Abstract

Osteoblastogenesis is the formation of osteoblasts from undifferentiated stem cells. The objective of this study was the identification of marker genes of early osteoblastogenesis, using DNA microarray technology. Human multipotent adipose tissue derived stem (hMADS) cells were used as the model cell line to study osteoblastogenesis. The use of DNA microarray technology and hMADS cells to study osteoblastogenesis was the first of its kind.

The experimental design included three biological replicates each consisting of an eight time point series and one reference. Reference sample (Day -3) was extracted three days before induction and consisted of mRNA from preconfluent undifferentiated cells. The remaining 8 time points were: TP1 (day -2), TP2 (day 0), both before induction and TP3 (+8hrs), TP4 (+24hrs), TP5 (+48hrs), TP6 (day +8), TP7 (day+15) and TP8 (day+24), all after induction. In order to measure the transcriptome level of the cells before and after induction, mRNA was extracted from each time point, reverse transcribed to cDNA, labelled with Cy3 and Cy5 and then hybridized twice with reverse labelled reference.

Using this dye swap technique for normalization, 48 genome wide human oligonucleotide microarrays were hybridized with the cDNA. The microarrays were scanned using GenePix Pro 5.0 and the resulting data set was normalized using Array Norm 1.7 software. Genesis 1.5.0 Beta 1 software was then used for clustering the normalised data. Data was clustered according to **different cellular functions** (ECM, signalling factors, transcription factors, cytoskeleton), **specific gene families** (RHO, G-proteins, Wnt pathway), **degree of differential gene expression** from one time point to another (time point 2 to time point 3, time point 3 to time point 4 and so on) and finally **similarity of gene expression patterns**. Quantitative PCR was performed to validate the microarray data.

The up regulation of several known markers genes of osteoblastogenesis could be confirmed by both microarray and q-PCR methods. These include alkaline phosphatase, osteoprotegerin, Matrilin 3, BMP6, BMP5, BMP receptor 1b, BMP receptor1a, FOSL1, MATN4 and Osterix. Some known markers of adipogenesis were shown to be down regulated like LPL, FASN, PPAR gamma, SREBF1and FABP5. Several other osteoblastogenesis specific genes showed expected expression profiles by at least one method. These included BMP4, BMP7, BMP10, Sortilin, RunX2/Cbfa1, VCAM, OSTF1, PTHlh, DLK/PREF1 and SOX4. Potential candidate genes for early markers of osteoblastogenesis were identified.

Key words: osteoblastogenesis, osteoblast differentiation, hMADS, oligonucleotide microarray, differential gene expression