

Ewing sarcoma fusion oncogene: At the crossroads of transcription and DNA damage response

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Ewing sarcoma is an aggressive pediatric cancer that is driven by a fusion oncogene, *EWS-FLI1*. While the transcriptional targets of *EWS-FLI1* have been widely studied, the systemic impact of *EWS-FLI1* transcription is relatively less understood. Here, we comment on our recent findings, published in *Nature*, regarding *EWS-FLI1* driven transcription dysregulation and its impact on DNA damage response, *BRCA1* function and R-loop accumulation.

Ewing sarcoma is the prototypical example of a solid tumor that is driven by chimeric oncogenes derived from chromosomal translocation. In over 85% of patients, the translocation is between chromosomes 22 and 11, fusing the N-terminal transactivation domain of the EWS RNA binding protein 1 (*EWSR1*) gene to the C-terminal DNA binding domain of the weak transcription factor Fli-1 proto-oncogene, ETS transcription factor (*FLI1*) gene.¹ The resultant *EWS-FLI1* oncogene is a highly expressed transcription factor that modulates both gene and protein-regulatory networks to drive tumor initiation and maintenance. Despite the presence of this unique genetic event, conventional chemotherapy remains the treatment of choice for Ewing sarcoma. While effective in a significant number of cases, the long-term ramifications of the aggressive treatment regime necessitate understanding the basis of chemosensitivity and identifying alternative therapeutic targets.² We recently published in *Nature*³ that *EWS-FLI1* drives increased global transcription to cause a widespread accumulation of R-loops. This provides an obvious explanation to the increased replication stress that we and others have observed. However, we also chanced upon some unusual observations that provide insight into the PARP1 inhibitor sensitivity and mechanisms of chemoresistance.

A new class of BRCAness tumors

DNA damage induces profound changes to the transcriptional landscape, with both a targeted transcriptional program and a global shut down of general transcription.⁴ We

provide evidence of an active link between transcription stress and recombinational repair; *EWSR1* dependent recruitment of the BRCA1, DNA repair associated (*BRCA1*) protein from transcription complexes to sites of damage is necessary for efficient homologous recombination. We speculate that in Ewing sarcoma, *BRCA1* is retained not only at canonical target gene loci but also in *EWS-FLI1* driven genes, further depleting *BRCA1* availability. However, the mechanism by which this occurs remains an open question. Work in the 1990s demonstrated that *BRCA1* is associated with the active transcription complex⁵ and was later shown to release from this complex following DNA damage. Without *EWSR1*, or in the presence of *EWS-FLI1*, damage-induced global transcription shut down does not occur, and *BRCA1* cannot relocate to sites of damage.

BRCA1 retention with active transcription complexes in Ewing sarcoma precludes its participation in homologous recombination, thereby explaining the acute sensitivity to poly (ADP-ribose) polymerase 1 (*PARP1*) inhibitors.⁶ Therefore, this study also reveals a new class of *BRCA1* deficient tumors that could benefit from *PARP1* inhibitor treatments. However, chemoresistance mechanisms that arise in *BRCA1* deficient carcinomas and circumvent the need for *BRCA1* promoting recombination would also likely confer both chemotherapy and *PARP1* inhibitor resistance in Ewing sarcoma. In fact, depletion of tumor protein p53 binding protein 1 (*TP53BP1*) both restores homologous recombination despite the presence of *EWS-FLI1*, and confers chemotherapy resistance to Ewing sarcoma cells. These observations may explain the lack of response observed in single agent *PARP1* inhibitor clinical trials which were conducted with relapsed, chemoresistant patients.⁷

EWS-FLI1: Dominant-negative over *EWSR1*

The *EWS-FLI1* fusion protein is known to drive an oncogenic transcriptome, but here we demonstrate that it also interferes with *EWSR1* function, thereby contributing to Ewing sarcoma pathogenesis. The most extensive knowledge

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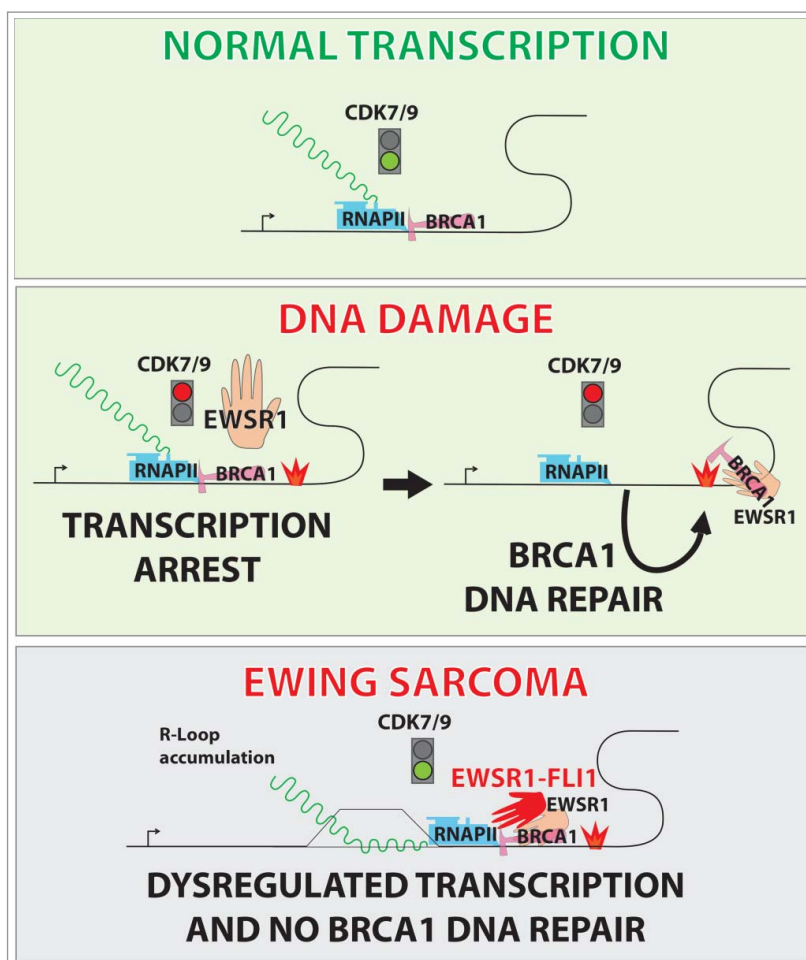


Figure 1. Altered control of transcription in Ewing sarcoma. Transcription elongation is triggered by cyclin dependent kinase 9 (*CDK9*) dependent phosphorylation of RNA Polymerase II (*RNAPII*) C-terminal domain. *EWS* RNA binding protein 1 (*EWSR1*) inhibits this phosphorylation event thereby controlling transcription rates. *BRCA1*, DNA repair associated protein (*BRCA1*) is associated with hyper-phosphorylated *RNAPII* under basal conditions but is released upon infliction of DNA damage to sites of break repair. However in Ewing sarcoma, *EWS-FLI1* allows aberrant transcription by lifting *EWSR1* dependent inhibition of *CDK9* activity resulting in R-loop accumulation. This, in turn, prevents *BRCA1* release from transcription complexes thus impairing homologous recombination repair of exogenously induced double strand breaks.

of *EWSR1* function stems from protein interaction studies that suggest a bridging function between transcription, RNA metabolism and genome surveillance.⁸ There are mixed opinions in the field regarding its proto-oncogenic versus tumor suppressor role. We observed that *EWSR1* inhibits the activation of RNA Polymerase II whereas *EWS-FLI1* promotes the same, even in the presence of wild type *EWSR1*. Biochemical data indicated this was via the regulation of cyclin dependent kinase 9 (*CDK9*) (Fig. 1). These findings indicate a potential dominant-negative function of *EWS-FLI1* over *EWSR1*. We also report that *EWSR1* inhibits R-loop accumulation and facilitates homologous recombination. *EWSR1* may be important for the release of *BRCA1* from those transcribed genes with which it associates, and the concerted involvement of both proteins is essential for efficient recombination upon damage.

Lack of genome instability

One paradoxical observation from our work is that R-loops are thought to promote mutagenesis,⁹ but genomic instability is

largely absent in Ewing sarcoma,¹⁰ with an average of five mutations found per primary tumor beyond the translocation. This is further compounded by the presence of high levels of replication stress and the lack of homologous recombination. In fact, the high levels of transcriptional stress, replication stress and the lack of homologous recombination conferred by *EWS-FLI1* explain why expression of this oncogene is highly toxic to almost every cell type. This leads to a key question in the field regarding the cell-of-origin of Ewing sarcoma. It will be interesting to ask if neural crest stem cells or bone marrow mesenchymal stem cells, the putative cells of origin, provide a special molecular context that is permissive to the increased R-loops, replication stress and homologous recombination deficiency. Irrespective, these findings raise significant questions about the consequences of these defects, the associated genome instability and whether genomic instability in of itself is important for cancer development in all instances, or a secondary consequence in some cases. It is also important to note that genomes of tumors that survive chemotherapy are not stable. This suggests a “ceiling-effect” for the damage tolerance of Ewing sarcoma.

In conclusion, the present study highlights the contribution of *EWSR1* loss to tumorigenesis and the unexpected outcomes of elevated R-loops in Ewing sarcoma. Pediatric cancers have comparatively stable backgrounds. This gives us the opportunity to study the biological processes that contribute to tumorigenesis or present therapeutic targets in relative isolation. We believe that Ewing sarcoma stands as an ideal system to study key processes involved in cancer biology without confounding mutations. Overall this work raises more questions than answers, and we hope that our findings will lead to asking the right questions to better understand this disease as well as identifying novel treatment strategies.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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