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HEMATOPOIESIS AND STEM CELLS

# *SLFN11* promotes stalled fork degradation that underlies the phenotype in Fanconi anemia cells

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KEY POINTS

- **A DNA damage sensitizing gene *SLFN11* promotes stalled fork degradation caused by DNA2 and MRE11 nucleases by inhibiting RAD51 accumulation.**
- **Suppression of *SLFN11* in Fanconi anemia cells attenuates FA phenotypes such as chromosomal breakages or cell cycle arrest on DNA damage.**

**Fanconi anemia (FA) is a hereditary disorder caused by mutations in any 1 of 22 FA genes. The disease is characterized by hypersensitivity to interstrand crosslink (ICL) inducers such as mitomycin C (MMC). In addition to promoting ICL repair, FA proteins such as RAD51, BRCA2, or FANCD2 protect stalled replication forks from nucleolytic degradation during replication stress, which may have a profound impact on FA pathophysiology. Recent studies showed that expression of the putative DNA/RNA helicase SLFN11 in cancer cells correlates with cell death on chemotherapeutic treatment. However, the underlying mechanisms of SLFN11-mediated DNA damage sensitivity remain unclear. Because *SLFN11* expression is high in hematopoietic stem cells, we hypothesized that *SLFN11* depletion might ameliorate the phenotypes of FA cells. Here we report that *SLFN11* knockdown in the FA patient-derived *FANCD2*-deficient PD20 cell line improved cell survival on treatment with ICL inducers. *FANCD2*$^{-/-}$*SLFN11*$^{-/-}$ HAP1 cells also displayed phenotypic rescue, including reduced levels of MMC-induced chromosome breakage compared with *FANCD2*$^{-/-}$ cells. Importantly, we found that *SLFN11* promotes extensive fork degradation in *FANCD2*$^{-/-}$ cells. The degradation process is mediated by the nucleases MRE11 or DNA2 and depends on the SLFN11 ATPase activity. This observation was accompanied by an increased RAD51 binding at stalled forks, consistent with the role of RAD51 antagonizing nuclease recruitment and subsequent fork degradation. Suppression of *SLFN11* protects nascent DNA tracts even in wild-type cells. We conclude that *SLFN11* destabilizes stalled replication forks, and this function may contribute to the attrition of hematopoietic stem cells in FA. (*Blood*. 2021;137(3):336-348)**

## Introduction

The *Schlafen* (*SLFN*) family was first described as a set of homologous genes that are involved in T-cell development and inhibit cell growth.[1] These genes are found only in mammals and a small number of nonmammalian species.[2] A member of the SLFN family, SLFN11, is a putative DNA/RNA helicase ubiquitously expressed in the human body.[3] Notably, its expression is often lost in primary cancers and in commonly used cancer cell lines by epigenetic silencing.[4,5] Recent studies indicated that SLFN11 expression in cancer correlate with a favorable response to widely used anticancer drugs, such as irinotecan, cisplatin, etoposide, and poly(ADP-ribose) polymerase inhibitors, with a better prognosis for the patients.[5-7] *SLFN11* expression has the strongest association among the DNA repair proteins with the sensitivities to DNA damaging cancer chemotherapy drugs but not to non-DNA damaging agents.[8] It has also been reported that SLFN11 associates with replication protein A (RPA) and negatively regulates RPA loading to chromatin, thereby disfavoring homologous recombination (HR) repair.[9] Another study found that SLFN11 accumulates at stalled replication forks and blocks replication.[10] SLFN11 may also affect protein translation by cleavage of a specific group of tRNAs, resulting in the abrogation of ATR kinase expression during the DNA damage response.[11,12] These studies define SLFN11 as a guardian of the genome that controls cell fate decisions in response to DNA damage and replication stress. However, how *SLFN11* exerts this function remains unclear.

Fanconi anemia (FA) is a rare hereditary disorder that is caused by mostly recessive mutations in any 1 of 22 FA genes identified thus far (*FANCA-W*), leading to hematopoietic stem cell failure and cancer predisposition.[13,14] FA proteins act in the common FA pathway to repair interstrand crosslink (ICL) damage, and therefore FA cells are hypersensitive to ICL-inducing agents such