

## Introduction:

Mesenchymal stem cells (MSCs) are a group of pluripotent cells that are characterized by their self-renewing and differentiation capacity into mesodermally-derived tissues, including cartilage, bone, fat and muscle. In addition, these cells expressed stromal cell-associated markers CD73, CD90, CD29, CD105, CD44 and CD13. The same population was negative for CD31, CD34, HLA DR, CD45 and CD14 according to the international society for cellular therapy (ISCT)(1). In the past decade, MSC acquired huge interest due to their unique properties for homing to injured tissue and repairing tissue damage (2). Thus in order to enable the clinical use of MSC in cell therapy, it is highly important to fully characterize MSC(3). In this context, despite the increasing number of available CD markers expressed by MSC, identifying unique specific surface markers that can selectively distinguish MSC from other cell types, still elusive.

### Abbreviation:

AT-MSC - Adipose Tissue derived Mesenchymal Stem Cell, SVF - Stromal Vascular Fraction, hDSFs - human Dermal Skin Fibroblasts, hFSFs - human Foreskin Fibroblasts, hPBMcs - human Peripheral Blood Mononuclear Cells, hTERT - immortalized human Telomerase Reverse Transcriptase Bone marrow MSCs.

### Aim

The aim of this study to identify a group of specific surface markers that can be used to isolate a pure population of mesenchymal stem cells from other heterogeneous cell populations. Thus, we investigate the expression pattern of hematopoietic and mesenchymal stem cell surface markers between MSC-derived from human bone marrow and adipose tissue, versus fibroblast cells derived from dermal skin and foreskin fibroblasts.

### Materials and Methods:

In this study, we used our telomerized human bone marrow derived MSC (MSC-TERT) as a model of BM-hMSCs. These cells have unlimited life span and maintained their differentiation capacity into bone, fat and cartilage (4). In Aseptic condition, AT-MSCs were isolated by collagenase enzymatic digestion and fibroblasts from dermal and foreskins were derived by explant culture as described before (5). All cells were cultured in Dulbecco's Modified Eagle Medium(DMEM) supplemented with 10%FCS and Penicillin-Streptomycin. Immunofluorescent staining was performed using purified mouse Anti Vimentin, BD Pharmingen

### Flow cytometry analysis (FACS):

Cells were harvested in 0.05% trypsin-EDTA (Gibco) from the adherent culture. Trypsinized cells were suspended at a concentration of  $1 \times 10^3/\mu\text{L}$  in PBS

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and then incubated for 30 minutes at 4°C with fluorochrome conjugated monoclonal antibodies for the respective CD's as shown in Table 1. Mouse iso types were used as control. All antibodies were from BD Biosciences, except that the monoclonal antibody against human CD105, was from R&D systems. All analyses were performed on a BD FACS Caliber flow cytometer (BD Biosciences). The data were analyzed with Cell Quest Pro Software Version 3.3 (BD Biosciences).

Antigen Specificity	Fluorochrome	Isotype	Description of Fluorochrome – Conjugated Monoclonal Antibodies Used for Flow Cytometry
CD34	FITC	Mu IgG1	
CD146	PE	Mu IgG1	
CD105	APC	Mu IgG1	
CD90	FITC	Mu IgG1	
CD73	PE	Mu IgG1	
CD14	APC	Mu IgG2a	
CD45	FITC	Mu IgG1	
CD29	PE	Mu IgG1	
CD13	FITC	Mu IgG1	
CD31	FITC	Mu IgG1	
HLADR	PE	Mu IgG2a	
CD44	APC	Mu IgG2b	

### Results:

AT-MSCs, hDSFs, hFSFs and hMSC-TERT-20 cells were adhered to plastic culture dishes during the cultured period and showed spindle-shaped fibroblast like morphology at all passage level. hDSFs and hFSFs had greatest proliferation rate, similarly equivalent to hTERT-20 cell line.

Immunocytochemistry images were taken in Leica fluorescence microscope. Images here demonstrates Vimentin (Green, shows cytoplasmic localization in MSCs and Fibroblast) and DAPI (blue-nuclear counter stain). The images illustrate the filamentous nature of the vimentin and confirm the significant role of vimentin plays in supporting and anchoring the position of the organelles in the cytosol. After having observed the vimentin expression of all four types of adherent cell populations, features were acquired in a similar fashion. (Figure 1)

Trypsinized adherent cells (AT-MSCs, hDSFs, hFSFs and hTERT-20) and hPBMcs were tested with flow cytometry for the presence or absence of characteristic hematopoietic, endothelial and stromal cells-associated markers by multicolour immunophenotype method. Representative flow histograms are shown in Figure 2. Stromal cells-associated markers CD13 (>98%), CD29 (>93%), CD44 (>99%), CD73 (>98%), CD90 (>99%) and CD105 (>98%) were positive for hTERT-20, hAT-MSCs, hDSFs and hFSFs (Table.3). They were negative for hematopoietic and endothelial markers CD34, CD45, CD14, CD31, as well as for the MHC class II marker HLA DR (Table.2). In this experiment hPBMcc (Lymphocyte and Monocyte) population used as reagent control, hTERT 20 used as positive control.

Stromal cells-associated markers expressions in the different groups of cells are shown in the Table 2.

The levels of CD13, CD29, CD44, CD73, CD90 and CD105 expression mean values were significantly

(P<0.001, P<0.01 and P<0.05) higher in hDSFs and hFSFs than AT-MSCs. The mean values of hTERT-20 (positive control) were significantly higher than AT-MSCs Figure 3.

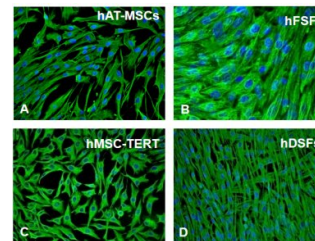


Figure 1: Cell morphology as shown by Immunofluorescence localization of vimentin. Magnification 20.

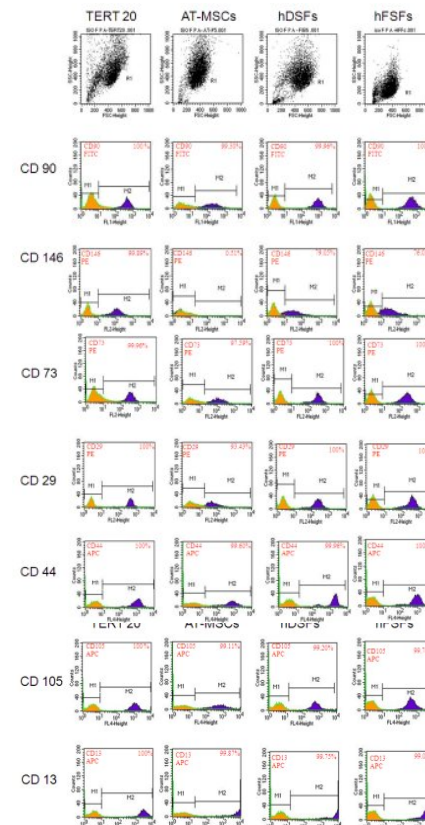


Figure 2. FACS analysis of characteristic stromal cells-associated markers present on MSCs and typical hematopoietic and endothelial markers which are not expressed by MSC.

Sample	CD31 F	HLADR P	CD34 F	CD45 F	CD14 A
PBMc-Lympho	40.77±0.11	13.17±0.02	0.48±0.003	98.39±0.001	0.93±0.01
PBMc-Mono	9.82±0.01	0.84±0.11	0.48±0.01	98.39±0.002	92.8±0.02
TERT 20	0.26±0.23	0.25±0.1	1.75±0.002	0.25±0.6	0.45±0.003
AT-MSCs	0.64±0.01	0.6±0.01	4.98±0.1	0.53±0.01	0.98±0.01
hDFb	0.13±0.001	0.09±0.001	0.26±0.001	0.59±0.002	0.63±0.002
hFFb	0.03±0.001	0.04±0.001	0.11±0.001	0.06±0.0002	0.81±0.003

Table 2. Mean Levels of typical hematopoietic and endothelial markers expression on Different Cell Population

Sample	CD44 A	CD146 F	CD105 A	CD29 P	CD13 A	CD90 F	CD73 P
PBMc-Lympho	99.56±0.003	1.95±0.005	8.72±0.05	92.95±0.1	3.84±0.01	17.57±0.2	18.01±0.03
PBMc-Mono	99.76±0.003	1.85±0.002	8.72±0.1	92.95±0.002	3.84±0.2	17.57±0.2	18.01±0.01
TERT 20	99.89±0.6	44.7±0.2	99.37±0.05	99.37±0.54	99.34±0.02	99.95±0.1	99.49±0.1
AT-MSCs	99.74±0.002±	4.52±0.1±	99.41±0.01	98.13±0.02±	98.23±0.04	99.62±0.008±	98.61±0.01±
hDFb	99.98±0.003±	44.56±0.30±	99.74±0.004	100±0±	100±0.01	99.96±0.006±	99.71±0.001±
hFFb	99.98±0.001±	65.97±0.31±	98.91±0.01	99.98±0.002±	99.83±0.04	99.97±0.004±	99.95±0.0004±

Table 3. Mean Levels of stromal cells-associated markers expression on Different Cell Population

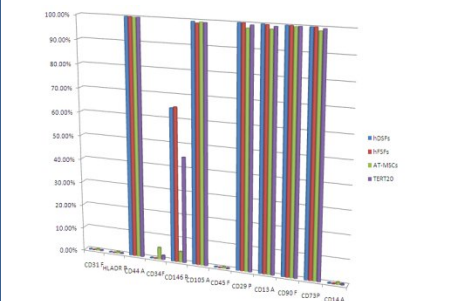


Figure 3. Expression level of stromal cells-associated markers, hematopoietic and endothelial markers expression on hDSFs, hFSFs, AT-MSCs and TERT-20

### Conclusion:

We isolated and cultured the mesenchymal stem cells from adult adipose tissue regardless of the regions. Our data the expression of well known MSC surface markers (CD73, CD90, CD29, CD146, CD44 and CD13) on human dermal and foreskin fibroblast fibroblastic cells similarly to their expression on bone marrow and adipose derived MSC cells. This finding suggests the critical needs for identifying novel specific surface markers that can selectively identify MSC population among other cell types.

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