UNIVERSITY OF
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Dissertation Seminar

MECHANISMS OF RAD51D-DEPENDENT REPAIR OF DNA AND TELOMERE DAMAGE INDUCED BY INTERSTRAND CROSSLINKING AGENTS AND THIOPURINES

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Mutations in homologous recombination (HR) genes increase genomic instability, an enabling characteristic of cancer. However, the status of these same genes can also determine chemotherapy outcomes. RAD51D is a breast and ovarian cancer susceptibility gene that is an important component of HR. Mammalian cells defective for RAD51D have extensive chromosomal aberrations, and are more sensitive to the interstrand crosslink-inducing agent mitomycin C (MMC) and the thiopurine 6-thioguanine (6TG). Previously, the RNF138 E3 ubiquitin ligase was identified to promote RAD51D ubiquitination, and loss of RNF138 also increased cellular sensitivity to MMC. Ubiquitination assays were used to show that a 3-ubiquitin modification occurs along the RAD51D wild-type protein. To identify potential sites of ubiquitination, amino acid substitutions were generated at all thirteen lysine residues along RAD51D. Arginine substitutions at Lys235 (K235R) and Lys298 (K298R) were found to confer cellular sensitivity to MMC. In addition, protein stability of K235R and K298R were 2 to 3-fold higher as compared with wild-type RAD51D. Current data also suggest that neither Lys235 nor Lys298 are required for homology-directed repair of DNA double strand break damage, implying that these residues are necessary specifically for RAD51D-dependent ICL repair.

RAD51D is also known to contribute to telomere maintenance, although its precise function at the telomeres remains unclear. In this dissertation, I investigated the activity of RAD51D at telomeres and the contribution of RAD51D to protect against 6TG-induced telomere damage. As measured by γ-H2AX induction and foci formation, the extent of γ-H2AX telomere localization following 6TG treatment was higher in *Rad51d*-deficient cells than in *Rad51d*-proficient cells. In the final portion of this dissertation, *Rad51d*-deficient cells were used as a model for genome unstable mammalian cells to identify genetic compromises that support cell proliferation. Gene expression profiles of *Rad51d*-proficient and -deficient primary mouse embryonic fibroblasts were analyzed by microarray and RNA Seq. In both analyses, the highest proportion of genes were associated with cellular growth and proliferation. In summary, the data presented in this dissertation identified potential regulatory sites along RAD51D that mediate its function during ICL repair, elucidated the role of RAD51D in maintaining telomere integrity in the presence of thiopurine-induced DNA damage, and revealed genetic compromises in *Rad51d*-deficient cells that promote cell proliferation.