Check for updates

Cell Transplantation, Vol. 24, pp. 339–347, 2015
Printed in the USA. All rights reserved.
Copyright © 2015 Cognizant Comm. Corp.

0963-6897/15 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096368915X686841
E-ISSN 1555-3892
www.cognizantcommunication.com

Review

Human Umbilical Cord Mesenchymal Stem Cells: A New Era for Stem Cell Therapy

Dah-Ching Ding,*† Yu-Hsun Chang,†‡ Woei-Cherng Shyu,§¶ and Shinn-Zong Lin§¶#

*Department of Obstetrics and Gynecology, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan
†Graduate Institute of Medical Science, Tzu Chi University, Hualien, Taiwan
‡Department of Pediatrics, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan
§Center for Neuropsychiatry, China Medical University and Hospital, Taichung, Taiwan
¶Graduate Institute of Immunology, China Medical University, Taichung, Taiwan
#China Medical University Beigang Hospital, Yunlin, Taiwan

The human umbilical cord is a promising source of mesenchymal stem cells (HUCMSCs). Unlike bone marrow stem cells, HUCMSCs have a painless collection procedure and faster self-renewal properties. Different derivation protocols may provide different amounts and populations of stem cells. Stem cell populations have also been reported in other compartments of the umbilical cord, such as the cord lining, perivascular tissue, and Wharton's jelly. HUCMSCs are noncontroversial sources compared to embryonic stem cells. They can differentiate into the three germ layers that promote tissue repair and modulate immune responses and anticancer properties. Thus, they are attractive autologous or allogenic agents for the treatment of malignant and nonmalignant solid and soft cancers. HUCMCs also can be the feeder layer for embryonic stem cells or other pluripotent stem cells. Regarding their therapeutic value, storage banking system and protocols should be established immediately. This review critically evaluates their therapeutic value, challenges, and future directions for their clinical applications.

Key words: Umbilical cord; Wharton's jelly; Clinical application; Stem cells

INTRODUCTION

Mesenchymal stem cells (MSCs) are attractive cells due to their capacity for proliferation, multilineage differentiation, and immunomodulatory properties. These cells were first identified and isolated from bone marrow (BMMSCs) and have since emerged as important components in regeneration therapy (53). However, the process of isolating MSCs from bone marrow is complex and painful. As such, derived stem cells from dumped fetal tissue are preferred (9–11).

Embryonic stem cells (ESCs) can differentiate into almost all tissues in the human body and are thus labeled as pluripotent. Recently, induced pluripotent stem cells (iPSCs) have been developed (76) and have pluripotent properties like ESCs. Pluripotency is defined as the ability of these cells to produce tissues from all three germ layers (ectoderm, mesoderm, and endoderm) when transplanted into immunodeficient mice. However, the use of ESCs generated from surplus embryos has raised ethical concerns. Moreover, the clinical applications of iPSCs have been criticized because of the possibility of forming tumors by integrated oncogenes, particularly c-myc (51), by insertional mutagenesis (28), or by disrupting tumor suppressor genes (3). Epigenetic memories and genomic aberrations in the reprogrammed cells have also been noted (26). Thus, in the manufacturing of ESCs or iPSCs for clinical applications, precautions regarding safety and efficacy need to be taken. Finding another useful source of stem cells is imperative.

Recently, two papers have drawn attention to the so-called vampire therapy (63,70). They used blood from young mice and transplanted it into old mice. The young stem cells in the blood can help the aged mice recover their muscle and neuron functions. The growth differentiation factor 11 (GDF11) and cAMP response element binding protein (CREB) were shown to be responsible for this effect.

Received December 9, 2014; final acceptance January 21, 2015. Online prepub date: January 23, 2015.
Address correspondence to Shinn-Zong Lin, M.D., Ph.D., Center for Neuropsychiatry, China Medical University and Hospital, Taichung, Taiwan, ROC. Tel: +886-3-8561825; Fax: +886-3-8577161; E-mail: shinnzong@yahoo.com.tw

To date, younger stem cells include fetal stem cells obtained from amniotic fluid, the umbilical cord (UC), and placenta (4,9,66). Fetal stem cells are mostly MSCs, and fetal MSCs from birth-associated tissues are gaining popularity. MSCs derived from the umbilical cord can be obtained from the amniotic membrane, cord lining, Wharton's jelly, and perivascular region (Fig. 1). They are the focus of this review (Fig. 2).

STEM CELLS DERIVED FROM DIFFERENT PARTS OF UMBILICAL CORD

The human umbilical cord starts developing on the fifth week of gestation and continues to grow until 50 cm in length (75). Stem cells can be derived from various parts of the umbilical cord. All of these compartments have been described in the literature, including Wharton's jelly, cord lining, and the perivascular region.

Wharton's Jelly

Most studies use UC MSCs from Wharton's jelly (2,9,74). Regarding isolation, there are two kinds of methods: the explant method and the enzymatic digestion method (50). In the explant method, the Wharton's jelly is manually minced into 1–2-mm^3 fragments after removal of UC vessels. The fragments are undisturbed for 7 days to allow the stem cells to come out (9). However, the downside of the explant method is that the fragments often float in the medium. Moreover, this method may not provide a consistent number of MSCs.

In the enzymatic digestion method, the enzymes used for digestion vary from collagenase to a combination of collagenase and hyaluronidase with or without trypsin (12,59,65). This method can provide more homogenous cell populations and more consistent cell numbers compared to the explant method.

Cord Lining

Umbilical cord lining cells have been isolated by the explant method (25,56,57). Two kinds of cells, MSCs (CLMCs) and epithelial cells (CLECs), can be isolated from cord lining. The CLMCs are isolated from the subamnion region by dissecting out the Wharton's jelly. Pieces of the outer envelope membranes are cultured with Connaught Medical Research Laboratories (CMRL) 1660 containing L-glutamine and 10% fetal bovine serum (FBS) (33). Around 20 million cells can be generated at passage 1 (38).

The CLECs can be used for treating persistent corneal epithelial defects and as a skin cosmetic improvement, whereas CLMCs have been used for burn and diabetic ulcer wound healing (38). Since preclinical studies reveal successful disease treatment, further exploration of the utility of these cells is warranted.

BIOMARKER OF HUCMSCs

Surface Markers

MSCs are positive for cluster of differentiation 29 (CD29), CD44, CD90, CD73, CD105, and human leukocyte antigen (HLA)-ABC. Conversely, MSCs are negative for the endothelial cell marker CD31; hematopoietic cell markers CD34, CD45, and CD117; and HLA-DR (14,62). The surface markers of HUCMSCs are similar to those of MSCs (31), but they are negative for CD133 (31). Nevertheless, phenotypic characterization of HUCMSCs may be influenced by the culture passage number, medium, and method.

Embryonic Stem Cell Markers

Octamer-binding transcription factor 4 (Oct4), Nanog, sex-determining region Y box 2 (Sox2), and Kruppel-like factor 4 (KLF4) are expressed only at low levels in HUCMSCs (27), suggesting that MSCs are primitive

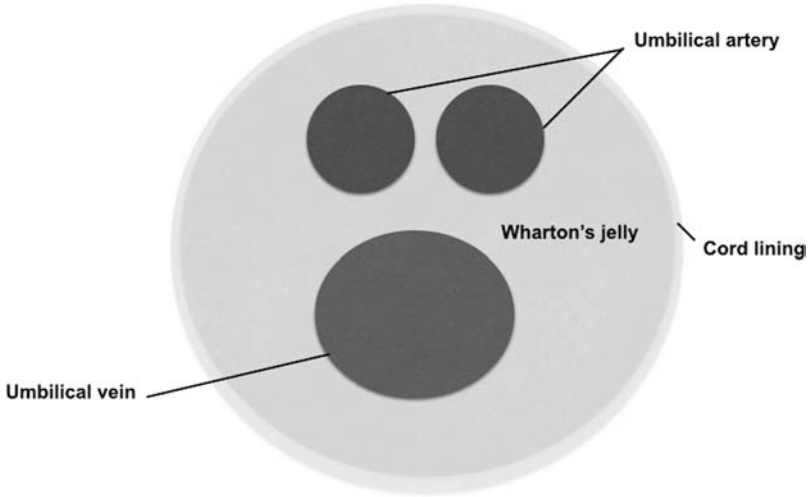

Figure 1. Diagrammatic illustration of cross-section of the human umbilical cord showing different compartments (cord lining, Wharton's jelly, and perivascular region) from where stem cells can be derived.

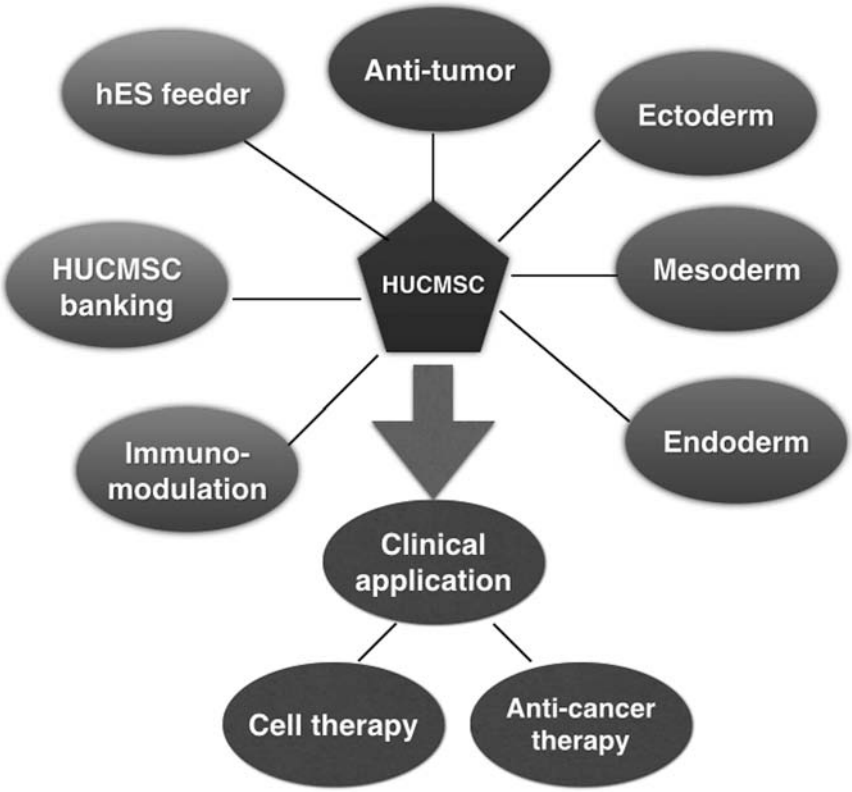

Figure 2. Diagrammatic illustration of this review of human umbilical cord mesenchymal stem cells, including three germ layer differentiation, antitumor effect, immunomodulation, and clinical application.

stem cells, between ESCs and adult mature cells. Nonetheless, the isolation of pluripotent MSCs using specific markers remains a challenge. HUCMSCs can also express stage-specific embryonic antigen 4 (SSEA4) in medium supplemented with FBS, whereas SSEA3 is conversely correlated (29). The gene profile of HUCMSCs is reported to be close to those of ESCs (30).

DIFFERENTIATION CAPABILITY

Mesoderm

Adipocytes. HUCMSCs can produce small lipid vacuoles, whereas BMMSCs produce more mature adipocytes (48). HUCMSCs can undergo more than 40 passages (12), and they maintain their multipotency for longer periods compared to BMMSCs (18). Recently, HUCMSCs were shown to be able to differentiate into adipocytes using different induction chemical combinations (58). The presence of indomethacin greatly enhances their adipogenic potential beyond that of rosiglitazone.

Osteocytes. Regarding osteogenic differentiation, HUCMSCs show delayed and insufficient differentiation into osteocytes (30). HUCMSCs from Wharton's jelly present defective osteogenesis ability (31). In contrast, another work demonstrated that HUCMSCs have the best osteogenesis differentiation when obtained from different regions of the UC (48). Recently, HUCMSCs have shown better osteogenesis than stem cells derived from periodontal tissue (32,77).

Cartilage. Regarding chondrogenic differentiation, HUCMSCs reveal the same potential regardless of the portion of the UC from which they were isolated (48). Moreover, the chondrogenic potential of HUCMSCs is thrice that of BMMSCs in producing collagen (18). An unpublished study also reveals that HUCMSCs can effectively repair the monoiodoacetic acid (MIA)-injured cartilage via a decrease in the interleukin (IL)-1 levels induced by MIA treatment. Nevertheless, HUCMSCs possess chondrogenesis compared to stem cells derived from the infrapatellar fat pad (13).

Ectoderm

A previous study reveals that HUCMSCs can differentiate into neurons, astrocytes, and glial cells and can rescue the stroke rat model via an increase in 1-integrin and neurotrophic factors (9,41). When HUCMSCs are exposed to rat neuronal conditioned medium, they differentiate into microglial cells, generate neuronal proteins, and upregulate the astrocyte protein glial fibrillary acidic

protein (GFAP) (21,42). Several neuronal differentiation protocols have been published (19,49,71). A 5–6-h exposure to neuronal induction chemicals like potassium chloride, valproic acid, forskolin, hydrocortisone, and insulin can result in long-term neuronal differentiation (19,20).

Endoderm

Liver. HUCMSCs for rescue of liver fibrosis has been previously reported (2,40,67). Firstly, HUCMSCs can express hepatic markers and differentiate into hepatocyte-like cells in vitro and in vivo (2). HUCMSCs transplanted into the CCl_4-injured rat model have proven to be able to rescue liver fibrosis (67) and differentiate into hepatocytes in a chemically injured liver rat model (40). An unpublished study also reveals that HUCMSC-differentiated hepatocytes can engraft successfully into injured liver.

Islet Cells. HUCMSCs can differentiate into insulin-producing cells in vitro (68,72). Using a portal vein injection, HUCMSC-differentiated islet cells can alleviate hyperglycemia in diabetic rats (68) and mice (72).

IMMUNOMODULATION

The HUCMSCs that show an absence or low expression of major histocompatibility complex (MHC) class II and costimulatory molecules may be considered immunoprivileged cells (8). HUCMSCs can alter immune cell function by inhibiting T-cell, B-cell, and natural killer (NK) cell proliferation and by steering monocytes and dendritic cells to an immature state (16,69).

The immunomodulation of HUCMSCs on T-cells, B-cells, NK cells, and dendritic cells has been comprehensively reviewed previously (15).

Nonclassical type I HLA molecules are interesting but only partly explored in HUCMSC function. HUCMSCs express the HLA-G molecule, at both the mRNA and protein levels and in its soluble form, HLA-G5 (34,60,74). The HLA-G6 isoform is also reported to be involved in the HUCMSC immunosuppressive function (34). HLA-G5 is involved in the induction of regulatory cells (37) and suppression of NK cell production of interferon (IFN)- (61). Since fetal expression of high levels of HLA-G isoforms can inhibit maternal alloreactivity, the HLA-G isoforms need to be evaluated in detail (54).

In addition, HUCMSCs can express HLA-E and HLA-F, both of which are implicated in the tolerogenic process occurring in the fetal–maternal interface, along with HLA-G (35). A recent study also demonstrated HLA-G5 expression in all four HUCMSC cell lines responsible for decreasing lymphocyte proliferation during mixed lymphocyte reaction. HUCMSCs, but not BMSCs or placenta MSCs, can maintain low HLA-DR expression under IFN- stimulation (in revision).

BANKING

Banking of umbilical cord blood is common worldwide, including public and private banking services (45). Similarly, banking of HUCMSCs can also fulfill the need for transplantation purposes. HUCMSCs are an alternative source of stem cells for patients seeking an unrelated donor. Advantages of HUCMSCs compared to other sources of stem cells include procurement, less stringent requirements for HLA matching, reduced graft-versus-host disease (GVHD), and improved access to transplantation. The differentiation of HUCMSCs is better than that of umbilical cord blood cells, not only due to restricted hematopoietic lineage but also for differentiation into other germ layer lineages.

There are several cord blood banks that include storage of MSCs derived from Wharton's jelly and placenta in Taiwan. The potential of banking MSCs is full of hope for the future. Nonetheless, building standard isolation and freezing–thaw protocols is necessary. Deriving stem cells from frozen umbilical cord is not possible (6). A fresh UC tissue fragment is necessary for deriving stem cells. For the isolation of stem cells in good manufacturing practice (GMP) laboratories, several tests should be performed before using these products. These include checking the sterility of cells by 14-day microbial culture protocol, Gram staining to rule out bacterial infection, culture method to rule out mycoplasma infection, and checking cell surface marker and differentiation ability of stem cells.

The endotoxin level and viability after thawing must also be checked. Cell counts would also need to be performed (24). A banking system using the above procedures has been established, with 70 cell lines and HLA typing in a GMP laboratory, for transplantation purposes.

FEEDER FOR HUMAN EMBRYONIC STEM CELLS

The HUCMSCs can be a feeder layer for ESCs, with a nontumorigenic effect (12). Such nontumorigenic effect may be caused by the downregulation of the c-myc signaling (12). There are also various fetal stem cells that can also act as a feeder layer, such as stem cells derived from the placenta and amniotic fluid (7,36). Human feeder cell layers would eliminate the xenoprotein (*n*-glycolyneuraminic acid) (46) caused by using mice fibroblast feeder layers.

ANTICANCER EFFECT OF HUCMSCs

Most studies using HUCMSCs for anticancer research are on solid cancers. One recent report is on soft cancer.

Solid Cancer

Human breast cancer cell line MDA MB-231 (derived from a 51-year-old female) is the most tested cell line (1,5,43,64). An in vivo study has revealed that three

weekly intravenous injections of HUCMSCs can attenuate tumor growth (1). Another study used a rat breast cancer cell line to test the anticancer effect of rat umbilical stem cells (rUSCs) (22). Intravenous or intratumor injection of rUSCs can attenuate tumor growth. Moreover, complete tumor regression can be achieved after 1 month, and this was maintained for more than 3 months (22).

Chao and coworkers reported using HUCMSCs to treat MDA MB-231 cells. The tumor attenuation effect is dependent on cell–cell contact and internalization (5). This phenomenon may be present both in vitro and in vivo. Another study used umbilical cord blood (UCB) MSCs to treat the MDA MB-231 cell line (64). They found that dickkopf (DKK1) secreted by UCBMSCs can inhibit tumor growth via the phosphatase and tensin homolog (PTEN) pathway. Ma et al. reported the use of HUCMSCs to treat the breast cancer cell lines MDA MB-231 and MCF-7 (69-year-old female) (43).

Cancer stem cells (CSCs) are sorted using the surface markers of epidermal surface antigen positive (ESA^+), $CD44^+$, and $CD24^{-/low}$. The CSCs reveal a high degree of apoptosis when cocultured with HUCMSCs. HUCMSCs also decrease the xenograft tumor size, and the phosphoinositide-3-kinase (PI3K) and Akt signaling pathways play roles in this anticancer effect. Another study has used MDA-231 and TOV-112D cells (latter is from an endometrioid carcinoma of a 42-year-old female) as a tumor formation model (23). Conditioned medium and cell lysates of HUCMSCs have been used for treating cancer cell lines. Both the conditioned medium and cell lysates can inhibit tumor cell proliferation and make them stay at the sub-G_1 going to the apoptosis stage. Upregulation of BCL2-associated X protein (BAX) and downregulation of B-cell CLL/lymphoma 2 (BCL2) and survivin genes are observed. Autophagy genes are also upregulated upon treatment. The conclusion is that HUCMSCs possess tumor inhibitory properties via secretory agents.

Aside from breast cancer, lung cancer is the second most studied cancer. Maurya et al. reported using rUSCs to inhibit murine lung adenocarcinoma (47). rUSCs can attenuate the proliferation and colony formation of cancer. A coculture experiment revealed that most cancer cells stay at the G_0/G_1 phase, while cyclin A and cyclin-dependent kinase 2 (CDK2) downregulation is noted.

Treatment with rUSCs can decrease tumor size and weight. Homing of rUSCs to the tumor site is also noted. Another study reported by Rachakatla et al. describes the use of HUCMSCs modified to express the human interferon gene to treat a MDA-231-formed lung tumor (55). Following intravenous injection of HUCMSCs, the cells can migrate to the lung tumor site. The engineered HUCMSCs significantly reduced the MDA-231 tumor burden.

The preliminary results of another study reveal that HUCMSCs have effects on SKOV3 cells (ovarian cancer cell line) via suppression of the cyclin-dependent kinase inhibitor 1/retinoblastoma (P21/Rb) signaling pathway (unpublished data).

Nonsolid Cancer

Recently, HUCMSCs have been used to treat Burkitt's lymphoma (39). Cell proliferation, viability, and death of lymphoma cells were significantly inhibited after 48 h of exposure to HUCMSCs or its extracts, suggesting that HUCMSCs secreted molecules that inhibited lymphoma cell growth via oxidative stress pathways.

SAFETY

In early phase MSC preclinical and clinical trials, the safety of transplanted MSCs is well documented in animal models and in human trials. However, in vivo efficacy is controversial in humans (52). Moreover, HUCMSCs have been injected intravenously into nonhuman primates to test safety (73). Cells have been injected once every 2 weeks for 6 weeks into cynomolgus monkeys. No stem cell transplantation-related toxicity has been reported, and all injection sites and organs studied are normal, with no tumor noted.

Furthermore, HUCMSCs injected into xenograft disease rat models result in good engraftment and functional outcome. No immunorejection or tumorigenesis have been noted (17). Further long-term in vivo studies must be conducted to assure the safety of MSCs and can increase the homing and therapeutic efficacy of transplanted MSCs derived from adult tissues.

FUTURE APPLICATIONS

Cell-Based Therapy

The HUCMSCs can be used for specific cell-based therapies (15,17). Preclinical validation of HUCMSCs or their derived tissue in disease models have been reviewed (17). In all of these studies, the HUCMSCs can be differentiated and engrafted with successful functional outcome in vivo in rat models for cerebral ischemia (9), Parkinson's disease, Alzheimer's disease, multiple sclerosis, retinal disease, type 1 and type 2 diabetes, and myogenic disease. Immunity is always a hazard for transplantation. The HUCMSCs have low immunity and immunomodulatory effects that can increase the survival of transplanted cells and decrease the risk of GVHD (44).

Anticancer Therapy

Many studies have proven that HUCMSCs have an anticancer effect. Therefore, it is necessary to perform clinical trials to determine the real effects on tumor abolishment. Dose–time studies on larger nonhuman primates are also warranted. The administration of GMP-grade HUCMSCs to shrink the tumor first, followed by chemotherapy or surgery, can be done. CSCs are currently a hot

Table 1. Pros and Cons of Each Application

Application	Pros	Cons
Cell therapy	1. Good differentiation capability, successful engraftment in rat model, stroke, Parkinson's disease, Alzheimer's disease, multiple sclerosis, retinal disease, diabetes, myogenic disease. 2. Low immunity and immunomodulation	Less differentiation capability than embryonic stem cells and induced pluripotent stem cells
Anticancer	Effective anticancer effect on breast cancer, lung cancer, ovarian cancer and Burkitt's lymphoma	Dose–time study not yet performed
Banking	Easy collecting procedure at delivery or operating room, complete tests before storing, for cell transplantation purpose	High cost

topic. Whether HUCMSCs can target CSCs is an interesting study for further investigation.

HUCMSC Banking

The HUCMSC banking system and protocol is important. The establishment of a banking protocol and various microbial testing warrants further studies. The simultaneous storage of UCBs and HUCMSCs has already been performed in some cord blood banks in Taiwan. Storage of HUCMSCs provides an opportunity for use in various applications.

The pros and cons of each application are listed in Table 1.

CONCLUSIONS

The use of HUCMSCs has many attractive advantages, including a noninvasive collection procedure, low risk of infection, nontumorigenesis, multipotency, and low immunogenicity. But whether HUCMSCs are the best for clinical use is not yet known. Nevertheless, the era of clinical use of HUCMSCs has arrived and has full potential.

ACKNOWLEDGMENTS: The authors sincerely thank the National Science Council of the Republic of China, Taiwan, for financially supporting this research (Contract No. NSC 98-2314-B-303 -009 -MY3) and the Buddhist Tzu Chi General Hospital for the financial support (Contract Nos. TCRD99-12 and TCSP98-07). The authors declare no conflicts of interest.

REFERENCES

1. Ayuzawa, R.; Doi, C.; Rachakatla, R. S.; Pyle, M. M.; Maurya, D. K.; Troyer, D.; Tamura, M. Naive human umbilical cord matrix derived stem cells significantly attenuate growth of human breast cancer cells in vitro and in vivo. Cancer Lett. 280(1):31–37; 2009.
2. Campard, D.; Lysy, P. A.; Najimi, M.; Sokal, E. M. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. Gastroenterology 134(3):833–848; 2008.
3. Cavazzana-Calvo, M.; Payen, E.; Negre, O.; Wang, G.; Hehir, K.; Fusil, F.; Down, J.; Denaro, M.; Brady, T.; Westerman, K.; Cavallesco, R.; Gillet-Legrand, B.; Caccavelli, L.; Sgarra, R.; Maouche-Chretien, L.; Bernaudin, F.; Girot, R.; Dorazio, R.; Mulder, G. J.; Polack, A.; Bank, A.; Soulier, J.; Larghero, J.; Kabbara, N.; Dalle, B.; Gourmel, B.; Socie, G.; Chretien, S.; Cartier, N.; Aubourg, P.; Fischer, A.; Cornetta, K.; Galacteros, F.; Beuzard, Y.; Gluckman, E.; Bushman, F.; Hacein-Bey-Abina, S.; Leboulch, P. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 467(7313): 318–322; 2010.
4. Chang, C. J.; Yen, M. L.; Chen, Y. C.; Chien, C. C.; Huang, H. I.; Bai, C. H.; Yen, B. L. Placenta-derived multipotent cells exhibit immunosuppressive properties that are enhanced in the presence of interferon-gamma. Stem Cells 24(11):2466–2477; 2006.
5. Chao, K. C.; Yang, H. T.; Chen, M. W. Human umbilical cord mesenchymal stem cells suppress breast cancer tumourigenesis through direct cell-cell contact and internalization. J. Cell. Mol. Med. 16(8):1803–1815; 2012.
6. Chatzistamatiou, T. K.; Papassavas, A. C.; Michalopoulos, E.; Gamaloutsos, C.; Mallis, P.; Gontika, I.; Panagouli, E.; Koussoulakos, S. L.; Stavropoulos-Giokas, C. Optimizing isolation culture and freezing methods to preserve Wharton's jelly's mesenchymal stem cell (MSC) properties: An MSC banking protocol validation for the Hellenic Cord Blood Bank. Transfusion 54(12):3108–3120; 2014.
7. Cho, M.; Lee, E. J.; Nam, H.; Yang, J. H.; Cho, J.; Lim, J. M.; Lee, G. Human feeder layer system derived from umbilical cord stromal cells for human embryonic stem cells. Fertil. Steril. 93(8):2525–2531; 2010.
8. De Miguel, M. P.; Fuentes-Julian, S.; Blazquez-Martinez, A.; Pascual, C. Y.; Aller, M. A.; Arias, J.; Arnalich-Montiel, F. Immuno-suppressive properties of mesenchymal stem cells: Advances and applications. Curr. Mol. Med. 12(5):574–591; 2012.
9. Ding, D. C.; Shyu, W. C.; Chiang, M. F.; Lin, S. Z.; Chang, Y. C.; Wang, H. J.; Su, C. Y.; Li, H. Enhancement of neuroplasticity through up-regulation of beta1-integrin in human umbilical cord-derived stromal cell implanted stroke model. Neurobiol. Dis. 27(3):339–353; 2007.
10. Ding, D. C.; Shyu, W. C.; Lin, S. Z. Mesenchymal stem cells. Cell Transplant. 20(1):5–14; 2011.
11. Ding, D. C.; Shyu, W. C.; Lin, S. Z.; Li, H. Current concepts in adult stem cell therapy for stroke. Curr. Med. Chem. 13(29):3565–3574; 2006.
12. Ding, D. C.; Shyu, W. C.; Lin, S. Z.; Liu, H. W.; Chiou, S. H.; Chu, T. Y. Human umbilical cord mesenchymal stem cells support non-tumorigenic expansion of human embryonic stem cells. Cell Transplant. 21(7):1515–1527; 2012.
13. Ding, D. C.; Wu, K. C.; Chou, H. L.; Hung, W. T.; Liu, H. W.; Chu, T. Y. Human infra-patellar fat pad-derived stromal

cells have more potent differentiation capacity than other mesenchymal cells and can be enhanced by hyaluronan. Cell Transplant. 2014.
14. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4): 315–317; 2006.
15. El Omar, R.; Beroud, J.; Stoltz, J. F.; Menu, P.; Velot, E.; Decot, V. Umbilical cord mesenchymal stem cells: The new gold standard for mesenchymal stem cell-based therapies? Tissue Eng. Part B Rev. 20(5):523–544; 2014.
16. English, K.; French, A.; Wood, K. J. Mesenchymal stromal cells: Facilitators of successful transplantation? Cell Stem Cell 7(4):431–442; 2010.
17. Fan, C. G.; Zhang, Q. J.; Zhou, J. R. Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. Stem Cell Rev. 7(1):195–207; 2011.
18. Fong, C. Y.; Gauthaman, K.; Cheyyatraivendran, S.; Lin, H. D.; Biswas, A.; Bongso, A. Human umbilical cord Wharton's jelly stem cells and its conditioned medium support hematopoietic stem cell expansion ex vivo. J. Cell. Biochem. 113(2):658–668; 2012.
19. Fong, C. Y.; Richards, M.; Manasi, N.; Biswas, A.; Bongso, A. Comparative growth behaviour and characterization of stem cells from human Wharton's jelly. Reprod. Biomed. Online 15(6):708–718; 2007.
20. Fong, C. Y.; Subramanian, A.; Biswas, A.; Gauthaman, K.; Srikanth, P.; Hande, M. P.; Bongso, A. Derivation efficiency, cell proliferation, freeze-thaw survival, stem-cell properties and differentiation of human Wharton's jelly stem cells. Reprod. Biomed. Online 21(3):391–401; 2010.
21. Fu, Y. S.; Shih, Y. T.; Cheng, Y. C.; Min, M. Y. Transformation of human umbilical mesenchymal cells into neurons in vitro. J. Biomed. Sci. 11(5):652–660; 2004.
22. Ganta, C.; Chiyo, D.; Ayuzawa, R.; Rachakatla, R.; Pyle, M.; Andrews, G.; Weiss, M.; Tamura, M.; Troyer, D. Rat umbilical cord stem cells completely abolish rat mammary carcinomas with no evidence of metastasis or recurrence 100 days post-tumor cell inoculation. Cancer Res. 69(5): 1815–1820; 2009.
23. Gauthaman, K.; Yee, F. C.; Cheyyatraivendran, S.; Biswas, A.; Choolani, M.; Bongso, A. Human umbilical cord Wharton's jelly stem cell (hWJSC) extracts inhibit cancer cell growth in vitro. J. Cell. Biochem. 113(6):2027–2039; 2012.
24. Gong, W.; Han, Z.; Zhao, H.; Wang, Y.; Wang, J.; Zhong, J.; Wang, B.; Wang, S.; Wang, Y.; Sun, L.; Han, Z. Banking human umbilical cord-derived mesenchymal stromal cells for clinical use. Cell Transplant. 21(1):207–216; 2012.
25. Gonzalez, R.; Griparic, L.; Umana, M.; Burgee, K.; Vargas, V.; Nasrallah, R.; Silva, F.; Patel, A. An efficient approach to isolation and characterization of pre- and post-natal umbilical cord lining stem cells for clinical applications. Cell Transplant. 19(11):1439–1449; 2010.
26. Gore, A.; Li, Z.; Fung, H. L.; Young, J. E.; Agarwal, S.; Antosiewicz-Bourget, J.; Canto, I.; Giorgetti, A.; Israel, M. A.; Kiskinis, E.; Lee, J. H.; Loh, Y. H.; Manos, P. D.; Montserrat, N.; Panopoulos, A. D.; Ruiz, S.; Wilbert, M. L.; Yu, J.; Kirkness, E. F.; Izpisua Belmonte, J. C.; Rossi, D. J.; Thomson, J. A.; Eggan, K.; Daley, G. Q.; Goldstein, L. S.; Zhang, K. Somatic coding mutations in human induced pluripotent stem cells. Nature 471(7336):63–67; 2011.
27. Greco, S. J.; Liu, K.; Rameshwar, P. Functional similarities among genes regulated by OCT4 in human mesenchymal and embryonic stem cells. Stem Cells 25(12):3143–3154; 2007.
28. Hacein-Bey-Abina, S.; Von Kalle, C.; Schmidt, M.; McCormack, M. P.; Wulffraat, N.; Leboulch, P.; Lim, A.; Osborne, C. S.; Pawliuk, R.; Morillon, E.; Sorensen, R.; Forster, A.; Fraser, P.; Cohen, J. I.; de Saint Basile, G.; Alexander, I.; Wintergerst, U.; Frebourg, T.; Aurias, A.; Stoppa-Lyonnet, D.; Romana, S.; Radford-Weiss, I.; Gross, F.; Valensi, F.; Delabesse, E.; Macintyre, E.; Sigaux, F.; Soulier, J.; Leiva, L. E.; Wissler, M.; Prinz, C.; Rabbitts, T. H.; Le Deist, F.; Fischer, A.; Cavazzana-Calvo, M. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302(5644):415–419; 2003.
29. He, H.; Nagamura-Inoue, T.; Tsunoda, H.; Yuzawa, M.; Yamamoto, Y.; Yorozu, P.; Agata, H.; Tojo, A. Stage-specific embryonic antigen 4 in Wharton's jelly-derived mesenchymal stem cells is not a marker for proliferation and multipotency. Tissue Eng. Part A 20(7–8):1314–1324; 2014.
30. Hsieh, J. Y.; Fu, Y. S.; Chang, S. J.; Tsuang, Y. H.; Wang, H. W. Functional module analysis reveals differential osteogenic and stemness potentials in human mesenchymal stem cells from bone marrow and Wharton's jelly of umbilical cord. Stem Cells Dev. 19(12):1895–1910; 2010.
31. Ishige, I.; Nagamura-Inoue, T.; Honda, M. J.; Harnprasopwat, R.; Kido, M.; Sugimoto, M.; Nakauchi, H.; Tojo, A. Comparison of mesenchymal stem cells derived from arterial, venous, and Wharton's jelly explants of human umbilical cord. Int. J. Hematol. 90(2):261–269; 2009.
32. Kim, S. S.; Kwon, D. W.; Im, I.; Kim, Y. D.; Hwang, D. S.; Holliday, L. S.; Donatelli, R. E.; Son, W. S.; Jun, E. S. Differentiation and characteristics of undifferentiated mesenchymal stem cells originating from adult premolar periodontal ligaments. Korean J. Orthod. 42(6):307–317; 2012.
33. Kita, K.; Gauglitz, G. G.; Phan, T. T.; Herndon, D. N.; Jeschke, M. G. Isolation and characterization of mesenchymal stem cells from the sub-amniotic human umbilical cord lining membrane. Stem Cells Dev. 19(4):491–502; 2010.
34. La Rocca, G.; Anzalone, R.; Corrao, S.; Magno, F.; Loria, T.; Lo Iacono, M.; Di Stefano, A.; Giannuzzi, P.; Marasa, L.; Cappello, F.; Zummo, G.; Farina, F. Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: Differentiation potential and detection of new markers. Histochem. Cell Biol. 131(2): 267–282; 2009.
35. La Rocca, G.; Corrao, S.; Lo Iacono, M.; Corsello, T.; Farina, F.; Anzalone, R. Novel immuno-modulatory markers expressed by human WJ-MSC: An update review in regenerative and reparative medicine. Open Tissue Eng. Reg. Med. J. 5:50–58; 2012.
36. Lai, D.; Cheng, W.; Liu, T.; Jiang, L.; Liu, T.; Huang, Q.; Guo, L. Optimization of culture conditions to support undifferentiated growth of human embryonic stem cells. Cell. Reprogram. 12(3):305–314; 2010.
37. Lee, J. M.; Jung, J.; Lee, H. J.; Jeong, S. J.; Cho, K. J.; Hwang, S. G.; Kim, G. J. Comparison of immuno-modulatory effects of placenta mesenchymal stem cells with bone marrow and adipose mesenchymal stem cells. Int. Immunopharmacol. 13(2):219–224; 2012.
38. Lim, I. J.; Phan, T. T. Epithelial and mesenchymal stem cells from the umbilical cord lining membrane. Cell Transplant. 23(4–5):497–503; 2014.

39. Lin, H. D.; Fong, C. Y.; Biswas, A.; Choolani, M.; Bongso, A. Human Wharton's jelly stem cells, its conditioned medium and cell-free lysate inhibit the growth of human lymphoma cells. Stem Cell Rev. 10(4):573–586; 2014.
40. Lin, S. Z.; Chang, Y. J.; Liu, J. W.; Chang, L. F.; Sun, L. Y.; Li, Y. S.; Luo, G. H.; Liao, C. H.; Chen, P. H.; Chen, T. M.; Lee, R. P.; Yang, K. L.; Harn, H. J.; Chiou, T. W. Transplantation of human Wharton's Jelly-derived stem cells alleviates chemically induced liver fibrosis in rats. Cell Transplant. 19(11):1451–1463; 2010.
41. Liu, S. P.; Ding, D. C.; Wang, H. J.; Su, C. Y.; Lin, S. Z.; Li, H.; Shyu, W. C. Non-senescent Hsp27-upregulated MSCs implantation promotes neuroplasticity in stroke model. Cell Transplant. 19(10):1261–1279; 2010.
42. Ma, L.; Feng, X. Y.; Cui, B. L.; Law, F.; Jiang, X. W.; Yang, L. Y.; Xie, Q. D.; Huang, T. H. Human umbilical cord Wharton's Jelly-derived mesenchymal stem cells differentiation into nerve-like cells. Chin. Med. J. 118(23): 1987–1993; 2005.
43. Ma, Y.; Hao, X.; Zhang, S.; Zhang, J. The in vitro and in vivo effects of human umbilical cord mesenchymal stem cells on the growth of breast cancer cells. Breast Cancer Res. Treat. 133(2):473–485; 2012.
44. Manochantr, S.; U-pratya, Y.; Kheolamai, P.; Rojphisan, S.; Chayosumrit, M.; Tantrawatpan, C.; Supokawej, A.; Issaragrisil, S. Immunosuppressive properties of mesenchymal stromal cells derived from amnion, placenta, Wharton's jelly and umbilical cord. Intern. Med. J. 43(4):430–439; 2013.
45. Martin, A.; Badell, M. Umbilical cord blood banking. Postgrad. Obstet. Gynecol. 34(15):1–6; 2014.
46. Martin, M. J.; Muotri, A.; Gage, F.; Varki, A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat. Med. 11(2):228–232; 2005.
47. Maurya, D. K.; Doi, C.; Kawabata, A.; Pyle, M. M.; King, C.; Wu, Z.; Troyer, D.; Tamura, M. Therapy with un-engineered naive rat umbilical cord matrix stem cells markedly inhibits growth of murine lung adenocarcinoma. BMC Cancer 10: 590; 2010.
48. Mennan, C.; Wright, K.; Bhattacharjee, A.; Balain, B.; Richardson, J.; Roberts, S. Isolation and characterisation of mesenchymal stem cells from different regions of the human umbilical cord. Biomed. Res. Int. 2013:916136; 2013.
49. Mitchell, K. E.; Weiss, M. L.; Mitchell, B. M.; Martin, P.; Davis, D.; Morales, L.; Helwig, B.; Beerenstrauch, M.; Abou-Easa, K.; Hildreth, T.; Troyer, D.; Medicetty, S. Matrix cells from Wharton's jelly form neurons and glia. Stem Cells 21(1):50–60; 2003.
50. Nagamura-Inoue, T.; He, H. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility. World J. Stem Cells 6(2):195–202; 2014.
51. Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of germline-competent induced pluripotent stem cells. Nature 448(7151):313–317; 2007.
52. Parekkadan, B.; Milwid, J. M. Mesenchymal stem cells as therapeutics. Annu. Rev. Biomed. Eng. 12:87–117; 2010.
53. Pittenger, M. F.; Mackay, A. M.; Beck, S. C.; Jaiswal, R. K.; Douglas, R.; Mosca, J. D.; Moorman, M. A.; Simonetti, D. W.; Craig, S.; Marshak, D. R. Multilineage potential of adult human mesenchymal stem cells. Science 284(5411): 143–147; 1999.
54. Prasanna, S. J.; Jahnavi, V. S. Wharton's jelly mesenchymal stem cells as off-the-shelf cellular therapeutics: A closer look into their regenerative and immuno-modulatory properties. Open Tissue Eng. Reg. Med. J. 4:28–38; 2011.
55. Rachakatla, R. S.; Marini, F.; Weiss, M. L.; Tamura, M.; Troyer, D. Development of human umbilical cord matrix stem cell-based gene therapy for experimental lung tumors. Cancer Gene Ther. 14(10):828–835; 2007.
56. Reza, H. M.; Ng, B. Y.; Gimeno, F. L.; Phan, T. T.; Ang, L. P. Umbilical cord lining stem cells as a novel and promising source for ocular surface regeneration. Stem Cell Rev. 7(4):935–947; 2011.
57. Reza, H. M.; Ng, B. Y.; Phan, T. T.; Tan, D. T.; Beuerman, R. W.; Ang, L. P. Characterization of a novel umbilical cord lining cell with CD227 positivity and unique pattern of P63 expression and function. Stem Cell Rev. 7(3):624–638; 2011.
58. Saben, J.; Thakali, K. M.; Lindsey, F. E.; Zhong, Y.; Badger, T. M.; Andres, A.; Shankar, K. Distinct adipogenic differentiation phenotypes of human umbilical cord mesenchymal cells dependent on adipogenic conditions. Exp. Biol. Med. 239(10):1340–1351; 2014.
59. Salehinejad, P.; Alitheen, N. B.; Ali, A. M.; Omar, A. R.; Mohit, M.; Janzamin, E.; Samani, F. S.; Torshizi, Z.; Nematollahi-Mahani, S. N. Comparison of different methods for the isolation of mesenchymal stem cells from human umbilical cord Wharton's jelly. In Vitro Cell Dev. Biol. Anim. 48(2):75–83; 2012.
60. Selmani, Z.; Naji, A.; Gaiffe, E.; Obert, L.; Tiberghien, P.; Rouas-Freiss, N.; Carosella, E. D.; Deschaseaux, F. HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. Transplantation 87(9 Suppl):S62–66; 2009.
61. Selmani, Z.; Naji, A.; Zidi, I.; Favier, B.; Gaiffe, E.; Obert, L.; Borg, C.; Saas, P.; Tiberghien, P.; Rouas-Freiss, N.; Carosella, E. D.; Deschaseaux, F. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells 26(1):212–222; 2008.
62. Seo, K. W.; Lee, S. R.; Bhandari, D. R.; Roh, K. H.; Park, S. B.; So, A. Y.; Jung, J. W.; Seo, M. S.; Kang, S. K.; Lee, Y. S.; Kang, K. S. OCT4A contributes to the stemness and multi-potency of human umbilical cord blood-derived multipotent stem cells (hUCB-MSCs). Biochem. Biophys. Res. Commun. 384(1):120–125; 2009.
63. Sinha, M.; Jang, Y. C.; Oh, J.; Khong, D.; Wu, E. Y.; Manohar, R.; Miller, C.; Regalado, S. G.; Loffredo, F. S.; Pancoast, J. R.; Hirshman, M. F.; Lebowitz, J.; Shadrach, J. L.; Cerletti, M.; Kim, M. J.; Serwold, T.; Goodyear, L. J.; Rosner, B.; Lee, R. T.; Wagers, A. J. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science 344(6184):649–652; 2014.
64. Sun, B.; Yu, K. R.; Bhandari, D. R.; Jung, J. W.; Kang, S. K.; Kang, K. S. Human umbilical cord blood mesenchymal stem cell-derived extracellular matrix prohibits metastatic cancer cell MDA-MB-231 proliferation. Cancer Lett. 296(2): 178–185; 2010.
65. Tsagias, N.; Koliakos, I.; Karagiannis, V.; Eleftheriadou, M.; Koliakos, G. G. Isolation of mesenchymal stem cells using the total length of umbilical cord for transplantation purposes. Transfus. Med. 21(4):253–261; 2011.
66. Tsai, M. S.; Lee, J. L.; Chang, Y. J.; Hwang, S. M. Isolation of human multi-potent mesenchymal stem cells from second-trimester amniotic fluid using a novel two-stage culture protocol. Hum. Reprod. 19(6):1450–1456; 2004.
67. Tsai, P. C.; Fu, T. W.; Chen, Y. M.; Ko, T. L.; Chen, T. H.; Shih, Y. H.; Hung, S. C.; Fu, Y. S. The therapeutic

potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis. Liver Transpl. 15(5):484–495; 2009.
68. Tsai, P. J.; Wang, H. S.; Shyr, Y. M.; Weng, Z. C.; Tai, L. C.; Shyu, J. F.; Chen, T. H. Transplantation of insulin-producing cells from umbilical cord mesenchymal stem cells for the treatment of streptozotocin-induced diabetic rats. J. Biomed. Sci. 19:47; 2012.
69. Uccelli, A.; Moretta, L.; Pistoia, V. Mesenchymal stem cells in health and disease. Nat. Rev. Immunol. 8(9):726–736; 2008.
70. Villeda, S. A.; Plambeck, K. E.; Middeldorp, J.; Castellano, J. M.; Mosher, K. I.; Luo, J.; Smith, L. K.; Bieri, G.; Lin, K.; Berdnik, D.; Wabl, R.; Udeochu, J.; Wheatley, E. G.; Zou, B.; Simmons, D. A.; Xie, X. S.; Longo, F. M.; Wyss-Coray, T. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat. Med. 20(6):659–663; 2014.
71. Wang, H. S.; Hung, S. C.; Peng, S. T.; Huang, C. C.; Wei, H. M.; Guo, Y. J.; Fu, Y. S.; Lai, M. C.; Chen, C. C. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 22(7):1330–1337; 2004.
72. Wang, H. S.; Shyu, J. F.; Shen, W. S.; Hsu, H. C.; Chi, T. C.; Chen, C. P.; Huang, S. W.; Shyr, Y. M.; Tang, K. T.; Chen, T. H. Transplantation of insulin-producing cells derived from umbilical cord stromal mesenchymal stem cells to treat NOD mice. Cell Transplant. 20(3):455–466; 2011.
73. Wang, Y.; Han, Z. B.; Ma, J.; Zuo, C.; Geng, J.; Gong, W.; Sun, Y.; Li, H.; Wang, B.; Zhang, L.; He, Y.; Han, Z. C. A toxicity study of multiple-administration human umbilical cord mesenchymal stem cells in cynomolgus monkeys. Stem Cells Dev. 21(9):1401–1408; 2012.
74. Weiss, M. L.; Anderson, C.; Medicetty, S.; Seshareddy, K. B.; Weiss, R. J.; VanderWerff, I.; Troyer, D.; McIntosh, K. R. Immune properties of human umbilical cord Wharton's jelly-derived cells. Stem Cells 26(11):2865–2874; 2008.
75. William, P. L.; Banister, L. H. Gray's anatomy. London, UK: Churchill Livingstone; 1995.
76. Yamanaka, S.; Takahashi, K. [Induction of pluripotent stem cells from mouse fibroblast cultures]. Tanpakushitsu Kakusan Koso 51(15):2346–2351; 2006.
77. Yu, S.; Long, J.; Yu, J.; Du, J.; Ma, P.; Ma, Y.; Yang, D.; Fan, Z. Analysis of differentiation potentials and gene expression profiles of mesenchymal stem cells derived from periodontal ligament and Wharton's jelly of the umbilical cord. Cells Tissues Organs 197(3):209–223; 2013.