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BRIEF ARTICLE

7-difluoromethoxyl-5,4′-di-n-octylgenistein inhibits growth of gastric cancer cells through downregulating forkhead box M1

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Abstract

AIM: To investigate whether the 7-difluoromethoxyl-5, 4′-di-n-octylgenistein (DFOG), a novel synthetic genistein analogue, affects the growth of gastric cancer cells and its mechanisms.

METHODS: A series of genistein analogues were prepared by difluoromethylation and alkylation, and human gastric cancer cell lines AGS and SGC-7901 cultured *in vitro* were treated with various concentrations of genistein and genistein analogues. The cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cells were incubated by DFOG at different concentrations. The growth inhibitory effects were evaluated using MTT and clonogenic assay. The distribution of the phase in cell cycle was analyzed using flow cytometric analysis with propidium iodide staining. The expression of the transcription factor forkhead box M1 (FOXM1) was analyzed by reverse transcription-polymerase chain reaction and Western blotting. The expression levels of CDK1, Cdc25B, cyclin B and $p27^{KIP1}$ protein were detected using Western blotting.

RESULTS: Nine of the genistein analogues had more effective antitumor activity than genistein. Among the tested analogues, DFOG possessed the strongest activity against AGS and SGC-7901 cells *in vitro*. DFOG significantly inhibited the cell viability and colony formation of AGS and SGC-7901 cells. Moreover, DFOG efficaciously arrested the cell cycle in G2/M phase. DFOG decreased the expression of FOXM1 and its downstream genes, such as CDK1, Cdc25B, cyclin B, and increased $p27^{KIP1}$ at protein levels. Knockdown of FOXM1 by small interfering RNA before DFOG treatment resulted in enhanced cell growth inhibition in AGS cells. Up-regulation of FOXM1 by cDNA transfection attenuated DFOG-induced cell growth inhibition in AGS cells.

CONCLUSION: DFOG inhibits the growth of human gastric cancer cells by down-regulating the FOXM1 expression.

Key words: Gastric cancer; 7-difluoromethoxyl-5,4′-di-n-octylgenistein; Genistein; Forkhead box M1; Therapeutic action

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INTRODUCTION

Genistein, 5,7,4'-trihydroxylisoflavone, as one of the active constituents of soybean products, has been reported to possess anti-cancer activities[1,2]. But probably because of its low solubility in both water and organic solvent, genistein has a very low bioavailability. The introduction of the fluorine or fluoride-bearing alkyl such as CF_3 or HCF_2 into organic molecules dramatically changes their physiological, physical and chemical properties[3]. To our best knowledge, the introduction of fluorine moiety into the aryl part of the flavonoid molecule can enhance their biological activities, including anti-bacterial, anti-fungal and anti-viral activities[3]. Fluorinated 3, 4-dihydroxychalcones have illustrated interesting biological activities, including anti-peroxidation and antitumor activity *in vitro*[4]. We have previously reported that introduction of CF_3 or HCF_2 group into chrysin (5,7-dihydroxylflavone) molecule can improve their anticancer activities[5-7]. Recently, we synthesized a series of difluoromethoxylated genistein analogues and determined their protective effects against vascular endothelial cells[8]. However, there are few studies which reported the anti-cancer effect of fluorinated genistein analogues.

Gastric cancer is one of the most common malignancies in the world and its incidence and mortality rank first in China[9]. Recent data indicate that the mortality of gastric cancer in China tends to increase and it severely threatens the health and life of people[10]. At present, surgery and chemotherapy remain as the main modalities in the management of gastric cancer, but the curative effect of the existing chemotherapeutic drugs is not satisfactory, which cause numerous side effects. Therefore, it has been a focus to search for new drugs capable of preventing and treating gastric cancer and other malignancies. Gastric cancer has been shown to have activated forkhead box protein M1 (FOXM1) signaling pathway[11]. The FOXM1 belongs to a family of evolutionary conserved transcriptional regulators that were characterized by the presence of a DNA-binding domain called the forkhead box or winged helix domain[12]. It has been shown that FOXM1 signaling plays an important role in cellular developmental pathways, and activation of FOXM1 signaling is associated with carcinogenesis[13]. FOXM1 signaling is frequently up-regulated in cancers, including lung, breast, pancreatic and gastric cancer[11,14-16]. Moreover, FOXM1 has been shown to regulate transcription of cell cycle genes, including Cdc25B, CDK1, cyclin B and $p27^{KIP1}$[13,17]. Recently, it has been reported that FOXM1 expression could serve as an independent predictor of a poor survival of gastric cancer patients[11]. Studies by Wang *et al*[18] have shown that genistein may inhibit FOXM1 activation in pancreatic cancer cells, leading to apoptotic cell death. Therefore, it is believed that the targeted inactivation of FOXM1 could represent a promising strategy for the development of novel selective anti-cancer therapies.

In the present study, we investigated whether the growth inhibitory effects of genistein and the novel synthetic genistein analogue 7-difluoromethoxyl-5,4'-di-n-octylgenistein (DFOG) on gastric cancer cells could be attributed to modulation of FOXM1 activity. We found that DFOG and genistein down-regulated the FOXM1 expression and its downstream genes, including cdc25B, CDK1, cyclin B and up-regulated $p27^{KIP1}$, resulting in the growth inhibition of gastric cancer cells. These results provide strong evidences to support that FOXM1 is a rational target in gastric cancer, and the targeted inactivation of FOXM1, especially by genistein and its analogue DFOG, may provide new insight into the strategy development for better prevention of tumor progression and/or treatment of gastric cancer.

MATERIALS AND METHODS

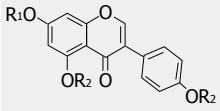
Cell culture and reagents

Human gastric cancer cell lines AGS and SGC-7901 were purchased from China Center for Type Culture Collection (CCTCC, Wuhan, China). Cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA, United States) supplemented with 10% fetal bovine serum, 2 mmol/L glutamine, 100 mg/L penicillin, and 100 mg/L streptomycin, and incubated in a humidified atmosphere of 5% CO_2 at 37 ℃. The difluoromethoxylated genistein analogues 2,4a-4h were prepared by the method described elsewhere[8] (Table 1). Primary antibodies for FOXM1, CDK1, cyclin B, $p27^{KIP1}$ and Cdc25B were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, United States). Anti-β-actin antibody and Horseradish peroxidase-conjugated rabbit anti-mouse secondary antibody were purchased from Santa Cruz Biotechnology. Lipofectamine 2000 was purchased from Invitrogen. Protease inhibitor cocktail, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and all other chemicals were obtained from Sigma (St. Louis, MO, United States). Genistein (Sigma) and the difluoromethoxylated genistein analogues were dissolved in dimethyl sulfoxide (DMSO) to make a 10 mmol stock solution and were added directly to the media at different concentrations before use.

MTT assay

Cells were seeded in a 96-well plate at a density of 5000 cells/well as described previously[19]. After incubation for 24 h to allow cell attachment, different concentrations of genistein and genistein analogues (0.1, 0.3, 1.0, 3.0, 10.0 and 30.0 μmol/L) were added to each well and cultured for 24 h. Medium was removed and then incubated with 5.0 mg/mL MTT for 4 h. Then supernatant was removed after centrifugation. Finally, 100 μL of DMSO was added and absorbance at 570 nm wavelength (A_{570}) was measured by means of an Enzyme-labeling instrument (ELX-800 type; Bio-Tek, Shanghai, China). Relative cell proliferation inhibition rate = (1 - average A_{570} of the experimental group/average A_{570} of the control group) × 100%. The IC_{50} (defined as the drug concentration which 50% cell viability was inhibited) was as-

Table 1 Structures of genistein and its difluoromethylated derivatives



	Compound	R1	R2
1	Genistein (5,7,4′-trihydroxylisoflavone)	H	H
2	7-difluoromethyl genistein	CHF_2	H
4a	7-difluoromethyl-5,4′-dimethyl genistein	CHF_2	CH_3
4b	7-difluoromethyl-5,4′-diethyl genistein	CHF_2	CH_3CH_2
4c	7-difluoromethyl-5,4′-di-n-propyl genistein	CHF_2	n-C_3H_7
4d	7-difluoromethyl-5,4′-di-benzyl genistein	CHF_2	$C_6H_5CH_2$
4e	7-difluoromethyl-5,4′-diheptyl genistein	CHF_2	n-C_7H_{15}
4f	7-difluoromethyl-5,4′-di-n-octyl genistein	CHF_2	n-C_8H_{17}
4g	7-difluoromethyl-5,4′-didecyl genistein	CHF_2	n-$C_{10}H_{21}$
4h	7-difluoromethyl-5,4′-diisobutyl genistein	CHF_2	iso-C_4H_9

sessed from the dose-response curves using GraphPad Prism program (Version 4, GraphPad Software).

Clonogenic assay

Cells were plated in 24-well plates at a density of 300 cells/well for 24 h, prior to the addition of various concentrations of DFOG (1, 5 and 10 μmol/L) and 10 μmol/L genistein. After 48 h of treatment, the drug-containing medium was removed and replaced with complete growth medium. Medium was changed every 3 d for 8-10 d until visible colonies formed. Colonies were simultaneously fixed and stained with 0.5% crystal violet in methanol, and manually counted. Individually stained colonies in each well were counted and the colony formation fraction was calculated as follows: colony number/(number of cells seeded × plating efficiency), where plating efficiency is equivalent to the colony number divided by the number of cells seeded in the drug-free medium.

Cell cycle analysis by flow cytometry

Cells were plated in 6-well plates at a density of 1 000 000 cells/well for 24 h, prior to the addition of various concentrations (1, 5 and 10 μmol/L) of DFOG and 10 μmol/L genistein. After 24 h of treatment, cells were harvested, and DNA content was stained for 15 min at 37 ℃ with a solution containing 0.4% Triton X-100 (Sigma), 50 μg/mL of propidium iodide (Sigma), and 2 μg/mL of DNase-free RNase (Roche, United States). The cells were then analyzed for cell cycle perturbation using a FACSCalibur (FACS 420, Becton Dickinson, United States). The CellQuest program was used to quantitate the distribution of cells in each cell cycle phase: G1, S and G2/M.

Reverse transcription-polymerase chain reaction

Total RNA was extracted using Trizol reagent (Life Technologies, Gaithersburg, MD, United States). The integrity of the RNA was checked by 2% agarose gel electrophoresis. Approximately 2 μg RNA was reversely transcribed following the protocol of the Super Script™ first-strand synthesis system (Invitrogen Corporation, Carlsbad, CA, United States). The cDNAs encoding FoxM1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were amplified by polymerase chain reaction (PCR) as follows: denaturation at 94 ℃ for 30 s, annealing at 63 ℃ for 30 s and elongation at 72 ℃ for 45 s. Primer sequence was designed using Primer. For FoxM1, the forward primer was 5'-AACCGCTACTTGACATTGG-3' and reverse primer 5'-GCAGTGGCTTCATCTTCC-3'. A housekeeping gene, GAPDH was used as the internal control. The forward primer was 5'-ACCCAGAAGACTGTGG ATGG-3', and the reverse primer was 5'-TGCTGTAGCCAAATTCGTTG-3'. PCR products were analyzed by agarose (2%) gel electrophoresis.

Plasmids and transfections

FOXM1 small interfering RNA (siRNA) and control siRNA were obtained from Santa Cruz Biotechnology. The FOXM1 cDNA plasmid was purchased from OriGene Technologies Inc (Rockvile, MD, United States). Human gastric cancer AGS cells were transfected with FOXM1 siRNA and cDNA, respectively, using Lipofectamine 2000 (Invitrogen) as described by Wang *et al*[20].

Western blotting analysis

Western blotting analysis was carried out as previously described[21]. Cells were lysed in lysis buffer by incubation for 20 min at 4 ℃. The protein concentration was determined using the Bio-Rad assay system (Bio-Rad, Hercules, CA, United States). Total proteins were fractionated using sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto Polyvinylidene fluoride membrane (Millipore, United States). Anti-FOXM1, anti-CDK1, anti-cyclin B, anti-$p27^{KIP1}$, anti-cdc25B and anti-β-actin rabbit polyclonal antibodies were used as primary antibodies. The signals were detected using an ECL Advance Western blotting analysis system (Amersham Pharmacia Biotech Inc., Piscataway, NJ, United States).

Statistical analysis

The SPSS 15.0 software package (SPSS Inc, Chicago, IL, United States) was used for the statistical analysis. Data were expressed as mean ± SD. The means of multiple groups were compared with one-way analysis of variance, after the equal check of variance, and the pairwise comparisons among the means were performed using the least significant difference method. Statistical comparison was also performed with two-tailed *t* test when appropriate. A $P < 0.05$ was considered as statistically significant.

RESULTS

Effects of genistein and genistein analogues on the cell viability of gastric cancer cells

First, we examined the effects of genistein and the genistein analogues on the viability of AGS and SGC-7901

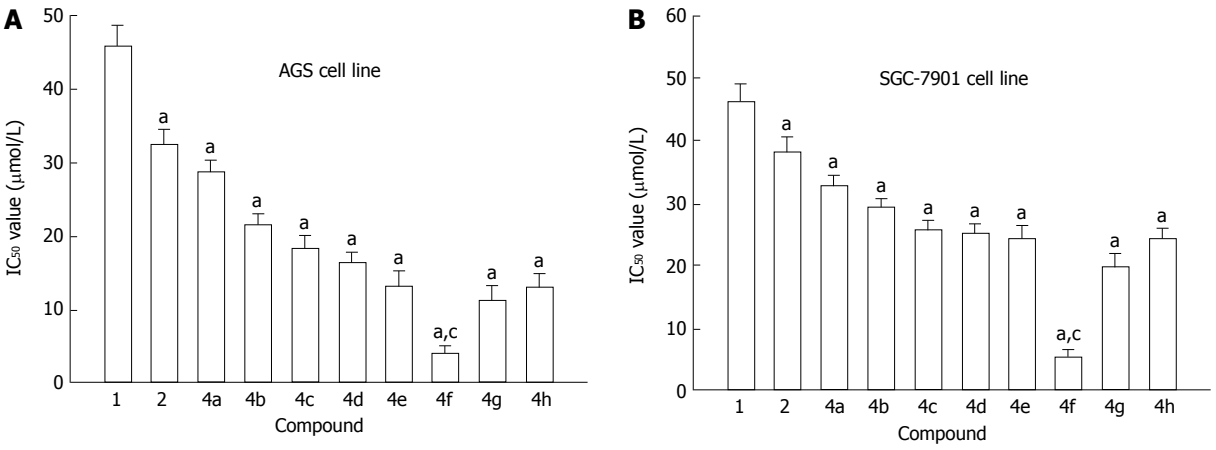


Figure 1 Inhibition of cell viability by genistein and genistein analogues. A: AGS cell line; B: SGC-7901 cell line. $^{a}P < 0.05$ *vs* treatment with genistein; $^{c}P < 0.05$ *vs* treatment with genistein or other genistein analogues. 1: Genistein (5,7,4'-trihydroxylisoflavone); 2: 7-difluoromethyl genistein; 4a: 7-difluoromethyl-5,4'-dimethyl genistein; 4b: 7-difluoromethyl-5,4'-diethyl genistein; 4c: 7-difluoromethyl-5,4'-di-n-propyl genistein; 4d: 7-difluoromethyl-5,4'-di-benzyl genistein; 4e: 7-difluoromethyl-5,4'-diheptyl genistein; 4f: 7-difluoromethyl-5,4'-di-n-octyl genistein; 4g: 7-difluoromethyl-5,4'-didecyl genistein; 4h: 7-difluoromethyl-5,4'-diisobutyl genistein.

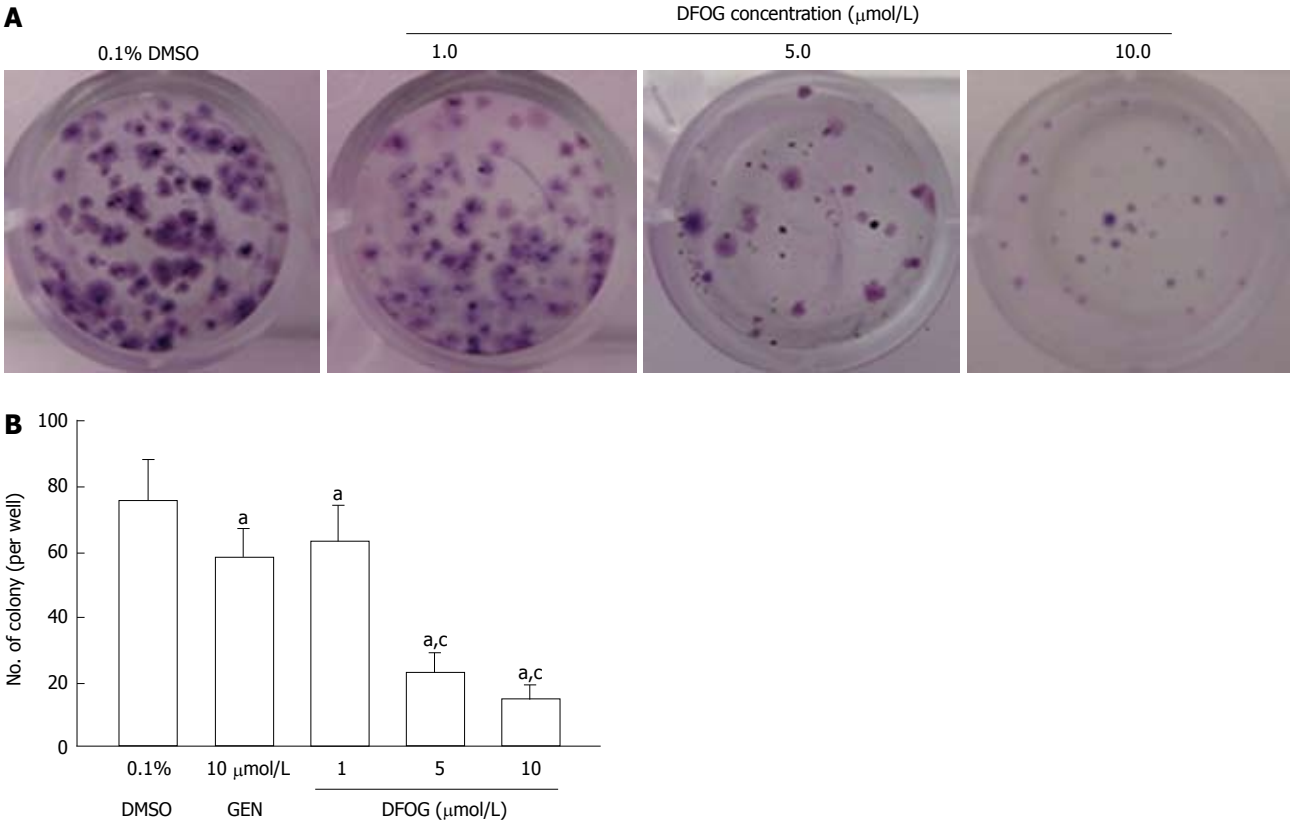


Figure 2 Decrease of colony number and inhibition of colony formation by 7-difluoromethoxyl-5,4'-di-n-octylgenistein. A: Decrease of colony number by 7-difluoromethoxyl-5,4'-di-n-octylgenistein (DFOG); B: Inhibition of colony formation by DFOG and genistein in AGS cell line. $^{a}P < 0.05$ *vs* treatment with dimethyl sulfoxide (DMSO); $^{c}P < 0.05$ *vs* treatment with 10 μmol/L genistein (GEN) or 1 μmol/L DFOG.

cells using MTT assay. Nine of difluoromethylated genistein analogues had higher effective antitumor activities than genistein. Among the aforementioned analogues, DFOG showed the strongest activity against AGS and SGC-7901 *in vitro* (Figure 1A and B). IC_{50} of DFOG was 3.9 μmol/L for AGS cells and 5.2 μmol/L for SGC-7901 cells, the potency of DFOG was 11.7 and 8.9 as much as that of the lead compound, genistein (IC_{50} was 45.9 μmol/L for AGS cells and 46.3 μmol/L for SGC-7901 cells).

Effects of DFOG on the colony formation of gastric cancer cells

Next, we tested the effects of DFOG on cell growth by clonogenic assay. DFOG treatment resulted in a significant inhibition of colony formation of AGS cells

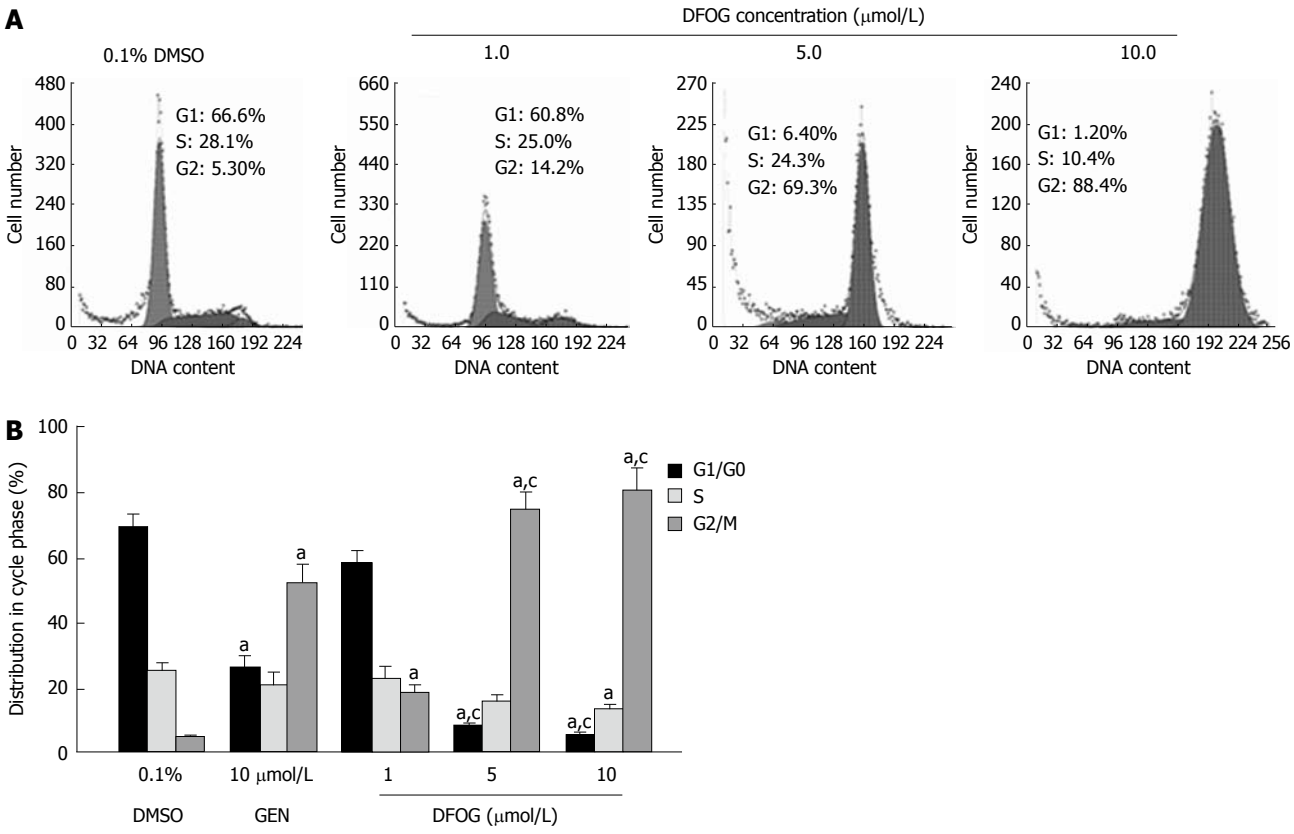


Figure 3 Increase of cells in G2/M phase and induction of cell cycle arrest in G2/M phase by 7-difluoromethoxyl-5,4'-di-n-octylgenistein. A: Increase of cells in G2/M phase by 7-difluoromethoxyl-5,4'-di-n-octylgenistein (DFOG); B: Induction of cell cycle arrest in G2/M phase by DFOG and genistein in AGS cell line. $^{a}P < 0.05$ *vs* treatment with dimethyl sulfoxide (DMSO); $^{c}P < 0.05$ *vs* treatment with 10 μmol/L genistein (GEN) or 1 μmol/L DFOG.

compared with controls (Figure 2A and B). Similar results were observed in SGC-7901 cells (data not shown). These data suggests that DFOG inhibits the growth of gastric cancer cells.

Effects of DFOG on the distribution of cell cycle phase in gastric cancer cells

To assess whether the loss of cell survival could in part be attributed to the induction of cell cycle arrest, we evaluated the effects of DFOG treatment on the distribution in cell phase using flow cytometry with propidium iodide staining. As shown in Figure 3A and B, in gastric cancer cell line AGS, DFOG treatment caused a significant accumulation of cells in the G2/M phase and a marked decrease in the G1/G0 phase compared with control cells. Similar results were observed in SGC-7901 cells (data not shown). These results provided convincing data that DFOG could induce the growth inhibition and arrest of cell cycle in G2/M phase in gastric cancer cells.

Effects of DFOG on FOXM1 expression in gastric cancer cells

The studies by Wang *et al*[18] have shown that FOXM1 signaling is over-expressed in pancreatic cancer and is involved in promotion of cell growth, and thus considered as a putative target for drug development. Therefore, we investigated whether DFOG could regulate FOXM1 signaling pathway. FOXM1 mRNA and protein expression in AGS cell line treated with DFOG and genistein for 24 h were decreased in a concentration-dependent manner (Figure 4A and B). We also found that FOXM1 protein expression was down-regulated by DFOG and genistein in SGC-7901 cells (Figure 4C).

Effects of DFOG on the expression of FOXM1 downstream target genes in gastric cancer cells

It is well known that FOXM1 has several downstream target genes, such as CDK1, Cdc25B, cyclin B, and $p27^{KIP1}$. We used Western blotting analysis to determine the expression of these genes, and found that DFOG and genistein inhibited the expression of CDK1, Cdc25B, cyclin B, and increased $p27^{KIP1}$ at the protein levels in AGS and SGC-701 cells (Figure 5A and B).

Effects of down-regulation of FOXM1 expression by siRNA on DFOG-induced cell growth inhibition in AGS cells

Down-regulation of FOXM1 by siRNA transfection showed less expression of FOXM1 protein in AGS cells, as confirmed by Western blot (Figure 6A).The down-regulation of FOXM1 expression significantly inhibited cell viability induced by DFOG (Figure 6B). DFOG plus FOXM1 siRNA inhibited cell growth to a greater degree compared with DFOG alone. FOXM1 siRNA transfection induces arrest of cell cycle in G2/M phase in AGS cells (Figure 6C). These results provide some molecular evidences suggesting that the DFOG-induced inhibition

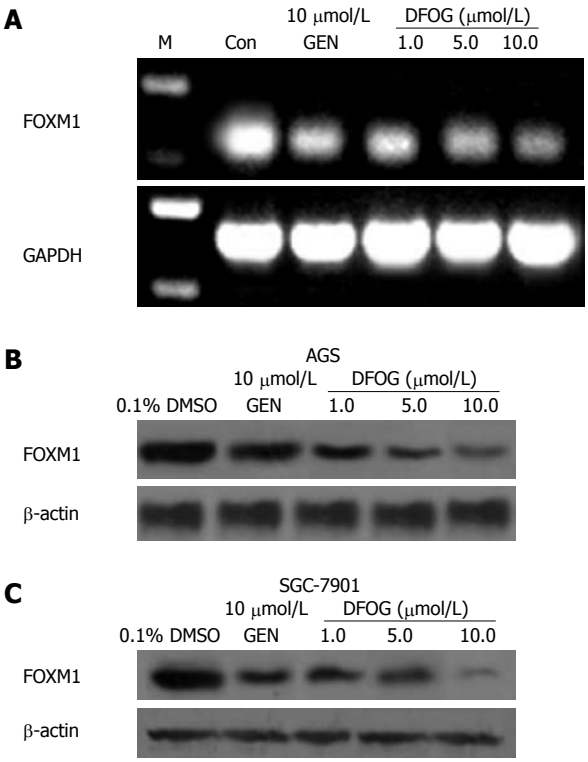


Figure 4 Down-regulation of forkhead box M1 expression by 7-difluoromethoxyl-5,4'-di-n-octylgenistein and genistein in AGS and SGC-7901. A: mRNA level using reverse transcription-polymerase chain reaction in AGS cell line; B: Protein level using Western blotting in AGS cell line; C: Decrease of forkhead box M1 (FOXM1) protein expression in SGC-7901 cell line. DMSO: Dimethyl sulfoxide; GEN: Genistein; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

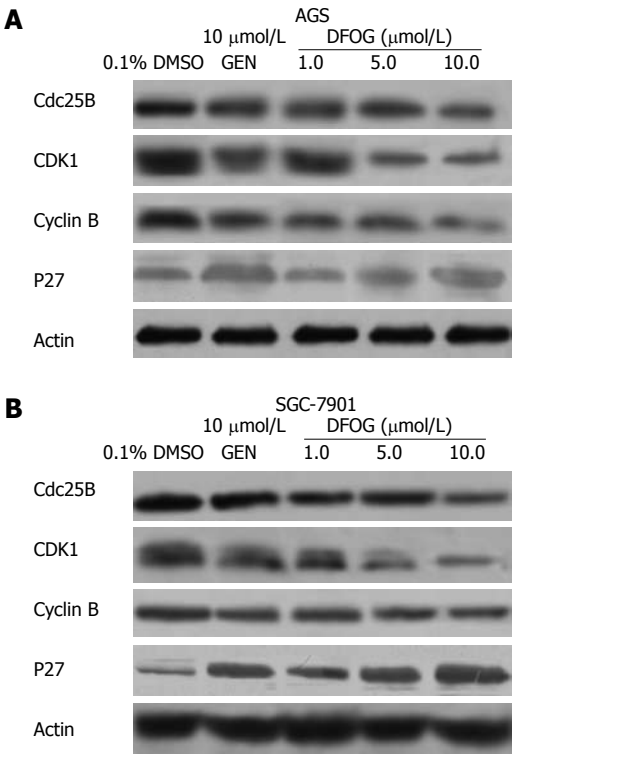


Figure 5 Modulation of the protein expressions of forkhead box M1 downstream target genes by 7-difluoromethoxyl-5,4'-di-n-octylgenistein and genistein. A: AGS cell line; B: SGC-7901 cell line. DMSO: Dimethyl sulfoxide; GEN: Genistein; DFOG: 7-difluoromethoxyl-5,4'-di-n-octylgenistein.

of the growth is mediated *via* inactivation of FOXM1 in gastric cancer cells.

Effects of over-expression of FOXM1 by cDNA transfection on DFOG-induced cell growth inhibition in AGS cells

Up-regulation of FOXM1 by cDNA transfection showed over-expression of FOXM1 protein in AGS cells, as confirmed by Western blotting (Figure 7A). The over-expression of FOXM1 rescued DFOG-induced cell viability inhibition to a certain degree (Figure 7B). These findings suggested that DFOG-inhibited cell growth is in part attributed to inactivation of FOXM1 signaling pathway in gastric cancer cells.

DISCUSSION

FOXM1 signaling has been demonstrated to maintain a balance between cell proliferation, differentiation and apoptosis, suggesting that abnormal activation of *FOXM1* gene is one of the characteristics of human cancers[22]. Increasing studies have shown over-expression of *FOXM1* gene in human cancer cells and tissues[14,23,24]. Thus, the development of agents targeting FOXM1 is likely to have a significant therapeutic impact on the treatment of human cancers, including gastric cancer. FOXM1 could be down-regulated by some drugs, such as antibiotic thiazole compound Siomycin A, thiostrepton, and epidermal growth factor receptor inhibitor gefitinib[25-27]. These observations clearly suggest that chemical compounds that target FOXM1 may act as anti-cancer drugs[27]. Wang *et al*[18] have shown that genistein may inhibit FOXM1 activation in pancreatic cancer cells, leading to apoptotic cell death. In the present study, we used two human gastric cancer cell lines, AGS and SGC-7901, which have high expression of FOXM1, and found that DFOG, a novel synthetic genistein analogue, and genistein could induce significant growth inhibition in the two cell lines, as evidenced by both MTT and clonogenic assay. Furthermore, DFOG and genistein inhibited the expression of FOXM1 and its target genes. Therefore, DFOG and genistein could mediate the cell growth inhibition partly *via* inactivation of FOXM1. Down-regulation of FOXM1 by siRNA together with DFOG treatment inhibited cell growth to a greater degree in AGS cells as compared with DFOG treatment alone. Up-regulation of FOXM1 by cDNA transfection showed over-expression of FoxM1 protein as confirmed by Western blotting analysis, and this over-expression in FOXM1 attenuated DFOG-induced cell growth inhibition in AGS cells. In view of these findings, we strongly believe that inactivation of FOXM1 by DFOG and genistein results in the down-regulation of its target genes, which are mechanistically linked with DFOG and genistein induced cell growth inhibition.

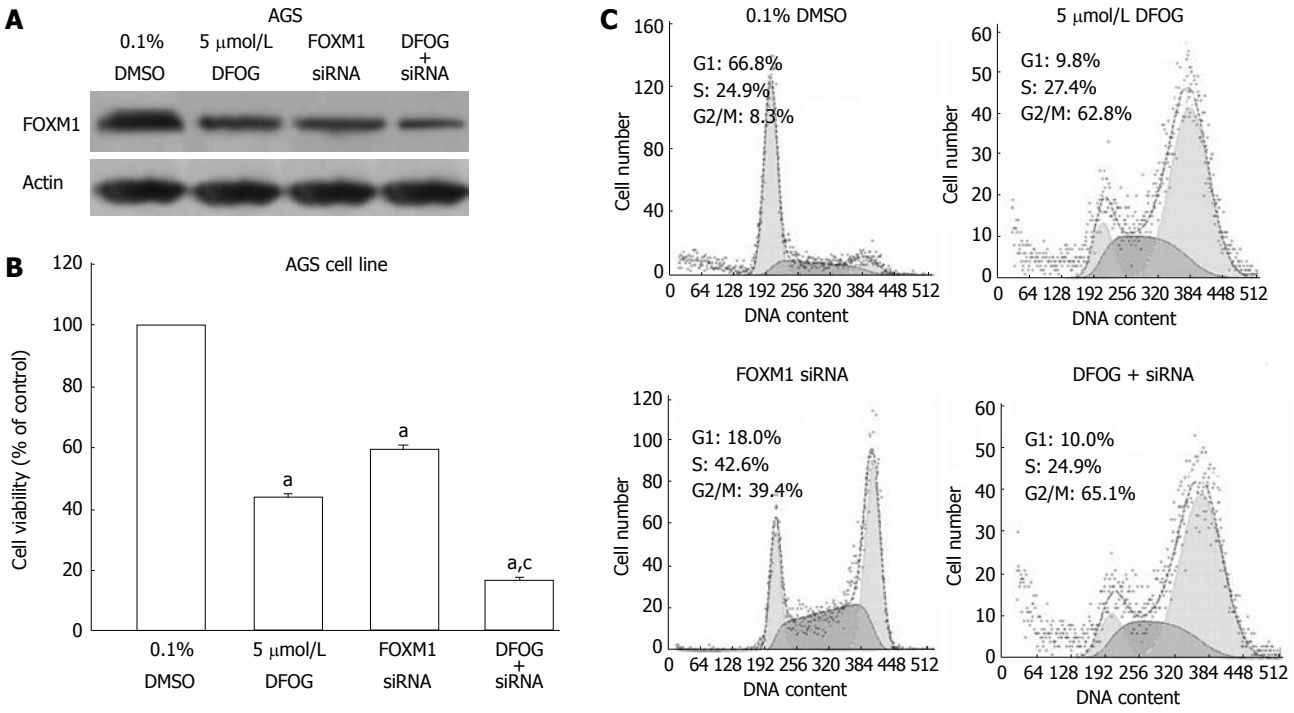


Figure 6 Forkhead box M1 small interfering RNA enhances the down-regulation of forkhead box M1 protein expression by 7-difluoromethoxyl-5,4'-di-n-octylgenistein and effects of forkhead box M1 small interfering RNA transfection or 7-difluoromethoxyl-5,4'-di-n-octylgenistein in AGS cell line. A: Western blotting analysis; B: The cell viability inhibitory effects of 7-difluoro methoxyl-5,4'-di-n-octylgenistein (DFOG) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte trazolium bromide assay; C: Both on cell cycle distribution in AGS cells. $^{a}P < 0.05$ *vs* treatment with dimethyl sulfoxide (DMSO); $^{c}P < 0.05$ *vs* treatment with 5 μmol/L DFOG or forkhead box M1 (FOXM1) small interfering RNA (siRNA) alone.

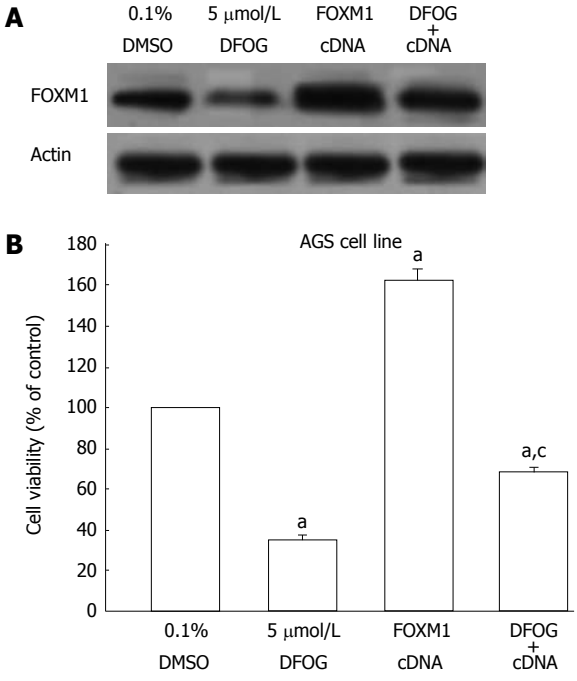


Figure 7 Forkhead box M1 cDNA transfection reduces the down-regulation of forkhead box M1 protein expression by 7-difluoromethoxyl-5,4'-di-n-octylgenistein in AGS cell line. A: Western blotting analysis; B: The cell viability inhibitory effects of 7-difluoro methoxyl-5,4'-di-n-octylgenistein (DFOG) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte trazolium bromide assay. $^{a}P < 0.05$ *vs* treatment with dimethyl sulfoxide (DMSO); $^{c}P < 0.05$ *vs* treatment with 5 μmol/L DFOG or forkhead box M1 (FOXM1) cDNA transfection alone.

Genistein has been found to induce G2 arrest and inhibit proliferation in a variety of cancer cell lines[28]. Because down-regulation of FOXM1 by DFOG reduced cell growth, we postulated that cell growth inhibition might result from cell cycle arrest in any specific phase of the cell cycle. We did find that FOXM1 down-regulation increased cell population in the G2/M phase and decreased cells in the G1/G0 and S phase. We also observed a marked reduction in cyclin B, Cdc25B and CDK1 expression in DFOG-treated cells. It is known that Cdc25B, cyclin B and CDK1 are the major effectors of the G2/M checkpoint response[29]. Diminished G1-S progression and growth rate was associated with increased expression of the *Cdk*I protein $p21^{CIP}$ and $p27^{KIP1}$, which have negative effects on cell cycle machinery by binding to various cyclin-Cdk complexes and inhibiting their activities[24,30]. In our study, the decreased cyclin B, cdc25B and Cdk1 and the increased expression of CdkI proteins, $p27^{KIP1}$, were strongly correlated with the altered cell cycle distribution phenotype and growth suppression. These results suggest that FOXM1 affects the gastric cancer cell cycle by regulating the expression levels of some cyclins (cyclin B), CDK (CDK1), CDK modulator (cdc25B) and CDK inhibitors ($p27^{KIP1}$).

In summary, we presented experimental evidence which strongly supports the role of DFOG, a novel synthetic genistein analogue, as an anti-tumor agent mediated through inactivation of FOXM1 signaling pathway.

However, further in-depth studies are needed to identify how DFOG could regulate the FOXM1 pathway, and to assess the anti-tumor activity mediated by the inactivation of FOXM1 either by genistein and DFOG or other synthetic compounds in pre-clinical animal models for the successful treatment of gastric cancer in the future. It is also tempting to speculate that the inactivation of FOXM1 together with the treatment of gastric tumor cells with conventional agents could be a useful strategy toward better treatment of human malignancies, especially gastric cancer.

COMMENTS

Background

Gastric cancer is one of the most common malignancies in the world and its incidence and mortality rank first in China. Recent data indicate that the mortality of gastric cancer in China tends to increase and it severely threatens the health and life of people. New therapeutic agents for this malignant disease are urgently needed.

Research frontiers

Genistein, 5,7,4'-trihydroxylisoflavone, a major component of soybean products, has been reported to possess anticancer activities. The authors synthesized a series of difluoromethoxylated genistein analogues and determined their protective effects against vascular endothelial cells. There have been few studies focusing on the anticancer effect of fluorinated genistein analogues.

Innovations and breakthroughs

The authors investigated whether the inhibitory effects of genistein and the novel synthetic genistein analogue 7-difluoromethoxyl-5, 4'-di-n-octylgenistein (DFOG) on the growth of gastric cancer cells could be attributed to modulation of Forkhead Box M1 (FOXM1) activity. It was found that DFOG and genistein down-regulated the FOXM1 expression and its downstream genes, including Cdc25B, CDK1, cyclin B and up-regulated $p27^{KIP1}$, resulting in the inhibition of gastric cancer cell growth.

Applications

These results provide strong evidences for the first time to support that FOXM1 is a rational target in gastric cancer, and the targeted inactivation of FOXM1, especially by genistein and its analogue DFOG, and provided new insight into strategies for better prevention of tumor progression and/or treatment of gastric cancer.

Terminology

Genistein, 5,7,4'-trihydroxylisoflavone, as one of the active constituents of soybean products, has been reported to possess anti-cancer activities. The DFOG is a novel synthetic genistein analogue.

Peer review

The novel anti-cancer drugs are mandatory to overcome cancer cells resistant to conventional anti-cancer drugs. In this paper the authors identified DFOG to be a good anti-cancer drug in gastric cancer cells. In addition, they also obtained the results to show FOXM1 as one of target molecules of DFOG. All data presented in this paper is acceptable.

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