



RESEARCH PROGRESS REPORT SUMMARY

Grant 01759: Disrupting the Differentiation of Cancer Stem Cells to Prevent the Spread of Hemangiosarcoma

Principal Investigator: Dr. Jaime F Modiano, VMD PhD

Research Institution: University of Minnesota

Grant Amount: \$233,914.00

Start Date: 1/1/2013

End Date: 12/31/2015

Progress Report: Mid-Year 3

Report Due: 6/30/2015

Report Received: 7/6/2015

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:

Hemangiosarcoma is a rapidly fatal disease. The lifetime risk is alarmingly high for some breeds like Golden Retrievers (~20% will die of this disease) and Portuguese Water Dogs (~15% will die of this disease). Furthermore, the risk of hemangiosarcoma is not limited to a single breed. In fact so many dogs are at risk to develop hemangiosarcoma that 40 Breed Clubs designated it as a research priority for 2012. Despite considerable efforts to find effective treatments, the outcome for dogs with hemangiosarcoma has changed very little over the past 30 years. We believe this is because our understanding of this disease is still rudimentary, but that is changing. Recent evidence suggests hemangiosarcoma conforms to the "cancer stem cell" model, where a defined subset of cells is responsible for initiating and maintaining the tumor. These cells are resistant to conventional therapies and they also are very adaptable, being able to survive in a variety of niches. In the case of hemangiosarcoma, the cancer stem cells also retain or acquire the potential to differentiate along several different lineages. For this project, we will use this property against the tumor by modulating factors that support the self-renewal of the stem cell compartment and by inducing their terminal differentiation along alternate pathways that have reduced malignant potential. We propose that disrupting the interactions between hemangiosarcoma cancer stem cells and their microenvironment will enhance the sensitivity of these cells to conventional and targeted therapies and improve the outcomes of dogs with this disease.



Grant Objectives:

For this project, we will examine the potential to use the multipotency of hemangiosarcoma cells to our advantage by forcing them to differentiate into lineages with reduced malignant potential.

Publications:

Abstracts (since 01/2013):

1. Graef AJ, Kim JH, Sarver AL, Frantz AM, O'Brien TD, Sharkey LC, Dickerson EB, Modiano JF (2013). Gene expression profiling reveals a role of CXCR4/7 in canine hemangiosarcoma. Proceedings of the 2013 International Conference on Advances in Canine and Feline Genomics and Inherited Diseases
2. Kim JH, Sarver AL, Frantz AM, Scott MC, Graef AJ, Tonomura N, Elvers I, Thomas R, Lewellen M, Dickerson EB, Breen M, Lindblad-Toh K, Modiano JF (2013). Germ-line risk factors are associated with upregulation of genes mediating cell cycle arrest and stem cell activity in canine hemangiosarcoma. Proceedings of the 2013 International Conference on Advances in Canine and Feline Genomics and Inherited Diseases
3. Kim JH, Anderson KL, Frantz AM, Graef AJ, Dickerson EB, Modiano JF (2013). IL-8 and Slug regulate cancer cell self-renewal and microenvironment interactions in hemangiosarcoma. Proceedings of the 2013 Veterinary Cancer Society Meeting
4. Graef AJ, Kim JH, Modiano JF. Inhibition of CXCR4 in canine hemangiosarcoma (2013). Proceedings Masonic Cancer Center Research Symposium
5. Kim JH, Rodriguez A, Graef AJ, Modiano JF. Sphingosine 1-phosphate signaling pathway in canine hemangiosarcoma (2013). Proceedings Masonic Cancer Center Research Symposium (also supported by CHF 2081-A)
6. Witschen P, Graef AJ, Im KS, Modiano JF, Kim JH (2014). Role of CD44 signaling in canine hemangiosarcoma. Proceedings of the 2014 Merit NIH National Veterinary Scholars Symposium
7. Im KS, Graef AJ, Kim JH, Modiano JF. CXCL12 binding to CXCR4 regulates canine hemangiosarcoma cell migration and invasion (2014). Points of Pride Proceedings (UMN CVM Research Symposium)



8. Graef AJ, Kim HK, Im KS, Modiano JF. CXCR4/CXCL12 regulates inflammatory response to promote progression of canine hemangiosarcoma (2014). Points of Pride Proceedings (UMN CVM Research Symposium)
9. Rodriguez A, Graef AJ, Im KS, LeVine D, Modiano JF, Kim JH. S1P/S1P1 pathway promotes cell proliferation and survival in canine hemangiosarcoma (2014). Proceedings Masonic Cancer Center Research Symposium (also supported by CHF 2081-A)
10. Im KS, Graef AJ, Kim JH, Modiano JF. CXCL12-CXCR7 axis regulates maintenance of canine hemangiosarcoma progenitor cells (2014). Proceedings Masonic Cancer Center Research Symposium
11. Modiano JF. Spontaneous Animal Models: Unsung Heroes, Proceedings 2015 ACVIM Forum
12. Im KS, Graef AJ, Kim JH, Modiano JF. (2015). CXCR4 and its ligand CXCL12 regulate canine hemangiosarcoma cell migration and invasion. Proceedings of the Annual Meeting of the American College of Veterinary Pathology

Papers

1. Gorden BH, Kim JH, Sarver AL, Frantz A, Breen M, Lindblad-Toh K, O'Brien TD, Sharkey LC, Modiano JF, Dickerson EB. (2014). Identification of three molecular and functional subtypes in canine hemangiosarcoma through gene expression profiling and progenitor cell characterization. *Am J Pathol*, 184(4), 985-95. PMID: 24525151
2. Kim JH, Frantz AM, Anderson KL, Graef AJ, Scott MC, Robinson SR, Sharkey LC, O'Brien TD, Dickerson EB, Modiano JF. (2014). Interleukin-8 promotes canine hemangiosarcoma growth by regulating the tumor microenvironment. *Exp Cell Res*, 323(1), 155-164. PMID: 24582862
3. Rodriguez AM, Graef AJ, LeVine D, Cohen IR, Modiano JF, Kim JH. (2015). Association of sphingosine-1-phosphate (S1P)/S1P receptor-1 pathway with cell proliferation and survival in canine hemangiosarcoma. *J Vet Intern Med*, published online: 25 JUN 2015 DOI: 10.1111/jvim.13570

Report to Grant Sponsor from Investigator:

We have made substantial progress. Our results confirm and extend the notions that interactions between the tumor and its local environment regulate hemangiosarcoma progression. Yet, variability in cells within tumors can reduce the predictability of hemangiosarcoma behavior, and possibly contribute to therapy resistance. For example, hemangiosarcomas respond to the degradation of their supporting matrix by recruiting



inflammatory cells and blood vessels. But the magnitude of this effect is variable among different hemangiosarcomas, which requires us to consider that these tumors might adapt efficiently to very different microenvironments. The hemangiosarcoma microenvironment also tends to be rich in a molecule called CXCL12, which is used as a means of communication between the tumor cells and the normal supporting cells. Only some of the tumor cells have the receptors that transmit the signals from CXCL12. These cells help to support the tumor, and also can be efficient mediators of metastasis. But in their absence, other mechanisms might perform these functions. This suggests that blocking this pathway and others like it could have a positive therapeutic effect, but managing this disease will require combining strategies that lower the capacity of cells to simply switch their behavior to use alternate pathways to survive and thrive.