Molecular basis for the impaired adipogenic differentiation potential of cord-blood derived mesenchymal stromal cells

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Introduction
Mesenchymal stromal cells (MSCs) have received considerable attention for their potential role in cell-based regenerative therapy. For their clinical application, a better understanding of the behaviour of cells, e.g. MSCs from different tissue sources, including differentiation, proliferation, migration and the disparities among them is required. MSCs have the ability to undergo mesodermal differentiation (osteogenic, chondrogenic and adipogenic differentiation). Cord blood (CB) derived MSCs, in contrast to theMSCs isolated from liposapirate (LA) and bone marrow (BM), differ with respect to low or absent adipogenic but higher osteogenic differentiation potential in vitro. Preadipocyte factor 1 (Pref-1, DLK-1) has been shown via reverse transcription quantitative PCR (RT-qPCR) to be coexpressed in CB MSCs in comparison to LA and BM MSCs during adipogenesis. Within this study we investigated the role of Pref-1 in the adipogenic differentiation process via upregulation using CB plasma and siRNA mediated downregulation of Pref-1.

Methods
Pref-1 upregulation: CB plasma has been shown via an enzyme linked immunosorbent assay to contain high levels of Pref-1, whereas no Pref-1 concentration was detectable in BM plasma. Adipogenic differentiation was induced in liposapirate derived MSCs under three different conditions: addition of 10 % CB plasma or 10% BM plasma to the differentiation medium, as well as under standard conditions, containing 10% FCS as control. Adipogenic differentiation capacity was detected with oil red O staining. The mRNA of CB plasma treated and control cells has been analyzed via RT-qPCR.

siRNA knockdown of Pref-1: Adipogenic differentiation was induced in cord blood derived MSCs with 12nM siRNA and without siRNA as control. SiRNA treatment was performed with every medium exchange. The gene expression of adipogenic marker genes was analyzed with RT-qPCR.

Immunofluorescence staining: Adipogenic differentiated LA and CB MSCs were fixed with methanol/aceton and stained with mouse anti-Pref-1 antibody (1:3000) for 1 hour at room temperature (RT). The secondary anti-mouse Cy3 antibody (1:1500) was incubated for 30 min at RT and the nuclei were stained with DAPI (1:10 000).

RT-qPCR: RT-qPCR reaction was performed using the Light Cycler 480 instrument with the universal probe library system by Roche. Reference genes (RG) were included to normalise PCR plates, whereas RNase-free water was used as negative control.

Results

Discussion
Further studies should ascertain whether Pref-1 protein is still abundant after 21 days of siRNA treatment to inhibit adipogenic differentiation in CB MSCs. In addition a screening for Pref-1 interaction partners will be performed to define whether Pref-1 requires specific proteins for their inhibitory role in adipogenesis.

Our studies have identified Pref-1 as one candidate to be responsible for the impaired adipogenic differentiation potential of CB MSCs. It needs to be investigated if Pref-1 competitively balances the differentiation into adipogenic and osteogenic lineage. Thereby high Pref-1 expression that yields a lower or absent adipogenic differentiation, may consequence a higher osteogenic differentiation potential.