Journal of Dermatological Science 82 (2016) 75–83

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

The comparison of microRNA profile of the dermis between the young and elderly

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ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form 19 December 2015
Accepted 14 January 2016

Keywords:
MicroRNA
microRNA-34
microRNA-29
Skin aging
Dermis
Human

ABSTRACT

Background: Skin aging is a process of structural and compositional remodeling that can be manifested as wrinkling and sagging. Remarkably, the dermis plays a dominant role in the process of skin aging. Recent studies suggest that microRNAs (miRNAs) may play a role in the regulation of gene expression in organism aging. However, studies about age-related miRNAs in human skin remain limited.
Objective: To obtain an overall view of miRNAs expression in human aged dermis by comparison of dermis samples between young and elderly, construct the miRNA-gene-network and reveal the pivotal miRNAs in the regulatory network.
Methods: Human dermis tissue was obtained from 12 donors, including 6 of young group and 6 of elderly one. The miRNA microarray and data analysis were performed. Target genes of differentially expressed miRNAs were predicted, followed by a gene ontology and pathway enrichment analysis. A miRNA-gene-network was then constructed, and the pivotal miRNAs in the network was revealed. Primary human dermal fibroblasts (HDFs) were isolated, and the cellular senescence was induced by serial passaging. Alteration in the expression of miRNAs between young and senescent fibroblasts was evaluated by real-time quantitative RT-PCR. MiR-34b-5p mimics were transfected into primary HDFs. Subsequent cell cycle analysis was performed and expression level of COL1A1, elastin and MMP-1 were evaluated.
Results: The expression of a total of 40 miRNAs (25 upregulated and 15 downregulated) was found to be significantly altered in aged dermis compared with young dermis. Real-time quantitative PCR results confirmed the differential expression of miR-34 family and miR-29 family between young and aged dermis. A computational approach demonstrated that predicted target genes of the miRNA profile were found to be mainly involved in processes including cell adhesion, collagen synthesis, positive or negative regulation of transcription, as well as pathways such as insulin signaling pathway, ErbB (Erythroblastic Leukemia Viral Oncogene Homolog) signaling pathway and Focal adhesion pathway. The miRNA-Gene-Network revealed that miR-34 family, miR-29 family and miR-424 may play a dominant role in the regulatory network. A similar miRNA alteration was observed in senescent fibroblasts in vitro, and the age-related miRNA profile may interact with p16 pathway to regulate the fibroblasts' senescence. Additionally, transfection of miR-34b-5p mimics induced cell cycle arrest in HDFs, decreased the expression of both COL1A1 and elastin and increased MMP-1 expression.
Conclusion: The miR-34 family and miR-29 family expressed differentially in young and aged dermis. MiR-34 in HDFs modulated the cell function and expression of MMP-1, COL1A1 and elastin. The miRNAs may play critical roles in affecting dermis aging.

Abbreviations: ErbB, erythroblastic leukemia viral oncogene homolog; ECM, extracellular matrix; TGF, transforming growth factor; CTGF, connective tissue growth factor; ROS, reactive oxygen species; MMP, matrix metalloproteinases; HDF, human dermal fibroblasts; GO, gene ontology; SA-β-gal, senescence-associated β-galactosidase; BP, biology process; MF, molecular function; CC, cellular component; SIRT1, sirtuin type1; VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; COL1A1, collagen, type I, alpha 1.

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http://dx.doi.org/10.1016/j.jdermsci.2016.01.005
0923-1811/

1. Introduction

Skin is the largest organ of the human body and fulfills many important functions. In addition to protecting the inner organism from external environmental factors, it also plays an important role in the physical attractiveness of the human beings. Recently, skin aging has become a big concern, because not only does it significantly affect a person's appearance, but also it leads to a series of diseases that affect the person's quality of life. Therefore, modulating the progress of skin aging is becoming more and more important for dermatologists. From the point of view of physiology, skin aging is a structural and compositional remodeling associated with phenotypic changes in skin cells. In aged skin, the epidermis becomes thinner and both the quantity and quality of the extracellular matrix (ECM) in the dermis decrease. Remarkably, owing to the complicated nature of the components of the ECM, the dermis plays a dominant role in the process of skin aging. While this process is subjected to both intrinsic and extrinsic factors, the consequences of both involve abnormal synthesis of matrix proteins, increased degradation of collagen and elastin, and senescence of dermal fibroblasts. These molecular events have been shown to be mediated by cell-signaling pathways for collagen synthesis and ECM degradation, as well as by the senescence-associated gene pattern that controls cell-cycle progression and cell proliferation [1]. Moreover, during the last decade, global gene-expression profiling has been applied in the research field of skin aging, and hundreds of genes have then been found to participate in various cellular activities that regulate skin homeostasis, revealing that the aging process of the dermis is extremely complex [2].

Recent studies suggest that microRNAs (miRNAs) may also play a role in the regulation of gene expression in organism aging [3,4]. MicroRNAs are a class of short, endogenous, single-stranded, non-coding RNA molecules. By binding the specific 3′-UTRs sequence of the target mRNAs, they can regulate the expression of multiple genes at the post-transcriptional level through degradation or translational inhibition of the targeted transcripts [5]. Some recent studies have compared young and aged organs or tissue such as heart [6], cornea [7], kidneys [8] etc., indicating clues about the role of miRNA in regulation of aging at the tissular level, whereas for skin, especially dermis, studies about age-related miRNAs remain limited, and most of them were carried out at the cellular level [9,10].

To obtain an overall view of the miRNAs expression and the network that regulates the skin aging, we compared the miRNA profile of human dermis of a young group with that of an elderly group, predicted their targeted mRNAs, analyzed their gene ontology and signaling pathways, constructed the miRNA-gene-network, and performed function studies by miRNA mimics transfections, all of which might help better understand the mechanism of dermis aging and provide innovative views for anti-aging therapies. Moreover, to provide a view of the similarities and differences between tissue aging and cellular senescence in the skin, we also examined the expression of miRNAs in senescent human dermal fibroblasts.

2. Material and methods

2.1. Tissue collection from donors

Following the pre-approval from the Independent Ethics Committee of Huashan Hospital affiliated to Fudan University, normal human skin tissue ($8 \times 5 \times 3$ mm) from sun-protected abdomen was obtained from 12 consenting individuals who were undergone Miles surgeries or excisions of benign skin neoplasms. Since previous study discovered an age-dependent increase of senescent cells in dermis [11], a widened age gap between two groups in this study was designed in order to achieve distinctive difference. The donors with the age over 60 years old were defined as the elderly group, and ones with the age below 10 years old were defined as the young group, respectively; each group was constituted of 6 individuals. Adipose tissue and epidermis was carefully removed (on ice, by forceps and scissors, with the aid of dissecting microscope). The remained dermal tissue was stored in the liquid nitrogen at −196 °C for subsequent RNA analysis.

2.2. Primary cultures of human dermal fibroblast and the induction of senescence

Primary human dermal fibroblasts (HDFs) were obtained from the foreskin of a healthy man (Huashan Hospital, Shanghai, China). Cells were cultured in high glucose DMEM (Gibco) medium supplemented with 10% FBS (Invitrogen) at 37 °C in 5% CO_2 humidified air. Serial passaging to induce replicative cellular senescence was performed twice weekly at early passage and at decreasing dilution with increasing passage. Cells from either passage 5 or passage 20, referred as young or senescent cells, respectively, were used in subsequent experiments. The proliferative potential of the culture of different passages was evaluated by cell morphology, survival curve and senescence-associated β-galactosidase staining.

2.3. RNA extraction

Total RNA of dermal tissue or dermal fibroblasts was extracted using mirVana™ miRNA Isolation Kit (Ambion) according to the manufacturer's instructions. The quantity and quality was evaluated using a bioanalyzer (Agilent 2100 Bioanalyzer, LabChip Kits).

2.4. MicroRNA microarray and data analysis

MicroRNA microarray study, including labeling, hybridization, scanning, normalization, and data analysis, was performed by Shanghai Biotechnology Corporation (Shanghai, China) on Agilent Human miRNA Genechip V16.0, which held 1205 human-related miRNAs. Briefly, miRNA molecules were labeled according to the manufacturer's protocol, followed by the hybridization reaction for 20 h at 55 °C. Microarray results were obtained and extracted by Agilent Microarray Scanner and Feature Extraction software 10.7 (Agilent). Raw data were subsequently normalized by Quantile algorithm with Gene Spring Software 11.0 (Agilent). The array output was presented on Excel spreadsheets giving lists of miRNA profile in each young and aged human dermal sample. The lists were sorted based on the most differentially expressed miRNAs between the two sample groups. Filtering criterial set for defining up- or downregulated miRNAs was a fold change ≥ 2 and a p value ≤ 0.01.

2.5. MicroRNA target gene prediction, annotation and function analysis

Target genes regulated by the miRNAs can be predicted theoretically by applying an advanced computational approach. With different algorithms, the prediction of target genes of either up-regulated or down-regulated miRNAs in this study was performed within the databases of miRDB [12] and TargetScan [13]. Gene ontology (GO) and pathway enrichment of target genes were retrieved from the database for annotation, visualization, and integrated discovery (DAVID Bioinformatics Resources, http://david.abcc.ncifcrf.gov/home.jsp). The enrichment was calculated with the modified Fisher's exact test in the DAVID Bioinformatics