c-ABL tyrosine kinase stabilizes RAD51 chromatin association

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HRR is a major pathway for the resolution of DNA double-strand breaks (DSBs) in the somatic cells of higher eukaryotes. HRR is mediated by RAD51, the eukaryal orthologue of bacterial RecA. A key step in HRR involves the assembly of RAD51 onto DNA substrates at the site of DNA breakage to form an ordered, helical nucleoprotein filament, which catalyzes homologous pairing and the strand exchange reaction. In vertebrate cells of avian, rodent or human origin, RAD51 assembly is marked in cells by the formation of nuclear foci containing RAD51. An important unresolved question concerns the nature of the signaling process that triggers RAD51 assembly at sites of DNA breakage. A c-ABL, ubiquitously expressed non-receptor-type tyrosine kinase, is activated by ionizing radiation (IR) in an ATM-dependent manner and plays important roles in growth arrest and cell death. The results of previous studies indicate that c-ABL is involved in HRR through the phosphorylation of RAD51. We provide evidence that c-ABL, a tyrosine kinase activated by DNA damage which phosphorylates RAD51 on Tyr-315, works at a previously unrecognized, proximal step to initiate RAD51 assembly. We first show that c-ABL associates with chromatin after DNA damage in a manner dependent on its kinase activity. Using RAD51 mutants that are unable to oligomerize to form a nucleoprotein filament, we separate RAD51 assembly on DNA to form foci into two steps: stable chromatin association followed by oligomerization. We show that phosphorylation on Tyr-315 by c-ABL is required for chromatin association of oligomerization-defective RAD51 mutants, but is insufficient to restore oligomerization. Our findings suggest a new model for the regulation of early steps of HRR. We also found that imatinib, a specific inhibitor for the c-ABL tyrosine kinase, effectively inhibited c-ABL-mediated enhancement of RAD51 chromatin association, raising a possibility that the inhibition of RAD51 functions is one of mechanisms by which imatinib exerts its anti-cancer activity as a molecular targeting drug. Our work thus provides useful information for future clinical application of imatinib and related compounds as direct and/or adjuvant therapeutic drugs for haematopoietic malignancies and solid d tumors.