Investigation of EGCg distribution in HUVECs

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Summary

Since epigallocatechin gallate (EGCg) shows inhibitory effect of tumor angiogenesis, EGCg is expected as a functional component for cancer therapy and prevention. In the present study, to elucidate the mechanism of antiangiogenic activity of EGCg, some EGCg derivatives were synthesized and the distribution in human umbilical vein endothelial cells (HUVECs) was observed. When *trans*- or *cis*-aminopentyl dehydroepigallocatechin gallate (APDOEGCg) was synthesized and examined the antiangiogenic activity of them, both derivatives showed higher inhibitory effect on the proliferation, migration, and invasion of HUVECs than EGCg. Then, a fluorescence agent, Tokyo Green was conjugated with APDOEGCg, and the distribution in HUVECs was observed under a confocal laser scanning microscope. As a result, the fluorescence was mainly distributed in cytoplasm. These results suggest that EGCg was incorporated into the HUVECs.

Introduction

(-)-Epigallocatechin-3-*O*-gallate (EGCg) exhibits various biological activities such as anti-viral, anti-microbial, anti-oxidative and anti-cancer ones. We previously reported that EGCg suppresses tumor growth through the inhibition of tumor angiogenesis (Yamakawa, *et al.*, 2004). Since 5,7-deoxyepigallocatechin gallate (DOEGCg) possesses more potent anti-viral activity than the original EGCg (Furuta, *et al*., 2007), modification of EGCg A-ring does not abolish but rather enhances the functional ability of EGCg. Based on structure-relationship, we synthesized (±)-6-(5-aminopentyl)-5,7-deoxyepigallo-catechin gallate (*cis*-APDOEGCg) and (±)-6-(5-aminopentyl)-5,7- deoxygallo-catechin gallate (*trans*-APDOEGCg) as EGCg derivatives. We compared their anti-angiogenic activity with that of EGCg by examining the inhibitory effect on proliferation, migration, invasion, and tube formation by HUVECs. Then, we synthesized fluorescence-labeled EGCg by conjugating the fluorescent reagent TokyoGreen (TG) to (±)-*cis*-APDOEGCg (EGCg-TG) and examined the intracellular distribution of it in HUVECs by confocal laser scanning microscopy.

Materials and methods

Biological assays

EGCg derivatives, i.e., *cis*- and *trans*-APDOEGCg, and TokyoGreen (TG) were synthesized (Yoshida, *et al*., submitted). Anti-proliferation activity of EGCg or EGCg derivatives *(cis*- or *trans*-APDOEGCg) on human umbilical vein endothelial cells (HUVECs) was monitored with TetraColor One. Motility and invasion assays were performed with fluorescence-labeled HUVECs by use of FluoroBlok Insert (BD). Tube formation by HUVECs on Matrigel was also examined in the presence of EGCg or its derivatives.

Intracellular distribution of EGCg-TG in HUVECs

HUVECs were cultured on a chamber slide for 24 h at 37°C. After incubation with EGCg-TG or TG, the cells were fixed with paraformaldehyde, permeabilized, and stained with rhodamine/phalloidin and DAPI for staining *F*-actin and cell nuclei, respectively. The distribution of EGCg-TG was observed under a confocal laser scanning microscope.

esults and discussion

Effect of EGCg derivatives (APDOEGCg) on HUVECs

We firstly examined the inhibitory effect of *cis*- or *trans*-APDOEGCg on HUVEC proliferation. EGCg and its derivatives effectively inhibited HUVEC proliferation when tested at more than 30 μM, and the anti-proliferative effect of *trans*-APDOEGCg was the most potent among them. However, HUVEC proliferation was not affected at less than 10 μM EGCg or its derivatives. To elucidate the biological effect of EGCg, a concentration of EGCg or its derivatives at 1 μM was used for the following experiments.

The motility assay revealed that the EGCg derivatives inhibited the migration of the HUVECs at the concentration of 1 μM, whereas EGCg did not inhibit it at the same concentration. Furthermore, the inhibitory effect of *trans*-APDOEGCg was the highest among them. A similar effect was observed in the invasion assay. To confirm the anti-angiogenic effect of EGCg derivatives, we performed a tube formation assay with HUVECs and observed the inhibition of tube formation by HUVECs at even 1 μM (Fig.1); and the EGCg derivatives clearly inhibited it more so than did the EGCg. These data indicate that the EGCg analogs *cis*- and *trans*-APDOEGCg showed anti-angiogenic activity similar to but stronger than that of EGCg *in vitro*.

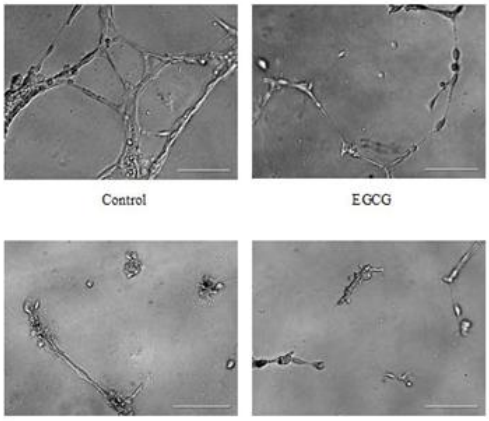

Fig.1 Tube formation by HUVECs in the presence of 1 μM EGCG or its derivatives

Observation of distribution of EGCg

To understand the intracellular distribution of EGCg, we synthesized fluorescence-labeled (±)-*cis*-APDOEGCg derivative, EGCg-TG, by conjugating it to the fluorescence agent TokyoGreen, and examined the distribution of EGCg-TG in HUVECs. EGCg-TG fluorescence appeared as dots in the cytoplasm of HUVECs, suggesting that it was distributed in organelles. On the other hand, the fluorescence of free TG was localized on the surface and in intracellular regions of the cells as a filamentous shape co-localized with *F*-actin. Fluorescent images also indicated that EGCg was incorporated into the cells within 3 h after the treatment.

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