Original Article

Hydroxyflavanone inhibits gastric carcinoma MGC-803 cell proliferation

Haiyan Zhang, Zhuo Zhan, Mingfu Cui, Yongjian Gao, Dayu Wang, Ye Feng

Department of Gastrointestinal Surgery, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin Province, China

Received June 7, 2015; Accepted September 6, 2015; Epub September 15, 2015; Published September 30, 2015

Abstract: Gastric carcinoma (GC) is the most common primary malignancy of the digestive tract, with increasing incidence in many countries. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess inhibition of HepG2 cell proliferation by 2'-hydroxyflavanone. The STAT3 pathway was performed. 2'-hydroxyflavanone reduced inhibitory effects on MGC-803 cell proliferation. 2'-hydroxyflavanone exhibited the highest inhibition rate. Treatment of MGC-803 cells with 400, 200, and 100 µg/ml 2'-hydroxyflavanone resulted in 88.9±0.7%, 81.2±0.5%, 68.4±0.5% decrease in cell viability, respectively, indicating an IC

50 of 9.3 µg/ml. The 100 µg/ml 2'-hydroxyflavanone can significantly inhibit the STAT3 pathway activation. 2'-hydroxyflavanone inhibits MGC-803 cell proliferation by inhibiting STAT3 pathway activation. This extract is therefore a potential drug candidate for treatment of liver cancer.

Keywords: 2'-hydroxyflavanone, gastric carcinoma, STAT3

Introduction

Although the incidence of gastric cancer (GC) was still decreasing in the worldwide, the GC-related deaths were 3% to 10% [1-4]. Unfortunately, GC incidence is increasing in many countries with the estimated number of new cases annually over 500,000, and the yearly incidence comprised between 2.5 and 7% of patients with liver cirrhosis. Most radical treatment option for GC, surgical resection, embolization, ablation, and chemotherapy are important as well, but limited to a significant extent by toxicity, significant resistance to available chemotherapeutic agents, side effects and complexities [5-7].

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor, it can be activated by various cytokines and growth factors, and modulating cell proliferation, angiogenesis and migration [8, 9]. Up to now, it is clear that STAT3 has been found to be overexpressed in many human cancers including solid tumor such as liver cancer, lung cancer, breast cancer and prostate cancer [10]. Moreover, the apoptosis pathway can be activated after STAT3 activity is inhibited [11].

The previous studies revealed that 2'-hydroxyflavanone has anticancer role in kinds of tumor, including colon cancer and renal cell carcinoma cells [12, 13]. However, whether it has an anti-tumor role in other tumor such as gastric carcinoma is unclear. In here, we found 2'-hydroxyflavanone inhibited MGC-803 cell proliferation, suggesting that 2'-hydroxyflavanone is a potential drug candidate for treatment of gastric cancer.

Materials and methods

Cell culture

The human gastric cancer cell line MGC-803 was provided by Jilin University. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) purchased from Boster (Wuhan, China), and supplemented with 10% fetal bovine serum (Thermo Fisher Scientific Inc., USA) and 100 U/ml penicillin/streptomycin. Cells were incubated in a humid environment containing 5% CO

Hydroxyflavanone inhibits cancer cell

Table 1. Effect of 2'-Hydroxyflavanone on MGC-803 cell proliferation

<table>
<thead>
<tr>
<th>2'-Hydroxyflavanone (g/ml)</th>
<th>Cell death rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>88.9±0.7</td>
</tr>
<tr>
<td>200</td>
<td>81.2±0.5</td>
</tr>
<tr>
<td>100</td>
<td>68.4±0.5</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Data were expressed in % as means ± standard deviation (SD) of three independent experiments. *P<0.05 vs. 100 g/ml. **P>0.05 vs control 250 μg/mL group.

Figure 1. 2'-hydroxyflavanone inhibited the STAT3 activation. MGC-803 cells were treated with 100, 250, 500 μg/mL 2'-hydroxyflavanone for 24 h. 0.05% PBS was used as negative control. *P<0.05 vs control, **P>0.05 vs control 250 μg/mL group.

Western blot

After treatment with different concentration of 2'-hydroxyflavanone, cells were lysed in the RIPA (P0013B, Beyotime, Suzhou, Jiangsu, China) with a protease inhibitor (No. 1187-3580001, Roche, Penzberg, Germany). The BCA method (P0010, Beyotime, Suzhou, Jiangsu, China) was used to determine protein concentration. A total of 40 μg of protein was boiled for 10 min before loading, then was separated using 10% SDS-PAGE. Then the proteins were transferred onto nitrocellulose membranes (0.45 μm, Whatman, Clifton, NJ, USA) by the semi-dry transfer system (Bio-Rad, USA). After blocking with 5% BSA, the membrane was probed with STAT3, p-STAT3, phospho-Src, Src, phosphor-JAK2 (Tyr1007/1008), anti-JAK2 antibodies, Mcl-1, Bcl-2 or CCND2 (Cell Signaling Technology) (1:300), β-actin antibody (ImmunoCreate, USA) (1:500). Secondary antibodies against rabbit (No. 31460, Thermo Fisher, USA) (1:40,000) IgG were used. Protein band signals were amplified by ECL detection reagents (No. 34079, Thermo Fisher, USA). Protein levels were determined semi-quantitatively using the image software (Quantity one, version 4.4.0).

Statistical analysis

All statistical analyses were conducted using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± standard deviation (SD) of three independent experiments. Statistical significance was evaluated by one-way analysis of variance (ANOVA) with Student-Newman-Keuls (SNK) test used for post hoc analysis. A P-value of less than 0.05 was considered statistically significant.

Results

Effect of 2'-hydroxyflavanone on MGC-803 cell proliferation

The different concentration of 2'-hydroxyflavanone on MGC-803 cells proliferation were performed (Table 1). We found cell death rate that cancer cell death rate after treatment with different concentration of 2'-hydroxyflavanone included 400, 200 and 100 g/ml was 8.9±...
Hydroxyflavanone inhibits cancer cell

0.7%, 81.2±0.5% and 68.4±0.5% respectively. Meanwhile, our data shown the IC_{50} of 2'-hydroxyflavanone for MGC-803 cells was 9.3 g/ml. However, there were no difference between 400 and 200 g/ml on the cell death rate (P>0.05).

2'-hydroxyflavanone inhibited the STAT3 activation

As shown in Figure 1, we found that 2'-hydroxyflavanone could inhibit the STAT3 activation. With the increasing 2'-hydroxyflavanone concentration, the p-STAT3 concentration was inhibited significantly (P<0.05).

Moreover, in here, we have explored that role of 2'-hydroxyflavanone in upstream signals included JAK2 and c-Src and downstream signals included MCL-1, Bcl-2 and CCND2 of STAT3. As shown in Figure 2A and 2B, 2'-hydroxyflavanone inhibited significantly the upstream and downstream signals of STAT3 in here (P<0.05).

Discussion

Gastric cancer, the most common primary malignancy [1, 2], continue to be a challenge to public health. Current treatments include liver transplantation, surgical resection, embolization, ablation, and chemotherapy, which are limited by toxicity, resistance, side effects and complexity of procedures. Therefore, there is a growing research studies aiming to find more effective antitumor drugs.

The strategy of targeting tumor therapy has been happening fundamental shift [15]. In tumor occurrence and progress, transcription factor plays an important role, which provides an effective targeted tumor therapy, such as STAT3. STAT3 is involved in a lot of cancer cells progress included cell proliferation, growth, angiogenesis, and cancer cells invasion [16]. In lots of tumor included liver cancer, constitutively activated IL-6/STAT3 signaling has been detected and is known as play an important factor for cancer initiation and progression [17, 18]. Inhibition of STAT3 signal way may have potential therapeutic role in treatment of liver cancer by modulating Bcl-2, cyclin D2 expression [19-21]. In the recent years, Bcl-2 family proteins are thought to maintain cell survival likely by dragging caspases into mitochondrial membrane, or alternatively, Bcl-2 would regulate the release of some caspases activators from mitochondria. In addition, mitochondria contribute to apoptosis signaling via the production of reactive oxygen species [22]. With increase in 2'-hydroxyflavanone concentration, decreased red fluorescence accompanied by green fluorescence intensity increase were observed, indicating depolarization of mitochondrial transmembrane potential by 2'-hydroxyflavanone, a possible mechanism for the
2'-hydroxyflavanone induced inhibition of STAT3 activation.

In conclusion, 2'-hydroxyflavanone is a new STAT3 inhibitor, and there is no relative research shown the role in gastric cancer therapy. Here, our research showed that 2'-hydroxyflavanone can inhibit the gastric cancer cells proliferation by inhibiting the STAT3 activation, which provided a new therapy method for human liver cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Ye Feng, Department of Gastrointestinal Surgery, China-Japan Union Hospital of Jilin University, No. 126 at Xian Tai Street, Changchun, Jilin Province, China. Tel: +86-431-84995138; Fax: +86-431-84995138; E-mail: ye_fenggs@163.com

References

Hydroxyflavanone inhibits cancer cell
