Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 334 (2005) 947–953
BBRC
www.elsevier.com/locate/ybbrc

EGCG inhibits activation of the insulin-like growth factor-1 receptor in human colon cancer cells ☆

Masahito Shimizu [a,c], Atsuko Deguchi [a], Yukihiko Hara [b], Hisataka Moriwaki [c], I. Bernard Weinstein [a,*]

[a] *Herbert Irving Comprehensive Cancer Center and Department of Medicine, Columbia University Medical Center, New York, NY 10032, USA*
[b] *Polyphenon Division, Mitsui Norin Co. Ltd., Tokyo 160-8381, Japan*
[c] *Department of Medicine, Gifu University School of Medicine, Gifu 501-1194, Japan*

Received 29 June 2005
Available online 12 July 2005

Abstract

The IGF/IGF-1R system, which includes the IGF, IGF-1R, and IGFBPs proteins, plays an important role in the development and growth of colorectal cancer. We previously reported that in the HT29 human colon cancer cell line EGCG, the major biologically active component of green tea, inhibits activation of the RTKs EGFR, HER2, and HER3, and that this is associated with inhibition of multiple downstream signaling pathways. Since IGF-1R is also a RTK, in this study we examined the effects of EGCG on the activity of IGF/IGF-1R system in human colon cancer cells. We found that the colon cancer cell lines Caco2, HT29, SW837, and SW480 express high levels of the IGF-1R receptor, and that both SW837 and SW480 cells display constitutive activation of this receptor. Treatment of SW837 cells with 20 μg/ml of EGCG (the IC_{50} concentration for growth inhibition) caused within 6 h a decrease in the phosphorylated (i.e., activated) form of the IGF-1R protein. At 12 h, there was a decrease in the levels of both IGF-1 protein and mRNA and within 3–6 h there was an increase in the levels of both IGFBP-3 protein and mRNA. The increased expression of the latter protein was sustained for at least 48 h. When SW837 cells were treated with EGCG for a longer time, i.e., 96 h, a very low concentration (1.0 μg/ml) of EGCG also caused inhibition of activation of IGF-1R, a decrease in the IGF-1 protein, and an increase in the IGFBP-3 protein. EGCG also caused a decrease in the levels of mRNAs that encode MMPs-7 and -9, proteins that proteolyze IGFBP-3. In addition, treatment with EGCG caused a transient increase in the expression of TGF-β2, an inducer of IGFBP-3 expression. These findings expand the roles of EGCG as an inhibitor of critical RTKs involved in cell proliferation, providing further evidence that EGCG and related compounds may be useful in the chemoprevention or treatment of colorectal cancer.

Keywords: Colon cancer cells; EGCG; IGF/IGF-1R; IGFBP-3; MMPs; Tea catechins; Insulin-like growth factor receptor

IGF-1R,[1] a membrane associated RTK, plays a crucial role in normal development [1]. It is a transmembrane heterotetramer consisting of two-α and two-β subunits linked by disulfide bridges. The receptor has

☆ This work was supported by funds from the EIF-National Colorectal Cancer Research Alliance, the National Foundation for Cancer Research, and the T.J. Martell Foundation (to I.B.W.).
* Corresponding author. Fax: +1 212 305 6889.
E-mail address: ibw1@columbia.edu (I.B. Weinstein).

[1] *Abbreviations:* IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor-1 receptor; IGFBP, insulin-like growth factor binding protein; RTK, receptor tyrosine kinase; EGCG, (−)-epigallocatechin-3-gallate; GTP, green tea polyphenol; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; AP-1, activator protein-1; NF-κB, nuclear factor-κB; MMP, matrix metalloproteinase; TRAMP, transgenic adenocarcinoma of mouse prostate; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor; FGFR, fibroblast growth factor; TGFα, transforming growth factor-α; EGF, epidermal growth factor; COX-2, cyclooxygenase-2; PGE_2, prostaglandin E_2; HNSCC, head and neck squamous cell carcinoma.

0006-291X/$ - see front matter
doi:10.1016/j.bbrc.2005.06.182

two major ligands, IGF-1 and IGF-2. The biologic activities of these ligands are negatively controlled by a family of high affinity IGFBPs, of which IGFBP-3 is the most important since in the serum it can sequester IGFs in an inactive form [2]. The binding of free IGFs to IGF-IR results in intramolecular receptor autophosphorylation and phosphorylation of critical downstream targets. This leads to activation of several signaling pathways, including the PI3K/Akt pathway and the Ras/MAPK pathway, thus inducing activation of specific genes, DNA synthesis, and cell proliferation [3–5]. In addition to its role in regulating the growth of normal cells, the IGF-1/IGF-1R system also plays an important role in carcinogenesis [3–5]. Thus, epidemiologic studies have demonstrated that an increase in circulating levels of IGF-1 and a decrease in levels of IGFBP-3, are frequently associated with the development of several types of epithelial cancers, including colorectal cancer [6,7]. In addition, IGF-1R is frequently overexpressed in colon cancers when compared to its level of expression in normal colonic mucosa [8]. Blockade of the IGF/IGF-1R axis by a soluble IGF-1R derivative inhibited IGF-1-induced activation of Akt and also inhibited the growth of human colon cancer xenografts in mice [9]. Therefore, targeting the IGF/IGF-1R signaling pathway may be an effective strategy for the prevention and treatment of colorectal cancer.

Numerous studies indicate that green tea or its catechin constituents can exert anticancer effects in various experimental systems (for review, see [10,15]). In addition EGCG, a major biologically active constituent of green tea, can inhibit several critical pathways of signal transduction [10–16]. Treatment of cancer cells with EGCG can inhibit activation of EGFR (erbB1), HER2 (neu/erbB2), and HER3, receptors that belong to subclass I of the RTK superfamily [10–16]. This is associated with inhibition of multiple downstream signaling pathways in human HNSCC, breast cancer, and colon cancer cell lines [11–16]. Thus, treatment of HT29 colon cancer cells with EGCG results in inhibition of activation of both ERK and Akt, inhibits basal and TGFα-stimulated transcriptional activity of the AP-1, c-*fos*, and NF-κB promoters, and induces apoptosis [14]. Liang et al. [16] found that in human A431 epidermoid carcinoma cells, EGCG also inhibits the tyrosine kinase activities of two additional RTKs, PDGFR, and FGFR [10,15]. Therefore, several RTKs and their downstream signaling pathways may be critical molecular targets of EGCG with respect to growth inhibition and induction of apoptosis.

In view of the above-described importance of the IGF-1/IGF-R system in cancer cells, the present study was designed to examine possible effects of EGCG on activation of the RTK IGF-R, and on expression of the related molecules IGF-1 and IGFBP-3, in human colon cancer cells. In these studies, we used the SW837 human colon cancer cell line since we found that these cells express high levels of IGF-1R and also display constitutive activation of this receptor.

Materials and methods

Chemicals. EGCG was provided by the National Cancer Institute (NCI, Bethesda, MD).

Cell lines and cell culture. The Caco2, HCT116, HT29, SW480, and SW837 human colorectal cancer cell lines were obtained from American Type Culture Collection (Manassas, VA). All of the cell lines were maintained in DF10 medium containing DMEM (Invitrogen, San Diego, CA) supplemented with 10% FBS (Invitrogen). Cells were cultured in an incubator with humidified air at 37 °C with 5% CO_2.

Protein extraction and Western blot analysis. Total cellular protein was extracted and equivalent amounts of protein were examined by Western blot analysis, as previously described [14,17]. Cell lysates were separated by SDS–PAGE using 7.5–15% polyacrylamide gels and transferred onto Immobilon-P transfer membranes (Millipore, Bedford, MA). The primary antibodies for IGF-1Rα (#3022) and p-IGF-1R (#3021) were purchased from Cell Signaling Technology (Beverly, MA). The primary antibodies for IGF-1Rβ (sc-713) and IGFBP-3 (sc-6003) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The primary antibodies for IGF-1 (clone Sm1.2) and actin (A2066) were purchased from Upstate Cell Signaling Solutions (Charlottesville, VA) and Sigma Chemical (St. Louis, MO), respectively. An antibody to actin was used as a loading control. Anti-mouse IgG (Amersham Pharmacia Biosciences, Piscataway, NJ), anti-rabbit IgG (Amersham Pharmacia Biosciences), or anti-goat IgG (Santa Cruz Biotechnology) antibodies were used as the secondary antibodies. Each membrane was developed using an ECL-enhanced chemiluminescence system (Amersham Biosciences).

RNA extraction and semiquantitative RT-PCR analysis. RNA extraction and semiquantitative RT-PCR analysis were also performed as described previously [17]. Total RNA was isolated from SW837 cells using the Trizol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was amplified from 1 μg of total RNA using SuperScript one-step RT-PCR with the platinum Taq system (Invitrogen). The primers used for amplification of *IGFBP-3*, *IGF-1*, *MMP-7*, *MMP-9*, and *TGF-β2* specific genes are shown in Table 1. Actin specific primer sets were described previously [17]. By using a thermal controller (Programmable Thermal Controller; MJ Research, Watertown, MA), 25-, 35-, 32-, 40-, and 30-cycle rounds of PCR were chosen for optimum analysis of expression of IGFBP-3, IGF-1, MMP-7, MMP-9, and TGF-β2 mRNAs, respectively, since a semiquantitative assessment indicated that the reactions had not reached a plateau and were still in the log phase. Each amplification cycle consisted of 0.5 min at 94 °C for denaturation, 0.5 min at 55 °C for primer annealing, and 1.0 min at 72 °C for extension. After PCR amplification, the fragments were analyzed by agarose gel electrophoresis and stained with ethidium bromide. The intensities of bands were quantified with NIH Image software version 1.62.

Results

Expression of IGF-1R and related proteins in colon cancer cell lines

Initially, we examined by Western blot analysis cellular levels of the IGF-1R, p-IGF-1R, IGF-1, and IGFBP-3 proteins in five human colon cancer cell lines (Fig. 1). When compared to HCT116 cells, the levels of the IGF-1Rα and IGF-1Rβ proteins were increased in the Caco2,