Research Square

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METADICHOL: A natural nano lipid emulsion that expresses all 48 Nuclear receptors in the Stem, somatic, and Cancer cells.

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Research Article

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Abstract

The human nuclear receptor (NR) consists of 49 ligand-dependent transcription factors that play a critical role in development, metabolism, reproduction, cell cycle, differentiation, and diseases, e.g., cancer and cardiovascular disease, to name a few. Today, we have gene sequencing data on NR presence in many organs, disease cells, and tissues. We do not have a simple universal method to study the expression of NR that small molecules can induce in various cell diseased and non-diseased states. Here in we present systematic profiling of NRs in human umbilical cord stem cell lines and Expression of the 48 human NRs was assessed by quantitative real-time PCR. using Metadichol a nano-emulsion natural lipid alcohol treated Umbilical cord cells, Fibroblasts, cells expressed all the nuclear receptors at a concentration of 1 picogram per ml to 100 nanogram per ml in a concentration-dependent manner as seen by Q-RT-PCR and qualified by Western blot. This method will allow the study of many organs and tissues and expand our understanding of the role of NR in normal and disease states.

Introduction

Nuclear receptors (1) are ligand-activated transcription factors. There are 49 if including AHR (Aryl Hydrocarbon receptor) in the human genome Nuclear receptors are subdivided into seven subfamilies (NOR, NR1, NR2, NR3, NR4, NR5, and NR6) based on amino acid sequence and are highly conserved across species (2). Many of these nuclear receptors are classified as orphan receptors as they lack a specific ligand for activation (3). Nuclear receptors in their un-liganded state can be found in the cytosol and the nucleus. Unliganded nuclear receptors are bound to HSP90 (heat shock proteins). Receptors may also bind to co-repressors without ligand (4).

Small lipophilic substances like 1,25 Dihydroxy Vitamin D3 (5) can diffuse past the cell membrane and bind to nuclear receptors located in the cytosol or nucleus of the cell. The binding of Vitamin D3 leads to several downstream events that eventually result in up or down-regulation of gene expression. Nuclear receptor ligands exhibit a broad spectrum of full, partial, or inverse agonist or antagonist activities, and selective nuclear receptor modulators that activate only a subset of the functions induced by the ligand or that act in a cell-type-selective manner (6).

Chromatin plays a crucial role in nuclear receptor action (7) by controlling its genomic localization and interactions with regulatory elements. When receptor binding occurs, chromatin changes, which impacts receptor signaling. Nuclear receptors have been shown to bind to specific DNA sequences and recruit cofactors that modify the local chromatin structure, which in turn modulate the recruitment and activity of RNA polymerases to repress or enhance transcription (8). Chromatin is, therefore, a key component of pathways that impact nuclear receptor action in cell-type-specific and cell-state-dependent manners. The activity of chromatin remodeling complexes is integral to numerous biological processes, including maintenance of pluripotency, cellular differentiation, inflammation, DNA damage and repair, and tumor suppression (9)

Regulation of NRs and the tissue-expression profile of NR itself and associated cofactors are needed to separate desirable therapeutic efficacy from undesirable side effects. There is a need to understand feedback and feed forward regulation of nuclear receptor ligands by nuclear receptors themselves.

Chang-Qing Xie et al. (10) reported on NR expression profiles in human and mouse embryonic stem cell (ESC) lines and during their early differentiation into embryoid bodies. The expression of the 48 human and mouse NRs was assessed by quantitative real-time PCR. Work by Hong teal details works evaluating the expression of receptors for estrogen, progesterone, and glucocorticoids (11).

Very little is known today about systemic NR expression in human cell lines. We present research that explores the specific roles of NRs in humans cell lines by characterizing the mRNA expression profile of the NR superfamily by treating Stem cells, Fibroblasts, with Metadichol, a Nano-emulsion lipped of long-chain alcohols (12) using a high-throughput quantitative real-time PCR (QPCR) methods.

Experimental Procedures

All work was carried out by a commercial service provider, Skanda Life sciences, Bangalore, India.

Quantitative PCR (qPCR), Western blotting, and cell culture.

Chemicals and reagents

Human Mesenchymal Stem Cells (HMSC) Human Dermal Fibroblast Cells (NHDF**and** Human dermal Fibroblast cells were procured from ATCC, USA .Primary antibodies were procured form either ABclonal, Woburn Massachusssets USA and E-labscience, Maryladn USA. . Primers were from Sahagene, Hyderabad India. Other molecular biology reagents were procured form Sigma Aldrich India.

Maintenance and seeding

Cells were maintained in appropriate medium with or without the required supplements and 1% antibiotics in a humidified atmosphere of 5% CO_2 at 37 °C. The medium was changed every other day until the cells reached confluency. The viability of the cells was assessed using a hemocytometer.

When the cells reached 70-80% confluency, single cell suspensions containing 10^6 cells/ml were prepared and seeded in 6-well plates at a density of 1 million cells per well. The cells were incubated for 24 hr at 37 °C in 5% CO_2. After 24 hr, the cell monolayer was rinsed with serum-free medium and treated with Metadichol at predetermined concentrations.

Cell treatment

Metadichol was at various concentrations (1 pg/ml, 100 pg/ml, 1 ng/ml and 100 ng/ml) was prepared in serum-free media Metadichol-containing medium was added to predesignated wells. Control cells received medium without the drug. The cells were incubated for 24 hr. Post treatment; cells were gently rinsed with sterile PBS. Whole cell RNA was isolated using Trizol according to the manufacturer's instructions and cDNA was prepared followed by qPCR analysis of various biomarkers was analyzed using qPCR and Western blotting.

Quantitative real-time PCR (qRT–PCR)

RNA isolation

Total RNA was isolated from each treatment group using TRIzol reagent (Invitrogen). Approximately ~1×10^6 cells were collected in 1.5 ml micro centrifuge tubes. The cells were centrifuged at 5000 rpm for 5 min at 4 °C, and the cell supernatant was discarded. Then, 650 µl of TRIzol was added to the pellet, and the contents were mixed well and incubated on ice for 20 min. Subsequently, 300 µl of chloroform was added to the mixture, and the samples mixed well by gentle inversion for 1-2 min and incubated on ice for 10 min. The samples were centrifuged at 12000 rpm for 15 min at 4 °C. The upper aqueous layer was carefully transferred to a new sterile 1.5 ml centrifuge tube, an equal amount of prechilled isopropanol was added to the tube, and the samples was incubated at -20 °C for 60 min. After incubation, the mixture was centrifuged at 12000 rpm for 15 min at 4 °C. The supernatant was discarded carefully, and the RNA pellet was retained. The pellet was washed with 1.0 ml of 100% ethanol, followed by 700 µl of 70% ethanol via centrifugation as described above after each step. The RNA pellet was air dried at RT for approximately 15-20 min and then resuspended in 30 µl of DEPC-treated water. The RNA concentration was quantified using a Spectradrop (Spectramax i3x, USA) spectrophotometer (Molecular Devices), and cDNA was synthesized using reverse transcription PCR (RT-PCR).

cDNA synthesis

cDNA was synthesized from 2 µg of RNA using the Prime Script cDNA synthesis kit (Takara) and 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 85 °C for 5 min on an Applied Biosystems instrument (Veritii). The cDNA was used for qPCR.

Primers and qPCR

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

Fold change was calculated using the following equation.

(ΔΔ CT Method)

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

Delta CT for each treatment was calculated using the formula.

Delta Ct = Ct (target gene) – Ct (reference genes.

To compare the individual sample from treatment with untreated control delta CT of sample was subtracted from control to get a delta delta CT. Deltadelta Ct = delta Ct (treatment group) – delta Ct (control group)

Fold change in target gene expression for each treatment was calculated using the formula. Fold change = 2^ (-deltadelta Ct)

Protein isolation

Total protein was isolated from 10^6 cells using RIPA buffer supplemented with the protease inhibitor PMSF. The cells were lysed for 30 min at 4 °C with gentle inversion. The cells were centrifuged at 10,000 rpm for 15 min, and the supernatant was transferred to a fresh tube. The protein concentration was determined by the Bradford method, and 25 µg of protein was mixed with 1X sample loading dye containing SDS and loaded on a gel. The proteins were separated under denaturing conditions using Tris-glycine running buffer.

Western blotting

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

Table 1; Primer Details.

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	

Summary Of Results

Table 2; Metadichol and HMSC

Metadichol concentration	1pg	100pg	1ng	100ng	
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 Human Dermal Fibroblast Cells (NHDF)

Common Name	1pg	100pg	1ng	100ng	Nomenclature name
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

Results And Discussion

IN both stem cells and fibroblast all 49 nuclear receptors assuming a threshold of Plus minus 50% we see that 25 Nuclear receptors are unregulated with stem cells and 36 with Fibroblasts at a concentration of 1 pico gram . SF1 (NR5A1) showed increase of 27 fold in Fibroblasts at 1 and 5 fold in Stem cells.

NR5A1(13) directs somatic cells to fetal sertoli cells.(4). Sertoli cells are essential nurse cells in the testis that regulate the process of spermatogenesis and establish the immune-privileged environment of the blood-testis-barrier which is required for male germ cell development. Sertoli cells also secrete cytokines and growth factors . They also modulate immune processes that protect germ cell from immunological onslaught(14,15,16). Sertoli cells secrete factors that suppress the proliferation of T cells, B cells and NK cells This finds use in reducing immune response in cell transplantation in diabetes and neuro-generative, skin grafts and other diseases (17) . Defects in Sertoli cells can cause infertility Our in vitro induced system now provides potentially a platform for research of of male infertility caused by Sertoli cells. Also Oct4 encodes a transcription factor that is involved in the maintenance of self-renewal in stem cells as well as

somatic cells (18,19). SF-1 plays a crucial role in the regulation of hOct4 transcription. Nr5a2 (also known as Lrh-1) can replace Oct4 in the derivation of iPSCs from mouse somatic cells, and it can also enhance reprogramming efficiency (20)

SHP (NROB2) was not seen expressed with Fibroblast and down regulated in Stem Cells. A possible explanation is provided by the work Huang et al (20) who showed that forced expression and activation of Nrf2 consistently induced SHP expression, and Nrf2 was identified as a novel activator of the SHP gene transcription. Nuclear factor-erythroid 2 p45-related factor 2 (NRF2, also known as Nfe2l2) plays a critical role in regulating cellular defense against electrophilic and oxidative stress by activating the expression of an array of antioxidant response element-dependent genes. On one hand, NRF2 activators have been used in clinical trials for cancer prevention and the treatment of diseases associated with oxidative stress; on the other hand, constitutive activation of NRF2 in many types of tumors contributes to the survival and growth of cancer cells, as well as resistance to anticancer therapy. In this review, we provide an overview of the NRF2 signaling pathway and discuss its role in carcinogenesis.We also introduce the inhibition of NRF2 by nuclear receptors. Namani et al (21) showed that the NRF2 signaling pathway can be regulated Nuclear receptors such as RARα, RXRα, PPARγ, ERα, ERRβ, and GR inhibit Nrf2. Since these are expressed by both Stem and Fibroblasts it is not surprising that SHP is down regulated in stem cells and not expressed in Fibroblasts.

The skin is the first line of defense against a multitude of pathogens and environmental threats.AHR expression is increased multifold in Skin fibroblast cells (10 fold increase) compared to the stem cells (60% down regulated). AHR is an important regulator of skin barrier function. It also controls immune-mediated skin responses. The AHR modulates various physiological functions by acting as a sensor that mediates environment–cell interactions, particularly during immune and inflammatory responses. AHR is an important player in skin integrity and immunity in both homeostasis and disease. This is a likely explanation of why we see that huge increase in AHR expression.

To further understand the direct interactions between the Nuclear receptors we carried Analysis of gene networks using Pathway Studio (22,23) and protein-protein interaction maps shows the formation of a loop feedback network, as shown in Figure 1. The top 25 most highly significant (p<E-9) cell processes regulated by the gene set are shown in Table 3. A complete list of processes regulated by the subset of genes is available in the supplementary material.

Table 3

cell processes are 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,NR
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,NR1I
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,N
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,NR4A
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,NR2C
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,N
Protein regulators of steroidogenesis	879	steroidogenesis	29	3	PPARD,PPARG,NR2F1,NR2F2,NR1H4,ESR1,ESR2,ESRRA,NR1D2,ESRRB,NR5A2,NR0B2,ESRRG,THRB,VDR,NR1H2,NR1I
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,A
Protein regulators of gluconeogenesis	1164	gluconeogenesis	30	2	PPARD,PPARG,HNF4A,HNF4G,NR1I3,NR1H4,ESR1,ESRRA,NR1D2,NR3C2,NR0B2,ESRRG,VDR,NR1H2,NR1D1,NR2C2,N
Protein regulators of lipogenesis	1428	lipogenesis	32	2	PPARD,RXRG,PPARG,HNF4A,HNF4G,NR1I3,NR1H4,ESR1,ESR2,ESRRA,NR1D2,NR5A2,NR3C2,NR0B2,ESRRG,NR6A1,T
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,NR4
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,NR2C
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,PPA
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,NR3
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,N
Protein	2019	adipogenesis	33	1	PPARD,RXRG,PPARG,NR2F1,NR2F2,NR1H4,ESR1,ESR2,ESRRA,NR5A2,NR3C2,ESRRG,NR4A2,NR2F6,THRB,VDR,NR1D1

regulators of adipogenesis					
Protein regulators of circadian rhythm	906	circadian rhythm	24	2	PPARD,PPARG,HNF4A,NR1I3,ESR1,ESRRA,NR1D2,NR3C2,NR0B2,NR4A2,THRB,NR1H2,NR1D1,NR1I2,NR3C1,RORA,RO
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,NR1
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,V
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,NR1
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,NR1

One of the cell processes regulated is chromatin modeling. We can further refine the process of the 32 NR's that are involved in Chromatin modeling (see fig 2) . It shows a tight network of Nr's that interact in chromatin modelling DNA of eukaryotic genomes is complexed with histones to form higher-order chromatin structures, the basic unit of which is the nucleosome, which consists of ∼146 bp of DNA wrapped around a histone Octamer (24). Chromatin has a crucial regulatory role in DNA replication, repair, cell division, and transcription (25). Moreover, it has a vital role in the nuclear receptor-signaling axis,

and it affects nuclear receptor action by specifying its genomic localization and interactions with regulatory elements. Chromatin acts as a regulator of selective nuclear receptor interactions with DNA to drive specific transcriptional programs.

Nuclear receptors bind to specific DNA sequences and recruit cofactors that modify the chromatin structure, which leads to RNA polymerase actions to repress or enhance transcription (26,27,28). The activity of chromatin remodeling complexes is key to biological processes that include Pluripotency and its maintenance, cellular differentiation, inflammation, DNA damage and repair, and tumor suppression. Chromatin is, therefore, an integral component of the pathways that guide nuclear receptor action in a cell-type-specific and cell-state-dependent manner (29) Understanding the role of cofactors and mechanisms regulating nuclear receptor actions on chromatin and transcription could provide novel druggable pathways, as epigenetic and chromatin regulators have emerged as novel targets in disease treatment.

Conclusions

This work shows for the first time that we can express Nuclear Receptors from undifferentiated uncommitted, and differentiated ones. Our procedure is straightforward and can be applied to study various cells.

Metadichol seems to be a natural ligand for all 49 Nuclear Receptors. We have already shown that it binds to VDr, AHR, THRA, THRB, and RORC (12,30,31,32).

Many other genes are differentially expressed in the 2 sets of cells studies (stem and fibroblasts). To understand the differences and why, more experiments with different types of cells, like to see the pattern of expression of NR, would lead to understanding the nature of Nuclear expression induced in various cells by treatment with Metadichol. This is ongoing and will be reported soon.

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Figures

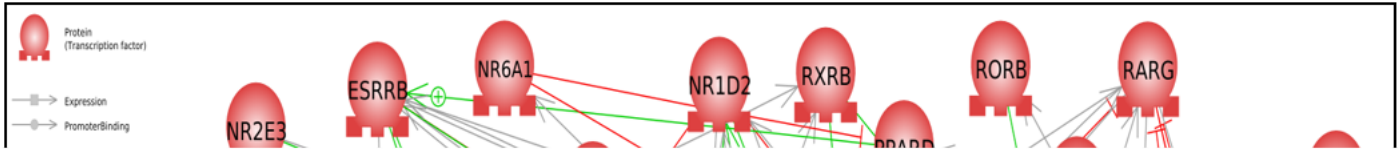

Figure 1

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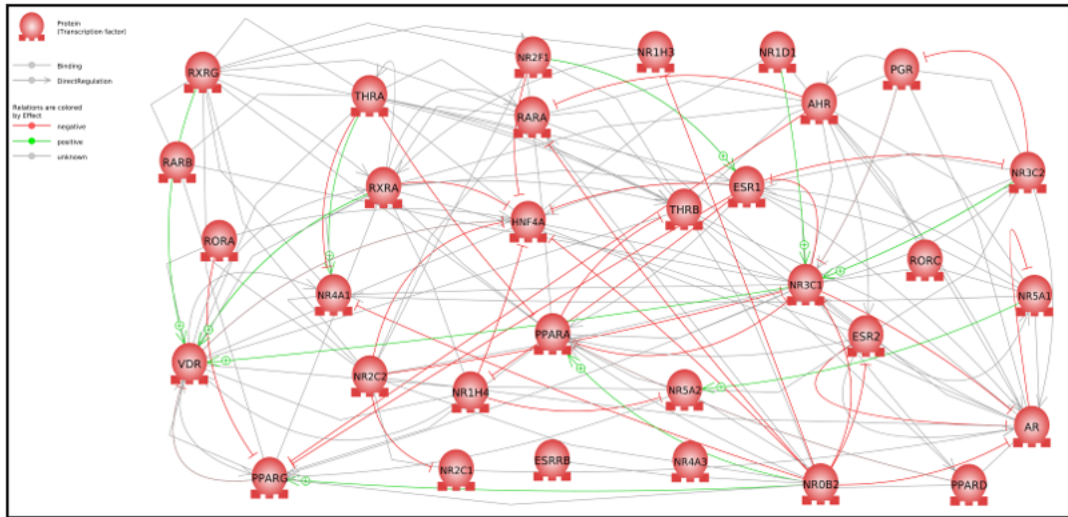

Name	Total # of Neighbors	Gene Set Seed	Overlap	Percent Overlap	Overlapping Entities	p-value
Protein regulators of chromatin remodeling	1626	chromatin remodeling	32	1	PPARD;RXRG;PPARG;NR2F1;HNF4A;NR1H4;ESR1;ESR2;ESRRB;NR5A2;NR3C2;NR0B2;THRB;VDR;NR1D1;NR2C1;NR2C2;NR4A3;NR3C1;AR;PGR;RORA;RORC;NR5A1;NR4A1;RARA;RXRA;PPARA;RARB;AHR;THRA;NR1H3	3.14494E-31

Figure 2

Figure legend not available with this version.

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