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TRAIL and Triptolide: An Effective Combination that Induces Apoptosis in Pancreatic Cancer Cells

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Dr. Daniel Borja-Cacho, Presenter (University of Minnesota, Minneapolis, MN)

Discussant

Dr. Jeffrey Matthews (University of Chicago Medical Center, Chicago, IL): Thank you for that presentation.

Just a few questions about triptolide. Do you know if the doses that you are using are similar to the kinds of levels that you would get from eating a Chinese herb? Or at what level are you starting to see the effects of the triptolide, and do you know of its toxicity profile when given in vivo?

Secondly, do you have any insight into what the actual target of triptolide is that might be upstream from some of these changes in gene expression?

Finally, I am wondering if you have had the chance to go back and look at archived surgical specimens or specimens that you obtain fresh in your pancreatic surgical program to know whether there is a difference in the pattern of XIAP expression in pancreatic cancer cells versus other cancer cell types that are perhaps less resistant to this therapy? This might help understand whether that accounts for the unusual resistance of pancreatic cancer to standard therapies.

Discussion of Paper #19

Title of Paper: Triptolide and TRAIL: An Effective Combination that Activates Both the Intrinsic and Extrinsic Apoptotic Pathways in Pancreatic Cancer Cells

Closing Discussant

Dr. Daniel Borja-Cacho: The first question: in vitro, the optimal dose that we have used to study the mechanism of action of triptolide is 200 nM. The equivalent dose that we have used for in vivo with mice is $0.2 \text{ mg kg}^{-1} \text{ day}^{-1}$. The toxic dose in vivo that we found so far is 0.8. Therefore, we have a therapeutic window.

Since triptolide is very efficacious in treating pancreatic tumors in mice, the University of Minnesota is planning to undertake a phase I trial for Triptolide in the near future.

About this work, the interesting finding is that a lower dose of Triptolide, 50 nM, is working with the combination. This is very encouraging because it seems that, for synergistic studies, a lower dose is enough. We still need to find the optimum dose in vivo for this combination.

Most of the toxicity that has been reported regarding triptolide is confined to the liver. Hepatotoxicity is the main concern. This is present when doses higher than 0.8 mg/kg are used. However, the doses we are using are much lower than this and do not appear to have any toxic effects.

Regarding your second question, we are definitely very interested to see why XIAP expression is decreased and why the expression of other antiapoptotic proteins is coming down. We are currently studying other transcription factors such as heat shock factor 1, which gives resistance to cells and regulate different antiapoptotic proteins such as heat shock protein 70 and gives resistance to chemotherapeutic agents. That is the main reason why we are not emphasizing that triptolide inhibits XIAP expression. We are also looking for other transcription factors.

Finally, we know that XIAP is overexpressed in pancreatic cancer cells. However, we have not so far evaluated it in our patients. It will be an interesting study to do to try to predict which patients are going to respond to the treatment, similar to patients with breast cancer.

Discussion of Paper #19

Title of Paper: Triptolide and TRAIL: An Effective Combination that Activates Both the Intrinsic and Extrinsic Apoptotic Pathways in Pancreatic Cancer Cells

Discussant

Dr. Daniela Basso (Padua, Italy): A quick question about toxicity. For the combination of this therapy, did you test whether in vitro is safe for normal cells? How do normal cells respond to this treatment?

Discussion of Paper #19

Title of Paper: Triptolide and TRAIL: An Effective Combination that Activates Both the Intrinsic and Extrinsic Apoptotic Pathways in Pancreatic Cancer Cells

Closing Discussant

Daniel Borja-Cacho: Anti-death receptor therapy including recombinant TRAIL is not toxic to normal cells because these receptors are only expressed in cancer cells. In general, normal cells do not express them. For example, normal pancreatic duct cells are not known to express these receptors.

Multiple phases 1 and 2 trials have shown the safety of this therapy with minimal toxicity. Similarly, our studies indicate that triptolide is also safe both in vivo and in vitro at doses, which are efficacious.

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Abstract

Introduction—An emerging therapy in oncology is the induction of apoptotic cell death through anti-death receptor therapy. However, pancreatic cancer is resistant to apoptosis including anti-death receptor therapy. We have previously described how triptolide decreases resistance to apoptosis in pancreatic cancer cells in vitro and in vivo. We hypothesized that triptolide decreases tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in pancreatic cancer cells. The aim of this study was to evaluate the effects that combined therapy with TRAIL and triptolide have on different parameters of apoptosis.

Methods—Four different pancreatic cancer cell lines were exposed to triptolide, TRAIL, or a combination of both drugs. We assessed the effects that combined therapy with TRAIL and triptolide has on cell viability, apoptosis, caspase-3 and caspase-9 activities, and poly(ADP)-ribose polymerase cleavage.

Results—Pancreatic cancer cells were resistant to TRAIL therapy; however, combined therapy with triptolide and TRAIL significantly decreased the cell viability in all the cell lines and increased apoptotic cell death as a result of caspase-3 and caspase-9 activation.

Conclusions—Pancreatic cancer is highly resistant to anti-death receptor therapy, but combined therapy with TRAIL and triptolide is an effective therapy that induces apoptotic cell death in pancreatic cancer cells.

Keywords

Death receptor therapy; TRAIL; Triptolide; Pancreatic cancer; Apoptosis

Introduction

Pancreatic cancer is associated with a poor prognosis that has not significantly changed over the past 30 years. Major pancreatectomies are now considered a safe procedure with a low mortality; however, less than 10–15% of the patients with pancreatic cancer are candidates for surgery because most patients present with locally advanced tumors or systemic disease. In addition, most patients will develop a locoregional or distant recurrence within the next 2 years after surgery.¹ Given the high recurrence rate, adjuvant chemotherapy with or without radiotherapy is an important component in the treatment of pancreatic cancer. Multiple drugs have been approved as a standard of care (i.e., gemcitabine, 5-fluorouracil, and erlotinib). Despite the use of these drugs, the long-term outcome for pancreatic cancer remains very poor, with current 5-year survival rates of less than 5%.^{2–4}

The poor response associated with conventional chemo-therapy has created a shift in pancreatic cancer research in order to identify mutations that are responsible for the aggressive biologic behavior or confer resistance to treatment. At the same time, this trend

has led to the development of multiple targeted drugs that induce apoptosis, restore the cell cycle in cancer cells, or restore or block the deleterious effects of the mutations that give resistance to apoptosis.

One of the most promising approaches to targeted therapies in oncology is the induction of apoptosis in cancer cells. Apoptosis or programmed cell death can be induced by two different mechanisms. The first pathway is triggered by different stimuli such as DNA damage, radiotherapy, or chemotherapy that induce the mitochondrial release of cytochrome *c* and apoptosis-inducing factor. Once cytochrome *c* is present in the cytoplasm, it binds to APAF-1 and procaspase-9 to form the apoptosome complex, which in turn activates caspase-9. Caspase-9 is an initiator caspase that amplifies the signal by activating effector procaspase-3, procaspase-7 and procaspase-6.⁵ In contrast, the extrinsic or death receptor pathway is mediated by different ligands and their receptors. Upon binding to their ligands, these receptors recruit both Fas-associated death domain and procaspase-8 to form the death-inducing signaling complex (DISC). After the DISC is formed, procaspase-8 gets activated; active caspase-8 can directly activate the effector caspases or, through BID cleavage, induce the activation of the mitochondrial pathway.⁶

As previously mentioned, the tumor necrosis factor (TNF) superfamily has different ligands that induce apoptosis. The first two members described were TNF- α and Fas ligand. Upon ligation to their receptors, these two peptides trigger apoptosis in normal and cancer cells; therefore, systemic therapy with these peptides induces a systemic shock response manifested with hypotension, severe liver failure, and death. TNF-related apoptosis-inducing ligand (TRAIL) was more recently discovered.⁷ The main difference between this relatively new member and TNF- α or Fas ligand is that TRAIL is more selective to cancer cells; it only induces apoptosis in tumor cells. Multiple preclinical and clinical studies have shown that TRAIL is a safe therapy associated with minimal toxicity to normal cells.⁸

Pancreatic cancer cells are known to be highly resistant to apoptosis, including TRAIL therapy. We have previously demonstrated that triptolide, a diterpene triepoxide extracted from the Chinese plant *Tripterygium wilfordii*, induces apoptosis in pancreatic cancer cells, both in vitro and in vivo.⁹ Consequently, we hypothesized that triptolide therapy decreases resistance to TRAIL therapy in human pancreatic cancer cells. The aim of this study was to assess the effects that combined therapy with TRAIL and triptolide has on different markers of apoptosis in pancreatic cancer cells.

Materials and Methods

Cells Culture and Drugs

MIA-PaCa2 and PANC-1 cells were obtained from the American Type Culture Collection. Both cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PS). S2-013 and S2-VP10 cells were kindly provided by Dr. Buchsbaum (University of Alabama at Birmingham). S2-013 and S2-VP10 cells were grown in RPMI supplemented with 10% FBS and 1% PS. All cell lines were grown under standard conditions at 37°C in a humidified atmosphere containing 5% CO₂. DMEM, RPMI, PS, and FBS were obtained

from Invitrogen Corporation. Triptolide (Calbiochem) was diluted in dimethyl sulfoxide. Recombinant human TRAIL (also known as Apo2 ligand; Invitrogen Corporation) was diluted in sterile water. All drugs were stored in aliquots according to manufacturer's recommendations.

Determination of Cell Viability

Four different pancreatic cancer cell lines were seeded into 96-well plates (5×10^3 /well) and allowed to adhere for 24 h. Cells were treated with vehicle (control), increasing concentrations of TRAIL (0–20 ng/ml) alone, or in the presence of a low dose of triptolide (50 nM). Cell viability was measured using Dojindo Cell Counting Kit-8 according to manufacturer's protocol. After 48 h of treatment, 10 μ l of tetrazolium substrate were added into each well. The plates were protected from light and incubated for 1 h in a humidified atmosphere at 37°C containing 5% CO₂. Absorbance was measured in a plate reader (BioTek) at an absorbance of 450 nm. Cell viability was measured in triplicates; each experiment was repeated four times.

Determination of Apoptosis

Pancreatic cancer cells (2.5×10^5 cells/well) were seeded into six-well plates. After a 24-h incubation, cells were treated with vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of TRAIL plus triptolide (using the same doses). After 24 h of treatment, the externalization of phosphatidylserine was measured by flow cytometry using the Guava Nexin Kit as previously described.⁹ Apoptosis was measured in duplicates; each experiment was repeated four times.

Quantification of Caspase-3 and Caspase-9 Activities

Caspase-3 and caspase-9 activities were analyzed using the Caspase-Glo luminescent-based assays (Promega) according to the manufacturer's instructions. Cells (1×10^4 cells/well) were seeded in 96-well white opaque plates and a corresponding optically clear 96-well plate. Cells were allowed to adhere for 24 h and treated with vehicle, TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs. After 4, 8, 12, and 18 h of treatment, 100 μ l of Caspase-Glo-3 and Caspase-Glo-9 were added into each well. Plates were gently mixed for 1 min, and after 30 min of incubation, plates were read using a luminometer (BMG Labtech). The corresponding 96-well clear well plate was used to measure the number of viable cells with CCK-8 reagent. Caspase activity was normalized to the number of viable cells. Caspase activity was measured in triplicates and repeated four times.

Determination of PARP Cleavage by Western Blotting

Cells (8×10^5) were plated in 10-cm dishes. Once cells were 70% confluent, they were treated with vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs. After 24 h of treatment, cells were harvested and washed with 1 $\times$ phosphate-buffered saline. Cells were resuspended in lysis buffer (Boston bioproducts Inc.; 65 mmol/L Tris-HCL (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Nonidet P40, 1% sodium deoxycholate, 1 μ g/ml aprotinin, and 100 μ g/mL phenylmethylsulfonyl fluoride) with freshly added protease inhibitor cocktail (Roche) for 30 min at 4°C and stored at –80°C. The

next day, cell lysates were cleared by centrifugation for 20 min at 13,000×g. Total protein concentration was measured using the bicinchoninic acid protein assay according to manufacturer's protocol (Pierce). Equal amount of protein (10 µg) were resolved over 10% Tris–HCl polyacrylamide gels and transferred onto nitrocellulose membranes (Bio-Rad laboratories). Membranes were incubated in blocking buffer (5% bovine serum albumin) for 1 h. The blot was subsequently incubated with polyclonal rabbit anti-human poly(ADP)-ribose polymerase (PARP; Santa Cruz Biotechnology) or polyclonal rabbit anti-human Actin (Santa Cruz Technology). After three washes, membranes were incubated for 1 h with the appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Technology). Blots were detected with chemiluminescence. Actin expression was used as an internal control.

Statistical Analysis

Values are expressed as mean ± SE. The significance of the difference between control and each experimental test condition was analyzed by one-way ANOVA using GraphPad InStat Software. The difference was considered statistically significant if $p < 0.05$.

Results

Effect of Triptolide and TRAIL on Cell Viability

We selected four different pancreatic cancer cell lines based on the level of aggressiveness and resistance to TRAIL therapy. We used two metastatic cancer cell lines (S2-013 and S2-VP10), which are known to be TRAIL resistant, one non-metastatic with intermediate sensitivity to TRAIL (PANC-1), and finally, one non-metastatic TRAIL-sensitive cell line (MIA-PaCa2). These cell lines were exposed to increasing concentrations of TRAIL (0–20 ng/ml). After 48 h of treatment, most pancreatic cancer cells were resistant to TRAIL therapy; only MIA-PaCa2 cell line exhibit a significant decrease in the cell viability (Fig. 1a). However, when all cell lines were co-incubated with TRAIL and triptolide (50 nM), the cell viability in all the cell lines tested significantly decreased. This effect was seen in MIA-PaCa2 (Fig. 1a), Panc-1 (Fig. 1b), S2-VP10 (Fig. 1c), and S2-013 (Fig. 1d).

Combined Therapy with Triptolide and TRAIL Increases Apoptosis

Apoptosis is the main mechanism by which TRAIL induces cell death.^{6,7,10} We have previously demonstrated that triptolide enhances apoptotic cell death in pancreatic cancer cells.⁹ Therefore, we decided to assess the effect that combined therapy with TRAIL and triptolide has on apoptosis. We used Annexin V staining to measure the externalization of phosphatidylserine as a marker of apoptosis. Four different pancreatic cancer cell lines were exposed to vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both (TRAIL 1.25 ng/ml+ Triptolide 50 nM). After 24 h of treatment, single therapy with TRAIL or triptolide had a minimal effect on apoptosis; however, if pancreatic cancer cells are exposed to combined therapy with TRAIL and triptolide, the number of cells undergoing apoptosis significantly increases. This effect was statistically significant as compared to single therapy with each drug alone (Fig. 2).

Combined Therapy with Triptolide and TRAIL Increases Caspase-3 Activity

Caspases are the main enzymes that mediate apoptosis.^{8,11} Any stimuli that triggers apoptosis eventually leads to the activation of the effector (also known as executioner) caspases, which include caspase-3, caspase-6, and caspase-7. In order to corroborate that apoptosis is the main type of cell death that occurs when pancreatic cancer cells are exposed to combined therapy with TRAIL and triptolide, we measured the activation of caspase-3 and caspase-7 using a luminescence assay. For this purpose, we exposed our four pancreatic cancer cell lines to vehicle (control), TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs for 18 h. Figure 3 illustrates how a low dose of TRAIL or triptolide induces minimal activation of caspase-3 and caspase-7; however, if both drugs are combined using the same low doses, the activation of caspase-3 and caspase-7 considerably increases. This effect can be seen in all the cell lines tested: MIA-PaCa2, PANC-1, S2-VP10, and S2-013.

In order to validate the previous result, we evaluated the effect that combined therapy with TRAIL and triptolide have on PARP cleavage. PARP is one of the caspase-3 substrates that mediate apoptosis; once caspase-3 gets activated, it cleaves PARP. We exposed MIA-PaCa2, S2-013, and S2-VP10 cells to vehicle, TRAIL (1.25 ng/ml), triptolide (50 nM), or a combination of both drugs. As indicated in Fig. 4, PARP cleavage occurs in all cell lines only when both drugs are co-administered. The results of this set of experiments validate that combined therapy with TRAIL and triptolide increases the activity of caspase-3 and caspase-7. This effect is present in all cell lines regardless of TRAIL sensitivity.

Effect of TRAIL and Triptolide on the Mitochondrial Apoptotic Pathway

Effector caspases (3 and 7) are activated directly by caspase-9 (intrinsic pathway). We decided to measure caspase-3 and caspase-9 at different time points to assess if the mitochondrial pathway is being activated by TRAIL and triptolide. As seen in Fig. 5, combined therapy with both drugs induced an increase in the activity of caspase-9 and caspase-3. This result was seen in the four cell lines evaluated, which suggests that when pancreatic cancer cells are incubated with both drugs, there is a time-dependent activation of the mitochondrial apoptotic pathway. It is also evident in Fig. 5 that the decrease in cell viability occurs at the same time that caspase-9 and caspase-3 activation is occurring. Finally, we exposed our cell lines to vehicle (control), TRAIL 1.25 ng/ml, triptolide (50 nM), and combined therapy with both drugs. After 18 h of exposure, we measured caspase-9 activity. As seen in Fig. 6, the activity of caspase-9 was increased significantly when MIA-PaCa2 and S2-VP10 cells were treated with both drugs as compared to each drug alone.

Discussion

Pancreatic adenocarcinoma remains a devastating tumor with a poor prognosis because it has an aggressive biological behavior. As a result, up to 80% of patients with pancreatic cancer will not be able to undergo resection and require the administration of chemotherapy. The current drugs considered as the standard of care for pancreatic cancer have a minimal impact in the long-term survival of patients with pancreatic cancer, which is reflected by the pronounced lethality of this tumor.¹² Since the chemotherapy that is considered as the

standard of care for pancreatic cancer has not been able to induce a significant impact in the overall survival of patients with pancreatic cancer, new forms of therapies that specifically target pancreatic cancer are required.

Our group has previously described that triptolide, a diterpenoid triepoxide present in a Chinese herb, induces the release of cytochrome *c* from the mitochondria, which in turn induces sequential activation of procaspase-9 and procaspase-3. Once caspase-9 and caspase-3 are activated, they induce apoptotic cell death in pancreatic cancer cells in vitro. We have also shown that triptolide decreases the tumor growth and locoregional invasion in an orthotopic model of pancreatic cancer in vivo, which suggests that triptolide is a good candidate for pancreatic cancer therapy.⁹ Nevertheless, the clinical experience and treatment of other solid tumors tell us that only a few solid tumors respond to single agent-based chemotherapy. Chronic exposure to chemotherapeutic agents can induce the selection of clones that are resistant to that particular agent; therefore, overtime tumor resistance can occur. Additionally, solid organ tumors sometimes have intrinsic resistance to the drug before any treatment has started. It cannot be overemphasized that the probability that drug resistance develops over the course of the disease decreases if different agents are combined. Combined therapy also allows decreasing drug doses, decreasing the likelihood of toxicity.

Anti-death receptor therapy is a relatively new form of cancer treatment; this type of therapy has proven to induce apoptosis of multiple cancer cell lines in vitro and tumor regression in some xenograft models without affecting normal cells.^{6,10,13} Since the preclinical evidence has been promising, this therapy is now being evaluated in phase I and II clinical trials. Results from these trials suggest that anti-death receptor therapy is safe in humans because it is not associated with significant toxicity.¹⁴ While death receptor therapy has been promising in solid tumors, the majority of human pancreatic cancer cell lines are known to be highly resistant to drugs that induce apoptosis, including anti-death receptor therapy.^{15,16} Our initial experiments also validate that most pancreatic cancer cells are resistant to TRAIL therapy.

Since both TRAIL and triptolide induce apoptosis in pancreatic cancer cells, we formulated the hypothesis that combined therapy with these two compounds increases the effectiveness as compared to single therapy. Our results prove the fact that low doses of TRAIL and triptolide induce a significant increase in apoptosis as compared to single therapy either with TRAIL and triptolide. It is important to mention that the doses of both drugs are considerably lower than the doses used in single therapy. All our results showed the same trend: Combined therapy with both drugs increases the externalization of phosphatidylserine, procaspase-3 and procaspase-9 activation, and PARP cleavage. Taken together, these results suggest that combined therapy with TRAIL (death receptor therapy) and triptolide is a promising therapy that requires further investigation. Cells can be classified into two types according to the main pathway that induces apoptosis. If cancer cells do not require activation of the mitochondrial pathway, they are considered type I cells, but if tumor cells require activation of the intrinsic or mitochondrial pathway, cells are classified as type II. In a similar way to what has been described, we found that pancreatic cancer cells are type II because they require activation of the intrinsic pathway to undergo apoptosis after TRAIL therapy.

Conclusions

Combined therapy with TRAIL and triptolide is a new promising therapy for pancreatic cancer that increases the activation of caspase-3 and caspase-9. As a result, this therapy increases the number of cells undergoing apoptotic cell death as compared to monotherapy with TRAIL or triptolide. This effect is not exclusive of TRAIL-sensitive cell lines because it is also seen in cell lines that are known to be highly aggressive and resistant to TRAIL. Combined therapy with TRAIL and triptolide is a novel therapy in pancreatic cancer that requires further investigation.

Acknowledgments

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Figure 1.

Effect of TRAIL and triptolide on cell viability and apoptosis in pancreatic cancer cells. After 48 h of treatment, most pancreatic cancer cell lines are TRAIL-resistant. TRAIL therapy alone only decreases the cell viability in MIA-PaCa2 cells (a). However, combined therapy with increasing concentrations of TRAIL in the presence of triptolide (50 nM) significantly increases the number of cells dying. This effect is also present in TRAIL-resistant cell lines, as is shown in b–d (PANC-1, S2-VP10, and S2-013, respectively). Points means, bars SE ($n=4$, run in triplicates).

Figure 2.

Effect of TRAIL and triptolide on annexin V. After 24 h of single therapy with a low dose of TRAIL or triptolide, there was no significant externalization of phosphatidylserine; however, combined therapy using a low dose of both drugs increased the number of cells that stained positive for annexin V, indicating that apoptosis is occurring in the cells. This effect is seen in TRAIL-sensitive (MIA-PaCa2, **a**) and TRAIL-resistant cell lines (**b** PANC-1, **c** S2-VP10, and **d** S2-013). Column mean, bar SE, * $p < 0.01$ and ** $p < 0.001$ as compared to control, TRAIL, or triptolide.

Figure 3.

Effect of TRAIL and triptolide on caspase-3 activity. A low dose of TRAIL (1.25 ng/ml) or triptolide (50 nM) has minimal effect on the activation of caspase-3 and caspase-7, but if both drugs are combined, the activities of caspase-3 and caspase-7 significantly increase after 18 h of exposure. This effect is seen in MIA-PaCa2 (a), PANC-1 (b), S2-VP10 (c), and S2-013 (d). Column mean, bar SE ($n=4$ run in triplicates) * $p<0.0001$ or ** $p<0.0009$ as compared to control, triptolide, or TRAIL.

Figure 4.

Effect of TRAIL and triptolide on PARP cleavage. Single therapy with low doses of either TRAIL or triptolide did not induce any cleavage of PARP but combined therapy with both drugs increased PARP cleavage. The results of this experiments correlate with the increase activity of caspase-3 and caspase-7 in the previous figure. This experiment was repeated at least three times with each cell line, all with similar results.

Figure 5.

Effect of combined therapy with TRAIL and triptolide on the activity of caspases (3 and 9). Combined therapy with TRAIL (1.25 ng/ml) and triptolide (50 nM) induces an increase in the activity of procaspase-3 and procaspase-9. This increase is time-dependent. The increase seen in the activity of caspase-3 and caspase-9 occurs when the cell viability starts to decrease. This effect was seen in all the cell lines tested (**a** MIA-PaCa2, **b** PANC-1, **c** S2-VP10, **d** S2-013). Points, mean; Bar, SE ($n=4$, run in triplicates for each time point).

Figure 6.

Effect of TRAIL and triptolide on caspase-9 activity. Monotherapy with TRAIL or triptolide induces minimal caspase-9 activation; however, combined therapy with both drugs induces a significant increase in the activation of caspase-9. This effect is seen in all cell lines. *Column mean, bar SEM; * $p < .001$ ($n = 4$, run in triplicates) as compared to control, TRAIL, and triptolide.*