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RESEARCH ARTICLE

Influence of cell culture media conditions on the osteogenic differentiation of cord blood-derived mesenchymal stem cells

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Summary

In this study the critical parameters directing osteogenic differentiation of umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) were investigated, key factors and conditions identified and improved protocols for a more cell-type adapted differentiation developed. Today only little information about the specific conditions directing osteogenic development is available and current protocols for cultivation and differentiation of UCB-MSCs are based mainly on experience with bone marrow-derived MSCs (BM-MSCs) without further adaptation. Thus, protocols for improved osteoinduction are of particular interest.

The goal of this study was to investigate the influence of three different culture media (A) alpha MEM, 15% FBS, (B) DMEM, 15% FBS and (C) MSCGM, 10% SingleQuot growth supplement on the osteogenic differentiation of UCB-MSCs. Moreover, a systematic analysis of two concentrations of dexamethasone (10^{-8} M/10^{-7} M) in combination with or without BMP-2 (10^{-7} M) was carried out by detecting the expression of alkaline phosphatase (ALP), collagen-1 and the mineralization of ECM. We found that MSCGM, 10% SingleQuot had a supportive effect on the osteogenic differentiation of UCB-MSCs. In case of treatment with 10^{-8} M dexamethasone, mineralization occurred in combination with BMP-2 exclusively, while a concentration of 10^{-7} M dexamethasone led to a high amount of mineralized ECM and the expression of collagen-1 independent of BMP-2 addition. According to this data dexamethasone is the leading osteoinductive factor, but BMP-2 seems to have supportive properties in UCB-MSCs. In conclusion, MSCGM supplemented with 10% SingleQuot and 10^{-7} M dexamethasone was the condition identified to be best for inducing the osteogenic differentiation of UCB-MSCs.

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Introduction

Due to their self renewal capacity and potential of multipotent differentiation, adult stem cells are promising candidates for use in cell-based therapies and tissue engineering. Stem cells exist in various tissues of the adult organism where they play an essential role in tissue repair and regeneration processes. The best characterized adult stem cells are the mesenchymal stem cells isolated from bone marrow (BM-MSCs), alternatively named bone marrow stromal cells (BMSCs). This population of non-hematopoietic cells was first isolated by Friedenstein in 1968 and has been reported to have the potential to replicate and differentiate into osteogenic precursor cells. Thereafter, a large number of investigators reported on additional differentiation lineages of BM-MSCs e.g. into osteoblasts, chondrocytes, adipocytes, tendon and stromal cells (Pittenger et al., 1999; Owen and Friedenstein, 1988; Mackay et al., 1998; Caplan, 1991; Young et al., 1998) elucidating their multipotent characteristics. Moreover, differentiation into myogenic (Wakitani et al., 1995; Prockop, 1997; Ferrari et al., 1998; Dezawa et al., 2005) and neuronal cells (Choong et al., 2007; Pacary et al., 2007) has been described.

The bone marrow is a common source of stem cells, but isolation of BM-MSCs requires surgical procedures associated with risks, including infection (Romanov et al., 2003). Moreover, the differentiation potential of BM-MSCs is age dependent (Nakahara et al., 1991; Digirolamo et al., 1999; Baxter et al., 2004) limiting their applicability for research and clinical use, thus alternative sources are of particular interest. Nowadays, it is known that adipose tissue (Zuk et al., 2001), skeletal muscle, deciduous teeth (Barry and Murphy, 2004), pancreas (Kruse et al., 2006), fetal pancreas (Hu et al., 2003), lung, liver (In't Anker et al., 2003), testes (Guan et al., 2006), menstrual blood (Meng et al., 2007), amniotic fluid (Coppi et al., 2007) and umbilical cord blood (Chang et al., 2006; Kang et al., 2006) harbor stem cells, which are assumed to have future potential for cell-based therapies.

The umbilical cord is a postnatal organ discarded after birth; hence umbilical cord blood (UCB) is readily accessible particularly regarding an excess of one million births per year. UCB is a high class source of hematopoietic stem cells, since those isolated cells show a reduced risk of acute graft-versus-host disease after transplantation (Barker et al., 2001; Rocha et al., 2004). Recently, isolation of MSCs-like stem cells (UBC-MSCs) has also been described (McGuckin and Forraz, 2008; Markov et al., 2007; Bieback et al., 2004; Feldmann et al., 2005). There is a general consensus that MSCs are found in several fetal organs (Campagnoli et al., 2001), the placenta (Li et al., 2005; Fukuchi et al., 2004), the umbilical cord tissue (Romanov et al., 2003; Can and Karahuseyinoglu, 2007; Lu et al., 2006), including blood vessels and Wharton's jelly (Covas et al., 2005; Romanov et al., 2003; Wang et al., 2004). MSC-like stem cells derived from full-term deliveries of UCB (Erices et al., 2000; Goodwin et al., 2001) have been demonstrated to differentiate into adipogenic, chondrogenic, osteogenic, hepatogenic and neurogenic lineages (Bieback et al., 2004; Lee et al., 2004; Kögler et al., 2004).

Today, only little information is available on the specific conditions necessary to direct the osteogenic development of UCB-MSCs and current protocols for cultivation and differentiation of UCB-MSCs are mainly based on earlier experiments with BM-MSCs. As the UCB-MSCs have the potential to become an important future stem cell source for basic research as well as clinical application, protocols for an improved osteoinduction of UCB-MSCs are of particular interest.

To better understand the differentiation processes in UCB-MSCs and improve osteoinduction *in vitro*, this study has been focused on the identification of critical parameters directing osteogenic differentiation and the development of optimized protocols. As differentiation inductors, two different concentrations of dexamethasone, 10^{-8} and 10^{-7} M, in combination with and without 10^{-7} M BMP-2, respectively, were evaluated for their capacity to induce osteogenic differentiation. To investigate the influence of the growth media, the differentiation process was carried out in three different standard culture media (A) alpha MEM, 15% FBS, (B) DMEM, 15% FBS and (C) MSCGM, 10% SingleQuot growth supplement.

Material and methods

Materials

Culture slides were obtained from BD Falcon (Erembodegem, Belgium). All other cell culture materials were purchased from Greiner Bio-one GmbH (Frickenhausen, Germany). Phosphate buffered saline (PBS), alpha MEM (without desoxyribonucleotides or ribonucleotides, with GlutaMAX), DMEM (low glucose, with GlutaMAX), penicillin/streptomycin, trypsin/EDTA solution and goat serum were obtained from Gibco (Invitrogen Karlsruhe, Germany). FBS was purchased from PAA Laboratories GmbH (Pasching, Austria). MSCGM

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