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YOU ARE INVITED TO ATTEND THE

DEFENSE OF THE DOCTORAL

DISSERTATION

"Identification of a novel role of NKX2.2 as a transcriptional co-regulator of EWS/FLI1 in Ewing's sarcoma pathogenesis"

by

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Thursday, September 6th, 2018
10:00 A.M.
PHRI Board Room

ABSTRACT

Ewing's sarcoma (ES) is a prevalent pediatric, bone and soft tissue cancer which is caused by a chromosomal translocation between the Ewing's sarcoma breakpoint region 1 (EWSR1) gene on chromosome 22 and Friend leukemia integration 1 (FLI1) gene on chromosome 11. This translocation leads to the formation of the fused *EWS/FLI1* gene, which codes for an aberrant transcription factor. EWS/FLI1 is an internally disordered protein that relies on protein-protein interactions to alter gene expression. We identified a novel functional interaction between the EWS/FLI1 fusion protein and the NKX2.2 transcription factor which may play an important role in ES oncogenesis. Previous studies have examined the role of NKX2.2 as a transcriptional repressor in maintaining ES oncogenesis, but no mechanism of action between EWS/FLI1 and NKX2.2 was described. In this study, we hypothesize that EWS/FLI1 interacts with NKX2.2 to alter its transcriptional activity to promote oncogenesis. Co-immunoprecipitation (CoIP) experiments identified a DNA-dependent interaction between NKX2.2 and EWS/FLI1. Additional sequential chromatin immunoprecipitation (Seq-ChIP) experiments followed by sequencing showed numerous direct targets of individual EWS/FLI1 and NKX2.2 binding, and also targets of simultaneous binding by EWS/FLI1 and NKX2.2. Targets of interest that showed co-occupancy of EWS/FLI1 and NKX2.2 on their promoter region were explored further; these include the six transmembrane epithelial antigen of the prostate 1 (STEAP1) and S-phase kinase associated protein 2 (SKP2). Both STEAP1 and SKP2 were dependent on EWS/FLI1 for their upregulation in ES, but the role of NKX2.2, and potentially the EWS/FLI1-NKX2.2 protein complex, in regulating these two genes is not known. EWS/FLI1 and NKX2.2 knockdown ES cell lines were designed using a retroviral vector expressing a short hairpin RNA (shRNA) to further examine the relationship between these two transcription factors and STEAP1 and SKP2

expression. Results from these experiments indicated that STEAP1 expression was affected by the loss of EWS/FLI1 and NKX2.2; the downstream products of STEAP1 such as reactive oxygen species (ROS) production and ROS-dependent genes were also affected. The same trend was observed for SKP2; the expression of SKP2 and cell cycle regulation were affected by the loss of EWS/FLI1 and NKX2.2 in ES. From this study, we conclude that NKX2.2 acts as a transcriptional co-regulator of EWS/FLI1 at promoter sites, which augments the normal activity of the latter transcription factor. Based on our data, we propose that the presence of NKX2.2 at the promoter sites facilitates the recruitment of EWS/FLI1 and thus causes a dysregulation of target genes which are normally under the regulation of NKX2.2 alone. Currently, there are no direct treatments for ES because EWS/FLI1 cannot be targeted. This analysis will pave the way for future studies to investigate small molecules that can disrupt its functional interactions with other proteins and hopefully curb the progress of sarcoma.