OUTLOOK

Microsatellite enhancers can be targeted to impair tumorigenesis

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Dysregulation of repetitive elements has been implicated in many cancers and other human diseases; however, the role of repetitive elements remains largely unexplored. In this issue of *Genes & Development*, Boulay and colleagues (pp. 1008–1019) explore the ability of GGAA repeats to act as alternative enhancers activated by EWS-FLI1 in Ewing sarcoma and contribute to tumorigenesis. Using CRISPR-mediated epigenome editing, repression of EWS-FLI1 targeted microsatellite enhancers halted aberrant gene expression and impaired the growth of Ewing sarcoma xenografts in vivo. The study reveals the regulatory capacity of repetitive elements in cancer and offers insight into therapeutic targets for Ewing sarcoma.

Repetitive elements comprise ~50% of the human genome; however, due to the difficulty of studying these elements both experimentally and computationally, their roles in gene expression remain largely unexplored (Lander et al. 2001). Recent studies have shown that dysregulation of repetitive elements is associated with cancers and other diseases; however, their contribution to disease progression has been unclear (Burns 2017).

In Ewing sarcoma, the second most common pediatric bone cancer, active enhancer-like GGAA repeats are a hallmark of the disease (Gangwal et al. 2008). Ewing sarcoma is commonly characterized by a chromosomal translocation that creates the fusion protein EWS-FLI1 from a fusion of the *EWSR1* gene and the *FLI1* transcription factor gene (Delattre et al. 1992). In Ewing sarcoma, EWS-FLI1 recruits the BRG1/BRM-associated factor (BAF) chromatin remodeling complex to tumor-specific enhancers and activates target genes (Boulay et al. 2017). The fusion protein gains the ability to activate GGAA microsatellite repeat elements as aberrant enhancers, induces changes in global H3K27ac, and reprograms enhancers genome-wide (Riggi et al. 2014). While the GGAA microsatellite regions acquire H3K4me1 and H3K27ac (Tomazou et al. 2015), which are epigenetic marks of active enhancers, the functional consequences on gene expression and tumorigenesis resulting from these changes remain unexplored.

In this issue of *Genes & Development*, Boulay et al. (2018) sought to understand how the usage of these alternative microsatellite enhancers can promote tumorigenesis and present an approach for selectively silencing repetitive elements. The investigators first confirmed the nascent activity of EWS-FLI1-bound GGAA repeat enhancers with NRO-seq (nuclear run-on experiments [NRO] combined with high-throughput sequencing), which confirmed the presence of repeat-derived enhancer RNAs that were sensitive to a knockdown of EWS-FLI1. They also silenced the GGAA repeats by targeting a CRISPR-based dCas9-KRAB, which led to local chromatin deacetylation and deposition of H3K9me3. By targeting repeat enhancers acting upstream of critical Ewing sarcoma genes, including *SOX2* and *NKX2-2*, they were able to greatly reduce both transcript and protein levels of these targets while avoiding disruption of neighboring repeat-associated genes. These findings demonstrate the ability to epigenetically target and silence specific GGAA repeats.

The investigators also sought to confirm the specificity of EWS-FLI1-bound GGAA repeat enhancers usage to Ewing sarcoma and indeed were able to confirm this by silencing these repeats in the NCI-H810 lung cancer cell line, which lacks EWS-FLI1. Upon dCas9-KRAB-mediated silencing of the repeats in NCI-H810 cells, no epigenetic marks of active enhancers were observed at the repeats, and no changes were observed in NKX2-2 or SOX2 transcript or protein levels. The investigators also showed that in mesenchymal stem cells where the NKX2-2 GGAA repeat enhancer had been initially silenced with dCas9-KRAB, when subsequently transduced with EWS-FLI1, NKX2-2 induction was greatly impaired. However, expression of other genes targeted by EWS-FLI1 remained unchanged. Thus, EWS-FLI1 can convert silent GGAA repeats to active enhancers, and the GGAA enhancers are active only in Ewing sarcoma.

[*Keywords*: Ewing sarcoma; pediatric cancer; epigenetics; EWS-FLI1; enhancer therapy; epigenome editing]
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Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.318105.118.

Importantly, the investigators explored the effects of silencing GGAA repeats on tumorigenesis in vivo. In A673 and SKNMC Ewing sarcoma cell lines, they targeted and silenced an intergenic GGAA repeat enhancer that regulates, from a distance of 470 kb, the *SOX2* locus, a gene critical for oncogenesis in Ewing sarcoma. The human cells were then injected into NOD-*scidγ* (NSG) mice. The xenografts derived from these cancer cells exhibited striking reductions in both weight and volume compared with xenografts that lacked enhancer silencing. These findings show that the activation of the GGAA repeat enhancers by EWS-FLI1 significantly contributes to tumor growth in vivo.

In summary, the study from Boulay et al. (2018) showed that GGAA microsatellite enhancers that are activated by EWS-FLI1 act as critical regulators of the oncogenic program in Ewing sarcoma and that they can be selectively inhibited through epigenome-editing techniques. The study adds a significant example of the ability of repetitive elements to regulate gene expression and demonstrates how cancer cells can use novel regulatory networks to promote tumorigenesis. The approach to silence specific repetitive elements using epigenome editing ultimately impacted Ewing sarcoma tumor growth. Further research will be able to determine the applicability of these findings and approaches to additional cancer types.

Acknowledgments

Relevant work in the our laboratory is supported by National Institutes of Health (NIH) T32 HD083185 to K.E.K. and NIH 2P01GM099134 to K.S.Z.

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