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 the MSCs isolated from lipoaspirate (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 contrarily expressed 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 lipoaspirate 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.

Immunfluorescence staining: Adipogenic differentiated LA and CB MSCs were fixed with methanol/acetone and stained with mouse anti-Pref-1 antibody (1:300) for 1 hour at room temperature (RT). The secondary anti-mouse Cy3 antibody (1:1000) 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 gene expression of different samples. The geNorm software was used to identify the most stable RG from a set of five. Primer efficiency was determined in a previous experiment. Synthetic cDNA served as positive control to normalise PCR plates, whereas RNase-free water was used as negative control.
To determine adipogenesis-associated marker expression, we selected peroxisome proliferator-activated receptor gamma (PPARγ), Perilipin and Pref-1 and calculated their expression relative to the reference genes glyceraldehyd 3-phosphate dehydrogenase (GAPDH), TATA-binding protein (TBP) and beta-2-microglobuline (B2M). Relative expression of the genes of interest was calculated as described in Vandesompele *et al.* in 2002.

Results

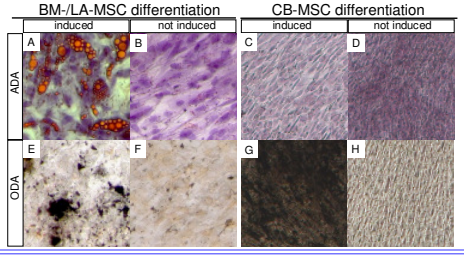

Fig.1 Differentiation potential of LA and BM MSCs in comparison to CB MSCs.

The lipid vacuoles of adipogenic differentiated cells (ADA) are stained with oil red O (A,B,C,D) and osteogenic differentiated cells (ODA) with von Kossa (E,F,G,H). CB MSCs fail to differentiate into the adipogenic lineage (C), but have a higher osteogenic differentiation potential (G) in comparison to LA and BM MSCs (A,E). MSCs without induction medium do not differentiate spontaneously (B,D,F,H).

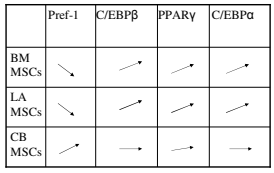

Fig.2 Relative expression of adipogenic marker genes in LA and BM MSCs compared with CB MSCs.

The adipogenic-associated marker genes PPARγ, CCAAT/enhancer-binding protein alpha and beta (C/EBPα and β) were upregulated during adipogenic differentiation in LA and BM MSCs, but not in CB MSCs. This is consistent with the absent adipogenic differentiation potential of CB MSCs. In contrast to this, Pref-1 expression increases in CB MSCs, but decreases in LA and BM MSCs during adipogenic differentiation.

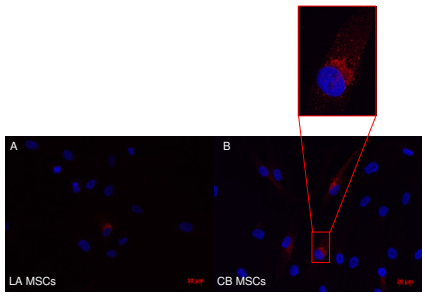

Fig.3 Pref-1 immunofluorescence staining in LA and CB MSCs.
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LA MSCs are barely positive for Pref-1 (A), whereas CB MSCs have indicate higher Pref-1 expression (B) (n=2, magnification 40x). Mouse anti-Pref-1 Cy3, red, nuclei DAPI, blue.

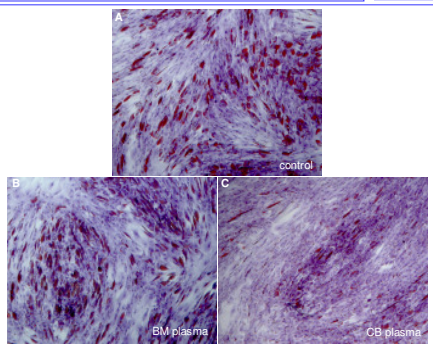


Fig.4 CB plasma diminishes adipogenic differentiation in LA MSCs.

Oil red O staining of adipogenic differentiated LA MSCs: control (A), addition of 10% BM plasma (B) and 10% CB plasma (C). Only CB plasma, containing high levels of Pref-1, diminishes adipogenic differentiation in LA MSCs (n=2, magnification 5x).

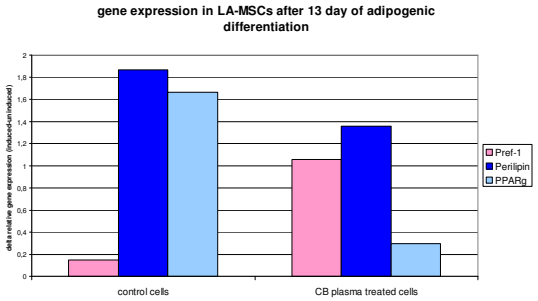
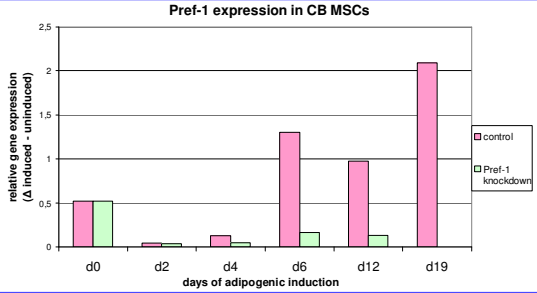


Fig.5 CB plasma downregulates gene expression of adipogenic marker PPARg and Perilipin in LA MSCs.

RT-qPCR data shows relative expression of Pref-1, PPARγ and Perilipin in LA MSCs adipogenic differentiated under standard conditions (control cells) and with 10% CB plasma. In CB plasma treated cells Pref-1 expression is upregulated, whereas adipogenic marker genes PPARγ and Perilipin are downregulated, compared to control cells (preliminary data).



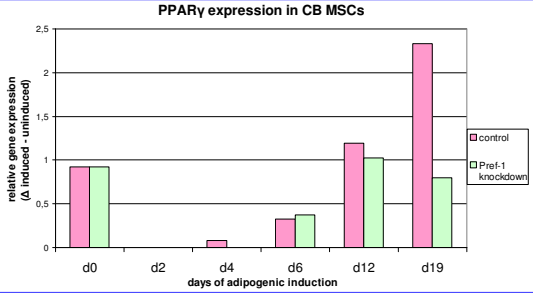


Fig.6 Pref-1 and PPARg expression in Pref-1 downregulated cells.

Pref-1 and PPARγ expression in CB MSCs after adipogenic induction with and without siRNA treatment. Unexpectedly, it was not possible to detect adipogenic differentiation after 21 days of Pref-1 knockdown, neither on RNA level nor as lipid vacuoles (preliminary data).

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.