

FINAL REPORT

Project Title: Targeting the Ras-Pathway for the
Treatment of Embryonal Rhabdomyosarcoma (ERMS)

Project Number: SFA09-13

1. Date project was initiated: July 1, 2009
2. Period covered by this report: From July 1, 2009 To June 30, 2010
3. Publications, Abstracts, and Presentations:

- a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry must include the author(s), article title, journal [book, editors(s), publisher, volume number, page number(s), and date.]

(1) Peer-Reviewed Scientific Journals:

High-throughput cell transplantation establishes that tumor-initiating cells are abundant in zebrafish T-cell acute lymphoblastic leukemia. Smith AC, Raimondi AR, Salthouse CD, Ignatius MS, Blackburn JS, Mizgirev IV, Storer NY, de Jong JL, Chen AT, Zhou Y, Revskoy S, Zon LI, Langenau DM. *Blood*. 2010 Apr 22;115(16):3296-303. – Rhabdomyosarcomas were also made in this publication, although not the focus of this paper.

High-throughput Methods for Imaging Adult Fluorescent Zebrafish. Jessica S. Blackburn, Sali Liu, Aubrey R. Raimondi, Myron S. Ignatius, Chris D. Salthouse, David M. Langenau. *Nature Protocols* (under review).

Combination inhibition of S6 Kinase and MAPK suppresses RAS induced tumor proliferation. Xiuning Le, Emily K. Pugach, Simone Hettmer, Narie Y. Storer, Jianing Liu, Airon Wills, Kenneth D. Poss, Amy Wagers, David M. Langenau, Leonard I. Zon. *Nature Chemical Biology* (under review).

Discrete subsets of myogenic and mesenchymal precursors give rise to soft tissue sarcomas of distinct lineage differentiation. Simone Hettmer, Jianing Liu, Roderick T. Bronson, David M. Langenau, Amy J. Wagers. *Cancer Cell* (under review).

(2) Invited Articles:

Zebrafish as a model for cancer self-renewal.
Ignatius MS, Langenau DM.
Zebrafish. 2009 Dec;6(4):377-87.

- b. List presentations made during the last year (international, national, local societies, etc.). Use an asterisk (*) if presentation produced a manuscript.

In vivo imaging identifies that myf5+ embryonal rhabdomyosarcoma-initiating cells are dynamically reorganized into distinct tumor niches during various stages of growth. – Presented by Dr. Myron Ignatius (Fellow in the lab) at the International Society for Stem Cell Research, San Francisco June 2010.

Looking inside embryonal rhabdomyosarcoma self-renewal – Presented by David Langenau at the Zebrafish Disease Models III International Meeting, Boston June 2010.

High-throughput imaging of adult fluorescent transgenic zebrafish – Presented by Sali Liu from my lab at the Zebrafish Disease Models III International Meeting, Boston June 2010.

4. Provide a brief list of keywords: (limit to 20 words)

Rhabdomyosarcoma, RAS, zebrafish, satellite cells, cancer stem cell.

5. Summarize the progress during the period of this report and its impact on your plans for the remainder of the project. Include a summary of the progress toward the achievement of the originally stated aims and list the significant results:

Aim 1. Identify the mechanism by which mTOR and/or Map-kinase pathways regulate tumor growth in a transgenic zebrafish model of embryonal rhabdomyosarcoma.

Aim 2. Validate zebrafish results in human ERMS cell lines.

Great progress was made toward these aims in this one year grant and resulted in the production of a manuscript co-authored by Drs. Wagers, Zon, and myself. This highly collaborative paper addresses both aims 1 and 2. Chemical suppressors of RAS targets in embryos were identified and further evaluated for their therapeutic effects in a KRAS-induced rhabdomyosarcoma zebrafish model. Treatment with MEK inhibitor PD98059 and chymotrypsin-like serine protease inhibitor TPCK resulted in inhibition of tumor proliferation in tumor-bearing fish respectively. The combinational treatment of PD98059 and TPCK further enhanced inhibitory effect with significantly lower doses of each single agent. Such synergistic anti-proliferative effect was also observed in human rhabdomyosarcoma cell lines. PD98059 suppress MAPK/ERK signaling whereas TPCK suppress S6K1 signaling in zebrafish embryos and in cell lines, combinational treatment lead to inhibition phosphorylation of eIF4B. These results suggest that S6K signaling is an important target in RAS induced tumors and indicate that combining MAPK/ERK inhibitors with S6K1 suppression presents a potential therapeutic approach.

Future project development:

Based on the remarkable success of this grant application, we have now applied for supplemental funding to expand our screen to identify new compounds that directly kill tumor-initiating cells in zebrafish and human ERMS. This work is now funded in part by the Harvard Stem Cell Institute and a pending R21 application entitled "Chemical Inhibitors of Rhabdomyosarcoma Self-renewal" that will be awarded December 2010. An R01 entitled "Genetics of Rhabdomyosarcoma" has also been submitted.

6. In layperson's terms, summarize the progress during the period of this report. Explain any medical significance or implications of your results to date:

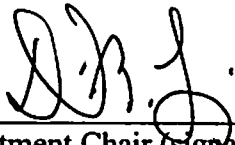
In collaboration with the Zon and Wagers groups, we have discovered that combinatory inhibition of two molecular pathways found to be active in rhabdomyosarcoma leads to reduction of tumor growth in a zebrafish model of disease and human ERMS cell lines. Our results suggest that targeting these two pathways in ERMS may curb tumor growth and could lead to new treatments for human disease.

David M. Langenau

July 6, 2010

Principal Investigator (signature)

Date



Department Chair (signature)

7/9/10

Date