Trends in Cell Biology

CellPress OPEN ACCESS

Review

The Wnt–NAD$^+$ axis in cancer, aging, and tissue regeneration

Jilian Lamprecht[1,4], Sundos Abu Sanad[1,4], Anjali P. Kusumbe[2,3], and Shukry J. Habib[1,*]

The intricate interplay between Wnt signaling and nicotinamide adenine dinucleotide (NAD$^{+)}$ biosynthesis has emerged as a crucial axis that influences aging and tissue regeneration. Wnt signaling, a key regulator of cellular proliferation, differentiation, and tissue homeostasis, intersects with NAD$^+$ metabolism, a cornerstone of cellular energy balance and genomic stability. This relationship is mediated through shared regulatory pathways involving sirtuins, poly(ADP-ribose) polymerases (PARPs), and metabolic enzymes which are sensitive to cellular NAD$^+$ levels. Dysregulation of either pathway is implicated in cancer, age-related decline, and impaired regenerative capacity. This review consolidates current knowledge of the Wnt–NAD$^+$ axis and highlights its cooperative roles in maintaining tissue integrity and combating the effects of aging. Furthermore, it explores therapeutic approaches targeting this axis to restore tissue health and enhance the capacity for repair, thereby offering promising avenues for addressing age-associated pathologies.

Highlights

The Wnt–NAD$^+$ axis is a fundamental regulatory hub in which metabolic state meets developmental signaling and it acts as a metabolic sensor that coordinates tissue regeneration with cellular energy status through compartment-specific NAD$^+$ pools.

Wnt signaling regulates NAD$^+$ metabolism by controlling the expression of key biosynthetic enzymes and NAD$^+$ consumers, while NAD$^+$-dependent proteins modulate Wnt activity through direct interactions and epigenetic modifications.

Sirtuins exhibit tissue-specific and subcellular compartment-dependent roles in Wnt regulation where they function as activators or suppressors depending on the cellular bioenergetic state.

The Wnt–NAD$^+$ axis maintains stem cell function and self-renewal capacity through metabolic/signaling integration, and its disruption during aging leads to declining regenerative capacity.

The progressive dysregulation of compartment-specific Wnt–NAD$^+$ coordination contributes to stem cell exhaustion and multiple pathological conditions, indicating that therapeutic strategies must consider tissue-specific and subcellular targeting.

Ancient pathways, emerging connections

Cellular energy drives all life processes, and conserved bioenergetic pathways such as glycolysis and the citric acid cycle are present in most organisms. NAD$^+$ is a vital cofactor in these pathways that supports redox reactions, electron transport, and ATP production. NAD$^+$ is synthesized through *de novo* (tryptophan), salvage (nicotinamide mononucleotide, NMN), and Preiss–Handler [**nicotinic acid (NA)**; see Glossary] pathways, and can be phosphorylated to NADP$^+$. NAD$^+$ primarily drives catabolism, while NADP$^+$ supports anabolism. Both also serve as substrates for enzymes that regulate DNA repair, gene expression, cell signaling, and protein modifications, underscoring their essential, conserved roles in cellular function [1–3].

As life evolved from single-celled organisms to complex multicellular entities, new regulatory pathways such as Wnt signaling (a portmanteau of the mouse *Int1* and *Drosophila Wingless* genes) emerged to coordinate cell communication, development, and tissue homeostasis [4]. The Wnt signaling network includes the canonical Wnt/β-catenin pathway, which activates gene transcription for cell proliferation and differentiation, and two noncanonical pathways: planar cell polarity (PCP) that regulates cell polarity and migration, and the Wnt/Ca^{2+} pathway, which modulates cell adhesion and migration. These pathways are interlinked and play critical roles in embryogenesis, stem cell maintenance, and tissue homeostasis [5,6]. Wnt signaling and NAD$^+$ metabolism are crucial in health and disease because they influence development, regeneration, aging, and cancer. While Wnt/β-catenin signaling controls cell fate and tissue maintenance [4], NAD$^+$ biosynthesis governs metabolic balance [7]. Age-related NAD$^+$ decline [8] and dysregulated Wnt signaling contribute to impaired tissue regeneration and increased disease susceptibility.

Although traditionally studied separately, emerging research suggests NAD$^+$ metabolism intersects with Wnt signaling, linking metabolic states to developmental and regenerative processes

[1]Department of Biomedical Sciences, University of Lausanne, Rue du Bugnon 7a, CH-1005 Lausanne, Switzerland
[2]Tissue and Tumor Microenvironments Laboratory, Cancer Discovery and Regenerative Medicine Program, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
[3]Multidisciplinary Institute of Ageing (MIA-Portugal), Coimbra, Portugal
[4]Contributed equally to this work

*Correspondence: shukryjames.habib@unil.ch (S.J. Habib).

(Figure 1). For example, activation of the Wnt–NAD^+ axis successfully rejuvenated aged osteoprogenitors and promoted bone repair to levels seen in younger mice [9]. This review consolidates current knowledge on their molecular crosstalk, explores their roles in cancer, aging and regeneration, and discusses therapeutic strategies for tissue repair.

Molecular crosstalk between Wnt signaling and NAD+ biosynthesis

NAD^+ metabolism and Wnt signaling exhibit compartmentalized organization – NAD^+ pools in the cytosol, mitochondria, and nucleus rely on localized enzymes, while canonical Wnt/β-catenin spans extracellular to nuclear domains. This compartmentalization creates interaction points between the pathways, enabling coordinated regulation of cellular functions through processes such as signal transduction, protein stability, and gene expression (Figures 1,2).

Two key families of NAD^+-consuming enzymes, **poly(ADP-ribose) polymerases (PARPs)** and sirtuins, act as molecular sensors that link cellular energy status to Wnt signaling [7]. PARPs transfer poly(ADP-ribose) to proteins (PARylation), altering their stability and function, while sirtuins primarily deacetylate proteins and mediate modifications such as ADP-ribosylation. These enzymes establish a crucial link between cellular metabolic status and Wnt signaling, where fluctuations in NAD^+ availability can profoundly impact on developmental and homeostatic processes [10]. Importantly, Wnt signaling influences the expression of enzymes for NAD^+ biosynthesis and consumption, creating a regulatory network that is crucial for cellular function [11,12] (Figures 1,2). The following sections explore the specific mechanisms underlying this crosstalk.

Sirtuins as NAD^+ consumers in Wnt pathway regulation: context-dependent crosstalk

Sirtuins (SIRT1–7) play diverse roles in cellular regulation, including metabolism, aging, and signaling pathways [13,14]. Their involvement in the Wnt/β-catenin signaling pathway reveals context-dependent mechanisms that vary depending on the cell type.

Sirtuins regulate Wnt signaling through three main mechanisms: direct protein modification, epigenetic regulation, and control of protein localization (Figure 2). SIRT1 shuttling between nucleus and cytoplasm enables versatile regulation of the Wnt/β-catenin pathway. In mouse mesenchymal stem cells, SIRT1 promotes Wnt signaling by direct deacetylation of β-catenin, leading to its nuclear accumulation [15], as well as through epigenetic silencing of Wnt antagonists [**Dickkopf-1 (DKK1)**, **secreted Frizzled-related proteins 1/2 (SFRP1/2)**, and **DACT1**] [15,16] (Figure 2B). SIRT1-mediated deacetylation of histone H3 lysine 9 (H3K9) and histone H4 lysine 16 (H4K16) at Wnt antagonist gene promoters reduces their expression. The stimulatory role of SIRT1 extends to osteoblasts, where nuclear SIRT1 deacetylates **Forkhead box O (FOXO)** transcription factors to prevent β-catenin sequestration, thereby promoting osteogenesis [17]. Its knockdown in HEK293T (an immortalized human embryonic kidney cell line) and various cancer cell lines reduces **Dishevelled (Dvl)** protein levels and Wnt signaling [16]. Conversely, in human colon carcinoma cells, SIRT1 deacetylates nuclear β-catenin to export it from the nucleus, thereby attenuating Wnt signaling [18]. Tissue-specific cofactors, localization, and modifications influence SIRT1 context-dependent effects and fine-tune Wnt signaling in physiological and cancerous states.

SIRT2, which is predominantly localized in the cytoplasm, similarly displays context-dependent regulation of Wnt signaling. In mouse embryonic fibroblasts, SIRT2 inhibits Wnt signaling by binding to β-catenin and blocking its nuclear translocation [19] (Figure 2A). During mouse embryonic stem cell differentiation, SIRT2 expression increases, and its knockdown leads to elevated levels of **glycogen synthase kinase 3β (GSK3β)**, promoting β-catenin degradation [20]. In CRC, active Wnt signaling suppresses SIRT2 expression, forming a feedback loop that ties sirtuin regulation to tumor progression [21] (Figure 2B).

Glossary

Ataxia telangiectasia: a rare inherited neurodegenerative disorder caused by mutations in the *ATM* gene that is essential for DNA repair. The disease is characterized by premature aging.
BIO: 6-bromoindirubin-3′-oxime, a GSK-3β inhibitor that is used to promote Wnt signaling.
CD38: an immune receptor with NADase and ADP-ribosyl-cyclase enzymatic activities.
Cyclin D1: a cell-cycle regulator that acts downstream of Wnt/β-catenin signaling and facilitates the transition from G1 to S phase in the cell cycle.
DACT1: Dishevelled binding antagonist of β-catenin 1, a Wnt signaling pathway antagonist that binds to Dishevelled to downregulate β-catenin activity.
Dickkopf-1 (DKK1): a Wnt signaling pathway antagonist that associates with the LRP5/6 coreceptor to block Wnt binding, leading to β-catenin degradation.
Dishevelled (Dvl): a key Wnt signaling transducer that associates with the Frizzled receptor.
DPQ: 3,4-dihydro-5-[4-(1-piperidinyl) butoxy]-1(2H)-isoquinolinone, a PARP1 inhibitor.
Forkhead box O (FOXO): a group of transcription factors that are involved in multiple important cellular processes including proliferation, resistance to oxidative stress, and metabolism.
Glycogen synthase kinase 3β (GSK3β): a serine/threonine kinase that is involved in multiple cellular processes, including Wnt signaling, by phosphorylating target proteins such as β-catenin for degradation.
Hypoxia-inducible factor 1α (HIF-1α): a transcription factor that regulates cellular responses to low oxygen levels by promoting the expression of genes involved in survival under hypoxic conditions, notably *VEGF*, to induce angiogenesis.
Inflammaging: chronic low-grade inflammation that develops with age.
Low-density lipoprotein receptor-related protein 6 (LRP6): LRP6 and LRP5 are coreceptors for Wnt/β-catenin signal transduction.
MYC: a transcription factor induced by Wnt/β-catenin signaling that promotes cell growth, proliferation, and metabolism.
Nicotinamide (NAM): a form of vitamin B3 and key precursor in NAD^+ biosynthesis through the salvage

SIRT3 bidirectionally regulates Wnt signaling. In human heart-derived fibroblasts, SIRT3 deacetylates GSK3β, activating it to phosphorylate β-catenin for degradation and inhibiting Wnt signaling. Consequently, TGF-β1 signaling and tissue fibrosis are reduced [22](Figure 2A). SIRT3 also suppresses β-catenin levels in prostate cancer cells and inhibits epithelial–mesenchymal transition and metastasis [23]. Knockdown of SIRT3 increases the expression of β-catenin and Wnt target genes such as that encoding **MYC**, and thus promotes a more aggressive cancer phenotype. Demonstrating the bidirectional nature of this regulation, β-catenin downregulates SIRT3 through promoter inhibition in *PIK3CA*-mutated cervical cancer cells, thereby enhancing glucose metabolism and proliferation (Figure 2B) [24].

SIRT4, which is typically localized in mitochondria, translocates to the cytoplasm upon Wnt activation, where it deacetylates Axin1, and thus interferes with the ability of β-TrCP (BTRC) to target β-catenin for degradation (Figure 2B) [25]. As a result, β-catenin accumulates in the nucleus, leading to increased activation of Wnt target genes. SIRT5 uniquely influences bone metabolism by enhancing β-catenin stability through demalonylation [26], showcasing noncanonical sirtuin activities (Figure 2B).

Through a different mechanism, SIRT6 mediates epigenetic regulation by deacetylating H3K56 at Wnt target gene promoters and binding to **T cell factor/lymphoid enhancer factor (TCF/LEF1)** transcription factors to reduce their activity (Figure 2A) [27]. SIRT7 exhibits complex regulation of Wnt signaling across different tissues. During osteogenesis, SIRT7 acts as a Wnt pathway inhibitor and its knockdown promotes β-catenin upregulation and differentiation of bone marrow-derived skeletal stem cells [28]. By contrast, in cancer contexts, SIRT7 can promote Wnt signaling through multiple mechanisms. In human hepatocellular carcinoma cells, SIRT7 upregulates transcription factor PU.1, which leads to increased expression of the Wnt receptor Frizzled 7 and enhanced Wnt/β-catenin signaling (Figure 2B) [29]. Similarly, in human ovarian cancer, SIRT7 promotes Wnt/β-catenin signaling by deacetylating GATA4, thereby decreasing its transcriptional activity [30].

The regulation of Wnt signaling by sirtuins illustrates how NAD^+-dependent metabolic sensors can fine-tune developmental pathways through diverse mechanisms. While the context-dependent nature of these effects is now evident, the roles of NAD^+ availability and the coordination between different sirtuins remain to be fully understood.

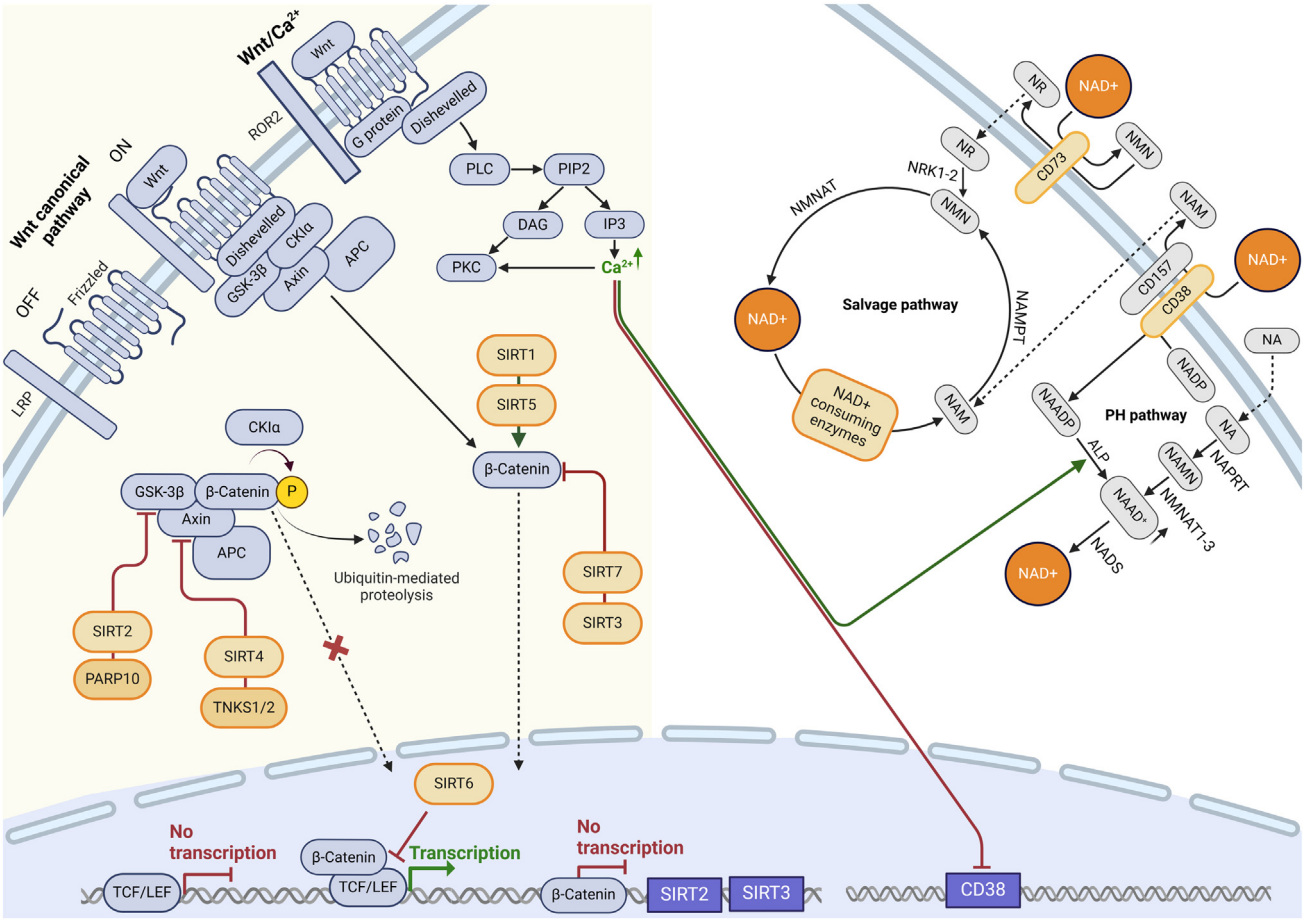
PARPs and NAD^+-dependent regulation of Wnt signaling

While sirtuins primarily influence Wnt signaling through protein deacetylation, the PARP family employs distinct NAD^+-dependent mechanisms to regulate this pathway. A key example is the tankyrase subfamily (TNKS1 and TNKS2) of PARPs which function as regulators of Wnt/β-catenin signaling through their ADP-ribosyltransferase activity [31,32]. These enzymes regulate Axin through ADP-ribosylation to promote Wnt signaling.

Axin plays a dual role in Wnt signaling. In the absence of Wnt ligands, it acts as a scaffold protein in the β-catenin destruction complex, facilitating β-catenin phosphorylation and subsequent degradation, thereby suppressing Wnt signaling. Conversely, upon Wnt activation, Axin is recruited to the plasma membrane where it contributes to receptor complex activation and signal transduction [4] (Figure 2).

TNKS-mediated ADP-ribosylation affects Axin in two ways [31,32]. First, it marks Axin for degradation via RNF146-dependent ubiquitination, thereby reducing available Axin for destruction complex formation. Second, ADP-ribosylation enhances the binding affinity of Axin for phosphorylated

pathway. NAM is generated by NAD^+-consuming enzymes.
Nicotinic acid (NA): a precursor for the biosynthesis of NAD^+ through the Preiss–Handler pathway. NA is absorbed from the diet or produced in the body from tryptophan.
Nicotinic acid adenine dinucleotide ($NAAD^+$): an intermediate precursor of NAD^+ biosynthesis from NA in the Preiss–Handler pathway.
Olaparib: a PARP inhibitor commonly used in the treatment of *BRCA*-mutated ovarian cancer.
PJ34: *N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-(*N*,*N*-dimethylamino)acetamide hydrochloride, a PARP1 and PARP2 inhibitor.
Poly(ADP-ribose) polymerase (PARP): a family of proteins that catalyze the transfer of ADP-ribose from NAD^+ to target proteins.
Polyphosphate: a linear polymer of phosphate residues linked by phosphoanhydride bonds.
SBI-797812: a nicotinamide phosphoribosyltransferase (NAMPT) activator that enhances NAD^+ biosynthesis.
Secreted Frizzled-related proteins 1/2 (SFRP1/2): secreted Wnt antagonists that inhibit the Wnt/β-catenin pathway by binding to Wnt ligands.
Superoxide dismutase 2 (SOD2): a mitochondrial enzyme that converts superoxide radicals into hydrogen peroxide, and thus protects cells from oxidative stress.
T cell factor/lymphoid enhancer factor (TCF/LEF): a transcription factor that orchestrates Wnt/β-catenin target gene expression by binding to β-catenin upon its nuclear accumulation.
Telomerase reverse transcriptase (TERT): an enzyme that maintains telomere length and is crucial for genomic stability.
Vascular endothelial growth factor (VEGF): a key factor in angiogenesis that is essential for tissue repair and regeneration.
Vitamin D: a fat-soluble vitamin that occurs naturally in some foods and is also produced by the body when exposed to UV light. It modulates SIRT1 activity and suppresses Wnt signaling in some cancer contexts.

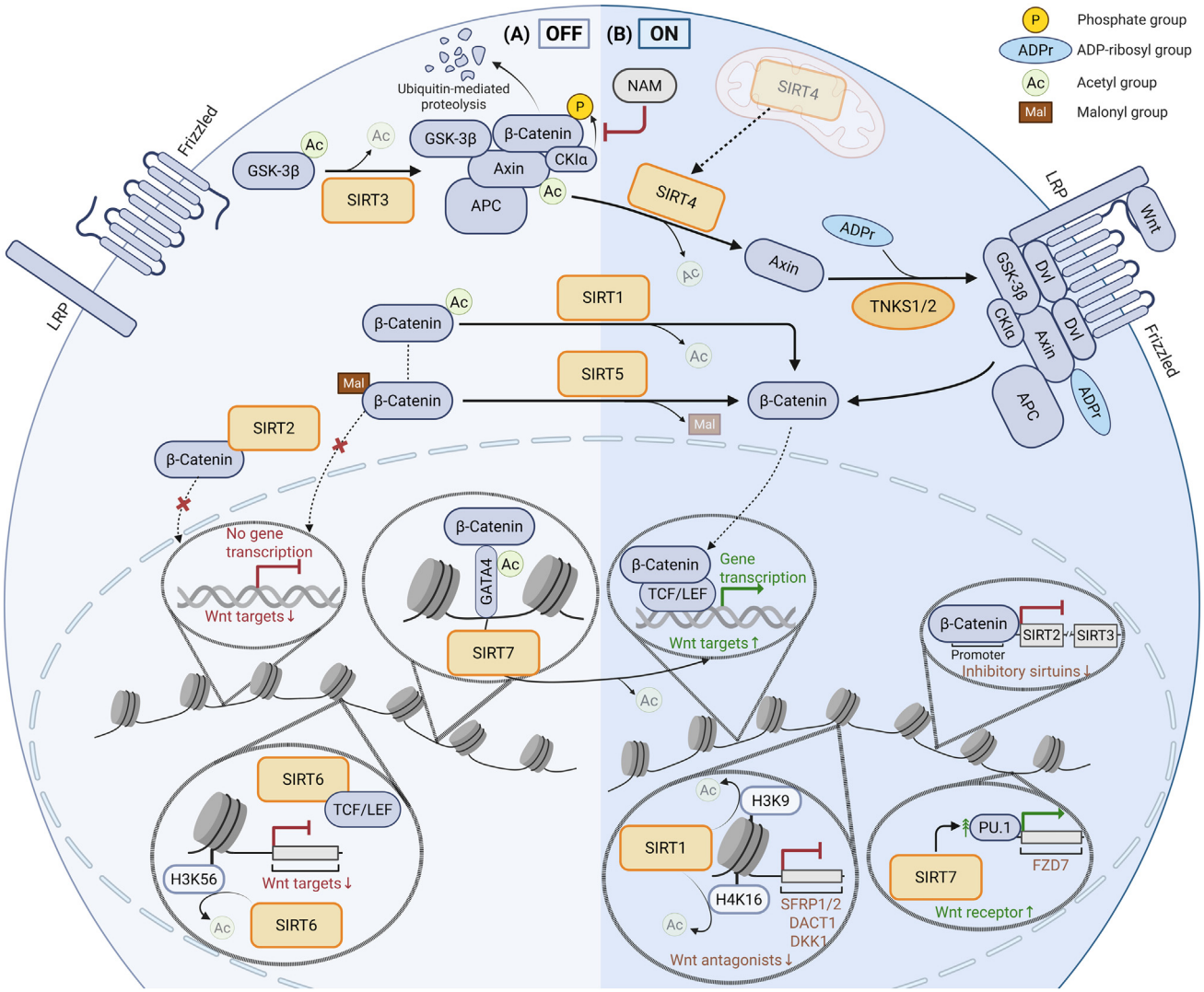


Trends in Cell Biology

Figure 1. An overview *of* Wnt–NAD$^+$ crosstalk. The interactions between NAD$^+$ and Wnt pathways are shown, highlighting the canonical (β-catenin-dependent) and noncanonical (calcium-dependent) branches of Wnt signaling. NAD$^+$-consuming enzymes such as sirtuins, PARPs, and CD38 influence Wnt activity through regulation of β-catenin stability, transcriptional activity, and nuclear factor pathways. Green arrows denote activation, while red arrows indicate inhibitory interactions, emphasizing the dual regulation between NAD$^+$- and Wnt-dependent cellular processes. Dotted lines symbolize translocation. Protein color code*:* light blue*,* components of the Wnt pathway; light orange, NAD$^+$ consumers; light gray, others. Abbreviations: ALP, alkaline phosphatase; APC, adenomatous polyposis coli; CD38, cluster of differentiation 38; CD157, cluster of differentiation 157; CKIα, casein kinase 1α; DAG, diacylglycerol; GSK-3β, glycogen synthase kinase 3β; IP3, inositol 1,4,5-trisphosphate; LRP, low-density lipoprotein receptor-related protein; NA, nicotinic acid; NAADP, nicotinic acid adenine dinucleotide phosphate; NAD$^+$, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); NADS, NAD$^+$ synthetase; NAM, nicotinamide; NAMN, nicotinic acid mononucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NAPRT, nicotinic acid phosphoribosyltransferase; NMNAT, nicotinamide mononucleotide adenylyltransferase; NR, nicotinamide riboside; NRK1/2, nicotinamide riboside kinases 1 and 2; PARP10, poly(ADP-ribose) polymerase 10; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; ROR2, receptor tyrosine kinase-like orphan receptor 2; SIRT1–7, sirtuins 1 to 7; TCF/LEF, T cell factor/lymphoid enhancer factor; TNKS1/2, tankyrases 1 and 2.

low-density lipoprotein receptor-related protein (LRP6), and thus promotes its recruitment to the active Wnt receptor complex (Figure 2B) and triggers Wnt signaling transduction.

These opposing effects – degrading Axin while enhancing its receptor-binding capability – may fine-tune Wnt signaling intensity and duration. Because TNKS activity requires NAD$^+$ as a substrate, cellular NAD$^+$ levels directly influence this regulation. In a related but potentially distinct mechanism, research in CRC has demonstrated that knockdown of nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme in the NAD$^+$ salvage pathway, promotes β-catenin degradation by upregulating Axin expression, thereby suppressing Wnt/β-catenin



Trends in Cell Biology

Figure 2. Graphical summary of molecular interactions in the NAD$^+$–Wnt axis. (A) Wnt signaling OFF: the absence of Wnt ligand binding to LRP/Frizzled leads to assembly of the β-catenin destruction complex, further stabilized through SIRT3-mediated deacetylation of GSK-3β, resulting in β-catenin degradation and preventing the transcription of Wnt/β-catenin target genes. Additional mechanisms such as β-catenin acetylation, malonylation, and direct interaction with SIRT2, as well as SIRT6-mediated deacetylation of H3K56 at Wnt target gene promoters combined with TCF/LEF capture, all contribute to inhibition of Wnt target gene transcription. (B) Wnt signaling ON: the destruction complex is not assembled, but its components instead colocalize to the membrane to form the signalosome that is necessary for Wnt signal transduction. In this configuration, NAM directly inhibits the phosphorylation of β-catenin by CK1, preventing it from being tagged for degradation. This is further stabilized by SIRT4 translocation from the mitochondria and its subsequent deacetylation of Axin, as well as ADP-ribosylation of Axin by tankyrases 1 and 2. SIRT1 and SIRT5 respectively mediate the deacetylation and demalonylation of β-catenin which enable its translocation and accumulation within the nucleus. Once in the nucleus it associates with TCF/LEF to transcribe Wnt/β-catenin target genes. β-catenin also binds to the promoter regions of the genes encoding Wnt-inhibitory SIRT2 and SIRT3 and represses their transcription. Finally, SIRT1 can deacetylate H3K9 and H4K16 at the promoter regions of genes encoding Wnt antagonists, thereby inhibiting their transcription. (Top right) Legend for post-translational modifications. Protein color code: light blue, components of the Wnt pathway; light orange, NAD$^+$ consumers. Abbreviations: APC, adenomatous polyposis coli; CK1α, casein kinase 1α; FZD7, Frizzled 7 receptor; GSK-3β, glycogen synthase kinase 3β; H3K9, histone H3 lysine 9; H4K16, histone H4 lysine 16; LRP, low-density lipoprotein receptor-related protein; NAM: nicotinamide; SIRT1–7, sirtuins 1 to 7; TCF/LEF, T cell factor/lymphoid enhancer factor; TNKS1/2, tankyrases 1 and 2.

signaling activity. However, whether this effect occurs through TNKS-dependent mechanisms requires further investigation [12]. Overall, sirtuins and PARPs integrate metabolic signals with Wnt pathway control, linking energy status to development and homeostasis.

Wnt-mediated regulation of NAD^+ metabolism

Wnt signaling regulates NAD^+ metabolism through multiple mechanisms ranging from transcriptional control of NAD^+-consuming enzymes to broader metabolic pathways (Figures 1,2). At the transcriptional level, activated β-catenin directly suppresses the promoters of the *SIRT2* and *SIRT3* genes in cancer cells, driving metabolic shifts that favor proliferation [21,24]. In CRC, Wnt/β-catenin activation is linked to SIRT1 acetylation and reduced activity. **Vitamin D** promotes SIRT1 auto-deacetylation [33], enabling SIRT1 to deacetylate β-catenin, limiting its nuclear accumulation and suppressing Wnt target genes [18]. Elevated NAD^+ levels enhance tankyrase activity and thus promote β-catenin stabilization, amplifying the Wnt signal [34]. This forms a positive feedback loop in specific cell types where Wnt signaling increases NAD^+ levels which in turn further enhance Wnt pathway activation [11,12].

In addition, the Wnt/Ca^{2+} signaling pathway contributes to broader changes in NAD^+ biosynthesis. In human breast cancer cells, it increases alkaline phosphatase levels, driving the production of **nicotinic acid adenine dinucleotide ($NAAD^+$)**, a precursor in NAD^+ synthesis [11]. Simultaneously, Wnt/Ca^{2+} signaling reduces the levels of **CD38**, an NAD^+-degrading enzyme, and thus helps to maintain intracellular NAD^+ levels during metabolic stress [35] (Figure 1). In parallel, CD38 itself modulates calcium signaling by converting NAD^+ into cyclic ADP-ribose (cADPR) and ADP-ribose, which activate ryanodine receptors (RyRs) on the endoplasmic reticulum (ER) to release Ca^{2+} into the cytosol [36]. Further studies will be necessary to clarify how CD38 and Wnt signaling interact and are regulated by Ca^{2+} dynamics.

In summary, the Wnt–NAD^+ axis represents a sophisticated regulatory network across cellular compartments. Sirtuins modulate this crosstalk through protein modification and epigenetic regulation, while PARPs (especially tankyrases) control Wnt components via PARylation. Reciprocally, Wnt signaling modulates NAD^+ metabolism through transcriptional control of key enzymes and the establishment of feedback loops that are responsive to cellular metabolic status. While many of these mechanisms have been initially characterized in cancer models, their roles in development and tissue homeostasis are increasingly being recognized. Understanding their context-dependent roles could unlock therapeutic potential. Given their impact on aging and regeneration, exploring the Wnt–NAD^+ axis is crucial for advancing interventions in tissue repair. The following sections examine how the Wnt–NAD^+ axis influences aging and regeneration.

The Wnt–NAD axis in aging

Aging involves multistage decline in cellular and tissue dysfunction characterized by genomic instability, telomere attrition, and stem cell exhaustion [37]. A central feature of this decline is the age-related reduction in NAD^+ levels [38–40] that is primarily due to decreased NAMPT-mediated synthesis and increased NAD^+ consumption by CD38 and PARP enzymes [14,38,39,41,42]. In particular, accumulated oxidative DNA damage with age activates PARP1, further depleting NAD^+ stores [43,44]. NAD^+ depletion impairs sirtuin activity and stress responses, and is further exacerbated by chronic low-grade inflammation ('**inflammaging**') [8,45].

Inflammaging exacerbates NAD^+ loss, in part by increasing CD38 expression in immune cells such as macrophages and in tissues including liver, spleen, skeletal muscle, and adipose tissue [42,46–48]. In macrophages, NAD^+ depletion promotes NLRP3 inflammasome activation, which can be reversed by NAD^+ precursor supplementation [49].

However, not all age-related NAD^+ dysregulation is reversible by NAD precursor supplementation alone. Calcific aortic valve disease (CAVD), the most prevalent form of heart valve pathology,

exemplifies a distinct CD38-driven mechanism. Aging is a major risk factor for CAVD, and studies have shown that calcified valves exhibit elevated degradation of NAD^+ and NMN, without compensatory increases in NMN or nicotinamide riboside (NR), accompanied by significant accumulation of NAM. Crucially, this NAM buildup is not corrected by NAD^+ precursor supplementation but is abolished by CD38 inhibition, implicating CD38 as a central driver of aberrant NAD^+ metabolism in CAVD [50].

Concurrently, Wnt/β-catenin signaling shows age-dependent dysregulation [51,52]. In most tissues, reduced Wnt activity impairs adult stem cell maintenance [53], contributing to a decline in tissue regeneration and homeostasis [54]. However, the relationship between aging and Wnt signaling is complex because excessive activation can promote pathological outcomes such as fibrosis [55] and cancer [56]. This dual nature is evident in tissue-specific contexts. For example, elevated Wnt activity drives muscle progenitor cells toward a fibrogenic rather than myogenic fate [57]. In the skin, dysregulated Wnt signaling affects wound healing, and reduced activity impairs keratinocyte proliferation and migration, while excessive activation can lead to hypertrophic scarring or skin tumors [58].

Beyond their individual roles, the convergence of Wnt and NAD^+ pathways in aging is particularly evident in two key areas.

(i) Stem cell maintenance: age-related decline in both pathways compromises stem cell function across various organs (Figure 3A,B). For example, in the aging intestine, mitochondrial DNA mutations deplete NAD^+ through the activation of the ATF5-dependent mitochondrial unfolded protein response (UPRmt), triggering impaired Wnt signaling and reduced intestinal stem cell number and regenerative capacity [59]. Similarly, an age-related decline in the Wnt–NAD^+ axis in osteoprogenitors is linked to compromised bone structure and health in mice [9]. In muscle stem cells, Wnt signaling and NAD^+ metabolism interact uniquely. The NAD precursor **nicotinamide (NAM)** regulates Wnt signaling by directly activating cytoplasmic β-catenin independently of NAD^+ synthesis. NAM stimulates the proliferation of the muscle stem cells by inhibiting CK1α-mediated phosphorylation, which facilitates β-catenin acetylation and nuclear translocation (Figure 2B). This leads to improved muscle regeneration in aged mice and restored function of aged human muscle progenitors [60]. Research into the Wnt–NAD^+ axis in stem cell/progenitor biology remains nascent, but dysregulation of this axis may be a common feature of stem cell aging across multiple tissues.

(ii) Telomere maintenance: telomere attrition involves the Wnt–NAD^+ axis (Figure 3C,D), which acts through mechanisms including telomerase activity, telomere protection, and DNA damage responses. The Wnt/β-catenin pathway targets **telomerase reverse transcriptase (TERT)**, a key enzyme that is responsible for telomere length maintenance in multiple cell lines from human cancers including stomach (AGS), breast (MCF7/10A), kidney (293T), colon (HCT116, LS174T), testis (NTera2), and large intestine (SW480), as well as in mouse embryonic stem cells [61,62]. TERT also interacts with the promoter of the gene encoding β-catenin (*Ctnnb1*) and also with some β-catenin target genes such as *Myc* and **cyclin D1** *(Ccnd1)* in small intestine crypts, colon, and embryonic stem cells of mice. Moreover, in human CRC, hepatocellular carcinoma cell lines, patient-derived hepatoma cells, and IMR90 (a normal fibroblast cell line), as well as in mouse intestinal mucosa and skin keratinocytes, the Wnt/β-catenin pathway upregulates **telomeric repeat-binding factor 2 (TRF2)** [63], a crucial shelterin complex component that protects telomeres from being misidentified as double-strand breaks, thereby preventing telomere shortening and fusion [64,65]. Reducing β-catenin levels leads to telomere dysfunction, which can be rescued by TRF2 overexpression.

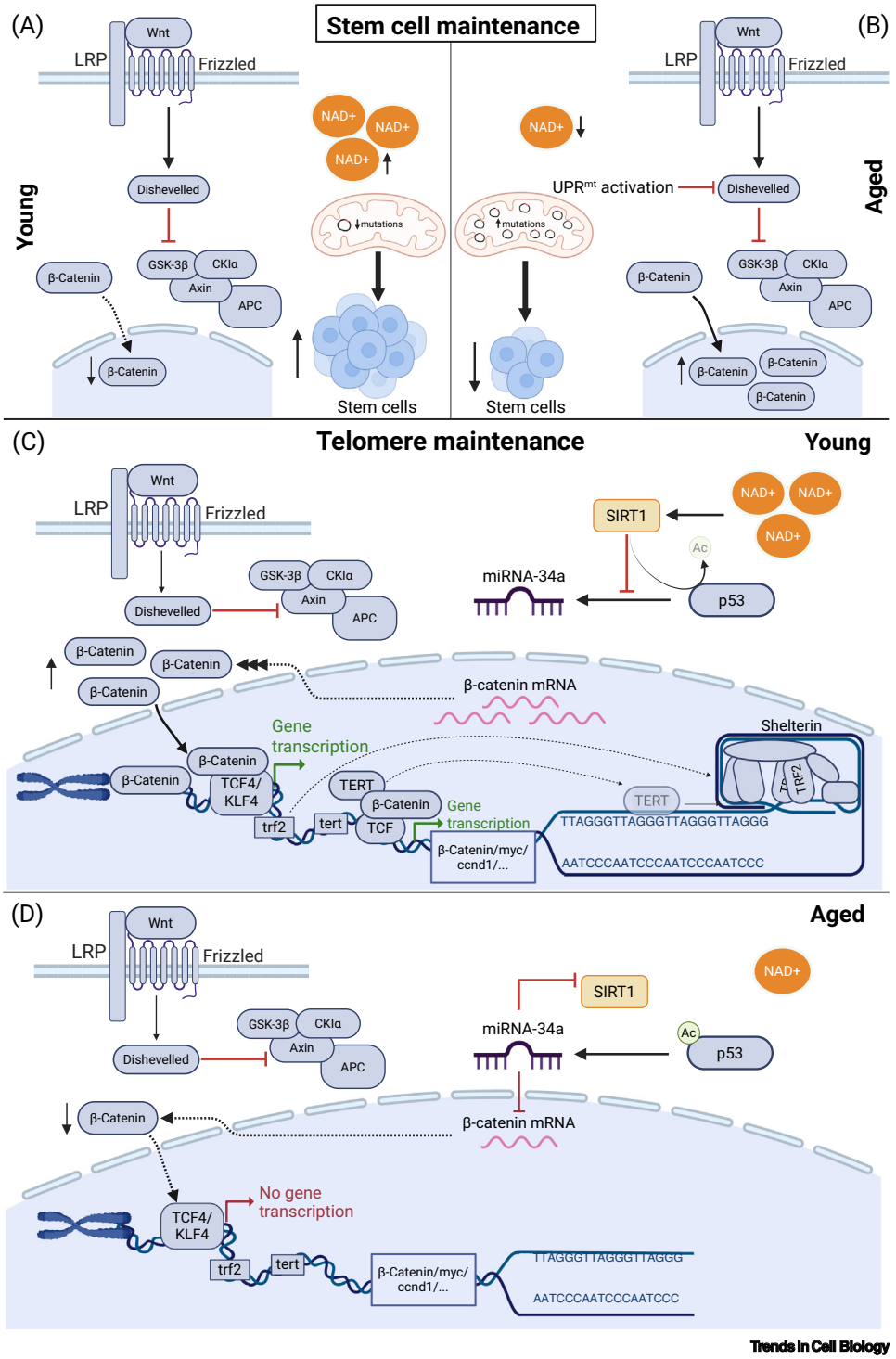


Figure 3. Convergence of the Wnt signaling and NAD$^+$ signaling pathways in regulating stem cell function and telomere maintenance. (A) In young cells, optimal NAD$^+$ levels maintain robust mitochondrial function and active Wnt signaling, supporting normal stem cell populations. (B) During aging, accumulated mitochondrial DNA (mtDNA) mutations

(Figure legend continued at the bottom of the next page.)

When telomeres become uncapped, p53 is activated and induces the expression of miR-34a, which then acts as a key regulatory element through multiple mechanisms. First, miR-34a inhibits Wnt signaling [66], potentially as a protective response to telomere dysfunction. Second, miR-34a targets *SIRT1* transcripts [67] and reduces SIRT1 levels. This creates a regulatory circuit because when NAD^+ levels are higher, SIRT1 deacetylates and inactivates p53, thereby suppressing miR-34a expression [68]. This complex interplay establishes a molecular bridge between Wnt signaling and NAD^+-dependent pathways in telomere maintenance [69] where NAD^+ availability can modulate the severity of the telomere dysfunction response. Indeed, boosting NAD^+ levels, either through NR supplementation or CD38 inhibition, reduces telomere dysfunction-induced DNA damage foci in telomerase-deficient mice and fibroblasts derived from patients with dyskeratosis congenita, a premature aging disease [70,71]. Overall, the Wnt–NAD^+ axis is emerging as a crucial regulator in aging that maintains cellular function and tissue homeostasis. Dysregulation of these pathways contributes to several hallmark features of aging, and more insights are expected as research advances.

The Wnt–NAD^+ axis in regeneration

Tissue regeneration involves a specialized regenerative niche that orchestrates stem cell function and tissue repair while maintaining stem cell populations [53]. This process integrates multiple cellular mechanisms: signaling pathways that control cell fate and organization, epigenetic reprogramming that governs cellular plasticity, and balanced immune responses and coordination of extracellular matrix remodeling with angiogenesis [72].

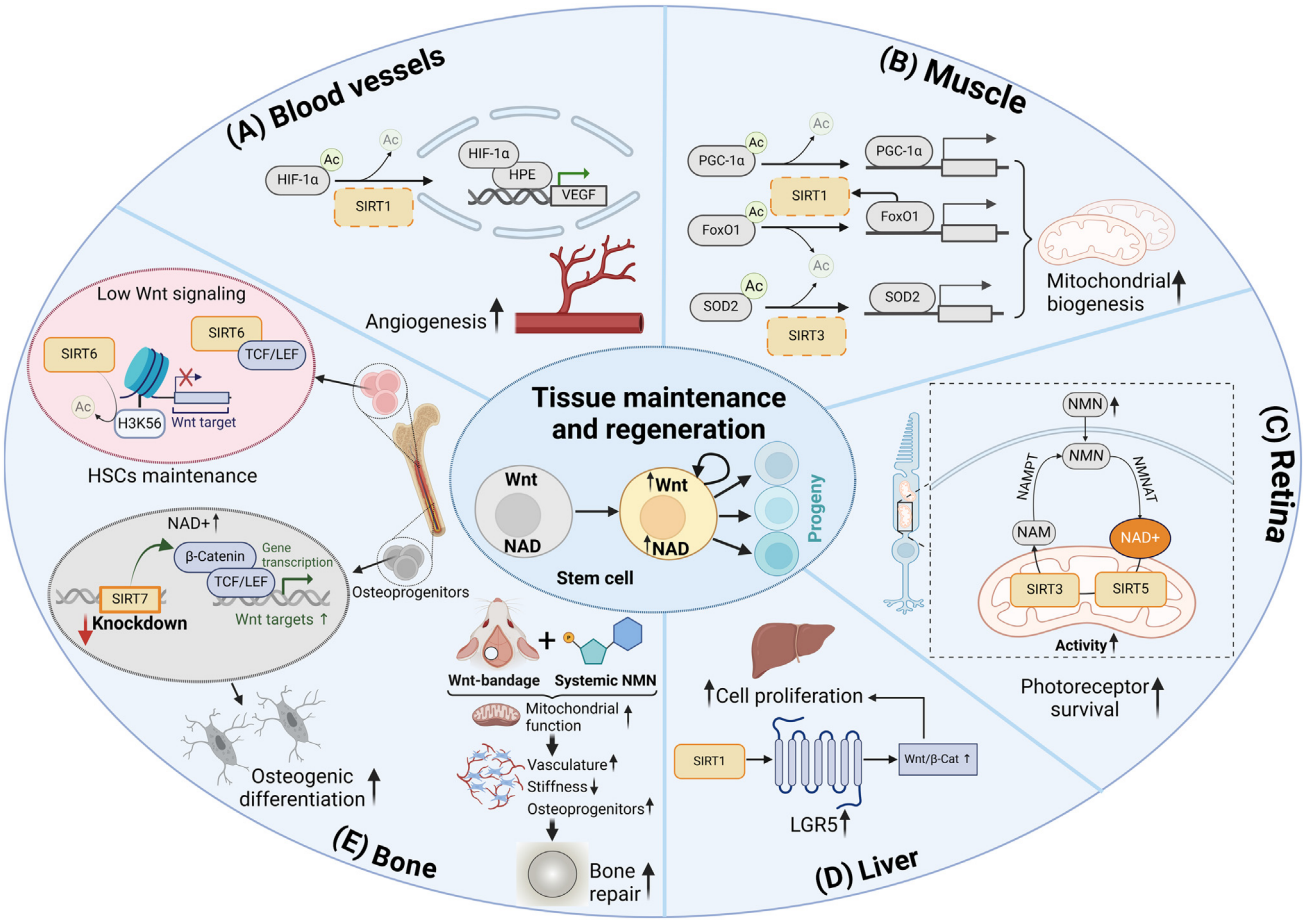
In regeneration, NAD^+ plays a pivotal role beyond energy metabolism and supports DNA repair, genomic stability, and angiogenesis through sirtuins and PARP-dependent mechanisms (Figure 4). For example, SIRT1 deacetylation of **hypoxia-inducible factor 1α (HIF-1α)** promotes angiogenesis through **vascular endothelial growth factor (VEGF)** expression (Figure 4A) [73]. In muscle tissue, NAD^+ restoration enhances stem cell function and mitochondrial performance through sirtuin-dependent deacetylation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a key regulator of mitochondrial biogenesis (Figure 4B) [74]. Similarly, in retinal tissue, NAD^+ deficiency reduces SIRT3 and SIRT5 activity, thereby compromising photoreceptor survival, which NMN supplementation can restore (Figure 4C) [75].

NAD^+ also modulates systemic processes, particularly inflammation [76]. Controlled inflammation is vital in the early stages of regeneration because it enables clearance of damaged cells and activation of repair mechanisms. However, chronic or excessive inflammation, often associated with NAD^+ depletion, can disrupt this delicate balance and impede regeneration. Replenishing physiological levels of NAD^+ reduced proinflammatory signaling in the brain and restored the balance necessary for effective tissue repair [77].

The Wnt pathways operate alongside NAD^+ metabolism in tissue regeneration. Upon injury, Wnt pathways regulate inflammation, genomic stability, and mobilization of stem cells [78]. The short-

lead to reduced NAD^+ levels, resulting in impaired Wnt signaling and subsequent decline in stem cell numbers. Restoration of NAD^+ levels through nicotinamide mononucleotide (NMN) supplementation reactivates Wnt signaling, effectively reversing the age-related decline in stem cell populations. (C) In young cells, high NAD^+ levels enable SIRT1 to deacetylate p53 effectively. This deacetylation prevents p53 from activating miR-34a expression. In the absence of miR-34a repression, β-catenin can be translated normally and translocates to the nucleus where it activates transcription of Wnt target genes. Key targets for telomere maintenance include TRF2 and telomerase reverse transcriptase (TERT). TERT functions by adding TTAGGG repeats to maintain telomere length, while TRF2 works with other shelterin complex components to protect telomere ends from fusion. (D) In aged cells, low NAD^+ levels impair the ability of SIRT1 to deacetylate p53. As a result, acetylated p53 activates miR-34a expression, which then inhibits β-catenin activity by

(See figure legend at the bottom of the next page.)



Trends in Cell Biology

Figure 4. Overview of the Wnt–NAD$^+$ axis in tissue maintenance and regeneration, with select tissue examples. Activation of Wnt signaling and increased NAD$^+$ availability are crucial for the self-renewal of adult stem cells and their ability to generate differentiating progeny that are required for tissue maintenance and repair. (A) In blood vessels, SIRT1-mediated deacetylation of HIF-1α upregulates the transcription of proangiogenic genes such as *VEGF*, a process that is required for tissue regeneration. (B) In muscle tissue, SIRT3 and SIRT1 respectively deacetylate superoxide dismutase 2 (SOD2) and the transcription factors PGC-1α and FOXO1, leading to upregulation of mitochondrial biogenesis. (C) In the retina, mitochondrial SIRT3 and SIRT5 upregulate mitochondria bioenergetics upon nicotinamide mononucleotide (NMN) supplementation, ensuring photoreceptor survival and counteracting NAD$^+$ decline. (D) In the liver, SIRT1 has been shown to upregulate the expression of LGR5, a key receptor for canonical Wnt signaling, leading to hepatocyte proliferation. (E) In bone, SIRT6 deacetylation of histone H3 lysine 56 (H3K56) on Wnt target gene promoters ensures a reduced level of Wnt signaling that is essential for hematopoietic stem cell (HSC) maintenance. Knockdown of SIRT7 in osteoprogenitors led to heightened Wnt/β-catenin (β-cat) signaling and increased osteogenic differentiation. Combining a Wnt-bandage with systemic supplementation of NMN rejuvenates osteoprogenitors and their niche, and promotes calvarial bone repair to the levels seen in young mice. Protein color code: light blue, components of the Wnt pathway; light orange, NAD$^+$ consumers; light gray, others.

range nature of Wnt signaling enables cell–cell communication within the stem cell niche and promotes localized maintenance of stem cells inside a spatially delimited zone [4,53,79]. Conversely, progenies leaving this area differentiate and contribute to tissue repair [4]. While Wnt/β-catenin signaling has been extensively studied, noncanonical Wnt pathways are also emerging as key regulators of tissue homeostasis, including stem cell quiescence in neural and hematopoietic systems [80].

Recent studies have highlighted the Wnt–NAD$^+$ axis in regeneration (Figure 4). For example, SIRT1 enhances liver regeneration by activating Wnt/β-catenin signaling (Figure 4D) [81]. Accordingly, SIRT1 upregulates Wnt pathway components and target genes, including LGR5 (a receptor

for R-spondin1), which boosts Wnt signaling. This activation promotes hepatic progenitor cell proliferation and activity, and facilitates liver repair after injury. Similarly, SIRT6 modulates Wnt signaling to maintain hematopoietic stem cell (HSC) quiescence under normal conditions, and its deficiency results in hyperactive Wnt signaling, excessive HSC proliferation, and eventual exhaustion under stress (Figure 4E) [27].

SIRT7 regulates the osteogenic differentiation of human bone marrow mesenchymal stem cells (hBMSCs) (Figure 4E). SIRT7 knockdown enhances osteogenic differentiation, partly through activation of the Wnt/β-catenin signaling pathway [26]. Indeed, applying SIRT7-knockdown hBMSCs to rat tibial defects markedly improved bone repair. Moreover, with age there is a significant decline in osteoprogenitor number and function in mouse calvarial bone. This decline is accompanied by reduced Wnt signaling and NAD^+ metabolism within these cells that contribute to impaired bone repair capacity. Combining a Wnt-based intervention (Wnt-bandage) with short-term NAD^+ supplementation can rejuvenate osteoprogenitors and restore their repair capabilities to youthful levels [9]. Overall, growing evidence positions the Wnt–NAD^+ axis as a key regulator of tissue repair, offering promising targets for regenerative therapies.

Approaches to targeting the Wnt–NAD^+ axis

Boosting NAD^+ availability

Cellular NAD^+ levels can be enhanced through multiple approaches that target different aspects of NAD^+ metabolism (Figure 5). These include supplementation with NAD^+ precursors, modulation of NAD^+-related enzymes, regulation of gut microbiota, and lifestyle interventions such as exercise and intermittent fasting.

NAD^+ precursors are the primary intervention for boosting NAD^+ levels (Table 1). The two main precursors are NMN and NR which are preferred over direct NAD^+ supplementation because of better bioavailability [82]. NR features smaller molecular size, direct cellular uptake in humans, an established safety profile, and superior stability [83]. By contrast, NMN requires extracellular conversion to NR before cellular entry in humans, although mice can directly absorb it via the SLC12A8 transporter [84].

The effects of NAD^+ precursors on cellular function operate through multiple mechanistic pathways, predominantly through activation of SIRT1, PARPs, and CD38. In murine pancreatic β cells, SIRT1 increases insulin secretion following glucose availability [85]. NMN supplementation in diabetic mouse models increases insulin sensitivity and secretion through SIRT1-mediated deacetylation of NF-κB [86–88], reducing proinflammatory cytokines that induce insulin resistance [89]. Similarly, NMN restores arterial SIRT1 activity levels in aged mice and improves vascular function [90]. In endothelial cells, SIRT1 activation induces proangiogenic signals [91] and can rescue age-related decreases in endurance and capillary density. NMN further supports microvasculature and exercise capacity by increasing skeletal muscle capillary density. In premature aging models of **ataxia telangiectasia**, that is characterized by increased PARylation, low NAD^+ levels, and mitochondrial dysfunction, NAD^+ repletion via NR supplementation or PARP inhibitors reduces neuronal DNA damage, enhances mitophagy, restores neuromuscular function, and extends lifespan [92]. These benefits are partly mediated by SIRT1 activation.

Other sirtuin family members also contribute to the effects of NAD^+. For instance, SIRT2 upregulation through NMN supplementation increases lifespan in mice by stabilizing BUBR1, a mitotic checkpoint kinase whose decline with age promotes cellular senescence and aging phenotypes [93]. NAD^+ precursors also improve DNA repair mechanisms through modulation of protein–

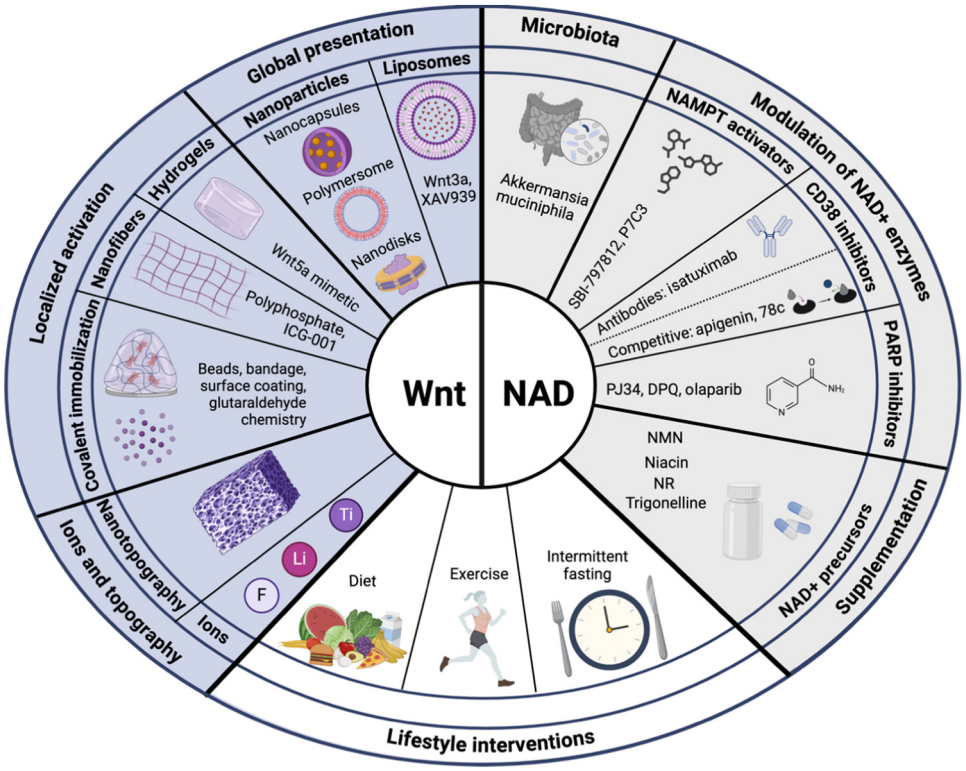


Figure 5. Overview of therapeutic strategies targeting Wnt and NAD$^+$ signaling. This schematic summarizes diverse methods to modulate Wnt and NAD$^+$ signaling. Wnt-related methods are shown in purple. The approaches are categorized into three main strategies: localized diffusion, localized covalent immobilization, global presentation, and metal-based modulation. Localized diffusion methods, such as hydrogels and nanofibers, enable controlled release of Wnt proteins or inhibitors. Localized covalent immobilization ensures spatially specific delivery through tethering Wnt-related molecules onto beads or surfaces. Global presentation utilizes nanoscale delivery systems such as liposomes, nanoparticles, and nanodisks to distribute Wnt proteins, activators, or inhibitors broadly. Metal-based strategies, including the use of metallic ions (e.g., titanium, lithium, fluoride) and nanostructured surfaces, provide additional means of influencing Wnt signaling activity. NAD-related methods are shown in gray. NAD$^+$ precursors such as nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), niacin, and trigonelline are highlighted as supplementation methods. Microbiota-based approaches involve probiotics such as *Akkermansia muciniphila* which support NAD$^+$ homeostasis. Nicotinamide phosphoribosyltransferase (NAMPT) activators, including compounds such as SBI-797812 and P7C3, enhance NAD$^+$ biosynthesis by upregulating the activity of the rate-limiting enzyme NAMPT. Finally, strategies to inhibit NAD$^+$ consumers target enzymes such as CD38 and PARPs by using inhibitors (e.g., apigenin and **olaparib**, respectively) or antibodies (e.g., isatuximab) to reduce NAD$^+$ degradation. Collectively, these interventions offer diverse pathways to regulate NAD$^+$ levels and support cellular health and longevity. White, common interventions. Lifestyle interventions include dietary strategies such as caloric restriction and intermittent fasting, as well as exercise, which are known to enhance NAD$^+$ metabolism and Wnt signaling.

protein interactions [94]. For example, NAD$^+$ binding to DBC1 (deleted in breast cancer 1) [95] prevents it from inhibiting PARP1, a key DNA repair enzyme [94]. In aging mice, declining NAD$^+$ levels lead to increased DBC1–PARP1 interaction and subsequent accumulation of DNA damage. Restoring NAD$^+$ levels by NMN supplementation reverses this effect, rescuing PARP1 activity and reducing DNA damage.

Aging-related declines in mitochondrial bioenergetics are linked to NAD$^+$ metabolism. A single dose of NMN boosts hippocampal NAD$^+$ levels in mice, resulting in SIRT3-mediated deacetylation of mitochondrial proteins [96] including **superoxide dismutase 2 (SOD2)**. Subsequently, oxidative stress levels and mitochondrial fragmentation are reduced. The shift towards

Table 1. NAD^+-boosting strategies in disease: list of select *in vivo* NAD^+ supplementation experiments in animal models of age-related diseases followed by select completed and ongoing human clinical trials[a]

Animal models: *in vivo*						
Model, target	Age	Tissue	Treatment	Length	Effect	PMID
Age-induced type II diabetes mice	15–26 months		NMN intraperitoneal, 500 mg/kg bw/day	11 days	Increased insulin secretion and sensitivity	21982712
Old C57Bl/6 mice, impaired carotid artery endothelium-dependent dilation	26–28 months	Aorta	NMN 300 mg/kg bw/day in drinking water,	8 weeks	Reversed vascular dysfunction and oxidative stress, rescued SIRT1 expression	26970090
APP^{swe}/*PS1*$^{\Delta E9}$ double transgenic mouse Alzheimer's disease model	3–12 months	Brain	NMN subcutaneously, 100 mg/kg bw/ 48 h	28 days	Rescued mitochondrial respiration, reduction in mutated APP levels	25884176
APP^{swe}/*PS1*$^{\Delta E9}$ mice	7–12 months	Brain	NR in drinking water, 12 mM	5 months	Reduced neuroinflammation, DNA damage, and senescence	34497121
APP^{swe}/*PS1*$^{\Delta E9}$ mice	6 months	Brain	NMN subcutaneously, 100 mg/kg bw/ 48 h	28 days	Improvement of cognitive impairments, decreased β-amyloid production, synaptic loss, and inflammatory responses	28330719
APP^{swe}/*PS1*$^{\Delta E9}$ mice	6 months	Brain, gut	NMN by gavage, 300 mg/kg/day	16 weeks	Increase in relative abundance of SCFA-producing bacteria	37271037
Aged C57BL/6J mice, reduced DNA repair	20–26 months		NMN intraperitoneal, 500 mg/kg/day	7 days	Reversed DNA damage	28336669
Aged C57BL/6J mice, vascular aging	18 months	Skeletal muscle	NMN in drinking water, 400 mg/kg/day	2 months	Improved blood flow, capillary density, and exercise capacity	29570999
Aged C57BL/6J mice, skeletal aging	12–15 months	MSCs	NMN in drinking water, 300 mg/kg bw/day	3 months	Increased osteogenesis, decreased adipogenesis	31000692
B6;129S4-*Atm*^{tm1Bal}/J mice, ataxia telangiectasia	1 month	Brain, liver, muscle	NMN in drinking water, 3.5 mg/ml (12 mM)	2 weeks	Increased lifespan, upregulation of mitophagy and DNA repair	27732836
B6;129S4-*Atm*^{tm1Bal}/J mice, ataxia telangiectasia	1.5 months		NMN in drinking water 3.5 mg/ml (12 mM)	2 months	Reduced neuroinflammation and senescence, improved motor functions	33734555
C57BL/6N mice, normal aging	5 months until 17 months	Skeletal muscle, WAT, liver	NMN in drinking water, 100 or 300 mg/kg/day	12 months	Reduced age-related weight gain, improved energy metabolism, insulin sensitivity, eye function, and functional activity	28068222

(continued on next page)

Table 1. (continued)

Animal models: *in vivo*						
Model, target	Age	Tissue	Treatment	Length	Effect	PMID
C57Bl/6 mice on a high-fat diet	8 weeks	Muscle, WAT, BAT	Resveratrol 4 g/kg food	15 weeks	Increased SIRT1 enzymatic activity and muscle aerobic capacity, upregulation of OXPHOS genes, improved insulin sensitivity, reduced weight gain	17112576
Tg2576 mice, Alzheimer's disease model	7–8 months	Brain	NR in drinking water, 250 mg/kg/day	3 months	Improved cognitive function	23312803
APP$^{swe/ind}$ mice (J20 line)	15 weeks	Brain	40% caloric restriction (3 g/day)	6 weeks	Decreased amyloid plaque frequency and size	15748777
C57BL/6 mice	30 months	Testes	NMN injection, 500 mg/kg/day	7 days	Increased SIRT2 activity, stabilization of BUBR1	24825348
C57BL/6 mice	6 months	Liver	Caloric restriction, water only	24 h	Increased NAD^+ levels and SIRT1 expression following PPARα activation	20148352
Fisher 344 rats	12 months	Brain, kidney, liver, muscle, adipose tissue	Caloric restriction, 60% of ad lib	12 months	Increased SIRT1 expression, decreased Ku70 acetylation, reduced stress-induced apoptosis	15205477
Human: completed clinical trials						
Age	Target	Treatment	Length	Effect	Study design	Clinical trial ID or PMID
55–79 years	Cardiovascular function	NR 1000 mg/day oral supplementation	6 weeks	Increased NAD^+ metabolism, reduction in systolic blood pressure and aortic stiffness	Phase 1 and 2, double-blind randomized crossover trial	NCT02921659
70–80 years	Skeletal muscle	NR 1000 mg/day oral supplementation	21 days	Decrease in circulatory inflammatory cytokines	Phase 2, double-blind randomized crossover trial	NCT02950441
Mean 64 +/– 8 years	Parkinson's disease	NR 1000 mg/day oral supplementation	30 days	Increased brain NAD levels, improved physical and mitochondrial function	Phase 1, double-blind randomized parallel trial	NCT03816020
Mean 59 +/– 8 years	Heart failure with reduced ejection fraction	NR 2000 mg/day oral supplementation	12 weeks	Reduced inflammation and improved mitochondrial function	Phase 1 and 2 double-blind randomized single-arm trial	NCT03423342
65+ years	Healthy older men	NMN 250 mg/day oral supplementation	12 weeks	Improved muscle performance (gait speed and grip strength)	Double-blind randomized parallel trial	UMIN000036321

Table 1. (continued)

Human: completed clinical trials						
Age	Target	Treatment	Length	Effect	Study design	Clinical trial ID or PMID
62 +/– 4 years	Prediabetic post-menopausal women	NMN 250 mg/day oral supplementation	10 weeks	Improved muscular insulin sensitivity	Double-blind randomized parallel trial	NCT03151239
71–83 years	Older people with mild cognitive impairment	NR oral capsules, dose escalation, 250–500–750–1000 mg/day	10 weeks	Reduced cerebral blood flow and DNA methylation	Double-blind randomized factorial trial	NCT02942888
71.5 +/– 1 years	Healthy older people	NR acute supplementation 2 × 250 mg	11 days	Higher NADH and NADPH levels, improved isometric peak torque and fatigue index	Double-blind randomized crossover trial	PMID 30725213
Human: ongoing clinical trials						
Age	Target	Treatment	Length	Outcomes	Study design	Clinical trial ID
55–85 years	Older adults with Alzheimer's disease	MIB-626 2 × 1000 mg daily	90 days	Change in cerebrospinal fluid	Double-blind randomized parallel trial	NCT05040321
>55 years	Older adults with peripheral artery disease	NR 1 g daily	4 weeks	Change in endothelial function	Single-group assignment	NCT06534944
>75 years	Older frail adults	NR 2 × 1000 mg daily	52 weeks	Gait speed	Double-blind randomized parallel trial	NCT06208527
60–85 years	Healthy older adults	NR 1000 mg daily	8 weeks	Neurovascular coupling and endothelial function	Double-blind randomized parallel trial	NCT05483465
65–85 years	Older veterans	NR 2 × 500 mg daily	12 weeks	VO2max, muscle strength, gait speed	Double-blind randomized parallel trial	NCT04691986
18–75 years	Non-small cell lung carcinoma patients	NMN 1 ×, 2 ×, or 3 × 150 mg daily, with radiotherapy and PD-1 inhibitor	Until dose toxicity is observed	Adverse effects and dose toxicity	Single-group assignment	NCT06966583
<18 years	Stage IV breast cancer under anthracycline therapy	NR 2 × 500 mg daily	3 or 6 months	Reduction in left ventricular systolic function	Double-blind randomized parallel trial	NCT05732051

[a]Abbreviations: BAT, brown adipose tissue; bw, body weight; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; OXPHOS, oxidative phosphorylation; SCFA, short-chain fatty acid; WAT, white adipose tissue; VO2max, maximal oxygen consumption.

mitochondrial fusion is attributed to reduced interaction of phosphorylated dynamin-related protein 1, a key mitochondrial fission protein, with mitochondria. Taken together, NMN supplementation improves mitochondrial health by modulating mitochondrial dynamics through a SIRT3-dependent mechanism.

Recent research has expanded the toolkit for NAD^+ enhancement beyond traditional precursors. Trigonelline, an NAD^+ precursor that is metabolized via the Preiss–Handler pathway, demonstrates NAD^+-boosting capabilities [97], while nicotinamide mimetics such as AZ6102 show promise through inhibition of tankyrase [98,99]. Lifestyle interventions such as intermittent fasting and calorie restriction also increase NAD^+ levels and activate sirtuins [100–104]. Orally

administered NMN can also be metabolized by the gut microbiota, leading to its incorporation into the *de novo* pathway and supporting intestinal homeostasis [105]. Long-term NMN treatment enhances probiotic levels, including *Akkermansia muciniphila* (*AKK*), which increases longevity in mice [106]. Of note, supplementation with *AKK* bacteria improved bone repair in aged mice, mimicking the effects of NMN supplementation and intermittent fasting [9].

NAD^+ levels can be maintained by targeting major NAD^+-consuming enzymes. CD38, a key contributor to age-related NAD^+ depletion, can be inhibited through multiple approaches:

(i) Small-molecule inhibitors: the competitive inhibitor 78c increases murine lifespan and rescues age-induced metabolic dysfunction and telomere-associated DNA damage [40,107]. However, it has also limitations including potential off-target effects at high concentrations [108], including selective inhibition of CD38 hydrolase activity (but not its cyclase function) and immune modulation [109].
(ii) Natural compounds: apigenin, a flavonoid found in fruits, acts as a CD38 inhibitor, increasing NAD^+ levels and counteracting metabolic syndromes [110]. However, its use is limited by moderate potency, lack of specificity, and broad off-target effects [110,111].
(iii) Therapeutic antibodies: isatuximab, that was developed for multiple myeloma treatment [112,113], inhibits the NAD^+-consuming activities of CD38 [114,115], suggesting potential applications in age-related diseases. However, it may cause immunosuppression and increase the risk of infection [116].

Similarly, PARP inhibitors (**DPQ**, **PJ34**) effectively increase intracellular NAD^+ levels and prevent cellular senescence [44,117,118]. Notably, senescence outcomes depend on the stress context [119], and PARP1 inhibition preserves NAD^+ levels and improves recovery following chronic stress [44], while shifting necrosis to senescence enhances healing in acute injury models [120]. Complementing these approaches, NAMPT activators such as **SBI-797812** [121] and P7C3 [122] upregulate NAD^+ biosynthesis and have shown therapeutic potential in diabetes and muscle function in mice.

While NAD^+ enhancement strategies show therapeutic promise, their implementation requires careful consideration of context-specific effects. Increased NAD^+ availability may support tumor growth because cancer cells depend on NAD^+ for energy [123]. This complexity extends to CD38 regulation where its NAD^+-depleting activity must be balanced against its role in calcium homeostasis. Moreover, the long-term safety of chronic NAD^+ precursor use in humans remains poorly understood [124,125]. Individual differences in metabolism, gut microbiota, and transporter expression can affect NAD^+ bioavailability and efficacy, and the possibility of negative feedback mechanisms – such as suppression of endogenous NAD^+ synthesis or sirtuin activity – has not been ruled out. Potential drug interactions and effects on immune function further underscore the need for personalized and well-monitored therapeutic approaches that are tailored to the health status, age, and treatment objectives of each individual.

Targeting Wnt signaling

Recent studies have revealed multiple approaches for modulating Wnt pathways to enhance tissue repair and regeneration. These approaches can be categorized into distinct groups: ionic modulators, Wnt ligand delivery systems, small-molecule interventions, and lifestyle modifications (Figure 5).

Ionic approaches modulate Wnt/β-catenin signaling primarily through inhibition of GSK-3β, a key component of the β-catenin destruction complex. This inhibition prevents β-catenin degradation,

enabling its nuclear translocation and activation of Wnt target genes. Lithium ions exemplify this mechanism and, when incorporated into mesoporous bioactive glass scaffolds, enhance cell proliferation and cementogenic differentiation of human periodontal ligament-derived cells [126]. Similarly, strontium and fluoride ions promote osteoblast differentiation and bone regeneration through this mechanism [127–132]. Nanotopography has also emerged as a complementary material-based approach because it can influence stem cell behavior through biophysical cues and enhance canonical Wnt signaling when combined with materials such as titanium [133–135].

The delivery of Wnt ligands represents another category of pathway modulation [53]. Due to their hydrophobic nature, Wnt proteins require specialized delivery systems. Liposomal delivery of Wnt3a, for example, can enhance bone repair when injected into bone defects [136], and Wnt3a nanodisks support the expansion of HSCs [137]. Hydrogels with Wnt5a mimetic ligands promote chondrogenesis in human mesenchymal stem cells [138].

Immobilization of Wnt ligands onto surfaces and microbeads offers another promising strategy because it enables localized Wnt signaling activation [139–142]. A particularly promising example is the 'Wnt-bandage' composed of Wnt3a immobilized onto polycaprolactone. This approach has demonstrated efficacy in activating endogenous osteoprogenitors for bone defect repair [9,142] and facilitates the delivery of engineered osteogenic tissue to promote healing in critical-sized bone defects [142].

Small-molecule modulators constitute a distinct approach to Wnt pathway regulation. These include the GSK-3β inhibitor **BIO** that can be delivered via synthetic amphiphilic polymersomes to human bone marrow stromal cells to promote osteogenesis [143]. The tankyrase inhibitor XAV939, delivered through liposomal systems, has demonstrated anti-inflammatory and antiangiogenic effects in ocular applications [144]. Other small molecules such as alsterpaullone have been successfully delivered via nanocapsules in various model systems [145]. Hydrogels and nanofibers have also been designed to deliver both Wnt activators such as **polyphosphate** [146] and inhibitors such as ICG-001 [147].

Lifestyle interventions provide non-pharmacological approaches to Wnt pathway modulation. Exercise promotes neurogenesis and myelin repair through upregulation of the Wnt/β-catenin signaling pathway [148]. Similarly, intermittent fasting activates this pathway in aged osteoprogenitors and can enhance bone repair capacity [9]. These interventions also influence NAD^+ metabolism, suggesting potential mechanistic overlap between these pathways.

While these approaches offer promising therapeutic potential, each has advantages and limitations [53,142]. Ionic approaches lack pathway specificity because GSK-3β inhibition affects multiple signaling cascades beyond Wnt. Both ionic and liposomal delivery systems face challenges with tissue specificity and diffusion beyond target cells, potentially requiring multiple administrations that increase the risk of infection. Small molecules face similar challenges in specificity and delivery. By contrast, newer technologies show improved targeting capabilities. For instance, Wnt surrogates – engineered water-soluble proteins that activate Wnt receptors – offer enhanced handling properties while maintaining pathway specificity [149]. Local presentation strategies, such as the Wnt-bandage, demonstrate a particular promise by enabling precise spatial control of Wnt signaling activation. This approach has shown remarkable efficacy in bone repair without adverse effects [9,142]. The controlled spatiotemporal activation of Wnt signaling is crucial because pathway dysregulation is frequently associated with tissue abnormalities including cancer development [4]. Understanding these limitations and advantages will be key for optimizing therapeutic applications.

Concluding remarks

The NAD–Wnt axis represents an emerging frontier in regenerative medicine and aging biology. While both pathways have been extensively studied individually, their interconnection in development, homeostasis, and repair remains incompletely understood. Recent findings suggesting crosstalk between NAD^+ metabolism and Wnt signaling open intriguing questions about their coordinated regulation of stem cell function and tissue regeneration. The convergence of these pathways may provide novel therapeutic opportunities for addressing age-related decline and enhancing tissue repair capacity. Future research on the molecular mechanisms linking these pathways, their tissue-specific roles, and optimal therapeutic strategies for their modulation will be crucial for advancing regenerative medicine and developing effective anti-aging interventions (see Outstanding questions).

Outstanding questions

What are the unidentified molecular checkpoints that coordinate NAD^+ metabolism with Wnt-dependent transcriptional programs during tissue regeneration?

How does compartmentalization of NAD^+ pools affect local Wnt signaling responses?

How do age-related changes in NAD^+ availability mechanistically alter the stem cell response to Wnt signals?

What mechanisms regulate the balance between NAD^+ consumption and synthesis in aging stem cells, and how does this affect their regenerative capacity?

What are the spatial and temporal requirements for coordinated Wnt and NAD^+ signaling in tissue regeneration?

How do tissue-specific metabolic states influence the effectiveness of combined Wnt- and NAD^+-targeting therapies?

Acknowledgments

This work was supported by the University of Lausanne. We thank Professor Virginie Mansury-Aubert, Professor Nicola Vannini, and Dr Flortent Allagnat for critically reading the manuscript and providing valuable feedback.

Declaration of interests

S.J.H is an inventor on patent application P6125GB00, Tissue regeneration patch. The other authors declare no competing interests.

References

1. Chini, C.C.S. *et al.* (2024) NAD metabolism: role in senescence regulation and aging. *Aging Cell* 23, e13920
2. Amjad, S. *et al.* (2021) Role of NAD^+ in regulating cellular and metabolic signaling pathways. *Mol. Metab.* 49, 101195
3. Nikiforov, A. *et al.* (2011) Pathways and subcellular compartmentation of NAD biosynthesis in human cells: from entry of extracellular precursors to mitochondrial NAD generation. *J. Biol. Chem.* 286, 21767–21778
4. Garcin, C.L. and Habib, S.J. (2017) A comparative perspective on Wnt/β-catenin signalling in cell fate determination. *Results Probl. Cell Differ.* 61, 323–350
5. Komiya, Y. and Habas, R. (2009) Wnt signal transduction pathways. *Organogenesis* 4, 68–75
6. Liu, J. *et al.* (2022) Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. *Signal Transduct. Target. Ther.* 7, 3
7. Cantó, C. *et al.* (2015) NAD^+ metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. *Cell Metab.* 22, 31–53
8. Covarrubias, A.J. *et al.* (2021) NAD^+ metabolism and its roles in cellular processes during ageing. *Nat. Rev. Mol. Cell Biol.* 22, 119–141
9. Reeves, J. *et al.* (2024) Rejuvenating aged osteoprogenitors for bone repair. *Elife* 13, RP104068
10. Pavlova, N.N. and Thompson, C.B. (2016) The emerging hallmarks of cancer metabolism. *Cell Metab.* 23, 27–47
11. Lee, J. *et al.* (2016) Integrated omics-analysis reveals Wnt-mediated NAD^+ metabolic reprogramming in cancer stem-like cells. *Oncotarget* 7, 48562–48576
12. Ye, C. *et al.* (2020) Targeting the NAD^+ salvage pathway suppresses APC mutation-driven colorectal cancer growth and Wnt/β-catenin signaling via increasing Axin level. *Cell Commun. Signal.* 18, 16
13. Kupis, W. *et al.* (2016) The role of sirtuins in cellular homeostasis. *J. Physiol. Biochem.* 72, 371
14. Imai, S. and Guarente, L. (2016) It takes two to tango: NAD^+ and sirtuins in aging/longevity control. *NPJ Aging Mech. Dis.* 2, 16017
15. Zhou, Y. *et al.* (2016) SIRT1 suppresses adipogenesis by activating Wnt/β-catenin signaling in vivo and in vitro. *Oncotarget* 7, 77707–77720
16. Holloway, K.R. *et al.* (2010) SIRT1 regulates Dishevelled proteins and promotes transient and constitutive Wnt signaling. *Proc. Natl. Acad. Sci.* 107, 9216–9221
17. Iyer, S. *et al.* (2014) Sirtuin1 (Sirt1) promotes cortical bone formation by preventing β-catenin sequestration by FoxO transcription factors in osteoblast progenitors. *J. Biol. Chem.* 289, 24069–24078
18. García-Martínez, J.M. *et al.* (2024) SIRT1 mediates the antagonism of Wnt/β-catenin pathway by vitamin D in colon carcinoma cells. *Int. J. Biol. Sci.* 20, 5495–5509
19. Nguyen, P. *et al.* (2014) SIRT2 Interacts with β-catenin to inhibit Wnt signaling output in response to radiation-induced stress. *Mol. Cancer Res.* 12, 1244–1253
20. Si, X. *et al.* (2013) Activation of GSK3β by Sirt2 is required for early lineage commitment of mouse embryonic stem cell. *PLoS ONE* 8, e76699
21. Li, C. *et al.* (2021) Regulation of SIRT2 by Wnt/β-catenin signaling pathway in colorectal cancer cells, *Biochimica et Biophysica Acta (BBA) - Molecular. Cell Res.* 1868, 118966
22. Sundaresan, N.R. *et al.* (2015) SIRT3 blocks aging-associated tissue fibrosis in mice by deacetylating and activating glycogen synthase kinase 3β. *Mol. Cell. Biol.* 36, 678–692
23. Li, R. *et al.* (2018) SIRT3 inhibits prostate cancer metastasis through regulation of FOXO3A by suppressing Wnt/β-catenin pathway. *Exp. Cell Res.* 364, 143–151
24. Jiang, W. *et al.* (2018) The PIK3CA E542K and E545K mutations promote glycolysis and proliferation via induction of the β-catenin/SIRT3 signaling pathway in cervical cancer. *J. Hematol. Oncol.* 11, 139
25. Wang, Y. *et al.* (2022) SIRT4-catalyzed deacetylation of Axin1 modulates the Wnt/β-catenin signaling pathway. *Front. Oncol.* 12, 872444
26. Ke, K.-X. *et al.* (2024) Leptin attenuates the osteogenic induction potential of BMP9 by increasing β-catenin malonylation modification via Sirt5 down-regulation. *Aging (Albany NY)* 16, 7870–7888
27. Wang, H. *et al.* (2016) SIRT6 controls hematopoietic stem cell homeostasis through epigenetic regulation of Wnt signaling. *Cell Stem Cell* 18, 495–507
28. Chen, E.E.M. *et al.* (2017) Knockdown of SIRT7 enhances the osteogenic differentiation of human bone marrow mesenchymal stem cells partly via activation of the Wnt/β-catenin signaling pathway. *Cell Death Dis.* 8, e3042
29. Gu, Y. *et al.* (2024) SIRT7 stabilizes β-catenin and promotes canonical Wnt activation via upregulating FZD7. *Life Sci.* 359, 123240

30. Li, G. *et al.* (2023) Oncogenic SIRT7 inhibits GATA4 transcriptional activity and activates the Wnt signaling pathway in ovarian cancer. *Gynecol. Oncol.* 171, 39–48
31. Yang, E. *et al.* (2016) Wnt pathway activation by ADP-ribosylation. *Nat. Commun.* 7, 11430
32. Huang, S.-M.A. *et al.* (2009) Tankyrase inhibition stabilizes Axin and antagonizes Wnt signalling. *Nature* 461, 614–620
33. García-Martínez, J.M. *et al.* (2023) Vitamin D induces SIRT1 activation through K610 deacetylation in colon cancer. *eLife* 12, RP86913
34. Mariotti, L. *et al.* (2017) Regulation of Wnt/β-catenin signalling by tankyrase-dependent poly(ADP-ribosyl)ation and scaffolding. *Br. J. Pharmacol.* 174, 4611–4636
35. Aksoy, P. *et al.* (2006) Regulation of intracellular levels of NAD: a novel role for CD38. *Biochem. Biophys. Res. Commun.* 345, 1386–1392
36. Howard, M. *et al.* (1993) Formation and hydrolysis of cyclic ADP-ribose catalyzed by lymphocyte antigen CD38. *Science* 262, 1056–1059
37. López-Otín, C. *et al.* (2023) Hallmarks of aging: an expanding universe. *Cell* 186, 243–278
38. Camacho-Pereira, J. *et al.* (2016) CD38 dictates age-related NAD decline and mitochondrial dysfunction through a SIRT3-dependent mechanism. *Cell Metab.* 23, 1127–1139
39. Mouchiroud, L. *et al.* (2013) The NAD^+/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. *Cell* 154, 430–441
40. Tarragó, M.G. *et al.* (2018) A potent and specific CD38 inhibitor ameliorates age-related metabolic dysfunction by reversing tissue NAD^+ decline. *Cell Metab.* 27, 1081–1095
41. Stein, L.R. and Imai, S. (2014) Specific ablation of NAMPT in adult neural stem cells recapitulates their functional defects during aging. *EMBO J.* 33, 1321–1340
42. Hogan, K.A. *et al.* (2019) The multi-faceted ecto-enzyme CD38: roles in immunomodulation, cancer, aging, and metabolic diseases. *Front. Immunol.* 10, 1187
43. Massudi, H. *et al.* (2012) Age-associated changes in oxidative stress and NAD^+ metabolism in human tissue. *PLoS ONE* 7, e42357
44. Murata, M.M. *et al.* (2019) NAD^+ consumption by PARP1 in response to DNA damage triggers metabolic shift critical for damaged cell survival. *Mol. Biol. Cell* 30, 2584
45. Franceschi, C. *et al.* (2018) Inflammaging: a new immune–metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14, 576–590
46. Chini, C.C.S. *et al.* (2019) The NADase CD38 is induced by factors secreted from senescent cells providing a potential link between senescence and age-related cellular NAD^+ decline. *Biochem. Biophys. Res. Commun.* 513, 486–493
47. Chini, C.C.S. *et al.* (2020) CD38 ecto-enzyme in immune cells is induced during aging and regulates NAD^+ and NMN levels. *Nat. Metab.* 2, 1284–1304
48. Covarrubias, A.J. *et al.* (2020) Senescent cells promote tissue NAD^+ decline during ageing via the activation of $CD38^+$ macrophages. *Nat. Metab.* 2, 1265–1283
49. Shim, D.-W. *et al.* (2021) Intracellular NAD^+ depletion confers a priming signal for NLRP3 inflammasome activation. *Front. Immunol.* 12, 765477
50. Jablonska, P. *et al.* (2021) The new insight into extracellular NAD^+ degradation-the contribution of CD38 and CD73 in calcific aortic valve disease. *J. Cell. Mol. Med.* 25, 5884–5898
51. Hofmann, J.W. *et al.* (2014) The effects of aging on the expression of Wnt pathway genes in mouse tissues. *Age* 36, 9618
52. García-Velázquez, L. and Arias, C. (2017) The emerging role of Wnt signaling dysregulation in the understanding and modification of age-associated diseases. *Ageing Res. Rev.* 37, 135–145
53. Mills, K.M. *et al.* (2017) Wnt ligand presentation and reception: from the stem cell niche to tissue engineering. *Open Biol.* 7, 170140
54. Gruber, J. *et al.* (2016) Developmental drift and the role of Wnt signaling in aging. *Cancers (Basel)* 8, 73
55. Hu, H.-H. *et al.* (2020) Wnt signaling pathway in aging-related tissue fibrosis and therapies. *Ageing Res. Rev.* 60, 101063
56. Zhan, T. *et al.* (2017) Wnt signaling in cancer. *Oncogene* 36, 1461–1473
57. Brack, A.S. *et al.* (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* 317, 807–810
58. Bai, R. *et al.* (2023) The roles of WNT signaling pathways in skin development and mechanical-stretch-induced skin regeneration. *Biomolecules* 13, 1702
59. Yang, L. *et al.* (2024) NAD^+ dependent UPRmt activation underlies intestinal aging caused by mitochondrial DNA mutations. *Nat. Commun.* 15, 546
60. Ancel, S. *et al.* (2024) Nicotinamide and pyridoxine stimulate muscle stem cell expansion and enhance regenerative capacity during aging. *J. Clin. Invest.* 134, e163648
61. Zhang, Y. *et al.* (2012) Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/β-catenin pathway in human cancer. *J. Biol. Chem.* 287, 32494–32511
62. Hoffmeyer, K. *et al.* (2012) Wnt/β-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* 336, 1549–1554
63. Diala, I. *et al.* (2013) Telomere protection and TRF2 expression are enhanced by the canonical Wnt signalling pathway. *EMBO Rep.* 14, 356–363
64. van Steensel, B. *et al.* (1998) TRF2 protects human telomeres from end-to-end fusions. *Cell* 92, 401–413
65. de Lange, T. (2005) Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev.* 19, 2100–2110
66. Kim, N.H. *et al.* (2011) p53 and microRNA-34 are suppressors of canonical Wnt signaling. *Sci. Signal.* 4, ra71
67. Navarro, F. and Lieberman, J. (2015) miR-34 and p53: new insights into a complex functional relationship. *PLoS One* 10, e0132767
68. Yamakuchi, M. *et al.* (2008) miR-34a repression of SIRT1 regulates apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* 105, 13421–13426
69. Yang, T.-L.B. *et al.* (2017) Mutual reinforcement between telomere capping and canonical Wnt signalling in the intestinal stem cell niche. *Nat. Commun.* 8, 14766
70. Sun, C. *et al.* (2020) Re-equilibration of imbalanced NAD metabolism ameliorates the impact of telomere dysfunction. *EMBO J.* 39, e103420
71. Stock, A.J. *et al.* (2023) Boosting NAD ameliorates hematopoietic impairment linked to short telomeres in vivo. *GeroScience* 45, 2213–2228
72. Julier, Z. *et al.* (2017) Promoting tissue regeneration by modulating the immune system. *Acta Biomater.* 53, 13–28
73. Joo, H.-Y. *et al.* (2015) SIRT1 deacetylates and stabilizes hypoxia-inducible factor-1α (HIF-1α) *via* direct interactions during hypoxia. *Biochem. Biophys. Res. Commun.* 462, 294–300
74. Cantó, C. *et al.* (2012) The NAD^+ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet induced obesity. *Cell Metab.* 15, 838
75. Lin, J.B. *et al.* (2016) NAMPT-mediated NAD^+ biosynthesis is essential for vision in mice. *Cell Rep.* 17, 69–85
76. Pugel, A.D. *et al.* (2024) The role of NAD^+ and NAD^+-boosting therapies in inflammatory response by IL-13. *Pharmaceuticals* 17, 226
77. Lee, H.J. and Yang, S.J. (2019) Supplementation with nicotinamide riboside reduces brain inflammation and improves cognitive function in diabetic mice. *Int. J. Mol. Sci.* 20, 4196
78. Whyte, J.L. *et al.* (2012) Wnt signaling and injury repair. *Cold Spring Harb. Perspect. Biol.* 4, a008078
79. Habib, S.J. and Acebrón, S.P. (2022) Wnt signalling in cell division: from mechanisms to tissue engineering. *Trends Cell Biol.* 32, 1035–1048
80. Sarabia-Sánchez, M.A. and Robles-Flores, M. (2024) WNT signaling in stem cells: a look into the non-canonical pathway. *Stem Cell Rev. Rep.* 20, 52–66
81. Li, Q. *et al.* (2021) Sirt1 promotes the restoration of hepatic progenitor cell (HPC)-mediated liver fatty injury in NAFLD through activating the Wnt/β-catenin signal pathway. *Front. Nutr.* 8, 791861
82. Yoshino, J. *et al.* (2018) NAD^+ intermediates: the biology and therapeutic potential of NMN and NR. *Cell Metab.* 27, 513–528

83. Zhan, J. *et al.* (2024) Regulation of CD73 on NAD metabolism: unravelling the interplay between tumour immunity and tumour metabolism. *Cell Commun. Signal.* 22, 387
84. Grozio, A. *et al.* (2019) Slc12a8 is a nicotinamide mononucleotide transporter. *Nat. Metab.* 1, 47–57
85. Moynihan, K.A. *et al.* (2005) Increased dosage of mammalian Sir2 in pancreatic β cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab.* 2, 105–117
86. Shoelson, S.E. *et al.* (2006) Inflammation and insulin resistance. *J. Clin. Invest.* 116, 1793
87. Yeung, F. *et al.* (2004) Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.* 23, 2369
88. Kauppinen, A. *et al.* (2013) Antagonistic crosstalk between NF-κB and SIRT1 in the regulation of inflammation and metabolic disorders. *Cell. Signal.* 25, 1939–1948
89. Cai, D. *et al.* (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-β and NF-κB. *Nat. Med.* 11, 183–190
90. de Picciotto, N.E. *et al.* (2016) Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. *Aging Cell* 15, 522–530
91. Das, A. *et al.* (2018) Impairment of an endothelial NAD^+–H_2S signaling network is a reversible cause of vascular aging. *Cell* 173, 74–89
92. Fang, E.F. *et al.* (2016) NAD^+ replenishment improves lifespan and healthspan in ataxia telangiectasia models via mitophagy and DNA repair. *Cell Metab.* 24, 566–581
93. North, B.J. *et al.* (2014) SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. *EMBO J.* 33, 1438–1453
94. Li, J. *et al.* (2017) A conserved NAD^+ binding pocket that regulates protein–protein interactions during aging. *Science* 355, 1312–1317
95. Kim, J.-E. *et al.* (2008) DBC1 is a negative regulator of SIRT1. *Nature* 451, 583–586
96. Klimova, N. *et al.* (2019) Nicotinamide mononucleotide alters mitochondrial dynamics by SIRT3-dependent mechanism in male mice. *J. Neurosci. Res.* 97, 975–990
97. Membrez, M. *et al.* (2024) Trigonelline is an NAD^+ precursor that improves muscle function during ageing and is reduced in human sarcopenia. *Nat. Metab.* 6, 433–447
98. Johannes, J.W. *et al.* (2015) Pyrimidinone nicotinamide mimetics as selective tankyrase and Wnt pathway inhibitors suitable for in vivo pharmacology. *ACS Med. Chem. Lett.* 6, 254–259
99. Fujimura, T. *et al.* (2024) Targeting ErbB and tankyrase1/2 prevent the emergence of drug-tolerant persister cells in ALK-positive lung cancer. *npj Precis. Onc.* 8, 1–16
100. Cohen, H.Y. *et al.* (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305, 390–392
101. Hayashida, S. *et al.* (2010) Fasting promotes the expression of SIRT1, an NAD^+-dependent protein deacetylase, via activation of PPARα in mice. *Mol. Cell. Biochem.* 339, 285–292
102. Bordone, L. and Guarente, L. (2005) Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat. Rev. Mol. Cell Biol.* 6, 298–305
103. Wang, Y. (2014) Molecular links between caloric restriction and Sir2/SIRT1 activation. *Diabetes Metab. J.* 38, 321–329
104. Someya, S. *et al.* (2010) Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell* 143, 802–812
105. Huang, P. *et al.* (2021) NMN maintains intestinal homeostasis by regulating the gut microbiota. *Front. Nutr.* 8, 714604
106. Bárcena, C. *et al.* (2019) Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. *Nat. Med.* 25, 1234–1242
107. Peclat, T.R. *et al.* (2022) CD38 inhibitor 78c increases mice lifespan and healthspan in a model of chronological aging. *Aging Cell* 21, e13589
108. Lory, W. *et al.* (2024) CD38 inhibitor 78c attenuates pro-inflammatory cytokine expression and osteoclastogenesis in macrophages. *Cells* 13, 1971
109. Benton, T.Z. *et al.* (2021) Selective targeting of CD38 hydrolase and cyclase activity as an approach to immunostimulation. *RSC Adv.* 11, 33260–33270
110. Escande, C. *et al.* (2013) Flavonoid apigenin is an inhibitor of the NAD^+ase CD38: implications for cellular NAD^+ metabolism, protein acetylation, and treatment of metabolic syndrome. *Diabetes* 62, 1084–1093
111. Lauritzen, K.H. *et al.* (2025) Apigenin inhibits NLRP3 inflammasome activation in monocytes and macrophages independently of CD38. *Front. Immunol.* 15, 1497984
112. Moreno, L. *et al.* (2019) The mechanism of action of the anti-CD38 monoclonal antibody isatuximab in multiple myeloma. *Clin. Cancer Res.* 25, 3176–3187
113. Morandi, F. *et al.* (2018) CD38: a target for immunotherapeutic approaches in multiple myeloma. *Front. Immunol.* 9, 2722
114. Martin, T.G. *et al.* (2019) Therapeutic opportunities with pharmacological inhibition of CD38 with isatuximab. *Cells* 8, 1522
115. Qiu, Y. *et al.* (2023) NAD^+ exhaustion by CD38 upregulation contributes to blood pressure elevation and vascular damage in hypertension. *Signal Transduct. Target.* 8, 353
116. Favas Karimbanathottathil, M. *et al.* (2024) Comprehensive safety evaluation of isatuximab in multiple myeloma using disproportionality analysis of FAERS and meta-analysis of randomized controlled trials. *Sci. Rep.* 14, 31859
117. Bai, P. *et al.* (2011) PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* 13, 461–468
118. Zha, S. *et al.* (2018) PARP1 inhibitor (PJ34) improves the function of aging-induced endothelial progenitor cells by preserving intracellular NAD^+ levels and increasing SIRT1 activity. *Stem Cell Res Ther* 9, 224
119. Di Micco, R. *et al.* (2021) Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat. Rev. Mol. Cell Biol.* 22, 75–95
120. Nehme, J. *et al.* (2024) Converting cell death into senescence by PARP1 inhibition improves recovery from acute oxidative injury. *Nat. Aging* 4, 771–782
121. Gardell, S.J. *et al.* (2019) Boosting NAD^+ with a small molecule that activates NAMPT. *Nat. Commun.* 10, 3241
122. Manickam, R. *et al.* (2022) Nampt activator P7C3 ameliorates diabetes and improves skeletal muscle function modulating cell metabolism and lipid mediators. *J. Cachexia. Sarcopenia Muscle* 13, 1177–1196
123. Palmer, R.D. and Vaccarezza, M. (2021) Nicotinamide adenine dinucleotide and the sirtuins caution: pro-cancer functions. *Aging Med. (Milton)* 4, 337–344
124. Ferrell, M. *et al.* (2024) A terminal metabolite of niacin promotes vascular inflammation and contributes to cardiovascular disease risk. *Nat. Med.* 30, 424–434
125. Poljšak, B. *et al.* (2022) Current uncertainties and future challenges regarding NAD^+ boosting strategies. *Antioxidants* 11, 9
126. Han, P. *et al.* (2012) The cementogenic differentiation of periodontal ligament cells via the activation of Wnt/β-catenin signalling pathway by Li^+ ions released from bioactive scaffolds. *Biomaterials* 33, 6370–6379
127. Cui, X. *et al.* (2020) Strontium modulates osteogenic activity of bone cement composed of bioactive borosilicate glass particles by activating Wnt/β-catenin signaling pathway. *Bioact. Mater.* 5, 334–347
128. Müller, W.E.G. *et al.* (2017) Fabrication of amorphous strontium polyphosphate microparticles that induce mineralization of bone cels *i vitro* and *in vivo*. *Acta Biomater.* 50, 89–101
129. Yang, F. *et al.* (2011) Strontium enhances osteogenic differentiation of mesenchymal stem cells and in vivo bone formation by activating Wnt/catenin signaling. *Stem Cells* 29, 981–991
130. Borkowski, L. *et al.* (2020) Fluorapatite ceramics for bone tissue regeneration: Synthesis, characterization and assessment of biomedical potential. *Mater. Sci. Eng. C* 116, 111211
131. Cooper, L.F. *et al.* (2006) Fluoride modification effects on osteoblast behavior and bone formation at TiO_2 grit-blasted c.p. titanium endosseous implants. *Biomaterials* 27, 926–936
132. Rybchyn, M.S. *et al.* (2011) An Akt-dependent increase in canonical Wnt signaling and a decrease in sclerostin protein levels are involved in strontium ranelate-induced osteogenic effects in human osteoblasts. *J. Biol. Chem.* 286, 23771–23779
133. Abuna, R.P.F. *et al.* (2019) The Wnt/β-catenin signaling pathway is regulated by titanium with nanotopography to induce osteoblast differentiation. *Colloids Surf. B: Biointerfaces* 184, 110513

134. Barlian, A. and Vanya, K. (2022) Nanotopography in directing osteogenic differentiation of mesenchymal stem cells: potency and future perspective. *Future Sci. OA* 8, FSO765
135. Jagannathan, C. *et al.* (2024) Nanoparticle and nanotopography-induced activation of the wnt pathway in bone regeneration. *Tissue Eng. Part B Rev.* 30, 270–283
136. Minear, S. *et al.* (2010) Wnt proteins promote bone regeneration. *Sci. Transl. Med.* 2, 29ra30
137. Al Moubarak, A. *et al.* (2021) Curcumin-polyallyhydrocarbon nanocapsules potently suppress 1,2-dimethylhydrazine-induced colorectal cancer in mice by inhibiting Wnt/β-catenin pathway. *BioNanoScience* 11, 518–525
138. Deng, Y. *et al.* (2022) Biomaterial-mediated presentation of wnt5a mimetic ligands enhances chondrogenesis and metabolism of stem cells by activating non-canonical Wnt signaling. *Biomaterials* 281, 121316
139. Lowndes, M. *et al.* (2017) Constructing cellular niche properties by localized presentation of Wnt proteins on synthetic surfaces. *Nat. Protoc.* 12, 1498–1512
140. Lowndes, M. *et al.* (2016) Immobilized WNT proteins act as a stem cell niche for tissue engineering. *Stem Cell Rep.* 7, 126–137
141. Habib, S.J. *et al.* (2013) A localized Wnt signal orients asymmetric stem cell division in vitro. *Science* 339, 1445–1448
142. Okuchi, Y. *et al.* (2021) Wnt-modified materials mediate asymmetric stem cell division to direct human osteogenic tissue formation for bone repair. *Nat. Mater.* 20, 108–118
143. Scarpa, E. *et al.* (2018) Polymersome nanoparticles for delivery of Wnt-activating small molecules. *Nanomedicine* 14, 1267–1277
144. Zhong, Y. *et al.* (2021) Ocular Wnt/β-catenin pathway inhibitor XAV939-loaded liposomes for treating alkali-burned corneal wound and neovascularization. *Front. Bioeng. Biotechnol.* 9, 753879
145. Ambrosone, A. *et al.* (2020) Glycogen synthase kinase 3β inhibitor delivered by chitosan nanocapsules promotes safe, fast, and efficient activation of Wnt signaling in vivo. *ACS Biomater. Sci. Eng.* 6, 2893–2903
146. Sohrabi, A. *et al.* (2021) *Wnt* pathway activator delivery by poly(lactide-co-glycolide)/silk fibroin composite nanofibers promotes dental pulp stem cell osteogenesis. *J. Drug Deliv. Sci. Technol.* 61, 102223
147. Zhang, K. *et al.* (2015) Application of Wnt pathway inhibitor delivering scaffold for inhibiting fibrosis in urethra strictures: in vitro and in vivo study. *Int. J. Mol. Sci.* 16, 27659
148. Cheng, J. *et al.* (2020) Treadmill exercise promotes neurogenesis and myelin repair via upregulating Wnt/β-catenin signaling pathways in the juvenile brain following focal cerebral ischemia/reperfusion. *Int. J. Mol. Med.* 45, 1447–1463
149. Janda, C.Y. *et al.* (2017) Surrogate Wnt agonists that phenocopy canonical Wnt and β-catenin signalling. *Nature* 545, 234–237