USP1 Maintains Replication Fork Stability In BRCA1-Deficient Tumour Cells

Homologous-recombination (HR) deficient tumors with BRCA1 and BRCA2 mutations frequently exhibit replication fork stability defects. To date, PARP inhibitors are the only targeted therapy available in the clinic against HR deficient tumors. In this study, we found a deubiquitinase, USP1, to be significantly upregulated in tumors with mutations in BRCA1. SiRNA mediated silencing or small molecule inhibition of USP1 resulted in fork destabilization and decreased viability of BRCA1 deficient cells. The cofactor of USP1, UAF1, had previously been shown to have DNA-binding activity. USP1 independently binds to and is stimulated by fork DNA and is the first known deubiquitinase to be directly regulated by DNA binding. A truncated form of USP1, lacking its DNA binding region, was not stimulated by DNA and failed to localize and protect the replication fork. Persistence of monoubiquitinated PCNA at the replication fork was the mechanism of fork destabilization and cell death. Loss of monoubiquitinated PCNA, resulting from RAD18 siRNA, rescued the sensitivity and replication fork instability induced by USP1 inhibition. USP1 therefore is the first DUB enzyme exhibiting DNA-mediated activation at the replication fork, and is important in fork protection in BRCA1 deficient cells. We propose that small molecule inhibitors against USP1 could be used as a potential therapeutic option in BRCA1 deficient cancers.