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Development of a Novel Fluorophore-Based Drug Screening Assay by Targeting Endonuclease Activity of Parvovirus B19 NS1

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Human parvovirus B19 (B19V), a member of the genus Erythroparvovirus of the family Parvoviridae, is a small non-enveloped virus that has a single-stranded DNA (ssDNA) genome of 5.6 kilobases with two identical inverted terminal repeats at the ends. B19V exhibits a remarkable tropism for human erythroid progenitor cells in the bone marrow and fetal liver. B19V infection often results in severe hematological disorders and fetal death in humans. B19V replication follows a model of rolling hairpin-dependent DNA replication, in which the large non-structural protein NS1 introduces a site-specific single strand nick into the viral DNA replication origins, which locate at the inverted terminal repeats. NS1 executes endonuclease activity through the N-terminal origin binding domain. Nicking of the viral replication origin is a pivotal step in rolling hairpin-dependent parvoviral DNA replication. We have developed a fluorophore-based in vitro nicking assay of the replication origin using the origin binding domain of the NS1 and compared it with the radioactive in vitro nicking assay. We used both assays to screen a set of small molecule compounds that have anti-nuclease activity. We found that the fluorophore-based in vitro nicking assay possesses a specificity and sensitivity as high as the radioactive assay. Among the 90 anti-nuclease compounds tested, we identified 3 compounds with have an IC50 ~of 1-3 µM under in- vitro conditions., These three compounds which also exhibited potent inhibition of B19V DNA replication in UT7/Epo-S1 cells and of ex vivo expanded human erythroid progenitor cells. Thus, our study suggests that the fluorophore-based in vitro nicking assay, which is a single step reaction, can be employed to high throughput screening of large library of small molecule compounds for anti-B19V drug candidates.