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hMSC differentiation marker detection using Thermo Scientific Solaris™ qPCR Gene Expression Assays

Human mesenchymal stem cells have become an important resource in developing strategies for regenerative therapies, owing to their ease of use and differentiation potential. Analytical tools, such as whole-genome expression array and validation with Solaris™ qPCR Assays, are essential to fully understand the key molecular events, such as microRNA-mediated gene modulation, that mark stem cell differentiation.

Although microarrays are useful for rapid whole-genome profiling, a complementary method with improved sample throughput, sensitivity and dynamic range is needed for follow-up studies. Quantitative real-time PCR (qPCR) is often the method of choice to validate gene expression results from whole-genome microarrays. Solaris™ qPCR Gene Expression Assays are predesigned on a genome-wide scale using a novel, tier-based algorithm to detect all variants of a given gene and distinguish among closely related family members. Solaris assays incorporate minor groove binder (MGB™)[1] and Superbase™ technologies (Epoch Biosciences, Inc) for increased sequence design space and enhanced specificity. Combining these two chemical strategies with a fluorescent (FAM) reporter dye and corresponding Dark Quencher™ fluorochrome (Epoch Biosciences, Inc) results in highly specific and sensitive assays that consistently function under universal thermocycling conditions. Here we describe an application of Solaris technology to validate the microarray expression data from early-stage osteogenic human mesenchymal stem cells (hMSCs).

microRNAs (miRNAs) are involved in many aspects of cellular processes; however, little is known about their role in the regulation of adult stem cell differentiation. In a recently published screen using a Thermo Scientific Dharmacon miRIDIAN microRNA Inhibitor and Mimic library, miR-148b was shown to increase alkaline phosphatase (ALPL) activity, an early marker of osteoblast differentiation[2]. Here we show how the novel Solaris platform was used to further characterize gene expression in hMSCs treated with differentiation medium or with miRNA mimics.

We assessed osteogenic differentiation in hMSCs treated with medium or with miRNA mimics (**Fig. 1**). For the medium treatments, hMSCs were grown in osteoblast differentiation medium or propagation medium. For the mimic treatment, we transfected miRIDIAN miR-148b mimic or miRNA mimic negative control 1 into hMSCs. Six days after induction of osteogenic differentiation, we collected the cells and assessed the culture for ALPL-positive cells[2]. Using the Thermo Scientific Cellomics VTi ArrayScan high-content imaging system, we observed an approximately eightfold increase in ALPL-positive cells treated with either differentiation medium or miRNA mimics relative to controls (data not shown).

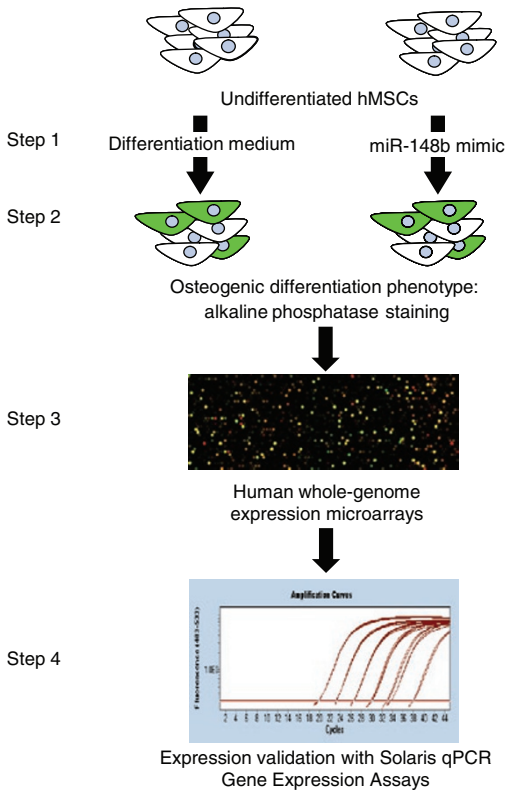

Figure 1 | Experimental workflow for characterization of gene expression changes in human mesenchymal stem cell (hMSC) osteogenic differentiation. Step 1: hMSCs (Lonza) are treated with differentiation medium or miRIDIAN miR-148b mimic for 6 d[2]. Step 2: cells are assessed for the ALPL early osteogenic marker. Step 3: RNA is isolated and microarray expression analysis performed to identify genes that are differentially regulated with each treatment. Step 4: Solaris qPCR Gene Expression Assays are used to validate differentially expressed gene targets identified from the microarray data.

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APPLICATION NOTES

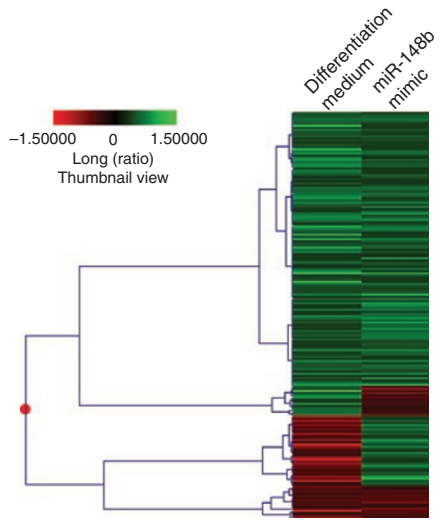

Figure 2 | Microarray analysis identifies genes that are differentially regulated in hMSC osteogenic differentiation by differentiation medium treatment and miRNA treatment. Isolated total RNA from treated hMSCs was hybridized against RNA from undifferentiated cells or mimic control transfected cells on Human Whole Genome (4x44k) Expression Microarrays (Agilent) per manufacturer's instructions. Three technical replicates were combined for each treatment, and a twofold cutoff (log ratio of greater than 0.3 or less than –0.3) and *P* values <0.001 were applied to identify genes that were differentially regulated. Agglomerative hierarchical clustering was performed using cosine correlation distance metrics. Each row of the heat map represents a gene.

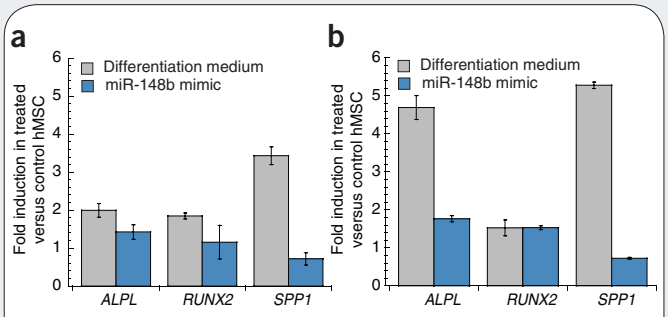

Figure 3 | Expression of three characterized osteoblast differentiation markers for differentiation medium–treated or miR-148b mimic–treated hMSCs. (**a**) Expression of the three gene markers as measured by microarray analysis (Agilent). (**b**) Expression of the three gene markers based on qPCR data obtained using Solaris qPCR Gene Expression Assays. qPCRs were carried out in a 12.5-µl reaction volume (final oligonucleotide concentrations of 800 nM of each primer and 200 nM of MGB probe) in Solaris Gene Expression master mix using a standard thermal cycling protocol on a Roche LightCycler480. Expression data were normalized to a *GAPDH* reference gene using the $\Delta\Delta Cq$ method[6]. All data are presented as fold change over the reference samples (differentiation medium/propagation medium– or miR-148b/mimic control–treated cells) for *ALPL*, *SPP1* and *RUNX2*.

Microarray expression analysis identified 891 genes as differentially regulated as a result of treatment with differentiation medium, and 686 as differentially regulated by the miR-148b mimic treatment (analyzed using Rosetta Resolver software). Among these, 190 genes were regulated by both treatments (**Fig. 2**). The majority of these genes (143) were regulated in the same direction (up or down) by both treatments.

We examined differential expression of three characterized early osteoblast marker genes[3] in more detail: *ALPL* (alkaline phosphatase), *SPP1* (secreted phosphoprotein 1) and *RUNX2* (runt DNA-binding domain transcription factor). Based on the microarray analysis, *ALPL* and *RUNX2* were modestly induced approximately two fold under medium treatment and only modestly under miRNA treatment (**Fig. 3a**). *SPP1* expression was induced only by the medium treatment, by approximately 3.5-fold, and was slightly reduced by the miRNA treatment.

We then validated the expression levels of the same early osteogenic markers using Solaris qPCR Gene Expression Assays. We observed upregulation of all three osteogenic markers in differentiation medium–treated hMSCs: *ALPL* and *SPP1* were induced ~4.5-fold and >5-fold, respectively, whereas *RUNX2* was only mildly induced (**Fig. 3b**). The relatively low induction of *RUNX2* expression on day 6 is not surprising as this transcription factor is typically upregulated at the onset of osteogenic differentiation[4]. *ALPL* and *RUNX2* were mildly induced in miR-148b mimic–treated hMSCs. *SPP1* gene expression, however, was slightly decreased in miR-148b mimic–treated cells, in contrast to the marked induction observed with the differentiation medium treatment. This supports previously published data demonstrating a decrease in the *SPP1* expression caused by the miR-148b mimic[2].

The microarray and qPCR detection methods revealed relatively similar expression levels for both treatments, with the exception of *ALPL* (for which higher expression was indicated with qPCR detection). Although these two gene expression detection methods are commonly used for identification and validation, discrepancies between them are sometimes observed owing to the differences in sensitivity and dynamic range[5]. The similarities in gene expression for *ALPL* and *RUNX2* osteogenic markers and the 143 genes identified in the expression profiling that are commonly regulated between differentiation medium– and miRNA mimic–treated cells further support a role for miR-148b in the stimulation of osteogenic differentiation of hMSCs.

Follow-up qPCR studies using Solaris qPCR Gene Expression Assays will provide a more robust and quantitative assessment of these gene expression changes and a foundation for further study of the osteoblast differentiation mechanism and miRNA involvement in this process.

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