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Avicularin reversed multidrug-resistance in human gastric cancer through enhancing Bax and BOK expressions

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ABSTRACT

5-Fluorouracil (5-Fu) and cisplatin (DDP) as important therapies in treatment of human gastric cancer have been widely determined. However, the therapeutic effects are usually hampered due to drug resistance or toxicity at high concentrations for application. Avicularin (AL, quercetin-3-α-L-arabinofuranoside), a bio-active flavonol isolated from a number of plants, has been reported to display diverse pharmacological properties. In this study, we explored the hypothesis by which AL reversed 5-Fu or DDP resistance in gastric cancer and the underlying molecular mechanism. Here, in vitro, the drug-resistant cancer cells were incubated to AL or DDP alone or the combination of AL and DDP. Then, MTT, colony formation, Hoechst 33258, flow cytometry and western blot analysis were used to investigate the effects of AL in the regulation of drug-resistance gastric cancer cells. The results indicated that AL treatment markedly re-sensitizes the drug resistant cells (SGC-7901/5-Fu and SGC-7901/DDP) to cytotoxicity of 5-Fu or DDP. Molecular mechanism analysis indicated that AL and DDP combination treatment enhanced apoptosis in SGC-7901/DDP cells, accompanied with the up-regulation of cleaved Caspase-3 and PARP, as well as the activation of pro-apoptotic signals, including Bax and BOK. Significantly, down regulation of Bax or BOK expressions using Bax siRNA or BOK siRNA decreased the inhibitory role of DDP in apoptosis of SGC-7901/DDP cells pretreated with AL, demonstrating that AL-reversed DDP resistance was associated with Bax and BOK expression. In vivo, AL and DDP combination significantly reduced gastric tumor growth. Immunohistochemical analysis indicated that co-treatment of AL and DDP significantly induced apoptosis, and reduced tumor cell proliferation in tumor tissue samples. Furthermore, we also found that the Bax, BOK, cleaved Caspase-3 and PARP expression in tumor tissues were highly induced by AL and DDP co-treatment. Together, our findings may provide a novel combination therapeutic strategy in treatment of human gastric cancer.

1. Introduction

Gastric cancer is reported as the fourth most commonly diagnosed cancer and is the second most common cause of cancer-associated death in the world [1]. Accordingly, gastric cancer is a heterogeneous disease with various molecular and histological subtypes [2]. Although advances in chemotherapy and surgical approaches, as well as its declining incidence were widely detected, the gastric cancer is still a major global public health problem [3]. Accumulating evidences regarding molecular mechanism of 5-Fu in inhibition of cancer has resulted in the development and progression of therapeutic strategies. Despite these improvements, drug resistance still remains a significant limitation in the clinical application of 5-Fu [4,5]. In addition, cisplatin (DDP) is a commonly used drug for cancer treatment through cross-linking DNA, resulting in apoptosis of tumor cells [6]. However, resistance to DDP treatment often occurs, which contributes to relapse [7,8]. Thus, it is necessary for the development of more effective therapeutic strategies that could overcome chemoresistance.

Many dietary flavonoids exist as glycosides in fruits and vegetables, and they are considered as bioactive food components potentially responsible for various health benefits. Avicularin (AL, Fig. 1A), quercetin-3-α-L-arabinofuranoside, is a glycoside of quercetin, possessing a variety of biological properties, including anti-oxidant, anti-allergic, anti-inflammatory, hepatoprotective, and even anti-tumor activities

Abbreviation: 5-Fu, 5-fluorouracil; AL, avicularin; Bax, B-cell lymphoma 2 associated X; BCA, bicinchoninic acid; BOK, BCL-2-related ovarian killer; DDP, cisplatin; H&E, hematoxylin and eosin; Mcl-1, myeloid cell leukemia 1; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; PARP, poly (ADP-ribose) polymerase; TUNEL, terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling

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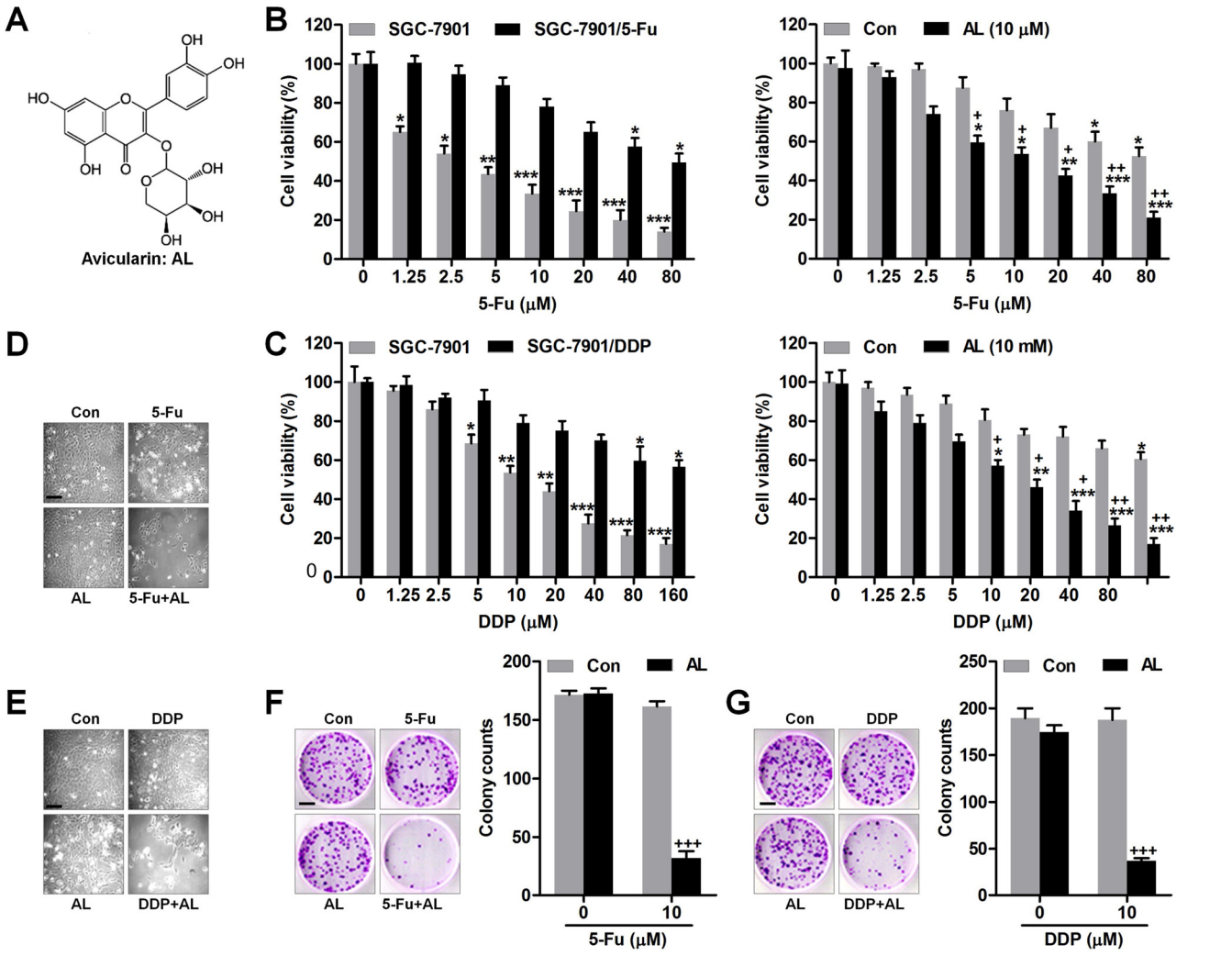

Fig. 1. Avicularin sensitizes SGC-7901/5-Fu and SGC-7901/DDP cells to 5-Fu- and DDP-induced cytotoxicity. (A) The chemical structure of aviculairn (AL). (B) Left, SGC-7901 and SGC-7901/5-Fu cells were treated with 5-Fu at the indicated concentrations for 48 h, followed by MTT analysis. Right, SGC-7901/5-Fu cells were treated with AL for 12 h, and then subjected to the described concentrations of 5-Fu for 36 h, followed by MTT analysis. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.01$ vs Con group without any treatments; $^{+}P < 0.05$, and $^{++}P < 0.01$ vs each corresponding 5-Fu-treated group. (C) Left, SGC-7901 and SGC-7901/DDP cells were treated with DDP at the indicated doses for 48 h, followed by MTT analysis. Right, SGC-7901/DDP cells were treated with AL for 12 h, and then administered with the described concentrations of 5-Fu for 36 h, followed by MTT analysis. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.01$ vs Con group without any treatments; $^{+}P < 0.05$, and $^{++}P < 0.01$ vs each corresponding DDP-treated group. SGC-7901/5-Fu and SGC-7901/DDP were pre-treated with 10 μM AL for 12 h, followed by 5-Fu (10 μM) or DDP (10 μM) treatment for another 36 h. (D and E) Then, the morphology of cells was captured. (F and G) Colony formation analysis of gastric cancer cells treated as indicated. Data were shown as mean ± S.E.M. And n = 6 in each group. $^{+++}P < 0.001$ vs 5-Fu or DDP alone group.

[9–11]. As reported, AL showed significant ability to attenuate type 2 diabetes process [12]. The biological activities of quercetin and aglycone of avicularin, have been also well explored [13]. However, the biological properties of AL were not fully understood. Here, in our study, we attempted to investigate the role of AL in regulating gastric cancer development.

In this study, we explored the effects of AL, combined with 5-Fu or DDP treatment on different regulatory parameters, and the characterization of molecular mechanisms in drug-resistant gastric cancer cell line in vitro and in vivo. Avicularin sensitized drug-resistant SGC-7901 cells to 5-Fu- and DDP-induced cytotoxicity and apoptosis. Co-treatment of AL and DDP significantly increased Caspase-3 and PARP expressions. Meanwhile, pro-apoptotic signals, including Bax and BOK, were sharply expressed in AL and DDP co-treated cells. Of note, knockdown of Bax or BOK proteins eliminated the effects of AL and DDP combination-induced cell death. In vivo, AL and DDP co-treatment reduced the tumor growth without toxicity. The combination regimen held promise as a potential therapeutic strategy for patients with gastric cancer that is refractory to standard chemotherapy.

2. Materials and methods

2.1. Cells and culture

The cell lines SGC-7901, and its DDP-resistant cell lines, SGC-7901/DDP, were purchased from Bioleaf Biotech (Shanghai, China). The 5-Fu-resistant cell line, SGC-7901/5-Fu, was obtained from Cellbio (Shanghai, China). The gastric cancer cell lines were cultured in RPMI 1640 medium containing penicillin (100 U/mL), streptomycin (100 μg/mL) and 10% (v/v) fetal bovine serum (Gibco Life Technologies, USA) in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were treated with different concentrations of AL, 5-Fu or DDP [14–17]. And the cells were also transfected with Bax si-RNA, BOK siRNA or NC si-RNA (Genescript Co., Ltd, China) using lipofectermin 3000 (Life Technology, USA). The cells were allowed to grow for 24 h for the following experiments. 5-Fu and DDP were purchased from Sigma (USA).