

Targeting the HU177 cryptic collagen epitope with humanized antibody TRC093 functions cooperatively with anti-VEGF therapy to inhibit tumor growth.

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Abstract

The ability of tumor and endothelial cells to respond to external stimuli such as growth factors, anti-angiogenic agents and chemotherapeutic drugs may depend in part on communication with the extracellular matrix (ECM). Thus, selective disruption of communication links with the ECM may represent an effective approach to enhance the clinical efficacy of current anti-tumor strategies. Structural alterations in the integrity and molecular composition of the ECM are a hallmark of tumor progression. However, the molecular mechanisms by which these changes contribute to angiogenesis and tumor growth are incompletely understood. We identified a number of cryptic sites within ECM proteins that play roles in angiogenesis and tumor growth. In particular, the HU177 cryptic collagen epitope was shown to be selectively expressed within the ECM of tumors and targeting this cryptic epitope inhibited angiogenesis and tumor growth *in vivo*. To this end, the humanized antibody TRC093, directed to the HU177 cryptic collagen site is currently being evaluated in a phase-1 clinical trial of metastatic cancer patients.

To gain a more in depth molecular understanding of the potential mechanisms by which TRC093 may inhibit angiogenesis and tumor growth, we examined signaling pathways by which TRC093 may regulate cellular proliferation and apoptosis. Here we provide evidence that TRC093-mediated inhibition of melanoma cell interactions with the HU177 cryptic collagen epitope resulted in reduction of phosphorylated Erk1/2 and elevated levels of FOXO3A, P27KIP1 and Bax. These important regulatory molecules have been suggested to play functional roles in mediating the inhibitory activity of certain chemotherapy and anti-angiogenic drugs. In this regard, we examined the effects of TRC093 alone and in combination with the anti-VEGF drug bevacizumab on melanoma tumor growth *in vivo*. While TRC093 and bevacizumab inhibited tumor growth by approximately 50% as monotherapies, a combination of TRC093 and bevacizumab significantly ($P < 0.05$) inhibited tumor growth by approximately 85%. Collectively these novel findings suggest that selective disruption of cellular communication with a unique cryptic collagen epitope may function cooperatively with bevacizumab to enhance anti-tumor activity. Further other chemotherapy, studies are underway to examine whether TRC093 may enhance the sensitivity of other tumor types to targeted agents and

Background

Background and Introduction

The extracellular matrix (ECM) is an integrated network of macromolecules which helps regulate key signaling events important to tumor growth and chemoresistance including the PI3K/Akt and MAP/Erk pathways (1,2). Conformational changes within the ECM are associated with angiogenic blood vessel development, tumor growth and metastasis. Given that cellular interactions with the ECM have been suggested to help control the response of cells to chemotherapy, it is possible that selective disruption of tumor cell interactions with the ECM may function cooperatively with existing anti-angiogenic and/or chemotherapeutic agents to enhance their therapeutic efficacy. In this regard, our previous studies have identified a cryptic epitope in collagen that is selectively exposed within the ECM during proteolytic remodeling. This cryptic epitope termed HU177 has been shown to regulate endothelial cell adhesion, migration, proliferation and angiogenesis *in vivo* (3). However, little is known concerning the effects of cellular interactions with this cryptic epitope has directly on melanoma cell behavior. Given the selective expression of the HU177 epitope during tumor development; targeting this unique non-cellular cryptic regulatory site may represent a way to selectively enhance the anti-tumor activity of anti-angiogenic and chemotherapeutic drugs. Based on previous studies, a humanized function-blocking antibody (TRC093) directed to the HU177 epitope was developed (4,5). To gain a more complete understanding of the molecular mechanism by which cellular interactions with the HU177 epitope may regulate tumor cell behavior, we sought to examine the impact of TRC093 on human melanoma cell-ECM signaling events thought to control malignant tumor progression and chemoresistance.

Methods

Cell Adhesion Assay:

Non-tissue culture treated 48 well plates were coated with native or denatured collagen IV (10µg/ml) and incubated overnight at 4°C. M21 human melanoma cells were suspended in adhesion buffer in the presence or absence of TRC093 (50µg/ml) or a control antibody and incubated at 37°C for 15-30min. Non attached cells were washed off and attached cells were stained with crystal violet. Adhesion was quantified by measuring the optical density of eluted stain.

Western Blot Analysis:

6 well plates were coated with denatured collagen type IV (10µg/ml). Equal numbers of M21 melanoma cells were added to the plates in the presence or absence of TRC093 or a control antibody (50µg/ml) and incubated for 60 min. Cells were harvested, washed and lysed in RIPA buffer plus protease and phosphatase inhibitors. Equal amounts total cell lysates were loaded per lane in an SDS-page gel. Membranes were probed with specific antibodies and visualized by chemiluminescence.

Immunohistochemistry:

Frozen sections of M21 tumors were sectioned (4µm) and blocked for non-specific binding with 1.0% BSA in PBS. Tumor sections were stained for apoptosis using tunnel method (Millipore).

Tumor Growth Assay:

M21 human melanoma cells (4x10⁶) were subcutaneously injected into nude mice. Five days later TRC093 alone (50µg/ms), bevacizumab alone (50µg/ms), a combination of TRC093 and bevacizumab (25µg/ms of each) or control antibody (50µg/ms) was injected into the mice (3X/week). Tumor growth was monitored for 28 days by measuring tumor volumes.

Results

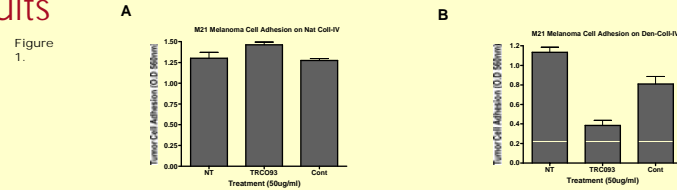


Figure 1. TRC093 Inhibits M21 Human Melanoma Cell Adhesion to Denatured Collagen IV. Culture plates were coated with either native or denatured collagen IV (10µg/ml). M21 human melanoma cells were allowed to attach in the presence or absence of TRC093 or control antibody (50µg/ml). (A). TRC093 has little effect on M21 melanoma cell adhesion to native collagen IV. (B). TRC093 inhibits M21 melanoma cell adhesion to denatured collagen IV by greater than 50% as compared to no treatment or control antibody.

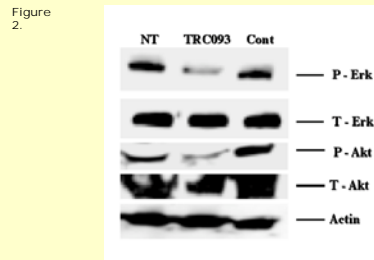


Figure 2. TRC093 Inhibits Phosphorylation of ERK and AKT In M21 Human Melanoma Cells. M21 melanoma cells seeded on denatured collagen IV were treated with TRC093 or control antibody (50µg/ml). After a one-hour incubation period total cell lysates were prepared and analyzed by immunoblotting. TRC093 treated M21 melanoma cells were associated with reduced levels of phosphorylated Erk and Akt, while little change was detected in the total levels of Erk or Akt.

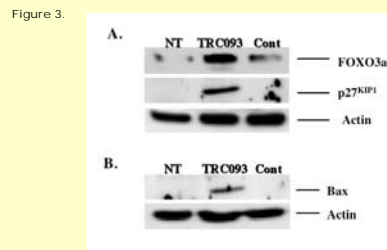


Figure 3. TRC093 Enhances Expression of FOXO3A, P27KIP1 and Bax. M21 melanoma cells seeded on denatured collagen IV were treated with TRC093 or control antibody (50µg/ml). Total cell lysates from M21 human melanoma cells were analyzed by western blot. (A). TRC093 increased the expression of FOXO3A and P27KIP1. (B). TRC093 increased the expression of Bax.

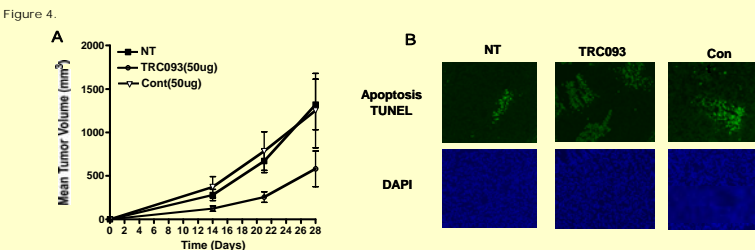


Figure 4. TRC093 Inhibits M21 Human Melanoma Tumor Growth In vivo and Increases Apoptosis. Nude mice were injected with M21 human melanoma cells (4 x 10⁶). TRC093 (50µg/mouse) or control antibody (50µg/mouse) was injected 3X/week. Tumor growth was monitored for 28 days by measuring tumor volumes. (A). TRC093 significantly ($P < 0.05$) inhibited M21 melanoma tumor growth as compared to the control treatment. (B). M21 human melanoma tumors from mice were sectioned (4µm) and stained via the tunnel method. Tumors from TRC093 treated mice exhibited increases apoptosis as compared to control tumors.

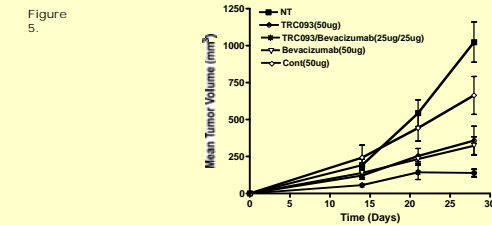


Figure 5. TRC093 Enhances the Anti-Tumor Activity of Bevacizumab in M21 Human Melanoma In vivo. Nude mice were injected with M21 human melanoma cells (4 x 10⁶). TRC093 (50µg/mouse), Bevacizumab (50µg/mouse), control antibody (50µg/mouse) or a combination of TRC093 (25µg/mouse) and Bevacizumab (25µg/mouse) was injected i.p. (3X/week) for 28 days. Tumor growth was monitored by measuring tumor volumes. TRC093 significantly ($P < 0.05$) enhanced the anti-tumor activity of Bevacizumab in M21 human melanoma growth *in vivo*.

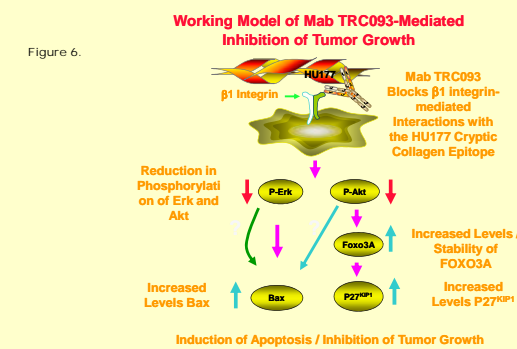


Figure 6. Summary Model of TRC93 Mediated Inhibition of M21 Human Melanoma Tumor Growth. Proposed working model by which selective blocking of β1 integrin-mediated interaction with the HU177 cryptic collagen epitope may inhibit tumor growth. The inhibition of angiogenesis and tumor growth may function cooperatively with antagonists of VEGF signaling to selectively enhance anti-tumor activity at sites of tumor growth.

Conclusion

CONCLUSION:

- TRC093 selectively inhibits M21 human melanoma cell interaction with denatured but not native collagen-IV.
- TRC093 significantly inhibits M21 human melanoma tumor growth *in vivo*, which was associated with elevated levels of apoptosis.
- TRC093 selectively inhibits phosphorylation of Erk and Akt in M21 human melanoma cells.
- TRC093 significantly increases levels of Pro-apoptotic FOXO3A and Bax and increases expression of the cell cycle inhibitor P27KIP1 in M21 human melanoma cells.
- TRC093 enhances the anti-tumor activity of bevacizumab in M21 human melanoma tumors *in vivo*.

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