A novel approach to induce HBV-infected cell death as a potential cure

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BACKGROUND

Approximately 5 percent of the world’s population carry Hepatitis B Virus (HBV) and, despite an effective vaccine, nearly 1 million people die per year. Several promising avenues for treatment have been pursued including depleting the pool of covalently circular DNA and inhibiting HBV pol gene (HBV pol), which is essential both for reverse transcriptase function and packaging. In this study, rather than trying to inhibit the HBV life cycle, we utilize the virus machinery to kill the infected cells as a potential cure.

MECHANISM OF ACTION

AAV particles were packaged with a novel and proprietary vector construct that expresses a non-functional non-coding (ncRNA) flanked between sequences specific to the reverse transcriptase (RT) domain of HBV pol (HBV pol/RT). HBV pol/RT would recognize these sequences and reversely transcribe the ncRNA into a double stranded (ds)DNA. The dsDNA engages with host polymerases to overexpress caspase-9 and induce apoptosis of the infected cell. In an uninfected cell, our non-functional ncRNA would go through degradation immediately upon transcription.

EXPERIMENTAL APPROACH

- HBV-infected and uninfected hepatoma cell lines and primary human hepatocytes (PHH) were treated with AAV2 particles carrying the “test vector” or GFP expressing “mock vector”. To confirm the function of action, we treated HBV-infected HepAD38 cells with the test AAV in the presence or absence of RT inhibitors tenofovir or entecavir, caspase-9 inhibitor Z-LEHD-FMK, or pan-caspase inhibitor Z-VAD-FMK. Daily cell viability and proliferation were evaluated.

- HBV-transgenic mice were intra-peritoneally injected with 1011 AAV8 particles packaged with test or mock vector. Day 14 post-injection liver and kidneys tissues were evaluated.

RESULTS

- AAV particles were packaged with a novel and proprietary vector construct that expresses a non-functional non-coding (ncRNA) flanked between sequences specific to the reverse transcriptase (RT) domain of HBV pol (HBV pol/RT). HBV pol/RT would recognize these sequences and reversely transcribe the ncRNA into a double stranded (ds)DNA. The dsDNA engages with host polymerases to overexpress caspase-9 and induce apoptosis of the infected cell. In an uninfected cell, our non-functional ncRNA would go through degradation immediately upon transcription.

- Experimental approach included:
  - Co-opting HBV pol to reversely transcribe a non-coding RNA to express caspase-9, which triggers cell death.
  - RT inhibitors or caspase-9 inhibitors confirmed the efficiency of the mechanism of action.

CONCLUSIONS

- Co-opting HBV pol to reversely transcribe a non-coding RNA to express caspase-9 has induced significant cell death in HBV-infected but not in HBV-uninfected cells.
- RT and caspase inhibition obviated the result, validating the impact of the anticipated mode of action.
- Preliminary data from in vivo studies confirm the anticipated PK and vector expression efficacy with no visible off-target effects.
- The specific HBV-infected cell death could lead to a depletion of the pool of HBV cccDNA over time, without significant damage to uninfected hepatocytes or other important organs.
- These results validate a novel mechanism of action for a potential cure for HBV infection.

DISCLOSURE: On November 25th, 2019, Enochian Biosciences Inc has entered into an agreement in principle to acquire an exclusive license for this technology.