GRANT PROGRESS REPORT SUMMARY

Grant: 01429: *Mechanistic Relationship of IL-8 in Cell Proliferation and Survival of Canine Hemangiosarcoma*

Principal Investigator: Dr. Jaime F Modiano, VMD PhD

Research Institution: University of Minnesota

Grant Amount: $100,000.00

Start Date: 1/1/2011 **End Date:** 6/30/2013

Progress Report: Mid-Year 3

Report Due: 6/30/2013 **Report Received:** 6/9/2013

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

New insights into the mechanisms that control tumor progression have provoked considerable interest in the interaction of cancer cells with their microenvironment. Specifically, a molecule called IL-8 that can support tumor growth and survival, also recruits inflammatory cells and promotes blood vessel formation in the local tumor environment, enhances resistance to therapy, and facilitates metastasis in various aggressive cancers. Hemangiosarcoma (HSA) is an incurable, highly metastatic cancer that occurs commonly in dogs. There is virtually nothing known about how tumor cells and the microenvironment interact with each other in HSA, and more specifically, a role for IL-8 has not been investigated. In a recent study funded by CHF grant 422, we showed upregulation of IL-8 was a consistent feature that distinguished HSA cells from non-malignant endothelial cells, suggesting IL-8 might play a significant role in this disease. For this project, we will characterize the direct effects of IL-8 on HSA cells, an essential first step in the process to establish if and how this pleotropic molecule modulates disease progression. Our results will begin to clarify the importance of IL-8 production by HSA cells, and provide the foundation for subsequent studies to define its role regulating interactions between HSA cells and their microenvironment.

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Grant Objectives:

Objective 1: To establish the requirement for IL-8 in proliferation of cultured hemangiosarcoma cells

Objective 2: To define a role for IL-8 in survival of cultured hemangiosarcoma cells

Publications:

Manuscript submitted.

Report to Grant Sponsor from Investigator:

The hypothesis tested in this project was that interleukin-8 (IL-8) promotes growth and survival of hemangiosarcoma cells. This hypothesis was based on our previous results showing significant enrichment of IL-8 gene expression in hemangiosarcoma cells compared to normal endothelial cells isolated from non-malignant hematomas. Here, we confirmed that IL-8 is constitutively expressed by canine hemangiosarcoma cells in laboratory culture, as well as by primary tumors (fresh frozen samples). However, the levels of IL-8 are moderately variable among tumors.

Hemangiosarcoma cells in culture and primary hemangiosarcoma tumors also express IL-8 receptors (IL-8Rs). The receptors are expressed at comparable levels by virtually all the cultured cells and all the tumors, suggesting changes in expression of the receptor are unlikely to contribute to malignant behavior. We also confirmed that IL-8 binds to IL-8 receptors, and this interaction has functional consequences: IL-8 promotes signal transduction (calcium mobilization) in cultured HSA cells, and when we added IL-8 to cultured cells, they were able to "sense" this IL-8 excess and downregulated the expression of their own IL-8 gene. In contrast, if we blocked the interaction of their own secreted IL-8 with the receptor, they increased the amount of IL-8 gene expression. This is a classic response of compensatory regulation to negative feedback. Expression of a gene whose protein product turns on IL-8 gene expression followed the same pattern. It was downregulated when IL-8 was present in excess and induced when IL-8 was prevented from interacting with its receptor.

Despite its biological activity, IL-8 did not promote growth of hemangiosarcoma cells in culture, and IL-8 blockade did not hinder IL-8 growth in culture. When cells were deprived of nutrients and growth factors, they did not compensate by increasing production of IL-8; instead, IL-8 expression was reduced. And the addition of IL-8 did not prevent these nutrient-deprived cells from dying, and neither did it prevent cells treated with chemotherapeutic drugs from dying. Together, the data suggested that IL-8 did not directly mediate growth or survival of hemangiosarcoma cells in culture, refuting the initial hypothesis.

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We then compared the gene expression profiles of cells and tumors that expressed high levels of IL-8 (and thus were adapted to growing in an environment rich in IL-8) with those of cells and tumors that expressed lower levels of IL-8 (adapted to growing in environments with relatively scant IL-8). The data show that cells adapted to high IL-8 environments had gene expression profiles indicative of greater inflammation, coagulation, fibrosis, and angiogenesis. These data suggested that IL-8 could be important to modulate the microenvironment and provide a suitable tumor niche. Experiments from an independent, complementary project funded by the National Canine Cancer Foundation showed that indeed, blocking IL-8 hindered the ability of hemangiosarcoma cells to establish a tumor niche in vivo. Finally, preliminary data suggest that IL-8 also may be necessary to maintain the tumor-initiating populations of canine hemangiosarcoma, by enhancing self-renewal. This hypothesis is under investigation in our newly funded project supported by AKC CHF.

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