Supplementary data

Expression of serum amyloid A transcripts in human bone tissues, differentiated osteoblast-like stem cells and human osteosarcoma cell lines

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Figure legends

Figure I

Alizarin Red staining of hMSC: hMSCs cells were cultured either in expansion (A, B) or in osteogenic medium (C, D) for 3 weeks and stained with Alizarin Red S. A staining solution (1% Alizarin Red S in 2% (w/v) EtOH) was prepared. The medium was removed, the cells were washed once with preheated 37°C PBS, fixed with 10% formaldehyde for 10 min, washed again with distilled water and incubated with the staining solution for 5 min. After incubation the staining solution was removed and the cultures were washed 5 times with distilled water to get rid of excessive color.

Figure II

RT-PCR of cytokine receptors, SAA and SAF-1 transcripts *in human osteosarcoma cells:* SAOS-2 cells were stimulated with different cytokines (10 ng/ml) for 24 h. (**A**) Cytokine receptor transcripts as well as (**B**) *SAA* and *SAF-1* transcripts were amplified using specific

oligonucleotide primers (Table I). The PCR products were separated on 1.5% agarose gels. NS (non stimulated); P (positive control: RNA was isolated from HUH-7 cells for SAF-1, SAA, cytokine receptors except IL-1R2, where RNA from THP-1 cells was used); N1 (negative control, RNA template: negative controls were done for all samples); N2 (negative control, water template). To ensure that equal amount of cDNA was used as a template RT-PCR for GAPDH was made as a control. One representative experiment out of three is shown.

Figure III

Time-dependent expression of SAA in osteosarcoma cells: (A) MG-63 and (B) SAOS-2 cells, non-treated or stimulated with 10 ng/ml IL-1β for 24 h, were cultured on Lab-Tek chamber slides as described in Materials and Methods. Labelling was performed using sequence-specific polyclonal anti-human SAA1/2 (position 89-104) or SAA4 peptide (position 94-112) antisera as primary antibodies, followed by 30 min incubation with cyanine-3-labeled goat anti-rabbit IgG. One representative experiment out of three is shown. The x/y dimension of the scanned field is 120 μm.

Figure IV

RT-PCR of SR-BI/II and FPRL-1/ALX in human osteosarcoma cells: SAOS-2 were stimulated with different cytokines (10 ng/ml) for 24 h. Human *SR-BI/II,* and *FPRL-1/ALX* transcripts were amplified using specific oligonucleotide primers (Table I). The PCR products were separated on 1% agarose gels. NS (non stimulated); P (positive control: RNA was isolated from THP-1 cells for *SR-BI/II*; for *FPRL-1/ALX*, HUH-7 genomic DNA was used); N1 (negative control, RNA template: negative controls were done for all samples); N2 (negative control, water template). To ensure that equal amount of cDNA was used as a template RT-PCR for GAPDH was made as a

control. One representative experiment out of three is shown.

Figure V.

RT-PCR of FPRL-1/ALX in human osteosarcoma cells in the presence of dexamethasone : MG-63 and SAOS-2 were were seeded in 6-well plates and, upon reaching 80% confluency, stimulated for 24 h by adding dexamethasone (Dex, 1 or 2 μM) in the absence or presence of 10 ng/ml IL-1β or IL-6. Human *FPRL-1/ALX* transcripts were amplified using specific oligonucleotide primers (*Table I*). The PCR products were separated on 1% agarose gels. NS (non stimulated); P (positive control, HUH-7 genomic DNA); N1 (negative control, RNA template: negative controls were done for all samples); N2 (negative control, water template). To ensure that equal amount of cDNA was used as a template RT-PCR for GAPDH was made as a control. One representative experiment out of three is shown.

Table I

Primers, expected amplicon size (bp), and unique PCR properties (cycle number and annealing temperature)

Gene Accession Nr.	**Primers (start position on + strand)**	**bp**	**cycles**	**°C**
SAA1 ***NM_000331***	F 5' CAG ACA AAT ACT TCC ATG CT 3' (373) R 5' ATT GTG TAC CCT CTC CCC 3' (658)	303	40	53
SAA2 ***NM_030754***	F 5' CAG ACA AAT ACT TCC ATG CT 3' (172) R 5' ATT ATA TGC CAT ATC TCA GC 3' (479)	327	40	55
SAA4 ***NM_006512***	F 5' CCA GTG AAA GCT GGC GTT CG 3' (125) R 5' GAG AAG TGT GTG GCT CAC AGC C 3' (500)	397	40	55
SAF-1/2 ***NM_002383*** **AF489858** *	F 5' GCC AGG GTC CTC ACC ATG TCT G 3' (1407/1353*) R 5' CAA CTT GGA GCT CAC CAG GG 3' (1577/1761*)	190/428	30	59
IL-1R1 ***NM_000877***	F 5' TGC TTA CTG GAA GTG GAA TGG 3' (853) R 5' CCT CAG GCA AGA CTT TAA ACA C 3' (1301)	470	30	58
IL-1R2 ***NM_004633***	F 5' GCA ATG TTG CGC TTG TAC GTG 3' (227) R 5' GTC TTT ATC CAA AAG AAG AG 3' (747)	540	30	59
IL-6R ***NM_000565***	F 5' AGA GGC GTG CTG ACC AGT CTG 3' (531) R 5' ACG GCT CCT GGA AGT CTT CGG 3' (910)	400	40	63
TNFR1 ***NM_001065***	F 5' TCG ATT TGC TGT ACC AAG T 3' (447) R 5' GAA AAT GAC CAG GGG CAA CAG 3' (918)	492	30	62
SR-BI/II ***NM_005505***	F 5' TCT ACC CAC CCA ACG AAG GCT 3' (1007) R 5' AGA AGC GGG GTG TAG GGA CTG G 3′ (1655)	669/540	30	58
FPRL-1 ***NM_001005738***	F 5' CTG CTG GTG CTG CTG GCA AG 3' (26) R 5' AAT ATC CCT GAC CCC ATC CTC A 3' (1099)	1095	30	62
GAPDH **M17851**	F 5' ACA GTC CAT GCC ATC ACT GCC 3'(562) R 5' GCC TGC TTC ACC ACC TTC TTG 3' (827)	265	30	58

***** refers to *SAF-2*

Figure I.

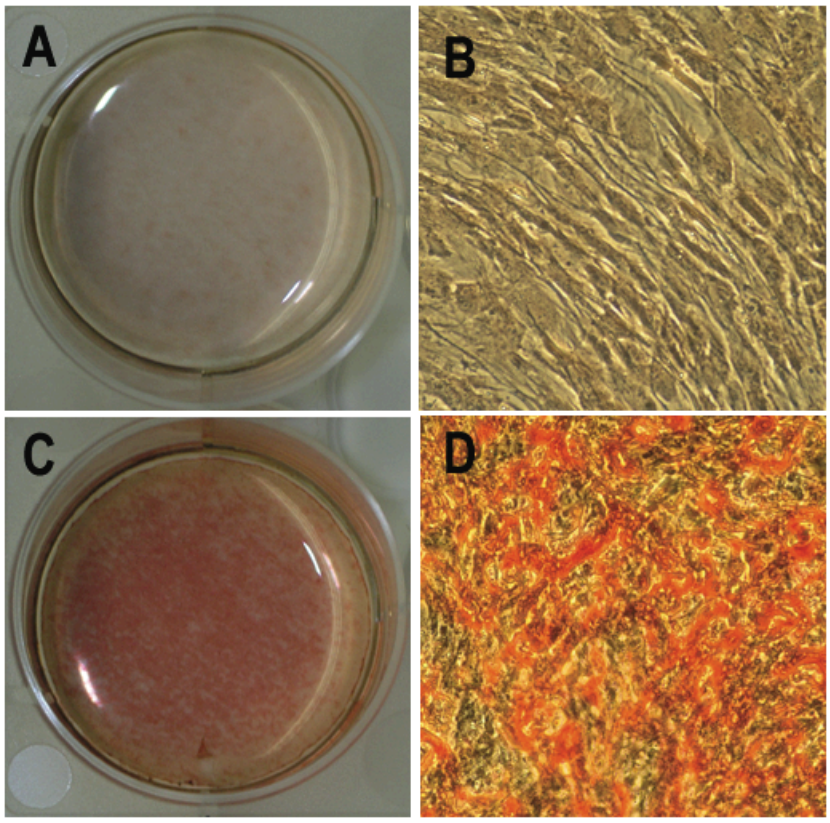


Figure II.

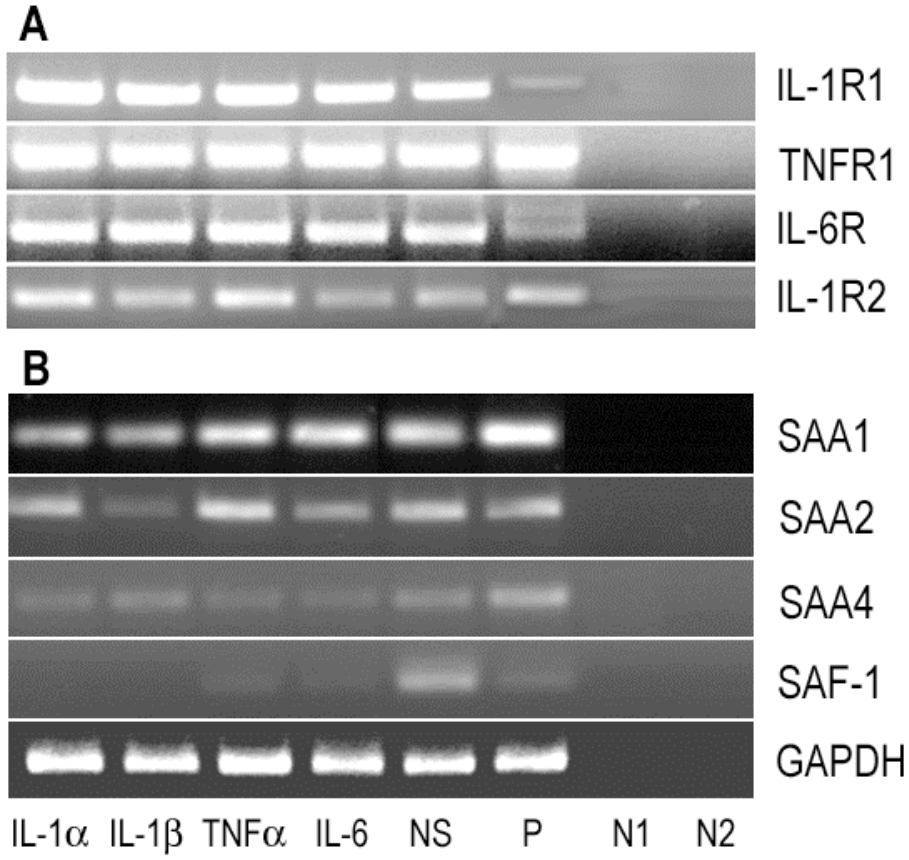
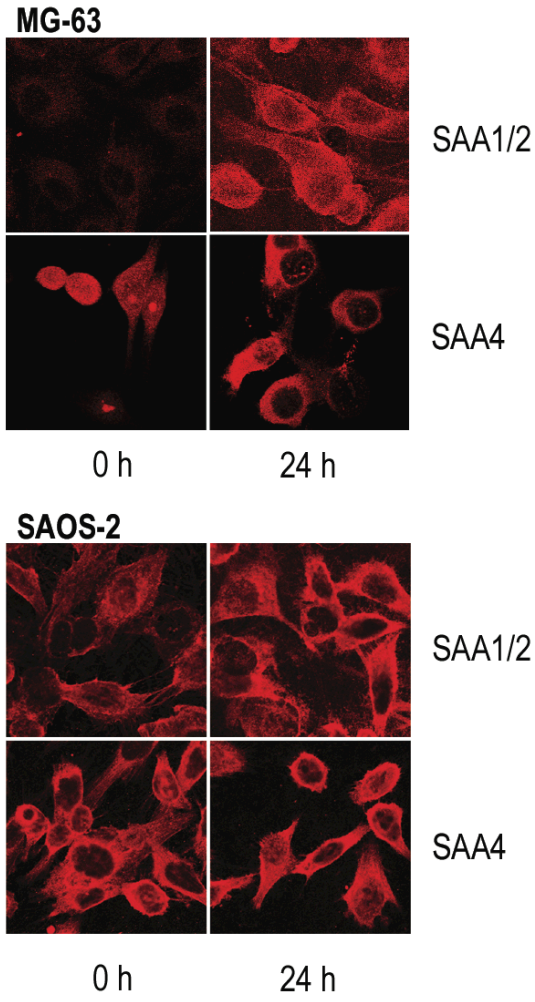

A
IL-1R1
TNFR1
IL-6R
IL-1R2
B
SAA1
SAA2
SAA4
SAF-1
GAPDH
IL-1α IL-1β TNFα IL-6 NS P N1 N2

Figure III.



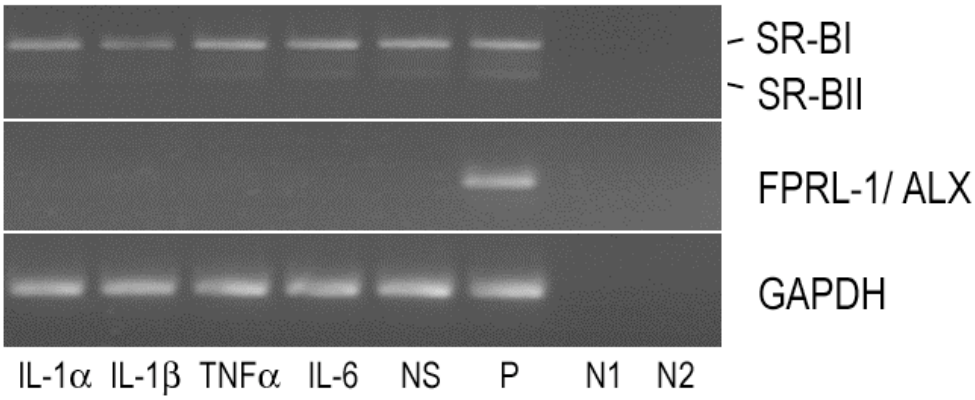
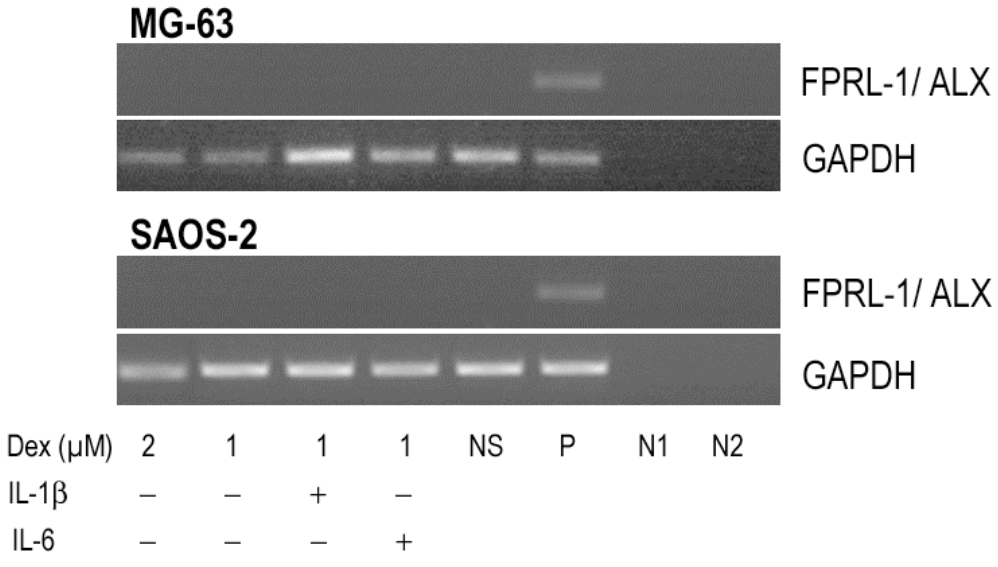

SR-BI
SR-BII
FPRL-1/ ALX
GAPDH
IL-1α
IL-1β
TNFα
IL-6
NS
P
N1
N2

Figure V.


MG-63
FPRL-1/ ALX
GAPDH
SAOS-2
FPRL-1/ ALX
GAPDH
Dex (μM) 2 1 1 1 NS P N1 N2
IL-1β – – + –
IL-6 – – – +