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Adult telomerase positive stem cells: Effects of biological agents and characterization criteria on genomically-labeled clones and unlabeled clones of adult cells

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Abstract

The adult human body is composed of trillions and trillions of cells. These cells can be divided into three categories: telomerase negative differentiated cells, telomerase negative progenitor cells, and telomerase positive stem cells (aTPSCs). The aTPSCs are uniquely different from differentiated cells and progenitor cells, due mainly to not conforming to standard tissue culture practices, their limited number in the body, and the presence of the telomerase enzyme. Therefore, special technologies were developed to examine aTPSCs in vitro, which have been extensively outlined in recent publications. The aTPSCs and a Tripotent progenitor cell, e.g., mesenchymal stem cell (MSC), were cloned from single cells using repetitive single cell clonogenic analysis with 5-6x exosome-conditioned medium. These clones were examined with proliferation, progression, induction, and anti-differentiation biological agents to assess their particular responses. As noted, both aTPSCs and MSC clones responded similarly to proliferation and anti-differentiation agents, but differently with respect to progression and induction agents. The aTPSC clones were subsequently genomically-labeled with the Lac-Z gene for beta-galactosidase, using lipofection, to track the cells both in vitro and in vivo. This study was undertaken to determine if genomically labelling of aTPSCs caused any alteration in their response to 1. human recombinant proteins, morphogenetic proteins isolated from both demineralized bone matrix and sera, and cell-specific exosome conditioned media for the four activities of biological agents, e.g., proliferation, progression, induction, and anti-differentiation, and 2. standardized procedures for characterizing aTPSCs and MSCs.

Keywords: Stem Cells; aTPSCs; MSCs; Genomic Label; Biological Agents; Characterization

1. Introduction

The adult human body is composed of trillions and trillions of cells. These cells can be divided into three categories: telomerase negative differentiated cells, telomerase negative progenitor cells, and telomerase positive stem cells (aTPSCs) [1]. Both progenitor cells and aTPSCs are involved in restoring and/or regenerating worn-out or damaged cells and tissues [2]. To do these activities, they respond to local environmental cues (exosomes) emanating from adjacent undamaged cells and tissues [3].

Four categories of biological agents have been identified, e.g., proliferative, progressive, inductive, and anti-differentiative. Various biological factors were utilized in this study, e.g., recombinant proteins [4-26], morphogenetic

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proteins isolated from both demineralized bone matrix [2,27-42] and sera [43], and cell-specific exosome conditioned medium [44]. These biological agents [45] were used to ascertain the response of clonal populations of aTPSCs and the tripotent MSCs to these compounds using the ELICA high throughput screening assay [46,47]. Individual clones of aTPSCs and MSCs were reported to react both similarly and differently to biological agents, e.g., proliferation, progression, induction, and anti-differentiation (Table 1) [44,45].

Table 1 Similarities and Differences between aTPSC clones and MSC clone

Agent	**TSC**	**PSC**	**EctoSC**	**MesoSC**	**EndoSC**	**MSC**
Anti-Differentiative[1]	+++	+++	+++	+++	+++	+
Proliferative[2] (Population Doublings)	Unlimited	Unlimited	Unlimited	Unlimited	Unlimited	R: 6-8
Progressive[3]	-	-	-	-	-	+++
Inductive[4]	All Cells	All Somatic Cells Only	Ectodermal Lineage Cells Only	Mesodermal Lineage Cells Only	Endodermal Lineage Cells Only	Bone Cartilage Fat

Table 1. Similarities and Differences between adult telomerase stem cell (aTPSC) clones and mesenchymal stem cell (MSC) clone. Anti-differentiative1, prevents differentiation of cells in the presence of inductive agents; Proliferative2, stimulates proliferation in cells, for aTPSCs that contain the telomerase enzyme, proliferation is essentially unlimited as long as the cells remain undifferentiated, once they begin to differentiate into progenitor cells, they lose the telomerase enzyme and assume the cell doubling number associated with particular species, e.g., rodents = 6-8 population doublings before cell senescence and cell death [47]; Progressive3, accelerates the phenotypic expression of cell-committed progenitor cells, but has no response on uncommitted stem cells; Inductive4, is dependent on subcategory of aTPSCs, TSCs will form all cells similar to day-four blastomeres, PSCs will form all cells similar to the inner cell mass, EctoSCs will form all cells of the ectodermal lineage, MesoSCs will form all cells of the mesodermal lineage, EndoSCs will form all cells of the endodermal lineage, MSCs will only form bone, cartilage, and fat cells. Reprinted with permission from Young HE. Adult telomerase positive stem cells: effects of biological agents on single cell clones of adult cells. GSC Advanced Research and Reviews. 2025; 25(03): 028-047 [44].

A series of protocols were developed to identify characteristics with respect to adult stem cells (aTPSCs) and progenitor cells (tripotent progenitor MSCs) (Table 2) [48].

Table 2 Comparison/Contrast Characterization of Fresh isolates of Adult Telomerase-Positive Stem Cells with Adult Telomerase-Negative Tripotent Progenitor Cell, MSC

Characteristics Tested	**us-TSCs[1]**	**s-TSCs[2]**	**PSCs[3]**	**MesoSCs[4]**	**MSCs[5]**
Size, microns	0.1-1	1-2	6-8	10-12	10-20
Trypan Blue Viability Dye	Positive	Positive	Negative	Negative	Negative
Telomerase	Positive	Positive	Positive	Positive	Negative
Life Span Population Doublings	>300	>300	>400	>300	6-8
Species	Rat	Rat	Rat	Rat	Rat
Clones	Rat-Scl-44β, Rat-Scl-4β	Rat-Scl-9β	Rat-Scl-40β	Rat-A_2A_2β	Rt-MSC[6], Rt-Adip[7], Rt-Chon[8], Rt-Os[9]

Contact Inhibited	No	No	No	Yes	Yes
Survived Contact Inhibition	Not Applicable	Not Applicable	Not Applicable	Yes	No
Growth in Culture	Suspension[10]	Adherent[11]	Adherent	Adherent	Adherent
Substrate	None	CollagenT1[12]	CollagenT1	CollagenT1	None
Serum-Free Defined Medium	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent
No Factors	Quiescent	Quiescent	Quiescent	Quiescent	Quiescent
Inhibitory Factors[13]	Respond	Respond	Respond	Respond	Respond
Proliferative Factors[14]	Yes	Yes	Yes	Yes	Yes
Progression Factors[15]	No	No	No	No	Yes
Inductive Factors[16]	Yes	Yes	Yes	Mesodermal Lineage Only	Fat, Cartilage, Bone Only
# Cells Identified	75	74	70	37	3
Proliferation Rate, Hours	12-14	12-14	14-16	18-24	Days to weeks
Cryopreservative Agent	DMSO[17]	DMSO	DMSO	DMSO	DMSO
Concentration Cryopreservative Agent	7.5%	7.5%	7.5%	7.5%	10%
# Cells Cryopreserved	1-10^9	1-10^9	1-10^6	1-10^6	1-10^6
Optimum Freezing Temperature	-80°C	-80°C	-80°C	-70°C	-196°C
Freezing Protocol	Slow	Slow	Slow	Slow	Flash
Optimum Storage Temperature	-80°C	-80°C	-80°C	-70°C	-196°C
Thaw Temperature	37°C	37°C	37°C	37°C	37°C
% Recovery of Viable Cells	>98%	>98%	>98%	>98%	>95%
Cell Surface Markers	CEA-CAM-1[18]	CEA-CAM-1	SSEA-4[19]	Thy-1[20]	Thy-1

Table 2. us-TSCs1, ultra-small totipotent stem cell; s-TSCs2, small totipotent stem cell; PSCs3, pluripotent stem cells; MesoSC4, mesodermal stem cell – will form all cell types of the mesodermal germ layer lineage; MSCs5, mesenchymal stem cells; Rt-MSC6, rat-derived tripotent progenitor cell clone, MSC; Rt-Adip7, rat unipotent adipogenic progenitor cell; Rt-Chon8; rat-chondrogenic unipotent progenitor cell; Rt-Os9, rat-osteogenic progenitor cell; Suspension10, ability to grow in culture suspended in the medium without any attachment to a substratum; Adherent11, ability to grow in culture when attached to a substratum; CollagenT112, collagen type-I substratum needed to be provided for cell attachment; Inhibitory Factors13, e.g., LIF, leukemia inhibitory factor and ADF, anti-differentiation factor; Proliferation Factors14, e.g., platelet-derived growth factors-AA (PDGF-AA), PDGF-AB, PDGF-BB; Progression Factors15, e.g., Insulin, IGF-1, IGF-2, added to the medium to accelerate the phenotypic expression of lineage-committed progenitor cells; Induction Factors added to the medium to induce the expression of multiple phenotypes in culture. We routinely used dexamethasone at 10^-6, 10^-7, 10^-8, 10^-9, and 10^-10 molar concentrations as a non-specific induction agent for mesodermal lineage cells. We also used specific induction agents to induce specific cellular phenotypes, e.g., recombinant proteins, morphogenetic proteins, and cell-specific exosome conditioned medium [#]; DMSO17, Dimethyl Sulfoxide; CEA-CAM-118, carcinoembryonic antigen-cell adhesion molecule-1; SSEA-419, stage specific embryonic antigen-4; Thy-120, heavily N-glycosylated, glycophosphatidylinositol anchored conserved cell-surface protein with a single V-like immunoglobulin domain. Reprinted in part with permission from Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. Stem Cell Regen Med 2020; 4(2):1-14 [48].

The current study was undertaken to determine if genomically labelling aTPSCs with a Lac-Z gene for beta-galactosidase, using lipofectin, caused any alteration in their response to 1. human recombinant proteins, morphogenetic proteins isolated from both demineralized bone matrix and sera, and cell-specific exosome conditioned media for the four activities of biological agents, e.g., proliferation, progression, induction, and anti-differentiation, and 2. standardized procedures for characterizing aTPSCs and telomerase negative progenitor cells, including MSCs.

2. Materials And Methods

2.1. Assay Reagents: [3,40-56]

2.1.1. Species-specific buffers

- Non-human mammals – Phosphate Buffered Saline, PBS (Sigma)
- Non-human mammals – Ca+2, Mg+2-free Phosphate Buffered Saline, PBS (Sigma)

2.1.2. Medium

- OptiMEM + GlutaMax, GIBCO
- Heat Inactivated Serum, Atlas Biologicals
- Antibiotic, Penicillin/Streptomycin, #600-514AG, GIBCO

2.1.3. Anti-Differentiative

- Leukemia Inhibitory Factor (LIF) – R&D Systems
- Anti-Differentiation Factor (ADF) – DFRD
- Caffeine (CAF) – Fisher Scientific
- Scar Inhibitory Factor (SIF) – DFRD

2.1.4. Proliferative

- Platelet-derived Growth Factor-AA (PDGF-AA) – R&D Systems
- Platelet-derived Growth Factor-AB (PDGF-AB) – R&D Systems
- Platelet-derived Growth Factor-BB (PDGF-BB) – R&D Systems

2.1.5. Progression

- Insulin-Like Growth Factor-1 (IGF-1) – Cell Sciences
- Insulin-Like Growth Factor-2 (IGF-2) – Cell Sciences
- Insulin (Ins) - Sigma

2.1.6. Inductive

- Bone morphogenetic protein-2 (BMP-2) – Genetics Institute
- Osteogenic morphogenetic protein (OMP) – Isolated from demineralized bone matrix
- Osteogenic conditioned medium (OCM) – generated from bone explant cultures
- Cartilage morphogenetic protein (CMP) – Isolated from demineralized bone matrix
- Cartilage conditioned medium (CCM) generated from cartilage explant cultures
- Adipocyte morphogenetic protein (AMP) – isolated from demineralized bone matrix
- Adipocyte conditioned medium (ACM) generated from adipocyte explant cultures
- Bone morphogenetic protein-4 (BMP-4) – Genetics Institute,
- Acidic fibroblast growth factor (a-FGF) – R&D Systems
- Endothelial cell growth factor (ECGF) – Sigma
- Vascular endothelial growth factor (VEGF) – Genentech
- Blood Vessel Morphogenetic protein (BVMP) – Isolated from serum
- Blood Vessel Conditioned Medium (BVCM) generated from blood vessel explant cultures
- Fibroblast morphogenetic protein (FMP) – Isolated from demineralized bone matrix
- Fibroblast Conditioned Medium (FCM) generated from fibroblast explant cultures
- Scar Fibroblast morphogenetic protein (ScFMP) – Isolated from serum
- Transforming growth factor beta (TGF-b) – Genentech
- Basic fibroblast growth factor (b-FGF) – R&D Systems

- Erythropoietin (EPO) – Amgen
- c-Kit (c-Kit) – R&D Systems
- Interleukin-6 (IL-6) – R&D Systems
- Nerve growth factor (NGF) – Genentech
- Brain Morphogenetic Protein (BrnMP) – Isolated from serum
- Brain Conditioned Medium (BrnCM) – generated from brain explant cultures
- Hepatocyte growth factor (HGF) – Genentech
- Liver Morphogenetic Protein (LivMP) – Isolated from serum
- Liver Conditioned Medium (LivCM) – generated from liver explant cultures
- Lung Morphogenetic Protein (LngMP) – Isolated from serum
- Lung Conditioned Medium (LngCM) – generated from lung explant cultures
- Pancreatic Morphogenetic Protein (PanMP) – Isolated from serum
- Pancreatic Conditioned Medium (PanCM) – generated from pancreas explant cultures
- Keratinocyte Morphogenetic Protein (KerMP) – Isolated from serum
- Keratinocyte Conditioned Medium (KerCM) – generated from keratinocyte explant cultures
- Sertoli Cell Conditioned Medium (SerCCM) – generated from Sertoli cell explant cultures
- Skeletal Muscle Morphogenetic Protein (SkMMP) – Isolated from demineralized bone matrix
- Skeletal Muscle Conditioned Medium (SkMCM) – generated from skeletal muscle explant cultures
- Smooth muscle morphogenetic protein (SmMMP) – Isolated from demineralized bone matrix
- Smooth muscle Conditioned Medium (SmMCM) – generated from smooth muscle explant cultures
- Cardiac muscle morphogenetic protein (CdMMP) – Isolated from demineralized bone matrix
- Cardiac muscle Conditioned Medium (CdMCM) – generated from cardiac muscle explant cultures
- Tendon morphogenetic protein (TenMP) – Isolated from demineralized bone matrix
- Tendon Conditioned Medium (TenCM) – generated from tendon explant cultures
- Ligament morphogenetic protein (LigMP) – Isolated from demineralized bine matrix
- Ligament Conditioned Medium (LigCM) – generated from ligament explant cultures

2.2. ELICA Fixative Reagents

- Species-Specific Buffer
- Paraformaldehyde, #P6146, Sigma
- Glutaraldehyde, #G5882, Sigma
- Sodium Azide, #S2002, Sigma
- D-Glucose, # G-6138, Sigma

2.2.1. Inhibition and Exhaustion of Endogenous Peroxidases

- 5% Sodium Azide, #S2002 Sigma
- 30% Hydrogen Peroxide, #H-1009, Sigma

2.2.2. Blocking Agents for ELICA

- Horse serum, #H7889, Sigma
- Goat serum, #200-6210-AG, DSHB
- Porcine Serum, #P9783 Sigma
- Bovine serum albumin, #A-7906, Sigma
- Neonate Bovine Serum, #2002, BioCell
- Fetal Calf Serum, #F0392, Sigma
- Human Serum, #H4522, Sigma
- Tween 20 (polyoxyethylenesorbitan), ChemPure, Curtain Matheson Scientific, Houston, TX
- Nonidet P-40, P-40, #56007, BDH
- Triton-X-100, #T-6878, Sigma
- Powdered non-fat dry milk, Kroger's
- Gelatin (cold water fish soluble collagen), #935425, Sigma

2.2.3. Positive Standards

- Calf thymus DNA type-I, #D-1501, Sigma
- Myosin from chicken muscle, #M-7266, Sigma

- Rabbit anti-myosin (skeletal-smooth), #M-7648, Sigma

2.2.4. Primary Probes

- 12/101, Developmental Studies Hybridoma Bank (DSHB)
- 31-2, DSHB
- 5D2-27, DSHB
- MF-1, DSHB
- MF-5, DSHB
- C3/1, DSHB
- M3F7, DSHB
- 22/18, DSHB
- ALD-58, DSHB
- MF-20, DSHB
- B3/D6, DSHB
- M-38, DSHB
- D76, DSHB
- ALD-66, DSHB
- JLA-20, DSHB
- SP1.D8, DSHB
- CHI, DSHB
- 5C6, DSHB
- 33-2, DSHB
- 2E8, DSHB
- D3, DSHB
- ID4B, DSHB
- MF-30, DSHB
- ABL-93, DSHB
- 5-D-4, #69-625, ICN
- Anti-myosin, #65-790-1, ICN
- Anti-desmin, #65-793-1, ICN
- Anti-vimentin, #69-127-2, ICN
- Anti-type IV collagen, #69-108-1, ICN
- Anti-type II collagen, #1320-01, Southern Biotechnology
- Anti-heparan sulfate proteoglycan, #MAB459, Chemicon
- CEA-CAM-1, DH (D. Hixson, Providence, RI)
- HCEA, RB
- CEA, #111518, Sigma
- CD66e, BD (Beckton-Dickinson)
- Dh-TuAg1, DH
- MC-480 (SSEA-1), DSHB
- MC-631 (SSEA-3), DSHB
- MC-813 (SSEA-4), DSHB
- CD10, BD
- CD90, BD
- Thy-1, DSHB
- CD56, BD
- Pax-6, DSHB
- FORSE-1, DSHB
- Vimentin, DSHB
- Nestin, DSHB
- R401, DSHB
- HNES, R&D
- MAB353, Sigma
- RT-97, DSHB
- NF68, Prospec
- S-100, DSHB

- NF-145, R&D
- N-200, R&D
- 8A2, DSHB
- NG2, DSHB
- TH, DSHB
- SV2, DSHB
- DOPA, Thermo-Fisher
- T8660, Sigma
- Tuj1, DSHB
- GFAP, Santa Cruz
- CNPase, Abcam
- Rip, DSHB
- MOSP, Sigma
- MAB, DSHB
- 40E-C, DSHB
- VM-1, DSHB
- CD13, BD,
- OP-137, Antibodies Inc
- F5D, DSHB
- MF-20, DSHB
- ALD-58, DSHB
- A4.74, DSHB
- IA4, Thermo-Fisher
- Calp, Abcam
- MAB-3252,
- MAB1548,
- WV1D1,
- MP111,
- CIIC1, DSHB
- II-4CII, DSHB
- HC-II, Abcam
- D1-9, DSHB
- 9/30, DSHB
- 12/21, DSHB
- 12C5, DSHB
- H-CD34, BD
- CD31, BD
- P1H12, DSHB
- P2B1, DSHB
- P2H3, DSHB
- H-endo, Fisher Scientific
- H5A4, DSHB
- H4C4, DSHB
- Hermes-1, DSHB
- CD45, BD
- CD63, BD
- CD95, BD
- H5A5, DSHB
- H5C6, DSHB
- HFSP, DSHB
- 1B10, DSHB
- H-AFP, R&D Systems
- R-AFP, Abcam
- DESMO, Abcam
- LAP, DSHB
- 151-Ig, DSHB

- HA4c19, DSHB
- OC2, DH
- OC3, DH
- OC4, DH
- OC5, DH
- OC10, DH
- H.4, DH
- H.1, DH
- DPPIV, DH
- OV6, DH
- HESA, DSHB
- YM-PS087, Sigma
- INS, Sigma
- YM-PS5088, Sigma
- SOMA, Sigma
- 11180, Sigma
- Telom, Abcam
- PI, Abcam
- DAPI, Abcam
- Gal-19, Abcam

2.2.5. Secondary Probes

- Biotin, goat anti-mouse IgG, #OB1126-21, Fisher
- Biotin, goat anti-mouse IgG (fab specific), #B-7151, Sigma
- Biotin, goat anti-rabbit IgG, #OB1316-21, Sigma
- Biotin-SP, goat anti-mouse IgG, #JGM-065003, Accurate
- Biotin-SP, goat anti-mouse fab-specific, #JGM-066003, Accurate
- Biotinylated affinity purified, rat adsorbed anti-mouse IgG (H + L) (BA-2001, Vector Laboratories)
- Goat anti-rat osteocalcin, #BT-413, Biomedical Technology

2.2.6. Tertiary Probes

- Avidin-peroxidase, #JSA-030083, Accurate
- Horseradish peroxidase conjugated Avidin-D, #A-2004, Vector
- Monoclonal anti-goat IgG clone GT-34 biotin, #B-3148, Sigma
- Peroxidase Standard PK-4000 Vastatin ABC Reagent Kit, Vector Laboratories
- Streptavidin-peroxidase, #JSA-030084, Accurate

2.2.7. Reagents to Quantify or Visualize Tertiary Probes

- ABTS kit, #506201, Kirkegaard and Perry
- 4-Cloro-1-napthol, #C-8890, Sigma
- 3,5-Diaminobenzoic acid dihydrochloride, #11-383-2, Aldrich
- Vastatin ABC Reagent Kit, Vector Laboratories Inc., Burlingame, CA

2.2.8. Histochemical Stains

- Von Kossa, Chroma-Gesellschaft
- Alphas, Sigma
- Mallory Heidenheim One-Step reagents, Chroma-Gesellschaft
- Alcian blue, Chroma-Gesellschaft
- Alcec blue, Chroma-Gesellschaft
- Safranin-O, Chroma-Gesellschaft
- Sudan Black-B, 199664, Sigma
- Oil Red-O, 00625, Sigma

2.3. Verification of Histochemical Staining

- Toluene, removes hydrocarbons from fat cells, Sigma
- Hyaluronidase, Sigma
- Chondroitinase-AC, Sigma
- Chondroitinase-ABC, Sigma
- Keratanase, Sigma
- Heparanase, Sigma
- EDTA, divalent cations (Ca+2, Mg+2, Zn+2, Ba+2), Sigma
- EGTA, univalent cation (Ca+2 only), Sigma

2.3.1. Enzymes

- Trypsin, Sigma
- Collagenase type-1, Worthington Biochemicals
- Dispase, R&D Laboratories

2.3.2. Miscellaneous Supplies

- Aqua-Mount (mounting coverslips), Vector Laboratories
- 100% Pure Dimethyl sulfoxide (DMSO), Alfa Catalog Chemicals, Morton Thiokol
- 99-100% Pure DMSO, Allied Chemical
- 99-100% Pure DMSO, Sigma
- Glycerin, Tissue Culture Grade, #830-951, Curtain Matheson Scientific
- Sodium Bicarbonate, Sigma
- Sodium Hydroxide, Sigma
- Penicillin/Streptomycin, GIBCO
- Hydrochloric Acid concentrated 12N, Sigma
- Perfix, #CS514-1, Fisher
- HPLC Water, Sigma
- Phenol, #2859-1, Baker
- Parafilm, #13-374-12, Diagger
- Ethanol, Sigma
- Isopropyl alcohol, Sigma
- Nitrile Gloves, Diagger
- 0.1-micron sterile bottle-top filter, Thermo-Fisher
- 0.2-micron sterile bottle-top filter, Thermo-Fisher
- 50-ml polypropylene centrifuge tubes, Falcon, Thermo-Fisher
- 15-ml polypropylene centrifuge tubes, Falcon, Thermo-Fisher
- 1.2-ml polypropylene cryopreservation tubes, Corning
- Trypan Blue, C.I. 23850, Kodak
- Tissue Culture Bottles, Corning
- Pipets: 1-ml, 5-ml, 10-ml, 25-ml, Diagger
- Hemocytometer, Thermo-Fisher
- 96-well plates, Costar
- 6-well, 24-well, and 48-well plates, Corning
- T-25 and T-75 flasks, Falcon, Thermo-Fisher
- Gelatin, # GX0045-3, EM Sciences
- Stir bars, Diagger
- Aluminum foil, Diagger
- Sterilization bags, #1310-L75, Thomas Scientific
- Autoclave Tape, Diagger
- Boxes for low temperature storage, Diagger
- Cryopreservation canes, Diagger
- Ranin micro-pipettors and micropipette tips, Mettler Toledo

2.4. Equipment

- Class-2 Biosafety Cabinet, Thermo-Fisher

- Centrifuge, floor model #J21, Beckman
- Blender, #7011G, Waring, Thermo-Fisher
- Blender Cylinders, #MC-1 and MC-3, Thermo-Fisher
- -4°C Refrigerator, Thermo-Fisher
- -70°C Ultralow Freezer, Forma Scientific
- -80°C Ultralow Freezer, Forma Scientific
- Liquid Nitrogen Dewer, Thermo-Fisher
- Liquid Nitrogen, AirGas
- Stir Plate, Diagger
- Steam Autoclave, Thermo-Fisher
- Freezing Chamber, #9001, Biotech Research
- Pipet-Aid #148, Drummond Scientific
- Vortex Mixer, #S8223, Scientific Products
- Watchmaker's Forceps, #72700D, Electron Microscopy Sciences
- Hemostats, scissors, forceps, Diagger
- Bunsen Birner, Diagger
- Weigh Balance, Mettler Toledo
- Crushed ice machine
- Variable temperature water bath, Fisher Scientific
- Variable temperature glass bead heating unit, Fisher-Scientific
- Vortex Mixer, Thermo-Fisher
- Flow Cytometer, Fisher Scientific
- 96-well plate reader, R&D

2.4.1. Suppliers

- Abcam, Cambridge, MA
- Accurate, Accurate Chemical and Scientific Corporation, Westbury, NY
- Air Gas, Air Gas, Local Store
- Aldrich, Aldrich Chemical Co, Milwaukee, WI
- Aldrich, Aldrich Chemical Co., Sigma-Aldrich, St. Louis, MO
- Antibodies Inc, Davis, CA
- Atlas, Atlas Biologicals, Fort Collins, CO
- BDH, BDH Chemicals Ltd, Poole, England
- BTH, Biomedical Technology Incorporated, Cambridge, MA
- Chem, Chemicon, El Segundo, CA
- Chem Pure, Curtain Matheson Scientific, Houston, TX
- Chroma-Gesellschaft, Roboz Surgical Co, Washington, DC
- DFRD, Dragonfly Foundation for Research and Development, Macon, GA
- DH, Douglas Hixson, Department of Medicine, Brown University, Providence, RI
- Diagger Scientific, Vernon Hills, IL
- DSHB, Developmental Studies Hybridoma Bank under the auspices of the NICHD and maintained at the University of Iowa, Department of Biological Sciences, Iowa City, IA
- Fisher, Fisher Scientific Co., Norcross, GA
- Fisher-Biotech, Waltham, MA
- GIBCO, GIBCO, Grand Island, NY
- ICN, ICN Biomedicals, Costa Mesa, CA
- Jackson, Jackson Laboratories, Bar Harbor, ME
- Kodak, Kodak, Rochester, NY
- KP, Kirkegaard and Perry Laboratories, Gaithersburg, MD
- Kroger's, Kroger's, Macon, GA
- Morton Thiokol, Morton International, Rohm and Haas, Philadelphia, PA
- Prospec, Prospec, Rehovot, Israel
- R&D, R&D Systems, Minneapolis, MN
- RB, Ray Biotech, Atlanta, GA
- Santa Cruz, Santa Cruz Biotechnologies, Santa Cruz, CA
- SBA, Southern Biotechnology Associates, Birmingham, AL

- Sigma, Sigma Chemical Co., St Louis, MO
- TF, Thermo-Fisher Scientific, Waltham, MA
- Vector, Vector Laboratories, Burlingame, CA

3. Procedure

3.1. Biological Activity

Clonal populations of adult telomerase positive stem cells, e.g., totipotent stem cells: SCl-4β, Scl-9β, SCl-44β; pluripotent stem cells: Scl-40β; mesodermal stem cells: Scl-A2A2β; unlabeled mesenchymal stem cell clone: Rt-MSC; and unlabeled unipotent progenitor clones: Rt-Adip, Rt-Cart, Rt-Bone, were incubated with physiological to pharmaceutical concentrations of biological agents to determine their response. Four categories of biological agents were identified: anti-differentiating agents, proliferative agents, progression agents, and inducing agents. Ninety-six well plates were utilized to devise a high throughput screening assay for the testing system [45,46]. One thousand cells were plated per well on a 1% type-1 collagen substratum for the aTPSCs and on uncoated plastic for the progenitor cells [45]. The cells were washed with incomplete culture medium (e.g., OptiMem + GlutaMax, 1% antibiotic/antimycotic, pH 7.4 [45], and then incubated with particular agents from physiological to pharmaceutical doses, e.g., nanogram to microgram quantities per ml, in complete medium containing 10% heat inactivated serum. Medium was changed, dependent on the color of the medium [3]. We utilized 130 immunocytochemical and histochemical staining procedures for specific phenotypic expression markers, using high throughput enzyme-linked immuno-culture assay (ELICA) [45], to screen aTPSCs, MSCs, and unipotent progenitor cells with biological agents to determine their response to these agents [44,45].

3.2. Processing Procedures

Clonal populations of genomically-labeled adult telomerase positive stem cells, , e.g., totipotent stem cells: SCl-4β, Scl-9β, SCl-44β; pluripotent stem cells: Scl-40β; mesodermal stem cells: Scl-A2A2β; unlabeled mesenchymal stem cell clone: Rt-MSC; and unlabeled unipotent progenitor clones: Rt-Adip, Rt-Cart, Rt-Bone, were characterized with respect to multiple criteria using the previously developed procedures (Table 2) [44,45,48].

4. Results

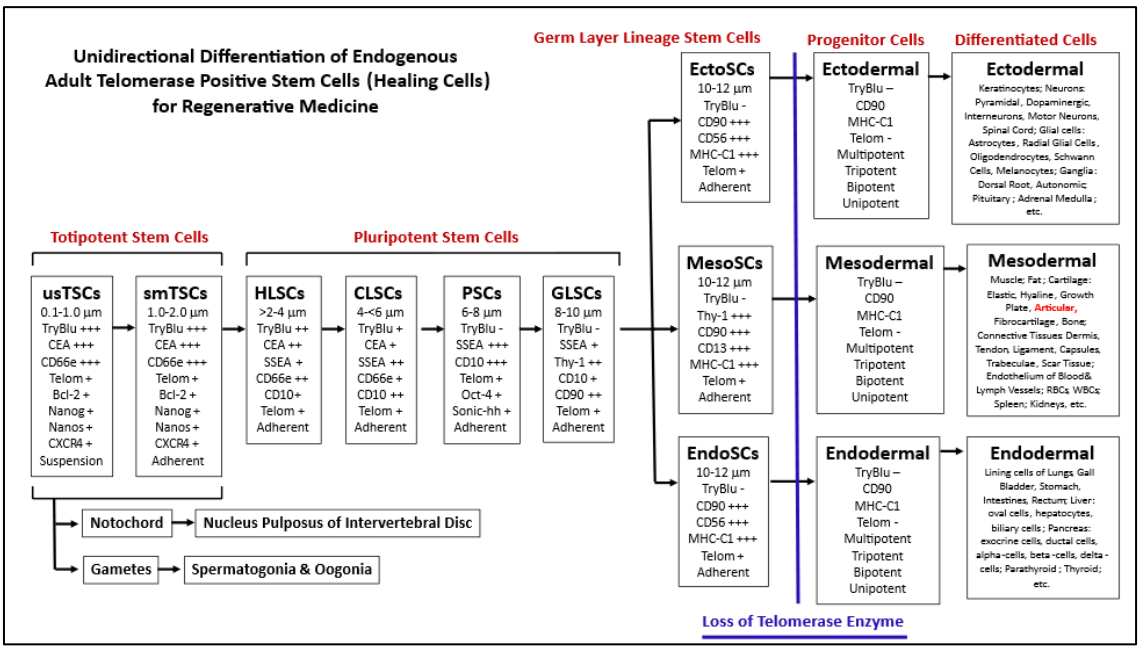

Figure 1 Summary of unidirectional differentiation potentials of aTPSCs with respect to subcategories, size, Trypan blue staining, cell surface markers, expressed genes, growth in culture, and differentiation potential. Reprinted with permission of Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. Stem Cell Regen Med 2020; 4(2):1-14 [48]

Table 3 Similarities and Differences between unlabeled and genomically-labeled ATPSC clones

Agent	**TSC**	**TSC-β**	**PSC**	**PSC-β**	**Meso SC**	**MesoSC-β**
Anti-Differentiative[1]	+++	+++	+++	+++	+++	+++
Proliferative[2] (Population Doublings)	Unlimited	Unlimited	Unlimited	Unlimited	Unlimited	Unlimited
Progressive[3]	-	-	-	-	-	-
Inductive[4]	All Cells	All Cells	All Somatic Cells Only	All Somatic Cells Only	Mesodermal Lineage Cells Only	All Somatic Cells Only

Table 3. Similarities and Differences between unlabeled aTPSC clones and genomically-labeled aTPSC clones. Anti-differentiative1, prevents differentiation of cells in the presence of inductive agents; Proliferative2, stimulates proliferation in cells, for aTPSCs that contain the telomerase enzyme, proliferation is essentially unlimited as long as the cells remain undifferentiated, once they begin to differentiate into progenitor cells, they lose the telomerase enzyme and assume the cell doubling number associated with particular species, e.g., rodents = 6-8 population doublings before cell senescence and cell death [47]; Progressive3, accelerates the phenotypic expression of cell-committed progenitor cells, but has no response on stem cells; Inductive4, is dependent on subcategory of aTPSCs, TSCs will form all cells similar to day-four blastomeres of a developing embryo, PSCs will form all cells similar to the inner cell mass of a developing embryo, EctoSCs will form all cells of the ectodermal lineage, MesoSCs will form all cells of mesodermal lineage, EndoSCs will form all cells of endodermal lineage. Reprinted in part with permission from Young HE. Adult telomerase positive stem cells: effects of biological agents on single cell clones of adult cells. GSC Advanced Research and Reviews. 2025; 25(03): 028-047 [44].

Table 4 Similarities and Differences between aTPSC clones, Tripotent MSC clone, and Unipotent Progenitor Clones

Category[1]	**TSC[2] Scl-4β**	**TSC Scl-9β**	**TSC Scl-44β**	**PSC[3] Scl-40β**	**Meso-SC[4] Scl-A_2A_2β**	**Rt-MSC[5]**	**Rt-Adip[6]**	**Rt-Cart[7]**	**Rt-Bone[8]**
Anti-Differentiation[9]	+++	+++	+++	+++	+++	++	+	+	+
Proliferation Population Doublings[10]	Unlim[11]	Unlim	Unlim	Unlim	Unlim	R: 6-8	R: 6-8	R: 6-8	R: 6-8
Progression[12]	-	-	-	-	-	+++	+++	+++	+++
Induction[13]	All Cells	All Cells	All Cells	All Somatic Cells	Meso Lineage Only	Fat Cartilage Bone	Fat	Cartilage	Bone

Table 4. Similarities and Differences between aTPSC clones, tripotent MSC clone, and unipotent progenitor clones. Category1, categories examined; TSC2 totipotent stem cell clones: Scl-40β, Scl-9β, Scl-44β; PSC3, pluripotent stem cell clone: Scl-40β; MesoSC4, mesodermal stem cell clone: Scl-A2A2β; Rt-MSC5, rat-derived tripotent mesenchymal stem cell clone; Rt-Adip6, rat-derived unipotent adipoblast clone; Rt-Cart7, rat-derived unipotent chondroblast clone; Rt-Bone8, rat-derived unipotent osteoblast clone; Anti-Differentiation9, prevents differentiation of cells in the presence of inductive agents; Proliferation Population Doublings10, stimulates proliferation in cells with respect to population doublings, for aTPSCs that contain the telomerase enzyme, proliferation is essentially unlimited (Unlim11) as long as the cells remain undifferentiated; once they begin to differentiate into progenitor cells, they lose the telomerase enzyme and assume the cell doubling number associated with particular species, e.g., rodents = 6-8 population doublings [47] before cell senescence and cell death; Progression12, accelerates phenotypic expression of cell-committed progenitor

cells, but has no response on stem cells; Induction13, is dependent on subcategory of aTPSCs, TSCs will form all cells similar to day-four blastomeres of a developing embryo, PSCs will form all somatic cells, similar to inner cell mass of a developing embryo; EctoSCs will form all cells of the ectodermal lineage; MesoSCs will form all cells of mesodermal lineage; EndoSCs will form all cells of endodermal lineage; MSCs will only form fat, cartilage, and bone; Rt-Adip will only form fat; Rt-Cart will only form cartilage, and Rt-Bone will only form bone.

Table 5 Biological Activity of Genomically-Labeled aTPSCs, Unlabeled MSCs, and Unlabeled Unipotent Progenitor Cells

Agent	**TSC Scl-4β**	**TSC Scl-9β**	**TSC Scl-44β**	**PSC Scl-40β**	**MesoSC Scl-A_2A_2β**	**Rt-MSC**	**RT-Adip**	**Rt-Cart**	**Rt-Bone**
LIF[1]	InDiff[2]	InDiff	InDiff	InDiff	InDiff	InDiff	InDiff	InDiff	InDiff
ADF[3]	InDiff	InDiff	InDiff	InDiff	InDiff	InDiff	InDiff	InDiff	InDiff
CAF[4]	InDiff	InDiff	InDiff	InDiff	InDiff	No Eff[5]	No Eff	No Eff	No Eff
SIF[6]	PrScF[7]	PrScF	PrScF	PrScF	PrScF	No Eff	No Eff	No Eff	No Eff
-AA[8]	Prolif[9]	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif
-AB[10]	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif
-BB[11]	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif
IGF-1[11]	No Eff	No Eff	No Eff	No Eff	No Eff	Progres[12]	Progres	Progres	Progres
IGF-2[13]	No Eff	No Eff	No Eff	No Eff	No Eff	Progres	Progres	Progres	Progres
INS[14]	No Eff	No Eff	No Eff	No Eff	No Eff	Progres	Progres	Progres	Progres
BMP-2[15]	Bone	Bone	Bone	Bone	Bone	Bone	No Eff	No Eff	Bone
OMP[16]	Bone	Bone	Bone	Bone	Bone	Bone	No Eff	No Eff	Bone
OCM[17]	Bone	Bone	Bone	Bone	Bone	Bone	No Eff	No Eff	Bone
CMP[18]	Cart[19]	Cart	Cart	Cart	Cart	Cart	No Eff	Cart	No Eff
CCM[20]	Cart	Cart	Cart	Cart	Cart	Cart	No Eff	Cart	No Eff
AdipMP[21]	Fat	Fat	Fat	Fat	Fat	Fat	Fat	No Eff	No Eff
AdipCM[22]	Fat	Fat	Fat	Fat	Fat	Fat	Fat	No Eff	No Eff
BMP-4[23]	Endo[24]	Endo	Endo	Endo	Endo	No Eff	No Eff	No Eff	No Eff
a-FGF[25]	Endo	Endo	Endo	Endo	Endo	No Eff	No Eff	No Eff	No Eff
ECGF[26]	Endo	Endo	Endo	Endo	Endo	No Eff	No Eff	No Eff	No Eff
VEGF[27]	Endo	Endo	Endo	Endo	Endo	No Eff	No Eff	No Eff	No Eff
BVMP[28]	Bld Vs[29]	Bld Vs	Bld Vs	Bld Vs	Bld Vs	No Eff	No Eff	No Eff	No Eff
BVCM[30]	Bld Vs	Bld Vs	Bld Vs	Bld Vs	Bld Vs	No Eff	No Eff	No Eff	No Eff
FMP[31]	Fibro[32]	Fibro	Fibro	Fibro	Fibro	No Eff	No Eff	No Eff	No Eff
FCM[33]	Fibro	Fibro	Fibro	Fibro	Fibro	No Eff	No Eff	No Eff	No Eff
ScFMP[34]	ScFib[35]	ScFib	ScFib	ScFib	ScFib	No Eff	No Eff	No Eff	No Eff
TGF-β[36]	ScFib	ScFib	ScFib	ScFib	ScFib	No Eff	No Eff	No Eff	No Eff
b-FGF[37]	ScFib	ScFib	ScFib	ScFib	ScFib	No Eff	No Eff	No Eff	No Eff
EPO, c-kit, IL-6[38]	RBC CFU[39]	RBC CFU	RBC CFU	RBC CFU	RBC CFU	No Eff	No Eff	No Eff	No Eff

NGF[40]	Neuron[41]	Neuron	Neuron	Neuron	No Eff	No Eff	No Eff	No Eff	No Eff
BrnMP[42]	Neuron	Neuron	Neuron	Neuron	No Eff	No Eff	No Eff	No Eff	No Eff
BrnCM[43]	Neuron	Neuron	Neuron	Neuron	No Eff	No Eff	No Eff	No Eff	No Eff
HGF[44]	Liver Cs[45]	Liver Cs	Liver Cs	Liver Cs	No Eff	No Eff	No Eff	No Eff	No Eff
LivMP[46]	Liver Cs	Liver Cs	Liver Cs	Liver Cs	No Eff	No Eff	No Eff	No Eff	No Eff
LivCM[47]	Liver Cs	Liver Cs	Liver Cs	Liver Cs	No Eff	No Eff	No Eff	No Eff	No Eff
LngMP[48]	Lung Cs[49]	Lung Cs	Lung Cs	Lung Cs	No Eff	No Eff	No Eff	No Eff	No Eff
LngCM[50]	Lung Cs	Lung Cs	Lung Cs	Lung Cs	No Eff	No Eff	No Eff	No Eff	No Eff
PanMP[51]	Pan Cs[52]	Pan Cs	Pan Cs	Pan Cs	No Eff	No Eff	No Eff	No Eff	No Eff
PanCM[53]	Pan Cs	Pan Cs	Pan Cs	Pan Cs	No Eff	No Eff	No Eff	No Eff	No Eff
KerMP[54]	Kera Cs[55]	Kera Cs	Kera Cs	Kera Cs	No Eff	No Eff	No Eff	No Eff	No Eff
KerCM[56]	Kera Cs	Kera Cs	Kera Cs	Kera Cs	No Eff	No Eff	No Eff	No Eff	No Eff
SerCCM[57]	Spermat[58]	No Eff	No Eff	No Eff	No Eff	No Eff	No Eff	No Eff	No Eff
SkMMP[59]	Sk Mus[60]	Sk Mus	Sk Mus	Sk Mus	Sk Mus	No Eff	No Eff	No Eff	No Eff
SkMCM[61]	Sk Mus	Sk Mus	Sk Mus	Sk Mus	Sk Mus	No Eff	No Eff	No Eff	No Eff
SmMMP[62]	Sm Mus[63]	Sm Mus	Sm Mus	Sm Mus	Sm Mus	No Eff	No Eff	No Eff	No Eff
SmMCM[64]	Sm Mus	Sm Mus	Sm Mus	Sm Mus	Sm Mus	No Eff	No Eff	No Eff	No Eff
CdMMP[65]	Car Mus[66]	Car Mus	Car Mus	Car Mus	Car Mus	No Eff	No Eff	No Eff	No Eff
CdMCM[67]	Car Mus	Car Mus	Car Mus	Car Mus	Car Mus	No Eff	No Eff	No Eff	No Eff
TenMP[68]	Tendon	Tendon	Tendon	Tendon	Tendon	No Eff	No Eff	No Eff	No Eff
TenCM[69]	Tendon	Tendon	Tendon	Tendon	Tendon	No Eff	No Eff	No Eff	No Eff
LigMP[70]	Ligament	Ligament	Ligament	Ligament	Ligament	No Eff	No Eff	No Eff	No Eff
LigCM[71]	Ligament	Ligament	Ligament	Ligament	Ligament	No Eff	No Eff	No Eff	No Eff

Table 5. Biological Agents Effecting Clones of aTPSCs, e.g., TSCs (Scl-4β, Scl-9β, SCl-44β), PSCs (Scl-40β), MesoSCs (Scl-A2A2β) and progenitor cells (Rt-MSCs, Rt-Fat, Rt-Cart, Rt-Bone). LIF1, Leukemia Inhibitory Factor, cell number dependent; InDiff2, Inhibit Differentiation; ADF3, anti-differentiation factor, cell number independent; CAF4, Caffeine; No Eff5, No Effect; SIF6, Scar Inhibitory Factor; PrScF7, prevent scar formation; -AA8, Platelet-Derived Growth Factor-AA; Prolif9, Proliferation; -AB10, Platelet-Derived Growth Factor-AB; -BB11, Platelet-Derived Growth Factor-BB; IGF-I11, Insulin-Like Growth Factor-1; Progres12, Progression, accelerates phenotypic expression in progenitor cells; IGF-213, Insulin-like Growth Factor-2; INS14, Insulin; BMP-215, Bone Morphogenetic Protein-2; OMP16, Osteogenic Morphogenetic Protein; OCM17, Osteogenic Conditioned Medium; CMP18, Cartilage Morphogenetic Protein; Cart19, Cartilage; CCM20, Cartilage Conditioned Medium; AdipMP21, Adipocyte Morphogenetic Protein; AdipCM22, Adipocyte Conditioned Medium; BMP-423, Bone Morphogenetic Protein-4; Endo24, Endothelium lining of blood vessel; a-FGF25, acidic-Fibroblast Growth Factor; ECGF26, Endothelial Cell Growth Factor; VEGF27, Vascular Endothelial Growth Factor; BVMP28, Blood Vessel Morphogenetic Protein; Bld Vs29; Blood Vessels; BVCM30, Blood Vessel Conditioned Medium; FMP31, Fibroblast Morphogenetic Protein; Fibro32; Fibroblasts/Fibrocytes; FCM33, Fibroblast Conditioned Medium; ScFMP34, Scar Fibroblast Morphogenetic Protein; ScF35; Scar Fibroblast/ Fibrocyte; TGF-β36, Transforming Growth Factor-beta; b-FGF37, basic-Fibroblast Growth Factor; EPO, Erythropoietin, c-Kit, IL-6, Interleukin-638 cocktail; RBC CFU39; Red Blood Cell Colony Forming Units; NGF40, Nerve Growth Factor; Neuron41, neuronal lineage cells, e.g., Neurons, Astrocytes, Oligodendrocytes, Schwann Cells, Ganglion cells, Radial Glial Cells; BrnMP42, Brain Morphogenetic protein; BranCM43, Brain Conditioned Medium; HGF44, Hepatocyte Growth Factor; Liver Cs45, e.g., oval cells, canalicular cells, biliary cells, hepatocytes; LivMP46, Liver Morphogenetic Protein; LivCM47, Liver Conditioned Medium; LngMP48, Lung Morphogenetic Protein; Lung Cs49, e.g., type-1 pneumocytes; LngCM50, Lung Conditioned Medium; PanMP51, Pancreatic Morphogenetic Protein; Pan Cs52, e.g., pancreatic ductal cells, alpha-cells, beta-cells, delta-cells, PP-cells, epsilon-cells; PanCM53, Pancreatic Conditioned Medium; KerMP54, Keratinocyte Morphogenetic

Protein; Ker Cs55, Keratinocytes; KerCM56, Keratinocyte Conditioned Medium; SerCCM57, Sertoli Cell Conditioned Medium; Spermat58, spermatogonia; SkMMP59, Skeletal Muscle Morphogenetic Protein, Sk Mus60, Skeletal Muscle; SkMCM61, Skeletal Muscle Conditioned Medium; SmMMP62, Smooth Muscle Morphogenetic Protein; Sm Musc63, Smooth Muscle; SmMCM64, Smooth Muscle Conditioned Medium; CdMMP65, Cardiac Muscle Morphogenetic Protein; Car Mus66, Cardiac Muscle; CdMCM67, Cardiac Muscle Conditioned Medium; TenMP68, Tendon Morphogenetic Protein; TenCM69, Tendon Conditioned Medium; LigMP70, Ligament Morphogenetic Protein; LigCM71, Ligament Conditioned Medium.

Table 6 Characterization of Genomically-Labeled aTPSCs, Unlabeled MSCs, and Unlabeled Unipotent Progenitor Cells

Character Tested[1]	**TSC[2] Scl-4β**	**TSC Scl-9β**	**TSC Scl-44β**	**PSC[3] Scl-40β**	**Meso-SC[4] Scl-A_2A_2β**	**Rt-MSC[5]**	**Rt-Adip[6]**	**Rt-Cart[7]**	**Rt-Bone[8]**
Size, microns	0.1-1	1-2	0.1-1	6-8	10-12	15-30	15-30	15-30	15-30
Trypan Blue	Pos[9]	Pos	Pos	Neg[10]	Neg	Neg	Neg	Neg	Neg
Telomerase	Pos	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg
Life-Span PDs[11]	>300	>300	>300	>400	>300	50	50	50	50
Species	Rat	Rat	Rat	Rat	Rat	Rat	Rat	Rat	Rat
Contact Inhibited	No	No	No	No	Yes	Yes	Yes	Yes	Yes
Survive Contact Inhibited	NA[12]	NA	NA	NA	Yes	No	No	No	No
Growth	Suspen[13]	Adhes[14]	Suspen	Adhes	Adhes	Adhes	Adhes	Adhes	Adhes
Substrate	None	T1-Collag[15]	None	T1-Collag	T1-Collag	Bare Plastic	Bare Plastic	Bare Plastic	Bare Plastic
SFM[16]	Quies[17]	Quies	Quies	Quies	Quies	Quies	Quies	Quies	Quies
No Factors	Quies	Quies	Quies	Quies	Quies	Quies	Quies	Quies	Quies
Inhibition	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Proliferation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Progression	No	No	No	No	No	Yes	Yes	Yes	Yes
Induction	Yes	Yes	Yes	Yes	Meso-dermal only	Fat Cartilage Bone	Fat	Cart	Bone
# Cells ID'[18]	75	74	75	70	37	3	1	1	1
Proliferation Rate Hours	12-14	12-14	12-14	14-16	18-24	Days	Days	Days	Days
Cryo Ag[19]	DMSO[20]	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
Cryo %[21]	7.5	7.5	7.5	7.5	7.5	10	10	10	10
# Cells Cryo[22]	1-10^9	1-10^9	1-10^9	1-10^9	1-10^6	1-10^6	1-10^6	1-10^6	1-10^6
Op Fr T[23]	-80°C	-80°C	-80°C	-80°C	-70°C	-196°C	-196°C	-196°C	-196°C
Freeze Prot[24]	Slow	Slow	Slow	Slow	Slow	Flash	Flash	Flash	Flash
Op St T[25]	-80°C	-80°C	-80°C	-80°C	-70°C	-196°C	-196°C	-196°C	-196°C
Thaw T[26]	37°C	37°C	37°C	37°C	37°C	37°C	37°C	37°C	37°C

Recovery	>98%	>98%	>98%	>98%	>98%	>95%	>95%	>95%	>95%
Cell Surface Markers	CEA-CAM-1[27]	CEA-CAM-1	CEA-CAM-1	SSEA-4[28]	Thy-1[29]	Thy-1	Thy-1	Thy-1	Thy-1

Table 6. Character Tested1, characteristics tested; TSC2, totipotent stem cell clone; PSC3, pluripotent stem cell clone; Meso-SC4, mesodermal stem cell clone; Rt-MSC5, tripotent progenitor mesenchymal stem cell clone; Rt-Adip6, unipotent adipogenic progenitor cell clone; Rt-Cart7, unipotent chondrogenic progenitor cell clone; Rt-Bone8, unipotent osteogenic progenitor cell clone; Pos9, Positive; Neg10, Negative; Life-Span PDs11, life span population doublings; NA12, Not Applicable; Suspen14 , growth in suspension culture; Adhes11, growth in two-dimensional adherent cultures; T1-Collag15, type-1 collagen; SFM16, serum free defined medium; Quies17, quiescent, default state without outside influences; # Cells ID18, Number of cells identified; Cryo Ag19, cryopreservative agent; DMSO20, ultra-pure dimethyl sulfoxide; Cryo %21, percent DMSO used to cryopreserve cells; # Cells Cro %23, number of cells per vial cryopreserved; Op Fr T23, Optimum Freezing temperature; Freeze Prot24, Freezing protocol; Op St T25, Optimum storage temperature; Thaw T26, thawing temperature; CEA-CAM-127, carcinoembryonic antigen-cell adhesion molecule-1; SSEA-428, stage-specific embryonic antigen-4; Thy-129, aka CD90, a glycosylphosphatidylinositol (GPI)-anchored glycoprotein on the outer surface membrane.

5. Discussion

The current study was undertaken to determine if genomically labelling aTPSCs with the Lac-Z gene beta-galactosidase utilizing lipofectin caused any alteration in their response to 1. human recombinant proteins, morphogenetic proteins isolated from both demineralized bone matrix and sera, and cell-specific exosome conditioned media for the four activities of biological agents, e.g., proliferation, progression, induction, and anti-differentiation, and 2. standardized procedures for characterizing aTPSCs and telomerase negative progenitor cells, including MSCs. As shown, there were no observable differences with respect to the response of unlabeled TSC, PSC, and MesoSC clones to genomically-labeled clones of TSC-β, PSC-β, and MesoSC-β for the four observed biological activities of proliferation, progression, induction, or anti-differentiation (compare Tables 1,3,4). There were apparent differences in response to biological activities, comparing Table 5 seen (this study) to Table 2 in unlabeled clone study [44]. There were also no differences with respect to the characterization criteria comparing Table 1 to Table 5.

6. Conclusion

The hypothesis tested was that there was no difference in responses to biological activities, e.g., proliferation, progression, induction, and/or anti-differentiation or characterization criteria between clones of aTPSCs and MSCs, either before or after genomically labeling. The results demonstrated that the hypothesis was a true statement.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest was disclosed.

Statement of ethical approval

Animal Use The use of animals in this study complied with the guidelines of Mercer University's Institutional Animal Care and Use Committee (ACUC). These guidelines reflect the criteria for humane animal care of the National Research Council as outlined in "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health

Statement of informed consent

Human Use The use of human biopsy specimens in this study complied with the guidelines of Mercer University's Institutional Review Board (IRB). These guidelines reflect the Federal Regulations for Protection of Human Research Subjects, HHS Office for Human Research Protections – 45 and 46 CFR: 46.102(I). Informed consent was obtained from all individual participants included in the study.

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