

# Identifying a Gene Orchestrating Skin Regeneration via Tissue Rebuilding ~ Inspiration from Aesthetic Treatments ~

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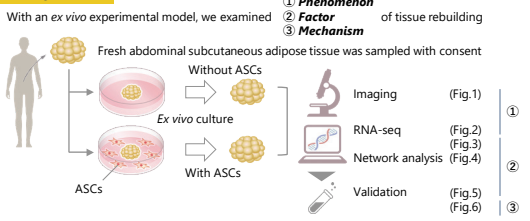
## Introduction



**Hypothesis**  
Subcutaneous injection of adipose-derived stem cells (ASCs) occur biological responses that trigger tissue remodeling

**Aim**  
Clarify "Rebuilding Induction"  
- What factor induces rebuilding?  
- What is the mechanism?

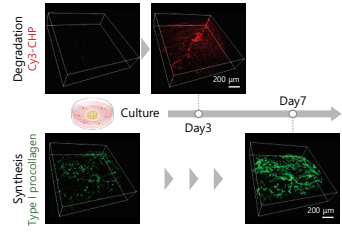
## Study flow



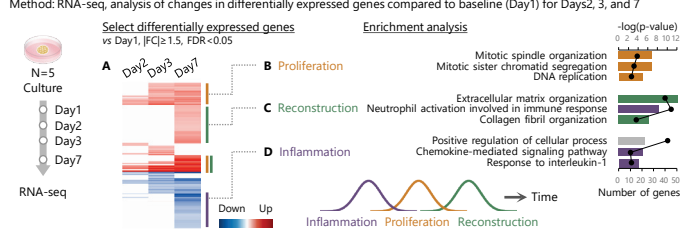
## Result

① **Phenomenon:** In an ex vivo co-cultured experimental model, not only rebuilding but also wound healing was observed

### 1. Rebuilding, sequential collagen degradation and synthesis, occurs in the presence of ASCs



### 2. Alongside rebuilding, wound healing processes, inflammation, proliferation and reconstruction, occurred

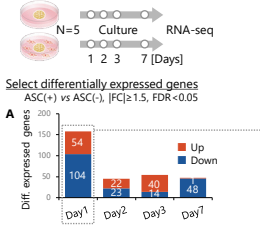


### ② Factor: TSG-6 was identified as a key factor in triggering tissue rebuilding instead of scarring

#### 3. ASCs mediate suppression of pro-fibrotic signals and early termination of inflammation

Purpose: To understand the influence of ASCs co-culture in the ex vivo subcutaneous adipose tissue

Method: RNA-seq, analysis of changes in differentially expressed genes compared to ASC(-) for each culture day

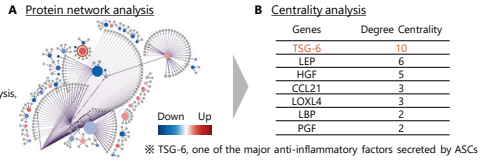


#### 4. TSG-6 was selected as a key paracrine factor involved in rebuilding based on the high degree of centrality in the network analysis

Purpose: To identify the genes involved in tissue rebuilding

Methods: A protein-protein interaction network was generated

- A protein from differentially expressed gene analysis, ASC(+) vs ASC(-) at day1
- A protein directly interacting with red/blue from the database
- Direct interaction



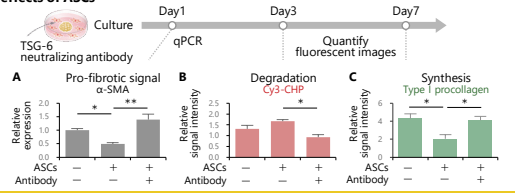
#### 5. TSG-6 released by ASCs mediates the effects of ASCs

Purpose: To reveal the role of TSG-6 in inducing ASC's biological responses

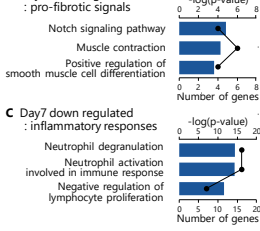
Method: After treatment with a TSG-6 neutralizing antibody, either qPCR or imaging was performed.

(A) qPCR for α-SMA, a marker of myofibroblast.  
(B,C) Imaging analysis of (B) degradation and (C) synthesis of fibrous structures.

N=4, Mean ± SE, Tukey HSD test. \*p < 0.05, \*\*p < 0.01



#### Enrichment analysis



### ③ Mechanism: NET Inhibition by TSG-6 reduces fibrosis, shifts to rebuilding

#### 6. TSG-6 dependent inhibition of NET formation suppresses fibrosis

We hypothesized that TSG-6 regulates rebuilding through neutrophil extracellular traps (NETs).

A Purpose: To verify the link between TSG-6 and NETs status

Method: NETs in ex vivo model were analyzed immunohistologically

B Purpose: To investigate the effects of NETs on the fibrous structures

Method: By qPCR, gene expression changes in subcutaneous adipose tissue exposed to NETs were examined

NET induction by PMA

NETs +

NETs -

Count the number of NETs

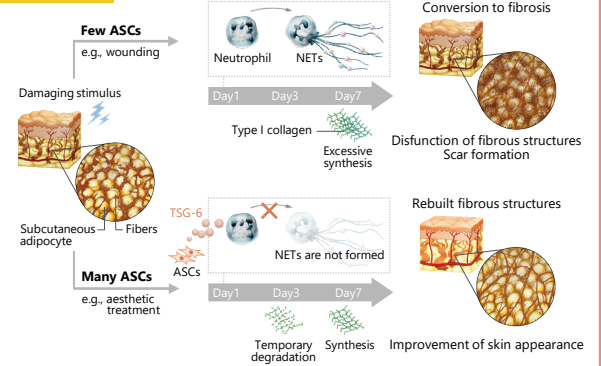
N=6, Mean ± SE, Tukey HSD test. \*\*\*p < 0.001

Synthesis Type I procollagen

Pro-fibrotic signal α-SMA

N=4, Mean ± SE, paired t-test. \*p < 0.05, \*\*p < 0.01

## Conclusion



## Impact

**Transformation of approach methods:**

- Current paradigm: Elucidation of the causes of skin problems
- New paradigm: Elucidation of the mechanisms underlying skin improvement

"Stop the aging process"

"Restore the skin to its younger state"

**Our finding is useful for:**

- Cosmetics: to enhance efficacy
- Aesthetic treatments: to expand applications for fibrotic skin problems
- Medical fields: to reduce downtime/to maximize efficacy to reduce scarring

**In the future:** "Rebuilding Induction" by ASC injection in a bottle

