Epigallocatechin gallate inhibits IGF signalings in mitogenesis of 3T3-L1 preadipocytes via the 67-kilodalton laminin receptor pathway

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**ABSTRACT**

Green tea catechins, polyphenolic flavonoids, also known as vitamin P, have been proposed as co-preservative agents for both acute and chronic inflammation. In particular, epigallocatechin gallate (EGCG) has been shown to reduce body weight and body fat in vivo. One in vivo study has shown that EGCG, or an EGCG-containing green tea extract, reduced food intake, lipid absorption, basal triglyceride levels, and cholesterol levels. Furthermore, EGCG has been shown to stimulate energy expenditure and inhibit lipogenesis in vivo. These effects may have important implications for the treatment of obesity and fatty liver disease. These in vivo observations may be explained by the following in vitro findings: 1) EGCG, caffeine, and naphthylphenol synergistically stimulated thermogenesis in brown adipose tissue; 2) EGCG regulated various elements related to lipid metabolism and inflammation, including acyl-CoA synthetase, fatty acid synthase (FAS), peroxisome proliferator-activated receptor-γ, and PPAR-γ. 3) EGCG blocked the differentiation of preadipocytes into adipocytes, and 4) EGCG induced mitogen-activated protein kinase (MAPK) signaling in human preadipocytes (3T3-L1). These results indicate that EGCG may be a promising candidate for the treatment of obesity, and that its effects may be AMPK-independent.

**MATERIALS and METHODS**

Cell culture. 3T3-L1 preadipocytes (American Type Culture Collection, Manassas, VA, USA; ATCC-CL-177) and NIH3T3 embryonic mouse fibroblasts (ATCC-CL-1638) and human A431 cancer cells (purchased from the Cancer Research Center, National Taiwan University, Taiwan) were grown in DMEM (pH 7.4) containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C and 80% relative humidity (85% humidity in a humidified atmosphere of 95% air and 5% CO2 at 37°C). Media was replaced every 48 h. Growth inhibition experiments. 3T3-L1 cells (10,000-20,000) were plated in triplicate wells of 6-well plates. After incubation for 1 day, the cells were counted in a hemocytometer using the 0.4% trypan blue exclusion method. Cell proliferation was measured according to the method described by Hwang et al. (1) with a commercially available tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT)-based instrument assay kit (BioLine Applied Science, Marshalltown, Germany). Determining and demonstrating the monochromaticity of the measuring molecules were performed as described by Hwang et al. (1). Statistical data were analyzed using the Microsoft Excel and Minitab statistical software packages. All data are presented as the mean ± standard deviation.

**RESULTS**

Fig. 2. EGCG dose-dependently blocked IGF-I and IGF-II-induced increase in the total number of pancytopenia (M) and in BrdU incorporation (B).

Fig. 3. The effect of EGCG on IGF-I-induced phosphorylation of IRS and association of IRS with IGF-I receptor.

Fig. 4. EGCG dose-dependently blocked IGF-I-stimulated MEK activity (A), and EGCG dose-dependently blocked IGF-I-enhanced MEK activity (B).

Fig. 5. The effect of EGCG on IGF-I-induced phosphorylation of IRS and association of IRS with IGF-I receptor.

Fig. 6. The effect of EGCG on IGF-I-induced phosphorylation of Shc and association of Shc with IGF-I receptor.

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