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Solasodine reverses stemness and epithelial-mesenchymal transition in human colorectal cancer

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ABSTRACT

Adverse side effects of conventional chemotherapy, acquired resistance and fatal tumor metastasis of human colorectal cancer (CRC) are propelling the exploration for novel selective anticarcinogens. Solasodine is a main active component isolated from Solanum incanum L that exhibited a potent stemness and invasion inhibitory effect on human colorectal cancer HCT116 cells. Colony Spheroid formation assay showed that solasodine dose-dependently prohibited HCT116 cell stemness. CD133, CD44, Nanog, Oct-4 and Sox-2 were inhibited by solasodine to reverse stemness and similar mechanism was stimulated in vivo. Transwell and scratch wound assays revealed that solasodine impeded HCT116 cell invasion and migration potential strengthened by TGF-β1. Moreover, solasodine attenuated TGF-β1-induced EMT and decreased MMPs while in vivo study showed the same trend. The results of this study implied that solasodine may be a novel therapeutic drug for CRC treatment.

1. Introduction

Colorectal cancer (CRC) is one of the most common types of malignant tumors, causing the deaths of 50,260 Americans annually [1], while 191,000 Chinese people succumb to this disease [2]. At present, selection of the treatment for CRC, including appropriate surgery, adjuvant chemotherapy and radiation, largely depended on the tumor stage. Survival also varies based on the degree of disease; the 5-year survival rate of localized CRC is 90%, but this declines to 71 and 14% for those diagnosed with regional and distant disease respectively [3]. Despite novel therapies for metastasis and the use of imaging to improve detection of metastatic lesions, the survival of patients with distant-stage disease remains poor, novel drugs that may prevent CRC metastasis are urgently required in order to advance treatment for subtypes with low response rates to current therapies.

Malignant transformation is the result of an abnormal growth of cells, and the tumor growth speed is usually determined by the ratio of cell death to cell division. Cancer stem cell (CSC) is a group of cells with talent of self-renewal, multi-directional differentiation and unlimited growth, which is primarily responsible for recurrence as well as poor prognosis of cancers [4]. The potential of cancer cell stemness depends on transduction of development-related signals and the controlled expression of those transcriptional activators [5]. The epithelial-mesenchymal transition (EMT) is a fundamental process governing not only embryonic development and organ formation, but also cancer progression [6]. During the course of EMT, polar epithelial cells undergo considerable phenotypic alteration and lose the polarity needed to transform into mesenchymal cells, weakening contact with ambient matrix and cell-cell junctions while initiating infiltrative and metastatic characteristics [7]. Therefore, the investigation of stemness and EMT as therapeutic targets in CRC has attracted increasing interest recently [8,9].

Solasodine is the main active ingredient isolated from solanaceous species, which performs a wide range of functions, including anti-oxidant, anti-infection and neurogenesis promotion effects

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[10–12]. Recent studies have proposed that solasodine exhibits anticancer activities in human lung and ovarian cancer cells through blocking the expression of microRNA 21 (miR-21) and matrix metalloproteinases (MMPs), inhibiting the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathways, inducing apoptosis, and modifying autophagy [13,14]. The present study aimed to investigate the effect of solasodine on human colorectal cancer cells and its potential underlying mechanism.

2. Materials and methods

2.1. Reagents and antibodies

Solasodine was obtained from Sigma-Aldrich (St. Louis, MO), dissolved in 100% DMSO, kept at −20 °C. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and monoclonal mouse β-actin antibody were brought from Sigma-Aldrich (St. Louis, MO). The antibodies against Stemness kit, MMPs, EMT kit and the horseradish peroxidase (HRP) labeled goat anti-mouse or anti-rabbit IgG antibody were purchased from Cell Signaling Technology (Beverly, MA). TRIzol reagent and Power SYBR Green PCR Master Mix were from Life Technologies (Grand Island, NY). PrimeScript RT reagent Kit with gDNA Eraser was from TaKaRa (Dalian, China). All chemicals were of the highest grade available.

2.2. Cell lines and culture

The human colorectal carcinoma HCT116 cells were obtained from the Type Culture Collection, Chinese Academy of Sciences (Shanghai, China). Cells were cultivated in RPMI-1640 medium with 10% fetal bovine serum (FBS) (both from Gibco-BRL, Gaithersburg, MD, USA) in a humidified incubator at 37 °C containing 5% CO_2 atmosphere.

2.3. Colony spheroid formation assay

Cells treated with TGF-β1 or solasodine were seeded into ultralow attachment six-well plates at a density of 1×10^3 per well and filled with serum free medium contains 2% B27, 20 ng/ml EGF and bFGF at 37 °C in 5% CO_2. Medium was renewed every 6 days. Assorted pictures of respective group of cell colony formation were taken by an inverted microscope after twenty-day incubation.

2.4. Invasion and migration assay

Cell invasion ability was examined by transwell membrane filter inserts (pore size, 8-μm; Costar, Corning, NY, USA). Approximately 1×10^4 HCT116 cells in 200 μl of serum-free medium mixed with solasodine were seeded into the upper chambers, and 500 μl of complete medium was furnished in the lower chamber. After 48 h, the non-migrated cells on the upper side were wiped off and then take a view of invaded cells which were fixed in 4% paraformaldehyde and stained in 0.05% crystal violet by an inverted microscope (Bio-Tek, Winooski, VT, USA). Cell migration ability was observed by scratch wound assay. All cells were seeded into 6-well plates as confluent monolayers and then scratched by a pipette tip. The cells were then washed twice with PBS to remove detached cells and underwent incubation with various doses of solasodine for 48 h. Wound images were acquired by use of an inverted microscope.

2.5. In vivo tumor xenograft experiments

BALB/c/nu/nu nude mice (6–8 week old, 18–22 g body weight, half male and half female) were brought from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal studies were accomplished in line with the National Institutes of Health guide for the care and use of Laboratory animals and ratified by the Animal Ethics and Research Committee of Jinling Hospital. The HCT116 cells (1×10^6) were suspended in 100 μl PBS and injected subcutaneously into the right flank of all mice (n = 24). Mice were randomly divided into four groups including control (PBS), low-dose (30 mg/kg), high-dose (50 mg/kg). When the tumors reached a volume of approximately 150 mm^3, PBS or solasodine were intraperitoneally delivered once daily for 5 weeks, during which the mean tumor volumes were figured weekly following the formula: volume = (length × $width^2$)/2. Mice were sacrificed 24 h after the last dose and tumors were excised for weight and volume computation and then stored at −80 °C for RNA or protein isolation.

2.6. Total RNA extraction and RT-qPCR

Total RNA was extracted from HCT116 cells and tumor tissues with TRIzol reagent. Reverse-transcription was carried out by using TaKaRa RT reagent kit on the basis of manufacturer's protocol. Gene expression levels were quantitatively measured by ABI 7500 fast RT-qPCR System through DNA-binding dye SYBR-Green by ΔΔCt, ACTB was served as the internal control. The sequences of primer are listed in Table 1.

2.7. Western blot analysis

The HCT116 cells and xenograft tumor tissues were lysed by RIPA buffer which contains protease inhibitor cocktail. The lysates were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA, USA). After incubated with BSA one hour at room temperature, membranes were probed with primary antibodies directed against corresponding proteins overnight at 4 °C. After washing thrice with TBST, membranes were incubated with secondary antibodies and detected by ECL detection kit.

2.8. Immunohistochemistry

With the purpose of further histological research, formaldehyde-fixed portions of tumor tissues were embedded in paraffin and cut into 4-mm thick for immunohistochemical detection of Bax, E-cadherin according to the routine protocols.

2.9. Statistical analysis

All data were presented as means ± standard deviation (SD) and analyzed by the one-way ANOVA analysis of variance test by SPSS 20.0 (SPSS, Inc., Chicago, IL, USA) software. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Solasodine inhibits stemness in HCT116 cells

Our previous research proved that solasodine (Fig. 1A) significantly inhibited cell growth in a time- and dose-dependent manner and the calculation of IC_{50} value for HCT116 cells at 48 h was 39.43 μmol/L [15]. To estimate the influence of solasodine treatment on HCT116 cell stemness, colony spheroid formation experiments was conducted. TGF-β1 could effectively enhanced HCT116 cell potential of colony spheroid formation. However, after 48 h solasodine treatment in different concentration (20, 40 μmol/L), the spheroid constitution rate was significantly decreased in a concentration-dependent manner (Fig. 1B).

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