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Augmentation of Sebaceous Lipogenesis by an Extract of *Grifola frondosa* (Maitake Mushroom) *In Vivo* and *In Vitro*

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Introduction

Sebaceous lipids (sebum) play an important role in maintaining physiological functions by forming a biological barrier in the skin (Fig. 1). The decrease of sebum levels in the skin is thought to depress the barrier functions, and thereafter may be associated with the development of dry skin (xerosis) with a variety of complaints including a rough or scaly skin surface, and pruritus (Fig. 1). Therefore, a novel aspect to control sebaceous lipogenesis might be beneficial for the prevention of dry skin and sequential itching. In the present study, we investigated the effects of an ethanol extract of *Grifola frondosa* (Maitake) fruit body (Gripin®) (Fig. 2) on sebum production in hamsters and humans and compared these effects with those of an ethanol extract of *Agaricus blazei murrill* (Agaricus) *in vivo* and *in vitro*.

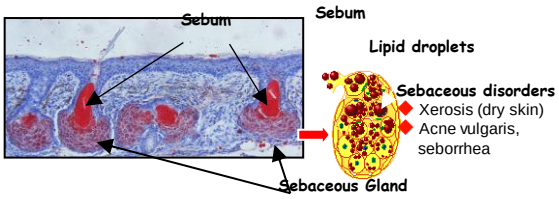

Fig. 1 Sebaceous glands in the skin
Left panel, oil red O and hematoxylin staining in the skin of hamster.

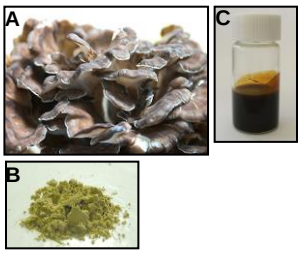

Fig. 2 Ethanol extract of *Grifola frondosa* (Maitake) fruiting body (Gripin®)
A, *Grifola frondosa* (Maitake); B, dry powder of Maitake; and C, ethanol extract of Maitake, designated Gripin®.

Results

1. When hamster sebocytes were treated with Gripin®, the intracellular lipid droplet formation was augmented in a dose-dependent manner (100-400 μg/ml) (Fig. 3).
2. Gripin® preferentially augmented the synthesis of triacylglycerols (TG), a major sebum component, in hamster sebocytes (Figs. 4 and 5). The augmentation of TG production resulted from the increase of cellular DGAT activity in hamster sebocytes (Fig. 5, *inserted panel*).
3. The topical treatment of hamster auricles with 1-4% Gripin® augmented sebum accumulation in sebaceous glands (Fig. 6).
4. Another ethanol extract prepared from *Agaricus blazei murrill* (Agaricus) showed less or no effect on sebaceous lipogenesis in hamsters *in vivo* and *in vitro* (Figs. 3, 4, and 6).
5. In most of the volunteers treated with 0.1 and 1% Gripin® cream, the levels of sebum components including TG, squalene, free-fatty acids, and cholesterol were augmented when compared with the vehicle cream treated skin.

Conclusion

These results provide novel evidence that Gripin® augments sebaceous lipogenesis in hamsters and humans *in vivo* and *in vitro*. Thus, Gripin® is likely to be a unique anti-dry skin agent with lipogenic actions for sebocytes.

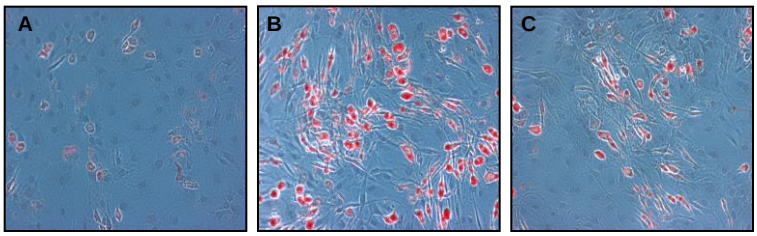

Fig. 3 Gripin® augments the formation of lipid droplets in hamster sebocytes
A, control; B, Gripin® (400 μg/ml); and C, the ethanol extract prepared from *Agaricus blazei murrill* (400 μg/ml). The cells were stained with oil red O.

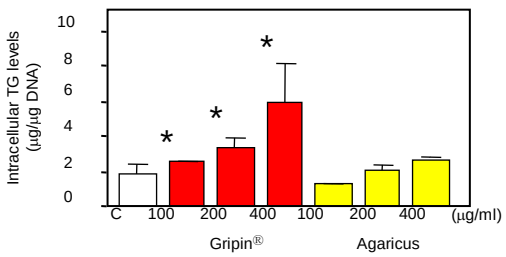

Fig. 4 Gripin® increases the intracellular levels of TG in hamster sebocytes
*, significantly different from untreated cells (C) ($p<0.05$).

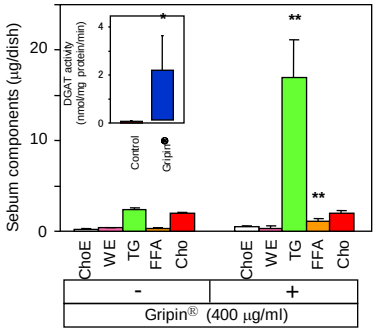

Fig. 5 Gripin® preferentially augments TG synthesis by increasing DGAT activity in hamster sebocytes
Inserted panel, DGAT activity. * and **, significantly different from untreated cells (Control) ($p<0.05$ and 0.01, respectively). ChoE, cholesterol ester; WE, wax ester; TG, triacylglycerols; FFA, free-fatty acids; and Cho, cholesterol.

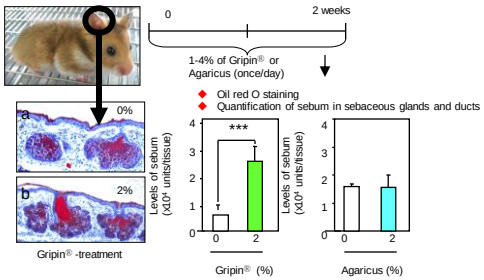

Fig. 6 Gripin®, but not Agaricus, augments the accumulation of sebum in sebaceous glands and ducts in hamsters
Treatments of Gripin® and Agaricus at 1-4% were performed and similar results were obtained under these experimental conditions. Thus, typical data of 2% Gripin® and Agaricus treatment are shown. ***, significantly different from vehicle-treated cells ($p<0.001$).

Table 1 The augmentation of sebum components in Gripin® cream-treated skin of healthy volunteers

Sebum components	The number of volunteers whose lipid levels in the skin were increased	
	0.1% Gripin®	1% Gripin®
Squalene	6/7	6/7
Wax ester	4/7	4/7
Triacylglycerols	3/7	3/7
Free-fatty acids	4/7	6/7

When 0.1 and 1% Gripin® cream, and vehicle cream were topically treated three times a day for 14 days on the antebrachial regions of seven healthy volunteers (six males and one female, age: 22-41 years) , a thin-layer chromatographic analysis was performed to quantify the levels of sebum components on the skin surface.

Materials & Methods

Treatment. Hamster sebocytes were treated with Gripin® (100-400 μg/ml) or the ethanol extract of *Agaricus blazei murrill* (Agaricus) (100-400 μg/ml) in DMEM/F12 supplemented with the serums for up to 7 days.

Oil red O staining. After the treatment of sebocytes with Gripin® or Agaricus, cells were stained with 0.3% oil red O in isopropanol:distilled H_2O (3:2, vol:vol), and then viewed with a light microscope furnished with a digital camera. The cells were also counterstained with Mayer's hematoxylin solution.

Quantitative analysis of sebaceous lipids. The sonicated-cell lysates were subjected to an automatic thin-layer chromatography, Iatroscan, as previously described (1). Otherwise, the sonicated-cell lysates were used to measure the level of intracellular TG using Liquitech TG-II according to the manufacturer's instructions. The intracellular DNA content was measured using authentic salmon sperm DNA (6.25-100 μg/ml) and 3,5-diaminobenzoic acid dihydrochloride as previously described (2).

Diacylglycerol acyltransferase (DGAT) activity. DGAT activity in hamster sebocytes treated with Gripin® was measured using 1,2-dioleoyl glycerol, and [^{14}C]palmitoyl-CoA as previously described (3, 4).

In-vivo experiments in hamsters. Auricles of 3 week-old male golden hamsters were topically treated with 1-4% Gripin® or Agaricus extract in 95% ethanol and 5% glycerol, or with the same volume of vehicle for 14 days. After the treatments, the frozen tissue sections were stained with 0.3% oil red O and counterstained with Mayer's hematoxylin solution as described above. The relative intensity (units/tissue) of oil red O staining in sebaceous glands and ducts was quantified using an imaging analysis system, Lumina Vision (Mitani Co., Japan). The animals had free access to food and water according to the Guidelines of Experimental Animal Care issued by the Prime Minister's Office of Japan. The experimental protocol was approved by the Committee of Animal Care and Use of Tokyo University of Pharmacy and Life Sciences.

In-vivo trial of Gripin® cream in volunteers. Gripin® creams (0.1 and 1%) and vehicle one were topically treated three times a day for 14 days on the posterior antebrachials of seven healthy volunteers (six male and one female; age: 22-41 years). The skin was wiped with cotton and acetone, and then the sebum on the skin surface was extracted twice with acetone using stainless cups at 1 h after the wiping. The sebum extracts were subjected to Iatroscan and the amounts of lipid components were measured as described above.

Statistical analysis. Data are presented as mean ± standard deviation (SD), and were analyzed by a one-way analysis of variance (ANOVA) and by the Fisher test for multiple comparisons. A value of $p<0.05$ was considered to indicate a statistically significant difference.

References

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