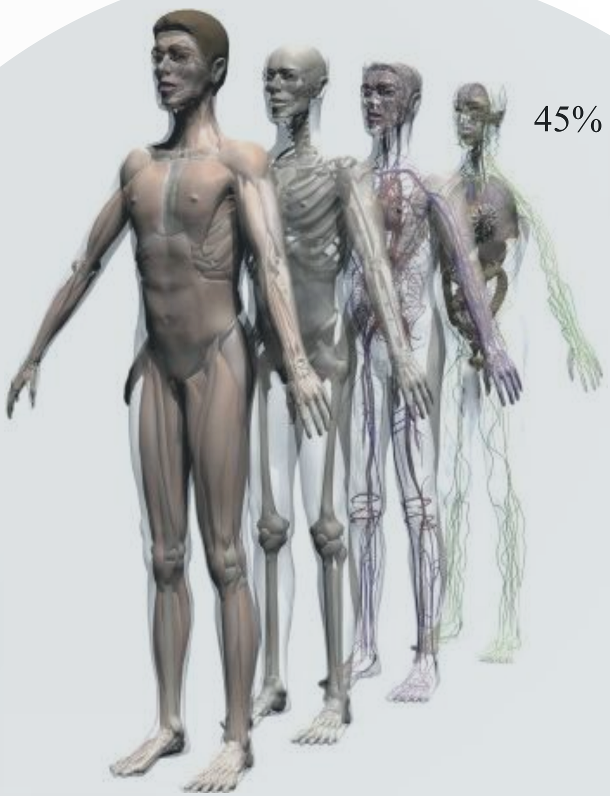
Epigenetics in Human Adult Stem Cell Aging

Victoria Lunyak, PhD
Buck Institute for Research on Aging

Human Body is in a State of Constant Flux

50-70 million cells are discarded and new ones are generated **daily**



Heart
45% through the life

Epidermus
4 weeks

Gut
15.9 years

Muscle
15.1 years

Intestinal epithelial cells
5 days

Fat cells
10% a year

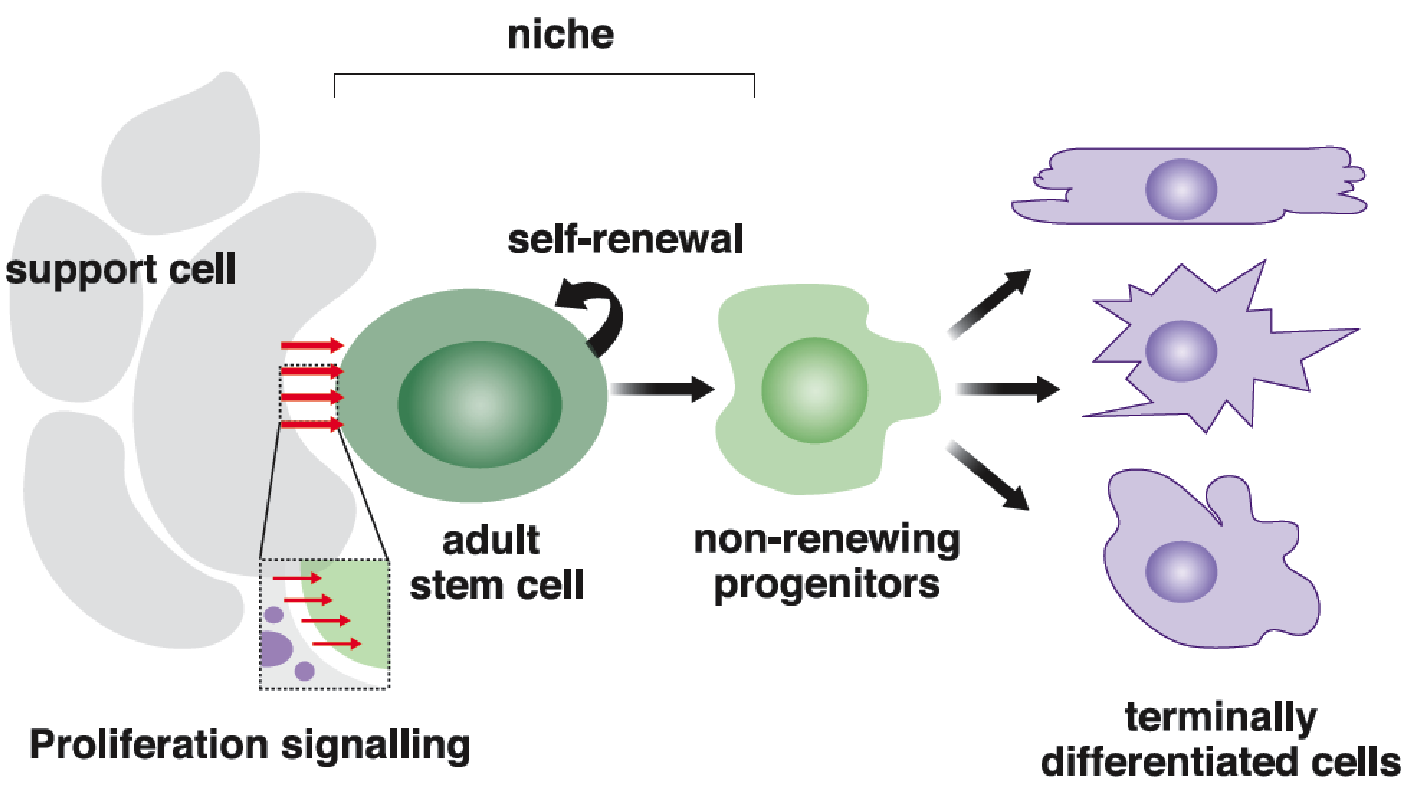
Liver cells
300-500 days

Human skeleton
10 years

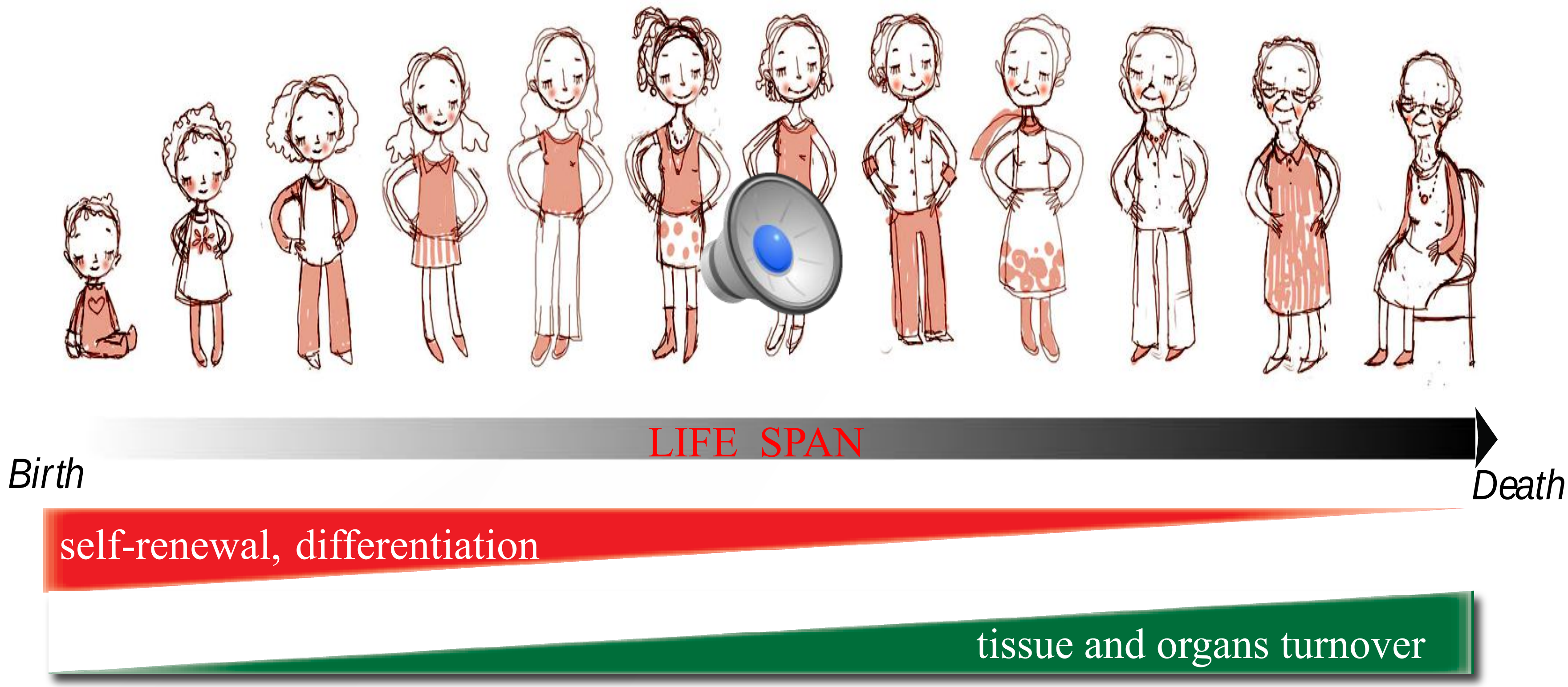
Red blood cells
2 weeks

Frisen et. al Cell, 2005
Science, 2009

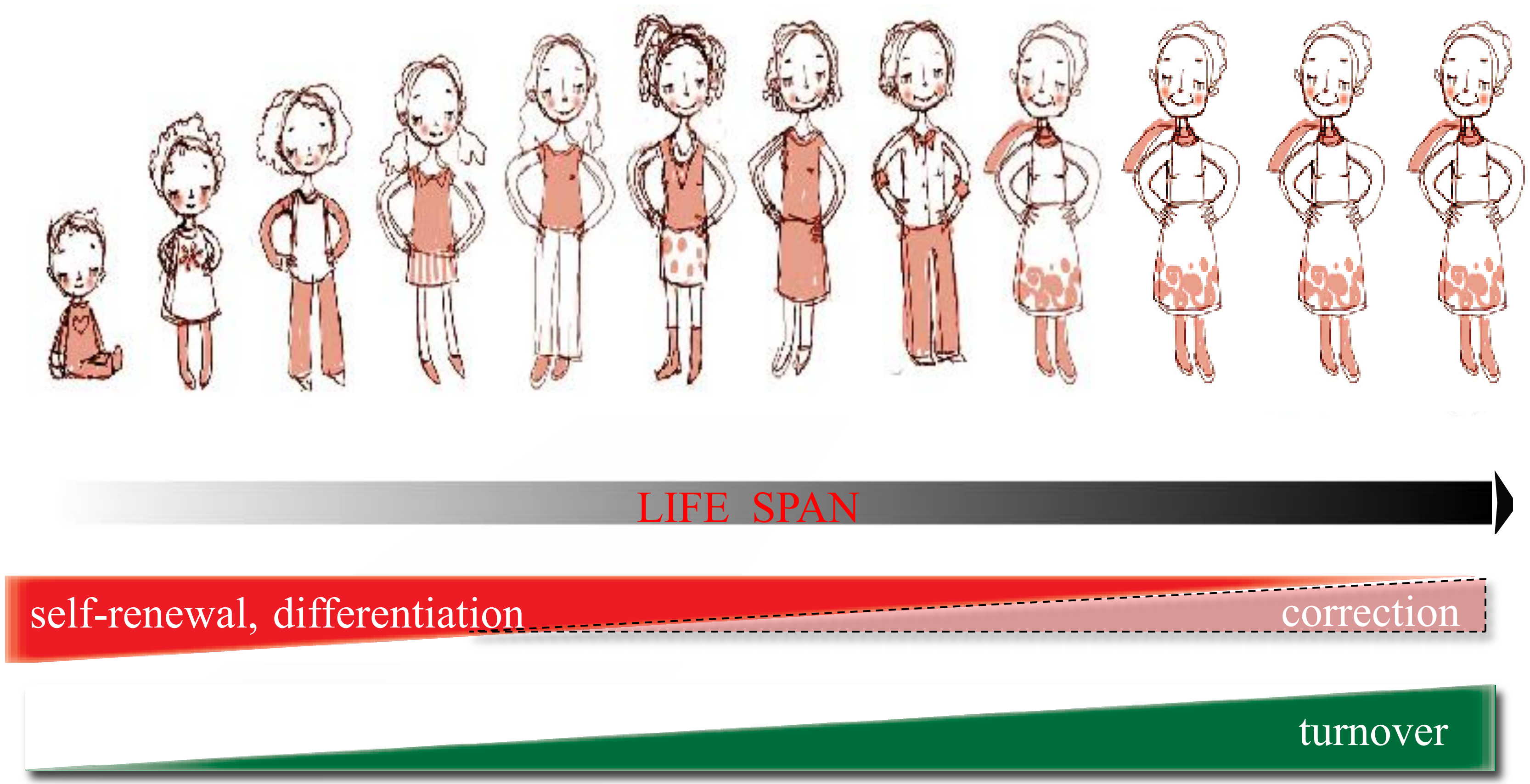
Adult Stem Cells and Niche Concept



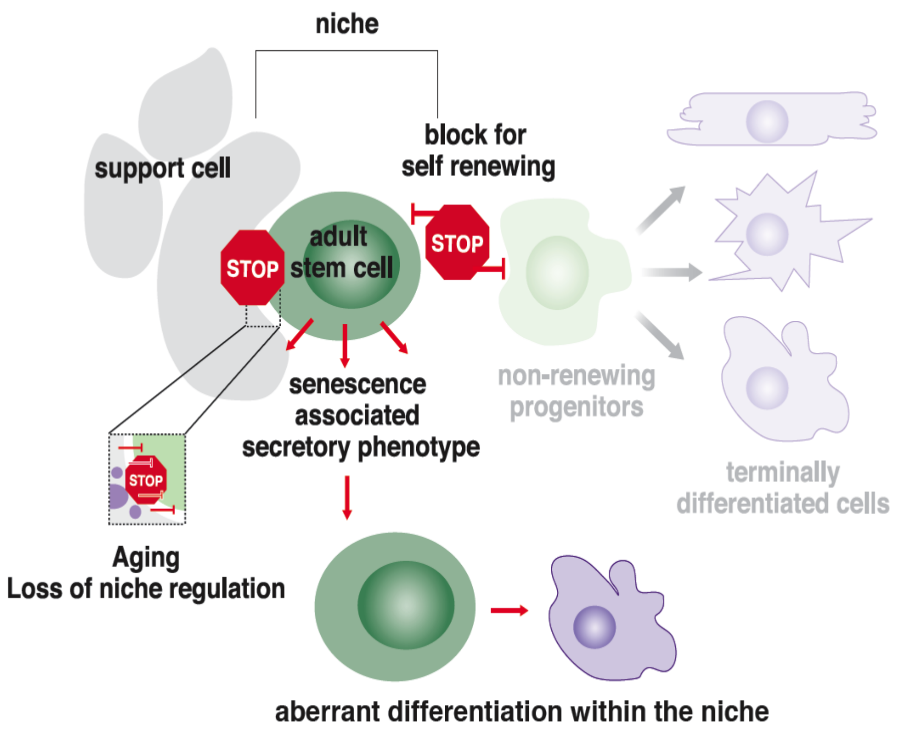
Adult Stem Cells and Aging Paradigm



Correction of Aging Pathologies with Rejuvenated Adult Stem Cells



Senescence of Adult Stem Cells



Oxidative Stress

Accumulation of DNA Damage

Shortened Telomeres

Loss of Epigenetic Regulation

Altered Signaling Within Niche

Are these changes reversible?

Integrated Approach for Stem Cells Study

Epigenomics
ChIPseq
DNA Metseq

Transcriptomics
RNAseq

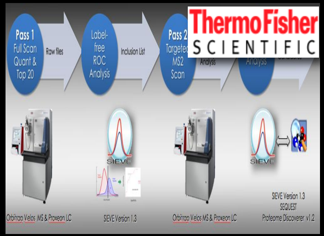
Proteomics
Protein Biomarkers
Post Translational Modification (PTMs)

Clinical application

NG DATA GENERATION

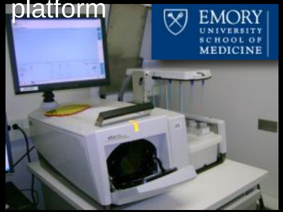
SOLID III NGS platform

ChIPseq
DNA Metseq



Proteomics
Histone PTMs

ROSHE 454 NGS platform

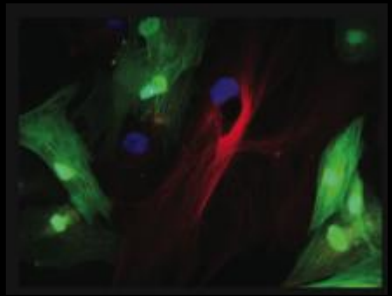


RNAseq

miRNA, lnc RNA
protein coding RNA

COMPUTATIONAL ANALYSIS

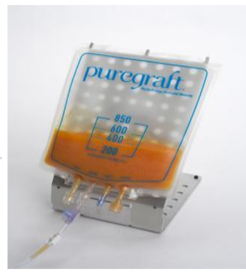
FUNCTIONAL STUDY



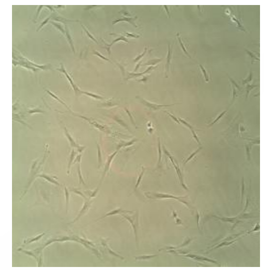
Isolation and Maintenance of Human Adipose Tissue Derived Stem Cells (ADSC)

fat tissue extraction

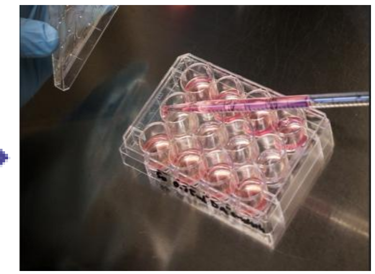
aspirated fat

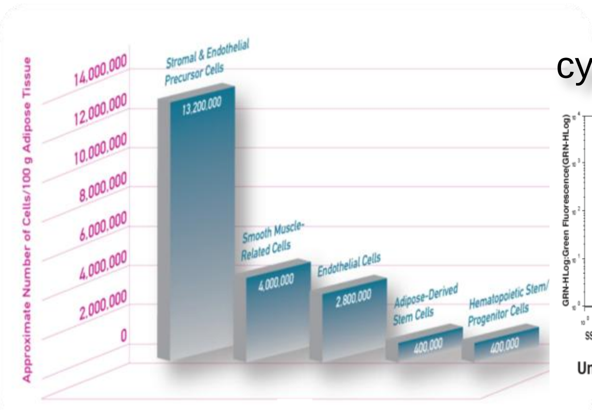


stem cells isolation

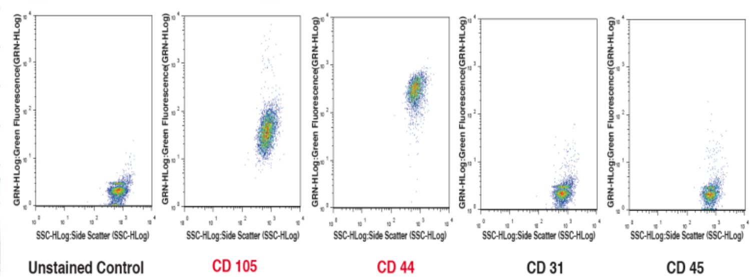


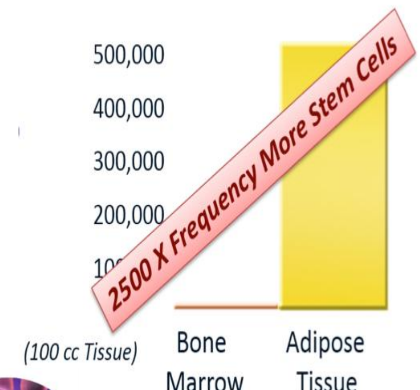
cultured ADSC

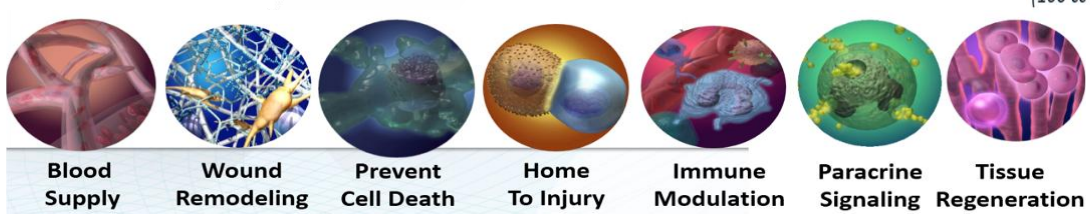




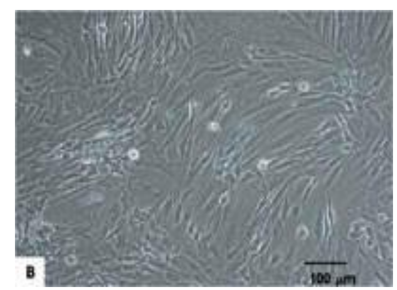
cytometric analysis of isolated ADSC







Isolation and Maintenance of Human Adipose Tissue Derived Stem Cells (ADSC)



Adipose Tissue Derived Stem Cells (ADSCs) are Important Regulators of Metabolic, Immunomodulatory,Inflamatory and Angiogenic Pathways

1264 publications from 2006-2011 based on Pubmed

NATURE | ARTICLE

◂ previous article next article ▸

CRTC3 links catecholamine signalling to energy balance

Youngsup Song, Judith Altarejos, Mark O. Goodarzi, Hiroshi Inoue, Xiuqing Guo, Rebecca Berdeaux, Jeong-Ho Kim, Jason Goode, Motoyuki Igata, Jose C. Paz, Meghan F. Hogan, Pankaj K. Singh, Naomi Goebel, Lili Vera, Nina Miller, Jinrui Cui, Michelle R. Jones, CHARGE Consortium, GIANT Consortium, Yii-Der I. Chen, Kent D. Taylor, Willa A. Hsueh, Jerome I. Rotter & Marc Montminy

Nat Protoc. 2011 March ; 6(3): 346–358. doi:10.1038/nprot.2010.199.

Feeder-independent iPS cell derivation from human and mouse adipose stem cells

Shigeki Sugii[1,2,4], Yasuyuki Kida[2,4], W. Travis Berggren[3], and Ronald M. Evans[1,2]
[1]Howard Hughes Medical Institute, La Jolla CA 92037, USA
[2]Gene Expression Laboratory, La Jolla CA 92037, USA
[3]Stem Cell Core, The Salk Institute for Biological Studies, La Jolla CA 92037, USA

Experimental Cell Research
Volume 314, Issue 3, 1 February 2008, Pages 603-615
doi:10.1016/j.yexcr.2007.10.007 | How to Cite or Link Using DOI
Permissions & Reprints
Research Article

Stem molecular signature of adipose-derived stromal cells

Daniele Peroni[a], Ilaria Scambi[a], Annalisa Pasini[b], Veronica Lisi[b], Francesco Bifari[b], Mauro Krampera[b], Gino Rigotti[c], Andrea Sbarbati[a] and Mirco Galiè[a]

Review

Nature Reviews Endocrinology **6**, 195-213 (April 2010) | doi:10.1038/nrendo.2010.20

Transplantation of adipose tissue and stem cells: role in metabolism and disease

Thien T. Tran & C. Ronald Kahn About the authors

Concise Review: Adipose-Derived Stromal Cells for Skeletal Regenerative Medicine[†‡§]

Benjamin Levi[1], Michael T. Longaker[1,2,*,¶]

Article first published online: 5 APR 2011
DOI: 10.1002/stem.612

Issue

STEM CELLS
Volume 29, Issue 4, pages 576–582, April 2011

OPEN ACCESS Freely available online PLoS one

Adipose-Derived Stem Cells Stimulate Regeneration of Peripheral Nerves: BDNF Secreted by These Cells Promotes Nerve Healing and Axon Growth *De Novo*

Tatiana Lopatina[1*], Natalia Kalinina[1], Maxim Karagyaur[1], Dmitry Stambolsky[1], Kseniya Rubina[1], Alexander Revischin[2], Galina Pavlova[2], Yelena Parfyonova[1], Vsevolod Tkachuk[1]

Human Adipose Tissue Is a Source of Multipotent Stem Cells

Patricia A. Zuk,[*†] Min Zhu,[*] Peter Ashjian,[*] Daniel A. De Ugarte,[*] Jerry I. Huang,[*] Hiroshi Mizuno,[*] Zeni C. Alfonso,[‡] John K. Fraser,[‡] Prosper Benhaim,[*] and Marc H. Hedrick[*]

[*]Departments of Surgery and Orthopedics, Regenerative Bioengineering and Repair Laboratory, UCLA School of Medicine, Los Angeles, California 90095; and [†]Department of Medicine and the Jonsson Comprehen
Angeles, California 90095

Submitted February 25, 2002; Revised June 21, 2002; Accepted August 23, 2002
Monitoring Editor: Martin Raff

OPEN ACCESS Freely available online PLoS one

Genome-Wide Profiling of MicroRNAs in Adipose Mesenchymal Stem Cell Differentiation and Mouse Models of Obesity

Lena Bengestrate[1¤], Sam Virtue[2], Mark Campbell[2], Antonio Vidal-Puig[2], Dirk Hadaschik[1], Peter Hahn[1], Wolfgang Bielke[1*]

Nature Cell Biology **7**, 601 - 611 (2005)
Published online: 15 May 2005 | doi:10.1038/ncb1259

Prediction of preadipocyte differentiation by gene expression reveals role of insulin receptor substrates and necdin

Yu-Hua Tseng[1,5], Atul J. Butte[2,4,5], Efi Kokkotou[1], Vijay K. Yechoor[1], Cullen M. Taniguchi[1], Kristina M. Kriauciunas[1], Aaron M. Cypess[1], Michio Niinobe[3], Kazuaki Yoshikawa[3], Mary Elizabeth Patti[1] & C. Ronald Kahn[1]

Clinical Trials With Human Adipose Tissue Derived Stem Cells (ADSC)

Condition	Delivery of ASCs	End points	Design	Follow-up	Patients	Site or company
I. Reconstructive surgery						
Lumpectomy (RESTORE-2)	Transplantation of autologous ASCs to reconstruct breast deformities	Cosmetic and functional results of reconstructive surgery	Phase IV non-randomized	1 year	70	Cytori Therapeutics Inc.
Cell-assisted lipotransfer strategy	Transplantation of lipoaspirates with additional ASCs from a separate volume of lipoaspirates	Cosmetic and functional results of reconstructive breast surgery and facial remodeling	NA	NA	400	University of Tokyo
Renal failure (vesico-ureteral reflux)	Transplantation of autologous adipocytes to treat defective volume	Radiography of urethra and bladder. Presence of kidney or ureter infection	Phase III non-randomized	10 years	14	Strasbourg University Hospital
Perianal fistulas without Crohn disease (FATT-1)	Fibrin adhesive only, autologous ASCs only, or fibrin adhesive with autologous ASCs during surgery	Closure of fistulas (abnormal connection between structures)	Phase III randomized double blinded	26 weeks and 6 months	214 and 150	Cellerix Ltd
Complex perianal fistula	Fibrin adhesive only or fibrin adhesive with autologous ASCs from lipoaspirates	Closure of fistulas Recurrence of fistulas	Phase II randomized	1 year	50	Cellerix Ltd
Diabetic lower extremity & venous stasis wounds	Subcutaneous injection of lipoaspirate into wounds	Wound healing	Phase I/II randomized single blinded	1 year	250	Washington D.C. Veterans Affairs Medical Center
Depressed scar	Subcutaneous injection of autologous cultured adipocytes	Cosmetic and safety	Phase II/III interventional single group	12 weeks	36	Anterogen Co. Ltd

Tran TT and Kahn CR. Nat Rev Endocrinol. 2010 Apr;6(4):195-213.

Clinical Trials With Human Adipose Tissue Derived Stem Cells (ADSCs)

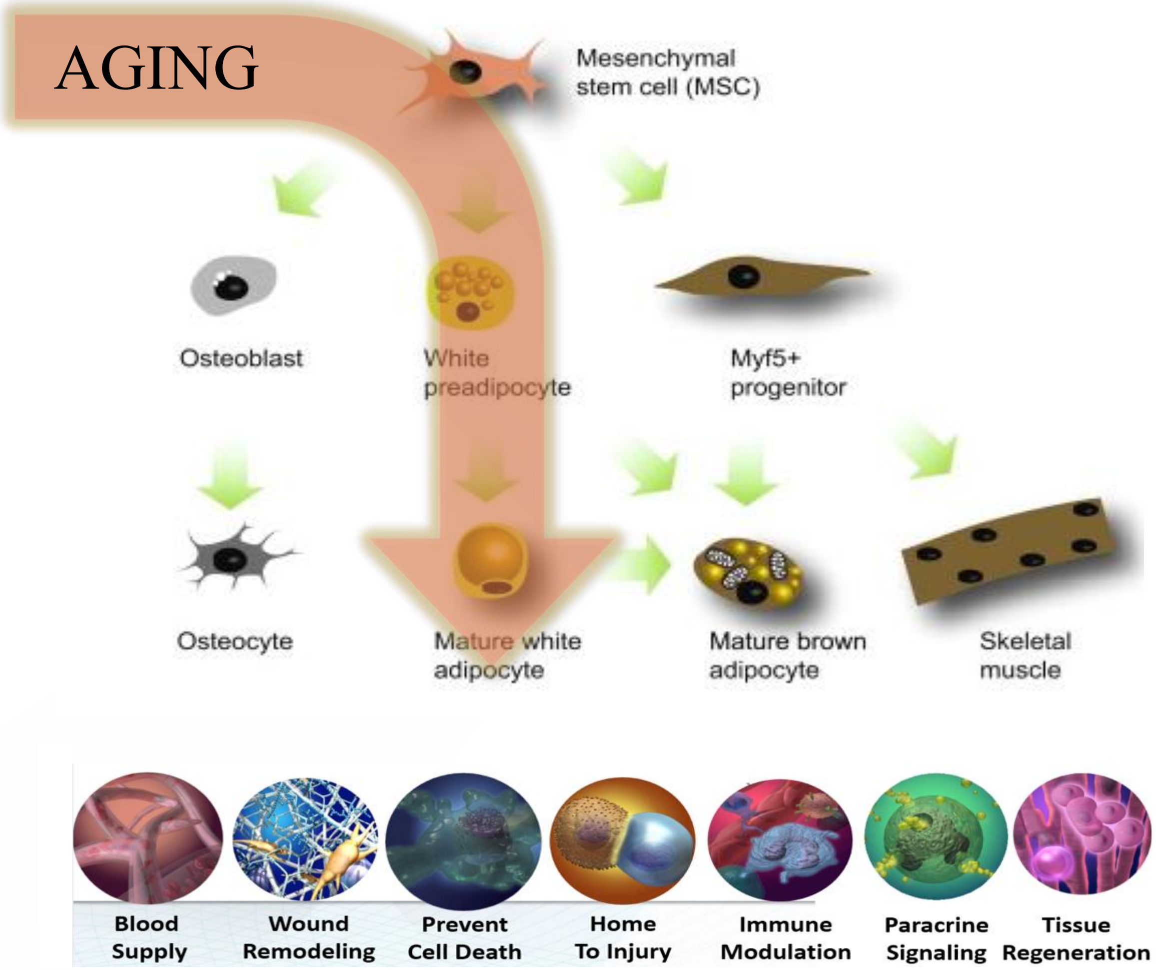
Condition	Delivery of ASCs	End points	Design	Follow-up	Patients	Site or company
II. Metabolic						
Non-revascularizable ischemic myocardium (PRECISE-01)	Injection of autologous ASCs or placebo into left ventricle	Cardiac function, major adverse cardiac and cerebral events	Phase I randomized double blinded placebo	3 years	36	Cytori Therapeutics Inc.
Acute myocardial infarction and coronary artery disease (APOLLO)	Injection of autologous ASCs or placebo	Cardiac function, major adverse cardiac and cerebral events	Phase I randomized double blinded placebo	6 months	48	Cytori Therapeutics Inc.
Lipodystrophy (AADSCTPL)	Transplantation of autologous lipoaspirate enriched with ASCs	Clinical evaluation of transplanted area. Tissue viability, neovascularization, degree of resorption of adipose tissue graft	Phase I interventional single group	1 year	10	Hospital Irmandade Santa Casa de Misericordia de Porto Alegre
Survivors of leukemia or lymphoma	Transplantation of ASCs after total body irradiation versus no treatment	Abdominal MRI, blood pressure, cholesterol, presence of diabetes	Observational prospective	1 year	60	Memorial Sloan-Kettering Cancer Center

Very promising source of cells for autologous therapies with one limitation- age of patient!

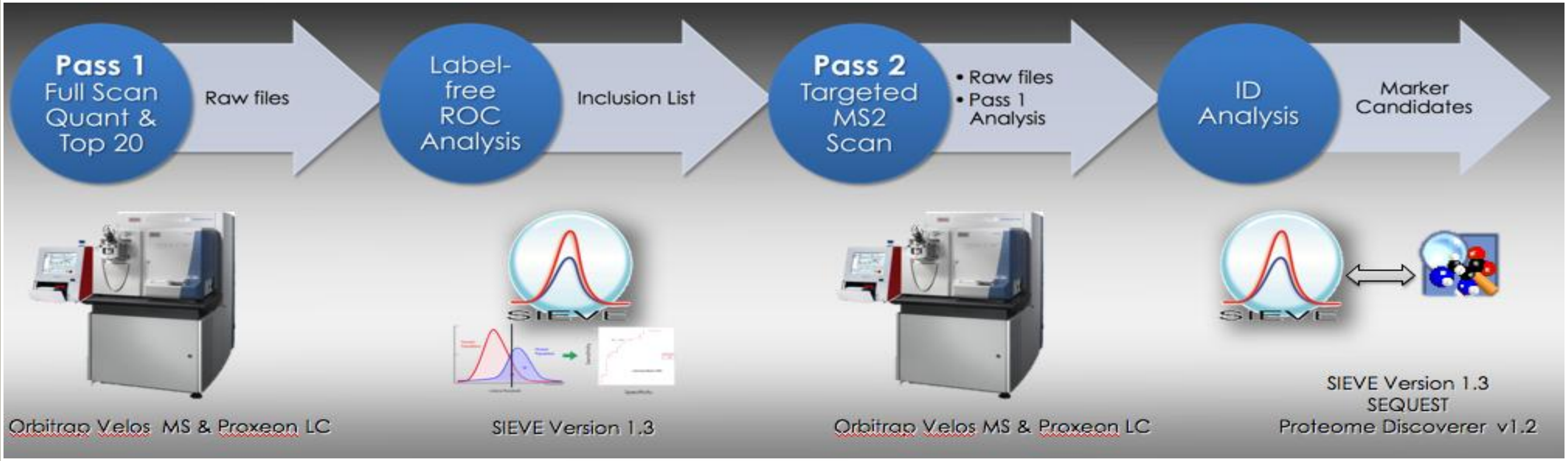
Expansion and Differentiation capacity of ADSCs declines with age.

Tran TT and Kahn CR. Nat Rev Endocrinol. 2010 Apr;6(4):195-213.

MSCs lineage Commitment and Differentiation



Discovery-driven LS-MS Pipeline For Identification of Novel Post-translational Protein Modifications in Senescent Human ADSCs



Comparative Analysis of hADSCs

self-renewal *vrs* replicative senescence

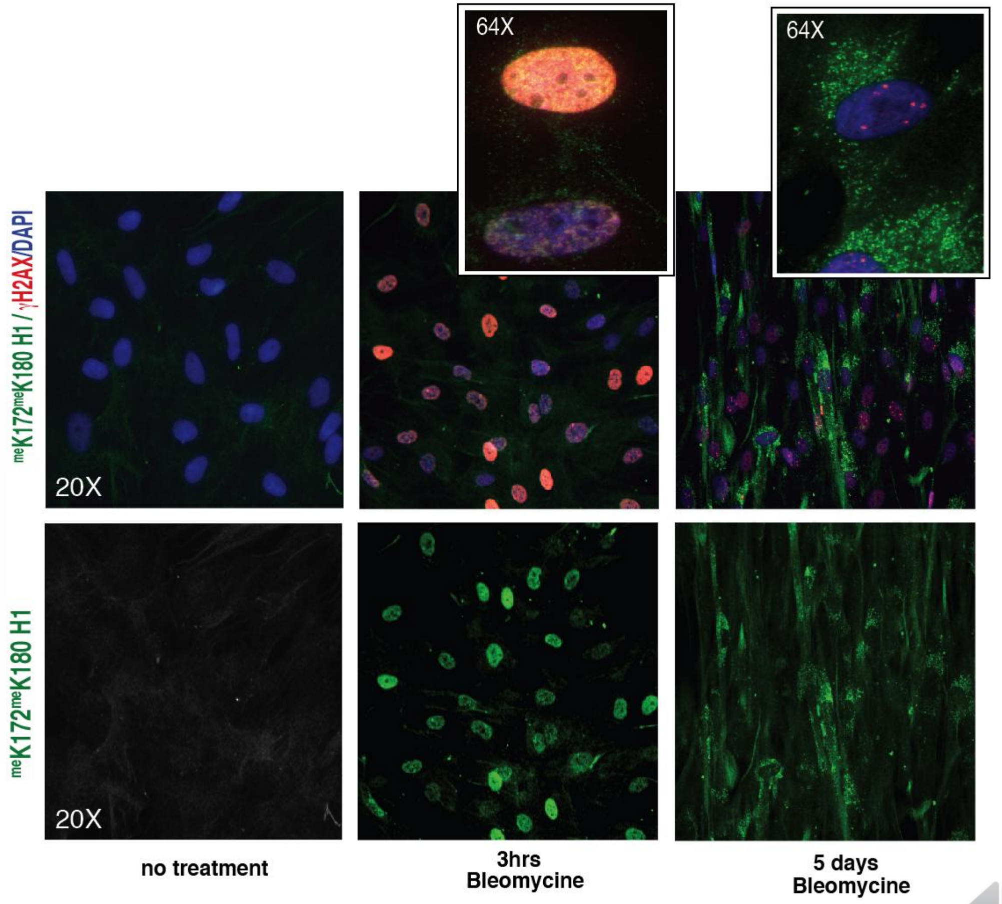
4 different genotypes

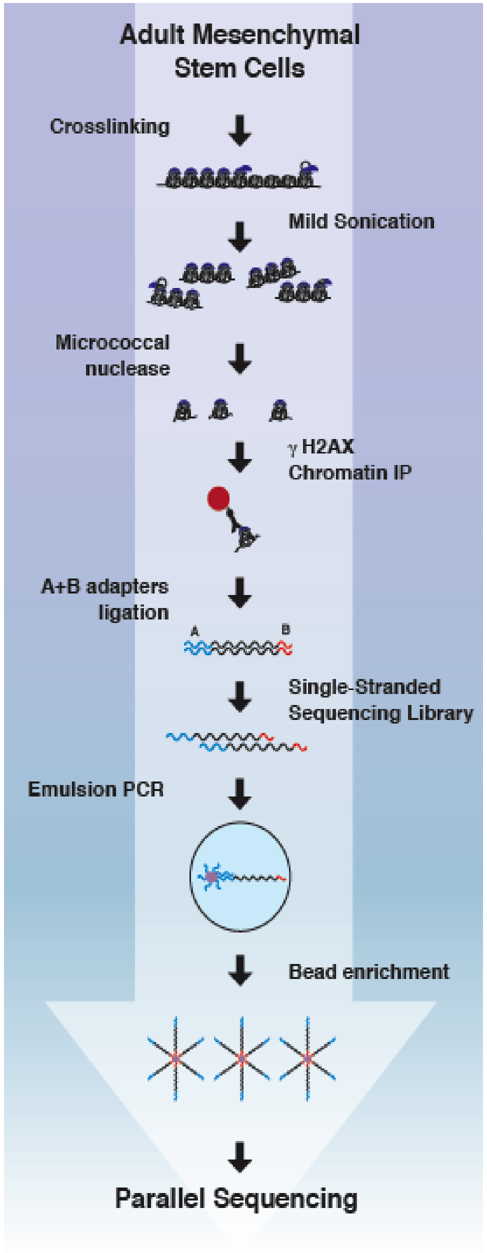
36 NOVEL Histone PTMS associated with *ex-vivo* stem cell aging

Eviction of metH1.0 from Chromatin Upon Genotoxic Stress Induced Senescence of hADSCs

Are novel histone H1.0 PTMs better markers of cellular aging?

On going studies include:
-IHC of the old mice, rat tissue
-Biopsy samples
- Analysis of progeria models





Genome-wide Mapping of DNA Damage (CHiP-Seq) in ADSC

Two genotypes

4 samples of self-renewing (SR) vrs 4 samples of senescent (SEN) ADSCs

Sequencing platform: SOLiD

Sequencing read 35bp

Depth of sequencing 120 million tags

Algorithms for assembly:

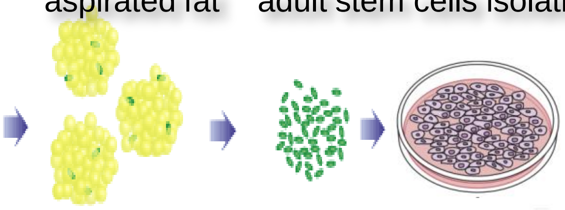
MAQ and Gibbs sampling

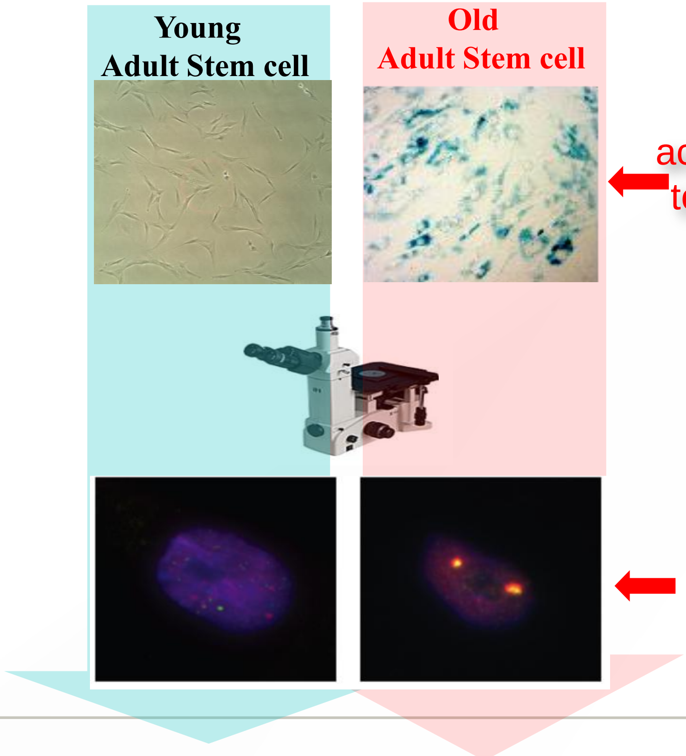
Aging of human adult stem cells

fat tissue extraction

aspirated fat

adult stem cells isolation

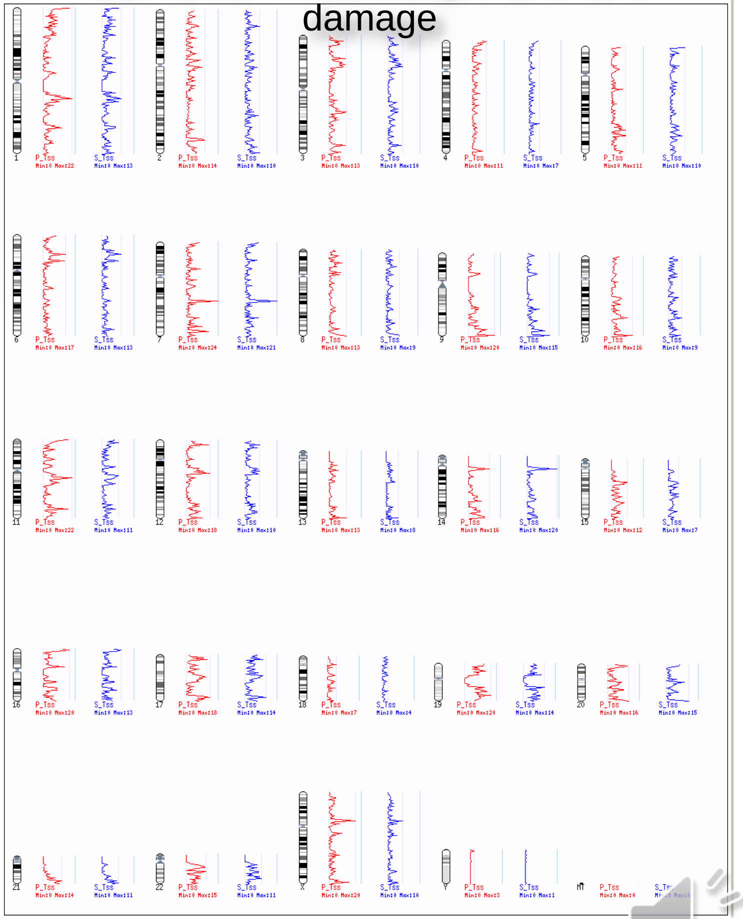




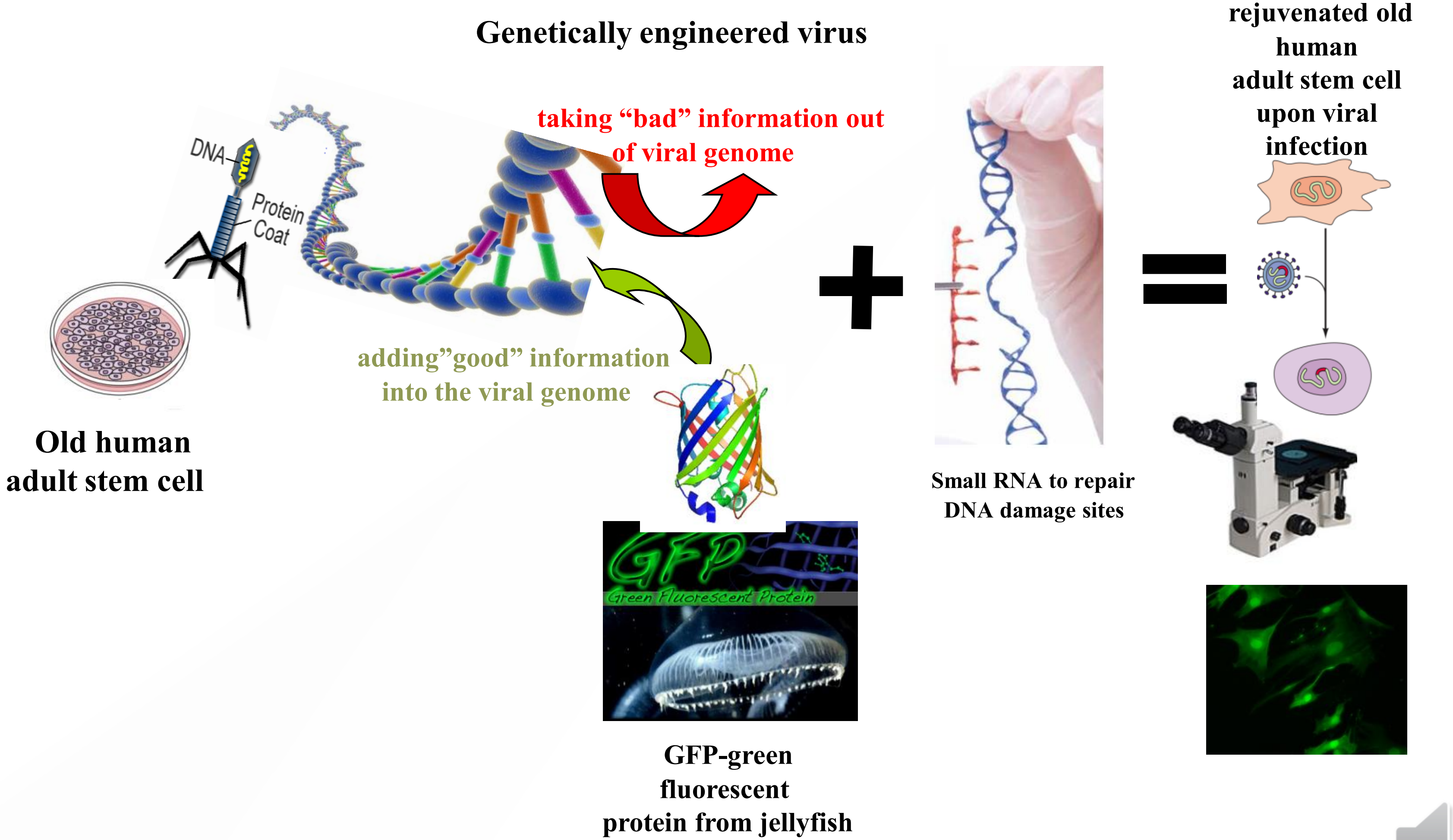
accumulation of toxic products

DNA damage accumulation

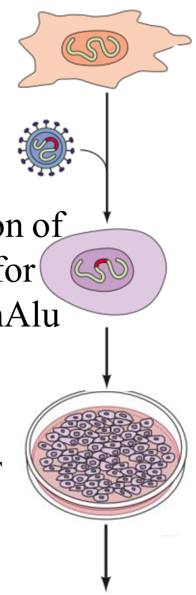
GENOME-WIDE blueprints of DNA damage



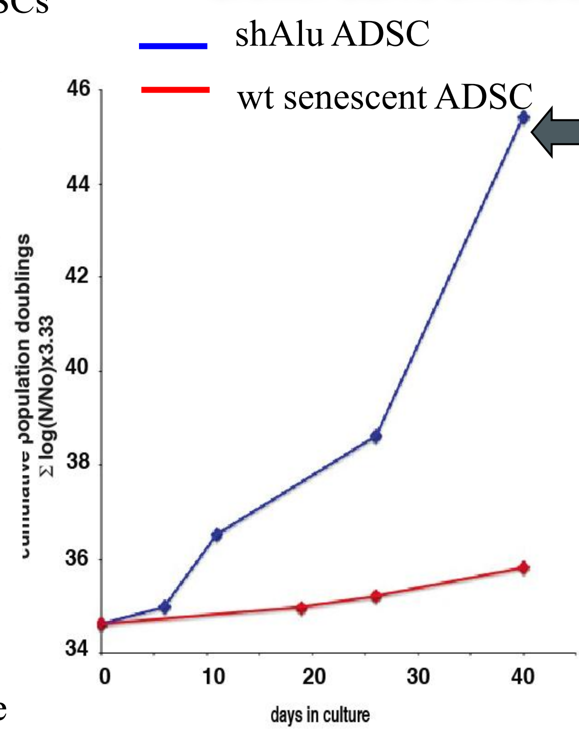
Genetic engineering to fight adult stem cell aging



Lentiviral shRNA-Mediated Knockdown of Generic Alu Transcript in Senescent hADSCs

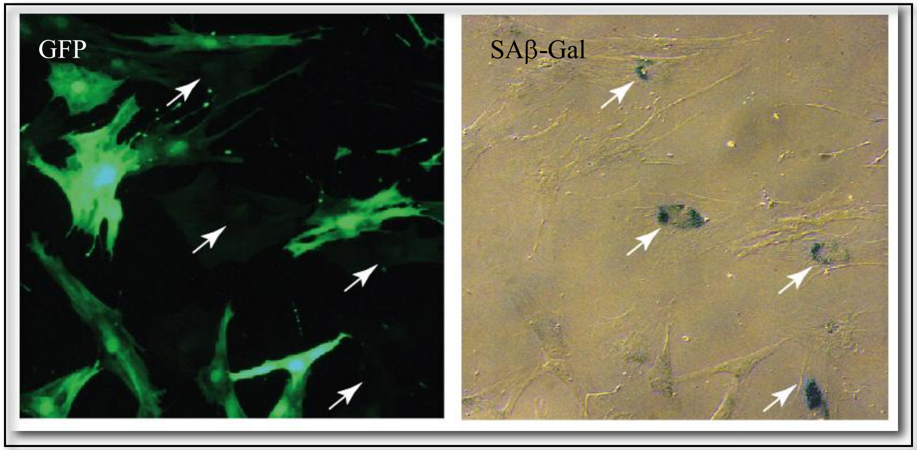


Growth Curve of ADSC



Reinstated proliferation of prior senescent cells

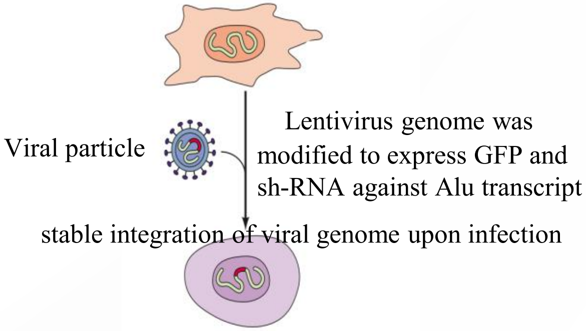
Lentiviral shRNA-Mediated Knockdown of Generic Alu Transcript in Senescent hADSCs



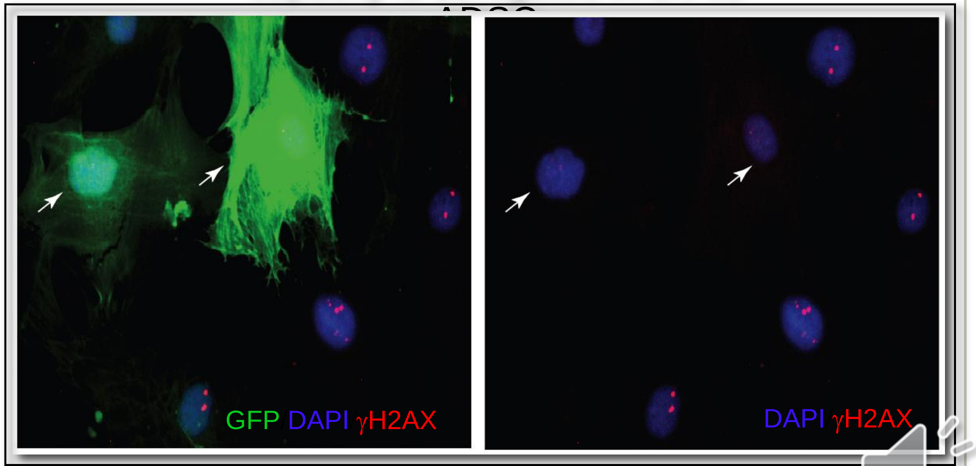
shAlu-ADSCs are negative for senescence-associated SAβ-gal

Persistent γH2AX foci forming capacity is abrogated in shAlu-

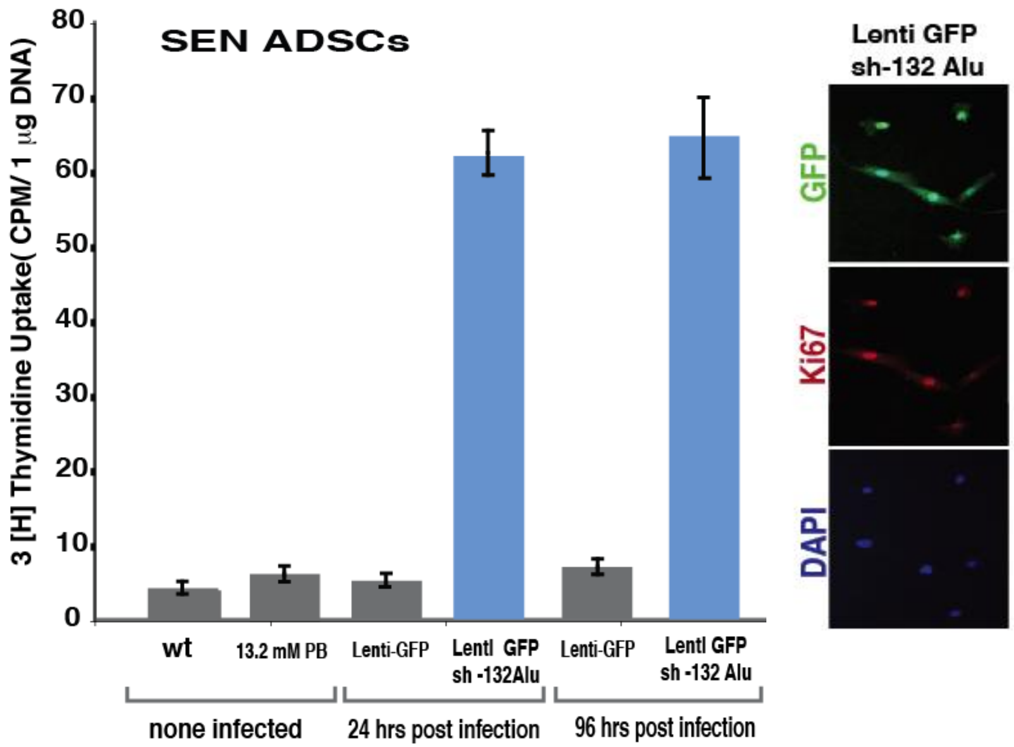
Senescent hADSCs



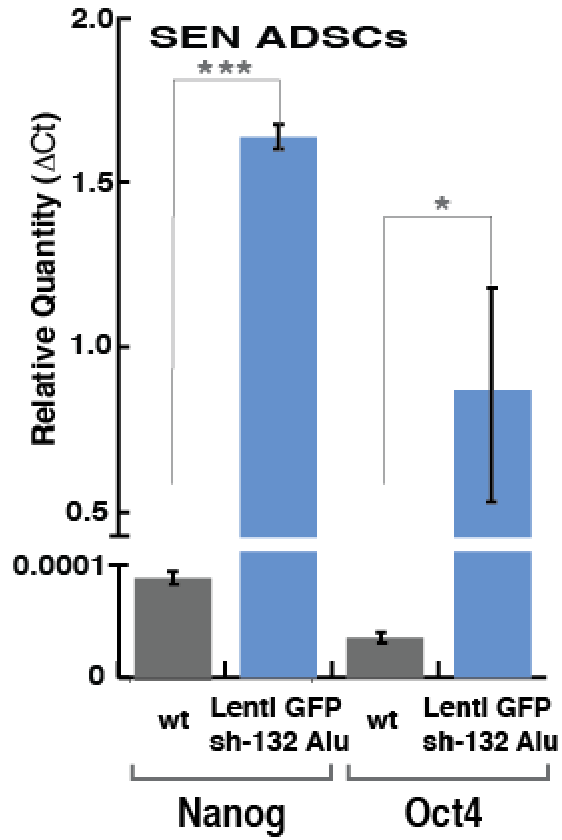
GFP expressing hADSCs with stable knockdown of Alu RNA



Lentiviral shRNA-Mediated Knockdown of Generic Alu Transcript in Senescent hADSCs



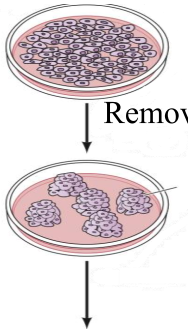
DNA Replication Assay



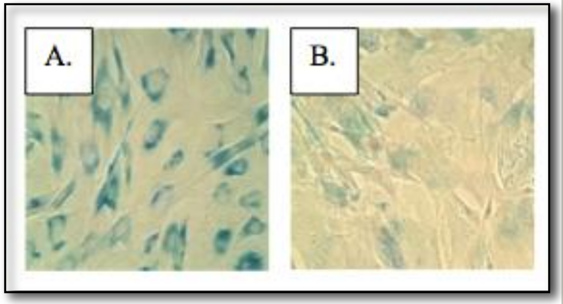
qPCR Analysis

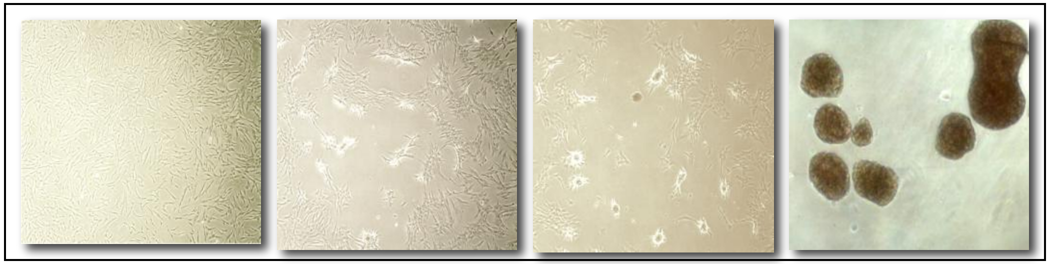
Morphology of Alu shRNA mutant ADSC in culture and their initial characterization

Infection of senescent ADSC



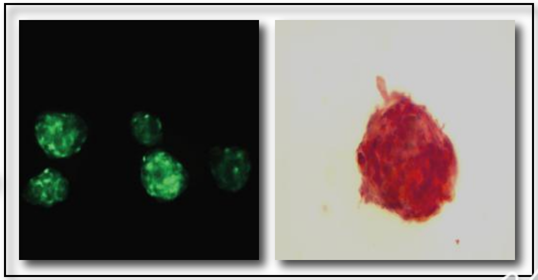
Senescence-associated β-Gal staining
A-control lenti-GFP
B- ALU shRNA lenti-GFP





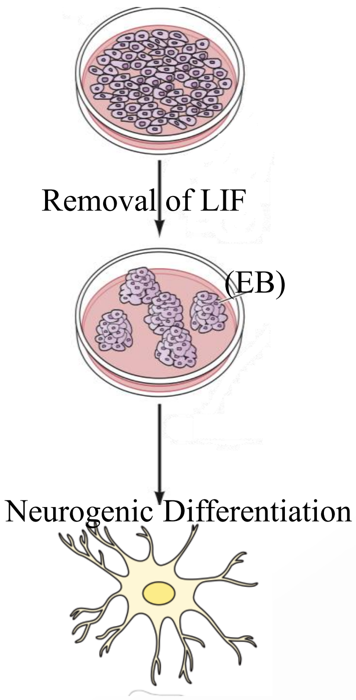
2 days 3 days 4 days 7 days

ALU shRNA lenti-GFP mutant cell display EB-like phenotype after 7days in culture and are positive for Alkaline Phosphatase

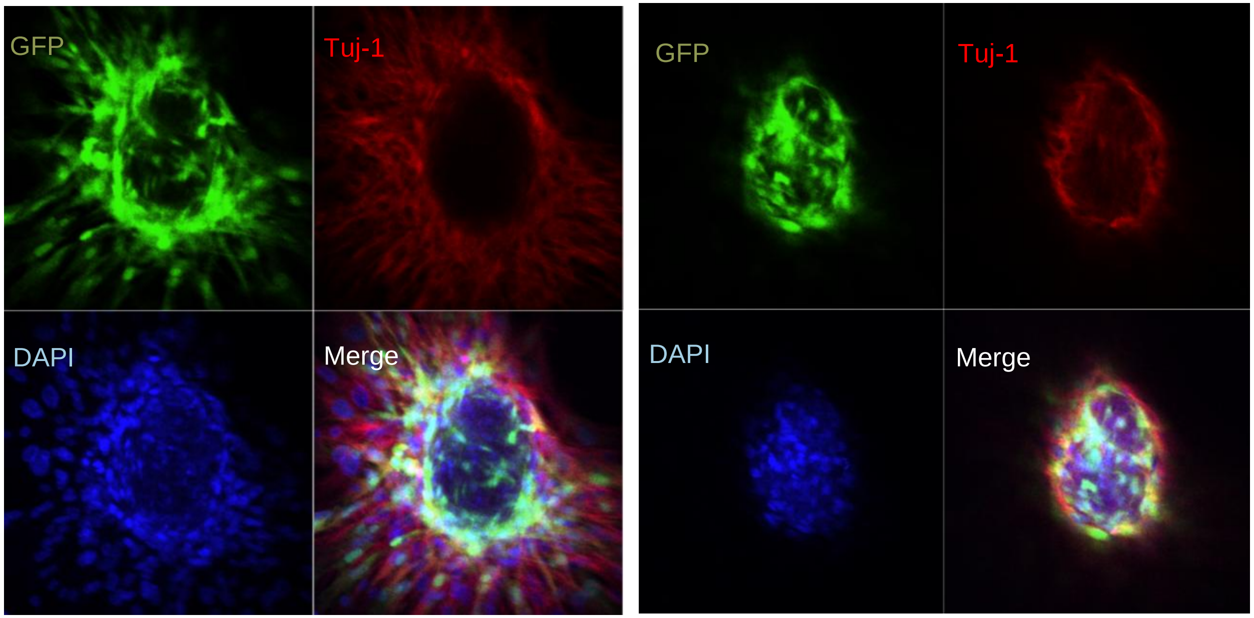


Neurogenic Differentiation of shAlu mutant EB

sh-Alu
expressing ADSC



Immunohistochemical analysis after 2 days in neurodifferentiation media.

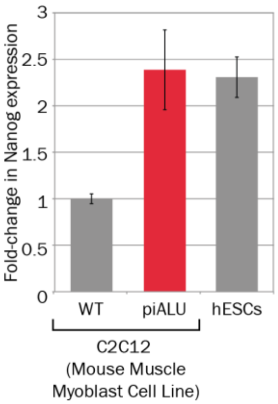


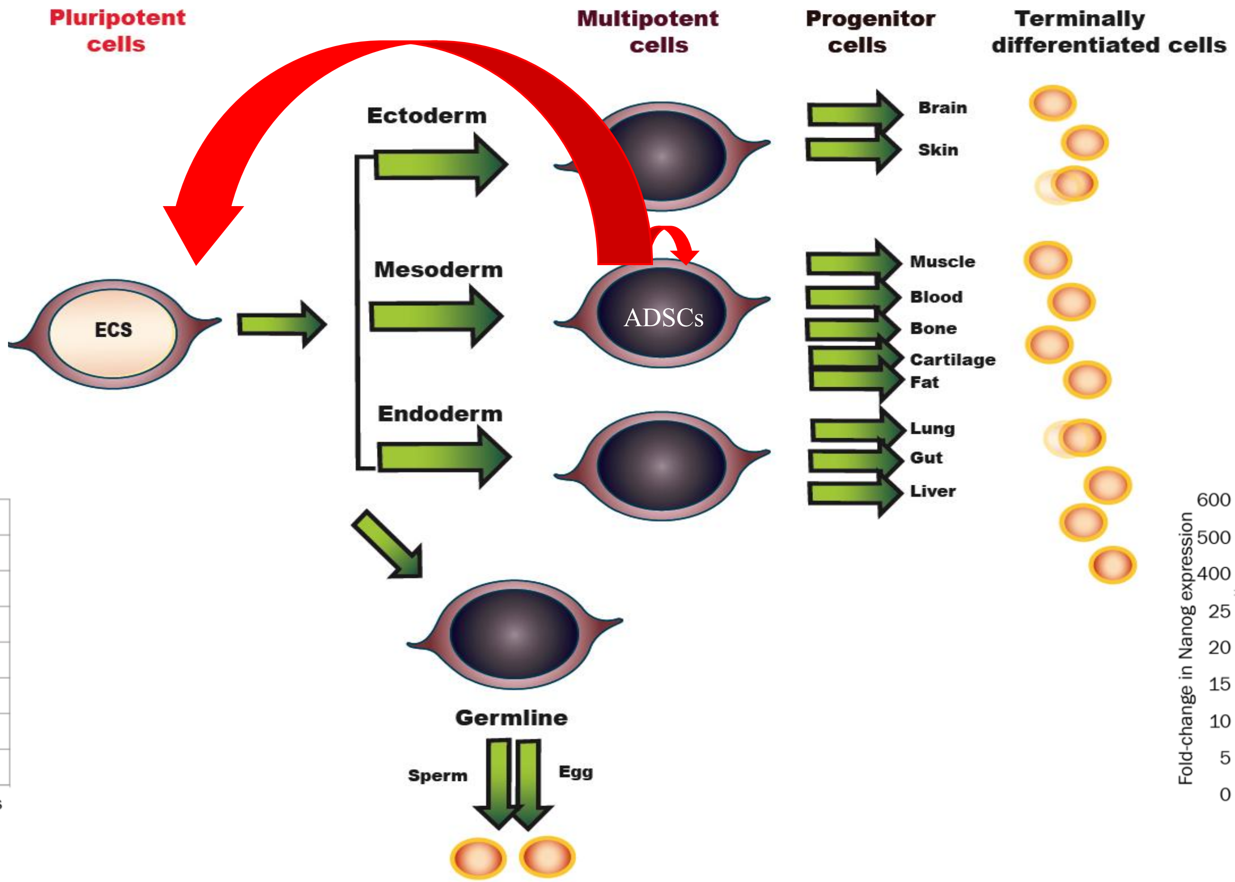
Section 24

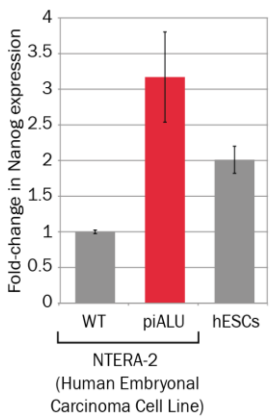
Section 11

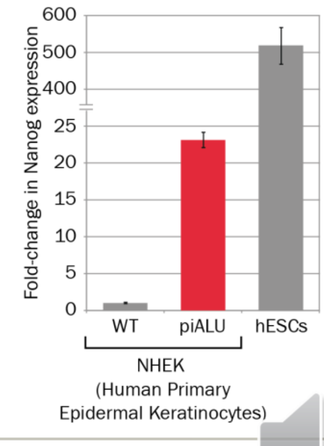
Optical sections

Rejuvenation of senescent hADSCs erases developmental memory



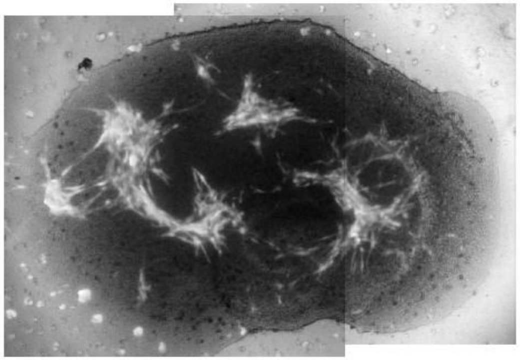




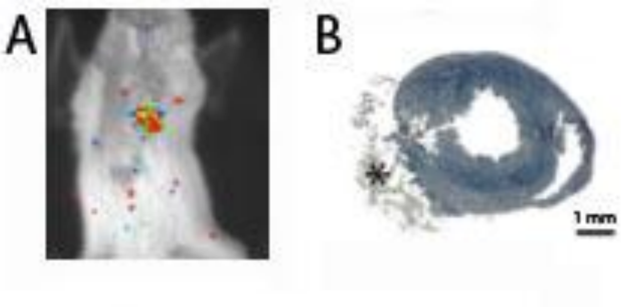


What is Next for REjev ADSC?

Neurogenic differentiation on brain slices



Myocardial repair



Collaboration with European Stem Cell Consortia

Carlos Semino Lab

Conclusions:

1. Understanding of stem cell cellular epigenetic regulations is incomplete (does not include the aging component).

2. Novel biomarkers for monitoring of organismal aging can be obtained through the investigation of the stem cells.

3. We can use genetic engineering to rejuvenate old human stem cells. This allows for enhancement of cellular therapies and understanding of the biology of cellular and organismal aging.

Regenerative Medicine: a modern days Fountain of Youth?

edgments

LUNYAK Lab
James Tollervey,PhD
Sirkka Liisa Hostikka, PhD
Benjamin Blackwell,M.Sc
Glenn Geesman M.Sc.
Christina Vicente Garcia

<u>Undergraduate students:</u>
Michelle Attallah
Garrett Peck
Ben Lerch
Daniel Ross Eth

Kennedy Lab

<u>Georgia Institute of Technology:</u>
School of Biology
King I. Jordan Lab
<u>Emory University Genomic Core:</u>
Tim Reed Lab
<u>UCSD:</u>
School of Medicine
Geoff Rosenfeld Lab
Department of Plastic Surgery
Marek Dobke , MD

<u>TEXAS MD Anderson Cancer Center:</u>
Mark Bedford Lab
<u>Life Technologies:</u>
<u>Biomarkers Research Initiave (BRIMS)TermoFisher Scientific:</u>
Mary Lopez lab, PhD
<u>European Stem Cell Consortia:</u>
Carlos Eduardo Semino Lab, PhD

<u>Funding Sources:</u>

Buck Institute Start-up Fund
BUCK institute Geroscience Consortium pilot (NIA)