**Novel Fanconi anemia E3 ligase RFWD3 promotes removal of both RPA and RAD51 from DNA damage sites during ICL repair**

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The Fanconi anaemia (FA) pathway repairs DNA interstrand crosslinks (ICLs) in the genome, and 20 responsible genes have been identified so far. Identification of a novel FA gene would provide a great opportunity to further unveil the mechanism of ICL repair. The whole exome sequencing in a German FA patient has identified biallelic mutations (p.L69Pfs12X and p.I639K) in relatively uncharacterized ring-finger E3 ligase gene, *RFWD3*. The patient is hematologically stable with modestly reduced bone marrow cellularity. The E3 ligase activity of RFWD3 toward its interactor RPA has been shown to be critical for homologous recombination (HR) at stalled replication forks (Elia et al. Mol Cell 2015). However, how RFWD3-mediated ubiquitination affects HR remains unclear.

Here we identified RAD51, the central HR player, as another target of RFWD3 ubiquitination. We show that MMC triggers ATR/ATM-dependent RFWD3 phosphorylation that is necessary for polyubiquitination of RPA and RAD51. RFWD3 interacts with and polyubiquitinates both RPA and RAD51 *in vitro* and *in vivo*. Following MMC treatment, RFWD3 promotes degradation of RPA and RAD51 and VCP/p97-mediated protein turnover in their foci. Depletion of *BRCA2* impairs RPA ubiquitination and *RFWD3* functions epistatically with *BRCA2* in RPA foci turnover. Furthermore, MMC-induced chromatin loading of both RAD54 and MCM8 are defective in *RFWD3*-deficient cells. Collectively, we propose that timely removal of RPA and RAD51 from DNA damage sites by RFWD3 ubiquitination is a critical step for the late phase HR to proceed.