



## FINAL REPORT

Project Title: Regulation of sarcomagenesis by the Piwi proteins and their interacting small RNAs (piRNAs)

Project Number: SFA10-07

1. Date project was initiated: \_\_\_9/1/08\_\_\_\_\_
2. Period covered by this report: From\_\_\_7/10/10\_\_\_\_\_ To\_\_\_6/31/11\_\_\_\_\_
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:
    - (2) Peer-Reviewed Scientific Journals:  
Submitted to Clinical Cancer Research:  
Hiwi mediated tumorigenesis via DNA-hypermethylation
    - (3) Invited Articles:  
Journal of Cellular Biochemistry:  
Piwis and piwi-interacting RNAs in the epigenetics of cancer
    - (4) Abstracts:  
ASCO 2011  
AACR 2010
  - b. List presentations made during the last year (international, national, local societies, etc.). Use an asterisk (\*) if presentation produced a manuscript.
4. Provide a brief list of keywords: (limit to 20 words)

mesenchymal stem cells, piRNAs, Piwi, HIWI, sarcomas

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:

Expression of Piwi proteins is confined to early development and stem cells during which they suppress transposon migration via DNA methylation to ensure genomic stability. In addition to being absent from all mature tissues, Piwi's genomic protective function conflicts with reports that its human ortholog, Hiwi, is expressed in numerous cancers and prognosticates shorter survival. Here we demonstrate that (1) over-expressing Hiwi in sarcoma precursors inhibit their differentiation in vitro and generates sarcomas in vivo; (2) transgenic mice expressing Hiwi (mesodermally restricted) develop sarcomas; and (3) inducible down-regulation of Hiwi in human sarcomas inhibits growth and re-establishes differentiation. Our data indicates that Hiwi is directly tumorigenic and Hiwi-expressing cancers may be addicted to Hiwi expression. We further show that Hiwi mediates DNA methylation and cyclin-dependent kinase inhibitor (CDKI) silencing, which is reversible along with Hiwi-induced tumorigenesis, via DNA-methyltransferase inhibitors. Our studies reveal for the first time not only a novel oncogenic role for Hiwi as a driver of tumorigenesis, but also suggest that the use of epigenetic agents may be clinically beneficial for treatment of tumors that express Hiwi. Additionally, our data showing that Hiwi-mediated methylation results in DNA hyper-methylation with subsequent genetic and epigenetic changes favoring a tumorigenic state via epigenetic silencing of CDKIs, reconciles the conundrum of how Hiwi may act appropriately to promote genomic integrity during early development (via transposon silencing) and inappropriately in adult tissues with subsequent tumorigenesis.

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:

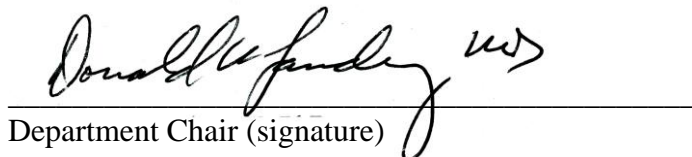
An increasing body of evidence suggests that cancer cells acquire "stem-like" epigenetic and signaling characteristics during the tumorigenic process, including global DNA hypo-methylation, gene-specific DNA hyper-methylation, and small RNA deregulation. RNAs have been known to be epigenetic regulators, both in stem cells and in differentiated cells. A novel class of small RNAs, called piwi-interacting RNAs (piRNAs), maintains genome integrity by epigenetically silencing transposons via DNA methylation, especially in germline stem cells. piRNAs interact exclusively with the Piwi family of proteins. The human Piwi ortholog, Hiwi, has been found to be aberrantly expressed in a variety of human cancers and in some, its expression correlates with poor clinical prognosis. However, there has been little investigation into the potential role that Piwi and piRNAs might play in contributing to the "stem-like" epigenetic state of a cancer



Principal Investigator (signature)

9/09/11

Date



Department Chair (signature)

9/10/11

Date