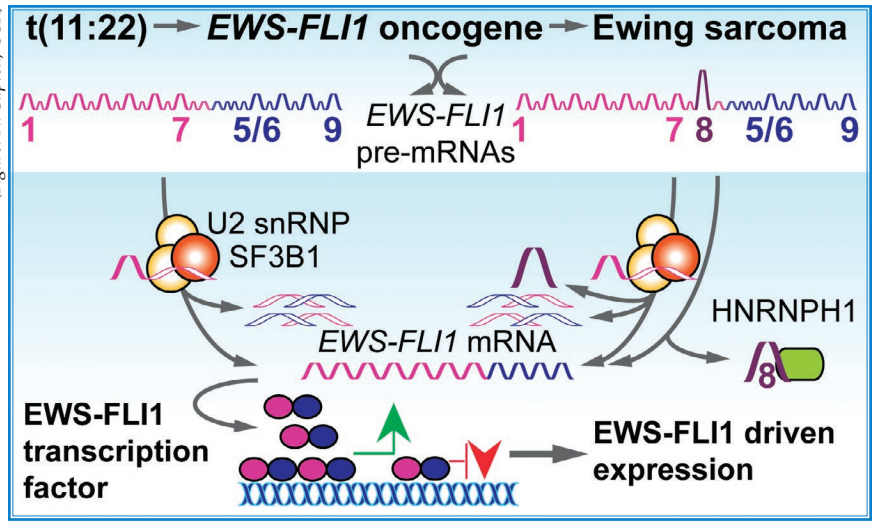
Defusing a Fusion Gene

Genome-wide RNAi screening discovers splicing is a vulnerability in Ewing sarcoma.

(Figure: N. Caplen, CCR)



Splicing factors SF3B1 and HNRNPH1 are critical for the correct expression of the Ewing sarcoma protein EWS-FLI1.

Pediatric oncologists know what causes the majority of Ewing sarcomas (ES), cancers of the bone and soft tissue: a chromosomal translocation that joins together the opening sequences of the *EWSR1* gene on chromosome 22 with the closing sequences of the *FLI1* gene on chromosome 11. The resulting gene expresses a fusion protein, EWS-FLI1, responsible for tumor initiation and maintenance. Despite this knowledge, targeted therapies for ES have lagged, because drugs against the EWS-FLI1 protein have proven difficult to develop.

To discover new targets for the treatment of ES, researchers from CCR's Pediatric Oncology Branch and Genetics Branch worked with a trans-NIH team at the National Center for Advancing Translational Sciences (NCATS) to conduct a genome-wide RNAi screen designed to identify genes needed for EWS-FLI1 activity. As recently described in *Cell Reports*, the researchers homed in on genes that, when silenced by RNAi, selectively reduced EWS-FLI1 activity. Among the activities ascribed to these genes, the most common were RNA splicing and RNA processing, including SF3B1, a component of the spliceosome and HNRNPH1, an alternative splicing factor. Follow-up studies, conducted by Postdoctoral Fellow Suntae Kim, Ph.D., Investigator Natasha Caplen, Ph.D., and colleagues in CCR's Genetics Branch, in collaboration with a former CCR Pediatric Oncology Fellow and Assistant Clinical Investigator, Patrick Grohar, M.D., Ph.D., focused on testing the hypothesis that the *EWS-FLI1* transcript is vulnerable to the loss of proteins required for specific steps in splicing.

Previous studies have characterized the exact position of the chromosomal breakpoints that occur in ES and the structure of the *EWS-FLI1* transcripts expressed in ES cells. When the breakpoint is within *EWSR1* intron 8, exon 8 of *EWSR1* must be removed by splicing to generate a *EWS-FLI1* mRNA that will encode the fusion protein. Caplen and colleagues established that HNRNPH1 is required for this splicing event. Depleting HNRNPH1 in ES cells with a breakpoint in *EWSR1* intron 8 blocked the expression and the activity of the EWS-FLI1 protein and reduced cell survival.

The researchers also determined that silencing *SF3B1* results in mis-splicing of *EWS-FLI1*, irrespective of the position of the breakpoints in either fusion gene partner. This mis-splicing disrupts expression of full-length EWS-FLI1 protein, resulting in a change in EWS-FLI1 activity; however, variant EWS-FLI1 proteins were also generated. These variant EWS-FLI1 proteins still need to be studied in more detail, but targeting SF3B1 in ES cells may be an interesting therapeutic approach as this study reported a substantial decrease in the growth of ES cells when a compound that inhibits spliceosome activity was used.

"Our goal was to identify therapeutic vulnerabilities in ES that might be more amenable to drug development. We found that the *EWS-FLI1* transcript is vulnerable to the loss of specific RNA-processing proteins, particularly those required to ensure the splicing of exons at and downstream of the fusion breakpoint," said Caplen. "This study opens up a potential strategy for the treatment of ES through disruption of the processing of the *EWS-FLI1* transcript itself."

To learn more about Dr. Caplen's research, please visit her CCR website at https://ccr.cancer.gov/genetics-branch/natasha-j-caplen.