PROSPECT
Journal of Cellular Biochemistry 108:762–768 (2009)

Journal of Cellular Biochemistry

Cord Blood for Tissue Regeneration

Anja Buchheiser,[1] Stefanie Liedtke,[1] Leendert H.J. Looijenga,[2] and Gesine Kögler[1*]

[1]*Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich-Heine-University Medical Center, 40225 Düsseldorf, Germany*

[2]*Department of Pathology, Erasmus MC-University Medical Center Rotterdam, Daniel den Hoed Cancer Center, Josephine Nefkens Institute, Rotterdam, the Netherlands*

ABSTRACT

Umbilical cord blood (CB) has become a commonly accepted source of hematopoietic stem cells for transplantation in children and adults. It is readily available and outperforms bone marrow (BM) as well as peripheral blood stem cells in terms of tolerance for HLA-mismatches between donor and recipient and its decreased graft-versus-host disease. Clinical use has been expanded from hematological malignancies to various areas such as treatment of metabolic genetic disorders or to induce angiogenesis. For the last years CB has been under intense experimental investigation in in vitro differentiation models as well as in preclinical animal models. Since CB-derived stem cells offer multiple advantages over adult stem cells from other sources like BM, CB may provide a future source of stem cells for tissue repair and regeneration. To facilitate the use of CB-derived stem cells in clinical scenarios, the biology of these cells needs to be further explored in detail particularly with regard to the fact that different non-hematopoietic stem cell populations occur within CB. Here we explore the most consistent and the most contradictory data referring to the differentiation potential of CB-derived stem cells and give an outlook on their potential clinical value including and possible reprogramming into IPS cells. J. Cell. Biochem. 108: 762–768, 2009.

KEY WORDS: CORD BLOOD; REGENERATIVE MEDICINE

It has been shown that in contrast to its adult bone marrow (BM) counterpart, the stem cell compartment in cord blood (CB) is less mature. This has been documented extensively for the hematopoietic system, including a higher proliferative potential in vitro and in vivo, associated with an expanded life span and longer telomeres. The biological immaturity and increased frequency of hematopoietic stem cells already indicated that CB might also be an attractive source of non-hematopoietic stem cells. Indeed, various non-hematopoietic stem cell populations have been described. It is generally accepted that CB contains endothelial colony forming cells (ECFC) [Yoder et al., 2007], multipotent mesenchymal stromal cells (MSC) [Erices et al., 2000], and unrestricted somatic stem cells (USSC) [Kogler et al., 2004]. Other populations like very small embryonic-like (VSEL) stem cells [Halasa et al., 2008] or multilineage progenitor cells (MLPC) [Berger et al., 2006] have been described. Some groups have specified pluripotent stem cells from CB which are positive for OCT4, SOX-2, and NANOG [McGuckin et al., 2008]. All reports agree on the multipurpose application of CB, due to the fact that CB-derived cells exhibit a variety of differentiation potentials and many key benefits compared to other adult stem cells. However, some aspects have been discussed controversially (Fig. 1).

ENDOTHELIAL PROGENITOR CELLS

Since 1997 EPC have frequently been studied according to their morphology and surface antigen expression even though the obtained data often lacks a detailed characterization of cellular function and lineage of origin. Owing to this, cell populations of different origin including myeloid, lymphoid, or endothelial cells have been embraced by the term EPC. It has to be taken into consideration that in contrast to colony forming unit-Hill (CFU-Hill) ECFC are the only circulating cells within CB that posses all characteristics of an EPC [Yoder et al., 2007]. Plating MNC or isolated $CD34^+$ or $CD133^+$ enriched cells on fibronectin coated surfaces results in the appearance of distinct, so called CFU-Hill colonies. CFU-Hill have been shown to express the endothelial markers CD31, CD105, CD144, CD146, vWF, and KDR. They display the ability to ingest acetylated low-density lipoprotein (AcLDL). This

Grant sponsor: DFG; Grant number: KO2119/6-1.

*Correspondence to: Prof. Dr. Gesine Kögler, Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich-Heine-University Medical Center, Moorenstr. 5 Geb.14.88, 40225 Düsseldorf, Germany.
E-mail: koegler@itz.uni-duesseldorf.de

Received 23 July 2009; Accepted 24 July 2009 • DOI 10.1002/jcb.22320 • © 2009 Wiley-Liss, Inc.
Published online 26 August 2009 in Wiley InterScience (www.interscience.wiley.com).

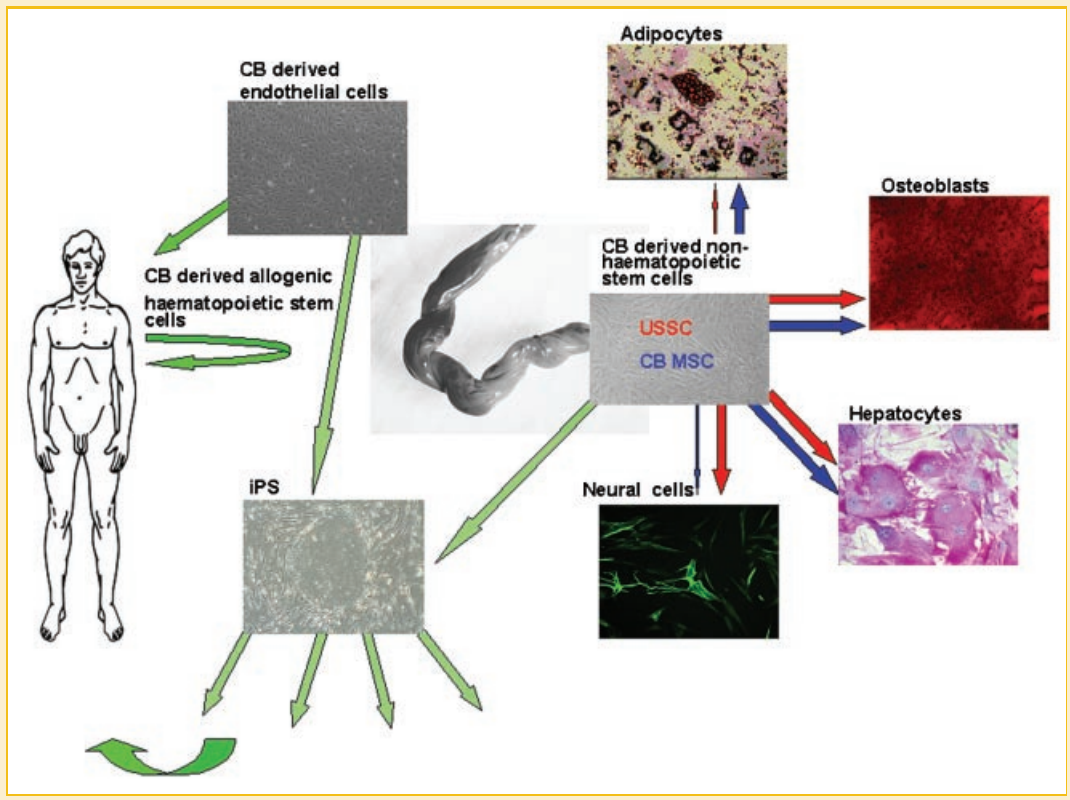

Fig. 1. Cord blood as a cell source for hematopoietic cells for transplantation, endothelial cells, USSC, and MSC and in the future for the generation of IPSC. USSC are able to differentiate towards the osteogenic, neural, and endodermal lineage (red arrows), CB MSC differentiate preferentially to adipocytes, the osteogenic lineage, and the endoderm. CB MSC lack neural differentiation (blue arrows). The green arrow indicates possible cell sources for reprogramming.

feature is exhibited not only by endothelial cells, but also by macrophages. Furthermore, in contrast to ECFC CFU-Hill express many monocytes/macrophage markers including CD14, CD45, and CD115, ingest bacteria, and display non-specific esterase activity [Yoder et al., 2007]. CFU-Hill progeny and ECFC progeny exhibit different tube formation potential when plated on Matrigel. Although often used as a specific test of endothelial cell function, the Matrigel tube formation assay is inappropriate as a specific discriminator of endothelial cell function since Matrigel induces cord formation from non-endothelial cells as well. ECFC progeny, but not CFU-Hill progeny, form de novo blood vessels when seeded into collagen fibronectin matrix in vivo in immunocompromised mice [Yoder et al., 2007].

MULTIPOTENT MESENCHYMAL STROMAL CELLS IN CB

Since 2000 several groups have identified adherently growing fibroblast-like cells in CB revealing an immunophenotype (CD45−, CD13+, CD29+, CD73+, CD105+) similar to bone marrow (BM−)-derived MSC [Erices et al., 2000]. None of these markers is MSC specific, neither individually nor combined. MSC have been defined as multipotent cells with an osteogenic, chondrogenic, and adipogenic differentiation potential. In addition, some controversial data suggest that BM MSC may give rise to other cells from mesodermal origin such as sarcomeric muscle [Planat-Benard et al., 2004], and even cells of non-mesodermal origin such as hepatocytes [Chagraoui et al., 2003], and neural cells [Woodbury et al., 2000]. The mesodermal differentiation potential of CB MSC has been confirmed by different groups. Further investigations show an even wider differentiation spectrum of CB MSC characterized by the combination of different germinal layers. Goodwin et al. [2001] described a cell population, which can give rise to cells containing features ascribed to adipocytes, osteocytes, and neural cells, indicating a mesodermal/ectodermal differentiation. MSC bear various properties of clinical interest, including their wide-ranging differentiation potential, their capacity for engraftment [Dominici et al., 2008], their immunosuppressive effects [Di Nicola et al., 2002], and their expansion through culture.

UNRESTRICTED SOMATIC STEM CELLS

In 2004, our group described a multipotent cell population within CB, which we termed USSC. While the differentiation potential of MSC remains partly controversial, USSC can be differentiated into cells of all three germ layers in vitro and in vivo [Kogler et al., 2004] as discussed below. Specific immunophenotypic markers are still unknown, but recently we have been able to demonstrate that the adipogenic differentiation potential is a functional marker to discriminate between USSC and CB MSC. After analyzing a large

scale of CB-derived cell lines we have been able to clearly show that USSC do not exhibit any adipogenic differentiation potential except for those passing through a high number of population doublings.

MLPC, VSELs, AND OTHER "EMBRYONIC LIKE"-CELL POPULATIONS

A multipotent cell (MLPC) has recently been identified in CB as a CD45+, CD34+, CD9+, nestin+ plastic adherent population [Berger et al., 2006]. These cells have shown extensive expansion capacity, while maintaining normal genetic stability and the ability to differentiate into cells representing all three germ layers. MLPC can be isolated from full-term CB as well as from MSC by single cell cloning. MLPC distinguish themselves from MSC by showing a more extensive expansion and differentiation potential [van de Ven et al., 2007].

VSEL stem cells had initially been identified by the group of Ratajczak in adult murine BM as a rare population of stem cells with embryonic characteristics Moreover, an appropriate cell population has been found in the human CB [Kucia et al., 2006; Ratajczak et al., 2009]. VSELs exhibit very small size and express SSEA4, OCT4, and NANOG. VSELs have the potential to differentiate into cells of all three germ layers such as ectodermal neural cells, endodermal pancreatic cells, and mesodermal cardiomyocytes [Kucia et al., 2006]. Furthermore, a subset of CD45+/lin− cells has been described [Rogers et al., 2007].

Moreover, other studies report on multipotent progenitor cells (MPCs) capable of differentiating into neural cells and hepatic cells [Moon et al., 2008].

Besides the introduction of several acronyms, which may themselves cause confusion, some aspects of the biology of these cells are still being questioned. The same problems occurred, whenever different cell populations within BM were analyzed [Dominici et al., 2009].

MULTIPOTENCY, PLURIPOTENCY, TUMORIGENICITY

CB contains multipotent cell populations with a broad differentiation potential. Different groups report on pluripotent or embryonic like stem cells. Pluripotency is defined as a regeneration of all tissues including germ line. To our knowledge this criterion has not been shown for CB so far. The concept of ES-cells or ES-like cells circulating in the neonate and the CB remains speculative. Most of these propositions are based on the expression of the so-called embryonic stem cell (ESC) markers like OCT4 (also known as OCT3/4 or POU5F1), NANOG, or SSEA4. Some of these previously published data remain questionable due to data misinterpretation.

FACS analysis show that USSC and MNC from CB or BM do not express SSEA3, SSEA4, or SSEA1 [Buchheiser et al., 2008].

The first marker described as essential for self-renewing or maintenance of the ESC phenotype was OCT4. At least six pseudogenes and several alternatively spliced transcripts which can be ascribed to OCT4 are known [Takeda et al., 1992]. Due to the high homology of the pseudogene sequences to their parental gene, a very high possibility exists to amplify a pseudogene-derived PCR product [Liedtke et al., 2008]. Furthermore, two main splice variants OCT4A and OCT4B, which show different spatial expression patterns during human preimplantation exist [Cauffman et al., 2006]. Hence, false positive results of OCT4 can also occur on the protein level. The C-terminal part of OCT4A and OCT4B is identical. In the meantime most researchers have taken this issue into consideration.

The pluripotency markers OCT4, NANOG, and the transcription factor SOX2 build a transcriptional network that regulates a large number of genes involved in cellular differentiation in ES cells [Cauffman et al., 2006]. Analyses of human ES cells revealed that all three factors occupy and activate genes that promote ES cell growth and self-renewal while simultaneously repressing genes that promote differentiation [Lee et al., 2006]. Downregulation of OCT4 and NANOG results in differentiation in physiological and malignant ES cells [Matin et al., 2004]. In vivo, OCT4 is expressed within the inner cell mass of blastocysts from which ES cells are derived. OCT4 expression is downregulated in somatic cells around the time of gastrulation, but retained in primordial germ cells only and presumably in some adult stem cell populations. Using specific antibodies for the type 1 variant clearly demonstrates that OCT4A is highly specific only for the seminomas and embryonal carcinoma, independent of anatomical localization, stage of progression, and therapy response. A large study including non-germ cell tumors and normal tissues did not indicate nuclear OCT4 [Looijenga et al., 2003]. Pluripotent germ cell tumors are in fact preferentially diagnosed in the gonads, both ovary and testis, where the OCT4 positive (germ) cells migrate to, and under normal conditions lose their embryonic features [Stoop et al., 2005]. The extra-gonadal pluripotent germ cells tumors are predominantly identified along the midline of the body, which is likely to be related to a mis-migration of embryonic germ cells [Oosterhuis et al., 2007]. The presence of pluripotent cells at other anatomical localizations can be hazardous because of the potential of generating both embryonic and extra-embryonic lineages of differentiation, including trophoblast, and placental development. Using RT-PCR, real-time PCR, and immunohistochemistry the expression of OCT4A, NANOG, and SOX2 which was absent from any tested cell population from CB and BM, was studied [Buchheiser et al., 2008]. A strict regulation of pluripotency, both temporally and spatially is required for normal development and maintenance of the adult individual. One of the defining characteristics of the ESC phenotype is the capacity of unlimited, indefinite self-renewing division to produce identical progeny. Recently, OCT4 has been described to be expressed in several cell types, questioning the universal role of OCT4 in identification of pluripotent self-renewing stem cells. However, Lengner et al. [2007] recently demonstrated that OCT4 is not even essential for self-renewal and maintenance of mouse somatic stem cells. It has been argued by Howe et al. [2009] that the expression of OCT4A mRNA or protein neither implies nor confers embryonic like phenotype of $CD133^{+}$ stem cells from CB.

Stem cell tumorigenicity, especially of pluripotent ESC and induced pluripotent stem cells (IPSC), represents a key obstacle to the safe use of these cells in regenerative medicine. IPSC are predicted to exhibit tumorigenic potential equal to that of ESC while use of adult multipotent stem cells appears to be safe. It is

remarkable that to date one of the most common assays for attesting pluripotency of stem cells, including IPSC is a teratoma assay. The greater the pluripotency and the self-renewal properties a stem cell possesses the probability it will cause tumors is invariably higher [Knoepfler, 2009]. Furthermore, it is important to study the global epigenetic changes associated with pluripotency. Epigenetic alterations may in part confound the efficacy of moving away from genetic changes through promoting tumor genesis themselves. Developing safer regenerative medicine by using pluripotent stem cells could benefit from characterizing the relationship among the epigenome, pluripotency, and tumorigenicity [Knoepfler, 2009]. Human IPS cells might be an ideal source for cell therapy in the future, but their production has to be standardized and must be safe in terms of potential tumorigenicity. Since the first report from Takahashi and Yamanaka [2006] on the reprogramming of adult mouse fibroblasts by inducing the expression of OCT4, SOX2, c-MYC, and KLF4 into ES-like cells termed as IPSC, different groups have tried to improve this method. It had been shown that reprogramming is possible without introduction of the oncogene c-MYC [Pera and Hasegawa, 2008]. Furthermore, Kim et al. [2008] were able to reprogram adult neural stem cells (NSC) by introducing two factors, and currently it has been shown that simply OCT4 is a sufficient means [Kim et al., 2009]. It has been shown that NSC represents a more advanced stage in the reprogramming process, and hence can be used for reprogramming more easily and more efficiently than fibroblasts, which are terminally differentiated. Since CB-derived MSC and USSC also represent an early developmental stage and express basal KLF4 and cMYC, they also might be easily reprogrammed. To make reprogramming safer, some groups showed that IPSC could be generated without using viral vectors. Stadtfeld et al. [2008] generated IPSC by using non-integrating adenoviruses transiently expressing Oct4, SOX2, c-Myc, and KLF4. Furthermore, this group was able to generate IPSC by using a single lentiviral vector expressing a stem cell cassette comprised of the four transcription factors and a combination of 2A peptide and IRES technology [Sommer et al., 2009]. Whereas retroviral transduction increases the risk of tumorigenicity, transient expression methods have considerably lower reprogramming efficiencies. Yusa et al. [2009] have avoided this issue by using a piggyBac transposon-based approach to generate integration free IPSC. Another issue concerning the clinical use of IPS cells is to safely obtain them from either a potential donor or established sources such as banked CB [Yamanaka, 2009]. In the meantime, the clinical use of adult stem and progenitor cells from different sources including CB will be expanded. In the next chapters we focus on the CB-derived cell populations that could have therapeutical applications in the near future due to the conformance to requirements for a clinical use.

BASIC REQUIREMENT FOR CLINICAL APPLICATION

Before the regular use of MSC and USSC from CB as a therapeutic product in patients can be implemented main challenges remain. The production of large scales of cells even linked to biomaterials is mandatory for clinical purposes. The cell production must adhere to good manufacturing practices (GMP), to ensure the delivery of a product that is safe, reproducible, and efficient. For the clinical GMP grade production of MSC from different sources, different protocols already exist [Sensebe, 2008]. We have established the GMP grade generation and expansion of USSC from fresh CB [Radke et al., 2007]. MNC were isolated by using the automated cell processing system Sepax (BIOSAFE) with the CS900 separation kit. For the subsequent generation of USSC colonies 30% GMP grade fetal calf serum (EMEA certified), low glucose DMEM/10^{-7} dexamethasone was used. Cell expansion was performed in a nearly closed system applying the cell stack system (Corning). Cell numbers of 1×10^9 could be obtained within four passages. The USSC product could be cryopreserved, thawed, and expanded further in clinical grade quality. We must comply with district requirements on the safety of the original donor material as well as on the biological safety of the product. The final product is tested for endotoxins and mycoplasms. Each cell line is tested for the immunophenotype as well as for their differentiation potential.

WHY ARE ADULT STEM CELLS FROM CB SUPERIOR TO STEM CELLS FROM OTHER SOURCES?

As described by our group in cooperation with A. Dickinson (Newcastle) USSC are conditionally immunosuppressive, and IFNγ and TNFα constitute a switch that regulates their immunological properties. They either suppress T-cell responses in the presence of both cytokines or in their absence block dendritic cell differentiation and function. These activities may contribute to an adaptation of the immune system especially at sites of tissue damage. Recently van den Berk et al. [2009] have shown that USSC differ from BM MSC with regard to the Toll-like receptor ligands.

Our group has studied cytokine production and in vitro hematopoiesis-supporting stromal activity of USSC and MSC from CB in comparison to BM-derived MSC [Kogler et al., 2005]. USSC produce significant amounts of hematopoiesis-supporting cytokines and are superior to BM MSC in supporting the expansion of $CD34^+$ cells from CB.

MESODERMAL TISSUE REGENERATION

Tissue engineering for bone and chondrocytes is becoming an additional alternative in the treatment of traumatic and skeletal diseases and in oral and maxillofacial surgery. At present autologous BM or BM-derived MSC are used. This procedure bears some major disadvantages. The intervention is a burden for the patients and denotes an operative risk. Furthermore, in elderly patients the growth and differentiation potential of the BM MSC is restricted. Any alternative to autologous MSC from BM would be a welcome benefit. Since all MSC and USSC populations derived from CB were capable of differentiating along the osteogenic and chondrogenic lineage this cell source could be an option. The in vivo chondrogenic potential was shown in a mouse model. Since USSC also show a high production of VEGF and FGF2 and express VEGF receptor 2 the transplantation into a bone or tissue defect might support the repair process not only by initiating mineralization but

also by creating an angiogenetic environment [Degistirici et al., 2008].

APPLICATION IN WOUND HEALING AND NEOVASCULARIZATION

One potential use of ECFC is the treatment of patients with defective wound healing due to impaired neoangiogenesis. It was shown that CB ECFC form de novo blood vessels when seeded into a collagen fibronectin matrix and implanted in vivo. Shepherd et al. [2006] seeded tissue engineered human skin substitutes with keratinocytes and ECFC progeny, transplanted them onto immunocompromised mice and demonstrated the formation of human endothelial cell vessels within the skin substitute. CB-derived ECFC progeny yielded more human vessels in the skin substitutes than either adult blood-derived ECFC or HUVEC. The skin substitutes also exhibit an ingrowth of host vessels. In addition to various in vitro studies, promising preclinical data still exist for vascular engineering strategies using ECFC also concerning the differentiation potential of CB-derived MSC and USSC. This suggests that ECFC represent an excellent cell source for vascular engineering strategies.

CARDIOVASCULAR APPLICATION

Cell-based therapy is a promising approach for cardiac repair in patients with coronary artery disease. In preclinical and early clinical studies, investigators have preliminary evidence showing that stem cell therapy can safely and effectively improve myocardial perfusion and left ventricular function. Cardiac stem cell therapy may decrease left ventricular remodeling in cases of myocardial infarction (MI) and may alleviate symptoms and prevent cardiac enlargement in chronic ischemic heart disease.

Another area of interest for tissue engineering is the production of heart valves. Heart valve disease is a significant cause of mortality, which occurs when one, or more of the heart valves are stenosed or become insufficient, failing to make a tight seal during diastole. To date, the most common treatment is implantation of prosthetic valves. Several attempts have been made to create functional heart valve replacements, with the ability to grow, repair, and to remodel. In the tissue engineering approach, the patient's own cells isolated, for example, from a blood vessel and expanded, are seeded onto appropriate starter matrices in the shape of a heart valve. Currently, two types of starter matrices have been applied: xenogenic or allogenic decellularized fixed heart valves and synthetic biodegradable polymers. In most of the approaches cells are harvested from donor tissues. However, several human cell sources have been investigated. Recently, cells derived from BM or CB have successfully been used to generate heart valves [Schmidt et al., 2006].

Various mechanisms, including paracrine effects, are believed to contribute to stem cell-mediated cardiac repair. Recently, autologous BM stromal cells or skeletal myoblasts have been tested to prevent damage to heart muscle caused by a heart attack as an addition to angioplasty. Analysis of the first randomized controlled trials in 2007 indicates that this new treatment may lead to some improvements over conventional therapy as measured by surrogate tests of heart function.

In vitro differentiation of CB-derived cells towards cardiomyocytes has been described [Nishiyama et al., 2007]. In vivo differentiation of CB-derived cells into human cardiomyocytes was first described by our own group in 2004 applying the preimmune fetal sheep model [Kogler et al., 2004]. Subsequent studies in porcine addressed the question whether USSC can engraft in other more clinically relevant conditions like postinfarction. Kim et al. [2005] showed in a porcine model of chronic MI that transplanted USSC survived 1 month after intra-myocardial transplantation and were able to adopt cardiac phenotypes accompanied by improvement of myocardial function. In collaboration with A. Ruhparwar and the department of Thoracic and Cardiovascular Surgery in Hannover our group was able to show in a porcine model of acute MI that after 8 weeks no USSC were detectable in the infarct area as demonstrated by FISH and immunohistochemistry. Although no human cells were detectable, the USSC preserved the recipient myocardium and prevented scar formation after acute ischemia. This was associated with a significant improvement of left ventricular ejection fraction and it prevented left ventricular dilation. We could not confirm USSC graft survival and USSC differentiation towards cardiomyocytes in this model. However, probable reasons for the observed functional recovery and prevention of scar formation exist. The induction of cytokines can lead to the de novo formation of myocardium or the preservation of the recipient myocardium by paracrine effects. USSC can release a variety of cytokines including VEGF, FGF2, SDF, SCF, and hepatocyte growth factor (HGF). It has been described by other groups that CB-derived CD133+ enhance function and repair of extensive MI in athymic nude rats, confirming that cells improve the left-ventricular function by self-repair through autologous myofibroblasts that prevent scar thinning and dyskinesis instead of differentiation of the human cells towards cardiomyocytes. The use of off-the-shelf-CB populations that repair the myocardium might be important to elderly patients whose functional stem cells are limited.

POTENTIAL FOR NEURAL DIFFERENTIATION

Different kinds of neuronal damage are a major threat to patients, especially on account of the fact that effective treatments still need to be developed. Stem cell therapy is a promising means of replacing or regenerating neuronal tissue after stroke or spinal cord injuries as well as neurodegenerative diseases such as Parkinson's. Several studies have shown how subsets of CB-derived cells differentiate under defined conditions into neurons, astrocytes, and microglia. Most animal studies using CB-derived stem cells for the treatment of neurological disorders were performed using models for cerebral ischemia, reflecting the importance of stroke research since cerebrovascular diseases remain the third leading cause of death in the European Union and in the United States. Studies in different animal models examining the effects of stroke demonstrate the beneficial effects of infusion as well as intra-cerebral transplantation of CB-derived stem cells [Bliss et al., 2007]. Transplanted cells

may secret neurotrophic or neuroprotective factors counteracting degeneration or promoting regeneration. Further it is possible that an inflammatory-modulating action of stem cells reduces stroke-initiated damage. Using a rodent stroke model it has been shown that applying USSC leads to cells being attracted by HGF secreted by ischemia-damaged brain tissue and by apoptotic neurons in both, in vitro and in vivo [Trapp et al., 2008]. A number of neurological diseases exhibit an activation of proapoptotic signal transduction and hence, it is likely that USSC can be attracted by brain lesions resulting from those diseases. Moreover, USSC could act as potential vehicles since their tropism for neural injury might deliver neuroprotective factors in a targeted way towards a lesion. Because of their broad neural differentiation potential CB-derived cell populations were also investigated with regard to their regenerative potential in animal models of amyotrophic lateral sclerosis, Parkinson's disease, cerebral palsy, traumatic brain injury, and in spinal cord injury. Infusion of CB stem cells repeatedly resulted in observable behavioral improvement compared to control groups. Moreover, stem cells expressed neural markers and caused significant reductions of the lesions compared to controls.

ENDODERMAL REGENERATION

A current means for treating pancreas and liver diseases is an organ transplantation providing a definitive cure. However, this kind of treatment still bears considerable risks and limitations, foremost the limited availability of transplantable organs remains the greatest problem for this therapy. Unlike transplantations, stem cell therapies are minimally invasive procedures and could offer a potentially unlimited source of cells for tissue replacement. We already showed that USSC and MSC from CB have the potential to differentiate into the endodermal lineage in vitro and in vivo. Endodermal differentiation in vitro applying MSC from CB was also confirmed by Lee et al. [2004]. To evaluate the potential of USSC to differentiate into liver cells in vivo, in utero transplantation into fetal sheep was employed. Livers of the sheep were taken 14-month post-USSC transplantation. More than 20% of total liver cells were of human origin. No fusion to host cells occurred [Kogler et al., 2004]. At present CD133+ cells from autologous BM in combination with portal vein embolization are used to substantially increase hepatic regeneration in patients with large hepatic malignancies. Preliminary data applying USSC in an appropriate sheep model look promising (A. Ruhparwar, Medical Center, University of Heidelberg, personal communication).

FUTURE DIRECTIONS

In summary, CB-derived stem cells represent a potential source of cells for regenerative medicine. Key questions concerning the differences between the described cell populations concerning their origin, differentiation potential, tumorigenicity, and availability still need to be answered. Moreover, CB could be a source of cells for reprogramming into IPS cells facilitating a future therapeutical use.

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