Characterization of Mouse and Human HSD17B13 Structure and Activity

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BACKGROUND

Hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) belongs to a 15-member family of NAD(P)H/NAD(P)+-dependent oxidoreductase enzymes which are involved in various metabolic processes. Loss-of-function mutations in the human HSD17B13 gene confer a protective effect against chronic liver injury. The human HSD17B13 gene encodes a 300 amino acid (aa) protein, whereas mouse HSD17B13 is annotated to encode two different isoforms, a 300 aa (iso1) and a 304 aa protein (iso2). To date, the biochemical activity of human HSD17B13 and the orthologous mouse enzyme remain poorly characterized. In this study, we compare mouse and human HSD17B13 (hHSD17B13) protein structure and activity.

METHODS

- **Materials.** Recombinant $(His)_6$-ENLYFQSG- HSD17B13 was expressed in Sf9 insect cells using a baculoviral expression system and purified using metal affinity purification and size exclusion chromatography. NAD-Glo assay kit was purchased from Promega. HSD17B13 inhibitor compounds (HSD17B13i) were synthesized at Enanta.

- **Substrate Screening and Biochemical Assays.** Bioactive molecule libraries (Cayman Chemical and Sigma Aldrich) were screened for candidate human and mouse HSD17B13 substrates using a coupled-enzyme luminescence assay which detects NADH production (NAD-Glo). Potential substrates were confirmed using RapidFire mass spectrometry (RF-MS)-based assays to measure conversion of substrate to product by HSD17B13.

- **Cell based Assays.** HSD17B13 activity was monitored by RapidFire mass spectrometry for conversion of substrates in HEK293 cells either stably or transiently expressing human or mouse isoform 1 or isoform 2 HSD17B13, respectively.

- ***In vivo* Liver Injury Models.** HSD17B13i compounds were evaluated in mouse models of acute (adenoviral) or chronic liver injury (choline deficient, L-amino acid defined, high fat diet; CDAA-HFD; A16092201). Gene and protein markers of inflammation, injury and fibrosis were measured in plasma and liver of mice treated with HSD17B13i or sh-adenoviral mediated knockdown.

- **Structural Analysis.** Structure determination using x-ray crystallography was performed with human HSD17B13 co-crystallized in the presence of NAD^+.

RESULTS

Mouse isoform 1 is active and expressed in the liver, whereas isoform 2 is not.

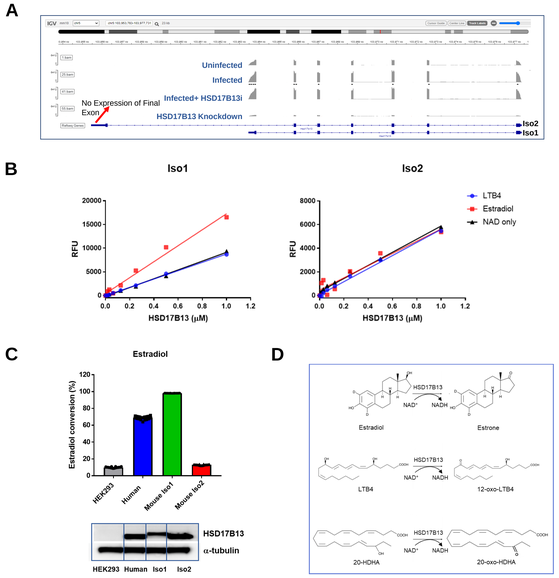

Figure 1. (A) RNA-seq analysis of mouse liver tissue from the adenoviral injury model show expression of isoform 1. (B) Biochemical NAD-Glo HSD17B13 activity assay with mouse isoform 1 or isoform 2 demonstrate activity for mouse isoform 1. (C) Cellular HEK293 HSD17B13 activity assay with human, mouse isoform 1 or mouse isoform 2 show that mouse isoform 2 is inactive.(D) HSD17B13 substrates/products. NAD: Nicotinamide adenine dinucleotide; LTB4: Leukotriene B4; 20-HDHA: 20-Hydroxy docosahexaenoic acid.

Differential substrate preference for mouse isoform 1 vs human HSD17B13.

Table 1. Number of candidate substrates for human vs mouse isoform 1 HSD17B13 identified from screening of lipid libraries.

In vitro Substrate Screens		
Bioactive Lipid Library	**Human HSD17B13**	**Mouse HSD17B13**
Cayman #10506 >800 lipids [10 μM]	4	-
Cayman #10507 >190 lipids [2 μM]	26	-
Sigma Bile Acid/Carnitine/Sterol Metabolite Library [20 μM]	0	-
In-house Handpicked Library of 14 lipids	5	2

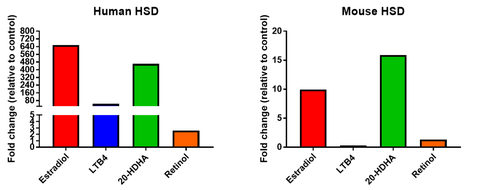

Figure 2. Biochemical RF-MS assay measuring conversion of various substrates by human vs mouse isoform 1 HSD17B13 show differential substrate preference.

RESULTS

Differences in compound structure-activity relationship for mouse vs human proteins parallel differential efficacies *in vivo*.

Table 2. Potencies of HSD17B13 inhibitor compounds from different chemical series against human vs mouse isoform 1 HSD17B13 in biochemical or cellular HSD17B13 activity assays.

HSD17β13	Human	Mouse iso1	Human	Mouse iso1
Assay	**Biochemical/RF-MS**		**Cellular**	
Compound	**IC_{50} (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**
Chemical Series I	0.014	0.0025	0.071	0.055
Chemical Series II	0.0825	0.0737	0.034	1.083

In vivo studies demonstrate differential efficacies of the two compounds correlating to differences in compound structure-activity relationship for mouse isoform 1 vs human HSD17B13.

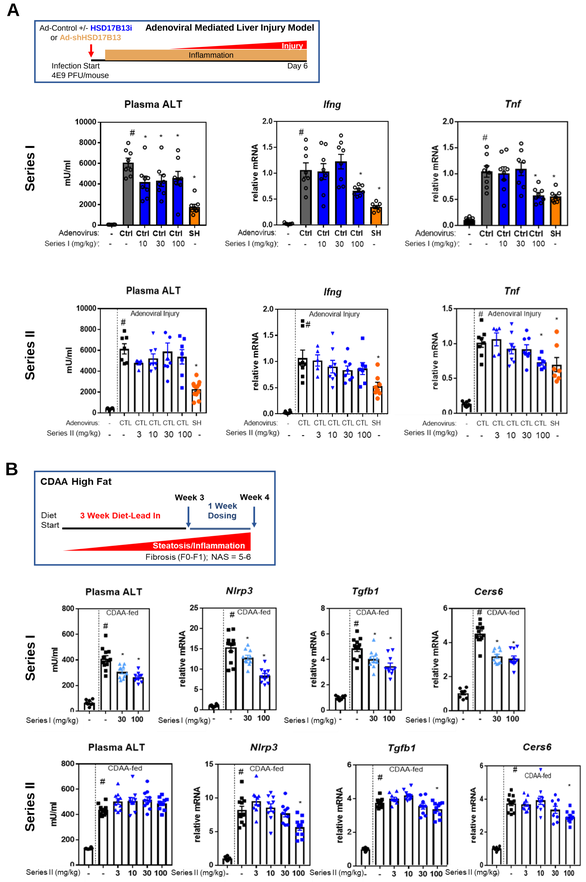

Figure 3. Plasma and liver markers of inflammation, injury and fibrosis in adenoviral injury (A) or CDAA-HFD (B) mice treated with HSD17B13i compounds demonstrate differential efficacy of the two compounds. ALT: alanine transaminase; Cers6: Ceramide synthase 6; Ifng: Interferon gamma; Nlrp3: NOD-, LRR- and pyrin domain-containing protein 3; Tgfb1: transforming growth factor-beta 1; Tnf: tumor necrosis factor.
*Anova; # p<0.05 vs Uninfected (A) or Healthy (B); * p<0.05 vs Ad-Control (A) or CDAA vehicle (B).*

RESULTS

Structural analysis of human HSD17B13 reveal conformational changes in the C-terminal helix upon NAD^+ binding.

Sequence alignment of human, mouse isoform 1 and isoform 2 proteins reveal differences in the C-terminus.

Figure 4. (A) Sequence alignment of human, mouse isoform 1 and isoform 2 proteins show differences in the C-terminus. (B) Crystal structure of the human HSD17B13 protein bound to NAD+ demonstrate conformational changes involving the C-terminus helix upon ligand binding.

CONCLUSION

- We have identified differential substrate preferences for mouse vs human HSD17B13.
- Characterization of the mouse vs human HSD17B13 proteins highlights potential differences in the physiological function of HSD17B13 in rodents and humans.
- Differences in substrate utilization and in residues located near the C-terminal helix highlight potential functional differences between the mouse and human proteins.

ACKNOWLEDGEMENTS

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