Evaluating the Expression of Angiogenic Mediators in a Mouse Model of Tumor Metastasis

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biomedical and Veterinary Sciences

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July 3, 2008
Blacksburg, Virginia

Key words: Angiogenesis, Vascular Endothelial Growth Factor, Flt-1, sFlt-1, Solid Tumors, Metastasis

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ABSTRACT

Solid tumors typically require angiogenesis, the development of new blood vessels, for growth and metastasis. Vascular endothelial growth factor (VEGF) induces angiogenesis by activating receptors on host endothelial cells. One such receptor, Flt-1, occurs as either a membrane bound or a secreted form (sFlt-1) that can inhibit angiogenic signaling. Previous studies have shown that variation in mRNA expression of VEGF and its receptors KDR, sFlt-1 and Flt-1 occurs in pathological angiogenesis, *i.e.* metastatic tumorigenesis. We hypothesize that the ratio of sFlt-1:Flt-1 mRNA will be altered in the presence of solid tumors. The objective of this study was to evaluate the expression of sFlt-1 and Flt-1 mRNAs in a mouse metastatic tumor model using CT26.CL25 cells. CT26.CL25 cells are VEGF-producing murine colon carcinoma cells transfected with the lacZ gene, which expresses □-galactosidase activity. These cells, injected intravenously, form tumor nodules in the lung. A pilot study revealed development of lung nodules in mice nine days after intravenous injection with 10^5 cells. In a second study, twenty-five 10- week-old female Balb/c mice were injected intravenously, via tail vein, with 2 x 10^5cells, and fifteen with vehicle control. Lung nodules developed in all mice injected with cells. Tissues were harvested by routine necropsy and either formalin-fixed for routine histology/histochemistry or stored for quantitative RT-PCR (QPCR) analysis of gene expression. Under microscopic evaluation, sections of lungs stained with Hematoxylin & Eosin (H&E) revealed nodules composed of polygonal neoplastic cells. cDNA from lungs (14 tumor-bearing, 10 controls) and cultured CT26.CL25 cells was analyzed by QPCR using primers and TaqMan probes directed against sFlt-1, Flt-1, KDR, $VEGF_A$, PlGF (Placental Growth Factor), Angiotensin Converting Enzyme (ACE), 18S ribosomal RNA and neo^R (neomycin phosphotransferase). We observed an increased sFlt-1:Flt-1 ratio in tumor-bearing versus control lungs, suggesting that tumor-derived signals may influence sFlt-1 and Flt-1 expression differentially. Additionally, there was increased expression of Flt-1, sFlt-1 and KDR in tumors versus controls, but not in VEGF expression in tumors versus controls. Interestingly, expression of PlGF was increased in tumors versus controls, suggesting its role as an enhancer of tumor progression in the presence of other angiogenic factors. Together, these findings indicate that solid tumor angiogenesis results from an intricate balance of various angiogenic factors.

DEDICATION

I dedicate this work and all that comes from it first of all to God, who has given me the strength to make it through what has been the most challenging, yet rewarding time of my life. Also to my husband, Earl, and my son, Josiah, without their support, sacrifice and love I would not have been able to fully reach my potential as a wife, mother, and student. And lastly, but definitely not least, to my mother, Doris Durrett, who has given me immeasurable love, support, encouragement and purpose. Thank you Mom! I love each of you more than you could imagine and appreciate you allowing me to be me and pursue my goals.

ACKNOWLEDGEMENTS

I would like to acknowledge everyone that has contributed to my success over the past eight years, including all my professors, advisors, classmates, resident mates and students. I would especially like to thank Dr. William Huckle, for his guidance as my graduate advisor. Dr. Geoff Saunders, Dr. Phillip Sponenberg and the late Dr. Bob Duncan for their instruction on what it means to be a veterinary pathologist. Dr. Tanya LeRoith for being such a great role model and for setting the bar for me as a pathologist, friend and individual. And to my resident mates, Dr. Ron Tyler, Dr. Anya Hawthorn, Dr. Bonnie Brenseke and Dr. Elizabeth Besteman. I am so thankful for you and will miss you all greatly. Each of you played a crucial role in my development and in making my time as a resident fun and so worthwhile. You all are going to do great things in the field of pathology and in your personal lives. And for those that I am inadvertently leaving out, everyone at Virginia Tech that I have worked with, Thank You!!!

Go HOKIES!!!!!

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