Kirsten M. Johnson
PhD Candidate

“Characterization of length-dependent GGAA-microsatellites in EWS/FLI mediated Ewing sarcoma oncogenesis”

March 1, 2018
105 Biomedical Research Tower
2:00 p.m.
VITA

October 1989 .......................... Born, El Paso, TX

December 2011 ................. B.S. Molecular Biology, Brigham Young University

2012-present .......................... M.D. Ph.D. Candidate, The Ohio State University

COMMITTEE MEMBERS

Stephen L. Lessnick, MD, PhD, Advisor

Michael A. Freitas, PhD

Denis C. Guttridge, PhD

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ABSTRACT

Ewing Sarcoma is a pediatric bone malignancy initiated by a t(11;22) chromosomal translocation that produces the EWS/FLI oncoprotein. EWS/FLI transcriptionally activates and represses its target genes to mediate oncogenic reprogramming. Expression of its up-regulated targets correlates with EWS/FLI binding to associated GGAA-microsatellites, which show length polymorphisms. These microsatellite polymorphisms may critically affect EWS/FLI-responsiveness of key gene targets. For example, NR0B1 is necessary for EWS/FLI mediated oncogenic transformation, and we found a “sweet-spot” of 20-26 repeat length as optimal for EWS/FLI mediated transcriptional activity at NR0B1 through clinical observations and in vitro studies. The mechanism underlying this optimal length, however, is unknown.

We explored the stoichiometry and binding affinity of EWS/FLI for different GGAA-repeat lengths through biochemical studies, including fluorescence polarization, ChiP-seq, and RNA-seq, combined with bioinformatics analysis. Additionally, use of EWS/FLI deletion constructs has been critical for elucidating the particular binding behavior of EWS/FLI at different microsatellite repeat lengths. Luciferase reporter assays, anchorage-independent growth and proliferation assays, as well as CRISPR technology have extended our findings to the in vivo setting. Finally, microscopy studies including use of confocal and transmission electron microscopy (TEM) have contributed visual characterization of the specific biochemical mechanisms we are investigating.

CRISPR-mediated deletion of the NR0B1 GGAA-microsatellite in Ewing sarcoma cells provided our field with the first in vivo evidence for the necessity of EWS/FLI binding at GGAA-microsatellites for anchorage dependent growth. Our biochemical studies, using recombinant Δ22 (a version of EWS/FLI containing only the FLI portion) demonstrate a stoichiometry of one monomer binding every two consecutive GGAA-repeats on shorter microsatellite sequences. Surprisingly,
the affinity for Δ22 binding to GGAA-microsatellites significantly decreased, and was unmeasureable when the size of the microsatellite was increased to the “sweet-spot” length. In contrast, a fully-functional EWS/FLI mutant (Mut9, retaining approximately half of the EWS portion) showed low affinity for smaller GGAA-microsatellites, but instead significantly increased its affinity at “sweet-spot” microsatellite lengths. Single-gene ChIP and genome-wide ChIP-seq and RNA-seq studies extended these findings to the in vivo setting. Additionally, through bioinformatics analysis, we defined GGAA-microsatellites in a Ewing sarcoma setting, and showed GGAA-microsatellite length is predictive of EWS/FLI responsiveness (binding and transcriptional activation) at “promoter-like” EWS/FLI targets.

Together, these data demonstrate the necessity for EWS/FLI binding at GGAA-microsatellites in Ewing sarcoma, and characterize their role in oncogenesis. These data also reveal an unexpected novel role for the EWS portion of the EWS/FLI fusion in DNA-binding. Overall, our results suggest a length-dependent biochemical mechanism for EWS/FLI binding and transcriptional regulation at GGAA-microsatellites.
RECENT ABSTRACTS AND PRESENTATION

Oral Presentations:

Johnson, Kirsten M., EWS/FLI transcriptional activation via GGAA Microsatellites. Oral Presentation at The Ohio State University Medical Scientist Training Program (MSTP) Faculty Forum; 2016 Dec 1; Columbus, Ohio.

Johnson, Kirsten M., GGAA: The 4-letter word of Ewing sarcoma. Oral Presentation at The Ohio State University Biomedical Sciences Graduate Program Annual Retreat, Speaker Competition; 2016 Dec. 8; Columbus, Ohio.

Johnson, Kirsten M., GGAA: The 4-letter word of Ewing sarcoma. Oral Presentation at Phase Separation and RNA Processing as Drivers of Cancer and Neurodegenerative Disease; 2017 Feb. 24-26; San Diego, California.

Johnson, Kirsten M., GGAA: The 4-letter word of Ewing sarcoma. Oral Presentation at CTOS; 2017 Nov. 9-11; Maui, Hawaii.

Abstracts:


Johnson, Kirsten M., Lessnick, Stephen L. EWS/FLI regulates Transcriptional activation in Ewing sarcoma via length dependent
GGAA microsatellites. Poster session presented at CTOS 21st Annual Meeting; 2016 Nov 9-12; Lisbon, Portugal.

**Johnson, Kirsten M.,** Lessnick, Stephen L. EWS/FLI regulates Transcriptional activation in Ewing sarcoma via length dependent GGAA microsatellites. Poster session presented at AACR Annual Meeting; 2017 April 1-5; Washington, D.C.

**Johnson, Kirsten M.,** Lessnick, Stephen L. EWS/FLI regulates Transcriptional activation in Ewing sarcoma via length dependent GGAA microsatellites. Poster session presented at OSUWMC Trainee Research Day April 13, 2017

**Johnson, Kirsten M.,** Lessnick, Stephen L. EWS/FLI regulates Transcriptional activation in Ewing sarcoma via length dependent GGAA microsatellites. Poster session presented at NCH Research Retreat; 2017 Nov. 28, OSU

**Johnson, Kirsten M.,** Lessnick, Stephen L. EWS/FLI regulates Transcriptional activation in Ewing sarcoma via length dependent GGAA microsatellites. Poster session presented at AACR Advances in Pediatric Cancer Research; 2017 Dec 3-6; Atlanta, Georgia


Johnson KM* and Taslim C* (*co-first authors), Saund RS, Lessnick SL. Identification of two types of GGAA-microsatellites and their roles in EWS/FLI binding and gene regulation in Ewing sarcoma. PLOSOne U S A. 2017. doi: 10.1371/journal.pone.0186275
AWARDS AND HONORS

2016-2019  F30 Ruth L. Kirschstein National Research Service Fellowship Award, NCI/NIH

2016  BSGP Speaker Competition 2nd place & Travel Award recipient, OSU

2017  Travel Award for oral presentation at Phase Separation and RNA Processing as Drivers of Cancer and Neurodegenerative Disease Conference, San Diego, CA

2017  Travel Award for oral presentation at Connective Tissue Oncological Society Annual Meeting, HI

FUTURE PLANS

I plan to return to medical school to complete my medical training, after which I will pursue a research residency in pediatrics, and fellowship in pediatric hematology/oncology.