PARP-1 Regulates Resistance of Pancreatic Cancer to TRAIL Therapy

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Abstract

Purpose: Activating extrinsic apoptotic pathways targeting death receptors (DR) using agonistic antibodies or TNF-related apoptosis-inducing ligand (TRAIL) is promising for cancer therapy. However, most pancreatic cancers are resistant to TRAIL therapy. The present studies aimed to identify combination therapies that enhance the efficacy of TRAIL therapy and to investigate the underlying mechanisms.

Experimental Design: A xenograft model in nude mice was used to determine pancreatic cancer tumorigenesis and therapeutic efficacy of TRA-8, a monoclonal agonistic antibody for DR5. Pancreatic cancer cells were used to characterize mechanisms underlying PARP-1 regulation of TRA-8–induced apoptosis in vitro.

Results: PARP-1 was found highly expressed in the TRA-8–resistant PANC-1 and Suit-2 cells, compared with TRA-8–sensitive BxPc-3 and MiaPaca-2. Inhibition of PARP-1 with a pharmacologic inhibitor sensitized PANC-1 and Suit2 cells to TRA-8–induced apoptosis in a dose-dependent manner. Furthermore, siRNAs specifically knocking down PARP-1 markedly enhanced TRA-8–induced apoptosis in vitro and augmented the efficacy of TRA-8 therapy on tumorigenesis in vivo. PARP-1 knockdown increased TRA-8–induced activation of caspase-8 in the death-induced signaling complex (DISC). Immunoprecipitation with DR5 antibody identified the recruitment of PARP-1 and PARP-1–mediated protein poly-ADP-ribosylation (pADPr) modification in the DR5-associated DISC. Further characterization revealed that PARP-1–mediated pADPr modification of caspase-8 inhibited caspase-8 activation, which may contribute to its function in regulating TRA-8 resistance.

Conclusions: Our studies provide molecular insights into a novel function of PARP-1 in regulating the extrinsic apoptosis machinery and also support interventions combining PARP-1 inhibitors with DR agonists for pancreatic cancer therapy. Clin Cancer Res; 19(17); 4750–9. ©2013 AACR.

Introduction

Pancreatic cancer is an important therapeutic challenge with nearly similar incidence and mortality rates. It is the most lethal cancer of the digestive system with a 5-year survival rate of 5% (1). Because of the resistance of pancreatic cancer to chemotherapy and radiotherapy, current treatments for pancreatic cancer are generally not curative with modest benefit (2, 3). We and others have shown that modulating death receptor (DR)-activated extrinsic apoptosis pathways may provide potential novel avenues for cancer therapy (4, 5). Upon ligand stimulation, DRs (Fas or death receptor 4/5, DR4/5) recruit FADD and the initiator caspases, caspase-8 or caspase-10, resulting in formation of the death-inducing signaling complex (DISC). The recruited caspase-8 or caspase-10 undergoes autocatalytic cleavage and activation to trigger the caspase cascade that ultimately leads to apoptosis (4, 5). We have previously reported that regulating the DISC formation promotes Fas DR–induced apoptosis of cancer cells, including pancreatic cancer cells (6–10).

Activating the DR4/5-mediated extrinsic apoptotic signaling pathway by TNF-related apoptosis-induced ligand (TRAIL) induces apoptosis of a variety of cancer cells including melanoma, colon, lung, breast, kidney, and pancreatic cancers (11, 12). With virtually no toxicity for normal cells, recombinant TRAIL or agonistic antibodies specifically targeting DR4/5 are currently being tested in several phase I–III clinical trials (12–14). Although TRAIL is a promising anticancer agent, many cancer cells, including pancreatic cancer cells, develop resistance to TRAIL-induced apoptosis. The resistance of cancer cells to TRAIL-induced apoptosis has been associated with mutations of DR5 receptor (15), increased expression of the decoy DRs DcR1 or DcR2 (16, 17), decreased expression of apoptotic protein domain (FADD) and the initiator caspases, caspase-8 or caspase-10, resulting in formation of the death-inducing signaling complex (DISC). The recruited caspase-8 or caspase-10 undergoes autocatalytic cleavage and activation to trigger the caspase cascade that ultimately leads to apoptosis (4, 5).
caspase-8 activation, which is independent of its known regulating DR5-induced DISC recruitment and subsequent more, we have uncovered a novel mechanism of PARP-1 in pancreatic cancers in a mouse tumor xenograft model. Further-enhances the efficacy of DR5 antibody therapy for pancreatic cancer cells to DR5-activated apoptosis and the first evidence that PARP-1 inhibition sensitizes resistant signaling pathways is unknown. The present study provides herein the potential use of combination therapies that enhance the efficacy of TNF-related apoptosis-inducing ligand (TRAIL) therapy of resistant pancreatic cancer, a fatal disease with very limited therapeutic options. Using a pancreatic cancer xenograft model in nude mice, we have shown that PARP-1 knockdown sensitizes the resistant pancreatic cancer cells to TRA-8–induced apoptosis and thus enhances the efficacy of TRA-8 therapy. PARP inhibitors are already used in clinical trials for different tumors because of its known function in DNA repair. The studies presented here highlight a novel function of PARP-1 in regulating the extrinsic apoptosis machinery that contributes to pancreatic cancer resistance and support interventions combining PARP-1 inhibitors with death receptor agonists for pancreatic cancer therapy. Thus, these studies set up a stage for a possibility of clinical applications of the combination therapy in patients with pancreatic cancer.

Translational Relevance
The present studies explore the potential use of combination therapies that enhance the efficacy of TNF-related apoptosis-inducing ligand (TRAIL) therapy of resistant pancreatic cancer, a fatal disease with very limited therapeutic options. Using a pancreatic cancer xenograft model in nude mice, we have shown that PARP-1 knockdown sensitizes the resistant pancreatic cancer cells to TRA-8–induced apoptosis and thus enhances the efficacy of TRA-8 therapy. PARP inhibitors are already used in clinical trials for different tumors because of its known function in DNA repair. The studies presented here highlight a novel function of PARP-1 in regulating the extrinsic apoptosis machinery that contributes to pancreatic cancer resistance and support interventions combining PARP-1 inhibitors with death receptor agonists for pancreatic cancer therapy. Thus, these studies set up a stage for a possibility of clinical applications of the combination therapy in patients with pancreatic cancer.

caspase-8 (18), and increased expression of a number of antiapoptotic proteins, such as Bcl-2, Bcl-XL (19, 20) and the FLICE-like inhibitory protein (FLIP; refs. 21, 22).

Combination therapy with other anticancer agents has been shown to enhance the efficacy of TRAIL treatment for the resistant cancers (23–25). We and others have found that activating intrinsic apoptotic signaling pathways using gemcitabine (26), doxorubicin (27), or ionizing radiation (28) sensitizes resistant cancer cells to DR-induced apoptosis. These intrinsic apoptotic activators function via inducing mitochondria leakage or DNA damage (26–28).

The PARPs are key to repairing damaged DNA by modifying proteins associated with DNA repair (29). Inhibition of PARPs results in accumulation of irreparable damaged DNA and facilitates triggering of the intrinsic apoptosis signaling pathway in cancer therapy (29). PARP-1 is the most abundant of PARPs, accounting for 75% to 90% of the PARPs (29). Inhibition of PARP-1 through siRNA knockdown or chemical inhibitors for PARP has been used to treat cancers with DNA repair deficiencies, such as BRCA-deficient breast and ovarian cancer (30) as well as BRCA2-associated pancreatic cancer (31). In addition, combined use of PARP inhibitors with DNA-damaging reagents or radiation sensitizes glioma, ovarian, and pancreatic cancers to therapy (32–34).

The role of PARP-1 in DR-mediated extrinsic apoptotic signaling pathways is unknown. The present study provides the first evidence that PARP-1 inhibition sensitizes resistant pancreatic cancer cells to DR5-activated apoptosis and enhances the efficacy of DR5 antibody therapy for pancreatic cancers in a mouse tumor xenograft model. Furthermore, we have uncovered a novel mechanism of PARP-1 in regulating DR5-induced DISC recruitment and subsequent caspase-8 activation, which is independent of its known function in DNA repair. Our studies provide novel molecular insights into the function PARP-1 in the DR-activated apoptosis machinery and support the use of combined therapy with DR5 agonists and PARP inhibitors for resistant pancreatic tumors.

Materials and Methods
Cell culture, antibodies, and reagents
The human pancreatic cancer cell lines BxPc-3, MiaPaCa-2, PANc-1, and Suit-2 were from the American Type Culture Collection. BxPc-3 and Suit-2 cells were grown in RPMI-1640; MiaPaCa-2 and PANc-1 cells were grown in Dulbecco’s Modified Eagle’s Media (DMEM; Invitrogen) supplemented with penicillin (5 units/mL), streptomycin (5 μg/mL), and 10% heat-inactivated FBS. DR5 agonist antibody TRA-8 was generated as previously described (35). All antibodies used were commercially available, including FADD (Millipore), caspase-3 and pADPr (Enzo Life), caspase-8 (BD Bioscience and Cell Signaling Technology), PARP-1 and GAPDH (Santa Cruz Biotech), and DR5 (Prosci).

Protein G agarose was from Invitrogen. Recombinant human PARP-1 protein was purchased from Enzo. β-NAD was from Sigma-Aldrich. Active human recombinant caspase-8 was from Research & Diagnostics. Full-length mouse recombinant caspase-8 was from Abcam.

Gene knockdown with lentivirus-delivered short hairpin RNA
Lentiviral constructs expressing a 21-nucleotide short hairpin RNA (shRNA) targeting human PARP-1 gene (GenBank accession number NM_001618.3) or human FADD gene (GenBank accession number NM_003824.3) were purchased from Open Biosystems. The constructs were packaged into lentivirus-like particles pseudotyped with the vesicular stomatitis virus glycoprotein as we previously described (10). Transduction was conducted by incubating cancer cells with lentivirus, and stably transduced cells were selected with puromycin (2 μg/mL).

Assessment of apoptosis
Apoptosis was determined by Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining (BD Biosciences) and analyzed by flow cytometry (BD Biosciences).

Western blot analysis
Proteins were extracted, quantified with a BCA protein Detection Kit (Millipore), and then electrophoresed on SDS-PAGE and transferred to Immobilon P membranes (Millipore) as described previously (10). Membranes were blocked in 5% non-fat milk and incubated with primary antibodies overnight at 4°C and then with horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. Signals were detected using the Immobilon Western Chemiluminescent Horseradish Peroxidase Substrate Detection Kit (Millipore).
**Immunoprecipitation**

Five hundred micrograms of extracted proteins was incubated with 1 μg of antibodies as indicated in figure legends for 1 hour and subsequently incubated with 50 μL of 1:1 slurry of protein G-agarose beads overnight at 4°C. Beads were then washed, and 20 μL 2× Laemmli sample buffer was added to the beads followed by heating at 95°C for 5 minutes and chilling on ice. After brief centrifugation, proteins in the supernatant were analyzed by Western blotting with specific antibodies.

**In vitro PADPr modification and caspase-8 activation assays**

Recombinant full-length mouse Caspase-8 (300 ng, Abcam) was immunoprecipitated using antibody for caspase-8. Immunoprecipitated beads were incubated in 100 μL reaction buffer [100 mmol/L Tris-HCl (pH 8), 10 mmol/L MgCl₂, 1 mmol/L dithiothreitol] with or without 100 ng of reaction buffer [100 mmol/L Tris-HCl (pH 8), 10 mmol/L MgCl₂, 1 mmol/L dithiothreitol] with or without 100 ng of PARP-1 and 500 μmol/L β-NAD for 30 minutes at 37°C and then washed 3 times.

For caspase-8 activation analysis, immunoprecipitated beads were incubated in 100 μL reaction buffer [20 mmol/L HEPES (pH 7.5), 5 mmol/L dithiothreitol] with or without 100 ng of recombinant active human caspase-8 (Research & Diagnostic) at 37°C for 60 minutes. Beads were then washed and 20 μL 2× Laemmli sample buffer was added to the beads followed by heating at 95°C for 5 minutes and chilling on ice. After brief centrifugation, proteins in the supernatant were analyzed by Western blotting with specific antibodies.

**Mouse xenograft model**

The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham (Birmingham, AL). Male athymic nu/nu mice (6 weeks old, NCI-Frederick) were used for tumor inoculation. Briefly, control PANC-1 pancreatic cancer cells or PANC-1 cells with PARP-1 knockdown were inoculated subcutaneously into the flanks of mice (2×10⁶ cells in 200 mL PBS/site). Three weeks following tumor inoculation, mice were divided into 2 groups (8 mice per group), mice in the treatment group were intraperitoneally injected with TRA-8 once a week for 6 weeks (200 μg/mice), and mice in the control group were injected with 0.9% sodium chloride.

Tumor size and body weight were measured every week, and chilling on ice. After brief centrifugation, proteins in the supernatant were analyzed by Western blotting with specific antibodies.

**Statistical analysis**

Results are expressed as means±SD. Differences between 2 groups were identified with the Student t test. For multiple groups, one-way ANOVA and Student–Newman–Keuls tests were conducted to identify differences. Significance was defined as P<0.05.

**Results**

**Inhibition of PARP-1 activity sensitizes resistant pancreatic cancer cells to TRA-8**

Using TRA-8, a monoclonal agonistic antibody specifically targeting DR5 (35), we determined DR-activated apoptosis in four pancreatic cell lines. BxPc-3 and MiaPaCa-2 cells were highly sensitive to TRA-8–induced apoptosis, whereas PANC-1 and Suit-2 cells are resistant to TRA-8 (Fig. 1A). To understand the underlying mechanisms, we analyzed the expression of the receptor DR5, as well as the expression of an antia apoptotic protein FLIP (Fig. 1B) that has been shown to inhibit DR5-induced apoptosis (36). The expression of DR5 is higher in MiaPaCa-2 and Suit2 cells compared with that in BxPc-3 and PANC-1 cells, which was not correlated with their sensitivity to TRA-8–induced apoptosis. Similarly, the expression level of FLIP was not correlated to the resistance of the cells to TRA-8–induced apoptosis, suggesting that additional mechanisms may contribute to TRA-8 resistance.

We found that the expression of PARP-1 was correlated with TRA-8 resistance of the pancreatic cancer cells. The expression of PARP-1 was markedly higher in the TRA-8–resistant cell PANC-1 and Suit-2 cells, compared with that in the TRA-8–sensitive BxPc-3 and MiaPaCa-2 cells (Fig. 1B), suggesting a role of PARP-1 in TRA-8 resistance. Using a pharmacologic inhibitor for PARPs, PJ-34, we found that inhibition of PARP activity enhanced TRA-8–induced apoptosis in PANC-1 and Suit-2 cells in a concentration-dependent manner (Fig. 1C). Such an effect of PJ-34 was not due to its toxicity, as PJ-34 did not induce apoptosis at all concentrations used (0–40 μmol/L). Consistent with its effects on TRA-8–induced apoptosis, PJ-34 dramatically enhanced TRA-8–induced activation of caspase-8 and its downstream apoptotic effector, caspase-3 (Fig. 1D).

**Knockdown of PARP-1 sensitizes TRAIL-resistant pancreatic cancer cells to TRA-8**

The specific role of PARP-1 in mediating TRA-8 resistance of pancreatic cancer cells was further determined using shRNAs specific for PARP-1. Two lines of PANC-1 cells with PARP-1 knockdown were generated using shRNAs specifically targeting different regions of the PARP-1 gene. Knockdown of PARP-1 by the 2 shRNAs was confirmed by Western blot analysis (Fig. 2A, inserts). Knockdown of PARP-1 per se did not affect cell viability (Fig. 2A, white bars) but significantly increased the sensitivity of PANC-1 cells to TRA-8–induced apoptosis (Fig. 2A, black bars).
Similar to the observations with PJ-34 (Fig. 1D), PARP-1 knockdown increased TRA-8–induced activation of caspase-8 and caspase-3 (Fig. 2B).

**PARP-1 knockdown enhances efficacy of TRA-8 therapy in vivo**

To determine the effects of PARP-1 knockdown on the efficacy of TRA-8 therapy on tumorigenesis in vivo, we characterized tumorigenesis and response to TRA-8 treatment of PARP-1 knockdown PANC-1 cells in a mouse xenograft model. PANC-1 cells with scrambled shRNAs were used as controls. In the control groups, TRA-8 treatment did not reduce tumor size, further showing resistance of these cancer cells to TRA-8 therapy (Fig. 3A). In contrast, TRA-8 treatment effectively reduced tumor size of the PARP-1–knockdown cells (Fig. 3A). Western blot analysis determined that the expression of PARP-1 was maintained at a lower level in xenograft tumors of PARP-1–knockdown cells, which was not affected by TRA-8 treatment (Fig. 3B). Activation of caspase-8 and its downstream apoptosis effector caspase-3, indicated by cleaved caspase-8 and caspase-3, was shown in PARP-1–knockdown tumors from the TRA-8–treated mice (Fig. 3B). Furthermore, TUNEL staining of tumor sections showed significant increases in cell death in the PARP-1–knockdown tumors from TRA-8–treated mice (Fig. 3C).

**Activation of caspase-8 is responsible for PARP-1 knockdown–rendered TRA-8 sensitivity**

Because inhibition of PARP-1, by PJ-34 (Fig. 1) or by shRNAs (Fig. 2), increased TRA-8–induced activation of the upstream apoptosis initiator, caspase-8, we determined whether inhibition of caspase-8 may block the effects of PARP-1 inhibition. Z-IETD-FMK, a caspase-8 inhibitor, blocked TRA-8–induced apoptosis in the control PANC-1 cells. Moreover, inhibition of caspase-8 significantly reduced TRA-8–induced apoptosis in the PARP-1–knockdown cells (Fig. 4A). Consistently, caspase-8 inhibition resulted in
marked decreases in TRA-8–induced cleavage/activation of caspase-8 and its downstream caspase-3 in the PARP-1–knockdown cells (Fig. 4B).

Furthermore, knockdown of PARP-1 did not affect the expression of the death receptor DR5, the adaptor protein FADD, or the procaspase-8 (Fig. 4C). As procaspase-8 is...
known to be proteolytically cleaved and activated in the DR5-induced DISC, we analyzed DR5-recruited DISC proteins, including FADD and caspase-8, in response to TRA-8 stimulation. PARP-1 knockdown did not affect the recruitment of FADD into DR5-associated DISC in response to TRA-8 stimulation (Fig. 4C). As expected, TRA-8 induced the recruitment and activation of caspase-8 into the DR5-associated DISC in the control cells (Fig. 4C, shScr). TRA-8–induced DR5 recruitment of procaspase-8 appeared to be similar in the control and PARP-1–knockdown cells. However, increased activation of caspase-8 was shown in the PARP-1–knockdown cells (Fig. 4C, p41/43 and p24/26). Furthermore, PARP-1 was identified in the DR5-associated DISC complex in the control cells under the basal condition, which was not affected by TRA-8 treatment (Fig. 4C, PARP-1).

**Activation of DR5 induces pADPr modification of DISC component proteins**

A key function of PARP-1 is to modify proteins by adding poly(ADP-ribose) (pADPr) chain to target proteins. Accordingly, we analyzed pADPr modification profile of the DR5-associated DISC proteins. Using anti-pADPr antibody, we determined that knockdown of PARP-1 significantly reduced overall protein pADPr modification in the PANC-1 cells (Fig. 5A). TRA-8 treatment did not affect the overall protein pADPr modification profile (Fig. 5A, pADPr). However, increased pADPr modification on DISC components, DR5, FADD, and caspase-8, was detected in the TRA-8–treated control cells (Fig. 5A, shScr). Furthermore, PARP-1 knockdown decreased TRA-8–induced pADPr modification on DR5, FADD, and caspase-8 (Fig. 5A, PARP-1 KD).

To further determine that pADPr modification of DR5, FADD, and caspase-8 occurred in the DR5-associated DISC, we knocked down FADD, the adaptor protein of DISC formation, with shRNA specifically targeting FADD (Fig. 5B). Knockdown of FADD did not affect the overall protein pADPr modification profile but decreased TRA-8–induced pADPr modification on DR5 and caspase-8 (Fig. 5B, pADPr), indicating that TRA-8–induced pADPr modification on DR5 and caspase-8 is dependent on FADD-mediated DISC formation (Fig. 5B).

**pADPr modification inhibits caspase-8 activation**

Using purified recombinant PARP-1 and caspase-8 proteins, a direct effect of PARP-1 on pADPr modification of caspase-8 was shown (Fig. 6A). Furthermore, the effect of pADPr modification of caspase-8 on its activation was characterized. In the absence of PARP-1, active caspase-8 cleaved procaspase-8 into cleaved p10/18 fragments (Fig. 6B). However, decreased caspase-8 cleavage was observed when PARP-1 was added (Fig. 6B, last lane), indicating that pADPr modification of caspase-8 inhibited caspase-8 activation.

**Discussion**

TRAIL therapies have shown low toxicity in patients in several clinical trials (12–14). However, therapeutic effects
Activation of DR5 induces pADPr modification of DISC proteins. A, pADPr modification of DISC proteins. Control (shScr) and PARP-1 KD cells were exposed to TRA-8 (1 μg/mL) for 60 minutes. Immunoprecipitation was conducted with pADPr antibody, and Western blotting was conducted to determine pADPr modification of DR5, caspase-8, and FADD. B, the effects of FADD knockdown on pADPr modification of DISC proteins. Expression of FADD in the control (shScr) and FADD KD cells was determined by Western blot analysis. Immunoprecipitation conducted with pADPr antibody and Western blot analysis of pADPr-modified DR5, caspase-8, and FADD. Representative blots from 3 independent experiments are shown.

The expression of PARP-1 was identified in both nucleus and cytoplasm of the PANC-1 and Suit-2 cells (data not shown). PARP-1 is an important regulator of the DNA base excision repair pathway and is essential for the maintenance of genomic integrity and cell survival in response to genotoxic insults (37, 38). PARP-1 elevation is associated with adverse outcomes of breast and ovarian cancers (39, 40). Consistently, we found that the expression of PARP-1 was increased in 2 TRA-8-resistant pancreatic cell lines, PANC-1 and Suit-2 cells, and inhibition of PARP-1 rendered the resistant cells more sensitive to TRA-8-induced apoptosis, showing the engagement of PARP-1 in TRA-8-resistant pancreatic cancer cells.

Consistent with its essential role in DNA repair, PARP-1 antagonists, used as a monotherapy for tumors with DNA repair deficiencies or in combination with DNA damage-inducing agents, increase accumulation of damaged DNA that triggers intrinsic apoptotic pathways (31–34). In pancreatic cancer, PARP inhibitors enhance the effects of chemotherapy with DNA-alkylating agents or radiation therapy, both via promoting DNA damage-induced intrinsic apoptotic signaling pathways. The present studies have provided the first evidence that PARP-1 inhibition alone did not induce apoptosis of pancreatic cancer cells but enhanced TRA-8-induced apoptosis, suggesting that PARP-1 regulates TRA-8-induced extrinsic apoptosis via a mechanism independent of its function in DNA repair.
PARP-1 is also supported by the observation that caspase-8 increased, and TRA-8–induced apoptosis was enhanced. In support of this concept, when PARP-1 residues may contribute to the inhibition of caspase-8 cleavage (29), pADPr modification on these aspartate residues at 216, 374, and 384 (47, 48). Because the cleavage of caspase-8 into the p18/p10 active form are the active form (Fig. 6B). The amino acids that are critical for modification of caspase-8; and (iii) recombinant PARP-1 of DISC formation by FADD knockdown blocked pADPr modification on caspase-8, thereby inhibiting caspase-8 activation. Such an observation is consistent with previous studies showing that pADPr modification inhibits protein function. PARP-1 did not appear to modify DR5 directly, although an association between PARP-1 and DR5 was shown under basal conditions. In response to TRA-8 treatment, pADPr modification of the DISC complex, DR5/caspase-8/FADD, was identified, which was dependent on the DISC recruitment of FADD. Therefore, PARP-1 may not directly modify DR5 at basal conditions but regulates the pADPr modification of the DR5-associated DISC complex and thus modulating downstream apoptotic signaling.

Our studies revealed that PARP-1 modified caspase-8 and determined a novel function of PARP-1 in regulating pADPr modification of caspase-8, thereby inhibiting caspase-8 activation. Such an observation is consistent with previous studies showing that pADPr modification inhibits protein function. For instance, pADPr modification of NFKB(p65) decreases its interaction with the nuclear export factor and thus retains it in the nucleus (46). The activation of procaspase-8 is believed to occur in the DR-activated DISC, which provides a platform that allows 2 procaspase-8 homodimers to be in close proximity necessary to initiate cleavage and activation of procaspase-8 (47–49). By the same token, the presence of PARP-1 in the DR5-associated complex provides the opportunity for PARP-1 to modify procaspase-8 in the DISC. This concept is supported by several lines of evidence: (i) pADPr modification of caspase-8 was identified in the DR5-associated DISC induced by TRA-8; (ii) inhibition of DISC formation by FADD knockdown blocked pADPr modification on caspase-8; and (iii) recombinant PARP-1 induced pADPr modification of caspase-8 and inhibited caspase-8 cleavage/activation directly. We found PARP-1 inhibited the cleavage of procaspase-8 into the p18/p10 active form (Fig. 6B). The amino acids that are critical for the cleavage of caspase-8 into the p18/p10 active form are the aspartate residues at 216, 374, and 384 (47, 48). Because aspartate is one of the PARP-1 targeting modification residues (29), pADPr modification on these caspase-8 aspartate residues may contribute to the inhibition of caspase-8 cleavage and activation. In support of this concept, when PARP-1 was inhibited, TRA-8–induced activation of caspase-8 was increased, and TRA-8–induced apoptosis was enhanced. This is also supported by the observation that caspase-8 inhibitor blocked the effect of PARP-1 inhibition on TRA-8–induced apoptosis.

In summary, we have found that PARP-1 knockdown sensitized resistant pancreatic cancer cells to TRA-8–induced apoptosis in vitro and enhanced the efficacy of TRA-8 therapy of pancreatic tumors in vivo. PARP-1 was identified in the DR5-associated complex, which induced pADPr modification on caspase-8 in the TRA-8–activated DR5-associated DISC and modulated caspase-8 activation. Therefore, inhibition of PARP-1 blocks pADPr modification of caspase-8, which facilitates TRA-8–mediated activation of caspase-8 in the DISC, and thus sensitizing resistant pancreatic cancer cells to TRA-8–induced apoptosis.
Grant Support
This work was supported in part by UAB Department of Pathology start-up funds (to Y. Chen) and a VA Merit Review Award (to I.M. McDonald).

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Received February 22, 2013; revised June 10, 2013; accepted June 25, 2013; published OnlineFirst August 9, 2013.

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