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Proliferating effect of extracts from woods and fungi on rat vibrissa dermal papilla cells

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Abstract Hair growth is a highly regulated cyclical process. Three distinct phases have been defined for the mammalian cycle: anagen (growing phase), catagen (regression phase), and telogen (resting phase). Although little is known about the mechanism that regulates the hair cycle, it is believed that dermal papillae (DP) derived from mesenchymal cells play an essential role in controlling the established hair follicle and hair cycle. The purpose of this investigation was to find the components of woods and fungi that exert a proliferative activity on DP cells. Results show that the fungus YL161 (ethyl acetate extract, 1 ppm), *Agaricus blazei* (ethyl acetate extract, 0.1 ppm), and the bark of *Camptotheca cuminata* (methanol extract of bark, 0.1 ppm) exhibit higher growth-promoting activity than pentadecanoic acid. These components that have a proliferative effect on DP cells may be useful hair growth-stimulating materials and can be used to understand the mechanism of hair growth.

Key words Dermal papilla · Hair cycle · Wood · Fungus

Introduction

Hair growth is a highly regulated cyclical process. Three distinct phases have been defined for the mammalian cycle: anagen (growing phase), catagen (regression phase), and telogen (resting phase).[1,2] Hair follicles are formed from epidermal and dermal tissues derived from ectodermal and mesenchymal cells, respectively.[3] The dermal papilla (DP), which is formed during embryonic development from a stable population of specialized fibroblasts, exists in the base of the hair follicle and is surrounded by epidermal cells.[3] Although little is known about the mechanism that regulates the hair cycle, it is believed that the DP plays an essential role in controlling the established hair follicle and hair cycle. DP implanted in ear dermis can induce the formation of hair follicles and hair growth in rats.[4] Cultured DP cells can induce the growth of hair when implanted into the bases of follicles from which the lower halves had been removed.[5] This suggests that DP cells have specific properties that modulate follicular epidermal cell activity and thus control the growth of hair. When the follicles from which the DP had been removed are cultured with DP, the spikes elongate toward the DP, finally reaching and surrounding it.[6] This suggests that DP cells produce a factor(s) that enhances the growth of follicular epithelial cells and attracts those cells as well.

Hair follicle size correlates with DP volume,[7,8] and its volume is due to cell numbers and extracellular matrix.[9] Generally the hair follicle that exhibits alopecia is smaller than normal.

Taking into consideration the essential roles of DP mentioned above, the extracts that have the proliferating effect on the DP cells may be useful not only as hair growth-stimulating materials but also as biochemical reagents in tests to help us understand the mechanism of hair growth. The purpose of this investigation is to identify components in wood and fungi that have proliferative activity on DP cells.

Materials and method

Samples

A total of 366 extracts were prepared from fruiting bodies (n = 12) and mycelia (n = 188) of fungi, woods (bark 57, heartwood 76), and others (n = 37).

Extract preparation

The heartwood and bark portions were chipped and milled to pass a no. 40 screen. The milled heartwoods

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and barks were extracted with methanol for 10 h at room temperature.

The fungi samples were prepared as follows. An erlenmeyer flask containing 200 ml standard basal medium (low nitrogen)[10] or PMY [peptone (5 g/l)/malt extract (5 g/l)/yeast extract (2 g/l)/glucose (15 g/l)] was inoculated with 10 agar plugs obtained from 5-day-old potato dextrose agar plates of fungi. Incubation was carried out at 30°C and 150 rpm until mycelia grew well, after which the cultures were homogenized. The homogenates were suspended in water and partitioned with ethyl acetate successively to give an ethyl acetate-soluble (ethyl acetate extract) portion and an aqueous portion (water extract).

Fruiting bodies were prepared as follows. The sample was freeze-dried and chipped into small portions. The portions were refluxed with water, and residue was extracted by ethyl acetate, resulting in an aqueous portion (water extract) and an ethyl acetate-soluble portion (ethyl acetate extract).

Cultivation of rat vibrissa dermal papilla cells

Rat vibrissa papilla cells were purchased from Toyobo (Osaka, Japan). DP cells were cultured in special medium (Toyobo, Osaka, Japan) for proliferation of DP cells. The fifth or sixth passage cells were used for the following study. DP cells were inoculated on a type 1 collagen-coated plastic dish at 2×10^5 cells/ml (96-well plate; Falcon Labwaer, Becton Dickinson, Oxford, UK) or 3×10^4 cells/ml (24-well plate, Falcon Labwaer). After a 24-h incubation (37°C, 5% CO_2) the sample was dissolved in ethanol (1:1000 v/v to medium) for ethyl acetate and methanol extracts or in water (1:100 v/v to medium) for water extracts and added to the culture medium to result in sample concentrations of 10, 1.0, and 0.1 ppm. The control was prepared with ethanol or water without a sample.

Measuring the growth of dermal papilla cells by tetrazolizum salt WST-1

The degree of cell growth was determined by a cell counting kit (Dojindo Laboratories, Kumamoto, Japan). After incubating the samples for 3 days on the 96-well plate described above, the aquaous mixture of WST-1 and 1-methoxy-5-methylphenazinium methylsufate (1-methoxy PMS) was added in a volume of 1:10 to the culture medium and further incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 3 h. After incubation, the absorbance was measured at 415 nm.

Measuring the growth of dermal papilla cells by uptake of [methyl-^{3}H]thymidine

After incubation for 1 day on the 24-well plate described above, [methyl-^{3}H]thymidine (3.7 kBq/well) (statistic ability, 1.81 TBq/mmol) (Amersham International, Bucks, UK) was added to the plate and then incubated for 6 h at 37°C in 5% CO_2. The medium was then removed and washed three times with phosphate-buffered saline. The proteins in each well were precipitated by adding a 10% solution of trichloroacetic acid for 30 min at 4°C before digestion in 500 μl of 0.5 N sodium hydroxide for 1 h at 37°C. The incorporation rate of [methyl-^{3}H]thymidine was counted by a liquid scintillation counter. Pentadecanoic acid was used as a positive control.

Results and discussion

We investigated the proliferative activity of extracts from woods and fungi on DP cells by the tetrazolium salt WST-1 assay as the first screening. After screening 366 samples, the mycelia of fungus YL161 (ethyl acetate extract) and *Agaricus blazei* (ethyl acetate extract) (obtained from Dr. M. Iwahara, Sojo University, Kumamoto, Japan) and the wood bark of *Camptotheca cuminata* (methanol extract) showed proliferative activity (Fig. 1). The proliferative activity of these extracts was not dose-dependent but might have a suitable range of concentration. For example, the optimum concentration of YL161 mycelium that exhibits maximum proliferative activity is 1 ppm.

Subsequently, the proliferative activities of these three samples were evaluated by [methyl-^{3}H]thymidine uptake. [Methyl-^{3}H]thymidine uptake is a convenient method that indicates DNA synthesis in DP cells. We used pentadecanoic acid as a positive control, as it had been reported to have both hair growth activity and proliferative activity on DP cells.[11] These samples showed more proliferative activity than pentadecanoic acid (Fig. 2).

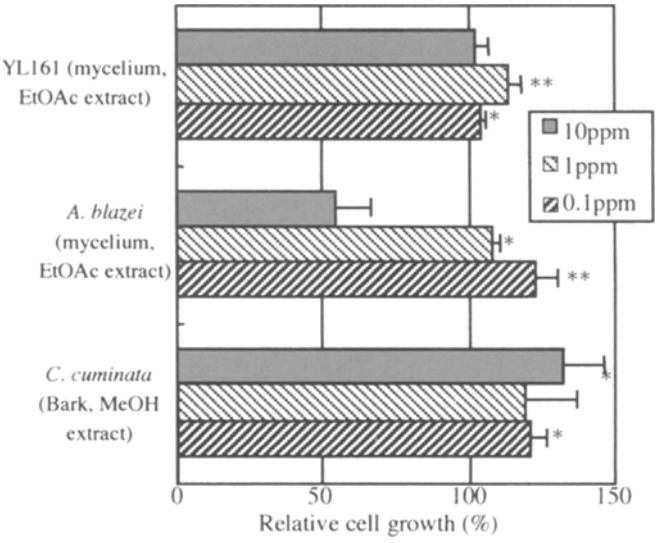

Fig. 1. Effects of extracts on dermal papilla (DP) cell growth. The growth rate was determined by the tetrazolium WST-1 assay. Growth-promoting activities relative to controls (100%) are shown. For the control, we used ethanol (1:1000 ethanol/medium). Values are means ± SD. Asterisks indicate a statistically significant difference compared with the control using Student's *t*-test. $*P < 0.05$, $**P < 0.01$

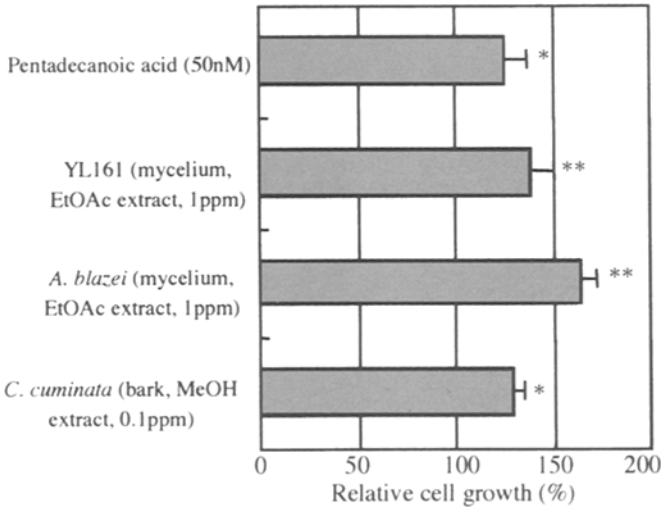

Fig. 2. Promotion effects of extracts on DP cell growth. The growth rate was determined by uptake of the [methyl-^{3}H]thymidine assay. Maximum growth-promoting activities relative to controls (100%) are shown. For the control, we used ethanol (1:1000 ethanol/medium). Pentadecanoic acid was used as the positive standard. Values are means ± SD. Asterisks indicate a statistically significant difference compared with the control using Student's *t*-test. $*P < 0.05$, $**P < 0.01$

In this study we took advantage of the distinctive features of cultured cells. Comparative in vivo experiments using animals and adopting a cultured cell system has some merit, including the simplicity of the assay, saving time and cost, and studying the function of cells originating from tissue or organs. DP has been reported to play an important role in hair growth, hair follicle morphogenesis, and the hair cycle; thus cultured DP cells from hair follicles are useful for investigating their function and the effects of several substances. The DP has receptors for several growth factors, cytokines, and hormones, and it secretes several growth factors that act in an autocrine or paracrine manner.[12,13] Moreover, cultured DP cells have the ability to induce hair follicles and growth of hair.[4,5] Thus it appears that the specific functions of the DP is maintained even in cultured DP cells. Morphologically, the DP of the hair follicle that shows symptoms of male pattern baldness is markedly smaller than that of the normal follicle, and hair follicle size has been clearly related to DP volume.[7,8] Furthermore, Elliott et al. illustrated that DP volume is due to the cell number and the extracellular matrix.[9] These data suggest that if such DP can be made larger, it will promote hair growth. Hence we took advantage of cultured DP cells suitable for screening the bioactive compound. Ethyl acetate extracts of mycelia of fungi YL161 and *A. blazei* and wood bark of *C. cuminata* showed higher proliferating activity in DP cells than PDA. The result indicates that these samples could be candidates not only as hair growth-promoting agents in vivo but also biochemical agents that can help us understand the mechanism of hair growth and alopecia. This is the first time these samples have been reported to stimulate the proliferation of DP cells. *A. blazei* has been reported to have an antitumor effect[14,15] and is used with a health food diet.

Minoxidil, a piperidinopyrimidine derivative that acts as a smooth muscle vasodilator for treatment of hypertension, is a Food and Drug Administration (FDA)-approved topical medication for curing male-pattern baldness.[16,17] Recently, finasteride, a type 2 of 5α-reductase inhibitor that was initially used to cure prostatic hypertrophy, has been approved by the FDA as an oral drug for curing male-pattern baldness.[18] Several side effects have been reported with both agents.[19–21] On the other hand, several plant extracts have been used traditionally to cure male-pattern boldness (e.g., swertia herb extract[22] and capsicum tincture[23]), although their role in enhancing hair growth has been uncertain. Takahashi et al. investigated hair epithelial cells' growth-promoting activity from more than 1000 plant extracts and found proanthocyanidins from grape seeds to have hair growth activity.[24]

In this study, we found three extracts (YL161, *A. blazei*, *C. cuminata*) that have proliferative activity on DP cells. Additional studies of hair regrowth-stimulating effects of these extracts in vivo are in progress.

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