“A successful high-throughput screening campaign for new small molecule inhibitors of BLM Helicase”

Abstract

BLM (Bloom syndrome protein) is an essential RECQ-family helicase involving DNA replication and repair via homologous recombination repair pathways. Synthetic lethality study showed BLM as a promising target in a range of cancers with defects in the DNA damage response. Unfortunately, selective small molecule inhibitors are still lacking.

We have established easy, cheap, fast and robust assays to measure duplex DNA unwinding and ATPase activity in a high-throughput fashion. The primary assay employs förster resonance energy transfer (FRET) methods utilised a labelled forked DNA with a fluorophore and a quencher. The assay carried out in a 1536-well plate format and provided robust and good quality (Z prime = 0.85) for large-scale and high-throughput applications.

In this study, approximately 333,440 small molecules were screened to measure inhibition of duplex DNA unwinding by a catalytically active BLM helicase domain. In addition, selected molecules are screened using a bioluminescent approach to validate their ATPase activity. The second assay employs firefly luciferase to detect ATP turnover. As a result, we observed 277 compounds have a dose-response profile against BLM, while 87 compounds showed a selective inhibition on BLM compared to another member helicase (RECQ1). Hence, this paired fluorescence and bioluminescence methods are suitable for screening against helicase family members.