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Anti-tumor activity of the TRA-8 anti-DR5 antibody in combination with cisplatin in an *ex vivo* human cervical cancer model

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Abstract

Objectives. To investigate the cytotoxicity of TRA-8, an antibody that specifically binds death receptor 5 (DR5), alone and in combination with cisplatin, using an *ex vivo* human cervical cancer model.

Methods. Fifteen cervical cancer specimens were obtained at the time of radical hysterectomy and tumor slices were prepared with the Krumdieck tissue slicer. Tumor slices were exposed to varying concentrations of TRA-8, cisplatin, or the combination of TRA-8 and cisplatin. Using non-linear modeling, dose response curves and IC_{50} values were generated for each specimen treated with TRA-8. The additive cytotoxic effect of combination treatment was evaluated as well. In addition to ATP viability assays, treated and untreated slices were assessed by immunohistochemistry (IHC) and western blot analysis to confirm apoptosis induction via the extrinsic pathway.

Results. Eleven patient specimens yielded TRA-8-induced IC_{50} values. Sixty-four percent were found to be sensitive to TRA-8-induced cytotoxicity at IC_{50} doses less than 1000 ng/ml. Seven patient specimens underwent combination treatment with TRA-8 and cisplatin. Of these specimens, 86% exhibited additive cytotoxicity in comparison to treatment with either agent alone. IHC revealed an increase in DR5 expression in tumor slices treated with cisplatin for 24 h. IHC and Western blotting demonstrated TRA-8-induced cell death via apoptosis and activation of caspase 3 and 8.

Conclusions. This study confirms the utility of an *ex vivo* human cervical cancer model, to evaluate the anti-tumor activity of TRA-8 and cisplatin. This model may be a useful pre-clinical tool to assess cytotoxicity and mechanistic properties of novel agents in cervical cancer.

Keywords: TRAIL; Tumor necrosis factor-related apoptosis-inducing ligand; Cervix cancer; Cisplatin; Anti-DR5 monoclonal antibody

Introduction

Although the incidence of cervical cancer in the United States has declined over the past three decades, a worldwide reduction in the incidence of cervical cancer has not been achieved with approximately 500,000 women diagnosed with invasive cervical cancer annually [1]. If cervical cancer is diagnosed early, prognosis is excellent with either radical surgery or chemoradiation. Patients with metastatic or recurrent disease have poor outcomes with limited therapeutic options. The development of novel agents for patients with advanced or metastatic disease is crucial to improving outcomes. Recently, translational research has focused on improving the specificity and toxicity of chemotherapeutic agents. Molecular targeted therapies, often used in conjunction with traditional cytotoxic agents, hold promise to achieve this goal. A host of FDA-approved monoclonal antibodies directed towards specific targets in a variety of tumors have elicited improvements in cancer outcomes [2–4]. The role of novel therapeutic agents in the treatment of cervical cancer has only recently been evaluated. To date, no monoclonal antibody has been established as efficacious in the treatment of cervical cancer.

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Selective apoptosis of cancer cells, with minimal adverse effects on non-cancerous tissues, can be achieved with TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) [5]. TRAIL is a type 2 transmembrane protein which is expressed in normal tissues and can be cleaved by proteases to release a soluble form [6]. The soluble ligand is then capable of binding any of the four membrane receptors and one soluble receptor for TRAIL. Death receptors 4 and 5 (DR4 and DR5) function with cytoplasmic domains that initiate apoptosis by activation of downstream caspases in the extrinsic apoptotic pathway [7–10].

Early experiences with recombinant TRAIL were marked by significant toxicity to human hepatocytes [11,12]. Thus, researchers at our institution developed a monoclonal antibody, TRA-8, that binds specifically to DR5 [13]. TRA-8 was created by immunizing BALB/c mice with a fusion protein containing the extracellular domain of human DR5 and the Fc portion of human IgG1. Preliminary data suggested that TRA-8 possesses potent anti-tumor properties without inducing cytotoxicity in human hepatocytes and other normal tissues [13].

Several studies have demonstrated increased DR5 expression in cervical cancer cell lines and human cervical cancer tissue [14–16]. The presence of DR5 on the surface of a variety of cancer cells confers cytotoxicity via an apoptotic mechanism when exposed to TRA-8 [16–18]. This cytotoxicity can be seen with TRA-8 treatment alone, but is enhanced when combined with conventional cytotoxic agents [16–18]. Most studies evaluating the apoptotic effects and mechanisms of TRA-8 utilize traditional *in vitro* cancer cell line experiments followed by *in vivo* human tumor xenograft models. We sought to investigate the therapeutic potential of TRA-8 with cisplatin using a three-dimensional *ex vivo* human model of primary cervical cancer.

Materials and methods

Human cervical cancer specimens

Following IRB approval from the University of Alabama at Birmingham, patients with clinical stage IB1 to IIA biopsy-proven cervical carcinoma (visible lesions) undergoing radical hysterectomy were enrolled in the study. Patients with a history of neoadjuvant chemotherapy or radiation therapy were ineligible. All histologic subtypes were allowed. Patient demographics were recorded for each patient specimen, and final histologic diagnosis was confirmed by a gynecologic pathologist.

Specimen processing

Immediately following removal of the radical hysterectomy specimen from the patient, the uterine cervix was evaluated for tumor availability. Patients with insufficient tumor were ineligible for the study. 5 mm cylindrical cores were created by random sampling of grossly visible cervical tumor using specialized coring tools compatible with the Krumdieck tissue slicer (Alabama Research and Development, Munford, AL). The 5 mm tumor cores were placed in complete culture media (DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin B, and 50 μg/ml gentamycin) on ice and taken immediately to the 4 °C cold room for subsequent tissue slicing. Within 30 min of specimen retrieval, the Krumdieck tissue slicer was used according to previous reports to prepare tissue slices from the collected tumor cores [18–20]. Each tumor core was cut into multiple slices of 300 μm thickness, using a reciprocating blade, while submerged in media. Tissue slices were collected and randomly placed into individual wells of 24-well plates in 1.5 ml of complete media. The 24-well plates were incubated at 37 °C with 5% CO_2 and atmospheric oxygen for 48 h.

Cell viability

To assess cell viability, ATP assays were performed on six replicate slices for each treatment variable. Tumor slices were exposed to varying concentrations of TRA-8 (10, 30, 100, 300, 500, and 1000 ng/ml) and cisplatin (10, 30, and 100 μM) as tumor size and slice number would permit. Since prior studies have revealed that chemotherapy has the ability to enhance DR5 expression, tumor slices were pre-treated for 24 h with cisplatin prior to the addition of TRA-8 [21–23]. After a total of 48 h treatment, slices were sonicated for 15 s in a 50:50 mixture of complete media and ATP mammalian cell lysis buffer. ATP levels were measured in four aliquots from each tissue slice via ATP-dependent light emission in counts per second, and mean ATP levels were determined for each slice [24]. Fractional tumor slice survival was then calculated for each of the six untreated control slice specimens (each with four replicate aliquots) and each treated specimen consisting of six slices (also with four replicate aliquots per slice) as the ratio of mean ATP levels in the TRA-8 treated slices versus the mean of untreated control slices. IC_{50} values were established from the dose response curve created from these fractional ratios as described in the statistical section. When applicable, patient specimens were assessed for interactive effects of TRA-8 and cisplatin combination therapy.

To assess viability of tumor slices using this model, a slice viability time-course study was performed. Six replicate control slices from time points 24, 48, and 96 h post-slicing were processed in an identical manner as previously described. Viability plots were generated from the fractional ratios of the individual time points compared to time point zero.

Immunohistochemistry

Treated and untreated tumor slices from available specimens were assessed by immunohistochemistry (IHC) and compared to representative tumor slices fixed immediately after slicing. The methods we employed for bright-field immunohistochemical analysis have been previously published [25–27]. Tumor slices were mounted on sialinized slides (Bond-Rite), and soaked in Tris buffer. Paraffin sections were cut at 5 μm thickness and mounted on sialinized microscopic slides (Bond-Rite). In a similar fashion as reported by Estes *et al*, slices and paraffin sections were evaluated with the TUNEL assay and antibody staining for DR5 and cleaved caspase 8, respectively [18]. The tumor cells were classified by one of the co-authors (WG) with respect to the intensity of immunostaining, with the percent of cells determined at each staining intensity from 0 to +4 as previously described [25,26,28,29].

Western blot analysis

Treated and untreated patient specimens were analyzed for caspase 3 and caspase 8 cleavage. Tumor tissue slices were washed twice with cold PBS and solubilized in 300 μl lysis buffer and the lysates were subsequently prepared for Western blotting in a similar fashion as reported previously [18]. A total of 20 μg of protein was loaded and separated on 15% SDS-PAGE gels, and electrophoretically transferred to PVDF membranes. The blots were blocked with 5% nonfat dry milk in TBST buffer (100 mM Tris–HCl at pH 7.4, 150 mM NaCl, and 0.1% Tween 20) and incubated with primary antibody in blocking buffer at 4 °C overnight. The blots were then washed three times with TBST and probed with HRP-conjugated secondary antibodies for 1 h at room temperature. After washing four times with TBST, the probed proteins were visualized using the ECL Western blotting detection system (Amersham Biosciences, Piscataway, NJ). Mouse anti-human caspase 8 antibody (BD Pharmingen, San Diego, CA) and rabbit anti-human caspase 3 antibody (Stressgen, Ann Arbor, MI) recognize the cleaved and proforms of caspase 8 and caspase 3.

Statistical analysis

The dose-response curve of TRA-8 treated slices was created by plotting the fractional tumor slice survival against the logarithm of TRA-8 concentration. The data was fit by nonlinear regression to a sigmoidal dose-response function with variable slope using Prism 3.01. Log IC_{50} values, defined as the logarithm of the TRA-8 concentration producing 50% reduction in ATP levels (counts per second) compared to the untreated tumor slices, were determined for each

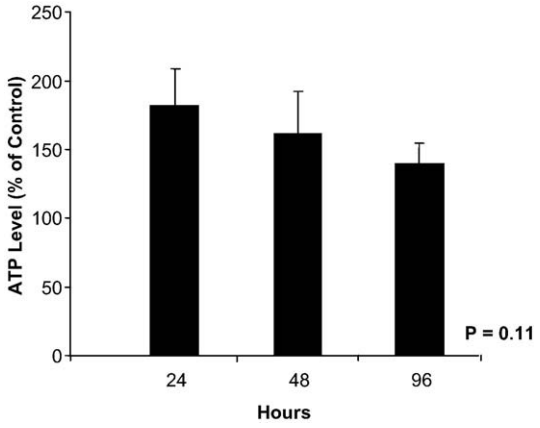

Fig. 1. Viability of untreated *ex vivo* cervical cancer tissue slices over time as compared to the mean ATP level of timepoint zero control slices.

tumor. To estimate the IC_{50} of TRA-8, we used a non-linear model: $y = Min + (Max - Min)/(1 + \text{dose}/\beta)^{\alpha}$, was applied to calculate IC_{50}, where y is the response, the parameter β represents IC_{50}, the parameter α is used to scale concentration for proper transformation, and *Min* and *Max* represent the minimum and the maximum of response [30,31]. NLIN procedure in SAS® version 9.1 (SAS Institute Inc., Cary, NC) was utilized for estimation. A correlation coefficient of $R^2 \geq 0.85$ was used as a cut-off value to ensure data quality control for each assay. The cytotoxicity data were evaluated to assess whether the combination cytotoxic effects were additive, less than additive (antagonistic), or greater than additive (synergistic). The dose response relationships for the treatment combinations were modeled using a second-order response surface model with linear, quadratic and interaction terms [32]. A significant interaction term was classed as synergistic if it was positive and antagonistic if negative. If the interaction term was not significant, then the relationship between TRA-8 and cisplatin would be considered additive, provided the additive terms were significant.

Results

Patient characteristics

Fifteen cervical cancer specimens were collected from patients undergoing radical hysterectomy at the University of Alabama at Birmingham from January 2006 to April 2007. Eleven specimens were assayed for TRA-8-induced cytotoxicity. Ten of eleven were squamous cell carcinomas, and one was adenocarcinoma. Seven specimens were treated with TRA-8 alone, cisplatin alone, and the combination of TRA-8 and cisplatin. Six of seven were squamous cell carcinomas, and one was adenocarcinoma. The median patient age was 43 years (range 29–53), and the majority of patients were Caucasian. All patients had visible cervical lesions, and 53% were clinical stage IB1.

Cytotoxic tissue slice assay

Untreated cervical cancer slices exhibited excellent viability over 96 h of *ex vivo* culture (Fig. 1). TRA-8-induced IC_{50} values were generated for 11 specimens. Sensitivity to TRA-8 did not correlate with stage or histology. Insufficient slice number in four patients resulted in the absence of IC_{50} data for these four specimens. TRA-8-induced cytotoxicity, depicted

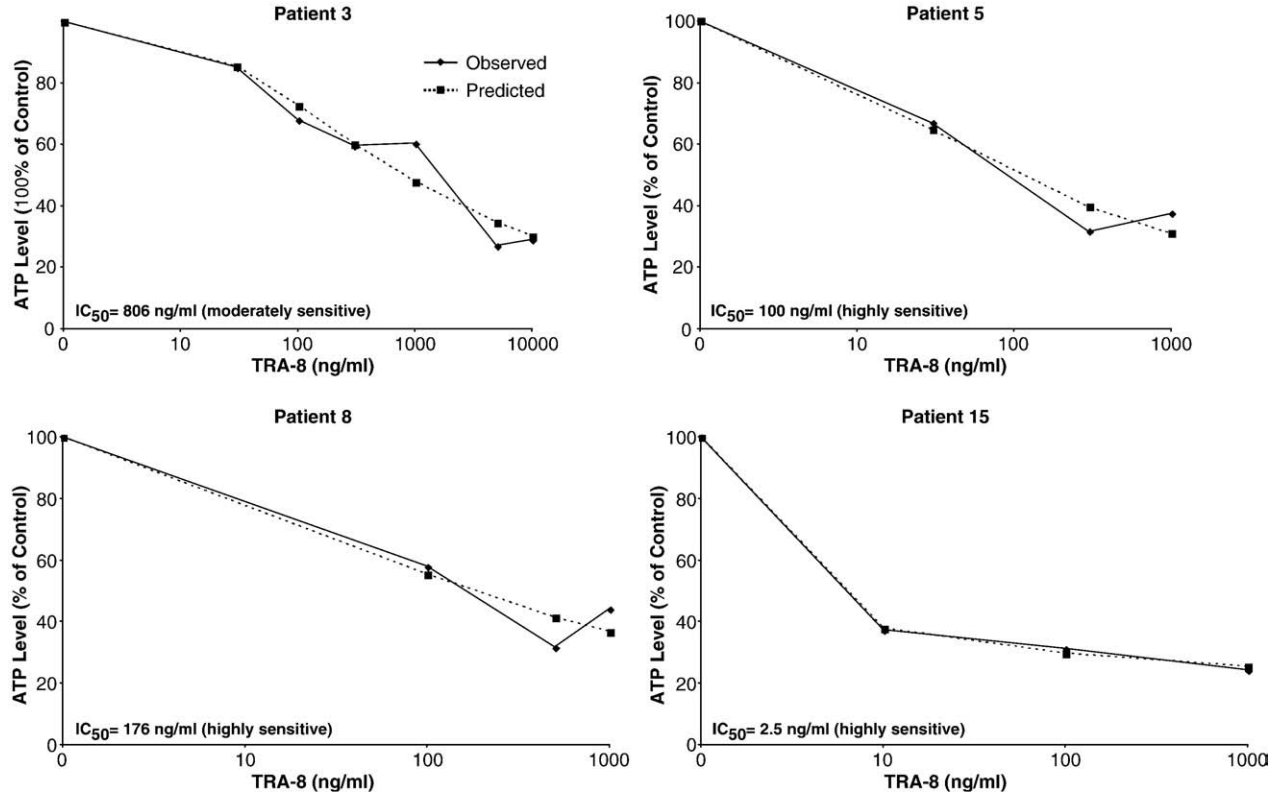

Fig. 2. TRA-8-induced cytotoxicity of cervical cancer tissue slices. Results are plotted as ATP level (% of untreated control) versus log TRA-8 concentrations (hatched line). Non-linear modeling was used to determine IC50 values for each individual curve (solid line).

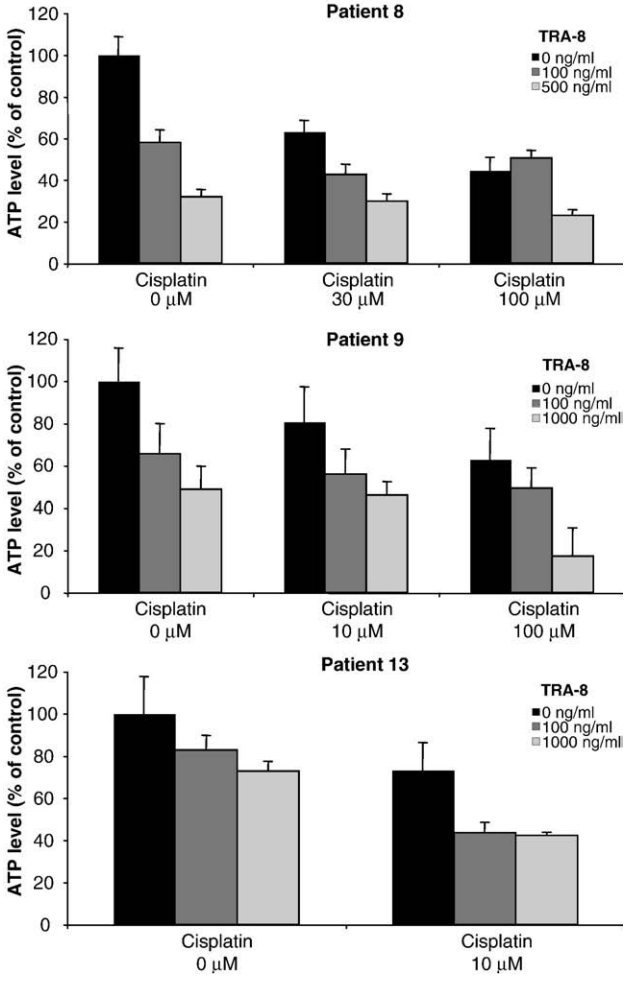

Fig. 3. TRA-8, cisplatin, and combination therapy cytotoxicity assays for 3 cervical cancer tissue slice experiments. Additive cytotoxicity is evident when TRA-8 and cisplatin are combined.

with dose response curves as well as the calculated IC_{50} values, is shown in Fig. 2 for four patient specimens. There was a broad range of IC_{50} values in the 11 specimens (2.5 to >1000 ng/ml). Patient specimens 5, 6, 8, and 15 were highly sensitive to TRA-8-induced apoptosis, with IC_{50} values ranging from 2.5 to 300 ng/ml. Patient specimens 3, 9, and 10 were moderately sensitive to TRA-8 treatment, with IC_{50} values ranging from 787 to 813 ng/ml. Four patient specimens with IC_{50} values greater than 1000 ng/ml were resistant to TRA-8.

Cytotoxicity of combination therapy with TRA-8 and cisplatin was evaluated for seven patients that had adequate slice numbers. Six patient specimens treated with TRA-8 and cisplatin exhibited additive cytotoxicity in comparison to treatment with either agent alone. Fig. 3 graphically represents three patient specimens exhibiting variable additive cytotoxicity at different drug and TRA-8 concentrations. In patient 8, the increase in viability seen in slices treated with the combination of TRA-8 100 ng/ml and cisplatin 100 μM likely reflects tumor heterogeneity. Specimens with a greater proportion of stromal tissue than tumor appear to be less susceptible to cytotoxic agents.

Immunohistochemical and Western blot analysis

Available specimens underwent IHC utilizing staining for DR5 expression, TUNEL assays, caspase 3 and caspase 8 evaluation. In order to evaluate the effect of cisplatin chemotherapy on DR5 expression, slices were stained for DR5. Fig. 4 demonstrates an increase in DR5 expression in slices treated with 100 μM of cisplatin compared to control slices. The apoptotic effect of cisplatin and TRA-8 monotherapy was evaluated using the TUNEL assay. Increased TUNEL staining was seen with each therapy compared to controls (Fig. 5).

Additional specimens underwent Western blot analysis to confirm that the cytotoxicity seen in treated slices resulted from the induction of apoptosis and not necrosis. In order to evaluate TRA-8 activation of the extrinsic apoptotic pathway, caspase 8 and caspase 3 were evaluated (Fig. 6). The presence of cleaved caspase 8, was evident with combination utilizing cisplatin and TRA-8 or TRA-8 alone. Apoptotic cell death was further confirmed by staining for caspase 3 which demonstrated caspase 3 cleavage products in slices treated with 1000 ng/ml TRA-8 alone and in combination with cisplatin.

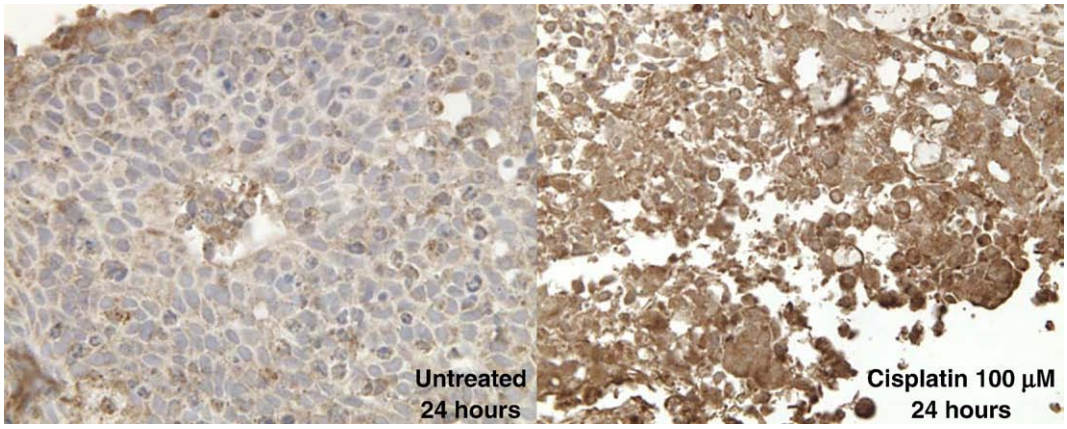

Fig. 4. Immunohistochemistry analysis of untreated and cisplatin treated cervical cancer slices 24 h post-slicing. A marked increase in DR5 expression is seen in the cisplatin treated tissue using the TRA-8 antibody for staining.

Discussion

Patients with locally advanced or recurrent cervical cancer have limited therapeutic options and poor outcomes. In 1999, the National Cancer Institute issued a clinical announcement on cervical cancer advising clinicians that concomitant chemotherapy and radiotherapy should be strongly considered for all patients with locally advanced cervical cancer. Five randomized trials which led to this announcement exhibited improved survival when chemotherapy was combined with radiation therapy [33–37]. Since that time, no novel treatment regimen has been identified to further improve survival of patients with advanced or recurrent cervical cancer.

Currently, cancer research is focused on targeted therapeutics to enhance treatment of cancer while limiting the toxicities which have plagued conventional cytotoxic drugs and radiation therapy. TRAIL receptor activating agents have established themselves as promising candidates for novel biological therapy development. The monoclonal antibody, TRA-8, has the ability to target death receptor 5 on the surface of cancer cells and trigger an apoptotic response while sparing normal cells.

Several studies have demonstrated substantial efficacy of the TRA-8 antibody, particularly when coupled with chemotherapy and/or radiation therapy [16–18,38]. Furthermore, the *ex vivo* technique utilized in this study has previously been evaluated in patients with ovarian cancer [18]. Therefore, we sought to evaluate the cytotoxic effects of TRA-8 alone and in combination with cisplatin in an *ex vivo* tumor slice model of human cervical cancer.

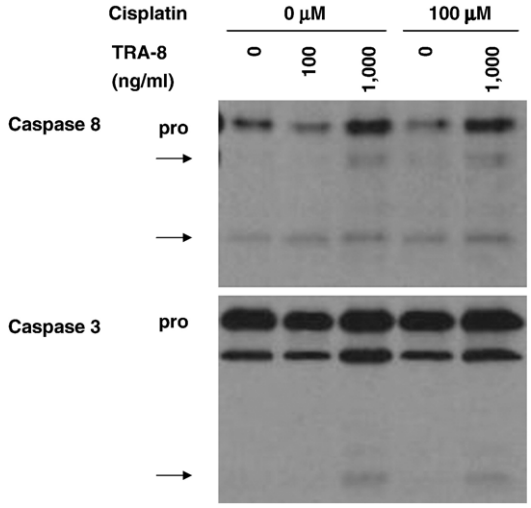

Fig. 6. TRA-8 activation of caspase 8 and caspase 3 in cervical cancer tissue slices. Following 48 h of treatment, tissue lysates were separated in SDS-PAGE and blotted. Blots were probed with antibodies specific to human caspase 8 and caspase 3. Arrows point to caspase cleavage products.

This *ex vivo* tissue slice model provides a unique experimental approach to evaluate fresh human tissue. Unlike cell line and

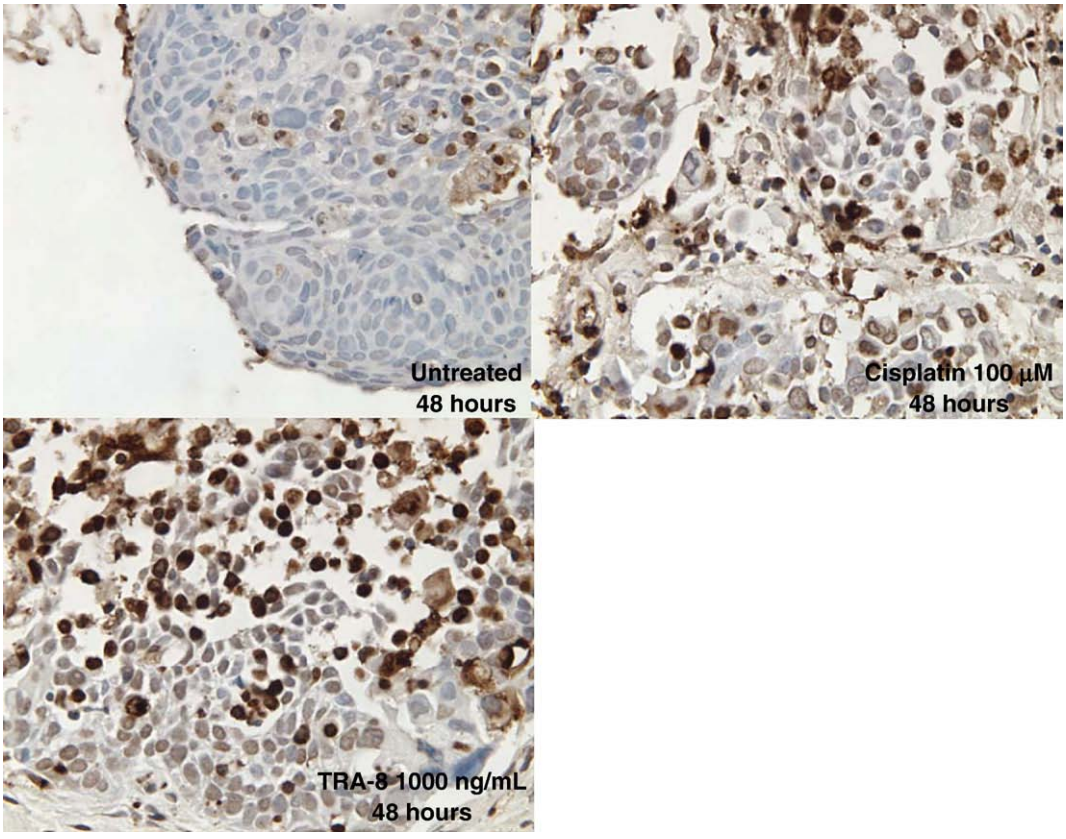

Fig. 5. Immunohistochemistry analysis of treated and untreated cervical cancer slices after 48 h. An increase in staining via the TUNEL assay demonstrates marked apoptosis in the slices treated with cisplatin or TRA-8.

xenograft studies, fresh tissue slices maintain all the components indigenous to human tumors. These components comprise a complex three-dimensional structure of tumor, stroma, endothelium, and lymphocytic infiltrate which may influence the therapeutic outcome of a drug or antibody treatment. Similar to previous studies, we found remarkable viability of untreated cervical cancer tissue slices in *ex vivo* culture [18]. It appears that the cytotoxic effects seen in these experiments were due to drug/antibody treatment and not loss of cell viability over time.

The majority of targeted agents are utilized in conjunction with traditional cytotoxic drugs possessing known efficacy against the tumor type. There is significant data to support the use of cisplatin as an active agent for cervical cancer [33–37]. Moreover, Nagane *et al*. found that cisplatin enhanced TRAIL-mediated apoptosis of glioma cells by upregulating DR5 expression [39]. Therefore, we designed our experiments to assess the cytotoxic response of TRA-8 alone and in combination with cisplatin. Approximately two-thirds of the specimens exhibited a response to TRA-8 monotherapy, while one-third were resistant to TRA-8 (IC_{50} >1000 ng/ml). Importantly, additive cytotoxicity was seen in 86% of specimens treated with the combination of TRA-8 and cisplatin. Western blot analysis revealed a reduction of pro-caspase 8 as well as the presence of its cleavage product, indicating activation of the extrinsic apoptotic pathway. Immunohistochemistry revealed an increase in DR5 expression in tumor slices pre-treated with cisplatin. These results support our rationale to treat with chemotherapy prior to TRA-8 therapy in hopes that increased DR5 expression will enhance the cytotoxic effects of TRA-8.

Traditional chemotherapeutic agents initiate apoptosis via activation of the intrinsic, or p53-mitochondrial pathway. Treatment with chemotherapy or radiotherapy triggers translocation of p53 from the cytoplasm to the nucleus of the cell, thereby stimulating transcription of pro-apoptotic factors ultimately leading to cell death [40,41]. Approximately 50% of human cancers possess a p53 genomic mutation that may enable them to escape death induced by conventional therapeutic regimens [42]. More specific to cervical cancer, HPV E6 leads to down regulation of p53, thereby inhibiting apoptosis via the intrinsic pathway [43]. Thus, development of novel agents that exploit the extrinsic apoptotic pathway becomes paramount. Treatment with TRA-8 may provide an alternative treatment that can effectively activate the extrinsic apoptotic pathway, and circumvent chemoresistance resulting from p53 mutations or HPV-induced down regulation.

This pilot study demonstrated a therapeutic effect of TRA-8 and cisplatin in an *ex vivo* human cervical cancer tissue slice model. This *ex vivo* model not only provides cytotoxicity data on fresh tumor obtained from patients prior to therapy, but also allows for mechanistic studies to be performed. Future studies are planned to evaluate the response of cervical cancer to TRA-8 plus radiation and chemotherapy. The tissue slice model represents a new paradigm for translational research, as it allows human tissue to be evaluated both from a cytotoxic and mechanistic standpoint. A phase I study utilizing a humanized version of the TRA-8 antibody in the treatment of patients with solid malignancies is currently underway.

Acknowledgments

Conflict of Interest: DJB has intellectual property interest related to the TRA-8 antibody.

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References

[1] Franco EL, Schlecht NF, Saslow D. The epidemiology of cervical cancer. Cancer J 2003;9:348–59.
[2] Goldenberg MM. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. Clin Ther 1999;21:309–18.
[3] Monk BJ, Choi DC, Pugmire G, Burger RA. Activity of bevacizumab (rhuMAB VEGF) in advanced refractory epithelial ovarian cancer. Gynecol Oncol 2005;96:902–5.
[4] Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567–78.
[5] Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 1999;104:155–62.
[6] Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3:673–82.
[7] Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L. Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-κB pathway. Immunity 1997;7:821–30.
[8] Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. Curr Biol 1997;7:1003–6.
[9] Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, et al. The receptor for the cytotoxic ligand TRAIL. Science 1997;276:111–3.
[10] Walczak H, Degli-Esposti MA, Johnson RS, Smolak PJ, Waugh JY, Boiani N, et al. TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. EMBO J 1997;16:5386–97.
[11] Jo M, Kim TH, Seol DW, Esplen JE, Dorko K, Billiar TR, et al. Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. Nat Med 2000;6:564–7.
[12] Lawrence D, Shahrokh Z, Marsters S, Achilles K, Shih D, Mounho B, et al. Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions. Nat Med 2001;7:383–5.
[13] Ichikawa K, Liu W, Zhao L, Wang Z, Liu D, Ohtsuka T, et al. Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity. Nat Med 2001;7:954–60.
[14] Ryu HS, Chang KH, Chang SJ, Kim MS, Joo HJ, Oh KS. Expression of TRAIL (TNF-related apoptosis-inducing ligand) receptors in cervical cancer. Int J Gynecol Cancer 2000;10:417–24.
[15] Reesink-Peters N, Hougardy BM, van den Heuvel FA, Ten Hoor KA, Hollema H, Boezen HM, et al. Death receptors and ligands in cervical carcinogenesis: an immunohistochemical study. Gynecol Oncol 2005;96:705–13.
[16] Straughn Jr JM, Oliver PG, Zhou T, Wang W, Alvarez RD, Grizzle WE, et al. Anti-tumor activity of TRA-8 anti-death receptor 5 (DR5) monoclonal antibody in combination with chemotherapy and radiation therapy in a cervical cancer model. Gynecol Oncol 2006;101:46–54.
[17] Buchsbaum DJ, Zhou T, Grizzle WE, Oliver PG, Hammond CJ, Zhang S, et al. Antitumor efficacy of TRA-8 anti-DR5 monoclonal antibody alone or in combination with chemotherapy and/or radiation therapy in a human breast cancer model. Clin Cancer Res 2003;9:3731–41.
[18] Estes JM, Oliver PG, Straughn Jr JM, Zhou T, Wang W, Grizzle WE, et al. Efficacy of anti-death receptor 5 (DR5) antibody (TRA-8) against primary human ovarian carcinoma using a novel ex vivo tissue slice model. Gynecol Oncol 2007;105:291–8.

[19] Krumdieck CL, dos Santos JE, Ho KJ. A new instrument for the rapid preparation of tissue slices. Anal Biochem 1980;104:118–23.
[20] Kirby TO, Rivera A, Rein D, Wang M, Ulasov I, Breidenbach M, et al. A novel ex vivo model system for evaluation of conditionally replicative adenoviruses therapeutic efficacy and toxicity. Clin Cancer Res 2004;10:8697–703.
[21] Gibson SB, Oyer R, Spalding AC, Anderson SM, Johnson GL. Increased expression of death receptors 4 and 5 synergizes the apoptosis response to combined treatment with etoposide and TRAIL. Mol Cell Biol 2000;20: 205–12.
[22] Nimmanapalli R, Perkins CL, Orlando M, O'Bryan E, Nguyen D, Bhalla KN. Pretreatment with paclitaxel enhances Apo-2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of prostate cancer cells by inducing death receptors 4 and 5 protein levels. Cancer Res 2001;61:759–63.
[23] Singh TR, Shankar S, Chen X, Asim M, Srivastava RK. Synergistic interactions of chemotherapeutic drugs and tumor necrosis factor-related apoptosis-inducing ligand/Apo-2 ligand on apoptosis and on regression of breast carcinoma in vivo. Cancer Res 2003;63:5390–400.
[24] Cree IA, Andreotti PE. Measurement of cytotoxicity by ATP-based luminescence assay in primary cell cultures and cell lines. Toxicol In Vitro 1997;11:553–6.
[25] Grizzle WE. Tissue resources in the detection and evaluation of markers. In: Srivastava S, editor. Early Detection of Cancer: Molecular Markers. Futura Publishing Co., Inc.; 1997. p. 69–76.
[26] Grizzle WE, Myers RB, Manne U, Stockard CR, Harkins LE, Srivastava S. Factors affecting immunohistochemical evaluation of biomarker expression in neoplasia. In: Hanausek M, Walaszek Z, editors. John Walker's Methods in Molecular Medicine — Tumor Marker Protocols. Totowa: Humana Press, Inc.; 1998. p. 161–79.
[27] Poczatek RB, Myers RB, Manne U, Oelschlager DK, Weiss HL, Bostwick DG, et al. Ep-Cam levels in prostatic adenocarcinoma and prostatic intraepithelial neoplasia. J Urol 1999;162:1462–6.
[28] Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493–501.
[29] Li Y, Wang H, Wang Z, Makhija S, Buchsbaum D, LoBuglio A, et al. Inducible resistance of tumor cells to tumor necrosis factor-related apoptosis-inducing ligand receptor 2-mediated apoptosis by generation of a blockade at the death domain function. Cancer Res 2006;66:8520–8.
[30] Rodbard D. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clin Chem 1974;20: 1255–70.
[31] DeLean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose–response curves. Am J Physiol 1978;235:E97–E102.
[32] Gennings C. On testing for drug/chemical interactions: Definitions and inference. J Biopharm Stat 2000;10:457–67.
[33] Keys HM, Bundy BN, Stehman FB, Muderspach LI, Chafe WE, Suggs III CL, et al. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. N Engl J Med 1999;340:1154–61.
[34] Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, et al. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. N Engl J Med 1999;340:1137–43.
[35] Rose PG, Bundy BN, Watkins EB, Thigpen JT, Deppe G, Maiman MA, et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N Engl J Med 1999;340:1144–53.
[36] Whitney CW, Sause W, Bundy BN, Malfetano JH, Hannigan EV, Fowler Jr WC, et al. Randomized comparison of fluorouracil plus cisplatin versus hydroxyurea as an adjunct to radiation therapy in stage IIB–IVA carcinoma of the cervix with negative para-aortic lymph nodes: a Gynecologic Oncology Group and Southwest Oncology Group study. J Clin Oncol 1999;17:1339–48.
[37] Peters III WA, Liu PY, Barrett II RJ, Stock RJ, Monk BJ, Berek JS, et al. Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. J Clin Oncol 2000;18:1606–13.
[38] DeRosier LC, Huang Z-Q, Sellers JC, Buchsbaum DJ, Vickers SM. Treatment with gemcitabine and TRA-8 anti-death receptor-5 mAb reduces pancreatic adenocarcinoma cell viability in vitro and growth in vivo. J Gastrointest Surg 2006;10:1291–300.
[39] Nagane M, Pan G, Weddle JJ, Dixit VM, Cavenee WK, Huang H-JS. Increased death receptor 5 expression by chemotherapeutic agents in human gliomas causes synergistic cytotoxicity with tumor necrosis factor-related apoptosis-inducing ligand in vitro and in vivo. Cancer Res 2000;60:847–53.
[40] Lee JM, Bernstein A. Apoptosis, cancer and the p53 tumour suppressor gene. Cancer Metastasis Rev 1995;14:149–61.
[41] Orrenius S. Mitochondrial regulation of apoptotic cell death. Toxicol Lett 2004;149:19–23.
[42] Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2002;2:277–88.
[43] Chen QM, Merrett JB, Dilley T, Purdom S. Down regulation of p53 with HPV E6 delays and modifies cell death in oxidant response of human diploid fibroblasts: an apoptosis-like cell death associated with mitosis. Oncogene 2002;21:5313–24.