Discovering Characteristics of DNA Helicase B: A Promising Target for Cancer Therapy

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Helicases are enzymes that unwind double-stranded DNA during replication and repair. Human DNA helicase B (HELB) is critical for proper genome maintenance and recovery from replication stress. The importance of its function is underscored by the fact that high expression of HELB increases mortality in cancer patients. It has been observed that each helicase has a distinguishable preference for specific substrate characteristics; however, these characteristics have not been reported for HELB. Duplex DNA unwinding assays were used to measure unwinding of forked and non-forked duplexes over time to determine the preferred substrate of HELB. We demonstrate that human HELB has a strong preference for unwinding a forked duplex relative to a non-forked duplex. Additionally, an unwinding assay was performed with duplexes containing varying length single-stranded DNA overhangs. We conclude that longer single-stranded DNA overhangs increase duplex unwinding via HELB. The preference for a forked duplex and for a longer single-stranded DNA overhang was confirmed by DNA binding assays. Electrophoretic mobility shift assays were performed to determine the binding site size of one molecule of HELB; we found this size to be 10-13 nucleotides for HELB. Additionally, a plate based unwinding assay for HELB was optimized. This assay can be used to test HELB inhibitors, which would provide novel targets for cancer therapeutics. Knowledge of the preferred DNA substrates for unwinding by HELB will be beneficial for future studies of the enzymatic activity of HELB and testing of HELB inhibitors as potential anti-cancer drugs.

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