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). Tumor and endothelial cells are known to communicate with their surrounding ECM via a variety of mechanisms. Tumor growth, angiogenesis and metastasis are dependent on cellular interactions with the ECM. The 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 exposed within the ECM of tumors and targeting this site with antibody TRC093, directed to the HU177 cryptic collagen site is currently being evaluated in a phase-1 clinical trial of metastatic cancer patients.

Methods

Background and Introduction

The extracellular matrix (ECM) is an integrated network of molecules which regulates key signaling events important to tumor growth and chemoresistance including the PI3K/Akt and MAPK/ERK pathways (14). Conformational changes within the ECM are associated with angiogenic blood vessel development, tumor growth and metastatic progression. For example, the exposed and non-exposed cryptic sites within the ECM are known 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 85%, co-administration of TRC093 and bevacizumab significantly (p<0.05) inhibited tumor growth, by approximately 95%. 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 studies on other cell types are underway to examine whether TRC093 may enhance the sensitivity of other tumor types to targeted agents.

Background

Working Model of Mab TRC093-Mediated Inhibition of Tumor Growth

Figure 1. TRC093 Inhibits M21 Human Melanoma Cell Adhesion to Denatured Collagen IV. Culture plates were coated with native collagen type IV (10µg/ml) and incubated overnight at 4°C. M21 melanoma cells were seeded onto the plates in the presence or absence of TRC093 (10µg/ml) for 3 hours. Non-adherent cells were washed off and adherent cells were stained with crystal violet. Adhesion was quantified by measuring the optical density of eluted stain.

Figure 2. TRC093 Inhibits M21 Human Melanoma Cell Adhesion to Denatured Collagen IV. Culture plates were coated with native collagen type IV (10µg/ml) and incubated overnight at 4°C. M21 melanoma cells were seeded onto the plates in the presence or absence of TRC093 (10µg/ml). Total cell lysates from M21 human melanoma cells were analyzed by western blot. TRC093 increased the expression of Bax.

Figure 3. TRC093 Enhances the Anti-Tumor Activity of Bevacizumab in M21 Human Melanoma in vivo. Bevacizumab (25µg) or TRC093 (25µg) was injected i.p. into 4-week old mice for 28 days. Tumor growth was monitored by measuring tumor volumes. TRC093 significantly (p<0.01) inhibited tumor growth.

Figure 4. TRC093 Enhances the Anti-Tumor Activity of Bevacizumab in M21 Human Melanoma in vivo. Bevacizumab (25µg) or TRC093 (25µg) was injected i.p. into 4-week old mice for 28 days. Tumor growth was monitored by measuring tumor volumes. TRC093 significantly (p<0.01) inhibited tumor growth.

Figure 5. TRC093 Enhances the Anti-Tumor Activity of Bevacizumab in M21 Human Melanoma in vivo. Bevacizumab (25µg) or TRC093 (25µg) was injected i.p. into 4-week old mice for 28 days. Tumor growth was monitored by measuring tumor volumes. TRC093 significantly (p<0.01) inhibited tumor growth.

Figure 6. Summary Model of TRC93 Mediated Inhibition of M21 Human Melanoma Tumor Growth. Proposed working model which recapitulates the data obtained from xenograft tumor growth in vivo, suggests that selective disruption of cellular interactions with the HU177 cryptic collagen epitope may inhibit tumor growth. The inhibition of angiogenesis and tumor growth may function cooperatively with anti-VEGF signaling in selecting tumor and endothelial activity at sites of tumor growth.

Conclusion

•TRC093 selectively inhibits M21 human melanoma cell interaction with denatured but not native collagen-IV.

•TRC093 sensitively inhibits M21 melanoma tumor growth in vivo, suggesting that disruption of the cryptic collagen epitope may inhibit tumor growth.

•TRC093 sensitively inhibits M21 melanoma angiogenesis.

•TRC093 increases the expression of Bax.

References


4. TRC093 enhances the anti-tumor activity of brevacin in M21 human melanoma in vivo.