Secretome of human dental pulp stem cells (DPSCs): differential gene expression during induction of osteogenic/odontogenic and chondrogenic differentiation.

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ABSTRACT

Constant remodeling of extracellular matrix (ECM) is a hallmark during physiological conditions, such as stem cell differentiation, embryogenesis and tissue repair. MMPs, MMP-inhibitors (TIMPs and RECK) and MMP-inducer (EMMPRIN) play a key role in these processes, being part of cell proteolytic secretome. Transforming Growth Factor Beta superfamily members, such as TGF-β and BMPs, are responsible for bone, tooth and cartilage formation and modulate the expression of molecules involved on matrix turnover. In this way, we evaluated gene expression of MMPs, TIMPs, RECK and EMMPRIN during induction of osteogenic/odontogenic and chondrogenic differentiation from DPSCs in vitro by qPCR. DPSCs were isolated from extracted human third molars and grown in clonogenic medium (α-MEM medium + 10% FBS + 100 µM ascorbate) and differentiation induction in presence of osteogenic medium (10 mM β-glycerophosphate, 1 µM dexamethasone and 100 µM ascorbate), odontogenic medium (10 mM β-glycerophosphate, 1 µM dexamethasone and 100 µM ascorbate + 50 ng/mL BMP-7) or chondrogenic medium (DMEM/F12 + 500 ng/mL insulin + 1 µM dexamethasone + 50 µM ascorbate + 10 ng/mL TGF-β1 + 1mM sodium pyruvate) for 35-days. For each differentiation, a differential gene expression pattern was observed and our results suggest that both TGF-β1 and BMP-7 may regulate MMPs, their inhibitors and inducer gene expression during osteogenic/odontogenic and chondrogenic differentiation in vitro from DPSCs.

Keywords: Dental pulp stem cells, MMPs, TIMPs, RECK, BMP-7, TGF-β1, Osteoblast/Odontoblast and Chondrogenic Differentiation.
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MATERIALS AND METHODS

- Dental pulp collection and DPSCs isolation: Normal human impacted third molar was collected from 2 adult (between 18 and 32-years old) at the Dental Clinic of University Hospital at University of São Paulo under approved guidelines set by the National Institutes of Health Office of Human Subjects Research. The pulp tissue was gently separated from the crown and root and then digested in a solution of 6 mg/ml collagenase type I (Gibco) and 8 mg/ml dispase (Gibco) for 1 h at 37°C. Single-cell suspensions were obtained by passing the cells through a 70-µm strainer. Cells were cultured in α-MEM + 10% SFB + 100 µM L-ascorbic acid 2-phosphate (clonogenic medium), and then incubated at 37°C in 5% CO_2 (adopted from Gronthos et al 2000; 2011).
- Cellular Differentiation induction: Osteogenic medium was constituting by α-MEM + 10% SFB + 1 µM dexamethasone + 10 mM β-glicerofhosfate + 50 µg/mL L-ascorbic acid 2-phosphate and Odontogenic medium by adding 50 ng/mL BMP-7. Chondrogenic medium was constituting by DMEM/F12 + 500 ng/mL insulin + 1 µM dexamethasone + 50 µM ascorbate + 10 ng/mL TGF-β1 + 1mM sodium pyruvate. Verification of calcification nodule was assessed by alizarin red stain and glycosaminoglycans by toluidine blue. Differentiation was evaluated by 28, 21 and 35-days, respectively.
- qRT-PCR: Total RNA was used as template for cDNA synthesis in a RT-PCR reaction. qRT-PCR reaction was performed by SYBR® Green Dye I (Applied Biosystems - 40 cycles at 60°C). Relative quantification was performed by Pfaffl method (2001). GAPDH was used as housekeeping gene and undifferentiated DPSCs as reference sample.

RESULTS AND DISCUSSION

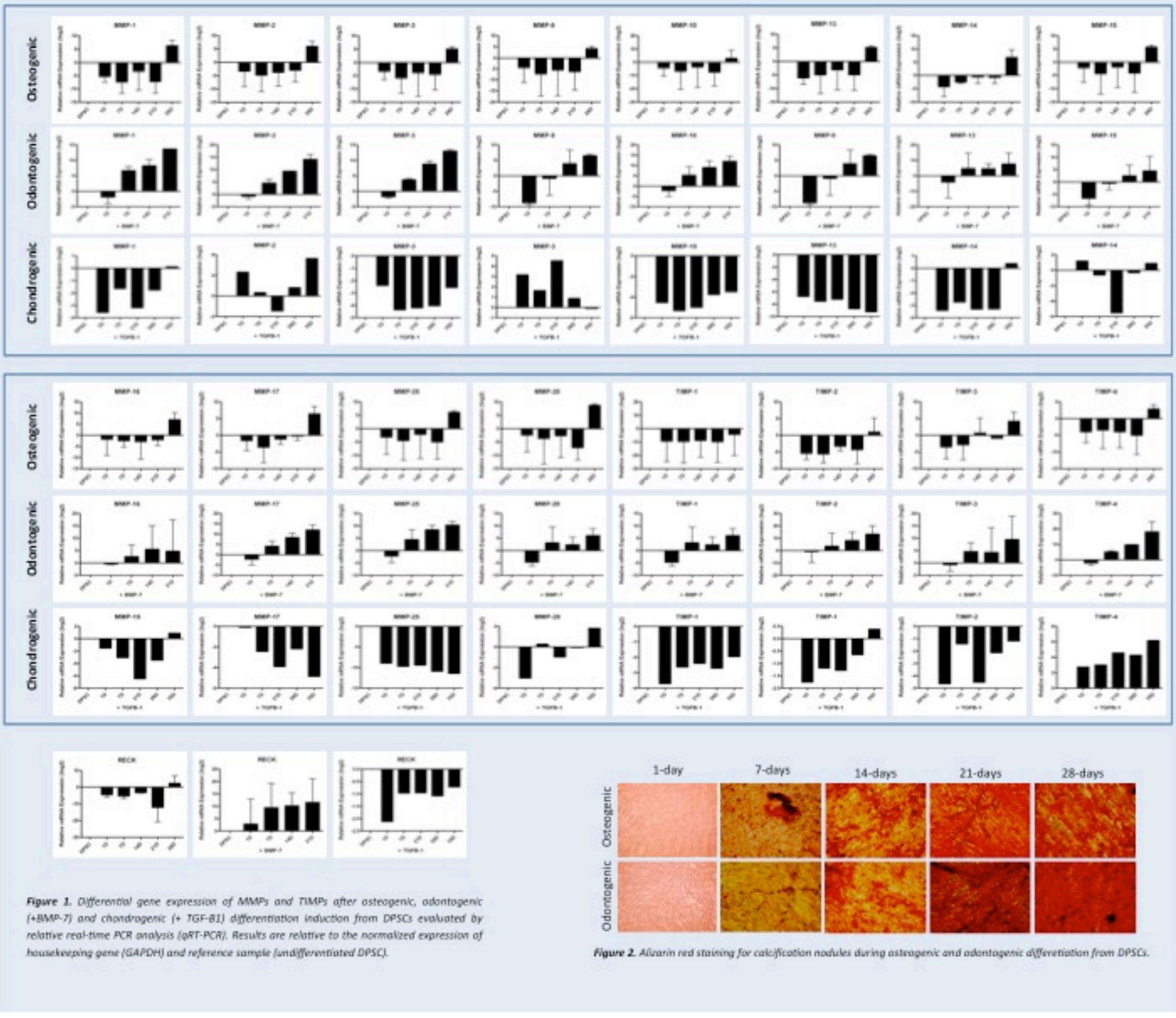

Figure 1. *Differential gene expression of MMPs and TIMPs after osteogenic, odontogenic (+BMP-7) and chondrogenic (+ TGF-B1) differentiation induction from DPSCs evaluated by relative real-time PCR analysis (qRT-PCR). Results are relative to the normalized expression of housekeeping gene (GAPDH) and reference sample (undifferentiated DPSC).*

Figure 2. *Alizarin red staining for calcification nodules during osteogenic and odontogenic differentiation from DPSCs.*