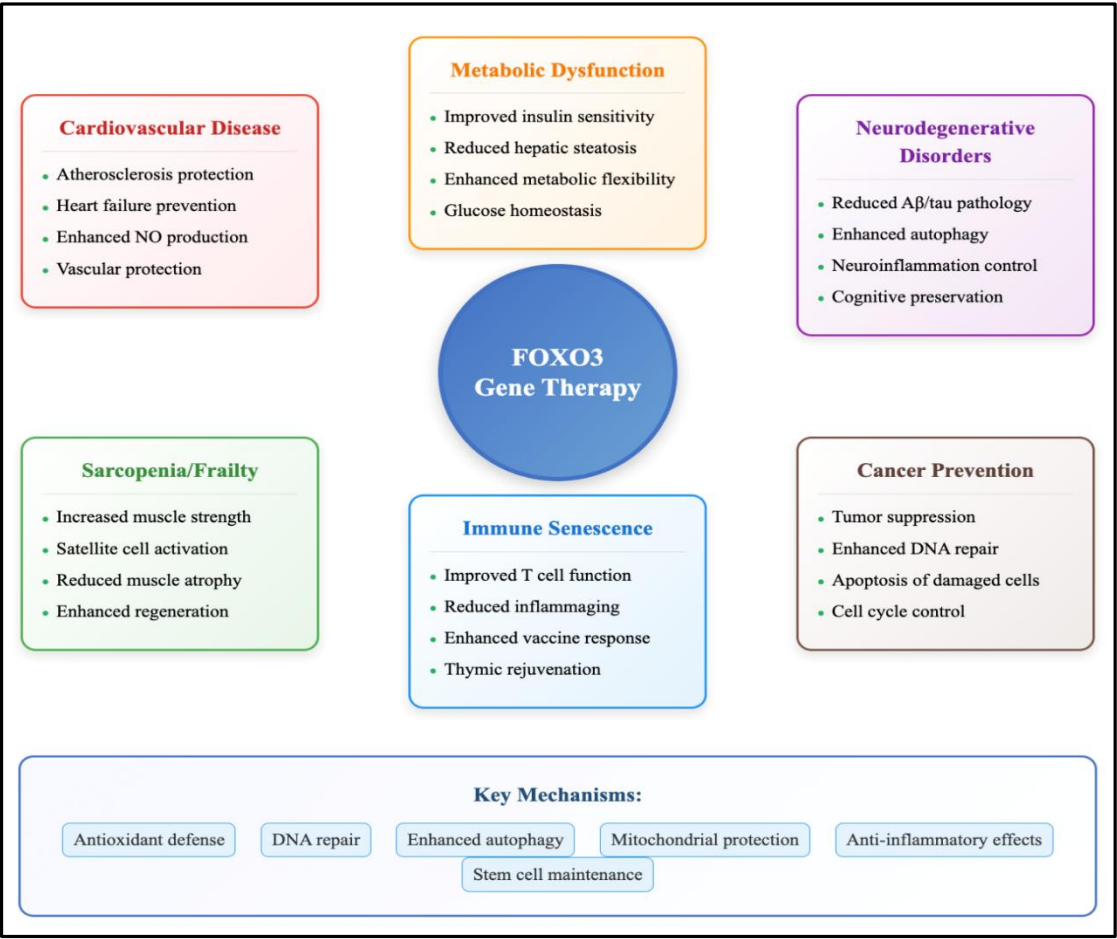


Figure 10: FOXO3 Gene Therapy Applications.

FOXO3 gene therapy targets multiple age-related diseases through common mechanisms of cellular protection and stress resistance. The central position of FOXO3 in aging biology enables a single intervention to address diverse pathological processes across multiple organ systems.

Enhanced Stress Resistance

Beyond disease prevention, FOXO3 gene therapy could enhance resilience against various stressors that contribute to age-related functional decline and acute medical events. This enhanced stress resistance could improve both daily quality of life and survival during medical crises.

Oxidative stress protection through FOXO3-mediated upregulation of antioxidant enzymes would benefit individuals exposed to high oxidative challenges. This includes those with chronic inflammatory conditions, individuals undergoing chemotherapy or radiation, and people exposed to environmental toxins [126]. Enhanced antioxidant capacity could reduce treatment side effects and improve tolerance to necessary medical interventions.

Ischemia resistance represents a particularly valuable clinical application. FOXO3 pre-activation could protect organs from ischemic damage during surgical procedures requiring temporary blood flow interruption [127].

In cardiac surgery, hepatic resection, or organ transplantation, FOXO3 gene therapy could provide prophylactic protection. For individuals with peripheral vascular disease, enhanced ischemia resistance could prevent tissue loss and improve wound healing.

Metabolic stress resistance conferred by FOXO3 could help individuals better tolerate the metabolic challenges of illness, surgery, or aging. Enhanced metabolic flexibility allows cells to switch between energy sources efficiently [128].

Improved mitochondrial function maintains cellular energetics under stress. These adaptations could reduce complications from critical illness, improve surgical outcomes, and enhance recovery from metabolic insults.

Inflammatory stress resistance through FOXO3's anti-inflammatory effects could benefit numerous conditions. By reducing NF- κ B signaling and inflammatory cytokine production, FOXO3 activation could dampen harmful inflammatory responses while preserving beneficial immunity [129]. This could prove valuable in conditions ranging from autoimmune diseases to COVID-19, where excessive inflammation drives pathology.

Physical stress resistance could help maintain functional capacity in elderly individuals. FOXO3 activation enhances muscle recovery from exercise, potentially allowing more effective physical therapy and rehabilitation [130]. Improved protein quality control reduces exercise-induced damage. Enhanced mitochondrial biogenesis improves exercise capacity. These effects could help elderly individuals maintain independence and quality of life through preserved physical function.

Neurodegenerative Conditions

FOXO3 gene therapy offers particular promise for neurodegenerative conditions characterized by protein aggregation, oxidative stress, and impaired proteostasis. The multifaceted neuroprotective mechanisms of FOXO3 address multiple pathological processes simultaneously.

In Alzheimer's disease, FOXO3 activation targets several key pathological mechanisms. Enhanced autophagy promotes clearance of amyloid- β peptides before they aggregate into plaques [131]. FOXO3 also enhances clearance of hyperphosphorylated tau, reducing neurofibrillary tangle formation.

The upregulation of antioxidant enzymes protects neurons from amyloid- β -induced oxidative damage. Anti-inflammatory effects reduce microglial activation that contributes to

neurodegeneration. These combined effects could slow disease progression and preserve cognitive function.

Parkinson's disease applications leverage FOXO3's ability to protect dopaminergic neurons. FOXO3 activation enhances α -synuclein clearance through autophagy induction, preventing formation of toxic aggregates [132].

Improved mitochondrial quality control through mitophagy is particularly relevant given mitochondrial dysfunction's central role in Parkinson's pathogenesis. FOXO3 also upregulates neurotrophic factors that support dopaminergic neuron survival. Early intervention with FOXO3 gene therapy could preserve dopamine production and motor function.

For Huntington's disease and other polyglutamine disorders, FOXO3 activation enhances clearance of mutant proteins with expanded polyglutamine repeats. The autophagy induction is particularly effective for clearing protein aggregates too large for proteasomal degradation [133].

FOXO3 also enhances neuronal stress resistance, potentially raising the threshold for polyglutamine toxicity. In mouse models, FOXO3 activation delays disease onset and reduces neuronal loss.

Amyotrophic lateral sclerosis (ALS) presents another potential application. FOXO3 activation in motor neurons enhances stress resistance and reduces vulnerability to excitotoxicity [134]. In astrocytes, FOXO3 reduces inflammatory activation that contributes to motor neuron death. The combined neuronal protection and reduced inflammation could slow disease progression. Additionally, FOXO3's effects on muscle satellite cells might help preserve neuromuscular junction integrity.

Beyond specific diseases, FOXO3 therapy could address age-related cognitive decline through multiple mechanisms. Enhanced adult neurogenesis in the hippocampus supports memory formation [135].

Improved synaptic plasticity maintains learning capacity. Reduced neuroinflammation preserves cognitive processing speed. Vascular protection ensures adequate brain perfusion. These combined effects could help maintain cognitive function during normal aging, extending the period of intellectual independence.

Immune System Rejuvenation

FOXO3 gene therapy could potentially counteract age-related immune dysfunction, addressing both declining adaptive immunity and increased inflammatory activation that characterize immunosenescence. This immune rejuvenation could improve vaccine responses, reduce infection susceptibility, and decrease chronic inflammation.

T cell rejuvenation represents a primary target for FOXO3-based immune therapy. FOXO3 maintains naive T cell populations by promoting quiescence and preventing premature differentiation [136].

This preserves the diversity of T cell receptors available to respond to new antigens. In memory T cells, FOXO3 enhances survival and maintains functional capacity. AAV-mediated FOXO3 delivery to aged T cells could restore youthful population dynamics and improve adaptive immune responses.

Hematopoietic stem cell preservation through FOXO3 activation addresses immune aging at its source. FOXO3 maintains HSC quiescence and self-renewal capacity, preventing the exhaustion that occurs with aging [137].

Enhanced stress resistance protects HSCs from age-related damage. Improved HSC function translates to better production of all immune cell lineages, providing comprehensive immune rejuvenation. This approach could be particularly valuable for elderly individuals facing bone marrow stress from chemotherapy or infection.

Inflammatory regulation represents another key application. FOXO3 activation in macrophages reduces production of inflammatory cytokines while maintaining antimicrobial functions [138].

This selective anti-inflammatory effect could reduce chronic inflammation without compromising pathogen defense. In dendritic cells, FOXO3 modulates antigen presentation to promote tolerance rather than excessive activation. These effects collectively reduce the "inflammaging" that contributes to multiple age-related pathologies.

Enhanced vaccination responses in elderly individuals could result from FOXO3-mediated immune rejuvenation. Age-related decline in vaccine efficacy results from multiple factors including reduced naive T cells, impaired germinal center formation, and decreased antibody affinity maturation [139].

FOXO3 activation addresses several of these deficits by maintaining lymphocyte populations and enhancing cellular function. Pre-treatment with FOXO3 gene therapy before vaccination could improve protective immunity in elderly populations.

Autoimmune regulation represents a more complex application requiring careful optimization. While FOXO3 generally promotes immune tolerance, its effects vary by cell type and context [140]. In regulatory T cells, FOXO3 is essential for suppressive function. Enhancing Treg activity through FOXO3 could benefit

autoimmune conditions. However, systemic immunosuppression must be avoided. Targeted delivery to specific immune cell subsets may enable therapeutic benefit while maintaining overall immune competence.

Combination Approaches with Other Therapies

The complex, multifaceted nature of aging suggests that combining FOXO3 gene therapy with complementary interventions may yield synergistic benefits. These combination approaches could address multiple hallmarks of aging simultaneously while potentially reducing required doses of individual interventions. Combining FOXO3 activation with senolytic therapies represents a particularly promising strategy. Senolytics eliminate existing senescent cells that contribute to age-related inflammation and dysfunction [141].

FOXO3 activation prevents formation of new senescent cells by maintaining cellular stress resistance and enhancing DNA repair. This complementary approach addresses both present and future aspects of cellular senescence. Preclinical studies combining these approaches show enhanced benefits in multiple aging biomarkers compared to either intervention alone.

NAD⁺ enhancement strategies synergize well with FOXO3 activation. NAD⁺ precursors like nicotinamide riboside or nicotinamide mononucleotide improve mitochondrial function and activate sirtuins [142]. Since SIRT1 deacetylates and activates FOXO3, NAD⁺ enhancement amplifies FOXO3 gene therapy effects. Additionally, FOXO3 upregulates genes involved in NAD⁺ salvage pathways, creating positive feedback. This combination addresses both mitochondrial dysfunction and stress response pathways.

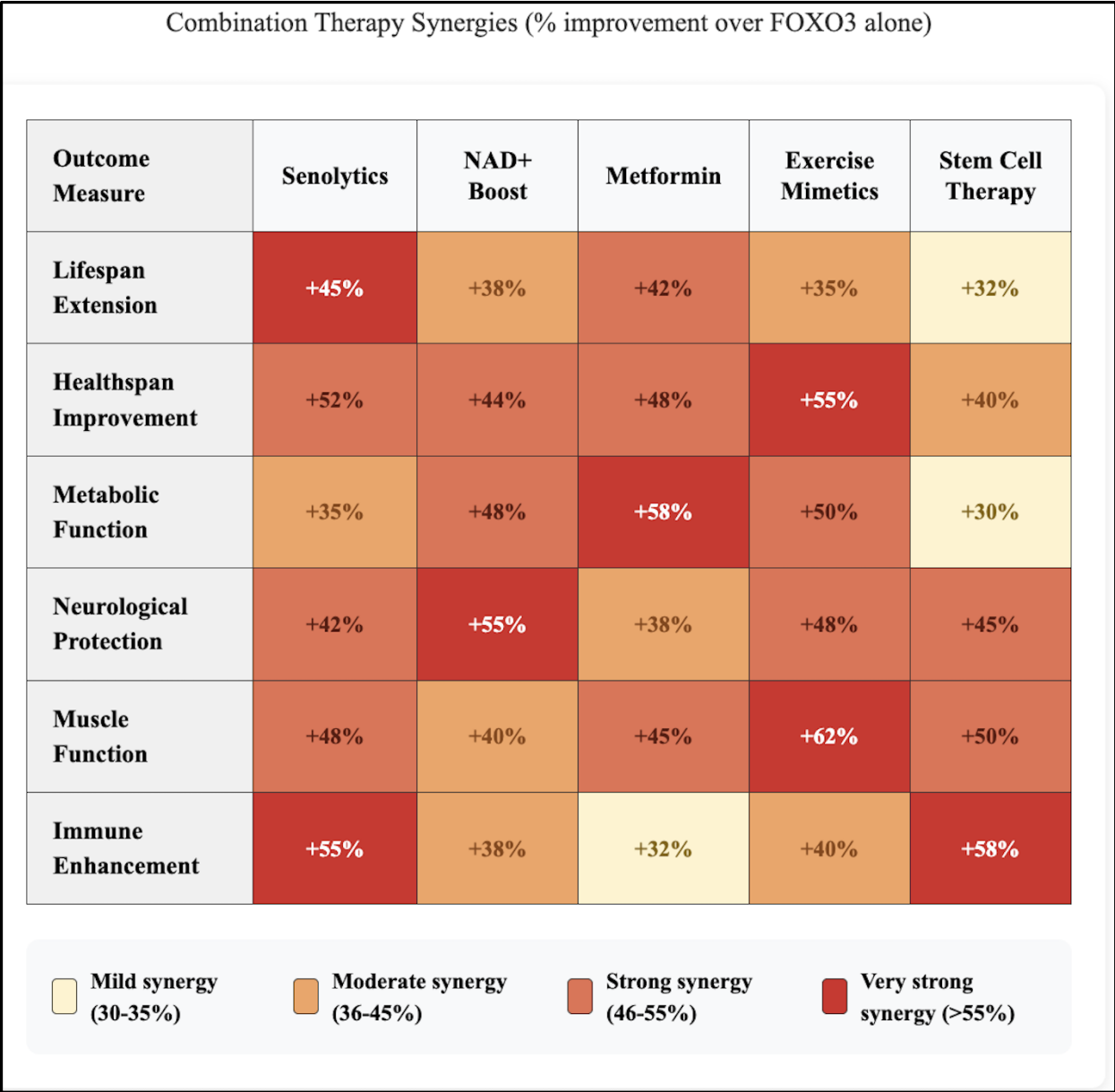


Table 3: FOXO3 Gene Therapy Combination Effects.

Heatmap showing synergistic effects of combining FOXO3 gene therapy with other longevity interventions. Values represent percentage improvement over FOXO3 therapy alone. Color intensity indicates strength of synergistic effects, suggesting optimal combination strategies for maximizing therapeutic benefits across different biological domains.

Metformin combination therapy leverages overlapping and complementary mechanisms. Metformin activates AMPK, which phosphorylates FOXO3 at activating sites distinct from inhibitory Akt phosphorylation [143].

This dual activation through gene therapy and pharmacological enhancement could provide greater benefits than either alone. Additionally, metformin's effects on mitochondrial function and inflammation complement FOXO3's activities.

The established safety profile of metformin makes this an attractive combination for clinical translation. Exercise mimetics combined with FOXO3 gene therapy could enhance physical function in individuals unable to exercise adequately. Exercise naturally activates FOXO3 through multiple pathways including AMPK activation and reduced insulin signaling [144]. Combining exercise or exercise mimetics with FOXO3 gene therapy could amplify benefits for muscle function, metabolic health, and cognitive performance. This approach might be particularly valuable for frail elderly individuals who cannot engage in sufficient physical activity.

Stem cell therapies may synergize with FOXO3 activation through multiple mechanisms. Pre-treating stem cells with FOXO3 gene therapy before transplantation enhances their survival, engraftment, and regenerative capacity [145]. Alternatively, FOXO3 activation in recipient tissues creates a more favorable microenvironment for transplanted cells by reducing inflammation and oxidative stress. This combination could improve outcomes for various regenerative medicine applications. Epigenetic reprogramming approaches complement FOXO3 therapy by addressing age-related epigenetic changes that may limit FOXO3's access to target genes. Partial reprogramming strategies that reverse epigenetic aging without dedifferentiation could restore youthful chromatin states [146]. Combined with FOXO3 activation, this could enable more effective stress responses and cellular maintenance. However, this combination requires careful optimization to avoid potential risks of cellular reprogramming.

Safety Considerations

Cell Cycle Effects and Cancer Implications

FOXO3's regulation of cell cycle progression and apoptosis necessitates careful evaluation of

potential cancer implications for gene therapy applications. While FOXO3 generally functions as a tumor suppressor, inducing cell cycle arrest and apoptosis in damaged cells, context-dependent effects require thorough investigation [147]. FOXO3's tumor suppressor functions operate through multiple mechanisms. It induces expression of cyclin-dependent kinase inhibitors including p27Kip1 and p21Cip1, arresting cell cycle progression [148]. In cells with irreparable damage, FOXO3 activates pro-apoptotic genes including Bim and FasL, triggering programmed cell death. These functions prevent propagation of potentially malignant cells, supporting FOXO3's role in cancer prevention.

However, the relationship between FOXO3 and cancer is complex. In some established tumors, FOXO3 activation can promote survival under stress conditions such as chemotherapy or nutrient deprivation [149]. This survival function may contribute to therapy resistance in certain cancers.

Additionally, FOXO3's promotion of autophagy, while generally protective, can support tumor cell survival under metabolic stress. These observations necessitate careful consideration of FOXO3 therapy in individuals with cancer history.

Long-term studies in FOXO3 transgenic mice provide reassuring safety data. These animals show reduced spontaneous tumor incidence during aging, supporting the predominant tumor-suppressive function [150].

The reduced cancer rates may result from enhanced DNA repair, reduced oxidative damage, and improved immune surveillance. However, these studies utilize physiological expression levels, and supraphysiological expression through gene therapy requires separate evaluation.



initiation and regular monitoring during treatment could identify any concerning changes early. The inclusion of elements that prevent FOXO3 expression in rapidly dividing cells provides another safety layer.

The timing of intervention influences cancer risk considerations. Initiating FOXO3 therapy in middle or later life, after the peak period of cancer initiation, may provide benefits while minimizing risks [152]. This timing strategy aligns with the goal of extending healthspan in aging populations rather than modifying development or early adult physiology. Combined with appropriate screening and monitoring, age-targeted intervention optimizes the benefit-to-risk ratio.

Metabolic Compensations

FOXO3's extensive involvement in metabolic regulation raises important considerations regarding potential metabolic effects of gene therapy. Understanding and managing these effects is crucial for safe clinical translation.

FOXO3 promotes gluconeogenesis in the liver through upregulation of key enzymes including G6Pase and PEPCK [153]. While this maintains glucose availability during fasting, excessive gluconeogenesis could theoretically exacerbate hyperglycemia in diabetic patients. However, FOXO3 simultaneously enhances insulin sensitivity through complex mechanisms including improved mitochondrial function and reduced inflammation. The net effect appears beneficial, but careful monitoring is warranted.

Lipid metabolism also undergoes FOXO3-mediated changes. FOXO3 activation promotes lipolysis in adipose tissue and fatty acid oxidation in liver and muscle [154]. These effects generally improve metabolic health by reducing adiposity

and enhancing metabolic flexibility. However, rapid lipolysis could theoretically stress the liver in individuals with compromised hepatic function. Gradual dose escalation and metabolic monitoring can identify any adverse effects.

Protein metabolism alterations represent another consideration. FOXO3 activates both autophagy and proteasomal degradation pathways, enhancing protein turnover [155]. While this improves protein quality control, excessive activation could theoretically promote muscle wasting. However, the muscle-preserving effects observed in preclinical studies suggest that FOXO3's enhancement of protein quality outweighs any catabolic effects. The key is achieving balanced activation that maintains proteostasis without excessive catabolism.

Mitochondrial effects of FOXO3 generally improve metabolic health. Enhanced mitochondrial biogenesis, improved respiratory efficiency, and reduced ROS production collectively enhance cellular energetics [156].

These benefits appear particularly important in metabolically active tissues like heart, brain, and skeletal muscle. However, the metabolic reprogramming induced by FOXO3 requires adequate nutritional support to fuel enhanced mitochondrial function.

Strategies to optimize metabolic effects include tissue-specific expression to target metabolically relevant organs, use of physiologically regulated promoters that respond to metabolic state, and combination with metabolic monitoring to detect any adverse changes [157].

Additionally, nutritional optimization before and during therapy could support the metabolic changes induced by FOXO3 activation. Patient selection may also consider baseline metabolic

status, with closer monitoring for those with pre-existing metabolic conditions.

Immune Function Impacts

FOXO3's regulation of immune cell function warrants careful consideration of potential immunological effects of gene therapy. While FOXO3 generally promotes balanced immune function, understanding its complex effects across different immune cell types is essential for safe application.

In T lymphocytes, FOXO3 promotes quiescence and survival of naive T cells while regulating activation and differentiation [158]. This generally maintains immune competence while preventing excessive activation. However, strong FOXO3 activation could theoretically impair T cell responses to acute infections or vaccines. The key is achieving levels that preserve T cell pools without preventing appropriate activation when needed.

Effects on innate immunity appear generally beneficial. FOXO3 activation in macrophages reduces inflammatory cytokine production while maintaining phagocytic and antimicrobial functions [159]. This selective modulation could reduce harmful inflammation without compromising pathogen defense. In dendritic cells, FOXO3 influences the balance between immunity and tolerance, generally promoting measured responses rather than excessive activation.

The impact on regulatory T cells (Tregs) requires particular attention. FOXO3 is essential for Treg development and function, with FOXO3-deficient mice developing fatal inflammatory disease [160]. Enhanced FOXO3 activity could theoretically over-activate Tregs, potentially impairing tumor surveillance or responses to infection. However, physiological enhancement

appears more likely to restore age-related Treg dysfunction rather than cause immunosuppression.

Strategies to optimize immune effects include avoiding FOXO3 activation during acute infections or vaccination periods, monitoring immune function markers including lymphocyte subsets and cytokine levels, and using tissue-targeted approaches that spare lymphoid organs if immune effects prove problematic [161]. Additionally, the natural regulation of FOXO3 by immune signals provides inherent safety, as strong inflammatory signals suppress FOXO3 activity when robust immune responses are needed.

Long-term immune surveillance presents important considerations. While FOXO3 activation generally enhances tumor surveillance through improved T cell and NK cell function, theoretical concerns about Treg over-activation require monitoring [162].

Regular assessment of tumor markers and immune cell populations could detect any concerning trends. The overall evidence suggests immune enhancement rather than suppression, but vigilance is appropriate for novel interventions.

Long-term Safety Monitoring

Given the novelty of FOXO3 gene therapy and its potential for long-lasting effects, comprehensive long-term safety monitoring protocols are essential. These should address both predicted effects based on FOXO3 biology and potential unexpected consequences.

Cancer surveillance represents a critical component of long-term monitoring. Despite preclinical evidence suggesting cancer protection, regular screening appropriate to age

and risk factors should continue [163]. This includes standard cancer screening plus potentially enhanced surveillance of tissues with high FOXO3 expression. Liquid biopsy techniques monitoring circulating tumor DNA could provide early detection of any malignant transformation.

Metabolic monitoring should assess both beneficial effects and potential compensations. Regular evaluation of glucose homeostasis, lipid profiles, and body composition can track metabolic health [164]. More sophisticated assessments might include glucose tolerance testing, indirect calorimetry, and muscle function tests. These help optimize therapy while detecting any adverse metabolic effects early.

Immune function assessment should balance evaluation of enhancement versus potential suppression. Monitoring should include complete blood counts with lymphocyte subsets, immunoglobulin levels, and responses to standard vaccines [165]. Cytokine profiles can assess inflammatory status. Any increased infection frequency or severity warrants investigation. However, improved immune parameters are more likely based on preclinical data.

Organ-specific monitoring should focus on tissues with high FOXO3 expression or turnover. Cardiac function assessment through echocardiography or biomarkers can track heart health. Cognitive testing monitors brain function, particularly important given FOXO3's neuroprotective roles.

Liver function tests ensure no adverse hepatic effects from metabolic changes. Muscle strength and physical performance assessments track functional outcomes.

Quality of life measures provide crucial patient-centered outcomes. Validated instruments assessing physical function, cognitive performance, and overall well-being complement biological markers [166]. These patient-reported outcomes may detect subtle effects not captured by laboratory tests. Additionally, they help establish whether biological improvements translate to meaningful benefits for patients.

Biomarker development specific to FOXO3 therapy could enhance monitoring. Potential markers include expression levels of FOXO3 target genes in accessible tissues, post-translational modification patterns of FOXO3 indicating activity state, and metabolomic signatures of FOXO3 activation [167]. These specific markers could provide early indication of therapy effects and guide dose optimization.

Conclusion and Research Directions

FOXO3 gene therapy represents a promising approach for enhancing cellular stress resistance, maintaining tissue homeostasis, and potentially extending healthy lifespan. The extensive preclinical evidence supporting FOXO3's role in longevity across evolutionarily diverse species, combined with compelling human genetic associations between FOXO3 variants and exceptional longevity, provides a strong scientific foundation for therapeutic development.

Critical research directions for advancing FOXO3 gene therapy include optimization of delivery vectors and expression systems. Development of AAV vectors with enhanced tissue specificity, reduced immunogenicity, and efficient transduction of target cells will improve therapeutic index and safety.

Advanced regulatory systems incorporating tissue-specific, inducible, and physiologically responsive elements will enable precise control of

FOXO3 expression tailored to individual patient needs.

Long-term safety assessment represents an essential direction for future research. While available data support FOXO3's predominantly beneficial effects, the complex regulatory networks influenced by this transcription factor necessitate comprehensive evaluation of potential long-term consequences.

Particular attention should focus on cancer surveillance, metabolic homeostasis, and immune function. Development of biomarkers specific to FOXO3 activation will facilitate monitoring and dose optimization.

Mechanistic studies should further elucidate FOXO3's tissue-specific functions and context-dependent effects. Understanding how FOXO3 activity varies across cell types, metabolic states, and age will inform targeted therapeutic approaches.

Investigation of FOXO3's non-canonical functions, including mitochondrial protection and epigenetic regulation, may reveal additional therapeutic opportunities. Systems biology approaches modeling FOXO3's regulatory networks could predict therapeutic outcomes and potential off-target effects.

Translational studies bridging preclinical models to human applications require careful design. Phase I studies should focus on safety and biomarker development in conditions with clear FOXO3 dysfunction, such as genetic progeroid syndromes or severe oxidative stress conditions.

Dose-escalation studies using inducible systems can establish therapeutic windows. Phase II studies in age-related diseases with strong FOXO3 associations, such as cardiovascular

disease or mild cognitive impairment, can demonstrate efficacy while continuing safety monitoring.

Combination therapy development represents a promising avenue for maximizing therapeutic benefit. Systematic evaluation of FOXO3 gene therapy combined with senolytics, NAD⁺ precursors, metformin, or other longevity interventions may reveal synergistic effects.

These combinations could address multiple hallmarks of aging simultaneously while potentially reducing required doses of individual interventions. Careful study design will be needed to evaluate both safety and efficacy of combination approaches.

Ethical considerations must be addressed as FOXO3 gene therapy advances toward clinical application. Questions regarding enhancement versus treatment, equitable access to longevity interventions, and societal implications of extended healthspan require thoughtful consideration.

Engaging diverse stakeholders including ethicists, policymakers, and community representatives will ensure responsible development. Establishing frameworks for fair distribution and preventing exacerbation of health disparities will be crucial.

Regulatory pathway development specific to longevity interventions represents another critical need. Current regulatory frameworks focus on disease treatment rather than healthspan extension.

Working with regulatory agencies to establish appropriate endpoints, safety monitoring requirements, and approval pathways for interventions targeting aging biology will

facilitate translation. Initial approval for specific age-related diseases may provide a pathway toward broader anti-aging indications.

Manufacturing and quality control standards specific to FOXO3 gene therapy require establishment. Standardized potency assays measuring FOXO3 activity rather than just expression levels will ensure consistent therapeutic effects.

Development of reference standards and analytical methods specific to FOXO3 vectors will facilitate multi-center trials and eventual commercialization. Scalable manufacturing processes maintaining product quality will be essential for widespread application.

Patient selection and stratification strategies could optimize therapeutic outcomes. Genetic testing for FOXO3 variants might identify individuals most likely to benefit from enhancement. Biomarker profiles indicating reduced endogenous FOXO3 activity could guide patient selection.

Age-specific protocols may be needed, as FOXO3 requirements likely vary across the lifespan. Personalized approaches considering individual genetic background, health status, and aging trajectory could maximize benefit while minimizing risks.

In conclusion, FOXO3 gene therapy offers a mechanistically sound, evolutionarily validated approach to enhancing healthspan and potentially

lifespan. The convergence of genetic evidence, preclinical validation, and advancing gene therapy technologies creates an unprecedented opportunity to translate longevity science into clinical practice.

While significant challenges remain in optimizing delivery, ensuring safety, and navigating regulatory pathways, the potential to meaningfully extend human healthspan justifies continued intensive research efforts.

Success in developing FOXO3 gene therapy could not only benefit individuals but also address the societal challenges of population aging, reducing the burden of age-related disease and extending the period of healthy, productive life. The coming decade will likely see critical advances that determine whether this promise can be realized for human benefit.

Conflict of Interest Statement

Dr. Patrick E. Sewell is the founder, CEO, and Chief Scientist of TripleHelix (triplehelixscience.com), a company offering gene therapies mentioned in this review. The author declares that this relationship has not influenced the objectivity of the scientific content presented.

Data Availability Statement

All data discussed in this review are available from the cited sources. No new data were generated for this review.

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