Supporting Information

Supporting Methods

Isolation and osteogenic differentiation of bone-marrow-derived stromal cells

Primary mouse bone marrow stromal cells (BMSCs) were were isolated and propagated from 6-week-old male C57BL/6 mice. Mice were euthanized; tibiae, femurs and spine were removed and excess soft tissue was eliminated. Using a pestle and mortar, the bones were crushed and washed in PBS with 0.5% FBS and passed through a 40-mm filter into a collection tube. Cells were spun at 1,500 r.p.m. for 5 min; the supernatant was removed, and washed once with PBS. After pelleting once again, the cells were resuspended and plated in αMEM, 15% FBS, and penicillin and streptomycin solution (Invitrogen) and incubated at 37°C in humidified air with 5% CO_2. After 3 weeks of culture and expansion, plastic adherent cells were then harvested and cultured in αMEM, 10% FBS, and penicillin and streptomycin solution (Invitrogen) and incubated at 37°C in humidified air with 5% CO_2. To assess osteogenic differentiation, BMSCs were plated at 5×10^4 cells per well in a 24-well plate at 37°C in osteogenic induction medium: αMEM, 10% FBS, 50 µg/ml ascorbic acid, 10 mM ß-glycerol phosphate, and 10 nM dexamethasone (Sigma).

Cell cultures

C3H/10T1/2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 1% penicillin/streptomycin. MC3T3-E1 cells were grown in α-MEM supplemented with 10% FBS and 1% penicillin/streptomycin to confluency (Day 0). To induce differentiation, the culture medium was replaced with α-MEM containing 50 µg/ml ascorbic acid and 5 mM ß-glycerophosphate. The culture medium was changed every 2-3 days.

Supporting Table 1.

Table 1. Primers used for Real time PCR

Gene		Primer (Forward/Reverse)
Cebpd	(F)	5'-ATCGCTGCAGCTTCCTATGT-3'
	(R)	5'-AGTCATGCTTTCCCGTGTTC-3'
Ccne1	(F)	5'-CAGAGCAGCGAGCAGGAGA-3'
	(R)	5'-CAGCTGCTTCCACACCACTG-3'
Runx2	(F)	5'-CAGGAAGACTGCAAGAAGGCTCTGG-3'
	(R)	5'-ACACGGTGTCACTGCGCTGAAGA-3'
OC	(F)	5'-CTCTGTCTCTCTGACCTCACAG-3'
	(R)	5'-GGAGCTGCTGTGACATCCATAC-3'
Osx	(F)	5'-CCTCTGCGGGACTCAACAAC-3'
	(R)	5'-AAAGGTCAGCGTATGGCTTCTT-3'
Nfatc1	(F)	5'-GGAGAGTCCGAGAATCGAGAT-3'
	(R)	5'-TTGCAGCTAGGAAGTACGTCT-3'
Ctsk	(F)	5'-AATACCTCCCTCTCGATCCTACA-3'
	(R)	5'-TGGTTCTTGACTGGAGTAACGTA-3'
Adra1a	(F)	5'-TCTACTTGGGGTGATCTTGGG-3'
	(R)	5'-CGAGTGCAGATGCCGATGA-3'
Adra1b	(F)	5'-AACCTTGGGCATTGTAGTCG-3'
	(R)	5'-CTGGAGCACGGGTAGATGAT-3'
Adra1d	(F)	5'-TTCCCCTTTTTCTTCGTCCT-3'
	(R)	5'-ATTGAAGTAGCCCAGCCAGA-3'
Gapdh	(F)	5'-TGGAGAAACCTGCCAAGTATG-3'
	(R)	5'-GGAGACAACCTGGTCCTCAG-3'

Forward (F) and Reverse (R) primers are listed.

Supporting Figure 1.

Gene expression of α_1-AR subtypes in bone cells.

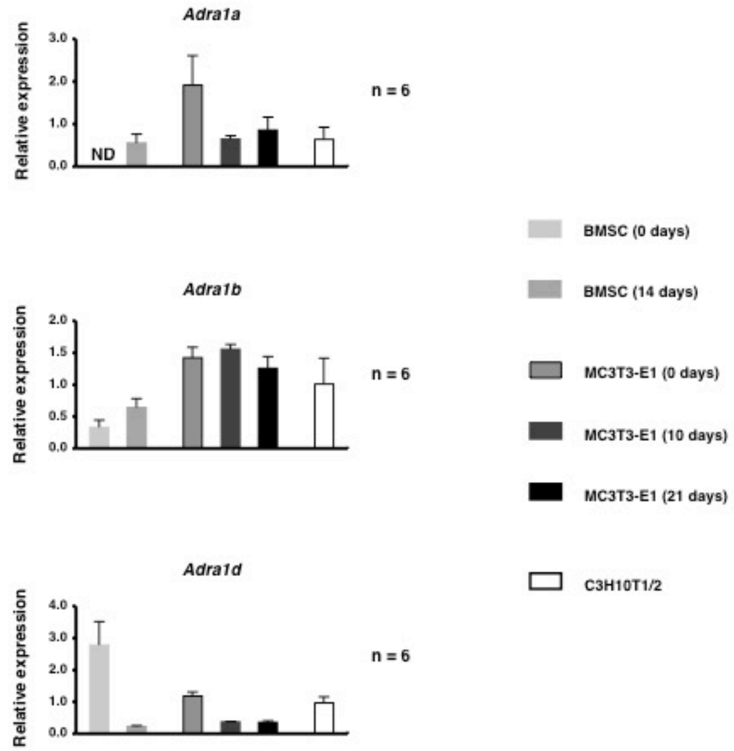
In order to determine the α_1-AR subtypes expressed by osteoblasts, we performed real-time PCR using primers specific for each receptor subtype in BMSCs and MC3T3-E1 cells. Relative mRNA expression was normalized to *Gapdh*. Figures are representative of data from six independent determinations. α_{1A}-AR, α_{1B}-AR, and α_{1D}-AR transcripts were detected in C3H10T1/2 cells as a positive control. ND, not detected.

Supporting Figure 2.

PHE stimulated cellular proliferation in BMSCs.

Cells were treated with PHE at 0.03 to 0.3 μM for 24 h, followed by the determination of BrdU incorporation (mean ± SEM). Each value represents the means ± SEM of six independent determinations. *, $P < 0.05$, significantly different from control value.

Supporting Figure 1.



Supporting Figure 2.