Chrysophanol Inhibits the Progression of Gastric Cancer by Activating NLRP3

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Abstract

Aim

Gastric cancer is one of the most common malignant tumors. Chrysophanol has been reported to have antitumor effects on a variety of cancers, but the role of chrysophanol in gastric cancer remains unclear. The aim of this study was to investigate the effects of chrysophanol on proliferation, pyroptosis, migration and invasion of gastric cancer cells.

Methods

MKN 28 and AGS cells were treated with different concentrations of chrysophanol, then cell proliferation, migration, invasion and pyroptosis were detected by CCK-8, Colony-forming assay, Wound Healing assay, Transwell and flow cytometry, respectively. Subsequently, NLRP3 siRNA was transfected into MKN 28 cells, cell proliferation, pyroptosis, migration and invasion were reassessed in these transfected cells. The expression of caspase-1 and IL-1 β in the downstream of NLRP3 was detected by qRT-PCR and Western blot.

Results

Chrysophanol significantly inhibited the proliferation of GC cells, promoted pyroptosis, inhibited cell migration and invasion, and up-regulated the expression level of NLRP3 inflammasome in GC cells. Silencing NLRP3 inhibited the effects of chrysophanol on proliferation, pyroptosis, migration and invasion of MKN 28 cells. Chrysophanol plays an anti-cancer role through high expression of NLRP3.

Conclusions

Chrysophanol can inhibit the proliferation, migration and invasion of gastric cancer cells by regulating NLRP3, promote the death of gastric cancer cells, and play an anti-tumor role, which is a clinical strategy with great potential for the treatment of gastric cancer.

1. Introduction

Gastric cancer (GC) is one of the most common malignant tumors in the digestive system, ranking the fifth in the global malignant tumor incidence and fourth in the case fatality rate^[1]. At present, the treatment of GC patients is mainly surgical operation, supplemented by radiotherapy and chemotherapy^[2]. Although various methods have been adopted to treat tumors, But the median survival time of patients with advanced gastric cancer is rarely more than 12 months, in the case of metastasis, 5-year survival is less than 10%^[3]. Although many anticancer drugs such as trastuzumab^[4], paclitaxel^[5], oxaliplatin^[6] have been used in the treatment of clinically common gastric cancer patients, their drug resistance and side effects urgently require us to further study the molecular biological mechanism of GC occurrence and development, so as to find targets for GC treatment.

In recent years, many drugs have been isolated from natural plants. As a common alternative and complementary treatment method, Chinese medicine has been established for a variety of cancer treatment methods, indicating that Chinese medicine may be a promising alternative treatment method in the future. Chrysophanol are the effective component of rheum officinale and widely exists in the nature of anthraquinone compounds, has a variety of rich biological activities, such as antibiosis, anti-inflammatory^[6], anti-tumor^[9]. Our previous research showed that chrysophanol inhibits the activity of colorectal cancer cells by targeting the Decorin protein^[10]. These results provide a reference for the study of anti-tumor activity of chrysophanol. However, the effect of chrysophanol on gastric cancer cells and its exact molecular mechanism are still unclear. Therefore, the purpose of this study is to determine the potential anti-gastric cancer effects of chrysophanol and its specific molecular targets.

Pyroptosis is a form of inflammatory programmed cell death^[11]. Different from other types of cell death, pyroptosis with characteristic morphological changes, including swollen cells, large bubbles, pore formation in the plasma membrane and subcellular organelles changes^[12]. NLRP3 inflammasome is a cytoplasmic protein complex composed of NLRP3, adaptor apoptosis-related speckle-like protein (ASC) and pro-caspase-1^[13]. Activation of NLRP3 inflammasomes leads to the maturation and secretion of IL-1 β and IL-18, which in turn leads to cell damage and pyrolysis^[14]. NLRP3 inflammasome plays a crucial role in the progression of various cancers such as lung cancer^[15], colorectal cancer^[16], breast cancer^[17]. Previous studies have shown that, NLRP3 was highly expressed in gastric cancer^[18]. In addition, antitumor agents such as resveratrol^[19], Parthenolide^[20] have been shown to induce cell death through NLRP3 inflammasomes^[21]. Therefore, regulating NLRP3 is a potential therapeutic strategy. In this study, we observed the effect of chrysophanol on gastric cancer cell pyroptosis, and further explored the regulation and mechanism of chrysophanol on NLRP3 in this process, in order to provide theoretical basis for its potential value in the treatment of gastric cancer.

2. Materials And Methods

2.1 Experimental materials

Chrysophanol was purchased from MCE(Monmouth Junction, NJ, USA). RPMI 1640 medium and fetal bovine serum were purchased from Gibco (CA,USA); FAM-FLICA Caspase-1 kit was purchased from ImmunoChemistry Technologies (Bloomington, MN, US); LDH kit was purchased from Jiancheng Biotechnology (Nanjing, China); NLRP3, Caspase-1, GSDMD, IL-1 β and IL-18 were purchased from ProteinTech (Wuhan, China). NLRP3 siRNA was purchased from Gemma Pharmaceutical Technology. (Shanghai, China); ELISA kit was purchased from Wuhan Huamei(Wuhan, China); RIPA cell lysate, PMSF,BCA protein quantitative kit, Trizol, Cell counting kit-8 (CCK-8)were purchased from Beyotime Biotechnology (Shanghai, China); Lipofectamine2000 were purchased from Invitrogen (Carlsbad, CA, USA); Retro-transcription Kit were purchased from TransGen Biotech (Beijing,China).

2.2. Patients and tissue samples

This study was approved by the Institutional Review Board of The First Affiliated Hospital of Bengbu Medical College. All the participants provided written informed consents. Tumorous tissue samples and adjacent tissue samples were collected from patients who underwent surgical resection. All the clinical tissue samples were immediately stored at -80°C for further use.

2.3. Cell lines and culture conditions

The human gastric lines MKN 28 and AGS were purchased from Chinese Academy of Medical Sciences (CAS, Beijing, China). Cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin in 5% CO_2 at 37°C . Culture medium was renewed every 2 days, and cells were passaged once they reached 70–80% confluence. Cells were seeded at an appropriate density according to each experimental design.

2.4. Cell viability assay

The cells were inoculated uniformly in 96-well plates with 5×10^3 cells per well. The cells were incubated overnight with chrysophanol at 0 μM , 2 μM , 5 μM and 10 μM for 24 and 48 h, respectively. 10 μl was added to each well for 2 h and the cells were incubated using a microplate (Bio-Tek USA) to determine the absorbance at 450 nm. Three parallel holes were set in each treatment group, Another negative control and blank zero hole, and the survival rate $\% = [\text{OD}(450) \text{ treatment group} - \text{OD}(450) \text{ empty self group}] / [\text{OD}(450) \text{ control group} - \text{OD}(450) \text{ empty self group}] \times 100\%$. With time as the abscissa and cell survival rate as the ordinate, the cell growth curve was drawn. All experiments were conducted independently and repeated three times.

2.5. Colony formation assay

Cells were inoculated evenly in a 6-well plate with 500 cells per well. The cells were cultured in different concentrations of chrysophanol (0 μM , 5 μM and 10 μM) for 2 weeks, during which the culture medium containing chrysophanol was changed once every 3 days for 2 weeks. After that, the medium was discarded, the cells were washed by PBS once, and fixed by 4% paraformaldehyde at room temperature for 20 weeks Min, and then stained with 0.1% crystal violet at room temperature for 30 min. After washing the cells for 3 times with PBS, the cells were observed and photographed under an inverted microscope. Image-pro Plus counted the number of clones and The number of cells > 15 was considered as a clone.

2.6. Flow cytometry analysis cell pyroptosis

Cells were uniformly inoculated in a 6-well plate with 5×10^5 cells/well. The cells were treated with different concentrations of chrysophanol (0 μM , 5 μM and 10 μM) for 24 h and then collected with reference to FAM-FLICA Caspase-1 assay kit describes double positive staining for activated caspase-1 and propidium iodide (PI) in cells to assess cell pyroptosis. All assays were performed independently and repeated three times.

2.7. LDH assay

Cells were uniformly cultured at 1×10^5 cells/well in a 24-well culture plate. The cells were treated with different concentrations of chrysophanol (0 μM , 5 μM and 10 μM) for 24 h. The absorbance value at 490nm was measured by a microplate and the release rate of LDH was calculated. LDH release rate % = (measured OD value - control OD value) / (standard OD value - blank OD value) $\times 100\%$.

2.8. Wound healing assay detects migration

Cells were evenly inoculated in 6-well plates with 5×10^5 cells/well, and cultured to 70%-80% of the fusion cells were treated with different concentrations of chrysophanol (0 μM , 5 μM and 10 μM) to scratch monolayer cells with disinfected pipet tips. The fragments were washed with PBS for 0 and 24 h to observe the migration of cells to the wound surface. Cell migration to the wound surface was observed under an inverted microscope and photographed. All experiments were performed independently and repeated three times.

2.9. Transwell invasion assay detects invasion

Cells were suspended in 100 μL RPMI 1640 containing 2% fetal bovine serum and then inoculated with Matrigel (BD Biosciences, New Jersey, USA), the bottom chamber was filled with 500 μL RPMI 1640 supplemented with 10% fetal bovine serum for 24h, the medium was discarded, and the PBS buffer was washed once. 4% paraformaldehyde was fixed at room temperature for 20 min, then the cells in the chamber were wiped with a wet cotton swab, and then stained with 0.5% crystalline violet at room temperature for 30 min. After washing the cells for 3 times with PBS, they were dried and observed under an inverted microscope and photographed. Image-J counted the number of cells in each group, and all experiments were conducted independently for 3 times.

2.10. Western blot assay

Cells were lysed for 30 min with RIPA+1%PMSF buffer. The protein concentration was determined by BCA protein assay kit. The proteins were electroblotted to a polyvinylidene fluoride membrane (Millipore, Massachusetts, USA). The membrane was incubated with a primary antibody against NLRP3, caspase-1, IL-1 β . β -actin as internal reference. All experiments were carried out independently in three times.

2.11. RNA extraction and qRT-PCR

Cells were uniformly seeded into 6-well plates at 5×10^5 cells/well with different concentrations of chrysophanol (0 μM , 5 μM and 10 μM) for 24 h. Total RNA from the culture cells was isolated using the TRIzol Reagent, and reverse transcribed into cDNA using a kit. Target gene expression was analyzed by RT-PCR using Power SYBR Green PCR Master Mix, with GAPDH as the internal control. The forward and reverse primers were synthesized by Sangon (Shanghai, China) according to previously published sequences. The relative expression of the target genes was calculated using the $2^{-\Delta\Delta C_t}$ method.

2.12. NLRP3 small interfering RNA (siRNA) transfection

NLRP3 si RNA (sense: 5 - CCUACCUUCUCUAUCAGAUTT -3;anti: 5 - AUCUGAUAGAGAAGGUA GGTT -3), NLRP3 siRNA was purchased from GenePharma (Shanghai, China).NLRP3 siRNA (100 nM) or negative control siRNA was mixed with Lipofectamine 2000. The cells were incubated with the transfection mixture for 6 h and then changed with MEM medium with 10% FBS. The cells were incubated for an additional 48 h before harvest.

2.13. Immunohistochemistry

Four percent paraformaldehyde-fixed tissues were carried out with paraffin and sliced into 4 μ m thick sections. Then 0.3% TritonX-100 penetrated tissues for 30 min and immunostained for Ki67, NLRP3 $\square$ caspase-1(Proteintech, Wuhan, China) primary antibodies at 4°C overnight and universal secondary antibodies at 37°C for 60 min. Next, tissues were visualized by using the 3-amino-9-ethylcarbazole was applied for 10 min. The tissues were washed with PBS for three times and tissues were counterstained with hematoxylin, then dehydrated and cover slipped according to the protocol.

2.14. ELISA assay

The supernatants of cell culture medium were collected and detected by human IL-1 β and IL-18 ELISA kit. Specific steps are carried out according to the kit instructions.

2.15. Statistical analysis

SPSS 25.0 software (SPSS Inc. USA) was used to student's t-test analysis on the experimental data. The results were expressed as the means $\pm$ standard deviation (SDS) of three independent experiments. P <0.05 was considered statistically significant.

3. Result

3.1. Chrysophanol inhibits gastric cancer cells proliferation

The chemical structure of chrysophanol is shown in the figure(Figure 1A).MKN 28 and AGS cells were treated with different concentrations of chrysophanol (0, 2, 5 and 10 μ M) for 24 and 48h, respectively. Cell viability was measured by CCK8 assay. Results show that gastric cancer cells viability of was prominently declined by chrysophanol at the concentrations of (0, 5 and 10 μ M) (P < 0.05 or P < 0.01), indicating that chrysophanol inhibits cell viability at a dose-dependent manner. (Figure 1B). The result of cell proliferation was detected by colony formation assay showed that the rate of proliferation of MKN 28 and AGS cells was significantly decreased by chrysophanol compared with control (P < 0.01, Fig. 1C) Colony formation experiments showed that chrysophanol treatment significantly reduced the number of human gastric cancer cell colonies. These results suggest that chrysophanol could inhibit proliferation of human gastric cancer cells at a dose-dependent manner.

3.2. Chrysophanol promotes pyroptosis in gastric cancer cells and induces G1 phase arrest.

Morphological changes of gastric cancer cells were induced by chrysophanol treatment, including decreased cell density, cell rounding and cell floating (Figure 2A). Further detected the release of LDH in the medium, Compared with the control group, the LDH release of MKN 28 and AGS was significantly increased in dose-dependent manner in the 5 and 10 μ M chrysophanol groups (Figure 2B). Furthermore, cell pyroptosis and cell cycle were measured by flow cytometry. The results was revealed in Figure 2C that chrysophanol significantly increased the activation percentage of caspase-1 cells in a dose-dependent manner. These results suggested that chrysophanol could promotes pyroptosis.

3.3. Chrysophanol suppresses MKN 28 and AGS cells migration and invasion

To investigate the effect of chrysophanol on metastasis of gastric cancer cells, wound healing assay and Transwell invasion assay were performed. Compared with the control group, the migration rate of MKN 28 and AGS cells was significantly decreased after 5 μ M and 10 μ M chrysophanol treatment ($P < 0.01$ or $P < 0.001$, Figure 3A). Similarly, The invasion ability of 5 μ M and 10 μ M chrysophanol to gastric cancer cells was also lower than that of control group ($P < 0.05$ or $P < 0.001$ Figure 3B). Taken together, chrysophanol can significantly suppressed the migration and invasion in MKN 28 and AGS cells.

3.4. Chrysophanol up-regulates the expression of NLRP3 inflammasome in gastric cancer cells

To explore the relationship between chrysophanol and NLRP3 inflammasome, the expression level of NLRP3 was measured by qRT PCR and western blot after treatment with different concentrations of chrysophanol (0, 5, 10 μ M). the results in Fig. 4A showed that the expression level of NLRP3 was significantly up-regulated after treatment with 5 μ M and 10 μ M chrysophanol in MKN 28 and AGS cells ($P < 0.01$). Meanwhile, the expression level of caspase-1 was increased by 5 μ M and 10 μ M chrysophanol in MKN 28 and AGS cells cells ($P < 0.01$, Fig. 4B). Additionally, the expression level of IL-1 β was also up-regulated by 5 μ M and 10 μ M chrysophanol in MKN 28 and AGS cells cells ($P < 0.01$, Fig. 4C). These data indicated that there was a positive regulation relationship between chrysophanol and NLRP3 in gastric cancer cells.

3.5. Chrysophanol induces pyroptosis by regulation of NLRP3

To explore the possible roles of NLRP3 in gastric cancer, we first searched the OncoMine database and found that NLRP3 was significantly up-regulated in gastric cancer ($P < 0.05$, Fig. 5A). NLRP3 expression in gastric cancer tissues was higher than adjacent tissues in TCGA datebase ($P < 0.001$, Fig. 5B). Through immunohistochemistry, we found that the expressions of NLRP3 $\square$ caspase-1 $\square$ Ki67 was low in paracarcinoma tissue but was significantly up-regulated in human gastric cancer tissue (Fig. 5C). Furthermore, Compared with GES-1, the expression of NLRP3 was significantly up-regulated in MKN 28 and AGS cells ($P < 0.01$ or $P < 0.001$, Fig. 5D-E). Moreover, NLRP3 expression was negatively correlated with survival through Kaplan-Meier survival analysis $\square$ $P < 0.05$, Fig. F. To further explore the effect of NLRP3 in GC occurrence and development, NLRP3 siRNA and NC were transfected into MKN 28 cells. The result showed that the expression level of NLRP3 was greatly down-regulated by NLRP3 siRNA knockout,

compared to control group ($P < 0.01$, or $P < 0.001$, Fig. 5G-H). These data indicated that the NLRP3 siRNA and NC had been successfully transfected into MKN 28 cells.

Subsequently, LDH assay and pyroptosis were assessed again. The result in Fig. 5I revealed that chrysophanol significantly increased LDH release and pyroptosis compared with control group ($P < 0.01$, Fig. 5I). But this results was reduced after NLRP3 silence. Silencing of NLRP3 together with chrysophanol significantly decreased LDH release in MKN 28 cells compared with chrysophanol ($10 \mu\text{M}$) group ($P < 0.01$). Meanwhile, flow cytometry assay results revealed that cell pyroptosis was markedly decreased after treatment with chrysophanol ($10 \mu\text{M}$)+NLRP3 siRNA compared with chrysophanol ($10 \mu\text{M}$) in MKN 28 cells ($P < 0.01$, Fig. 5J). These data suggested that the effects of chrysophanol on cell pyroptosis were abolished by NLRP3 siRNA in MKN28 cells.

The expression of NLRP3 in human gastric cancer tissues and adjacent tissues, (A) Data from OncoMine Database. (B) Data from TCGA, Database. (C) IHC staining for NLRP3/caspase-1/Ki 67. (D) The protein levels of NLRP3 in GES-1/MKN 28/AGS was detected by Western blot. (E) qRT-PCR analysis of NLRP3 in GES-1/MKN 28/AGS. (F) Kaplan-Meier analysis of NLRP3 expression in survival of GC patients. (G-H). MKN 28 cells were transfected with NLRP3 siRNA and NC, the expression level of NLRP3 siRNA in these transfected cells was detected by qRT-PCR and western blot. the transfected cells were treated with $10 \mu\text{M}$ chrysophanol for 24 h, then (I). LDH release was examined by LDH kit. (J). Cell pyroptosis was determined by flow cytometry. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3.6 Chrysophanol exerted antitumor effect via NLRP3 signaling pathway in gastric cancer cells

The effects of NLRP3 on cell migration and invasion were examined by Wound healing and Transwell assay again. As shown in Fig. 6A, the migration ability after treatment with chrysophanol was decreased compared with control group in MKN 28 cells ($P < 0.001$). Similarly, the invasion ability after treatment with chrysophanol was decreased compared with control group in MKN28 cells ($P < 0.001$, Fig. 6B). However, these results were reversed by NLRP3 si RNA suppression in MKN 28 cells ($P < 0.001$, Fig. 6A-B). The results indicated that NLRP3 abolished the inhibitory effects of chrysophanol on cell migration and invasion in gastric cancer cells.

To further investigate the underlying mechanism, NLRP3 downstream signal pathways were estimated in this study. As displayed in Fig. 7A and B, qRT PCR assay results showed that the mRNA level of IL-1 β was markedly increased compared with control group ($P < 0.01$, Fig. 7A). Western blot results displayed that the protein levels of supernatant IL-1 β and lysate IL-1 β and cleaved caspase-1 were significantly increased after treatment with chrysophanol compared with control group in MKN 28 cells ($P < 0.01$, Fig. 7C). The results showed that chrysophanol notably increased caspase-1 and IL-1 β expression levels in MKN 28 cells ($P < 0.01$, Fig. 7A-C). However, this results were reversed by suppression of NLRP3 ($P < 0.001$, Fig. 7A-C). Meanwhile, the results in Fig. 7D-E revealed that chrysophanol obviously increased IL-1 β and IL-18 expression levels in MKN 28 cells ($P < 0.001$). These inflammatory factors were declined by NLRP3 si RNA ($P < 0.001$). These results indicated that chrysophanol exerted antitumor effect via NLRP3 signaling pathway in gastric cancer cells.

4. Discussion

Chrysophanol is an important anthraquinone component isolated from rhubarb plants^[22]. Similar to other extracts of rhubarb, over the years, a number of scientific studies have confirmed the beneficial biological characteristics of chrysophanol, including its antiviral, antidiabetic, anti-inflammatory, hypolipidemic, hepatoprotective, neuroprotective, antiulcer and antiobesity effects^{[23] [24] [25]}. Chrysophanol attenuates airway inflammation and remodeling through nuclear factor-kappa B signaling pathway in asthma^[26]. At present, many pre-clinical researches have found that Chrysophanol inhibits the proliferation of different types of tumors by targeting various signaling pathways and is a potential drug for cancer prevention or treatment^[27]. Research findings show that Chrysophanol inhibits the proliferation of ovarian cancer cells by activating the MAPK signaling pathway^[28]. Chrysophanol inhibits the growth of breast cancer cells through the NF- κ B signaling pathway^[29]. Chrysophanol shows anticancer activity in lung cancer cells by regulating ROS/ HIF-1A /VEGF signaling pathway^[15]. The apoptosis of choriocarcinoma was induced by induction of ROS production and by Akt and ERK1/2 pathways^[30]. In addition, our previous research found that chrysophanol inhibits the activity of colorectal cancer by targeting decorin^[10]. However, the mechanism of chrysophanol on gastric cancer is still unclear. We investigated the anti-tumor effect of chrysophanol on gastric cancer. We found that chrysophanol could inhibit the proliferation, induce pyroptosis, and suppress cell migration and invasion.

Although there are new methods for cancer treatment every year, no matter traditional treatment methods such as surgery, radiotherapy and chemotherapy, or targeted drugs, immunotherapy and so on^[31], although they all play a role in killing cancer cells, one of the major difficulties in cancer treatment is that tumor metastasis has not been effectively solved^[32]. Migration and invasion are important components of cancer cell metastasis. Therefore, cancer cell metastasis can be controlled by reducing the ability of cell migration and invasion^[33]. Recent study showed that chrysophanol induces cell death and inhibits invasiveness via mitochondrial calcium overload in ovarian cancer cells^[28]. Chrysophanol decreased metastasis via a Wnt-3-dependent signaling pathway^[34]. Our results suggested that chrysophanol could inhibit the migration and invasion of gastric cancer cells.

Inflammation is a recognized marker of cancer, sustained inflammatory stimuli can promote tumorigenesis, and the inflammatory response is further aggravated after tumor development^[35]. In diagnosed cancers, there is increasing evidence that local immune response and systemic inflammation play an important role in tumor progression and survival of cancer patients^[36]. Studies have shown that the deletion of NF- κ B1 and the abnormal activation of STAT jointly promote the development of gastritis and gastric cancer^[37]. *Helicobacter pylori* infection activates a series of signaling cascade that causes epithelial and immune cells to secrete various cytokines (IL-1 β , IL-6, IL-8, IL-10) and chemokines (MCP-1), triggers chronic inflammatory response and drives the occurrence of cancer^[38]. The inflammatory mediators produced may induce tumor cell proliferation, and inhibit apoptosis^[39]. IL-1 β combined with *Helicobacter pylori* infection may be a key factor in gastric cancer, increased IL-1 β gene polymorphism

and IL-1 β level are associated with increased risk of gastric cancer^[40]. Blocking the production of endogenous IL-18, up-regulating the expression of CD70, down-regulating the expression of CD44 and VEGF can effectively inhibit tumor rejection and metastasis^[41].

The NLRP3 inflammasome is the most characterized and studied inflammasome, It responds to various activators (bacteria, pathogens) as well as dangerous molecular patterns^[42]. NLRP3 inflammasome is a multi-protein compound and is activated mainly by two different signals: One is the expression of NLRP3, IL-1 β and IL-18 mediated by NF- κ B; The other is the assembly of NLRP3 inflammasomes, activation of caspase-1, and secretion of IL-1 β and IL-18^[43]. Similar to most NLRs, As an important component of innate immunity, NLRP3 inflammasome plays an important role in the immune response and the occurrence of disease^[44]. Because it can be activated by multiple types of pathogens or danger signals, NLRP3 inflammasomes play an important role in the development of a variety of tumors^[45]. It has been found that fibroblasts (CAFs) sense damage-related molecular patterns (DAMPs) in breast cancer and accordingly activate the NLRP3 inflammasome pathway, leading to the secretion of pro-inflammatory signaling pathways and IL-1 β ^[46]. Activation of NLRP3 inflammasome can enhance the proliferation, invasion and migration of A549 lung cancer cells^[47]. PPVI induces caspase-1-mediated cell pyroptosis in non-small cell lung cancer by inducing reactive oxygen species/NF- κ B/NLRP3 / GSDMD signaling axis^[47]. Blocking NLRP3 signal can inhibit the migration ability of colorectal cancer cells in vitro and in vivo^[49].

In this study, it was found that the anti-tumor effect of chrysophanol may be significantly related to the up-regulation of NLRP3. First, we found that NLRP3, caspase-1 was upregulated in gastric cancer tissues compared with paracancerous tissues. Meanwhile, the expression of Ki67 related to cell proliferation was significantly higher in cancer tissues than in paracancerous tissues. The above results suggest that there may be a positive correlation between Ki67 expression and NLRP3 and caspase-1 expression, but there is still controversy due to the lack of large sample randomized control. Compared with GES-1, NLRP3 expression in MKN 28 and AGS cells was significantly increased. Chrysophanol induced morphological changes in MKN 28 and AGS cells and increased pyroptosis in gastric cancer cells and promoted LDH release in a dose-dependent manner. The results also showed that NLRP3 and its downstream caspase-1 and IL-1 β expression increased in MKN 28 and AGS cells after chrysophanol treatment. These results indicated that chrysophanol could activate the pyroptosis of gastric cancer cells via NLRP3. Above result suggests that chrysophanol plays an anti-tumor role in GC treatment by upregulation of NLRP3 expression.

Did chrysophanol exert antitumor effect in gastric cancer by regulation of NLRP3? NLRP3 siRNA was further used to detect the underlying mechanism in this study. We found that compared with the control group, chrysophanol treatment induced pyroptosis and increased LDH release, as well as downstream expression of Caspase-1, IL-1 β , and IL-18. However, the antitumor effect of chrysophanol was reversed after NLRP3 siRNA treatment. As the results showed, the combined effect of chrysophanol +NLRP3 siRNA could reduce pyroptosis and LDH release, and the downstream expressions of Caspase-1, IL-1 β

and IL-18 were also decreased. These results indicated that NLRP3 silencing could reduce the antitumor effect of chrysophanol. Furthermore, chrysophanol +NLRP3 siRNA treatment depressed the migration and invasion in gastric cancer. These results suggested that inhibiting NLRP3 could weaken the effect of chrysophanol on gastric cancer cells, and chrysophanol did exert antitumor effect by up-regulation of NLRP3.

Taken together, our results demonstrated that chrysophanol inhibited the growth of gastric cancer, increased cell pyroptosis, and inhibited migration and invasion of gastric cancer cells. Regarding to the molecular mechanism, chrysophanol exerts anti-tumor effects via up-regulation of NLRP3. NLRP3 might be a potential molecular target for gastric cancer therapy, chrysophanol will be expected to become a potential therapeutic scheme for the clinical treatment of gastric cancer. Further researches are still needed to uncover more potential mechanisms of chrysophanol on gastric cancer.

Abbreviations

GC Gastric cancer

ASC adaptor apoptosis-related speckle-like protein

DAMPs damage-related molecular patterns

Declarations

Ethics approval and consent to participate

The Ethics Committee of The First Affiliated Hospital of Bengbu Medical College (Bengbu, Anhui, China) approved this study, and all patients provided informed consent.

Consent for publication

This article was published with the consent of Bengbu Medical College. Data and materials availability

All data needed to evaluate the conclusions in this paper are available in the main text

Competing interests

None.

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Author contributions:

BF H performed experiments and generated, analysed and interpreted data. M D drafted the manuscript. L Z performed statistical analysis. M D conceived of the idea, designed experiments, assisted in data analyses, the drafting and critical review of the manuscript, and provided funding for the study. All authors critically revised the manuscript for important intellectual content.

Data availability statement:

All methods and protocols used were peer-reviewed and cited in the reference section.

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Figures

Figure 1

Chrysophanol inhibits MKN 28 and AGS cells proliferation . A. Chemical structure of chrysophanol.B. Two human gastric cancer lines(MKN28 and AGS)were treated with chrysophanol at different concentrations (0, 2, 5, 10 μ M) for 24 h and 48 h, respectively. Cell viability was measured by CCK8 .(C). Colony formation assay of MKN28 and AGS cells treated with chrysophanol at different concentrations (0, 5, 10 μ M), Colonies were quantified as the average percentage of cells in the control group. * P < 0.05,** P < 0.01.

Figure 2

Chrysophanol promotes pyroptosis of MKN28 and AGS, induces G2/M phase arrest.(A)MKN28 and AGS were treated with chrysophanol at different concentrations (0, 5, 10 μ M) ,cell morphology was observed and photographed under a 100 magnification microscope.(B). LDH release rate was detected by LDH assay.(C).cell pyroptosis was detected by Caspase-1 /PI flow cytometry. * P < 0.05,** P < 0.01.

Figure 3

Chrysophanol suppresses MKN28 and AGS cells migration and invasion. MKN28 and AGS were treated with different concentrations of chrysophanol (0, 5, 10 μ M) and then (A) cell migration was assessed by Wound healing assay. (B). cell invasion was detected by Transwell assay. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

Figure 4

Chrysophanol increases the expression of NLRP3 in inflammasome in MKN 28 and AGS. MKN28 and AGS cells were treated with different concentrations of chrysophanol (0, 5, 10 μ M). (A). The mRNA expression level of NLRP3 was detected by qRT-PCR (Left). The protein expression level of NLRP3 was detected by Western blot. (B) The mRNA expression level of caspase-1 was detected by qRT-PCR (Left). The protein expression level of cleaved caspase-1 was detected by Western blot (C). The mRNA expression level of IL-1 β was shown on the left; the protein expression levels of Supernatant IL-1 β , Lysate IL-1 β were shown on the right. * P < 0.05; ** P < 0.01.

Figure 5

Chrysophanol induces pyroptosis by regulation of NLRP3 The expression of NLRP3 in human gastric cancer tissues and adjacent tissues, (A) Data from OncoMine Database.(B) Data from TCGA, Database. (C) IHC staining for NLRP3, caspase-1, Ki 67. (D) The protein levels of NLRP3 in GES-1, MKN 28, AGS was detected by Western blot. (E) qRT-PCR analysis of NLRP3 in GES-1, MKN 28, AGS. (F) Kaplan-Meier analysis of NLRP3 expression in survival of GC patients. (G-H). MKN 28 cells were transfected with NLRP3 siRNA and NC, the expression level of NLRP3 siRNA in these transfected cells was detected by qRT-PCR and western blot. the transfected cells were treated with 10 μM chrysophanol for 24 h, then (I). LDH release was examined by LDH kit. (J). Cell pyroptosis was determined by flow cytometry. *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 6

Chrysophanol inhibited MKN 28 cells migration and invasion by regulation of NLRP3. MKN 28 cells were transfected with NLRP3 siRNA and NC and then treatment with chrysophanol(10μM) for 24h. (A) Cell migration was assessed by Wound healing assay. (B). Cell invasion was assessed by Transwell. *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 7

Chrysophanol exerted antitumor effect via NLRP3 signaling pathway in MKN 28 (A-B).the mRNA levels of IL-1 β was detected by qRT-PCR, the protein levels of supernatant IL-1 β and lysate IL-1 β were detected by Western blot. (C).the protein levels of cleaved caspase-1 was detected by Western blot. (D-E). Inflammatory factor IL-1 β , IL-18 were detected by ELISA. * $P < .05$, ** $P < .01$ and *** $P < .001$.

Figure 8

Schematic illustration of this study. Chrysophanol treatment activated NLRP3 in flamma some and induced cell pyroptosis.