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RESEARCH PAPER

FoxM1 is a Novel Target of a Natural Agent in Pancreatic Cancer

Zhiwei Wang • Aamir Ahmad • Sanjeev Banerjee • Asfar Azmi • Dejuan Kong • Yiwei Li • Fazlul H. Sarkar

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ABSTRACT

Purpose Pancreatic cancer remains the fourth most common cause of cancer-related death in the United States. Therefore, novel strategies for the prevention and/or treatment are urgently needed. Genistein has been found to be responsible for lowering the rate of pancreatic cancer. However, the molecular mechanisms by which genistein elicits its effects on pancreatic cancer cells has not been fully elucidated. Therefore, the purpose of the current study was to elucidate the anti-cancer mechanism(s) of genistein.

Methods Multiple molecular techniques, such as Real-time RT-PCR, Western blot analysis, invasion assay, immunofluorescence assay, gene transfection, MTT assay, and Histone/DNA ELISA, were used.

Results We found that genistein inhibited cell growth accompanied by induction of apoptosis with concomitant attenuation of FoxM1 and its downstream genes, such as survivin, cdc25a, MMP-9, and VEGF, resulting in the inhibition of pancreatic cancer cell invasion. We also found that down-regulation of FoxM1 by siRNA prior to genistein treatment resulted in enhanced cell growth inhibition and induction of apoptosis.

Conclusion This is the first report showing the molecular role of FoxM1 in mediating the biological effects of genistein in pancreatic cancer cells, suggesting that FoxM1 could be a novel target for the treatment of pancreatic cancer.

KEY WORDS FoxM1 · genistein · invasion · pancreatic cancer · proliferation

Z. Wang • A. Ahmad • S. Banerjee • A. Azmi • D. Kong • Y. Li • F. H. Sarkar (✉)
Department of Pathology, Karmanos Cancer Institute,
Wayne State University School of Medicine,
740 HWCRC, 4100 JohnR Street,
Detroit, Michigan 48201, USA
e-mail: fsarkar@med.wayne.edu

ABBREVIATIONS

ELISA	Enzyme-linked Immunosorbent Assay
FoxM1	Forkhead box protein M1
MMPs	Matrix metalloproteinases
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
RT-PCR	Reverse transcription-PCR
VEGF	Vascular endothelial growth factor

INTRODUCTION

Pancreatic cancer is one of the most common cancers and is the fourth leading cause of cancer-related death in the United States (1). Approximately, 42,500 people are expected to be diagnosed with pancreatic cancer, and about 35,250 people are expected to die from this disease in the United States in 2009. Presently, for all stages combined, the 1-year survival rate is only 20%, and the 5-year survival rate is less than 5% (1). In contrast, Asians who consume a diet high in soy products have a relatively low incidence of and mortality due to pancreatic cancer, suggesting that a high intake of soy products may protect people against pancreatic cancer (2). Genistein, a natural isoflavonoid found in soybean products, has been believed to be a chemopreventive agent because it has been reported to be associated with lower incidence of pancreatic cancer (3). It has been found that genistein can inhibit the growth of various cancer cell lines both *in vitro* and *in vivo* (4–6). However, the molecular mechanisms by which genistein elicits its effects on pancreatic cancer cells has not been fully elucidated, and it is an emerging area of research.

Pancreatic cancer has been shown to have activated Forkhead box protein M1 (FoxM1) signaling pathway (7). 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 (8,9). Studies have shown that FoxM1 signaling plays important roles in cellular developmental pathways, and thus, activation of FoxM1 signaling has been reported to be associated with carcinogenesis (9). FoxM1 signaling network is frequently up-regulated in human malignancies, including lung cancer, glioblastomas, prostate cancer, basal cell carcinomas, hepatocellular carcinoma, breast cancer and pancreatic cancer (10–14). Moreover, FoxM1 has been shown to regulate transcription of cell cycle genes, including cdc25A, cdc25B, cyclin B, cyclin D1, $p21^{cip1}$ and $p27^{kip1}$ (9,15–19). Furthermore, it has been shown that higher expression of FoxM1 was associated with poor prognosis of breast cancer patients (20). Recently, it has been reported that FoxM1b expression could serve as an independent predictor of poor survival in gastric cancer (21). These results suggest that FoxM1 may have a crucial role in the development and progression of human cancers including pancreatic cancer. Therefore, it is believed that the targeted inactivation of FoxM1 could represent a promising strategy for the development of novel and selective anti-cancer therapies, and it would be highly significant for pancreatic cancer because there is no curative treatment for this disease.

In the current study, we investigated whether genistein-induced inhibition of pancreatic cancer cell growth could be attributed to FoxM1 activity. Moreover, since cell invasion is an important process that is involved in tumor development and metastasis and because FoxM1 signaling is known to control this process, we also examined the effect of genistein on the processes of invasion of pancreatic cancer cells. We found that genistein down-regulated the FoxM1 expression and its downstream genes, including survivin, cdc25a, MMP-9 and VEGF, resulting in the inhibition of pancreatic cancer cell growth and invasion. These results provide supportive evidence for the first time that FoxM1 is a legitimate target in pancreatic cancer and that the targeted inactivation of FoxM1, especially by natural agents as shown here, would be highly relevant for designing novel strategies for the prevention of tumor progression and/or treatment of pancreatic cancer.

MATERIALS AND METHODS

Cell Culture and Experimental Reagents

Human pancreatic cancer cell lines BxPC-3, HPAC, MIA PaCa-2, and PANC-28 were used in this study. Human pancreatic ductal epithelial (HPDE) cells were obtained from University of Texas M. D. Anderson Cancer Center. All cells were cultured in RPMI-1640 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Primary antibodies for FoxM1, MMP-9, VEGF, Survivin and cdc25A were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All secondary antibodies were obtained from Pierce (Rockford, IL). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA). 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). Genistein (Toronto Research Chemicals, North York, ON, Canada) was dissolved in 0.1 M Na_2CO_3 to make a 10 mM stock solution and was added directly to the media at different concentrations.

Cell Growth Inhibition Studies by MTT Assay

Pancreatic cancer cells (5×10^3) were seeded in a 96-well culture plate and replaced next day with fresh medium containing genistein (0–100 μM) diluted from stock solution. After 72 h, MTT solution was added and incubated further for 2 h. MTT formazan formed by metabolically viable cells was dissolved in isopropanol, and absorbance was measured at 595 nm on a plate reader (TECAN) as described earlier (22).

Clonogenic Assay

To test the survival of cells treated with genistein, BxPC-3 and MIA PaCa-2 cells were plated (50,000–100,000 per well) in a six-well plate and incubated overnight at 37°C. After 72 h exposure to various concentrations of genistein, the cells were performed clonogenic assay as described earlier (23).

Histone/DNA Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of Apoptosis

The Cell Death Detection ELISA Kit was used for assessing apoptosis according to the manufacturer's protocol. Briefly, after genistein treatment, the cells were lysed, and the cell lysates were overlaid and incubated in microtiter plate modules coated with anti-histone antibody for detection of apoptosis as described earlier (23).

Real-Time Reverse Transcription-PCR Analysis for Gene Expression Studies

The total RNA from treatment cells was isolated by Trizol (Invitrogen, Carlsbad, CA) and purified by RNeasy Mini Kit and RNase-free DNase Set (QIAGEN, Valencia, CA) according to the manufacturer's protocols. The primers used in the PCR reaction, and real-time PCR amplications were performed as described earlier (7).

Western Blot Analysis

Cells were lysed in lysis buffer by incubating for 20 min at 4°C. The protein concentration was determined using the Bio-Rad assay system (Bio-Rad, Hercules, CA). Total proteins were fractionated using SDS-PAGE and transferred onto nitrocellulose membrane for Western blotting as described earlier (22).

Immunofluorescence Microscopy

MIA PaCa-2 Cells were fixed with 4% paraformaldehyde for 10 min, permeabilized in 0.5% Triton X-100 for 10 min, and incubated in PBS and 10% goat serum blocking solution for 1 h. The cells were incubated for 2 h with anti-FoxM1 in 5% goat serum and were stained for 1 h with Alexa Fluor 594-conjugated secondary antibody (1:500). The slides were mounted with mounting medium containing antifade reagent and 4,6-diamidino-2-phenylindole. Cells were viewed under fluorescence microscope, and images were analyzed using Advanced Sport software (Diagnostic Instruments, Sterling Heights, MI).

MMP-9 Activity Assay

The BxPC-3 or MIA PaCa-2 cells were seeded in 6-well plates and incubated at 37°C. After 24 h, the complete medium was removed, and the cells were washed with serum-free medium. The cells were then incubated in serum-free medium supplemented with genistein for 72 h. MMP-9 activity in the medium was detected using Human MMP-9 Activity Assay Kit (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's protocol.

VEGF Assay

The BxPC-3 or MIA PaCa-2 cells were seeded in 6-well plates and incubated at 37°C. After 24 h, the cells were incubated in medium supplemented with genistein for 72 h. The cell culture supernatant was harvested, and cell count was performed after trypsinization. The supernatant was assayed using commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN).

Cell Invasion Assay

The invasive activity of the BxPC-3 or MIA PaCa-2 cells after genistein treatment was tested using the BD BioCoat Tumor Invasion Assay System (BD Biosciences, Bedford, MA). Briefly, cells (5×10^4) with serum-free medium were seeded into the upper chamber of the system. Bottom wells in the system were filled with complete medium. After 24 h of incubation, the cells in the upper chamber were removed, and the cells that had invaded through matrigel matrix membrane were stained with 4 μg/ml Calcein AM for 1 h. The fluorescence of the invaded cells was read at excitation/emission wavelengths of 530/590 nm. These fluorescently labeled invasive cells were also photographed under a fluorescent microscope.

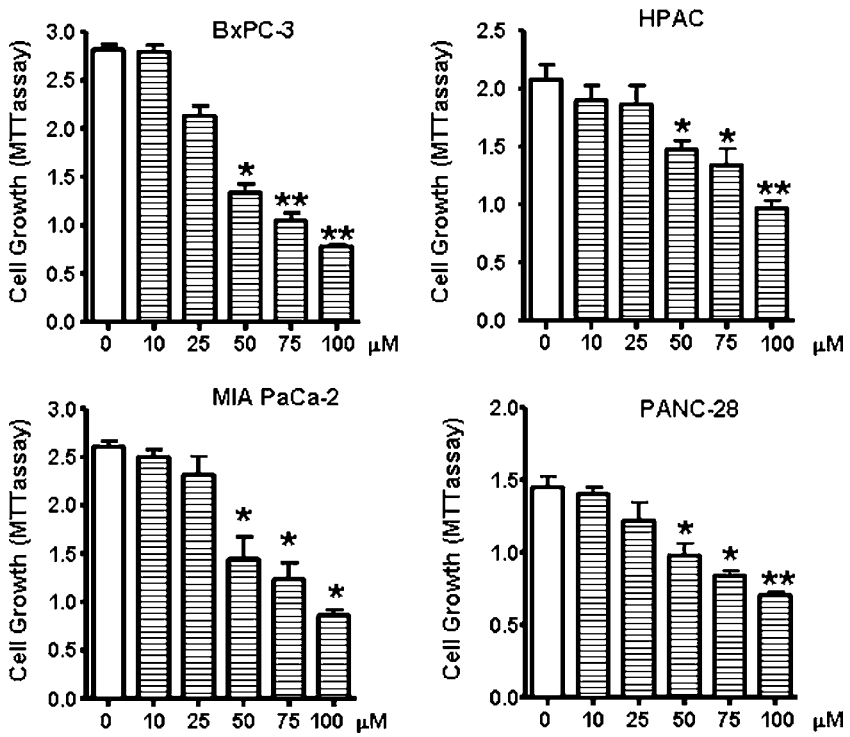

Fig. 1 Effect of genistein on pancreatic cancer cell growth. Cells were seeded in 96-well plates at 5,000 cells per well and treated with varied concentrations of genistein for 72 h. After treatment, MTT solution was added and incubated further for 2 h. MTT formazan formed by metabolically viable cells was dissolved in isopropanol, and absorbance was measured at 595 nm on a plate reader (TECAN). Each value represents the mean ± SD ($n=6$) of three independent experiments. *$P<0.05$, **$P<0.01$, compared to the control.

Plasmids and Transfections

FoxM1 siRNA and siRNA control were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The FoxM1 cDNA plasmid was purchased from OriGene Technologies Inc. (Rockville MD). Human pancreatic cancer cells were transfected with FoxM1 siRNA and cDNA, respectively, using Lipofectamine 2000 as described earlier (7).

Densitometric and Statistical Analysis

The cell growth inhibition after transfection was statistically evaluated using GraphPad StatMate software (GraphPad Software, Inc., San Diego, CA). Comparisons were made between control and treatment. $P<0.05$ was used to indicate statistical significance.

RESULTS

Genistein Induced Cell Growth Inhibition of Pancreatic Cancer Cells

First, we examined the growth inhibitory effects of genistein using the MTT assay in four human pancreatic cancer cell lines, such as BxPC-3, HPAC, MIA PaCa-2, and PANC-28. As shown in Fig. 1, the treatment of pancreatic cancer cells for 72 h with 10, 25, 50, 75 and 100 μM of genistein resulted in cell growth inhibition in a dose-dependent manner in all four pancreatic cancer cell lines (Fig. 1). However, the growth inhibition was highly significant in greater than 25 μM of genistein. In contrast, treatment of human pancreatic ductal epithelial cells resulted in minimal loss of viable cell when exposed to identical concentrations of genistein for a similar period (data not shown). In order to confirm our results, we have also tested the effects of genistein on cell viability by clonogenic assay as shown below.

Inhibition of Cell Growth/Survival by Clonogenic Assay

Since clonogenic assay is the "gold standard" for determining the transformed and tumorigenic potential of cancer cells, we assessed the effect of genistein on cell viability by clonogenic assay. Genistein treatment resulted in a significant inhibition of colony formation of BxPC-3 and MIA PaCa-2 cells when compared with control (Fig. 2A). Overall, the results from clonogenic assay was consistent with the MTT data as shown in Fig. 1, suggesting that genistein inhibits the growth of pancreatic cancer cells.

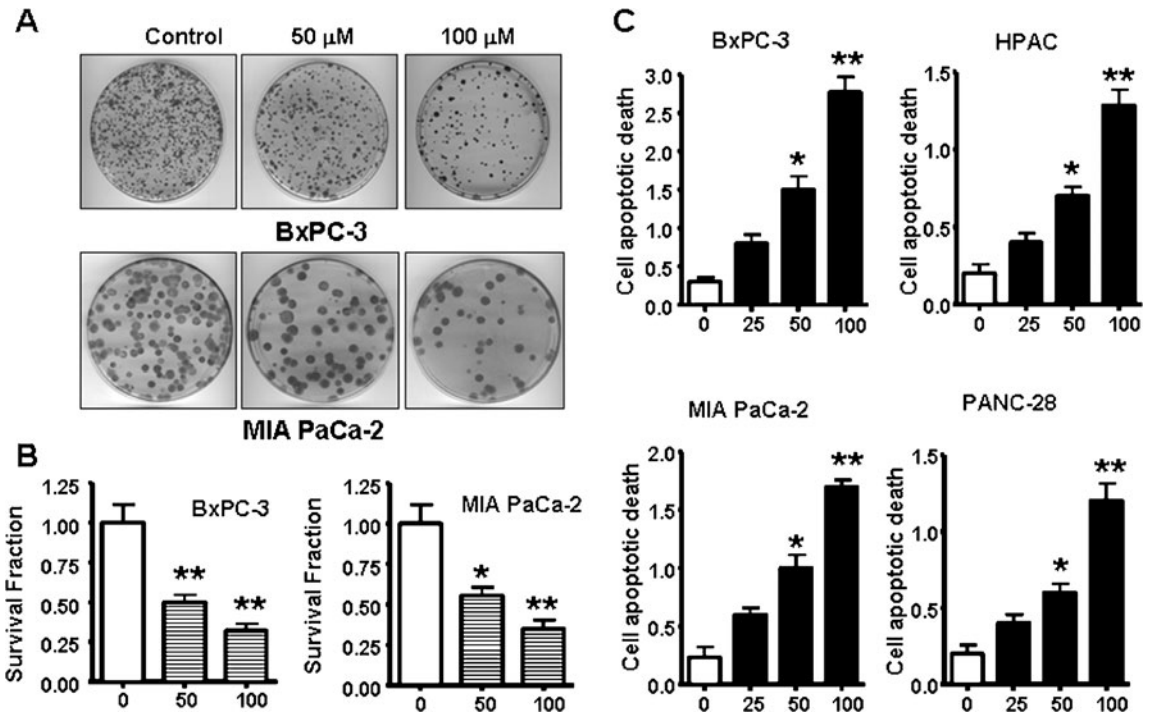

Fig. 2 Effect of genistein on pancreatic cancer cell growth and apoptosis. **A** Cell survival of human pancreatic cancer cell lines BxPC-3 and MIA PaCa-2. Cells treated with varied concentrations of genistein for 72 h were evaluated by the clonogenic assay. Photomicrographic difference in colony formation in cells untreated and treated with genistein are shown. **B** There was a significant reduction in the colony formation in BxPC-3 and MIA PaCa-2 cells treated with genistein compared with control cells. *P* values represent comparisons between cells treated by genistein and control using the paired *t*-test. $^{*}P<0.05$, $^{**}P<0.01$, compared to the control. **C** Cell death assay for measuring apoptosis induced by genistein. BxPC-3, HPAC, MIA PaCa-2 and PANC-28 cells were cultured in RPMI containing 5% FBS and exposed to different dose genistein for 72 h. Apoptosis was measured by Histone DNA ELISA. Values are reported as mean ± SD. $^{*}P<0.05$, $^{**}P<0.01$, compared to the control.

Further, to assess whether the loss of cell viability could in part be due to the induction of apoptosis, we evaluated the effects of genistein treatment on apoptosis using histone-DNA ELISA.

Genistein Induced Apoptosis in Pancreatic Cancer Cell Lines

BxPC-3, HPAC, MIA PaCa-2, and PANC-28 cells were treated with 25, 50 and 100 μM genistein for 72 h. After treatment, the degree of apoptosis was measured in all four cell lines. The induction of apoptosis was found to be dose-dependent (Fig. 2C). These results provided convincing data showing that genistein could induce apoptosis in pancreatic cancer cells. In order to further understand the molecular mechanism involved in genistein-induced apoptosis of pancreatic cancer cells, alterations in the cell survival pathway genes were investigated. Our previous studies have shown that FoxM1 signaling is over-expressed in pancreatic cancer and is involved in the inhibition of apoptosis, potentiation of cell growth, and angiogenesis, and thus considered as a putative target for drug development (7). Therefore, we investigated whether genistein could regulate FoxM1 signaling pathways using molecular and mechanistic approaches in this study.

Down-regulation of the FoxM1 Expression by Genistein

The baseline expression of FoxM1 was determined using real-time RT-PCR and Western blotting analysis, respectively. The results showed that FoxM1 was frequently but

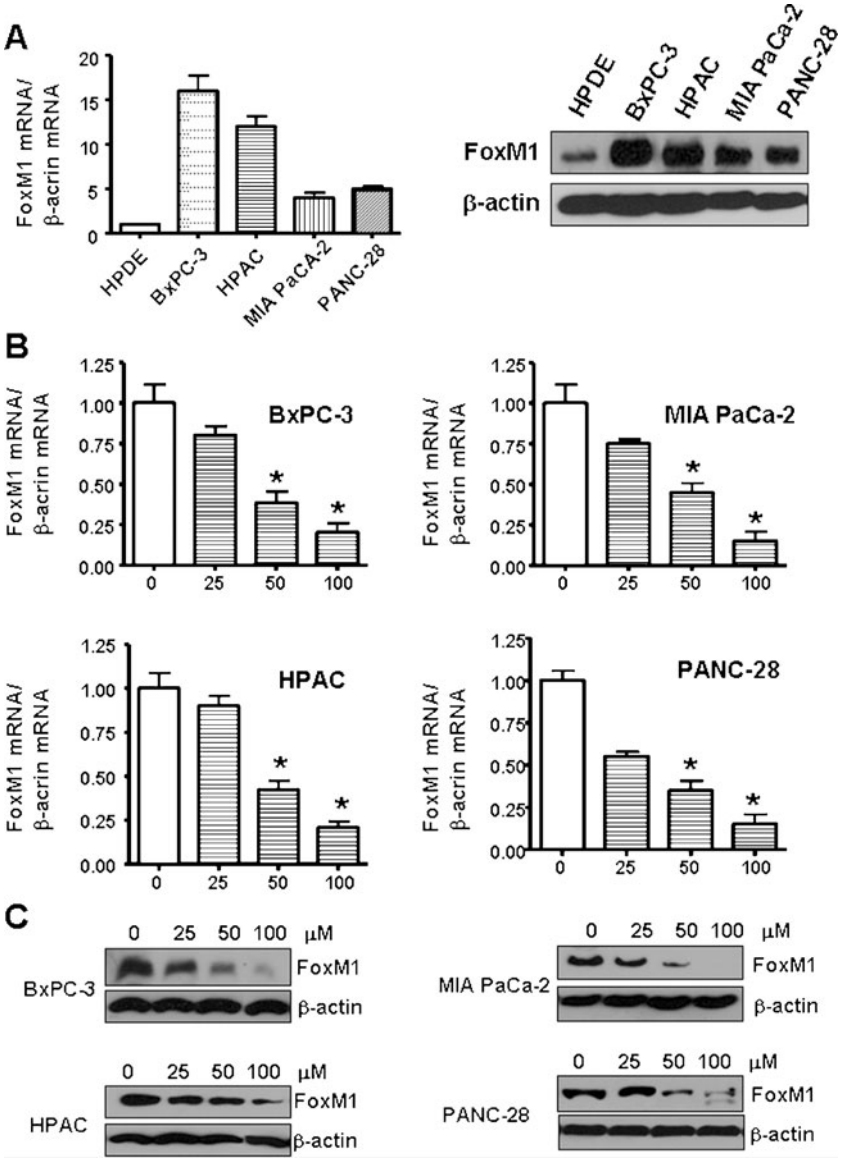

Fig. 3 Inhibition of FoxM1 expression by genistein. **A** The baseline expression of FoxM1 was determined among the panel of pancreatic cancer cell lines using real-time RT-PCR and Western Blotting analysis, respectively. FoxM1 was over-expressed in different human pancreatic cancer cell lines compared to "so-called" normal human pancreatic ductal epithelial (HPDE) cells. **B** The FoxM1 mRNA was detected by real-time RT-PCR in pancreatic cancer cells treated with varied concentrations of genistein for 72 h. **C** The FoxM1 protein was detected by Western Blotting analysis in pancreatic cancer cells treated with varied concentrations of genistein for 72 h.

differentially over-expressed in different human pancreatic cancer cell lines compared to HPDE cells, the "so-called" normal pancreatic ductal epithelial cells (Fig. 3A). FoxM1 mRNA and protein expression in pancreatic cancer cell lines treated with genistein for 72 h were assessed. We found that FoxM1 was down-regulated by genistein in all four cell lines (Fig. 3B, C). In order to confirm our results, we also did immunofluorescent staining. Indeed, we observed a lower level of FoxM1 protein in genistein-treated cells (Fig. 4A). To further confirm the effect on FoxM1 by genistein, we also assessed the expression of FoxM1 downstream target genes in BxPC-3 and MIA PaCa-2 cells after genistein treatment as shown below.

Genistein Inhibited the Expression of FoxM1 Downstream Target Genes

It is well known that FoxM1 has several downstream target genes, such as Survivin, cdc25a, MMP-9, and VEGF. To determine the expression of these genes, we used real-time RT-PCR and Western blotting analysis. We found that genistein inhibited the expression of survivin, cdc25a, MMP-9 and VEGF at mRNA and protein levels in BxPC-3 and MIA PaCa-2 cells (Fig. 4).

Genistein Decreased MMP-9 Activity

Our previous study has shown that MMP-9 was regulated by FoxM1 in pancreatic cancer (7), and since we found that genistein can down-regulate the expression of FoxM1, we tested whether genistein could decrease MMP-9 activity as assessed by ELISA. We found about 2–3-fold decrease in the activity of MMP-9 in genistein-treated pancreatic cancer cell lines (Fig. 5A). These results are consistent with our observation on the down-regulation of FoxM1, which indeed leads to the down-regulation of both MMP-9 levels and its activity as reported earlier (7).

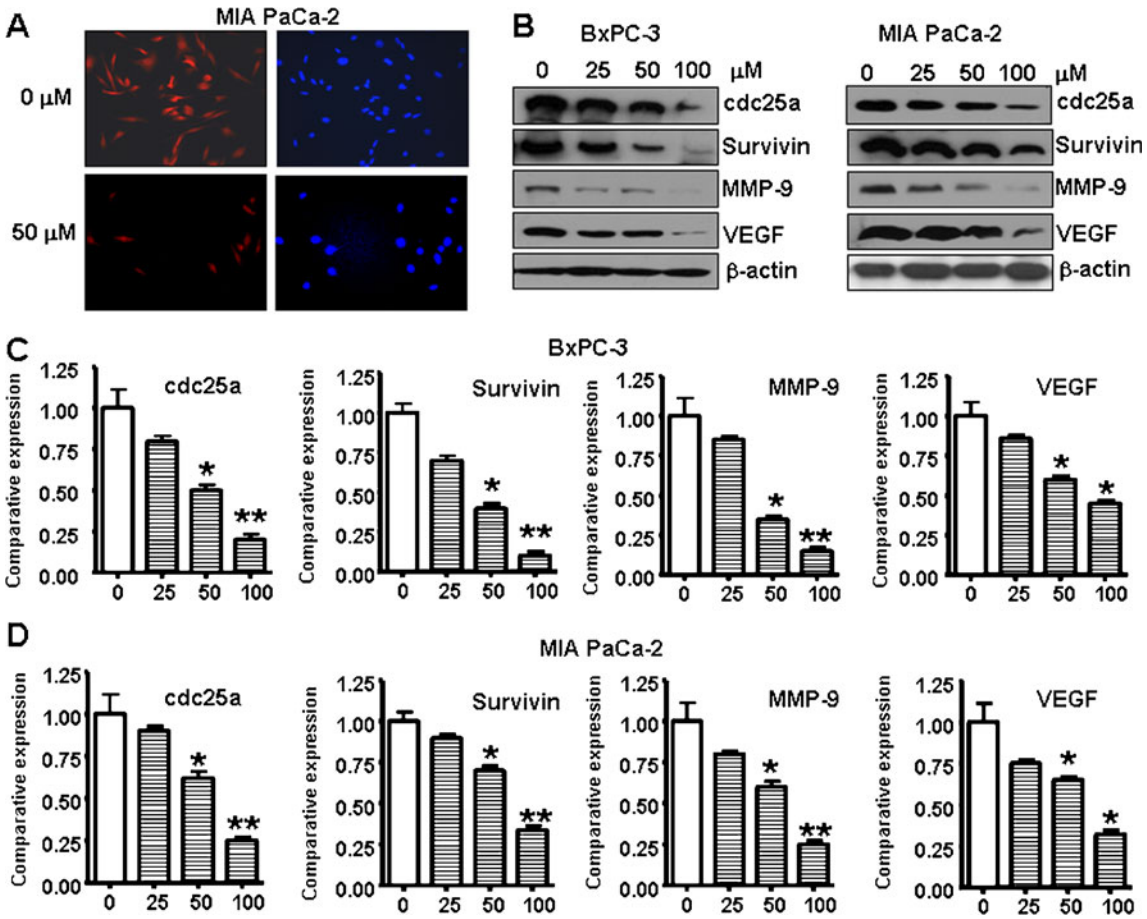

Fig. 4 Inhibition of expression of FoxM1 target genes by genistein. **A** The MIA PaCa-2 pancreatic cancer cells treated with 50 μM genistein for 72 h were subjected to immunofluorescent staining using anti-FoxM1 antibody. **B** The expression of FoxM1 target genes was detected by Western blotting analysis in pancreatic cancer cells treated with varied concentrations of genistein for 72 h. We found that genistein inhibited the expression of survivin, cdc25a, MMP-9 and VEGF in BxPC-3 and MIA PaCa-2 cells. **C**–**D** The FoxM1 target gene mRNA levels were detected by real-time RT-PCR in pancreatic cancer cells treated with varied concentrations of genistein for 72 h. Genistein inhibited the transcription levels of survivin, cdc25a, MMP-9 and VEGF in BxPC-3 and MIA PaCa-2 cells.

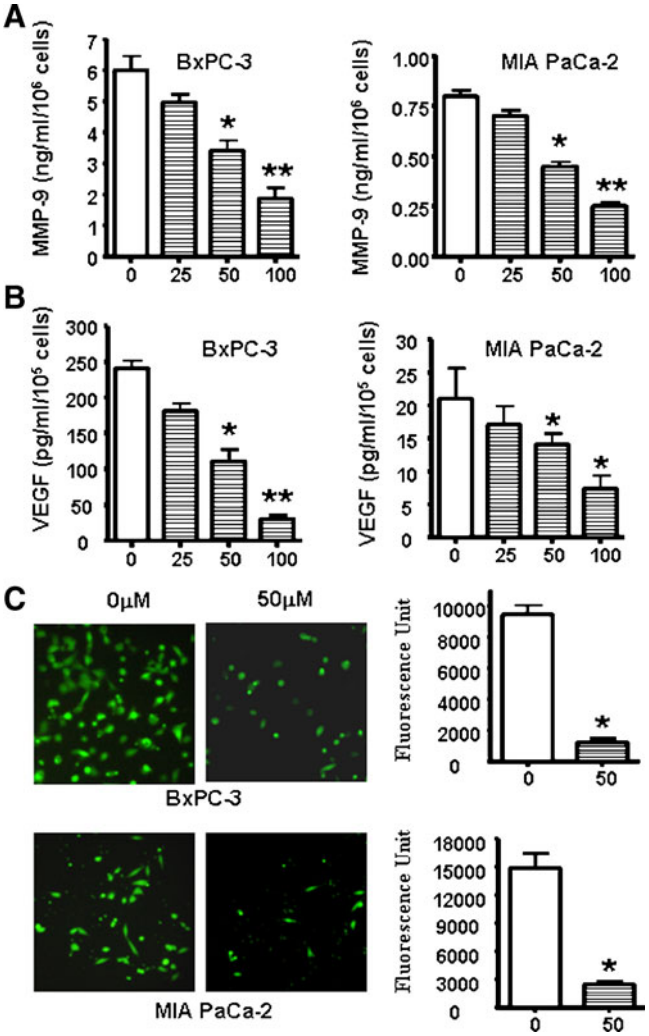

Fig. 5 Genistein inhibited the MMP-9 and VEGF activities, thus decreasing pancreatic cancer cell invasion. **A** Genistein inhibited the activity of MMP-9 in BxPC-3 and MIA PaCa-2 pancreatic cancer cells. **B** Genistein inhibited the activity of VEGF. VEGF activity assay showing that VEGF level in the culture medium was inhibited by genistein. **C** *Left panel*, invasion assay showing that genistein-treated cells resulted in low penetration through the Matrigel-coated membrane, compared with control cells; *right panel*, value of fluorescence from the invaded cells, and these values indicate the comparative amount of invaded cells.

Genistein Reduced VEGF Secretion

To further investigate whether genistein has any effect on VEGF, whose expression is transcriptionally regulated by FoxM1 (7,21,24), ELISA was performed to examine the activity of VEGF. We found that genistein could lead to a decrease in the levels of VEGF secreted in the culture medium (Fig. 5B). Since inhibition of MMP-9 and VEGF could result in the inhibition of invasion and angiogenesis, we tested the effects of genistein on invasive characteristics of pancreatic cancer cells.

Genistein Decreased Pancreatic Cancer Cell Invasion

Because genistein inhibited the expression and activity of MMP-9 and VEGF, we tested the effects of genistein on cancer cell invasion, and the results are shown in Fig. 5C, documenting that 50 μM genistein led to decreased penetration of pancreatic cancer cells through the matrigel-coated membrane compared with the control cells. Further quantitation of the value of fluorescence from the invaded pancreatic cancer cells was significantly decreased compared with that of control cells (Fig. 5C). The above results prompted us to assess the mechanistic basis of whether FoxM1 down-regulation by genistein could be responsible for such results or not; thus, the following experiments were conducted.

Down-regulation of FoxM1 Expression by siRNA Potentiates Genistein-Induced Cell Growth Inhibition and Apoptosis

Down-regulation of FoxM1 by siRNA transfection showed less expression of FoxM1 protein, as confirmed by Western blotting (Fig. 6A). We have also found that the down-regulation of FoxM1 expression significantly inhibited cell growth induced by genistein (Fig. 6B). Genistein plus FoxM1 siRNA inhibited cell growth to a greater degree compared to genistein alone. Moreover, FoxM1 siRNA-transfected MIA PaCa-2 cells were significantly more sensitive to spontaneous and genistein-induced apoptosis (Fig. 6B). These results provide some molecular evidence suggesting the genistein-induced effects are indeed mediated via inactivation of FoxM1, which is further supported by the following data.

Over-Expression of FoxM1 by cDNA Transfection Reduced Genistein-Induced Cell Growth Inhibition and Apoptosis

Up-regulation of FoxM1 by cDNA transfection showed over-expression of FoxM1 protein as confirmed by Western blot analysis (Fig. 6C), and this over-expression in FoxM1 rescued genistein-induced cell growth inhibition and abrogated genistein-induced apoptosis to a certain degree (Fig. 6D). These results provide mechanistic evidence suggesting that genistein inhibited cell growth and induction of apoptosis is in part due to inactivation of FoxM1 signaling pathway in pancreatic cancer cells.

DISCUSSION

FoxM1 signaling has been demonstrated to maintain a balance between cell proliferation, differentiation and

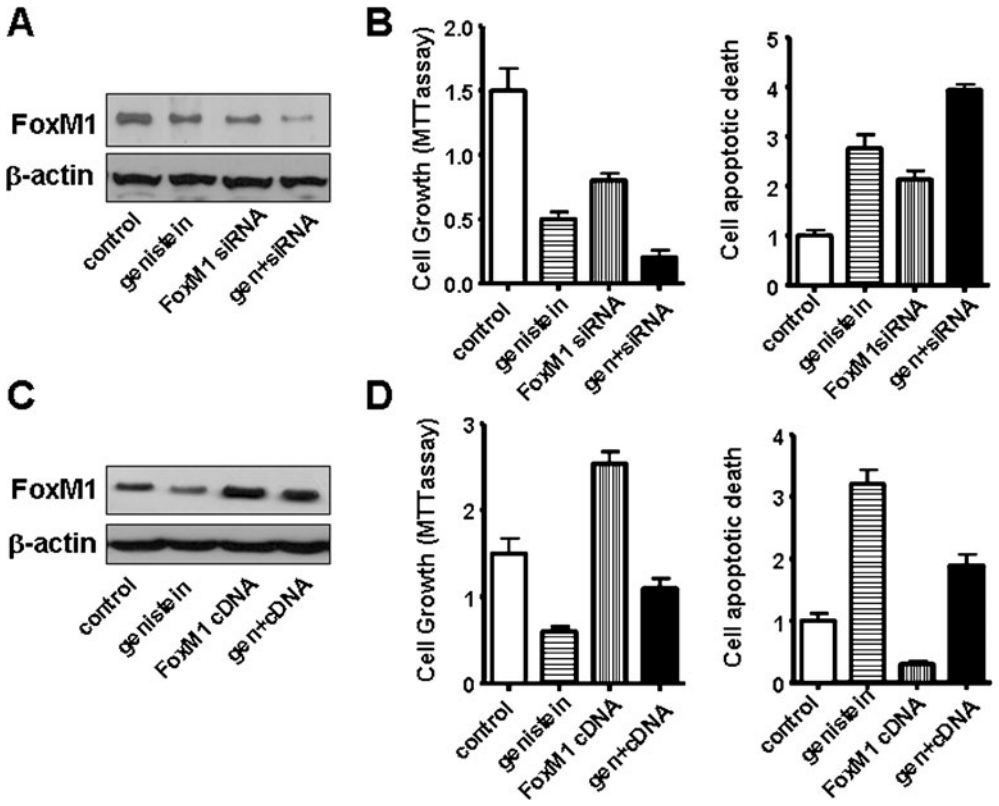

Fig. 6 Down-regulation of FoxM1 by siRNA promotes genistein-induced cell growth inhibition and apoptosis in MIA PaCa-2 cells. gen+siRNA: 50 μM genistein + FoxM1 siRNA; gen+cDNA: 50 μM genistein + FoxM1 cDNA. **A** The expression of FoxM1 was detected by Western blotting to check the FoxM1 siRNA transfection efficacy. **B** *Left panel*, down-regulation of FoxM1 by siRNA significantly inhibited MIA PaCa-2 cell growth. Genistein plus FoxM1 siRNA inhibited cell growth to a greater degree compared to genistein alone. *Right panel*, down-regulation of FoxM1 expression significantly increased apoptosis induced by genistein. FoxM1 siRNA transfected cells were significantly more sensitive to spontaneous and genistein-induced apoptosis. **C** The expression of FoxM1 was detected by Western blotting to check the FoxM1 cDNA plasmid transfection efficacy. **D** Over-expression of FoxM1 expression significantly promoted cell growth. Over-expression of FoxM1 rescued cells from genistein-induced cell growth inhibition. Over-expression of FoxM1 by FoxM1 cDNA transfection abrogated genistein-induced apoptosis to a certain degree.

apoptosis, suggesting that abnormal activation of FoxM1 gene is one of the "hallmarks" of human malignancies (9). Many studies have shown over-expression of FoxM1 gene in human cancer cells and tissues, including pancreatic cancer (7,10,12,14,25–27), and this emerging evidence suggests that inactivation of FoxM1 by novel approaches could have a significant impact in cancer therapy. Thus, it is conceivable that the development of agents that will target FoxM1 is likely to have significant therapeutic impacts on the treatment of human cancers, including pancreatic cancer. Given the emerging data describing the important roles of FoxM1 in the progression of human cancers, Radhakrishnan *et al.* have rightly pointed out that it should be possible to target multiple facets of tumorigenesis by inhibiting only this single transcription factor (28), which by itself is a strong statement but also points towards further in-depth molecular investigations.

In recent years, many investigators have reported the role of several FoxM1 inhibitors. For example, FoxM1 could be down-regulated by some drugs, namely antibiotic thiazole compound Siomycin A, thiostrepton, and EGFR inhibitor Gefitinib (28,29). We have also found that docetaxel (taxotere) alone or in combination with estramustine down-regulated the expression of FoxM1 in prostate cancer leading to cell growth inhibition and induction of apoptosis (30,31). Recently, Bhat *et al.* found that proteasome inhibitors, such as MG115, MG132 and bortezomib could inhibit FoxM1 transcriptional activity and its expression, and interestingly, FoxM1 inhibitors, including Siomycin A, thiostrepton, could serve as proteasome inhibitors (11). These observations clearly suggest that chemical compounds that target FoxM1 may act as anti-cancer drugs. Studies from our laboratory have shown that chemopreventive agents, such as 3,3′-diindolylmethane, may inhibit FoxM1 activation in breast cancer cells, leading to apoptotic cell death (32), suggesting that chemopreventive agents could be useful for the inhibition of FoxM1 and are likely to have beneficial effects toward cancer therapy because chemopreventive agents are typically known to be non-toxic, unlike many synthetic compounds described above.

In the current study, we used four human pancreatic cancer cell lines, BxPC-3, HPAC, PANC-28 and MIA PaCa-2, which have high expression of FoxM1, and we found that genistein could induce significant growth inhibition in these cells, as documented by both MTT and clonogenic assay. Moreover, genistein also induced apoptotic cell death in pancreatic cancer cell lines. Furthermore, genistein inhibited the expression of FoxM1 and its target genes. Therefore, genistein-mediated cell growth inhibition could be partly mediated via inactivation of FoxM1 activity. Indeed, we found that down-regulation of FoxM1 by siRNA together with genistein treatment inhibited cell growth and induced apoptosis to a greater degree in pancreatic cancer cells compared to genistein treatment alone. In view of these findings, we strongly believe that inactivation of FoxM1 by genistein results in the down-regulation of its target genes, which are believed to be mechanistically linked with genistein-induced cell growth inhibition, induction of apoptosis and cell invasion.

In summary, we presented experimental evidence which strongly supports the role of genistein as an anti-tumor agent mediated through inactivation of FoxM1 signaling pathway. However, further in-depth studies are needed to ascertain how genistein could regulate the FoxM1 pathway, and further studies are also needed to assess the anti-tumor activity mediated by the inactivation of FoxM1 either by genistein or other synthetic compounds in pre-clinical animal models for the successful treatment of pancreatic cancer in the future. It is also tempting to speculate that the inactivation of FoxM1 together with the treatment of pancreatic tumor cells with conventional agents could be a useful strategy toward better treatment of human malignancies, especially pancreatic cancer.

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