International Journal of Stem Cells Vol. 2, No. 2, 2009

SPECIAL ISSUE

Non-Hematopoietic Stem Cells in Umbilical Cord Blood

Taro Matsumoto[1], Hideo Mugishima[2]

[1]*Division of Cell Regeneration and Transplantation, School of Medicine, Advanced Medical Research Center, Nihon University,* [2]*Department of Pediatrics, School of Medicine, Nihon University, Tokyo, Japan*

Allogeneic umbilical cord blood (UCB) transplantation has been used to treat a variety of malignant and non-malignant diseases. Recent studies show convincing evidence that UCB contains not only hematopoietic progenitors, but also several types of stem and progenitor cells providing a high proliferative capacity and a variety of differentiation potentials. UCB-derived cells offer multiple advantages over adult stem cells from other sources like bone marrow (BM), because UCB can be collected without painful procedure, easily available in virtually unlimited supply, and has not been exposed to immunologic challenge. In addition, cord blood transplantation is now an established field with great potential and no serious ethical issue by establishment of public UCB banks throughout the world. Therefore UCB-derived non-hematopoietic stem cells may provide an attractive cell source for tissue repair and regeneration. It is generally accepted that UCB contains endothelial progenitor cells (EPC), mesenchymal stromal cells (MSC), unrestricted somatic stem cells (USSC), very small embryonic-like stem cells (VSEL), multilineage progenitor cells (MLPC), and neuronal progenitor cells. This review focuses on biological properties of these non-hematopoietic stem/progenitor cells derived from human UCB and their potential use in cell based therapies.

Keywords: Endothelial progenitor cell, Mesenchymal stem cell, Unrestricted somatic stem cell, Embryonic stem-like cell, Neuronal progenitor cell, Umbilical cord transplantation

Introduction

Umbilical cord blood (UCB) has serve as a source of hematopoietic stem and progenitor cells as similar as bone marrow (BM). Allogeneic UCB transplantation has been used to treat a variety of malignant diseases like leukemia and lymphoma, and non-malignant diseases such as severe hematological diseases and inherited metabolic diseases (1). Recent studies provide convincing evidence that UCB contains not only hematopoietic progenitors, but also several types of stem and progenitor cells (Table 1). They include highly immature cell fractions like embryonic-like cells providing a high proliferative capacity and a variety of differentiation potentials (2-4). UCB-derived stem cells have several advantages over adult stem cells like BM, because UCB can be collected without painful procedure, easily available in virtually unlimited supply, and has not been exposed to immunologic challenge. In addition, cord blood transplantation is now an established field with great potential and no serious ethical issue by establishment of public UCB banks throughout the world. Therefore UCB-derived non-hematopoietic stem cells may provide an attractive cell source for tissue repair and regeneration. It is generally accepted that UCB contains endothelial progenitor cells (EPCs) (5), mesenchymal stromal cells (MSCs) (6), unrestricted somatic stem cells (USSC) (2), very small embryonic-like stem cells (VSELs) (3), multi-lineage progenitor cells (MLPCs) (4), and neuronal progenitor cells (7). This review focuses on the isolation, characterization, and differentiation capacity of the non-hematopoietic stem/progenitor cells derived from human UCB and their potential use in cell based therapies.

Accepted for publication October 16, 2009

Correspondence to **Hideo Mugishima**
Department of Pediatrics, School of Medicine, Nihon University, 30-1, Oyaguchi-Kamimachi, Itabashiku, Tokyo 173-8610, Japan
Tel: +81-3-3972-8111 ext 2440, Fax: +81-3-3958-9864
E-mail: mugishim@med.nihon-u.ac.jp

Table 1. Characterization of non-hematopoietic stem/progenitor cells in UCB

Cell type	Morphology	Culture procedure	CD antigens	Differentiation potential
Endothelial cell colony-forming unit (CFU-ECs) (11)	Adherent round cells centrally and sprouts of spindle-shaped cells at periphery	Culture UCB MNCs on fibronectin-coated dishes in endthelial growth media for 5 days (Nonadherent cells were replated at 48 hours)	Endothelial cell markers positive ($CD31^+$, $CD105^+$, $CD144^+$, $CD146^+$, vWF^+, KDR^+, $UEA\text{-}1^+$), Myeloid cell markers positive ($CD14^+$, $CD45^+$, $CD115^+$)	Vascular endothelial cells Monocytes/Macrophages
Endothelial-colony forming cells (ECFCs) (12)	Adherent spindle-shaped cells	Culture UCB MNCs on collage I-coated dishes in endthelial growth media for 5～10 days (Nonadherent cells were discarded daily)	Endothelial cell markers positive ($CD31^+$, $CD105^+$, $CD144^+$, $CD146^+$, vWF^+, KDR^+, $UEA\text{-}1^+$) Myeloid cell markers negative ($CD14^-$, $CD45^-$, $CD115^-$)	Vascular endothelial cells
Unrestricted somatic stem cells (USSCs) (2)	Adherent spindle-shaped cells (20～25 μm)	Culture UCB MNCs on plastic dishes in myelocult medium for 6～25 days	MSC markers positive ($CD13^+$, $CD29^+$, $CD44^+$, $CD90^+$, $CD105^+$), Hematopoietic markers negative ($CD14^-$, $CD34^-$, $CD45^-$), $CK8^+$, $CK18^+$, KDR^+, $CD50^-$, $CD62L^-$, $CD106^-$, $HAS1^-$, $HLA\text{-}DR^-$, $SSEA\text{-}3^-$, $SSEA\text{-}4^-$	Osteoblastss, Chondro blasts, Adipocytes, Hematopoietic cells, Neuroglial cells, Parenchymal liver cells, Cardiomyocytes
Mesenchymal stromal cells (MSCs) (6, 16)	Adherent spindle-shaped cells	Culture UCB MNCs on plastic dishes in IMDM/20% FBS in the presence of bFGF (Nonadherent cells were discarded)	MSC markers positive ($CD13^+$, $CD29^+$, $CD44^+$, $CD49e^+$, $CD90^+$, $CD105^+$, $SH2^+$, $SH3^+$, $SH4^+$), Hematopoietic markers negative ($CD14^-$, $CD34^-$, $CD45^-$, $AC133^-$, $CD117^-$), $CD31^-$, vWF-	Osteoblastss, Chondroblasts, (Adipocytes), Neuroglial cells, Hepatocyte-like cells
Very small embryonic-like stem cells (VSELs) (3)	Small round cells (3～5 μm) with relatively large nuclei	Remove erythrocytes from UCB by hypotonic lysis followed by multiparameter FACS sorting (lin^-, $CD45^-$, $CXCR4^+$, $CD34^+$, $AC133^+$)	$CXCR4^+$, $AC133^+$, $CD34^+$, lin^-, $CD45^-$, $Oct\text{-}4^+$, $Nanog^+$, $SSEA\text{-}4^+S$	Neuroglial cells, Cardiomyocytes, Pancreatic cells, Hematopoietic cells (From mouse BM VSEL-derived spheres)
Multilineage progenitor cells (MLPCs) (4)	Initially leukocyte-like morphology, then convert to fibroblast-like morphology during adherent culture	Isolate from UCB by non-particle based negative cell selection methodology (PrepaCyte-MLPC) followed by plastic adherence	$CD45^+$, $CD34^+$, $AC133^+$, $SSEA\text{-}3^+$ $SSEA\text{-}4^+$ (These markers are expressed in freshly isolated cells but lost in cultured cells) MSC markers positive ($CD13^+$, $CD29^+$, $CD44^+$ $CD73^+$, $CD90^+$, $CD105^+$), $CD9^+$, $Nestin^+$	Hepatopancreatic precursor cells, Mature hepatocytes, Type II alveolar cells, Adipocytes, Chondrocytes, Osteoblasts. Myocytes, Vascular endothelial cells, Neuroglial cells
Neuronal progenitor cells (7)	Mid-sized round cells (8～10 μm) forming non-adherent nurosphere-like aggregates	Remove $CD34^+$, $CD45^+$ cells from UCB MNCs by immunomagnetic sorting and by plastic adherence, then reseed the cells in the presence of EGF	$Nestin^+$, $AC133^+$, $Oct3/4^+$, $NF200^+$, $GFAP^+$, $CD34^-$	Neuroglial cells

Endothelial progenitor cells (EPCs)

Circulating EPCs in adult human peripheral blood were originally identified from $CD34^+$ mononuclear cells (MNCs) in 1997 by Asahara et al. (8). $CD34^+$ cells from human peripheral blood form clusters of cells comprised of round cells centrally and sprouts of spindle-shaped cells at the periphery within 5 days, when the cells were plated on fibronectin-coated dishes in endothelial growth media. Murohara et al. (9) has shown that UCB is a valuable source of EPCs, and transplantation of UCB-derived cultured EPCs augments neovascularization and blood flow in a rat ischemic hind limb. Cultured EPCs from cord blood have a greater proliferative activity than those from other sources (5). A number of experimental and clinical studies have revealed that ischemic cardiovascular diseases

such as peripheral artery disease, acute myocardial infarction, brain infarction, and diabetic neuropathy can effectively be treated by UCB-derived EPC transplantation (5, 10) . It has been speculated that the paracrine effect of cultured EPCs are responsible for the modest effects in patients because there is no evidence of long-term engraftment of EPCs into newly formed vessels. The cultured EPCs characterized by endothelial cell-colony forming units (CFU-ECs) express not only endothelial markers CD31, CD105, CD144, CD146, vWF, UEA-1 and KDR, but also monocyte/macrophage markers CD14, CD45, and CD115. In addition, the cultured EPCs possess myeloid progenitor cell activity, differentiate into phagocytic macrophages, and fail to form perfused vessels *in vivo* (11). These findings suggest that the cultured EPCs are hematopoietic myeloid progenitor cells. Recently, Ingram et al. (12) have identified other EPCs with blood vessel-forming ability, termed endothelial colony-forming cells (ECFCs), which are also referred to as blood outgrowth endothelial cells (13), from human peripheral blood and UCB. ECFC colonies appear on days 5 to 10 for UCB or day 14 to 21 for adult peripheral blood samples when the MNCs are cultured on collagen-coated tissue dishes in endothelial growth media. The number of colonies generated from 10^6 MNCs was significantly smaller in ECFCs (0.017±0.04) compared to that in CFU-ECs (4.35±2) (11). ECFCs express endothelial markers CD31, CD105, CD144, and CD146, but not hematopoietic cell markers CD45 and CD115. ECFCs are characterized by robust proliferative potential and by their ability to form perfused blood vessels *in vivo* when transplanted with collagen fibronectin matrix into immunodeficient mice (11, 12). ECFCs are enriched in UCB compared to adult peripheral blood. In addition, UCB-derived ECFCs have greater proliferative activity and enhance vessel forming ability compared to adult peripheral blood-derived ECFCs (12, 14). Thus, UCB-derived ECFCs may more effectively contribute to vascular regeneration.

Mesenchymal stromal cells (MSCs)

MSCs are adherent stromal cells, initially isolated from the bone marrow (15). MSCs have been defined by their ability to self-renew and differentiate into the cells that form mesodermal tissue. Recent studies demonstrated that MSCs also show ability to suppress immune responses in vitro and *in vivo*. In contrast to hematopoietic stem cells, MSCs lack an unique surface antigen for positive selection. MSCs are suggested to be positive for CD13, CD29, CD44, CD73, CD90 (Thy-1), CD105 (endoglin), and CD166 and negative for CD14, CD34, CD38, and CD45. From 2000, several researchers reported that UCB contains multipotent MSCs (6). Subsequently, Lee et al. (16) has shown that clonally expanded MSCs from UCB exhibit multilineage differentiation potential including osteogenic and chondrogenic lineages. It has been shown that UCB MSCs also can differentiate into cardiomyocytes (17), hepatocyte (16, 18), and neuroglial cells (16, 19). Interestingly, UCB MSCs exhibit no or less adipogenic differentiation capacity (20-22). Recent studies demonstrated that frequency of MSC colony in UCB is much lower than that in the other tissue such as BM, adipose tissue, and umbilical cord tissue (Wharton's jelly) (21, 23, 24). The ability to obtain MSCs is from only 30% to 60% of UCB units with a frequency of up to 2.3 MSC clones/108 mononuclear cells (25, 26). Javed et al. (27) reported that 24～28 week gestational age UCB generate predominantly MSC colonies, although MSCs are rarely identified in 37～40 week gestational age UCB, suggesting circulating concentration of MSCs is rapidly decreased in full-term UCB. In addition, the isolation efficiency of UCB MSCs depends on time from collection to isolation and volume/cell number of UCB (20). Additional studies on development of the efficient isolation and expansion procedures for UCB MSCs are expected. MSCs possess various properties of clinical interest, including their wide-ranging differentiation potential, their capacity for engraftment (28, 29), their immunosuppressive effects (30) and their supporting effects on *in vivo* expansion of HSCs (31).

Unrestricted somatic stem cells (USSCs)

In 2004, Kögler et al. (2) reported a pluripotent cell population within UCB, which they named USSC. USSCs can be isolated and expanded from UCB MNCs based on their capacity to adhere to a plastic surface as same as MSCs. USSCs are adherent, spindle-shaped cells with high proliferative activity, although they have a very low primary frequency in UCB. Cell surface antigens profile of USSCs is similar to MSCs ($CD13^+$, $CD29^+$, $CD44^+$, $CD90^+$, $CD105^+$, $CD14^-$, $CD34^-$, and $CD45^-$). Differences between MSCs and USSCs include absent expression in USSCs of CD50, CD62L, CD106, and HAS1, all of which are present in MSCs. USSCs can differenciate into cells of all three germ layers including osteoblasts, chondroblasts, adipocytes, hematopoietic cells and neuroglial cells in vitro and *in vivo*. Furthermore, transplantation of USSCs in non-injured fetal sheep resulted in engraftment of USSC-derived hematopoietic cells, albumin-producing hepatic cells and cardiomyocytes (2). The high adipogenic

differentiation activity is likely to be a functional marker to discriminate between USSCs and UCB MSCs (32). Since USSCs express typical MSCs markers and expand in MSC-supporting media, these findings suggest that USSCs are of an earlier cell type than MSCs. USSCs also have hematopoiesis-supporting stromal activity (33) and capacity to enhance the homing of CB CD34-positive cells *in vivo* (34). The hematopoiesis-supporting activity and related cytokines expression levels of USSCs are significantly higher than those of BM MSCs (33). Thus, USSCs may be a potential cell source for cell-based therapy in facilitating homing and engraftment for cord blood transplant recipients.

Very small embryonic-like stem cells (VSELs)

VSELs were initially isolated from mouse BM as a population of small Sca-1^{+}lin^{-}CD45^{-} cells that express CXCR4 receptor with embryonic characteristics (3). The cells are characterized by their very small in size (2～4 μm) that are smaller than red blood cells. Subsequently, the VSELs were also identified in human UCB by same research group (35). To isolate the VSELs from human UCB, two-step isolation procedure (removal of erythrocytes by hypotonic lysis combined with multiparamtor FACS sorting) has been developed (36). The VSELs in UCB are also very small (3～5 μm) and enriched in a population of CXCR4^{+}AC133^{+}CD34^{+}lin^{-}CD45^{-}MNCs. The cells express several markers for embryonic pluripotent stem cells such as SSEA-4, Oct-4, and Nanog (35). Mouse BM-derived VSELs form cellular clusters like embryoid bodies when they plate over C2C12 feeder cells and differentiate into cells from all three germ layers including neural cells, pancreatic cells, and cardiomyocytes (3). Recently, McGukin et al. (37) reported that UCB-derived embryonic-like stem cells could be efficiently differentiated into neuronal cells in their refined culture system. *in vivo*, VSELs transplantation reconstitutes hematopoiesis in lethally irradiated mice (38) and improves cardiac function and remodeling in the mouse acute myocardial infarction model (39). It is conceivable that VSELs are originated from epiblast on the basis of phenotypical similarities with primordial germ cells. It is still obscure that UCB-derived VSELs also possess the pluripotent capacity as similar as BM-derived VSELs.

Multilineage progenitor cells (MLPCs)

MLPCs were identified as a multipotent stem cell population which rarely exist in UCB (40). The cells can be isolated from UCB units by using an antibody-based cell separation medium (PrepaCyte-MLPC; BioE Inc.) followed by plastic adherence. Freshly isolated MLPCs exhibit a leukocyte-like morphology and express CD45, CD34, CD133, CD9, nestin, SSEA-3, SSEA-4 and several MSC markers such as CD13, CD29, CD44, CD73, CD90, and CD105. After expansion by culturing on plastic surface, the cells change their morphology into fibroblast-like and lose hematopoietic stem cell makers CD34 and CD133, and embryonic stem (ES) cell markers SSEA-3 and SSEA-4 but continuously express MSC markers. Interestingly, MLPCs also can be isolated from UCB MSCs by single cell cloning (4). MLPCs distinguish themselves from MSCs by showing more extensive expansion and higher plasticity. A phenotypic feature of MLPCs is higher expression of CD9 compared with UCB MSCs and BM MSCs. The microarray analysis suggests that MLPCs are relatively quiescent primitive cells that are capable of wide plasticity yet are not committed to lineage, compared to BM MSCs (40). It has been reported that MLPCs differentiate into cells from all three germ layers such as endodermal hepatopancreatic precursor cells, mature hepatocytes and type II alveolar cells, mesodermal adipocytes, chondrocytes, osteoblasts, myocytes and endothelial cells, and ectodermal neurons, astrocytes and oligodendrocytes. Despite of their extensive expansion ability and high plasticity, the cells do not have abilities of spontaneous differentiation and teratoma formation like ES cells, suggesting the cells provide an attractive transplantation cell source for regenerative medicine.

Neuronal progenitor cells

In 2002, Buznska et al. (7) isolated UCB-derived cells with ability of neurosphere formation and neural differentiation potential. They used a combination of CD34-immunomagnetic depletion strategy and subfractionation according to cell surface adhesive properties to enrich the cells with neuronal progeny. The procedure efficiently removes the cells expressing CD34 and CD45. Subsequently, culturing the cells at a low cell density in the presence of epidermal growth factor (EGF) results in the neurosphere formation. The same group also established a clonogenic non-immortalized UCB neural stem cell line (UCB-NSC) that could be maintained in culture at different stages of neural progenitor development (41). The neurospheres derived from UCB-NSC express neural stem/progenitors markers nestin and glial fibrillary acidic protein (GFAP) and differentiate into neuronal, astrocytic and oligodendroglial lineages. McGuckin et al. (42) has

been reported the similar type of neurospheres can be induced by discrete hematopoietic lineage (CD45, CD33, CD7 and glycophorin-A) negative cell population from CB MNCs, followed by culturing in medium containing thrombopoietin, flt-3 ligand, and c-kit ligand (TPOFLK). Recently, our research group found that $p75NTR^{+}$ cell fraction in UCB MNCs efficiently form neurospheres and differentiate into neural cells, astrocytes and oligodendroglial cells. The freshly isolated $p75NTR^{+}$ cells express various neural crest specific marker genes such as Slug, Snail, Twist, Wnt-1, and Sox9, suggesting the cell fraction is originated from neural crest stem cells. Many studies have demonstrated that human UCB transplantation enhance functional recovery in animal models for stroke (43), amyotrophic lateral sclerosis (44, 45), traumatic brain injury (46), and spinal cord injury (47, 48), while it is not clear which of these cells are important for functional recovery. Because very few transplanted cells are found in the brain, even when delivered intracerebrally, it is more feasible that they secrete trophic factors that enhance endogenous mechanisms of brain repair.

Future perspectives

The recent studies provide convincing evidence that UCB contain not only hematopoietic progenitors, but also several other types of stem/progenitor cells from very primitive embryonic-like cells to relatively mature neuronal and endothelial progenitor cells. Some of these cells may be the overlapping populations of stem cells that have been described by different investigators and given various names due to their different isolation and expansion strategies. Important questions concerning their origin, differentiation potential, tumorigenicity, and availability still need to be elucidated. In addition, methods to expand specific population in sufficient numbers for transplantation must be established. It is interesting that UCB contains embryonic-like pluripotent stem cells lacking tumorigenesis, while efficient isolation and expansion strategies of these cells are not yet determined. Recently it was reported that somatic cells can be reprogrammed to a pluripotent state by forced expression of four transcriptional factors, including Oct4, Sox2, c-Myc and Klf4 (49). These induced pluripotent stem (iPS) cells are expected to be an attractive cell source for regenerative medicine because of their pluripotent ability, their lack of an immunological rejection reaction, and eliminating of current ethical issues surrounding ES cell research. However, low efficiency of reprogramming and tendency to induce malignant transformation compromise the clinical utility of iPS cells. Because of the lower incidence of tumorigenesis, more efficient preparation, and no use retroviral vectors and gene transmission, UCB-derived stem cell based therapy may be more useful for the clinical application of regenerative medicine compared to iPS cell based therapy. Further studies to evaluate the possibilities for both research and therapeutic applications for UCB-derived stem cells are required.

Potential Conflict of Interest

The authors have no conflicting financial interest.

References

1. Broxmeyer HE, Srour E, Orschell C, Ingram DA, Cooper S, Plett PA, Mead LE, Yoder MC. Cord blood stem and progenitor cells. Methods Enzymol 2006;419:439-473
2. Kögler G, Sensken S, Airey JA, Trapp T, Müschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Müller HW, Zanjani E, Wernet P. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. J Exp Med 2004;200:123-135
3. Kucia M, Reca R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, Ratajczak MZ. A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia 2006;20: 857-869
4. Berger MJ, Adams SD, Tigges BM, Sprague SL, Wang XJ, Collins DP, McKenna DH. Differentiation of umbilical cord blood-derived multilineage progenitor cells into respiratory epithelial cells. Cytotherapy 2006;8:480-487
5. Murohara T. Therapeutic vasculogenesis using human cord blood-derived endothelial progenitors. Trends Cardiovasc Med 2001;11:303-307
6. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 2000; 109:235-242
7. Buzańska L, Machaj EK, Zablocka B, Pojda Z, Domańska-Janik K. Human cord blood-derived cells attain neuronal and glial features in vitro. J Cell Sci 2002;115:2131-2138
8. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999;85:221-228
9. Murohara T, Ikeda H, Duan J, Shintani S, Sasaki K, Eguchi H, Onitsuka I, Matsui K, Imaizumi T. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest 2000;105:1527-1536
10. Naruse K, Hamada Y, Nakashima E, Kato K, Mizubayashi R, Kamiya H, Yuzawa Y, Matsuo S, Murohara T, Matsubara T, Oiso Y, Nakamura J. Therapeutic neovascularization using cord blood-derived endothelial progenitor cells for dia-

betic neuropathy. Diabetes 2005;54:1823-1828
11. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood 2007;109: 1801-1809
12. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 2004;104:2752-2760
13. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest 2000;105:71-77
14. Au P, Daheron LM, Duda DG, Cohen KS, Tyrrell JA, Lanning RM, Fukumura D, Scadden DT, Jain RK. Differential *in vivo* potential of endothelial progenitor cells from human umbilical cord blood and adult peripheral blood to form functional long-lasting vessels. Blood 2008; 111:1302-1305
15. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-147
16. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 2004;103:1669-1675
17. Planat-Bénard V, Menard C, André M, Puceat M, Perez A, Garcia-Verdugo JM, Pénicaud L, Casteilla L. Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. Circ Res 2004;94:223-229
18. Chagraoui J, Lepage-Noll A, Anjo A, Uzan G, Charbord P. Fetal liver stroma consists of cells in epithelial-to-mesenchymal transition. Blood 2003;101:2973-2982
19. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000;61:364-370
20. Bieback K, Kern S, Klüter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells 2004;22:625-634
21. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24:1294-1301
22. Chang YJ, Shih DT, Tseng CP, Hsieh TB, Lee DC, Hwang SM. Disparate mesenchyme-lineage tendencies in mesenchymal stem cells from human bone marrow and umbilical cord blood. Stem Cells 2006;24:679-685
23. Secco M, Zucconi E, Vieira NM, Fogaca LL, Cerqueira A, Carvalho MD, Jazedje T, Okamoto OK, Muotri AR, Zatz M. Multipotent stem cells from umbilical cord: cord is richer than blood! Stem Cells 2008;26:146-150
24. Chang YJ, Tseng CP, Hsu LF, Hsieh TB, Hwang SM. Characterization of two populations of mesenchymal progenitor cells in umbilical cord blood. Cell Biol Int 2006; 30:495-499
25. Kögler G, Sensken S, Wernet P. Comparative generation and characterization of pluripotent unrestricted somatic stem cells with mesenchymal stem cells from human cord blood. Exp Hematol 2006;34:1589-1595
26. Watt SM, Contreras M. Stem cell medicine: umbilical cord blood and its stem cell potential. Semin Fetal Neonatal Med 2005;10:209-220
27. Javed MJ, Mead LE, Prater D, Bessler WK, Foster D, Case J, Goebel WS, Yoder MC, Haneline LS, Ingram DA. Endothelial colony forming cells and mesenchymal stem cells are enriched at different gestational ages in human umbilical cord blood. Pediatr Res 2008;64:68-73
28. Javazon EH, Beggs KJ, Flake AW. Mesenchymal stem cells: paradoxes of passaging. Exp Hematol 2004;32:414-425
29. Dominici M, Marino R, Rasini V, Spano C, Paolucci P, Conte P, Hofmann TJ, Horwitz EM. Donor cell-derived osteopoiesis originates from a self-renewing stem cell with a limited regenerative contribution after transplantation. Blood 2008;111:4386-4391
30. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002;99:3838-3843
31. Wang JF, Wang LJ, Wu YF, Xiang Y, Xie CG, Jia BB, Harrington J, McNiece IK. Mesenchymal stem/progenitor cells in human umbilical cord blood as support for *ex vivo* expansion of CD34(+) hematopoietic stem cells and for chondrogenic differentiation. Haematologica 2004;89:837-844
32. Buchheiser A, Liedtke S, Looijenga LH, Kögler G. Cord blood for tissue regeneration. J Cell Biochem 2009.
33. Kögler G, Radke TF, Lefort A, Sensken S, Fischer J, Sorg RV, Wernet P. Cytokine production and hematopoiesis supporting activity of cord blood-derived unrestricted somatic stem cells. Exp Hematol 2005;33:573-583
34. Chan SL, Choi M, Wnendt S, Kraus M, Teng E, Leong HF, Merchav S. Enhanced *in vivo* homing of uncultured and selectively amplified cord blood $CD34^+$ cells by co-transplantation with cord blood-derived unrestricted somatic stem cells. Stem Cells 2007;25:529-536
35. Kucia M, Halasa M, Wysoczynski M, Baskiewicz-Masiuk M, Moldenhawer S, Zuba-Surma E, Czajka R, Wojakowski W, Machalinski B, Ratajczak MZ. Morphological and molecular characterization of novel population of $CXCR4^+$ $SSEA\text{-}4^+$ $Oct\text{-}4^+$ very small embryonic-like cells purified from human cord blood: preliminary report. Leukemia 2007;21:297-303
36. Halasa M, Baskiewicz-Masiuk M, Dabkowska E, Machalinski B. An efficient two-step method to purify very small embryonic-like (VSEL) stem cells from umbilical cord blood (UCB). Folia Histochem Cytobiol 2008;46:239-243
37. McGuckin C, Jurga M, Ali H, Strbad M, Forraz N. Culture of embryonic-like stem cells from human umbilical cord blood and onward differentiation to neural cells in vitro. Nat Protoc 2008;3:1046-1055
38. Ratajczak MZ, Zuba-Surma EK, Wysoczynski M, Ratajczak

J, Kucia M. Very small embryonic-like stem cells: characterization, developmental origin, and biological significance. Exp Hematol 2008;36:742-751
39. Dawn B, Tiwari S, Kucia MJ, Zuba-Surma EK, Guo Y, Sanganalmath SK, Abdel-Latif A, Hunt G, Vincent RJ, Taher H, Reed NJ, Ratajczak MZ, Bolli R. Transplantation of bone marrow-derived very small embryonic-like stem cells attenuates left ventricular dysfunction and remodeling after myocardial infarction. Stem Cells 2008;26:1646-1655
40. van de Ven C, Collins D, Bradley MB, Morris E, Cairo MS. The potential of umbilical cord blood multipotent stem cells for nonhematopoietic tissue and cell regeneration. Exp Hematol 2007;35:1753-1765
41. Buzańska L, Jurga M, Domańska-Janik K. Neuronal differentiation of human umbilical cord blood neural stem-like cell line. Neurodegener Dis 2006;3:19-26
42. McGuckin CP, Forraz N, Allouard Q, Pettengell R. Umbilical cord blood stem cells can expand hematopoietic and neuroglial progenitors in vitro. Exp Cell Res 2004;295:350-359
43. Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. Stroke 2007;38:817-826
44. Chen R, Ende N. The potential for the use of mononuclear cells from human umbilical cord blood in the treatment of amyotrophic lateral sclerosis in SOD1 mice. J Med 2000; 31:21-30
45. Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, Sanberg PR. Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. J Hematother Stem Cell Res 2003;12:255-270
46. Lu D, Sanberg PR, Mahmood A, Li Y, Wang L, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. Cell Transplant 2002;11:275-281
47. Saporta S, Kim JJ, Willing AE, Fu ES, Davis CD, Sanberg PR. Human umbilical cord blood stem cells infusion in spinal cord injury: engraftment and beneficial influence on behavior. J Hematother Stem Cell Res 2003;12:271-278
48. Zhao ZM, Li HJ, Liu HY, Lu SH, Yang RC, Zhang QJ, Han ZC. Intraspinal transplantation of $CD34^+$ human umbilical cord blood cells after spinal cord hemisection injury improves functional recovery in adult rats. Cell Transplant 2004;13:113-122
49. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861-872