Life Sciences 91 (2012) 279–283

Contents lists available at SciVerse ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Beta-catenin mediates the apoptosis induction effect of celastrol in HT29 cells

Wenzong Lu *, Guangfeng Jia, Xiangyan Meng, Chen Zhao, Liang Zhang, Yumiao Ren, Haixian Pan, Yuan Ni

Department of Biomedical Engineering, Xi'an Technological University, Xi'an, Shaanxi Province, 710032, People's Republic of China

ARTICLE INFO

Article history:
Received 26 March 2012
Accepted 16 July 2012

Keywords:
Celastrol
Apoptosis
Beta-catenin
Colon cancer

ABSTRACT

Aim: We evaluated the apoptosis induction effects of celastrol in human colorectal cancer cell line HT29 in WNT/beta-catenin pathway.
Main methods: HT29 cells were treated with various concentrations (10–100 μM) for 24 h, MTT assay was performed to examine the effect of celastrol on growth inhibition of HT29 cells. Beta-catenin siRNA was used for transfection of cells. Cell apoptosis was detected through both DNA laddering analysis and Tdt-mediated dUTP nick end labeling (TUNEL) assay. Western blot analysis and real-time reverse transcription polymerase chain reaction technologies were applied to assess the expression level of c-Myc, Bax, and Bcl-2 in HT29 cells.
Key findings: Treatment of HT29 cells with celastrol resulted in a growth inhibition effect, and the IC_{50} value was 56 μM. Celastrol induced HT29 cells apoptosis, and increased the nuclear translocation of beta-catenin. Apoptosis induction effects of celastrol were significantly attenuated by beta-catenin siRNA transfection. Beta-catenin siRNA markedly increased mRNA and protein levels of c-Myc compared with control siRNA. Beta-catenin siRNA significantly inhibited the expression of Bax and Bcl-2 in celastrol-treated HT29 cells.
Significance: Beta-catenin mediates the apoptosis induction effects of celastrol in HT29 cells.

Introduction

Colon cancer is one of the most common gastrointestinal cancers in the world today. In addition, synchronous colorectal cancers have also risen globally, challenging the current treatment strategies and adversely affecting the overall prognosis of colorectal cancer (Yang et al., 2011b). A human colorectal cancer cell line, HT29 has been commonly used as an in vitro model system to investigate the molecular mechanisms of apoptosis (Lan et al., 2010; Reyes-Zurita et al., 2009, 2011; Singh et al., 2009; Sze et al., 2010). The process of apoptosis is controlled by a diverse range of cell signals. Two theories of the direct initiation of apoptotic mechanisms in mammals have been suggested: the TNF-induced (tumor necrosis factor) model and the Fas–Fas ligand-mediated model (Chen and Goeddel, 2002; Wajant, 2002). Regardless of the various means used to initiate apoptosis, it progresses according to a general program involving a set of cystein-proteases termed caspases (Kihlmark et al., 2001). There also exists a caspase-independent apoptotic pathway that is mediated by AIF (apoptosis-inducing factor) (Susin et al., 1999). Bcl-2 functioned as a major inhibitor of cell death, therefore guarding against apoptosis while Bax acted as a promoter of apoptosis. Apoptosis occurrence always depended on the correct ratio of Bcl-2/Bax (Thomadaki and Scorilas, 2006).

Dysregulation of the Wnt signaling pathway is believed to play an important role in the pathogenesis of colorectal cancer. Wnt signaling regulates the assembly of a complex consisting of Axin, adenomatous polyposis coli, beta-catenin, and glycogen synthase-3-beta kinase. Activation of the Wnt signaling pathway inhibits degradation of beta-catenin, which enhances the nuclear translocation of beta-catenin. In the nucleus, beta-catenin binds to T-cell factor (Tcf)-lymphoid enhancer factor (Lef) family of transcription factors and changes the transcriptional activity of specific genes such as proto-oncogene and cell cycle regulator c-Myc, the G_1/S-regulating cyclin D1. Recently, beta-catenin has been suggested as an important target for drug design for preventing growth and metastasis of cancer cells through multiple pathways including Wnt signaling.

Celastrol [3-hydroxy-24-nor-2-oxo-1(10),3,5,7-friedelatetraen-29-oic acid] is an active ingredient of the traditional Chinese medicinal plant Tripterygium wilfordii Hook F., which exhibits significant antitumor activity in different cancer models in vitro and in vivo (Dai et al., 2010; Davenport et al., 2010; Ge et al., 2010; Pang et al., 2010; Raja et al., 2011); however, the lack of information on the target and mechanism of action of this compound has impeded its clinical application. This study was conducted to elucidate the detailed mechanism for the modulations of c-Myc and Bax by celastrol, and it was found that beta-catenin is a mediator.

Material and methods

Chemical and reagents

Dulbecco's Modified Eagle's Medium (DMEM) was supplied by Thermo-Fisher Biochemical Products (Beijing) Co., Ltd., (Thermo Fisher,

* Corresponding author. Tel.: +86 29 86173097; fax: +86 29 86173097.
E-mail address: wenzonglu@126.com (W. Lu).

0024-3205/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2012.07.032

USA). In Situ Cell Death Detection Kit was purchased from Roche Diagnostics (Mannheim, Germany). Antibodies (c-Myc, Bcl-2, Bax, and beta-catenin) and secondary antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). SYBR-Green PCR Master mix was purchased from Applied Biosystems. Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Celastrol (purity > 98%; Fig. 1) was purchased from Genetimes Technology Inc. (Shanghai, China). A 5 mmol/L solution was prepared in 100% dimethyl sulfoxide according to celastrol molar mass (450 g/mol), stored as small aliquots at −20 °C, and then diluted as needed in cell culture medium.

Cell line

HT29 cells, one human colorectal cancer cell line, were purchased from the Cell Center of Chinese Academy of Medical Sciences. Cells were incubated in DMEM medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2 atmosphere.

MTT assay

The cytotoxicity of celastrol was assessed by MTT assay. Briefly, HT29 cells were treated with various concentrations (10, 20, 40, 60, 80, and 100 μM) of celastrol in a 96-well flat-bottom plate for 24 h and then incubated with MTT (5 mg/mL, 20 μL/well) for 4 h. The medium was replaced with dimethyl sulfoxide (150 μL/well) and after 10 min incubation the plate was read on the Automated Microplate Reader (Multiskan MK3, Finland) with wavelength of 570 nm. The cytotoxicity of celastrol was expressed as IC_{50}.

DNA laddering analysis

After being treated with the effective celastrol concentration, about 10^7 cells were harvested and pelleted by centrifugation (Eppendorf 5417R, Germany) as described previously (Lu et al., 2009). Briefly, DNA extract was dissolved in gel loading buffer, and separated by electrophoresis in 1% agarose gel. DNA in gel was visualized under UV light after staining with ethidium bromide (0.5 μg/μL).

Preparations of the nuclear protein extracts

HT29 cells were treated with 60 μM celastrol for 24 h, and then were scraped, suspended in PBS and centrifuged for 10 min at 2000× *g*. Nuclear protein was extracted from the pelleted cells using the EpiQuik Nuclear Extraction Kit according to the manufacturer's instructions.

Transfection with siRNA

HT29 cells were plated in each well of 6-well plates and allowed to adhere for 24 h. On the day of transfection, 10 μL of lipofectamine transfection reagent was added to 50 nmol/L siRNA (beta-catenin siRNA or control siRNA) in a final volume of 100 μL of culture medium. After 24 h of transfection, cells were treated with celastrol for 24 h. Whole cell extracts were prepared for relevant protein analysis by Western blotting, and for DNA fragmentation analysis.

TUNEL assay

Apoptosis was determined by assessment of TUNEL assay in parallel to DNA laddering using the in situ Cell Death Detection kit, according to the manufacturer's (Roche Applied Science) instructions as described previously (Lu et al., 2009). Briefly, TUNEL reaction mixture was added on the cell samples in 6-well plate after rinsing the slides with PBS, subsequently incubated for 60 min at 37 °C in a humidified atmosphere in the dark. Finally, a fluorescence microscopy (Nikon, Japan) was used to identify the apoptotic cells nuclei, as indicated by their distinct green color change. The number of apoptotic cells was counted in five equal-sized fields and expressed as a percentage of the total number of cells in the same field (apoptosis index).

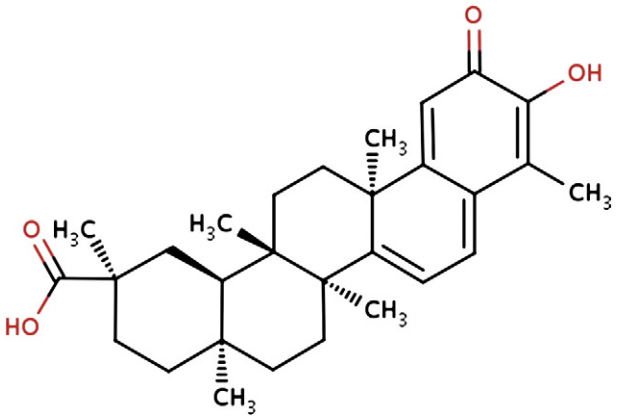

Fig. 1. The chemical structure of celastrol.

Real-time reverse transcription polymerase chain reaction

Total RNA was isolated from cells using TRIzol reagent (Invitrogen) as instructed by the manufacturer. One microgram of total RNA was converted to cDNA using Superscript reverse transcriptase and then amplified by platinum Taq polymerase using the Superscript One Step RT-PCR kit (Invitrogen). Real-time RT-PCR was performed in 25-μL volumes containing reaction buffer, Taq, 25 mM $MgCl_2$, 2 mM dNTP, DEPC-H_2O, cDNA, 100 pmol specific primers and SYBR Green I. The primer pairs (sense, antisense) were used for PCR as follows: c-Myc (5′-cgtctccacacatcagcacaa-3′, 5′-tcttggcagcaggatagtcctt-3′), Bax (5′-gctctgaacagatcatgaagacag-3′, 5′-caatccaaagtggacctgagg-3′), Bcl-2 (5′-cctggcacctggcggatagc-3′, 5′-cgactgaagagtgagcccagcagaac-3′), beta-catenin (5′-ttccagacacgctatcatgc-3′, 5′-aatccactggtgaaccaaaagc-3′), beta-actin 5′-gaacggtgaaggtgacag-3′, 5′-tagagagaagtggggtgg-3′). Beta-actin was used as an internal control. For each specimen, the mRNA level was normalized to beta-actin and was determined by the comparative Ct method. The thermal cycler (ABI PRISM 7900, Applied Biosystems, CA, USA) was used to measure mRNA expression of c-Myc, Bax, Bcl-2, and beta-catenin in MicroAmp optical 96-well plates.

Western blot analysis

Western blot analysis has been described previously (Narayan and Jaiswal, 1997). Briefly, the specimen proteins were separated by SDS-polyacrylamide gel electrophoresis. After electrophoresis, the proteins were electrotransferred onto nitrocellulose membranes, blotted with each antibody, and detected by an ECL reagent. The density of the bands was measured using Glyko BandScan 4.5 and beta-actin was used for an internal control.

Statistical analysis

The data were expressed as the mean ± SD. A one-way analysis of variance (ANOVA) and the Duncan test were used for multiple comparisons (SPSS 12.0). $p<0.05$ was considered statistically significant.

Results

MTT assay

Celastrol concentrations ranging from 10 to 100 μM were monitored within 24 h of cultivation in HT29 cell line (Fig. 2), and the IC_{50} value was 56 μM. On the basis of the results, HT29 cells treated