

## Original Research Article

# Quinolinone inhibits proliferation of gastric cancer cells and induces their apoptosis via down-regulation of the expression of pro-oncogene c-Myc

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### Abstract

**Purpose:** To determine the anti-proliferative potential of quinolinone against gastric cancer cells, and the underlying mechanism of action.

**Methods:** Quinolinone-mediated proliferative changes were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, while its effect on apoptosis was determined by flow cytometry. Transwell and wound healing assays were used for the determination of the effect of quinolinone on cell invasion and migration. The effect of quinolinone on protein expression levels were assayed with western blotting.

**Results:** Quinolinone caused reduction in gastric cancer cell viability, but it had no effect on normal (GES-1) cells. Treatment with 8  $\mu$ M quinolinone reduced the viability of SNU-5 and SGC-7901 cells to 32 and 27 %, respectively. Moreover, 8  $\mu$ M quinolinone induced 67.90 and 71.54 % apoptosis in SNU-5 and SGC-7901 cells, respectively. Quinolinone significantly increased the population of cells in G1 phase, and suppressed migration potential ( $p < 0.05$ ). Furthermore, in quinolinone-treated cells, the expression levels of p-PI3K, c-Myc and p-AKT were much lower than those in untreated cells ( $p < 0.05$ ). Quinolinone also downregulated the expressions of MMP-2 and MMP-9, while it upregulated p21 expression in SNU-5 and SGC-7901 cells.

**Conclusion:** Quinolinone suppresses the growth of SNU-5 and SGC-7901 gastric cancer cells via cell cycle arrest, induction of apoptosis and downregulation of the expressions of c-Myc and metalloproteinases. Thus, quinolinone may be developed as a potential drug candidate for the treatment of gastric cancer.

**Keywords:** Gastric cancer, Apoptosis, Metalloproteinases, Phosphorylation

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## INTRODUCTION

Gastric cancer, a life-threatening disease worldwide, has very poor prognosis [1,2]. It has been reported that the incidence of gastric

cancer is much higher in the developing nations of South America, East Asia and some European countries than in developed countries [3]. According to statistical data, approximately 70 % of gastric cancer cases are diagnosed in

developing nations of the world [3]. China accounts for 25 % of deaths due to gastric cancer, and the average 5-year survival of the patients is 40 % because the disease is diagnosed at advanced stage [4]. Gastric cancer ranks third in cancer-related mortalities worldwide, with the highest incidence in East Asia [5]. The techniques for diagnosis of gastric cancer at early stage need to be improved in order to overcome the high mortality associated with advanced stage of the disease [6]. Gastric cancer is a heterogeneous disease caused by multiple factors such as dietary salt intake, environmental factors, vitamin C intake, and infection with *Helicobacter pylori* [3]. The survival of gastric cancer patients is very unsatisfactory due to rapid recurrence and metastasis, despite improved surgical techniques, and advances in radiotherapy and chemotherapies [7,8]. Therefore, there is need for identification of novel bioactive anticancer compounds, as well as regulatory pathways for use in development of effective treatment for gastric cancer.

The phosphoinositide-3-kinase (PI3K)/AKT is an important signaling route associated with cancer cell proliferation, apoptosis and metastasis [9]. Studies have shown that PIK3R3, which is a regulating subunit of Class IA PI3Ks, is involved in human cancer tissues [10]. There is markedly enhanced phosphorylation of PI3K/AKT in cancer cells, when compared to normal control cells. Therefore, the inhibition of tumorigenesis by targeting PI3K/AKT activation constitutes an important therapeutic strategy for different types of cancers [11]. Quinoline, a nitrogen-bearing heterocyclic compound with aromatic nature, has been used for synthesis of various compounds of pharmacological importance [12]. The present study evaluated the anti-proliferative potential of quinolinone against gastric cancer cells, and the associated mechanism.

## EXPERIMENTAL

### Cell lines and culture

Gastric cancer cells (SNU-5 and SGC-7901) and normal cell line (GES-1) were provided by the Cell Bank of Chinese Academy of Sciences (Shanghai, China). The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % FBS, 1% penicillin and 1 % streptomycin at 37°C in a 5 % CO<sub>2</sub> incubator.

### Cell proliferation assay

The GES-1, SNU-5 and SGC-7901 cells were seeded separately, each at a density of  $1 \times 10^6$

cells/per well in 96-well plates, and cultured for 24 h. The cells were treated with graded doses of quinolinone (0.25, 0.5, 1.0, 2.0, 4.0 and 8.0  $\mu\text{M}$ ) for 48 h. Then, 20  $\mu\text{L}$  of MTT solution (5 mg/mL) was added to each well, and incubation was carried out for 4 h. Thereafter, the medium was discarded, followed by addition of 130  $\mu\text{L}$  of DMSO to dissolve the formazan crystals formed. The plates were shaken for 20 min at room temperature before optical density of each well content was read at 487 nm in a multi-well spectrophotometer.

### Apoptosis assay

The SNU-5 and SGC-7901 cells were seeded separately, each at a density of  $2 \times 10^6$  cells/well, and treated with 8.0  $\mu\text{M}$  quinolinone in 6-well plates for 48 h. Untreated cells served as controls. The cells were washed in PBS twice before staining with 5  $\mu\text{L}$  of Annexin V-FITC and 10  $\mu\text{L}$  of PI in 430  $\mu\text{L}$  of binding buffer. The staining was carried out for 20 min in the dark at room temperature for analysis of apoptosis using a flow cytometer (Cytomics FC 500; Beckman Coulter Inc., Miami, FL, USA).

### Cell cycle analysis

The SNU-5 and SGC-7901 cells were seeded separately, each at a density of  $2 \times 10^6$  cells/well and treated with 8.0  $\mu\text{M}$  quinolinone in 6-well plates for 48 h. The harvested cells were washed thrice with PBS prior to fixing with 70 % methanol for 3 h. Then, the cells were centrifuged for 4 min at 800 x g, followed by washing in PBS and subsequent treatment with 480  $\mu\text{L}$  of buffer mixed with RNase (10  $\mu\text{L}$ ) and PI (25  $\mu\text{L}$ ). Then, the cells were incubated in the dark for 13 min at room temperature, after which cell cycle analysis was done using a flow cytometer (Cytomics FC 500; Beckman Coulter Inc.).

### Cell invasion assay

The invasive potential of SNU-5 and SGC-7901 cells were determined in 24-well Transwell plates with 8-mm pore size. The chamber was coated with 200 mg/mL Matrigel, followed by overnight drying. The SNU-5 and SGC-7901 cells were treated with 8.0  $\mu\text{M}$  quinolinone or kept untreated for 48 h. Then,  $2 \times 10^5$  cells in DMEM were placed on the upper chamber, while the medium containing 20 % FBS was placed in the lower chamber. After incubation of the plates at 37°C for 48 h, non-invasive cells were cleaned off with cotton swab. Then, the cells were fixed with 100 % methyl alcohol for 15 min at room temperature, after which they were stained for 30 min with hematoxylin and eosin (H&E). Cell invasion was

determined using a light microscope (Olympus Corporation, Tokyo, Japan).

### Cell migration assay

The SNU-5 and SGC-7901 cells were seeded and treated with 8.0  $\mu\text{M}$  quinolinone as described under apoptosis assay. Following monolayer formation, a 100- $\mu\text{L}$  plastic pipette tip was used to scratch a wound through the center of the wells. Loose cells were washed off with PBS, and cell migration was determined using an inverted microscope at a magnification of x200.

### Western blot analysis

The SNU-5 and SGC-7901 cells were seeded and treated with 8.0  $\mu\text{M}$  quinolinone as described under apoptosis assay. Then, the cells were lysed by treatment with RIPA buffer [40 mM Tris-HCl (pH 7.4) along with 150 mM sodium chloride and 1% (v/v) Triton X-100 in combination with protease inhibitors. The protein contents of the lysates were determined with BCA protein assay method. Then, 30- $\mu\text{g}$  protein samples were resolved on 10 % SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes which were previously blocked using 5 % skimmed milk and 0.1% Tween-20 for 2 h at room temperature. The membranes were probed using anti-c-Myc, anti-p-AKT, anti-p-PI3K, anti-P21, anti-MMP-2, anti-MMP-9 and anti-GAPDH (1 Cell Signaling Technology, Inc.). After washing in 1X PBST, the blots were incubated for 2 h with horseradish peroxidase-conjugated secondary antibody at room temperature. Visualization of the blots was made using ECL Reagent system.

### Statistical analysis

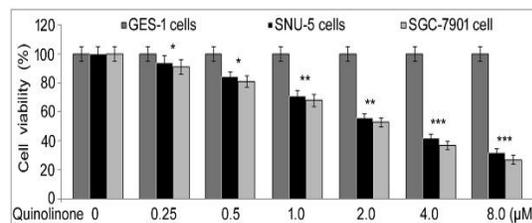
Data are expressed as mean  $\pm$  standard deviation of triplicate experiments carried out individually. The data analysis was made with one-way analysis of variance, along with Tukey's post-hoc test Student's t-test. For analysis of data, SPSS version 17.0 software (SPSS, Inc, Chicago, IL, USA) was used. Differences were taken as significant at  $p < 0.05$ .

## RESULTS

### Cytotoxicity of quinolinone on SNU-5 and SGC-7901 cells

Quinolinone reduced the viabilities of SNU-5 and SGC-7901 cells, but did not affect the viability of GES-1 cells (Figure 1). The viability of SNU-5 cells decreased to 32 %, whereas that of SGC-7901 cells was reduced to 27 % on treatment with 8  $\mu\text{M}$  quinolinone. Treatment with

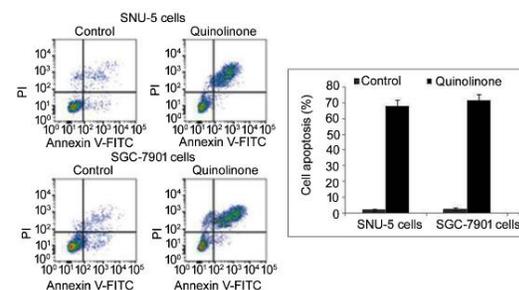
0.25  $\mu\text{M}$  quinolinone for 48 h reduced the viability of SNU-5 cells to 94 %, while the viability of SGC-7901 cells was decreased to 91 %. Thus, there were significant decreases in cell viability as quinolinone concentration was increased from 0.25 to 8.0  $\mu\text{M}$ .



**Figure 1:** Effect of quinolinone on the viabilities of GES-1 (normal), SNU-5 and SGC-7901 (cancer) cells. Treatment of cells with quinolinone at doses of 0.25 - 8.0  $\mu\text{M}$  for 48 h was followed by determination of cell viability using MTT assay. \* $P < 0.05$ ; \*\* $p < 0.02$ ; \*\*\* $p < 0.01$  vs normal control group

### Quinolinone promoted apoptosis in SNU-5 and SGC-7901 cells

Quinolinone (8.0  $\mu\text{M}$ ) induced apoptosis in SNU-5 and SGC-7901 cells after 48 h of treatment (Figure 2). In quinolinone-treated and untreated SNU-5 cells, apoptosis was detected in 67.90 and 1.86 % cells, respectively, after 48 h. Quinolinone (8.0  $\mu\text{M}$ ) treatment of SGC-7901 cells resulted in 71.54 % apoptosis, relative to 2.43 % apoptosis in control cells. Thus, quinolinone induced apoptosis in gastric cancer cells, thereby suppressing their viability.

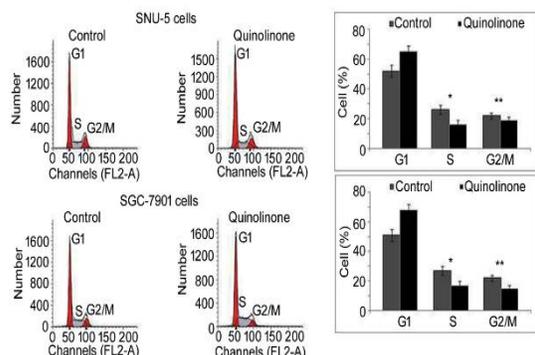


**Figure 2:** Apoptotic effect of quinolinone on the cell lines. Treatment of the cells with 8.0  $\mu\text{M}$  quinolinone for 48 h was followed by flow cytometric analysis of apoptosis. \* $P < 0.0456$  vs normal control group

### Quinolinone caused cell cycle arrest in SNU-5 and SGC-7901 cell lines

In 8.0  $\mu\text{M}$  quinolinone-treated cells, the count of G1 phase cells was significantly higher than the corresponding count in untreated cells ( $p < 0.05$ ). The count of SNU-5 and SGC-7901 cells in S and G2/M phases was significantly lower in

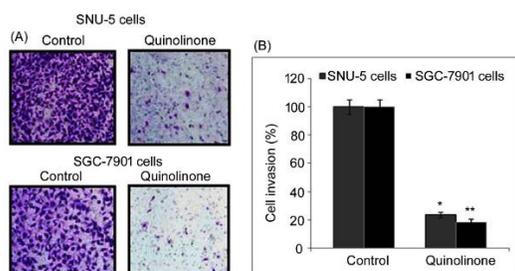
quinolinone-treated cell cultures than in control cells ( $p < 0.05$ ). These results are shown in Figure 3.



**Figure 3:** Effect of quinolinone on cell cycle. (A) Treatment of cells with 8.0  $\mu\text{M}$  quinolinone for 48 h was followed by flow cytometric analysis of DNA content. (B) Percentages of cells in the various phases of the cell cycle. \* $P < 0.05$  vs normal control group

#### Quinolinone inhibited the invasion of SNU-5 and SGC-7901 cells

The invasiveness of SNU-5 and SGC-7901 cells was suppressed significantly by treatment with 8.0  $\mu\text{M}$  quinolinone for 48 h ( $p < 0.05$ , Figure 4). The invasion of SNU-5 cells was decreased to 26.67 %, while the invasion of SGC-7901 cells was suppressed to 22.47 %, relative to control cells.

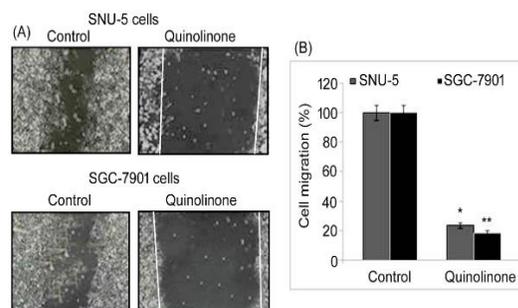


**Figure 4:** Effect of quinolinone on the invasiveness of SNU-5 and SGC-7901 cancer cells. (A) treatment of the cells with 8.0  $\mu\text{M}$  quinolinone for 48 h was followed by Transwell assay to determine invasion potential. (B) % invasion of cells. \* $P < 0.046$  vs normal control group. (x200)

#### Quinolinone inhibited the migration of SNU-5 and SGC-7901 cells

Treatment of SNU-5 and SGC-7901 cells with 8.0  $\mu\text{M}$  quinolinone suppressed their migration potential to 23.43 and 18.84 %, respectively, relative to controls (Figure 5). Thus, quinolinone treatment regulated the invasiveness and

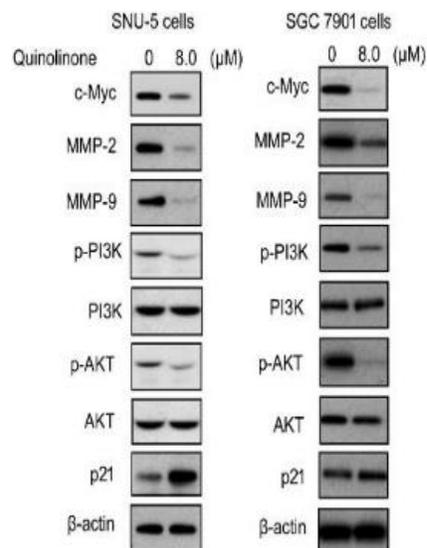
migration potential of SNU-5 and SGC-7901 gastric cancer cells.



**Figure 5:** Inhibitory effect of quinolinone on migration potential of SNU-5 and SGC-7901 gastric cancer cells. (A) treatment of the cells with 8.0  $\mu\text{M}$  quinolinone for 48 h was followed by wound healing assays for determination of migration potential. (B) % migration of the cells. \* $P < 0.046$  vs normal control group (x200)

#### Quinolinone regulated PI3K/AKT signal pathway

In quinolinone-treated cells, the expression levels of p-PI3K, c-Myc and p-AKT were much lower than the corresponding expressions in untreated cells. Quinolinone treatment also suppressed MMP2 and MMP9 levels in both cell lines, when compared to control. Moreover, treatment of SNU-5 and SGC-7901 cells with 8.0  $\mu\text{M}$  quinolinone resulted in upregulation of p21 expression, when compared to control cells. These results are presented in Figure 6.



**Figure 6:** Effect of quinolinone on protein expressions of PI3K/AKT. Treatment of the cells with 8.0  $\mu\text{M}$  quinolinone for 48 h was followed by assay of protein expression levels using western blotting assay

## DISCUSSION

The incidence of gastric cancer has shown gradual increase over the years, and the disease continues to rank among the top malignancies worldwide [13]. Studies have reported that gastric cancer is diagnosed in more than one million people globally every year [13]. The present study has demonstrated the anti-proliferative effect of quinolinone on SNU-5 and SGC-7901 cells, without any adverse effect of the viability of normal gastric cells. The enormous cell division potential of cancer cells is due to their ability to avoid apoptosis via downregulation of expressions of pro-apoptotic proteins [14]. However, oxidative stress leads to cleavage of caspases and increases in efflux of cytochrome *c* from mitochondria through enhancement of membrane permeability [14]. These processes initiate development of apoptotic complexes, and subsequently cleave effector caspases, leading to induction of apoptosis [15].

The present study found that quinolinone exerted anti-proliferative potential against SNU-5 and SGC-7901 cells. Flow cytometry data showed that quinolinone reduced cell viabilities by increasing the proportions apoptotic cells. Moreover, treatment of SNU-5 and SGC-7901 cells with quinolinone led to blockage of the cell cycle at the G1 phase. Cell cycle arrest was evident in the marked enhancement of cell population in G1 phase and subsequent reduction in S and G2/M phases in quinolinone-treated cells. Furthermore, it was shown that quinolinone treatment significantly suppressed invasiveness of SNU-5 and SGC-7901 cells, relative to control cells. Moreover, cell migration abilities were markedly lower in quinolinone-treated SNU-5 and SGC-7901 cells than in untreated controls. Thus, quinolinone acted as tumor suppressor of gastric cancer. The oncogene *c-Myc* is associated with cellular proliferation and transformation, as well as regulation of death [16]. Studies have revealed that the inhibition of expression of *c-Myc* blocks the progression of cell cycle, implying that targeting the expression of *c-Myc* would be beneficial for enhancement of apoptosis [17-19]. The present study showed that treatment of SNU-5 and SGC-7901 cells with quinolinone resulted in downregulation of the expression of *c-Myc*. The PI3K/AKT pathway is linked to survival and proliferation of cells, as well as angiogenesis [20, 21]. In the quinolinone-treated SNU-5 and SGC-7901 cells, the expressions of p-PI3K and p-AKT were much lower than those in untreated cells. Thus, quinolinone arrested proliferative potential of SNU-5 and SGC-7901 cells through

down-regulation of the PI3K/AKT pathway. The matrix metalloproteinases (MMP2 and MMP9) catalyze type IV collagen degradation, thereby contributing to tumor metastasis and vascularization [22]. The current study showed that quinolinone inhibited the activities of MMP2 and MMP9 in SNU-5 and SGC-7901 cells.

## CONCLUSION

This study has demonstrated that quinolinone arrests growth of gastric cancer cells by blocking cell cycle and activating apoptosis. The expressions of *c-Myc* and metalloproteinases are suppressed in SNU-5 and SGC-7901 cells on treatment with quinolinone. Thus, quinolinone has a good potential for development into a novel drug for treatment of gastric cancer.

## DECLARATIONS

### *Conflict of interest*

No conflict of interest is associated with this work.

### *Contribution of authors*

We declare that this work was done by the author(s) named in this article, and that all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Baoli Xu - conceived and designed the study. Wenguang Liu and Jing Ma collected and analyzed the data. Xinrui Chen, Wenguang Liu and Baoli Xu wrote the manuscript. All authors read and approved the manuscript for publication.

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