Stem Cell Research (2013) 11, 1393–1406

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

Inferior ectopic bone formation of mesenchymal stromal cells from adipose tissue compared to bone marrow: Rescue by chondrogenic pre-induction

J. Brocher[a,1], P. Janicki[a,1], P. Voltz[a], E. Seebach[a], E. Neumann[b,c], U. Mueller-Ladner[b,c], W. Richter[a,*]

[a] *Research Centre for Experimental Orthopaedics, Heidelberg University Hospital, Schlierbacher Landstrasse 200a, D-69118 Heidelberg, Germany*
[b] *Department of Internal Medicine and Rheumatology, Justus-Liebig University Giessen, D-61231 Bad Nauheim, Germany*
[c] *The Kerckhoff Klinik, D-61231 Bad Nauheim, Germany*

Received 28 February 2013; received in revised form 22 July 2013; accepted 29 July 2013
Available online 3 August 2013

Abstract Human mesenchymal stromal cells derived from bone marrow (BMSC) and adipose tissue (ATSC) represent a valuable source of progenitor cells for cell therapy and tissue engineering. While ectopic bone formation is a standard activity of human BMSC on calcium phosphate ceramics, the bone formation capacity of human ATSC has so far been unclear. The objectives of this study were to assess the therapeutic potency of ATSC for bone formation in an ectopic mouse model and determine molecular differences by standardized comparison with BMSC. Although ATSC contained less $CD146^+$ cells, exhibited better proliferation and displayed similar alkaline phosphatase activity upon osteogenic *in vitro* differentiation, cells did not develop into bone-depositing osteoblasts on β-TCP after 8 weeks *in vivo*. Additionally, ATSC expressed less BMP-2, BMP-4, VEGF, angiopoietin and IL-6 and more adiponectin mRNA, altogether suggesting insufficient osteochondral commitment and reduced proangiogenic activity. Chondrogenic pre-induction of ATSC/β-TCP constructs with TGF-β and BMP-6 initiated ectopic bone formation in >75% of samples. Both chondrogenic pre-induction and the osteoconductive microenvironment of β-TCP were necessary for ectopic bone formation by ATSC pointing towards a need for inductive conditions/biomaterials to make this more easily accessible cell source attractive for future applications in bone regeneration.

Introduction

Large bone defects resulting from non-healing fractures, osteomyelitis or tumor resection are still a therapeutic challenge to clinicians. Autografts are one solution for such problems but are limited in size, require a painful surgical procedure and necessitate the dissection of autologous bone from an intact site, hence creating an additional lesion. Implantation of a combination of autogenous cells with bone

* Corresponding author at: Research Centre for Experimental Orthopaedics, Heidelberg University Hospital, Schlierbacher Landstrasse 200a, D-69118 Heidelberg, Germany. Fax: +49 6221 5629288.
E-mail address: Wiltrud.Richter@med.uni-heidelberg.de (W. Richter).
[1] Authors contributed equally.

1873-5061/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scr.2013.07.008

replacement material might thus provide a less invasive therapeutic approach. Multipotent skeletal progenitor cells, harboring the ability to self-renew and establish new bone organs, are found in bone marrow (Owen, 1988; Sacchetti et al., 2007). However, common mesenchymal progenitor cells, not just of skeletal tissues but of most mesenchymal tissues, were proposed to reside in many tissues outside of bone marrow, including adipose tissue (Caplan, 1994; Zuk et al., 2002; da Silva Meirelles et al., 2006). Their intrinsic capacity to initiate new bone organs and foster equivalent sources of cells for efficient bone formation has so far, however, not been proven. Easier retrieval and larger quantities are clear advantages of adipose tissue, making adipose tissue-derived stromal cells (ATSC) a preferable cell source over bone marrow-derived stromal cells (BMSC), given they are equally suitable to support *de novo* bone formation together with bone replacement material.

Comparative *in vitro* characterization of human ATSC and BMSC revealed overlapping as well as diverging characteristics of both cell populations. Expanded human BMSC and ATSC were highly positive for MSC markers CD73 and CD90 (Wagner et al., 2005, 2008; Mitchell et al., 2006; Dickhut et al., 2009; van der Bogt et al., 2009) and negative for the haematopoietic cell surface marker CD45. While BMSC contained, however, less than 2% of CD34-positive cells (Zuk et al., 2002; Im et al., 2005; Dominici et al., 2006), some studies detected more CD34-positive cells in ATSC populations (Noel et al., 2008; Suga et al., 2009), while others did not (Zuk et al., 2002; Im et al., 2005). ATSC further contained a lower fraction of CD146-positive cells compared to BMSC (Dmitrieva et al., 2012), suggesting that ATSC populations most likely contain fewer pericytes than BMSC.

While *in vitro* multilineage differentiation potentials of ATSC and BMSC were reported to be similar (De Ugarte et al., 2003; Winter et al., 2003), some studies suggested a lower osteogenic *in vitro* potential of ATSC compared to BMSC, according to mineral deposition, alkaline phosphatase (ALP) activity as well as osterix and osteocalcin gene expression (Im et al., 2005; Liu et al., 2007; Hayashi et al., 2008; Shafiee et al., 2011). Others found no significant differences in calcium deposition and osteocalcin secretion (De Ugarte et al., 2003; Hattori et al., 2004). *In vitro* osteogenic differentiation assays are, however, prone to artifacts, since alizarin red S and van Kossa staining cannot distinguish between dystrophic calcification and matrix mineralization. Proteome and transcriptome profiles during expansion and differentiation, moreover, revealed significant differences between ATSC and BMSC populations (Noel et al., 2008). Expanded ATSC demonstrated lower expression of BMP-2, -4 and -6 mRNAs, showed lower chondrogenic potential *in vitro* and required BMP-6 beyond the TGF-β regimen to undergo successful chondrogenesis (Lin et al., 2005; Hennig et al., 2007).

Ectopic bone formation is a standard activity of human BMSC on calcium phosphate ceramics, such as β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA)/TCP (Krebsbach et al., 1997; Kasten et al., 2005, 2008; Mankani et al., 2006; Sacchetti et al., 2007; Janicki et al., 2010, 2011), particularly when granules with a size between 0.1 and 1 mm in diameter are applied (Mankani et al., 2001; Janicki et al., 2010). High BMSC proliferation at the time of transplantation correlates positively with new bone formation, explaining some degree of donor variability (Janicki et al., 2011). Bone quality can further be enhanced by chondrogenic pre-induction of BMSC/β-TCP constructs *in vitro*, since this triggers differentiation via the endochondral ossification pathway, attracted haematopoietic marrow more efficiently and allowed full ossicle formation (Janicki et al., 2010).

In contrast to BMSC, the ectopic bone formation capacity of expanded human ATSC on osteopermissive bone replacement materials was not unambiguously proven (Hattori et al., 2004, 2006; Scherberich et al., 2007; Schubert et al., 2011). Only Zannettino et al. showed a convincing ectopic bone formation by expanded ATSC from one donor on HA/TCP in NOD/SCID mice, when CD146-presorted cells were transplanted (Zannettino et al., 2008). In an orthotopic long bone defect model, sheep ATSC were inferior to BMSC in their ability to induce healing of a critical size defect when seeded on a mineralized collagen sponge (Niemeyer et al., 2010). While this indicates reduced performance of sheep ATSC versus BMSC, the osteogenic *in vivo* capacity of human ATSC has yet to be elucidated. Beyond a more simple retrieval procedure and higher yield of progenitors, one functional advantage of ATSC over BMSC may be a greater proliferation capacity (Schubert et al., 2011; Dmitrieva et al., 2012), an activity which supported bone formation of BMSC (Janicki et al., 2011). Since chondrogenic pre-induction was a means to improve *de novo* bone formation of BMSC (Janicki et al., 2010), this process may also enhance the response of ATSC.

The primary aim of this study, therefore, was to assess the therapeutic potency of human ATSC for bone formation by direct comparison with BMSC under standardized conditions. We expanded human ATSC and BMSC from similar donors and characterized them regarding *in vitro* osteogenicity and ectopic bone formation capacity on osteopermissive β-TCP in immune-deficient mice. Beta-TCP granules were selected to deploy the osteogenicity of the cells, since this calcium phosphate ceramic performed superior to HA/ß-TCP granules in a recent study (Janicki et al., 2010). Since stimulating the endochondral differentiation pathway led to enhanced results for BMSC, part of the samples were subjected to chondrogenic pre-induction to evaluate whether this would also promote bone formation by ATSC.

Materials and methods

Isolation and cultivation of MSC

Human BMSC from 7 donors were isolated from iliac crest marrow aspirates of seven donors (aged 22–76 years). Briefly, nucleated cells were washed and purified on a Ficoll-Paque™ Plus density gradient (GE Healthcare, Munich, Germany) (De Bari et al., 2001). Human ATSC of 7 donors were retrieved after surgical procedure or liposuction (aged 34–73). Isolation was carried out as described (Winter et al., 2003). Briefly, lipoaspirates were digested with Krebs–Ringer solution buffered with 25 mM HEPES, 20 mg/ml bovine serum albumin (BSA) and 1.5 mg/ml collagenase B (Roche Diagnostics, Mannheim, Germany) and filtered with a 150-μm nylon mesh. Erythrocytes were removed using erythrocyte lysis buffer (0.154 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA). Written consent was obtained from all subjects, and the study received approval from the local ethics committee.