The Protein Digestion and Absorption Pathway in the Epidermis of Women Differed Across Different Age-Groups Proved by RNA-Seq

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Abstract

Skin plays a important role in protecting our physical body, on the other side, it also suffer from inner or outer threatens, which may cause the degeneration and senescence of skin. Actually, various changes could occur in the skin when it experience aging. Given that prominent macroscopic signs of senescence of skin include being drier, rougher, thinner, less glossy and so on, for microscopic views, free radical, mitochondria, lysosome, telomere in cells were proved have been changing with the process of aging, even some special genes which directly modulate cells' fate also expressed differently between young and senior human's skin. However, there are few articles for more detail and thorough cognition to transcriptome level differences across different age groups in epidermal cells so far. So we decided to detect and compare the overall mRNA levels in 7 cases of liquid nitrogen frozen woman's epidermal sections which divided into young, middle-aged, senior groups by using RNA-Seq, for further investigation into what exact biological pathways may be affected by age change. Interestingly, we found that the protein digestion and absorption pathway associated genes expression were significantly disturbed by senescence in cells of epidermis finally. We hope our study can provide a brighter map for scientists to explore other relationship between senescence and mRNA expression.

Keywords

Epidermis, RNA-Seq, senescence, protein digestion and absorption pathway.

1. Introduction

Skin is the largest organs that directly contact the natural environment, continuously suffer from stress and damage, so complete recovery, senescence or death of epidermal cells or dermal cells are the normal responses to the stimulus at anytime [1]. During the senescence, transcripts and proteins in epidermal cells may have been changing to adjust to the deteriorating biological physical state and harsh environment. For example, the structure and function of the golgi apparatus in human dermal fibroblasts start to change due to replicative senescence [2]. Both the epidermal cells and dermal cells displayed significant reduced expression of the genes related to mitochondrial morphology and function [3].

However, there have not been any reports about RNA variation information happened in epidermis during the aging process. To investigate what alteration may bring to the overall gene and biological function in epidermis which have been experiencing senescence, so we detected the differences of every kind of mRNA contents in epidermal cells across young women, middle-age women and old women groups by RNA-Seq. Interestingly, we found much significantly differently expressed genes (DEGs) enriched in protein digestion and absorption pathway due to age differences.

2. Materials and Methods

2.1 Materials

Epidermis samples of 2 cases of young women (Y), 4 cases of mid-aged women (M), 1 case of senior women (S), which were cut off from the eyelid and collected by Qingyuan People's Hospital in Guangzhou and were stored in -80 ℃ refrigerator, and every volunteers of sample has been informed

consent. TRzol Total RNA Extraction Reagent Kit (DP405-02) purchased from Tiangen for RNA extraction. PrimeScript™ RT Master Mix (RR036A) purchased from Takara for cDNA synthesis.

2.2 RNA extraction and cDNA synthesis

Epidermal tissue section was cut into pieces of less than 100 mg and polished into slags immediately after frozen by liquid nitrogen, and then extracted the RNA from tissues as the manufacturer's protocol (DP405-02). The cDNA was synthesised by 200 ng RNA according to the manufacturer's instructions (RR036A).

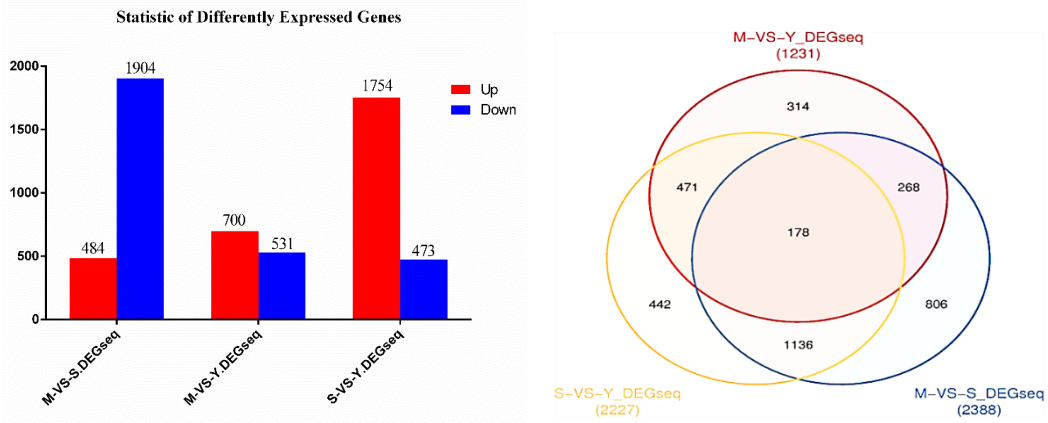
2.3 RNA-Seq

The cDNA that was synthesis as mentioned above was sent to BGI company in Shenzhen to take RNA sequencing(RNA-Seq) on BGISEQ-500, computed the expression levels of genes or transcripts using RSEM, and analysis the differentially expressed genes (DEGs), enriched KEGG pathway, DEGs number of the most enriched pathway across Y, M and S groups. RNA-Seq data from n=7 samples were deposited in SRA of NCBI(PRJNA554552).

3. Results

3.1 Statistics of DEGs in the epidermis across different age groups

Clean reads were filtered from original RNA-Seq data of 7 cases of samples, and then normalized to the refrence sequences by using *Bowtie 2* for caculating the relative expression levels of genes and transcripts on RESM and acquire a FPKM value. Total genes expressed in three groups were included in the statistical analysis are N= 18,525. According to DEGseq method, we checked the differently expressed genes(DEGs) across different age groups based on their genes expression levels. Next, column diagrams presented the exact numbers of significant DEGs across Y, M, S groups (Fig 1. A) including upreulating or downregulating genes. And the number of common DEGs and specfic DEGs across Y, M, S groups were shown by a Venn map, for example, there were 471 genes which expressed significantly differently between M and Y groups consist with the ones between S and Y groups, but there were 314 unique DEGs between M and Y groups(Fig 1. B) .



A B

Fig. 1 Detection of differentially expressed genes among different age groups of epidermis The number of DEGs counted across M, S and Y groups including up-regulated and down regulated genes; (B) The Venn map of common and specific DEGs numbers among different age groups.(Up: Fold change ≥ 2, q value ≤ 0.001; Down: Fold chang ≤ -2, q Value ≤ 0.001)

3.2 Aging of epidermis affect protein digestion and absorption pathway

According to the detection results based on the DEGs, we classified its KEGG biological pathways and analysised the enrichment degree. The enriched KEGG pathways results showed as Fig. 2(left panel), significant differences existed in the protein digestion and absorption way when compared the S group to the M group or the Y group, nevertheless there was less significant difference between the

M and Y goups, but the nitrogen metabolism pathway was affected by aging from young time to middle time, as we know, nitrogen is one of the major elements in protein. What's more, we listed top 10 KEGG pathways that upregulated and downregulated DEGs were enriched (Fig.2, right panel). Consistent with the enriched KEGG pathways, the enriched DEGs involved in protein digestion and absorption way occupied the large part of DEGs when compared the S group to the M group or the Y group, but there's no such significant changes from young to middle-age.

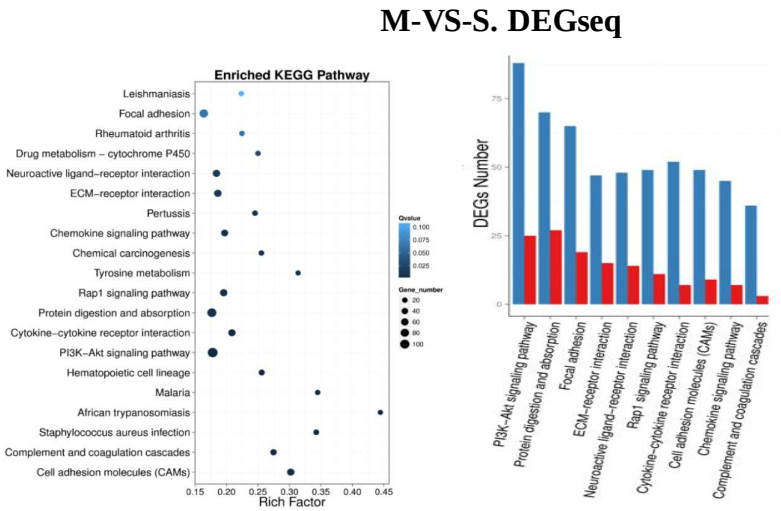
3.3 DEGs associated with protein digestion and absorption enriched in epidermis

At last, we listed all significant DEGs involved in protein digestion and absorption pathway(Table 1), and sequential of the listed genes name was from the top to the bottom based on expression levels differences. It showed that collagen genes in epidermis such as *COL17A1*, *COL6A2*, *COL1A2*, which were associated with protein digestion and absorption function, were largely affected by aging of the periods range from young or middle-age to old age, *COL26A1* and some of other collagens gene expression differed between the Y group and the M group. *SCARA5*, a kind of ferritin receptor that mediates cellular uptake of ferritin-bound iron by stimulating ferritin endocytosis from the cell surface with consequent iron delivery within the cell, differently expressed across the three different aging groups. Similarly, we also found that mRNA about amino acid transport varied significantly with the age change, including *SLC38A3*.

4. Discussion

As the RNA-Seq data presented, except for the protein digestion and absorption pathway genes were significantly disturbed by age, there were also genes included in PI3K-AKT, cell adhesion molecules(CAMs) signaling pathways which largely differently expressed across the age groups, and later results were consistent with the previous finds. For example, The PI3K/AKT pathway is commonly recognized as a key signaling pathway controlling autophagy[4], metabolism[5] and oxidative stress[6], the changes of which have been proved involved in aging process of skin cells. The matricellular protein periostin, a kind of cell adhesion molecules, can contribute to proper collagen function and is downregulated during skin aging[7].

The protein digestion and absorption pathway hasn't been reported any direct relationship linked with skin aging, however, apparent digestibility of protein showed lower for the old cats when compared to young ones[8]. And the dietary rates of protein digestion and absorption response do not differ between young and elderly men, which didn't correspond to what happened in the skin[9]. Even though the cases of our collected epidermis samples are limited to 7, and made our RNA-Seq data hard to convince every reviewers thoroughly, it may give us the more clear guide to lead us to find out how the protein digestion and absorption pathway work in the process of aging in skin.



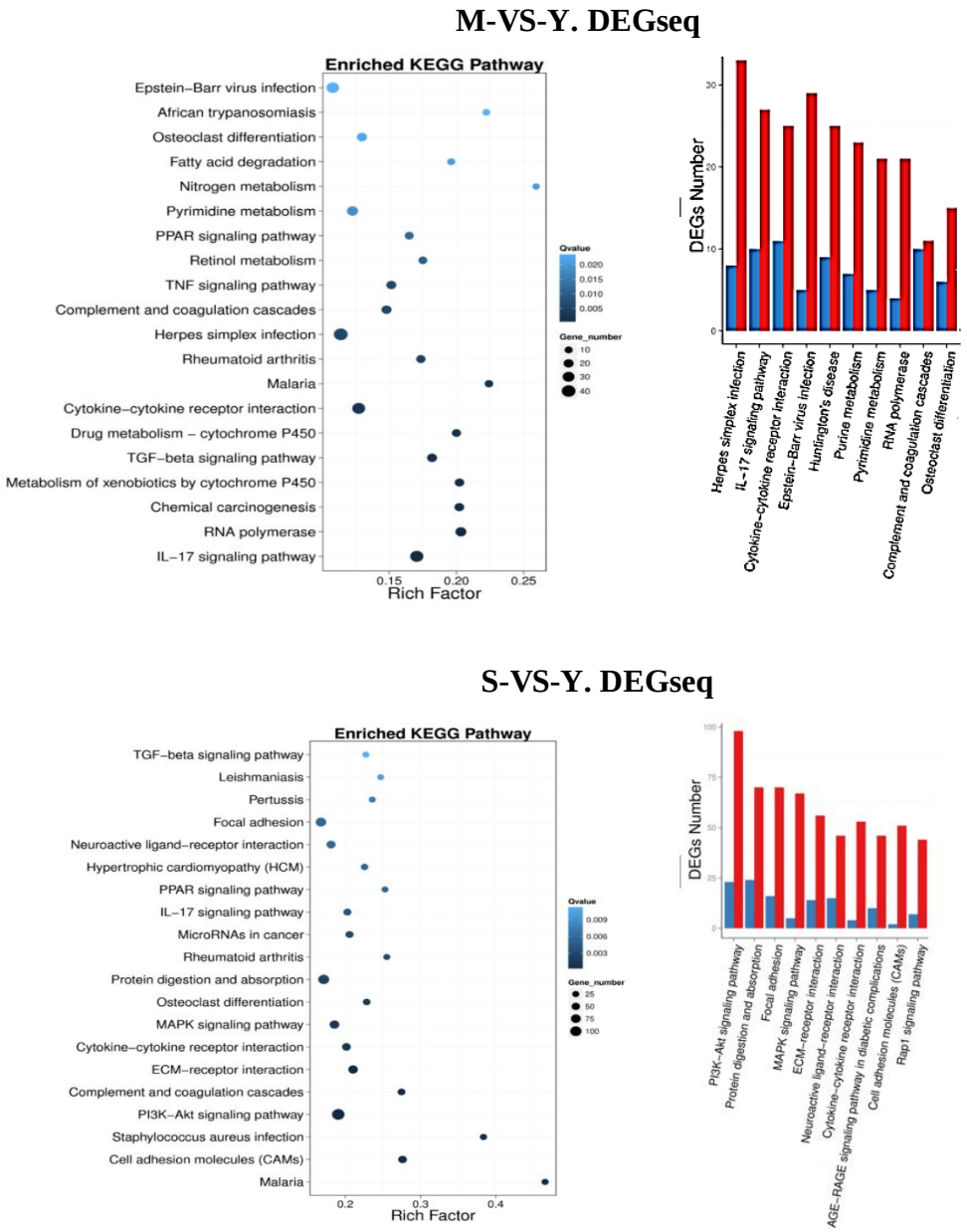


Fig. 2 Age affected the protein digestion and absorption pathway significantly in epidermis

Table 1 The top affected genes associated with protein digestion and absorption enriched in epidermis

Methods	Differentially expressed genes
M-VS-S. DEGseq	COL17A1, COL6A2, COL1A2, COL7A1, COL6A1, SCARA5, CLSTN1, COL12A1, C1QC, C1QA, MARCO, C1QB, MXRA8, CCDC3, ELN, MXRA7, COL14A1, ANKRD33B, TTC22, KANK2, COL27A1, C1QTNF5, EMILIN1, CPA3, EMILIN2, COL4A5, MMRN2, XPNPEP2, DPP4, GLDN, CCBE1, COCH, OLFM1, CTHRC1, GRASP, VIT, RNF225, VGLL3, C1QTNF2, TMEM200B, RIPOR3, COL4A6, C11orf96, THEMIS2, KANK3, C1QTNF7, EFCC1, COL6A5, SLC7A7, CDC42EP5, C1QTNF3, COL6A6, C2CD4B, VGF, FAM171A2, DPP6 , CYS1, SFTPD, CFAP73, LYNX1-SLURP2, NAT14, MSR1, TMEM88B, TMEM178A, OSGIN1, LRRC75A, TMEM88, NUTM2E, MME, PROB1, C2CD4A, NUTM2G, FAM110D, KCNJ13, TMEM198, TMEM61, LCNL1, SAMD11, SHISA8, FXYD2, C1QTNF4, PGA5, KRTAP21-2, PGA3, AJM1, SHISA7, SLC8A3, CTRL, FAM43B, ATP1A4, SLC8A2, C1QL3, CPA2, TMEM59L, C17orf82, COLEC11, STRA6

M-VS-Y. DEGseq	NRARP, SLC38A2, MIDN, C11orf96, COCH, MARCO, XIRP1, CASQ1, CCDC71L, COL9A2, GLDN, ADIPOQ, VGF, EPDR1, C2CD4C, NUTM2G, GLTPD2, MISP, PGA3, CCDC184, CTRL, C14orf180, COL26A1, C1QTNF9, LYNX1-SLURP2, COL22A1, C22orf34, C9orf163, COL4A4, MROH5, CPA2, LRRC14B, COL8A1, SYNDIG1, SLC7A9
S-VS-Y. DEGseq	COL6A2, COL1A2, COL17A1, IER2, COL1A1, NRARP, 1291(COL6A1), MIDN, COCH, C11orf96, SCARA5, COL12A1, ELN, MXRA8, XIRP1, MXRA7, C1QA, GRASP, C1QC, TTC22, C1QB, CPA3, C1QTNF5, ANKRD33B,OLFM1, VGLL3, COL15A1, COL14A1, CCDC71L, EMILIN2, C1QTNF2, EMILIN1, FNDC10, CASQ1, COL9A3, XPNPEP2, ZBED6CL, VIT, CCBE1, COL9A2, MARCO, PNMA8B, ADIPOQ, GAREM2, PLEKHO1, C1QTNF3, THEMIS2, CYS1, ATP1A2, TMEM200B, COL6A5, DPP4, SFTPD, C1QTNF7, MME, CDC42EP5, EPDR1, GLDN, SAMD11, CFAP73, BRI3BP, PROSER2, SRL, FAM110D, C1QTNF4, C2CD4A, PPFIA4, CTHRC1, PROB1, RIPOR3, C14orf180, SLC7A7, TMEM88B, MISP, COL28A1, CCDC184, GLTPD2, MSR1, KCNJ13, SLC8A3, TMEM88, WDR97, LRRC75A, NUTM2E, LCNL1, PGA5, SHISA8, TMEM178A, LYNX1-SLURP2, COL22A1, C1QL1, C1QTNF9, CPB2, ALKAL2

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References

[1] Matic. I, Ajinkya, Chen. J, et al. Identification of Salvia haenkei as gerosuppressant agent by using an integrated senescence-screening assay[J]. AGING. 8 (2016), 3223-40.

[2] Despres J, Ramdani Y, di Giovanni M, et al. Replicative senescence of human dermal fibroblasts affects structural and functional aspects of the Golgi apparatus[J]. Experimental dermatology. (2019).

[3] Kelly J, Murphy JE. Mitochondrial gene expression changes in cultured human skin cells following simulated sunlight irradiation[J]. Journal of photochemistry and photobiology B, Biology. 179 (2018), 167-74.

[4] Yu. X, Long. YC, Shen. H-M. Differential regulatory functions of three classes of phosphatidylinositol and phosphoinositide 3 kinases in autophagy[J]. Autophagy. 11 (2015), 1711-28.

[5] Tang D, Chen Q-B, Xin X-L, et al. Anti-diabetic effect of three new norditerpenoid alkaloids in vitro and potential mechanism via PI3K/Akt signaling pathway[J]. Biomedicine & Pharmacotherapy. 87 (2017), 145-52.

[6] Noh EM, Park J, Song HR, et al. Skin Aging-Dependent Activation of the PI3K Signaling Pathway via Downregulation of PTEN Increases Intracellular ROS in Human Dermal Fibroblasts[J]. Oxidative medicine and cellular longevity. 2016 (2016), 1-9.

[7] Egbert M, Ruetze M, Sattler M, et al. The matricellular protein periostin contributes to proper collagen function and is downregulated during skin aging[J]. Journal of dermatological science. 73 (2014), 40-48.

[8] Teshima E, Brunetto MA, Vasconcellos RS, et al. Nutrient digestibility, but not mineral absorption, is age-dependent in cats[J]. Journal of animal physiology and animal nutrition. 94 (2010), 251-258.

[9] Koopman R, Walrand S, Beelen M, et al. Dietary protein digestion and absorption rates and the subsequent postprandial muscle protein synthetic response do not differ between young and elderly men[J]. The Journal of nutrition. 139 (2009), 1707-13.