Metadichol® is a nano lipid emulsion that expresses all 48 nuclear receptors in stem and somatic cells

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Research Article

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EDITORIAL NOTE:

10 October, 2023 Editorial note (correction). This submission initially contained a declaration of no competing interests for this submitted work; however, the author has now provided a corrected competing interest declaration as follows: The author, PR Raghavan, is the CEO of Nanorx Inc., the manufacturer of the Metadichol® supplement mentioned in this work, and, as such, has professional and financial interests related to research outcomes of this supplement.

Abstract

Human nuclear receptors (NRs) involve 49 ligand-dependent transcription factors that are important for regulating the cell cycle and processes. There are many literature references to work on NR expression in many organs, abnormal cells, and tissues. However, a simple universal method to study the expression of NR is still missing. Here, we present systematic profiling of NRs in human umbilical cord stem cell lines and assess the expression of the 48 human NRs by quantitative real-time (qRT)-PCR using Metadichol, a nanoemulsion made of natural lipid alcohol. Metadichol-treated umbilical cord cells and fibroblasts, where all cells expressed NRs at a concentration range of 1 pg-100 ng/mL in a dependent manner, were detected by qRT-PCR and qualified by Western blotting. This method will allow the study of many organs and tissues and expand our understanding of the role of NRs and their role in mitigating diseases.

Highlights

Metadichol treatment of somatic cells leads to nuclear receptor expression

Depending on the cell and concentration of Metadichol used, fold changes are different

The method could be used to study many cells and disease cells to better understand NR expression patterns and implications.

The results suggest that Metadichol is a universal ligand to all nuclear receptors.

Introduction

Nuclear receptors (NRs) are transcription factors usually activated by small lipophilic molecules. (McEwan and Kumar, 2015). The 49 AHR (aryl hydrocarbon receptor) NRs, which are highly conserved in the human genome, are subdivided into seven subfamilies based on amino acid sequences (NOR, NR1, NR2, NR3, NR4, NR5, and NR6) (Frigo et al., 2021). Many NRs are classified as orphan receptors because they lack a specific ligand for activation (Sladek, 2011). Unliganded NRs are bound to heat shock protein 90 (HSP90) and can be found in the cytosol and nucleus. Receptors may also bind to corepressors without ligands (Li et al., 2013).

1,25-dihydroxyvitamin D3 is a small molecule that can diffuse through the cell membrane and bind to NRs located in the cytosol or nucleus of the cell (Pike and Meyer, 2010). This binding leads to several downstream events that eventually result in up- or downregulation of gene expression. The NR ligands exhibit a broad spectrum of full, partial, inverse agonist, or antagonist activities. Selective NR modulators activate only a subset of the functions induced by the ligand or act in a cell-type-selective manner (Burris et al., 2013).

Chromatin plays a crucial role in the actions of NR by modulating interactions with regulatory elements in the genome. However, when receptor binding occurs, chromatin changes that impact receptor signaling (Biddie and John, 2014). NRs bind to sDNA sequences, leading to modification of the chromatin structure. The final outcome leads to modulation of RNA polymerase activity resulting in increased or decreased transcription. transcription (Zaret and Yamamoto, 1984). Chromatin modification leads to pathways that impact NR action in various cells. Chromatin remodeling leads to numerous processes that involve pluripotency, cellular differentiation, inflammation, DNA damage and repair and tumor suppression (Xie et al., 2009).

NR regulation and its tissue-expression profile along with the associated cofactors are necessary to separate desirable therapeutic efficacies from undesirable side effects. One of the least studied areas is the regulation of NR ligands by NRs themselves.

Chang-Qing Xie et al. reported NR expression profiles in human and mouse embryonic stem cell lines and during their early differentiation into embryoid bodies (Klemm et al., 2019). The expression of the 48 human and mouse NRs was assessed by quantitative real-time (qRT)-PCR. Hong et al. evaluated the expression of the receptors for estrogen, progesterone, and glucocorticoids (2004). Very little is known about systemic NR expression in human cell lines. In this research, we present the specific roles of NRs in human cell lines by characterizing the RNA and cDNA expression profiles of the NR superfamily while treating stem cells and fibroblasts with metadichol, which is a nanoemulsion of long-chain alcohols (Raghavan, 2015), using qRT-PCR and Western blotting methods.

Methods

The methods, including qRT-PCR, Western blotting, and cell culture, were carried out by a commercial service provider—Skanda Life sciences, Bangalore, India.

The procurement of chemicals and reagents was as follows. Human mesenchymal stem cells and normal human dermal fibroblast cells were procured from ATCC (USA). Primary antibodies were from either ABclonal, Woburn, (both in Massachusetts, USA) or Elabscience, Maryland, USA. The primers were from Sahagene, Hyderabad, India. Other molecular biology reagents were from Sigma-Aldrich, India.

Cell maintenance and seeding

We preserved the cells in a suitable medium with 1% antibiotics in a wet atmosphere of 5% CO_2 at 37 °C. We changed the medium every two days until the cells reached confluency, and we assessed their viability using a hemocytometer.

At 70%-80% confluency, we prepared and seeded single-cell suspensions containing 10^6 cells/mL in six-well plates at a density of 10^6 cells/well. We incubated the cells for 24 h at 37 °C in 5% CO_2, rinsed the cell monolayer with serum-free medium and treated it with Metadichol at preset concentrations.

Cell treatment

We prepared Metadichol in serum-free media at different concentrations (1 pg/mL, 100 pg/mL, 1 ng/mL, and 100 ng/mL) and then added them to predesignated wells. Note that the control cells were drug-free. We incubated the cells for 24 h. Next, we washed them gently with sterile PBS. We isolated RNA using TRIzol following the manufacturer's instructions and prepared cDNA followed by the analysis of several biomarkers using qPCR and Western blotting.

RNA isolation

We separated total RNA using TRIzol reagent (Invitrogen) and collected approximately 10^6 cells in 1.5 mL microcentrifuge tubes. The cells were centrifuged at 5,000 rpm for 5 min at 4 °C, and the supernatant was removed. Next, we added 650 µL of TRIzol to the pellet and mixed the solution to be incubated on ice for 20 min. Subsequently, we added 300 µL of chloroform and mixed the samples well by gentle inversion for 1–2 min and then incubated them on ice for 10 min. We centrifuged the samples at 12,000 rpm for 15 min at 4 °C and carefully transferred the upper aqueous layer to a new sterile 1.5 mL centrifuge tube, to which we added an equal amount of prechilled isopropanol and incubated the samples at −20 °C for 60 min. Afterward, we centrifuged the mixture at 12,000 rpm for 15 min at 4 °C and prudently removed the supernatant. The RNA pellets were retained and washed with 1.0 mL of 100% ethanol, followed by 700 µL of 70% ethanol via centrifugation as described previously after each step. We air-dried the RNA pellets at room temperature for almost 15–20 min and then resuspended them in 30 µL of DEPC-treated water. We quantified the RNA concentration using a SpectraMax i3x - SpectraDrop Micro-Volume Microplate (Molecular Devices, USA) and synthesized cDNA using reverse transcription-PCR.

cDNA synthesis

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

Primers and qPCR

The PCR blend (final volume of 20 µL) contained 1 µL of cDNA, 10 µL of SYBR green Master Mix, and 1 µM complementary primers specific for the particular target genes. We ran the samples at the following settings: primary denaturation at 95 °C for 5 min followed by 30 cycles of a secondary at 95 °C for 30 s, then hardening at the optimized temperature for 30 s, with an extension at 72 °C for 1 min. We identified the number of cycles that allowed amplification in the exponential range without reaching a plateau as the optimal number of cycles. We evaluated the results using CFX Maestro software and computed the fold change via the following equation.

ΔΔCT method

We determined the relative expression of the target gene in relation to the housekeeping gene (β-actin) and untreated control cells by the comparative CT method.

The ΔCT for each treatment was calculated using the formula:

ΔCT = CT (target gene) – CT (reference genes).

To obtain a ΔΔCT, we subtracted the individual samples between the treated and control groups as follows:

ΔΔCT = ΔCT (treatment group) – ΔCT (control group).

Similarly, we calculated the fold change in the target gene expression for each treatment using the formula.

Fold change = 2^ (-ΔΔCT)

Protein isolation

We isolated total protein from 10^6 cells using RIPA buffer supplemented with the protease inhibitor PMSF. We applied a mild inversion for 30 min at 4 °C to lyse the cells and then centrifuged them at 10,000 rpm for 15 min. Finally, we transferred the supernatant to a fresh tube and determined the protein concentration using the Bradford method, where 25 µg of protein was mixed with 1X sample loading dye containing SDS and loaded on a gel. Under denaturing conditions, we separated the proteins using Tris-glycine running buffer.

Western blotting

We transferred the proteins to methanol-activated PVDF membranes (Invitrogen, USA) using a Turbo transblot system (Bio Rad, USA). We blocked the membranes with 5% BSA for 1 h and incubated them with the respective primary antibody overnight at 4 °C followed by a species-specific secondary antibody for 1 h at room temperature. We rinsed the blots and incubated them with ECL substrate (Merck, USA) for 1 min in the dark and captured the images at suitable exposure settings using a ChemiDoc XRS system (Bio Rad, USA).

Table 1 List of primers

Gene	Primers		Base pairs
NR1C2/PPARD	F	CCTTCTCAAGTATGGCGTGC	226
	R	GATGGCCGCAATGAATAGGG	
RXRG	F	CAGGAAAGCACTACGGGGTA	254
	R	CCTCACTCTCAGCTCGCTCT	
PPARG	F	AGAAGCCTGCATTTCTGCAT	236
	R	TCAAAGGAGTGGGAGTGGTC	
NR2F1	F	CATTTTTGGGCGATCTCCAGG	261
	R	GCCTTCTTCTTTCGGGAGGT	
HNF4A	F	ACTGCCACGTACCTGTGCCT	274
	R	AGGCATGCGAGTTGTGACCA	
HNF4G	F	AGCTGGCATATCTCAGCTGGC	185
	R	AACACCTGGCTGGCAATCGG	
NR2F2	F	CTCAACTGCCACTCGTACCT	253
	R	TCAACACAAACAGCTCGCTC	
NR1I3	F	CAGCAAACACCTGTGCAACT	189
	R	TGCGAAGTGTGTGACCAGAG	
NR1H4	F	AACAGAACAAGTGGCAGGTC	201
	R	AGAGTCTCAGCTGGCATACG	
ESR1	F	GATGTGGGAGAGGATGAGGA	165
	R	TCAGGCATGCGAGTAACAAG	
ESR2	F	TTCAGCCTGTGACCTCTGTG	178
	R	CTTGGTTTGTCCAGGACGTT	
ESRRA	F	CAGGGGAGCATCGAGTACAG	303
	R	CTTCTCAGGCTCAACCACCA	
NR1D2	F	AGTTCTTCCAGCTCAGCCTC	226
	R	TTGTCATCCCAGGTGCACTC	
ESRRB	F	CTTGGTTTGTCCAGGACGTT	264
	R	TTTTCCATCATGGCTTGACA	
NR5A2	F	TCCAGCTTCCAGGCAGCCTC	234
	R	GATCTGTGAATCTGCGTT	
NR3C2	F	CTGCCTCGTTTCCCTTTTCC	231
	R	CCATGATCTGTGCGTTCCTG	
NR0B2	F	GCTGTCTGGAGTCCTTCTGG	164
	R	CTGGGTATGAATCCCAGCAC	
ESRRG	F	GACTTGACTCGCCACCTCTC	174
	R	GTGGTACCCAGAAGCGATGT	
NR4A2	F	CCGGTGTCTAGTTGCCAGAT	275
	R	ACGCCGTAGTGTTGGCAG	
NR2F6	F	GGACTCTGGCTTCTCTCCTC	187
	R	TAGGGGTGCTGAGGAACAAG	
NR6A1	F	GAGGAACAGGTGCCAGTACT	175
	R	GGCCTCTTCCTCAAACTCCT	
THRB	F	GCCTCCAATAGCTCCAGGAT	201
	R	CACCCAGTTCCAGGATTCCT	
VDR	F	GACGCCCACCATAAGACCTA	247
	R	AGATTGGAGAAGCTGGACGA	
NR1H2	F	CCTCCTGAAGGCATCCACTA	261
	R	GAACTCGAAGATGGGGTTGA	
NR1D1	F	AGGCAGCAAGCAAGCAGT	291
	R	ACAGCGCATCCTTCCCCATA	
NR2C1	F	CCCAAGGCAAGCAGTTCATT	157
	R	GCAGACAGATCAGGAGTGGT	

NR2C2	F	TCACCACCTCAGACAACCTC	164
	R	ACTGACAGCCCCATAGTGAC	
NR1I2	F	AACGCAGATGAGGAAGTCGG	103
	R	AGCCCTTGCATCCTTCACAT	
NR4A3	F	GCCCAATATAGCCCTTCCCC	224
	R	TGCATTTGGTACACGCAGGA	
NR3C1	F	CTTGCATATTTGTGCCTTCA	174
	R	CTTGATGATTTGTGTTGTGC	
AR	F	GGGGCTAGACTGCTCAACTG	169
	R	GCCAAGTTTTGGCTGAAGAG	
NR0B1	F	CAGAGGCCAGGGGGTAAAG	137
	R	TGCGCTTGATTTGTGCTCGT	
PGR	F	ACATGGTAGCTGTGGGAAGG	198
	R	GCTAAGCCAGCAAGAAATGG	
RORA	F	TCGAACCAGTAGAAACCGCT	219
	R	TTGGCCGAGATGTTGTAGGT	
RORB	F	CTCACTTCTCCACCTGCTCA	212
	R	GGAGTTGGTGGCTGGGATAT	
RORC	F	AGTCGGAAGGCAAGATCAGA	204
	R	CAAGAGAGGTTCTGGGCAAG	
NR2E3	F	GGAGTCCAACACTGAGTCCC	289
	R	GGCCATGAAGAGTAGGCGAG	
NR5A1	F	AGGCACCAGGGAAGATCA	241
	R	TGCCAGGCCAGGGAATACA	
NR2E1	F	CAAGTGGGCTAAGAGTGTGC	158
	R	CGTTCATGCCAGATACAGCC	
NR4A1	F	GCCAATCTCCTCACTTCCCT	202
	R	CAGCAAAGCCAGGGATCTTC	
RARA	F	GTGTCACCGGGACAAGAACT	146
	R	CGTCAGCGTGTAGCTCTCAG	
RXRA	F	CTCTGTTGTGTCCTGTTGCC	155
	R	CTTCTCCCTTTGCGTGTTCC	
PPARA	F	CTGTCTGCTCTGTGGACTCA	247
	R	AGAACTATCCTCGCCGATGG	
RARB	F	GGTTTCACTGGCTTGACCAT	216
	R	GGCAAAGGTGAACACAAGGT	
AHR	F	GGTTTCACTGGCTTGACCAT	274
	R	CAGAGGACCAAATCCAGCAT	
RARG	F	GAAGACCGCGACACAACTTCC	180
	R	GTTGAGTTAAGACATGAGGG	
RXRB	F	GCAGGAGTAGGAGCCATCTT	188
	R	GCATACACTTTCTCCCGCAG	
THRA	F	ACCTCCATCCCACCTATTCC	242
	R	CTCTTCAGGAGTGGGCTCTG	
NR1H3	F	GAGATCCTCCCGTGGCATTA	151
	R	GAGAACCCTGTGCAAAGTGG	

F, forward; R, reverse

Table 2 Metadichol and Human Mesenchymal Stem Cells

Metadichol concentrations	1 pg	100 pg	1 ng	100 ng	
Common name					Nomenclature name
DAX1	0.19	0.33	0.38	0.11	NR0B1
SHP	1.39	0.75	1.06	0.29	NR0B2
TRα	16.16	12.24	7.7	5.32	NR1A1
TRβ	7.71	1.94	15.11	8.71	NR1A2
RARα	1.27	0.79	0.52	0.44	NR1B1
RARB	1.67	1.39	0.48	0.73	NR1B2
RARγ	2.52	1.04	0.96	0.82	NR1B3
PPARα	4.66	3.48	3.92	1.35	NR1C1
PPAR-β/δ	3.74	4.5	4.5	0.44	NR1C2
PPARG	1.82	1.7	1.03	1	NR1C3
Rev-ErbAα	1.93	1.29	0.8	0.6	NR1D1
Rev-ErbAβ	0.89	0.47	0.35	0.15	NR1D2
RORα	1.77	1.39	0.94	0.67	NR1F1
RORβ	0.81	0.84	0.7	0.33	NR1F2
RORγ	0.52	0.74	1.19	1.08	NR1F3
LXRB	1.28	0.97	0.55	0.19	NR1H2
LXRα	1.28	1.17	0.84	0.18	NR1H3
FXR	1.98	1.09	0.53	0.6	NR1H4
VDR	2.03	0.92	3.67	0.54	NR1I1
PXR	0.6	0.74	1.11	0.39	NR1I2
CAR	8.03	1.49	2.91	10.61	NR1I3
HNF4A	0.99	0.72	0.51	0.13	NR2A1
HNF4γ	1.39	1.51	0.36	0.26	NR2A2
RXRA	1.4	1.21	0.99	0.79	NR2B1
RXRB	1.87	1.13	1.05	0.69	NR2B2
RXRG	2.15	2.2	1.5	0.76	NR2B3
TR2	1.3	1.27	0.74	0.39	NR2C1
TR4	1.6	1.5	0.74	0.51	NR2C2
TLX	0.95	1.37	1.18	0.57	NR2E1
PNR	2.18	1.23	1.51	1.25	NR2E3
COUP-TFI	1.78	1.57	1.04	0.65	NR2F1
COUP-TFII	1.81	1.48	1.15	1.07	NR2F2
EAR-2	0.98	0.95	0.43	0.08	NR2F6
ERα	1.86	1.17	1.94	0.4	NR3A1
ERβ	1.81	1.37	1.09	0.66	NR3A2
ERRα	1	0.88	0.59	0.35	NR3B1
ERRβ	1.11	2.32	1.45	1.36	NR3B2
ERRγ	1.84	1.02	0.55	0.18	NR3B3
GR	0.99	0.86	0.82	0.09	NR3C1
MR	1.15	0.78	0.52	0.21	NR3C2
PR	1.19	0.94	0.67	0.12	NR3C3
AR	1.15	0.37	0.11	0.33	NR3C4
NGFIB	1.82	0.67	1.16	0.61	NR4A1
NURR1	1.06	0.61	0.45	0	NR4A2
NOR1	5.43	1.89	0.35	0.5	NR4A3

SF1	3.2	2.56	1.92	5.09	NR5A1
LRH1	1.3	0.72	0.29	0.15	NR5A2
GCNF	1.7	0.86	0.65	0.15	NR6A1
AHR	0.39	0.58	0.79	0.51	AHR

Table 3 Metadichol and normal human dermal fibroblasts

Metadichol concentrations	1pg	100pg	1ng	100ng	
Common Name					Nomenclature
DAX1	1.76	0.14	0.44	0.24	NR0B1
SHP	No Detectable expression	No Detectable expression	No Detectable expression	No Detectable expression	NR0B2
TRα	1.2	0.65	0.55	0.69	NR1A1
TRβ	1.15	1.18	2.4	0.92	NR1A2
RARα	2.15	1.81	0.63	1.06	NR1B1
RARB	2.68	1.3	1.18	1.1	NR1B2
RARγ	2.84	2.95	3.9	1.09	NR1B3
PPARα	1.9	1.24	0.72	1.8	NR1C1
PPAR-β/δ	2.48	2.59	2.83	0.84	NR1C2
PPARG	3.78	6.11	7.31	3.07	NR1C3
Rev-ErbAα	1.5	0.64	0.07	0.75	NR1D1
Rev-ErbAβ	2.19	1.18	1.8	1.82	NR1D2
RORα	0.9	1.14	1.33	1.01	NR1F1
RORβ	2.71	0.99	0.69	0.88	NR1F2
RORγ	1.86	1.02	0.91	1.04	NR1F3
LXRB	4.95	1.03	0.03	1.71	NR1H2
LXRα	1.63	1.79	3.84	0.71	NR1H3
FXR	2.69	1.32	1.27	0.87	NR1H4
VDR	1.83	2.34	2.35	1.54	NR1I1
PXR	1.81	0.37	0.97	1	NR1I2
CAR	0.98	0.67	0.49	0.53	NR1I3
HNF4A	6.03	3.4	2.69	3.64	NR2A1
HNF4γ	2.15	1.39	1.2	1.95	NR2A2
RXRA	2.5	0.86	1.32	0.98	NR2B1
RXRB	4.21	1.65	1.03	2.7	NR2B2
RXRG	2.84	2.95	3.9	1.09	NR2B3
TR2	1.08	1.16	1.7	0.85	NR2C1
TR4	3.66	1.38	1.09	4.17	NR2C2
TLX	3.38	1.49	1.69	0.88	NR2E1
PNR	1.43	1.46	2.48	1.27	NR2E3
COUP-TFI	0.16	0.9	4.18	0.5	NR2F1
COUP-TFII	1.05	1.19	2.98	0.59	NR2F2
EAR-2	2.84	1.22	1.66	1.02	NR2F6
ERα	2.42	1.58	0.73	2.02	NR3A1
ERβ	3.04	3.04	1.25	1.52	NR3A2
ERRα	10.31	8.3	6.58	11.1	NR3B1
ERRβ	0.92	0.74	0.48	1.76	NR3B2
ERRγ	0.81	1.07	1.86	0.45	NR3B3
GR	1.71	0.14	0.77	0.45	NR3C1
MR	3.7	0.26	1.04	0.42	NR3C2
PR	0.71	1.25	2.19	0.51	NR3C3
AR	2.54	0.15	0.57	0.11	NR3C4
NGFIB	2.36	1.55	0.93	3.42	NR4A1
NURR1	3.56	6.02	8.63	7.62	NR4A2

NOR1	**2.14**	0.72	1.48	1.59	NR4A3
SF1	**27.27**	13.78	13.28	2.69	NR5A1
LRH1	**3.32**	1.79	1.74	2.02	NR5A2
GCNF	**2.46**	0.83	0.39	2.19	NR6A1
AHR	**10.17**	4.32	3.79	1.52	AHR

The highlighted cell in yellow shows the highest expression recorded at 1 picogram/ml.

Discussion

Assuming a threshold of + or - 50% for the fold increase/decrease, we obtained 25 NRs that were unregulated with stem cells and 36 with fibroblasts at a 1 pg concentration. SF1 (NR5A1) showed an increase of 27-fold in fibroblasts and 5-fold in stem cells.

NR5A1 guides somatic cells to fetal Sertoli cells (Liang et al., 2019; Li et al., 2013). The latter are vital nurse cells located in the testis that aim at controlling spermatogenesis and set the immune-privileged environment of the blood-testis barrier required for the growth of male germ cells. Sertoli cells also secrete cytokines and growth factors; therefore, they control immune processes that defend germ cells from immunological attack (Rotgers et al., 2018; Kaur et al., 2014; Kaur et al., 2015). Additionally, they aid in blocking the multiplication of T, B, and NK cells. These findings are deployed in lessening the immune response in cell transplantation in diabetes and neurogenerative diseases, skin implants, and other illnesses (Mital et al., 2010). However, defects in Sertoli cells can cause infertility.

Alternately, Oct4, a pluripotent transcription factor, is involved in the maintenance of self-renewal in stem cells as well as somatic cells (Luca et al. 2018; Yang et al., 2007). In this context, SF1 is critical in regulating the transcription of Oct4. However, NR5A2 (LRH-1) can program somatic cells, leading to iPSCs, and thus can replace Oct4i and result in a better yield of iPSCs (Heng et al., 2010).

We did not detect SHP (NROB2) expressed in fibroblasts and downregulated in stem cells. Huang et al. provided a possible explanation, which proved that SHP gene transcription is constantly induced by the expression and activation of nuclear factor-erythroid 2 p45-related factor 2 (NRF2), which encodes NFE2l2 (Heng et al., 2010). In addition, NRF2 is key in controlling the negative effects of electrophilic and oxidative stress (Xue D et al., 2020) and does so by activating the expression of a wide array of antioxidant response genes. On the one hand, applying NRF2 activators in clinical trials (Yagishita et al, 2020) could prevent cancer and treat some diseases related to oxidative stress; on the other hand, integral triggering of NRF2 in many cancers can lead to the survival and proliferation of tumor cells, as well as resistance to anticancer therapy (Dornas et al, 2019). In this review, we presented the NRF2 signaling pathway, discussed its role in carcinogenesis, and introduced the inhibition of NRF2 by NRs. In this context, (Namani et al. 2014) revealed that the NRF2 signaling pathway can be regulated and inhibited with NRs such as RARα, RXRα, PPARγ, ERα, ERRβ, and GR (Raghavan, 2022). Since these are expressed by both stem cells and fibroblasts, SHP is expected to be downregulated in stem cells and not expressed in fibroblasts. The expression of AHR is increased in skin fibroblasts (10-fold increase) compared to stem cells (60% downregulated). In fact, AHR (Nakatsuji et al, 2019) is involved in the function of the skin as the first barrier against many pathogens and environmental threats. Moreover, AHR controls (Roztocil et al 2020) immune-mediated skin reactions and many physiological functions by acting as a sensor that mediates environment–cell interactions, predominantly during immune and inflammatory responses. Consequently, AHR plays a crucial role in skin integrity and immunity (Rothhammer et al 2019) in both homeostasis and disease. This might explain the reason behind the enormous growth of AHR expression.

To further understand the direct interactions among NRs, we analyzed gene networks using Pathway Studio (Huang et al., 2010; Fernández-Gallego et al., 2021). Nuclear receptor interaction maps showed the presence of a feedback-loop network, as shown in Figure 1. The 25 most significantly enriched cell processes regulated by the gene set ($p<E-9$) are shown in Table 4. A complete list of processes regulated by the gene subsets is available in the supplementary material.

Table 4; Cell processes regulated by the nuclear receptors

Cell processes regulated by entities enriched in the input	Total # of Neighbors	Gene Set Seed	Overlap	Percent Overlap	Overlapping Entities
Protein regulators of energy homeostasis	1185	energy homeostasis	39	3	PPARD,RXRG,PPARG,HNF4A,NR2F2,NR1I3,NR1H4,ESR1,ESR2,ESRRA,NR1D2,ESRRB,NR5A2,NR3C2,NR0B2,ESRRG,NR4A
Protein regulators of gene repression	737	gene repression	33	4	PPARD,PPARG,NR2F1,HNF4A,NR2F2,NR1H4,ESR1,ESR2,NR1D2,NR5A2,NR3C2,NR0B2,NR2F6,NR6A1,THRB,VDR,NR1D1,
Protein regulators of transcription activation	3198	transcription activation	48	1	PPARD,PPARG,HNF4A,HNF4G,NR1I3,NR1H4,ESR1,ESR2,ESRRA,NR1D2,ESRRB,ESRRG,THRB,NR1H2,NR2C1,NR2C2,NR1I
Protein regulators of liver metabolism	524	liver metabolism	27	5	PPARD,RXRG,PPARG,HNF4A,NR1I3,NR1H4,ESR1,ESRRA,NR5A2,NR0B2,ESRRG,THRB,VDR,NR1H2,NR1D1,NR1I2,NR4A3,
Protein regulators of lipid homeostasis	652	lipid homeostasis	28	4	PPARD,RXRG,PPARG,HNF4A,NR1I3,NR1H4,ESR1,ESR2,ESRRA,NR1D2,NR5A2,NR3C2,NR0B2,VDR,NR1H2,NR1D1,NR2C2,
Protein regulators of lipid metabolism	2420	lipid metabolism	41	1	PPARD,RXRG,PPARG,HNF4A,HNF4G,NR2F2,NR1I3,NR1H4,ESR1,ESR2,ESRRA,NR1D2,NR5A2,NR3C2,NR0B2,ESRRG,NR6A
Protein regulators of steroidogenesis	879	steroidogenesis	29	3	PPARD,PPARG,NR2F1,NR2F2,NR1H4,ESR1,ESR2,ESRRA,NR1D2,ESRRB,NR5A2,NR0B2,ESRRG,THRB,VDR,NR1H2,NR1D1,
Protein regulators of cholesterol homeostasis	393	cholesterol homeostasis	22	5	PPARD,PPARG,NR1I2,HNF4A,HNF4G,NR3C1,AR,NR1I3,NR1H4,RORA,ESR1,NR1D2,NR5A2,NR0B2,RXRA,PPARA,THRB,AHR
Protein regulators of gluconeogenesis	1164	gluconeogenesis	30	2	PPARD,PPARG,HNF4A,HNF4G,NR1I3,NR1H4,ESR1,ESRRA,NR1D2,NR3C2,NR0B2,ESRRG,VDR,NR1H2,NR1D1,NR2C2,NR1
Protein regulators of lipogenesis	1428	lipogenesis	32	2	PPARD,RXRG,PPARG,HNF4A,HNF4G,NR1I3,NR1H4,ESR1,ESR2,ESRRA,NR1D2,NR5A2,NR3C2,NR0B2,ESRRG,NR6A1,THRB
Protein regulators of fatty acid oxidation	1046	fatty acid oxidation	28	2	PPARD,PPARG,HNF4A,NR2F2,NR1I3,NR1H4,ESR1,ESRRA,NR0B2,ESRRG,NR4A2,THRB,VDR,NR1H2,NR1D1,NR1I2,NR4A3,
Protein regulators of chromatin remodeling	1626	chromatin remodeling	32	1	PPARD,RXRG,PPARG,NR2F1,HNF4A,NR1H4,ESR1,ESR2,ESRRB,NR5A2,NR3C2,NR0B2,THRB,VDR,NR1D1,NR2C1,NR2C2,N
Protein regulators of fatty acid beta-oxidation	537	fatty acid beta-oxidation	22	4	PPARD,RXRG,PPARG,NR1I2,NR2F1,HNF4A,AR,NR1I3,NR1H4,PGR,ESR1,ESRRA,NR0B2,ESRRG,NR4A1,RARA,RXRA,PPARA,
Protein regulators of adipocyte differentiation	1455	adipocyte differentiation	30	2	PPARD,PPARG,NR2F2,NR1H4,ESR1,ESR2,ESRRA,NR1D2,NR5A2,NR3C2,ESRRG,NR4A2,NR2F6,VDR,NR1D1,NR4A3,NR3C1
Protein regulators of cell development	4071	cell development	43	1	PPARD,PPARG,NR2F1,HNF4A,NR2F2,NR1H4,ESR1,ESR2,ESRRA,NR1D2,ESRRB,NR5A2,NR3C2,NR0B2,ESRRG,NR4A2,NR2
Protein regulators of adipogenesis	2019	adipogenesis	33	1	PPARD,RXRG,PPARG,NR2F1,NR2F2,NR1H4,ESR1,ESR2,ESRRA,NR5A2,NR3C2,ESRRG,NR4A2,NR2F6,THRB,VDR,NR1D1,N
Protein regulators of	906	circadian rhythm	24	2	PPARD,PPARG,HNF4A,NR1I3,ESR1,ESRRA,NR1D2,NR3C2,NR0B2,NR4A2,THRB,NR1H2,NR1D1,NR1I2,NR3C1,RORA,RORB

circadian rhythm					
Protein regulators of lipid storage	2174	lipid storage	33	1	PPARD,PPARG,HNF4A,NR2F2,NR1I3,NR1H4,ESR1,ESR2,ESRRA,NR3C2,NR0B2,ESRRG,NR2F6,NR6A1,VDR,NR1H2,NR1D1
Protein regulators of fatty acid metabolism	733	fatty acid metabolism	21	2	PPARD,RXRG,PPARG,NR1I2,HNF4A,NR4A3,NR2F2,AR,NR1I3,NR1H4,ESR2,ESRRA,NR5A2,NR4A2,NR4A1,RXRA,PPARA,VDI
Protein regulators of cellular aging	1834	cellular aging	29	1	PPARD,PPARG,HNF4A,NR2F2,NR1H4,ESR1,ESR2,ESRRA,ESRRB,NR5A2,NR3C2,ESRRG,NR4A2,THRB,VDR,NR1H2,NR1D1,
Protein regulators of cellular senescence	1563	cellular senescence	27	1	PPARD,PPARG,HNF4A,NR2F2,NR1H4,ESR1,ESR2,ESRRA,ESRRB,NR5A2,NR3C2,ESRRG,NR4A2,THRB,VDR,NR1H2,NR1D1,

We can further refine the process of the 32 NRs involved in chromatin remodeling, which is one of the regulated cell processes. Figure 2 shows a rigid system of NRs that interrelate in chromatin remodeling. DNA of eukaryotic genomes complexed with histones forms higher-order chromatin structures, of which the basic unit is the nucleosome involving ~146 bp of DNA wrapped around a histone octamer (Nikitin et al., 2003). Chromatin has an important role in DNA replication, repair, cell division, transcription (Sivachenko et al., 2007), and the nuclear receptor-signaling axis. It affects NR action by specifying its genomic localization and interactions with regulatory elements. Consequently, chromatin acts as a regulator of selective NR interactions with DNA to drive specific transcriptional programs.

To repress or enhance transcription, NRs bind to particular DNA sequences and recruit cofactors that alter the chromatin structure, which leads to RNA polymerase actions (Hebbar and Archer, 2003; Luger et al., 1997; Lee et al., 1993). The activity of chromatin remodeling complexes is fundamental to biological processes that include pluripotency and its maintenance, cellular differentiation, inflammation, DNA damage and repair, and tumor suppression. Therefore, chromatin is an integral component of the pathways that guide NR action in a cell-type-specific and cell-state-dependent manner (Zaret and Yamamoto, 1984). Understanding the roles of cofactors and the mechanisms regulating NR actions on chromatin and transcription could provide original druggable pathways due to the emergence of epigenetic and chromatin regulators as novel targets in disease treatment.

Conclusions

To the best of our knowledge, this research is the first to show that NRs can be expressed from undifferentiated, and differentiated cells. Our procedure is generic and can be applied to study variety of cells.

Metadichol, is potentially a natural ligand for all 49 NRs. Previously we have shown that it binds to to VDR, AHR, THRA, THRB, and RORC. (Raghavan, 2015; Raghavan, 2017; Raghavan, 2019;;; Raghavan, 2017-1).

NR's are differentially expressed in the two groups of cells (stems and fibroblasts. More studies need to be carried out with different types of cells to better understand the nature of nuclear expression by treatment with Metadichol. Ongoing research based on this approach will be reported soon.

Declarations

Declaration of Interest

The author declares no competing interests.

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Figures

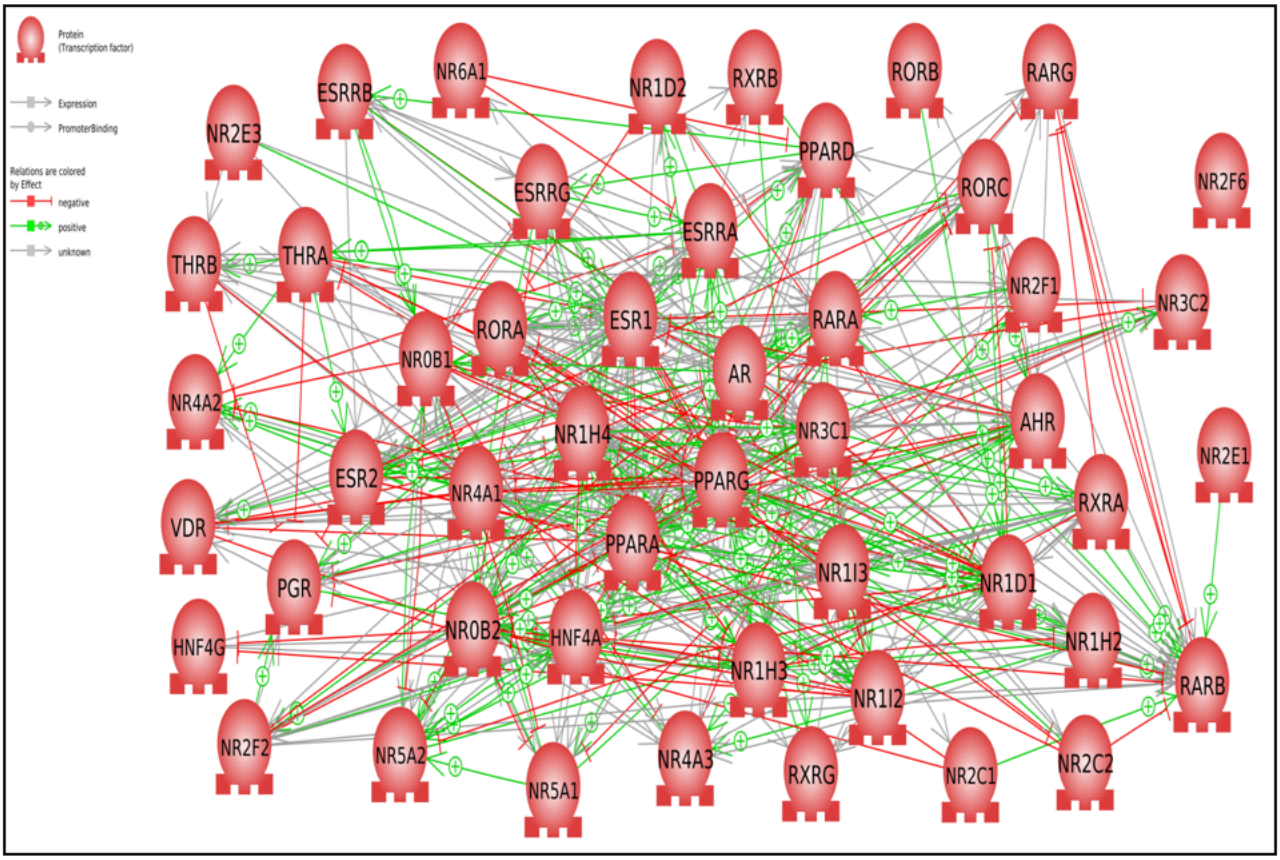

Figure 1

Feedback-loop Network Nuclear Receptor Interactions

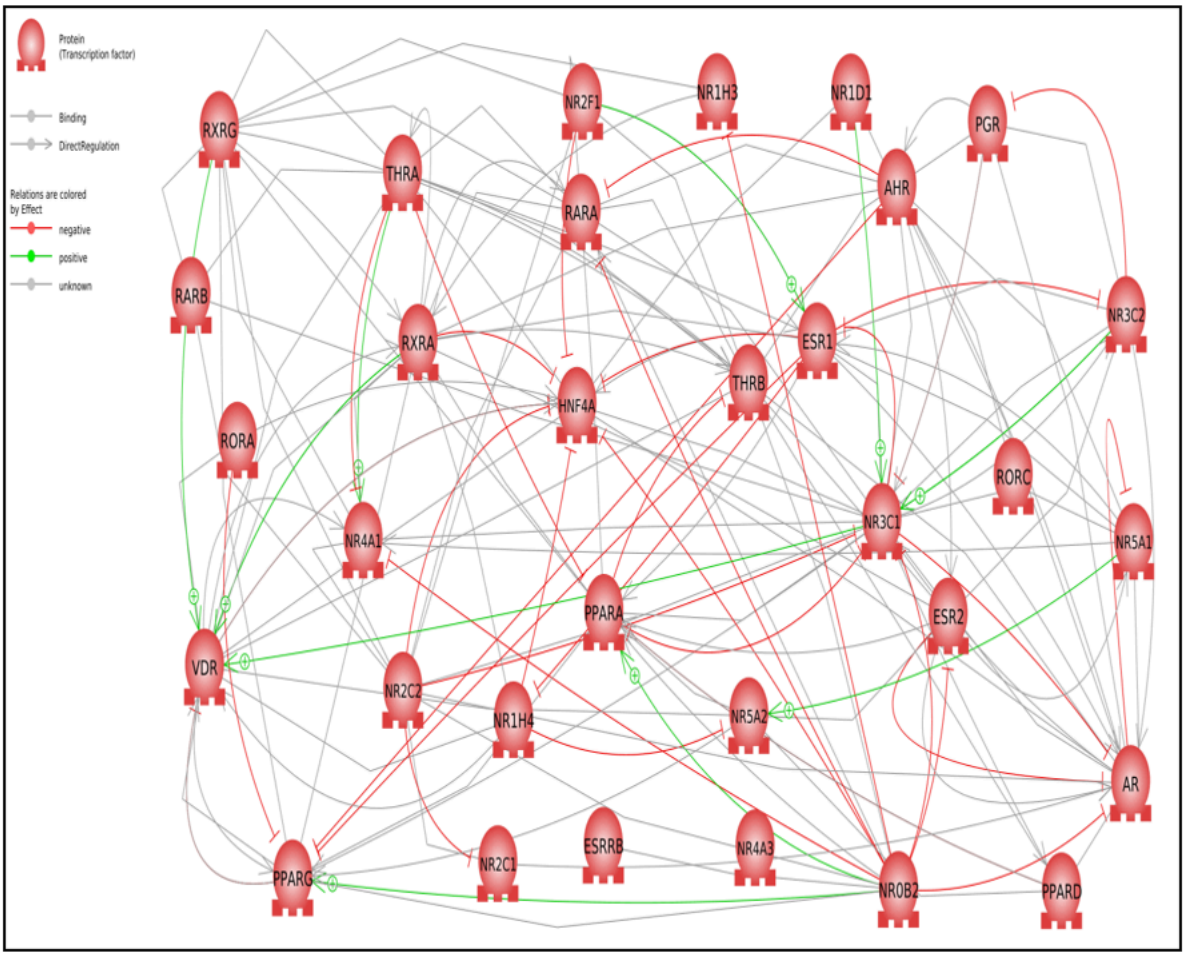

Figure 2

Network of 32 NRs interrelated in chromatin remodeling

Supplementary Files

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