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# Cisplatin-induced non-apoptotic death of pancreatic cancer cells requires mitochondrial cyclophilin-D-p53 signaling

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ABSTRACT

The pancreatic cancer remains a fatal disease for the majority of patients. Cisplatin has displayed significant cytotoxic effects against the pancreatic cancer cells, however the underlying mechanisms remain inconclusive. Here, we found that cisplatin mainly induced non-apoptotic death of the pancreatic cancer cells (AsPC-1 and Capan-2), which was associated with a significant p53 activation (phosphorylation and accumulation). Further, activated p53 was found to translocate to mitochondria where it formed a complex with cyclophilin D (Cyp-D). We provided evidences to support that mitochondrial Cyp-D/p53 complexation might be critical for cisplatin-induced non-apoptotic death of pancreatic cancer cells. Inhibition of Cyp-D by its inhibitor cyclosporine A (CsA), or by shRNA-mediated knockdown suppressed cisplatin-induced pancreatic cancer cell death. Both CsA and Cyp-D knockdown also disrupted the Cyp-D/p53 complex formation in mitochondria. Meanwhile, the pancreatic cancer cells with p53 knockdown were resistant to cisplatin. On the other hand, HEK-293 over-expressing Cyp-D were hyper-sensitive to cisplatin. Interestingly, camptothecin (CMT)-induced pancreatic cancer cell apoptotic death was not affected CsA or Cyp-D knockdown. Together, these data suggested that cisplatin-induced non-apoptotic death requires mitochondria Cyp-D-p53 signaling in pancreatic cancer cells.



## 1. Introduction

The pancreatic cancer remains a fatal disease for the majority of patients. Due to its predominantly late diagnosis, most patients present with the advanced diseases [1]. The current standard treatments for this disease include radiation and chemotherapy (gemcitabine) [2]. However, the pancreatic cancers are among the most intrinsically resistant tumors to both therapies, with a median survival of 6–12 months [3]. Hence, research scientists and oncologists are currently searching for novel and efficient anti-pancreatic cancer agents [4,5].

Cisplatin is a potent inducer of cell death in most cancer cells. It is among the most effective and widely used chemotherapeutic agents employed for the cancer treatments. Meanwhile, cisplatin is currently undergoing clinical and pre-clinical evaluations for the treatment of pancreatic cancer [6–8]. As a matter of fact, cisplatin has displayed significant cytotoxic effects against pancreatic cancer cells, however the underlying mechanisms are not fully understood [6–8].

The earlier studies have shown that cisplatin-induced cancer cell death was associated with p53 activation [9]. Pifithrin-α, an inhibitor of p53 suppressed cisplatin-induced cell death [10]. However, how p53 mediates cisplatin-induced cancer cell death remains to be explored. Although one main consequence of p53 activation is cell apoptosis, a recent study by Vaseva et al. showed that p53 is also important for cell necrosis. Oxidative stress-activated p53 translocates to the inner membrane of mitochondria, where it forms a complex with cyclophilin D (Cyp-D). This Cyp-D/p53 mitochondrial association is important for mitochondrial permeability transition pore (mPTP) opening and subsequent cell necrosis [11,12]. In the current study, we aimed to understand the potential role of Cyp-D and p53 in cisplatin-induced death of pancreatic cancer cells.

## 2. Materials and methods

### 2.1. Chemical and reagents

Cisplatin, camptothecin (CMT) and cyclosporine A (CsA) were obtained from Sigma (Sigma, St. Louis, MO); Z-VAD-fmk (ZVAD)

*Abbreviations:* MTT, 3-[4,5-dimethylthylthiazol-2-yl]-2,5 diphenyltetrazolium bromide; CMT, camptothecin; Cyp-D, cyclophilin D; CsA, cyclosporine A; mPTP, mitochondrial permeability transition pore.

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was purchased from Calbiochem (Shanghai, China). Anti-Erk1/2, p53, tubulin and rabbit/mouse IgG-horseradish peroxidase (IgG-HRP) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other antibodies used in this study were purchased form Cell Signaling Tech (Denver MA).

### 2.2. Cell culture

HEK-293 cells, pancreatic cancer AsPC-1 (p53 WT) and Capan-2 (p53 WT) cells were maintained in a DMEM medium (Invitrogen, Shanghai, China), supplemented with 10% fetal bovine serum (FBS, Sigma, Shanghai, China), penicillin/streptomycin (1:100, Sigma, Shanghai, China) and 4 mM L-glutamine (Sigma), in a $CO_2$ incubator at 37 °C.

### 2.3. Cell viability detection

Cell viability was measured by the 3-[4,5-dimethylthylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT, Sigma) assay [13]. The results were expressed as a percentage of absorbance measured in the control cells.

### 2.4. Cell apoptosis quantification by histone DNA–ELISA

The Cell Apoptosis ELISA Detection Kit (Roche, Palo Alto, CA) was used to detect the pancreatic cancer cell apoptosis after indicated treatments according to the manufacturer's protocol [14].

### 2.5. Clonogenic survival

The pancreatic cancer cells were suspended in 1 ml of DMEM containing 0.25% agar (Sigma, St. Louis, MO), 10% FBS and indicated treatments. The cell suspension was then added on the top of a pre-solidified 100 mm culture dish. The drug-containing medium was switched every 2 days. After 8 days of incubation, the survival colonies were photographed at 4×. The remaining large colonies were manually counted, and the number was normalized to that of control group.

### 2.6. Flow cytometry detecting Annexin V positive ("apoptotic") cells

The pancreatic cancer cell apoptosis was determined by the Annexin V In Situ Cell Apoptosis Detection Kit (Beyotime, Shanghai, China) according to the manufacturer's instruction. Pancreatic cancer cells were also stained with propidium iodide (PI, Molecular Probes). Annexin $V^+/PI^-$ cells (the apoptotic cells) were recorded through a flow cytometry (BD Bioscience).

### 2.7. Western blots

The cells were washed with ice-cold PBS before lysed with the lysis buffer (Beyotime, Shanghai, China). The lysates were separated by the 10% SDS–polycrylamide gel, and were electro-transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). The membranes were blocked with 10% milk in PBS plus Tween-20 (0.5%) (TBST), incubated overnight at 4 °C with the primary antibody, and then incubated with HRP-conjugated secondary antibody. The detection was performed by Supersignal West Pico Enhanced Chemiluminescent (ECL, Pierce, Rockville, IL). The blot intensity was quantified by Image J software. The intensity of each phosphorylated band was normalized to the intensity of non-phosphorylated kinase band (the loading control).

### 2.8. Mitochondrial immunoprecipitation (Mito-IP)

As reported [11], mitochondria of cultured pancreatic cancer cells were isolated using "Mitochondria Isolation Kit for Cultured Cells" from Thermo Scientific (Bremen, Germany). The mitochondria were then lysed with lysis buffer (20 mM Tris, pH 7.4, 135 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 10% glycerol and 1% Triton X-100). Immunoprecipitation (IP) was performed using anti-Cyp-D antibody (see [11]), and the immune complexes were captured with protein A/G-Sepharose (Santa Cruz). Proteins were resolved by SDS–PAGE, transferred onto the PVDF membrane, and detected by the antibody against p53 (Santa Cruz).

### 2.9. Real-time polymerase chain reaction (RT-PCR)

The total RNA in pancreatic cancer cells was prepared by RNA-TRIZOL extraction (Gibco, Grand Island, NY). The concentration of the extracted RNA was measured spectrophotometrically at A260 and A280. Real time-reverse transcription-polymerase chain reaction (RT-PCR) was performed by using Qiagen real-time RT-PCR kit (Hilden, Germany) according to the manufacturer's instructions. Primers were 5′-GCA CCGAATTCATGCTAGCTCTGC-3′ and 5′-GGCTTGAATTCTTAGCTCAACTGGCC-3′ for human Cyp-D [13]. The PCR reactions were carried out on a Bio-Rad real-time PCR detection system (Bio-Rad, Shanghai, China) by using 2 μg of synthesized cDNA. The Cyp-D mRNA expression level in stable cells transfected with Cyp-D shRNA was expressed as percentage of that in control cells.

### 2.10. Cyp-D vector and transfection

The Cyp-D plasmid and the empty vector (pSuper-puromycin) were gifts from Dr. Zhi-gang Bi [13]. Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) was used to transfect Cyp-D plasmid or the empty vector (1 μg/well) into HEK-293 cells according the manufacturer's protocol. The Cyp-D expression in the transfected cells was examined by Western blots.

### 2.11. Cyp-D, p53 shRNA knockdown and the stable cells selection

Two patches of Cyp-D lentiviral partials containing shRNAs targeting different sequence of human Cyp-D mRNA were purchased from Santa Cruz Biotech (shRNA-1) [11] and Niu-en biotech company (shRNA-2) (Shanghai, China) respectively. The lentiviral partials containing p53 shRNA were purchased from Santa Cruz Biotech (Santa Cruz, CA). The lentiviral particles containing scramble (Santa Cruz) or the targeted shRNA (against Cyp-D or p53) were added to pancreatic cancer cells for 12 h, cell culture medium was then replaced by fresh growth medium (with FBS), and cells were cultured for additional 36 h. Stable clones expressing target shRNA were selected by puromycin (1–2 μg/ml). The expressions of target protein (p53 or Cyp-D) in the resistant colonies were detected by Western blots. Only the stable cells with significant knockdown of the target protein were used for further experiments.

### 2.12. Statistical analyses

The data were expressed as mean ± SD. Data were collected using three set of independent experiments. Statistical differences were analyzed by one-way ANOVA followed by multiple comparisons performed with post hoc Bonferroni test (SPSS version 16). Values of $p < 0.05$ were considered statistically significant. The significance of any differences between two groups was tested using paired-samples $t$ test when appropriated.

## 3. Results

### 3.1. Cyclosporine A (CsA) suppresses cisplatin-induced pancreatic cancer cell death

In the current study, we investigated the potential role of Cyp-D in cisplatin-mediated pancreatic cancer cell death. Cyclosporine A (CsA), the Cyp-D inhibitor [11,13] was utilized. Results in Fig. 1A and C showed that CsA significantly inhibited cisplatin-induced death in two pancreatic cancer cell lines (AsPC-1 and Capan-2). The cell death was reflected by cell viability loss (Fig. 1A and C). To further confirm the anti-cisplatin effect of CsA, clonogenic survival assay was performed. As shown in Fig. 1B and D, in both AsPC-1 and Capan-2 cells, cisplatin dose-dependently inhibited cancer cell survival. Such effects of cisplatin were alleviated by CsA co-administration. Taken together, these data suggested that cisplatin-induced pancreatic cancer cell death might be associated with Cyp-D signaling.

### 3.2. Cisplatin fails to induce significant apoptosis in pancreatic cancer cells

We have shown that cisplatin induced significant cell death in cultured pancreatic cancer cells. Next we tested whether such a cytotoxic effect by cisplatin was due to cell apoptosis. The cell apoptosis was detected by histone-DNA apoptosis ELISA assay (Fig. 2A), Annexin V assay (Fig. 2B) [14] and cleaved-caspase-3 Western blotting assay (Fig. 2C). The results from these three assays showed that cisplatin failed to induce significant cell apoptosis in AsPC-1 cells, indicating that cell death-induced by cisplatin was not dependent on apoptosis. As a matter of fact, the apoptosis inhibitor z-VAD-fmk (ZVAD) only slightly reduced cisplatin-induced AsPC-1 cell death (Fig. 2D). On the other hand, camptothecin (CMT), an apoptosis inducer [11], caused significant apoptosis (Fig. 2A–C) and viability loss (Fig. 2D) in AsPC-1 cells, the latter was almost reversed by z-VAD-fmk co-administration (Fig. 2D). The results in Fig. 2E demonstrated that z-VAD-fmk had almost no effect on cisplatin-induced death of Capan-2 cells, once again indicating that apoptosis may not play a significant role in cisplatin-induced cell death.

### 3.3. Cisplatin-induced pancreatic cancer cell death is determined by Cyp-D expression

To further confirm the role Cyp-D in cisplatin-induced cancer cell death, we created two stable AsPC-1 lines expressing Cyp-D shRNAs (Cyp-D-RNAi-1 and Cyp-D-RNAi-2) (see Section 2) (Fig. 3A). As shown in Fig. 3A, the Cyp-D mRNA and protein expressions were dramatically down-regulated in the Cyp-D shRNA-expressing stable AsPC-1 cells. The cell viability assay and colonial survival assay results in Fig. 3B and C demonstrated that Cyp-D shRNA knockdown dramatically inhibited cisplatin-induced AsPC-1 cell death. The Cyp-D-shRNAs also successfully knocked-down Cyp-D in Capan-2 cells (Fig. 3D), and the amount of cell death by cisplatin was also significantly lower in Cyp-D knock-down stable Capan-2 cells (Fig. 3E). We also exogenously expressed Cyp-D in HEK-293 cells. As shown in Fig. 3F, HEK-293 cells with Cyp-D over-expression were hypersensitive to cisplatin. These results once again confirmed that Cyp-D is required for cisplatin-induced pancreatic cancer cell death. It should be noted that camptothecin (CMT)-induced apoptotic cell death (see Fig. 2) was not affected by Cyp-D knockdown (Fig. 3B and C) or over-expression (Fig. 3F).

### 3.4. Cisplatin induces p53 activation and translocation to mitochondria, where it forms a complex with Cyp-D

In an effect to explore the role of Cyp-D in cell death, Vaseva et al. demonstrated that Cyp-D is required for necrotic but not some apoptotic cell death. The authors showed that cellular stresses cause p53 mitochondrial translocation, where it binds to Cyp-D. The p53/Cyp-D complexation in mitochondria appears to be important for mPTP opening and cell necrosis [11]. We tested whether similar situation was also happening in cisplatin-treated pancreatic cancer cells. As expected, Western blot results in Fig. 4A and D confirmed p53 activation (phosphorylation and

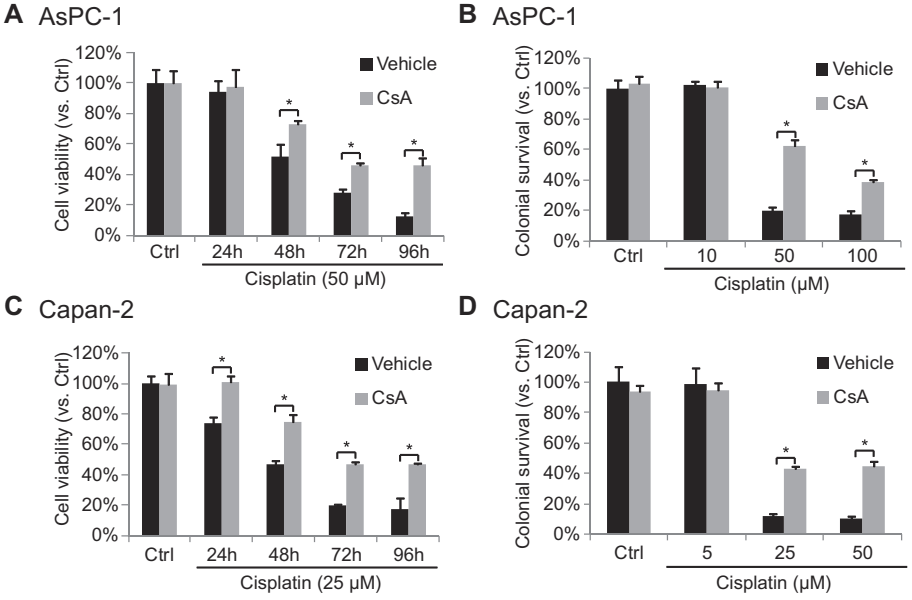


**Fig. 1.** Cyclosporine A (CsA) suppresses cisplatin-induced pancreatic cancer cell death. AsPC-1 and Capan-2 pancreatic cancer cells were treated with CsA (10 μM) and/or cisplatin (50 μM for AsPC-1 cells, and 25 μM for Capan-2 cells), cells were further cultured for indicated time points, cell viability was then analyzed by MTT assay (A and C). AsPC-1 and Capan-2 cells were maintained in culture medium containing CsA (10 μM) and/or indicated concentration of cisplatin, drug-containing medium was switched every 2 days for 8 days, colonial survival assay was performed and the survival colonies were counted manually (B and D). Experiments in this figure were repeated three times. Data were presented as mean ± SD. $^{*}p < 0.05$.

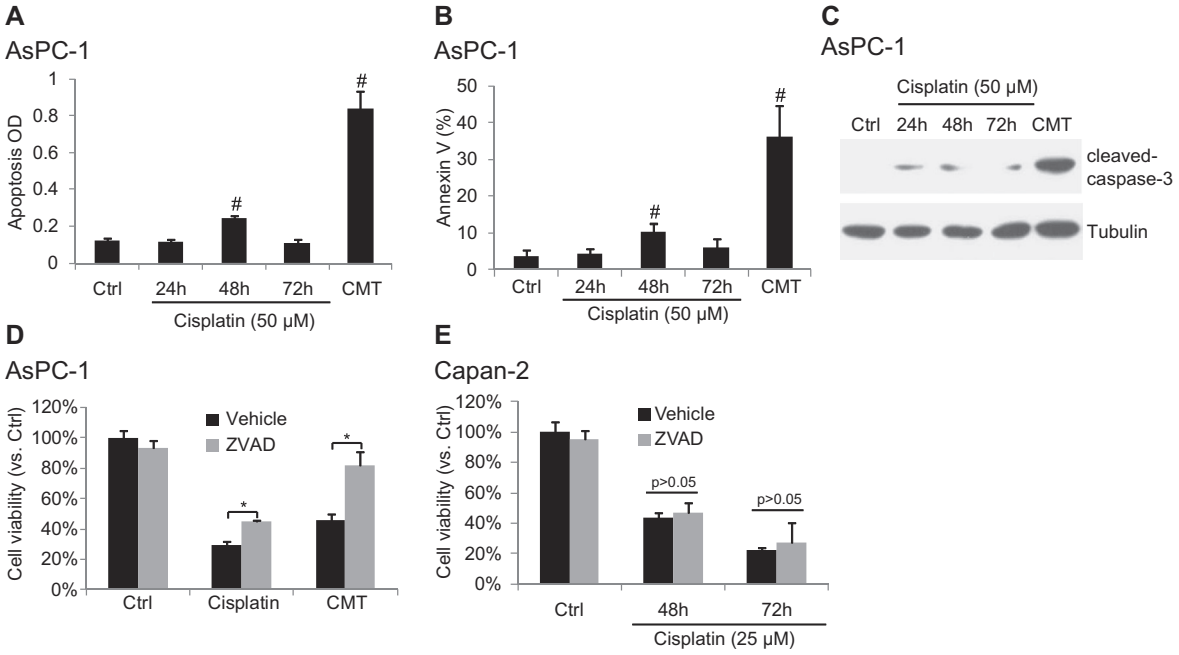


**Fig. 2.** Cisplatin fails to induce significant apoptosis in pancreatic cancer cells. AsPC-1 cells were either left untreated or treated with cisplatin (50 μM) for 24, 48 and 72 h, or treated with camptothecin (CMT, 5 μM) for 48 h, cell apoptosis was analyzed by histone-DNA apoptosis ELISA assay (A) and Annexin V assay (B), expressions of cleaved-caspase-3 and tubulin in above cells were tested by Western blots (C). AsPC-1 cells were pre-treated with z-VAD-fmk (ZVAD, 40 μM) for 1 h, followed by cisplatin (50 μM) or camptothecin (CMT, 5 μM) stimulation, cells were further cultured for 72 h, cell viability was then tested by MTT assay (D). Capan-2 cells were pre-treated with z-VAD-fmk (ZVAD, 40 μM) for 1 h, followed by cisplatin (25 μM) stimulation, cells were further cultured for 48 or 72 h, cell viability was tested (E). Experiments in this figure were repeated three times. Data were presented as mean ± SD. $^{\#}p < 0.05$ vs. control group (A and B). $^{*}p < 0.05$ (D).

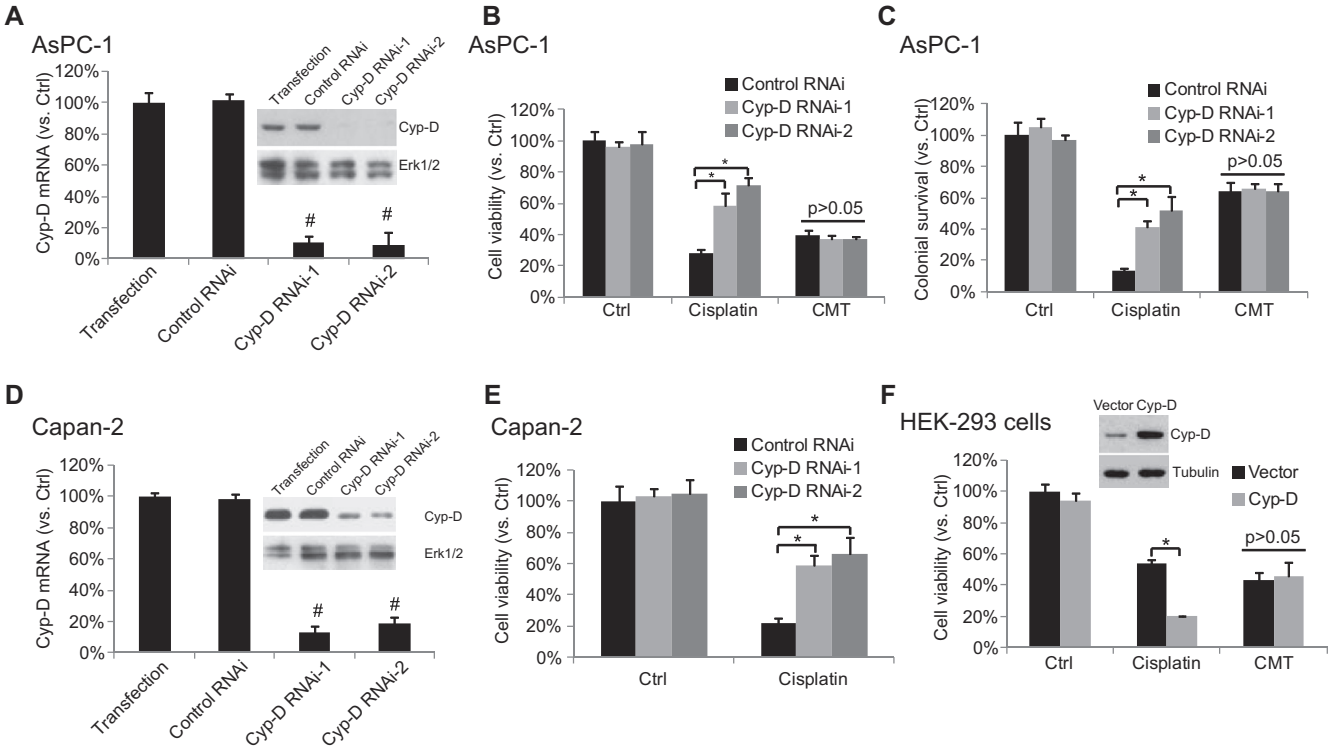


**Fig. 3.** Cisplatin-induced pancreatic cancer cell death is determined by Cyp-D expression. The Cyp-D mRNA and protein levels in stable AsPC-1 cells expressing scramble shRNA or Cyp-D shRNAs (RNAi-1 was from Santa Cruz, and RNAi-2 was from Niu-en biotech) were shown in (A). The scramble or Cyp-D shRNA-transfected stable AsPC-1 cells were treated with cisplatin (50 μM) or camptothecin (CMT, 5 μM), cells were further cultured, cell viability was analyzed by MTT assay after 72 h (B), colonial survival assay was also performed (C). The Cyp-D mRNA and protein expression levels in stable Capan-2 cells expressing scramble control shRNA or Cyp-D shRNAs were shown (D). The scramble or Cyp-D shRNA-transfected stable Capan-2 cells were treated with cisplatin (25 μM), cells were further cultured for 72 h, cell viability was analyzed (E). The vector or Cyp-D cDNA transfected HEK-293 cells were treated with cisplatin (50 μM) or camptothecin (CMT, 5 μM), cells were further cultured for 48 h and MTT cell viability was detected (F). Experiments in this figure were repeated three times. Data were presented as mean ± SD. $^{\#}p < 0.05$ vs. transfection control group (A and D). $^{*}p < 0.05$.

accumulation) after cisplatin treatment in AsPC-1 and Capan-2 cells. Interestingly, in both cell lines, p53 was found to translocate to mitochondria (Fig. 4B and E), where it also formed a complex with Cyp-D (Fig. 4C and F). Note that Cyp-D was exclusively expressed in mitochondria, and its expression level was not affected by cisplatin treatment (Fig. 4A, B, D and E). Importantly, CsA or shRNA knockdown of Cyp-D, which inhibited cisplatin-induced cell death (see Figs. 2 and 3), also abolished p53/Cyp-D mitochondrial association (Fig. 4C and F). These results indicated that Cyp-D/p53 mitochondrial complexation was required for cisplatin-induced pancreatic cancer cell death. The fact that cell death by cisplatin was significantly inhibited by p53 knockdown (Fig. 4G and H) further supported our hypothesis.

## 4. Discussion

An emerging theory for cancer therapy is to induce apoptotic cell death through anti-cancer drugs. However, pancreatic cancers are extremely resistant to apoptosis inducing drugs. As shown in Fig. 2, although cisplatin induced significant death of pancreatic cancer cells, apoptosis did not play a role. We found that cisplatin induced p53 activation (phosphorylation and accumulation) and translocation to mitochondria, where it formed a complex with Cyp-D. Our evidence supported that the association between p53/Cyp-D in mitochondria appeared required for cisplatin-induced non-apoptotic cell death. Inhibition of Cyp-D by its inhibitor (CsA), or by shRNA-mediated knockdown significantly suppressed cisplatin-induced pancreatic cancer cell death. Both CsA and knockdown of Cyp-D disrupted Cyp-D/p53 complex formation in mitochondria. Meanwhile, the pancreatic cancer cells with p53 deficiency were resistant to cisplatin. On the other hand, HEK-293 cells with Cyp-D overexpression were hyper-sensitive to cisplatin. Together, these data suggested that cisplatin-induced non-apoptotic pancreatic cancer cell death requires mitochondrial Cyp-D–p53 signaling.

The mitochondria serve as a "powerhouse" and provide over 90% of ATP necessary for cell life. However, recent studies confirmed that mitochondria also play a central role in cell death [15]. It is now known that mitochondrial mPTP, a channel composed of the adenine nucleotide translocator (ANT), the voltage-dependent anion channel (VDAC) Cyp-D and other necessary components [16], serves as the central hub for both apoptotic and non-apoptotic (necrotic) cell death [11,15,17,18]. Oxidative and other cellular stresses cause mPTP opening, mitochondrial swelling and outer membrane rupture, a necessary step for necrotic cell death [16]. Data from transgenic mice supported that Cyp-D is critical for the function of mPTP [17,18]. Cyp-D null mice were

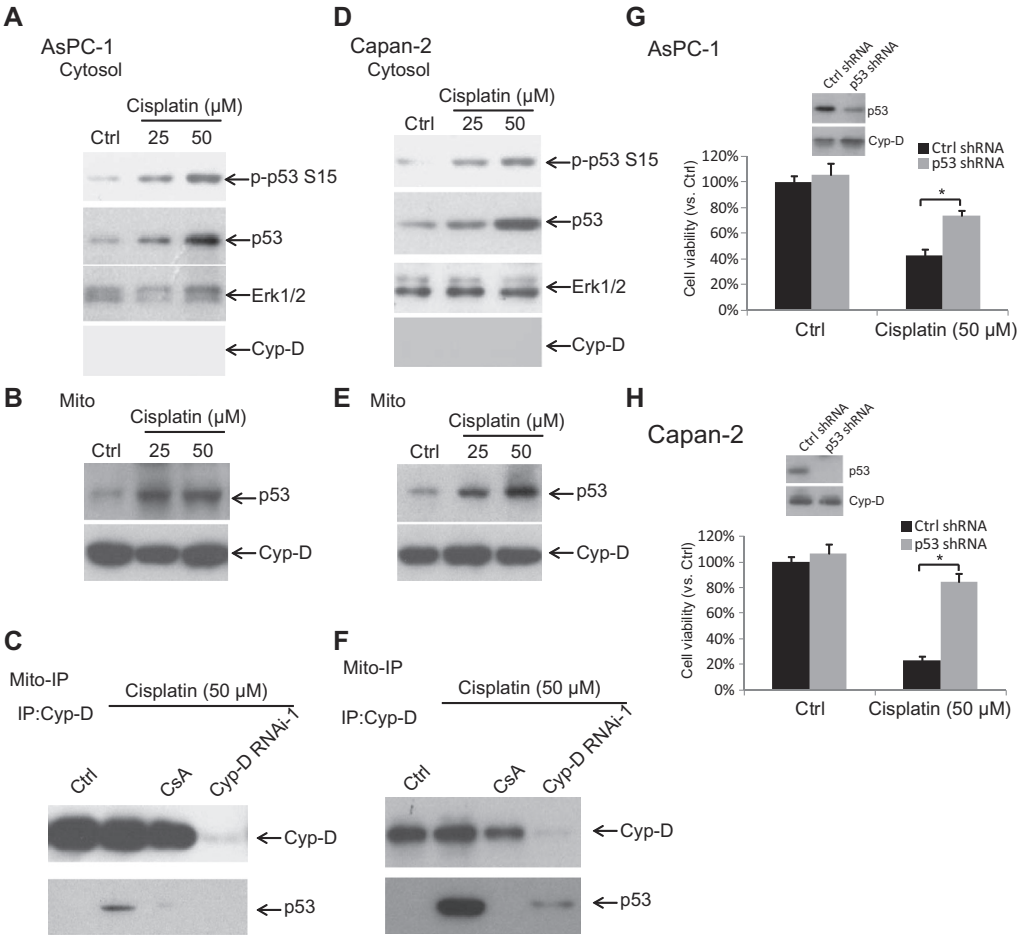


**Fig. 4.** Cisplatin induces p53 activation and translocation to mitochondria, where it forms a complex with Cyp-D. AsPC-1 (A and B) or Capan-2 (D and E) cells were treated with indicated concentration of cisplatin, cells were further cultured for 12 h, expressions of p53, phospho-p53 (Ser 15), Erk1/2 (loading) or Cyp-D in cytosol (A and D) or mitochondria (B and E) were tested by Western blots. The stable AsPC-1 (C) or Capan-2 (F) cells expressing scramble or Cyp-D shRNA (RNAi-1, Santa Cruz) were pretreated with CsA (10 μM), followed by the indicated cisplatin stimulation, cells were further cultured for 6 h, the association between Cyp-D and p53 in mitochondria in both cell lines was tested by mito-IP as described (C and F). The scramble control shRNA or p53 shRNA-transfected stable AsPC-1 (G) or Capan-2 (H) cells were stimulated with cisplatin, cells were further cultured for 72 h, cell viability was then detected by MTT assay. Experiments in this figure were repeated three times. Data were presented as mean ± SD. $^{*}p < 0.05$.

resistant to ischemia/reperfusion-induced cell death, whereas mice over-expressing Cyp-D demonstrated spontaneous cell death with swelling mitochondria [17,18]. Further, cells with Cyp-D deficiency were protected from $Ca^{2+}$-overload or oxidative stress-induced necrotic cell death, but not TNF-α/CMT-induced cell apoptosis [11,17,18]. In the current study, we found that cisplatin mainly induced non-apoptotic death in pancreatic cancer cells, and such effects appeared to be dependent on Cyp-D. Both Cyp-D inhibitor CsA and shRNA knockdown significantly inhibited cisplatin-induced pancreatic cancer cell death.

p53, the cancer suppressor gene, is involved in the maintenance of the genome integrity [19]. Following exposure to DNA damage and other possible stresses, cells show a rapid increase in p53 protein level as a consequence of its stabilization by post-transcriptional modifications [19]. Phosphorylation in several serine/threonine residues of p53 is the most common post-transcriptional modification, leading to p53 activation and stabilization. Previous studies have shown that cisplatin-induced cancer cell death is associated with p53 activation [9,10,20], but how p53 mediates cell death, especially the non-apoptotic cell death (as seen in this study), is not fully understood. As discussed, the earlier study has shown reactive oxygen species-activated p53 translocates to the mitochondria, and forms a complex with Cyp-D to regulate mPTP opening and necrotic cell death. In the current study, using two pancreatic cancer cells with wild-type p53 [21,22], we found that cisplatin-activated p53 also formed a complex with Cyp-D in mitochondria. Disrupting this complex formation by CsA, or by p53/Cyp-D shRNA knockdown significantly inhibited cisplatin-induced cell death. Hence, the p53/Cyp-D complex formation in mitochondria is required for cisplatin-induced cell death.

## References


[1] S. Rosewicz, B. Wiedenmann, Pancreatic carcinoma, Lancet 349 (1997) 485–489.

[2] G. Colucci, R. Labianca, F. Di Costanzo, V. Gebbia, G. Carteni, B. Massidda, E. Dapretto, L. Manzione, E. Piazza, M. Sannicolo, M. Ciaparrone, L. Cavanna, F. Giuliani, E. Maiello, A. Testa, P. Pederzoli, M. Falconi, C. Gallo, M. Di Maio, F. Perrone, Randomized phase III trial of gemcitabine plus cisplatin compared with single-agent gemcitabine as first-line treatment of patients with advanced pancreatic cancer: the GIP-1 study, J. Clin. Oncol. 28 (2010) 1645–1651.

[3] S. Jenks, AACR highlights: promise for treating pancreatic cancer, J. Natl. Cancer Inst. 103 (2011) 786–787.

[4] E. Costello, J.P. Neoptolemos, Pancreatic cancer in 2010: new insights for early intervention and detection, Nat. Rev. Gastroenterol. Hepatol. 8 (2011) (2010) 71–73.

[5] H.H. Wong, N.R. Lemoine, Pancreatic cancer: molecular pathogenesis and new therapeutic targets, Nat. Rev. Gastroenterol. Hepatol. 6 (2009) 412–422.

[6] V. Heinemann, D. Quietzsch, F. Gieseler, M. Gonnermann, H. Schonekas, A. Rost, H. Neuhaus, C. Haag, M. Clemens, B. Heinrich, U. Vehling-Kaiser, M. Fuchs, D. Fleckenstein, W. Gesierich, D. Uthgenannt, H. Einsele, A. Holstege, A. Hinke, A. Schalhorn, R. Wilkowski, Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer, J. Clin. Oncol. 24 (2006) 3946–3952.

[7] S. Banerjee, Y. Zhang, Z. Wang, M. Che, P.J. Chiao, J.L. Abbruzzese, F.H. Sarkar, In vitro and in vivo molecular evidence of genistein action in augmenting the efficacy of cisplatin in pancreatic cancer, Int. J. Cancer 120 (2007) 906–917.

[8] Z. Symon, M. Davis, C.J. McGinn, M.M. Zalupski, T.S. Lawrence, Concurrent chemoradiotherapy with gemcitabine and cisplatin for pancreatic cancer: from the laboratory to the clinic, Int. J. Radiat. Oncol. Biol. Phys. 53 (2002) 140–145.

[9] P. Bragado, A. Armesilla, A. Silva, A. Porras, Apoptosis by cisplatin requires p53 mediated p38alpha MAPK activation through ROS generation, Apoptosis 12 (2007) 1733–1742.

[10] M. Zhang, W. Liu, D. Ding, R. Salvi, Pifithrin-alpha suppresses p53 and protects cochlear and vestibular hair cells from cisplatin-induced apoptosis, Neuroscience 120 (2003) 191–205.

[11] A.V. Vaseva, N.D. Marchenko, K. Ji, S.E. Tsirka, S. Holzmann, U.M. Moll, p53 opens the mitochondrial permeability transition pore to trigger necrosis, Cell 149 (2012) 1536–1548.

[12] K. Baumann, Cell death: multitasking p53 promotes necrosis, Nat. Rev. Mol. Cell Biol. 13 (2012) 480–481.

[13] C. Ji, B. Yang, Z. Yang, Y. Tu, Y.L. Yang, L. He, Z.G. Bi, Ultra-violet B (UVB)-induced skin cell death occurs through a cyclophilin D intrinsic signaling pathway, Biochem. Biophys. Res. Commun. 425 (2012) 825–829.

[14] C.-H. Wu, C. Cao, J.H. Kim, C.-H. Hsu, H.J. Wanebo, W.D. Bowen, J. Xu, J. Marshall, Trojan-horse nanotube on-command intracellular drug delivery, Nano Lett. 12 (2012) 5475–5480.

[15] S. Javadov, A. Kuznetsov, Mitochondrial permeability transition and cell death: the role of cyclophilin D, Front. Physiol. 4 (2013) 76.

[16] C.P. Baines, The molecular composition of the mitochondrial permeability transition pore, J. Mol. Cell. Cardiol. 46 (2009) 850–857.

[17] H. Du, L. Guo, F. Fang, D. Chen, A.A. Sosunov, G.M. McKhann, Y. Yan, C. Wang, H. Zhang, J.D. Molkentin, F.J. Gunn-Moore, J.P. Vonsattel, O. Arancio, J.X. Chen, S.D. Yan, Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease, Nat. Med. 14 (2008) 1097–1105.

[18] H. Du, L. Guo, W. Zhang, M. Rydzewska, S. Yan, Cyclophilin D deficiency improves mitochondrial function and learning/memory in aging Alzheimer disease mouse model, Neurobiol. Aging 32 (2011) 398–406.

[19] S. Crunkhorn, Anticancer drugs: stapled peptide rescues p53, Nat. Rev. Drug Discov. 10 (2011) 21.

[20] C. Yang, V. Kaushal, R.S. Haun, R. Seth, S.V. Shah, G.P. Kaushal, Transcriptional activation of caspase-6 and -7 genes by cisplatin-induced p53 and its functional significance in cisplatin nephrotoxicity, Cell Death Differ. 15 (2008) 530–544.

[21] C.M. Barton, S.L. Staddon, C.M. Hughes, P.A. Hall, C. O'Sullivan, G. Kloppel, B. Theis, R.C. Russell, J. Neoptolemos, R.C. Williamson, et al., Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer, Br. J. Cancer 64 (1991) 1076–1082.

[22] M. Mohiuddin, D. Chendil, S. Dey, R.A. Alcock, W. Regine, M.M. Ahmed, Influence of p53 status on radiation and 5-flourouracil synergy in pancreatic cancer cells, Anticancer Res. 22 (2002) 825–830.