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Schizandrin A enhances chemosensitivity of colon carcinoma cells to 5-fluorouracil through up-regulation of miR-195

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ABSTRACT

Nowadays 5-fluorouracil (5-FU)-based adjuvant chemotherapy is widely used for treating colon carcinoma. However, 5-FU resistance in the treatment of colon carcinoma has become more common and thereby new therapeutic strategies and new adjuvant drugs still need to be explored. Two 5-FU-resistant colon cancer cell lines, HCT116 and SW480, were used to investigate the effects of Schizandrin A (SchA), 5-FU, or their combination on cell viability and apoptosis. Besides, the role of miR-195 was studied to further clarify the specific function of SchA. CCK-8 assay and flow cytometry analysis were conducted to determine cell viability and apoptosis, respectively. miR-195 expression was determined by quantitative real-time PCR. Cell apoptosis-related proteins and factors of PI3K/AKT and NF-κB pathways were analyzed by Western blot. Cell viability assay showed that SchA treatment at non-toxic dosages caused a marked enhancement of 5-FU-induced cytotoxicity. Moreover, we explored that miR-195 was up-regulated by SchA; and overexpression of miR-195 reduced cell viability and sensitized 5-FU-resistant HCT116 and SW480 cells to 5-FU. The promoting effect of SchA on 5-FU susceptibility can be partly abolished by miR-195 knockdown. Thus it was speculated that SchA might enhance cell chemosensitivity to 5-FU by up-regulating miR-195. Finally, we found that PI3K/AKT and NF-κB pathways were inhibited by high expression of miR-195 reduced by SchA. Our results suggested that SchA sensitized 5-FU-resistant colon carcinoma cells to 5-FU by up-regulating miR-195. SchA combined with 5-FU could be a promising strategy for the adjuvant chemotherapy of colon cancer.

1. Introduction

Colon carcinoma, a common type of malignant tumor of the alimentary system, is one of the leading causes of cancer-related deaths worldwide [1]. In addition to surgical procedures, adjuvant chemotherapy is often used to prolong the survival time of patients, especially for patients at advanced stages of the disease. With respect to treatment of advanced colon cancer, there are many anticancer agents in clinical use, such as cisplatin, carboplatin, gemcitabine, 5-fluorouracil (5-FU) derivatives, and etc. [2,3].

5-FU is a chemotherapeutic drug used in the treatment of metastatic colorectal cancer worldwide. 5-FU is an analog of uracil with a fluorine atom in place of hydrogen at the C-5 position. It exerts the antitumor effects via inhibition of thymidylate synthase and incorporation of its active metabolites into RNA and DNA, so as to affect the uracil metabolism [4,5]. Furthermore, futile cycles of misincorporation stimulate the cellular excision system, subsequently cause the DNA strand to brake, and eventually lead to cancer cell apoptosis [6]. Recently, 5-FU resistance has become more common, which is an important cause of failure in colon carcinoma therapy [7]. In fact, fewer than 25% of patients with advanced colon carcinoma have shown major responses after 5-FU-based chemotherapy, whereas, in many cases, patients who initially responded to 5-FU ultimately become resistant [8]. Thus, it is an urgent job to resolve this problem.

Schizandrin is a lignan of the dibenzocyclooctadiene type which is found mainly in the fruit of *Schisandra chinensis (Turcz.) Baill.* This fruit has been used as an antiaging agent, stimulant, cough remedy, and tonic [9]. Pharmacological studies also indicate that the active extracts or constituents of this fruit display hepatoprotective, antioxidant, antiviral, neuroprotective, and cancer-chemopreventive activities [10–12]. Moreover, it was reported that schizandrin A (SchA), schizandrin B, and schizantherin A showed cytotoxicity in human cancer cells, enhanced the cytotoxic effect of some anticancer agents, and even reversed multidrug resistance of cancer cells [13–15].

In this study, the effects of SchA on 5-FU-resistant colon carcinoma cells were studied. SchA, also referred to wuweizisu A and deoxyschizandrin, is one of the most effective lignins isolated from *Schisandra chinensis* [16]. Previous studies have also demonstrated that

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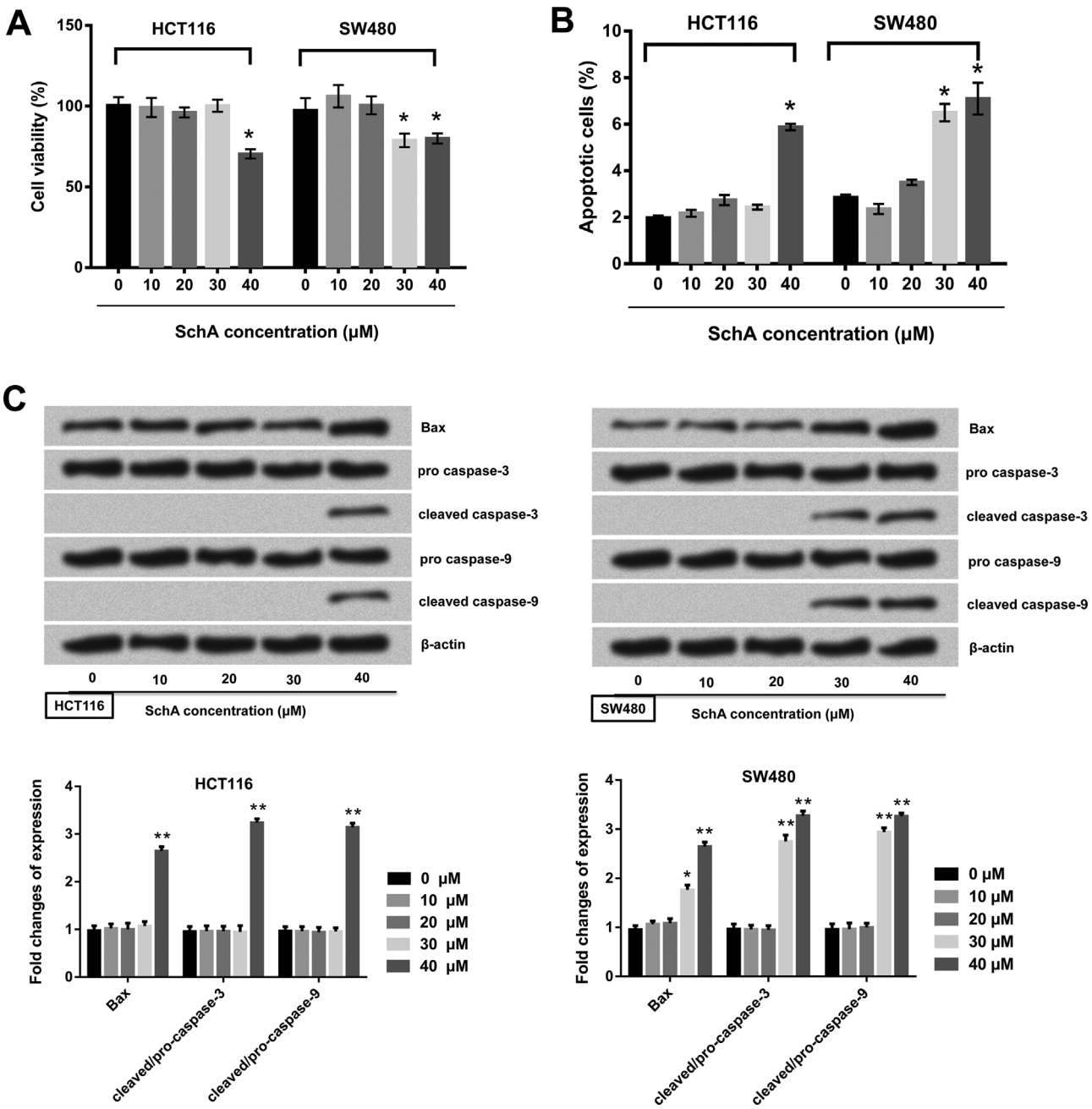

Fig. 1. Effect of SchA on viability and apoptosis of 5-FU-resistant colon carcinoma cells. After HCT116 and SW480 cells were treated by SchA with different concentrations ranging from 0 to 40 μM, (A) cell viability and (B) apoptosis were respectively determined by CCK-8 assay and flow cytometry analysis; (C) apoptosis related proteins were analyzed by Western blot. SchA, Schizandrin A. $^{*}P < 0.05$, $^{**}P < 0.01$ for significantly different from 0 μM.

SchA may possess the antitumor activities and enhance cancer treatment through overcoming multidrug resistance [17,18].

In this study, using two kinds of 5-FU-resistant colon cancer cells (HCT116 and SW480), we investigated the effect of SchA on 5-FU sensitivity of cells by evaluating cell viability and examined the underlying mechanism. The molecular regulatory mechanism of microRNA-195 (miR-195) in the action of SchA on regulating 5-FU sensitivity was emphatically studied.

2. Materials and methods

2.1. Cell culture and treatment

Human colonic cancer cell lines HCT116 and SW480, from American Type Culture Collection (Rockville, MD), were cultured in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Inc., Grand Island, NY) supplemented with 10% fetal calf serum (FCS; Life Technologies, Inc.). Cell lines were cultured at 37 °C in an incubator with humidified atmosphere and 5% CO_2. To generate 5-FU-resistant cell lines, cells were established by serial passages and incubations with increasing 5-FU concentrations as previously described [19]. In forthcoming experiments, cells were treated by 5-FU and SchA for 48 h. The concentrations of 5-FU were ranged from 0 to 8 μM and the concentrations of SchA were ranged from 0 to 40 μM. SchA and 5-FU were purchased from Wako Pure Chemical Industries, Ltd., Japan.

2.2. Cell viability assay

Cell viability was determined by Cell Counting Kit (CCK-8) assay. HCT116 or SW480 cells were seeded in 96-well plates at a density of