

Synergistic effect of arginine-specific ADP-ribosyltransferase 1 and poly(ADP-ribose) polymerase-1 on apoptosis induced by cisplatin in CT26 cells

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Abstract. Arginine-specific ADP-ribosyltransferase 1 (ART1) and poly(ADP-ribose) polymerase-1 (PARP-1) are both post-translational modification proteins. Inhibition of PARP1 induces apoptosis in cancer cells, and ART1 regulates RhoA which promotes apoptosis in hepatic cancer cells when inhibited. However, the interaction of ART1 and PARP-1 on the effect of apoptosis has not yet been elucidated. In the present study, lentiviral vector-mediated ART1-cDNA was transfected into CT26 cells, and the apoptosis rate was detected by flow cytometric assay and Hoechst 33342 staining. Relevant factors were detected by reverse transcriptase-PCR and western blotting. The results showed that the apoptosis rate in the ART1-cDNA CT26 cells treated with PARP-1 inhibitor 5-aminoisoquinoline (5-AIQ) and cisplatin increased, when compared with the ART1-cDNA CT26 cells treated with cisplatin only or the untreated ART1-cDNA CT26 cells. Further studies have shown that PARP-1 is in the downstream of ART1, and plays a role in ART1-mediated CT26 cell apoptosis through the ROCK1/NF- κ B/PARP-1 pathway when induced by cisplatin. We also found that in cisplatin-treated cells, activated caspase 3 cleaved PARP-1 and the decreased level of PARP-1 in turn decreased the expression of nuclear factor (NF)- κ B, Cox-2 and increased caspase 3, resulting in the enhanced ability of ART1 to regulate CT26 cell apoptosis. Our research provides initial sight into the synergistic effect of ART1 and PARP-1 on apoptosis induced by cisplatin in murine colon carcinoma CT26 cells.

Introduction

ADP-ribosylation reactions can be divided into four major groups: mono-ADP-ribosylation, poly-ADP-ribosylation, ADP-ribose cyclization and formation of *O*-acetyl-ADP-ribose (1). Mono-ADP-ribosylation and poly-ADP-ribosylation are common in both prokaryotes and eukaryotes and serve as post-translational modification proteins in which the ADP-ribose is transferred from NAD⁺ to a specific amino acid in a target protein (2,3).

Mono-ADP-ribosylation reactions transfer a single ADP-ribose to various amino acid (arginine, histidine, diphthamid, cysteine, asparagine) residues by mono-ADP-ribosyl transferases (ARTs) (4,5). Arginine-specific ADP-ribosyl transferase 1 (ART1), a member of the arginine-specific ARTs, was initially found to be expressed and activated in eukaryotes (6-8), and has been mainly researched in regards to inflammation (9). Yau *et al* reported that MIBG, a specific inhibitor of arginine-dependent mARTs, could inhibit smooth muscle cell (SMC) proliferation and migration via Rho-dependent signaling (10). However, it has been proven that the Rho kinase-associated coil-containing protein kinase (RhoA/ROCK) pathway plays a central role in tumor progression (11). Several reports indicate evidence that inhibition of the Rho/ROCK signaling pathway induces hepatocellular cancer (HCC) cell apoptosis (12,13).

Unlike mono-ADP-ribosylation, poly-ADP-ribosylation transforms NAD⁺ to nicotinamide and ADP-ribose to form long and branched (ADP-ribose) polymers on glutamic acid residues by poly(ADP-ribose) polymerase (PARP). PARP-1 is the principal member of the PARP enzymes and has been implicated in both the prevention and aggravation of various diseases (14). Expression of PARP-1 plays a central role in the cellular response to DNA damage (6), whereas the diverse physiological functions of PARP-1 in cell survival, several forms of cell death, gene transcription, immune responses, inflammation, learning, memory, synaptic functions, angiogenesis and aging have been widely appreciated recently (14). Our previous study demonstrated that downregulation of PARP-1 by RNA interference of PARG inhibits the migration, proliferation and metastatic potential of colon carcinoma

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cells (15-18). Ye reported that a PARP-1 inhibitor enhances the efficacy of DNA-damaging anticancer drugs by inducing cancer cell apoptosis, and inhibits necrosis in normal cells and neurons from the toxic effects of DNA-damaging anticancer drugs, both through processes that prevent the significant loss of ATP (19).

Pioneer observations found that mART could mediate the signal transduction from the cell surface to the nucleus (11), and our previously study demonstrated that both the expression of ART1 and PARP-1 was significantly increased in colon carcinoma tissues when compared with normal colon tissues, and inhibition of ART1 downregulated the expression of PARP-1 possibly by regulation of NF- κ B (20). However, the interaction of ART1 and PARP-1 in the process of apoptosis has not yet been elucidated. In the present study, we aimed to research their effect on apoptosis induced by cisplatin (CDDP) and the possible mechanism in murine colon carcinoma CT26 cells (21,22).

Materials and methods

Cell line and reagents. CT26, a murine colon carcinoma cell line, was kindly provided by Professor Y.Q. Wei, Huaxi Hospital, Sichuan University. ART1-shRNA and control-shRNA were successfully transfected in CT26 cells by us in early experiments (20). The lentiviral vector was obtained from GeneChem (Shanghai, China). The Annexin V-PE/7-AAD apoptosis detection kit was from KeyGen Biotechnology Co., Ltd. (Nanjing, China). Y-27632 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). CDDP was obtained from Sigma (St. Louis, MO, USA). 5-Aminoisoquinoline was kindly supplied by Professor M.D. Threadgill, Bath University, UK. The BCA protein assay kit was from Beyotime (Shanghai, China).

Cell culture. The murine CT26 colon carcinoma cell line was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μ g/ml streptomycin (Thermo Pierce) at 37°C in a 5% CO₂ incubator. CDDP was prepared in 1640 at a final concentration of 1 mM and stored at 4°C away from light.

Transfection of CT26 cells and identification. Mouse ART1-cDNA sequence (accession no. NC_000073) was obtained from GenBank, and the primers of ART1-cDNA were designed as follows: lentiviral vector-mediated ART1-cDNA primer 1, GAGGATCCCCGGGTACCGGTCGCCACCATGAAGATTCTTGCTATGATG; lentiviral vector-mediated ART1-cDNA primer 2, TCACCATGGTGGCGACCGGACATCGGGTAA GTTGCTG, and was successfully constructed by GeneChem. Transfection was carried according to the manufacturer's instructions. Cells were cultured in 12-well plates at a density of 3x10⁴ cells/well when they were in the logarithmic growth phase. Lentivirus particles (10 μ l) were added to each well while the cells covered 50% of each well. Transfection efficiency was optimized using green fluorescent protein and detected under a fluorescence microscope after 72 h. The efficiency of the ART1-cDNA lentivirus transfected into CT26 cells was determined by reverse transcriptase (RT)-PCR and western blot analysis.

RNA was extracted from ART1-cDNA CT26 cells with TRIzol reagent according to the supplied manual (Takara, Dalian, China), and RNAs were extracted from ART1-shRNA CT26 cells, control-shRNA CT26 cells and untransfected CT26 cells used as a control. ART1 (target gene) and β -actin (internal control gene) were detected using oligonucleotide primers which were designed and produced by Sangon Biotech Co. (Shanghai, China): *ART1*, 5'-ACCTTCTTCGGTATCTGGACCT-3' (F1) and 5'-TAAGTTGCTGGAGACCTGGATT-3' (R1); *β -actin*, 5'-ATATCGCTGCGCTGGTCTGTC-3' (F1) and 5'-AGGATGGCGTGAGGGAGAGC-3' (R1). The cycling conditions were as follows: the number of PCR cycles (94°C for 30 sec, 50-58°C for 30 sec, 72°C for 1 min and then 5 min for the last extension) was 30 for the amplification of reverse transcriptase products. Finally, the PCR amplification products were separated on 2.0% agarose gel (Genview, Tallahassee, FL, USA). This experiment was performed in triplicate.

Effect of ART1 and PARP-1 on CT26 cell apoptosis induced by CDDP. ART1-cDNA CT26 cells were treated with or without CDDP. ART1-shRNA CT26 cells, control-shRNA CT26 cells and untransfected CT26 cells were treated in the same manner as were the control. CT26 cells were cultured in 6-well plates at 1x10⁵ cells/well for 24 h and 30 μ M of CDDP for a further 48 h for inducing apoptosis. To confirm the role of PARP-1 in this apoptosis process, ART1-cDNA CT26 cells were treated with PARP-1 inhibitor 5-AIQ at 100 μ M for 24 h and CDDP at 30 μ M for 48 h or ART1-cDNA CT26 cells were treated with CDDP only at 30 μ M for 48 h or were untreated as control groups. The apoptosis rate was tested for each cell group by flow cytometric analysis and Hoechst 33342 staining.

For flow cytometric analysis, cells were trypsinized, washed twice with phosphate-buffered saline (PBS) before PE-conjugated Annexin V and 7-ADD were added, incubated for 30 min and analyzed by flow cytometry (Becton-Dickinson). As for Hoechst 33342 staining, cells were washed twice with PBS, and stained with 5 μ l Hoechst 33342 for 30 min according to the manufacturer's instructions (Beyotime). Stained nuclei were observed under a fluorescence microscope. The percentage of apoptotic cells, the apoptotic ratio (AR), was calculated using the formula: AR% = [apoptotic cells (A)/total cell count (T)] x 100. (Ten images from each sample were acquired and analyzed in three different experiments). All experiments were performed three times.

Detection of relevant apoptosis-related proteins. Western blotting was used to detect the expression of ART1, RhoA, ROCK1, NF- κ B, Cox-2, caspase 3, PARP-1 and PARP-1 cleaved fragments. Total and nuclear proteins in the above groups were extracted according to protein extraction protocols (Beyotime P0013 and P0028). Protein concentrations were determined using the BCA assay (Beyotime), and respective proteins (30 μ g/lane) were loaded onto 6% polyacrylamide gel (PARP-1, ROCK1) or 10% polyacrylamide gel (ART1, RhoA, Cox-2, caspase 3 and NF- κ B). Proteins were separated by electrophoresis, transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA) and blocked with 5% non-fat dry milk for 1 h at room temperature. Primary antibodies against ART1, PARP-1, NF- κ B (Bioworld, St. Louis Park, MN, USA); RhoA, ROCK1, Cox-2, caspase 3 (Proteintech Group,

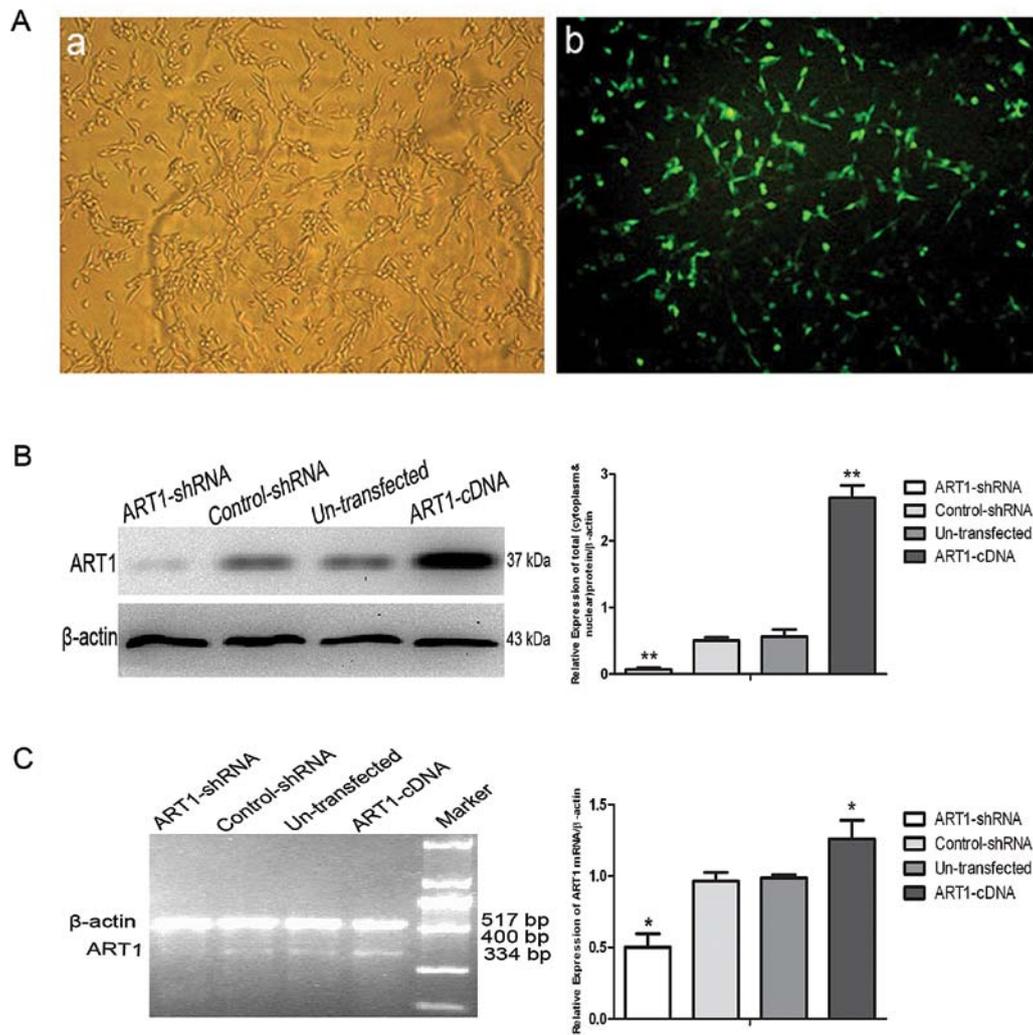


Figure 1. Lentiviral vector-mediated ART1-cDNA was successfully transfected into CT26 cells. (A) CT26 cells transfected with the lentiviral vector-mediated ART1-cDNA. The lentivirus (10 μ l) was transfected into CT26 cells, and the efficiency was observed under an optical microscope (a) and a fluorescence microscope (b) after 72 h. (B) Expression level of ART1 protein in the ART1-cDNA CT26 cells as compared with the ART1-shRNA CT26 cells, the control-shRNA CT26 cells and the untransfected CT26 cells. (C) Expression of ART1 mRNA in the ART1-cDNA CT26 cells as compared with the ART1-shRNA CT26 cells, the control-shRNA CT26 cells and the untransfected CT26 cells (** $P < 0.01$, * $P < 0.05$). ART1, arginine-specific ADP-ribosyltransferase 1.

Chicago, IL, USA); β -actin (Boster, Wuhan, China) were diluted to 1:500 and incubated at 4°C overnight. The next day, the membranes (37°C 30 min) were washed thoroughly 3 times for 10 min with Tris-buffered saline and Tween-20 (TBST). Secondary antibodies (peroxidase-conjugated goat or anti-rabbit IgG) (Boster) diluted to 1:1,000 were added and incubation was carried out for 2 h at 37°C, and washed thoroughly for 15 min with TBST. The bands were detected by enhanced chemiluminescence method (Beyotime).

To verify the relationship of ART1, PARP-1 and ROCK1, untransfected CT26 cells were treated with PARP-1 inhibitor 5-AIQ (100 μ M) and ROCK inhibitor Y-27632 (20 μ M) (23) for 24 h, respectively. Total protein was extracted and detected by western blotting. All experiments were repeated three times.

Data measurement and statistical analysis. Data are expressed as the means \pm standard deviation (SD) in the quantitative experiments. Statistical analysis was performed by Student's t-test or one-way ANOVA. Values of $P < 0.05$ were considered to indicate statistically significant results.

Results

Transfection of lentiviral vector-mediated ART1-cDNA in CT26 cells. The ratio of lentiviral vector-mediated ART1-cDNA transfected in the CT26 cells was detected by fluorescence microscope and indicated that ~80% of CT26 cells were successfully transfected (Fig. 1A). Western blot assay and RT-PCR were used to identify the efficiency of the ART1-cDNA lentivirus transfection in the CT26 cells. The cells transfected with the lentiviral vector-mediated ART1-cDNA revealed an increase in ART1 expression when compared to the expression level in the ART1-shRNA, control-shRNA and untransfected CT26 cells (Fig. 1B and C; $P < 0.01$ and $P < 0.05$, respectively).

Effect of ART1 and PARP-1 on CT26 cell apoptosis induced by CDDP. Based on the flow cytometric analysis, the results showed that the apoptosis rate increased in each group of CT26 cells induced by CDDP, while the ART1-cDNA CT26 cells induced by CDDP had a lower apoptosis rate (16.39%),

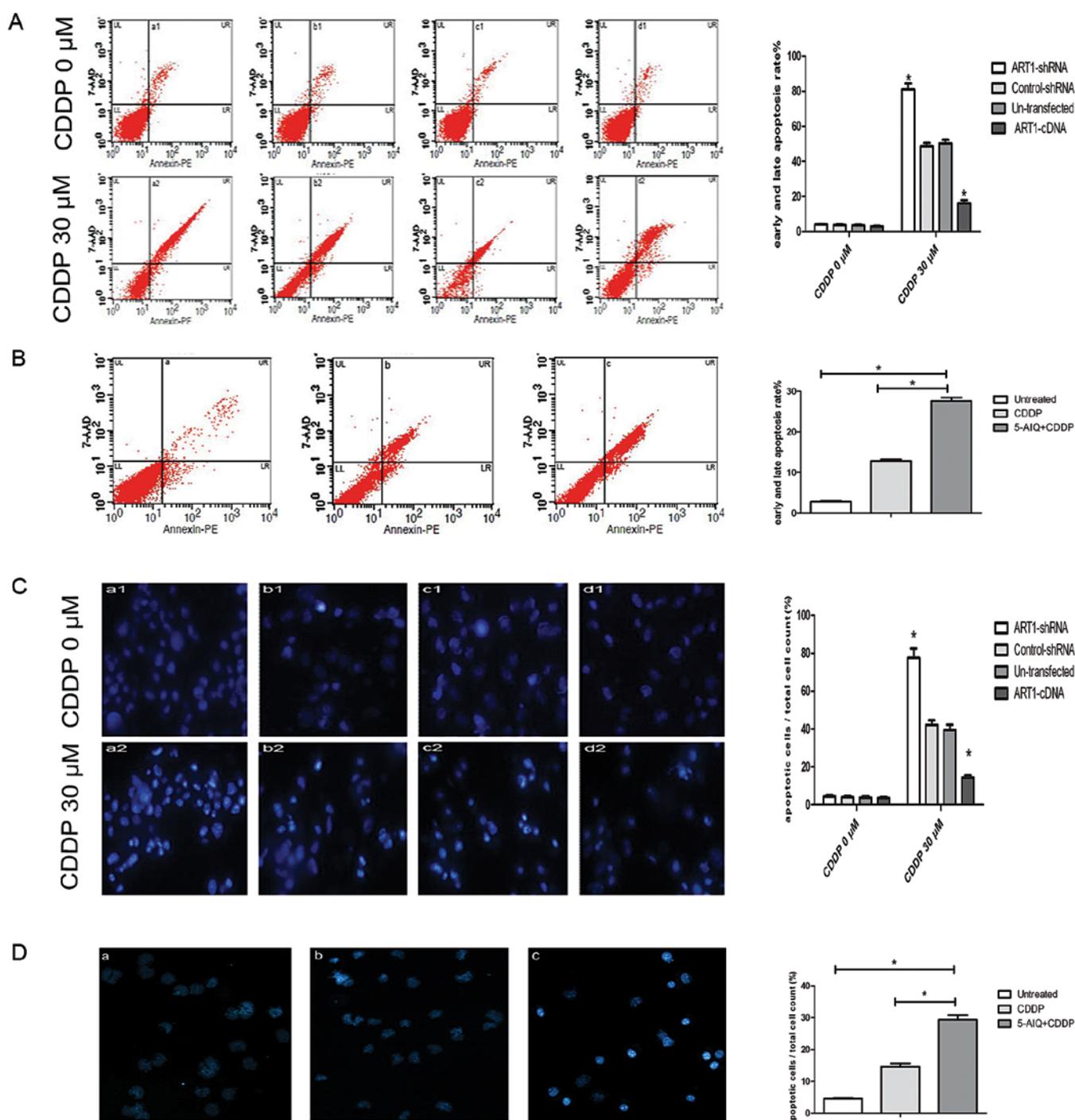


Figure 2. PARP-1 is involved in the ART1-mediated CT26 cell apoptosis induced by CDDP. (A and C) ART1-cDNA CT26 cells were untreated (a1) or treated with CDDP (a2), compared with ART1-shRNA CT26 cells (b1 and b2), control-shRNA CT26 cells (c1 and c2) and untransfected CT26 cells (d1 and d2) treated in the same manner. a1-d1, untreated cells; a2-d2, cells treated with CDDP (30 μM) for 48 h. The apoptosis rate was detected by flow cytometric analysis and Hoechst 33342 staining. (B and D) ART1-cDNA CT26 cells were treated with 5-AIQ (100 μM) for 24 h and then CDDP (30 μM) for 48 h (c) or ART1-cDNA CT26 cells were treated with CDDP (30 μM) for 48 h only (b), or ART1-cDNA CT26 cells were untreated (c) and apoptosis was detected by flow cytometric analysis and Hoechst 33342 staining. Diagrams represent the average of three independent experiments. All experiment were performed three times ($P < 0.05$). (In A and B: UL, necrotic cells; LL, viable cells; LR, early apoptosis; UR, late apoptosis, in percentages %). PARP-1, poly(ADP-ribose) polymerase-1; ART1, arginine-specific ADP-ribosyltransferase 1; CDDP, cisplatin; 5-AIQ, 5-aminoisoquinoline.

compared with the ART1-shRNA CT26 cells (80.03%), the untransfected CT26 cells (50.03%) and the control-shRNA CT26 cells (51.17%) treated in the same manner (Fig. 2A). The apoptosis rate of the ART1-cDNA CT26 cells treated with 5-AIQ and CDDP obviously increased (25.71%), compared to the ART1-cDNA CT26 cells treated with CDDP

only (13.23%) and the untreated ART1-cDNA CT26 cells (4.56%) ($P < 0.05$) (Fig. 2B).

The results of Hoechst 33342 staining showed that apoptotic bodies were significantly increased in each group of CT26 cells treated with CDDP. Moreover, after treatment with CDDP, the apoptosis rate in the ART1-cDNA CT26

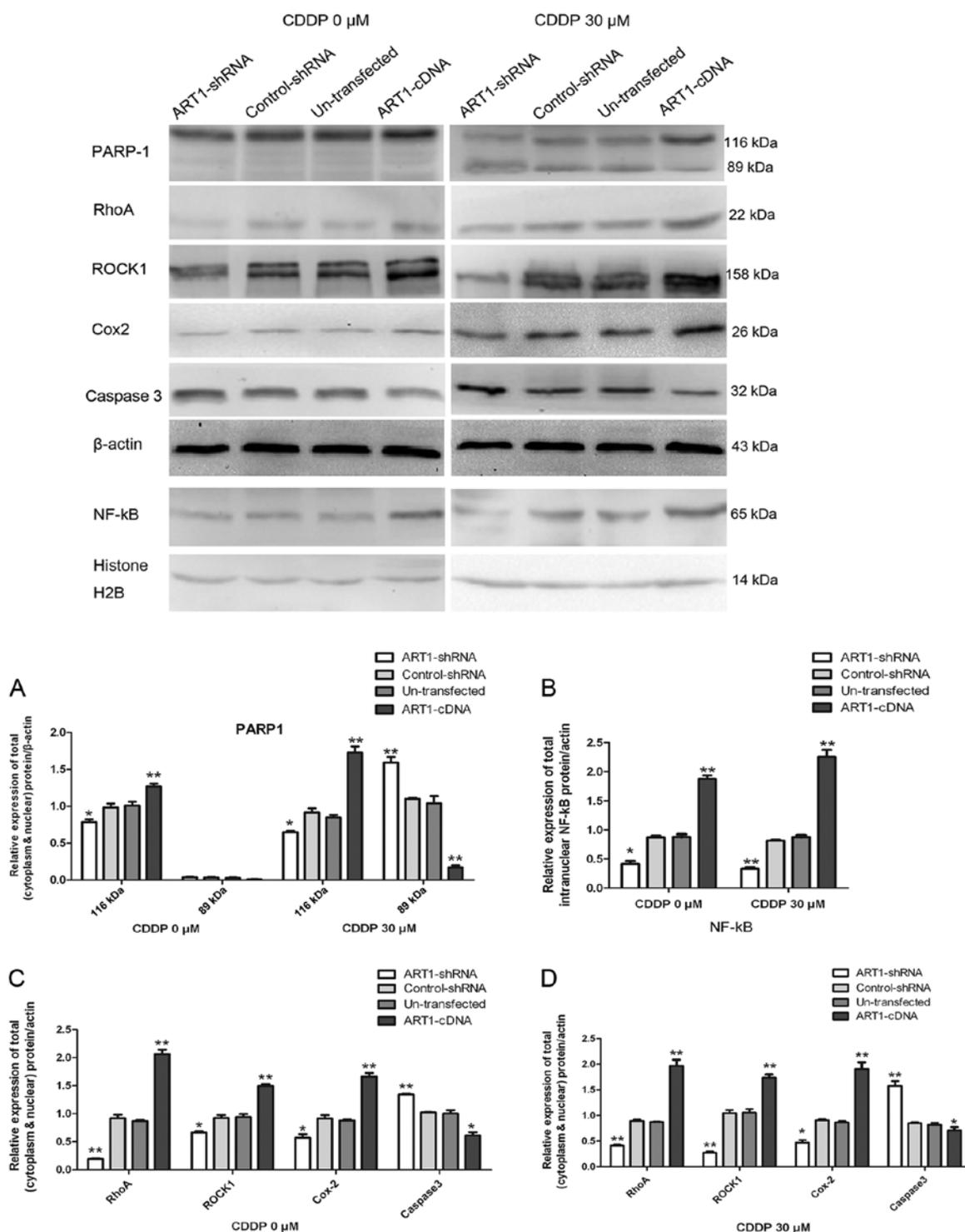


Figure 3. ART1 affects PARP-1, RhoA, ROCK1, NF- κ B, Cox2 and caspase 3 expression in CT26 cells. (A) Expression of PARP-1 and its cleavage fragment (p89) in ART1-cDNA CT26 cells treated with or without CDDP (30 μ M) for 48 h, compared with the expression level in the ART1-shRNA CT26 cells, the control-shRNA CT26 cells and the untransfected CT26 cells treated in the same manner. (B) The effect of ART1 on the expression of NF- κ B in the ART1-cDNA CT26 cells treated with or without CDDP (30 μ M) for 48 h, compared with the expression level in the ART1-shRNA CT26 cells, the control-shRNA CT26 cells and the untransfected CT26 cells treated in the same manner. (C and D) The effect of ART1 on the expression of RhoA, ROCK1, Cox-2, caspase 3 in the ART1-cDNA CT26 cells treated with or without CDDP (30 μ M) for 48 h, compared with the expression levels in the ART1-shRNA CT26 cells, the control-shRNA CT26 cells and the untransfected CT26 cells treated in the same manner. All experiments were repeated three times (*P<0.05, **P<0.01). ART1, arginine-specific ADP-ribosyltransferase I; PARP-1, poly(ADP-ribose) polymerase-1; ROCK, Rho kinase-associated coil-containing protein kinase; NF- κ B, nuclear factor- κ B; CDDP, cisplatin.

cells declined (15.2%), compared with the ART1-shRNA CT26 cells (77.8%), the control-shRNA CT26 cells (37.5%)

and the untransfected CT26 cells (35.8%) (Fig. 2C). ART1-cDNA CT26 cells treated with 5-AIQ and CDDP had a high

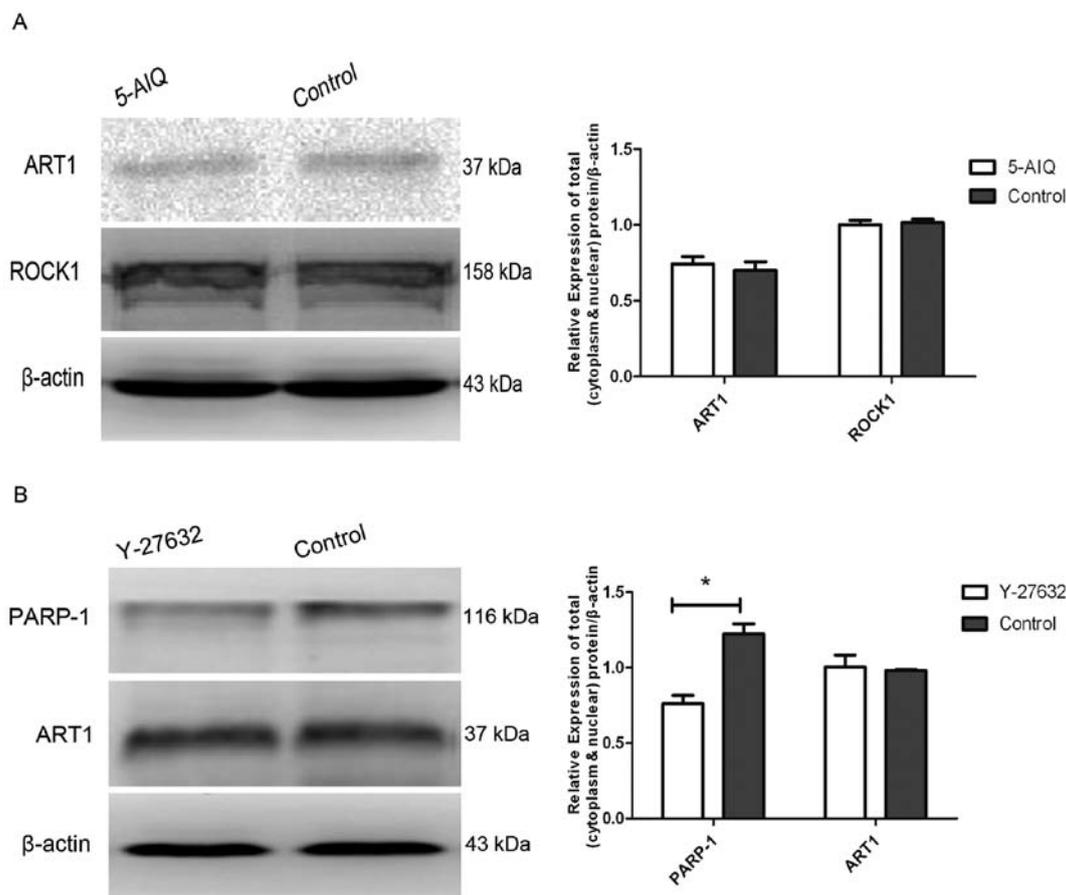


Figure 4. Relationship of ART1, ROCK1 and PARP-1 in untransfected CT26 cells. Untransfected CT26 cells were treated with 5-AIQ (100 μ M) or Y-27632 (20 μ M) for 24 h, respectively. Whole extracts were collected and subjected to western blot assay. (A) PARP-1 inhibitor 5-AIQ had no obvious effect on the expression of ROCK1 and ART1. (B) ROCK inhibitor inhibited the expression of PARP-1, while it had no significant influence on the expression of ART1 ($P < 0.05$). Both experiments were performed in triplicate. ART1, arginine-specific ADP-ribosyltransferase 1; ROCK, Rho kinase-associated coil-containing protein kinase; PARP-1, poly(ADP-ribose) polymerase-1.

apoptosis rate (25.3%), compared with the ART1-cDNA CT26 cells treated with CDDP only (14.03%) and the untreated ART1-cDNA CT26 cells (5.06%) ($P < 0.05$) (Fig. 2D).

Influence of ART1 on the expression of RhoA, ROCK1, NF- κ B, Cox-2, caspase 3, PARP-1 and PARP-1 cleavage fragments. Among the groups of cells treated with CDDP, the expression of RhoA, ROCK1, NF- κ B, Cox-2 and PARP-1 was increased in the ART1-cDNA CT26 cells, when compared to these levels in the ART1-shRNA CT26 cells, the control-shRNA CT26 cells and the untransfected CT26 cells, while the expression of caspase 3 and PARP-1 cleavage fragment (p89) had an opposite trend. The expression of RhoA, ROCK1, NF- κ B, Cox2 and PARP-1 had the same trend in cells not treated with CDDP, except for the PARP-1 cleavage fragment (p89) which was the cleavage substrate for activated caspase 3 which was noted in the CDDP-treated CT26 cells only ($P < 0.05$, $P < 0.01$) (Fig 3).

Influence of 5-AIQ and Y-27632 on the expression of ART1, ROCK1 and PARP-1 in the untransfected CT26 cells. The expression of ROCK1 and ART1 in the 5-AIQ treated untransfected CT26 cells exhibited no obvious changes when compared to the untreated untransfected CT26 cells. In the untransfected CT26 cells treated with Y-27632, the expression

of PARP-1 was significantly decreased while the expression of ART1 exhibited no obvious change ($P < 0.05$) (Fig. 4).

Influence of PARP-1 inhibitor 5-AIQ on the expression of NF- κ B, Cox-2 and caspase 3 in the ART1-cDNA CT26 cells. There was an obvious decrease in NF- κ B and Cox-2 and an increase in caspase 3 in the ART1-cDNA CT26 cells treated with 5-AIQ and CDDP, when compared to the ART1-cDNA CT26 cells treated with CDDP only and the untreated ART1-cDNA CT26 cells ($P < 0.05$) (Fig. 5).

Discussion

ADP-ribosylation reactions are currently topics of intense interest. A tremendous amount of research has been carried out to decipher the physiological and pathophysiological roles of ADP-ribosylation reactions at the molecular level. Many research groups with a wide range of expertise have become involved in mono-ADP-ribosylation or poly-ADP-ribosylation research (5). It has been determined that both ART1 and PARP-1 play important roles in reticular biological processes, and ART1 influences the expression of PARP-1 through NF- κ B. However, despite the progress made in recent years in the biochemistry, molecular biology, physiology and

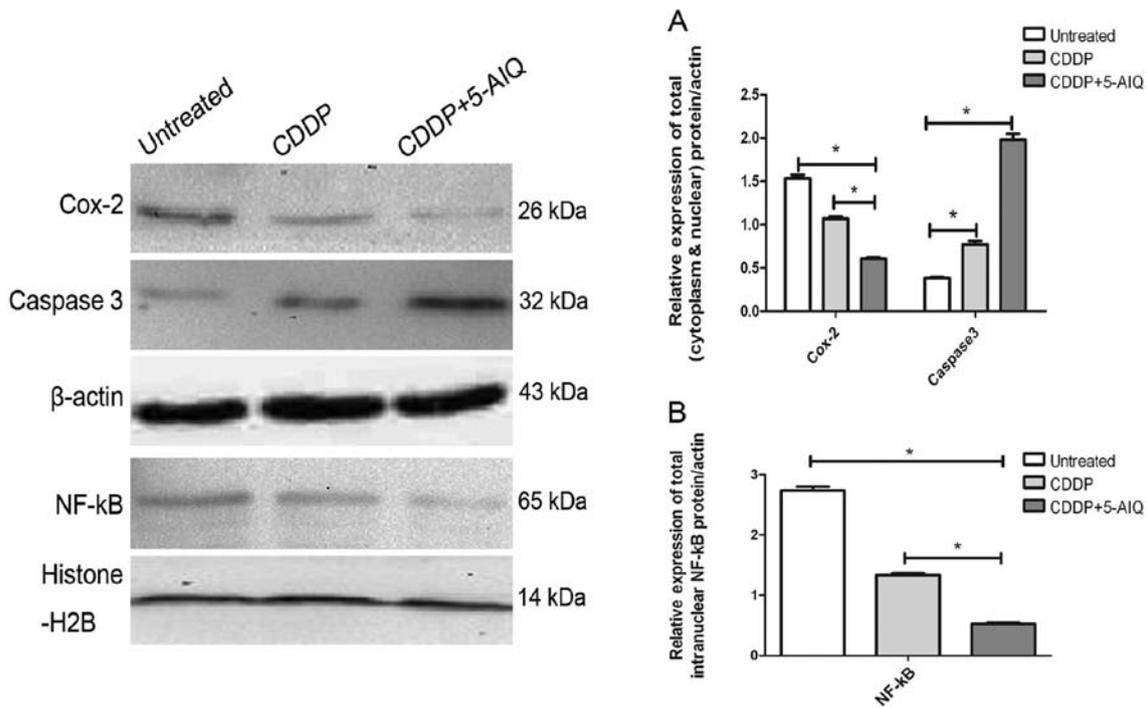


Figure 5. PARP-1 inhibitor affects the expression of Cox-2, NF- κ B and caspase 3 in ART1-cDNA CT26 cells. ART1-cDNA CT26 cells were treated with 5-AIQ (100 μ M) for 24 h and then CDDP (30 μ M) for 48 h or ART1-cDNA CT26 cells were treated with CDDP (30 μ M) only for 48 h or ART1-cDNA CT26 cells were untreated as control. (A) Expression of Cox-2 was reduced while caspase 3 expression was increased in the ART1-cDNA CT26 cells treated with 5-AIQ and CDDP. (B) Expression of NF- κ B in the nuclear extract was obviously decreased in the ART1-cDNA CT26 cells treated with 5-AIQ and CDDP ($P < 0.05$). All experiments were repeated thrice. PARP-1, poly(ADP-ribose) polymerase-1; NF- κ B, nuclear factor- κ B; ART1, arginine-specific ADP-ribosyltransferase 1; 5-AIQ, 5-aminoisoquinoline; CDDP, cisplatin.

pathophysiology of ADP-ribosylation, no unified image of the physiological and pathophysiological correlation of mono- and poly-ADP-ribosylation in the field of apoptosis has been noted (1). In the present study, the role of ART1 and PARP-1 in CDDP-induced CT26 cell apoptosis was studied. The results showed that a high level of ART1 expression decreased the apoptosis rate of CT26 cells induced by CDDP. Moreover, in the ART1-cDNA CT26 cells treated with 5-AIQ and CDDP an increase in the apoptosis rate was noted when compared with the ART1-cDNA CT26 cells treated with CDDP only and the untreated ART1-cDNA CT26 cells, which indicated that PARP-1 plays a role in the process of ART1-mediated apoptosis in CDDP-treated CT26 cells.

Several studies have shown that inhibition of the RhoA/ROCK pathway increases the apoptosis of human hepatocellular carcinoma cells (HCC) and endothelial cells through a mitochondrial apoptosis pathway (12,13). Benitah *et al* identified that ROCK is necessary for RhoA-induced expression of Cox-2 at the transcriptional level through the RhoA/ROCK/NF- κ B/COX-2 signaling pathway (24), and the Cox-2-specific inhibitor celecoxib induced apoptosis through a mitochondrial pathway to suppress Akt phosphorylation, to release cytochrome *c* to the cytosol and to activate caspase 3 (25,26). Caspase 3 mediates highly specific proteolytic cleavage events in dying cells, which collectively manifest the apoptotic phenotype (27). Moreover, inhibition of Cox-2 was also shown to inhibit NF- κ B by directly blocking the activity of I κ B kinase; these feedback loops of the inhibition of Cox-2 mediated inactivation of NF- κ B leading to a reconstituted sensitivity of cancer cells to apoptosis (28). Thus, RhoA/ROCK regu-

lates apoptosis through an NF- κ B/Cox2/caspase 3 pathway. Yau *et al* reported that the RhoA/ROCK pathways could be regulated by ADP-ribosyltransferase (10). However, the relationship of these factors with ART1 has not been reported in CDDP-induced colon carcinoma CT26 cell apoptosis. In the present study, we analyzed these factors, and the results showed that the expression of RhoA, ROCK1, NF- κ B and Cox-2 was increased in the ART1-cDNA CT26 cells when treated with CDDP, while caspase 3 had the opposite trend. Thus, we conclude that ART1 probably regulates apoptosis through the RhoA/ROCK1/NF- κ B/Cox2/caspase 3 pathway.

It has been demonstrated that PARP-1 acts as a co-activator of NF- κ B, and the PARP-1 inhibitor decreases the expression of NF- κ B and inhibition of NF- κ B could decrease PARP-1 expression through a feedback manner (29-31); whereas inhibition of PARP-1 is thought to impair DNA repair function and promote cellular dysfunction and death (32). In models of acute stress, the PARP-1 inhibitor may protect cellular NAD pools and prevent NF- κ B-dependent inflammatory signaling pathway. Research also found that PARP-1 inhibitor can trigger apoptosis in cancer cells and inhibit necrosis in normal cells and neurons through a mitochondrial pathway (33,34). Yau reported that mART could mediate the signal transduction from the cell surface to the nucleus (11). Our previous study showed that ART1 regulated the expression of PARP-1 probably through NF- κ B, while PARP-1 inhibitor 5-AIQ had no effect on ART1 (20). In the present study, we also found that the expression of PARP-1 was decreased in the ART1-shRNA CT26 cells and was increased in the ART1-cDNA CT26 cells, which is accordance to the trend of NF- κ B. From

these findings we can conclude that ART1 affects the expression of PARP-1 probably through the regulation of NF- κ B.

To clarify the relationship among ART1, ROCK1 and PARP-1, Y-27632 and 5-AIQ were used to treat the untransfected CT26 cells. Results showed that there was no obvious change in ROCK1 and ART1 in the 5-AIQ-treated untransfected CT26 cells, and the expression of PARP-1 in the Y-27632-treated untransfected CT26 cells was significantly decreased while the expression of ART1 had no obvious change. These results demonstrate that ROCK1 is in the downstream of ART1 while PARP-1 is regulated by ROCK1. To further verify the role of PARP-1 in ART1-mediated apoptosis in CT26 cells treated with CDDP, we used 5-AIQ to treat ART1-cDNA CT26 cells and determined the expression of NF- κ B, Cox-2 and caspase 3. Results showed that there was an obvious decrease in NF- κ B and Cox-2 and an increase in caspase 3 in the 5-AIQ-treated ART1-cDNA CT26 cells treated with CDDP, which demonstrated that PARP-1 is involved in CDDP-induced apoptosis in CT26 cells through the NF- κ B/Cox-2/caspase 3 pathway.

Moreover, Li and Yuan reported that PARP-1 was one of the first identified substrates of caspase 3 (35). During apoptosis, activated caspase 3 cleaves PARP-1 into two fragments, p89 and p24, resulting in the inactivation of PARP-1. The cleavage fragments lead to the suppression of PARP-1 activity by inhibiting the homoassociation and DNA binding of intact PARP-1, respectively (36-38), preserving cellular energy for certain ATP-sensitive steps of apoptosis (39-41). In the present study, the results also showed that caspase 3 was activated and PARP-1 cleavage fragments formed when the cells were treated with CDDP. However, in the untreated cells, caspase 3 was inactivated for there were no PARP-1 cleavage fragments (p89) observed. Compared to the control groups, more PARP-1 cleavage fragments were formed in the ART1-shRNA CT26 cells treated with CDDP and less were formed in the ART1-cDNA CT26 cells treated with CDDP. These results suggest that ART1 activates caspase 3 which can cleave PARP-1 into p24 and p89 fragments when induced by CDDP, and this reaction leads to a decreased level and inactivation of PARP-1, ending in the promotion of CT26 cell apoptosis.

In brief, this investigation is an initial research on the synergistic effect of ART1 and PARP-1 on apoptosis induced by CDDP in murine colon carcinoma CT26 cells. The results showed that PARP-1 is in the downstream of ART1, and plays a role in ART1-mediated CT26 cell apoptosis through the ROCK1/NF- κ B/PARP-1 pathway when induced by CDDP. In contrast, in CDDP-induced cells, activated caspase 3 cleaved PARP-1 and the decrease in PARP-1 in turn decreased the expression of NF- κ B, and Cox-2 and increased caspase 3, resulting in the enhanced ability of ART1-mediated CT26 cell apoptosis. However, despite our findings, further study is needed to detect the detail mechanism.

Acknowledgements

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