Abstract #4130

Dual targeting of VEGF and endoglin inhibits tumor angiogenesis and metastatic spread

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Introduction

Endoglin, a transforming growth factor-β co-receptor, is highly expressed on angiogenic endothelium in solid tumors. Therefore, targeting endoglin with the neutralizing antibody TRC105, is currently being explored in clinical trials for anti-angiogenic therapy, also in combination with other anti-angiogenic therapies like anti-vascular endothelial growth factor (anti-VEGF). In this project, the redundancy between endoglin and vascular endothelial growth factor (VEGF) signaling in angiogenesis and the effects of targeting both pathways on breast cancer metastasis were explored.

Aim

To explore the potential redundancy and efficiency of simultaneous targeting of endoglin and VEGF on breast cancer vascularization and metastasis.

Methods

Patient samples

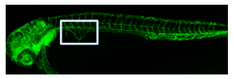
- pre- and post-bevacizumab treatment
- phosphorylated Smad1 (pSmad1), downstream target for endoglin signaling

In vitro

HUVEC endothelial cells; pSmad1 western blot (endoglin signaling), pERK1/2 (VEGF signaling)

Zebrafish experiments

TRC105 injected in blood island; SU5416 in fish water,
2 day experiment. Subintestinal vessels formation assessed



Breast cancer model

KEP1-11 mouse breast cancer cells, orthotopic injection
Treatment twice weekly with TRC105 or human IgG
Primary tumors resected and metastases followed by bioluminescent imaging

Conclusions

- VEGF targeting increases endothelial pSmad1 in cancer patients
- Endoglin targeting results in increased VEGF signaling *in vitro*
- Dual targeting of endoglin and VEGF inhibits angiogenesis and tumor vascularization to a higher extent than monotherapy
- Decreased tumor vascular density does not result in decreased tumor volume in the KEP1-11 breast cancer model
- Targeting endoglin, with a neutralizing antibody or ligand trap, decreases metastatic spread in a clinically relevant mouse model for invasive lobular breast cancer

Results

Increased pSmad1 after anti-VEGF in cancer patients

Pre-treatment biopsies and post -treatment resection samples were stained for pSmad1, downstream of endoglin. Increased nuclear accumulation of pSmad1 was seen in endothelial cells of cancer patients after anti-VEGF (Fig. 1).

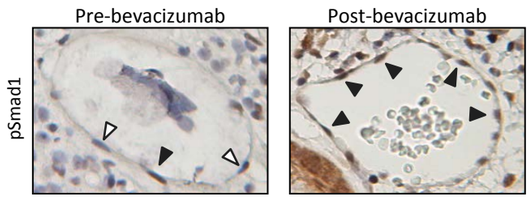


Figure 1 pSmad1 staining on rectal cancer tissues. White arrowheads; negative nuclei, black arrowheads; positive nuclei

Redundancy between endoglin and VEGF signaling *in vitro*

HUVEC endothelial cells were stimulated with BMP-9 or VEGF in presence of endoglin (TRC105) or VEGF (SU5416) inhibitors. TRC105 inhibits BMP-9-induced pSmad1 (Fig. 2A), while treatment with SU5416 inhibits VEGF-induced pERK. TRC105 increases VEGF-induced ERK phosphorylation (Fig. 2B).

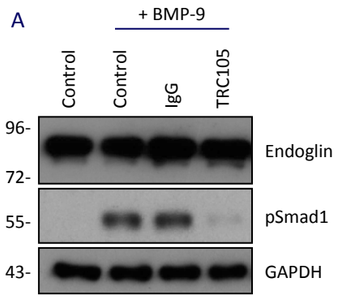


Figure 2A pSmad1 western blot

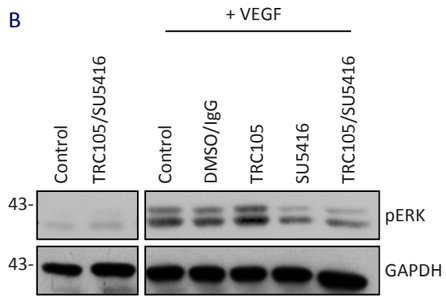


Figure 2B pERK western blot

TRC105/SU5416 combination treatment inhibits angiogenesis in zebrafish

TRC105 (red) binds to zebrafish endothelium (Fig. 3A). Zebrafish treated for two days with TRC105, SU5416 or combination therapy show phenotypic defects in development of the subintestinal vessels (Fig. 3B).

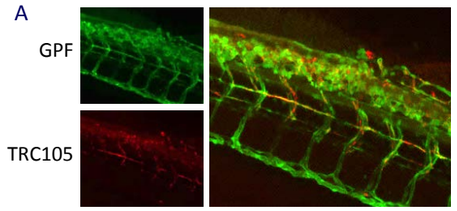


Figure 3A TRC105 binding to zebrafish endothelium

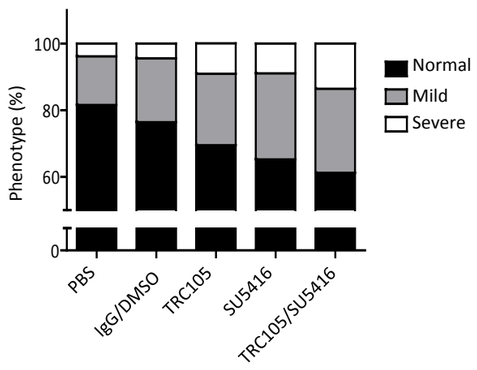


Figure 3B Phenotypes of SIV development after mono- or combination treatment

TRC105 treatment decreases tumor vascular density and metastatic spread of breast cancer *in vivo*

Mice bearing breast tumors were treated twice weekly with TRC105 or SU5416. Tumors showed decreased tumor vessel density, further enhanced by the combination treatment. Primary tumor size was unaffected. Metastatic spread of the tumors was strongly decreased in mice treated with TRC105, alone or with SU5416.

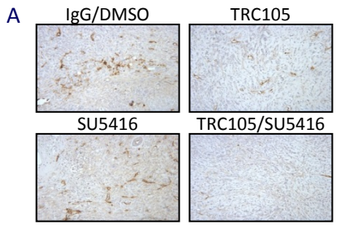
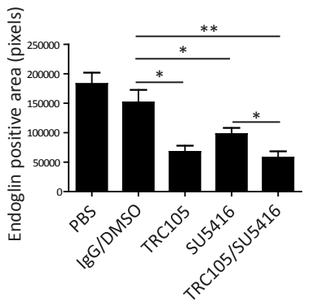


Figure 4A Endoglin staining on KEP1-11 breast tumors



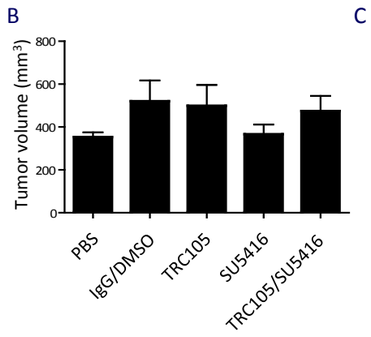


Figure 4B Tumor volume

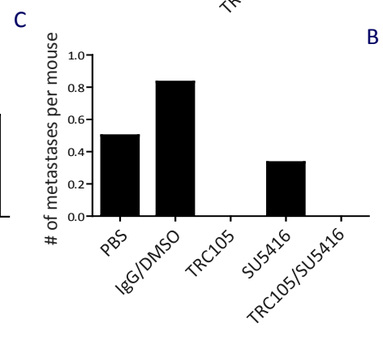


Figure 4C Number of metastases in mice

TRC105 inhibits metastatic spread after resection of the primary tumor

Tumors were resected and mice were adjuvantly treated with TRC105. Treatment with TRC105 significantly improved metastasis-free survival.

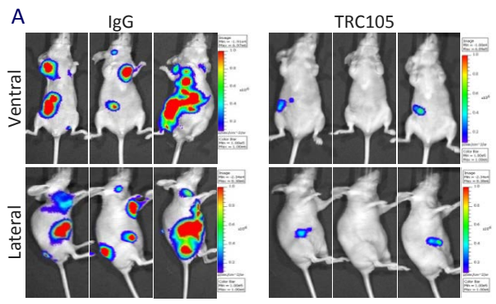


Figure 5A Bioluminescent signals from IgG and TRC105 treated mice

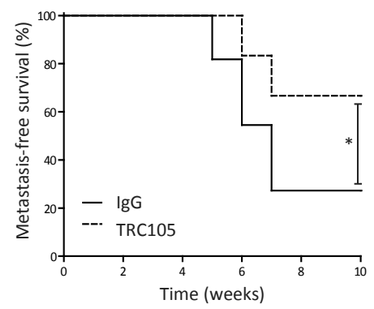


Figure 5B Metastasis-free survival

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