

Human hair growth enhancement in vitro by green tea epigallocatechin-3-gallate (EGCG)

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Abstract

Green tea is a popular worldwide beverage, and its potential beneficial effects such as anti-cancer and anti-oxidant properties are believed to be mediated by epigallocatechin-3-gallate (EGCG), a major constituent of polyphenols. Recently, it was reported that EGCG might be useful in the prevention or treatment of androgenetic alopecia by selectively inhibiting 5 α -reductase activity. However, no report has been issued to date on the effect of EGCG on human hair growth.

This study was undertaken to measure the effect of EGCG on hair growth in vitro and to investigate its effect on human dermal papilla cells (DPCs) in vivo and in vitro. EGCG promoted hair growth in hair follicles ex vivo culture and the proliferation of cultured DPCs. The growth stimulation of DPCs by EGCG in vitro may be mediated through the upregulations of phosphorylated Erk and Akt and by an increase in the ratio of Bcl-2/Bax ratio. Similar results were also obtained in in vivo dermal papillae of human scalps. Thus, we suggest that EGCG stimulates human hair growth through these dual proliferative and anti-apoptotic effects on DPCs.

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Introduction

Hair growth is controlled by a unique repetitive cycle comprised of anagen, catagen and telogen phases (Stenn and Paus, 2001). Dermal papilla cells (DPCs), a group of specialized fibroblasts within the hair follicle bulb, have an essential function in the control of hair growth not only in the normal hair cycle but also in the pathogenesis of certain conditions, for example in androgenetic alopecia (Inui et al., 2003). Therefore, factors affecting the functions of DPCs in hair loss are of great importance from the therapeutic viewpoint.

Green tea is a popular beverage worldwide, and its potential beneficial effects such as its anti-cancer and anti-oxidant properties are thought to be mediated by epigallocatechin-3-gallate (EGCG), a major constituent of polyphenols in green tea (Hsu, 2005; Wang and Bachrach, 2002). Moreover, EGCG has been reported to have a growth stimulatory effect on normal cells (Hsu et al., 2003): EGCG promotes human keratinocyte survival, and inhibits ultraviolet light-induced apoptosis by dual mechanism, namely, by phosphorylating Bad protein through Erk and Akt pathways, respectively, and by increasing the Bcl-2 to Bax ratio (Chung et al., 2003).

Recently, it was reported that EGCG may be useful in the prevention or treatment of androgenetic alopecia by

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selectively inhibiting 5 α -reductase activity (Hiipakka et al., 2002). However, no report has been issued on the effect of EGCG on human hair growth. This study was undertaken to investigate the effects of EGCG on the proliferation and apoptosis of human DPCs in vivo and in vitro. Cultured normal human DPCs and a hair follicle organ culture model were used as in vitro models, and scalp tissue specimens after the topical application of EGCG were used as an in vivo model. Here, we found that EGCG promoted in vitro hair growth probably by upregulating phosphorylated Erk and Akt and by increasing Bcl-2/Bax ratio.

Materials and methods

Drugs and reagents

EGCG from green tea (E4143, $\geq 95\%$ pure EGCG by HPLC) and 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO). EGCG was dissolved in dimethylsulfoxide (DMSO) at 0.5 mM and stored at -20°C . Antibodies recognizing phosphorylated-ERK-1/2 (Thr202/Tyr204), total ERK-1/2, phosphorylated-Akt (Ser473), and total Akt were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Antibodies to Bcl-2 and Bax were purchased from Dako (Glostrup, Denmark), and β -actin antibody from Santa Cruz Biotech Inc. (Santa Cruz, CA).

Isolation of human hair follicles and dermal papillae

Scalp tissue specimens were obtained from the occipital scalp regions of 5 healthy male volunteers (20–31 years). Tissue samples containing more than 100 hair follicles were dissected into single hair follicles using a surgical blade and a watchmaker's forceps under a stereodissecting microscope. Dermal papillae were microdissected individually from isolated hair follicles, as previously described (Messenger, 1984). Hair follicles considered to be in the anagen stage morphologically were used in this study. The study was approved by the Institutional Review Board of the Seoul National University Hospital, and all subjects gave written informed consent.

Hair follicle organ culture

Human scalp hair follicles were isolated and cultured in vitro, as described previously (Philpott et al., 1990). Briefly, dissected hair follicles were cut into small pieces of approximately 2.5 mm in length from the bottom of dermal papillae and cultured in 24-well dishes for 10 days in Williams E medium (Gibco BRL, Gaithersburg,

MD) containing 10 ng/ml hydrocortisone, 10 $\mu\text{g}/\text{ml}$ insulin, 2 mM L-glutamine, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 100 U/ml penicillin at 37°C in a 5% CO_2 atmosphere. EGCG was added to culture media at 0.1, 1, or 5 μM . In all experiments culture media and EGCG were changed every other day. A total of 30 anagen hair follicles from 3 different volunteers (10 follicles per subject) were cultured under each growth condition, respectively. The values shown are means \pm SEM of triplicate cultures.

Dermal papilla cell culture and MTT assay

The method used for isolating and culturing DPCs has been previously described (Randall et al., 1991). Briefly, DPCs were cultured in Dulbecco's modified eagle's medium (Gibco BRL, Gaithersburg, MD) containing 2 mM L-glutamine, 1 \times antibiotic antimycotic solution (1000 $\mu\text{g}/\text{ml}$ of streptomycin sulfate, 1000 units/ml of penicillin G sodium, and 2.5 $\mu\text{g}/\text{ml}$ of amphotericin B) and 10% fetal bovine serum (FBS; Hyclone, Logan, UT). Fourth-passage confluent DPCs were cultured for 24 h in serum-free DMEM, and then treated for 1 h or for 1–2 days with various concentrations of EGCG (i.e., 0 M (control), 0.01, 0.1, or 0.5 μM).

Cell proliferation was determined using MTT assay as previously described (Mosmann, 1983). DPCs (1.0×10^4 cells/well) were seeded into 96-well plates, and incubated for 24 h before adding EGCG at 0.01, 0.1, or 0.5 μM and then incubated for 5 days. Absorbance was measured at 570 nm using an ELISA reader. Results were expressed as percentages of untreated controls in six cultures. Values represent means \pm SEM.

In vivo trial of EGCG in human volunteers

Ten percent EGCG in ethanol or ethanol vehicle were applied daily to two regions of the occipital scalp of three normal human volunteer for 4 successive days, and then treated areas about 1×1.5 cm were excised. Tissue samples containing hair follicles were cautiously dissected into single hair follicles. Dermal papillae were selectively separated under a stereomicroscope and isolated into single cells for Western blot analysis.

Western blot analysis

Protein was extracted using a buffer containing 50 mM Tris-HCl (pH 7.4), 2 mM EDTA, 100 $\mu\text{g}/\text{ml}$ leupeptin, 20 $\mu\text{g}/\text{ml}$ aprotinin, and 100 mM NaCl. Fifty microgram of protein per lane was separated by 10% or 12% SDS-PAGE. Primary antibodies were incubated with at appropriate dilutions (anti-Bcl-2 monoclonal antibody, 1:500; anti-Bax monoclonal antibody, 1:500; anti-actin monoclonal antibody, 1:1000; anti-ERK

polyclonal antibody and anti-phosphorylated ERK polyclonal antibody, 1:500; anti-Akt polyclonal antibody, 1:1000; or anti-phosphorylated Akt polyclonal antibody, 1:500). Antibody–antigen complexes were detected using the ECL system (Amersham Pharmacia Biotech; Little Chalfont, UK), and results were analyzed using Bio-Rad GS-700 imaging densitometer (Hercules, CA).

Statistics

Statistical analyses were performed using the Wilcoxon-rank sum test, and p -values of less than 0.05 were considered statistically significant.

Results and discussion

In the present study, EGCG, the active component of green tea, was found to cause significant human hair follicle elongation *ex vivo*. EGCG at 0.1 or 1 μM induced hair follicle elongation by $123.0 \pm 9.0\%$ and $121.6 \pm 7.1\%$ compared with the vehicle-treated controls, respectively. Hair growth was significantly enhanced by EGCG at 5 μM by $181.2 \pm 15.8\%$ ($p < 0.05$). Moreover, in the concentration range 0.01 to 0.5 μM EGCG enhanced the proliferation of human DPCs *in vitro* in a dose-dependent manner ($p < 0.05$) (Fig. 1). To our knowledge, the present study is the first to evaluate the effect of EGCG on hair growth using human scalp hair follicles.

The present study also documents that EGCG affects the expressions of Erk, Akt, Bcl-2, and Bax in cultured human DPCs. EGCG induced the proliferation of cultured human DPCs possibly through the activation of Erk and Akt pathways. The roles of the Erk signaling pathway in mitogenesis and cell growth have been well established (Robinson and Cobb, 1997; Xia et al., 1995). Human DPCs treated with EGCG showed significant dose-dependent increases in the levels of phosphorylated Erk (P-Erk) compared with vehicle-treated controls ($p < 0.05$) (Fig. 2). Levels of phosphorylated Akt

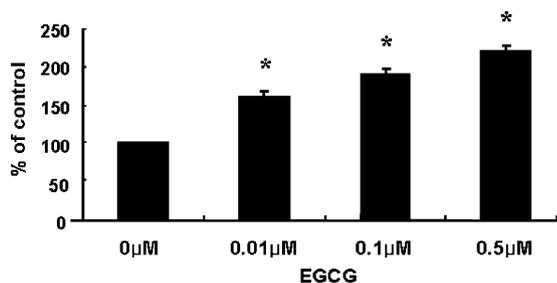


Fig. 1. EGCG enhanced the proliferation of cultured human DPCs as determined by MTT assay. Values are means \pm SEM. * $p < 0.05$, compared with the vehicle-treated control.

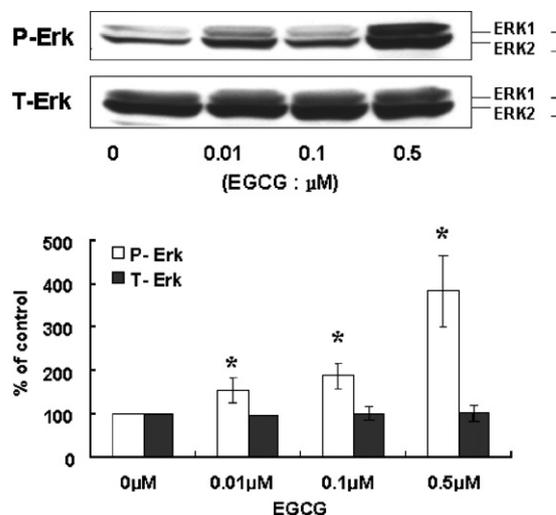


Fig. 2. EGCG increased the phosphorylation of Erk in cultured human DPCs. P-Erk level was significantly increased after 1 h EGCG treatment in a dose-dependent manner. The values shown are means \pm SEM of percentages vs. controls obtained using three different batches of DPCs. * $p < 0.05$, compared with the vehicle-treated control. P-Erk: phosphorylated Erk; T-Erk: total Erk.

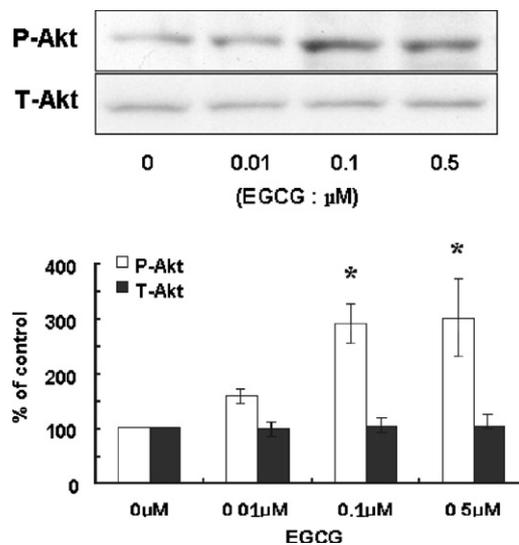


Fig. 3. P-Akt level increase by EGCG treatment in cultured human DPCs. EGCG treatment significantly enhanced the phosphorylation of Akt in a dose-dependent manner. The blotted bands are the representative of triplicate experiments. Values shown are means \pm SEM of percentages vs. controls from three different batches of DPCs. * $p < 0.05$, compared with the vehicle-treated control.

(P-Akt) were also increased significantly after EGCG treatment ($p < 0.05$) (Fig. 3). Recently, it was reported that Akt plays an critical role in mediating survival signals (Ahmad et al., 1999; Tang et al., 2000). And, it is also possible that the activation of the Akt pathway by EGCG is involved in regulating the DPCs survival.

EGCG was observed to increase Bcl-2 and to decrease Bax expression in cultured DPCs. EGCG treatment for 24h increased Bcl-2 expression but decreased Bax expression in a dose-dependent manner ($p < 0.05$)

(Fig. 4). The Bcl-2 family of proteins consist of more than a dozen members which are either anti- or pro-apoptotic in nature, and which appear to act as gatekeepers of the apoptotic process (Adams and Cory,

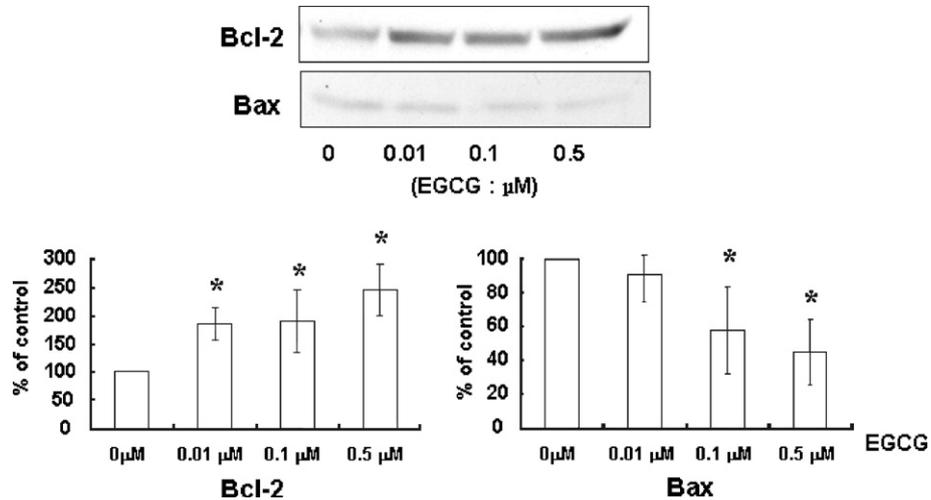


Fig. 4. The effect of EGCG on the expression of Bcl-2 and Bax proteins in cultured human DPCs. Compared with vehicle-treated control, the EGCG treatment significantly increased the expression of Bcl-2 and decreased Bax expression. Values shown are means \pm SEM of percentages vs. controls for three different batches of DPCs. * $p < 0.05$, compared with the vehicle-treated control.

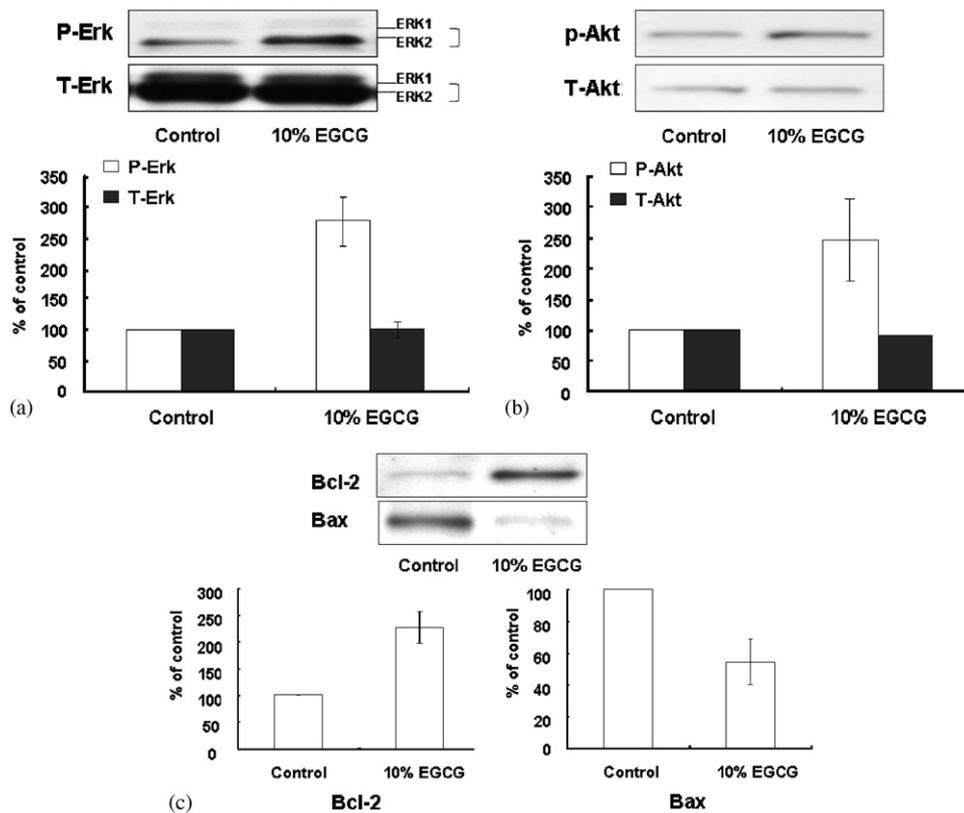


Fig. 5. Application of 10% EGCG on human occipital scalp: (a) P-Erk levels were significantly increased by 4-day successive EGCG treatment, (b) Akt phosphorylation was greatly enhanced by EGCG treatment, and (c) Bcl-2 expression was elevated but Bax was suppressed. The blotted bands are the representative of triplicate experiments. Values shown are means \pm SEM of percentages vs. controls.

1998). It is well known that Bcl-2 has an anti-apoptotic effect, whereas Bax induces apoptosis.

Finally, we applied 10% EGCG in ethanol directly onto human scalps to determine whether the changes observed in vitro occurred in vivo. DPCs excised from in vivo scalp hair follicles after treatment with 10% EGCG showed almost 3-fold P-Erk expressional increases versus vehicle-treated controls (Fig. 5a). Akt phosphorylation was also increased 2.5-fold in response to 10% EGCG treatment compared with the vehicle-treated controls (Fig. 5b). Bcl-2 expression increased over 2-fold whereas Bax expression reduced by 50% after topically treating 10% EGCG (Fig. 5c). Thus, it was confirmed that the events initially observed in vitro actually occurred in in vivo.

In summary, our data suggest that EGCG stimulates human hair growth via its proliferative and anti-apoptotic effects on DPCs, and may prolong anagen stage. The effects of EGCG on different hair follicle cell types and the molecular basis for its promotion of hair growth remain unclear and require further investigation.

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