Phosphoproteomic analysis of human mesenchymal stromal cells during osteogenic differentiation

INTRODUCTION:
Human mesenchymal stromal cells (hMSCs) are promising candidates for cell therapy and tissue regeneration. Knowledge of the molecular mechanisms governing hMSC commitment into osteoblasts is critical to the development of therapeutic applications for human bone diseases. Recently, several studies have focused on the proteomic profiles of MSCs undergoing osteogenesis using two-dimensional gel electrophoresis followed by mass spectrometry (MS) analysis. However, the information of biomarkers demonstrating osteogenic differentiation in hMSCs is limited, because the identified proteins from 2DE are restricted to no more than 50 proteins, and the selected time points after 14 days of induction can only identify osteogenic markers rather than osteogenic mediators. Since protein phosphorylation plays a critical role in signaling transduction network, the purpose of this study is to elucidate the phosphoproteomic changes in hMSCs during osteogenic differentiation.

METHODS:
hMSCs cultured in osteogenic induction medium for 0, 1, 3 and 7 days were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). A previous published label-free strategy which combined the pH-acid-controlled immobilized metal affinity chromatography (IMAC) protocol were used to enlarge the number of quantifiable phosphoproteins and the four-dimensional algorithm were used to evaluate the site-specific quantitation accuracy.3

RESULTS:
During osteogenic differentiation, hMSCs underwent morphological changes from spindle in shape to flattened osteoblast-like cells. Staining and Expression of osteogenic lineage marker genes suggested this defined osteogenic induction protocol worked well for the investigation of the osteogenic differentiation process. (Fig. 1)

To determine the regulatory networks in hMSCs during osteogenic differentiation, we performed a label-free method to investigate the global phosphoproteomic profile of hMSCs, we found the scatter plot of hMSCs after 1 day of induction exhibited an overall decreased protein phosphorylation level compared to undifferentiated hMSCs (D1/D0), while hMSCs after 3 days of induction compared to 1 day (D3/D1), and 7 days compared to 3 days (D7/D3) exhibited a continuous increase in protein phosphorylation. (Fig. 2)

In order to confirm the accuracy of our quantitative MS data, several phosphoproteins were chosen for further discussion due to their previously known functions within the cytoskeleton organization network during osteogenic differentiation. (Fig. 3) The similar pattern of the phosphorylation sites during osteogenic differentiation recognized by Western blot analyses and quantitative MS data indicated the high fidelity of our quantitative results from phosphoproteomic analyses.

DISCUSSION:
Our study provides the first and extended insight towards phosphoproteomic changes in hMSCs during osteogenic differentiation. This study unmasks several putative candidates that act as potential regulators in the transcriptional activation of the osteogenic phenotype of hMSCs and may shed light on the orchestrated networks mediating osteogenic commitment in hMSCs, which can serve as a basis for the development of new therapeutic regime for metabolic bone diseases such as osteoporosis.

SIGNIFICANCE:
To the best of our knowledge, this is the first report utilizing such a phosphoproteomic approach to address the signaling dynamics and explore potential new markers that play a functional role during the osteogenic differentiation process in hMSCs.

REFERENCES:
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Fig. 1 Osteogenic differentiation of hMSCs.

Fig. 2 Identified phosphoproteomic profiles during osteogenic differentiation.

Fig. 3 Partial summarization and verification of the selected phosphoproteins participating in the cytoskeleton organization network.

Fig. 4 Functional categorization of identified phosphoproteins with known functions in the Down-regulation group and the Up-regulation group were illustrated in a pie chart.

We summarized in this study several ion channel proteins and transcription regulators during osteogenic differentiation. These proteins are either correlated with osteogenic differentiation or are dysregulated in skeletal conditions, their phosphorylation status may render a positive regulatory role of the osteogenic phenotype during differentiation.