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SUMO conjugation and proteolysis regulate cell sensitivity to DNA topoisomerase I levels and poisons

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Abstract

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DNA topoisomerase I (Top1) constitutes the cellular target of camptothecin (CPT), which stabilizes a covalent Top1-DNA intermediate. During S-phase the CPT-Top1-DNA complexes are converted into lethal lesions that induce cell cycle arrest and cell death. To define cellular processes that protect cells from CPT-induced lesions, conditional mutants with enhanced sensitivity to Top1 poisons were isolated in yeast genetic screens. In each case, a self-poisoning Top1 mutant was used to avoid drug transporters: Top1N726H exhibits increased rates of DNA cleavage, while Top1T722A mimics the action of CPT by inhibiting DNA religation. Despite mechanistic differences in Top1 poisoning, the same hypomorphic allele of *UBC9*, *ubc9P123L*, was isolated that enhances sensitivity to Top1 poisons at 35°C. The essential gene *UBC9* encodes a unique SUMO E2 enzyme that conjugates the ubiquitin-like protein SUMO (Smt3 in yeast) to lysine residues in target proteins. Sumoylation alters protein activity, sub-cellular localization or ubiquitin-mediated degradation. SUMO is recycled for new rounds of sumoylation by the yeast Ulp1 and Ulp2 proteases. In *ubc9P123L*, a Pro123 to Lys substitution reduces the levels of SUMO conjugates detected at 35°C. This corresponds to enhanced cell sensitivity to Top1 poisons as well as a wide range of DNA damaging agents, independent of Top1. These results suggest a higher threshold of Ubc9 activity is required to maintain cell viability in the presence of genotoxic agents. In support of this interpretation, the *ubc9P123L* mutant complemented the temperature sensitive phenotype of cells deleted for the Ulp2 SUMO protease, yet *ubc9P123L, ulp2*Δ cells remained hypersensitive to hydroxyurea and Top1 poisons. Thus, compensatory alterations in SUMO conjugation were insufficient to alter cell sensitivity to DNA damaging agents, which suggests specific alterations in Ubc9 substrate specificity are induced by the *ubc9P123L*. Indeed within the structure of human Ubc9, the conserved Pro123 residue lies over the catalytic pocket of the enzyme and in a loop immediately N-terminal to residues implicated in substrate binding. As a Pro123 to Ala mutation had no effect on Ubc9 activity in vivo, structural perturbations in Ubc9P123L seem unlikely. A slow growth phenotype of *ulp2*Δ strains at 30°C was also detected, which was suppressed by deletion of *TOP1*. These strains did not tolerate increases in Top1, independent of Ubc9 function. In contrast to the results obtained with the *ubc9P123L* mutant, these data indicate that the accumulation of SUMO conjugates suffices to convert wild-type Top1 into a cellular poison. Whether this is a direct consequence of Top1 sumoylation has yet to be determined. Nevertheless, diverse effects on SUMO conjugation, induced by defects in Ubc9 or a SUMO specific protease, alter the cytotoxic consequences of Top1 activity. This work was supported by NIH grant CA58755 and ALSAC.

Footnotes

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