Identification of CD73, CD90 and CD105 by qPCR in Cryopreserved Human Cord Tissue

H. Pham[1,2], R. Tonai[1], Sam Cheng[1], J. Willert[1,3] and M. Chen[1]

[1]Processing Laboratory, StemCyte International Cord Blood Center, Baldwin Park, CA 91706, USA,
[2]Department of Medicine, University of California-Los Angeles, Los Angeles, CA 90095, USA,
[3]Department of Pediatrics, Stem Cell Transplant, School of Medicine, Stanford University, Palo Alto, CA 94305, USA

ABSTRACT

BACKGROUND: Mesenchymal stem cells (MSCs) are self-renewing, non-specialized cells capable of differentiating to adipogeinc, chrondrogenic and osteogenic lineages. Clinical applications involving MSCs include but not limited to tissue regeneration in myocardial ischemia and myocardial infarction and immunomodulation of graft-versus-host disease (GVHD). MSCs can be sourced from several types of tissue, including bone marrow, peripheral blood, umbilical cord blood and umbilical cord. Because of the tremendous therapeutic value MSCs possess, we would like to demonstrate the presence of MSCs in cryopreserved umbilical cord tissue stored long-term in the vapor phase of liquid nitrogen. Specifically, we show in this study that cryopreserved umbilical cord tissue test positive for mesenchymal stem cell markers CD73, CD90 and CD105 by qPCR.

AIM: This study tests whether qPCR can be utilized to quickly identify mesenchymal stem cell markers in cryopreserved umbilical cord tissue stored long-term in the vapor phase of liquid nitrogen.

METHODS: Donated human umbilical cord tissues were washed in ice-cold sterile PBS and sectioned into 1 cm squares prior to storage in CryoStor CS10 (BioLife Solutions) containing 10 % DMSO. Processed tissues were cryo-preserved using controlled rate freezing method and stored in liquid nitrogen vapor. After 4 weeks and 30 weeks, sample aliquots were quickly thawed and processed for RNA using Trizol (Invitrogen, Carlsbad, CA). RNA extracts were converted to cDNA using reverse transcriptase (Bio-Rad, Hercules, CA). Mesenchymal stem cell marker CD73, CD90 and CD105 were screened by SYBR Green with GAPDH as an internal standard (Bio-Rad). PCR reaction mixtures were resolved on 1.2 % agarose gel at 90V for 45 min and recorded on a Syngene PXi imager (Frederick, MD).

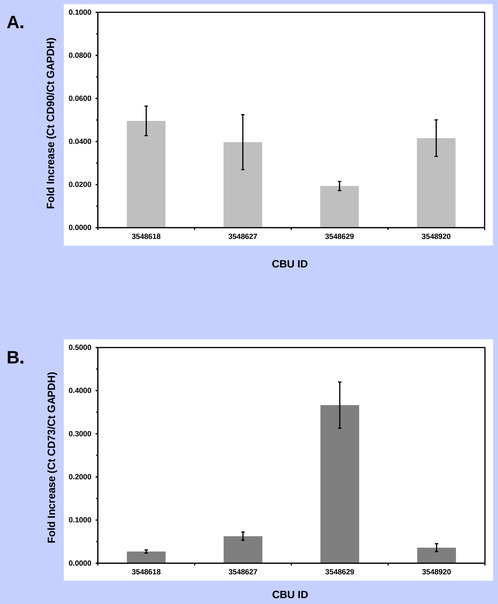
RESULTS: Donated umbilical cord tissues were manually dissected for cord lining, excluding blood vessels and Wharton's jelly. Sectioned umbilical cord lining were thoroughly rinsed in PBS and frozen in CryoStor CS10 (BioLife Solutions) containing 10 % DMSO. Sample sections were screened for CD73, CD90 and CD105 by SYBR green after 4 weeks and 300 weeks of storage time. The findings show that CD90 mRNA expression averaged 0.0376 ± 0.0129-fold, CD73 mRNA expression averaged 0.1230 ± 0.1630-fold and CD105 mRNA expression averaged 0.0452 ± 0.0136-fold expression after 4 weeks in storage. After 30 weeks in storage, expression levels for CD90 mRNA averaged 0.0345 ± 0.0132-fold, CD73 mRNA averaged 0.0639 ± 0.1016-fold and CD105 mRNA averaged 0.0496 ± 0.0068-fold. Recovery averaged 92 % for CD90, 52 % for CD73 and 100 % for CD105 but were not statistically significant.

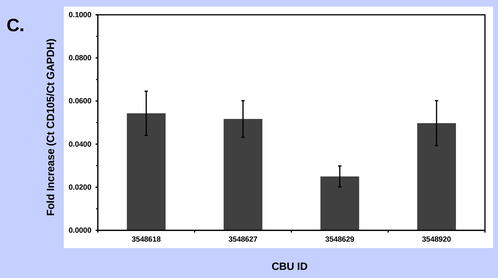
CONCLUSION: Mesenchymal stem cell markers CD73, CD90 and CD105 can be detected by qPCR in cryopreserved umbilical cord tissue after 4 weeks and 30 weeks of storage in liquid nitrogen vapor. Protein analysis and MSC in vitro expansion is required to confirm whether qPCR could be utilized as an inexpensive and quick method for identification of MSC and potential stability in long-term storage.

Financial Disclosure: All listed authors are employees or consultants of StemCyte Inc.

RESULTS

CD90, CD73 and CD105 can be detected by qPCR after 4 weeks in Storage





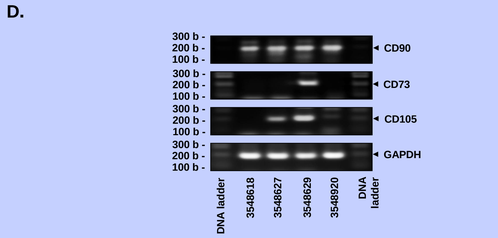
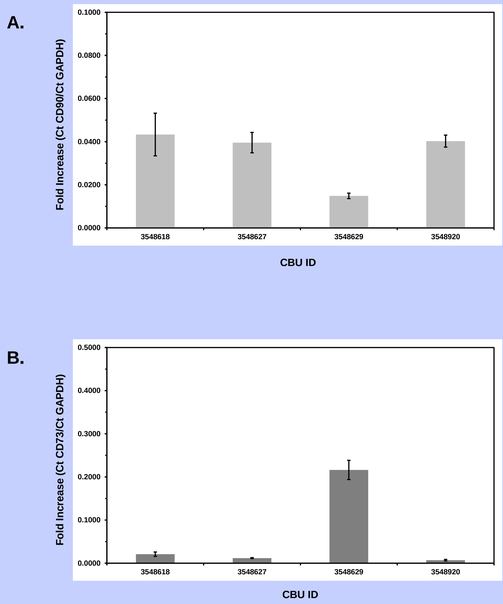
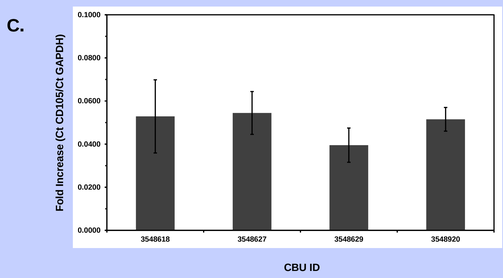


Figure 1. Cryopreserved human umbilical cord tissue express Cd90, CD73 and CD105 mRNA after 4 weeks in liquid nitrogen storage
Donated human umbilical cord tissue from 4 different donors were randomly de-identified with different seven-digit numbers. Umbilical cord tissue were sectioned for cord lining and quickly frozen in CryoStor CS10 containing 10 % DMSO. After 4 weeks, sample sections from all patients were processed for RNA using Trizol (Life Technologies) and converted to cDNA using iScript (Bio-Rad, Hercules, CA). Samples were screened for (**A**) CD90, (**B**) CD73 and (**C**) CD105 by qPCR for 40 cycles using Bio-Rad SYBR Green and quantitated on a CFX Connect Real-Time PCR (Bio-Rad). GAPDH was used as an internal control and data are expressed as fold expression against GAPDH ± SD. (**D**) Representative reactions were resolved on a 1.2 % agarose gel at 90V for 45 min and captured on an imager (Syngene PXi, Frederick, MD).

CD90, CD73 and CD105 can be detected by qPCR after 30 weeks in Storage





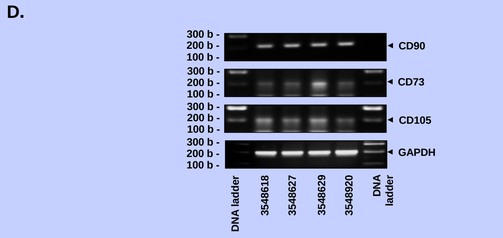


Figure 2. Cryopreserved human umbilical cord tissue express Cd90, CD73 and CD105 mRNA after 30 weeks in liquid nitrogen storage
Donated human umbilical cord tissue from 4 different donors were randomly de-identified with different seven-digit numbers. Umbilical cord tissue were sectioned for cord lining and quickly frozen in CryoStor CS10 containing 10 % DMSO. After 30 weeks, sample sections from all patients were processed for RNA using Trizol (Life Technologies) and converted to cDNA using iScript (Bio-Rad, Hercules, CA). Samples were screened for (**A**) CD90, (**B**) CD73 and (**C**) CD105 by qPCR for 40 cycles using Bio-Rad SYBR Green and quantitated on a CFX Connect Real-Time PCR (Bio-Rad). GAPDH was used as an internal control and data are expressed as fold expression against GAPDH ± SD. (**D**) Representative reactions were resolved on a 1.2 % agarose gel at 90V for 45 min and captured on an imager (Syngene PXi, Frederick, MD).

Percent Retention of CD90, CD73 and CD105 mRNA After 30 Weeks in Storage

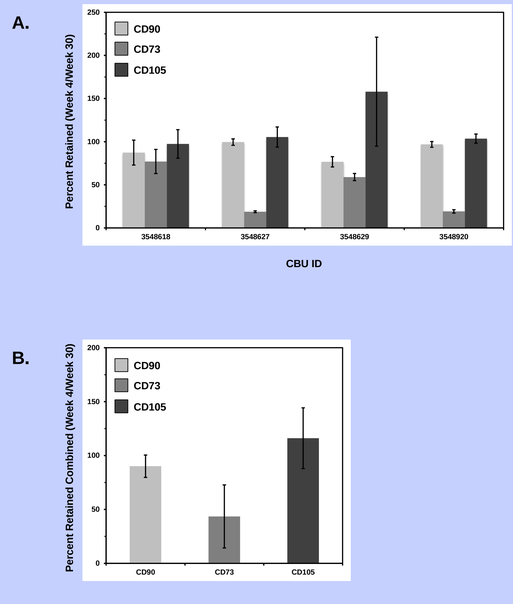


Figure 3. Percent Retention of CD90, CD73 and CD105 mRNA Expression
(**A**) Percent retention of CD90, CD73 and CD105 mRNA expression for individual CBUs at 30 weeks in storage compared to 4 weeks in storage were determined.
(**B**) Percent retention of CD90, CD73 and CD105 mRNA expression for combined CBUs at 30 weeks in storage compared to 4 weeks in storage were determined.

Primer Sequence used in qPCR Reaction

Human Primer	Sequence		Size (bases)
	Forward	Reverse	
CD73	CAGTACCAGGGCACTATCTGG	AGTGGCCCCTTTGCTTTAAT	194
CD90	ATGAACCTGGCCATCAGCA	GTGTGCTCAGGCACCCC	218
CD105	CCACTAGCCAGGTCTCGAAG	GATGCAGGAAGACACTGCTG	192
GAPDH	GAGTCAACGGATTTGGTCGT	TGGGATTTCCATTGATGACA	201

Table 1. Human CD73 (NM_002526) and CD90 (NM_006288) primers were designed to span exon 1 and exon 2. Human CD105 (NM_001114753) primer was designed to span exon 2 and exon 3.

CONCLUSIONS

- **Mesenchymal stem cell markers CD90, CD73 and CD105 mRNA could be detected by qPCR in cryopreserved umbilical cord tissue at 4 weeks and 30 weeks.**
- **CD90 and CD105 mRNA expression were statistically stable at week 30.**
- **Stability of cord tissue at 30 weeks could not be assessed due to the variability of CD73 mRNA expression.**
- **qPCR could be used for a quick screening of MSC markers in cord tissue but is not a definitive method for assessment of cord tissue stability and viability in long term storage.**