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Combination of salinomycin and gemcitabine eliminates pancreatic cancer cells

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ABSTRACT

Previous research has documented that a subpopulation of pancreatic cancer cells, named cancer stem cells (CSCs), harbor stem cell-like properties. Here, we examined the efficacy of combined treatments of salinomycin and gemcitabine in human pancreatic cancer cells. Salinomycin inhibited the growth of CSCs, while gemcitabine suppressed the viability of non-CSCs. Consistently, in vivo studies showed that salinomycin combined with gemcitabine could eliminate the engraftment of human pancreatic cancer more effectively than the individual agents. These data indicated that administration of salinomycin, which targets CSCs, may constitute a potential therapeutic strategy for improving the efficacy of gemcitabine to eradicate pancreatic cancer.

1. Introduction

Pancreatic cancer is the fourth most common cause of cancer death [1]. Despite significant improvements in diagnostic imaging and operative mortality rates during the past two decades, the 5-year survival rate remains lower than 5% [2]. Gemcitabine (GEM) has been the most commonly used chemotherapeutic agent over the past decade. Unfortunately, numerous phase III trials testing GEM combined with other cytotoxic drugs have failed to reveal any additional benefit compared with GEM alone [3–5].

Emerging data suggest that malignant tumors contain a small subset of distinct cells termed cancer stem cells (CSCs), which are responsible for tumor initiation and propagation [6]. Over the past few years CSCs have been discovered in several types of solid tumors, including pancreatic cancer [20]. Most current systemic therapies have been found ineffective in the treatment of solid tumors, and this may be due, at least in part, to the increased resistance of CSCs [7]. Therefore, it is important to discover new therapeutic modalities to eliminate CSCs in order to eventually develop protocols for more successful treatment [8].

Salinomycin (SAL) is a 751 Da monocarboxylic polyether antibiotic, which was originally used to kill bacteria, fungi, and parasites and fed to ruminants to improve nutrient absorption and feeding efficiency [9,10]. However, SAL has recently been shown to selectively deplete human breast cancer stem cells, which reduces the proportion of CSCs by >100-fold relative to paclitaxel [11]. Furthermore, SAL can overcome drug resistance in human cancer cells and activate a particular apoptotic pathway not accompanied by cell-cycle arrest and independent of p53, caspase activation, the CD95/CD95L system and the proteasome [12,13]. More interestingly, SAL may sensitize cancer cells to the effects

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of doxorubicin or etoposide treatment by enhancing apoptosis via increased DNA damage and reduced p21 protein levels through increased proteasome activity [14]. Thus, SAL may be a promising chemical for eradication of CSCs. Here we investigated whether SAL alone or combined with GEM was capable of eliminating pancreatic cancer cells both in vitro and in vivo.

2. Materials and methods

2.1. Cell culture and reagents

The highly metastatic human pancreatic cancer cell lines, SW1990 and AsPC-1, were maintained in RPMI 1640 (Gibco BRL, Grand Island, NY, USA) supplemented with 100 IU penicillin, 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA) and 10% fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA). SAL was purchased from Sigma–Aldrich (Saint Louis, MO, USA). GEM was purchased from Lilly (Bad Homburg, Germany). SAL was dissolved either in 95% ethanol or in DMSO. GEM was made fresh every day in 0.9% normal saline.

2.2. Fluorescence-activated cell sorting (FACS) analysis

Pancreatic cancer $CD133^+$ fraction sorting was performed as described previously [15]. Briefly, SW1990 and AsPC-1 cells were trypsinized, washed, and resuspended in PBS. The cells were then incubated with a monoclonal antibody labeled with phycoerythrin against the human CD133/2 epitope (clone 293C3; Miltenyi Biotec, Bergisch Gladbach, Germany) for 30 min at 4 °C. Cells were isolated using a BD FACS Arial II Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The side population (SP) assay was performed as described previously [16], in which cells were identified by exclusion of the vital dye Hoechst 33342. Both $CD133^+/CD133^-$ or SP/non-SP (NSP) cells were isolated by FACS from the two above-mentioned cell lines, resulting in a purity of >90%.

2.3. MTT Assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were performed in 96-well plates in triplicate using a Cell Proliferation Kit (I) (Roche Applied Science, Basel, Switzerland) following the manufacturer's instructions. The assays were repeated three times.

2.4. Annexin V analysis

Annexin V analysis was conducted by Annexin V and fluorescein isothiocynate (FITC) staining using a commercial Annexin V-FITC kit (BD Biosciences, Franklin Lakes, NJ, USA). Briefly, cells were harvested as described for FACS analysis. Cells (1×10^5) in 100 μl of binding buffer received 5 μl of Annexin V-FITC and 5 μl of propidium iodide (50 μg/ml), and they were then incubated for 15 min at room temperature. The stained cells were analyzed using a Cytomics FC 500 flow cytometer (Beckman Coulter, Brea, CA, USA). The experiments were repeated three times.

2.5. Colony and sphere formation assays

SW1990 cells were isolated by FACS and plated (50,000 per well) in a six-well plate. After 72 h of exposure to 5 μg/ml of GEM, 5 μmol/l of SAL, or their combination, cells were trypsinized, and 200 viable cells were plated into six-well plates in triplicate. The cells were then incubated for 12 days at 37 °C in a 5% CO_2 incubator. Colonies were stained with 0.05% crystal violet and counted. CSC spheres were cultured in DMEM/F12 (Gibco BRL, Grand Island, NY, USA) with 20 ng/ml EGF (R&D Systems, Minneapolis, MN, USA), 20 ng/ml bFGF (R&D Systems, Minneapolis, MN, USA), and B-27 supplement (Gibco BRL, Grand Island, NY, USA) for about 2 weeks. The spheres were counted with a light microscope. The assays were repeated three times.

2.6. Xenograft model of human pancreatic cancer

Nude mice were purchased from the animal institute of the Chinese Academy of Medical Science and maintained in microisolator cages. All experiments were approved by the animal care committee of Sun Yat-Sen University. SW1990 cells were suspended in serum-free RPMI media after FACS using a CD133 antibody, and both $CD133^+$ and $CD133^-$ cells were injected subcutaneously into the right flank of the mice using a 23-gauge needle at a 2×10^6 cells per mouse. The size and weight of the tumors were monitored every other day. Once the tumors reached a mean diameter of 2–4 mm, both groups of mice ($CD133^+$ and $CD133^-$) were randomized to four subgroups with six animals each. The mice were then treated with vehicle, GEM, SAL or their combination. GEM was administered twice a week (100 mg/kg), while SAL was administered every other day (4 mg/kg), and both were injected intraperitoneally over 25 days.

2.7. Statistical analysis

Data is presented as mean ± standard deviation (SD), where applicable, and differences were evaluated using the Student's *t*-test. A probability of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Separation of $CD133^+/CD133^-$ and SP/NSP cells by FACS from human pancreatic cell lines

The proportion of $CD133^+$ or SP cells in cultured pancreatic cancer cell lines is variable [17–19]. First we detected the percentage of $CD133^+$ and SP cells in several pancreatic cancer cell lines present in our laboratory by flow cytometry (data not shown), and chose two cell lines, SW1990 and AsPC-1, in which the proportion of either $CD133^+$ or SP cells were relatively high (Fig. 1).

3.2. Cytotoxic effects of SAL and GEM on pancreatic cancer cells separated by FACS

We evaluated the antiproliferative effects of SAL and GEM using MTT assays on both $CD133^+$ and $CD133^-$ cells isolated from the SW1990 and AsPC-1 cell lines. Cells were treated with a gradient of concentrations of SAL and GEM for 72 h. The ratio of viable cells compared with controls is delineated in Fig. 2A and B. With increasing concentrations, the survival rate of $CD133^+$ cells treated with SAL decreased more sharply than that of

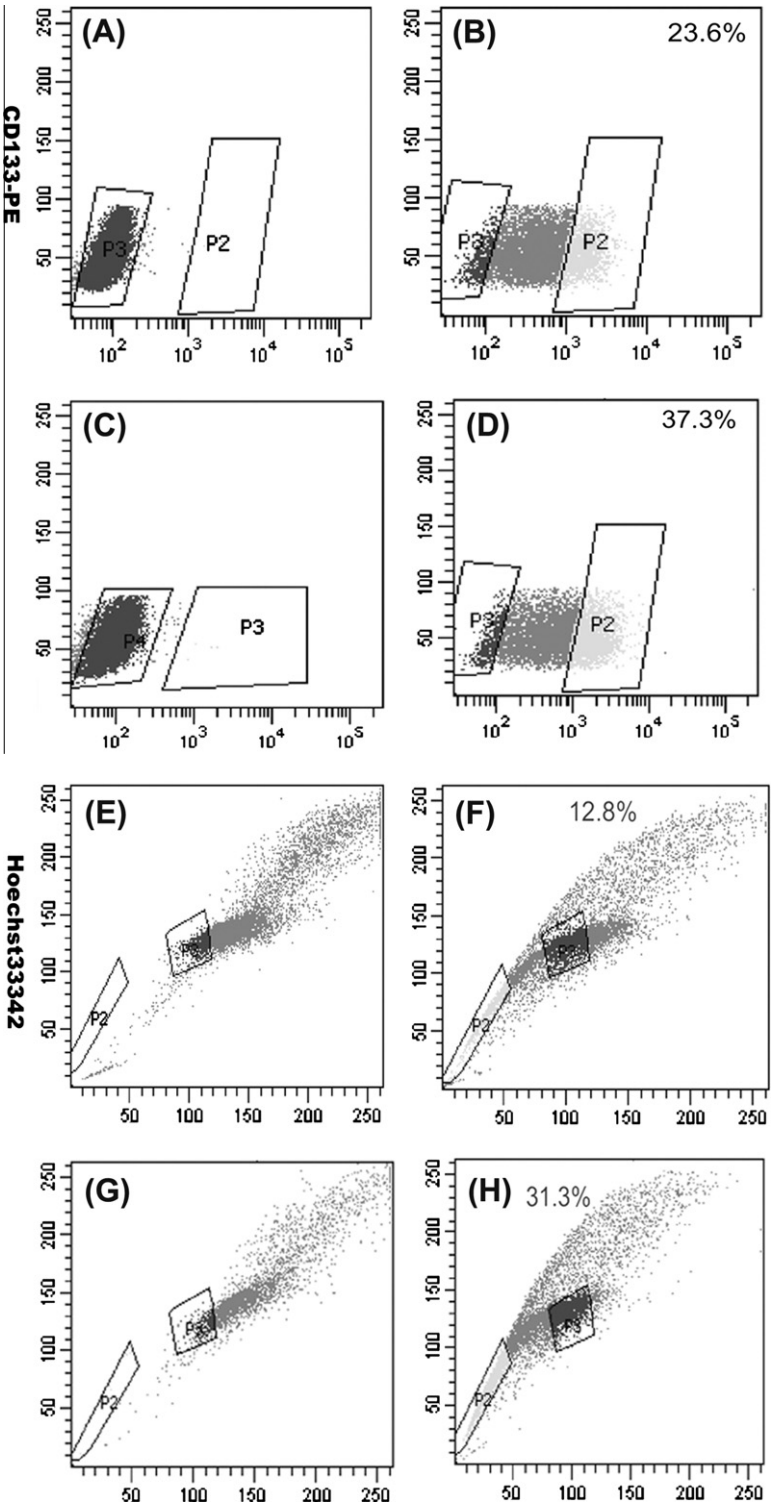

Fig. 1. Isolation of $CD133^+/CD133^-$ or SP/NSP cells from human pancreatic cancer cell lines SW1990 and AsPc-1. (A, B) SW1990 cells labeled with PE-conjugated antibody. (A) mouse anti-human IgG and (B) mouse anti-human CD133/2 antibody. (C and D) AsPc-1 cells labeled with PE-conjugated antibody. (C) mouse anti-human IgG and (D) mouse anti-human CD133/2 antibody. (E and F) Hoechst 33342 staining of SW1990 cells. (E) with 50 μM verapamil and (F) without verapamil. (G and H) Hoechst 33342 staining of AsPc-1 cells. (G) with 50 μM verapamil and (H) without verapamil. The percentage of $CD133^+$ subset or side population is indicated.

$CD133^+$ cells treated with GEM or $CD133^-$ cells treated with SAL, whereas the cytotoxic effects of GEM on $CD133^-$ cells was significantly better. Similarly, the viability of SP cells treated with SAL was inhibited more strongly compared with that of SP cells treated with GEM or NSP cells treated with SAL in both the SW1990 and AsPC-1 cell lines (Fig. 2C and D). These data implied that SAL was likely to preferentially target CSCs while GEM could eliminate further differentiated cancer cells.

3.3. Anti-tumor effects of SAL combined with GEM

Based on above results, we chose 5 μM SAL and 5 μg/ml GEM for subsequent experiments to test the antiproliferative effects of this drug combination. Cell growth inhibition was determined by MTT assay in SW1990 and AsPC-1 cell lines. As shown in Fig. 3A and B, combined treatment of SAL and GEM could eradicate both $CD133^+$ and $CD133^-$ cells more efficiently than either SAL or GEM alone ($P < 0.01$). Likewise, we performed the same assay on SP and NSP cells and observed similar findings, indicating that the combination of these two agents demonstrated the most efficient cytotoxic effect (Fig. 3C and D). Interestingly, at the same concentration, SAL eliminated $CD133^-$ or NSP cells less efficiently than either $CD133^+$ or SP cells (Fig. 3A–D), which was consistent with the observations published by Gupta et al. [11].

Furthermore, we used an Annexin V-FITC assay to test the extent of apoptosis in both $CD133^+$ and $CD133^-$ cells isolated from SW1990 and AsPC-1 cell lines following 72 h of treatment with SAL, GEM, or their combination. Administration of SAL alone enhanced apoptosis in $CD133^+$ cells, but not in $CD133^-$ cells. Conversely, GEM induced apoptosis in $CD133^-$ cells more efficiently than in $CD133^+$ cells. However, cotreatment with SAL and GEM significantly increased apoptosis in both $CD133^+$ and $CD133^-$ cells (Fig. 4A and B). We also evaluated the efficiency of combined treatment on SP and NSP cells. As shown in Fig. 4C and D, combined treatment induced significant apoptosis both in SP and NSP cells.

To evaluate whether single or combined treatment could suppress the formation of colonies in vitro, we exposed SW1990 cells isolated by FACS to GEM, SAL, or combined treatment for 72 h. As shown in Fig. 5A, compared with GEM, SAL alone could inhibit the colony-formation of $CD133^+$ cells, but showed a lower efficiency on $CD133^-$ cells. For combined treatment, neither $CD133^+$ or $CD133^-$ cells could effectively form colonies (both $P < 0.01$). To test the self-renewal feature further, we performed the sphere-forming assay after drug treatment. Interestingly, SAL alone almost completely abolished the sphere-formation of $CD133^+$ cells, and GEM could inhibit the formation of $CD133^-$ spheres. However, cotreatment with both SAL and GEM eliminated both isolated fractions, resulting in few surviving spheres of $CD133^+$ or $CD133^-$ cells (Fig. 5B).

3.4. Regression of human pancreatic cancer xenografts following combined treatment

To evaluate the anti-tumor effect of GEM, SAL or combined treatment in vivo, we subcutaneously inoculated $CD133^+$ or $CD133^-$ cells isolated from the cell line SW1990 into the flank of nude mice. Since the tumor formation of $CD133^-$ cells was much slower than that of $CD133^+$ cells, we began treatment after the tumors reached a mean diameter of 2–4 mm. Subsequently, tumor size and body weight of the mice were monitored every other day. In the $CD133^+$ group, administration of GEM or SAL alone retarded tumor growth, but the combined treatment of GEM and SAL almost completely inhibit tumor growth ($P < 0.01$ vs. GEM or SAL group; Fig. 6A). Surprisingly, in the $CD133^-$ group, tumors grew a little slower than the $CD133^+$ cells, and SAL only slightly suppressed the growth of $CD133^-$ tumors (not significant). However, either GEM alone or the combined treatment inhibited the tumor growth of $CD133^-$ cells significantly ($P < 0.01$ vs. SAL treatment). Importantly, there was no significant change in the body weight of the mice (data not shown). Taken together with the data of our in vitro experiments, combined treatment of SAL and GEM showed a promising potential to eradicate human pancreatic cancer.

4. Discussion

Although significant progress has been made in the treatment of pancreatic cancer, the 5-year survival has

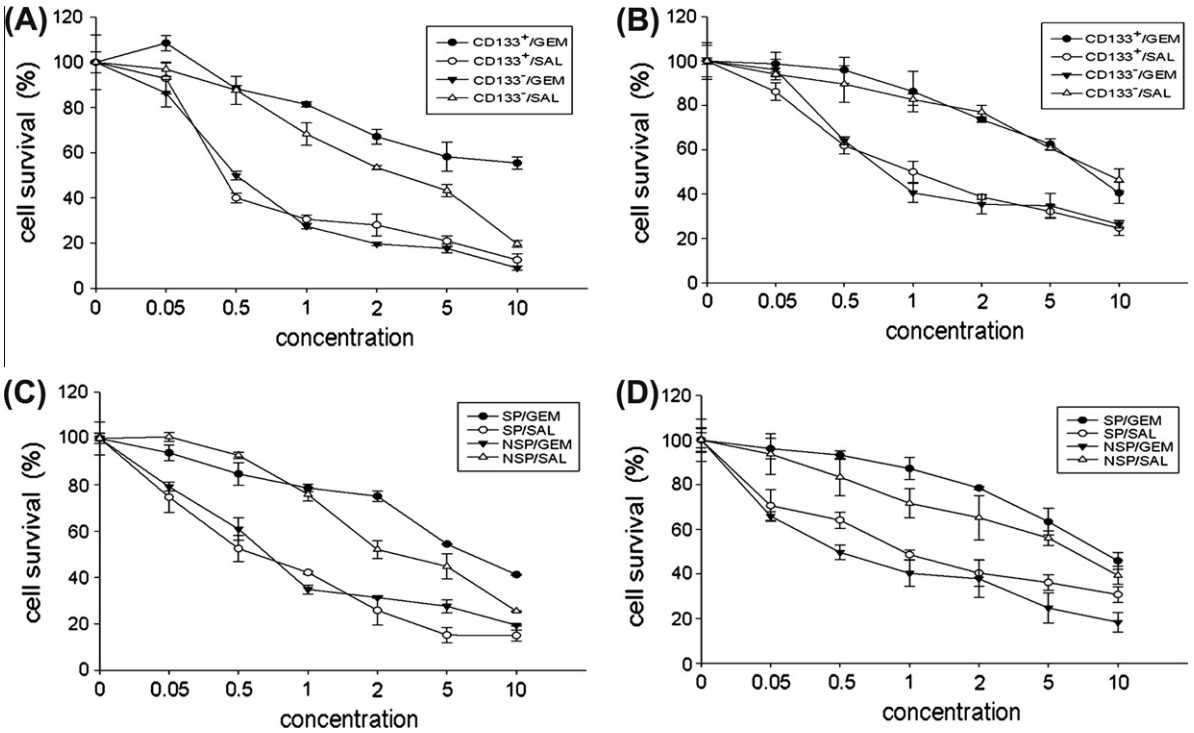

Fig. 2. Cytotoxic effects of salinomycin (SAL, μM) or gemcitabine (GEM, μg/ml) on human pancreatic cancer cells separated by FACS. (A and B) Survival rate with single treatment of SAL or GEM for 72 h in $CD133^+/CD133^-$ cells. (A) SW1990 cell line and (B) AsPc-1 cell line. (C and D) Survival rate with single treatment of SAL or GEM for 72 h in SP/NSP cells. (C) SW1990 cell line and (D) AsPc-1 cell line. $n = 3$, bars, standard deviation (SD).

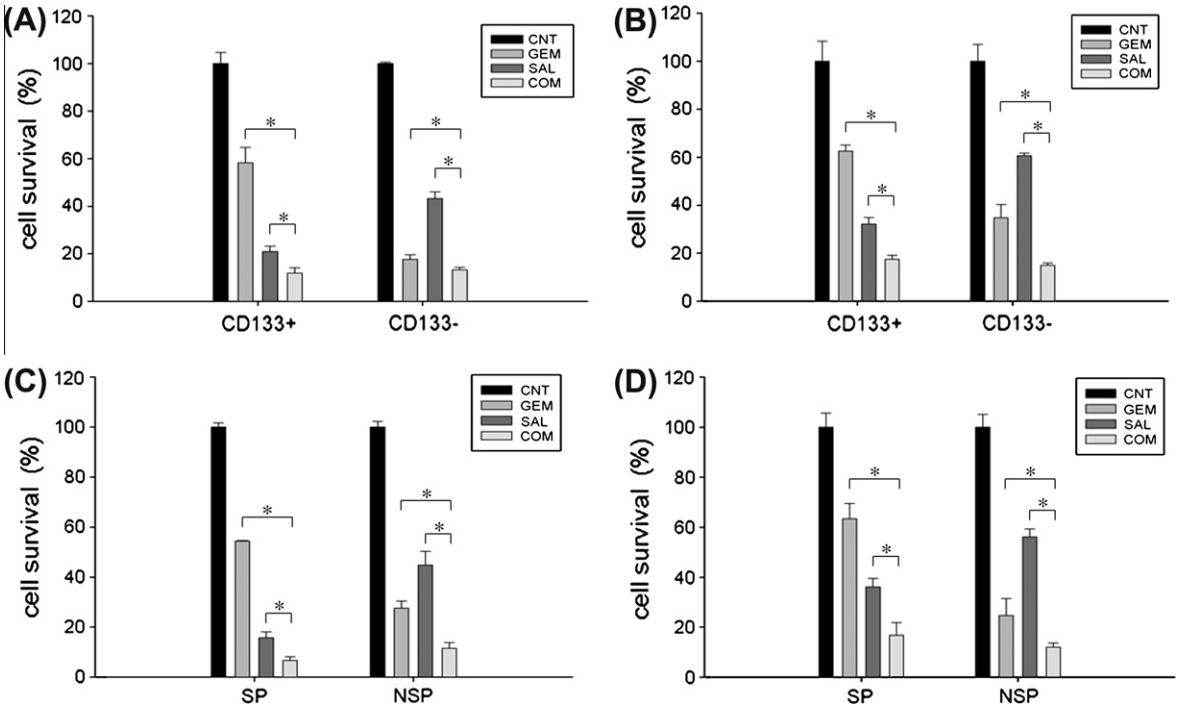

Fig. 3. Growth inhibition of $CD133^+/CD133^-$ or SP/NSP cells treated with vehicle (CNT), 5 μg/ml GEM, 5 μM SAL or their combination (COM). (A and B) Survival rate of $CD133^+/CD133^-$ cells isolated from human pancreatic cancer cells by FACS. (A) SW1990 cell line and (B) AsPc-1 cell line. (C and D) Survival rate of SP/NSP cells isolated from human pancreatic cancer cells by FACS. (C) SW1990 cell line and (D) AsPc-1 cell line. $^*P < 0.01$. $n = 3$, bars, standard deviation (SD).

not improved substantially over the past 30 years [1]. Gemcitabine (GEM), which has been the frontline chemotherapeutic agent against pancreatic cancer, has offered some relief over the past two decades. However, several agents of targeted therapy have been tested in combination with GEM and have similarly failed to confer any added benefit [2]. Emerging data have indicated that pancreatic CSCs are resistant to conventional chemotherapy [6]. However, treatment of pancreatic cancer with GEM is not capable of eliminating CSCs but rather leads to a relative increase in their numbers, indicating its preferential effect on more differentiated and rapidly proliferating tumor cells [8]. Thus, targeting both differentiated cancer cells and CSCs may provide advantages for treating pancreatic cancer and prevention of relapse.

Salinomycin (SAL), an antibiotic used to kill bacteria, fungi and parasites, has recently been shown to selectively deplete human breast cancer stem cells [11], overcome ABC transporter-mediated multidrug resistance in human acute myeloid leukemia and activate a particular apoptotic pathway [12,13]. Furthermore, SAL can sensitize cancer cells to the effects of doxorubicin or etoposide treatment by enhancing apoptosis via increased DNA damage and reduced p21 protein levels through increased proteasome activity [14]. Therefore, based on the antiproliferative effects of SAL and GEM, we used both compounds in vitro and in vivo to verify whether their combined treatment could eradicate human pancreatic cancer cells.

In this study, we used $CD133^{+/-}$ and side population (SP) cells to separate pancreatic CSCs and differentiated cells for further investigation. Li et al. previously deployed $CD44^+/CD24^+/EpCAM^+$ markers to define pancreatic CSCs [21]. However, it should be noted that these putative CSCs were compared to a population of cells negative for all three markers. Since EpCAM identifies epithelial cells within the tumor, the confinement to EpCAM negative cells as the control population may be too restrictive, as these cells should primarily represent non-epithelial inflammatory, stromal and vascular cells [22]. On the other hand, Hermann et al. showed that $CD133^+$ pancreatic cells isolated from primary pancreatic cancers and pancreatic cancer cell lines were highly tumorigenic [15]. However, it has been reported that CD133 is expressed on both CSC and differentiated tumor cells and is probably differentially folded as a result of differential glycosylation to mask specific epitopes [23]. CSCs can only be reliably identified by expression of AC133, which only binds to the modified form of CD133 [22]. Due to this present study, we used a monoclonal antibody against the human CD133/2 epitope to identify CSCs within the pancreatic cell lines.

Several researchers have demonstrated that SP cells found in a variety of tumor types, including leukemia, glioma, medulloblastoma, hepatocarcinoma, breast, prostate, thyroid, colorectal and ovarian carcinoma, are endowed with higher clonogenic, tumorigenic and chemoresistance potential relative to NSP cells. Due to these features, SP cells can be targeted as a therapeutic implication [24]. A recent study also observed that SP cells in pancreatic cancer were responsible for high resistance to chemotherapeutic agents [25]. Moreover, the most striking characteristic of

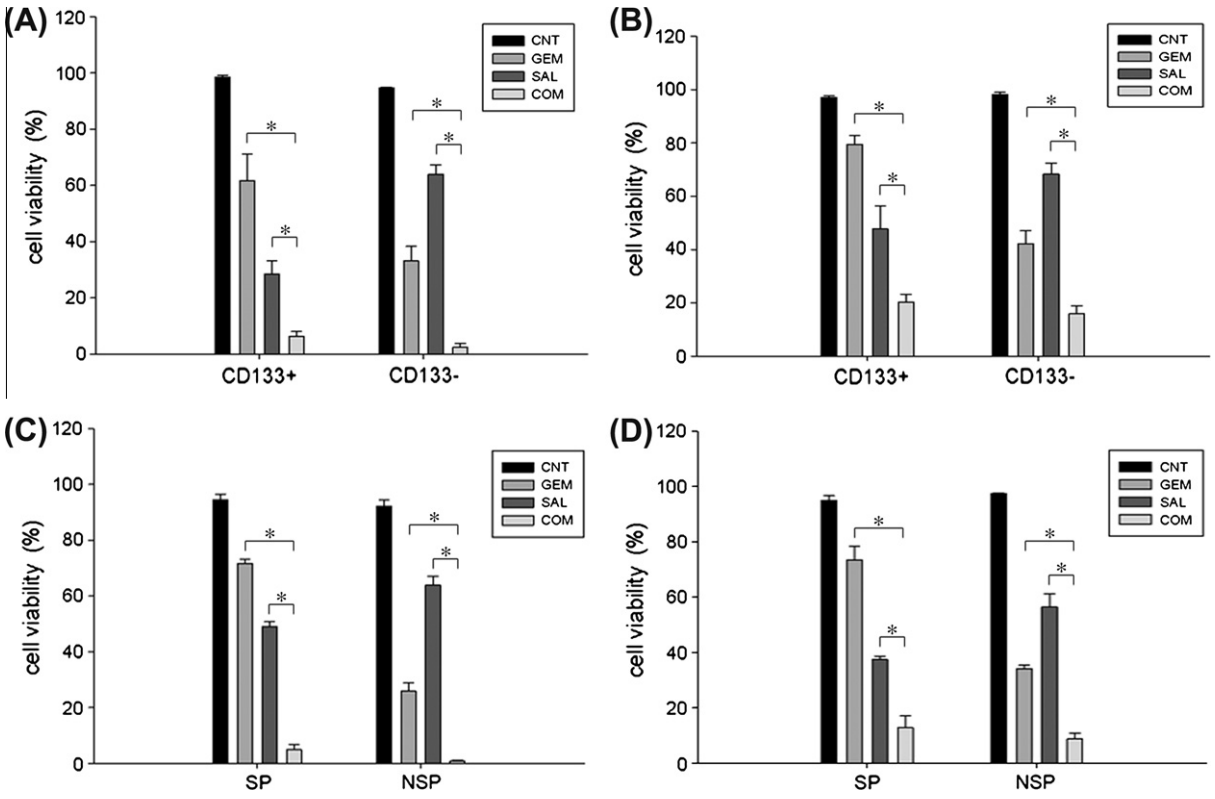

Fig. 4. Enhanced apoptosis in CD133$^+$/CD133$^-$ or SP/NSP cells treated with vehicle (CNT), 5 μg/ml GEM, 5 μM SAL or their combination (COM). (A and B) Cell viability rate of CD133$^+$/CD133$^-$ cells isolated from human pancreatic cancer cells by FACS. (A) SW1990 cell line and (B) AsPc-1 cell line. (C and D) Cell viability rate of SP/NSP cells isolated from human pancreatic cancer cells by FACS. (C) SW1990 cell line and (D) AsPc-1 cell line. $^*P < 0.01$. $n = 3$, bars, standard deviation (SD).

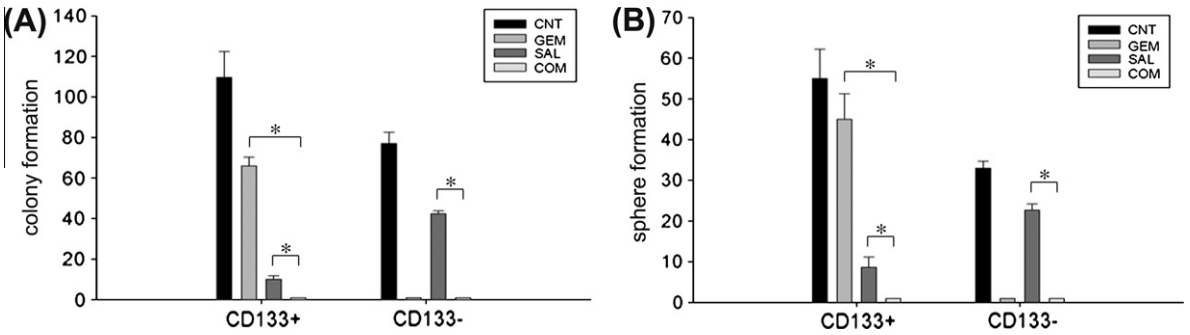

Fig. 5. Suppressed self-renewal of CD133$^+$/CD133$^-$ cells isolated from SW1990 cell line by FACS after treatment with vehicle (CNT), 5 μg/ml GEM, 5 μM SAL or their combinations (COM) for 72 h. (A) Statistical analysis of colony formation assay and (B) Statistical analysis of sphere formation assay. $^*P < 0.01$. $n = 3$, bars, standard deviation (SD).

SP cells is their invasiveness and metastatic potential [19]. In our study we also investigated the suppressive effect of SAL on SP cells and found that SAL could inhibit SP cells isolated from pancreatic cancer cell lines.

In this study, we provided strong evidence that SAL had a marked inhibition on CD133$^+$ and SP cells separated from human pancreatic cancer cell lines. Furthermore, GEM showed better antiproliferative effects on CD133$^-$ and NSP cells. However, neither of our tested drugs achieved complete elimination of CD133$^+$ or CD133$^-$ cells.

Recent studies have investigated a variety of compounds, including sulforaphane, cucurbitacin or its analog CDF, ceramide and masitinib, in combination with GEM, and those results indicate that such compounds may successfully eliminate pancreatic cancer CSCs or synergistically sensitize cells to GEM to abolish pancreatic cancer

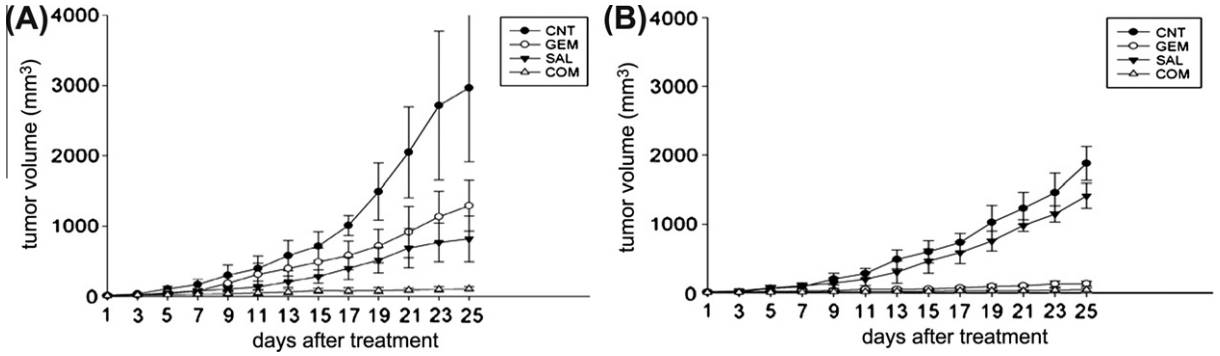

Fig. 6. Tumor regression of human pancreatic cancer xenograft with administration of vehicle (CNT), GEM, SAL or their combination (COM). (A and B) Inhibitive effects on xenografts formed by $CD133^+/CD133^-$ cells isolated from SW1990 cell line. (A) $CD133^+$ xenografts and (B) $CD133^-$ xenografts. The tumor growth curves were created from six mice in each group. Bars, standard deviation (SD).

cells [8,26–28]. Thus, we also investigated the combined treatment effects of SAL and GEM on separated pancreatic cancer cells. Interestingly, combined treatment led to a nearly complete abolishment of both CSCs and differentiated cells in vitro.

To validate our in vitro results, we observed our treatment effects in vivo. Consistent with our in vitro findings, our in vivo data showed that the combined treatment was superior against both the $CD133^+$ and $CD133^-$ cells groups. However, as compared with the in vitro data, SAL did not achieve a significant effect on $CD133^+$ engraftment.

In conclusion, our present data indicate that SAL can inhibit pancreatic CSCs. More importantly, our current results indicated combined treatment of SAL and GEM eliminated both CSCs and differentiated cells. As such, SAL could be a promising agent for novel combination therapy for the treatment of human pancreatic cancers.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] A. Jemal et al., Cancer statistics, CA: A Cancer J. Clin. 60 (5) (2010) 277–300.
[2] P.A. Philip et al., Consensus report of the national cancer institute clinical trials planning meeting on pancreas cancer treatment, J. Clin. Oncol. 27 (33) (2009) 5660–5669.
[3] C. Louvet et al., Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial, J. Clin. Oncol. 23 (15) (2005) 3509–3516.
[4] D. Cunningham et al., Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer, J. Clin. Oncol. 27 (33) (2009) 5513–5518.
[5] E. Van Cutsem et al., Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer, J. Clin. Oncol. 27 (13) (2009) 2231–2237.
[6] D.M. Simeone, Pancreatic cancer stem cells: implications for the treatment of pancreatic cancer, Clin. Cancer Res. 14 (18) (2008) 5646–5648.
[7] B. Sarkar, J. Dosch, D.M. Simeone, Cancer stem cells: a new theory regarding a timeless disease, Chem. Rev. 109 (7) (2009) 3200–3208.
[8] M.T. Mueller et al., Combined targeted treatment to eliminate tumorigenic cancer stem cells in human pancreatic cancer, Gastroenterology 137 (3) (2009) 1102–1113.
[9] N. Mahmoudi et al., Identification of new antimalarial drugs by linear discriminant analysis and topological virtual screening, J. Antimicrob. Chemother. 57 (3) (2006) 489–497.
[10] M. Mitani et al., Salinomycin effects on mitochondrial ion translocation and respiration, Antimicrob. Agents Chemother. 9 (4) (1976) 655–660.
[11] P.B. Gupta et al., Identification of selective inhibitors of cancer stem cells by high-throughput screening, Cell 138 (4) (2009) 645–659.
[12] D. Fuchs et al., Salinomycin induces apoptosis and overcomes apoptosis resistance in human cancer cells, Biochem. Biophys. Res. Commun. 390 (3) (2009) 743–749.
[13] D. Fuchs et al., Salinomycin overcomes ABC transporter-mediated multidrug and apoptosis resistance in human leukemia stem cell-like KG-1a cells, Biochem. Biophys. Res. Commun. 394 (4) (2010) 1098–1104.
[14] J.H. Kim et al., Salinomycin sensitizes cancer cells to the effects of doxorubicin and etoposide treatment by increasing DNA damage and reducing p21 protein, Br. J. Pharmacol. 00 (2010).
[15] P.C. Hermann et al., Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer, Cell Stem Cell 1 (3) (2007) 313–323.
[16] Y. Liang et al., Stem-like cancer cells are inducible by increasing genomic instability in cancer cells, J. Biol. Chem. 285 (7) (2010) 4931–4940.
[17] M. Olempska et al., Detection of tumor stem cell markers in pancreatic carcinoma cell lines, Hepatobiliary Pancreat. Dis. Int. 6 (1) (2007) 92–97.
[18] G. Sergeant et al., Role of cancer stem cells in pancreatic ductal adenocarcinoma, Nat. Rev. Clin. Oncol. 6 (10) (2009) 580–586.
[19] A. Kabashima et al., Side population of pancreatic cancer cells predominates in TGF-beta-mediated epithelial to mesenchymal transition and invasion, Int. J. Cancer 124 (12) (2009) 2771–2779.
[20] C. Li et al., Identification of pancreatic cancer stem cells, Cancer Res. 67 (3) (2007) 1030–1037.
[21] E. Lonardo, P.C. Hermann, C. Heeschen, Pancreatic cancer stem cells - update and future perspectives, Mol. Oncol. 4 (5) (2010) 431–442.
[22] K. Kemper et al., The AC133 epitope, but not the CD133 protein, is lost upon cancer stem cell differentiation, Cancer Res. 70 (2) (2010) 719–729.

[23] L. Moserle et al., Side population and cancer stem cells: therapeutic implications, Cancer Lett. 288 (1) (2010) 1–9.
[24] J. Zhou et al., Persistence of side population cells with high drug efflux capacity in pancreatic cancer, World J. Gastroenterol. 14 (6) (2008) 925–930.
[25] D.E. Modrak et al., Ceramide regulates gemcitabine-induced senescence and apoptosis in human pancreatic cancer cell lines, Mol. Cancer Res. 7 (6) (2009) 890–896.
[26] N.H. Thoennissen et al., Cucurbitacin B induces apoptosis by inhibition of the JAK/STAT pathway and potentiates antiproliferative effects of gemcitabine on pancreatic cancer cells, Cancer Res. 69 (14) (2009) 5876–5884.
[27] S. Ali et al., Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR-200 and miR-21 expression by curcumin or its analogue CDF, Cancer Res. 70 (9) (2010) 3606–3617.
[28] M. Humbert et al., Masitinib combined with standard gemcitabine chemotherapy: in vitro and in vivo studies in human pancreatic tumour cell lines and ectopic mouse model, PLoS ONE 5 (3) (2010) e9430.