Human PlGF Antibodies as a HNSCC Therapeutic

Ike Dingle¹,², Brian Hoel³, J. Kenneth Byrd⁴, M. Boyd Gillespie⁵,⁶, and Natalie Sutkowski⁷,⁸
¹College of Medicine, ²Department of Otolaryngology – Head and Neck Surgery, ³Department of Microbiology and Immunology, and ⁴Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA

Abstract
Placental Growth Factor (PlGF) was recently identified as a therapeutic target for inhibiting tumor angiogenesis. PlGF is upregulated in the tumor microenvironment and is only pathologically expressed in adults, suggesting the absence of effects if PlGF inhibition. Recent studies have identified PlGF blockade in a variety of head and neck squamous cell carcinomas (HNSCC), indicating that PlGF is a real therapeutic target for HNSCC. To date, several studies have shown that recombinant human PlGF expression in tumor xenografts results in robust tumor formation and an angiogenic phenotype. One potential target is to develop PlGF monoclonal antibodies (Mabs) that target the PlGF receptor and sequester PlGF activity.

We previously developed methods for isolating human monoclonal antibodies to elastin, but have not had success isolating antibodies to other extracellular matrix proteins. We developed a library of tonsil B cells containing PlGF reactive clones, and we identified a number of clones that can inhibit PlGF expression.

Our Goal
To generate fully human monoclonal antibodies (Hu Mab) reactive against PlGF for the blockade of tumor angiogenesis. Strategies aimed at neutralizing PlGF in the tumor environment might be safer and more effective than those directed at inhibiting VEGF.

Anti-angiogenic cancer for therapy

The great promise of anti-angiogenic strategies has led to a number of anti-VEGF therapeutic agents, some of which have been approved for breast and lung in clinical trials. However, side effects, tumor recurrence and vascular adverse events have tempered the initial enthusiasm surrounding them. New approaches for inhibiting angiogenesis are needed.

Placental Growth Factor (PlGF)

- PlGF is a member of the vascular endothelial growth factor (VEGF) superfamily.
- Enhances VEGF signaling, but also has a function to separate VEGF.
- Acts as a chemokine for neutrophils and monocytes, and causes endothelial and smooth muscle cell migration, and recruitment of angiogenic cells from bone marrow.
- Neutralized PlGF is strongly expressed in the absence of PlGF incorporation, and is expressed in a lower degree in heart, lungs, kidney and vertebral.
- PlGF is strongly regulated in normal tissues, including bone marrow, MC lung, and gastric.
- VEGF inhibitors can up-regulate PlGF expression in humans engaging angiogenesis and monocyte recruitment.

Generally, VEGF is essential in physiological angiogenesis, while PlGF is crucial only in pathological angiogenesis.

PIGF expression in cancer cell lines

PIGF expression in HNSCC and other cancer cell lines. (A) Western blotting analysis of HNSCC cell lines, UM-SCC1, UM-SCC12B, and UM-SCC14A, and other tumors 624-28MEL, U-130, U251MG and U434MG, and PC3 for PLGF and VEGF-R2 expression. B-FBUS (21O cells) served as negative control; 5 ng of recombinant PIGF (rPIGF) was spiked in 70 mg of B-LCL lysate for positive control. For relative quantification, results were normalized against GAPDH. (B) ELISA titration of recombinant PlGF (up to 4 ng/mL) for positive control. (C) For further quantification, results were normalized against IgM, and plotted as a correlation study.

Creation of PlGF specific cell lines

PIGF expression was measured in a variety of clonal cell lines. Anti-PlGF antibodies were used to block PlGF expression, and the effect on tumor angiogenesis was measured. Anti-PlGF antibodies were used to block PlGF expression, and the effect on tumor angiogenesis was measured.

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Summary and Conclusions

We isolated human antibodies specific to PlGF and will soon determine the biological activity of these antibodies. This sets the stage to identify:

1) The role of PlGF in HNSCC.
2) The therapeutic benefit of anti-PlGF therapy.

Our next steps include the following:

- Screen fresh tumor specimens for PlGF expression using immunohistochemistry and PCR.
- Characterize PlGF antibodies by type, affinity, and activity.
- Demonstrate antibody activity with angiogenic and chemotactic assays.
- Isolate anti-PlGF Mabs with sufficient activity will be cloned into expression vectors for transfer to stable production cell lines.
- Test the stabilized anti-PlGF antibody for neutralization activity.