Restoring Extracellular Matrix Synthesis in Senescent Stem Cells

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Abstract

Collagen type III (Col3) is one of the three major collagens in the body and loss of expression or mutations in the Col3 gene have been associated with the onset of vascular diseases such the Ehlers-Danlos syndrome. Previous work reported significant reduction of Col3 in tissues such as skin and vessels with aging. In agreement, we found that Col3 was significantly reduced in senescent human Mesenchymal Stem Cells (MSC) and myofibroblasts derived from patients with Hutchinson's Guilford Progeria Syndrome (HGPS), a premature aging syndrome. Most notably, we discovered that ectopic expression of the embryonic transcription factor, NANOG restored Col3 expression in the cells and tissue constructs prepared with these cells. RNA-Seq analysis showed that genes associated with the activation of the TGF-b pathway were upregulated, while negative regulators of the pathway were downregulated upon NANOG expression. ChiP-Seq and immunoprecipitation experiments revealed that NANOG bound to the SMAD2 and SMAD3 promoters, in agreement with increased expression and phosphorylation levels of both proteins. Using chemical inhibition, shRNA knockdown and gain of function approaches, we established that both Smad2 and Smad3 were necessary to mediate the effects of NANOG but only Smad3 was also sufficient for Col3 production. In conclusion, NANOG restored production of Col3, which was impaired by cellular aging, suggesting novel strategies to restore the impaired ECM production and biomechanical function of aged tissues, with potential implications for regenerative medicine and anti-aging treatments.

Background

A

Type	Constituent	Distribution	Pathology	Role
Ⅰ	α1 (Ⅰ), α2 (Ⅰ)	Skin,Vessels, Tendon, Ligament	OI, EDS(Ehlers-Danlos Syndrome), Osteoporosis	Stiffness
Ⅲ	α1 (Ⅲ)	Skin, Vessels, Tendon	EDS (vascular type), Arterial aneurysms	Control the size of Collagen 1 (crucial for Col1 fibrillogenesis)

B Type 1,3 collagen content in normal skin

Age Group	Type 1 collagen (ug/g)	Type 3 collagen (ug/g)	Type 1/3
Fetus	264.71±5.88	278.87±6.18	0.95±0.03
Adolescent	279.12±7.65	123.27±5.30	2.27±0.13
Adult	241.79±8.23	98.41±5.58	2.46±0.15
Elderly	209.50±14.31	71.30±7.41	2.97±0.40

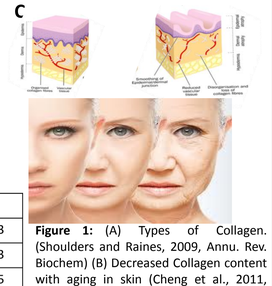


Figure 1: (A) Types of Collagen. (Shoulders and Raines, 2009, Annu. Rev. Biochem) (B) Decreased Collagen content with aging in skin (Cheng et al., 2011, African Journal of Biotechnology) (C) Schematic for skin changes with aging

Motivation

Myogenic function and mechanical properties are reversed by NANOG

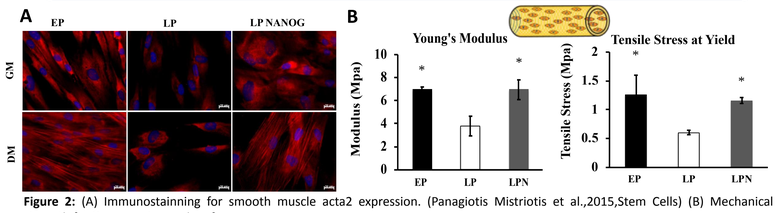


Figure 2: (A) Immunostainning for smooth muscle acta2 expression. (Panagiotis Mistriotis et al.,2015,Stem Cells) (B) Mechanical strength for tissue engineered grafts

EP: Early passage (4-7), LP: Late passage (13-16), LPN: Late passage plus 1μg/ml Dox. (*): p<0.05 compared to LP (n=3). Data are presented as mean ± standard deviation, n=3,: denotes statistical significance as compared to LP with p<0.05.*

Experiment Design

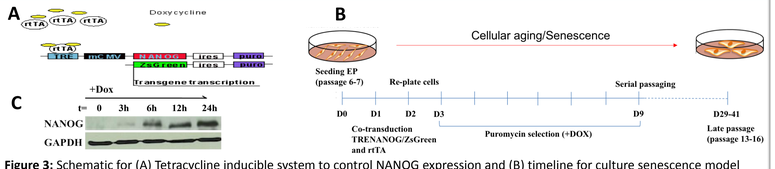


Figure 3: Schematic for (A) Tetracycline inducible system to control NANOG expression and (B) timeline for culture senescence model (C) NANOG expression is upregulated by addition of DOX within hours (Panagiotis Mistriotis et al.,2015,Stem Cells)

Hypothesis

NANOG restores Collagen deposition in senescent stem cells

Results

Senescence associated enzyme, growth arrest and DNA damage of aged cells are reversed by NANOG

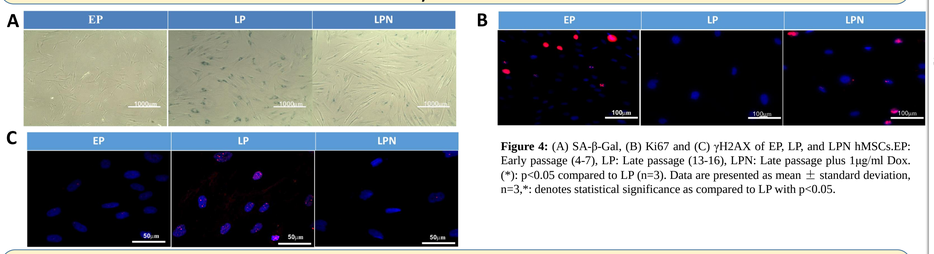


Figure 4: (A) SA-β-Gal, (B) Ki67 and (C) γH2AX of EP, LP, and LPN hMSCs.EP: Early passage (4-7), LP: Late passage (13-16), LPN: Late passage plus 1μg/ml Dox. (*): p<0.05 compared to LP (n=3). Data are presented as mean ± standard deviation, n=3,*: denotes statistical significance as compared to LP with p<0.05.

The loss of collagen content is rejuvenated by NANOG expression

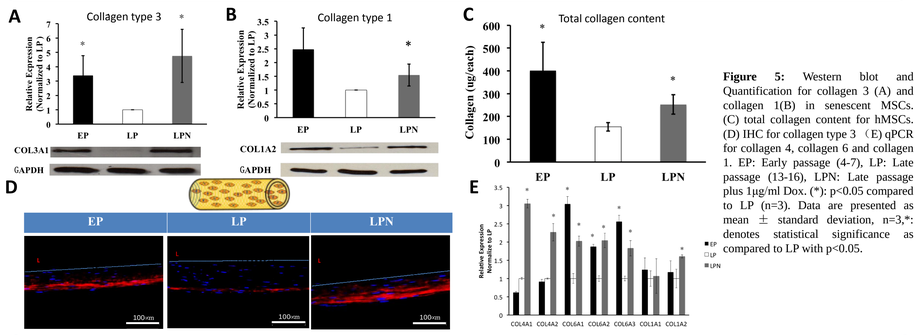


Figure 5: Western blot and Quantification for collagen 3 (A) and collagen 1(B) in senescent MSCs. (C) total collagen content for hMSCs. (D) IHC for collagen type 3 （E) qPCR for collagen 4, collagen 6 and collagen 1. EP: Early passage (4-7), LP: Late passage (13-16), LPN: Late passage plus 1μg/ml Dox. (*): p<0.05 compared to LP (n=3). Data are presented as mean ± standard deviation, n=3,*: denotes statistical significance as compared to LP with p<0.05.

TGFβ pathway is directly activated by NANOG expression

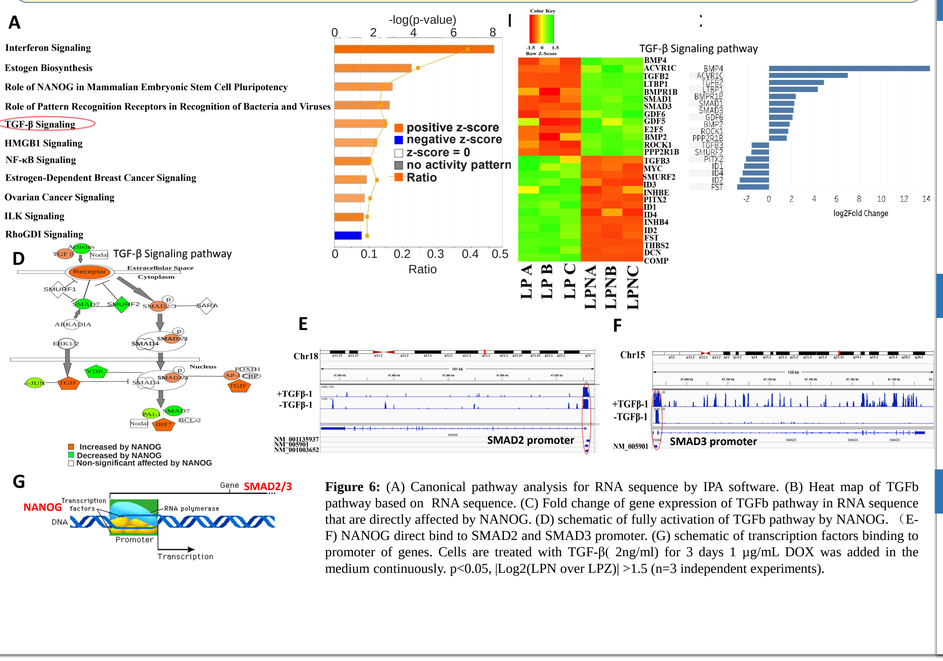


Figure 6: (A) Canonical pathway analysis for RNA sequence by IPA software. (B) Heat map of TGFb pathway based on RNA sequence. (C) Fold change of gene expression of TGFb pathway in RNA sequence that are directly affected by NANOG. (D) schematic of fully activation of TGFb pathway by NANOG. （E-F) NANOG direct bind to SMAD2 and SMAD3 promoter. (G) schematic of transcription factors binding to promoter of genes. Cells are treated with TGF-β(2ng/ml) for 3 days 1 μg/mL DOX was added in the medium continuously. p<0.05, |Log2(LPN over LPZ)| >1.5 (n=3 independent experiments).

NANOG maintains the activity of TGFβ pathway through binding to TGFβ key regulators-SMAD2/3

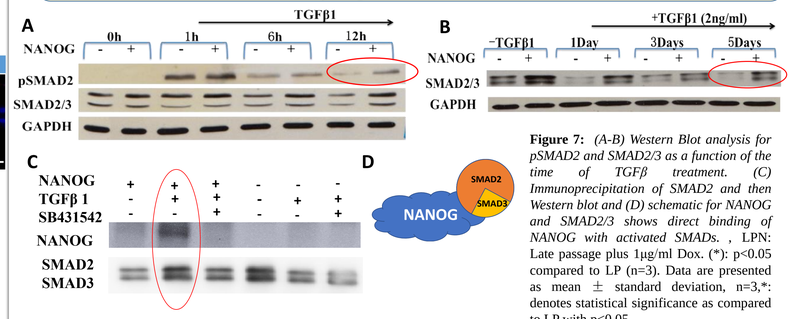


Figure 7: *(A-B) Western Blot analysis for pSMAD2 and SMAD2/3 as a function of the time of TGFβ treatment. (C) Immunoprecipitation of SMAD2 and then Western blot and (D) schematic for NANOG and SMAD2/3 shows direct binding of NANOG with activated SMADs.* , LPN: Late passage plus 1μg/ml Dox. (*): p<0.05 compared to LP (n=3). Data are presented as mean ± standard deviation, n=3,*: denotes statistical significance as compared to LP with p<0.05.

Both SMAD2/3 proteins are necessary, but only SMAD3 is sufficient in collagen restoration by NANOG

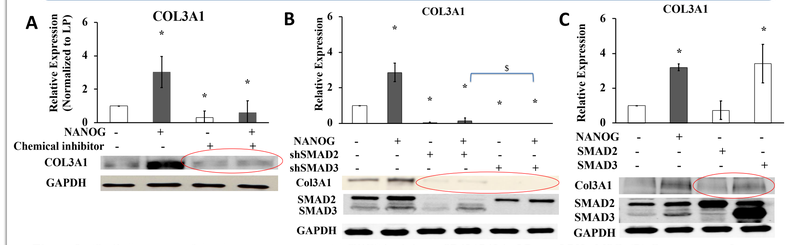
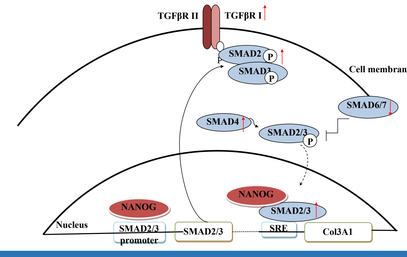


Figure 8: *(B) Collagen type 3 levels upon treatment with TGFβ inhibitor - SB431542 in LP and LPN hMSC. (B) Collagen type 3 levels after SMAD2 or SMAD3 knockdown in LP and LPN hMSC. (C) Collagen type 3 levels after SMAD2 or SMAD3 overexpress in LP and LPN hMSC. Late passage (13-16). LPN: Late passage plus 1μg/ml Dox. (*): p<0.05 compared to LP (n=3). Data are presented as mean ±std, n=3,*: denotes p<0.05 as compared to LP.*

Proposed mechanism



Conclusions

1. NANOG reverse the senescence phenotype of aged cells
2. With aging, senescent cells produce less col3, which can be restored by NANOG
3. Senescence decrease the activity of TGFb pathway, which is upregulated and maintained by NANOG through directly binding to SMAD2/3 promoters and proteins
4. Both SMAD2/3 are necessary, but only SMAD3 is sufficient in col3 reversal

Acknowledgements

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