BRG1 promotes chemoresistance of pancreatic cancer cells through crosstalking with Akt signalling

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Abstract Gemcitabine is a standard chemotherapeutic agent for locally advanced and metastatic pancreatic cancer. However, the chemoresistance of pancreatic cancer is the major barrier to efficient chemotherapy. Here, we reported that BRG1, a chromatin modulator, was exclusively overexpressed in human pancreatic ductal adenocarcinoma tissues. BRG1 knockdown inhibited PANC-1 and MIA PaCa-2 cell growth in vitro and in vivo, reduced the phosphorylation/activation of Akt and p21cip/waf, enhanced intrinsic and gemcitabine induced apoptosis and attenuated gemcitabine-induced downregulation of E-cadherin. Moreover, by establishing acquired chemoresistance of MIA PaCa-2 cells in vitro, we found that BRG1 knockdown effectively reversed the chemoresistance to gemcitabine. Surprisingly, inhibiting Akt phosphorylation resulted in BRG1 suppression in pancreatic cancer cells, indicating BRG1 as a new downstream target of Akt signalling. Taken together, our findings suggest that BRG1 promotes both intrinsic and acquired chemoresistance of pancreatic cancer cells, and BRG1 crosstalks with Akt signalling to form a positive feedback loop to promote pancreatic cancer development.

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1. Introduction

Pancreatic cancer (PC) is a highly aggressive malignancy with a 5-year survival rate less than 6% [1]. Despite recent advances in the diagnosis, surgery and chemotherapy of PC, the improvement in the prognosis of PC patients is still despairing. The high mortality of PC is largely due to the lack of early diagnosis markers, rapid...
lymphatic metastasis and aggressive vascular invasion. As a consequence, 80–85% of PC patients are diagnosed at advanced stage when surgical resection is not feasible [2]. Chemotherapy is a conventional regimen for unresectable case. The ribonucleotide reductase inhibitor gemcitabine has been used as a first-line chemotherapeutic agent for locally advanced and metastatic PC. However, treatment with gemcitabine merely results in a median survival of 5.65 months and 1-year survival rate of 18% [3]. The main reason for chemotherapy failure lies in the intrinsic and acquired chemoresistance of PC cells [4]. Therefore, interfering with particular genes that contribute to chemoresistance could provide an opportunity to overcome the chemoresistance of PC cells.

In mammalian, SWI/SNF complex is a chromatin modulator that mobilises nucleosome through ejecting and inserting histone octamer in an ATP-dependent manner. SWI/SNF complex comprises an ATPase subunits, either of BRM (also known as SMARCA2), or BRG1 (also known as SMARCA4). As a key component of SWI/SNF complex, BRG1 functions to control cell proliferation, cell lineage specification and to maintain cell pluripotency during early embryonic development [5]. Recent evidences suggest that BRG1 exhibits both tumour suppressing and tumour promoting functions, depending on the type of cancer [6]. Clinical studies showed that BRG1 expression was decreased in intraductal papillary mucinous neoplasm (IPMN) lesions but BRG1 expression level was high and correlated with worse clinicopathological features of PC [7,8]. A recent study showed that loss of BRG1 cooperates with oncogenic Kras to promote human IPMN lesions and the progression to ductal adenocarcinoma (PDA) [9]. These data indicate that BRG1 could inhibit or promote PC. In view of these controversies, in this study we aimed to investigate the potential role of BRG1 in regulating PC chemosensitivity. Our results demonstrated that BRG1 promoted chemoresistance of PC cells to gemcitabine via the activation of Akt and p21cip/waf, and the downregulation of E-cadherin expression.

2. Materials and methods

2.1. Reagents

Oxaliplatin, gemcitabine and 5-fluorouracil (5-FU) were obtained from Sanofi-aventis and Lilly. Foetal bovine serum (FBS) was from Gibeco technology. Crystal violet, dimethylsulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma–Aldrich, G418 was purchased from AMRESCO and bicinchoninic acid (BCA) protein assay reagent was from Thermo Fisher. Lipofectamine™2000 was from Invitrogen. FITC Annexin V Apoptosis Detection Kit I was from BD Pharmingen™. Antibodies for BRG1, BRG1, p21 (phospho T145), E-cadherin and vimentin were purchased from Abcam. Akt (pSer473) antibody was purchased from EPIT MICS®. Akt antibody was purchased from Cell signalling.

2.2. Cell culture

The pancreatic cancer cell lines (PANC-1, MIA PaCa-2, BxPC-3, Capan-1, T3M4 and colo357) were received from the American Type Culture Collection (ATCC). BxPC-3 cells were grown in RPMI-1640 containing 10% FBS and passage at a 1:4 ratio. PANC-1, MIA PaCa-2, T3M4 and colo357 were cultured in DMEM containing 10% FBS and passage at a 1:6 ratio. A retroviral packaging cell line (ATCC 293T) was maintained in DMEM supplemented with 10% FBS. All cell lines were grown in humidified 5% CO2, 95% air, 5% CO2 at 37°C. MIA PaCa-2/Gem and MIA PaCa-2/Oxa resistance cell lines were established by subjecting the cells to increased concentrations of oxaliplatin or gemcitabine. Resistance index (RI) was determined by MTT assay and calculated with the formula IC50R/IC50W = RI, with IC50R representing IC50 of resistance cells and IC50W representing IC50 of wild type cells. RI > 4 was considered as resistant cells. Resistant cells were not used beyond five passages following their subculturting.

2.3. Immunohistochemistry

Tissues were obtained from the resected specimens from 32 patients with pancreatic cancer who underwent the surgery at Department of Surgery, Peking University First Hospital, between 2005 and 2012. All patients received six courses of gemcitabine monotherapy with complete follow-up records (one course was defined as proceeding chemotherapy weekly for 3 weeks every 28 days). Clinical features of 32 patients in the study are shown in Table 1. The tissues were collected before the patients received subsequent chemotherapy. Tumours were classified according to the TNM staging system. Formalin-fixed, paraffin-embedded tissues were cut into 5-μm sections. After deparaffinisation and rehydration of each section, the sections were immersed in 0.3% hydrogen peroxide for 15 min. Sections were incubated with primary antibody overnight at 4°C, followed by incubation with HRP conjugated secondary antibody. Slides were then dehydrated and mounted for evaluation by two pathologists independently who were blind to the patients’ clinical status and outcome. Samples were classified into BRG1 high and low expression group according to the percentage of positive cells and the staining intensity of BRG1. Briefly, the percentage of positive cells was scored as: 0, no staining; 1, <10% of positive cells; 2, 10–49% of positive cells; 3, >50% of positive cells. The intensity of the positive cells was scored as: 0, negative; 1, light yellow; 2, yellow brown;
3, brown. The two individual parameters were summed resulting in a total score ranging from 0 to 6. Total score 0–4 was regarded as low expression of BRG1, score 5–6 was regarded as high expression of BRG1. The collection and use of human tissue was approved by Ethics Committee of Peking University First Hospital.

2.4. Cell cycle analysis

Cells were harvested by trypsinisation and resuspended in DMEM. Suspended cells (1 × 10^6/ml) were fixed in ice-cold 70% ethanol, then washed in PBS for three times and incubated with RNase at 37°C for 30 min. Thereafter, 400 μl propidium iodide solution was added to the mixture and kept in the dark at 4°C for 15 min. Samples were analysed by a FACSCalibur cytometer and CellQuest Software (Becton Dickinson).

2.5. Real-time polymerase chain reaction (PCR)

Total RNA was extracted from cells using TRIZOL Reagent (Invitrogen). First strand cDNA was amplified by using the ReverTra Ace qPCR RT kit according to the manufacturer’s protocol (TOYOBO). Real-time PCR was performed by using SYBR Green PCR Master Mix (TOYOBO). The primers for HMGAI were as follows: forward 5’-GAAGGAGCCAGCGAAGTG-3’; reverse 5’-GAGGCGTGTGATGGTG-3’. The primers for BRG1 were as follows: forward 5’-TCCCA AACTCGAGCAACCA-3’; reverse 5’-TCCGCTTCTGTTCCATC-3’.

Relative quantification of gene expression was calculated on triplicate reactions using the ΔΔCt method. Data were analysed by ABI 7500 FAST software v2.0.6 (Applied Biosystems).

2.6. Apoptosis detection assay

The cells were collected and stained by using Apoptosis Detection Kit according to the manufacturer’s protocol (BD Pharmingen). Then, cells undergoing apoptosis were quantified by calculating the percentage of cells positive for Annexin V. All experiments were carried out at least three times in duplicate.

2.7. Western blot analysis

Cells were lysed in lysis buffer containing 1 mmol/L Na_3VO_4, 1 mmol/L phenylmethylsulfonylfluoride (PMSF), 500 μM AEBSF, 150 nM Aprotinin, 1 μM E-64, 0.5 mM EDTA Na_2 and 1 μM leupepin. The lysates were cleared by centrifugation at 14,000 rpm and total protein concentration was measured with bicinchoninic acid assay Kit (Bio-Rad Laboratories). Cell lysates were separated in 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked in 10% goat serum in Tris-buffered saline with 0.1% Tween 20, incubated with primary antibodies, and then with HRP-conjugated secondary antibodies. The peroxidase activity was detected by using Immobilon Western Chemiluminescent HRP Substrate (Millipore) and exposed to X-ray films. Actin was used as a loading control.

2.8. siRNA transfection

Retrovirus harbouring siRNA against BRG1 was generated using pBJ-BRG1i, pSPAX2 and pMD2.G (Addgene) in 293T packaging cells. For transfection, PANC-1, BxPC-3 and MIA PaCa-2 cells were seeded in 6-well plates and transfected with either control RNA or BRG1 siRNA. Transfections were carried out using the Lipofectamine system according to the manufacturer’s protocol when cells had achieved about 50% confluence.

2.9. MTT and clonogenic assay

The cells were plated at a density of 1500 cells/well in 96-well plates. After exposure to drug for 72 h, MTT reagent was added and optical absorbance at 490 nm
was measured using a microplate reader. OD values of all experimental groups were normalised to control group. The IC_{50} of each cell line was determined by growth inhibition curve. For colony counting, cells were plated at a density of 10–50 cells/cm^2 in 100 mm^2 dishes and cultured for 14 days. Then cells were fixed and stained with crystal violet for clone number counting.

2.10. Mice xenograft model

1 × 10^6 cells transfected with control siRNA or BRG1 siRNA were resuspended in 100 mL of PBS and injected subcutaneously to the right of the dorsal midline in female 6-week-old nude mice (Charles River Laboratory). Tumours were measured every other five day. When the tumours reached a minimal diameter of 7 mm, the mice received i.p. injections of gemcitabine (50 mg/kg-d) or vehicle (PBS) twice a week. Tumour volumes were calculated with the formula \( V = A \times B^2/2 \), where \( A \) was the longest tumour axis, and \( B \) was the shortest tumour axis. Tumour inhibition rate was calculated with the formula \( TIR = (\text{average tumour weight of control group} - \text{average tumour weight of therapeutic group})/\text{average tumour weight of control group} \times 100\% \). All animal experiments followed the guidelines of Ethics Committee for Animal Experimentation.

2.11. Statistical analysis

Data were expressed as mean values and standard deviation (SD), and analysed using SPSS v.13.0 statistical software (SPSS, Inc.). One-way ANOVA, Kaplan–Meier survival calculations and the corresponding log-rank tests were performed to determine the differences in survival rates. \( P \) values <0.05 were considered statistically significant.

3. Results

3.1. PC cells with higher expression of BRG1 are more resistant to gemcitabine

First we assessed BRG1 protein level in six PC cell lines. BRG1 expression was strong in MIA PaCa-2, CaPan-1, T3M4, BxPC-3 and Colo357 cells but was weak in PANC-1 cells (Fig. 1A). Thus MIA PaCa-2 and PANC-1 cell lines were taken as complementary model to evaluate the role of BRG1 in regulating PC cell chemosensitivity. The results showed that in PANC-1 cells, gemcitabine induced significant upregulation of BRG1 in a dosage dependent manner, but oxaliplatin did not affect BRG1 expression (Fig. 1B–D). In MIA PaCa-2 cells, gemcitabine and oxaliplatin both caused the suppression of BRG1 expression (Fig. 1B). Furthermore, we evaluated the effects of 5-FU on BRG1 expression. Similar to gemcitabine, 5-FU upregulated BRG1 expression in PANC-1 cells but suppressed BRG1 expression in MIA PaCa-2 cells (Supplementary Fig. 1).

Notably, we found that the phosphorylation of Akt at 473 serine (pSer473-Akt) and the phosphorylation of p21^{cip/waf} at 145 threonine (pThr145-p21^{cip/waf}), a downstream target of pSer473-Akt, showed a positive correlation with BRG1 expression (Fig. 1B). Because the phosphorylation of p21^{cip/waf} promotes cell survival through the inhibition of cytoplasmically localised apoptosis-related proteins [10], we speculated that BRG1 may promote PC cell survival after chemotherapy.

MTT and apoptosis assay showed that compared to MIA PaCa-2, PANC-1 cells exhibited lower apoptosis level and higher cell viability upon gemcitabine treatment, but no significant differences in cell viability and apoptosis were observed between the two cell lines upon oxaliplatin treatment (Fig. 1E–G). Collectively, these data suggest that BRG1 promotes gemcitabine chemoresistance of PC cells.

3.2. BRG1 knockdown inhibits PANC-1 cell growth in vitro and in vivo

To confirm the role of BRG1 in PC cell chemoresistance, we used BRG1 siRNA to establish stable BRG1 suppressed PANC-1 cell line (PANC-1 BRG1i) and used PANC-1 cells transfected with control siRNA as control group (PANC-1 NC). We found that in PANC-1 BRG1i cells, BRG1 expression upon gemcitabine treatment was reduced, accompanied by reduced levels of pSer473-Akt and pThr145-p21^{cip/waf} (Fig. 2A). In addition, the apoptosis rate of PANC-1 BRG1i was significantly higher than that of PANC-1 NC (Fig. 2B and C).

To investigate whether BRG1 regulates gemcitabine chemosensitivity via the activation of Akt, we used LY294002 (a classical inhibitor of PI3K) to block Akt phosphorylation upon gemcitabine treatment in PANC-1 cells (Fig. 2D). Surprisingly, we observed that LY294002 inhibited BRG1 expression at both protein and mRNA levels in PC cells (Fig. 2D and E), indicating a positive feedback regulation between BRG1 and Akt phosphorylation. As expected, LY294002 treatment significantly facilitated gemcitabine induced apoptosis (Fig. 2F and G).

In vivo xenograft experiment showed that after gemcitabine treatment, the tumour growth of PANC-1 BRG1i group was significantly inhibited compared with control group (Fig. 2H–J). In addition, regression analysis on body weight during gemcitabine treatment showed that PANC-1 NC group lost weight at higher rate than PANC-1 BRG1i group (Fig. 2K). Taken together, these data demonstrate that BRG1 knockdown enhances gemcitabine induced apoptosis and inhibits PC cell growth in vitro and in vivo.
3.3. BRG1 knockdown inhibits MIA PaCa-2 cell growth in vitro and in vivo

Since BRG1 is abundantly expressed in MIA PaCa-2 cells, we also established stable BRG1 knockdown MIA PaCa-2 cell line (MIA PaCa-2 BRG1i) and control group (MIA PaCa-2 NC). We found that knockdown of BRG1 reduced the levels of pSer473-Akt and pThr145-p21cip/waf and increased endogenous apoptosis in MIA PaCa-2 cells (Fig. 3A–C). In addition, BRG1 knockdown resulted in restrained cell growth and induced S phase arrest in MIA PaCa-2 cells (Fig. 3D–F).

In vivo xenograft experiment showed that after gemcitabine treatment, the tumour growth of MIA PaCa-2 BRG1i group was significantly inhibited compared with control MIA PaCa-2 NC group (Fig. 3G and H).

Fig. 1. High expression of BRG1 confers gemcitabine resistance in pancreatic cancer (PC) cells. (A) BRG1 protein levels in six PC cell lines were detected by Western blot analysis. (B) PANC-1 and MIA PaCa-2 cell lines were exposed to 100 μM oxaliplatin or gemcitabine for 48 h, and the levels of BRG1, Ser473-Akt and Thr145-p21cip/waf were detected by Western blot analysis. (C) PANC-1 cell line was exposed to different concentrations of gemcitabine for 48 h, and the level of BRG1 was detected by Western blot analysis. (D) Densitometric analysis of BRG1 protein levels shown in C (n = 3). *P < 0.05 versus cells treated with 0 μM gemcitabine. (E) PANC-1 and MIA PaCa-2 cell lines were exposed to 100 μM oxaliplatin or gemcitabine for 48 h, and apoptosis was detected by flow cytometry. (F) Quantification of the data shown in E (n = 3). (G) The IC50 of PANC-1 and MIA PaCa-2 cell line was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Gem, gemcitabine; Oxa, oxaliplatin; *P < 0.05; **P < 0.01.
Furthermore, we compared chemotherapy-induced apoptosis in MIA PaCa-2 BRG1i group and MIA PaCa-2 NC group. The results showed that BRG1 knockdown significantly increased gemcitabine induced apoptosis in MIA PaCa-2 cells (Fig. 3I). Taken together, these data demonstrate that BRG1 knockdown enhances both endogenous and gemcitabine induced apoptosis and inhibits MIA PaCa-2 cell growth in vitro and in vivo.

3.4. BRG1 confers gemcitabine resistance in PC cells by promoting the activation of pSer473-Akt/pThr145-p21cip/waf axis

To confirm the role of BRG1 in conferring gemcitabine resistance in PC cells, we established a gemcitabine resistant MIA PaCa-2 cell line (MIA PaCa-2/Gem). Compared to parental cells, MIA PaCa-2/Gem cells became more flattened in shape and larger in size (Fig. 4A). MTT assay showed that IC50 of MIA PaCa-2/Gem was over ten times higher than that of MIA PaCa-2 (RI = 10.64) (Fig. 4B). Apoptosis assay confirmed that MIA PaCa-2/Gem cells were more resistant to gemcitabine than MIA PaCa-2 cells (Fig. 4C and D). Real-time PCR showed that the transcription of HMGA1, a biomarker of gemcitabine resistance in PC cells [11], was significantly upregulated in MIA PaCa-2/Gem cells compared to MIA PaCa-2 cells (Fig. 4E). Western blot analysis showed that BRG1 protein level had no significant difference between MIA PaCa-2/Gem and MIA PaCa-2 cells. However, upon gemcitabine treatment BRG1 protein level as well as the levels of pSer473-Akt and pThr145-p21cip/waf were significantly increased in MIA PaCa-2/Gem cells (Fig. 4F).
Knockdown of BRG1 significantly inhibited the levels of pSer473-Akt and pThr145-p21cip/waf and diminished the chemoresistance of MIA PaCa-2 upon gemcitabine treatment (Fig. 4 G–I). Similarly, LY294002 treatment significantly reduced the levels of pSer473-Akt and pThr145-p21cip/waf and diminished the chemoresistance of MIA PaCa-2 upon gemcitabine treatment (Fig. 4 J–L). In addition, LY294002 inhibited BRG1 expression in MIA PaCa-2 cells (Fig. 4 J). These data provide further evidences that BRG1 confers gemcitabine resistance in PC cells by promoting the activation of pSer473-Akt/pThr145-p21cip/waf axis, and there is a positive feedback between Akt activation and BRG1 expression.

3.5. BRG1 regulates epithelial-to-mesenchymal transition in PC cells

Epithelial-to-mesenchymal transition (EMT) has been implicated in the chemoresistance of different types of cancer. To further elucidate the mechanism by which BRG1 modulates the chemoresistance of PC, we investigated the relationship between BRG1 and EMT related markers, especially E-cadherin and vimentin in MIA PaCa-2 cells. First, we found that gemcitabine treatment led to reduced E-cadherin expression and increased vimentin expression in both MIA PaCa-2 and MIA PaCa-2/BRG1i cells. Furthermore, E-cadherin expression was lower while vimentin expression was higher in MIA PaCa-2 cells upon gemcitabine treatment, indicating the regulation of BRG1 on EMT in PC cells.
Fig. 4. BRG1 promotes the activation of pSer473-Akt/pThr145-p21 cip/waf axis in pancreatic cancer (PC) cells. (A) Phase contrast imaging of morphological variation of MIA PaCa-2/Gem compared with MIA PaCa-2. (B) The IC_{50} of MIA PaCa-2 and MIA PaCa-2/Gem was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. (C) MIA PaCa-2 and MIA PaCa-2/Gem were exposed to 10 μM gemcitabine for 48 h, and the apoptosis was detected by flow cytometry. (D) Quantification of the data shown in C (n = 3) represented as FACS plots of Annexin V. (E) mRNA expression of HMGA1 in MIA PaCa-2 and MIA PaCa-2/Gem cells was detected by real-time polymerase chain reaction (PCR), GAPDH was used as endogenous control. (F) Western blot analysis of the levels of BRG1, Ser473-Akt, Thr145-p21 cip/waf, total Akt and p21 cip/waf in MIA PaCa-2, MIA PaCa-2/Gem and gemcitabine treated MIA PaCa-2/Gem cells. (G) MIA PaCa-2/Gem BRG1i and negative control MIA PaCa-2/Gem NC were exposed to 100 μM gemcitabine for 48 h, and the levels of the indicated proteins were detected by Western blot analysis. (H) MIA PaCa-2/Gem NC and MIA PaCa-2/Gem BRG1i were exposed to 100 μM gemcitabine for 48 h, and the apoptosis was detected by flow cytometry. (I) Quantification of the data shown in H (n = 3). (J) MIA PaCa-2/Gem cells were exposed to 10 μM gemcitabine for 48 h with or without 20 μM LY294002, and the levels of BRG1, total Akt and Ser473-Akt were detected by Western blot analysis. (K) MIA PaCa-2/Gem cells were treated as in J and the apoptosis was detected by flow cytometry. (L) Quantification of the data shown in K (n = 3).
PaCa-2/Gem than in MIA PaCa-2 cells (Fig. 5A). These data indicate that the chemoresistance of PC cells is related to EMT.

Next, we examined the role of BRG1 in EMT induced by gemcitabine. When MIA PaCa-2/Gem cells were treated with gemcitabine, E-cadherin expression was reduced, but BRG1 knockdown partially abrogated gemcitabine-induced downregulation of E-cadherin expression (Fig. 5B). In contrast, the expression of vimetin was slightly upregulated by gemcitabine, but it was not significantly affected by BRG1 knockdown (Fig. 5B).

3.6. BRG1 is exclusively expressed in human pancreatic cancer

To further investigate the clinicopathological significance of BRG1 in PC, we examined BRG1 expression pattern in 32 PC tissues from patients before they received chemotherapy. BRG1 expression was detected in all PC tissues but not in adjacent benign tissues (Fig. 6A). The specimens were classified into BRG1 high expression group (18/32) and BRG1 low expression groups (14/32) according to immunohistochemical staining of BRG1 (Fig. 6B). Although no statistical differences were found in several clinical parameters between BRG1 high and low expression groups, we noted that BRG1 expression level had a potential correlation with tumour grade ($P = 0.064$) (Table 2). We next validated the correlation between BRG1 expression and patients' survival. While we found no statistical difference in postoperative prognosis between BRG1 high and low expression groups, we noted a trend that patients in BRG1 low expression group had higher survival rate than those in BRG1 high expression group (Fig. 6C).

4. Discussion

Chemotherapy is one of the conventional adjuvant therapies for PC patients. However, this approach often fails due to the intrinsic and acquired chemoresistance of PC cells. The components of SWI/SNF complex including BRG1 have been implicated in carcinogenesis. Silencing of BRG1 or BRM was found in various
tumours and human cancer cell lines including lung, prostate, breast, colon cancers and medulloblastoma [12–15]. These findings suggest that BRG1 functions as a tumour suppressor. However, other studies provided evidences that BRG1 may be an oncogene in lung, prostate, breast, colorectal, gastric cancer and melanoma [16,17]. It seems that the role of BRG1 in tumourigenesis is mainly dependent on the type of cancer or cell context [5].

Up to now only limited studies have investigated the role of BRG1 in PC. BRG1 and BRM were independently lost in PC cell lines. Among these cell lines, PANC-1 cells had weak BRG1 but high BRM expression. On the contrary, in MIA PaCa-2 cells BRG1 protein level was high while BRM was inhibited. Knockdown of BRG1 in MIA PaCa-2 led to actin cytoskeleton disruption and cell morphological alteration [18]. Re-expression of BRG1 in PANC-1 led to cell growth inhibition, reduced clonogenicity and cellular senescence, indicating that BRG1 functions as a tumour suppressor in PC [19]. Clinical studies showed that BRG1 expression decreased with increasing dysplasia in intraductal papillary mucinous neoplasm (IPMNs) lesions in 66 surgically resected IPMNs [7]. However, immunohistochemical analysis of 68 PC tissues showed that increased BRG1 expression was correlated with worse clinicopathological features [8].

In this study, we confirmed that BRG1 expression was strong in MIA PaCa-2 cells but was weak in PANC-1 cells. Furthermore, we found that gemcitabine and 5-FU upregulated BRG1 expression in PANC-1 cells but suppressed BRG1 expression in MIA PaCa-2 cells. The underlying mechanism is unclear.

For the first time we reported that BRG1 could promote PC chemosensitivity by enhancing the phosphorylation of Akt at 473rd (pSer473-Akt) serine and the phosphorylation of p21cip/waf at 145th threonine (pThr145-p21cip/waf). In clinical samples, we observed exclusive expression of BRG1 in PC tissues but not in adjuvant benign tissues. It is well known that pSer473-Akt is crucial for gemcitabine resistance in PC cells [20]. By using LY294002, we successfully inhibited Akt phosphorylation in PANC-1 upon gemcitabine treatment, and gemcitabine resistance of PANC-1 cells was significantly reversed, demonstrating that Ser473-Akt/Thr145-p21cip/waf is the main downstream mediator of BRG1. Unexpectedly, we observed a remarkable correlation between the suppression of BRG1 and the inhibition of Ser473-Akt. This finding indicates that BRG1 may be the downstream target of Akt signalling, and there is a positive feedback between BRG1 and Akt signalling.

Because BRG1 was abundant in MIA PaCa-2 cells in which BRM was silenced, MIA PaCa-2 cell line is an ideal model to investigate the biological function of BRG1 in PC cells. Silencing of BRG1 in MIA PaCa-2 led to pSer473-Akt inhibition. In addition, the phosphorylation of p21cip/waf, a downstream target of pSer473-Akt, was decreased. p21cip/waf phosphorylated by Akt and ERK at Thr145 resulted in multiple biochemical changes including the stabilisation of p21cip/waf and its translocation into the cytoplasm, where Thr145-p21cip/waf binds pro-apoptosis kinases ASK1 and protects cells from stress-activated or cytokines-induced apoptosis. However, p21cip/waf undergoes degradation once it is transported to the cytoplasm [21,22]. To our knowledge, this
is the first report of a parallel reduction of pSer473-Akt and pThr145-p21cip/waf after BRG1 suppression in PC cells.

Consistent with the biochemical changes described above, we found that BRG1 knockdown could impair in vivo and in vitro cell growth and promoted both endogenous and gemcitabine induced apoptosis in MIA PaCa-2 cells. BRG1 was able to mediate gemcitabine resistance in both PANC-1 and MIA PaCa-2 cells, indicating intrinsic chemoresistance. Next we created an acquired chemoresistance model by keeping MIA PaCa-2 cells exposed to increasing concentrations of gemcitabine, and finally established gemcitabine resistant cell line MIA PaCa-2/Gem. In MIA PaCa-2/Gem, we observed that gemcitabine induced the upregulation of BRG1, accompanied by the activation of Ser473-Akt/Thr145-p21cip/waf axis. BRG1 knockdown in MIA PaCa-2/Gem cells led to reduced activation of Ser473-Akt/Thr145-p21cip/waf axis and subsequently reversed the resistance to gemcitabine. In addition, LY294002 treatment inhibited gemcitabine resistance of MIA PaCa-2/Gem cells, confirming that Akt phosphorylation is crucial for BRG1 mediated chemoresistance. Interestingly, we found that BRG1 expression was reduced in MIA PaCa-2/Gem cells upon the inhibition of Akt phosphorylation, a similar phenomenon we found in PANC-1 cells. Taken together, these data clearly demonstrate that BRG1 promotes both intrinsic and acquired chemoresistance in PC cells by activating Akt signalling. Furthermore, BRG1 is a downstream target of Akt signalling pathway, suggesting that BRG1 crosstalks with Akt to form a positive feedback loop to promote PC tumourigenesis.

Growing evidence has confirmed molecular and phenotypic associations between chemoresistance and EMT in different cancers [23]. EMT is characterised by the loss of cell-to-cell adhesion with enhanced cell invasiveness and resistance to apoptosis. Cancer cells with responsive EMT phenotype upon drug treatment have developed chemoresistance [24]. Notably, BRG1 has been shown to act as a co-repressor to facilitate ZEB1 mediated E-cadherin transcriptional suppression [25]. Thus, we investigated the role of BRG1 in chemoresistance of PC cells may be related to EMT. We found that gemcitabine suppressed E-cadherin expression but stimulated vimentin expression in both MIA PaCa-2 and MIA PaCa-2/Gem cells. Moreover, E-cadherin expression was lower while vimentin expression was higher in chemoresistant MIA PaCa-2 cells. However, upon knockdown of BRG1 in MIA PaCa-2 cells we observed that gemcitabine-induced downregulation of E-cadherin was partially relieved, although vimentin expression was not significantly affected. Collectively, these data suggest that BRG1 regulates E-cadherin expression and may play a role in chemotherapy-induced EMT. Interestingly, a recent study found that Akt activation is crucially implicated in the induction of EMT [26]. Considering a positive feedback between BRG1 and Akt signalling we proposed above, the mechanism underlying BRG1 induced chemoresistance may involve the activation of Akt, which in turn induces EMT and chemoresistance. Further studies are needed to confirm our speculation.

Several limitations of this study should be pointed out. First, we only examined the role of BRG1 in chemoresistance of PC cells to gemcitabine. It is necessary to analyse other chemotherapies such as 5-fluorouracil to provide further support to our conclusion. Second, the size of clinical samples in this study is limited, which may prevent us from finding a significant correlation between BRG1 expression level and the survival of PC patients. Employing larger samples in our future studies will help determine prognostic and predictive value of BRG1 in PC.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2014.05.017.

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