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Astragaloside IV Enhances Cisplatin Chemosensitivity in Human Colorectal Cancer via Regulating NOTCH3

Tao Xie,* Yao Li,† Shi-Lei Li,* and Hai-Feng Luo‡

*Department of General Surgery, Rizhao People's Hospital, Shandong, P.R. China
†Department of Cardiology, Rizhao People's Hospital, Shandong, P.R. China
‡Department of General Surgery, The first Affiliated Hospital of Dalian Medical University, Dalian, Liaoning Province, P.R. China

Although astragaloside IV exhibits anti-inflammation, immunoregulatory, and anticancer properties, the chemosensitization effects of astragaloside IV in colorectal cancer have never been reported. Our study tested whether astragaloside could increase cisplatin sensitivity in colorectal cancer. CCK-8 assay was used to measure the cell viability of colorectal cancer cells. Quantitative real-time PCR and Western blot were performed to determine the mRNA and protein expression, respectively. Our data revealed that astragaloside IV administration significantly suppressed the cell growth of colorectal cancer cells, whereas no obvious cytotoxicity of astragaloside IV was observed in nonmalignant colonic cells. In addition, combined treatment with astragaloside IV dramatically elevated the chemosensitivity of colorectal cancer cells to cisplatin. Mechanical investigation revealed that the mRNA and protein expression of NOTCH3 was significantly lower in cisplatin and astragaloside IV-treated cells compared with cells treated with cisplatin alone. On the contrary, no obvious changes in tumor cell growth were shown after upregulation of NOTCH3 whether in the presence or absence of astragaloside IV. Thus, our data demonstrate that astragaloside IV increases the chemosensitivity of colorectal cancer cells to cisplatin, at least partly, through inhibition of NOTCH3. This study suggests that combined therapy with astragaloside IV might be a novel therapeutic approach for colorectal cancer.

Key words: Colorectal cancer (CRC); Astragaloside IV; Drug resistance

INTRODUCTION

Colorectal cancer is one of the most common causes of cancer-related deaths in the world with a poor prognosis (1). It is estimated that 241,600 European men and 205,200 women were diagnosed with colorectal cancer (2). In addition to surgical resection, chemotherapy is an important adjuvant therapy for the treatment of colorectal cancer. Nevertheless, acquired drug resistance has become one of the most challenging factors in the effective treatment of cancer (3,4).

Traditional Chinese herbs are regarded as abundant sources of drugs for the treatment of a variety of diseases. Radix astragali has been reported to possess cardioprotective, immunostimulant, and antihyperglycemic properties (5,6). Astragaloside IV, one of the bioactive components of Radix astragali, has been shown to reduce inflammation, oxidation, and apoptosis (7). For example, astragaloside IV treatment obviously inhibits the production of reactive oxygen species (ROS) and increases the Bax/Bcl-2 ratio and caspase 3 activity in SH-SY5Y cell exposed to MPP^+, making it a potential therapeutic agent for neurodegenerative disease (8). In addition, astragaloside IV suppresses the proliferation, migration, and invasion of lung cancer cells through regulation of regulatory T cells and ERK–NF-κB signaling pathway (9,10). It has also been reported that astragaloside IV could inhibit the expression of P-glycoprotein in multidrug-resistant hepatocellular carcinoma (HCC) cells and thus reverse drug resistance in HCC therapy (11). However, the chemosensitization role of astragaloside IV in colorectal cancer has never been reported. In the current study, we aimed to investigate the chemotherapeutic sensitization effects of astragaloside IV as well as its underlying mechanism.

Address correspondence to Hai-Feng Luo, Department of General Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian, 116011 Liaoning Province, P.R. China Tel: +86-411-83635963-2086; E-mail: luohaifeng1975@sina.cn

MATERIALS AND METHODS

Cell Culture and Reagents

Human colorectal cancer cells including HCT116, SW480, and a nonmalignant colonic epithelial cell line NCM460 were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator with 5% CO_2. Astragaloside IV and cisplatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). siRNAs were purchased from Invitrogen (Carlsbad, CA, USA). Cells were transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction.

Cell Viability Assay

Cells were seeded onto 96-well plates at 2×10^3 cells/well. The medium was replaced with the corresponding serum-free medium for 24 h, then serum-free medium was replaced with complete medium. Then 10 μl/well of CCK-8 (Beyotime Biotechnology, China) was added and incubated with the plates for 3 h. The absorbance was measured at 450 nm with a microplate reader (Dynex, Chantilly, VA, USA).

Quantitative Real-Time PCR

Total RNA was extracted, and cDNA was synthesized from 1 μg of RNA using the PrimeScript Kit (Takara, Tokyo, Japan). Quantitative real-time PCR was performed using the ABI 7500 system (Applied Biosystems, Foster, CA, USA). All mRNA quantification data were normalized to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) using the $2^{-\Delta\Delta Ct}$ method.

Western Blot Analysis

Cells were lysed in lysis buffer (Cell Signaling Technology, Danvers, MA, USA) containing protease inhibitors (Sigma-Aldrich). Whole-cell lysates were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After transferring to nitrocellulose filter membrane, proteins were incubated with primary antibodies at 4°C overnight. After being washed with TBST, membranes were incubated with the appropriate HRP-conjugated secondary antibodies for 1 h at room temperature. Protein expression was detected with a chemiluminescence detection kit (ECL kit; Amersham Bioscience, UK).

Statistical Analysis

Data were presented as means ± SD and analyzed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Differences among the groups were assessed by ANOVA. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effects of Astragaloside IV on Colorectal Cancer Cell Viability

First we assessed the cytotoxic effects of astragaloside IV on colorectal cancer cells with CCK-8 assay. Two tumor cell lines (HCT116 and SW480) and a nonmalignant colonic epithelial cell line (NCM460) were incubated with astragaloside IV (Fig. 1A) at different concentrations (1, 2.5, 5, 10, and 15 ng/ml). We found that the cell viability of the HCT116 and SW480 cell lines was obviously suppressed in the presence of astragaloside IV at doses of 10 and 15 ng/ml (Fig. 1B and C). Low concentrations of astragaloside IV (2.5, 5, and 7.5 ng/ml) had no obvious cytotoxicity effect on colorectal cancer cells (Fig. 1B and C). Moreover, treatment of nonmalignant colonic cells with astragaloside IV (2.5, 5, 7.5, 10, and 15 ng/ml) exhibited no dramatic inhibitory effects on cell viability (Fig. 1D).

Astragaloside IV Increased the Sensitivity of Cisplatin in Colorectal Cancer Cells

In order to evaluate the sensitization role of astragaloside IV, HCT116 and SW480 cell lines were incubated with cisplatin alone or in combination with astragaloside IV at a dose of 7.5 ng/ml. The CCK-8 assay indicated that cisplatin (10, 15, and 20 μM) treatment inhibited the cell viability of HCT116 and SW480 cells in a dose-dependent manner (Fig. 2A and B). In addition, combined treatment with astragaloside IV also increased the sensitivity of HCT116 and SW480 cells to cisplatin at a dose of 10 μM (Fig. 2C and D). Collectively, these results indicate that coadministration with astragaloside IV could sensitize colorectal cancer cells to cisplatin.

Astragaloside IV Suppressed the Expression of NOTCH3 in Cisplatin-Treated Colorectal Cancer Cells

In order to clarify the mechanism underlying the sensitization role of astragaloside IV in colon tumors, we assessed the molecular changes in colorectal cancer cells treated with cisplatin alone or combined with astragaloside IV. Compared with cisplatin-treated cells, our results revealed that the transcript levels of NOTCH3 were obviously downregulated in HCT116 cells in the presence of cisplatin and astragaloside IV (Fig. 3A). Moreover, astragaloside IV cotreatment inhibited the mRNA expression of NOTCH3 in SW480 cells (Fig. 3B). Western blot analysis showed that the protein levels of NOTCH3 were reduced in HCT116 and SW480 cells exposed to cisplatin and astragaloside IV (Fig. 3C and D). Taken together, these data suggested that astragaloside IV increased the cisplatin cytotoxicity, at least partly, through inhibition of NOTCH3.

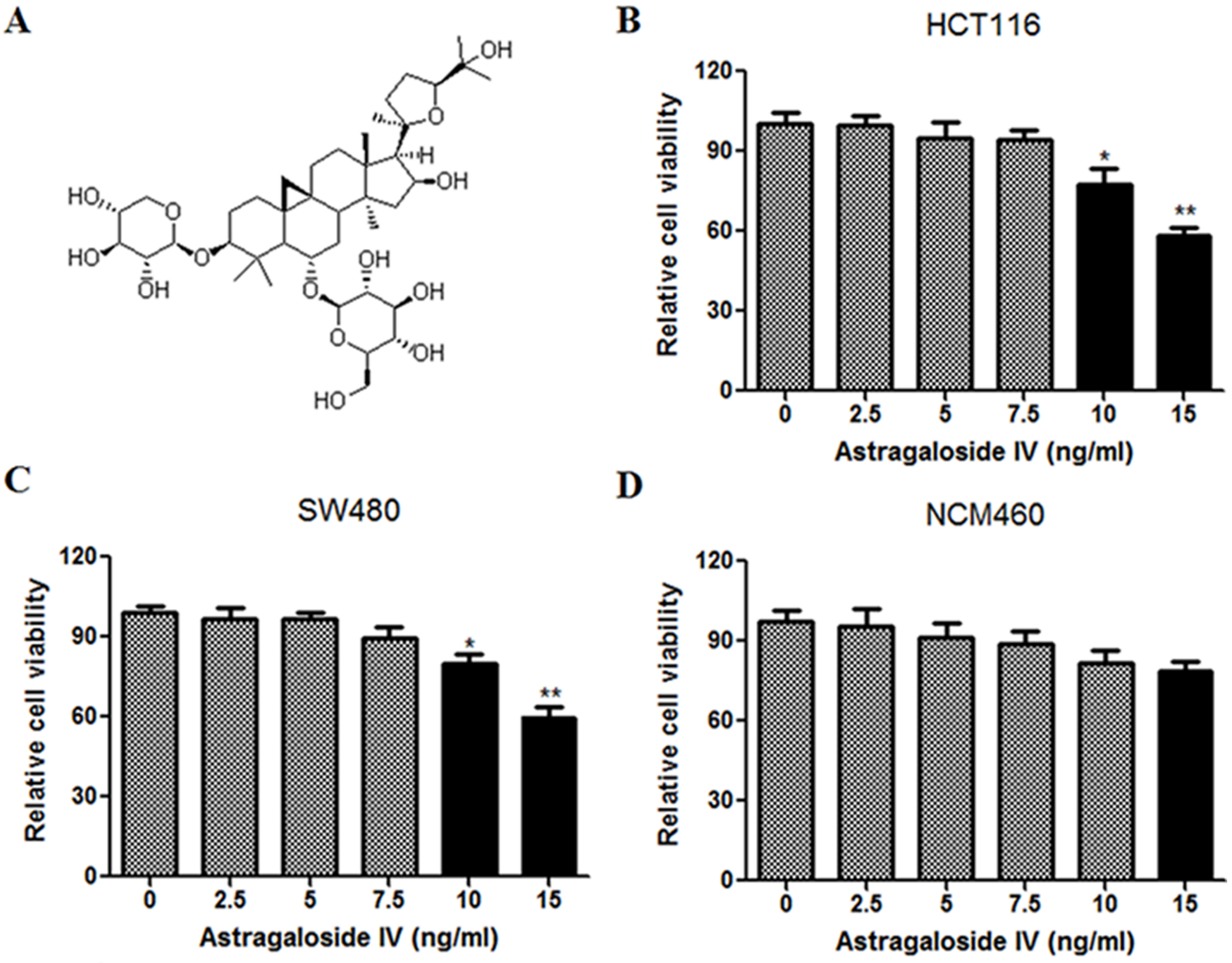

Figure 1. Effects of astragaloside IV on colorectal cancer cell viability. Structure of astragaloside IV (A). Two human colorectal cancer cell lines including HCT116 (B) and SW480 (C), and a nonmalignant colonic epithelial cell line NCM460 (D) were exposed to different doses of astragaloside IV (1, 2.5, 5, 10, and 15 ng/ml) for 48 h. The CCK-8 method was used to determine the cell viability. *$p<0.05$; **$p<0.01$.

Ectopic Expression of NOTCH3 Abolished the Chemosensitization Role of Astragaloside IV in Colorectal Cancer Cells

To ascertain the biological role of NOTCH3 in colon tumor cells, recombinant plasmid encoding NOTCH3 was constructed to increase the expression of NOTCH3. Quantitative real-time PCR demonstrated that NOTCH3 plasmid transfection significantly enhanced the mRNA expression of NOTCH3 in HCT116 cells (Fig. 4A). In addition, Western blot analysis showed that the protein levels of NOTCH3 were elevated in colorectal cancer cells transfected with NOTCH3-encoding plasmids (Fig. 4B and C). Consequently, the CCK-8 assay showed that ectopic expression of NOTCH3 enhanced HCT116 cell growth compared to the control group (Fig. 4D). Moreover, upregulation of NOTCH3 abolished the chemosensitization role of astragaloside IV in HCT116 cells (Fig. 4E). Taken together, our data demonstrated that astragaloside IV increased cisplatin sensitivity through modulation of NOTCH3 in colon tumor cells.

DISCUSSION

A large amount of evidence highlights the critical importance of chemotherapy in postoperative treatment of colorectal cancer (12,13). Nevertheless, acquired resistance to traditional chemotherapeutic drugs has greatly impaired the successful treatment of colorectal cancer (14). In the current study, our results demonstrated that astragaloside IV could be developed as a potential adjuvant drug to increase cisplatin sensitivity in colorectal cancer cells.

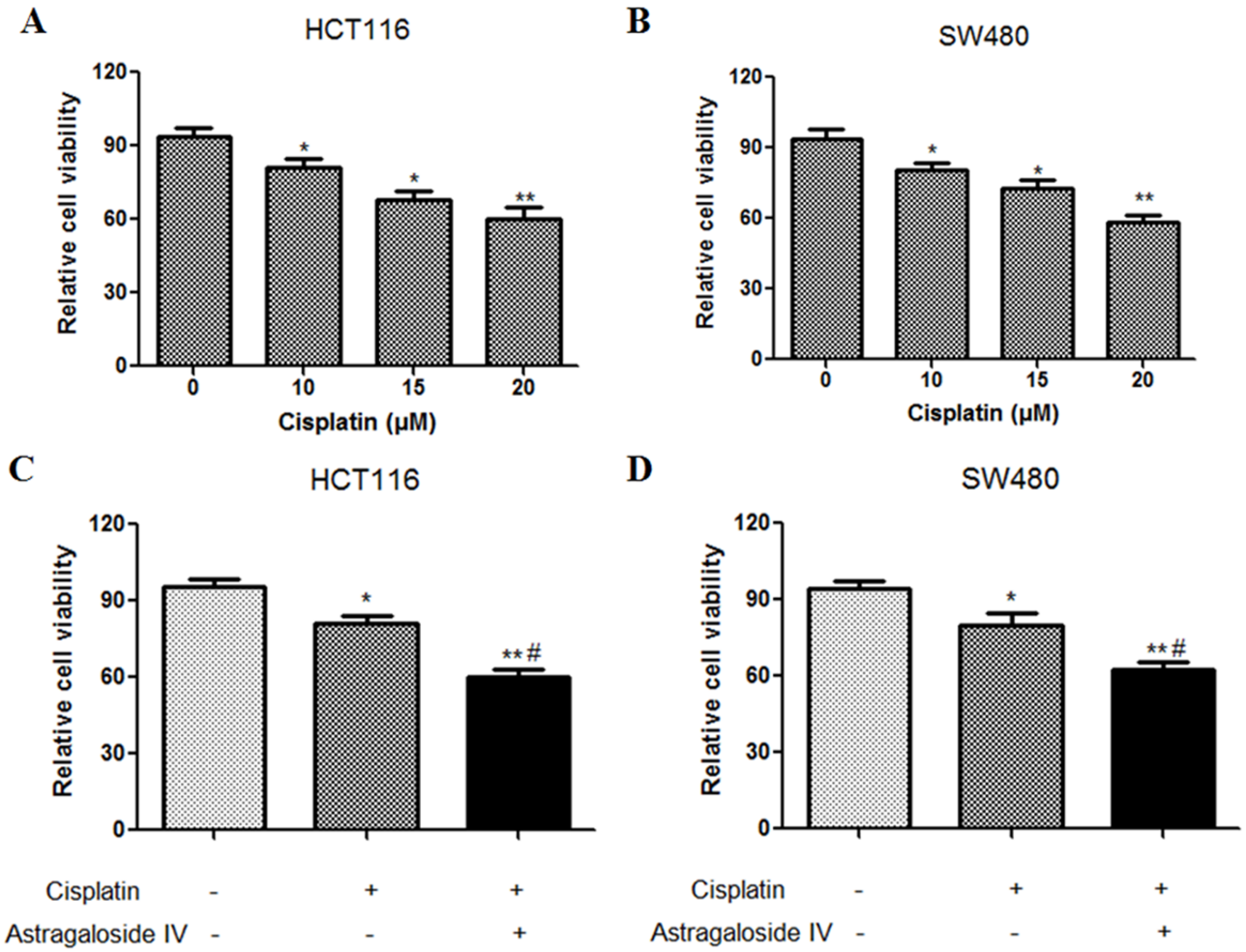

Figure 2. Astragaloside IV increased cisplatin sensitivity in colorectal cancer cells. Colorectal cancer cell lines including HCT116 (A) and SW480 (B) were incubated with cisplatin alone (10, 15, and 20 μM), and cell viability was determined using the CCK-8 method. Moreover, cancer cells were incubated with cisplatin alone (10 μM) or in combination with astragaloside IV at the dose of 7.5 ng/ml. The CCK-8 assay was applied to measure the cell viability of HCT116 (C) and SW480 (D) cells after exposure to cisplatin or combined with astragaloside IV. *$p < 0.05$, **$p < 0.01$, compared with control; #$p < 0.05$, compared with cisplatin-treated cells.

Astragaloside IV is a main component of Radix astragali, a commonly used medicinal plant in East Asia (7). Diverse biological roles of astragaloside IV have been documented such as antioxidative, anti-inflammatory, anti-infarction, and antifibrotic effects (15). For example, astragaloside IV administration ameliorates renal fibrosis through apoptosis inhibition dependent on mitogen-activated protein kinases (16). In addition, astragaloside IV combined with ginsenoside protects cerebral ischemia–reperfusion mice from oxidative stress injury. The underlying mechanism could be associated with activation of nuclear factor-erythroid 2-related factor 2/heme oxygenase-1 signaling pathway (17). The antitumor effects of astragaloside IV have also been reported in recent studies, including lung cancer and hepatic cancer (9,11,18). In our study, astragaloside IV was shown to suppress colorectal cancer cell growth, whereas no obvious cytotoxicity of astragaloside IV was observed in nonmalignant colonic cells, suggesting the potential application of astragaloside IV on colorectal cancer treatment. Accumulating evidence points out that combination treatment with traditional Chinese medicine could increase the chemotherapeutic efficacy in various types of cancer cells (19,20). Our study demonstrated that coadministration with astragaloside IV sensitized colorectal cancer cells to cisplatin, suggesting the therapeutic potential of astragaloside IV on cisplatin-resistant patients.

The NOTCH pathway is involved in intercellular communication, and it plays a critical role in self-renewal, proliferation, apoptosis, and differentiation of

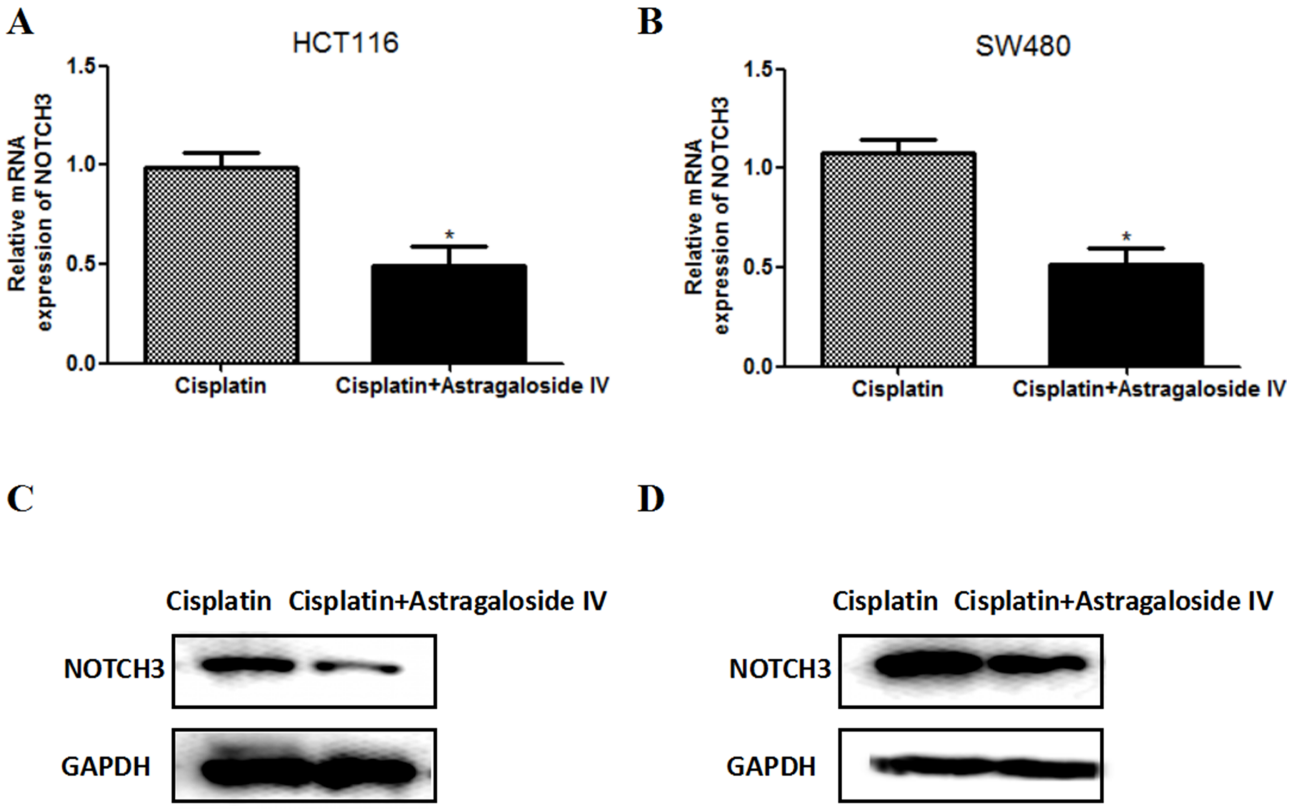

Figure 3. Downregulation of NOTCH3 by astragaloside IV in NSCLC cells. Determination of mRNA expression of NOTCH3 in HCT116 (A) and SW480 (B) cells exposed to cisplatin or combined with astragaloside IV. Western blot analysis was performed to detect the protein expression of NOTCH3 in HCT116 (C) and SW480 (D) cells exposed to cisplatin alone or in combination with astragaloside IV. *$p<0.05$.

cells (21). Aberrant expression of NOTCH genes including NOTCH1–4 has been associated with the initiation and progression of cancer cells (22). Accumulating evidence suggests that NOTCH3 regulates the growth, migration, cell cycle, and apoptosis in several cancers including HCC, colorectal cancer, ovarian cancer, and prostate cancer (23–25). Recently, the association between NOTCH3 and colorectal cancer has been established (26). Ozawa et al. points out that the expression of NOTCH3 is associated with tumor recurrence in stage II/III colorectal cancer patients, suggesting that NOTCH3 might be a potential predictive biomarker for recurrence of colorectal cancer (27). Increased levels of NOTCH3 have been observed in primary and metastatic colon cancer samples, and enforced expression of NOTCH3 promotes the clonogenic capacity and tumor growth (28). A recent study reveals that the feed-forward circuit involving NOTCH3 and MSI-1 may be relevant for the regulation of tumor development, highlighting that NOTCH3-specific drugs could represent a valuable strategy to overcome colorectal cancer (29). In our study, the mRNA and protein expression of NOTCH3 was obviously downregulated in colorectal cancer cells exposed to cisplatin and astragaloside IV. These data suggested that astragaloside IV coadministration increased cisplatin sensitivity through suppression of NOTCH3 expression. Conversely, we found that enforced expression of NOTCH3 abolished the chemosensitization effects of astragaloside IV in cellular responses to cisplatin in colorectal cancer cells.

In conclusion, our study demonstrates that astragaloside IV could sensitize colorectal cancer cells to cisplatin through inhibition of NOTCH3 expression. These findings indicate that combined therapy with astragaloside IV and downregulation of NOTCH3 might be novel therapeutic approaches for the treatment of colorectal cancer patients.

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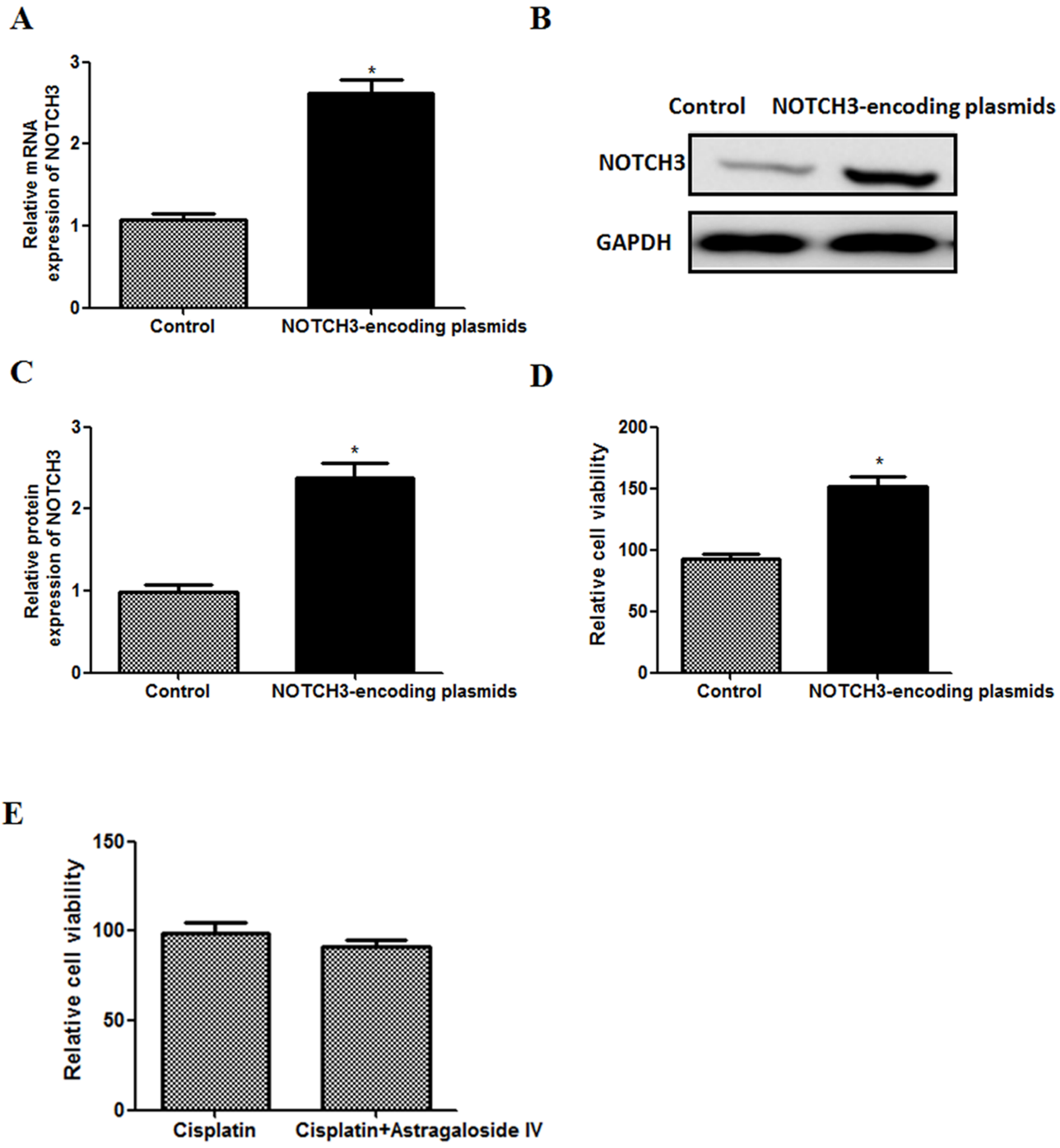

Figure 4. Enforced expression of NOTCH3 abolished the chemosensitization role of astragaloside IV. HCT116 cells were transfected with NOTCH3-encoding plasmids, and upregulation of NOTCH3 was confirmed by quantitative real-time PCR (A) and Western blot (B and C). (D and E) The CCK-8 assay was applied to measure the cell viability of HCT116 cells overexpressing NOTCH3 with or without chemical stimulation. *$p<0.05$.

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