Int J Clin Exp Med 2019;12(7):8599-8603
www.ijcem.com /ISSN:1940-5901/IJCEM0093818

Original Article

The role and mechanism of dandelionol in protecting gastric epithelial cells by regulating AMPK

Huayun Pu, Huichun Duan, Xin Zhou, Xiaofeng Yang, Zhujun Shi

Department of Gastroenterology, Gansu Gem Flower Hospital, Lanzhou 730000, Gansu, China

Received March 14, 2019; Accepted June 6, 2019; Epub July 15, 2019; Published July 30, 2019

Abstract: Digestive system disease caused by gastric mucosal lesion is gradually increased following social development and irregular sleeping. AMPK activation plays a critical role in many diseases. This study selected gastric mucosa GES-1 cells to establish gastric mucosal injury model. 5-aminooimidazole-4-formamide ribonucleotide (AICAR) was applied to activate AMPK, while different concentrations (0.5, 1.0, and 2.0 mg/ml) of taraxerol were adopted to treat the cells to investigate the protective role of taraxerol on GES-1 cells at the perspective of AMPK. AICAR was used to pretreat GES-1 cells followed by addition of different concentrations of taraxerol to the cells later. Cell proliferation and cell cycle were detected by MTT assay and flow cytometry, respectively. AICAR activated AMPK level and reached peak at 6 h ($P < 0.05$). Cell proliferation declined after AICAR treatment compared with blank group but was still higher than the control. Cell proliferation was significantly enhanced following elevation of taraxerol concentration and time extension ($P < 0.05$). Cell ratio in G0/G1 phase was declined, while in S and G2/M phase increased following elevation of taraxerol concentration ($P < 0.05$). AICAR can activate AMPK in gastric mucosa GES-1 cells, while taraxerol can protect gastric mucosal cell injury by enhancing GES-1 cell proliferation.

Keywords: AMPK, GES-1 cell, proliferation, cell cycle, taraxerol

Introduction

Gastric mucosa injury appears when the body in a state of stress. Gastric mucosa hyperemia, edema, erosion, and necrosis could be observed under microscope, thus forming the digestive tract ulcer [1]. It is thought that digestive tract mucosa barrier function is reduced under stimulus and stress condition, leading to defense capability declination [2]. Dandelion is a commonly used traditional Chinese medicine with multiple pharmacological effects, including anti-inflammation, anti-tumor, and antioxidation [3, 4]. The ratio of AMP/ATP rapidly elevates when the body was lack of energy, lack of oxygen, or even in ischemia environment, thus activating AMPK in a short period of time, regulating metabolism and cell cycle, and controlling gene transcription process [5-7]. This research established ethanol induced gastric mucosa GES-1 cell injury model, applied taraxerol for intervention and adopted AICAR to activate AMPK, to explore the protective role of taraxerol on gastric mucosa epithelial cell injury based on the AMPK level.

Materials and methods

Experimental cells

Gastric mucosa GES-1 cell line was provided by cell cultivation center of Wuhan University.

Reagents and instruments

Taraxerol was from ProSpec (USA). AICAR was from TRC. Centrifuge machine was provided by Beckman.

Conventional cell culture

Human gastric mucosa GES-1 cells were cultured in RPMI1640 medium and maintained at 37°C and 5% CO_2.

GES-1 cell injury model establishment

GES-1 cells were maintained in medium containing 4-8% ethanol for 2-5 h to keep the inhibitory rate at 40%.

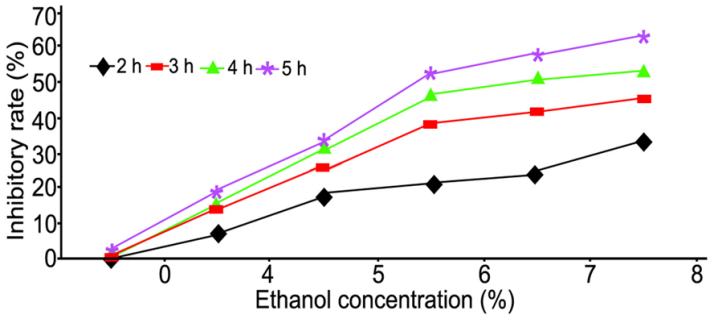

Figure 1. Establishment of ethanol induced GES-1 cell injury model.

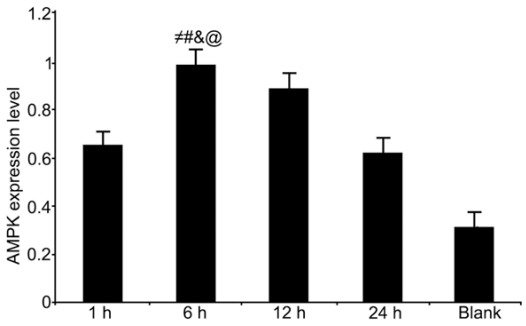

Figure 2. AMPK level in GES-1 cells after AICAR treatment. *P < 0.05, compared with blank group. #P < 0.05, compared with 1 h group. &P < 0.05, compared with 12 h group. @P < 0.05, compared with 24 h.

AICAR treatment on GES-1 cells

GES-1 cells in logarithmic phase were cultured for 24 h after ethanol intervention and treated by 0.5 mmol/L AICAR for 24 h. Conventional cultured GES-1 cells were selected as blank control.

Taraxerol treatment on GES-1 cells

After treated with AICAR for 24 h, GES-1 cells were added with different concentrations of taraxerol at 0.5 mg/ml, 1.0 mg/ml, and 2.0 mg/ml. GES-1 cells in control group received no taraxerol treatment. GES-1 cells in blank group received no AICAR intervention.

Western blot

Total protein was isolated from cells using RIPA lysis buffer. After quantification by BCA ASSAY, A total of 40 μg protein was separated on 10% SDS-PAGE, transferred to PVDF membrane, blocked with 5% silk milk and incubated with primary antibody (1:200, β-actin 1:500) at 4°C overnight. After PBST washing three times, the membrane was incubated with HRP-conjugated secondary antibody (1:2000) for 1 h at room temperature. At last, the membrane was developed for analysis after addition of ECL reagent.

MTT assay

GES-1 cells in logarithmic phase were collected to test cell viability at 24 h, 48 h, and 72 h. MTT solution at 5 mg/ml was added to the well for 4 h treatment followed by addition of 150 μl DMSO to stop the reaction. The OD value at a wavelength of 570 nm was measured by a microplate reader.

Flow cytometry

GES-1 cells in logarithmic phase were collected and cell cycle was measured according to the kit instructions. In brief, cells were fixed with 70% ethanol overnight and washed 2-3 times with PBS followed by addition of 100 μl RNase A, and incubation for 30 min at 37°C. Finally, 400 μL PI was added and incubated on ice for 30 min followed by analysis of cell cycle by flow cytometry.

Data analysis

SPSS 17.0 software was adopted for data analysis. The data was presented as mean ± standard deviation. Enumeration data was analyzed by chi-square test, while measurement data was compared by t test. P < 0.05 was depicted as statistical significance.

Results

Establishment of ethanol induced GES-1 cell injury model

Ethanol can damage GES-1 cells at different concentrations and time. It was found that 7% ethanol intervention for 3 h maintained the cell inhibitory rate at 40%, suggesting it was the best model (**Figure 1**).

AICAR induces AMPK activation in GES-1 cell

0.5 mmol/L AICAR was applied to treat GES-1 cell model established by ethanol. It was revealed that AMPK was activated after 1 h treatment and reached peak at 6 h (P < 0.05) (**Figures 2**, **3**).

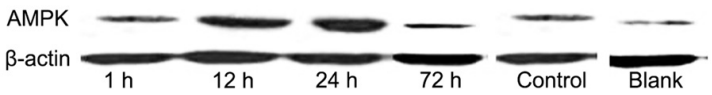

Figure 3. The effect of AICAR on GES-1 cells.

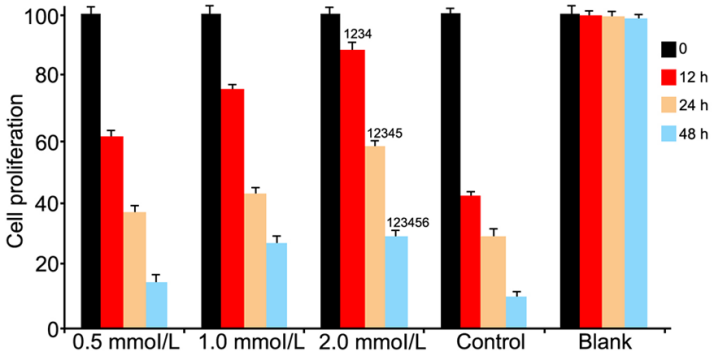

Figure 4. The impact of taraxerol on GES-1 proliferation after AICAR intervention. 1, $P < 0.05$, compared with control group. 2, $P < 0.05$, compared with blank group. 3, $P < 0.05$, compared with 0.5 mg/ml group. 4, $P < 0.05$, compared with 1.0 mg/ml group. 5, $P < 0.05$, compared with 12 h group. 6, $P < 0.05$, compared with 24 h group.

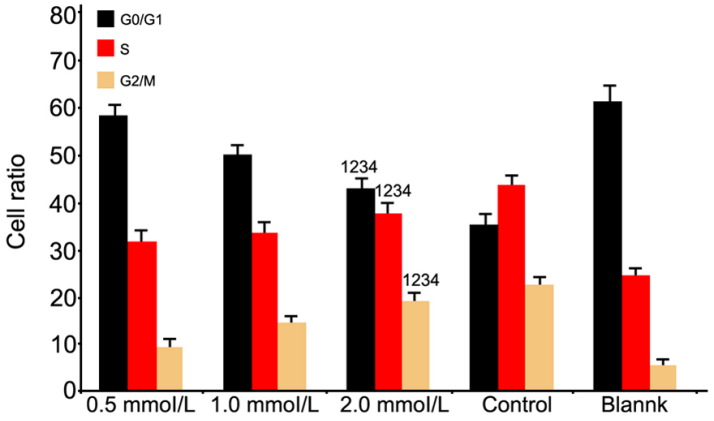

Figure 5. The effect of taraxerol on GES-1 cell cycle after AICAR intervention. 1, $P < 0.05$, compared with control group. 2, $P < 0.05$, compared with blank group. 3, $P < 0.05$, compared with 0.5 mg/ml group. 4, $P < 0.05$, compared with 1.0 mg/ml group. 5, $P < 0.05$, compared with 12 h group. 6, $P < 0.05$, compared with 24 h group.

Taraxerol increases GES-1 proliferation after AICAR intervention

Different concentrations of taraxerol were applied to treat GES-1 cells after AICAR intervention. It was found that GES-1 cell proliferation in 0.5 mg/ml, 1.0 mg/ml, and 2.0 mg/ml was obviously lower than the blank group ($P < 0.05$). Cell proliferation was increased in the same time point following elevation of taraxerol concentration ($P < 0.05$). Under the same taraxerol concentration, cell proliferation was elevated following time extension ($P < 0.05$) (**Figure 4**).

Taraxerol induces S and G2/M arrest in GES-1 cell after AICAR intervention

Cell ratio in G0/G1 phase was markedly declined, while in S and G2/M phase obviously increased in taraxerol group compared with blank group following elevation of taraxerol concentration ($P < 0.05$) (**Figure 5**).

Discussion

Overdose of ethanol increases the risk of upper gastrointestinal ulcer and threatens people's health [8]. AMPK is a kind of silk/threonine protein kinase presented as a heterologous trimer, containing α, β, and γ subunits [9]. AICAR is a type of AMPK activator by activating adenosine kinase phosphorylation [10]. It was found that deletion of AMPK expression increased cell energy metabolism, suggesting that AMPK can inhibit tumor cell growth [11]. As the rapid development of molecular diagnostic technology, the researchers investigated the related mechanism from the molecular and gene levels. More cytokines, genes, and related signaling pathway were gradually discovered, and some was applied for disease diagnosis and treatment in clinic. Traditional Chinese medicine also received exploration in recent years to expand the usage [12]. In this study, we adopted 0.5 mmol/l AICAR to treat gastric mucosa GES-1 cells and found that AMPK was activated at 1 h, reached peak at 6 h, and declined at 24 h. It indicated that AICAR can activate AMPK expression in GES-1 cells.

A variety of physical and chemical factors can lead to tissue cells acute or chronic injury, which presents cell membrane changes at first. Cell membrane damage lets LDH leaking out of the cell, resulting in increased LDH activity in

medium which was positively correlated with the degree of cell injury. SOD is type of antioxidant distributed in the tissue that can defense peroxidation damage. Tissue SOD level can reflect the balance of internal oxidation/antioxidation [13]. It was found that as an important extracted component, taraxerol presented extensive pharmacology role. Gastropathy investigation showed that taraxerol had good effect on various types of stomachache by anti-inflammation, relieving pain, and clearing heat with milder toxicity reaction [14]. AMPK is also an important molecule involved in the regulation of autophagy, cell survival, and apoptosis. AMPK activation can phosphorylate important factors in cell survival, apoptosis, and autophagy, such as P53 and mTOR. This research adopted different concentrations of taraxerol to interfere with GES-1 cells after AICAR treatment. After 12 h, 24 h, and 48 h, GES-1 cell proliferation in experimental group was lower than that in blank group but higher than the control. GES-1 cell proliferation was enhanced following concentration elevation and time extension. It indicated that activation of AMPK by AICAR can inhibit GES-1 cell proliferation, whereas taraxerol can enhance GES-1 cell proliferation after treated with AICAR.

To further clarify the impact of taraxerol on cell cycle, this study treated GES-1 cells intervened by AICAR with different concentrations of taraxerol for 48 h. Cell ratio in G0/G1 phase was markedly declined, while in S and G2/M phase obviously increased in taraxerol group compared with blank group following elevation of taraxerol concentration. It suggested that activation of AMPK by AICAR blocked cells in G0/G1 phase, while taraxerol facilitated cells entering S phase from G1 phase, thus accelerating mitosis. AMPK plays a strong inhibitory effect on endoplasmic reticulum stress mediated cell apoptosis in endothelial cells and myocardial cells. It was found that taraxerol can lead to leukemia cell apoptosis through external or receptor mediated cell apoptosis. Sung-Hyen Lee et al. discovered that taraxerol can affect a variety of innate immune parameters and tumor cell growth by antioxidation. Moreover, taraxerol demonstrated significant inhibitory effect on hepatoma carcinoma cells and colorectal cancer Lovo cells in vitro and in vivo. Taraxerol presented a significant enhancement effect on rabbit gastric smooth muscle contraction force in vitro, thus protecting mucosa integrity and avoid damage [15]. Gastrointestinal motility assay revealed that taraxerol showed stronger promoting effect than cisapride [16, 17]. Furthermore, it was found that taraxerol had different levels of protection on gastric ulcer and gastric mucosa lesion. It also played a certain inhibition on gastric hyperchlorhydria induced by histamine and gastrin [18-20].

Conclusion

Taraxerol can protect gastric mucosal GES-1 cell injury by enhancing GES-1 cell proliferation and cell cycle intervened by AICAR.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Huayun Pu, Department of Gastroenterology, Gansu Gem Flower Hospital, No. 733, Welfare Road, Xigu District, Lanzhou 730000, Gansu, China. Tel: +86-0931-7555317; Fax: +86-0931-7555317; E-mail: yuanmutu94@163.com

References

[1] SX Y. Moxibustion pretreatment on stress ulcer rat gastric mucosa proliferation effect of repair. The world combine traditional Chinese and western medicine 2007; 2: 21-24.

[2] JB H. Plasma of individuals undergoing moxibustion has protective effects against alcohol-induced injury to human gastric epithelial cells. The world Chinese people digest magazine 2010; 31: 3287-3293.

[3] Curlik J, Kolesar M, Durza O and Hiller E. Dandelion (Taraxacum officinale) and agrimony (Agrimonia eupatoria) as indicators of geogenic contamination of flysch soils in Eastern Slovakia. Arch Environ Contam Toxicol 2016; 70: 475-486.

[4] Martinez M, Poirrier P, Chamy R, Prufer D, Schulze-Gronover C, Jorquera L and Ruiz G. Taraxacum officinale and related species-An ethnopharmacological review and its potential as a commercial medicinal plant. J Ethnopharmacol 2015; 169: 244-262.

[5] Ramanjaneya M, Conner AC, Brown JE, Chen J, Digby JE, Barber TM, Lehnert H, Randeva HS. Adiponectin (15-36) stimulates steroidogenic acute regulatory (StAR) protein expression and cortisol production in human adrenocortical cells: role of AMPK and MAPK kinase path-

ways. Biochim Biophys Acta 2011; 1813: 802-809.
[6] Jansen M, Ten Klooster JP, Offerhaus GJ, Clevers H. LKB1 and AMPK family signaling: the intimate link between cell polarity and energy metabolism. Physiol Rev 2009; 89: 777-798.
[7] Liu LG, Yan H, Yao P, Zhang W, Zou LJ, Song FF, Li K and Sun XF. CYP2E1-dependent hepatotoxicity and oxidative damage after ethanol administration in human primary hepatocytes. World J Gastroenterol 2005; 11: 4530-4535.
[8] Albano E. Free radical mechanisms in immune reactions associated with alcoholic liver disease. Free Radic Biol Med 2002; 32: 110-114.
[9] Fogarty S and Hardie DG. Development of protein kinase activators: AMPK as a target in metabolic disorders and cancer. Biochim Biophys Acta 2010; 1804: 581-591.
[10] Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICAR boside increases AMP-activated protein kinase, fatty acid oxidation, and glucoseuptake in rat muscle. Am J Physiol 1997; 273: E1107-1112.
[11] Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, Dupuy F, Chambers C, Fuerth BJ, Viollet B, Mamer OA, Avizonis D, DeBerardinis RJ, Siegel PM, Jones RG. AMPK is a negative regulator of the warburg effect and suppresses tumor growth in vivo. Cell Metab 2013; 17: 113-124.
[12] Villiger A, Sala F, Suter A and Butterweck V. In vitro inhibitory potential of cynara scolymus, silybum marianum, taraxacum officinale, and peumus boldus on key enzymes relevant to metabolic syndrome. Phytomedicine 2015; 22: 138-144.
[13] Lai GH, Chao J, Lin MK, Chang WT, Peng WH, Sun FC, Lee MS, Lee MS. Rapid and sensitive identification of the herbal tea ingredient taraxacum formosanum using loop-mediated isothermal amplification. Int J Mol Sci 2015; 16: 1562-1575.
[14] Qureshi MN, Stecher G and Bonn GK. Quality control of herbs: determination of amino acids in althaea officinalis, matricaria chamomilla and taraxacum officinale. Pak J Pharm Sci 2014; 27: 459-462.
[15] Loh CH, Inbaraj BS, Liu MH and Chen BH. Determination of chlorophylls in taraxacum formosanum by high-performance liquid chromatography-diode array detection-mass spectrometry and preparation by column chromatography. J Agric Food Chem 2012; 60: 6108-6115.
[16] Ovadje P, Chatterjee S, Griffin C, Tran C, Hamm C and Pandey S. Selective induction of apoptosis through activation of caspase-8 in human leukemia cells (Jurkat) by dandelion root extract. J Ethnopharmacol 2011; 133: 86-91.
[17] Sigstedt SC, Hooten CJ, Callewaert MC, Jenkins AR, Romero AE, Pullin MJ, Kornienko A, Lowrey TK, Slambrouck SV, Steelant WF. Evaluation of aqueous extracts of taraxacum officinale on growth and invasion of breast and prostate cancer cells. Int J Oncol 2008; 32: 1085-1090.
[18] Chatterjee SJ, Ovadje P, Mousa M, Hamm C and Pandey S. The efficacy of dandelion root extract in inducing apoptosis in drug-resistant human melanoma cells. Evid Based Complement Alternat Med 2011; 2011: 129045.
[19] Jia YY, Guan RF, Wu YH, Yu XP, Lin WY, Zhang YY, Liu T, Zhao J, Shi SY and Zhao Y. Taraxacum mongolicum extract exhibits a protective effect on hepatocytes and an antiviral effect against hepatitis b virus in animal and human cells. Mol Med Rep 2014; 9: 1381-1387.
[20] Tahtamouni LH, Alqurna NM, Al-Hudhud MY, Al-Hajj HA. Dandelion (Taraxacum officinale) decreases male rat fertility in vivo. J Ethnopharmacol 2011; 135: 102-109.