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Development of HBV-targeting epigenetic repressors with deep, durable in vivo silencing of viral markers

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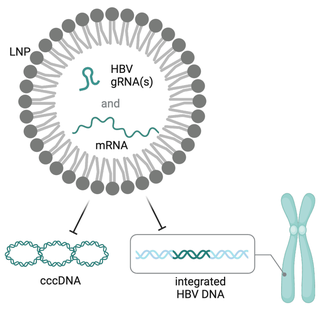
Hepatitis B Virus and Epigenetic Editing

Chronic HBV
- Nearly 300 million people live with chronic hepatitis B worldwide, with 1.5 million newly diagnosed each year.[1]
- There is no cure and current treatments are limited with patients rarely achieving loss of surface antigen

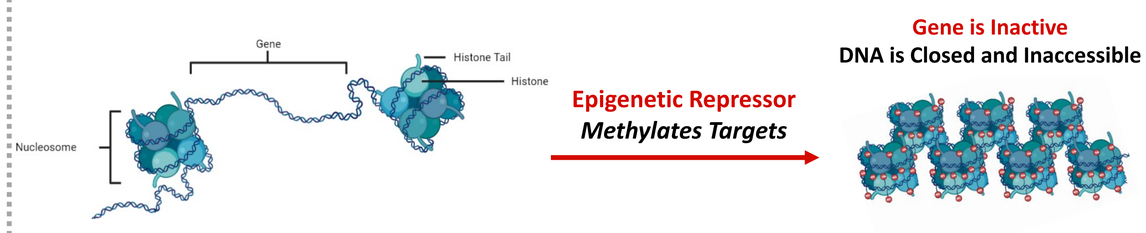
Epigenetic editing
- Harnesses the endogenous cellular mechanism for regulating gene expression
- Durably modulates transcription without affecting the DNA sequence

Chroma's multiplex HBV product designed as a functional cure

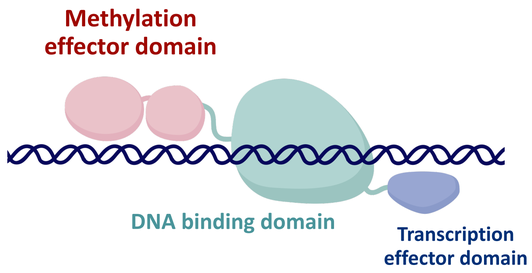


- ✓ Targets both HBV cccDNA and integrated DNA
- ✓ No cutting or nicking the DNA
- ✓ No viral RNA transcripts are produced
- ✓ Targets multiple genotypes in a single product
- ✓ Durable for lifetime of the patient

Chromatin packaging and epigenetic regulation of DNA transcription



Epigenetic editors



- **DNA binding domain** precisely localizes effector domains to target sequence
- **Transcription effector domain** transiently represses target gene
- **Methylation effector domain** durably silences target gene

Epigenetic Editors Designed to Cover the Entire HBV Genome

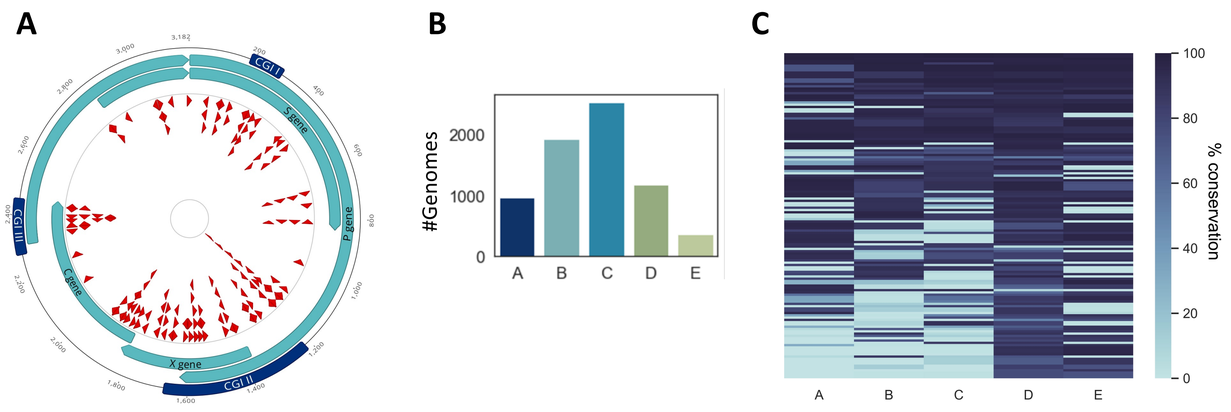


Figure 1: HBV epigenetic editors (EE) cover the entire HBV genome and are conserved for most common genotypes of HBV. **(A)** Representation of the HBV genome shows the target region of the 141 gRNAs designed for in vitro screening **(B)** ~7k sequences (genotype A-E) were evaluated for in silico conservation **(C)** Target sequences with a high degree of conservation across genotypes and CGI islands were prioritized. Conservation was defined as the percent of genomes in that genotype that contain the exact target sequence of the guide.

Epigenetic Editors Repress HBV Antigens from cccDNA and Integrated HBV DNA

Strong reduction of viral markers in HBV infected primary human hepatocytes

Epigenetic repression of HBsAg is maintained in vitro for nearly a month in dividing cells containing integrated HBV

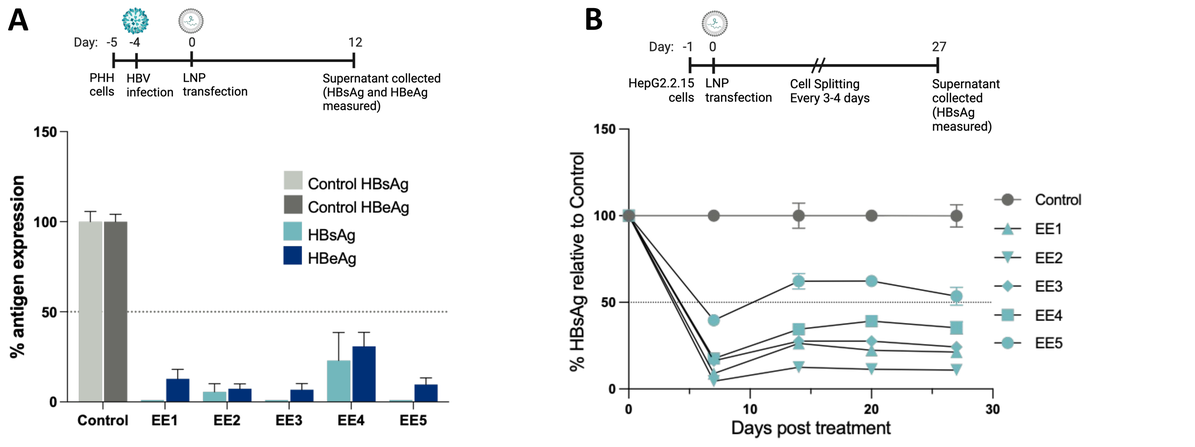


Figure 2: EEs robustly silence HBV antigens produced from cccDNA and integrated HBV DNA. EEs repress HBsAg and HBeAg secretion in **(A)** HBV infected PHH cells at Day 12 using research-grade LNPs containing 100 ng of payload (bars represent mean ± SEM; N=5) **(B)** or integrated cell line (HepG2.2.15) up to Day 27 using a submaximal payload dose (50 ng) (error bars represent mean ± SEM; N=3)

EE=epigenetic editor; HBeAg=hepatitis B e antigen; HBsAg=hepatitis B surface antigen; LNP=lipid nanoparticles; PHH=primary human hepatocytes; SEM=standard error of the mean

Epigenetic Editors are Highly Specific

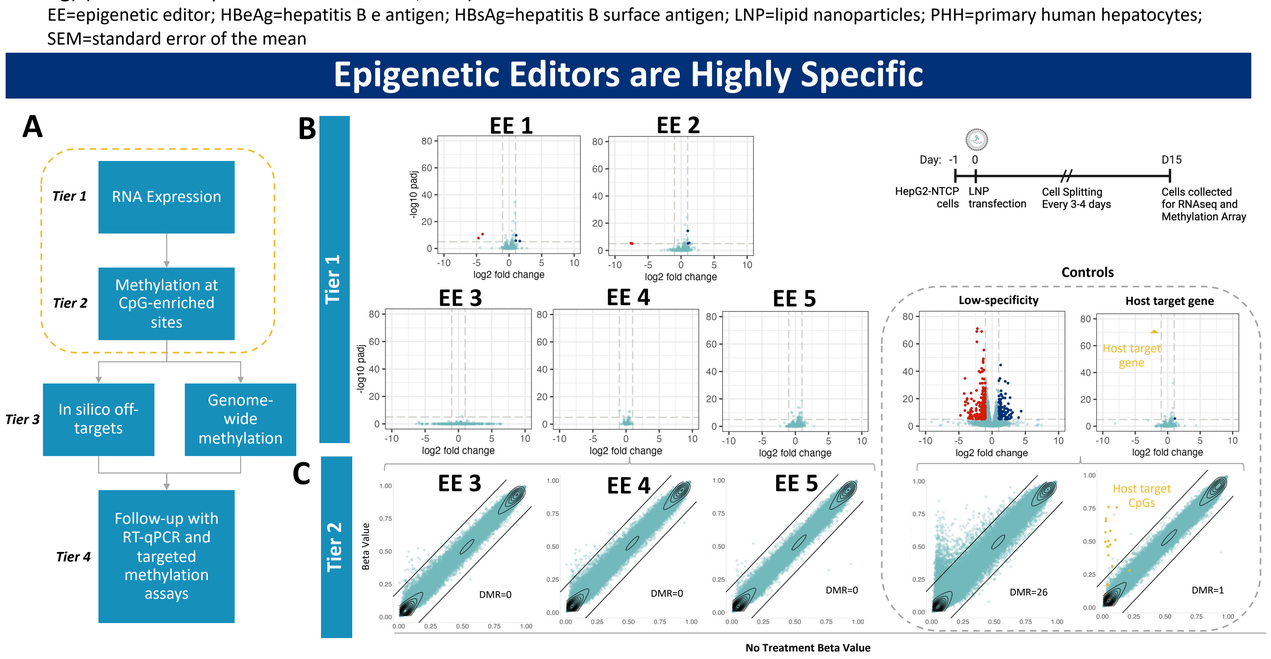


Figure 3: Tiered approach to evaluate off-targets of HBV EEs demonstrate high specificity. (A) EEs were profiled in HepG2-NTCPs with the first two specificity tiers to screen candidates **(B)** Differential gene expression identified through DESeq2[2] as volcano plots. Dots represent genes with their change in expression (x-axis) and statistical significance of that change (y-axis). **(C)** Scatterplots of methylation between treatment (y-axis) and control (x-axis) for 935,000 CpG sites (Infininium MethylationEPICv2.0) in the human genome. Lines represent thresholds for changes in methylation considered significant. DMRs were extracted using a statistical tool where the set of probes within a DMR show significant changes in methylation (yellow).

DMR=differentially methylated region; EE=epigenetic editor

Durable Repression of Viral Antigens

- A single dose of EE leads to > 5 month durable repression of serum viral markers in transgenic HBV mice
- This is our first in vivo experiment with this prototype payload
- Results show durable and progressive reduction of viral antigens achieving -2.7 log DNA and -2.8 HBsAg >5 months after single administration of EE
- Five out of six EE-treated animals (83%) had undetectable HBV DNA and HBsAg at the last time point evaluated

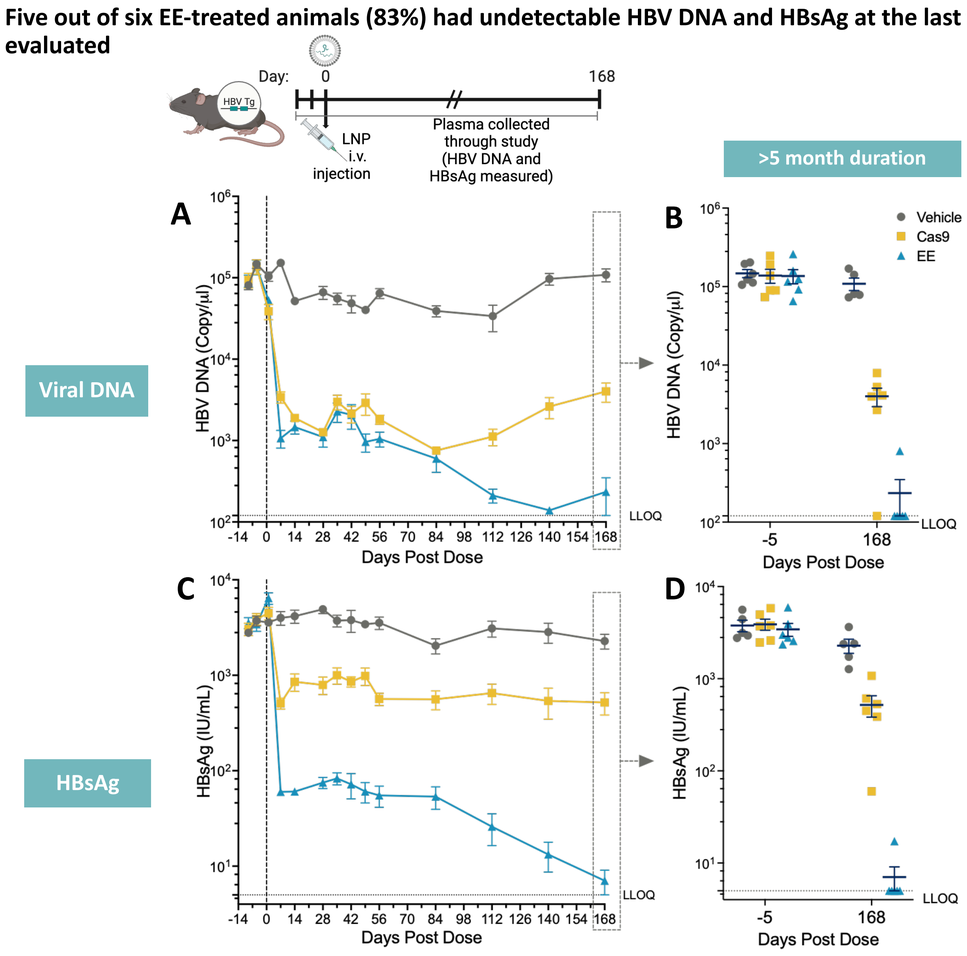


Figure 4: Single dose of EE leads to >5 month durable repression of serum viral markers in transgenic-HBV mice. HBV transgenic mice received an intravenous (iv) administration of vehicle (saline), control WT Cas9 mRNA and single gRNA, or mRNA encoding a prototype epigenetic editor and containing a single gRNA formulated in Acuitas LNPs. Circulating viral markers were evaluated at various timepoints through 168 days. **(A-B)** Circulating HBV DNA and **(C-D)** HBsAg were quantified by qPCR and ELISA, respectively. **(B,D)** show individual animals pre dose and 168 days post dose. (bars represent mean ± SEM; N=5-6; vertical dotted lines show dosing day [day 0]; horizontal dotted lines show LLOQ)

EE=epigenetic editor; HBsAg=hepatitis B surface antigen; LNP=lipid nanoparticles; SEM=standard error of the mean; WT=wild type; LLOQ=lower limit of quantitation; Tg=transgenic

Summary

- Epigenetic editors demonstrate deep and durable repression of HBV viral markers in multiple in vitro and in vivo models of HBV.
- Epigenetic editors are highly specific and do not cause off-target changes in host gene expression or methylation.
- A single administration of an HBV-targeting epigenetic editor in an HBV transgenic mouse model led to HBsAg and viral DNA loss in 83% of the animals for at least 5 months after treatment.
- Our study demonstrates in vivo proof-of-concept for efficacious, durable, and specific silencing of HBV using epigenetic editors.
- Epigenetic editing represents a new class of therapeutics designed for life-long repression of cccDNA and integrated HBV without cutting or nicking the human or viral genome.

Acknowledgements

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References

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