

Corynoxine and corynoxine B enhance the antitumor activity of gemcitabine in Panc-1 cells via ROS-p38 signaling pathway

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant tumors, and effective therapeutic interventions for PDAC are limited. While Corynoxine (Cory) and its isomer Cory B have been identified as autophagy inducers in neuronal cells, it remains unclear whether they exert a therapeutic effect on PDAC. Here, we performed cell counting kit-8 (CCK8), colony formation, 5-Ethynyl-2'-deoxyuridine (EDU) staining, TUNEL, and flow cytometry assays to evaluate the effects of Cory on PDAC. Western blotting was conducted to analyze the protein expression levels. We showed that Cory and Cory B enhanced cell growth arrest and pro-apoptotic effects of gemcitabine (Gem) on Gem-resistant Panc-1 cells. Mechanistic studies revealed that increased production of reactive oxygen species (ROS) and p38 activation were closely associated with Cory and Cory B-induced cell death. Pretreatment with ROS scavenger N-acetylcysteine blocked Cory and Cory B-induced cell death. Moreover, p38 inhibitor SB203580 prevented cell death induced by Cory and Cory B. Overall, Cory and Cory B increase the sensitivity of Gem-resistant Panc-1 cells to Gem through the activation of ROS-dependent p38 signaling pathway. Our results indicate that Cory and Cory B might be potential approaches for PDAC therapy.

Keyword: p38, Pancreatic ductal adenocarcinoma, Reactive oxygen species (ROS)

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive tumors with extremely poor prognosis¹. Only less than 10% of PDAC patients are appropriate for surgery because the most patients are in the advanced stage at the time of diagnosis. Gemcitabine (Gem) has been used for the first-line chemotherapy of PDAC. However, its efficacy is limited by development of drug resistance in cancer cells, posing a great challenge for PDAC treatment². Thus, development of efficient approaches for reversing Gem resistance remains one of the priorities for PDAC therapy.

Corynoxine (Cory) and its enantiomer corynoxine B (Cory B) are two natural compounds isolated from the hooks of *Uncaria macrophylla* wall (Rubiaceae)³. Both Cory and Cory B were originally identified as neuroprotective autophagy enhancers and may

potentially be used for prevention or treatment of Parkinson's disease⁴. It has been reported that Cory suppresses vascular smooth muscle cell proliferation and could be applied in treatment of relevant vascular diseases⁵. To date, it is unclear whether Cory and Cory B have effects on PDAC treatment and enhance the sensitivity of PDAC to Gem.

It has been shown that the drug resistance mechanisms behind the failure of chemotherapy involve the dysregulation of mitogen-activated protein kinases (MAPK)⁶. MAPKs act as key regulators of cellular responses such as proliferation, differentiation, and apoptosis. All three MAPKs including p38, ERK1/2, and JNK1/2 are implicated in tumor drug resistance⁶. Previous studies have revealed that p38 activation was involved in Gem-induced apoptosis in PDAC⁷. Like a tumor suppressor, p38 has been found to negatively regulate cell survival⁶. Besides, p38-induced phosphorylation of p53 protected p53 from mouse double minute 2 (MDM2)-mediated ubiquitylation and degradation⁸. p53 plays a pivotal role in tumor suppression, and p53 activation significantly improved the sensitivity of biliary tract cancer to Gem⁹. Elevated levels of intracellular Reactive oxygen species (ROS) have been implicated

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Abbreviations: Cory, Corynoxine; EdU, 5-Ethynyl-2'-deoxyuridine; Gem, Gemcitabine; MAPK, Mitogen-activated protein kinases; PDAC, Pancreatic ductal adenocarcinoma; ROS, Reactive oxygen species

in the expression of Bcl-2 family proteins, the canonical downstream effectors of p53 involved in regulation of cell apoptosis¹⁰. Meanwhile, ROS production contributed to p38-mediated pro-apoptotic effect^{11,12}. It remains unknown whether ROS and p38 mediate the antitumor effects of Cory and Cory B in combination with Gem on PDAC treatment.

In this study, we investigated the effect of Cory and Cory B in combination with Gem on PDAC cells in order to elucidate whether Cory and Cory B could improve the sensitivity of GEM in PDAC treatment, as well as the underlying mechanisms.

Materials and Methods

Cell culture

The human pancreatic cancer cell line Panc-1 was obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). To generate Gem-resistant Panc-1 (Panc-1-GR) cells, Panc-1 cells were initially exposed to 25 μ M Gem and subsequently treated with stepwise increasing concentrations of Gem (50, 100, 200 and 400 μ M) every two weeks. One single Gem-resistant cell clone was selected for the subsequent experiments. Cells were maintained in a humidified incubator with 5% CO₂ at 37°C.

Cell viability assay

The cell viability was determined by cell counting kit-8 (CCK-8) assay. Panc-1 or Panc-1-GR cells (5×10^3) were seeded into 96-well plates and grown overnight. Afterwards, the cells were treated with various concentrations of Gem, Cory, or Cory B for 24 h. And the culture medium was then replaced with 100 μ L fresh medium containing 10 μ L CCK-8 solution (Dojindo, Kumamoto, Japan). After incubation for 2 h, the optical density (OD) values were detected at a wavelength of 450 nm using a microplate reader¹³ (Thermo Fisher Scientific, Waltham, MA, USA).

Colony formation assay

Panc-1-GR cells (250) were seeded into 12-well plates and cultured for 7 days. Thereafter, the cells were incubated with Gem, Cory, or Cory B for another 4 days, followed by staining with 0.1% crystal violet (Solarbio, Beijing, China) in methanol for 20 min¹⁴. The images were captured and cell colonies (≥ 50 cells) were counted using Image J 1.46 (NIH, Bethesda, MD, USA).

5-Ethynyl-2'-deoxyuridine (EdU) staining and TUNEL assay

Panc-1-GR cells (2.5×10^4) were seeded into cell slides in 12-well plates, grown overnight and then

treated with Gem, Cory, or Cory B for 24 h. After the treatment, EdU or TUNEL solution (Beyotime, Shanghai, China) was added to the plates and incubated for 2 h at 37°C. Thereafter, cells were fixed in 4% paraformaldehyde, permeabilized with 0.3 % Triton X-100, and stained with DAPI (Solarbio). The images were captured by a fluorescence microscope (Olympus, Tokyo, Japan). The proliferation rate was defined as a ratio of EdU-positive cells (green) to DAPI-positive cells (blue), and the apoptosis rate was determined by the proportion of TUNEL-positive (red) cells¹⁵.

Cell cycle analysis

Panc-1-GR cells were incubated with Gem, Cory, or Cory B for 24 h and then fixed with 70% ethanol at -20°C overnight. Thereafter, cells were treated with RNase A (Solarbio) at 37°C for 30 min, stained with PI (BD Biosciences, San Diego, CA, USA), and analyzed using FACSCalibur flow cytometry (BD Biosciences, San Diego, CA, USA)¹⁶. The data were processed using Flowjo 10.0 software (Ashland, OR, USA).

Apoptosis assay

Apoptosis rate was determined using an Annexin V-PE/7-AAD kit (BD Biosciences) according to the manufacturer's protocol. Panc-1-GR cells were incubated with Gem, Cory, or Cory B for 24 h and then stained with Annexin V-PE (5 μ L) and 7-AAD (10 μ L) for 5 min in the dark. Apoptosis assay was performed by FACSCalibur flow cytometry (BD Biosciences), and the data were processed using Flowjo 10.0 software (Ashland). The PE (+)/7-AAD (-) cells and PE (+)/7-AAD (+) cells were defined as early apoptotic and late apoptotic cells, respectively¹⁶.

Western blotting analysis

Proteins were extracted from cells using RIPA lysis buffer (Solarbio) containing 1 \times phenylmethylsulfonyl fluoride (PMSF) (Beyotime) and 1 \times phosphatase inhibitor cocktail (Biomake, Houston, TX, US). Protein concentrations were determined using a BCA kit (Beyotime). Protein samples (20 μ g for each) were separated by SDS-PAGE gels electrophoresis and transferred to a PVDF membrane (Millipore Corporation, Billerica, MA, USA). β -actin was used as a loading control. After being blocked with skim milk (5%), the membrane was incubated at 4°C overnight with one of the following primary antibodies: anti-CDK1(1:1000, proteintech, Wuhan, China), anti-Cyclin B1 (1:1000, Proteintech), anti- γ -H2AX (1:1000, Abcam, Cambridge, UK), anti-Bax

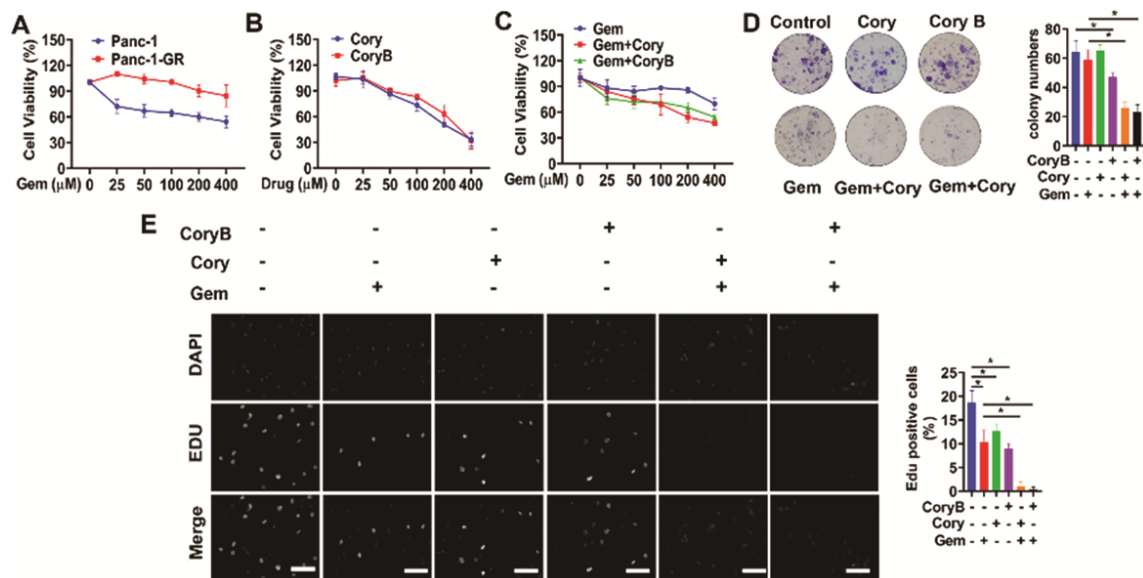


Fig. 1 — Cory and Cory B enhance the sensitivity of Panc-1-GR cells to gemcitabine (Gem). (A-C) Cell Counting Kit-8 (CCK8) assays to determine the effect of (A) Gem on the viability of Panc-1 or Panc1-GR cells; and (B & C) Cory or Cory B and Gem (100 μ M) in combination with Cory or Cory B (25 μ M), and on the viability of Panc1-GR cells; (D) Colony formation; and (E) EdU assays to assess the effect of Gem (100 μ M) combined with Cory or Cory B (25 μ M) on the proliferation rate of Panc-1-GR cells. [Scale bar: 100 μ m. All data are expressed as the mean \pm SD of three independent experiments. * P < 0.05, one-way ANOVA]

(1:1000, Proteintech), anti-Bcl-2 (1:1000, Proteintech), and anti- β -actin (1:5000; Proteintech), anti-p53 (1:1000; Proteintech), anti-Ki-67 (1:1000, Proteintech), anti-p38 (1:1000; Cell Signaling Technology, Danvers, MA, USA), anti-p-p38 (1:1000; Cell Signaling Technology), On the next day, the membrane was subjected to an incubation with HRP-conjugated secondary antibodies (1:1000; Cell Signaling Technology). The immunoreactive bands were visualized using an enhanced chemiluminescence (ECL) kit (Beyotime) under a gel imaging system (Bio-Rad, Hercules, CA, USA)¹⁶. The intensities of the protein bands were analyzed by Image J 1.46 (NIH).

ROS measurement

Panc-1-GR cells were treated with Gem, Cory, or Cory B for 24 h, followed by incubation with DCFH-DA probe (Beyotime) for 30 min at 37°C in the dark. Then, unbound DCFH-DA was removed and the fluorescence intensity was measured at 485/535 nm using a microplate reader¹⁷ (Thermo Fisher Scientific).

Statistical analysis

Data were statistically analyzed using Graphpad Prism 8.0 (San Diego, CA, USA). Statistical significance was assessed by one-way ANOVA. P < 0.05 indicated a statistical significance. All the experiments were performed in triplicates and the data are presented as the mean \pm SD.

Results and Discussion

Cory and Cory B increase sensitivity of Panc-1-GR cells to Gem

We first generated Gem-resistance Panc-1 cells (Panc-1-GR) and then performed CCK-8 assay to examine the cytotoxicity of Gem on both Panc-1 and Panc-1-GR cells. As shown in Fig. 1A, Gem at concentrations ranging between 25 and 400 μ M resulted in a lower growth inhibition rate in Panc-1-GR cells compared with the parental Panc-1 cells. Thus, we selected Panc-1-GR cells and 100 μ M Gem for the subsequent experiments. Moreover, we evaluated the cytotoxicity of Cory and Cory B on Panc-1-GR cells. As depicted in Fig. 1B, similar inhibitory effects of Cory and Cory B were observed in the cells. Strikingly, while no significant reduction in the cell viability was found at the concentration of 25 or 50 μ M, 100 μ M Cory or Cory B gave rise to an apparent cytotoxic effect. As a result, 25 μ M Cory or Cory B was chosen for subsequent assays. Furthermore, CCK-8 assay revealed that compared with Gem treatment alone, co-treatment of Gem and Cory or Cory B (GC/GB) potentiated the cytotoxic effects (Fig. 1C). Consistently, the combination treatment (GC/GB) led to a significant reduction in colony-forming (Fig. 1D) and proliferation ability of the cells (Fig. 1E). Taken together, these results indicate that Cory and Cory B enhance the chemosensitivity of Gem-resistant cells to Gem.

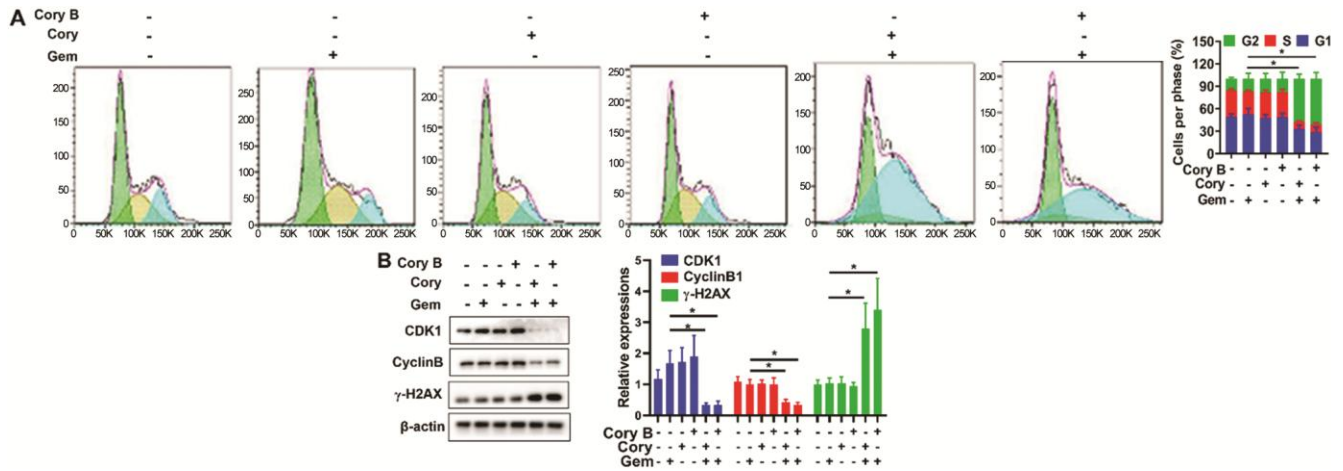


Fig. 2 — Cory and Cory B increase the chemosensitivity of Panc-1-GR cells to Gem by inducing G2/M arrest. (A) Flow cytometry to evaluate the effect of Gem (100 μ M) combined with Cory or Cory B (25 μ M) on cell cycle distribution of Panc-1-GR cells; (B) Western blotting to examine the expression levels of CDK1, CyclinB and γ -H2AX in Panc-1-GR cells. [β -actin was used as a loading control. * P < 0.05, one-way ANOVA]

Cory and Cory B increase chemosensitivity of Panc-1-GR cells to Gem by inducing G2/M arrest

As presented in Fig. 2A, no changes in the cell cycle distribution were found in the cells treated with 100 μ M Gem, 25 μ M Cory, or 25 μ M Cory B. On the contrary, the combination treatment (GC/GB) resulted in a decrease in the number of cells in G0/G1 and S phases as well as the accumulation of cells at the G2/M phase. Similarly, we observed that GC/GB treatment led to an enhanced inhibition on the expression of CDK1 and CyclinB in the cells (Fig. 2B). Given that cell cycle arrest is a typical response to DNA damage¹³, we further investigated whether GC/GB induces DNA damage in Panc-1-GR cells. As shown in Fig. 2B, Gem, Cory, or Cory B displayed no effect on the phosphorylation of DNA damage marker H2AX at Ser139 (γ -H2AX)¹⁴. Conversely, administration of GC/GB in Panc-1-GR cells caused an increase in γ -H2AX protein expression. These observations suggest that GC/GB triggered cell cycle arrest in Panc-1-GR cells through inducing DNA damage.

Cory and Cory B increase Gem-caused cell death in Panc-1-GR cells by inducing apoptosis

Given that apoptosis is commonly triggered by attenuated proliferation and cell cycle arrest¹⁵, we examined whether GC/GB induces apoptosis in Panc-1-GR cells. As depicted in Fig. 3A, no obvious apoptosis was observed in the cells treated with Gem, Cory, or Cory B, whereas GC/GB treatment significantly increased the proportion of early and late

apoptotic cells. Moreover, TUNEL assay identified more staining positive cells in GC/GB treated group as compared to the Gem group (Fig. 3B). Notably, we showed that Bax/Bcl-2 ratio was upregulated in Panc-1 GR cells treated with GC/GB, but not in those treated with Gem, Cory, or Cory B alone (Fig. 3C). All these data indicate that GC/GB promoted the sensitivity of Panc-1 GR cells to Gem through inducing apoptosis.

Cory and Cory B promote chemosensitivity of Panc-1 GR cells to Gem via ROS generation

As a chemotherapy-sensitizing effector, ROS has the capacity to damage DNA¹⁶. We then used DCFH-DA fluorescence probe to measure intracellular ROS levels in the Panc-1 GR cells. As shown in Fig. 4A, ROS production was elevated in the cells treated with GC/GB, while this effect was attenuated upon ROS scavenger N-acetylcysteine (NAC) co-treatment. Moreover, we found that NAC administration protected the cells from GC/GB-induced cell growth arrest (Fig. 4 B & C) and apoptosis (Fig. 4D). Notably, NAC administration attenuated the elevated expression of Bax/Bcl-2 ratio and reversed the decreased Ki-67 expression in the cells treated with GC/GB (Fig. 4E). Collectively, these results suggest that both Cory and Cory B enhance chemosensitivity of Panc-1 GR cells to Gem via ROS generation.

Cory and Cory B increase chemosensitivity of Panc-1 GR cells to Gem via ROS-induced p38 activation

MAPK signaling pathway plays an important role in ROS-mediated cell death^{17,18}. We further determined

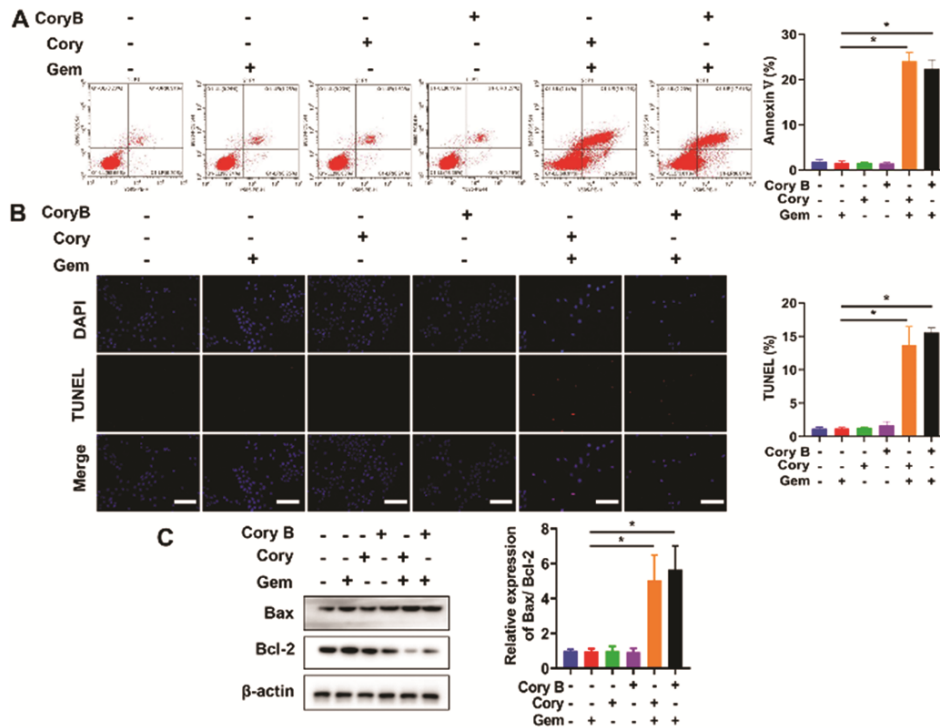


Fig. 3 — Cory and Cory B promote Gem-induced apoptosis in Panc-1-GR cells. (A) Flow cytometry; and (B) TUNEL assays to assess the effect of Gem (100 μ M) combined with Cory or Cory B (25 μ M) on the apoptosis rate of Panc-1-GR cells. Scale bar: 100 μ m; and (C) Expression levels of Bax and Bcl-2 were assayed by Western Blotting analysis. β -actin was used as a loading control. [All data are expressed as the mean \pm SD of three independent experiments. * P < 0.05, one-way ANOVA]

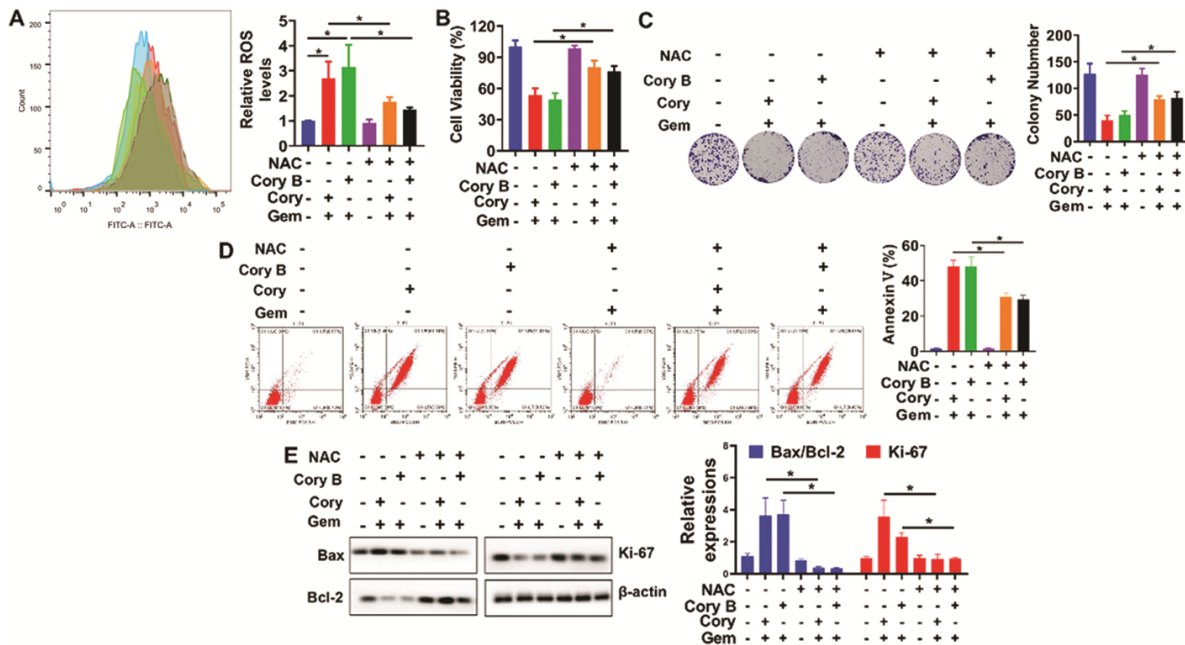


Fig. 4 — Cory and Cory B enhance chemosensitivity of Panc-1-GR cells to Gem via ROS generation. Panc-1-GR cells were pre-treated with NAC (5 mM) for 2 h, followed by incubation with Gem (100 μ M), Cory or CoryB (25 μ M) for another 22 h. (A) Flow cytometry to assess ROS levels; (B) CCK-8 assay to determine cell viability; (C) Colony formation assay to evaluate proliferation rate; (D) Flow cytometry to determine the apoptotic rate of Panc-1-GR cells; and (E) Western blotting assay to detect the expression levels of the related proteins. β -actin was used as a loading control. [All data are expressed as the mean \pm SD of three independent experiments. * P < 0.05, one-way ANOVA]

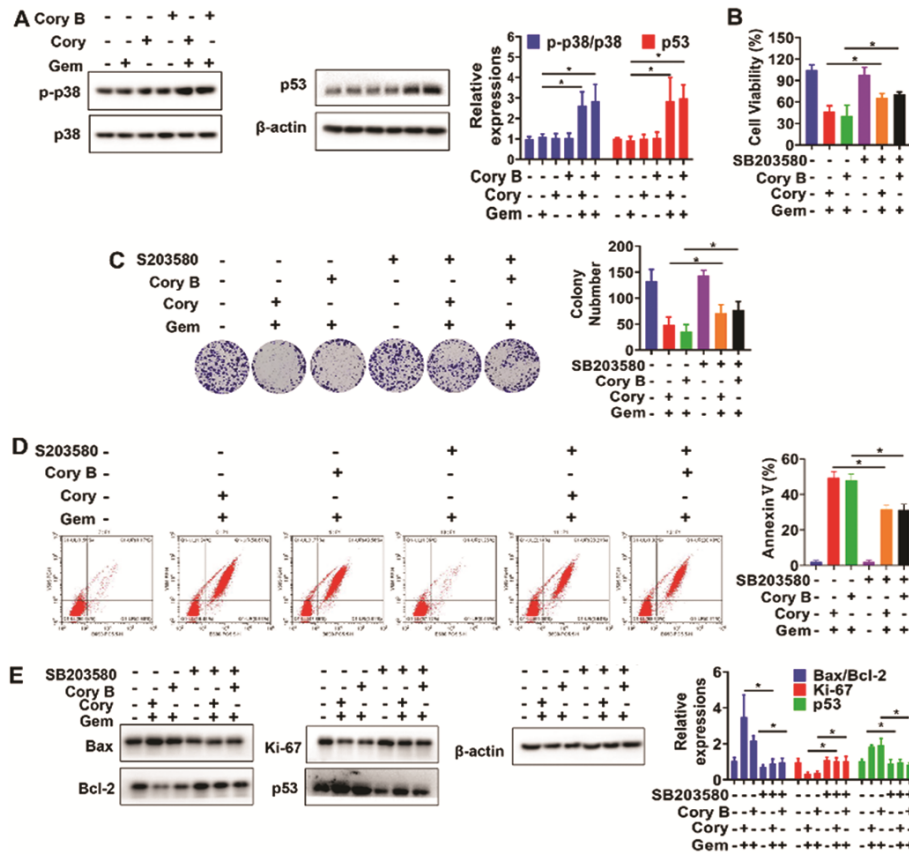


Fig. 5 — Cory and Cory B increase the chemosensitivity of Panc-1-GR cells to Gem via ROS-induced p38 activation. (A) Western blotting to detect expression level of p-p38 and p53 in Panc-1-GR cells treated with Gem (100 μ M), Cory or CoryB (25 μ M); (B) CCK-8 assay to determine the cell viability; (C) Colony formation assay to assess the proliferation rate; (D) Flow cytometry to determine the apoptotic rate of Panc-1-GR cells; and (E) Western blotting to examine the expression levels of the related proteins. [Panc-1-GR cells were pre-treated with SB203580 (10 μ M) for 4 h and then incubated with Gem (100 μ M), Cory or CoryB (25 μ M) for another 20 h. β -actin was used as a loading control. All data are expressed as the mean \pm SD of three independent experiments. * P < 0.05, one-way ANOVA]

whether MAPK signaling is involved in ROS-dependent cell death of Panc-1 GR cells treated with GC/GB. As depicted in Fig. 5A, GC/GB treatment markedly increased the phosphorylation level of p38, while no changes were observed upon Gem, Cory and CoryB treatment alone. p53, an established downstream mediator of p38 signaling, was also reduced after GC/GB treatment. Moreover, pretreatment with p38 inhibitor SB203580 reversed the reduced cell proliferation ability (Fig. 5 B & C) and enhanced apoptosis (Fig. 5D) in GC/GB-treated cells. Consistently, SB203580 pretreatment partially restored the altered expression of proteins implicated in cell survival such as Bax/Bcl-2, Ki-67 and p53 in cells incubated with GC/GB (Fig. 5E). These data led us to propose that Cory and Cory B increase the chemosensitivity of Panc-1 GR cells to Gem probably via ROS-dependent p38 activation.

In this study, we investigated whether Cory and Cory B have combinatory effects with Gem on PDAC treatment. The results have shown that Cory and Cory B could potentiate the effects of Gem on Panc-1 GR cells. Herein, we provided the first demonstration that Cory and Cory B enhanced the cell growth arrest and pro-apoptotic effects of Gem on Panc-1 GR cells. Furthermore, we found that the combined treatment (GC/GB) triggered cell death in Panc-1 GR cells via activation of ROS-mediated p38 signaling pathway.

ROS production in cancer cells serves as a promising strategy to fight cancer¹⁹. Unlike normal cells, most cancer cells exhibit elevated ROS generation because of the distorted cellular metabolism. Several antioxidant mechanisms have been developed to maintain intracellular ROS levels below a cytotoxic threshold²⁰. Hence, it is plausible that cancer cells are more susceptible to ROS-induced

cytotoxicity. Apoptosis is one of the known cell death mechanisms induced by ROS. ROS overproduction could disrupt mitochondrial homeostasis, leading to an imbalance between anti- and pro-apoptotic factors²¹. In this study, we found that ROS are responsible for GC/GB-induced apoptosis owing to the blunted apoptosis upon NAC pretreatment. Moreover, NAC administration reversed the elevated ratio of Bax/Bcl-2 in Panc-1 GR cells treated with GC/GB. It has been demonstrated that induction of ROS overload prompts DNA damage, eliciting cell cycle arrest and ultimately apoptosis²². Herein, we observed that GC/GB treatment increased the expression of phosphorylated H2AX (r-H2AX), a marker of DNA damage-induced DNA double-strand breaks²². While the CDK1-Cyclin B complex is required for G2/M transition in cell cycle¹³, suppression of both CDK1 and Cyclin B was associated with diminished growth of Panc-1 GR cells treated with GC/GB. Taken together, these data demonstrated that Cory and Cory B induce DNA damage response and subsequently cell cycle arrest and apoptosis by elevating intracellular ROS levels, thus augmenting the Gem efficacy in Panc-1 GR cells.

Multiple signaling pathways are regulated by ROS in cancer therapy. It has been reported that increased ROS generation could lead to activation of p38 and ERK1/2, which subsequently results in cell cycle arrest and cell death²³. In the present study, Gem treatment activated p38 signaling pathway, and this effect was increased further after combination with Cory and Cory B. Furthermore, administration with a p38 inhibitor prevented Panc-1-GR cells from GC/GB-induced cell growth impairment and apoptosis. ROS-mediated inhibition of PI3K/Akt signaling pathway was proved to be one of the critical mechanisms for several antitumor agents^{24,25}. Dual inhibition of PI3K and MAPK pathways enhances the response to Gem-based chemotherapy in preclinical models of pancreatic cancer²⁶. Moreover, both Cory and Cory B acted as an autophagy inducer via inactivation of Akt/mTOR pathway. Based on the above observations, we reckon that the antitumor effect of GC/GB may involve either PI3K/AKT signaling pathway or autophagy, investigation should be directed at in-depth understanding of the mechanisms underlying the antitumor effects of Cory and Cory B, as well as animal models and clinical trials.

Conclusion

Our results above have demonstrated that the reactive oxygen species (ROS) induced p38 activation underlay corynoxine or corynoxine B enhanced cellular sensitivity to gemcitabine (Gem) in pancreatic ductal adenocarcinoma (PDAC), and thereby indicate a promising therapeutic strategy for PDAC treatment.

Conflict of interest

Authors declare no competing interests.

References

- 1 Siegel RL, Miller KD, Wagle NS & Jemal A, Cancer statistics, 2023. *CA Cancer J Clin*, 73 (2023) 17.
- 2 Halbrook C, Lyssiotis C, Pasca di Magliano M & Maitra A, Pancreatic cancer: Advances and challenges. *Cell*, 186 (2023) 1729.
- 3 Chen L, Song J, Lu J, Yuan Z, Liu L, Durairajan S & Li M, Corynoxine, a natural autophagy enhancer, promotes the clearance of alpha-synuclein via Akt/mTOR pathway. *J Neuroimmune Pharmacol*, 9 (2014) 380.
- 4 Zhu Z, Liu LF, Su CF, Liu J, Tong BC, Iyaswamy A, Krishnamoorthi S, Sreenivasamurthy SG, Guan XJ, Kan YX, Xie WJ, Zhao CL, Cheung KH, Lu JH, Tan JQ, Zhang HJ, Song JX & Li M, Corynoxine B derivative CB6 prevents Parkinsonian toxicity in mice by inducing PI3K3C3 complex-dependent autophagy. *Acta Pharmacol Sin*, 43 (2022) 2511.
- 5 Kim T, Lee J, Lee J, Yu J, Hwang B, Ye S, Shujuan L, Gao L, Pyo M & Yun Y, Corynoxine isolated from the hook of *Uncaria rhynchophylla* inhibits rat aortic vascular smooth muscle cell proliferation through the blocking of extracellular signal regulated kinase 1/2 phosphorylation. *Biol Pharm Bull*, 31 (2008) 2073.
- 6 Lee S, Rauch J & Kolch W, Targeting MAPK Signaling in Cancer: Mechanisms of Drug Resistance and Sensitivity. *Int J Mol Sci*, 21 (2020) 1102.
- 7 Koizumi K, Tanno S, Nakano Y, Habiro A, Izawa T, Mizukami Y, Okumura T & Kohgo Y, Activation of p38 mitogen-activated protein kinase is necessary for gemcitabine-induced cytotoxicity in human pancreatic cancer cells. *Anticancer Res*, 25 (2005) 3347.
- 8 Zhu Y, Mao XO, Sun Y, Xia Z & Greenberg DA, p38 Mitogen-activated Protein Kinase Mediates Hypoxic Regulation of Mdm2 and p53 in Neurons. *J Biol Chem*, 277 (2002) 22909.
- 9 Wu C-E, Pan Y-R, Yeh C-N & Lunec J, Targeting P53 as a Future Strategy to Overcome Gemcitabine Resistance in Biliary Tract Cancers. *Biomolecules*, 10 (2020) 1474.
- 10 Wörmann SM, Song L, Ai J, Diakopoulos KN, Kurkowski MU, Görgülü K, Ruess D, Campbell A, Doglioni C, Jodrell D, Neeße A, Demir IE, Karpathaki AP, Barenboim M, Hagemann T, Rose-John S, Sansom O, Schmid RM, Protti MP, Lesina M & Algül H, Loss of P53 Function Activates JAK2-STAT3 Signaling to Promote Pancreatic Tumor Growth, Stroma Modification, and Gemcitabine Resistance in Mice and Is Associated With Patient Survival. *Gastroenterology*, 151 (2016) 180.

- 11 Jia XB, Zhang Q, Xu L, Yao WJ & Wei L, Lotus leaf flavonoids induce apoptosis of human lung cancer A549 cells through the ROS/p38 MAPK pathway. *Biol Res*, 54 (2021) 7.
- 12 Chen N, Wang J, He Y, Xu Y, Zhang Y, Gong Q, Yu C & Gao J, Trilobatin Protects Against A β 25–35-Induced Hippocampal HT22 Cells Apoptosis Through Mediating ROS/p38/Caspase 3-Dependent Pathway. *Front Pharmacol*, 11 (2020) 584.
- 13 Zhou X, Wang W, Li Z, Chen L, Wen C, Ruan Q, Xu Z, Liu R, Xu J, Bai Y & Deng J, Rosmarinic Acid Decreases the Malignancy of Pancreatic Cancer Through Inhibiting Gli1 Signaling. *Phytomedicine*, 9 (2022) 112.
- 14 Yin S, Zhao S, Li J, Liu K, Ma X, Zhang Z, Wang R, Tian J, Liu F, Song Y, Song M, Zhao R, Yang R, Lee MH & Dong Z, NUMA1 modulates apoptosis of esophageal squamous cell carcinoma cells through regulating ASK1-JNK signaling pathway. *Cell Mol Life Sci*, 8 (2023) 80.
- 15 Wu X, Zhang X, Liu P & Wang Y, Involvement of Ataxin-3 (ATXN3) in the malignant progression of pancreatic cancer via deubiquitinating HDAC6. *Pancreatology*, 7 (2023) 11.
- 16 Wu J, Ye F & Xu T, Celastrol impairs tumor growth by modulating the CIP2A-GSK3 β -MCL-1 axis in gastric cancer cells. *Aging (Albany NY)*, 15 (2023) 19.
- 17 Jambrovics K, Póliska S, Scholtz B, Uray IP & Balajthy Z, ATO Increases ROS Production and Apoptosis of Cells by Enhancing Calpain-Mediated Degradation of the Cancer Survival Protein TG2. *Int J Mol Sci*, 13(2023)240.
- 18 Matthews HK, Bertoli C & de Bruin RAM, Cell cycle control in cancer. *Nat Rev Mol Cell Biol*, 23 (2022) 74. doi: 10.1038/s41580-021-00404-3.
- 19 Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S & Pommier Y, γ H2AX and cancer. *Nat Rev Can*, 8 (2008) 957.
- 20 Lowe S, Cepero E & Evan G, Intrinsic tumour suppression. *Nature*, 432 (2004) 307.
- 21 Snezhkina AV, Kudryavtseva AV, Kardymon OL, Savvateeva MV, Melnikova NV, Krasnov GS & Dmitriev AA, ROS Generation and Antioxidant Defense Systems in Normal and Malignant Cells. *Oxid Med Cell Longev*, (2019) 1.
- 22 Zhan Y, Chen Q, Song Y, Wei X, Zhao T, Chen B, Li C, Zhang W, Jiang Y, Tan Y, Du B, Xiao J & Wang K, Berbamine Hydrochloride inhibits lysosomal acidification by activating Nox2 to potentiate chemotherapy-induced apoptosis via the ROS-MAPK pathway in human lung carcinoma cells. *Cell Biol Toxicol*, (2022). doi: 10.1007/s10565-022-09756-8.
- 23 Hao X, Feng P, Zhang Y, Wang F, Wang G & Fei H, Scutebarbatine A induces ROS-mediated DNA damage and apoptosis in breast cancer cells by modulating MAPK and EGFR/Akt signaling pathway. *Chem Biol Interact*, 378 (2023) 110487.
- 24 Raj L, Ide T, Gurkar AU, Foley M, Schenone M, Li X, Tolliday NJ, Golub TR, Carr SA, Shamji AF, Stern AM, Mandinova A, Schreiber SL & Lee SW, Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature*, 475 (2011) 231.
- 25 Pelicano H, Carney D & Huang P, ROS stress in cancer cells and therapeutic implications. *Drug Resist Updat*, 7 (2004) 97.
- 26 Tuli HS, Kaur J, Vashishth K, Sak K, Sharma U, Choudhary R, Behl T, Singh T, Sharma S, Saini AK, Dhama K, Varol M & Sethi G, Molecular mechanisms behind ROS regulation in cancer: A balancing act between augmented tumorigenesis and cell apoptosis. *Arch Toxicol*, 97 (2023) 103.
- 27 Renaudin X, Reactive oxygen species and DNA damage response in cancer. *Int Rev Cell Mol Biol*, 364 (2021) 139.
- 28 Hao Y, Huang Y, Chen J, Li J, Yuan Y, Wang M, Han L, Xin X, Wang H, Lin D, Peng F, Yu F, Zheng C & Shen C, Exopolysaccharide from *Cryptococcus heimaeyensis* S20 induces autophagic cell death in non-small cell lung cancer cells via ROS/p38 and ROS/ERK signalling. *Cell Prolif*, 53 (2020) e12869.
- 29 Li Z, Xu T, Fan X, Chen K, Wan C, Li X, Yin H & Li S, Bisphenol A aggravate selenium deficiency-induced apoptosis via miR-215-3p/Dio1 to activate ROS/PI3K/AKT pathway in chicken arterial. *J Cell Physiol*, 238 (2023) 1256. doi: 10.1002/jcp.31007.
- 30 Dong S, Liang S, Cheng Z, Zhang X, Luo L, Li L, Zhang W, Li S, Xu Q, Zhong M, Zhu J, Zhang G & Hu S, ROS/PI3K/Akt and Wnt/ β -catenin signalings activate HIF-1 α -induced metabolic reprogramming to impart 5-fluorouracil resistance in colorectal cancer. *J Exp Clin Cancer Res*, 41 (2022) 15.
- 31 Awasthi N, Kronenberger D, Stefaniak A, Hassan MS, von Holzen U, Schwarz MA & Schwarz RE, Dual inhibition of the PI3K and MAPK pathways enhances nab-paclitaxel/gemcitabine chemotherapy response in preclinical models of pancreatic cancer. *Cancer Lett*, 459 (2019) 41