MASSACHUSETTS GENERAL HOSPITAL
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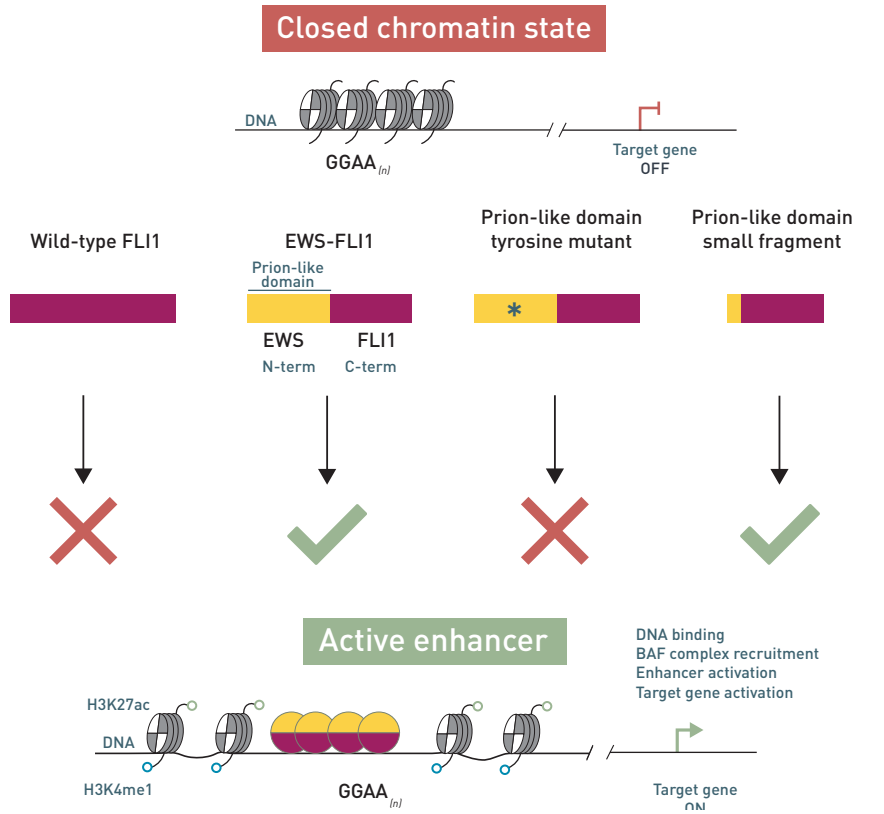
Prion-Like Domains Drive Tumor Growth in Ewing Sarcoma

What role does the EWS-FLI1 fusion protein play in activation in Ewing sarcoma?

Unlike adult cancers, pediatric cancers tend to feature few genetic mutations, and these mutations most often affect genes that play a role in gene expression and regulation. Ewing sarcoma is one such form of pediatric cancer. It grows aggressively, metastasizes frequently, and is driven by a single genetic defect: a fusion between the genes EWS and FLI1. How the EWS-FLI1 fusion protein promotes tumor formation is not fully understood and is the focus of new research from the lab of Miguel N. Rivera, MD, Assistant Professor of Pathology at Massachusetts General Hospital and Harvard Medical School, and an Investigator at the Center for Cancer Research at Mass General Cancer Center.

While the FLI1 is a well-known DNA-binding transcription factor involved in controlling gene expression, the function of EWS is less well defined. One prominent feature of EWS is that it contains prion-like domains that make it prone to pathological aggregation in neurodegenerative disease. In research published in *Cell* in September 2017, Dr. Rivera and his team showed that these prion-like domains, which are maintained by EWS-FLI1 fusion proteins, are instrumental in activating Ewing sarcoma oncogenes.

"It's very exciting to find that one of these prion-like domains is capable of turning on a whole set of genes that cause a tumor," said Dr. Rivera.



Enhancer Activation of Oncogenes

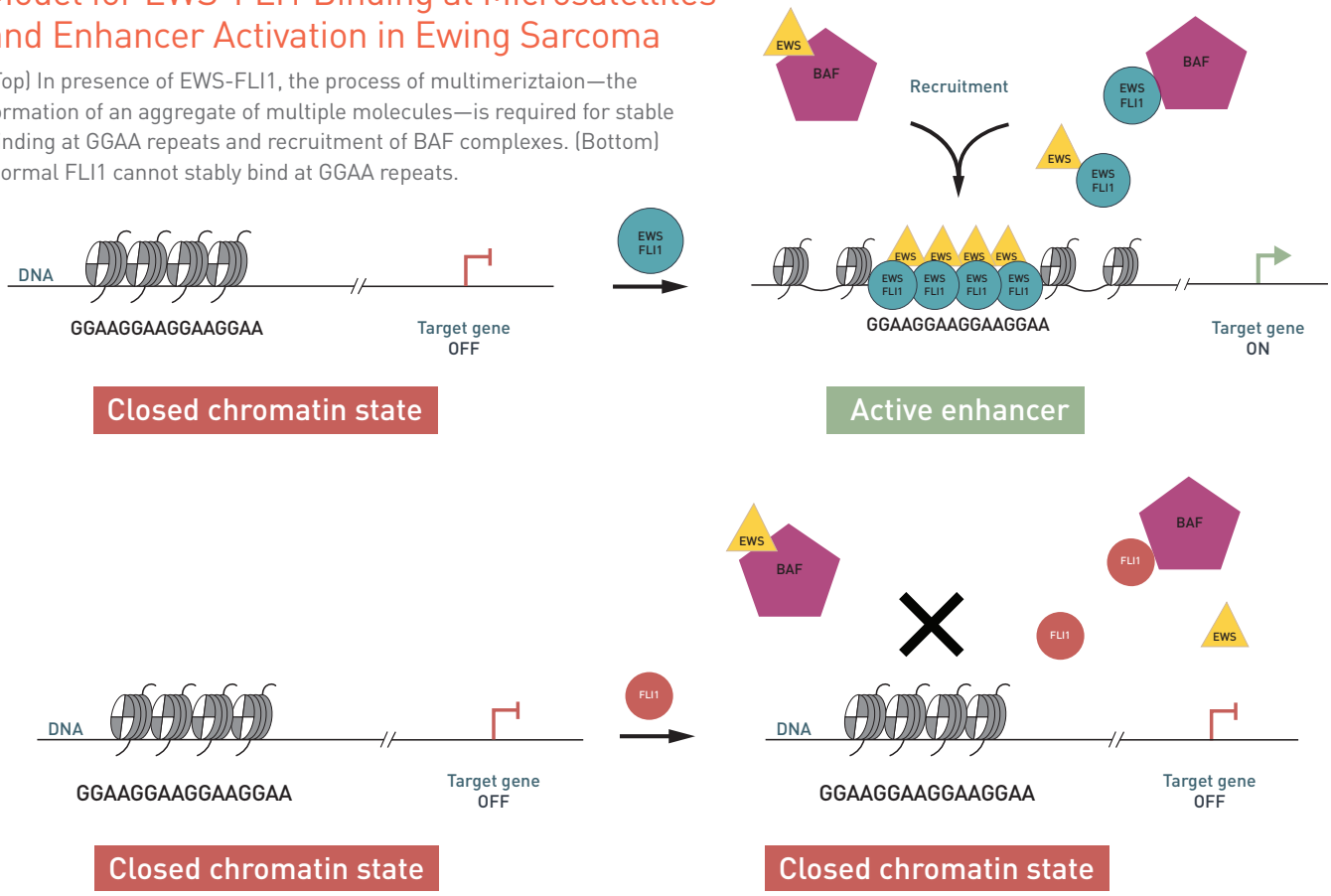
The phase transition properties of a prion-like domain in an oncogenic fusion protein called EWS-FLI1 are critical for remodeling chromatin complexes and activating GGAA enhancers, thereby driving the transcriptional program that promotes Ewing sarcoma.

RECRUITING THE BAF COMPLEX

In earlier research, Dr. Rivera's team found that the mutant EWS-FLI1 protein turns on repetitive, short sequences of DNA known as microsatellites, in this case GGAA repeats, by opening and modifying chromatin, a set of proteins that forms the packaging of DNA. These repeats become so-called active enhancers, which affect the expression of genes that are physically distant. The enhancers,

Model for EWS-FLI1 Binding at Microsatellites and Enhancer Activation in Ewing Sarcoma

(Top) In presence of EWS-FLI1, the process of multimeriztaion—the formation of an aggregate of multiple molecules—is required for stable binding at GGAA repeats and recruitment of BAF complexes. (Bottom) Normal FLI1 cannot stably bind at GGAA repeats.



they found, are only active in the presence of the EWS-FLI1 mutated protein and an Ewing sarcoma.

In their new study, the researchers found that activation of GGAA repeats is a novel property of the EWS-FLI1 fusion protein compared to the transcription factor FLI1 alone. In order to understand what accounts for this difference, Dr. Rivera's team worked with colleagues at the Kadoch laboratory at the Dana-Farber Institute to determine whether EWS-FLI1 is aided by the BAF complex in activating microsatellites. The BAF complex is critical to chromatin remodeling and dynamics, and is mutated in more than 20% of human cancers. Using mass spectrometry, they were able to show that BAF-remodeling complexes interact with normal EWS proteins in several cell types and with EWS-FLI1 fusion proteins in Ewing sarcoma. Furthermore, they found a striking overlap between BAF complexes and EWS-FLI1 sites genome-wide.

PHASE TRANSITION AND THE PRION-LIKE DOMAIN

Following these results, Dr. Rivera and his colleagues suspected that the phase transition properties of the EWS prion-like domain are instrumental in recruiting BAF complexes and other proteins that participate in the activation of GGAA repeats. Proteins that contain prion-like domains can promote phase transitions that allow for the formation of liquid compartments similar to non-membrane-bound organelles. The fact that the fusion of

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EWS is necessary for the activation of GGAA repeats suggested that this property of EWS may be important in the activity of EWS-FLI1.

To test their hypothesis, the lab made a number of tagged mutants of the EWS-FLI1 fusion protein that lacked the ability to undergo phase transition. They then introduced them into mesenchymal stem cells, which are a model for the cell of origin of Ewing sarcoma. Mutant EWS-FLI1 was unable to activate the GGAA enhancers or turn on the gene-expressing program of the tumor, said Dr. Rivera. The researchers also found that fusing small segments of the prion-like domain of the EWS protein to the FLI1 transcription factor was enough to enable phase transitions, GGAA repeat enhancer activation and changes in gene expression.

"Now that we understand that the phase transitions are important, we want to study more precisely how they lead to enhancer activation," said Dr. Rivera. "The BAF complex is one of several complexes that play important roles in activating enhancers. Understanding how EWS-FLI1 coordinates this process may reveal new targets for developing therapies." The findings have implications well beyond Ewing sarcoma, as EWS translocations are found in many kinds of cancers.

The Rivera group is now also working with the clinical team, led by Edwin Choy, MD, PhD, Clinical Director of the Center for Sarcoma and Connective Tissue Oncology and Director of Sarcoma Research at Mass General Cancer Center, and Gregory Cote, MD, PhD. The Center for Sarcoma and Connective Tissue Oncology specializes in the diagnosis and treatment of bone and soft tissue tumors, both malignant and benign. The sub-specialized team of experts, which includes pathologists, orthopedic surgeons, and medical and radiation oncologists focused on sarcoma, represents one of the largest sarcoma treatment groups in the country, caring for more than 400 patients per year. The program offers next day appointments, genetic sequencing for all patients with metastasis, and proton therapy for appropriate patients. The clinical team is dedicated to basic laboratory research, translational studies, and clinical trials all aimed at improving care for patients.

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