Hyaluronan synthase 2 antisense transcript level associates with human skin youthfulness as identified by transcriptome sequencing

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Introduction

1) The skin is an ideal model to study intrinisc 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 covariates (n=122).

2) Skin youthfulness (SY) was defined as the top 10% of individuals whose assessed skin aging features were most discrepant with their chronological ages.

3) Skin biopsies from sun-protected inner arm were obtained from SY (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

Quantitative Parameter	SY group (n=12)	no-SY group (n=33)	P-value
Chronological age, years (SD)	71(11)	69(9)	0.749
Skin age score (SAS), years (SD)	61(5)	81(8)	2.335x10
Body mass index kg/m	25(3.3)	25(4)	0.7108
VISIA WRINKLES (SD)	93(10.6)	73(22)	0.0003817
Smoking history: Yes (%)	3 (25%)	13 (39%)	0.7381
Previous Skin cancer: Yes	3 (25%)	10(30%)	1
Lifetime UV hours accounting for UV Index*, score (SD)	36(27)	49(44)	0.26

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

Verify expression data by skin aging genes

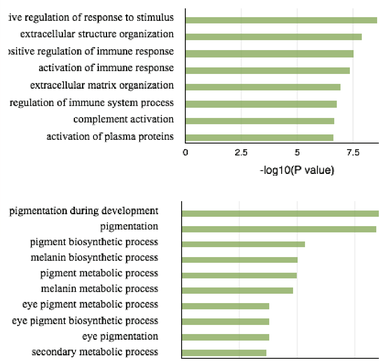

Figure1 Gene ontology analysis of age-effect genes. Biologic themes most significantly increased with age include biological adhesion, positive regulation of response to stimulus and extracellular structure organization and immune response genes. Biologic themes most significantly decreased with age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

Gene expression associated to skin youthful appearance

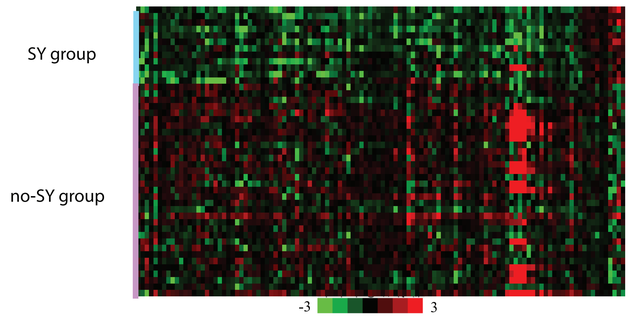

Figure 2 Heat map of gene expression profiles showing differences between SY group and non-SY group by unbiased clustering. A total of 114 genes were found to associate with SY phenotype, with 104 showing decreased levels in SY group and 10 showing increased levels in the SY group. A number of themes emerged from the SY genes identified. First, a group of genes involved in glycoprotein biosynthetic process and glycoprotein metabolic process were identified. Advanced glycation end products are known to associate with skin aging.

PHLDA1expression decreased in SY group

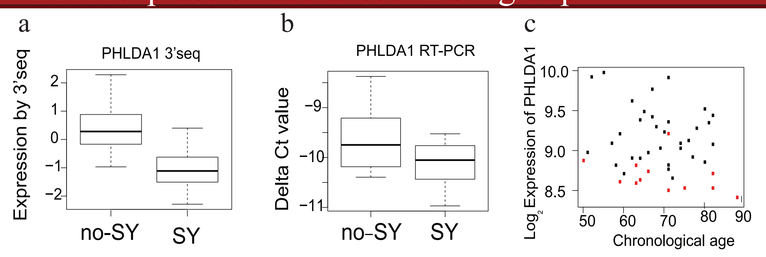

Figure 3 Expresion of PHLDA1, a hair follicle stem cell marker, decreased in SY group.(a) Expression difference of PHLDA1 between SY group (n=12) and no-SY (n=33) group by 3'seq (p=2.4E-5 by linear regression test). (b) Expression difference of PHLDA1 between SY group (n=10) and no-SY (n=10) by RT-PCR(p=0.07667, 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).

HAS2-AS1 expression is decreased in SY versus no-SY group.

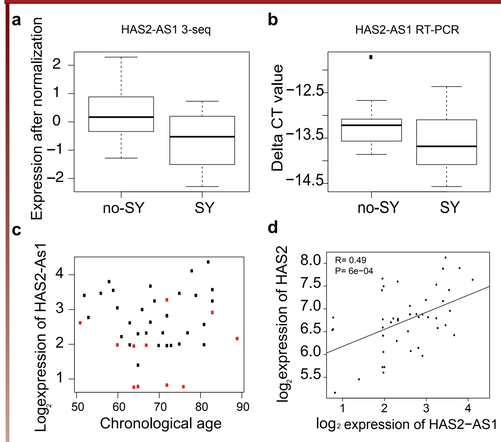

Figure 4 (a) Expression difference of HAS2-AS1 between SY group (n=12) and no-SY(n=33) group by 3'seq (p=0.00105, by linear regression). (b) Expression difference of HAS2-AS1 by RT-PCR (p=0.09067, Wilcoxon test) after normalized to actin-β.(c) Scatterplot of HAS2-AS1 expression by chronological age. There is no correlation between HAS2-AS1 and chronological age. Red points shows expression level of individuals with SY, which tend to be lower than individuals without SY, but at the same chronological age. (d) Positive correlation between HAS2-AS1 and HAS2 among SY and control patients (r=0.49, p=0.00063, n=45).

Immunofluorescence assay of HAS2

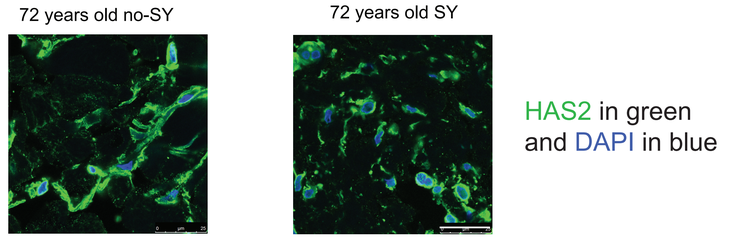

Figure 5 Immunofluorescence with antibody to HAS2 on sun-protected inner arm skin from individuals with and without SY,Scale bar=25μm. Typical fibroblasts are shown and did not show significant differences in signal.

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

The authors wish to thank Gefei Alex Zhu, Rashmi Tarhalkar and Hoa Nguyen for assistance with clinical data acquisition and curation.

Conflict of interest
DK and HEK are employees of NuSkin International.