Comparison of human bone marrow stromal cell lines HS-27a and HS-5

DNA spotted microarrays specifying over 17,000 cDNA sequences were used to compare gene expression of the human bone marrow stromal cell lines HS-27a and HS-5, described in Roecklein and Torok-Storb (1995) Functionally distinct human marrow stromal cell lines immortalized by transduction with the human papilloma virus E6/E7 genes. Blood 85: 997-1005.

Four independent RNA samples from each cell line were analyzed. Cells were plated in T75 flasks at 3,000,000 cells/flask. They were cultured in RPMI medium 1640 containing 10% fetal calf serum for 3 days and harvested by trypsinization followed by pelleting of the cells. All RNA isolation was accomplished with Qiagen RNeasy Mini Kit reagents. As a control RNA, Universal Human Reference RNA (Stratagene, La Jolla, CA) which is a mixture of RNA from 10 human cell lines approximating the expression profile of the majority of human genes was used. The RNA (30 µg) was annealed with 5 µg oligo dT12-18, and reverse-transcribed into cDNA with Superscript II reverse transcriptase for 2h at 42°C in the presence of 0.5mM dGTP, 0.5mM dCTP, 0.5mM dATP, 0.3mM dTTP, 0.2mM amino-allyl dUTP. After hydrolysis of RNA in 0.2M NaOH, Tris was removed from the reaction with a Microcon-30 concentrator. The cDNA from HS-27a or HS-5 RNA and Human Universal RNA was covalently coupled separately with Cy5 and Cy3 monoreactive fluors, respectively, in 50mM sodium bicarbonate, pH 9.0, followed by quenching with 2.7M hydroxylamine. The Cy5 and Cy3 labelled cDNAs were combined and purified with a QIAquick PCR purification kit and suspended in 36 µl of 3X SSC and 0.8 mg/ml of poly-A for hybridization to the microarray.

Microarrays were spotted from PCR-amplified sequence-verified clones from the publicly available Research Genetics IMAGE clone set by modifications of the procedures described in Derisi, Iyer, et al. 1997 Science 278:680-686.

Fluorescent array images were collected for both Cy3 and Cy5 with a GenePix 4000A fluorescent scanner and image intensity data were extracted and analyzed with GenePix Pro 3.0 analysis software. After background correction and removal of flagged values, log base 2 expression ratios were mean centered and linear transformed to obtain the log and linear values given in the data table.

More detailed descriptions of the procedures are found in Iwata, Graf, Awaya and Torok-Storb (2002) Functional interleukin-7 receptors (IL7R) are expressed by marrow stromal cells: Binding of IL-7 increases levels of IL-6 mRNA and secreted protein. Blood DOI 10.1182/blood-2002-01-0062.

Average_HS27a: tab-delimited text file includes the Research Genetics Clone ID, GenBank Accession number, Average and Sd (standard deviation) of the linear ratio of gene expression (HS27a/Universal human reference RNA) for four RNA samples.
Average_HS5: tab-delimited text file includes the Research Genetics Clone ID, GenBank Accession number, Average and Sd (standard deviation) of the linear ratio of gene expression (HS5/Universal human reference RNA) for four RNA samples.
Comparison_HSs27a_HS5: tab-delimited text file includes the Research Genetics Clone ID, GenBank Accession number, Average_HS27a, Average_HS5, p value, HS-27a/HS-5 (ratio of

Average_HS27a and Average_HS5). P value was derived from a Student's t-test for a statistical difference between the HS27a and HS5 values for each individual clone (n=4 of each cell line).

Datasets are also available from NCBI's GEO (Gene Expression Omnibus) data respository. Microarray Platform ID=GPL44, Sample IDs=GSM834, GSM835, GSM836, GSM837, GSM838, GSM839, GSM840, GSM841.

Title: HS-27a and HS-5
Type: dual channel
Organism: Homo sapiens
Target Source: human bone marrow stromal cell lines HS-27a and HS-5
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