Anti-VEGF induced reduction in microvessel density does not correlate with anti-tumor response in lung cancer xenografts

Laura A. Sullivan, Antonia J. Jacob, Jason E. Toombs, John D. Minna and Rolf A. Brekken

Division of Surgical Oncology, Department of Surgery and Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX 75390

ABSTRACT

Introduction

Vascular endothelial growth factor-A (VEGF) is a primary stimulant of angiogenesis in pathological conditions including tumor progression. Strategies to block VEGF activity prevent or slow tumor growth in preclinical settings; however, clinical studies with bevacizumab, a monoclonal antibody (mAb) specific for VEGF have resulted in only modest benefit to a subset of patients with lung cancer. Previous studies in our laboratory defined the therapeutic efficacy of bevacizumab and an alternative anti-VEGF mAb (r84) in 12 non small cell lung cancer (NSCLC) xenografts. Three NSCLC xenografts (Calu-6, A549 and Calu-3) showed intrinsic resistance to bevacizumab therapy.

Methods

In the present study we evaluated whether microvessel density (MVD) could be used to 1) demonstrate if the anti-VEGF mAbs were effective at reducing VEGF-driven angiogenesis and 2) if MVD changes induced by bevacizumab or r84 correlated with overall therapeutic efficacy as determined by tumor size after chronic therapy. 3-5 tumors from animals bearing NSCLC xenografts treated with a control mAb (XTL), bevacizumab or r84 were evaluated by immunohistochemistry for endothelial cells as a measure of microvessel density. Two independent endothelial cell markers were used, endomucin and CD31.

Results

In 11 of the 12 xenografts treatment with bevacizumab or r84 significantly reduced MVD compared to XTL treatment, suggesting that bevacizumab and r84 do reduce VEGF-driven angiogenesis. However, the reduction in MVD induced by anti-VEGF therapy did not correlate with overall tumor response to therapy.

Conclusions

These results strongly implicate resistance to anti-VEGF therapy is not mediated by activation alternative angiogenic programs to compensate for VEGF blockade. Further the results suggest that tumor cell adaptation to therapy-induced hypoxia underlies poor therapeutic response to anti-VEGF strategies. Microarray of gene expression analysis of control treated tumors revealed several genes associated with metabolism, proliferation, and metastasis were significantly increased in tumors that displayed intrinsic resistant to bevacizumab.
We conclude that response of tumor cells to therapy-induced hypoxia is a critical feature that drives the overall efficacy of anti-VEGF strategies.

Acknowledgements

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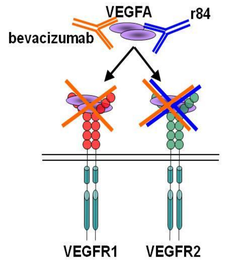
THERAPEUTIC ANTI-VEGF ANTIBODIES



	IgG	
	bevacizumab	r84
Species	humanized	human
Specificity	huVEGF	huVEGF msVEGF
Inhibition	VEGF:VEGFR1 VEGF:VEGFR2	VEGF:VEGFR2

Figure 1: Differences between the VEGF-neutralizing antibodies binding specificities. Bevacizumab (Avastin®, Genentech, Inc.), recognizes and blocks human VEGF binding to VEGFR1 and VEGFR2. r84 (Peregrine Pharmaceuticals, Affitech A/S) recognizes mouse and human VEGF. r84 only inhibits VEGF:VEGFR2 interactions, VEGF:VEGFR1 intact.

NSCLC RESPONSE TO BEVACIZUMAB IS TUMOR DEPENDENT

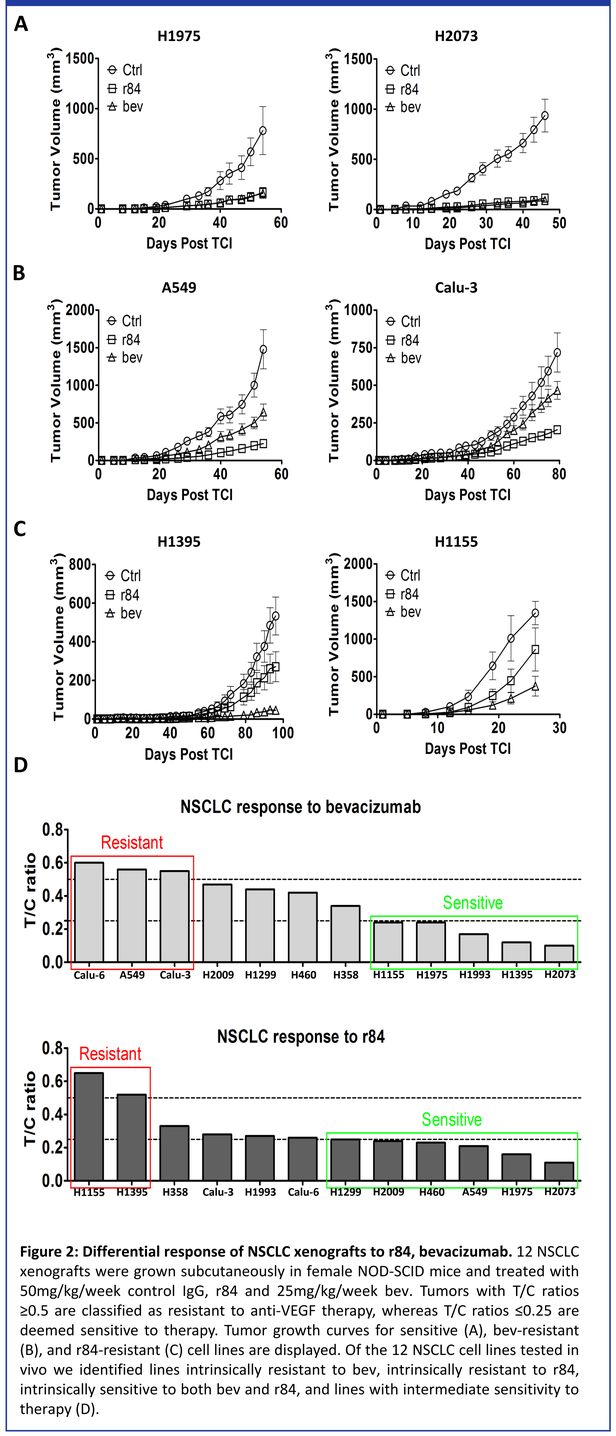


Figure 2: Differential response of NSCLC xenografts to r84, bevacizumab. 12 NSCLC xenografts were grown subcutaneously in female NOD-SCID mice and treated with 50mg/kg/week control IgG, r84 and 25mg/kg/week bev. Tumors with T/C ratios ≥0.5 are classified as resistant to anti-VEGF therapy, whereas T/C ratios ≤0.25 are deemed sensitive to therapy. Tumor growth curves for sensitive (A), bev-resistant (B), and r84-resistant (C) cell lines are displayed. Of the 12 NSCLC cell lines tested in vivo we identified lines intrinsically resistant to bev, intrinsically resistant to r84, intrinsically sensitive to both bev and r84, and lines with intermediate sensitivity to therapy (D).

BEVACIZUMAB, r84 THERAPY INDUCES VASCULAR CHANGES

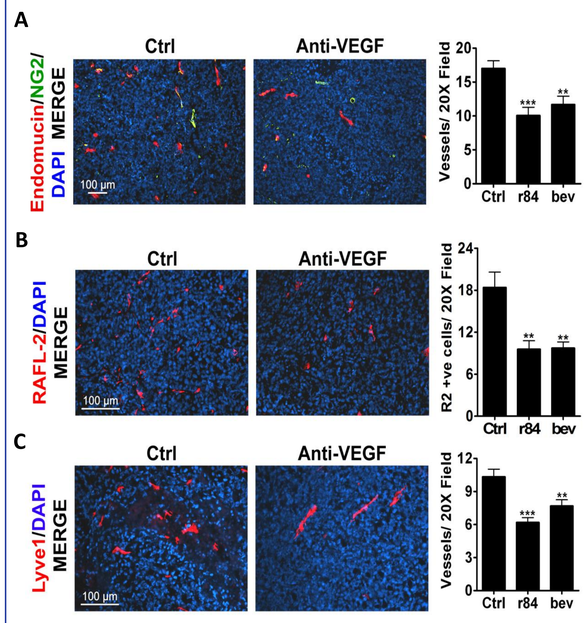


Figure 3: Bevacizumab, r84 therapy induces vascular changes within tumors. Bev, r84 treatment significantly decreases tumor microvessel density, as shown by a reduction in endomucin positive endothelial cells (A, red). Bev, r84 treatment also reduces the number of VEGFR2 positive cells as shown by RAFL-2 staining (B, red). Bev, r84 therapy significantly reduces tumor lymphatic vessels density as shown by lyve1 staining (red, C).

TUMOR GROWTH CONTROL BY ANTI-VEGF DOES NOT CORRELATE WITH INITIAL MVD

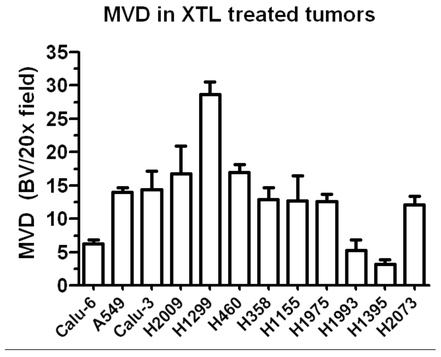


Figure 4: Tumor growth control by bevacizumab does not correlate with MVD in control-treated tumors. Microvessel density was measured in control-treated tumors. Above, the tumor lines are arranged in order from least sensitive to bevacizumab (Calu-6) to most sensitive to bevacizumab (H2073). There is no correlation between sensitivity and initial MVD, indicating that tumors are not resistant due to a predisposition to increased vascularization, independent of environment or treatment.

BEVACIZUMAB and r84 REDUCE MVD

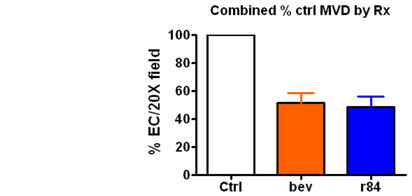


Figure 5: Bevacizumab and r84 reduce microvessel density in NSCLC tumor xenografts. Mean reduction in microvessel density as measured as a percent of control was significant with both antibodies for 12 xenografts.

TUMOR GROWTH CONTROL DOES NOT CORRELATE WITH MVD AFTER ANTI-VEGF

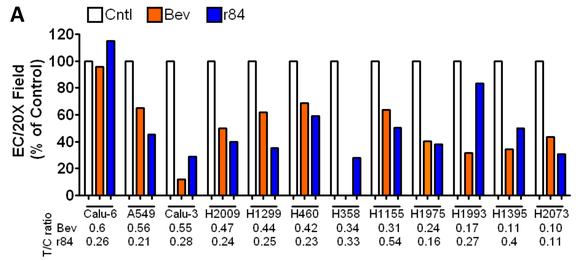


Figure 6: Decrease in MVD does not correlate with sensitivity to bevacizumab. Microvessel density was measured in tumors treated with bevacizumab and r84, and compared to control-treated tumors (A). Significantly, there was no correlation between the therapy induced change in microvessel density (%EC) and sensitivity to treatment (t/c), indicating that anti-VEGF therapy reduces blocks angiogenesis as designed.

CONCLUSIONS

- **Bevacizumab/r84 are effective at blocking blood vessel proliferation in tumors.**
- **Intrinsic resistance to anti-VEGF therapy does not appear to be related to induction of angiogenesis via unrelated angiogenic pathways.**
- **Other functions of VEGF, independent of angiogenesis, may be key for tumor growth, and *these* functions could be what resistant tumors are successfully compensating for.**

FUTURE DIRECTIONS

- **Microarray analysis of treated and resistant tumors resulted in some markers that may be relevant to methods of resistance.**
- **Further investigation into the metabolic and oncogenic effects of VEGF may enlighten our understanding of resistance to anti-VEGF therapy and aid in the design of future therapies.**