


WESTERN BLOT STUDIES OF NUCLEAR RECEPTOR PROTEIN EXPRESSION USING HMSC CELLS

HMSC cell line culture and treatment

HMSC cells were cultured in Mesenchymal Stem Cell Media supplemented with supplement mix given with the culture media that has low serum, 100units/ml penicillin G, and 100µg/ml streptomycin at 37°C, 5% CO₂ incubator.. The cells were then conventionally subcultured and counted using Hemocytometer. The cell count was adjusted to 5x10⁶ cells/2ml. 2ml of cell suspension is added to each dish in P35dishes and incubated until the cells reach 80% confluence. HMSC cells were then treated with various concentrations of test sample . Post incubation, the cells were harvested for isolation of protein using RIPA buffer (Sigma; R0-278)

Isolation of protein using RIPA buffer

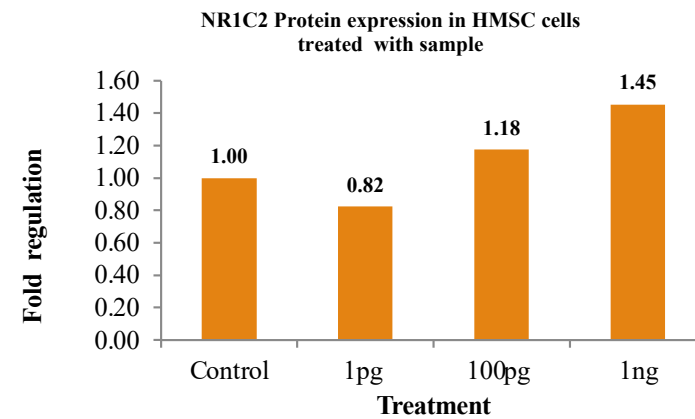
1. The cells, post harvesting, were washed twice using 1XPBS.
 2. The cell pellets were gently suspended in 500 μ l of RIPA buffer with 1X Protease Inhibitor (Sigma; P-8340)
 3. The cells were incubated for 30mins by gentle mixing every 5mins.
 4. Post incubation, the cells were centrifuged at 10,000rpm for 12-15 minutes.
 5. The protein lysates in the supernatant was transferred to fresh sterile tubes and stored in -20°C until further use.
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SDS-PAGE and Western Blot procedure

1. 100ug protein sample from each cell lysate was mixed with 5X loading dye and heated for 6 min at 98°C.
2. Protein samples were loaded and separated on 12% SDS-PAGE gel using Mini protean Tetra cell (Bio-Rad).
3. Methanol activated 0.45uM PVDF membrane was pre-wet in transfer buffer for 10 min @ RT.
4. Protein transfer was done for 10 min in Turbo Transblot (Bio-Rad) apparatus.
5. Blot was blocked in 5% BSA + TBST for 1 hr @RT.
6. Blot was incubated with 1^o Ab @ dilution: 1: 1000 for overnight @ 4°C on shaker.
7. Washed 3 times with TBST for 5min @ RT
8. Blot was incubated with 2^o Ab (Anti-Rabbit HRP- IgG) @ dilution 1: 1000 for 1hr @ RT.
9. Washed 3 times with TBST for 5min @ RT
10. Blot was rinsed with ECL reagent (two component system) for 1 min in dark and image was captured with 40 sec exposure in Chemidoc MP imaging system (Bio-Rad).

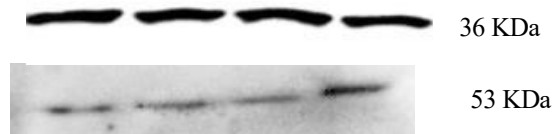
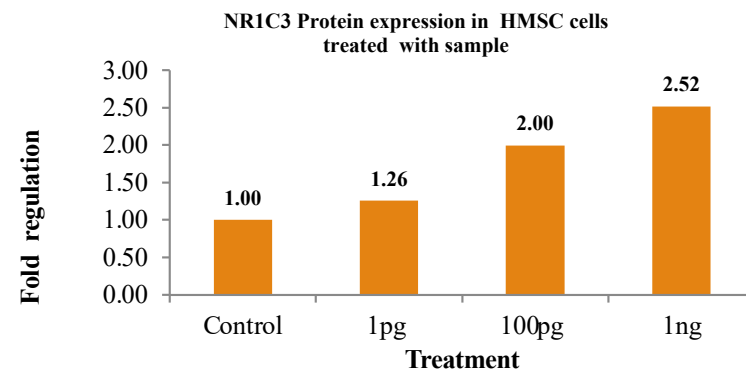
Western Blot for the expression of GAPDH, NR1C2 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1C2		
Control	23896.69	8955.14	0.37	1.00
1pg	21189.74	6534.79	0.31	0.82
100pg	24693.44	10880.08	0.44	1.18
1ng	22541.36	12281.05	0.54	1.45



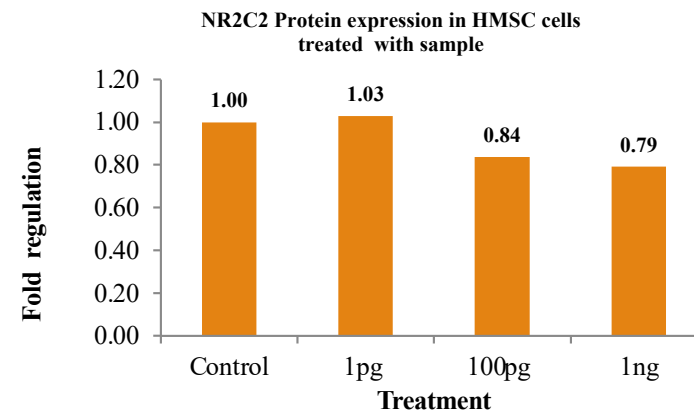
Western Blot for the expression of GAPDH, NR1C3 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1C3		
Control	23896.69	6825.79	0.29	1.00
1pg	21189.74	7602.08	0.36	1.26
100pg	24693.44	12092.09	0.49	1.71
1ng	22541.36	16203.8	0.72	2.52



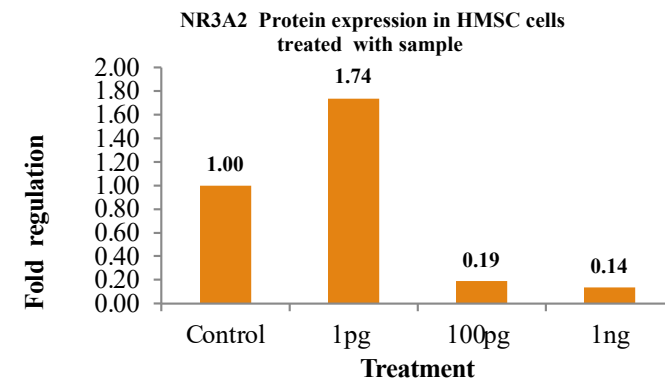
Western Blot for the expression of GAPDH, NR2C2 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR2C2		
Control	23896.69	20947.50	0.88	1.00
1pg	21189.74	19109.60	0.90	1.03
100pg	24693.44	18121.20	0.73	0.84
1ng	22541.36	15645.04	0.69	0.79



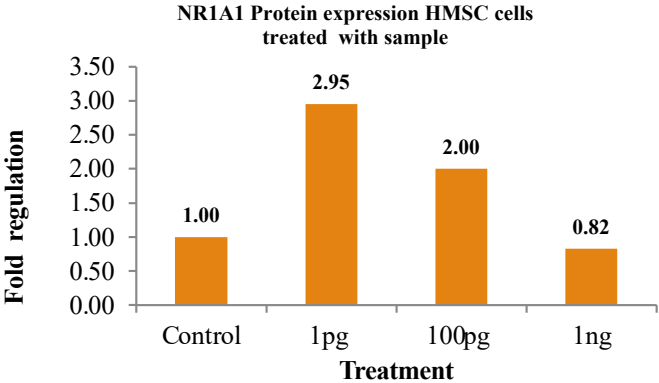
Western Blot for the expression of GAPDH, NR3A2 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR3A2		
Control	23896.69	6241.98	0.26	1.00
1pg	21189.74	9619.51	0.45	1.74
100pg	24693.44	1223.49	0.05	0.19
1ng	22541.36	799.65	0.04	0.14



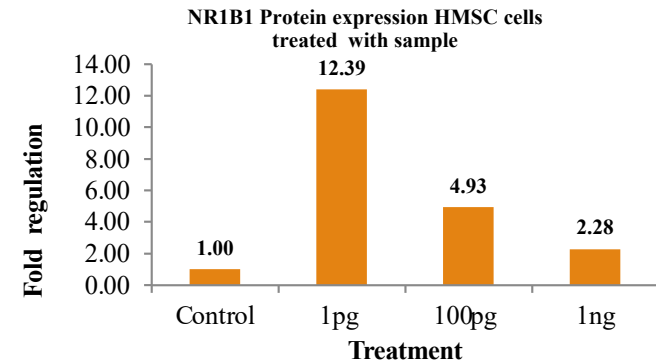
Western Blot for the expression of GAPDH, NR1A1 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1A1		
Control	23896.69	1546.66	0.06	1.00
1pg	21189.74	4050.39	0.19	2.95
100pg	24693.44	3202.52	0.13	2.00
1ng	22541.36	1202.28	0.05	0.82



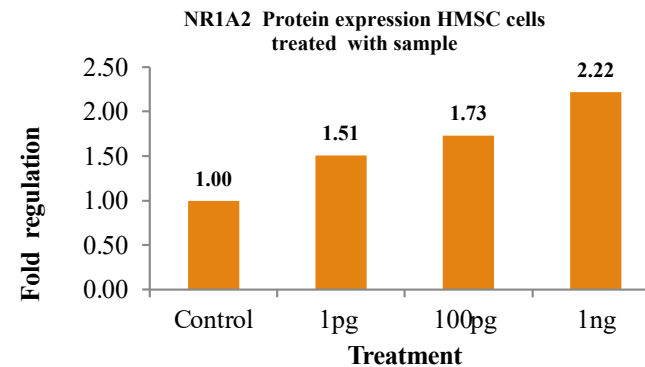
Western Blot for the expression of GAPDH, NR1B1 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1B1		
Control	23896.69	1698.45	0.07	1.00
1pg	21189.74	18654.27	0.88	12.39
100pg	24693.44	8652.79	0.35	4.93
1ng	22541.36	3654.99	0.16	2.28



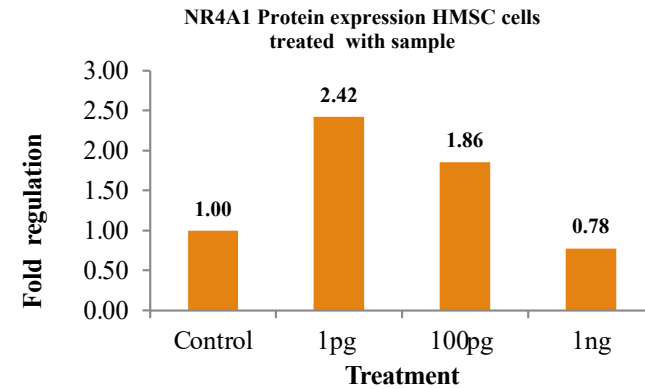
Western Blot for the expression of GAPDH, NR1A2 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1A2		
Control	23896.69	9236.46	0.39	1.00
1pg	21189.74	12346.98	0.58	1.51
100pg	24693.44	16539.62	0.67	1.73
1ng	22541.36	19363.54	0.86	2.22



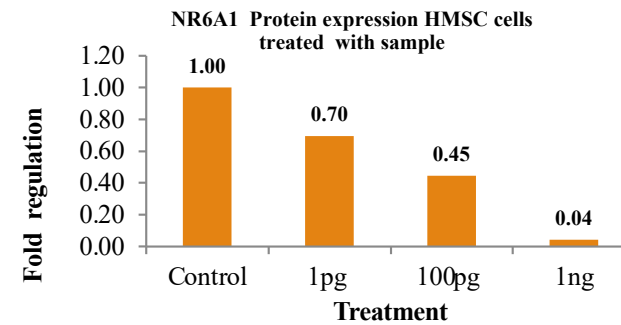
Western Blot for the expression of GAPDH, NR4A1 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR4A1		
Control	23896.69	7698.45	0.32	1.00
1pg	21189.74	16536.97	0.78	2.42
100pg	24693.44	14785.74	0.60	1.86
1ng	22541.36	5639.64	0.25	0.78



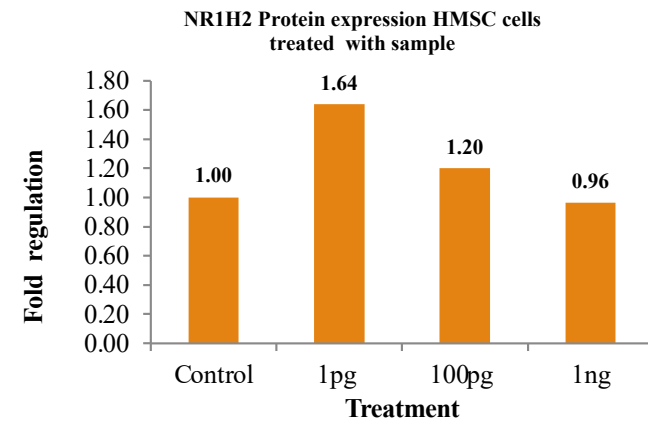
Western Blot for the expression of GAPDH, NR6A1 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR6A1		
Control	23896.69	14536.87	0.61	1.00
1pg	21189.74	8972.64	0.42	0.70
100pg	24693.44	6712.36	0.27	0.45
1ng	22541.36	563.26	0.02	0.04



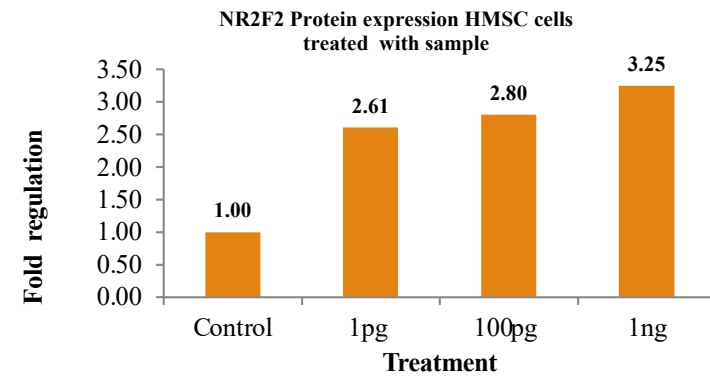
Western Blot for the expression of GAPDH, NR1H2 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1H2		
Control	23896.69	8694.69	0.36	1.00
1pg	21189.74	12636.64	0.60	1.64
100pg	24693.44	10789.36	0.44	1.20
1ng	22541.36	7896.36	0.35	0.96



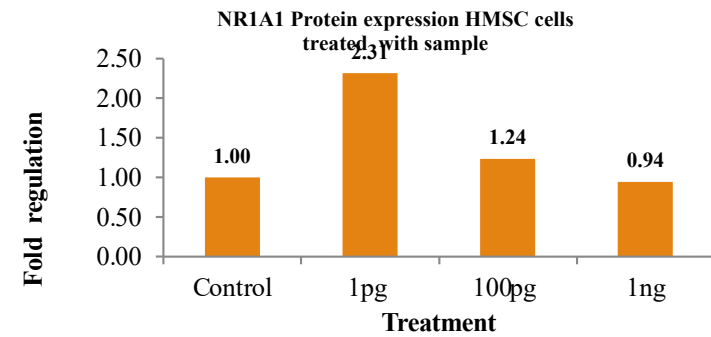
Western Blot for the expression of GAPDH, NR2F2 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR2F2		
Control	23896.69	5173.78	0.22	1.00
1pg	21189.74	11962.84	0.56	2.61
100pg	24693.44	14986.31	0.61	2.80
1ng	22541.36	15861.14	0.70	3.25



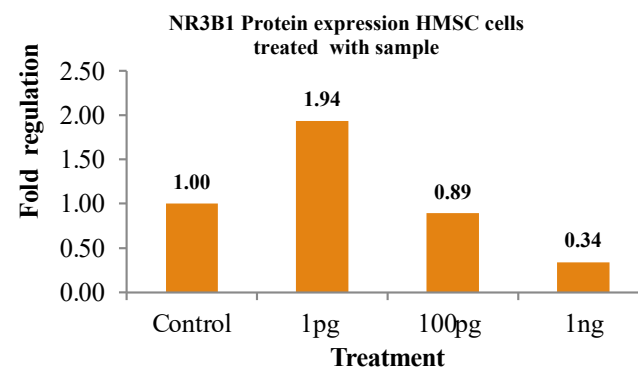
Western Blot for the expression of GAPDH, NR2A1 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR2A1		
Control	23896.69	4051.05	0.17	1.00
1pg	21189.74	8315.70	0.39	2.31
100pg	24693.44	5176.70	0.21	1.24
1ng	22541.36	3604.36	0.16	0.94



Western Blot for the expression of GAPDH, NR3B1 in HMSC cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR3B1		
Control	23896.69	6557.30	0.27	1.00
1pg	21189.74	11251.94	0.53	1.94
100pg	24693.44	6046.18	0.24	0.89
1ng	22541.36	2111.08	0.09	0.34



Conclusion

HMSC adult cells were treated with sample **Metadichol^R** at 1pg/ml, 100pg/ml, and 1ng/ml. The relative expression of target genes was quantified using GAPDH for normalization. **Metadichol^R** showed a dose dependent increase in NR1C2 , NR1C3 ,NR1H3 ,NR2F2 and NR1A2 expression. Rest of protein Dose dependent down regulation was observed in 1ng/ml NR2C2 ,NR2C1,NR3A2,NR1A1,NR4A1,NR6A1,NR1H2,NR3B1,NR2A1 are shown. NR2C1,NR1H3,NR2A2,NR2F1,NR5A1,NR1D1 and NR2F6 not expressed in HMSC adult cells. All in all 21nucelar receptors were expressed. We did not detect the rest as some of them likely need need co factors that are not used in the assay

