



## FINAL REPORT

Project Title: Identification of the target genes of  
the EWS/NR4A3 fusion protein expressed  
in extraskeletal myxoid chondrosarcoma

Project Number: SFA10-11

1. Date project was initiated: June 1st 2010
2. Period covered by this report: From June 1st 2010  
To Present
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: A manuscript is in preparation.
    - (3) Invited Articles:
    - (4) Abstracts:
  - 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)

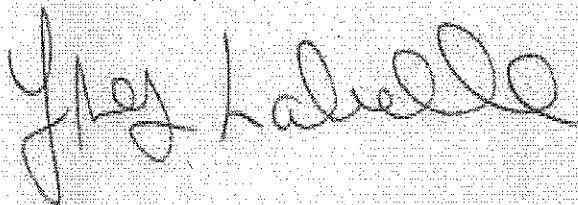
Extraskeletal myxoid chondrosarcoma; chromosome translocation; fusion protein; transcriptional regulation; target genes; human bone marrow stem cells

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:

The goal of this project was to identify the target genes of the EWS/NR4A3 fusion protein in extraskeletal myxoid chondrosarcoma (EMC) tumors. This fusion protein is thought to be essential for tumor development. Current evidence strongly suggest that one role of EWS/NR4A3 is to activate the transcription of specific target genes involved in tumor development. We have developed a unique human bone marrow stem cell (BMSC) model to study the transcriptional impact of EWS/NR4A3. We had proposed in the grant application to use this cellular model to identify target genes of the fusion protein that may play a role in the development of EMC tumors. We have performed microarray experiments on human bone marrow stem cell cell lines expressing EWS/NR4A3 as well as control cell lines not expressing the fusion protein. Statistical analyses identified 775 genes expressed 1.5 fold or more with a P value of 0.05 or less in cell lines expressing EWS/NR4A3. We compared those 775 genes to genes significantly over-expressed in EMC tumors published in three studies (Sjögren et al, *Am J Pathol* 2003, 162:781–792; Subramanian et al, *J Pathol* 2005, 206:433–444; Filion et al, *J Pathol* 2009, 217:83-93). A total of 15 genes were identified over-expressed in both the EN19 human bone marrow stem cell lines expressing EWS/NR4A3 and EMC tumors (Table 1). A reassuring result was that we found the PPARG gene in this list, which we have shown previously to be transcriptionally activated by EWS/NR4A3 (Filion et al, *J Pathol* 2009, 217:83-93). We next performed real-time PCR analyses of the transcripts of these 15 genes in 1) the EN19 cell line versus the original bone marrow stem cell line (column EN19/BMSC in Table 2); 2) the EN19 cell line in which expression of EWS/NR4A3 was knocked-down by siRNA versus a control siRNA (column si EN/si control in Table 2); and 3) three EMC tumors expressing EWS/NR4A3 versus four classical chondrosarcoma (CC) tumors which do not express EWS/NR4A3 (column EMC/CC in Table 2). These experiments identified 11 genes statistically significantly regulated by EWS/NR4A3 in bone marrow stem cells and over-expressed in EMC tumors compared to CC tumors (including PPARG). In addition, three of the remaining four genes not over-expressed in EMC versus CC tumors are nonetheless regulated by EWS/NR4A3 in bone marrow stem cells, indicating that they may be valid targets in EMC tumors but may also be over-expressed in CC through other mechanisms. Thus our studies have identified several putative target genes of the fusion protein in EMC tumors that may play a crucial role in their development, and which could be targeted to interfere with tumor progression. Also these results validate our cellular model, and we now wish to use this model to identify alternative mRNA splicing variants regulated by EWS/NR4A3. A study by Sanchez and collaborators (Sanchez et al, *Proc Natl Acad Sci USA* 2008, 105:6004-9) has shown that the EWS/FLI1 fusion protein regulates alternative mRNA splicing, and we suspect that EWS/NR4A3 may have the same ability since the EWS portion of the EWS/FLI1 fusion protein is suspected to be responsible for this effect. We therefore wish to perform microarray experiments using arrays designed to quantify alternative mRNA splicing variants.

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:

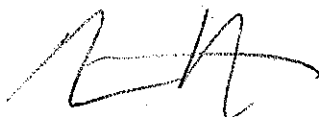
We have identified several genes that may play a crucial role in the development of EMC tumors, and which therefore could be targeted to interfere with the tumoral process. As numerous studies are currently attempting to interfere with gene expression as a means to circumvent tumor progression, our studies provide a rationale for choosing target genes to interfere with the development of EMC tumors.



\_\_\_\_\_  
Principal Investigator (signature)

August 5 2011 \_\_\_\_\_

Date



\_\_\_\_\_  
Department Chair (signature)

2011-08-09

\_\_\_\_\_  
Date

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