Osteogenic and Chondrogenic Differentiation of Umbilical Cord Blood-derived Mesenchymal Stem Cells
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Introduction: Mesenchymal stem cells (MSCs) found in many adult tissues are an attractive stem cell source for the regeneration of damaged tissues in clinical applications because they are characterized as undifferentiated cells, able to self-renew with a high proliferative capacity, and possess a mesodermal differentiation potential. Although bone marrow has been the main source for the isolation of multipotent MSCs, the harvest of bone marrow is a highly invasive procedure and the number, differentiation potential, and maximal life span of MSCs from BM decline with increasing age. Therefore, alternative sources from which to isolate MSCs are subject to intensive investigation. A recently reported potential alternative tissue source of MSCs is the connective tissue (Wharton's Jelly) of human umbilical cord. Umbilical cord blood-derived MSCs (UCBMSCs) are ontogenically primitive, less exposed to immunologic challenges, abundantly available, and can be harvested without risk to the donor. Our study is to study the ability of UCBMSCs to differentiate into osteogenic and chondrogenic cells.

Methods: UCBMSCs were isolated from umbilical cords (n=4) from consenting patients after extensive washing with phosphate derived saline and digesting with collagenase. After primary culture in basic medium and expanded to two passages, the cells were incubated in the osteogenic and the chondrogenic medium for 2-4 weeks to induce osteogenesis and chondrogenesis. Osteogenic differentiation was confirmed using the ALP and Von-Kossa Staining. Expressions of osteoblast-specific genes (ALP, Osteopontin, and Osteocalcin) were confirmed by RT-PCR. Chondrogenic differentiation was confirmed using the Alcian Blue Staining. Expressions of chondrocyte-specific genes (Collagen II, X, and Aggrecan) were confirmed by RT-PCR.

Results: UCBMSCs were able to be isolated from umbilical cords and expanded rapidly. They showed the highest proliferation capacity. UCBMSCs induced to osteogenesis were stained positively for alkaline phosphatase activity after 2 weeks and formed mineralized nodular structures, as conformed by Von Kossa staining. Expression of osteoblast specific genes, such as ALP, Osteopontin, Osteocalcin, were detected. ALP and Osteopontin, were expressed constitutively in osteogenic medium after 2 and 4 weeks of culture. Expression of Osteocalcin, was induced by osteogenic growth factors at 4 weeks. UCBMSCs induced to chondrogenesis were positive of Alcian blue staining under acidic conditions and expression of Aggrecan and Collagen II and X genes. Aggrecan and collagen II genes were abundant after 2 weeks in chondrogenic medium. Collagen X was detected at 4 weeks.

Discussion: UCBMSCs can be isolated from umbilical cords. Their biological characteristics are similar with bone marrow mesenchymal stem cells, and have the potential to differentiate into osteogenic and chondrogenic lineage. Interestingly, they showed the high proliferation capacity. This may prove to be an attractive strategy for bone formation and spinal fusion in humans. From the therapeutic indication, the clinical applications may be based on differentiation capacity, but more likely on the abundance, frequency, and expansion potential of the cells. Since alternative sources are intensely investigated, one day the new source may replace bone marrow.