Hydrolyzed soy protein prevents the wrinkle formation through regulating lysosomal function

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Guo Yang, Zhou Zheng, Guo Miao, Zhang Jinlong, Yang Fan

Mageline Biology Tech Co., Ltd. 20th Floor, Block A, Wandazun, Universal International Center, Wuchang District, Wuhan, Hubei Province, China

Introduction:

One of effective approaches to look skin healthy and youthful in women is the prevention of wrinkle formation because the wrinkle around their eyes and the other area on their faces changes the impression of face appearance of them. In particular, deep wrinkles, which formed by impairing extracellular matrix (ECM) such as collagen and elastin, is hard to be recovered by the treatment of only moisturizing substances, therefore it is needed to prevent the wrinkle formation by using the cosmetics containing some ingredients which have the suppressing effect regarding wrinkle formation.

In general, ECM is digested by lysosomes which have proteinases containing cathepsins when the ECM is impaired or denatured by stresses such as ultraviolet or reactive oxygen species and so on, and then digested ECM components are used to synthesize new one [1]. Thus, the regulation of lysosome activity is quite important to prevent skin-aging containing wrinkle formation. However, it is known that lysosomal quality is decreased with age or photo-aging [2], and which results in denatured ECM is not only accumulated in dermis but also induced oxidative stress by them. Since oxidative stress induces ECM degradation by matrix metalloproteinase 1 (MMP-1) via ERK/JNK pathway [3], it is suggested that wrinkle formation is accelerated due to impairing lysosomal function. From the facts, it considers that the maintain of lysosome activity is important matter to prevent the wrinkle formation.

Hydrolyzed protein (PPT), which derived from animal and vegetable proteins, has been used as a famous active ingredient in cosmetic market because they have various function for skin and hair such as anti-oxidant ability, anti-melanogenesis and so on. In this study, we investigated the improving effect of lysosome by PPT. From the result of this study, we found that PPT had attractive effect regarding lysosomal function through up-regulating sirtuin 1 (SIRT1) expression. We consider that the effect by PPT shows a potential to exert anti-wrinkle effect through improving lysosomal function.

Materials & Methods:

The evaluation of lysosome activity

Normal human dermal fibroblast (NHDFs; Kurabo) were incubated with or without samples for 24 h, and then were irradiated to UVA (2 J/cm²). After incubation for another 24 h, the cells were lysated by 0.5% Triton X-100. The lysate was mixed with Z-Phe-Arg-MCA as a substrate of cathepsin for 30 min at room temperature. The fluorescent intensity and the amount of protein was measured. In addition, the lysosome activity was measured using Lysosomal Intracellular Activity Assay Kit (Biovision) and the lysosomes were stained by Lysotracker Red DND-99 [4] (Thermo).

The evaluation of mRNA expression

NHDFs were incubated with or without samples for 24 h, and then were irradiated to UVA (2 J/cm²). After incubation for another 24 h, total RNA of them were extracted and synthesized cDNAs using a Power SYBR Green Cells-to-CT kit (Thermo). Real-time PCR was performed using the Real-Time PCR with specific primers.

Result & Discussion 1:

The lysosome activity of NHDF by PPT

We evaluated the cathepsin and lysosomal activity of fibroblast by five kinds of PPTs. As the results, all PPTs showed the up-regulation of cathepsin activity, in particular hydrolyzed soy protein (HSP) exerted the effect at low concentration (Fig. 1). Additionally, HSP also had high activity of lysosome compared with other PPTs (Fig. 2), suggested that HSP has a potential to activate lysosomal function effectively.

In order to investigate the effect of HSP in UVA-irradiated NHDFs regarding lysosome activity, the cathepsin activity of UVA-irradiated NHDFs which pre-treated with HSP for 24 h. Although the cathepsin activity of NHDFs were decreased by UVA, the pre-treatment with HSP suppressed the decrease of activity of them (Fig. 3), suggested that HSP maintains lysosome activity in UVA-irradiated NHDFs.

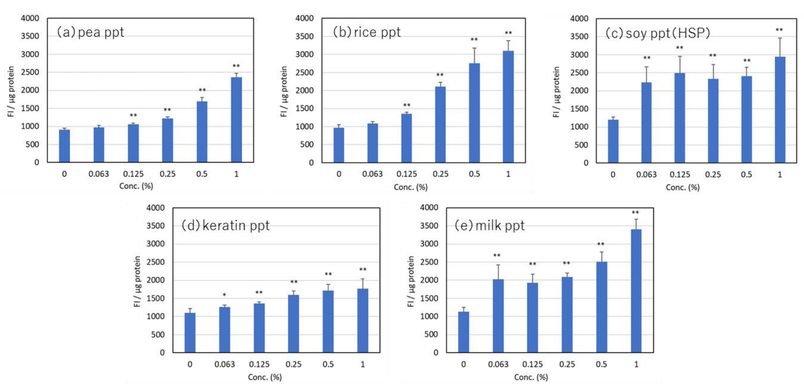


Fig. 1 The evaluation of cathepsin activity by PPT in NHDFs. (*p<0.05, **p<0.01 vs. 0%, n=4)

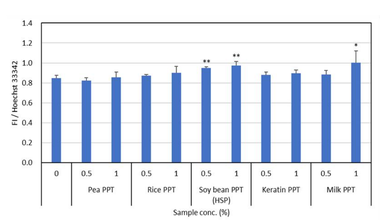


Fig. 2 The evaluation of lysosome activity by PPT in NHDFs. (*p<0.05, **p<0.01 vs. 0%, n=4)

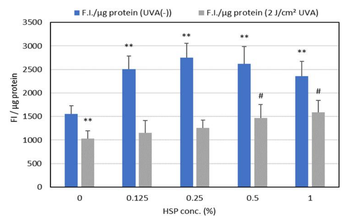


Fig. 3 The evaluation of cathepsin activity by HSP in UVA-irradiated NHDFs.(**p<0.01 vs. 0% (UVA(-)),#p<0.05 vs. 0% (UVA(+)), n=4)

Results & Discussion 2:

Influence of ECM related mRNA expression by HSP in NHDFs

To investigate the effect on ECM related substances by HSP, we evaluated the mRNA expression of ECM related substances of NHDFs treated with HSP. Treatment with HSP for 24 h significantly up-regulated the mRNA expressions of tropo-elastin (ELN) and sirtuin 1 (SIRT1), and the down-regulated the expression of MMP-1 (Fig. 4). Among of them, we focused on the effect of regulation of SIRT1 and MMP-1 by HSP since it is known that SIRT1 negatively regulates MMP-1 expression [5]. In UVA irradiated condition in NHDFs, the mRNA expression of SIRT1 was decreased and the MMP-1 expression was increased respectively. On the other hand, HSP pre-treatment significantly recovered these mRNA expressions (Fig. 5), suggested that HSP has a potential to prevent the up-regulation of MMP-1 by UVA via SIRT1 activation.

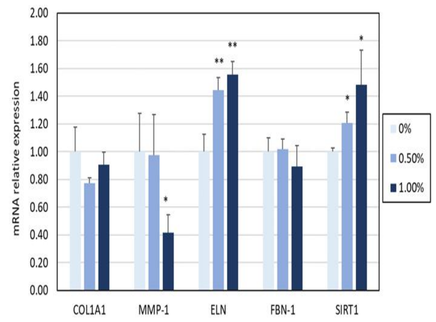
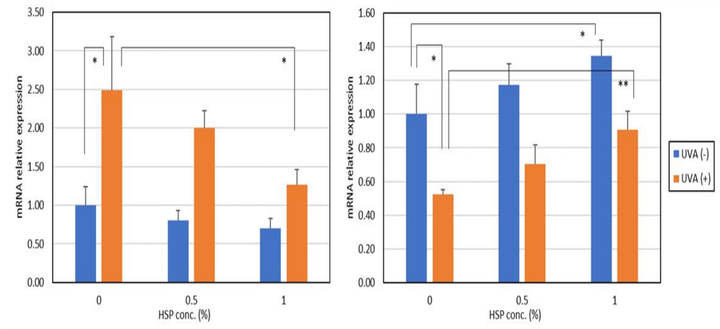


Fig. 4 The evaluation of mRNA expression by HSP in NHDFs. (*p<0.05, **p<0.01 vs. 0%, n=3) COL1A1; collagen 1A1, MMP-1; matrix metalloproteinase 1, ELN; tropo-elastin, FBN-1; fibrillin 1, SIRT1; sirtuin 1



(a) MMP-1 mRNA (b) SIRT1 mRNA

Fig. 5 The mRNA expression of MMP-1 (a) and SIRT1 (b) by HSP in UVA-irradiated NHDFs. (*p<0.05, **p<0.01, n=3)

The mechanism of the suppression of MMP-1 expression by HSP in NHDFs

From the above results, we considered that HSP maintained the lysosome activity and MMP-1 expression in UVA-irradiated NHDFs through up-regulating SIRT1. Thus, in order to clarify the relationship between SIRT1 and cathepsin activity and MMP-1 expression, we evaluated the influence of Ex-527, which is an inhibitor of SIRT1 [6], on cathepsin activity and MMP-1 expression in HSP treated NHDFs. The up-regulation of cathepsin activity and lysosome activity by HSP was significantly abrogated by Ex-527 in steady state and UVA-irradiated NHDFs (Fig. 6, 7). Furthermore, the down-regulation of MMP-1 mRNA expression by HSP also impaired by Ex-527 in those NHDFs (Fig. 8). The sum of these results, it is suggested that the effect of HSP in NHDFs was exerted by up-regulating SIRT1.

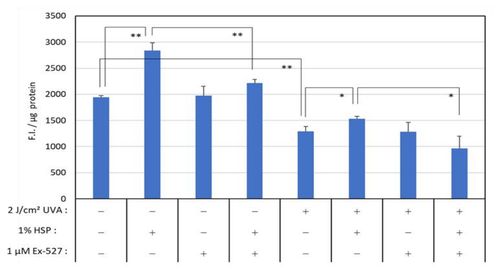


Fig. 6 Influence of Ex-527 in cathepsin activity in HSP-treated NHDFs. (*p<0.05, **p<0.01, n=3)

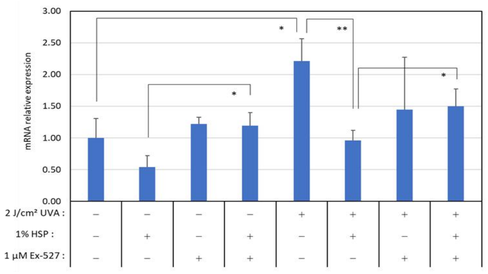


Fig. 8 Influence of Ex-527 in MMP-1 mRNA expression in HSP-treated NHDFs. (*p<0.05, **p<0.01, n=3)

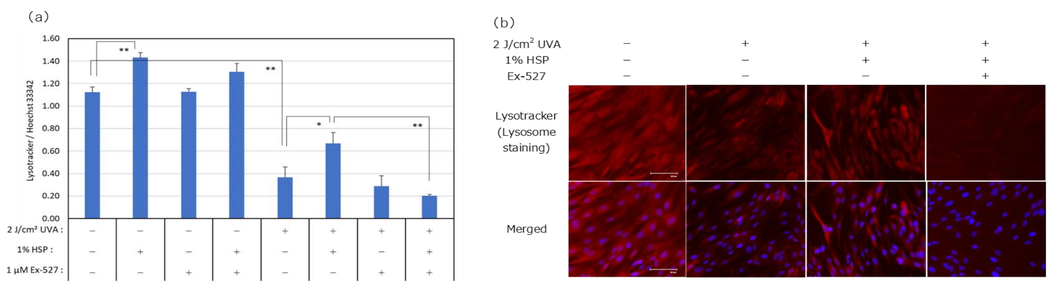


Fig. 7 Influence of Ex-527 in lysosome activity in HSP-treated NHDFs.
(a) The fluorescent intensity derived from lysosomes in NHDFs (*p<0.05, **p<0.01, n=3)
(b) The observation of lysosomes using the fluorescent microscopy (red; lysosome, blue; nucleus, bar scale; 100 μm)

Conclusions:

We conclude that HSP has a potential to prevent and/or improve wrinkle formation by not only the digestion of MMP-1 via activation of lysosome but also the suppression of MMP-1 expression via up-regulating SIRT1.

References:

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