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CELL ADHESION MOLECULE EXPRESSION PROFILING OF HUMAN UMBILICAL CORD MATRIX AND ADIPOSE STEM CELLS

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ABSTRACT

Introduction: The advancements and applications of redundant tissue sources such as human subcutaneous adipose tissue (SF) and human umbilical cord matrix (HUCM) tissue are gaining importance in recent years. Despite these advancements, certain stumbling blocks accounts for lack of functional improvement of the diseased. One major bottleneck for the prevailing failures relies on understanding the migratory and homing potential of stem cells. Cell adhesion molecules have been identified to play a vital role in vascular adhesion, migration, extravasations and ultimately homing. However, significance of cell adhesion molecules in therapeutic implications has not much concentrated upon in recent years unlike mesenchymal stem cells. Objective: The present study showcases the significance of cell adhesion molecules and addresses the major issue on identity of an ideal stem cell source with maximum benefits. Research methodology: To this end, the cells obtained from the human SF and HUCM were cultured until P3 and cultured cells were characterized for comparative expression profile analysis of certain cell adhesion molecules. Outcome of the study: Cultured MSCs derived from both these aforesaid sources exhibited a significant percentage of cell adhesion molecules, thereby substantiating its efficacy on tissue homing and migration. Thus, both the sources were found superior with regards to the expression of CAM and can be clinically exploited. However, umbilical cord matrix serves a better therapeutic option for allogenic transplantation, which is evident from the sparse expression of CD 34 at primary culture itself, thereby opening a gateway to circumvent the surgical complications in clinical transplantations.

Keywords: human umbilical cord matrix, human subcutaneous adipose tissue, stem cells, cell adhesion molecules, flowcytometry.

INTRODUCTION

Topical research directs clinical translation of its findings to assist medication for various diseases and disorders. However, there are bouquets of clinical conditions which are either stiff to treatment or give a sluggish response. Thus, there emerged the replacement therapy which is again hindered by the shortage of donors for the procurement of tissues or organs [1,2]. Thus regenerative medicine became a necessity in healthcare owing to its potency and ability to form any kind of cells. Stem cells possess ultimate and dynamic properties that make themselves a best tool for regenerative medicine and medical therapeutics. Research focus on stem cells and its multilineage potentials isolated from human adipose tissue and cord matrix tissues has been fascinating and gained wide interest in recent years. The rationale behind this is because of the selection of source on the basis of redundancy of tissue availability, allogeneic and autologous nature that suits well for clinical implications.

Human subcutaneous adipose tissue, a redundant tissue serves as an inexhaustible source of large

quantity of stem cells. Accumulating evidences report on its efficacy on the proliferative potency and plasticity, thereby substantiating its imperativeness in cell based therapies. Recently, large interest relies on using subcutaneous adipose tissue as a cue to cure wide range of diseases [3, 4,5]. In a similar manner, Umbilical cord matrix tissue, which is discarded during delivery, is a potent source of stem cells as no ethical concerns are involved. In addition, the beneficial properties of umbilical cord derived MSCs for instance high proliferation and multitude differentiation potential makes it a significant source [6,7,8]. Reckoning on its clinical application, several preclinical animal models of human disease such as neurodegenerative disease, cancer, diabetes and heart disease have been reported [9,10,11,12].

However, the ability of these cells to migrate, extravagate and ultimately home to the site of injury has not much concentrated upon. It is of prime importance to look into the credentials of homing in tissue specific stem cells, which will pave way for clinical translation. To take these research pursuits to cell based therapy, it is of vital importance to study the innate property of stem cells i.e. homing. Homing stands for the stem cell capture within the vasculature of a tissue followed by transmigration across the endothelium. Potential trafficking of stem cells in-vivo is a strenuous task and hence can be studied in-vitro to develop the cell based curative concepts [13]. Cell adhesion molecules (CAM) play a major role in vascular adhesion, migration, extravasations and ultimately homing of MSCs. Studies showed blocking the CD29 (Integrin-β1) on MSCs led to reduced engraftment in ischemic myocardium. Study also demonstrates that CD44 is instrumental in MSC homing to damaged kidney and in mechanisms governing MSC trafficking [14]. Therefore this study aims at comparing the stem cells derived from human subcutaneous fat and umbilical cord matrix for the expression of cell adhesion molecules (ALCAM, HCAM, Integrin β1, CD90, CD13 and CD34) at passage 0, 1 and 3.

MATERIALS AND METHODS

Sampling: Human umbilical cord matrix (HUCM) samples and subcutaneous fat tissue were obtained from 3 subjects (n=3) each undergoing delivery or exploratory bariatric surgery at Lifeline multi-speciality hospital, Chennai. Sampling and processing procedures were reviewed and approved by hospital ethical committee of Lifeline multi-speciality hospital and all samples were collected upon obtaining written informed consents from patients.

The HUCM samples were collected immediately after birth and transported in sterile collection bottles with 0.9% normal saline supplemented with gentamycin and 1% antibiotic-antimycotic solution at 4°C. The clamped cord samples were later transferred to sterile phosphate buffered saline (PBS) for processing. About 70-100g of subcutaneous fat samples were collected and transferred to PBS. All samples were processed with 24 hours of sample collection.

Explant culture

The cord tissue samples were washed in sterile PBS (without Ca^{2+} and Mg^{2+}) supplemented with antibiotic-antimycotic solution. The microbial contaminants were further removed by washing the samples with 70% ethanol for 30 seconds. The tissue samples were further washed in PBS and the cord vein as well as arteries was removed with the help of a surgical forceps. The cord tissues so obtained were washed to remove any blood clots and diced into fragments of 2-5mm size. The tissue fragments were placed in 6 well plates (3-4 explants per well) and dried for 10 minutes. The explants were further incubated in growth medium (DMEM-LG supplemented with 15% fetal bovine serum (FBS) and 1% antibiotic solution) at 5% CO_2, 95% humidity and 37°C. The plates were left undisturbed to allow the migration of cells from explants and the media were replaced twice every week. The explants were removed after 10 days and the cells were subcultured by trypsin-EDTA method until Passage 3 (P3).

Adipose cell culture: Solid fat tissues were minced and collagenase digested using 0.075% collagenase type-1, upon washing with 1x Phosphate Buffered Saline (PBS). Debris and lipocyte content from digested sample were removed by centrifugation to obtain the Stromal Vascular Fraction (SVF). Erythrocytes in cell fractions were lysed using 0.7% NH_4Cl solution. The cells were resuspended and pelleted in PBS. Viability and enumeration was done using Trypan Blue method. $3x10^5$ cells were seeded onto $25cm^2$ culture flasks (Nunc) and incubated in growth medium (DMEM-LG supplemented with 15% fetal bovine serum (FBS) and 1% antibiotic solution) at 37^OC, 5% CO_2 and 95% humidity for 2-4 days. The primary cultures were subcultured until passage 3 with media change twice every week.

Phenotypic characterization using flowcytometry About $1x10^5$ cells of each sample were phenotypically characterized for the expression of following cell surface markers; ALCAM, H-CAM, Integrin-β1, CD 13, CD 34 and Thy1. The cells were incubated with the antigenic cocktails (Table 1) at room temperature in dark for 20 minutes, washed with BD FACS wash buffer and pelleted by centrifugation. The pellets were resuspended in BD FACS flow and analyzed on the flowcytometer (BD FACS Aria) using BD FACS-Diva software.

Statistical Analysis: The expression profile of cell adhesion molecules obtained from subcutaneous fat tissue and umbilical cord matrix tissue samples (n=3) were represented as Mean ± Standard Error Mean (SEM). The data were analysed using two-tailed student *t*-test and the *p*-values were calculated to determine the statistically significant variations. Results were considered statistically significant when $p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$.

RESULTS

Cell Culture: The human umbilical cord matrix (n=3) and subcutaneous adipose tissue (n=3) derived stem cells were isolated and expanded till passage 3 in α-MEM (Alpha- Minimal Essential Media) based on their adherence. The *in vitro* experiments indicated that MSCs (Mesenchymal Stem Cells) derived from both HUCM (Human Umbilical Cord Matrix) and SF (Subcutaneous Fat) was easily isolated due to plastic adherence. The morphological appearance of the mesenchymal stem cells of these two sources were initially round epithelial in nature with a transition of long spindle shaped fibroblastic morphology at P1 (Passage 1) onwards (Fig.1).

Phenotypic expression We examined the expression of ALCAM (Activated Leukocyte Cell Adhesion Molecule) (CD166), Integrin β1 (CD29) and H-CAM (Hyluronate Cell Adhesion Molecule) (CD44), CD90 (Cluster of Differentiation 90), CD13, CD34 at passage 0, 1 and 3 by flowcytometry. The dot plot output of the flowcytometric analysis of the markers was illustrated (Fig.2). The comparison of surface antigenic expression profiles of cell adhesion molecules of these sources were comprehended in the form of Mean ± SEM (Standard Error Mean) and estimated *p* value symbolized evidence of significant variations among the expressions of HUCM and SF samples (Table 2). The MSCs derived from HUCM and SF shared a consistent positivity of Cell adhesion molecules, ALCAM (CD166), Integrin β1 (CD29) and H-CAM (CD44). The expression of the defined MSC as well as cell adhesion molecule marker CD90 was found to possess a remarkable expression of greater than 90%. Similarly, both the sources showed a constant positive expression for surface enzyme CD13 from passage 0 to passage 3. On the contrary, a marked variation was obtained in the expression profile of CD 34 positivity in MSCs derived from either of the sources. Interestingly, CD34 was found to be negligible in HUCM right from passage 0 opposite to the expression identified in SF (Fig.3), indicating imperativeness of HUCM in allogenic transplantation.

DISCUSSION

In the present research pursuit, we focus to showcase MSCs intriguing property of homing which makes them a strong candidate for cell-based therapy against various human diseases. MSC homing results in migration of stem cells to injured tissues, where they can encourage the survival of damaged cells by inhibiting the release of proinflammatory cytokines. MSC homing being a clinically relevant process has to be addressed in context to its most suitable sources. However, *in vivo* studies for mobilization of endogenous or exogenous MSCs are a strenuous task. Hence *in vitro* studies can be drawn to evaluate the MSCs on terms of their homing ability thereby providing an insight on its therapeutic potential. As the exact mechanism of MSC homing is still unearth, this is an attempt to correlate the concepts underlined in existing literature to the results we have arrived at. Literature reports MSC homing can be active by means of leukocyte-like cell-adhesion and transmigration mechanisms [15]. In lieu of the above, we propose to fabricate in vitro study on Human umbilical cord matrix (HUCM) and subcutaneous adipose tissue (SF) derived MSCs for expression of certain ALCAM (CD166), Integrin β1 (CD29) and H-CAM (CD44), CD90, CD13, CD34 by flowcytometry.

Integrin β1 (CD29) was found to be more than 90 % for both HUCM and SF at all culture time points under study. Literature reports that upregulation of VLA-4 β1integrin on MSCs prompt 10 folds increase in homing ability to bone marrow [16], thus a remarkable expression of Integrin β1 supports homing ability in both tissue sources. Another important cell adhesion molecule H-CAM (CD44) expressed remarkably (more than 90%) which can be related to a study stating CD44 negative MSC were unable to localize within the injured kidney and failed to accelerate morphological and functional recovery in contrast to CD44 positive MSCs [14]. Similarly, remarkable expression (< 90%) of ALCAM (CD166) also ropes to the homing property of MSC. CD90, ISCT defined MSC marker as well as CAM was also expressed in coherence with the literature as remarkable [17]. CD13, a surface enzyme was found deliberated to mimic action of a CAM and was highly expressed in MSCs from bone marrow and adipose tissue [18]. Likewise, CD13 showed remarkable expression for SF as well as HUCM. It is indicative from the results that both HUCM and SF equally possesses remarkable expressions of cell adhesion molecules, representative of an intriguing property of homing which makes them a strong candidate for cell-based therapy. On the other hand, negligible expressions of CD 34 in HUCM when compared to SF, indicates the MSC homogeneity of HUCM and its utilization for allogenic transplantation as well when compared to SF.

CONCLUSION

To conclude, this study augments in understanding homing property of tissue specific MSCs. Hence, this study will presumably prove beneficial to assess its therapeutic competence. Overall, this study accounts proof for trafficking behaviour of HUCM and SF derived MSCs and their probable positive role in regulating wound healing and inflammatory diseases due to homing action.

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