GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Check for updates

Adult telomerase positive stem cells: Effects of biological agents on single cell clones of adult cells

Henry E. Young [1, 2, 3, 4, 5, 6, 7, *]

[1] *Dragonfly Foundation for Research and Development, Macon, GA 31210 USA.*
[2] *Henry E Young PHD Regeneration Technologies, Macon, GA 31210 USA.*
[3] *Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA, 31210, USA.*
[4] *Department of Surgery, Mercer University School of Medicine, Macon, GA, 31210, USA.*
[5] *Department of Paediatrics, Mercer University School of Medicine, Macon, GA, 31210, USA.*
[6] *Department of Obstetrics and Gynaecology, Mercer University School of Medicine, Macon, GA, 31210, USA.*
[7] *Department of Anaesthesiology, Mercer University School of Medicine, Macon, GA, 31210, USA.*

GSC Advanced Research and Reviews, 2025, 25(03), 028-047

Publication history: Received 23 October 2025; revised on 30 November 2025; accepted on 02 December 2025

Article DOI: https://doi.org/10.30574/gscarr.2025.25.3.0369

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. Within the body, both aTPSCs and progenitor cells, including MSCs, respond to environmental cues (biological activities) dictating their ability to repair/regenerate damaged cells and tissues. Four biological activities were noted during the repair/regeneration process, e.g., proliferation, progression, induction, and anti-differentiation. This study was designed to test these four biological activities on the single cell generated clones to determine their response. We utilized human recombinant proteins, morphogenetic proteins isolated from both demineralized bone matrix and sera, and cell-specific exosome conditioned media to test the hypothesis that aTPSCs and MSCs react both similarly and differently to biological activities inherent in the body, e.g., proliferation, progression, induction, and anti-differentiation.

Keywords: Stem Cells; ATPSCS; MSCS; Proliferation; Progression; Induction; Anti-Differentiation

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, morphogenetic proteins

* Corresponding author: Henry E. Young

isolated from both demineralized bone matrix and sera, and cell-specific exosome conditioned medium. The recombinant proteins utilized were acidic-Fibroblast Growth Factor (a-FGF) [4-7], endothelial cell growth factor (ECGF) [8,9], vascular endothelial cell growth factor (VEGF) [10,11], transforming growth factor-beta (TGF-b) [12,13], basic-fibroblast growth factor (b-FGF) [14,15,], erythropoietin (EPO) [16-18], c-Kit [19,20], Interleukin-6 (IL-6) [21,22], Nerve Growth Factor (NGF) [23,24], and Hepatocyte Growth Factor (HGF) [25,26]. The morphogenetic proteins utilized were bone morphogenetic protein-2 (BMP-2) [27,28] and bone morphogenetic protein-4 (BMP-4) [29,30]; as well as additional morphogenetic proteins isolated from demineralized bone matrix, e.g., skeletal muscle morphogenetic protein (SkMMP) [2], smooth muscle morphogenetic protein (SmMMP) [2], cardiac muscle morphogenetic protein (CdMMP) [2], adipocyte morphogenetic protein (AMP) [2], fibroblast morphogenetic protein (FMP) [2], scar fibroblast morphogenetic protein (ScFMP), tendon morphogenetic protein (TenMP), and ligament morphogenetic protein (LigMP) [2]; and morphogenetic protein-activities isolated from different lots of serum, e.g., blood vessel morphogenetic protein (BVMP) [31], brain morphogenetic protein (BrnMP) [31], liver morphogenetic protein (LivMP) [31], lung morphogenetic protein (LngMP) [31], pancreas morphogenetic protein (PanMP) [31], and keratinocyte morphogenetic protein (KerMP) [31]. Cell-specific exosome conditioned medium was derived from explant cultures of the following tissues/organs [3] and designated as "organ" conditioned medium ("X"CM), e.g., skeletal muscle conditioned medium (SkMCM), smooth muscle conditioned medium (SmMCM), cardiac muscle conditioned medium (CdMCM), tendon conditioned medium (TCM), ligament conditioned medium (LCM), bone (osteogenic) conditioned medium (OCM), cartilage conditioned medium (CCM), adipocytes conditioned medium (ACM), blood vessel conditioned medium (BVCM), fibroblast/fibrocytes conditioned medium (FCM), brain conditioned medium (BrnCM), liver conditioned medium (LivCM), lung conditioned medium (LngCM), pancreas conditioned medium (PanCM), keratinocyte conditioned medium (KerCM), Sertoli cells conditioned medium (SertoliCellCM). These biological agents 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 [32]. The hypothesis tested was that aTPSCs and MSCs react both similarly and differently to biological agents with their own unique activities inherent in the body, e.g., proliferation, progression, induction, and anti-differentiation.

2. Materials And Methods

2.1. Assay Reagents: [2,31-33]

2.1.1. Species-specific buffers

- Amphibians and Reptiles – 10% Holtfreter's solution [34]
- Avians – Tyrode's balanced salt solution, #T-2145 (Sigma)
- Non-human mammals – Phosphate Buffered Saline, PBS (Sigma)
- Humans – Dulbecco's Phosphate-Buffered Saline (10X) #310-4080AJ (GIBCO)

2.2. Biological Agents

2.2.1. Anti-differentiative

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

2.2.2. 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

2.2.3. Progression

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

2.2.4. 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 Adipoblast/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/fibrocyte 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 (SertoliCellCM) – 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

2.3. ELICA Fixative Reagents

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

2.4. Inhibition and Exhaustion of Endogenous Peroxidases

- 5% Sodium Azide, #S2002 Sigma

- 30% Hydrogen Peroxide, #H-1009, Sigma

2.5. 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

2.6. 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

2.7. 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,
- 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

2.8. Histochemical Stains

- Von Kossa, Chroma-Gesellschaft
- AlkPhos, Sigma
- Mallory Heidenhain One-Step reagents, Chroma-Gesellschaft
- Alcian blue, Chroma-Gesellschaft
- pH 1.0
- pH 2.5
- Alcec blue, Chroma-Gesellschaft
- pH 1.0
- 2.5
- Safranin-O, Chroma-Gesellschaft
- pH 1.0
- pH 2.5
- Sudan Black-B, 199664, Sigma
- Oil Red-O, 00625, Sigma

2.9. 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

2.10. Secondary Probes

- Goat anti-rat osteocalcin, #BT-413, Biomedical Technology
- 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)

2.11. Tertiary Probes

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

2.12. 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

2.13. Miscellaneous Supplies

- Aqua-Mount (mounting coverslips), Vector Laboratories
- Parafilm, #13-374-12, Dagger
- Ethanol, Sigma
- Isopropyl alcohol, Sigma
- Micropipettors, Diagger
- Nitrile Gloves, Dagger
- Pipets: 1-ml, 2-ml, 5-ml, 10-ml, Diagger
- 96-well plates, Costar/Corning

2.14. Equipment

- Hera-2 Incubator, Thermo-Fisher
- 96-well plate reader, R&D
- Vortex Mixer, Thermo-Fisher
- -70^{o}C Ultra-Low Freezer, Thermo-Fisher
- -80^{o}C Ultra-Low Freezer, Thermo-Fisher
- Liquid Nitrogen Dewer, Thermo-Fisher

2.15. Suppliers

- Abcam, Cambridge, MA
- Accurate, Accurate Chemical and Scientific Corporation, Westbury, NY

- AirGas, AirGas, Local Sore
- 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
- ChemPure, Curtain Matheson Scientific, Houston, TX
- Chroma-Gesellschaft, Roboz Surgical Co, Washington, DC
- Costar/Corning, Life Sciences Div, Kennebunk, ME
- Diagger Scientific, Vernon Hills, IL
- DFRD, Dragonfly Foundation for Research and Development, Macon, GA
- DH, Douglas Hixson, Department of Medicine, Brown University, Providence, RI
- 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-Biotech, Waltham, MA
- Fisher, Fisher Scientific Co., Norcross, GA
- 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
- RB, Ray Biotech, Atlanta, GA
- R&D Systems, Minneapolis, MI
- SBA, Southern Biotechnology Associates, Birmingham, AL
- Sigma, Sigma Chemical Co., St Louis, MO
- Thermo-Fisher Scientific, Waltham, MA
- Vector, Vector Laboratories, Burlingame, CA

3. Procedure

Clonal populations of adult telomerase positive stem cells, e.g., totipotent stem cells (TSCs), halo-like stem cells (HLSCs), corona-like stem cells (CLSCs), pluripotent stem cells (PSCs), germ layer lineage stem cells (GLSCs), ectodermal stem cells (EctoSCs), mesodermal stem cells (MesoSCs), and endodermal stem cells (EndoSCs), and a clone of telomerase negative tripotent progenitor cell, MSCs, 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 [32]. One thousand cells were plated per well on a 1% type-1 collagen substratum for the aTPSCs and on uncoated plastic for the MSCs [35]. The cells were washed with incomplete culture medium (e.g., OptiMem + GlutaMax, 1% antibiotic/antimycotic, pH 7.4 [35], 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 (Table 1), using an enzyme-linked immuno-culture assay (ELICA), to screen aTPSCs and MSC with biological agents to determine their response to these agents [32].

Table 1 Antibodies, Immunocytochemistry, and Histochemistry for Phenotypic Expression Markers

Antibody	**Antigen**	**Embryological Origin**
CEA-CAM-1	Carcinoembryonic antigen-cell adhesion molecule-1	Totipotent
HCEA	Human Carcinoembryonic antigen	Totipotent

CEA	Carcinoembryonic antigen	Totipotent
CD66e	Carcinoembryonic antigen	Totipotent
DH-TuAg1	Spermatogonia	Male Gamete
MC-480	SSEA-1	Pluripotent
MC-631	SSEA-3	Pluripotent
MC-813	SSEA-4	Pluripotent
CD10	Neutral endopeptidase	Pluripotent
AlkPhos	Alkaline Phosphatase	Pluripotent
CD56	Neural cell adhesion molecule	Ectoderm
Pax-6	Neurogenic lineage	Ectoderm
FORSE-1	Neuronal precursor cells	Ectoderm
Vimentin	Cells of neurogenic lineage	Ectoderm
Nestin	Cells of neurogenic lineage	Ectoderm
R401	Nestin-neuronal lineage	Ectoderm
HNES	Nestin-neuronal lineage	Ectoderm
MAB353	Nestin-neuronal lineage	Ectoderm
RT-97	Neurofilaments = neurons	Ectoderm
NF68	Neurofilament-68 = neurons	Ectoderm
S-100	Neurofilaments-100 = neurons	Ectoderm
NF-145	Neurofilaments-145 = neurons	Ectoderm
N-200	Neurofilaments-200 = neurons	Ectoderm
8A2	Neurons	Ectoderm
NG2	Neurons	Ectoderm
TH	Tyrosine hydroxylase, precursor to neural transmitters	Ectoderm
SV2	Synaptic vesicles	Ectoderm
DOPA	Dopamine, transmitter of dopaminergic neurons	Ectoderm
T8660	Beta-tubulin-III	Ectoderm
Tuj1	Beta-tubulin	Ectoderm
GFAP	Glial-fibrillary acidic protein	Ectoderm
CNPase	Glial cells = oligodendrocytes and astrocytes	Ectoderm
Rip	Oligodendrocytes	Ectoderm
MOSP	Oligodendrocyte specific proteins	Ectoderm
MAB	Oligodendrocyte marker	Ectoderm
40E-C	Radial cells and radial glial cells	Ectoderm
VM-1	Keratinocytes	Ectoderm
M3F7	Type-IV collagen, basement membrane	Ectoderm and Mesoderm
31-2	Laminin, basement membrane	Ectoderm and Mesoderm
5D2-27	Cell adhesion molecule	Ectoderm and Mesoderm

B3/D6	Fibronectin, basement membrane	Ectoderm and Mesoderm
5C6	Type-IV collagen, basemen membrane	Ectoderm and Mesoderm
Anti-type IV	Type-IV collagen	Ectoderm and Mesoderm
33-2	Heparan sulfate proteoglycan	Ectoderm and Mesoderm
Anti-HSPG	Heparan Sulfate proteoglycan	Ectoderm and Mesoderm
5D4	Keratan sulfate proteoglycan	Ectoderm and Mesoderm
2E8	Laminin, basement membrane	Ectoderm and Mesoderm
D3	Desmin, in all 3 muscle groups	Ectoderm and Mesoderm
Anti-vimentin	Vimentin, lens of the eye	Ectoderm and Mesoderm
D76	Desmin, in all 3 muscle groups	Ectoderm and Mesoderm
CD13	Amino endopeptidase	Mesoderm
12/101	Skeletal Muscle	Mesoderm
C3/1	Glycoprotein of myoblast plasma membrane	Mesoderm
OP-137	MyoD	Mesoderm
F5D	Myogenin = skeletal muscle	Mesoderm
ALD-66	Slow twitch muscle fibers	Mesoderm
MF-1	Fast twitch muscle fibers	Mesoderm
MF-5	Myosin light chain-2 of fast muscle	Mesoderm
MF-20	Sarcomeric myosin = skeletal muscle	Mesoderm
MF-30	Neonatal and adult myosin	Mesoderm
ALD58	Myosin heavy chain	Mesoderm
CH1	Myosin tropomyosin	Mesoderm
A4.74	Myosin fast chain	Mesoderm
JLA-20	Actin	Mesoderm
Anti-Myosin	Skeletal muscle myosin	Mesoderm
IA4	Smooth muscle alpha actin = smooth muscle	Mesoderm
Calp	Calponin	Mesoderm
MAB-3252	Cardiotin = cardiac myocytes	Mesoderm
MAB1548	Myosin heavy chain of cardiac muscle	Mesoderm
M-38	Type 1 collagen	Mesoderm
SP1.D8	Procollagen type-III	Mesoderm
Anti-type-II	Type-II collagen	Mesoderm
WV1D1	Bone sialoprotein II = bone	Mesoderm
Anti-OsteC	Osteocalcin / Bone Gla-protein	Mesoderm
MP111	Osteopontine = bone	Mesoderm
EGTA	Leaches Calcium from bone, negative control	Mesoderm
CIIC1	Type-II collagen	Mesoderm
II-4CII	Type-II collagen	Mesoderm

Anti-type2	Type-II collagen	Mesoderm
HC-II	Human type-II collagen	Mesoderm
D1-9	Type-IX collagen = cartilage	Mesoderm
9/30	Cartilage link protein	Mesoderm
12/21	Cartilage proteoglycan hyaluronate binding region	Mesoderm
12C5	Versican hyaluronate binding region	Mesoderm
H-DC34	Sialomucin-containing hematopoietic/endothelial cells	Mesoderm
CD31	PECAM, Peripheral endothelial cell adhesion molecule	Mesoderm
P1H12	Human endothelial cell surface marker	Mesoderm
P2B1	Peripheral endothelial adhesion molecule	Mesoderm
P8B1	VCAM, vascular cell adhesion molecule	Mesoderm
P2H3	CD62e, E-selectin (vasculature)	Mesoderm
H-endo	CD146, Endothelial cells	Mesoderm
H5A4	CD11b, granulocytes, monocytes, NK-cells	Mesoderm
H4C4	CD44, hyaluronate receptor	Mesoderm
Hermes-1	CD44, hyaluronate receptor	Mesoderm
H5A5	CD45, all hematopoietic cells except RBCs	Mesoderm
H5C6	CD63, macrophages, monocytes, platelets	Mesoderm
HFSP	Human fibroblast specific protein	Mesoderm
1B10	Fibroblast-specific protein	Mesoderm
Sudan Black-B	Stains fat (adipocytes)	Mesoderm
Oil Red-O	Stains fat (adipocytes)	Mesoderm
H-AFP	Human alpha-fetoprotein = fetal liver	Endoderm
R-AFP	Rat alpha-fetoprotein = fetal liver	Endoderm
DESMO	Endodermal epithelial marker of liver	Endoderm
LAP	Canalicular cell surface protein of liver	Endoderm
151-Ig	Liver epithelial growth factor	Endoderm
HA4c19	Bile canalicular cells of liver	Endoderm
OC2	Progenitor cells, oval cell, and biliary cells of liver	Endoderm
OC3	Progenitor cells and biliary cells of liver	Endoderm
OC4	Progenitor cells and biliary cells of liver	Endoderm
OC5	Progenitor cells and biliary cells of liver	Endoderm
OC10	Progenitor cells and biliary cells of liver	Endoderm
H.4	Intracellular staining of liver hepatocytes	Endoderm
H.1	Liver hepatocytes cell surface marker	Endoderm
DPPIV	Progenitor, canalicular, and biliary cells of the liver	Endoderm
OV6	Biliary and oval cells of liver; biliary cells of liver	Endoderm
HESA	Human GI (Gastrointestinal) Epithelium	Endoderm

YM-PS087	Glucagon-secreting cells of endocrine pancreas	Endoderm
YM-PS5088	Insulin-secreting cells of endocrine pancreas	Endoderm
11180	Somatostatin-secreting cells of the endocrine pancreas	Endoderm
CK-19	Ductal cells of the exocrine pancreas	Endoderm
ABL-93	Lysosomal membrane glycoprotein	Ectoderm, Mesoderm, Endoderm
22/18	Regeneration cells	Ectoderm, Mesoderm, Endoderm
Telom	Telomerase positive cells	aTPSCs
CD90	Glycosylphosphatidylinositol anchoring membrane protein (Thy-1)	Transition: EctoSCs, MesoSCs, and EndoSCs to Progenitor Cells
Thy-1	Glycosylphosphatidylinositol anchoring membrane protein (CD90)	Transition: EctoSCs, MesoSCs, and EndoSCs to Progenitor Cells
CD95	Cells undergoing apoptosis	Dead Cells
PI	Propidium Iodide, measure of live cells, flow cytometry	Live Cells
DAPI	Fluorescent marker to visualize living and fixed DNA	Live and Dead Cells
Gal-19	Insect beta-galactosidase genomic marker	Cell tracking marker
Alcian Blue	Stains anions on carbohydrate groups	Extracellular Matrix
AB 1.0	Alcian Blue, pH 1.0 stains sulfate groups on GAGs	Extracellular Matrix
AB 2.5	Alcian Blue, pH 2.5 stains carboxyl groups on GAGs	Extracellular Matrix
Alcec Blue	Stains anions on carbohydrate groups	Extracellular Matrix
AcB 1.0	Alcec Blue, pH 1.0 stains sulfate groups on GAGs	Extracellular Matrix
AcB 2.5	Alcec Blue, pH 2.5 stains carboxyl groups on GAGs	Extracellular Matrix
Safranin-O	Stains anions on carbohydrate groups	Extracellular Matrix
SO 1.0	Safranin-O, pH 1.0 stains sulfate groups on GAGs	Extracellular Matrix
SO 2.5	Safranin-O, pH 2.5 stains carboxyl groups on GAGs	Extracellular Matrix
Von Kossa	Stains calcium in bone matrix	Extracellular Matrix
EDTA	Removes divalent cations, +2 charges Ca^{+2}, Mg^{+2}, Fe^{+2}, Zn^{+2}, Ba^{+2}	Extracellular Matrix
EGTA	Removes Ca^{+2} from tissues / bones	Extracellular Matrix
Enzyme	Streptomyces Hyaluronidase, negative staining control to verify presence of hyaluronic acid	Extracellular Matrix
Enzyme	Chondroitinase-AC, negative staining control to verify presence of non-sulphated chondroitin proteoglycans	Extracellular Matrix
Enzyme	Chondroitinase-ABC, negative control to verify presence of chondroitin sulfate proteoglycans	Extracellular Matrix
Enzyme	Keratanase, negative control to verify presence of keratan sulfate proteoglycans	Extracellular Matrix

Enzyme	Heparanase, to verify presence of heparan sulfate proteoglycans	Basement Membranes
PAS	Periodic Acid Schiff reaction for glycoproteins with vicinal hydroxyl groups	Extracellular Matrix

Table 1. Antibodies (left column) used to identify specific phenotypic expression markers (center column) for particular cell types (right column). Reprinted with permission from Young HE, Sippel J, Putnam LS, Lucas PA, Morrison DC. Enzyme-linked immuno-culture assay. Journal of Tissue Culture Methods, 14:31-36, 1992 [33]; Young HE, Black AC. Pluripotent Stem Cells, Endogenous versus Reprogrammed, a Review. MOJ Orthop Rheumatol 1(4): 00019, 2014 [36]; Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals. GSC Advanced Research and Reviews. 2025; 23(03): 149-157 [37].

4. Results

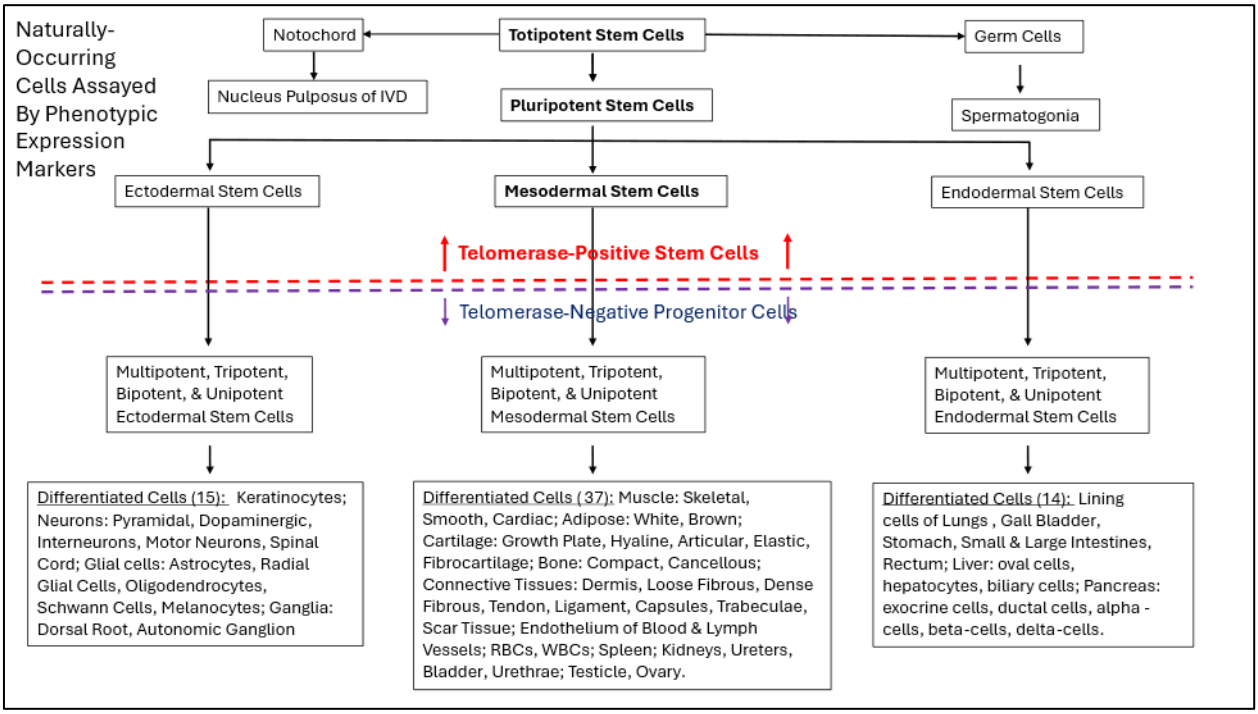

Figure 1 Unidirectional differentiation of primitive undifferentiated telomerase positive totipotent stem cells, through subsequent differentiation steps, i.e., telomerase positive pluripotent stem cells, telomerase positive ectodermal stem cells, telomerase positive mesodermal stem cells, and telomerase positive endodermal stem cells, to lose the telomerase enzyme and become telomerase negative multipotent, Tripotent, bipotent, and unipotent progenitor cells, which then differentiate into terminally differentiated adult cells. Reprinted 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 [38]

Table 2 Biological Agents Effecting Clones of ATPSCS and MSCs

Agent	**TSC**	**HLSC**	**CLSC**	**PSC**	**GLSC**	**EctoSC**	**MesoSC**	**EndoSC**	**MSC**
LIF[1]	ID[2]	ID	ID	ID	ID	ID	ID	ID	sID[3]
ADF[4]	ID	ID	ID	ID	ID	ID	ID	ID	sID
CAF[5]	ID	ID	ID	ID	ID	ID	ID	ID	NE[6]
SIF[7]	PScF[8]	PScF	PScF	PScF	PSCs	NE	PScF	NE	NE
PDGF- AA[9]	Prolif[10]	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif

PDGF-AB[11]	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif
PDGF-BB[12]	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif	Prolif
IGF-1[13]	NE	NE	NE	NE	NE	NE	NE	NE	Progre[14]
IGF-2[15]	NE	NE	NE	NE	NE	NE	NE	NE	Progre
INS[16]	NE	NE	NE	NE	NE	NE	NE	NE	Progre
BMP-2[17]	Bone	Bone	Bone	Bone	Bone	NE	Bone	NE	Bone
OMP[18]	Bone	Bone	Bone	Bone	Bone	NE	Bone	NE	Bone
OCM[19]	Bone	Bone	Bone	Bone	Bone	NE	Bone	NE	Bone
CMP[20]	Cart[21]	Cart	Cart	Cart	Cart	NE	Cart	NE	Cart
CCM[22]	Cart	Cart	Cart	Cart	Cart	NE	Cart	NE	Cart
AdipMP[23]	Fat	Fat	Fat	Fat	Fat	NE	Fat	NE	Fat
AdipCM[24]	Fat	Fat	Fat	Fat	Fat	NE	Fat	NE	Fat
BMP-4[25]	Endthe[26]	Endthe	Endthe	Endthe	Endthe	NE	Endthe	NE	NE
a-FGF[27]	Endthe	Endthe	Endthe	Endthe	Endthe	NE	Endthe	NE	NE
ECGF[28]	Endthe	Endthe	Endthe	Endthe	Endthe	NE	Endthe	NE	NE
VEGF[29]	Endthe	Endthe	Endthe	Endthe	Endthe	NE	Endthe	NE	NE
BVMP[30]	Vasc[31]	Vasc	Vasc	Vasc	Vasc	NE	Vasc	NE	NE
BVCM[32]	Vasc	Vasc	Vasc	Vasc	Vasc	NE	Vasc	NE	NE
FMP[33]	Fibro[34]	Fibro	Fibro	Fibro	Fibro	NE	Fibro	NE	NE
FCM[35]	Fibro	Fibro	Fibro	Fibro	Fibro	NE	Fibro	NE	NE
ScFMP[36]	ScF[37]	ScF	ScF	ScF	ScF	NE	ScF	NE	NE
TGF-b[38]	ScF	ScF	ScF	ScF	ScF	NE	ScF	NE	NE
b-FGF[39]	ScF	ScF	ScF	ScF	ScF	NE	ScF	NE	NE
EPO[40] + c-Kit[41] + IL-6[42]	RBCs[43]	RBCs	RBCs	RBCs	RBCs	NE	RBCs	NE	NE
NGF[44]	Nerve Cells[45]	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	NE	NE	NE
BrnMP[46]	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	NE	NE	NE
BrnCM[47]	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	Nerve Cells	NE	NE	NE
HGF[48]	Liver Cells[49]	Liver Cells	Liver Cells	Liver Cells	Liver Cells	NE	NE	Liver Cells	NE
LivMP[50]	Liver Cells	Liver Cells	Liver Cells	Liver Cells	Liver Cells	NE	NE	Liver Cells	NE
LivCM[51]	Liver Cells	Liver Cells	Liver Cells	Liver Cells	Liver Cells	NE	NE	Liver Cells	NE
LngMP[52]	Lung Cells	Lung Cells	Lung Cells	Lung Cells	Lung Cells	NE	NE	Lung Cells	NE

LngCM[53]	Lung Cells	Lung Cells	Lung Cells	Lung Cells	Lung Cells	NE	NE	Lung Cells	NE
PanMP[54]	Pan Cells[55]	Pan Cells	Pan Cells	Pan Cells	Pan Cells	NE	NE	Pan Cells	NE
PanCM[56]	Pan Cells	Pan Cells	Pan Cells	Pan Cells	Pan Cells	NE	NE	Pan Cells	NE
KerMP[57]	Kera Cells[58]	Kera Cells	Kera Cells	Kera Cells	Kera Cells	Kera Cells	NE	NE	NE
KerCM[59]	Kera Cells	Kera Cells	Kera Cells	Kera Cells	Kera Cells	Kera Cells	NE	NE	NE
Sertoli cell-CM[60]	Spermato-gonia[61]	NE	NE	NE	NE	NE	NE	NE	NE
SkMMP[62]	Skel Musc[63]	Skel Musc	Skel Musc	Skel Musc	Skel Musc	NE	Skel Musc	NE	NE
SkMCM[64]	Skel Musc	Skel Musc	Skel Musc	Skel Musc	Skel Musc	NE	Skel Musc	NE	NE
SmMMP[65]	Smth Musc[66]	Smth Musc	Smth Musc	Smth Musc	Smth Musc	NE	Smth Musc	NE	NE
SmMCM[67]	Smth Musc	Smth Musc	Smth Musc	Smth Musc	Smth Musc	NE	Smth Musc	NE	NE
CdMMP[68]	Card Musc[69]	Card Musc	Card Musc	Card Musc	Card Musc	NE	Card Musc	NE	NE
CdMCM[70]	Card Musc	Card Musc	Card Musc	Card Musc	Card Musc	NE	Card Musc	NE	NE
TenMP[71]	Tendon	Tendon	Tendon	Tendon	Tendon	NE	Tendon	NE	NE
TenCM[72]	Tendon	Tendon	Tendon	Tendon	Tendon	NE	Tendon	NE	NE
LigMP[73]	Lig[74]	Lig	Lig	Lig	Lig	NE	Lig	NE	NE
LigCM[75]	Lig	Lig	Lig	Lig	Lig	NE	Lig	NE	NE

Table 2. Biological Agents Effecting Clones of aTPSCs and MSCs. LIF1, Leukemia Inhibitory Factor, cell number dependent; ID2 Inhibit Differentiation; sID3, slight inhibition of differentiation; ADF4, anti-differentiation factor, cell number independent; CAF5, Caffeine; NE6, No Effect; SIF7, Scar Inhibitory Factor; PScF8, prevent scar formation; PDGF-AA9, Platelet-Derived Growth Factor -AA; Prolif10, Proliferation; PDGF-AB11, Platelet-Derived Growth Factor-AB; PDGF-BB12, Platelet-Derived Growth Factor-BB; IGF-113, Insulin-Like Growth Factor-1; Progre14, Progression; IGF-215, Insulin-like Growth Factor-2; INS16, Insulin; BMP-217, Bone Morphogenetic Protein-2; OMP18, Osteogenic Morphogenetic Protein; OCM19, Osteogenic Conditioned Medium; CMP20, Cartilage Morphogenetic Protein; Cart21, Cartilage; CCM22, Cartilage Conditioned Medium; AdipMP23, Adipocyte Morphogenetic Protein; AdipCM24, Adipocyte Conditioned Medium; BMP-425, Bone Morphogenetic Protein-4; Endthe26, Endothelium lining of blood vessel; a-FGF27, acidic-Fibroblast Growth Factor; ECGF28, Endothelial Cell Growth Factor; VEGF29, Vascular Endothelial Growth Factor; BVMP30, Blood Vessel Morphogenetic Protein; Vasc31; Vasculogenesis, blood vessel formation in culture; BVCM32, Blood Vessel Conditioned Medium; FMP33, Fibroblast Morphogenetic Protein; Fibro34; Fibroblast/Fibrocyte; FCM35, Fibroblast Conditioned Medium; ScFMP36, Scar Fibroblast Morphogenetic Protein; ScF37; Scar Fibroblast/Fibrocyte; TGF-b38, Transforming Growth Factor-beta; b-FGF39, basic-Fibroblast Growth Factor; EPO40, Erythropoietin; c-Kit41; IL-642, Interleukin-6; RBCs43; Red Blood Cells; NGF44, Nerve Growth Factor, neuronal lineage cells, e.g., neurons, astrocytes, oligodendrocytes, Schwann Cells, Ganglion cells; BrnMP47, Brain Morphogenetic protein; BranCM47, Brain Conditioned Medium; HGF48, Hepatocyte Growth Factor, Liver Cells49, e.g., oval cells, canalicular cells, biliary cells, hepatocytes; LivMP50, Liver Morphogenetic , Liver cells, e.g., oval cells, canalicular cells, biliary cells, hepatocytes; LivCM51, Liver Conditioned Medium, Liver Cells, e.g., oval cells, canalicular cells, biliary cells, hepatocytes; LngMP52, Lung Cells, e.g., type-1 pneumocytes; LngCM53, Lung Cells, e.g., type-1 pneumocytes; PanMP54, Pancreatic Cells55, e.g., pancreatic ductal cells, alpha-cells, beta-cells, delta-cells, PP-cells, epsilon-cells; PanCM56, Pancreatic

Conditioned Medium, Pancreatic cells, e.g., pancreatic ductal cells, alpha-cells, beta-cells, delta-cells, PP-cells, epsilon-cells; KerMP57, Ker Cells58, Keratinocytes; KerCM59, Ker Cells, Keratinocytes; Sertoli Cell-Conditioned Medium60, Spermaatogonia61, spermatogonia; SkMMP62, Skeletal Muscle Morphogenetic Protein, Skel Musc63, Skeletal Muscle; SkMCM64, Skeletal Muscle Conditioned Medium, Skel Musc, skeletal muscle; SmMMP65, Smooth Muscle Morphogenetic Protein, Smth Musc66, smooth muscle; SmMCM67, Smth Musc, smooth muscle; CdMMP68, Card Musc69, cardiac muscle; CbMCM70, Card Musc, cardiac muscle; TenMP71, Tendon Morphogenetic Protein; TenCM72, Tendon Conditioned Medium; LigMP73, Ligament Morphogenetic Protein, Lig74, ligament; LigCM75, Ligament Conditioned Medium, Lig, ligament.

5. Discussion

In recent years, considerable attention has focused on the isolation and characterization of endogenous bioactive factors and their importance in influencing aspects of tissue development, maturation, aging, replacement, and repair. Demineralized bone matrix has been shown to contain a number of factors that influence proliferation, chemotaxis, angiogenesis, chondrogenesis, and osteogenesis [39-42]. Of particular interest has been the family of bone morphogenetic proteins (BMP) in the transforming growth factor–beta (TGF-β) superfamily [43-45], cartilage morphogenetic protein [46], TGF-β, basic fibroblast growth factor (b-FGF), insulin, insulin-like growth factor–1 (IGF-1), and insulin-like growth factor–2 (IGF-2) [40,47]. On the basis of the presence of these compounds in bone matrix, we fractionated a water-soluble extract from a demineralized bone matrix and assayed for additional bioactive activities. We discovered activity corresponding to multiple factors within the water-soluble fractions, e.g., skeletal muscle morphogenetic protein (SkMMP), smooth muscle morphogenetic protein (SmMMP), cardiac muscle morphogenetic protein (CdMMP), fibroblast morphogenetic protein (FMP), adipocyte morphogenetic protein (AMP), tendon morphogenetic protein (TenMP), ligament morphogenetic protein (LigMP), osteogenic morphogenetic protein (OMP), cartilage morphogenetic protein (CMP), and scar inhibitory factor (SIF), and their ability to alter structure and function of aTPSCs [48-53].

We postulated that the bioactive factors isolated from demineralized bone matrix—that is, BMPs, TGF-β, IGF-1, IGF-2, insulin, Sk-MMP, SIF, and so forth, were produced by cells not belonging to the osteogenic lineage. The cells producing these factors would presumably secrete the compounds into the blood, which would in turn be sequestered in the bone matrix. We therefore began analysing various lots of commercially available serum to determine if these serum lots contained other inductive activities. On the basis of their respective interaction with aTPSCs in vitro [2,51-53], we identified the following activities from serum and designated the compounds responsible for their activity as platelet-derived growth factor (PDGF)-like (proliferative) activity, skeletal muscle morphogenetic protein (Sk-MMP), smooth muscle morphogenetic protein (Sm-MMP), adipocyte morphogenetic protein (AMP), fibroblast morphogenetic protein (FMP), SIF, Leukemia inhibitory factor (LIF)-like (inhibitory) activity, and antidifferentiation factor (ADF)- like (inhibitory) activity. Additional morphogenetic protein-like activity isolated from sera were blood vessel morphogenetic protein (BVMP), scar fibroblast morphogenetic protein (ScFMP), brain morphogenetic protein (BrnMP), liver morphogenetic protein (LivMP), lung morphogenetic protein (LngMP), pancreas morphogenetic protein (PanMP), keratinocyte morphogenetic protein (KerMP), cardiac morphogenetic protein (CdMMP), tendon morphogenetic protein (TenMP), and ligament morphogenetic protein (LigMP) [2,51-53]. In addition to biological agents identified from demineralized bone matrix and sera, we also generated cell-specific exosome-conditioned media utilizing cultured explants from the following tissues, e.g., osteoblasts/osteocytes, chondroblasts/chondrocytes, adipoblasts/adipocytes, blood vessels, fibroblasts/fibrocytes, brain, liver, lung, pancreas, keratinocytes, Sertoli cells, skeletal muscle myoblasts/myocytes, smooth muscle myoblasts/myocytes, cardiac muscle myoblasts/myocytes, tendon, and ligaments [3]. Utilizing these sources, four categories of biological agents were identified: Anti-Differentiating Agents, Proliferative Agents, Progression Agents, and Inducing Agents (Table 2). As shown, there were distinct similarities and differences with respect to clones of aTPSCs versus the clone of MSCs in their response to these biological agents (Fig. 1, Table 3).

Table 3. Similarities and Differences between aTPSC clones and 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., for rodents = 6-8 population doublings [54] before cell senescence and cell death; 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, PSCs will form all cells similar to inner cell mass, 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 bone, cartilage, and fat cells.

Table 3 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

6. Conclusion

The hypothesis tested was that ATPSCS and MSCs react both similarly and differently to biological activities inherent in the body, e.g., anti-differentiation, proliferation, progression, and induction. The results demonstrate that aTPSCs and MSCs reacted similarly with respect to anti-differentiative agents and proliferative agents, but differently with respect to progressive agents and inductive agents.

Compliance with ethical standards

Acknowledgments

This work was supported by grants from Rubye Ryle Smith Charitable Trust, Dragonfly Foundation for Research and Development, MedCen Community Health Foundation, and MorphoGen Pharmaceuticals, Inc. I like to thank my long-time collaborator Dr. Asa C. Black Jr for his mentorship with respect to teaching and research; my clinical collaborators; research associates and assistants, e.g., J Floyd-Collins-Coleman, GF Long-Black, NL Henson, LW Blake, KC Hawkins, N Walsh, C Alena, MBL Cole, V Krishna, S Ellis, J Hudson, WJ Butler, PE Kross, J Wang, J Wong, D Hixson, and C Duplaa; my multitude of co-authors, clinical attendings, clinical residents, foreign and domestic graduate students, medical students, undergraduate students, and a high school student, for their insight, strong work ethic, and help in conducting this research. The antibody CEA-CAM-1 was generously provided by D. Hixson (Providence, RI). The antibody to SSEA-4 was obtained from the Developmental Studies Hybridoma Bank under the auspices of the NICHD and maintained at the University of Iowa, Department of Biological Sciences, Iowa City, IA. MC813 (SSEA-4) antibody was developed by D. Solter. Leukemia Inhibitory Factor (LIF), Platelet-derived Growth Factor-AA (PDGF-AA), Platelet-derived Growth Factor-AB (PDGF-AB), Platelet-derived Growth Factor-BB (PDGF-BB), Acidic fibroblast growth factor (a-FGF), Basic fibroblast growth factor (b-FGF), c-Kit (c-Kit), and Interleukin-6 (IL-6) were the kind gifts of R&D Systems. Insulin-Like Growth Factor-1 (IGF-1) and Insulin-Like Growth Factor-2 (IGF-2) were generously provided by Cell Sciences. Bone morphogenetic protein-2 (BMP-2) and Bone morphogenetic protein-4 (BMP-4) were the kind gifts of Genetics Institute. Vascular endothelial growth factor (VEGF), nerve growth factor (NGF), hepatocyte growth factor (HGF), and Transforming growth factor beta (TGF-b) were the kind gifts from Genentech.

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.

References

[1] Young HE, Speight MO, Black AC Jr. Functional Cells, Maintenance Cells, and Healing Cells. J Stem Cell Res. 2017; 1(1): 003: 1-4.

[2] Young HE, Duplaa C, Romero-Ramos M, Chesselet M-F, Vourc'h P, Yost MJ, Ericson K, Terracio L, Asahara T, Masuda H, Tamura-Ninomiya S, Detmer K, Bray RA, Steele TA, Hixson D, El-Kalay M, Tobin BW, Russ RD, Horst MN, Floyd JA, Henson NL, Hawkins KC, Groom J, Parikh A, Blake L, Bland LJ, Thompson AJ, Kirincich A, Moreau C, Hudson J, Bowyer III FP, Lin TJ, Black Jr AC. Adult reserve stem cells and their potential for tissue engineering. Cell Biochem Biophys. 2004; 40(1):1-80.

[3] Young HE. Adult telomerase positive stem cells: Cell-Specific Exosome-Conditioned Medium, Repetitive Single Cell Clonogenic Analysis, and Genomic Labeling. GSC Advanced Research and Reviews. 2025; 25(2): 480-504.

[4] Wozney J. The Bone morphogenetic protein family and osteogenesis. Molec Repro Dev. 1992; 32(2): 160-167.

[5] Wozney JM. Bone morphogenetic proteins. Prog Growth Fac Res. 1989; 1(4): 267-280.

[6] Wozney JM. Bone morphogenetic proteins and their gene expression. Cell Molec Biol Bone. 1993; 131-167.

[7] Shore EM, Xu M-Q, Shah PB, Janoff HB, Hahn GV, Sovinsky L, Spinner NB. The human bone morphogenetic protein 4 (BMP-4) gene: molecular structure and transcriptional regulation. Calcified Tissue Intl. 1998; 63(3): 221-229.

[8] Kumar TRS, Krishnan LK. Endothelial cell growth factor (ECGF) enmeshed in fibrin matrix enhances proliferation of EC in vitro. Biomaterials. 2001; 22(20): 2769-2776.

[9] Burgess WH, Mehlman T, Friesel R, Johnson WV, Maciag TH. Multiple forms of endothelial cell growth factor. Rapid isolation and biological and chemical characterization. J Biol Chem. 1985; 260(21): 11389-11392.

[10] Cross MJ, Claesson-Welsh L. FGF and VEGF function in angiogenesis: signaling pathways, biological responses and therapeutic inhibition. Trends Pharmacol Sci. 2001; 22(4): 201-207.

[11] Poole TJ, Finkelstein EB, Cox CM. The role of FGF and VEGF in angioblast induction and migration during vascular development. Develop Dynamics. 2001; 220(1): 1-17.

[12] Branton MH, Kopp JB. TGF-b and fibrosis. Microbes Infection. 1999; 1(15): 1349-1365;

[13] Meng X-M, Nikolic-Paterson DJ, Lan HY. TGF-b: master regulator of fibrosis. Nature Rev Nephrology. 2016; 12(6): 325-338.

[14] Seitz T, Hellerbrand C. The role of fibroblast growth factor signaling in hepatic fibrosis. Liver Intl. 2021; 41(6): 1201-1215.

[15] Gonzalez A-M, Buscaglia M, Fox R, Ischii A, Sarmientos P, Farris J, et al. Basic fibroblast growth factor in Dupuytren's contracture. Amer J Path. 1992; 141(3): 661.

[16] Bhoopalan SV, Huang LJ-S, Weiss MJ. Erythropoietin regulation of red blood cell production: from bench to bedside and back. F1000 Research 9. 2020; F1000 Faculty Rev: 1153.

[17] Koury MJ, Bondurant MC. Control of red cell production: the roles of programmed cell death (apoptosis) and erythropoietin. Transfusion. 1990; 30(8): 673-674.

[18] Klinken SP. Red blood cells. Intl J Biochem Cell Biol. 2002; 34(12): 1513-1518.

[19] Munugalavadia V, Kapur R. Role of c-Kit and erythropoietin receptor in erythropoiesis. Critical Rev Oncol Hematol. 2005; 54(1); 63-75.

[20] Wessely O, Bauer A, Quang CT, Deiner E-M, Von Lindern M, et al. A novel way to induce erythroid progenitor self renewal: cooperation of c-Kit with the erythropoietin receptor. Biol Chem. 1999; 380 (2): 187-202.

[21] Sui X, Tsuji K, Tajima S, Tanaka R, Muraoka K, et al. Erythropoietin-independent erythrocyte production: Signals through gp 130 and c-Kit dramatically promote erythropoiesis from human CD34+ cells. J Exper Med. 1996; 183(3): 837-845.

[22] Ulich TR, del Castillo J, Guo KZ. In vivo hematologic effects of recombinant interleukin-6 on hematopoiesis and circulating numbers of RBCs and WBCs. Blood. 1989; 73(1): 108-110.

[23] Lindholm D, Castren E, Hengerer B, Zafra F, Berninger B, et al. Differential regulation of nerve growth factor (NGF) synthesis of neurons and astrocytes by glucocorticoid hormones. Euro J Neurosci. 1992; 4(5): 404-410.

[24] Cameron HA, McKay RDG. Restoring production of hippocampal neurons in old age. Nature Neurosci. 1999; 2(10: 894-897.

[25] Johnson M, Koukoulis G, Matsumoto K, Nakamura T, Iyer A. Hepatocyte growth factor induces proliferation and morphogenesis in nonparenchymal epithelial liver cells. Hepatology. 1993; 17(6): 1052-1061.

[26] Fausto N. Growth factors in liver development, regeneration, and carcinogenesis. Progress Growth Fac Res. 1991; 3(3): 219-234.

[27] Wozney J. The Bone morphogenetic protein family and osteogenesis. Molec Repro Dev. 1992; 32(2): 160-167.

[28] Wozney JM. Bone morphogenetic proteins. Prog Growth Fac Res. 1989; 1(4): 267-280.

[29] Wozney JM. Bone morphogenetic proteins and their gene expression. Cell Molec Biol Bone. 1993; 131-167.

[30] Shore EM, Xu M-Q, Shah PB, Janoff HB, Hahn GV, Sovinsky L, Spinner NB. The human bone morphogenetic protein 4 (BMP-4) gene: molecular structure and transcriptional regulation. Calcified Tissue Intl. 1998; 63(3): 221-229.

[31] Young HE. Endogenous Adult Telomerase Positive Stem Cells and/or Combinatorial Nutraceuticals, 50 Years in the Making, 50 Years of Discovery; In preparation

[32] Young HE. A high throughput screening assay to quantify, visualize, and standardize biological activities: Enzyme-Linked Immuno-Culture Assay (ELICA). GSC Advanced Research and Reviews. 2025; 24(02): 091-114.

[33] Young HE, Sippel J, Putnam LS, Lucas PA, Morrison DC. Enzyme-linked immuno-culture assay. Journal of Tissue Culture Methods, 14:31-36, 1992.

[34] Humason G. Animal Tissue Techniques. WH Freeman and Co., San Francisco, CA.

[35] Young HE. Adult telomerase positive stem cells: isolation, plating, and propagation. GSC Advanced Research and Reviews. 2025; 25(2): 407-440.

[36] Young HE, Black AC. Pluripotent Stem Cells, Endogenous versus Reprogrammed, a Review. MOJ Orthop Rheumatol. 2014; 1(4): 00019.

[37] Young HE. Carcinoembryonic antigen-cell adhesion molecule-1 and stage-specific embryonic antigen-4 are present in the reproductive organs of adult mammals. GSC Advanced Research and Reviews. 2025; 23(03): 149-157.

[38] 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.

[39] Urist, MR. (1965) Bone: formation by autoinduction. Science. 1965; 150: 893–899.

[40] Hauschka PV, Mavrakos AE, Iafrati MD, Doleman SE, and Klagsbrun M. Growth factors in bone matrix: isolation of multiple types by affinity chromatography on heparin-Sepharose. J Biol Chem. 1986; 261: 12665–12674.

[41] Linkhart TA, Jennings JC, Mohan S, Wakley GK, Baylink DJ. Characterization of mitogenic activities extracted from bovine bone matrix. Bone. 1986; 7(6): 479–487.

[42] Canalis E, McCarthy T, Centrella M. Growth factors and the regulation of bone remodeling. J Clin Invest. 1988; 81: 277–281.

[43] Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, et al. Novel regulators of bone formation: molecular clones and activities. Science. 1988; 242: 1528–1534.

[44] Urist, MR. Bone morphogenetic protein, bone regeneration, heterotopic ossification, and the bone-bone marrow consortium. In Bone and Mineral Research (Peck, W. A., ed.). Elsevier, New York, 1989, vol. 6, pp. 57–112.

[45] Wang EA, Rozen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, et al. Recombinant human bone morphogenetic protein induces bone formation (cartilage induction). Proc Natl Acad Sci USA. 1990; 87: 2220–2224.

[46] Syftestad, G. T., Lucas, P. A., and Caplan, A. I. (1985) The in vitro chondrogenic response of limb-bud mesenchyme to a water-soluble fraction prepared from demineralized bone matrix. Differentiation. 1985; 29: 230–237.

[47] Frolik CA, Ellis F, Williams C. Isolation and characterization of insulin-like growth factor-II from human bone. Biochem Biophys Res Commun. 1988; 151: 1011–1018.

[48] Young HE, Lucas PA. (1998) Pluripotent mesenchymal stem cells and methods of use thereof. US Patent No. 5,827,735, 1988.

[49] Young HE, Wright RP, Mancini ML, Lucas PA, Reagan CR, Black Jr AC. Bioactive factors affect proliferation and phenotypic expression in pluripotent and progenitor mesenchymal stem cells. Wound Rep Reg. 1998; 6: 65–75.

[50] Lucas PA, Young HE, Laurencin CT. Muscle morphogenetic protein and use thereof. US Patent No. 5,328,695, 1994.

[51] Young HE, Lucas PA. Pluripotent Embryonic-Like Stem Cells, Compositions, Methods, and Uses Thereof: a cell having the capability of extended self-renewal and the ability to form multiple ectodermal, mesodermal and endodermal lineages in vivo and in vitro. US Patent No. 9,404,895, 2003.

[52] Young HE. Non-Embryonic Totipotent Blastomere-Like Stem Cells and Uses Thereof. US Patent No. 9,616,515, 2017.

[53] Young HE. Method and System for Repairing Damaged Tissue Using Nucleated Plasma Particles (Nuc-P2s) and Mesodermal Stem Cells (MesoSCs); US Patent No. 10,213,465, 2019.

[54] Rohme D. Evidence for a relationship between longevity of mammalian species and life spans of normal fibroblasts in vitro and erythrocytes in vivo. Proc Natl Acad Sci USA 1981; 78: 5009-5013.