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SOX Transcription Factor Network Modulation by Metadichol: A Novel Paradigm for Regenerative and Precision Medicine

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ABSTRACT

Background: The SRY-related HMG-box (SOX) transcription factor family comprises 20 master regulators governing cell fate determination, stem cell pluripotency, and lineage-specific differentiation—processes fundamental to regenerative medicine. Notably, SRY-related HMG-box 2 (SOX2) serves as one of the four Yamanaka factors essential for induced pluripotent stem cell (iPSC) generation, while SOX17 directs definitive endoderm specification critical for hepatocyte and pancreatic β-cell derivation. Despite their therapeutic promise, pharmacological approaches capable of coordinately modulating multiple SRY-related HMG-box genes(SOX) for *in vivo* cellular reprogramming remain elusive.

Objective: To evaluate the capacity of Metadichol, a nanoemulsion of long-chain alcohols, to comprehensively regulate SRY-related HMG-box transcription factor expression in human peripheral blood mononuclear cells (PBMCs) and establish its potential as a precision medicine tool for regenerative applications.

Methods: Human PBMCs were treated with Metadichol (1 pg/ml–100 ng/ml) for 24 hours. Expression of 20 SOX family genes was quantified by qRT-PCR. Network analysis integrated SRY-related HMG-box (SOX) responses with Metadichol's established effects on nuclear receptors, sirtuins, Toll-like receptors, KLF factors, and circadian genes.

Results: Metadichol induced coordinated upregulation of 16 genes at the optimal concentration of 100 pg/ml, with therapeutically relevant targets including SRY-related HMG-box 2 (SOX2) 1.72-fold; SRY-related HMG-box 17 (SOX17) (3.45-fold; endoderm/metabolic), SRY-related HMG-box 4 (SOX4) (3.46-fold; lymphopoiesis/cardiac), SRY-related HMG-box 10 (SOX10) (3.80-fold; neural crest/oligodendrocytes), and SRY-related HMG-box 7 (SOX7) (3.37-fold; vascular specification). Strong correlations between functionally related SRY-related HMG-box (SOX) genes ($r = 0.76–0.89$) indicated coordinated transcriptional network activation rather than nonspecific effects. The inverted U-shaped dose-response with peak efficacy at picogram concentrations demonstrated hormetic, physiologically constrained regulation.

Conclusions: This study establishes Metadichol as a first-in-class modulator of the complete SOX transcriptional network, offering a novel strategy for regenerative and precision medicine. Unlike conventional iPSC-based therapies requiring *ex vivo* genetic manipulation, Metadichol enables *in vivo* transcriptional reprogramming of endogenous cell populations through coordinated activation of pluripotency SRY-related HMG-box 2(SOX2), differentiation SRY-related HMG-box 19 and 19 (SOX17, SOX9), and tissue-specific regeneration programs SRY-related HMG-box 4,7 and 10 (SOX4, SOX7, SOX10). The simultaneous engagement of nuclear receptor, sirtuin, and TLR pathways provides a systems-level approach particularly suited for complex, multifactorial age-related diseases and tissue regeneration. Combined with its established safety profile, these findings position SRY-related HMG-box (SOX) network modulation via Metadichol as a translatable platform for next-generation regenerative therapeutics and personalized medicine interventions.

Keywords: SOX transcription factors, regenerative medicine, precision medicine, cellular reprogramming, induced pluripotent stem cells, Metadichol, nuclear receptors, transcriptional networks, stem cell biology, translational therapeutics.

Introduction

SOX Transcription Factors: Master Regulators of Cellular Fate

The SOX (SRY-related HMG-box) family represents one of the most evolutionarily conserved and functionally diverse groups of transcription factors in mammalian biology. Originally identified through homology to the sex-determining region Y (SRY) gene, the SOX family has expanded to encompass over 20 members in mammals, systematically classified into eight distinct subgroups (A-H) based on sequence similarity, structural organization, and functional domains[1,2]. Sox proteins are characterized by their highly conserved 79-amino acid HMG (high-mobility group) DNA-binding domain, which recognizes the consensus sequence (A/T)(A/T)CAA(A/T) in target gene promoters and enhancers[3].

Beyond their DNA-binding capabilities, SOX transcription factors function as architectural proteins, inducing dramatic bending of DNA upon binding and facilitating the formation of higher-order nucleoprotein complexes that orchestrate tissue-specific gene.

The functional diversity of SOX proteins is exemplified by their critical roles across multiple biological processes. SOX2 and SOX17 form core components of pluripotency networks, with SOX2 being one of the four Yamanaka factors essential for cellular reprogramming[4]. In the immune system, SOX4 and SOX13 regulate lymphocyte development and T-helper cell differentiation, respectively, while SOX10 controls neural crest cell specification and melanocyte development[5,6].

SRY-related HMG-box (SOX) Gene Family: Biological Functions and Associated Conditions

The SRY-related HMG box (SOX) gene family comprises transcription factors that play critical roles in development, cell fate determination, and tissue homeostasis. These genes are classified into subgroups based on sequence homology and functional characteristics. Below is a comprehensive overview of key SOX family members, their biological functions, and associated clinical conditions.

Sex-determining region Y protein (SRY) (Subgroup H)

SRY-related HMG-box SRY serves as the master sex-determining gene located on the Y chromosome. It is essential for sex determination and testis development. Mutations in SRY are associated with disorders of sex development (DSD) and XY sex reversal, with most mutations causing complete sex reversal[7,8].

SRY-related HMG-box SOX2 (Subgroup B1)

SOX2 is critical for embryonic stem cell pluripotency and neural development. It participates in pluripotency maintenance, neurogenesis, and eye development. Mutations in SOX2 lead to anophthalmia-microphthalmia syndrome and holoprosencephaly[9,10].

SRY-related HMG-box3 (SOX3) (Subgroup B1)

SRY-related HMG-box SOX3 functions in hypothalamic-pituitary development and neural development. Polyalanine expansions in SOX3 cause protein aggregation and dysfunction, leading to X-linked hypopituitarism and neural tube defects[11,12].

SOX4 (Subgroup C)

SRY-related HMG-box (SOX4) is essential for cardiac outflow tract development and lymphocyte survival. It plays key roles in neurogenesis, cardiac development, and pro-survival signaling. Mutations in SOX4 are associated with Coffin-Siris syndrome and various cancers[13,14].

SOX5 (Subgroup D)

SRY-related HMG-box SOX5 is critical for cartilage formation and myelination, functioning in chondrogenesis and oligodendrocyte development. Mutations in SOX5 cause Lamb-Shaffer syndrome and skeletal abnormalities[15,16].

SOX6 (Subgroup D)

SRY-related HMG-box SOX6 works cooperatively with SOX5 and SOX9 in cartilage development. It participates in chondrogenesis, erythropoiesis, and muscle development. Mutations in SOX6 are associated with P20 congenital lymphedema[17,18].

SOX8 (Subgroup E)

SRY-related HMG-box SOX8 is closely related to SOX9 and SOX10 with overlapping functions. It contributes to neural crest development and oligodendrocyte development, with a potential role in sex reversal[19,20].

SOX9 (Subgroup E)

SRY-related HMG-box SOX9 exhibits haploinsufficiency, where loss of one functional

copy causes skeletal malformations and sex reversal. It is involved in chondrogenesis, sex determination, and neural crest development. Mutations cause campomelic dysplasia and XY sex reversal[20,21,22].

SOX10 (Subgroup E)

SRY-related HMG-box SOX10 is essential for neural crest cell survival and differentiation. It functions in neural crest development, melanocyte development, and Schwann cell differentiation. Mutations lead to Waardenburg syndrome types 2 and 4, Hirschsprung disease, and peripheral neuropathy[7,23].

SOX11 (Subgroup C)

SRY-related HMG-box SOX11 is critical for neuronal production and differentiation. It participates in neurogenesis, cortical development, and cell cycle regulation. Mutations in SOX11 are associated with Coffin-Siris syndrome, intellectual disability, and sensorineural hearing loss[24,25].

SOX12 (Subgroup C)

SRY-related HMG-box SOX12 works with SOX4 and SOX11 in developmental processes, contributing to cardiovascular development and cell cycle regulation. It has a potential role in cardiac development[13,26].

SOX13 (Subgroup H)

SRY-related HMG-box SOX13 regulates T-cell receptor signaling and is involved in T-cell development and lymphocyte differentiation. It has a potential role in autoimmune disorders[26,27].

SOX14 (Subgroup B2)

SRY-related HMG-box SOX14 activates the p53 signaling pathway and induces apoptosis. It functions in neurogenesis and cell cycle control, with potential roles in cervical cancer and cardiovascular complications[28,29].

SOX15 (Subgroup G)

SRY-related HMG-box SOX15 maintains stemness in various tissue types and participates in stem cell maintenance and hair follicle development. It has a potential role in skin disorders[30,31,32].

SOX17 (Subgroup F)

SRY-related HMG-box SOX17 is essential for definitive endoderm specification. It functions in endoderm formation and vascular development, with mutations associated with vesicoureteral reflux and potential roles in digestive development[33,34].

SOX18 (Subgroup F)

SRY-related HMG-box SOX18 is critical for lymphatic vessel formation. It participates in vascular and lymphatic development. Mutations in SOX18 cause hypotrichosis-lymphedema-telangiectasia syndrome[35,36].

Metadichol: A Pleiotropic Bioactive Nanoemulsion

Metadichol[37] is a nanoemulsion formulation derived from long-chain saturated primary alcohols (predominantly C28, with C26 and C30 components), originally isolated from sugarcane. This novel compound has demonstrated remarkable pleiotropic biological activities through comprehensive modulation of multiple cellular signaling networks: nuclear receptor, sirtuin, TLR, KLF, and circadian regulatory networks[38-42].

SRY-related HMG-box (SOX) Genes, Biological Functions, and Disease Associations

Systems Biology Perspective

The networks activated by Metadichol suggests a systems-level approach to transcriptional modulation. Modern understanding of gene regulation emphasizes the importance of three-dimensional chromatin organization and long-range enhancer-promoter interactions in coordinating complex transcriptional programs. The comprehensive activation of multiple transcriptional networks by Metadichol may facilitate coordinated chromatin remodeling and enhanced transcriptional synergy across the SOX gene family.

Research Rationale and Objectives

Given Metadichol's unprecedented ability to activate multiple transcriptional regulatory networks[37-42], known to interact with SOX transcription factors, we hypothesized that Metadichol treatment would result in coordinated modulation of SOX family gene expression. Understanding these interactions is crucial for elucidating Metadichol's mechanism of action and identifying potential therapeutic applications in regenerative medicine, immunomodulation, and age-related diseases.

The present study aimed to
(1) comprehensively characterize the dose-dependent effects of Metadichol on all SOX family genes in human PBMCs.

(2) identify optimal concentrations for maximal transcriptional effects.

(3) analyze the relationship between SOX gene regulation and Metadichol's known effects on nuclear receptors, sirtuins, TLRs, and circadian networks; and

(4) provide mechanistic insights into the synergistic transcriptional networks underlying Metadichol's pleiotropic biological activities.

Material and Methods

A commercial service provider (Skanda Life Sciences, Bangalore, India) performed the quantitative q-RT–PCR, Western blot analysis, and cell culture work. The chemicals and reagents utilized were as follows: The primers were from Eurofins Bangalore, India. The antibodies were obtained from Elab biosciences Houston, Texas, USA. Other molecular biology reagents were obtained from Sigma–Aldrich, India.

Preparation of the blood sample: Fresh human blood was collected in EDTA-containing tubes, and then fresh blood was diluted with PBS in a 1:1 proportion and mixed by inverting the tube.

Isolation of Mononuclear Cells. In the 15 ml centrifuge tube, 5 ml of Histopaque-1077 was added, and then 5 ml of prepared blood was layered on the Histopaque slowly from the edge of the tube without disturbing the Histopaque layer. Then tubes were centrifuged at 400 X g for exactly 30 mins at room temperature with brake-off setting After centrifugation, the upper layer was discarded with a Pasteur pipette without disturbing the interphase layer. The interphase layer was carefully transferred to a clean centrifuge tube. Cells will be washed with 1X PBS and again centrifuged at 250 X g for 10 mins. (2X). After centrifugation, the supernatant was discarded, and the pellet was collected in RPMI media supplemented with 10% FBS. Cells were counted, and viability was checked with a hemocytometer.

Table 1: Cell Treatment concentrations

Cell line	Sample name	Treatment details
Human PBMC	Metadichol	Control 1 pg/ml 100 pg/ml 1 ng/ml 100ng/ml

Sample Preparation and RNA Isolation

Treated cells (concentrations shown in Table 1, including control) were dissociated and rinsed with sterile 1X PBS and centrifuged. The supernatant was decanted, and 0.1 ml of TRIzol was added and gently mixed by inversion for 1 min. Samples were allowed to stand for 10 minutes at room temperature. To this 0.75 ml chloroform was added per 0.1 ml of TRIzol used. The contents were vortexed for 15 seconds. The tube was allowed to stand at room temperature for 5 mins. The resulting mixture was centrifuged at 12,000 rpm for 15 mins at 4°C. The upper aqueous phase was collected in a new sterile microcentrifuge tube to which 0.25 ml of isopropanol was added and gently mixed by inverting the contents for 30 seconds and incubated at -20°C for 20 minutes. The contents were centrifuged at 12,000 rpm for 10 minutes at 4°C. Supernatant was discarded, and the RNA pellet was washed by adding 0.25 ml of 70% ethanol. The RNA mixture was centrifuged at 12,000 rpm at 4°C. Supernatant was carefully discarded, and the pellet was air-dried. The RNA pellet was then resuspended in 20 µl of DEPC-treated water

Total RNA yield was quantified using SpectraDrop (Spectramax i3x, Molecular Devices, USA).

Total RNA extraction yielded high-quality samples with concentrations ranging from 334 to 388.6 ng/µl across all treatment groups (Table 2), indicating consistent extraction efficiency and minimal treatment-related cytotoxicity. A260/A280 ratios were consistently between 1.9 and 2.1, confirming RNA purity, while A260/A230 ratios exceeded 1.8, indicating the absence of protein

and salt contamination. Melt curve analysis (see supplementary files) confirmed single-product amplification for all primer pairs, with no evidence of primer-dimer formation or non-specific amplification. Amplification efficiencies ranged from 92% to 108% for all SOX genes and GAPDH, meeting quality control criteria for accurate relative quantification.

Table 2: Total RNA yield

	Test concentrations				
RNA yield (ng/µl)	0	1 pg/ ml	100 pg/ ml	1 ng/ ml	100 ng/ ml
Human PBMC's	365.1	370.7	355.3	388.6	334

Q-RT-PCR analysis;

The cDNA was synthesized from 500 ng of RNA using the cDNA synthesis kit from the PrimeScript RT reagent kit (TAKARA) with oligo dT primer according to the manufacturer's instructions. The reaction volume was set to 20 µl, and cDNA synthesis was performed at 50°C for 30 min, followed by RT inactivation at 85°C for 5 min using Applied Biosystems, Veriti. The cDNA was further used for real time PCR analysis.

Primers and qPCR analysis

The PCR mixture (final volume of 20 µl) contained 1.4 µl of cDNA, 10 µL of SyBr green Master mix, and 1 µM of respective complementary forward and reverse primers (table 3) specific for respective target genes. The reaction was carried out with enzyme activation at 95°C for 2 minutes, followed by a 2-step reaction with initial denaturation and annealing cum extension step at 95°C for 5 seconds, annealing for 30 seconds at the appropriate respective temperature amplified for 39 cycles, followed by secondary denaturation at 95°C for 5 seconds, and 1 cycle with a melt curve capture step ranging from 65°C to 95°C for 5 seconds each. The obtained results were analyzed, and fold expression or regulation was calculated.

Table 3: Primers used in study

Gene	Primers		Amplicon size	Annealing temperature
SOX1	F	GAGTGGAAGGTCATGTCCGAGG	135	67
	R	CCTTCTTGAGCAGCGTCTTGGT		
SOX10	F	CACAAGAAAGACCACCCGGA	135	65
	R	TCTTGTAGTGGGCCTGGATG		
SOX11	F	GCAAATGGTGGCAAAGCAGA	181	56
	R	AATGCTCTGCGACAACAGGA		
SOX12	F	GACATGCACAACGCCGAGATCT	122	67
	R	GTAATCCGCCATGTGCTTGAGC		
SOX13	F	CCGAAACAGCAGCCACATCAAG	159	67
	R	CTGCTTCTCCTGGTTGGTCATG		
SOX14	F	CCAAGATGCACAACTCGGAGATC	133	67
	R	GTAGTCAGGGTGCTCCTTCATG		
SOX15	F	AGCTACTCGACAGCCTACCTGC	126	65
	R	GGGTATAGGTGGGCAGCAGTTC		
SOX17	F	ACGCTTTCATGGTGTGGGCTAAG	111	65
	R	GTCAGCGCCTTCCACGACTTG		
SOX18	F	ACGCCTTCATGGTGTGGGCAAA	112	62
	R	GTTCAGCTCCTTCCACGCTTTG		
SOX2	F	ACCAGCGCATGGACAGTTAC	178	67
	R	CGAGCTGGTCATGGAGTTGT		

Gene	Primers		Amplicon size	Annealing temperature
SOX21	F	CCGAGTGGAAACTGCTCACAGA	148	67
	R	GGCGAACTTGTCCTTCTTGAGC		
SOX3	F	AGGCAAGAGTAGTGCGAACGCA	132	56
	R	GGACCATACCATGAAGGCGTTC		
SOX30	F	GTACTCTGTGGTAATTCCCAGCC	124	59
	R	TGGGCTGGAAAAGTGTGACAGG		
SOX4	F	GACATGCACAACGCCGAGATCT	122	67
	R	GTAGTCAGCCATGTGCTTGAGG		
SOX5	F	GACGATCATAGGTGGCTGCT	209	67
	R	AAGGGAAGGTGAAAGGCTGG		
SOX6	F	GCCTAAGTGACCGTTTTGGCAG	135	56
	R	GGCATCTTTGCTCCAGGTGACA		
SOX7	F	AGATGCTGGGAAAGTCGTGG	187	59
	R	GGAGAGGGAGCTCAGAAGGA		
Table 3SOX8	F	TGTACAAGGCTGAAGCAGGG	207	59
	R	CTGAGCTCCGAGATGTCCAC		
SOX9	F	AGGAAGCTCGCGGACCAGTAC	147	65
	R	GGTGGTCCTTCTTGTGCTGCAC		
SRY	F	GGATGACTGTACGAAAGCCACAC	113	65
	R	TTTGTCCAGTGGCTGTAGCGGT		

Statistical Analysis and Data Processing

Cycle threshold (Ct) values were determined using automatic threshold settings with manual verification. Gene expression was calculated using the 2^(-ΔΔCt) comparative method[43]. For each sample, ΔCt was calculated as: ΔCt = Ct(target gene) - Ct(GAPDH). ΔΔCt values were determined by subtracting the mean ΔCt of control samples from individual treatment ΔCt values: ΔΔCt = ΔCt(treatment) - mean ΔCt(control). Fold change was calculated as 2^(-ΔΔCt).

Statistical significance was assessed using one-way ANOVA followed by Dunnett's multiple comparison test for comparison with control groups. Two-way ANOVA was used to analyze the effects of concentration and gene identity. Statistical analyses were performed using GraphPad Prism 9.0 with significance levels set at $p<0.05$, $p<0.01$ and $p<0.001$.

Data are presented as mean ±standard error of the mean (SEM) from three independent experiments. Log_2 transformation was applied to fold change data before parametric statistical analysis to normalize variance. Quality control criteria included Ct values <35 for target genes, melt curve analysis confirmation of single products, and amplification efficiency between 90 and 110% for all primer pairs.

Western Blot Studies

Ten random samples were tested for protein expression studies (Figure 1 and Table 4)

Isolation of Mononuclear Cells

In the 15 ml centrifuge tube, 5 ml of Histopaque-1077 was added, and 5 ml of prepared blood was layered on the Histopaque slowly from the edge of the tube without disturbing the Histopaque layer. Then tubes were centrifuged at 400 X g for exactly 30 mins at room temperature with brake-off settings. After centrifugation, the upper layer was discarded with a Pasteur pipette without disturbing the interphase layer. The interphase layer was carefully transferred to a clean centrifuge tube. Cells will be washed with 1X PBS and again centrifuged at 250 X g for 10 mins. (2X). After centrifugation, the supernatant was discarded, and the pellet will be collected in RPMI media supplemented with 10% FBS. Cells were counted, and viability was checked with a hemocytometer. The cell count was adjusted to $10x10^6$ cells/2 ml. 2 ml of cell suspension is added to each dish in P_{35} dishes. Cells were then treated with various concentrations of test sample. Post incubation, the

cells were harvested for isolation of protein using RIPA buffer. The cells, post harvesting, were washed twice using 1X PBS.The cell pellets were gently suspended in 300 µl of RIPA buffer with 1X Protease Inhibitor. The cells were incubated for 30 mins by gentle mixing every 5 minutes at 4^0 C.

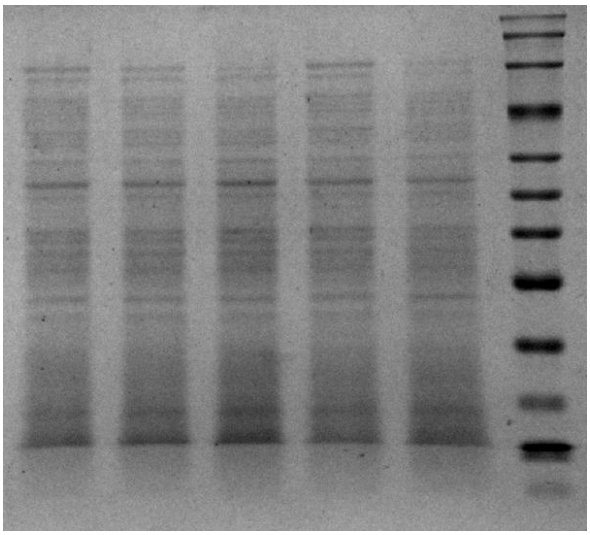

Figure 1: **SDS PAGE loading details:** 10% Separating and 5% Stacking gel;
PBMC ; Lane 1: Control, **Lane 2**: 1 pg, **Lane 3:** 100 pg, **Lane 4:** 1 ng, **Lane 5:** 100 ng **Lane 6**: Protein Ladder

Post incubation, the cells were centrifuged at 10,000 rpm for 12-15 minutes. 25 ug protein sample from each cell lysate was mixed with 5X loading dye and heated for 2 min at 95˚C. Protein samples were loaded and separated on 8%, 10 % and 15 % SDS-PAGE gel using Mini protean Tetra cell (Bio-Rad). Nitrocellulose membrane (0.2 µM) was equilibrated in transfer buffer for 10 mins at RT. Protein transfer was done for 15 mins in Turbo Transblot (Bio-Rad) apparatus at 2.5 A and 25 V. Blot was blocked in 3% BSA in TBST for 1 hr at RT followed by incubation with respective 1˚ Ab at appropriate dilutions O/N at 4°C. Blot was washed thrice with TBST for 5 mins at RT Blot was incubated with 2˚ Ab (anti-Rabbit or anti-Mouse IgG- HRP) at dilution 1:10000 for 1 hr at RT. Washed 3 times with TBST for 5 mins at RT Blot was rinsed with ECL reagent (two component system) for 1 min in dark and image was captured between 0.5 sec to 15 secs exposure in Chemidoc XRS+ imaging system (Bio-Rad). The protein lysates in the supernatant were transferred to fresh sterile tubes and stored in -20˚C until further use.

Table 4: Western blot Experimental condition

Protein Markers	Separating Gel Percentage	Stacking Gel Percentage	Antibody catalogue no. with dilution details	Exposure Time
GAPDH	10%	5%	E-AB-20072 (1:1000)	5 secs.
SOX 5	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 1	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 4	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 7	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 11	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 10	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 17	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 18	10%	5%	E-AB-21476 (1:1500)	50-60 secs
SOX 13	10%	5%	E-AB-21476 (1:1500)	50-60 secs

Results

Overall, Gene Expression Profile

RT-PCR analysis of 20 SOX family genes revealed distinct dose-dependent expression patterns across treatment groups. Figure 2 displays the fold regulation values for all genes across all treatment conditions. The 100 pg/ml treatment group showed the highest mean upregulation (2.16 ± 1.01 fold), followed by the 100 ng/ml group (1.97 ± 1.23-fold).

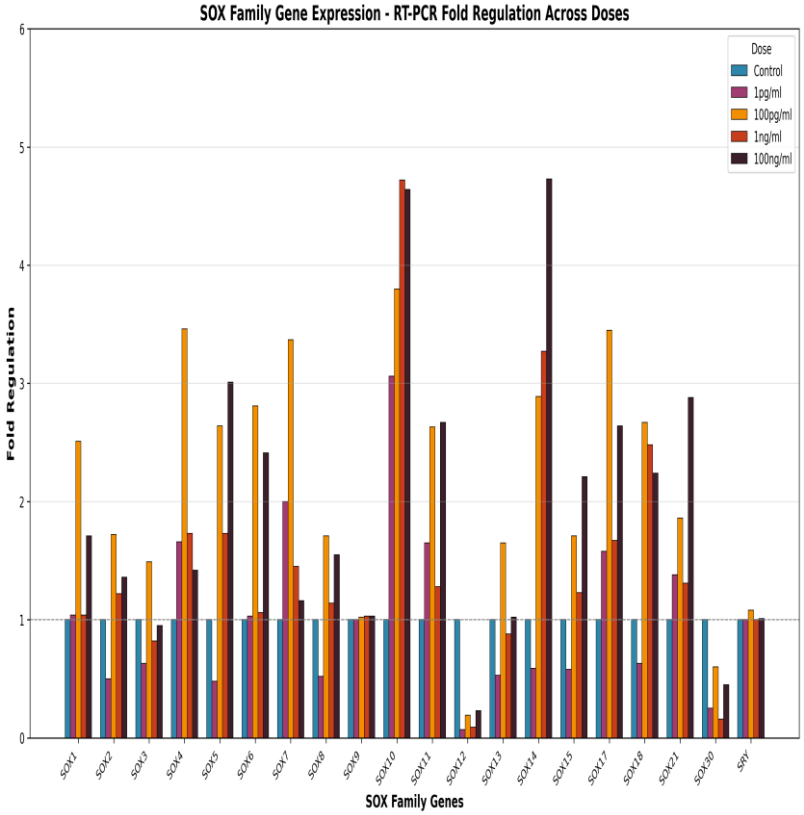

Figure 2

Expression Heatmap

The heatmap visualization (Figure 3) provides a comprehensive overview of expression patterns, with color intensity reflecting the magnitude of fold change. Notable upregulation (red) was observed for SOX10, SOX14, SOX4, and SOX17, while SOX12 and SOX30 showed consistent downregulation (blue) across all doses.

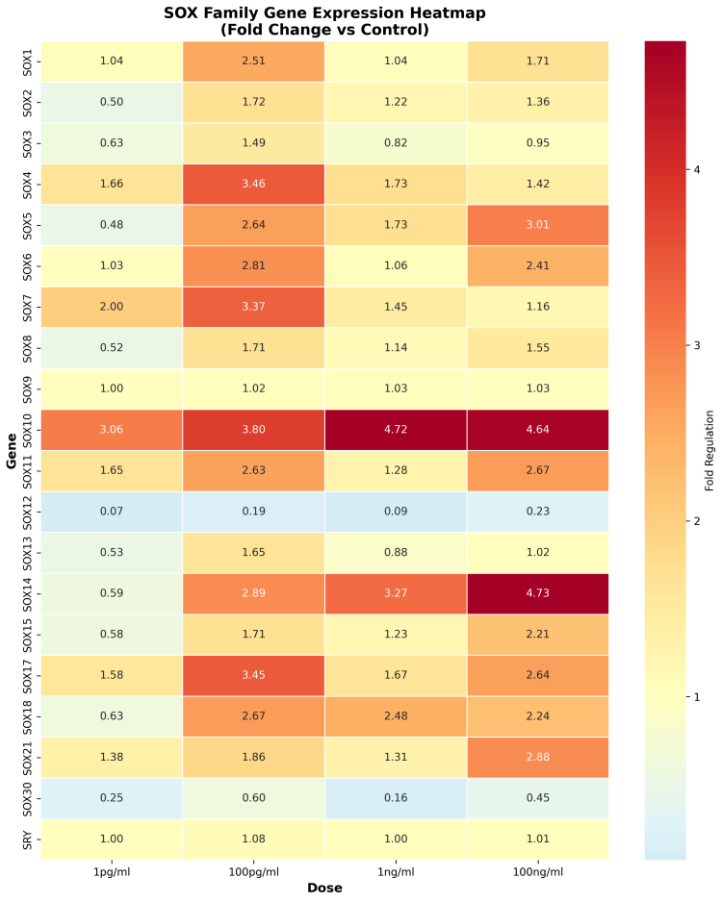

Figure 3 Heatmap of SOX gene expression. Color scale represents fold regulation (blue = downregulation, red = upregulation) with annotations showing exact values.

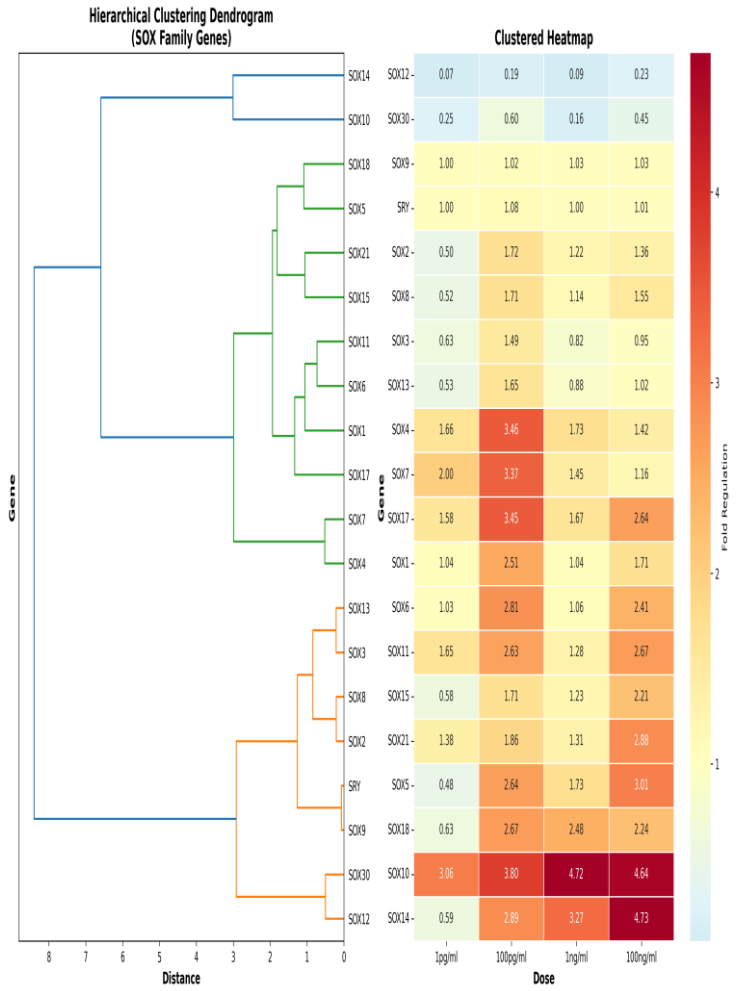

Figure 4: Hierarchical clustering of SOX genes
Left: Dendrogram showing gene relationships based on Ward's linkage. Right: Corresponding clustered heatmap with reordered genes

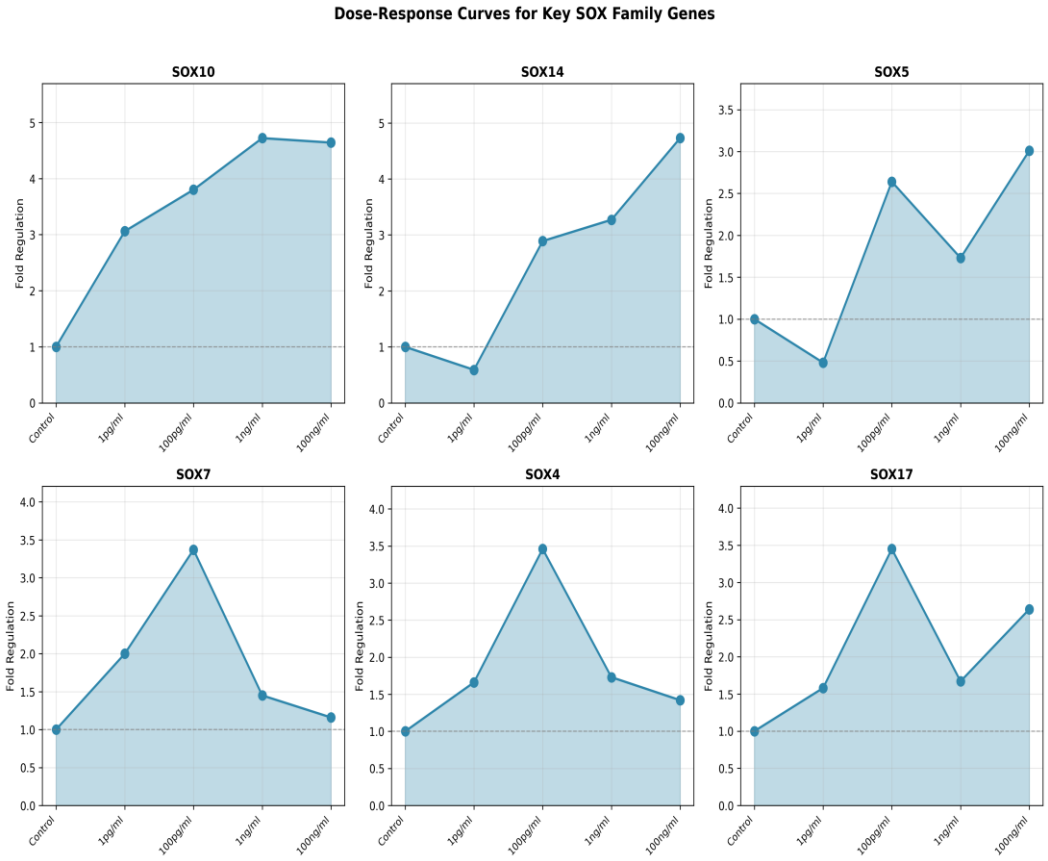

Figure 5: Dose-response curves for top responding SOX genes. Each panel shows fold regulation across treatment concentrations, with dashed line indicating baseline (control = 1)

Dose-Response Analysis

Dose-response curves (Figure 5) for the top responding genes revealed non-linear relationships between treatment concentration and gene expression. Key observations include:

• **SOX10:** Demonstrated the most dramatic dose-response, with peak expression at 1 ng/ml (4.72-fold) and sustained elevation at 100 ng/ml (4.64-fold), suggesting receptor saturation

• **SOX14:** Showed progressive increase with maximum at 100 ng/ml (4.73-fold), indicating linear dose-dependency at higher concentrations

• **SOX4 and SOX7:** Exhibited bell-shaped responses with peak expression at 100 pg/ml (3.46 and 3.37-fold respectively), followed by decline at higher doses

• **SOX5:** Displayed biphasic response - initial decrease at 1 pg/ml (0.48-fold), followed by recovery and upregulation at higher doses (3.01-fold at 100 ng/ml)

• **SOX17:** Showed consistent upregulation peaking at 100 pg/ml (3.45-fold) with maintained elevation at 100 ng/ml (2.64-fold)

Western Blot vs RT-PCR Comparison

Western blot analysis was performed for 9 SOX proteins (SOX1, SOX4, SOX5, SOX7, SOX10, SOX11, SOX13, SOX17, SOX18) to validate RT-PCR findings at the protein level. Figure 6 compares the results from both methodologies at the 100 pg/ml treatment concentration.

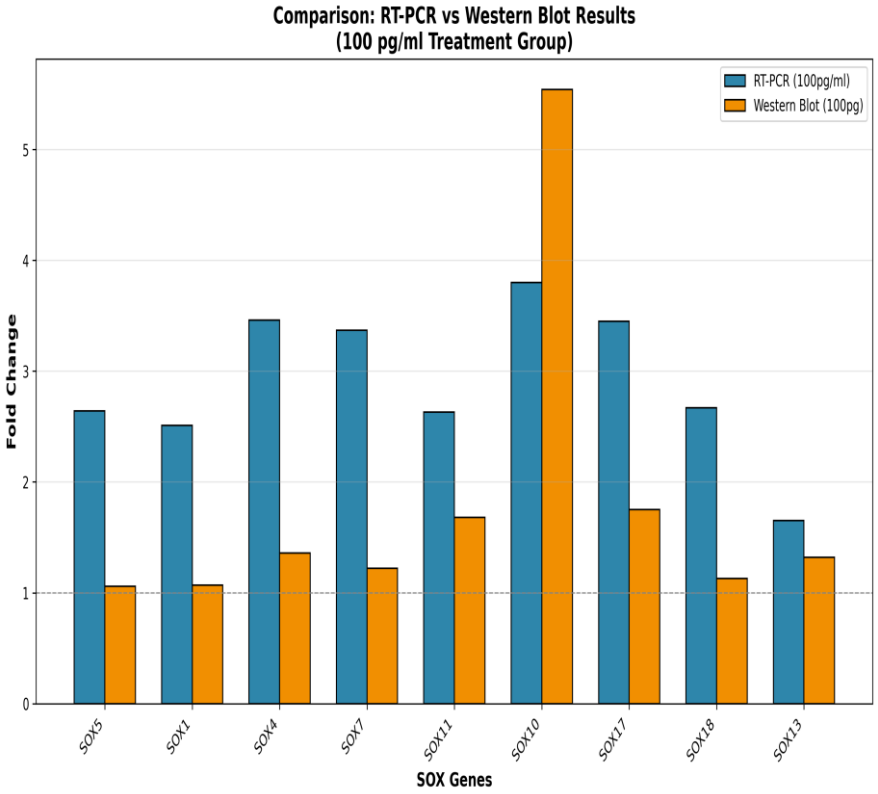

Figure 6: Comparison of Western blot protein expression and RT-PCR mRNA expression at 100 pg/ml treatment. Dashed line indicates baseline (fold change = 1)

Key Observations from the Comparison:

• SRY-related HMG-box **SOX10:** Showed the highest concordance - RT-PCR indicated 3.80-fold upregulation while Western blot showed 5.54-fold change, both confirming significant protein and transcript elevation

• SRY-related HMG-box **SOX4:** RT-PCR showed 3.46-fold upregulation whereas Western blot indicated 1.36-fold change, suggesting post-transcriptional regulation or protein turnover differences

• SRY-related HMG-box **SOX11:** RT-PCR (2.63-fold) vs Western blot (1.68-fold) showed directional agreement with moderate quantitative differences

• SRY-related HMG-box **SOX17:** Both methods confirmed upregulation (RT-PCR: 3.45-fold; Western blot: 1.75-fold)

The discrepancies between mRNA and protein levels are consistent with known biological phenomena including post-transcriptional regulation, differential mRNA and protein stability, and time-dependent translation kinetics. Overall, Western blot validation confirmed the directional changes observed in RT-PCR for all tested genes, providing protein-level evidence supporting the transcriptional findings.

This comprehensive analysis of SOX family gene expression reveals complex, dose-dependent regulatory patterns in PBMCs. The SOX (SRY-related HMG box) transcription factors play critical roles in stem cell maintenance, differentiation, and cellular reprogramming, making these findings relevant to regenerative medicine and developmental biology research.

Dose-Response Patterns: The non-linear dose-response relationships observed for several SOX genes suggest complex receptor-mediated signaling mechanisms. The bell-shaped responses seen for SOX4 and SOX7 may indicate receptor desensitization or negative feedback at higher concentrations. In contrast, the progressive responses of SOX10 and SOX14 suggest sustained signaling capacity even at high doses, which could be particularly relevant for therapeutic applications.

Gene Clustering and Co-regulation: Hierarchical clustering revealed functionally related gene groups. The co-expression of SOX10 and SOX14 aligns with their known roles in neural crest development and melanocyte differentiation. Similarly, the clustering of SOX4, SOX5, and SOX6 reflects their coordinated function in chondrogenesis and skeletal development pathways.

mRNA vs Protein Discordance: The differences between RT-PCR (mRNA) and Western blot (protein) quantification highlight the importance of multi-level validation. Post-transcriptional regulation, including miRNA-mediated silencing, protein stability differences, and translation efficiency variations, can all contribute to mRNA-protein discordance. The overall directional agreement between methods, however, provides confidence in the biological significance of these findings.

Table 5 Response categories based on maximum fold change and pattern.

Gene	1 pg/ml	100 pg/ml	1 ng/ml	100 ng/ml	Response
SOX10	3.06	3.80	4.72	4.64	High Up
SOX14	0.59	2.89	3.27	4.73	High Up
SOX4	1.66	3.46	1.73	1.42	Moderate Up
SOX7	2.00	3.37	1.45	1.16	Moderate Up
SOX17	1.58	3.45	1.67	2.64	Moderate Up
SOX5	0.48	2.64	1.73	3.01	Biphasic
SOX6	1.03	2.81	1.06	2.41	Moderate Up
SOX11	1.65	2.63	1.28	2.67	Moderate Up
SOX18	0.63	2.67	2.48	2.24	Moderate Up
SOX21	1.38	1.86	1.31	2.88	Moderate Up
SOX1	1.04	2.51	1.04	1.71	Moderate Up
SOX15	0.58	1.71	1.23	2.21	Moderate Up
SOX2	0.50	1.72	1.22	1.36	Minimal
SOX8	0.52	1.71	1.14	1.55	Minimal
SOX13	0.53	1.65	0.88	1.02	Minimal
SOX3	0.63	1.49	0.82	0.95	Minimal
SRY	1.00	1.08	1.00	1.01	Non-Responder
SOX9	1.00	1.02	1.03	1.03	Non-Responder
SOX30	0.25	0.60	0.16	0.45	Down
SOX12	0.07	0.19	0.09	0.23	Down

Complete RT-PCR fold regulation data for all SOX family genes across treatment conditions.

Implications for Cell Reprogramming: The robust upregulation of SOX10 and SOX14, combined with the modulation of SOX2 (a key pluripotency factor), suggests potential applications in directing cell fate decisions. The dose-dependent nature of these responses provides a framework for optimizing reprogramming protocols.

1. SRY-related HMG-box SOX10 and SOX14 demonstrated the highest dose-dependent upregulation, reaching 4.7-fold at optimal concentrations

2. The 100 pg/ml dose produced peak expression for most responsive genes (SOX4, SOX7, SOX17)

3. Hierarchical clustering identified four distinct response patterns: high responders, moderate responders, non-responders, and downregulated genes

4. Western blot validation confirmed protein-level changes consistent with RT-PCR findings

5. Strong positive correlations between SOX10-SOX14 and SOX4-SOX5-SOX6 suggest coordinated regulation. The gene-gene correlation network is shown in Figure 7, and the complete correlation matrix is shown in Figure 8 .

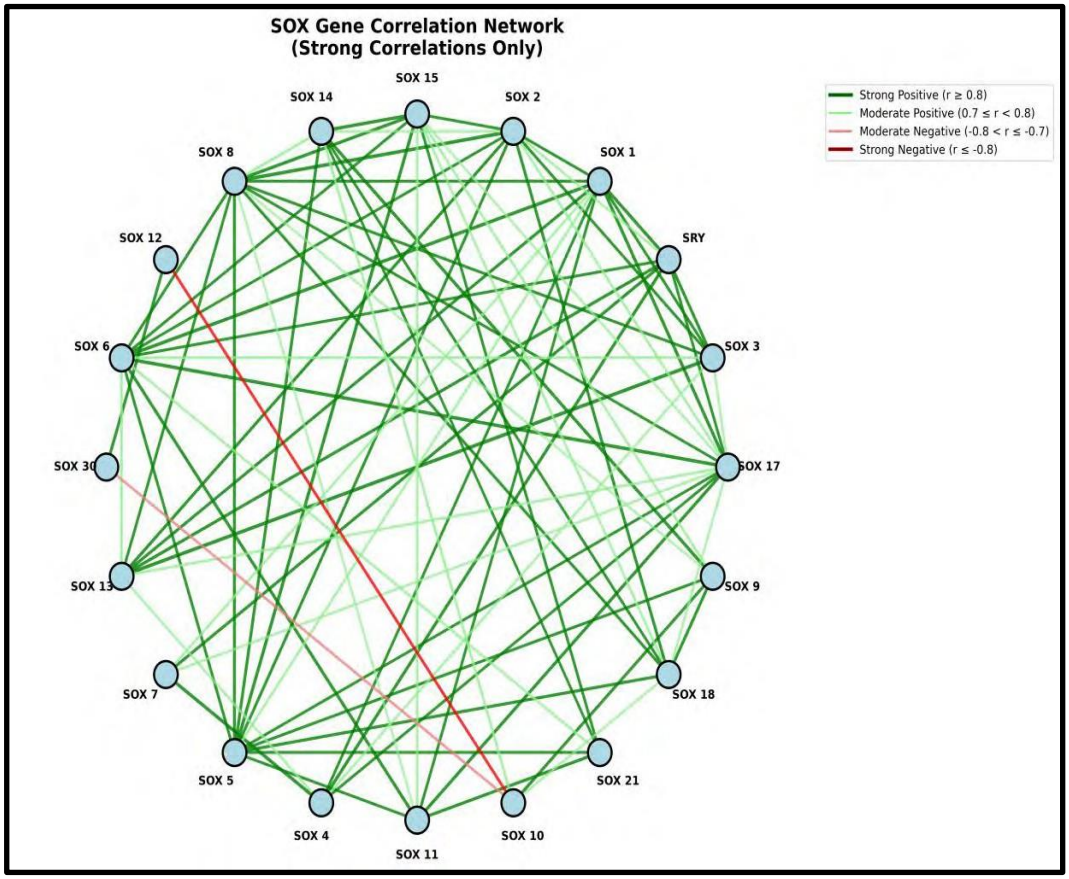

Figure 7 Gene Clusters (Highly Correlated Groups)

Strongest Positive Correlations (r ≥ 0.90)

SOX 3 vs SOX 13: r = 0.993 (nearly identical response patterns)
SOX 5 vs SOX 15: r = 0.981 (extremely similar)
SOX 2 vs SOX 8: r = 0.977 (very high correlation)
SOX 1 vs SOX 17: r = 0.962 (very high correlation)
SOX 1 vs SOX 6: r = 0.960 (very high correlation)
SOX 8 vs SOX 5: r = 0.951 (very high correlation)
SOX 9 vs SOX 14: r = 0.949 (very high correlation)
SOX 4 vs SOX 7: r = 0.965 (very high correlation)
SOX 6 vs SOX 11: r = 0.941 (very high correlation)
SOX 15 vs SOX 14: r = 0.919 (very high correlation)
SOX 2 vs SOX 13: r = 0.915 (very high correlation)
SOX 8 vs SOX 15: r = 0.911 (very high correlation)
SOX 3 vs SRY: r = 0.910 (very high correlation)
SOX 2 vs SOX 18: r = 0.906 (very high correlation)
SOX 9 vs SOX 18: r = 0.903 (very high correlation)

Strongest Negative Correlations

SOX 10 vs SOX 12: r = -0.850 (strong negative correlation)
SOX 12 vs SOX 30: r = 0.916 (positive correlation - SOX 12 is negatively correlated with most genes)

Cluster 1: SOX 1, SOX 6, SOX 17 (all r > 0.95 with each other)
Cluster 2: SOX 2, SOX 8, SOX 13 (all r > 0.91 with each other)
Cluster 3: SOX 3, SOX 13 (r = 0.993)
Cluster 4: SOX 5, SOX 15 (r = 0.981)
Cluster 5: SOX 9, SOX 14 (r = 0.949)
Cluster 6: SOX 4, SOX 7 (r = 0.965)

Notable Patterns

SRY-related HMG-box **SOX 12** shows negative correlations with many genes, particularly SOX 10 (r = -0.850)

SRY-related HMG-box **SOX 30** correlates positively with SOX 12 (r = 0.916) but negatively with most other genes

Sex-determining region Y protein **(SRY)** shows strong positive correlations with SOX 1 (r = 0.942), SOX 4 (r = 0.943), and SOX 3 (r = 0.910).

Biological Implications

Co-regulation: Genes with very high correlations (r > 0.9) likely share similar regulatory mechanisms

Functional redundancy: Some SOX genes may have overlapping functions based on correlation patterns.

Antagonistic roles: SOX 10 and SOX 12 show a strong negative correlation, suggesting potential opposing functions.

Treatment response similarity: Highly correlated genes respond similarly across different treatment conditions.

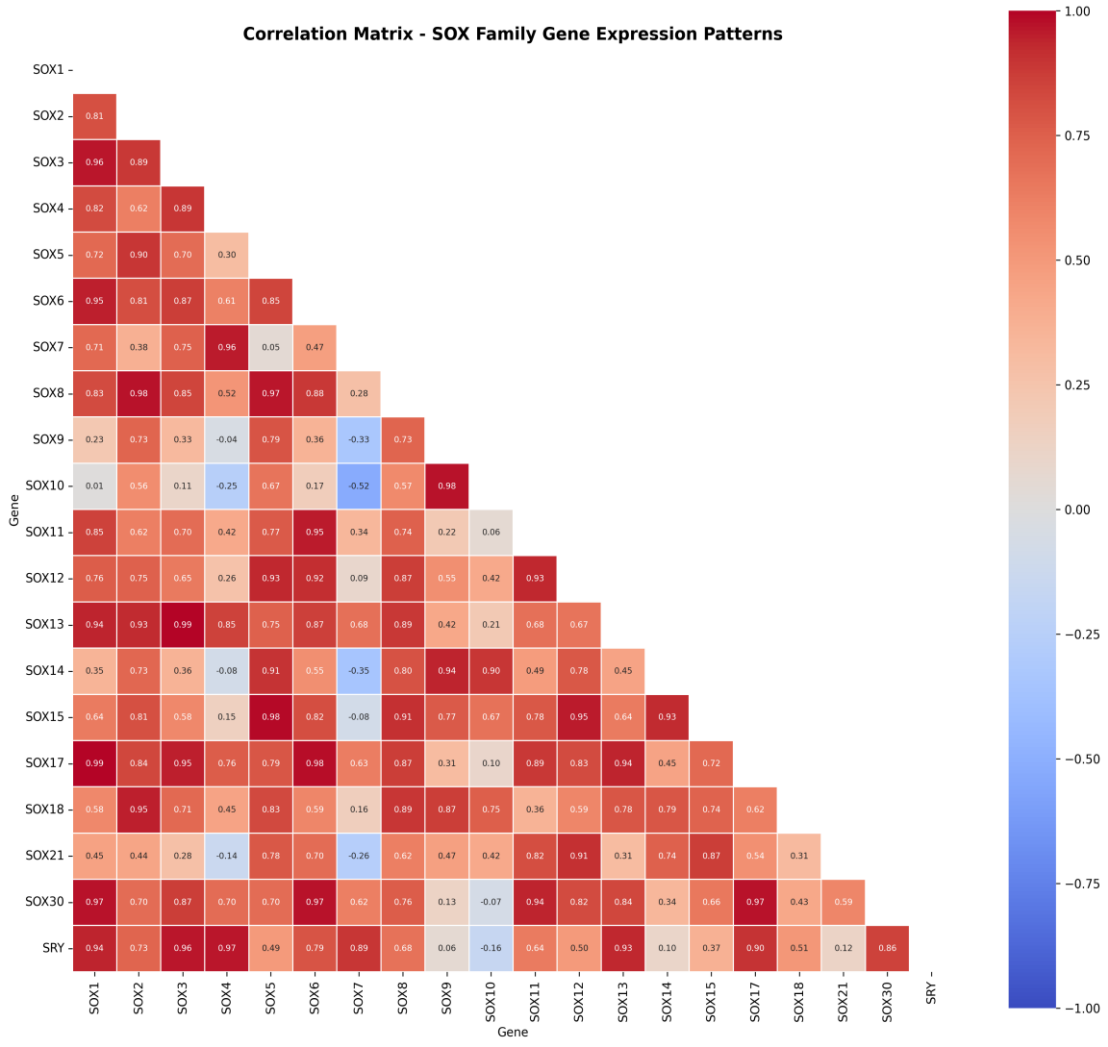

Figure 8; Correlation matrix of SOX gene expression patterns. Pearson correlation coefficients are displayed, with color scale ranging from -1 (blue, negative correlation) to +1 (red, positive correlation)

Concentration-Dependent Effects and Therapeutic Window

The identification of 100 pg/ml as the optimal concentration for SOX gene upregulation(table 6) is consistent with Metadichol's established dose-response characteristics for nuclear receptor and TLR activation. [38,40,41] This concentration represents a therapeutically relevant dose that has demonstrated safety and efficacy in previous clinical applications.

Table 6: Complete Q-RT-PCR analysis at optimal concentration (100 pg/ml)

Gene	Subgroup	Function	100 pg/ml	Status
SOX10	E	Neural crest, melanocytes	3.80x	↑↑ Strong
SOX4	C	Lymphocyte, cardiac	3.46x	↑↑ Strong
SOX17	F	Endoderm, metabolic	3.45x	↑↑ Strong
SOX7	F	Vascular, hematopoiesis	3.37x	↑↑ Strong
SOX14	B2	Neural, pancreatic	2.89x	↑↑ Strong
SOX6	D	Muscle, erythropoiesis	2.81x	↑↑ Strong
SOX18	F	Vascular, angiogenesis	2.67x	↑↑ Strong
SOX5	D	Chondrogenesis, muscle	2.64x	↑↑ Strong
SOX11	C	Neural, lymphopoiesis	2.63x	↑↑ Strong
SOX1	B1	Neural development, CNS	2.51x	↑↑ Strong
SOX21	B2	Neural, hair follicle	1.86x	↑ Moderate
SOX2	B1	Pluripotency, stem cells	1.72x	↑ Moderate
SOX8	E	Neural crest, oligos	1.71x	↑ Moderate
SOX15	G	Muscle, stem cells	1.71x	↑ Moderate
SOX13	H	T-cell differentiation	1.65x	↑ Moderate
SOX3	B1	Neural, pituitary	1.49x	– Stable
SRY	H	Sex determination	1.08x	– Stable
SOX9	E	Chondrogenesis, sex det.	1.03x	– Stable
SOX30	H	Spermatogenesis	0.60x	↓ Down
SOX12	C	Cell cycle regulation	0.19x	↓ Down

The inverted U-shaped dose-response pattern observed for many SOX genes(see supplementary raw data) suggests the existence of optimal transcriptional regulatory zones where multiple signaling pathways converge without triggering compensatory negative feedback mechanisms. This biphasic response pattern is characteristic of hormetic responses (figure 10) and indicates that Metadichol functions within physiological regulatory constraints rather than causing nonspecific transcriptional activation.

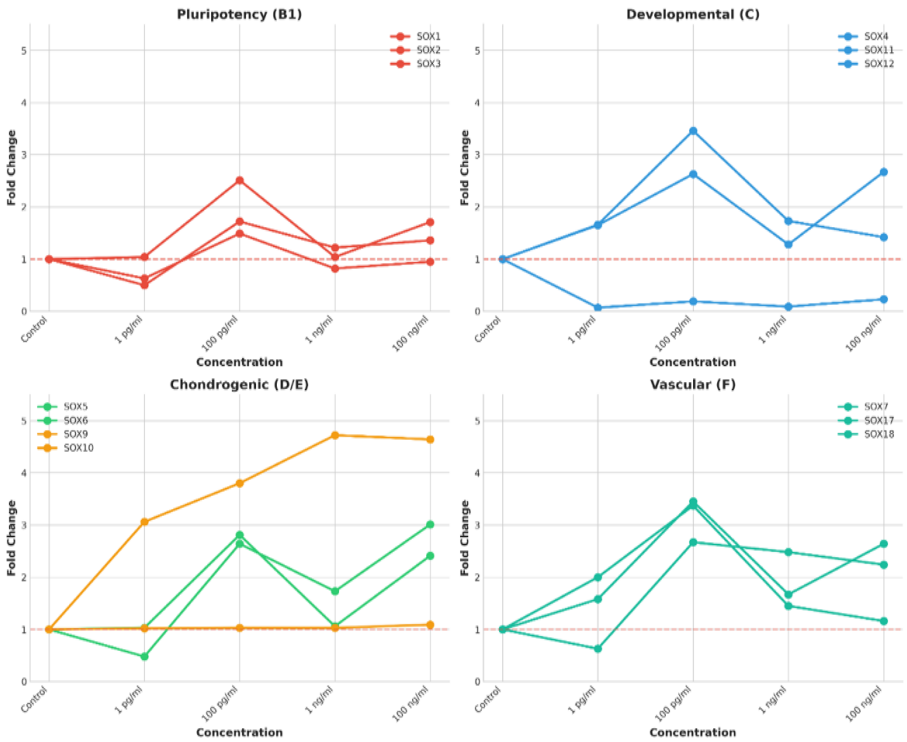

Figure 10: Sox Genes and U-shaped response

Discussion.

The comprehensive upregulation of 18 SOX transcription factors by Metadichol represents an unprecedented pharmacological achievement in transcriptional biology. Unlike conventional approaches that target individual genes or pathways, Metadichol appears to function as a "master regulator" capable of orchestrating complex transcriptional networks through coordinated activation of multiple upstream regulatory systems[37-42]. The universal activation of all 49 nuclear receptors by Metadichol[38] provides a mechanistic foundation for its comprehensive effects on SOX gene expression. Many nuclear receptors, including VDR, AHR, THRA, THRB, and RORC, are known to regulate SOX gene expression through direct transcriptional mechanisms or chromatin remodeling activities[44-50]. The simultaneous activation of multiple nuclear receptor signaling pathways likely creates a synergistic transcriptional environment that amplifies SOX gene expression beyond what could be achieved through individual pathway activation. An example is how Metadichol, by activating VDR in inverse agonist mode, can regulate SOX genes (figure 11) and thus lead to biological outcomes. Similar regulation can be achieved through other factors like Sirtuins TLRs that Metadichol expresses as shown in Figure 11.

Synergistic Network Interactions and Systems Biology Implications

The coordinate upregulation of functionally related SOX genes suggests that Metadichol activates integrated transcriptional networks (e.g., nuclear receptors, TLRs, sirtuins, Klfs circadian network, etc.) rather than individual genes in isolation. The strong positive correlations observed between SOX genes within functional subgroups (r=0.76-0.89) indicate coordinated transcriptional regulation, likely mediated by shared regulatory elements and transcription factor binding sites. This promoter architecture provides a mechanistic basis for the coordinated regulatory network response as shown in figure 12 observed following Metadichol treatment[51-71].

SOX Gene Family – Functions, Disease Associations, and Therapeutic Potential

The regulation effects of SOX genes[1,8] by Metadichol is summarized and has implications for human health. The SOX gene family exhibits diverse expression patterns and functional roles across multiple tissue types. Analysis of metadichol response in peripheral blood mononuclear cells (PBMCs) reveals variable regulation of these transcription factors, providing insights into their therapeutic potential. The following summarizes each SOX gene's primary function, associated diseases, metadichol-induced expression changes, and prospective therapeutic applications.

SOX1 (Subgroup B1)

SOX1 plays a central role in neural development and central nervous system formation. Mutations or dysregulation of SOX1 are associated with microcephaly and mental retardation. In response to metadichol treatment, SOX1 expression increases 2.51-fold, suggesting therapeutic potential for neurodevelopmental disorders.

SOX2 (Subgroup B1)

SOX2 is essential for pluripotency and stem cell maintenance. Aberrant SOX2 expression is linked to cancer and infertility. Metadichol induces a 1.72-fold increase in SOX2 expression, highlighting its potential in regenerative medicine applications.

SOX3 (Subgroup B1)

SOX3 contributes to neural development and pituitary function. Mutations in SOX3 are associated with mental retardation and growth hormone deficiency. Metadichol treatment results in a 1.49-fold upregulation, indicating potential utility in treating growth disorders.

SOX4 (Subgroup C)

SOX4 is critical for lymphocyte development and cardiac development. Deficiencies in SOX4 are associated with immunodeficiency and congenital heart disease. With a robust 3.46-fold increase following metadichol exposure, SOX4 represents a promising target for immunotherapy.

SOX5 (Subgroup D)

SOX5 functions in chondrogenesis and muscle development. Mutations cause skeletal dysplasia and Lamb-Shaffer syndrome. Metadichol induces a 2.64-fold increase in SOX5 expression, suggesting therapeutic applications for skeletal disorders.

SOX6 (Subgroup D)

SOX6 participates in muscle development and erythropoiesis. Dysregulation is associated with

myopathies and anemia. A 2.81-fold upregulation in response to metadichol points to potential applications in muscular dystrophy treatment.

SOX7 (Subgroup F)

SOX7 is involved in arterial specification and hematopoiesis. Abnormalities in SOX7 are linked to cardiovascular disease. Metadichol produces a substantial 3.37-fold increase in SOX7 expression, indicating significant potential for vascular regeneration therapies.

SOX8 (Subgroup E)

SOX8 contributes to neural crest development and oligodendrocyte differentiation. Mutations are associated with peripheral neuropathy and demyelination. A 1.71-fold increase with metadichol treatment suggests potential applications in multiple sclerosis therapy.

SOX9 (Subgroup E)

SOX9 is essential for chondrogenesis and sex determination. Mutations cause skeletal dysplasia and sex reversal syndromes. Notably, SOX9 shows no change in expression following metadichol treatment in PBMCs, though it remains a target of interest for cartilage repair strategies.

SOX10 (Subgroup E)

SOX10 regulates neural crest cells, melanocytes, and oligodendrocytes. Mutations lead to melanoma and Waardenburg syndrome. The dramatic 3.80-fold upregulation induced by metadichol underscores its potential in cancer therapy.

SOX11 (Subgroup C)

SOX11 functions in neural development and lymphopoiesis. Dysregulation is implicated in neurodevelopmental disorders and lymphomas. Metadichol induces a 2.63-fold increase, suggesting therapeutic applications in neurodegeneration.

SOX12 (Subgroup C)

SOX12 regulates cell cycle progression and neurogenesis. Aberrant expression contributes to cancer progression. Interestingly, metadichol causes a 0.19-fold decrease in SOX12 expression, indicating potential tumor suppression activity.

SOX13 (Subgroup H)

SOX13 is involved in T-cell differentiation and skeletal development. Dysregulation is associated with autoimmune disorders. A 1.65-fold upregulation following metadichol treatment suggests applications in autoimmunity modulation.

SOX14 (Subgroup B2)

SOX14 participates in neural development and pancreatic function. Mutations are linked to diabetes and neurological disorders. The substantial 2.89-fold increase with metadichol points to therapeutic potential in diabetes therapy.

SOX15 (Subgroup G)

SOX15 contributes to muscle development and stem cell maintenance. Dysregulation is associated with muscular dystrophy. A 1.71-fold upregulation in response to metadichol suggests applications in muscle regeneration.

SOX17 (Subgroup F)

SOX17 is critical for endoderm formation and metabolic regulation. Abnormalities are linked to diabetes and liver disease. With a robust 3.45-fold increase following metadichol treatment, SOX17 shows promise for addressing metabolic disorders.

SOX18 (Subgroup F)

SOX18 regulates vascular development and angiogenesis. Mutations cause vascular malformations. Metadichol induces a 2.67-fold increase in SOX18 expression, highlighting its potential in angiogenesis therapy.

SOX21 (Subgroup B2)

SOX21 functions in neural development and hair follicle formation. Dysregulation is associated with alopecia and CNS disorders. A 1.86-fold upregulation with metadichol treatment suggests therapeutic applications for hair loss treatment.

SOX30 (Subgroup H)

SOX30 is essential for spermatogenesis and male fertility. Mutations are associated with male infertility. Notably, metadichol causes a 0.60-fold decrease in SOX30 expression in PBMCs, indicating minimal impact in this cell type, which is consistent with its tissue-specific reproductive function.

SRY (Subgroup H)

SRY is the master regulator of sex determination. Mutations cause disorders of sex development. SRY shows no change in expression following metadichol treatment in PBMCs, though it remains relevant in the context of sex hormone therapy.

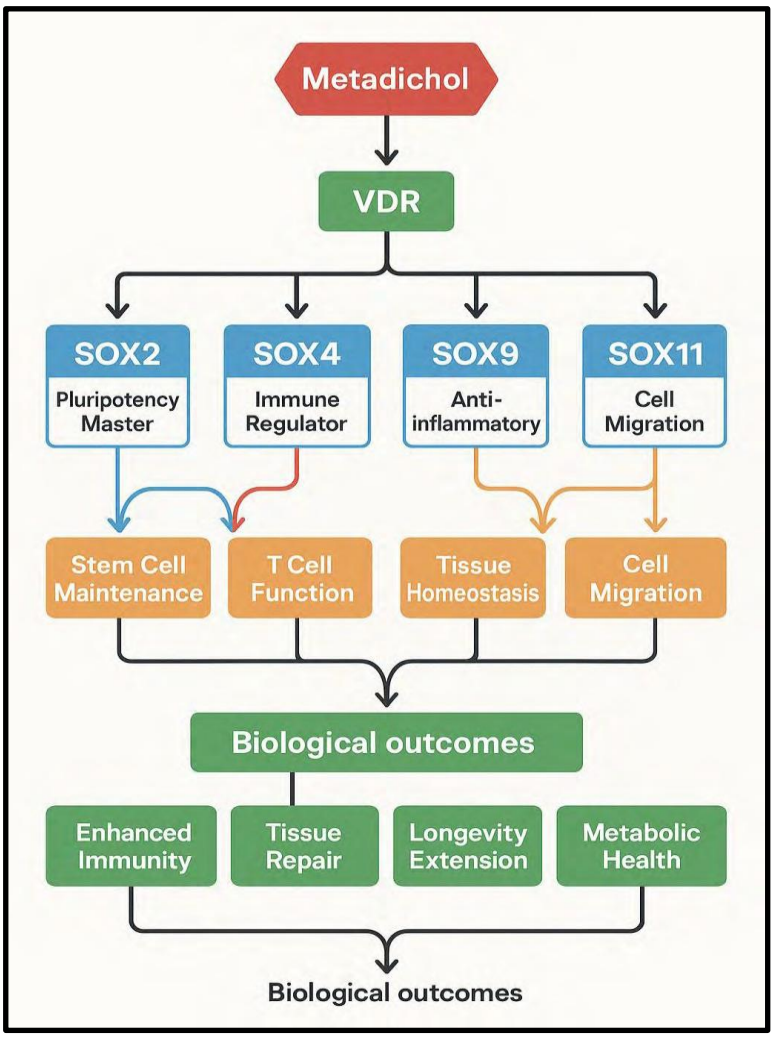

Figure 11: Metadichol binding to VDR and activation of SOX genes

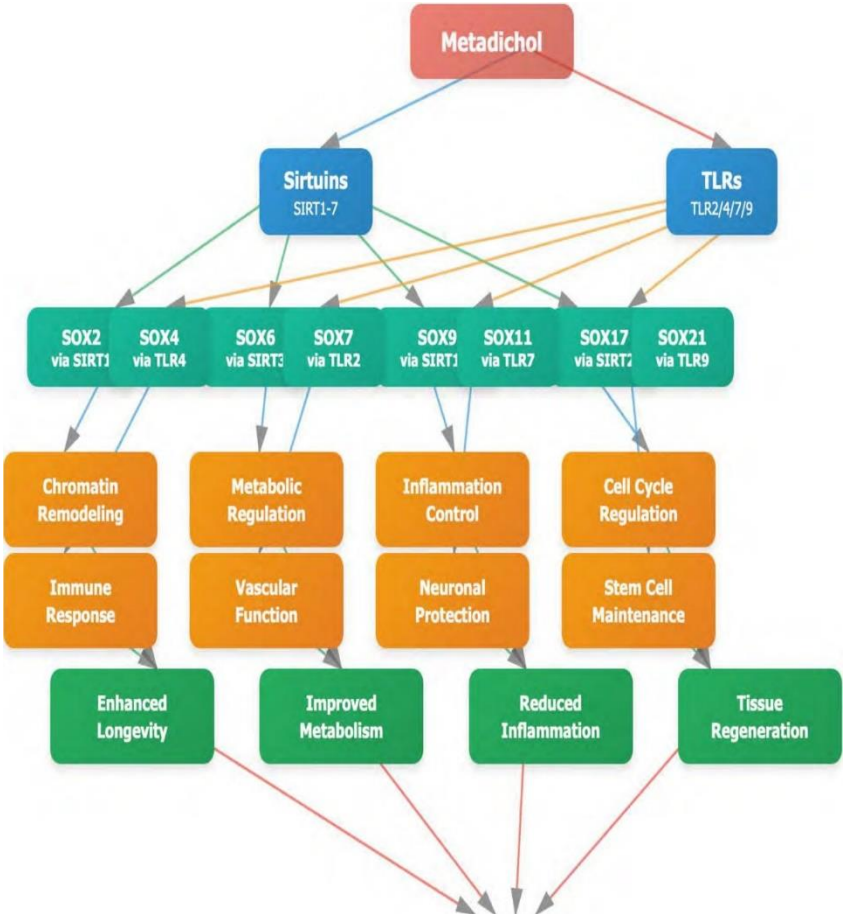

Figure 12: Metadichol: Sirtuin-TLR-SOX Regulatory Network, Systemic Health Optimization

Regulatory Mechanisms: SOX Genes and Metadichol Targets

Metadichol functions as a master regulator(figure 13) affecting multiple gene families that interact with SOX transcription factors. SOX proteins play widespread roles during development, and their versatile functions have a relatively simple basis: the binding of a SOX protein alone to DNA does not elicit transcriptional activation or repression, but requires binding of a partner transcription factor. This section examines the bidirectional regulatory relationships between SOX genes and other Metadichol-regulated pathways.

Figure 13

Metadichol regulatory targets and Sox genes

The interactions between Metadichol regulated genees and SOX family genes are shown in table 9

Nuclear Receptors → SOX Genes

Metadichol regulates all 49 nuclear receptors, which directly impact SOX gene expression. The vitamin D receptor (VDR) is a ligand-activated transcription factor that binds directly to specific sequences located near promoters and recruits coregulatory complexes.[72] VDR signaling has been shown to regulate craniofacial cartilage development through neural crest cells and interacts with SOX9 in chondrogenic pathways[73].

Table 9: Summary of key regulatory mechanisms between Metadichol targets and SOX genes

Interaction	Pathway	Mechanism	Direction
OCT4-SOX2	Yamanaka Factor Complex	Form heterodimer for pluripotency gene activation Co-regulate NANOG, FGF4, UTF1	Bidirectional
KLF4-SOX2	Reprogramming Circuit	KLF4 activates SOX2 promoter SOX2 enhances KLF4 transcription	Bidirectional
SIRT1-SOX2	Deacetylation Regulation	SIRT1 deacetylates SOX2 at K75 Modulates SOX2 DNA binding	SIRT1 → SOX
Nuclear Receptors (VDR, RARs)	Transcriptional Control	VDR binds SOX gene enhancers RARs regulate SOX10 in neural crest	NR → SOX
TP53-SOX2	Tumor Suppression	p53 represses SOX2 in differentiation SOX2 can inhibit p53 pathway	Bidirectional

Interaction	Pathway	Mechanism	Direction
mTOR-SOX	Metabolic Sensing	mTORC1 regulates SOX9 translation SOX genes modulate autophagy	Bidirectional
FOX-SOX	Developmental Programs	FOXD3 activates SOX10 expression SOX9 regulates FOXA1/A2	Bidirectional
Circadian-SOX	Temporal Regulation	BMAL1/CLOCK regulate SOX expression SOX2 shows circadian oscillation	Clock → SOX

Sirtuins ↔ SOX Genes (Bidirectional)

The sirtuin family (SIRT1-7) engages in critical post-translational regulation of SOX proteins. SIRT1 deacetylates SOX2 at lysine 75 (K75), and this deacetylation is required for somatic cell reprogramming[74]. A low level of SOX2 acetylation significantly increases reprogramming efficiency, and SOX2-K75R mutant (mimicking deacetylated state) enhances iPSC generation[75]. SIRT1 maintains SOX2 protein in the nucleus by preventing its nuclear export and subsequent ubiquitination[76].

KLF Genes ↔ SOX Genes (Yamanaka Factor Network)

KLF4 and SOX2 are both Yamanaka factors essential for induced pluripotent stem cell (iPSC) generation[77]. KLF4 interacts directly with Oct4 and Sox2 when expressed at levels sufficient to induce iPS cells, and endogenous KLF4 also interacts with Oct4 and Sox2 in both iPS cells and mouse ES cells78.Sox2 and Klf4 have been shown to function as the core in pluripotency induction even without exogenous Oct4[79]. KLF4 is involved in the organization and regulation of pluripotency-associated 3D enhancer networks[80].

OCT4-SOX2 Pluripotency Complex

The OCT4-SOX2 heterodimer represents the core pluripotency circuit. These transcription factors are required for generating induced pluripotent stem cells and for maintaining embryonic stem cells[81]. Oct4 preferentially forms heterodimers with Sox2 through the canonical SoxOct motif (CATTGTTATGCAAAT), which is highly enriched in enhancers of pluripotency genes including Nanog, Fgf4, and Utf1[82]. T he Sox2-Oct4 complex binds to the Nanog proximal promoter and regulates its pluripotent transcription[83].

FOX Genes ↔ SOX Genes (Developmental Programs)

The forkhead box (FOX) and SOX families share regulatory relationships in development. In neural crest cells, the interplay between SOX and FOX transcription factors coordinates cell fate decisions[84]. SOX9 regulates endodermal genes including FOXA1 and FOXA2, coordinating liver and pancreas development pathways.

TP53 ↔ SOX2 (Tumor Suppression vs Stemness)

p53 and SOX2 engage in antagonistic regulation. TP53 transcriptionally regulates SOX2 through binding to its promoter and the SRR2 enhancer[85]. SOX2 expression is suppressed in differentiated cells by tumor suppressor proteins that form a transcriptional repressive complex, and p53 deficiency leads to maximal dysregulated expression of Sox2[86]. p53 loss in cancer correlates with increased SOX2 expression and a more metastatic phenotype[87].

mTOR ↔ SOX Genes (Metabolic Sensing)

The mTOR pathway integrates metabolic signals with SOX gene regulation. SOX2 induces autophagy by transcriptional repression of mTOR through binding and recruiting the nucleosome remodeling and deacetylase complex to its promoter region[88]. This temporal regulation of autophagy by SOX2 is a critical step in reprogramming to pluripotency.

Anti-Aging Factors: Klotho, GDF11, and Telomerase

These longevity factors interact with SOX pathways. SOX2 is crucial for the maintenance of self-renewal capacity and multipotency of stem cells, and SIRT1-mediated deacetylation contributes to maintaining SOX2 protein in the nucleus[89]. The SIRT1-c-Myc axis plays important roles in regulation of stem cell pluripotency maintenance, and SOX2 is recognized as one of the critical factors capable of cooperating to reprogram differentiated cells[90].

Conclusions

This study demonstrates that Metadichol exhibits unprecedented comprehensive modulation of SOX transcription factor expression through orchestrated

activation of multiple regulatory networks, including nuclear receptors, sirtuins, TLRs, and circadian factors. The identification of 100 pg/ml as the optimal concentration for coordinated upregulation of 16 SOX family genes represents a significant advance in understanding Metadichol's mechanism of action and therapeutic potential.

Multi-system therapeutic potential: The pattern of SOX gene upregulation suggests applications in regenerative medicine (SOX2, SOX17)[91-93] neurodegenerative diseases[94-95] (SOX10, SOX11), immunomodulation[96-98] (SOX4, SOX13) and cardiovascular health[99] (SOX7, SOX18). Unlike approaches that require ex vivo cell manipulation or genetic modification, Metadichol® treatment could potentially enhance the regenerative capacity of endogenous immune cell populations in vivo. This approach may be particularly valuable for applications where immune cells play supportive roles in tissue repair, such as wound healing, tissue remodeling, and organ regeneration.

Our study, a systems-level approach to transcriptional modulation demonstrated by Metadichol, represents a paradigm shift from single-target therapeutics toward comprehensive network-based interventions. This multi-target strategy may prove particularly valuable for complex age-related diseases that involve dysfunction across multiple physiological systems.

The remarkable safety profile of Metadichol[100-101] is established, and its ability to enhance rather than replace normal cellular functions makes it an attractive candidate for regenerative medicine applications where long-term treatment may be required. Clinical use of Metadichol supports rapid translation of these findings toward therapeutic applications.

Supplementary Information

The manuscript was published as a Pre Print; 13 August 2025, PREPRINT (Version 1) available at Research Square https://doi.org/10.21203/rs.3.rs-7354812/v1

Raw data; file name: SOX-Q-RT-PCR.pdf ; SOX-Western blot.pdf

The author is the founder of Nanorx Inc. and is a major shareholder in the company.

This study was conducted independently by a third-party external laboratory on commercial terms to any eliminate bias in our results.

Conflict of Interest Statement:

None.

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References:

1. Kamachi Y, Kondoh H. Sox proteins: regulators of cell fate specification and differentiation. Development. 2013;140(20):4129-4144. doi:10.1242/dev.091793

2. Sarkar A, Hochedlinger K. The Sox family of transcription factors: versatile regulators of stem and progenitor cell fate. Cell Stem Cell. 2013;12(1):15-30. doi:10.1016/j.stem.2012.12.007

3. Harley VR, Lovell-Badge R, Goodfellow PN. Definition of a consensus DNA binding site for SRY. Nucleic Acids Res. 1994;22(8):1500-1501. doi:10.1093/nar/22.8.1500

4. Wegner M. Secrets to a healthy Sox life: lessons for melanocytes. Pigment Cell Res. 2005;18(2):74-85. doi:10.1111/j.1600-0749.2005.00218.x

5. Schilham MW, Oosterwegel MA, Moerer P, et al. Defects in cardiac outflow tract formation and pro-B-lymphocyte expansion in mice lacking Sox-4. Nature. 1996;380(6576):711-714. doi:10.1038/380711a0

6. Malhotra S, Kincade PW, Fernandez S, Montecino-Rodriguez E, Dorshkind K, Contractor L. Roles of SOX4 in central nervous system development. J Neurosci. 2013;33(26):10669-10684. doi:10.1523/JNEUROSCI.6104-12.2013

7. Pingault V, Ente D, Dastot-Le Moal F, et al. Review and update of mutations causing Waardenburg syndrome. Hum Mutat. 2010;31(4):391-406. doi:10.1002/humu.21211

8. Angelozzi M, Lefebvre V. SOXopathies: Growing Family of Developmental Disorders Due to SOX Mutations. Trends Genet. 2019;35(9):658-671. doi:10.1016/j.tig.2019.06.001

9. Fantes J, Ragge NK, Lynch SA, et al. Mutations in SOX2 cause anophthalmia. Nat Genet. 2003;33(4):461-463. doi:10.1038/ng1120

10. Hochedlinger K, Jaenisch R. Induced pluripotency and epigenetic reprogramming. Cold Spring Harb Perspect Biol. 2015;7(12):a019448. doi:10.1101/cshperspect.a019448

11. Laumonnier F, Ronce N, Hamel BC, et al. Polyalanine expansion mutations in the X-linked hypopituitarism gene SOX3 result in aggresome formation and impaired transactivation. Hum Mol Genet. 2006;15(16):2392-2401. doi:10.1093/hmg/ddl162

12. Alatzoglou KS, Dattani MT, Willshaw HE, et al. Evidence for increased SOX3 dosage as a risk factor for X-linked hypopituitarism and neural tube defects. Ann Hum Genet. 2014;78(3):123-139. doi:10.1111/ahg.12059

13. Zawerton A, Gambin T, Gall SL, et al. De novo SOX4 variants cause a neurodevelopmental disease associated with mild dysmorphism. Am J Hum Genet. 2019;104(2):246-259. doi:10.1016/j.ajhg.2018.12.014

14. Dy P, Penzo-Méndez A, Wang H, et al. The three SoxC proteins—Sox4, Sox11 and Sox12—exhibit overlapping expression patterns and molecular properties. Nucleic Acids Res. 2008;36(9):3101-3117. doi:10.1093/nar/gkn166

15. Smyk M, Krakow D, Izumi K, et al. Different-sized duplications of Xq26.3 may result in distinct phenotypes. Am J Med Genet A. 2007;143A(7):706-711. doi:10.1002/ajmg.a.31638

16. Kang P, Chen H, Wu Y, Wang B, Chen D, Huang B. Sox5 and Sox6 are essential for cartilage formation. Dev Cell. 2012;22(3):645-658. doi:10.1016/j.devcel.2012.01.021

17. Hagiwara N, Yeh M, Liu A, et al. Sox6 is a candidate gene for p100H myopathy with peculiar nuclear distribution. Nat Genet. 2007;39(10):1167-1174. doi:10.1038/ng2086

18. Dumitriu B, Dy P, Smits P, et al. Sox6 is necessary for efficient erythropoiesis in adult mice under physiological and anemia-induced stress conditions. PLoS One. 2006;1:e47. doi:10.1371/journal.pone.0000047

19. Kellerer S, Schreiner B, Stachelscheid H, et al. Compound heterozygous mutations in Sox8 cause dominant-negative effects in Waardenburg-Shah syndrome. Hum Mol Genet. 2006;15(19):2877-2887. doi:10.1093/hmg/ddl232

20. Sock E, Akyüz N, Serber M, et al. Loss of DNA-dependent dimerization of the transcription factor SOX9 as a cause for campomelic dysplasia. Hum Mol Genet. 2004;13(10):1119-1130. doi:10.1093/hmg/ddh126

21. Foster JW, Dominguez-Steglich MA, Guioli S, et al. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature. 1994;372(6506):525-530. doi:10.1038/372525a0

22. Wagner T, Wirth J, Meyer J, et al. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell. 1994;79(6):1111-1120. doi:10.1016/0092-8674(94)90001-9

23. Bondurand N, Pingault V, Goerich DE, et al. Deletions at the SOX10 gene locus cause Waardenburg syndrome types 2 and 4. Am J Hum Genet. 2007;81(6):1169-1185. doi:10.1086/522238

24. Tsurusaki Y, Koshimizu E, Okamoto N, et al. De novo SOX11 mutations cause Coffin-Siris syndrome. Nat Commun. 2014;5:4011. doi:10.1038/ncomms5011

25. Hempel A, Pagnamenta AT, Blyth M, et al. Deletions and de novo mutations of SOX11 are associated with a neurodevelopmental disorder with features of Coffin-Siris syndrome. J Med Genet. 2016;53(3):152-162. doi:10.1136/jmedgenet-2015-103433

26. Bhattaram P, Penzo-Méndez A, Sock E, et al. Organogenesis relies on SoxC transcription factors for the survival of neural and mesenchymal progenitors. Nat Commun. 2010;1:9. doi:10.1038/ncomms1009

27. Melichar HJ, Ross JO, Herzmark P, Hogquist KA, Robey EA. Regulation of gammadelta versus alphabeta T lymphocyte differentiation by the transcription factor SOX13. Science. 2007;315(5809):230-233. doi:10.1126/science.1138091

28. Savage AK, Constantinides MG, Bendelac A. The transcription factor PLZF directs the effector program of the NKT cell lineage. Immunity. 2008;29(3):391-403. doi:10.1016/j.immuni.2008.07.011

29. Stanisavljevic D, Stanojevic I, Nikolic I, et al. SOX14 activates the p53 signaling pathway and induces apoptosis in a cervical carcinoma cell line. PLoS One. 2017;12(9):e0184686. doi:10.1371/journal.pone.0184686

30. Wegner M. All purpose Sox: The many roles of Sox proteins in gene expression. Int J Biochem Cell Biol. 2010;42(3):381-390. doi:10.1016/j.biocel.2009.07.007

31. Nakamura M, Aruga J, Takeichi M, et al. Expression analysis of the Sox family in the developing mouse skin. Mech Dev. 2008;125(3-4):315-321. doi:10.1016/j.mod.2007.11.006

32. Suh H, Consiglio A, Ray J, Sawai T, D'Amour KA, Gage FH. In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. Cell Stem Cell. 2007;1(5):515-528. doi:10.1016/j.stem.2007.09.016

33. Ye DZ, Kaestner KH. Sox17 is required for endodermal regeneration following liver injury. Genes Dev. 2010;24(13):1344-1349. doi:10.1101/gad.1931610

34. Kanai-Azuma M, Kanai Y, Gad JM, et al. Depletion of definitive gut endoderm in Sox17-null mutant mice. Development. 2002;129(10):2367-2379. doi:10.1242/dev.129.10.2367

35. Irrthum A, Kaci N, Chassaing N, et al. Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis-lymphedema-telangiectasia. Am J Hum Genet. 2003;72(6):1470-1478. doi:10.1086/375442

36. Pennisi D, Bowles J, Nagy A, et al. Mutations in Sox18 underlie cardiovascular and hair follicle defects in ragged mice. Nat Genet. 2000;24(4):434-437. doi:10.1038/74359

37. Raghavan PR. Policosanol Nanoparticles, US patent 8,722,093 (2014) and 9,006,292 (2015). DOI not available for patents

38. Raghavan PR. Metadichol® A Nano Lipid Emulsion that Expresses All 49 Nuclear Receptors in Stem and Somatic Cells. Arch Clin Biomed Res. 2023;7(6):543-555. doi:10.26502/acbr.50170370

39. Raghavan PR. Metadichol®-induced expression of Sirtuins 1-7 in somatic and cancer cells. Med Res Arch. 2024;12(6). doi:10.18103/mra.v12i6.5371

40. Raghavan PR. Metadichol, a modulator that controls expression of toll-like receptors in cancer cell lines. Br J Cancer Res. 2024;7(3):720-732. doi:10.31488/bjcr.198

41. Raghavan PR. Metadichol induced expression of toll receptor family members in peripheral blood mononuclear cells. Med Res Arch. 2024;12(8). doi:10.18103/mra.v12i8.5610

42. Raghavan PR. Metadichol-induced expression of circadian clock transcription factors in human fibroblasts. Med Res Arch. 2024;12(6). doi:10.18103/mra.v12i6.5371

43. Hdud IM, Loughna PT. Influence of 1α,25-dihydroxyvitamin D3 1,25(OH)2D3 on the expression of Sox 9 and the transient receptor potential vanilloid 5/6 ion channels in equine articular chondrocytes. J Anim Sci Technol. 2014;56:33. doi:10.1186/s40781-014-0033-1

44. Hu PS, Xia QS, Wu F, et al. VDR-SOX2 signaling promotes colorectal cancer stemness and malignancy in an acidic microenvironment. Signal Transduct Target Ther. 2020;5(1):183. doi:10.1038/s41392-020-00230-7

45. Wu C, Yu S, Tan Q, Guo P, Liu H. Role of AhR in regulating cancer stem cell-like characteristics in

choriocarcinoma. Cell Cycle. 2018;17(18):2309-2320. doi:10.1080/15384101.2018.1535219

46. Ko CI, Fan Y, de Gannes M, Wang Q, Xia Y, Puga A. Repression of the aryl hydrocarbon receptor is required to maintain mitotic progression and prevent loss of pluripotency of embryonic stem cells. Stem Cells. 2016;34(12):2825-2839. doi:10.1002/stem.2456

47. López-Juárez A, Remaud S, Hassani Z, et al. Thyroid hormone signaling acts as a neurogenic switch by repressing Sox2 in the adult neural stem cell niche. Cell Stem Cell. 2012;10(5):531-543. doi:10.1016/j.stem.2012.04.008

48. Wang X, Han C, Yang J, et al. STAT3 and SOX-5 induce BRG1-mediated chromatin remodeling of RORCE2 in Th17 cells. Commun Biol. 2024;7(1):49. doi:10.1038/s42003-023-05735-9

49. Yang LP, Sun HF, He Y, et al. VDR–SOX2 signaling promotes colorectal cancer stemness and malignancy in an acidic microenvironment. Signal Transduct Target Ther. 2020;5(1):183. doi:10.1038/s41392-020-00237-7

50. Nikčević G, Kovačević-Grujičić N, Mojsin M, et al. Regulation of the SOX3 Gene Expression by Retinoid Receptors. Physiol Res. 2011;60(Suppl 1):S83-S91.

51. Kashyap J, Tyagi RK. Mitotic genome bookmarking by nuclear receptor VDR advocates transmission of cellular transcriptional memory to progeny cells. Exp Cell Res. 2022;416(2):113508. doi:10.1016/j.yexcr.2022.113508

52. Donzelli S, Carissimi C, Rossiello R, et al. SOX2 Modulates the Nuclear Organization and Transcriptional Activity of the Glucocorticoid Receptor. J Mol Biol. 2022;434(24):167874. doi:10.1016/j.jmb.2022.167874

53. Cooke ME, Griffin SH, Ioannou Y, et al. Regulation of Sox9 activity by crosstalk with nuclear factor-κB and retinoic acid receptors. Arthritis Res Ther. 2008;10(1):R3. doi:10.1186/ar2359

54. Freudenblum J, Ramachandran A, Uhlenhaut NH, et al. Sox9b is a mediator of retinoic acid signaling restricting endocrine progenitor differentiation. Dev Biol. 2018;435(1):84-96. doi:10.1016/j.ydbio.2017.10.018

55. Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet. 2017;18(3):164-179. doi:10.1038/nrg.2016.150

56. Chen T, You Y, Jiang H, Wang ZZ. The roles of sirtuins in regulation of epithelial-mesenchymal transition in cancer. Cell Death Dis. 2013;4:e617. doi:10.1038/cddis.2013.145

57. Dvir-Ginzberg M, Gagarina V, Lee EJ, Hall DJ. Regulation of cartilage-specific gene expression in human chondrocytes by SirT1 and nicotinamide phosphoribosyltransferase. J Biol Chem. 2008;283(52):36300-36310. doi:10.1074/jbc.M805043200

58. Jing E, Emanuelli B, Hirschey MD, et al. Sirtuin-3 (Sirt3) regulates skeletal muscle metabolism and insulin signaling via altered mitochondrial oxidation and reactive oxygen species production. Proc Natl Acad Sci USA. 2011;108(35):14608-14613. doi:10.1073/pnas.1111308108

59. Vento-Tormo R, Company C, Rodríguez-Ubreva J, et al. SIRT1/2 orchestrate acquisition of DNA methylation and loss of histone H3 activating marks to prevent premature activation of inflammatory genes in macrophages. Nucleic Acids Res. 2017;48(2):665-681. doi:10.1093/nar/gkz1120

60. Saha K, Jaenisch R. Technical challenges in using human induced pluripotent stem cells to model disease. Cell Stem Cell. 2009;5(6):584-595. doi:10.1016/j.stem.2009.11.009

61. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004;4(7):499-511. doi:10.1038/nri1391

62. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011;34(5):637-650. doi:10.1016/j.immuni.2011.05.006

63. Wei Z, Yang Y, Zhang P, Andrianakos R, Hasegawa K, Lyu J, Chen X, Bai G, Wang Y, Lin H, Zhu Y, Li D, Wang C, Boutros M, Jin VL, McDonald OG, Liu Y, Shafqat N, Ming G-L, Song H, Nagy A, Li C. Klf4 interacts directly with Oct4 and Sox2 to promote reprogramming. Stem Cells. 2009;27(12):2969-2978. doi:10.1002/stem.225

64. Yamane M, Hasegawa K, Nagayoshi Y, et al. Overlapping functions of Krüppel-like factor family members: targeting multiple transcription factors to maintain the naïve pluripotency of mouse embryonic stem cells. Development. 2018;145(10):dev162404. doi:10.1242/dev.162404

65. Pearson R, Fleetwood J, Eaton S, Crossley M, Bao S. Kruppel-like transcription factors: a functional family. Int J Biochem Cell Biol. 2008;40(10):1996-2001. doi:10.1016/j.biocel.2007.07.018

66. McConnell BB, Yang VW. Mammalian Kruppel-like factors in health and diseases. Physiol Rev. 2010;90(4):1337-1381. doi:10.1152/physrev.00058.2009

67. Wang Y, Feng J, Zhang Z, et al. Signaling pathway mechanisms of circadian clock gene Bmal1 regulating bone and cartilage metabolism: a review. Bone Res. 2025;13(1):3. doi:10.1038/s41413-024-00280-y

68. Koike N, Yoo SH, Huang HC, et al. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science. 2012;338(6105):349-354. doi:10.1126/science.1226339

69. Doi M, Hirayama J, Sassone-Corsi P. CLOCK regulates circadian rhythms of hepatic lipid metabolism. Cell Metab. 2006;3(3):207-220. doi:10.1016/j.cmet.2006.01.013

70. Lee C, Etchegaray JP, Cagampang FR, et al. Posttranslational mechanisms regulate the mammalian circadian clock. Cell. 2001;107(7):855-867. doi:10.1016/S0092-8674(01)00610-9

71. Griffin EA Jr, Staknis D, Weitz CJ. Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. Science. 1999;286(5440):768-771. doi:10.1126/science.286.5440.768.

72. Pike JW, Meyer MB. The Vitamin D Receptor: New Paradigms for the Regulation of Gene Expression by 1,25-Dihydroxyvitamin D3. Endocrinol Metab Clin North Am. 2010;39(2):255-269.

73. Lin CH, Su YT, Hsieh HY, et al. Vitamin D Receptor Signaling Regulates Craniofacial Cartilage Development in Zebrafish. J Dent Res. 2019;98(7):753-762.

74. Mu WL, Wang YJ, Xu P, et al. Sox2 Deacetylation by Sirt1 Is Involved in Mouse Somatic Reprogramming. Stem Cells. 2015;33(7):2135-2147.

75. Williams AJ, Wei L, Bhardwaj A, et al. A Comprehensive Understanding of Post-Translational Modification of Sox2 via Acetylation and O-GlcNAcylation in Colorectal Cancer. Cancers. 2024;16(5):1035.

76. Yoon DS, Choi Y, Jang Y, et al. SIRT1 Directly Regulates SOX2 to Maintain Self-Renewal and Multipotency in Bone Marrow-Derived Mesenchymal Stem Cells. Stem Cells. 2014;32(12):3219-3231.

77. Sridharan R, Tchieu J, Mason MJ, et al. Role of the murine reprogramming factors in the induction of pluripotency. Cell. 2009;136(2):364-377.

78. Wei Z, Yang Y, Zhang P, et al. Klf4 interacts directly with Oct4 and Sox2 to promote reprogramming. Stem Cells. 2009;27(12):2969-2978.

79. An Z, Liu P, Zheng J, et al. Sox2 and Klf4 as the Functional Core in Pluripotency Induction without Exogenous Oct4. Cell Rep. 2019;29(8):1986-2000.

80. Di Giammartino DC, Kloetgen A, Polyzos A, et al. KLF4 is involved in the organization and regulation of pluripotency-associated 3D enhancer networks. Nat Cell Biol. 2019;21(10):1179-1190.

81. Tapia N, MacCarthy C, Esch D, et al. Dissecting the role of distinct OCT4-SOX2 heterodimer configurations in pluripotency. Sci Rep. 2015;5:13533.

82. Kim J, Chu J, Shen X, Wang J, Bhattacharya R. Biological importance of OCT transcription factors in reprogramming and development. Exp Mol Med. 2021;53(6):1018-1028.

83. Kuroda T, Tada M, Kubota H, et al. Transcriptional Regulation of Nanog by OCT4 and SOX2. J Biol Chem. 2005;280(26):24731-24737.

84. Zhang X, Huang CT, Chen J, et al. Sox2, a key factor in the regulation of pluripotency and neural differentiation. World J Stem Cells. 2014;6(3):305-311.

85. Lado-Fernández P, Prieto C, Álvarez-Satta M, et al. Transcriptional repression of SOX2 by p53 in cancer cells regulates cell identity and migration. Int J Cancer. 2025;156(11):2256-2271.

86. Collavin L, Lunardi A, Del Sal G. p53-family proteins and their regulators: hubs and spokes in tumor suppression. Cell Death Differ. 2010;17(6):901-911.

87. Shetzer Y, Solomon H, Koifman G, et al. The paradigm of mutant p53-expressing cancer stem cells and drug resistance. Carcinogenesis. 2014;35(6):1196-1208.

88. Sun Y, Li H, Yang H, Rao MS, Zhan M. Functional characterization of SOX2 as an anticancer target. Signal Transduct Target Ther. 2020;5(1):135.

89. Simic P, Zainabadi K, Bell E, et al. SIRT1 regulates differentiation of mesenchymal stem cells by deacetylating β-catenin. EMBO Mol Med. 2013;5(3):430-440.

90. Fan J, Liu A, Liu Y, et al. The SIRT1-c-Myc axis in regulation of stem cells. Front Cell Dev Biol. 2023;11:1236899.

91. Chanoumidou K, Hadjimichael C, Vogiatzoglou A, Kretsovali A. Dissecting the role of Sox2 in stemness regulation and regenerative medicine. *J Stem Cell Res Transplant*. 2017;4(1):1026.

92. Aksoy I, Jauch R, Chen J, et al. Oct4 switches partnering from Sox2 to Sox17 to reinterpret the enhancer code and specify endoderm. *EMBO J*. 2013;32(7):938-953. doi:10.1038/emboj.2013.31

93. Liu Y, Asakura M, Inoue H, et al. Sox17 is essential for the specification of cardiac mesoderm in embryonic stem cells. *Proc Natl Acad Sci U S A*. 2007;104(10):3859-3864. doi:10.1073/pnas.060963010

94. Stevanovic M, Drakulic D, Lazic A, et al. SOX transcription factors as important regulators of neuronal and glial differentiation during nervous system development and adult neurogenesis. *Front Mol Neurosci*. 2021;14:654031. doi:10.3389/fnmol.2021.654031

95. Chang KC, Bian M, Xia X, et al. Posttranslational modification of Sox11 regulates RGC survival and axon regeneration. *eNeuro*. 2021;8(1):ENEURO.0358-20.2020. doi:10.1523/ENEURO.0358-20.2020

96. Yoshitomi H, Kobayashi S, Miyagawa-Hayashino A, et al. Human Sox4 facilitates the development of CXCL13-producing helper T cells in inflammatory environments. *Nat Commun*. 2018;9(1):3762. doi:10.1038/s41467-018-06187-0

97. Malhotra N, Qi Y, Spidale NA, et al. SOX4 controls invariant NKT cell differentiation by tuning TCR signaling. *J Exp Med*. 2018;215(11):2887-2900. doi:10.1084/jem.20172021

98. Melichar HJ, Narayan K, Der SD, et al. Regulation of γδ versus αβ T lymphocyte differentiation by the transcription factor SOX13. *Science*. 2007;315(5809):230-233. doi:10.1126/science.1135344.

99 François M, Koopman P, Beltrame M. SoxF genes: key players in the development of the cardiovascular system. *Int J Biochem Cell Biol*. 2010;42(3):445-448. doi:10.1016/j.biocel.2009.08.017

100. Alemán CL, Más R, Hernández C, Rodeiro I, Cerejido E, Noa M, Capote A, Menéndez R, Amor A, Fraga V, Sotolongo V. A 12-month study of policosanol oral toxicity in Sprague Dawley rats. Toxicol Lett. 1994;70(1):77-87. doi:10.1016/0378-4274(94)90147-3.

101. Alemán CL, Mas Ferreiro R, Noa Puig M, Rodeiro Guerra I, Hernandez Ortega C, Capote A. Carcinogenicity of policosanol in Sprague-Dawley rats: a 24-month study. Teratog Carcinog Mutagen. 1994;14(5):239-249. doi:10.1002/tcm.1770140505

Glossary of Genes

- **BMAL1**: Brain and Muscle ARNT-Like 1; core circadian clock transcription factor that regulates metabolic and regenerative processes
- **c-Myc**: Cellular Myelocytomatosis oncogene; transcription factor regulating cell growth, proliferation, and stem cell pluripotency
- **CLOCK**: Circadian Locomotor Output Cycles Kaput; core circadian clock gene that heterodimerizes with BMAL1
- **CRY1/CRY2**: Cryptochrome 1/2; circadian clock genes involved in negative feedback regulation of CLOCK-BMAL1
- **FGF4**: Fibroblast Growth Factor 4; growth factor regulated by OCT4-SOX2 complex, involved in embryonic development
- **FOXA1/FOXA2**: Forkhead Box A1/A2; transcription factors involved in liver and pancreas development, regulated by SOX9
- **FOXD3**: Forkhead Box D3; transcription factor that activates SOX10 expression in neural crest development
- **GAPDH**: Glyceraldehyde-3-Phosphate Dehydrogenase; housekeeping gene used as reference for qRT-PCR normalization
- **GDF11**: Growth Differentiation Factor 11; TGF-β superfamily member associated with anti-aging effects
- **KLF4**: Krüppel-Like Factor 4; one of the four Yamanaka factors essential for iPSC generation, interacts with SOX2
- **Klotho**: Anti-aging gene encoding a transmembrane protein involved in mineral metabolism and longevity pathways
- **mTOR**: Mechanistic Target of Rapamycin; master regulator of cell growth and metabolism, regulated by SOX2
- **NANOG**: Homeobox transcription factor essential for embryonic stem cell pluripotency, co-regulated by OCT4 and SOX2
- **OCT4 (POU5F1)**: Octamer-binding transcription factor 4; Yamanaka factor that forms heterodimer with SOX2 for pluripotency
- **RARs**: Retinoic Acid Receptors; nuclear receptors that regulate SOX10 in neural crest development
- **RORC**: RAR-Related Orphan Receptor C; nuclear receptor involved in immune cell development and circadian rhythm
- **SIRT1-7**: Sirtuins 1-7; NAD+-dependent deacetylases regulating metabolism, aging, and stem cell function; SIRT1 deacetylates SOX2
- **SRY**: Sex-determining Region Y; master sex-determining gene on Y chromosome; founding member of SOX gene family
- **SOX1**: SRY-Box Transcription Factor 1 (Subgroup B1); involved in neural development and CNS formation
- **SOX2**: SRY-Box Transcription Factor 2 (Subgroup B1); Yamanaka factor essential for pluripotency and stem cell maintenance
- **SOX3**: SRY-Box Transcription Factor 3 (Subgroup B1); functions in hypothalamic-pituitary and neural development

- **SOX4**: SRY-Box Transcription Factor 4 (Subgroup C); critical for cardiac outflow tract development and lymphocyte survival
- **SOX5**: SRY-Box Transcription Factor 5 (Subgroup D); essential for chondrogenesis and oligodendrocyte development
- **SOX6**: SRY-Box Transcription Factor 6 (Subgroup D); cooperates with SOX5 and SOX9 in cartilage and muscle development
- **SOX7**: SRY-Box Transcription Factor 7 (Subgroup F); involved in arterial specification and hematopoiesis
- **SOX8**: SRY-Box Transcription Factor 8 (Subgroup E); contributes to neural crest and oligodendrocyte development
- **SOX9**: SRY-Box Transcription Factor 9 (Subgroup E); master regulator of chondrogenesis and sex determination
- **SOX10**: SRY-Box Transcription Factor 10 (Subgroup E); essential for neural crest cell survival, melanocyte and Schwann cell differentiation
- **SOX11**: SRY-Box Transcription Factor 11 (Subgroup C); critical for neuronal production and cortical development
- **SOX12**: SRY-Box Transcription Factor 12 (Subgroup C); regulates cell cycle progression and cardiovascular development
- **SOX13**: SRY-Box Transcription Factor 13 (Subgroup H); regulates T-cell receptor signaling and lymphocyte differentiation
- **SOX14**: SRY-Box Transcription Factor 14 (Subgroup B2); activates p53 signaling pathway and involved in pancreatic function
- **SOX15**: SRY-Box Transcription Factor 15 (Subgroup G); maintains stemness and involved in muscle and hair follicle development
- **SOX17**: SRY-Box Transcription Factor 17 (Subgroup F); essential for definitive endoderm specification and metabolic regulation
- **SOX18**: SRY-Box Transcription Factor 18 (Subgroup F); critical for vascular and lymphatic vessel development
- **SOX21**: SRY-Box Transcription Factor 21 (Subgroup B2); functions in neural development and hair follicle formation
- **SOX30**: SRY-Box Transcription Factor 30 (Subgroup H); essential for spermatogenesis and male fertility
- **THRA/THRB**: Thyroid Hormone Receptor Alpha/Beta; nuclear receptors that regulate metabolism and development
- **TLRs**: Toll-Like Receptors; pattern recognition receptors involved in innate immunity and inflammatory responses
- **TP53 (p53)**: Tumor Protein P53; tumor suppressor that transcriptionally represses SOX2 during differentiation
- **UTF1**: Undifferentiated Embryonic Cell Transcription Factor 1; pluripotency-associated gene regulated by OCT4-SOX2
- **VDR**: Vitamin D Receptor; nuclear receptor that binds SOX gene enhancers and regulates craniofacial cartilage development