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Efficacy of anti-death receptor 5 (DR5) antibody (TRA-8) against primary human ovarian carcinoma using a novel *ex vivo* tissue slice model

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Abstract

Objectives. The purpose of this study was to evaluate the cytotoxicity of a death receptor 5 (DR5) targeting monoclonal antibody (TRA-8) in primary ovarian cancer specimens utilizing a tissue slice technique that allows for assessment of anti-tumor activity in a three-dimensional *ex vivo* model.

Methods. Nineteen primary ovarian tumor specimens were obtained at the time of cytoreductive surgery and tumor slices were prepared with the Krumdieck tissue slicer. Tumor slices were incubated with TRA-8 for 24 h and a dose–response curve was established for each specimen using non-linear modeling, with IC_{50} values used as the parameter of TRA-8 sensitivity. In parallel with ATP viability assays, TRA-8 treated and untreated tumor slices were assessed by immunohistochemistry (IHC) and western blot analysis to confirm apoptosis induction.

Results. Incubation with 0–1000 ng/ml TRA-8 resulted in a dose response with maximum killing observed at 1000 ng/ml compared to untreated control slices. IC_{50} values of 6.0 to >1000 ng/ml were calculated for individual tumor specimens. H&E, IHC, and western blot specimens demonstrated TRA-8-induced cellular death in a dose-dependent fashion via apoptosis and activation of caspases 3, 8, and 9. The apoptosis produced by varying concentrations of TRA-8 was confirmed using the TUNEL technique. Treatment with TRA-8 markedly reduced proliferation in the ovarian cancer cells as measured by expression of Ki-67/SP6.

Conclusions. This study demonstrates that targeting DR5 with TRA-8 decreases cellular proliferation, increases caspase activation, and induces apoptosis in this novel three-dimensional *ex vivo* model of primary ovarian cancer.

Keywords: TRAIL; DR5; Death Receptor 5; Tumor slices; Krumdieck slicer; TRA-8; Ovarian cancer slices

Introduction

Approximately 20,180 patients will be diagnosed with ovarian cancer in 2006, resulting in 15,310 deaths due to disease [1]. While relatively rare, ovarian cancer remains the most lethal of the gynecologic malignancies due to its lack of early symptoms and resultant detection at an advanced stage. Fortunately, nearly 80% of patients with advanced disease will respond to adjuvant taxane/platinum-based chemotherapy following cytoreductive surgery. Despite this encouraging initial response rate, most patients with advanced stage will relapse, resulting in a 35–40% 5-year survival for stage III disease [2–5]. The dismal 5-year survival is due in large part to the fact that patients with recurrent tumors only respond to traditional chemotherapy 20–30% of the time [6]. A concerted effort has been made to identify alternative agents that could be given in conjunction with, or in place of conventional therapy, while sparing some of the toxic side-effects. There is evidence that targeted therapies may be effective in ovarian cancer. Recently, bevacizumab, an anti-VEGF

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monoclonal antibody, has been reported to decrease ascites, and has resulted in moderate tumor response. However, cardiovascular and gastrointestinal complications have not been infrequent, and must be taken into account when using this therapy [7,8].

TRAIL, tumor necrosis factor-related apoptosis-inducing ligand, is an attractive therapeutic agent for cancer therapy due to its specificity for apoptosis induction in tumor cells as compared to normal cells [9,10]. TRAIL is a type 2 membrane protein that is expressed in a majority of normal tissues and can undergo protease cleavage resulting in a soluble form that is able to bind TRAIL receptors. There are five known TRAIL receptors: two death receptors (DR4 and DR5), and three decoy receptors (DcR1, DcR2, OPG). The DR4 and DR5 receptors have cytoplasmic death domains that, upon ligand binding, are capable of apoptosis induction via downstream caspase activation [11–14]. Because of the ability to selectively induce apoptosis in cancer cells, TRAIL has been in development as a potential anti-cancer agent [15].

Due to toxicity of normal hepatocytes and other human tissues in early recombinant TRAIL formulations, alternative TRAIL targeting therapies were developed [16,17]. One such therapy is TRA-8, a DR5 specific agonistic monoclonal antibody. TRA-8 has proven to be non-toxic to cultured human hepatocytes, and has produced significant anti-tumor activity alone and in combination with cytotoxic chemotherapy and radiation therapy in breast and cervical cancer models [18–20]. We hypothesize that TRA-8 will be efficacious against primary human ovarian cancer. Based on our previous findings, we sought to evaluate this antibody in a preclinical setting using a three-dimensional *ex vivo* model to determine the efficacy of TRA-8 against primary human epithelial ovarian cancer specimens.

Methods

Patient specimen collection

After obtaining IRB approval at the University of Alabama at Birmingham, all patients suspected to have advanced ovarian carcinoma undergoing initial cytoreductive surgery were enrolled in the study. Patients who received neoadjuvant chemotherapy or who had cancer from another organ site metastatic to the ovary were excluded. Only patients with bulky stage IIIC or IV disease were included to ensure sufficient specimen volume for evaluation and appropriate staging. All histologic subtypes were allowed with the exception of tumors of low malignant potential. Information regarding age, race, stage, and residual disease was recorded at the time of surgery. Final histologic confirmation was performed by a gynecologic pathologist.

Specimen processing and slicing

Tumor specimens obtained from ovaries or omentum were assessed for adequacy, based on size and tissue consistency immediately after surgical resection from the peritoneal cavity. If the specimen was acceptable for evaluation, a 2–3 cm portion of grossly appearing carcinoma was removed from the resected specimen (usually from the infracolic omentum) and placed on ice in complete culture media (DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 2.5 μg/ml amphoterecin B, and 50 μg/ml gentamycin). Within an hour, the Krumdieck tissue slicer (Fig. 1) was used according to previous reports to create multiple tissue slices from the collected tumor sample [21,22]. Briefly, 5 mm cylindrical cores were created from random sections of the 2–3 cm portion of tumor using coring devices provided by the manufacturer and stored in DMEM media until slices were created. Tumor slices 300–600 μm thick were then cut by the reciprocating blade of the slicer in DMEM media at 4 °C. Slices were removed from the collection basin of the apparatus, placed on ice in complete culture media where they underwent selection and sorting. Each slice was randomly selected and placed into individual wells of a 24-well plate (Fig. 2) in 1.5 ml of complete media and maintained in a 37 °C incubator at 5% CO_2 and atmospheric oxygen conditions.

A portion of the tumor slices were fixed in 10% buffered formalin and pressed to paraffin so that the slices would remain flat. The slices were grouped together according to the set of variables used and embedded in paraffin as a group on edge. The thickness of the slices was measured by photographing with the calibration micrometer at 100× magnification. The photographed micrometer was used to measure the thickness of the slices.

ATP viability assays

For ATP viability assays, six replicate slices were treated for each variable. The slices were exposed to varying concentrations of TRA-8 (0, 10, 30, 100, 300, 600, and 1000 ng/ml) for 24 h. After 24 h, 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 4 equal aliquots from each tissue slice via ATP-dependent light emission in counts per second, and mean ATP levels were

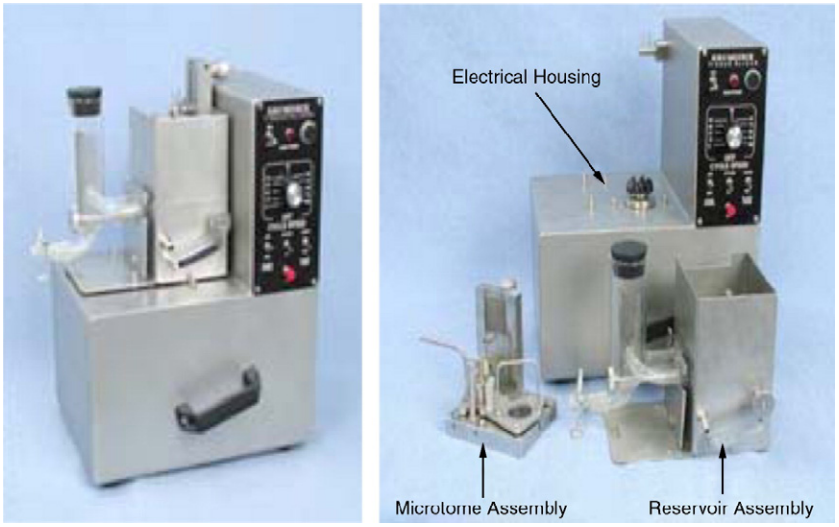

Fig. 1. The Krumdieck tissue slicer. Slices are prepared in DMEM media at 4 °C under sterile conditions.

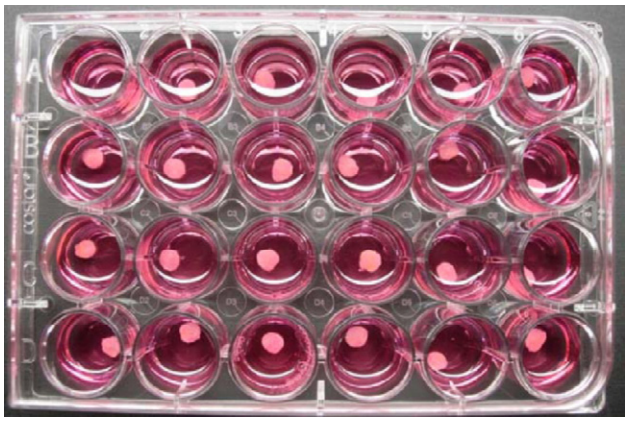

Fig. 2. Ovarian cancer patient tumor slices. Tissue cores were cut from an ovarian tumor using a 5 mm diameter coring tool then sliced with the Krumdieck tissue slicer set at ~300 μm slice thickness. Tumor slices were then transferred to culture medium in 24 well plates and photographed prior to initiating TRA-8 treatments and 37 °C incubation.

determined for each slice [23]. Fractional tumor slice survival was then calculated for each control slice specimen and each treated specimen as the ratio of mean ATP levels in 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 analysis section.

In addition to the cytotoxicity experiments, a slice viability time-course study was performed on three patient specimens. Six replicate control slices from time point 0, 24, 48, 72, and 96 h were processed in an identical manner as previously described and viability curves were generated from the fractional ratios of the individual time points compared to time point zero.

Immunohistochemistry

TRA-8 treated and untreated tumor slices from several specimens were assessed by immunohistochemistry (IHC) and compared to representative tumor slices fixed immediately after slicing. Our methods for bright-field immunohistochemical analysis have been previously published [24–26]. Specifically, tumor slices were mounted on sialinized slides (Bond-Rite), and soaked in Tris buffer. In brief, paraffin sections were cut at 5 μm thickness and mounted on sialinized microscopic slides (Bond-Rite). Paraffin was removed by soaking the tissue sections in three changes of xylene, followed by washing in graded ethanols (100% to 70%). Residual ethanol was removed by soaking in Tris buffer. Antigen retrieval was performed in a cooker at high pressure for 5 min using 10 mM EDTA at a pH of 8.0. The slides in solution were allowed to cool slowly to room temperature. Endogenous peroxidase was quenched using a fresh aqueous solution of 3% peroxide. Non-specific staining was blocked by soaking specimens for 20 min in 3% goat serum.

The slice sections were then incubated with the primary antibodies for 1 h. Antibody dilutions for Ki67 (1:500), DR5/TRA-8 (5 μg/ml), and cleaved caspase 8 (1:200) were prepared in PBE buffer (1% BSA, 1 mM EDTA, and 1.5 mM NAN_3 in PBS). Secondary detection methods for monoclonal antibodies used an anti-mouse anti-rabbit detection system from Signet (Multi-Species Ultra Streptavidin Detection System HRP, Signet Laboratories, Inc., Dedham, MA).

After the excess primary antibody was washed from the tissue slices with Tris buffer, the slices were incubated with biotinylated goat anti-mouse antibody for 20 min. The slices were then washed thoroughly with Tris buffer and then covered with peroxidase labeled streptavadin for 20 min. The slices were washed, drained, and the chromogen, 3,3′ BioGenex HK53 diaminobenzidine (DAB), at 1 mg/ml in Tris buffer with 0.016% fresh hydrogen peroxide, was added to the sections for 7 min. The DAB was washed from the slides with deionized water and lightly counterstained with hematoxylin. The TUNEL assay was performed with a Serologicals S7100 ApopTag Peroxidase *in situ* Apoptosis Detection Kit according to the manufacturer's recommendations. 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 [24–27].

Western blot analysis

TRA-8 treated and untreated patient specimens were analyzed for caspase activity. Tumor tissue slices were washed twice with cold PBS, then homogenized with 300 μl lysis buffer containing 10 mM Tris–HCl (pH 7.6), 150 mM NaCl, 0.5 mM EDTA, 1 mM EGTA, 0.1% SDS, 1 mM sodium orthovanadate, and a mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, and 2 μg/ml aprotinin). Total tissues lysates were sonicated for 10 s and then centrifuged for 20 min at 12,000×*g*. Equal amounts of total protein from each lysate were boiled for 5 min in SDS-PAGE sample buffer. A total of 50 μg of protein was loaded and separated on 12% SDS-PAGE gels, and electrophoretically transferred to nitrocellulose membranes. The blots were blocked with 5% nonfat dry milk in TBST buffer (20 mM Tris–HCl at pH 7.4, 500 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) according to the manufacturer's instructions.

Mouse monoclonal anti-human caspase 2 (clone: 2A3), anti-caspase 3 (clone: 1H6), anti-human caspase 8 (clone: 5G7), and anti-human caspase 9 (clone: 4B4) were prepared in our laboratories [28]. Anti-caspase 2 and caspase 8 antibodies primarily recognized the proforms of caspase 2 and caspase 8, whereas anti-caspase 3 and caspase 9 antibodies recognized both pro- and cleaved forms of caspases.

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 were 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 tumor. To estimate the IC_{50} of TRA-8, we used a non-linear model: $y = \text{Min} + (\text{Max} - \text{Min})/(1 + x/\beta)$ where y is the cell viability measured by ATP level, β represents IC_{50}, x is the $\log_{10}$(concentration), and Min and Max represent the minimum and the maximum cell viability. NLIN procedure in SAS 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. Slice values greater than two

Table 1
Patient characteristics

	N	%
Age (years)		
Median	65	
Range	(38–93)	
Race		
Caucasian	20	100
Histology		
Papillary Serous	13	63
Endometrioid	2	9
Adenocarcinoma	2	9
Carcinosarcoma	2	9
Small cell	1	5
Signet cell	1	5
Stage		
IIC	1	5
IIIC	19	90
IV	1	5

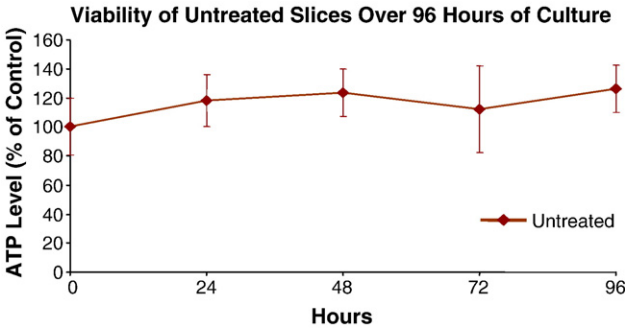

Fig. 3. Viability of *ex vivo* tissue slices at 24, 48, 72, and 96 h post-slicing as compared to the mean ATP level of the time point zero control slices.

standard deviations from the mean for each treatment were excluded from the analysis.

Results

Patient characteristics

Nineteen patient specimens were collected and assayed for tissue slice TRA-8-induced cytotoxicity at the University of Alabama at Birmingham at the time of primary cytoreductive surgery from February 2005 to April 2006. The median patient age was 65 years (range 38–93) and all patients were Caucasian. The majority of patients had stage III disease (90%) and papillary serous histologic subtype (63%) (Table 1). One patient with a final pathologic diagnosis of signet cell tumor was included in the analysis after a gastrointestinal tract evaluation revealed no primary tumor.

Cytotoxic tissue slice assay

As illustrated in Fig. 3, control (untreated) tissue slices maintained excellent viability over 96 h of *ex vivo* culture. Examples of TRA-8-induced cytotoxicity dose–response curves are provided in Fig. 4 and include the calculated IC_{50} value derived from each patient's curve. As further demonstrated in this figure, the treated slice specimens do not reach 100% cytotoxicity. This observation may reflect the persistent viability of fibroblasts and other normal stromal cells present in these three-dimensional tissue slices.

Table 2 provides the IC_{50} values for each of the 19 patients as well as their stage, histology, and debulking status. Sensitivity to TRA-8 (IC_{50}) did not correlate with stage, tumor histology, or debulking status. The individual patient specimens had a broad range of IC_{50}'s (6 to >1000 ng/ml). The specimens could be characterized as very sensitive (IC_{50} <100 ng/ml) including patients 3, 5, 7, 9, 10, 11, 13, and 18; moderately sensitive (101–500 ng/ml) including patients 1, 2, 4, 6, 8, 15, and 16; and resistant (>501 ng/ml) including patients 12, 14, 17, and 19. It is notable that, 15 of 19 patients (79%) were classified as either very or moderately sensitive to TRA-8 cytotoxicity.

Immunohistochemical analysis

Prior to evaluating patient specimens for immunohistochemical staining, H&E stained slices were examined for slice thickness and architecture as described previously. Slice thickness varied from 300 to 600 μm, with the majority of slices measuring 600 μm. As illustrated in Fig. 5, the tissue slices mimicked the tumor environment and architecture as would be expected *in vivo*. Both inflammatory and stromal cells

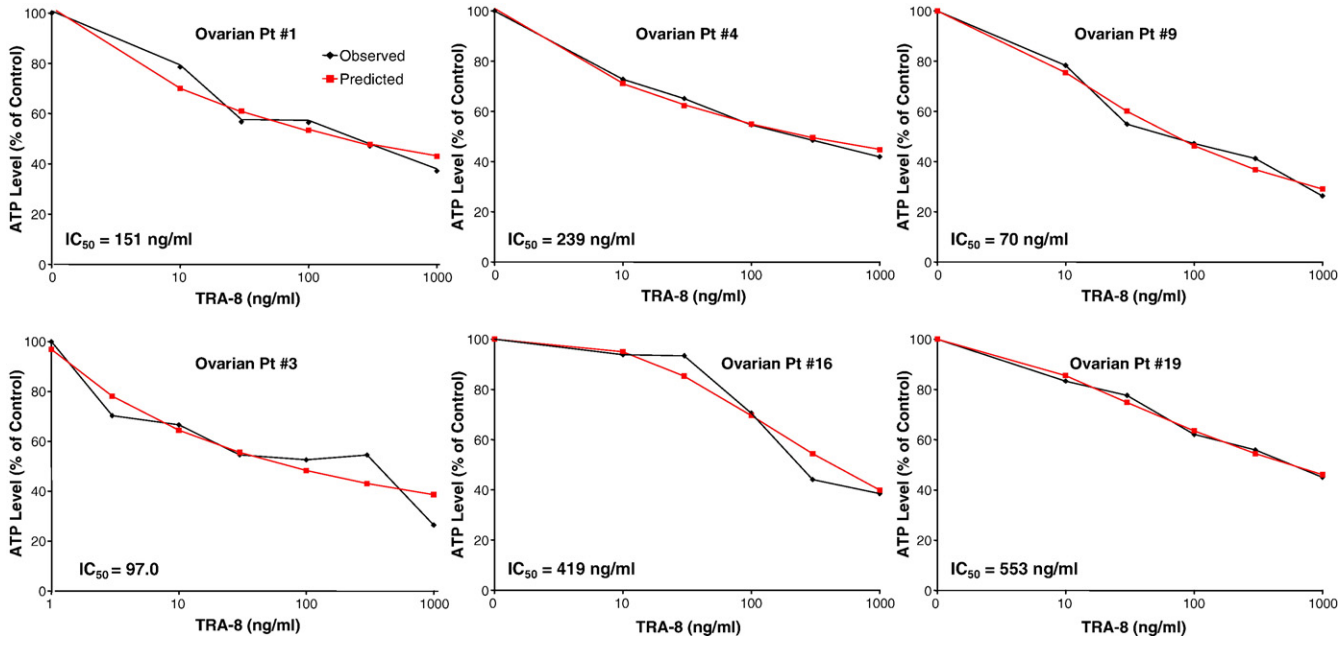

Fig. 4. Explant tissue slice cytotoxicity assay of TRA-8 activity against ovarian cancer patient specimens. IC_{50} values are reported for each individual curve. Results are plotted as ATP level (% of untreated control) versus log TRA-8 concentration (red line). Non-linear modeling of 6 replicate values was used to determine IC_{50} values (black line). In the above, patients 1, 3, 4, 9, and 16 would be considered sensitive to TRA-8-induced apoptosis.

Table 2
TRA-8 IC_{50} values of ovarian cancer patients

Patient ID	Stage	Histology	Debulking status	IC_{50} (ng/ml)
1	IIIC	Adenocarcinoma	Optimal	151
2	IIC	Papillary serous	Optimal	122
3	IV	Small cell carcinoma	Suboptimal	97
4	IIIC	Papillary serous	Optimal	239
5	IIIC	Carcinosarcoma	Optimal	30
6	IIIC	Endometrioid	Suboptimal	484
7	IIIC	Adenocarcinoma	Suboptimal	6
8	IIIC	Papillary serous	Suboptimal	207
9	IIIC	Papillary serous	Suboptimal	70
10	IIIC	Papillary serous	Optimal	27
11	IIIC	Signet cell	Optimal	47
12	IIIC	Papillary serous	Optimal	>1000
13	IIIC	Papillary serous	Optimal	53
14	IIIC	Papillary serous	Optimal	>1000
15	IIIC	Papillary serous	Optimal	128
16	IIIC	Papillary serous	Suboptimal	419
17	IIIC	Papillary serous	Optimal	>1000
18	IIIC	Carcinosarcoma	Optimal	44
19	IIIC	Endometrioid	Optimal	553

are present, as well as intact vasculature, identical to what would be encountered by a therapeutic *in vivo*.

A total of seven patient specimens underwent immunohistochemical analysis via TUNEL assay, Ki-67 staining, and caspase 8 evaluation. A dose response was observed when comparing untreated control slices compared to those treated with TRA-8 at a dose of 300 ng/ml as demonstrated in Figs. 6 and 7. Increased TUNEL staining was observed in a dose–response fashion as well as increased cleaved caspase 8 staining in the treated slices. In parallel, decreased cellular proliferation as evidenced by a decrease in Ki67 staining intensity was observed in the treated slices. The combination of decreased proliferation, increased caspase 8 activation, and apoptosis-induced DNA nicking supports TRA-8-induced tumor killing.

Western blot analysis

Four patient specimens underwent western blot analysis to confirm that the cytotoxicity observed in treated slices was due to apoptosis induction rather than tissue necrosis. These studies demonstrated activation of both the extrinsic (death receptor-mediated) and intrinsic (mitochondrial) pathways following TRA-8 exposure. Examples of TRA-8-mediated caspase activation are provided for two patients (Fig. 8) with the reductions of the pro-caspase forms of 8, 9, 3, and 2, and appearance of the cleaved forms of caspases 9 and 3.

Discussion

Despite acceptable response rates to initial surgery and taxane/platinum-based chemotherapy, patients with advanced ovarian cancer have a poor prognosis due to high recurrence rates. Recently, the NCI issued a clinical alert advocating the use of intraperitoneal chemotherapy in patients with stage III ovarian carcinoma who were optimally debulked. The 16-month survival benefit demonstrated in the intraperitoneal arm was the most significant survival advantage to date in a randomized chemotherapeutic trial in solid tumors [29]. However, significant toxicities were associated with this regimen and precluded completion of therapy in 60% of patients in the intraperitoneal arm. The toxicities associated with traditional cytotoxic regimens are well documented and may result in significant morbidity. Adverse events are attributable to cumulative effects on normal tissues, such as the bone marrow, as well as the relative lack of specificity for cancer cells. Given these significant limitations of cytotoxic chemotherapy, it is paramount that less toxic and more specific targeting therapies be developed.

TRAIL is an attractive anti-cancer agent and has been in drug development due to its selective apoptosis induction of transformed cancer cells as compared to normal cells. It has

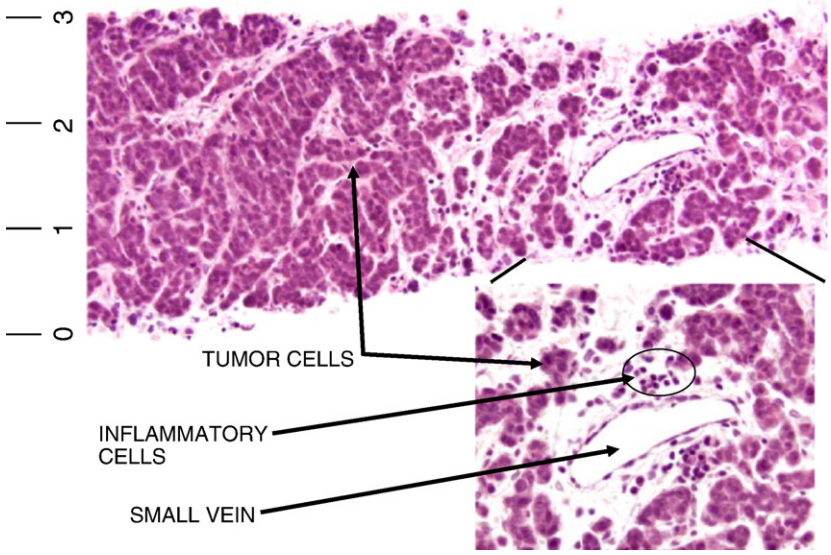

Fig. 5. H&E stained tumor slice cross-section. The slice measures 300 μm in thickness and contains inflammatory cells, tumor cells, and a small vein, which is further highlighted in the cutout.

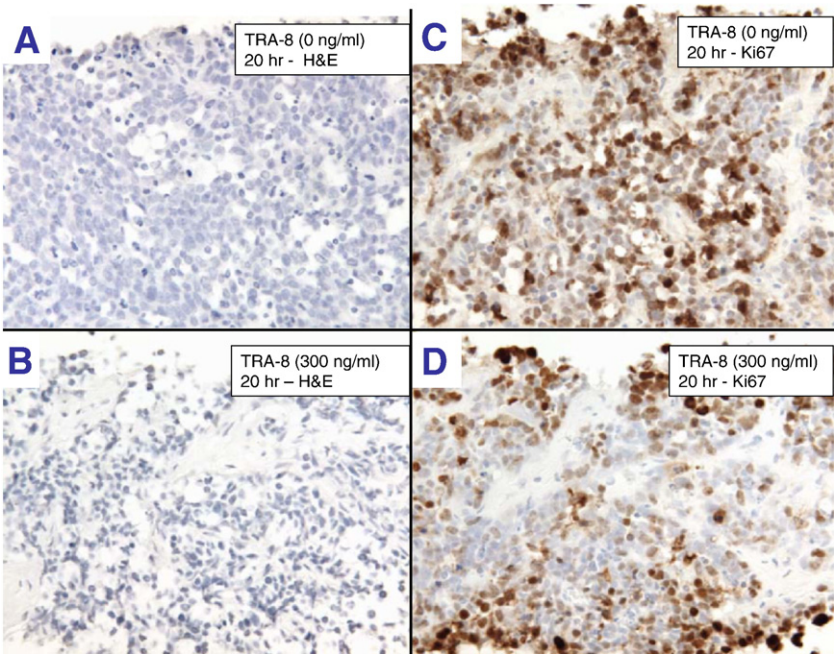

Fig. 6. H&E and Ki67 stained tissue slice sections. As demonstrated by the H&E stain, adequate tumor cells are present with decreased numbers due to apoptosis in the treated group. In parallel, decreased cellular proliferation in the treated slices, as evidenced by decreased Ki67 staining, is observed.

been previously demonstrated that ovarian cancer cell lines are sensitive to TRAIL-mediated apoptosis induction, and numerous chemotherapeutic agents have been shown to either enhance the effect of TRAIL or overcome resistance to TRAIL when exposed to cytotoxic therapy [30–34].

Currently, there are multiple therapeutic agents targeting TRAIL receptors in development. Initial soluble recombinant forms of TRAIL demonstrated significant cytotoxicity against cancer cells; however, cytotoxicity to normal human hepatocytes was also observed [16,17]. In addition, the short half-life in serum demonstrated by these initial recombinant molecules was an additional limitation. As a result of these limitations, monoclonal antibodies targeting the death receptors (DR4 and DR5) of the TRAIL complex have been developed. The use of a monoclonal antibody offers several advantages including increased specificity for the death receptor, avoiding the

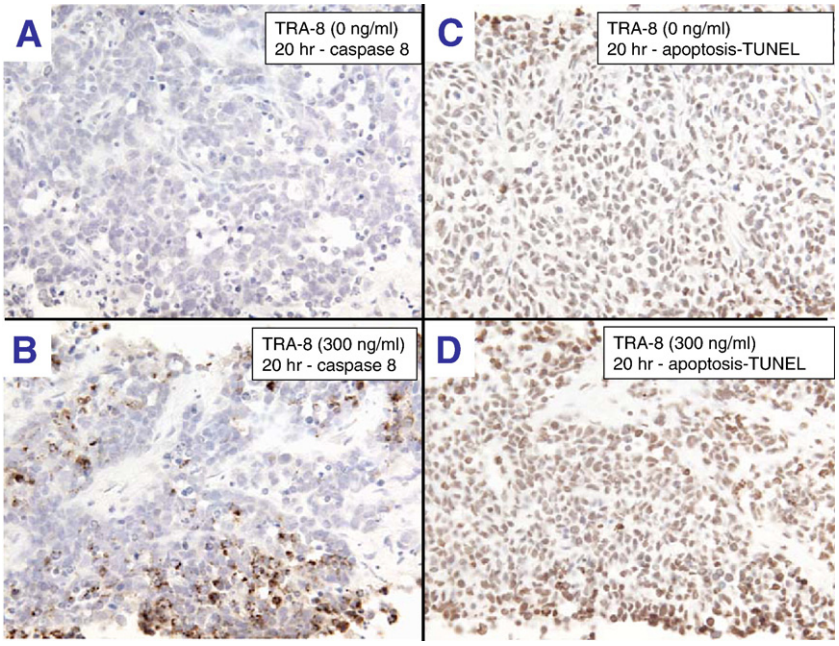

Fig. 7. Immunohistochemistry analysis of untreated and treated (300 ng/ml TRA-8) slices demonstrating an increase in caspase 8 activation and apoptosis induction via TUNEL assay in the treated slices.

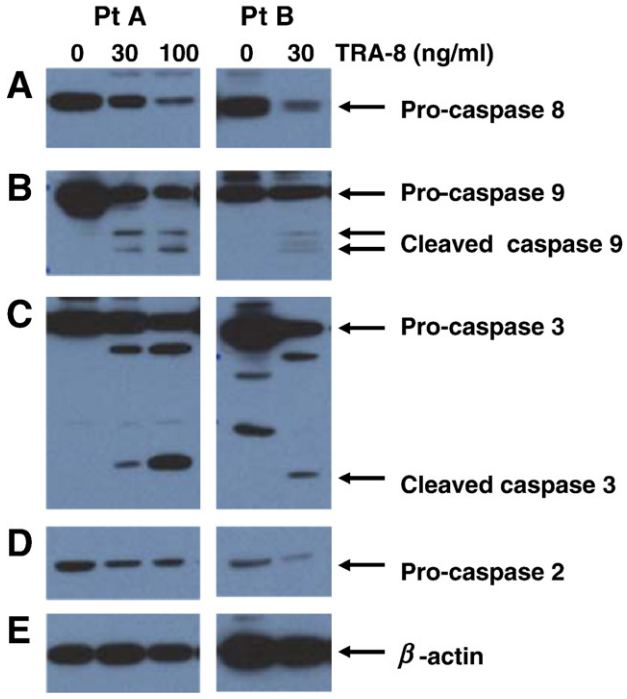

Fig. 8. TRA-8 induces activation of caspases in tissue slices. The tissue slices prepared from two patients were cultured with and without TRA-8 for 24 h. Total tissue lysates were separated in SDS-PAGE and blotted. The blots were probed with specific mAbs against indicated human caspase.

potential loss of effect by decoy receptor binding, longer serum half-life allowing for better pharmacokinetics and tumor delivery, and decreased toxicity to normal cells.

Preliminary reports of Phase I studies using human monoclonal antibodies agonistic to DR4 and DR5 have been presented, and encouraging findings from these early reports include minimal toxicity and modest evidence of efficacy [35]. Trials evaluating the combination of these TRAIL receptor agonistic monoclonal antibodies with traditional cytotoxic drugs have also been initiated and have demonstrated acceptable toxicity, with durable partial responses noted in some patients with advanced solid tumors [36]. A humanized version of the TRA-8 monoclonal antibody has been developed by Sankyo Co., Ltd., and a Phase I trial in patients with advanced tumors is underway.

To date, we have observed efficacy of TRA-8 antibody against cervical, breast, and ovarian cancer cell lines *in vitro*, and against tumor xenografts *in vivo* [19,20,37,38]. Given these encouraging findings, we wanted to evaluate the efficacy of the TRA-8 antibody against primary human ovarian cancer tissue. Kirby *et al.* have previously demonstrated the utility of an *ex vivo* tumor slice model in evaluating the therapeutic effectiveness of conditionally replicative adenoviruses in primary human ovarian cancer tissue [21]. A three-dimensional slice model provides advantages over more traditional *in vitro* techniques using human cancer cell lines. Fresh tumor samples are more likely representative of human tumors than are cell lines, and may be used immediately in short-term assays without the need to isolate or expand tumor cells. Tissue slices also maintain the normal three-dimensional architecture and stromal cell compartment that exists *in vivo*, and therefore allow a more realistic interplay of tumor cells and therapeutic moiety. The surrounding stromal cell structure is involved in tumor cell signal transduction and viability, which may affect the therapeutic efficacy of antibodies or drugs.

The tissue slice procedure generated specimens that had excellent viability over 96 h of *ex vivo* culture, although we used a 24-h incubation with TRA-8 given its rigid induction of apoptosis. Drug sensitivity studies using this technique may benefit from longer incubation times. The immunohistology studies demonstrated the three-dimensional structure of the slices (including stromal and inflammatory cells, vessels, and dense populations of tumor cells) and confirmed the presence of tumor cell induction of apoptosis. The western blot studies further supported the TRA-8 induction of both the extrinsic and intrinsic pathways of caspase activation.

These studies support the concept that this *ex vivo* assay system can estimate the sensitivity of fresh ovarian cancer cells to apoptosis induction by TRA-8. It has been our experience using tumor cell lines, that individual cell lines vary greatly in their sensitivity to TRAIL or agonistic anti-death receptor antibodies. In this study 79% of patients had tumor cells which were sensitive to TRA-8-induced cytotoxicity. Whether such a test could be used for selection of patients most likely to benefit from TRA-8 therapy will require future correlative studies. Given the fact that chemotherapy has been shown to upregulate death receptor expression and promote reversal of resistance in cancer cells [31,33–35], we anticipate greater sensitivity with the combination of TRA-8 and chemotherapy. We are currently evaluating this scenario with our tissue slice technology.

In conclusion, TRA-8 and other similar TRAIL-associated therapies have entered early clinical trials. These preliminary *ex vivo* tissue slice studies indicate that the majority of human ovarian cancers are susceptible to TRA-8-mediated apoptosis. Intracellular regulatory proteins are thought to be responsible for the sensitivity of cancer cells to DR5-mediated apoptosis. The elucidation of such mechanisms is a significant issue for the future development of translational studies focused on determining the therapeutic efficacy of humanized TRA-8 combined with chemotherapy in patients with advanced ovarian cancer. These findings in primary human tissue support further studies in patients with ovarian cancer of this DR5 targeted antibody.

References

[1] Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics. CA J Cancer Clin 2006;56:106–30.
[2] Chi DS, Sabbatini P. Advanced ovarian cancer. Curr Treat Options Oncol 2000;1:139–46.
[3] Bookman MA, McGuire III WP, Kilpatrick D, et al. Carboplatin and paclitaxel in ovarian carcinoma: a phase I study of the Gynecologic Oncology Group. J Clin Oncol 1996;14:1895–902.
[4] McGuire WP, Hoskins WJ, Brady MF, et al. Cycophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 1996;334:1–6.

[5] Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. J Clin Oncol 2003;21:3194–200.
[6] Herzog TJ. Recurrent ovarian cancer: how important is it to treat disease progression? Clin Cancer Res 2004;10:7439–49.
[7] Monk BJ, Choi DC, Pugmire G, Burger RA. Activity of bevacizumab (rhuMAB VEGF) in advanced refractory epithelial ovarian carcinoma. Gynecol Oncol 2005;96:902–5.
[8] Cohn DE, Valmadre S, Resnick KE, Eaton LA, Copeland LJ, Fowler JM. Bevacizumab and weekly taxane chemotherapy demonstrates activity in refractory ovarian cancer. Gynecol Oncol 2006;102:134–9.
[9] Wiley SR, Schooley K, Smolak PJ, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3:673–82.
[10] Daniel PT, Krammer PH. Activation induces sensitivity toward APO-1 (CD95)-mediated apoptosis in human B cells. J Immunol 1994;152: 5624–32.
[11] 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.
[12] Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, et al. A novel receptor for Apo2/TRAIL contains a truncated death domain. Curr Biol 1997;7:1003–6.
[13] 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.
[14] 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.
[15] Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev, Cancer 2002;2:420–30.
[16] Jo M, Kim T-H, Seol D-W, 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.
[17] Lawrence D, Shahrokh Z, Marsters S, Achilles K, Shih D, Mounho B, et al. Differential hepatocyte toxicity of recombinant Apo2/TRAIL versions. Nat Med 2001;7:383–5.
[18] 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 toxicity. Nat Med 2001;7:954–60.
[19] 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.
[20] Straughn JM, Oliver PG, Zhou T, Wang W, Alvarez RD, Grizzle WE, Buchsbaum DJ. Antitumor 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.
[21] Kirby TO, River A, Rein D, Wang M, Ulasov I, Breidenbach M, Kataram 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.
[22] Krumdieck CL, dos Santos JE, Ho KJ. A new instrument for the rapid preparation of tissue slices. Anal Biochem 1980;104:118–23.
[23] 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.
[24] Poczatek RB, Myers RB, Manne U, Oelschlager D, Weiss HL, Bostwick DG, Grizzle WE. EpCAM levels in prosatic adenocarcinoma and prostatic intraepithelial neoplasia. J Urol 1999;162:1462–6.
[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. Methods in molecular medicine - tumor marker protocols. Totowa, NJ: Humana Press, Inc.; 1998. p. 161–79.
[27] 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.
[28] Li Y, Wang H, Wang Z, Makhija S, Buchsbaum D, LoBuglio A, Kimberly R, Zhou T. 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.
[29] Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, Copeland LJ, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 2006;354:34–43.
[30] Enfeng Z, Zhou M, Caiying F, Beifen S, Zhang Q, Lijuan L. Effects of TNF alone or in combination with chemotherapeutic agents on human ovarian cancers in vitro and in nude mice. Chin Med J 1995;108:571–5.
[31] Cuello M, Ettenberg SA, Nau MM, Lipkowitz S. Synergistic induction of apoptosis by the combination of TRAIL and chemotherapy in chemoresistant ovarian cancer cells. Gynecol Oncol 2001;81:380–90.
[32] Lane D, Cartier A, L'Esperance S, Cote M, Rancourt C, Piche A. Differential induction of apoptosis by tumor necrosis factor-related apoptosis-inducing ligand in human ovarian carcinoma cells. Gynecol Oncol 2004;93:594–604.
[33] Tomek S, Horak P, Pribill I, Haller G, Rossler M, Zielinski CC, et al. Resistance to TRAIL-induced apoptosis in ovarian cancer cell lines is overcome by cotreatment with cytotoxic drugs. Gynecol Oncol 2004;94:107–14.
[34] Vignati S, Codegoni A, Polato F, Broggini M. TRAIL activity in human ovarian cancer cells: potentiation of the action of cytotoxic drugs. Eur J Cancer 2002;38:177–83.
[35] Sarantopoulos J, Wakelee H, Mita M, Fitzgerald A, Hill M, Fox NL, et al. HGS-ETR2-STO2: a phase I clinical trial of HGS-ETR2, a fully human activating monoclonal antibody to TRAIL-R2, in patients with advanced solid tumors. Abstract presented at the AACR-NCI-EORTC International Conference of Molecular Targets and Cancer Therapeutics. Philadelphia, PA. Nov 2005.
[36] Mom CH, Sleijfer S, Gietema JA, Sneller V, Fox NL, Lo L. A phase I study of HGS-ETR1, a fully human agonistic monoclonal antibody to the TRAIL-R1, in combination with gemcitabine and cisplatin in subjects with advanced solid malignancies. Abstract presented at the AACR-NCI-EORTC International Conference of Molecular Targets and Cancer Therapeutics. Philadelphia, PA. Nov 2005.
[37] Ohtsuka T, Buchsbaum D, Oliver P, Makhija S, Kimberly R, Zhou T. Synergistic induction of tumor cell apoptosis by death receptor antibody and chemotherapy agent through JNK/p38 and mitochondrial death pathway. Oncogene 2003;22:2034–44.
[38] Makhija S, Ohtsuka T, Li Y, Buchsbaum D, LoBuglio A, Alvarez R, Gercel-Taylor C, Zhou T. Expression and function of TRAIL-R1 (DR4) and TRAIL-R2 (DR5) in primary ovarian cancer cells. Abstr Gynecol Oncol 2003;88:180.