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Neonatal mesenchymal-like cells adapt to surrounding cells

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Abstract Hematopoietic cord blood (CB) transplantations are performed to treat patients with life-threatening diseases. Besides endothelial cells, the neonatal multipotent stromal cell subpopulations CDSCs (CB-derived stromal cells) and USSCs (unrestricted somatic stromal cells) are like bone marrow (BM) SCs interesting candidates for clinical applications if detailed knowledge is available. Clonal USSC compared to CDSC and BMSC lines differ in their developmental origin reflected by a distinct *HOX* expression. About 20 (out of 39) *HOX* genes are expressed in CDSCs (HOX+), whereas native USSCs reveal no *HOX* gene expression (HOX-). Moreover, USSCs display a lineage-specific absence of the adipogenic differentiation potential. As the specific HOX code can be ascribed to topographic bodysites it may be important to match the HOX code of transplanted cells to the tissue of interest. Herein co-culture experiments were performed, presenting a novel approach to modulate the differentiation potency of USSCs towards HOX positive stromal cells. After co-culturing native USSCs with CDSCs and BMSCs, USSCs adapt a positive HOX code and gain the adipogenic differentiation capacity. These results present for the first time modulation of a lineage-specific differentiation potential by co-culture. Finally, USSCs can be claimed as potential candidates to substitute unique progenitor cell populations in clinical approaches.

Introduction

Besides hematopoietic stem cells (HSCs), multipotent stromal cells are claimed to be a promising source for clinical applications, but their typical differentiation capacities depending on their origin (cord blood, CB; bone marrow, BM; adipose tissue, AT) might have an impact on their usefulness in specific clinical applications. Therefore, a detailed characterization of their developmental origin and their lineage-specific differentiation potential is mandatory to elaborate, if a specific cell source might be favourable for bone and cartilage formation. Questionable as well is, if mixed bulk cultures should be applied in clinical applications, as no detailed knowledge is available on the impact of mixed subpopulations of stromal cells. In cord blood, two distinct clonal neonatal subpopulations are described: USSCs (unrestricted somatic stromal cells) and CDSCs (cord blood derived stromal cells) (Kluth et al., 2010; Kogler et al., 2004; Liedtke et al., 2010). Regarding their immunophenotype both clonal neonatal cell types share the same pattern of

Abbreviations: CB, cord blood; BM, bone marrow; CD, cord blood derived; SCs, stromal cells; USSCs, unrestricted somatic stromal cells; UC, umbilical cord.

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surface molecules ($CD45^-$, $CD13^+$, $CD29^+$, $CD73^+$, $CD105^+$) similar to bone marrow-derived stromal cells (Erices et al., 2002) and are commonly referred to as "MSCs" phenotype (Dominici et al., 2006). However, the markers used to describe stromal cells are not specific and are expressed by many connective tissue cells that are not stem cells. To date, a marker set clearly distinguishing connective tissue stem cells from more mature cells is not available. As the term "MSCs", which can stand for mesenchymal stromal cells as well as multipotent stromal cells, is controversially discussed (Bianco, 2011a), we will refer to cells derived out of cord blood (CDSCs and USSCs) and out of bone marrow (BMSCs) as stromal cells in this paper.

A great advantage of these CDSCs and USSCs is their simple isolation and expansion *in vitro*. Likewise, USSCs produce functionally significant amounts of hematopoiesis-supporting cytokines and are superior to BMSCs in expansion of $CD34^+$ cells from cord blood (Kogler et al., 2005). USSCs are therefore a suitable candidate for stroma-driven *ex vivo* expansion of hematopoietic cord blood cells for short-term reconstitution or co-transplantation (Jeltsch et al., 2011). In the near future, these cells may be applied to patients to reduce the graft-versus-host disease, the most occurring side effect after transplantation of hematopoietic stem cells or to support hematopoiesis (Abdallah and Kassem, 2009). USSCs and CDSCs share the osteogenic and chondrogenic differentiation potential. In a recent study our group analysed in detail the expression of osteogenic and chondrogenic marker genes during differentiation defining the osteogenic signature of USSCs, CDSCs, BMSCs and (umbilical cord) UCSCs (Bosch et al., 2012). Based on the work of Kluth et al. (2010), it was demonstrated that USSCs in contrast to CDSCs do not differentiate naturally towards the adipogenic lineage, while expressing the adipogenic inhibitor *Delta-like 1 homolog* (*DLK1*) on a transcript but not on a secreted protein level. In addition, expression of *HOX* genes is absent in USSCs, whereas CDSCs revealed a typical positive *HOX* code similar to BMSCs (Liedtke et al., 2010).

HOX genes are essential for normal development of vertebrates by determining the positional identity along the anterior-posterior body axis (Krumlauf, 1994). In humans, the 39 known *HOX* genes are distributed among four paralogous clusters *HOXA* to *HOXD*, located in chromosomes 7, 17, 12 and 2, respectively. *HOX* genes are expressed sequentially 3´ to 5´ along the body axis during embryogenesis, termed "temporal and spatial colinearity" (Kmita and Duboule, 2003). The typical *HOX* code of a cell describes the specific expression of functional active *HOX* genes in distinct tissues (Gruss and Kessel, 1991). More importantly *HOX* genes may also have a therapeutic application in near future. It was found that HOXD3 protein is upregulated during normal wound repair (Hansen et al., 2003). The protein promotes angiogenesis and collagen synthesis, but is absent in poorly healing wounds of genetically diabetic mice. After adding HOXD3, the treatment resulted in faster diabetic wound closure and tissue remodeling.

While the facial skeleton is formed by HOX negative neural crest cells (Creuzet et al., 2002), the skeleton, originating from mesoderm-derived progenitor cells, is usually HOX positive in adults (Leucht et al., 2008). In bone regeneration experiments, Leucht et al. revealed that the HOX negative mandibular progenitor cells are favourable in bone repair as compared to the HOX positive tibial progenitor cells (Leucht et al., 2008). In their experiments, HOX negative mandibular progenitor cells started to express *HOX* genes after transplantation into a tibial bone defect leading to bone repair. In contrast to that, HOX positive tibial progenitor cells transplanted into a mandibular defect failed to regenerate bone. This data is supported by recent findings, suggesting the biological advantages of HOX negative cells isolated from endoral sites (Lohberger et al., 2012). The potency of a HOX negative cell to adapt the HOX code of surrounding cells or tissues therefore seems to be an important feature for regenerative approaches but also for the normal development in the skeleton of the fetus.

As *HOX* genes are able to translocate passively through biological membranes, a technique applying a co-culture method provides several advantages. In the work presented here, USSCs (HOX^-) with a restricted adipogenic potential were co-cultured with CDSCs and BMSCs (HOX^+) to test if the USSCs are able to adapt a *HOX* positive expression pattern. Additionally, changes of the lineage-specific cell fate modulated by co-culture were monitored for osteogenic, chondrogenic and adipogenic differentiation in this approach. Finally, following hypotheses were tested:

Do USSCs have the potential to adapt the *HOX* expression pattern of a surrounding celltype?
Can the differentiation capacity be switched to an adipogenic direction by exposing USSCs to *HOX* positive CDSCs able to generate adipocytes?
Is the osteogenic differentiation capacity of USSCs affected by co-culture with CDSCs and BMSCs?
Can USSCs be claimed as potential candidates to substitute unique progenitor cell populations?

Material and methods

Generation and expansion of CB-derived cells and BMSCs

USSCs and CDSCs were generated as described previously (Bosch et al., 2012; Kluth et al., 2010). In brief, CB was collected from the umbilical cord vein with informed consent of the mother. Mononuclear cells (MNC) were obtained by ficoll (Biochrom, density 1.077 g/cm^3) gradient separation followed by ammonium chloride lysis of RBCs. 5–7 *10^6 CB MNC/ml were cultured in DMEM low glucose (Cambrex) with 30% FCS (Perbio), 10^{-7} M dexamethasone (Sigma-Aldrich), penicillin/streptomycin and L-glutamine (PSG; Cambrex). Clonal populations were obtained from cord blood by applying special cloning cylinders. Cell lines were generated as described before and, as soon as distinct, separate colonies were observed, a cloning cylinder was attached on a single colony and cells were trypsinated according to the standard protocol. BMSCs were isolated using BM aspirated from the iliac crest of healthy unrelated donors as previously described (Kluth et al., 2010).

Transfection of USSCs

The transfection of USSCs was performed using the transfection reagent FuGENE® (Roche Applied Science, Mannheim, Germany). The production of lentiviral particles