Introduction

- Prior antiviral screening against HBV has been largely limited to HBV DNA replication inhibition in HBV stable cell lines
- Recent progresses in the successful infection of HepG2 cells expressing the HBV entry receptor sodium-taurocholate cotransporting polypeptide (NTCP), made it possible to develop an assay which is amenable to screen for HBV cccDNA inhibitors\(^1\)
- Branched DNA (bDNA) technology enables the visualization of cytosolic RNA in situ which can be multiplexed with nucleic acid targets to monitor cytotoxicity\(^2\)
- We use this technology to develop a 384-well multiplex screening assay for potential anti-HBV compounds

Background

Figure 1A. HBV Life Cycle. cccDNA Serves as the Template for HBV Transcripts

- Figure 1B. The Principle of Using Branched DNA Technology to Detect Target RNA in Situ

Methods

Figure 2. Design of HBV RNA Hybridization Probes for bDNA Signal Amplification

- Probes:
  - Negative strand sequence
  - 20bp long each probe
  - Derived from the HBx gene of AD38 virus
  - Hybridize to all species of HBV RNA
  - Designed by Affymetrix, Inc.

Figure 3. 384-well Format bDNA-based HBV RNA Assay Workflow

- Figure 3. 384-well format HBV RNA assay workflow using branched DNA technology. The assay workflow is described in Affymetrix ViewRNA assay instruction. In this assay, Cy3 (550/570) was used as the fluorophore of labeled probes. After the nuclei staining (Hoechst 33342), the cells were imaged under an automated imager, Cytomics ArrayScan (Thermo) Images were analyzed using HCS Studio software (Thermo)

Results

Figure 4. HBV RNA Assay Signal is Specific to HBV Infection

- Figure 4. HBV RNA assay signal is specific to HBV infection. HepG2-NTCP cells were infected with HBV AD38 virus, and were then fixed and proceeded to HBV RNA assay. The blue color shows nuclei staining and the red color shows HBV RNA staining. As shown, HBV RNA staining is specific to HBV infected cells. The amount of HBV RNA can be quantified by measuring the fluorescence intensity in the cytoplasmic region

Figure 5. HBV RNA Assay Signal is Sensitive to RNase Treatment but Not to DNase Treatment

- Figure 5. HBV RNA assay signal is sensitive to RNase treatment but not to DNase treatment. HepG2 cells stably expressing HBV AD38 virus was used in this study. After stable expression of HBV for 3 days, the cells were fixed and treated with RNAse A or DNase I. The treated cells were subjected to HBV RNA assay workflow. HBV RNA assay signal (red color in image: quantified as average fluorescence signal in the cytoplasmic region) is sensitive to RNase A treatment but insensitive to DNase I treatment. Each point is the mean of n=4 and error bars are standard deviation

Figure 6. Use HBV RNA Assay to Determine the HBV RNA Kinetics Post Infection

- Figure 6. HBV RNA and HBeAg expression kinetics post infection. HepG2-NTCP cells were infected with HBV AD38 virus and were fixed at indicated time points and processed with HBV RNA assay. The culture supernatants from the same well were harvested and analyzed using a MSD (Mesoscale Discovery) assay to measure the amount of HBeAg. HBV RNA signal started to increase from day 2 to day 4 post infection and maintained the top level at day 5. On day 7, there was a slight decrease of HBV RNA signal. HBeAg showed similar kinetics. Bar graphs show the mean of n=4 and error bars are standard deviation

Conclusions

- We have developed a 384-well HBV RNA assay using branched DNA technology to quantify HBV RNA in duplicate with nuclei count in HBV infected HepG2-NTCP cells
- HBV RNA assay signal is specific to HBV RNA, demonstrated by RNA signal decrease with RNAse but not DNase treatment, and siRNA-mediated HBV RNA knockdown
- The 384-well HBV RNA assay offers a signal-to-background ratio which is sufficient to screen compounds for potential anti-HBV agents

References

2. Horn T, Tiselj C, Udecsky MS. Chemical synthesis and characterization of branched oligodeoxyribonucleotides (bDNAs) for use as signal amplifiers in nucleic acid quantification assays. Nucleic Acids Res. 1997;25:4842-4849

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