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P44

Poster for you

HELZ2 is an interferon stimulated gene with antiviral properties against Hepatitis Delta Virus

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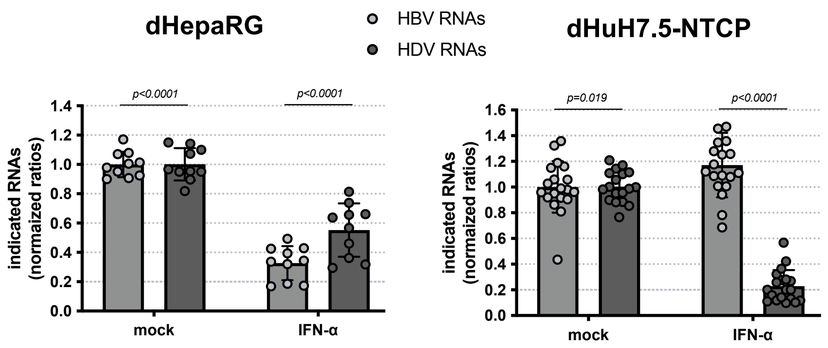
INTRODUCTION AND AIMS

Patients chronically co-infected by Hepatitis B Virus (HBV) and Hepatitis D Virus (HDV) suffer from the most aggressive form of viral hepatitis leading to severe liver disease such as cirrhosis, hepatocellular carcinoma and liver decompensation. Treatments options are very limited with only two drugs used in clinic (IFN-α and bulevertide) that rarely allow viral clearance. The molecular mechanisms leading to inhibition of HDV by IFN-α (in patients but also *in vitro*) are not known and mechanisms behind treatment failures in patients also remain elusive.

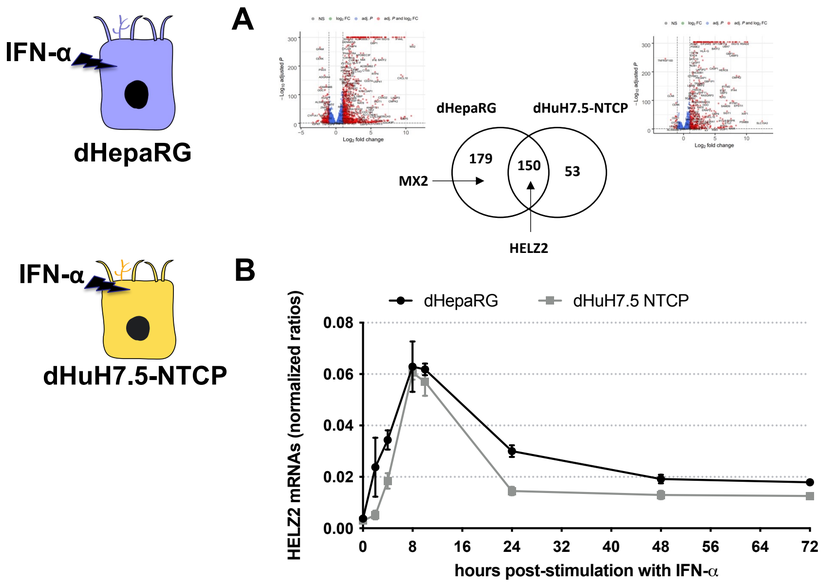
Here we aimed at the identification of interferon stimulated genes (ISGs) that can specifically inhibit intracellular HDV replication.

1 Differential antiviral effect of IFN-α on HBV-, but not on HDV-, infected dHuH7.5-NTCP cells and dHepaRG cells



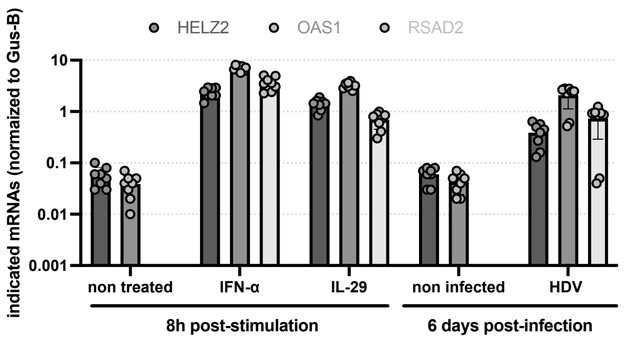
dHepaRG and dHuH7.5-NTCP cells were infected with HBV or HDV and treated or not 3 days later for 10 days with IFN-α. At 13 days post infection (dpi), cells were lysed and HBV and HDV total intracellular RNAs were analyzed by RT-qPCR. Bars are the means +/− SD of at least 3 independent experiments each performed with 3 biological replicates. Dots represent individual replicates. p values were calculated using the Prism software and Mann–Whitney U test.

2 Different subsets of ISGs are expressed in dHepaRG and dHuH7.5-NTCP upon stimulation with IFN-α



dHepaRG and dHuH7.5-NTCP cells were stimulated for 8h and total RNAs were extracted and genes expression was determined by RNA-seq. (A) X axis represents Log2 FC non treated vs IFN-α condition. Y Axis represents -log10 adjusted pvalue non treated vs IFN-α condition. Adjusted pvalue < 0,05 was considered statistically significant. Each dot represents a single gene. (B) dHepaRG and dHuH7.5-NTCP cells were stimulated with IFN-α and harvested at the indicated time points. Total RNAs were extracted and levels of HELZ2 mRNAs were quantified using a nCounter Nanostring.

3 HELZ2 is expressed upon IFN-α stimulation and HDV infection



dHepaRG cells were either treated with IFN-α (500IU/mL), IL-29 (100 ng/mL) or infected with HDV. At the indicated time, cells were harvested and levels of intracellular HELZ2, OAS1 and RSAD2 mRNAs were assessed by RT-qPCR.

FINANCIAL SUPPORTS AND CONTACTS

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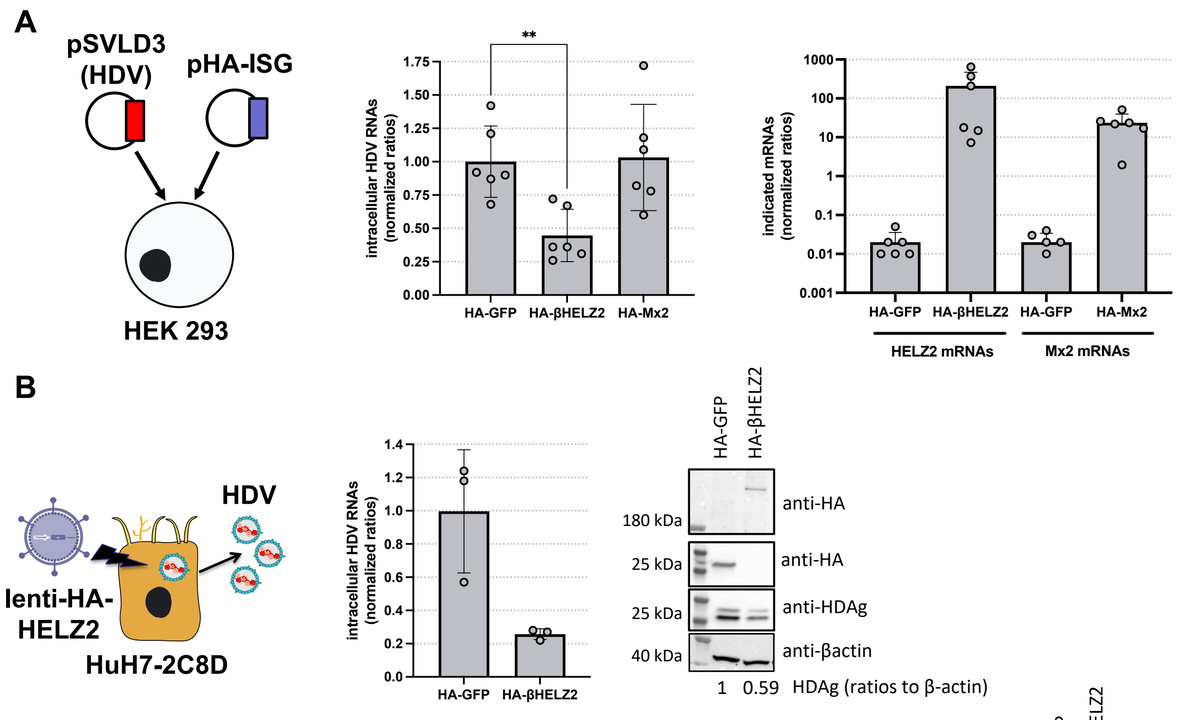
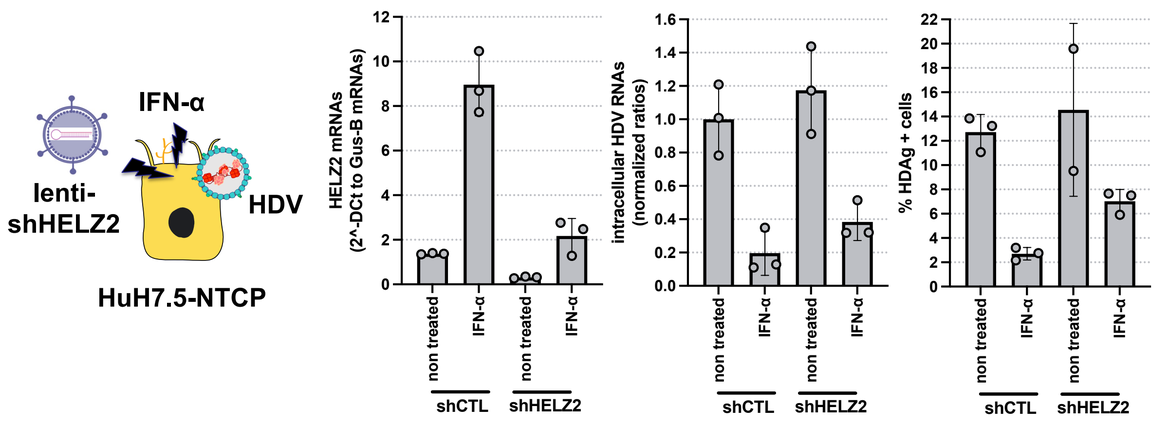
4 HELZ2 over-expression inhibits HDV infection



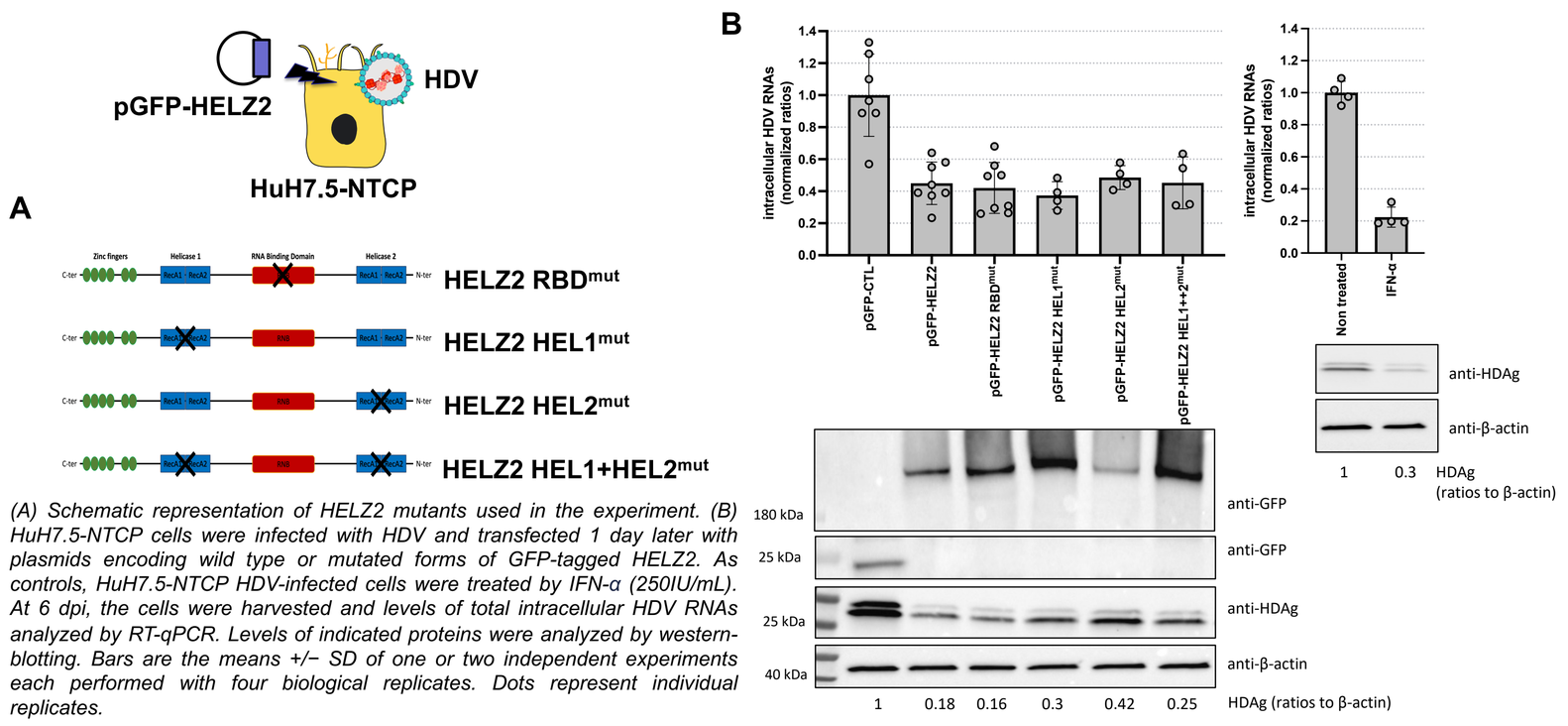
(A) HEK293 cells were co-transfected with pSVLD3 and plasmids encoding HA-tagged version of GFP, HELZ2 or Mx2. At day 4 post-transfection, cells were lysed and levels of total intracellular HDV RNAs or HELZ2 and Mx2 mRNA were analyzed by RT-qPCR. (B) HuH7-2C8D cells were transducted with lentivectors encoding HA-tagged version of GFP or HELZ2. At 6 post transduction, cells were lysed and levels of total intracellular HDV RNAs and proteins were analysed by RT-qPCR and by western blotting respectively. (C) Freshly isolated primary human hepatocytes (PHH) were infected with HDV and transduced 3 days later with a lentivector allowing the overexpression of an HA-tagged version of GFP or HELZ2. As controls, PHH were also infected with HDV and treated at 3 dpi with IFN-α (500IU/mL). At 10 dpi, cells were lysed and levels of total intracellular HDV RNAs analyzed by RT-qPCR. Levels of indicated proteins were analyzed by western-blotting. Bars are the means +/− SD of one or two independent experiments each performed with three biological replicates. Dots represent individual replicates. Statistic analyses were performed using the Prism software and Mann–Whitney U test.

5 Knock-down of HELZ2 partially inhibits the antiviral effect of IFN-α on HDV



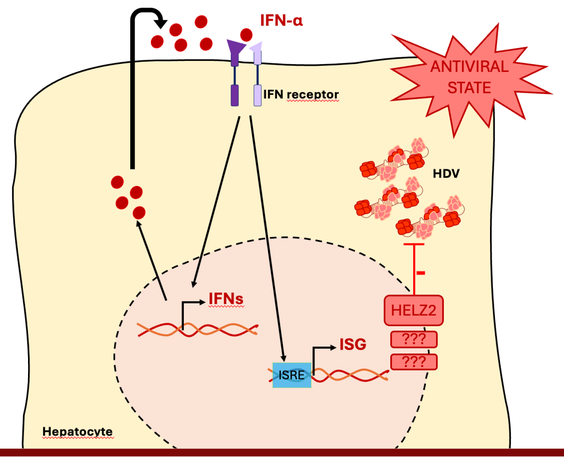
HuH7.5-NTCP cells were transducted with lentivectors encoding control (CTL) shRNA or shRNA directed against HELZ2 mRNA and seeded at the same time. Three days later, cells were infected with HDV or treated with IFN-α (500 IU/mL). At 6 dpi, the cells were either harvested and levels of total intracellular HDV RNAs or HELZ2 mRNA were analyzed by RT-qPCR or fixed for quantification of HDAg+ cells by immunofluorescent assays. Bars are the means +/− SD of one experiment performed with three biological replicates. Dots represent individual replicates.

6 The antiviral effect of HELZ2 on HDV is independent of its catalytic activity



(A) Schematic representation of HELZ2 mutants used in the experiment. (B) HuH7.5-NTCP cells were infected with HDV and transfected 1 day later with plasmids encoding wild type or mutated forms of GFP-tagged HELZ2. As controls, HuH7.5-NTCP HDV-infected cells were treated by IFN-α (250IU/mL). At 6 dpi, the cells were harvested and levels of total intracellular HDV RNAs analyzed by RT-qPCR. Levels of indicated proteins were analyzed by western-blotting. Bars are the means +/− SD of one or two independent experiments each performed with four biological replicates. Dots represent individual replicates.

CONCLUSION



WORKING HYPOTHESES