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 as 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 (MSCs) and myofibroblasts derived from patients with Heredofamilial Osteoarthropathy Syndrome (HROAs), 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 those cells. RNA-Seq analysis showed that these associations with the activation of the TGFβ pathway was 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, dnNANOG 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 reverse the impaired ECM production and biomechanical functions of aged tissues, with potential implications for regenerative medicine and anti-aging treatments.

**Motivation**

Myogenic function and mechanical properties are reversed by NANOG

**Background**

**Results**

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

**Hypothesis**

NANOG restores Collagen deposition in senescent stem cells

**Conclusions**

1. NANOG reverse the senescence phenotype of aged cells
2. With aging, senescent cells produce less collagen, which can be restored by NANOG
3. Senescence decrease the activity of TGFβ 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 cell reversal

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