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A new development in DNA repair modulation

Discovery of a BLM helicase inhibitor

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Bloom's syndrome (BS) is a rare autosomal recessive genetic disorder characterized by predisposition to a wide variety of cancers observed in the normal population.[1] The *BLM* gene defective in BS encodes a RecQ DNA helicase (BLM) that is important for genomic stability by suppressing sister chromatid exchanges (SCE) that arise during homologous recombination (HR).[2] In fact, SCE frequency of patient cells is used for clinical diagnosis of BS. BLM helicase is believed to suppress SCEs by channeling DNA molecules away from pathways leading to crossover products through its DNA unwinding function and interaction with protein partners (e.g., human topoisomerase IIIα).[3]

Targeting DNA helicases for therapeutic purposes has attracted interest, with the discovery of other DNA repair inhibitors, highlighted by poly(ADP)ribosylase (PARP) inhibitors used in synthetic lethal approaches to attenuate carcinogenesis in HR-defective BRCA1/2-deficient tumors.[4] Small molecules (< 800 Daltons) can penetrate cell membranes and represent a potentially suitable class of compounds for therapeutic use, such as anticancer drugs. In the January 24, 2013 issue of *Chemistry and Biology*, Nguyen et al. reported their discovery of a small-molecule inhibitor of BLM helicase.[5] From a high-throughput screen of a chemical compound library and medicinal chemistry optimization, a small molecule (ML216) was identified that inhibited BLM helicase activity on a forked duplex DNA substrate in vitro (IC_{50} ~3 μM) by preventing BLM binding to DNA.[5] Cultured human fibroblasts exposed to ML216 (50 μM) displayed reduced proliferation, a statistically significant increase in SCE frequency, and elevated sensitivity to aphidicolin, an inhibitor of replicative DNA polymerases. The specificity for ML216 targeting BLM in cell-based experiments was suggested, because BLM-deficient cells were resistant to the phenotypic effects of ML216.

The BLM helicase inhibitor discovery may provide a new strategy for understanding molecular functions of BLM required for its role in chromosomal stability, and also potential development of a new class of chemotherapy drugs to treat tumors that rely heavily on BLM for proliferation. From a biochemist's perspective, it is intriguing that ML216 potently inhibited BLM unwinding of a forked DNA duplex substrate but only modestly affected unwinding of other DNA substrates (G-quadruplex, Holliday Junction, or plasmid-based D-loop) at much higher concentrations of drug.[5] The specificity of ML216 (and conceivably other helicase inhibitors) may allow an experimental approach to dissect molecular requirements of the helicase for its role(s) in genome stability. Although ML216 inhibited unwinding by the sequence-related BLM and WRN helicases similarly in vitro, the apparent dependence on BLM for ML216 to exert its biological effects in human cells suggests BLM specificity for the drug's mechanism of action in vivo. A co-crystal structure of BLM in complex with inhibitor would be informative. Cellular cues in vivo may induce a specific conformation of WRN that makes it resistant to ML216. Direct or water-mediated contacts of the small molecule with poorly conserved amino acid residues of BLM that are distal in the primary structure but proximal in the tertiary structure may be critical for drug action.

Other studies reporting pharmacological inhibition of DNA repair protein function have also shown a dependence on target protein for the small molecule's cellular effect. An inhibitor of WRN helicase (NSC 19630) was discovered that inhibited proliferation and induced DNA damage and apoptosis in human cancer cells in a WRN-dependent manner.[6] Although the mechanism of action whereby NSC 19630 interferes with critical function(s) of WRN at the cellular level is unknown, there are several avenues to investigate. The WRN-inhibitor drug complex may prevent WRN from interacting favorably with its protein partners or cause formation of a static protein-DNA complex that is deleterious to normal biological DNA transactions. Since NSC 19630 exerted only a marginal effect on DNA-dependent WRN ATPase or exonuclease activity in vitro at very high drug concentrations,[6] WRN inhibitor is likely to operate by a mechanism distinct from that of the BLM inhibitor, which adversely affected BLM DNA binding and DNA-dependent ATPase activity at relatively low drug concentrations.[5] Our current hypothesis is that the biological effects of NSC 19630 may at least partly reflect an inactive WRN helicase-drug complex trapped on DNA repair or replication intermediates. Further studies will be necessary to determine if this is the case. However, a recent study of clinical PARP inhibitors that operate in a PARP-dependent manner hinted at a provocative scenario. Small molecule inhibition of PARP1 or PARP2 became more cytotoxic than genetic depletion of PARP by causing PARP to become trapped on DNA at damaged sites.[7] This finding suggests a reasonable mechanism

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for a class of DNA helicase inhibitors (like NSC 19630), but more research is necessary.

Understanding the mechanisms of DNA repair inhibitors has potential clinical significance. Chemo- and radiotherapy approaches to combat cancer are largely based on introducing DNA damage leading to double-strand breaks (DSB). Recently, a small-molecule inhibitor (SCR7) of DNA ligase IV responsible for nonhomologous end-joining (NHEJ) was discovered and found to inhibit NHEJ in a ligase IV-dependent manner,[8] reminiscent of the helicase and PARP inhibitors discussed above. Importantly, SCR7 impeded tumor progression in mouse models.[8] Hopefully, further research and clinical applications for helicase inhibitors will prove to be promising.

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