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A study on the cellular senescence-inducing function of aging markers acting on the 1st aging peak.

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Introduction:

The final goal of cosmetic research is to prevent skin aging and restore aging skin. In a previous study, we discovered four types of aging marker genes, FSTL3, GDF15, MMP12, and CCDC80, whose expression in skin cells changes significantly during the 1st aging peak that occurs around the age of 40 [1]. MMP12 as an elastase was confirmed to increase with aging of skin cells, and it can promote the degradation of elastin in the skin and cause a decrease in skin elasticity. However, in the case of FSTL3, GDF15 and CCDC80, except for MMP12 among the four aging markers, what function induces skin aging is not yet known.

Growth differentiation factor 15 (GDF15) is a stress molecule produced in response to mitochondrial, metabolic and inflammatory stress with a number of beneficial effects on metabolism [2]. Follistatin-like 3 (FSTL3) is a glycoprotein that binds and inhibits the action of TGFβ ligands such as activin. It mediates cell differentiation and growth, acts as a biomarker of tumors and participates in cancer development and progression. Coiled-coil domain containing 80 (CCDC80) is a secreted protein highly enriched in mouse and human white adipose tissue (WAT) that plays an important role during adipocyte differentiation. It is a modulator of glucose and energy homeostasis [5].

In this study, the mechanism of cellular senescence by aging markers was investigated. The GDF15 promotes skin cell aging through ROS generation, and CCDC80 suppresses ROS generation to suppress skin cell aging. This is done through p16 signaling associated with ROS generation. GDF15 also promotes melanin synthesis, which leads to skin darkening. Therefore, the novel skin aging markers we found may be involved in comprehensive skin aging control.

Materials & Methods:

Cell cultures and reagents

Human primary fibroblast cells (HDFn, PromoCell) and B16F10 melanoma cells (ATCC) were used.

Senescence-associated-β-galactosidase (SA-β-gal) staining and soluble enzyme assay

SA-β-gal activity was detected using a senescence cell histochemical staining kit (Sigma-aldrich, USA) as per the manufacturer's instructions. Stained cells were viewed under a microscope (Leica, Germany). ONPG (O-nitrophenyl-beta-D-galactopyranoside) enzyme analysis of SA β-gal activity followed experimental procedure of SensoLyte® NPG β-Galactosidase Assay Kit Colorimetric (AnaSpec, USA). The absorbance was measured at 420 nm with an EPOCH2 ELISA reader (Bio-Tek, USA).

ROS detection

The Abcam DCFDA ROS detection kit (ab113851, Abcam, Cambridge, MA) uses 2',7'-dichlorofluorescein diacetate reagent (DCFDA), a fluorogenic dye measuring hydroxyl, peroxyl and other intracellular ROS

Western blot analysis

The primary antibodies were as follows: anti-CDKN2A/p16INK4a antibody, anti-p21 antibody, (GeneTex, Irvine, USA)

Melanin content assay

The B16F10 melanoma cells were exposed to various concentrations (50, 100, 150 and 100 ng/ml) of the recombinant aging marker proteins for 48 h. The cells were lysed with 800 ul of 1 N NaOH (Merck, Germany) containing 10% DMSO for 1 h at 60 ◦C. The absorbance at 420 nm was measured using an EPOCH2 ELISA reader (Bio-Tek, USA).

Results & Discussion:

1. GDF15 promoted cellular senescence and CCDC80 inhibited cellular senescence.

As a result of SA-β-gal staining, it was confirmed that the dyed blue color increased when treated with rh GDF15 and rh FSTL3. This suggests that rh GDF15 and rh FSTL3 promote cellular senescence (Fig. 1A, Upper panel). Furthermore, SA-β-gal activity increased by H2O2 was enhanced when treated with rh rh GDF15, and weakened when treated with rh CCDC80 (Fig. 1A, Lower panel). In addition, to quantitatively measure SA-β- activity the ONPG test was performed. (Fig. 1B).

From these results, it was confirmed that rhGDF15 promoted cellular senescence and rh CCDC80 inhibited cellular senescence. However, the case of rh FSTL3 showed a different result from the staining result. This should be studied further later.

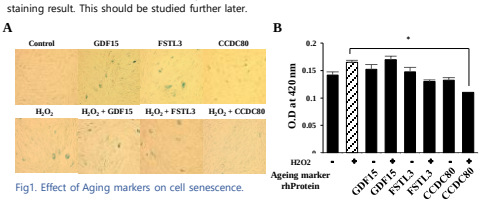


Fig1. Effect of Aging markers on cell senescence.

Results & Discussion:

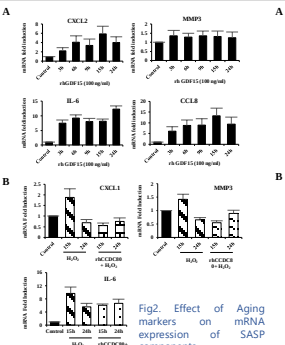


Fig2. Effect of Aging markers on mRNA expression of SASP components.

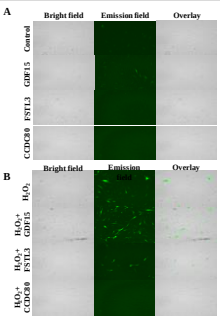


Fig3. Effect of Aging markers on ROS generation.

2. GDF15 promotes cellular senescence and CCDC80 inhibits cellular senescence caused by oxidative stress.

rhGDF15 was treated in HDFn cells and gene expression levels were measured for each time point (0h, 3h, 6h, 9h, 15h and 24h). As a result, expression levels of CXCL2, MMP3. IL-6, and Ccl8 genes were increased by rh GDF15 (Fig. 2A). In addition, rh CCDC80 suppressed the increase in gene expression of CXCL1, MMP3 and IL-6 by H2O2 (Fig. 2C). From the above results, it can be hypothesized that rh GDF15 promotes cellular senescence and rh CCDC80 inhibits cellular senescence caused by oxidative stress.

3. GDF15 and CCDC80 are involved in ROS generation and affect cell senescence.

It was speculated that the mechanisms of the aging-promoting effect of the aging marker GDF15 and the aging-inhibiting effect of CCDC80 are related to oxidative stress, and ROS generation was measured to confirm this.. As a result, it was confirmed that rh GDF15 induces ROS generation and promotes ROS generation under oxidative stress, and rhCCDC80 has the effect of inhibiting ROS generation due to oxidative stress. This means that GDF15 and CCDC80 are involved in ROS generation and affect cell senescence (Fig. 3).

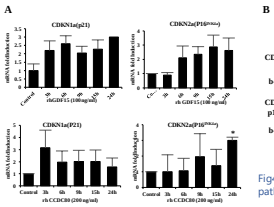
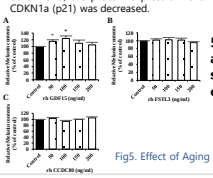


Fig4. Effect of Aging markers on p16 signaling pathway.

4. GDF15 and CCDC80 affect senescence through opposing actions on p21.

As a result, rh GDF15 increased the gene expression levels of CDKN1a (p21) and CDKN2a (p16^{INK4a}) in a time-dependent manner in HDFn cells. On the other hand, by rh CCDC80, it was confirmed that the gene expression level of CDKN2a (p16^{INK4a}) was significantly increased only at 24 hours (Fig. 4A). Furthermore, when the protein expression levels of CDKN2a (p16^{INK4a}) and CDKN1a (p21) were confirmed through western blot, the results were similar to those of mRAN expression (Fig. 4B). The rh GDF15 increased the prtoein expression levels of CDKN1a (p21) and CDKN2a (p16^{INK4a}). On the other hand, the protein expression level of CDKN2a (p16^{INK4a}) was increased by rh CCDC80, but CDKN1a (p21) was decreased.



5. GDF15 not only promotes cellular aging but also increases melanin synthesis, thereby causing overall aging of the skin (Fig. 5).

Fig5. Effect of Aging markers on the regulation of pigmentation.

Conclusions:

We developed a technology to dramatically prevent aging by using aging markers that act during the 1st aging peak, when the skin is rapidly aging. We found that the skin aging markers, GDF15 and FSTL3, promotes cell senescence by inducing ROS generation through the p16 signaling pathway. On the other hand, it was confirmed that CCDC80 can inhibit aging through the effect of inhibiting ROS generation. In addition, it was confirmed that melanin synthesis that darken the skin tone were promoted by the aging marker GDF15. Through this, the aging markers we discovered can be used as a new target for the development of ingredient that can comprehensively care for skin aging in various fields, from wrinkles and elasticity to whitening and cellular senescence.

References:

1. Lee, S., An, H., Park., H.W., Cho., J (2021) Identification of novel markers for skin senescence associated with 1st ageing peaks of life span (2021IFSCC conference)
2. Park, H., Kim, C. H., Jeong, J. H., Park, M., Kim, K. S (2016) GDF15 contributes to radiation-induced senescence through the ROS-mediated p16 pathway in human endothelial cells. Oncotarget 7(9): 9634–9644.