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RESEARCH ARTICLE

Metadichol®-induced expression of circadian clock transcription factors in human fibroblasts

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ABSTRACT

Circadian clock genes regulate many physiological processes, including sleep, metabolism, inflammation, and cancer. Disruptions in these cycles are associated with heightened vulnerability to cancer, psychiatric diseases, and neurological problems. Circadian clock genes play a vital role in the growth and progression of cancer. Manipulating the circadian clock mechanism can eliminate cancer cells, augment the effectiveness of chemotherapy, eliminate senescent cells, and reinstate regular circadian cycles.

The circadian clock genes that are crucial for regulating the daily physiological processes of mammals include *Cry1, Bmal1, Per1, Ppargc1a, and Clock*. Currently, there is a lack of information regarding small molecules that can effectively control and activate all five transcription factors. By subjecting fibroblasts to treatment with Metadichol, we can induce the expression of all the genes, as evidenced by the results obtained from quantitative real-time polymerase chain reaction (qRT–PCR). The administration of metadichol significantly enhanced the expression of the *Cry1, Clock, and Ppargc1a* genes in human fibroblasts, resulting in a four- to fivefold increase in expression. Metadichol successfully maintained the expression of the *Per1 and Bmal1* genes at a dose of 100 ng. *Clock and Bmal1* heterodimers initiate the activation of Cry1, Per1, and other clock genes via a feedback loop. The *Cry1 and Per1* proteins inhibit the function of Clock-Bmal1. Ppargc1a regulates the expression of Clock and metabolic genes. Metadichol did not result in elevated levels of *Per1 and Bmal1* expression, suggesting that it does not directly affect these genes. Metadichol is a nontoxic molecule, and its effects on circadian rhythm can have significant implications for human health to alleviate the progression of numerous ailments and diseases

Keywords: Circadian rhythm, Clock, Bmal1, Per1, Cry1, Ppargc1a, NRID1, Metadichol, Nanoemulsion, Long-Chain Alcohols, Sirt 1 and Sirt 6.

Introduction

The circadian rhythm is a 24-hour cycle, and it forms the basis for key physiological activities that occur in mammals and are necessary for maintaining equilibrium and health, as well as regulating sleep and wakefulness.[1,2]

To generate oscillations over a period of twenty-four hours, the biological clock is formed by proteins that are encoded by clock genes. These proteins turn on and off in a specific sequence. [1] All of the biological clocks in the body are controlled by the suprachiasmatic nucleus (SCN) of the brain, which is responsible for synchronizing them to exposure to light and darkness. [2]. This synchronization is essential for determining the timing of actions involving metabolism, the immune system, and hormones.

Through both positive and negative feedback loops, transcription factors are responsible for regulating circadian rhythms at the molecular level. Furthermore, clock genes, including *BMal1 (*basic helix-loop-helix ARNT like 1), *Clock (*clock circadian regulator), *Cry (*cryptochrome circadian regulator 1), *Per1* (period circadian regulator 1) *and Ppargc1a (*PPARG coactivator 1 alpha), are responsible for the regulation of this system. By regulating the pathways that are involved in cellular signaling, these core clock genes influence a number of physiological activities.

Circadian rhythm abnormalities such as a lack of sleep or a misalignment of the circadian rhythm result in several diseases, such as cancer, diabetes, mental health issues, cardiovascular disease, and obesity. [3] The *Clock-Bmal1* complex regulates gene expression as well as circadian rhythms while also having an impact on a significant portion of the genome. In cancer, these genes regulate the cell cycle, metabolism, and DNA repair, all of which are significant. [4]

Through a negative feedback loop, *Per1 and Cry1* contribute to the stability and output of the circadian clock, which is a necessary component of cell cycle control. Their expression influences the timing of the cell cycle as well as the proliferation and development of cancer cells.[5]

One of the transcriptional coactivators known as *Ppargc1a also called PGC-1α* is responsible for regulating genes related to energy metabolism through its interaction with the circadian clock. Cancer cells exhibit metabolic defects, and the expression of *Ppargc1a* can mitigate these defects. [6]

Circadian gene mutations or aberrant expression are linked to several different types of cancer and based on their ability to influence the occurrence of cancer, its growth, and the way it reacts to treatment, they are targets for cancer therapy. Understanding the patterns of expression of these genes could lead to the synchronization of cancer treatments with the circadian clock of the body, improving the effectiveness of treatments and minimizing adverse effects. [8]

Researchers in model organisms that have biological clock genes that are comparable to those found in humans have been able to better understand how circadian rhythms influence both health and illness. [9] Circadian transcription factor dysregulation leads to epilepsy. [10] It is possible that deregulation of circadian genes could result in mood disorders. [11,12] This list contains minute substances that influence the genes that control the circadian clock. The clock genes *Per1 and Bmal1* are regulated by resveratrol, a naturally occurring polyphenol, in rat-1 fibroblasts; however, this is not the case in human cells. [13] Resveratrol has numerous health benefits, such as antiaging, anticancer, and anti-inflammatory effects. Additionally, resveratrol modulates metabolism and the circadian clock through the mechanisms of *Sirt1 and PGC-1α*.

Cry proteins are activated by the synthetic small molecule KL001, which stabilizes and blocks the degradation of these proteins by the ubiquitin–proteasome system. Glucagon suppresses primary hepatocyte gluconeogenesis, which increases the duration of the circadian period. [14,15] KL001 blocks this process. It has been demonstrated that KL001 has antidiabetic effects in mouse models of type 2 diabetes.

The synthetic small molecule SR9009 can bind and activate the nuclear receptor *REV-ERBα* (*NR1D1*), hence suppressing the production of Bmal1 and clock genes. SR9009 has been shown to affect the circadian rhythm and metabolism of mice, which offers the possibility that it could be used to treat obesity, diabetes, and sleep disorders.[16]

Thus, circadian rhythm transcription factors are integral to the proper functioning of the biological clock and, by extension, to human health. These proteins regulate the timing of various biological processes, and their disruption can lead to a plethora of health issues. Herein, we show for the first time that Metadichol [17], a lipid emulsion of long-chain alcohols, can express all these factors and could serve as the basis for overcoming defects

in the circadian rhythm necessary for maintaining whole-body homeostasis.

EXPERIMENTAL

All experiments were designed and outsourced to the commercial service provider Skanda Biolabs Ltd., Bangalore, India. The pathway Studio was outsourced on commercial terms to Elsevier R&D Solutions, Inc. 18 The raw data are provided in the Supplemental files.

CELL TREATMENT

The cells were treated for 24 hours at the indicated concentrations (1 pg, 100 pg, 1 ng and 100 ng/ml). of growth media without FBS (fetal bovine serum).

SAMPLE PREPARATION AND RNA ISOLATION

The treated cells were harvested, rinsed with sterile 1X PBS, and centrifuged. The supernatant was decanted, and 0.4 ml of TRIzol was added and gently mixed by inversion for 1 min. Samples were allowed to stand for 10 min at room temperature. To this solution, 0.25 ml of chloroform was added per 0.4 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 min at 4°C. The upper aqueous phase was collected in a new sterile microcentrifuge tube, to which 0.5 ml of isopropanol was added, 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. The supernatant was discarded, and the RNA pellet was washed by adding 0.5 ml of 70% ethanol. The RNA mixture was centrifuged at 12,000 rpm at 4°C. The supernatant was carefully discarded, and the pellet was air-dried. The RNA pellet was then resuspended in 20 µl of diethyl polycarbonate (DEPC)-treated water. The total RNA yield (Table 1) was quantified using a Spectra Drop (SpectraMax i3x, Molecular Devices, USA).

Table 1: RT–qPCR analysis

NHDF cells Treatment	Test sample concentrations				
	0	1 pg/ ml	100 pg/ ml	1 ng/ ml	100 ng/ ml
RNA yield (ng/µl)	444.400	288.36	264.845	426.720	200.584

cDNA was synthesized from 500 ng of RNA using a cDNA synthesis kit from the Prime Script RT Reagent Kit (TAKARA) with oligo dT primers according to the manufacturer s instructions. The reaction volume was 20 µl, and cDNA synthesis was performed at 50°C for 30 min, followed by RT inactivation at 85°C for 5 min using the applied biosystem Veritii. The cDNA was further used for real-time PCR analysis.

cDNA SYNTHESIS

cDNA was synthesized from 2 µg of RNA using a PrimeScript cDNA synthesis kit (Takara) and oligo dT primers per the manufacturer's instructions. The reaction volume was 20 µL, and cDNA synthesis was performed on an Applied Biosystems instrument (Veritii). cDNA was used for qPCR (50°C for 30 min followed by 85°C for 5 min).

PRIMERS AND qPCR

The PCR mixture (final volume of 20 µL) contained 1 µL of cDNA, 10 µL of SYBR Green Master Mix, and one µM complementary forward and reverse primers specific for the respective target genes. The reaction conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of secondary denaturation at 95°C for 30 s, annealing at the optimized temperature for 30 s, and extension at 72°C for 1 min. The number of cycles that allowed amplification in the exponential range without reaching a plateau was selected as the optimal number of cycles. The obtained results were analyzed using CFX Maestro software.

The fold change was calculated using the ΔΔCT method.

The comparative CT method was used to determine the relative expression of target genes to that of the housekeeping gene (β-actin) in untreated control cells.

The delta CT for each treatment was calculated using the following formula: Delta Ct = Ct (target gene) – Ct (reference gene).

To compare the delta Ct of individually treated samples with that of the untreated control samples, the Ct of each group was subtracted from that of the control to obtain the delta CT.

Delta delta Ct = delta Ct (treatment group) – delta Ct (control group).

The fold change in target gene expression for each treatment group was calculated using the following formula: Fold change = 2^ (−delta delta Ct).

Results

Figure 1 shows the increased expression of all circadian transcription factors at a concentration of 100 ng of *metadichol*. *Bmal1* and *Per1* are maintained at normal levels, whereas *Ppargc1A*, *Cry1* and Clock exhibit increased expression.

Figure 1: Fold regulation of circadian transcription factors.

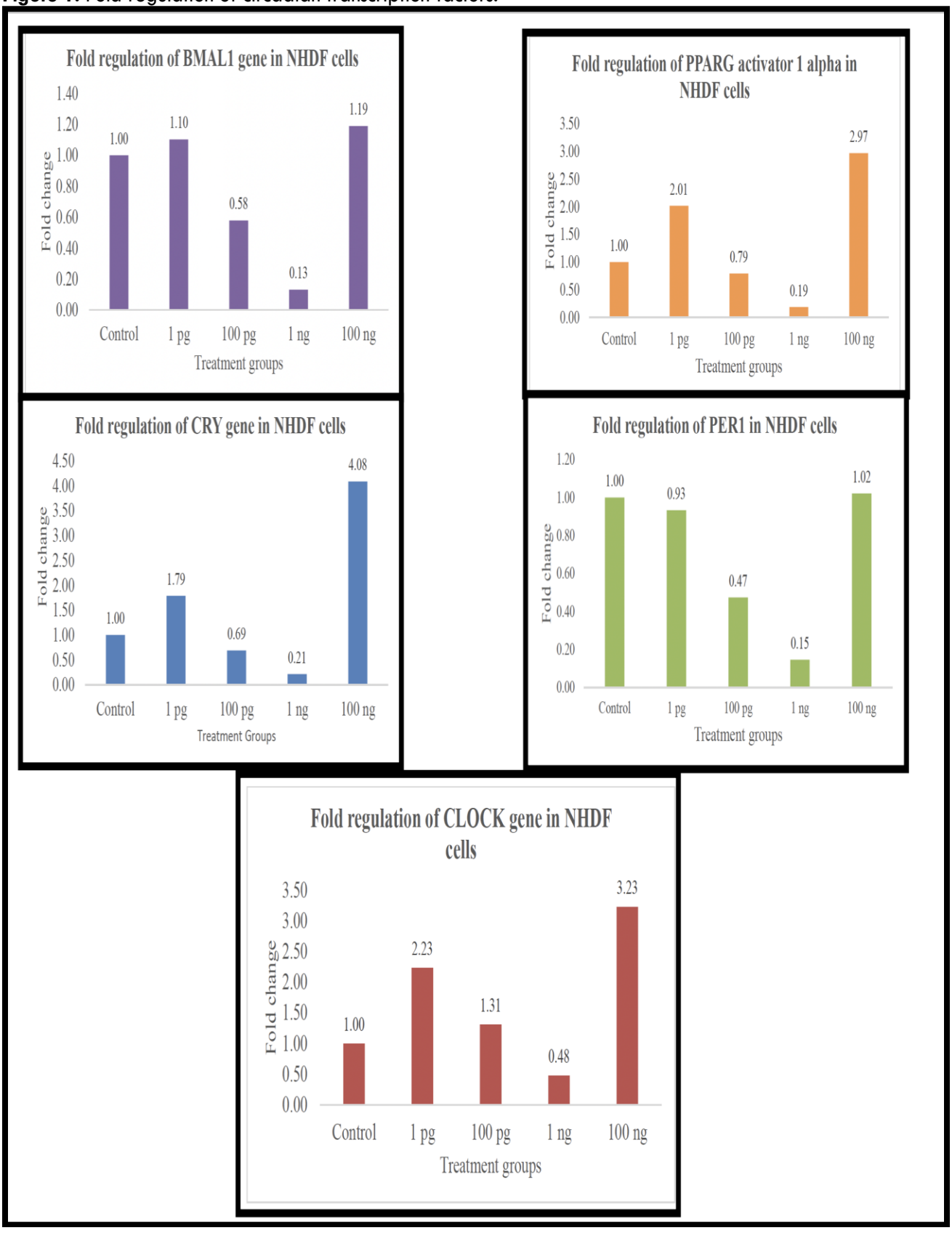

Table 2: Primer details

Primer	sequence Forward and reverse	Amplicon size	Annealing temperature
GAPDH	GTCTCCTCTGACTTCAACAGCG	186	60
	ACCACCCTGTTGCTGTAGCCAA		
BMAL1	GCTCAGGAGAACCCAGGTTATC	160	59
	GCATCTGCTTCCAAGAGGCTCA		
CLOCK	CAGGCAGCATTTACCAGCTCATG	119	65
	GTAGCTTGAGACATCACTGGCTG		
PER	GCAGGCCAACCAGGAATACT	157	67
	CAGGAAGGAGACAGCCACTG		
CRY	GTGGACAACCGCCTCTAACTT	163	56
	TCCAGTGAAGGGACTCCATATT		
(PPARGC1A)	ACGCACCGAAATTCTCCCTT	172	56
	TCTGCCTCTCCCTTTGCTTG		

The network formed by the transcription factors was generated using Pathway Studio [19,20,21] and is shown in Figure 2. The other key pathways and their impacts on diseases and organs are shown in Tables 3, 4, 5, and 6.

The circadian clock genes form a transcriptional-translational feedback loop in which the transcription factors *Clock and Bmal1* activate the expression of the genes *Cry1 and Per1*, which encode proteins that inhibit the activity of *Clock-Bmal1*.[22,23] Other transcription factors, such as *Ppargc1a and Bmal1*, modulate the expression of clock genes and metabolic genes.

Figure 2: Network interactions of circadian genes.

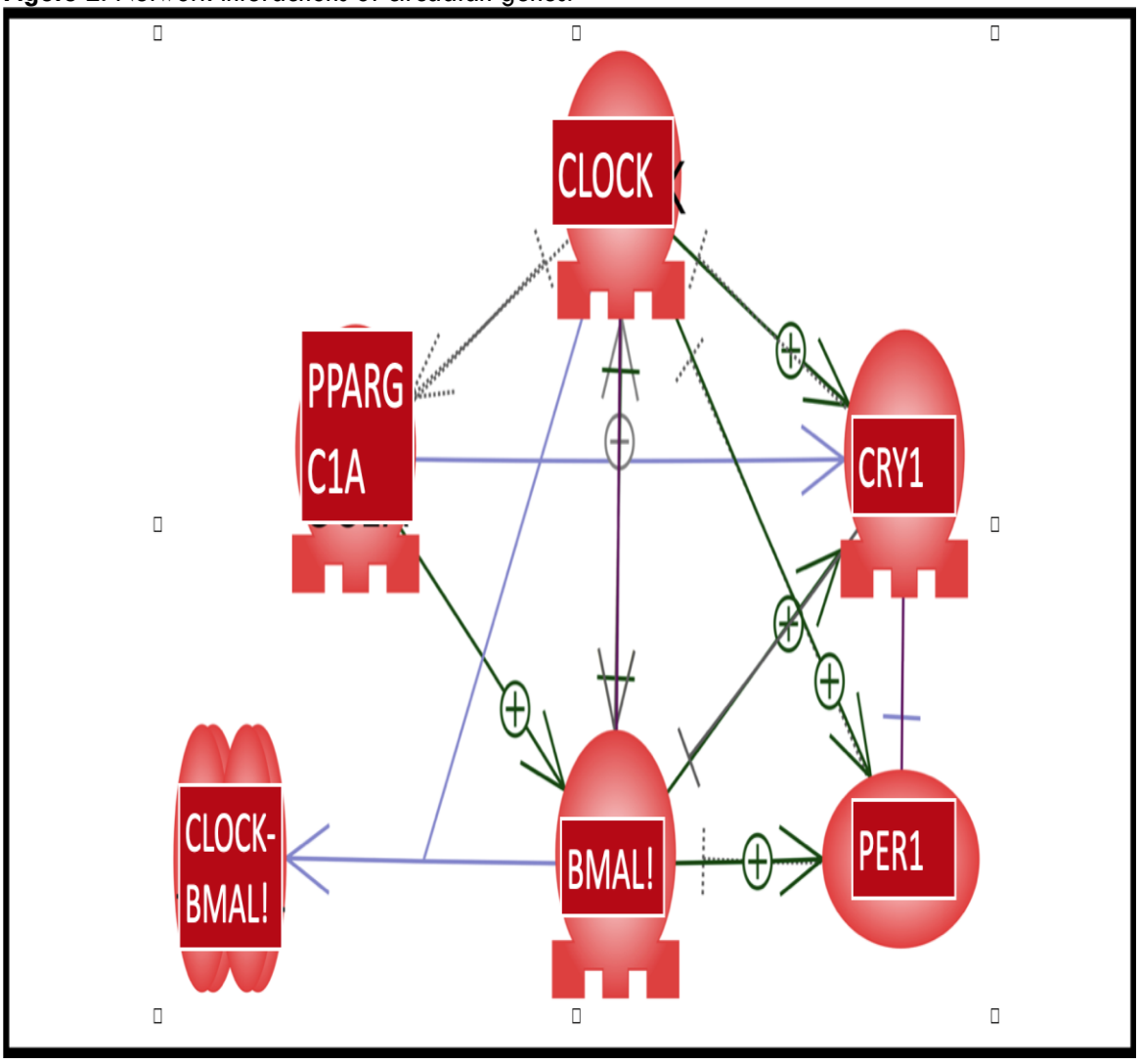

Table 3: Top Pathways; Biological Processes.
Several key biological processes are related to circadian rhythm and processes involving sirtuins In aging (highlighted).

Name	Parent Folder	# of Entities	Expanded # of Entities	Overlap	Percent Overlap	Overlapping Entities	p value
Circadian Clock Genes Mutations Cause Insomnia	Aging Related Diseases; Sleep Dysregulation; Sleep Dysregulation (Neurological Diseases); nerve tissue; nervous system	26	41	4	9	CRY1, CLOCK, PER1, ARNTL	6.34966E-10
Circadian Clock and Sleep Regulation by Melatonin	Amino Acid Derived Hormones; Sleep Regulation; Sleep Regulation (Biological Process); nerve tissue; nervous system	26	41	4	9	CRY1, CLOCK, PER1, ARNTL	6.34966E-10
Circadian Clock in Sleep Regulation	Sleep Regulation; Sleep Regulation (Biological Process); nerve tissue; nervous system	32	55	4	7	CLOCK, CRY1, PER1, ARNTL	2.13638E-09
Circadian Clock Genes in Suprachiasmatic Nuclei Neurons	Sleep Regulation; Sleep Regulation (Biological Process); nerve tissue; nervous system; nucleus	64	125	4	3	CLOCK, CRY1, ARNTL, PER1	6.04168E-08
SIRT1 Signaling in Aging	Genomic Instability Associated with Aging; Genomic Instability Associated with Aging (Aging Biology); generic; generic; nucleus	64	170	3	1	CLOCK, ARNTL, PPARGC1A	2.90853E-05
SIRT4 Signaling in Aging	Genomic Instability Associated with Aging; Genomic Instability Associated with Aging	26	31	1	3	PPARGC1A	1.31176E-02

Name	Parent Folder	# of Entities	Expanded # of Entities	Overlap	Percent Overlap	Overlapping Entities	p value
	(Aging Biology); generic; generic; nucleus						
Vitamin D Activates Transcription	Vitamins Biology; Vitamins Biology (Biological Process); generic; generic	16	55	1	1	CLOCK	2.31784E-02
Agomelatine Antidepressant Action	Sleep Regulation; Sleep Regulation (Biological Process); nerve tissue; nervous system	26	85	1	1	PER1	3.56387E-02
SIRT7 Signaling in Aging	Genomic Instability Associated with Aging; Genomic Instability Associated with Aging (Aging Biology); generic; generic; nucleus	27	98	1	1	PPARGC1A	4.09986E-02
TGFB-|COX2	Corneal stroma remodeling	47	115	1	0	PPARGC1A	4.79716E-02

Table 4. Organs that express molecules enriched in the input gene set
Highlights the presence of circadian genes in all key organs, such as the testis, brain, liver, lung nerve and kidney, that are necessary for human survival, with a P value of zero (highlighted)

Protein entities related to testis	10374	testis	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein entities related to cerebral cortex	8874	cerebral cortex	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein entities related to brain	11768	brain	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein entities related to neuron	9335	neuron	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein entities related to lung	10040	lung	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein entities related to kidney	10904	kidney	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein entities related to liver	9996	liver	4	0	ARNTL, CRY1, PER1, CLOCK	0.00000E+00
Protein entities related to GT1-7	277	GT1-7	5	1	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	3.37236E-08

Protein entities related to hypophysis pars tuberalis	111	hypophysis pars tuberalis	4	3	ARNTL, CRY1, PER1, CLOCK	1.30955E-07
Protein entities related to suprachiasmatic nucleus neuron	154	suprachiasmatic nucleus neuron	4	2	ARNTL, CRY1, PER1, CLOCK	4.90711E-07

Table 5 Diseases regulated by input genes
Most chronic disease impacts lead to aging and cancer, which are regulated by circadian genes.

Name	Total # of Neighbors	Gene Set Seed	Overlap	Percent Overlap	Overlapping Entities	p value
Protein regulators of glucose intolerance	1111	glucose intolerance	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	1.97088E-07
Protein regulators of elevated blood pressure	430	elevated blood pressure	4	0	ARNTL, PPARGC1A, CRY1, CLOCK	4.74904E-07
Protein regulators of hyperglycemia	1325	hyperglycemia	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	4.76213E-07
Protein regulators of circadian rhythm sleep disorder	6	circadian rhythm sleep disorder	2	28	CRY1, CLOCK	5.06905E-07
Protein regulators of intermittent hypoxia	142	intermittent hypoxia	3	2	ARNTL, CRY1, PER1	1.93132E-06
Protein regulators of seasonal affective disorder	14	seasonal affective disorder	2	13	ARNTL, CLOCK	3.07320E-06
Protein regulators of astrogliosis	731	astrogliosis	4	0	ARNTL, PPARGC1A, CRY1, CLOCK	3.94934E-06
Protein regulators of disorder of initiating and maintaining sleep	18	disorder of initiating and maintaining sleep	2	10	PPARGC1A, CLOCK	5.16533E-06
Protein regulators of sarcopenia	213	sarcopenia	3	1	ARNTL, PPARGC1A, CLOCK	6.53589E-06
Protein regulators of premature aging	221	premature aging	3	1	ARNTL, PPARGC1A, CLOCK	7.30046E-06

Table 6: Regulated cell processes
Key process involved is the regulation of apoptosis, cell proliferation and epigenetic modifications which is very important in cancer (highlighted)

Name	Total # of Neighbors	Overlap	Percent Overlap	Overlapping Entities	p value
Protein regulators of anagen	273	4	1	ARNTL, CRY1, PER1, CLOCK	8.49703E-07
Protein regulators of apoptosis	13703	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein regulators of biological rhythm	108	5	4	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	3.19377E-11
Protein regulators of cell proliferation	15725	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	0.00000E+00
Protein regulators of circadian behavior	103	4	3	ARNTL, CRY1, PER1, CLOCK	1.67667E-08
Protein regulators of epigenetic modification	641	5	0	ARNTL, PPARGC1A, CRY1, PER1, CLOCK	2.54371E-07
Protein regulators of light dark cycle	89	4	4	ARNTL, PPARGC1A, CRY1, PER1	9.26714E-09
Protein regulators of lipid absorption	212	4	1	ARNTL, PPARGC1A, PER1, CLOCK	3.08178E-07
Protein regulators of locomotor rhythm	105	4	3	ARNTL, CRY1, PER1, CLOCK	1.81257E-08
Protein regulators of sleep waking cycle	155	4	2	ARNTL, CRY1, PER1, CLOCK	8.74397E-08

Discussion.

The interactions and roles of *Clock*, *Cry1*, *Per1*, *Bmal11*, and Ppargc1a in regulating circadian rhythm are summarized below.

Clock and *Bmal1* form a heterodimer that activates the transcription of other clock genes, including Per1 and *Cry1*. This activation occurs by binding to E-box promoter elements in the genome, thus activating many clock-controlled genes. 24 This activation leads to the production of *Per* and *Cry* proteins, which accumulate and form complexes that repress their own transcription by binding to the *Clock:Bmal1* complex, creating a negative feedback loop. This cycle repeats approximately every 24 hours, contributing to the circadian rhythm. [25-27]

Per1 and *Cry1* form a complex that inhibits the activity of *Clock-Bmal1*, creating a negative feedback loop. This inhibition leads to the suppression of their own transcription, thus forming the negative arm of the circadian clock. [28] *Per1*, along with other *Per* and *Cry* proteins, is involved in the regulation of the expression of genes outside of the core clock genes. *Per1* and *Cry1/2* may regulate the expression of genes outside of the core clock, indicating a broader role in cellular function. [29]

The expression levels of *Per1* and *Bnal1* are maintained through a complex interplay of transcriptional, posttranscriptional, and posttranslational modifications. Disruptions in the circadian rhythms of *Per1* and *Bmal11* have been linked to various physiological and pathological conditions. For instance, circadian misalignment and disruptions in circadian rhythms have been associated with an increased risk of various diseases, including cancer. [30-33] Concentration 100 ng/ml of Metadichol maintains normal levels of *Perl1* and *Bmal1*.This is crucial for the proper functioning of the circadian rhythm, and disruptions in circadian rhythm expression can have significant implications for human health.

Ppargc1a is a transcriptional coactivator that plays a critical role in the maintenance of energy metabolism. Pgc-1alpha has been found to interact with a gene regulatory network of the circadian clock.34 Pgc-1alpha stimulates the expression of clock genes, notably *Bmal1* (*Arntl*) and *Rev-Erb alpha* (Nr1d1), through coactivation of the *ROR* family of orphan nuclear receptors. [35] *Ppargc1a*, *Bmal1*, and Clock are interconnected in regulating circadian rhythm and metabolic processes. *Bmal1* and *Clock* have been predicted to be significant upstream regulators, and they function as a heterodimer (*Bmal1:Clock*) that binds the enhancer box (E-box) regulatory element, regulating the transcription of the Period (Per) and Cryptochrome (*Cry*) genes, which form the negative arm of the circadian clock.

It is highly unlikely that metadichol can directly activate these transcription factors. This happens through upstream genes. Previously, we showed that metadichol is expressed on all 48 nuclei. Table 7 shows the fold changes in nuclear receptor levels induced by metadichol in fibroblasts. [36]. Table 7 shows the fold increases in the levels of the nuclear receptors *RXR, RORα, NR1F1, NR1D1 (REV-ErBα)* and *HNF4A* in fibroblasts. The highest expression occurred between 1 picogram and 1 nanogram per ml.

Table 7: Metadichol Nuclear receptor Fold increase in Fibroblasts

Common Names	IUPAC Name	1 pg	100 pg	1 ng	100 ng
Rev-ErbAα	NR1D1	1.5	0.64	0.07	0.75
RORα	NR1F1	0.9	1.14	1.33	1.01
HNF4A	NR2A1	6.03	3.4	2.69	3.64
RXRA	NR2B1	2.5	0.86	1.32	0.98
RXRB	NR2B2	**2.68**	1.3	1.18	1.1
RXRG	NR2B3	**2.84**	2.95	3.9	1.09
PPARG	NR1C3	3.76	**6.11**	7.31	3.07

The expression of the *Clock* gene is regulated [37] by the nuclear receptor *RORα* (retinoic acid receptor-related orphan receptor alpha) and transcriptional coregulator receptor interacting protein 140 (RIP140).

RORα is involved in a secondary feedback loop that regulates the *Clock* gene, and RIP140 has been identified as a modulator of *Clock*, participating in a feedback mechanism affecting the circadian clock.

Additionally, the nuclear receptor *NR1D1* (nuclear receptor subfamily 1 group D member 1) is involved in regulating the expression of target genes, including the *Clock* gene. *RORα* and *NR1D1* play roles in the expression of the *Clock* gene. [38]

The nuclear receptor responsible for the expression of the *Cry1* gene is *HNF4A*. [39] *HNF4A* (*NR2A1*) is critical for

circadian rhythm maintenance and period regulation in liver and colon cells. It acts differently from other *Clock:Bmal1* repressors and binds to *Cry* proteins, thereby inhibiting *Clock:Bmal1* via a mechanism independent of *Cry1*. On the other hand, the nuclear receptor *REV-ERBα*, also known as *NR1D1*, is involved in the regulation 40 of the Bmal1 gene, which is part of the core circadian clock. Although *REV-ERBα* regulates the expression of *Bmal1*, it does not directly regulate the expression of the *Cry1* gene. [41] *HNF4A* is likely the nuclear receptor responsible for the expression of the *Cry1* gene.

The nuclear receptor responsible for the expression of the *BMAL1* gene is retinoid-related orphan receptor α (*RORα*), also known as *NR1F1*. *RORα* stimulates transcription through the *BMAL1* promoter, thereby regulating the expression of *the* BMAL1 gene.[42] This regulation is part of the complex network of interactions involved in the circadian clock, where *RORα* plays a key role in activating the transcription of clock genes, including Bmal1. Therefore, *RORα* is the nuclear receptor responsible for the expression of the *Bmal1* gene.

The nuclear receptors responsible for the expression of the *Ppargc1a* (PGC-1α) gene are the retinoid X receptor (*RXR*) and peroxisome proliferator-activated receptor gamma (*Ppar-γ*). Pgc-1α binds to *Ppar-γ* and coactivates *Ppar-γ* to stimulate the transcription of genes involved in various metabolic processes, including mitochondrial biogenesis and energy metabolism. [43] The interaction between Pgc-1α and *Pparγ* is essential for regulating genes related to energy metabolism and other physiological functions. Therefore, *Pparγ* and its coactivator *PGC-1α* are crucial for regulating various metabolic pathways. Thus, the effect of metadichol on nuclear receptor [36] leads to the regulation of circadian gene expression.

Metadichol also induces the expression of *Sirtuin* 1 and 6 in fibroblasts. [44] The fold increases are shown in Table 8. Sirtuins are a family of NAD+-dependent protein deacetylases that play a crucial role in various cellular processes, including aging, metabolism, and the regulation of circadian rhythms. Sirtuins, particularly *Sirt1*, have been shown to interact with components of the circadian clock machinery directly, influencing the regulation of circadian rhythms. [45]

Table 8: Metadichol and Sirt1 and Sirt6-fold expression

Gene	1 pg	100 pg	1 ng	100 ng	Control
Sirt1	3.29	2.30	3.48	**6.92**	1.00
Sirt6	2.19	1.97	3.01	**4.26**	1.00

While *Sirt1* is the most studied sirtuin in the context of circadian rhythms, other members of the sirtuin family also contribute to circadian regulation. Sirt6 has been identified as a critical regulator of circadian transcription, suggesting that it also serves as an interface between the circadian clock and cellular metabolism.[46]

Sirt6 may also interact with Pgc-1α, a coactivator that regulates genes involved in energy metabolism. [47] This interaction is important for maintaining metabolic balance, which is often disrupted in cancer cells. [48,49]

The involvement of sirtuins in circadian regulation has significant implications for understanding the pathophysiology of various diseases, including metabolic disorders, aging-related diseases, and cancer. Disruptions in circadian rhythms have been linked to a range of health issues, and the role of sirtuins in this process highlights their potential as therapeutic targets. [50-63]

Sirt1 and *Sirt6* play a critical role in regulating circadian rhythms through their interactions with core clock components and modulation of metabolic processes. Their activity influences the stability and function of circadian proteins, aligning physiological processes with the environmental day–night cycle. This intricate relationship between sirtuins and the circadian clock underscores the importance of these proteins in maintaining cellular and organismal homeostasis.

Conclusions

Our findings show that metadichol, a small molecule, induces the expression of transcription factors such as *Clock, Cry1, Per1, Bmal1, Ppardc1a, and Arntl* is significant for several reasons. These genes have important role in and could be of therapeutic use in hypertension, insulin resistance aging and cancer.

Metadichol has been shown to regulate the expression of genes implicated in viral infections [64] and by blocking the entry of SARS-CoV-2 into host cells, indicating a potential function in antiviral responses It also expresses Yamanaka factors [65] in a variety of cell types which can lead to potential applications in stem cell therapy and regenerative medicine. Metadichol is nontoxic [66-68] and has been commercially accessible for a decade. Metadichol proven ability to regulate the expression multitude of transcription factors like Sirtuins, toll receptors

can potentially lead to a wide range of applications in cancer, neurology, chronic disorders, infectious diseases, and regenerative medicine.

Conflict of interest

The author is the founder and CEO of Nanorx, Inc., a privately held company.

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https://doi.org/10.1101/2024.02.21.581403
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