Umbilical Cord Tissue Offers the Greatest Number of Harvestable Mesenchymal Stem Cells for Research and Clinical Application: A Literature Review of Different Harvest Sites

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Variations in allogeneic mesenchymal stem cell harvest levels from human tissues reflect the evolving nature of the field, patient demographic characteristics, and differences in harvest and isolation techniques. At present, Wharton's jelly tissue yields the highest concentration of allogeneic mesenchymal stem cells whereas adipose tissue yields the highest levels of autologous mesenchymal stem cells per milliliter of tissue.

Isolation of multipotent mesenchymal stem cells from umbilical cord blood

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It is well accepted that umbilical cord blood has been a source for hematopoietic stem cells. However, controversy exists as to whether cord blood can serve as a source of mesenchymal stem cells, which can differentiate into cells of differ- ent connective tissue lineages such as bone, cartilage, and fat, and little success has been reported in the literature about the isolation of such cells from cord blood. Here we report a novel method to obtain single cell-derived, clonally expanded mesenchymal stem cells that are of multi- lineage differentiation potential by negative immunoselection and limiting dilution. The immunophenotype of these clonally expanded cells is consistent with that reported for bone marrow mesenchymal stem cells. Under appropriate induction conditions, these cells can differentiate into bone, cartilage, and fat. Surprisingly, these cells were also able to differentiate into neuroglial- and hepatocyte-like cells under appropriate induction conditions and, thus, these cells may be more than mesenchymal stem cells as evidenced by their ability to differentiate into cell types of all 3 germ layers. In conclusion, umbilical cord blood does contain mesenchymal stem cells and should not be regarded as medical waste. It can serve as an alternative source of mesenchymal stem cells to bone marrow. (Blood. 2004;103:1669-1675)

Comparative Analysis of Human Mesenchymal Stem Cells from Bone Marrow, Adipose Tissue, and Umbilical Cord Blood as Sources of Cell Therapy

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Various source-derived mesenchymal stem cells (MSCs) have been considered for cell therapeutics in incurable diseases. To characterize MSCs from different sources, we compared human bone marrow (BM), adipose tissue (AT), and umbilical cord blood-derived MSCs (UCB-MSCs) for surface antigen expression, differentiation ability, proliferation capacity, clonality, tolerance for aging, and paracrine activity. Although MSCs from different tissues have similar levels of surface antigen expression, immunosuppressive activity, and differentiation ability, UCB-MSCs had the highest rate of cell proliferation and clonality, and significantly lower expression of p53, p21, and p16, well known markers of senescence. Since paracrine action is the main action of MSCs, we examined the anti-inflammatory activity of each MSC under lipopolysaccharide (LPS)-induced inflammation. Co-culture of UCB-MSCs with LPS-treated rat alveolar macrophage, reduced expression of inflammatory cytokines including interleukin-1α (IL-1α), IL-6, and IL-8 via angiopoietin-1 (Ang-1). Using recombinant Ang-1 as potential soluble paracrine factor or its small interference RNA (siRNA), we found that Ang-1 secretion was responsible for this beneficial effect in part by preventing inflammation. Our results demonstrate that primitive UCB-MSCs have biological advantages in comparison to adult sources, making UCB-MSCs a useful model for

clinical applications of cell therapy

HIGHER TELOMERASE ACTIVITIES IN MESENCHYMAL STEM CELLS FROM UMBILICAL CORD BLOOD THAN FROM BONE MARROW AFTER CULTURE EXPANSION

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It is demonstrated in this study that MSCs from both UCB and BM possess telomerase activities after culture-expansion for 30 population doublings, and UCB-MSCs demonstrated greater telomerase activities and longer telomere length than BM-MSCs, as shown by TRAP assay and TRF measurement. Besides, greater telomerase activities in UCB-MSCs are associated with the shorter doubling time. Interestingly, it is found that a greater proportion of UCB-MSCs are in the quiescent (G0/G1) state than BM-MSCs. This may also be associated with greater telomerase activities of UCB-MSCs, which make the time required for cell division in each cell cycle shorter. Taken together, UCB-MSCs possess greater telomerase potential after culture-expansion after 30 population doublings while maintaining their osteogenic differentiation abilities. UCB-MSCs may therefore be excellent candidates for cell therapy and tissue engineering applications of bone reconstruction.

Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue

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Mesenchymal stem cells (MSCs) represent a promising tool for new clinical concepts in supporting cellular therapy. Bone marrow (BM) was the first source reported to contain MSCs. However, for clinical use, BM may be detrimental due to the highly invasive donation procedure and the decline in MSC number and differentiation potential with increasing age. More recently, umbilical cord blood (UCB), attainable by a less invasive method, was introduced as an alternative source for MSCs. Another promising source is adipose tissue (AT). We compared MSCs derived from these sources regarding morphology, the success rate of isolating MSCs, colony frequency, expansion potential, multiple differentiation capacity, and immune phenotype. No significant differences concerning the morphology and immune phenotype of the MSCs derived from these sources were obvious. Differences could be observed concerning the success rate of isolating MSCs, which was 100% for BM and AT, but only 63% for UCB. The colony frequency was lowest in UCB, whereas it was highest in AT. However, UCB-MSCs could be cultured longest and showed the highest proliferation capacity, whereas BM-MSCs possessed the shortest culture period and the lowest proliferation capacity. Most strikingly, UCB-MSCs showed no adipogenic differentiation capacity, in contrast to BM- and AT-MSCs. Both UCB and AT are attractive alternatives to BM in isolating MSC: AT as it contains MSCs at the highest frequency and UCB as it seems to be expandable to higher numbers. STEM CELLS *2006;24: 1294 –1301*

Bone marrow and umbilical cord blood human mesenchymal stem cells: state of the art

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Mesenchymal stem cells (MSCs) are multipotent adult stem cells present in all tissues, as part of the perivascular population. As multipotent cells, MSCs can differentiate into different tissues originating from mesoderm ranging from bone and cartilage, to cardiac muscle. MSCs are an excellent candidate for cell therapy because they are easily accessible, their isolation is straightforward, they can be bio-preserved with minimal loss of potency, and they have shown no adverse reactions to allogeneic versus autologous MSCs transplants. Therefore, MSCs are being explored to regenerate damaged tissue and treat inflammation, resulting from cardiovascular disease and myocardial infarction (MI), brain and spinal cord injury, stroke, diabetes, cartilage and bone injury, Crohn's disease and graft versus host disease (GvHD). Most of the application and clinical trials involve MSCs from bone marrow (BMMSCs). Transplantation of MSCs from bone marrow is considered safe and has been widely tested in clinical trials of cardiovascular, neurological, and immunological disease with encouraging results. There are examples of MSCs utilization in the repair of kidney, muscle and lung. The cells were also found to promote angiogenesis, and were used in chronic skin wound treatment. Recent studies involve also mesenchymal stem cell transplant from umbilical cord (UCMSCt). One of these demonstrate that UCMSCt may improve symptoms and biochemical values in patients with severe refractory systemic lupus erythematosus (SLE), and therefore this source of MSCs need deeper studies and require more attention. However, also if there are 79 registered clinical trial sites for evaluating MSC therapy throughout the world, it is still a long way to go before using these cells as a routinely applied therapy in clinics.

Potential Application of Cord Blood-Derived Stromal Cells in Cellular Therapy and Regenerative Medicine

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Neonatal stromal cells from umbilical cord blood (CB) are promising alternatives to bone marrow-(BM-) derived multipotent stromal cells (MSCs). In comparison to BM-MSC, the less mature CB-derived stromal cells have been described as a cell population with higher differentiation and proliferation potential that might be of potential interest for clinical application in regenerative medicine. Recently, it has become clear that cord blood contains different stromal cell populations, and as of today, a clear distinction between unrestricted somatic stromal cells (USSCs) and CB-MSC has been established. This classification is based on the expression of DLK-1, HOX, and CD146, as well as functional examination of the adipogenic differentiation potential and the capacity to support haematopoiesis *in vitro* and *in vivo*. However, a marker enabling a prospective isolation of the rare cell populations directly out of cord blood is yet to be found. Further analysis may help to reveal even more subpopulations with different properties, which could be useful for the directed application of these cells in preclinical models.

Comparison of the osteogenic differentiation potential of mesenchymal cells isolated from human bone marrow, umbilical cord blood and placenta derived stem cells

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Bone marrow has been considered for long time as the main source for mesenchymal stem cells. However, bone marrow aspiration is an invasive process that can be associated with morbidity as well as few numbers of obtained cells. Umbilical cord blood and placental tissues are other potential sources for the same type of cells. These sources are abundant, accessible and associated with no harm to the donor. This study aimed at determining the differentiation of the three cell types towards the osteogenic lineage in short term culture and in classical osteogenic conditions. The gene expression profile showed that bone marrow derived cells were the most responsive to the culture conditions while umbilical cord blood derived cells were next, as shown by the expression by the osteogenic key transcription factors 'Runx-2' and osterix. At the meantime, umbilical cord blood and placenta derived cells showed significant enhancement of the gene expression over the study course, which denoted potential response of the cells. Based on these results and the availability of these two sources, umbilical cord blood and placenta should still be considered as potential sources for mesenchymal stem cells in osteogenic research program. However their differentiation potential will need further enhancement.

Age-Related Intrinsic Changes in Human Bone Marrow-Derived Mesenchymal Stem Cells and Their Differentiation to Osteoblasts

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In vivo and *in vitro* studies indicate that a sub-population of human marrow-derived stromal cells (MSCs, a.k.a. mesenchymal stem cells) has potential to differentiate into multiple cell types, including osteoblasts. In this study, we tested the hypotheses that there are intrinsic effects of age in human MSCs (17 to 90 years). We tested the effect of age on senescence-associated β-galactosidase (SA-β-gal), proliferation, apoptosis, *p53* pathway genes, and osteoblast differentiation in confluent monolayers by alkaline phosphatase activity and osteoblast gene expression analysis. There were 4- fold more hMSCs positive for SA-β-gal in samples from older than younger subjects ($p<0.001$, $n=17$). Doubling time of hMSCs was 1.7-fold longer in cells from the older than the younger subjects and was positively correlated with age ($p=0.002$, $n=19$). Novel age-related changes were identified. With age, more cells were apoptotic ($p=0.016$, $n=10$). Further, there were age-related increases in expression of *p53* and its pathway genes, *p21* and *BAX*. Consistent with other experiments, there was a significant age-related decrease in generation of osteoblasts both in the STRO-1^+ cells ($p=0.047$, $n=8$) and in adherent MSCs ($p<0.001$, $n=10$). In sum, there is an age-dependent decrease in proliferation and osteoblast differentiation and an increase in SA-β-gal-positive cells and apoptosis in hMSCs. Upregulation of the *p53* pathway with age may have a critical role in mediating the reduction in both proliferation and osteoblastogenesis of hMSCs. These findings support the view that there are intrinsic alterations in human MSCs with aging that may contribute to the process of skeletal aging in humans.