

Functional Interaction between FANCD2 and PCNA via a Conserved PCNA-interaction Motif

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Objective: The objectives of this study were to a) determine if the FANCD2 protein interacts with key components of the DNA replisome and b) to establish a framework for how the FANCD2 protein is recruited to sites of DNA damage.

Methods: To address these questions, we have characterized the interaction between FANCD2 and the Proliferating Cell Nuclear Antigen (PCNA), the major cellular DNA polymerase processivity factor. PCNA is known to act as a mobile reaction platform for several DNA repair enzymes. We have identified a highly conserved PCNA-interaction motif (PIP-box) in FANCD2. Using PCR-mediated site-directed mutagenesis, we have mutated the critical FANCD2 PIP-box amino acids, and introduced mutant FANCD2 PIP394 into the FA-D2 patient-derived cell line, PD20F.

Results: Unlike wild-type FANCD2, FANCD2 PIP394 fails to efficiently interact with PCNA, as demonstrated by immunoprecipitation analyses and immunofluorescence microscopy. However, FANCD2 PIP394 retains the ability to interact with the FA core complex component FANCE. Significantly, FANCD2 PIP394 fails to become mono-ubiquitinated spontaneously as well as following exposure to DNA damaging agents. Furthermore, FANCD2 PIP394 fails to accumulate in discrete nuclear foci. However, like wild-type FANCD2, FANCD2 PIP394 is phosphorylated following exposure to X- and UV-irradiation. Finally, FANCD2 PIP394 fails to correct the MMC sensitivity, MMC-induced G2 accumulation, and the elevated DNA damage-induced chromosome breakage of FA-D2 patient-derived fibroblasts.

Conclusions: Our results strongly suggest that the FANCD2 protein associates with PCNA in a DNA damage surveillance capacity. Thus, PCNA promotes the localization of FANCD2 to sites of DNA damage, and functions as a molecular platform to facilitate the mono-ubiquitination of the FANCD2 protein during the DNA damage response.

Translational Applicability: The identification of critical functional domains of FANCD2 could lead to the design of specific FA pathway inhibitors, which could be used for adjuvant chemotherapy. Furthermore, a greater knowledge of the endogenous cellular function of the FA pathway will hopefully lead to improved preventative, *e.g.* nutritional supplementation, and therapeutic approaches to FA.