

FINAL REPORT

Project Title: How does a sarcoma circumvent
fusion oncoprotein-mediated toxicity?

Project Number: SFA10-15

1. Date project was initiated: 05/01/10
2. Period covered by this report: From 05/01/10 To 12/31/11
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) Lay Press: none
 - (2) Peer-Reviewed Scientific Journals: none
 - (3) Invited Articles: none
 - (4) Abstracts: none
 - b. List presentations made during the last year (international, national, local societies, etc.). Use an asterisk (*) if presentation produced a manuscript.

November 10, 2011 "When PAX met FOX – the strange world of fusion proteins in rhabdomyosarcoma" - Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD.
4. Provide a brief list of keywords: (limit to 20 words)
rhabdomyosarcoma, translocation, gene fusion, PAX3, FOXO1, oncogene, oncoprotein
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:

This research project proposed to investigate the mechanisms by which developing rhabdomyosarcoma cells tolerate the toxicity induced by high level expression of the PAX3-FOXO1 fusion generated by the 2;13 chromosomal translocation. As an experimental system, we developed a human myoblast line expressing an inducible form of PAX3-FOXO1. Using this line, we initially found that induction of PAX3-FOXO1 in individually plated cells resulted in growth suppression. However, when plated with fibroblasts, induction of PAX3-FOXO1 resulted in enhanced growth and the appearance of transformed foci. The original application consisted of two aims, one to study the role of stromal cells (such as fibroblasts) in suppressing PAX3-FOXO1-induced toxicity and a second aim to analyze the contribution of FGFR4, a PAX3-FOXO1 target gene, to PAX3-FOXO1-induced toxicity.

Our initial studies following submission of the proposal challenged our starting hypotheses and thereby changed our aims for this project. We initially focused on stromal cells as necessary agents for attenuating the toxicity of PAX3-FOXO1. However, subsequent studies with only inducible PAX3-FOXO1-expressing myoblasts in the culture indicated that PAX3-FOXO1-induced growth suppression could be lost with just manipulations of the microenvironment. Therefore, the effect does not require a second "helping" cell type but rather just requires that PAX3-FOXO1 act on myoblasts under specific microenvironmental conditions. We also had initial data from ARMS lines indicating that signaling through the PAX3-FOXO1 downstream target FGFR4 was needed for the growth suppression effect. However, subsequent additional studies of more ARMS lines and the inducible myoblast system did not confirm this finding, and we therefore no longer believe that FGFR4 signaling is needed for this toxicity effect.

Based on these findings, we changed our aims to conduct screens for pathways responsible for the toxicity effects and for rescue of toxicity in the presence of these microenvironmental alterations. We focused this screen at the level of RNA expression and initially examined a panel of 14 genes that are known downstream targets (direct or indirect) of PAX3-FOXO1. Various patterns of expression were observed as we manipulated the microenvironment with and without PAX3-FOXO1 induction. As expected, expression of most of these genes was modulated following PAX3-FOXO1 induction. For several genes, there was increased basal expression associated with the change in the microenvironment and then an additional additive increase following PAX3-FOXO1 induction. (Note that all reported expression levels were normalized for cell content.) However, for some genes such as MYCN (encoding a growth promoting transcription factor), there was much higher expression associated with a growth promoting or favorable microenvironment. This MYCN expression change may serve as a reporter of pathways that are changed by the microenvironment and permit growth to occur in the presence of PAX3-FOXO1 induction. In an unfavorable environment, DAPK1 (encoding a growth inhibitory protein) is induced from a low basal level. In contrast, there is increased basal expression in a favorable microenvironment and then a smaller increase following PAX3-FOXO1 induction to a relatively constant final level. These DAPK1 expression changes may indicate effects that precondition the myoblasts in the favorable environment to tolerate previously toxic levels of this PAX3-FOXO1 target.

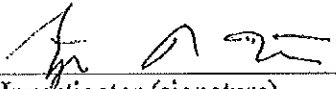
Based on these findings, we wanted to examine larger numbers of genes and use bioinformatic strategies to identify genes that fit specific predetermined categories. Using the information from the previously described experiments, sets of conditions to achieve optimal microenvironmental conditions were selected in which there were prominent differences in expression of these candidate target genes. These conditions were used to perform multiple replicates to isolate RNA for expression profiling studies. In particular, RNA was isolated from four independent cultures of cells at favorable and unfavorable microenvironments

treated with or without the inducing agent. This RNA was then analyzed on the Affymetrix Human Gene 1.0 ST Arrays to evaluate all known expressed genes in the human genome. Probe preparation from extracted total RNA, hybridization, and scanning was performed in the Penn Microarray Facility. The microarray data was normalized by established methods and analyzed by strategies such as 2-way ANOVA. This data analysis is ongoing.

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:

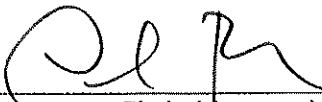
We previously determined that high levels of the PAX3-FOXO1 protein produced by the characteristic chromosomal translocation event in rhabdomyosarcoma result in growth suppression or cell death instead of the expected growth enhancement. We propose that specific changes in the rhabdomyosarcoma cell or conditions affecting this cell are needed to occur for a rhabdomyosarcoma cell to produce and tolerate high levels of this important protein. In the project funded by the Sarcoma Foundation of America, we used a model system to determine that cells expressing high levels of this protein can tolerate the toxic effects when the cells are present in specific environmental conditions. We propose that cells respond to changes in the local external environment by changes in their internal regulatory pathways, and we have started to unravel these changes by analyzing RNA levels in the cells. Some of these pathway changes allow the cells to tolerate the toxic effects of PAX3-FOXO1 when it is turned on, and then PAX3-FOXO1 can exert its cancer-causing effects. Our goal is to understand these pathway changes so that we can eventually find ways to reverse them and force PAX3-FOXO1 to exert its toxic effects on the cancer cell. In this way, we will have a new strategy by which the cancer-causing PAX3-FOXO1 protein turns into a cancer-fighting agent.



Principal Investigator (signature)

1/3/12

Date



Department Chair (signature)

1/3/12

Date