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ScienceDirect
Gynecologic Oncology 105 (2007) 291–298
Gynecologic Oncology
www.elsevier.com/locate/ygyno

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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Received 29 July 2006
Available online 15 February 2007

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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doi:10.1016/j.ygyno.2006.12.033

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

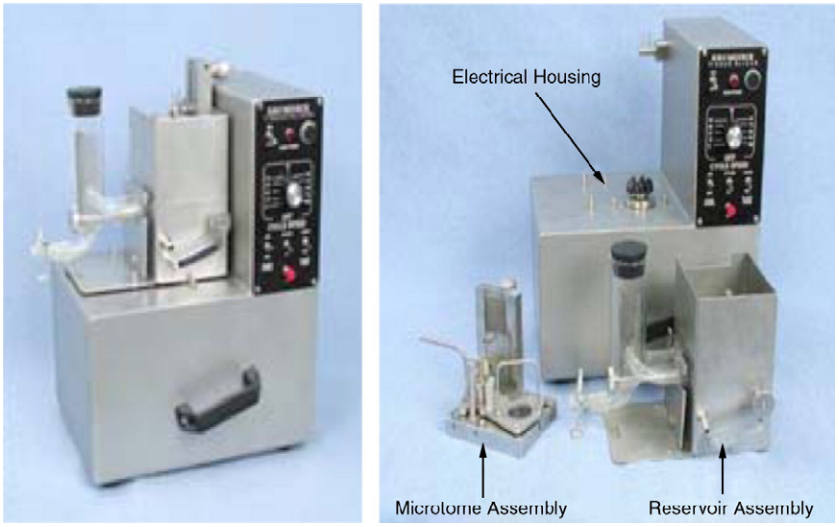

Fig. 1. The Krumdieck tissue slicer. Slices are prepared in DMEM media at 4 °C under sterile conditions.