Introduction

1) The skin is an ideal model to study intrinsic genes and pathways protecting against aging, due to the relative ease of clinical inspection and biopsy.

2) The main objective of this exploratory study is to identify gene expression profiles of older women with visibly youthful skin.

3) Our systematic analysis highlight a gene-pair, HAS2 and HAS2-AS1, associated with human skin youthfulness.

Methods and Materials

1) Healthy women of European descent, aged 18-89 years, and skin type I/II were assessed on facial skin aging parameters and biopsy.

2) Skin youthfulness (SY) was defined as the top 10% of individuals of a given age group.

3) Skin biopsies from sun-protected inner arm were obtained from SY group (n=12) and no-SY (n=33) participants and subjected to 3’-end sequencing for expression quantification.

4) SY accocited genes were verified by quantitative RT-PCR

Demographics of patients

<table>
<thead>
<tr>
<th>Quantitative Parameter</th>
<th>SY group (n=12)</th>
<th>no-SY group (n=33)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological age, years (SD)</td>
<td>71(11)</td>
<td>69(9)</td>
<td>0.749</td>
</tr>
<tr>
<td>Skin age score (SAS), years (SD)</td>
<td>61(5)</td>
<td>81(8)</td>
<td>2.35x10</td>
</tr>
<tr>
<td>Body mass index kg/m</td>
<td>25(3.3)</td>
<td>25(4)</td>
<td>0.7108</td>
</tr>
<tr>
<td>VISIA WRINKLES (SD)</td>
<td>93(10.6)</td>
<td>73(22)</td>
<td>0.0003817</td>
</tr>
<tr>
<td>Smoking history: Yes (%)</td>
<td>3 (25%)</td>
<td>13 (39%)</td>
<td>0.7381</td>
</tr>
<tr>
<td>Previous Skin cancer: Yes</td>
<td>3 (25%)</td>
<td>10 (30%)</td>
<td>1</td>
</tr>
<tr>
<td>Lifetime UV hours accounting for UV Index*, score (SD)</td>
<td>38(27)</td>
<td>49(44)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table1. Demographics of women enrolled and potential covariates. There were no significant changes in other covariance between SY and no-SY group, except the SAS and VISIA WRINKLES.

Verify expression data by skin aging genes

Figure1 Gene ontology analysis of age-effect genes. Biologic themes most significantly increased with age include biological adhesion, positive regulation of response to stimulus and extracellular structure organization and immune response genes. Biologic themes most significantly decreased with age include pigmentation related genes. These results are consistent with prior study on pigmentation related genes. These genes are known to associate with skin aging.

PHLDA1 expression decreased in SY group

(a) Expression difference of PHLDA1 between SY group (n=12) and no-SY (n=33) group by 3’seq (p=2.4E-5 by liner regression). (b) Expression difference of PHLDA1 between SY group (n=10) and no-SY (n=10) by RT-PCR(p=0.07667, Wilcoxon test) (c) Scatterplot of PHLDA1 expression by chronological age (n=45). Overall, there is a slight negative correlation between PHLDA1 and chronological age (R=-0.227, p=0.1345). SY individuals (red dots) tend to have lower expression levels of PHLDA1 compared to no-SY individuals (black dot).

Immunofluorescence assay of HAS2

Figure 5 Immunofluorescence with antibody to HAS2 on sun-protected inner arm skin from individuals with and without SY. Scale bar=25μm. Typical fibroblasts are shown and did not show significant differences in signal.

Discussion

1) The expression profiles reported here are a mix of epidermal and dermal cells, and different numbers of each type may influence the expression profiles. Future studies are underway to delineate the cell types that express HAS2-AS1 and assess if there are differences across chronological age and SY phenotype according to cell type

2) Knock-down of HAS2-AS1 in fibroblast cell showed non-significant decreases in HAS2 transcript levels by qRT-PCR. HAS2 protein did not show significant differences between SY and no-SY groups. Precise mechanism of HAS2-AS1 functions remains to be worked out.

Acknowledgments

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