Leucine-rich alpha-2-glycoprotein 1, which is down-regulated during skin aging, plays a role in the maintenance of extracellular matrix integrity

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Abstract

The skin aging process can be divided into intrinsic aging and photo-aging. Photo-aging has been studied extensively, but intrinsic aging is poorly understood. In an attempt to identify genes involved in the intrinsic skin aging, we analyzed differentially expressed genes (DEGs) by RNA-sequencing analysis of dorsal skin tissues from 3-month-old (young) and 24-month-old (old) SKH1 hairless mice. Transcriptome data showed that over 1500 genes are up-regulated or down-regulated during intrinsic skin aging. The transcript of the gene encoding leucine-rich alpha-2-glycoprotein 1 (LRG1) was significantly down-regulated in skin tissues of the old group. Although LRG1 is known as a positive regulator of transforming growth factor- β signaling pathway, its function and the detailed mechanism in the skin aging have not been well investigated. Our results showed that mRNA and protein levels of LRG1 significantly decreased in the skin of the old group compared with that of the young group. A recombinant human LRG1 (rhLRG1) was expressed and purified from HEK 293 cells. Treatment of rhLRG1 increased the synthesis of type I collagen and reduced the secretion of MMP-1 in human dermal fibroblasts. In addition, rhLRG1 increased the phosphorylation of Smads and an inhibitor of type I TGF- β receptor abolished the rhLRG1-induced phosphorylation of Smads, suggesting that LRG1 activates the TGF- β signaling pathway. In these regards, our results show that LRG1 has a potential to retard skin aging by inducing TGF- β signaling pathway and then maintaining extracellular matrix integrity.

Figure 1. Strategy to identify differentially expressed genes in mouse and human skin aging

Differentially expressed genes were screened from skin tissues from aging models of humans and mice by transcriptome analysis and bioinformatics approach. Target genes were selected by further analysis based of RT-qPCR, immunohistochemistry, and western blotting.

Table 1. Expression of LRG1 gene in young and old mouse and human skin tissues analyzed by microarray and RNA-Seq

RNA-seq	Mouse		
	Gene Symbol	log ₂ ratio (young vs. old)	q value
	Lrg1	-0.74	0.001
Human	Male		Female
	Gene Symbol	log ₂ ratio (young vs. old)	q value
	LRG1	-2.89	0.2576
Microarray	Mouse		
	Probe Set ID	Gene Symbol	log ₂ ratio (young vs. old)
	1417290 at	Lrg1	-1.68
Human	Male		Female
	Probe Set ID	Gene Symbol	log ₂ ratio (young vs. old)
	228648 at	LRG1	-2.26

Figure 2. mRNA and protein levels of LRG1 in young and old mouse skin tissues

(A) The mRNA level of the Lrg1 gene was analyzed by RT-qPCR in mouse skin tissues. (B) The protein level of LRG1 was analyzed by western blotting in mouse skin tissues. Expression levels of the Lrg1 gene were quantified using Multi-gauge densitometry software. Values are presented as means $\pm$ SD.

Introduction

Intrinsic skin aging is characterized by the progressive accumulation of senescent cells, thinning of the epidermis, decline in cell-to-cell adherence, reduction of collagen content, formation of fine wrinkles, and gradual loss of elastic materials.

Type I collagen is the most abundant structural protein in skin connective tissue and confers strength and resilience to skin. Type I collagen is primarily synthesized by dermal fibroblasts. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endoproteases that play pivotal roles in the dynamic remodeling of the extracellular matrix.

MMP-1 specifically degrades the interstitial collagens, types I, II and III and is also produced by fibroblasts. In our RNA-seq and microarray analysis, LRG1 is one of the down-regulated genes during skin aging.

Here we analyzed expression of LRG1 in young and old mouse skin tissues. Furthermore, we examined effect of LRG1 on expression of MMP-1 and type I collagen in human dermal fibroblasts. Based on these data, we suggest a potential role of Lrg1 in retardation of skin aging.

Figure 3. Histological analysis of LRG1 in paraffin-embedded sections of mouse skin tissues.

Sections of mouse skin tissues were stained with LRG1 antibody and counterstained with hematoxylin. Magnification, x 200.

Figure 4. Purification of the His-tagged recombinant human LRG1 (rhLRG1)

(A) Scheme of the rhLRG1 expression construct. (B) The rhLRG1 expression construct was stably expressed in HEK293 cells. The rhLRG1 was purified using Ni²⁺-NTA column chromatography. Purified protein was subjected to SDS-PAGE and stained with Coomassie brilliant blue.

Figure 5. Effect of LRG1 on expression of MMP-1 and type I collagen in young dermal fibroblasts

Serum-starved human dermal fibroblasts were incubated in the presence of various concentrations of LRG1 (0-8 μ g/ml) for 24 h. Expression levels of type I collagen, MMP-1, and GAPDH in cell lysates and conditioned media were evaluated by western blot analysis using antibodies against type I collagen, MMP-1, and GAPDH.

Figure 6. Time-dependent effects of LRG1 on Smad2 phosphorylation

Serum-starved human dermal fibroblasts were treated with LRG1 at concentration of 2 μ g/ml for the indicated time intervals. Cell lysates were examined by western blotting using antibodies against Smad2 and phospho-Smad2.

Figure 7. Effect of Role of a TGF- β receptor inhibitor on LRG1-mediated Smad2 phosphorylation

Human dermal fibroblasts were starved for 12 h, followed by treatment with LRG1 (2 μ g/ml) or TGF- β 1 (3 ng/ml) for 90 min in the presence of an inhibitor of type I TGF- β receptor, SB431542 (10 μ M), or vehicle for 90 min. Whole cell lysates were examined by western blotting with antibodies for Smad2, phospho-Smad2, and GAPDH.

Conclusions

1. RNA-seq and microarray analyses of mouse and human skin tissues showed that Lrg1 is significantly down-regulated during skin aging.
2. mRNA and protein levels of LRG1 were down-regulated in old mouse skin tissues compared with young mouse skin tissues.
3. Treatment of recombinant LRG1 reduces MMP-1 expression and promotes type I collagen expression in human dermal fibroblasts.
4. LRG1 enhances Smad2 phosphorylation in human dermal fibroblasts.
5. An inhibitor of TGF- β receptor abolished the LRG1-induced Smad2 phosphorylation, suggesting that LRG1 activates the TGF- β signaling pathway.
6. LRG1 has a potential to retard skin aging by inducing TGF- β signaling pathway and then maintaining extracellular matrix integrity.