Decreased Synthesis of Matrix Metalloproteinase-7 and Adhesion to the Extracellular Matrix Proteins of Human Colon Cancer Cells Treated with Troglitazone

Eiji Sunami1, Nelson Hirokazu Tsuno1,2, Joji Kitayama1, Shinsuke Saito1, Takuya Osada1,2, Hironori Yamaguchi1, Shigeru Tomezawa1, Takashi Tsuruo1, Yoichi Shibata1, and Hirokazu Nagawa1

1 Department of Surgical Oncology and 2 Department of Transfusion Medicine, Graduate School of Medical Sciences, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
3 Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

Abstract

Purpose. In the present study, we investigated the effect of troglitazone, a selective ligand and agonist of PPAR-γ, on the metastatic potential of human colon cancer cells.

Methods. High- and low-PPAR-γ expression clones of the colon cancer cell line, HT29, namely clones 21 and 3 respectively, were used. We investigated the effect of troglitazone on the proliferation, on the adhesion to extracellular matrix proteins and on the synthesis of matrix metalloproteinases (MMPs) of colon cancer cells.

Results. Troglitazone inhibited the proliferation of both subclones, in a dose-dependent manner, and the inhibitory effect correlated with the level of PPAR-γ expression. Troglitazone strongly inhibited the production of MMP-7, an enzyme associated with invasiveness of cancer cells, by both subclones. In addition, troglitazone caused a strong decrease in the adhesion of clone 21 to extracellular matrix (ECM) proteins, laminin and type IV collagen. This effect was independent of β1-integrins expression.

Conclusion. In addition to inhibition of cancer cell growth, troglitazone had an inhibitory effect on two important events associated with the metastatic potential of cancer cells, production of MMPs and adhesion to ECM proteins. Consequently, troglitazone is a promising agent for the treatment and prevention of colon cancer metastasis.

Key words Peroxisome proliferator-activated receptor gamma · Colon cancer · Matrix metalloproteinase-7 · Integrin

Introduction

Peroxisomal proliferator-activated receptor gamma (PPAR-γ), a nuclear hormone receptor, is the downstream transcriptional mediator for prostaglandins and fatty acids. It provides a direct link between fatty acid metabolism and control of gene transcription. PPAR-γ is predominantly expressed in adipose tissue, the adrenal glands, and the spleen, and has a dominant regulatory role in the differentiation of cells of adipose lineage.1-2

The development of colorectal cancer is profoundly influenced by prostaglandins and fatty acids, and decreased prostaglandin synthesis prevents or attenuates colon cancer development. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to lower the relative risk of colorectal cancer in humans,3 and decrease tumor yield in rodents treated with carcinogens3 or implanted with tumors.4 A well-known target of NSAIDs is prostaglandin endoperoxide synthase (cyclooxygenase, COX) and two isoforms of this enzyme have been identified, namely, COX-1 and COX-2. COX enzymes catalyze the production of eicosanoids, some of which have recently been shown to activate the transcription mediated by PPAR-γ. NSAIDs are also known to directly bind to the PPAR-γ receptor which has been shown to be highly expressed in the normal colonic mucosa,5,6 in colon cancer cell lines,5,8 and in both well- and poorly-differentiated adenocarcinomas.5,6

Troglitazone (TZD) has recently been developed as a selective ligand and agonist of PPAR-γ which has been shown to inhibit colon cancer cell growth by the induction of G1 cell-cycle arrest5,8 and cell differentiation,6,7 and the inhibition of anchorage-independent cell growth.8 These facts suggest that TZD could play a role in colorectal cancer tumorigenesis and progression.

In the present study, we investigated the effect of TZD on the adhesion of colon cancer cells to extracellular matrix proteins, and on the synthesis of matrix metalloproteinases (MMPs), which are essential events in the development of cancer metastasis.
Materials and Methods

Troglitazone (TZD)

The PPAR-γ activator, TZD (Noscal), was kindly donated by Sankyo (Tokyo, Japan).

Monoclonal Antibody

The monoclonal antibodies (mAbs) to human integrin β1 (CD29), α2, α3, α5, and α6, were obtained from Dako Japan (Kyoto, Japan); the mAb to human PPAR-γ was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); and mAb to MMP-7 (Matrilysin) was obtained from Fuji Chemical (Toyama, Japan).

Human Colon Cancer Cell Line

The human colon cancer cell line HT29 was cultured in RPMI-1640 containing 5% fetal calf serum (FCS) and 1% antibiotics-antimycotics in an atmosphere containing 5% CO2 at 37°C. Cells were routinely passaged by trypsinization before achieving confluence. The HT29 cell line was cloned in our laboratory by serial dilution, and among the 31 clones obtained, clones 3 and 21 were chosen for the low- and high-PPAR-γ expressions, respectively, and used in all the experiments.

Western Blotting

Samples for the Detection of PPAR-γ

HT29 clones 3 and 21 were cultured in RPMI-1640 containing 5% FCS, then washed twice with phosphate-buffered saline without Ca2+ and Mg2+ [PBS(–)], and lysed in lysis buffer, containing 1% Tween-20, 1% NP-40, and different kinds of protease inhibitors, for 60 min on ice. After adding 1 ml per 1.0 × 107 cells, the cells were harvested using a cell scraper, centrifuged at 15000 rpm for 20 min, and the supernatant collected and used as the cell protein extract.

Samples for the Detection of MMP-7

Western blotting for MMP-7 was carried out on supernatant from the two subclones with a 24-h treatment of TZD, with 0 and 20 μM in RPMI 1640 medium containing 0.1% bovine serum albumin (BSA) as described. In each group, cells were washed with PBS and cultured with 5 ml of serum-free DMEM:F12 medium for 5 h. Harvested media were collected, concentrated into 200 μl/1 × 107 cells in each group, then analyzed by Western blotting.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed as previously described10 using a Laemmli buffer system and 10% polyacrylamide resolving slab gels. Proteins were then transferred electrophoretically to polyvinylidene difluoride (PVDF, Immobilon P; Millipore, Bedford, MA, USA). The PVDF membrane was first equilibrated in transfer buffer (192 mM glycine, 25 mM Tris, 20% methanol, pH 8.3). The cell membrane protein fraction samples were either reduced, or not reduced, with 2% 2-mercaptoethanol at an ambient temperature. Blotting was carried out at 150 V for 1 h (4°C). After blotting, the remaining reactive sites of the PVDF membrane were blocked by incubating the sheets in Tris-buffered saline (TBS) containing 3% gelatin. The membrane was then washed three times for 15 min in TBS. The same buffer containing 10% calf serum (CS) was used to dilute either the antiserum or the purified antibodies. The membrane strips were incubated with the primary antibody overnight (16–20 h) at an ambient temperature in a shaker, then washed three times for 15 min and incubated with a 1:500 dilution of alkaline phosphatase-conjugated antibodies against human or mouse IgG (Zymed Laboratories, South San Francisco, CA, USA). After 1 h, the strips were washed an additional three times, then incubated with a freshly prepared substrate solution. The substrate solution consisted of 66 ml of a stock solution of nitroblue tetrazolium (NBT; 50 mg/ml in 70% dimethylformamide) and 33 ml of a stock solution bromo-4-chloro-3-indolylphosphate (BCIP; 50 mg/ml in 100% dimethylformamide) in 10 ml of 100 mM Tris-HCl, 100 mM NaCl, 5 mM MgCl2, pH 9.5. To stop color development, the membranes were rinsed in distilled water for 10 min and allowed to dry.

Flow Cytometry

For analysis of the nuclear antigen (PPAR-γ), cells were harvested by trypsinization, washed in PBS(–), and fixed in 4% paraformaldehyde (PFA) for 30 min at 4°C. Cells were then permeabilized by incubation with PBS containing 0.1% Tween-20 for another 30 min at 4°C. The primary antibody was added and the samples were incubated for 30 min at 4°C. Cells were then washed with PBS containing 0.2% bovine serum albumin (BSA) and 0.1% sodium azide, and the secondary FITC-labeled antibody was added, followed by incubation for another 30 min at 4°C. After washing in 0.2% BSA, 0.1% NaN3-PBS, cells were analyzed in the flow cytometer (FACSCalibur, Becton Dickinson, San Diego, CA, USA).

Proliferation Assay

For proliferation studies, the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay system (Promega, Madison, WI, USA) was used according to the manufacturer’s instructions.