Transcriptome Analysis of Human Livers Explants from Alcohol-Associated Liver Disease Highlights a Loss of Pericentral and Proliferation Gene Expression

H. Baribault*, Chenggang Lu, Shalaka Deshmukh, Darshini Shah, Russell Fletcher, Wen-Chen Yeh, Jay Tibbitts and Geertrui F. Vanhove

All authors: Surrozen, Inc. South San Francisco, CA94080, USA; * presenting author

Introduction

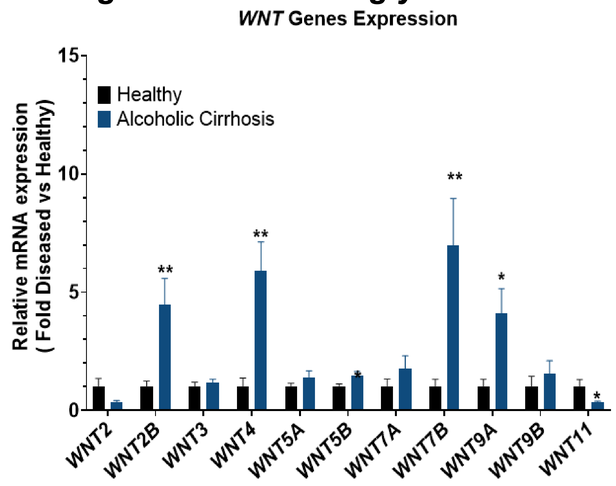
Severe alcohol-associated liver disease is characterized by reduced hepatocyte proliferation and impaired hepatic regeneration. As there is evidence that increased beta-catenin signaling is linked to increased regeneration and survival, we wanted to evaluate whether the molecular components of the Wnt signaling pathway were affected in livers of patients with alcohol-associated liver disease.

Methodology

Human liver tissue remnants from liver resections and explants from patients with alcohol-associated liver disease were used for histological processing and total mRNA extraction. Non-tumorous liver tissue from hepatic resections was used as a healthy control and compared to liver explants from patients with alcohol-associated liver disease (n=12 per group). Genes encoding Wnt and RSPO ligands, FZD receptors, RSPO and SZN-043 target receptors, and Wnt target genes were analyzed by qPCR. Global transcriptome profiling of human liver tissue was performed by RNA-Seq. Sequencing libraries were prepared following TruSeq Stranded mRNA Sample Preparation Guide using the TruSeq Stranded mRNA Library Prep kit and TruSeq RNA Single Indexes (Illumina Inc.). Libraries were then sequenced in a HiSeq2500 System (Illumina) with a single-end type read and a read length of 101 nucleotides, 36M of reads. Immunohistochemical analyses of CYP1A2 and double immunofluorescence of the proliferation marker, Ki-67, and hepatocyte marker, HNF4A, were also performed.

Results

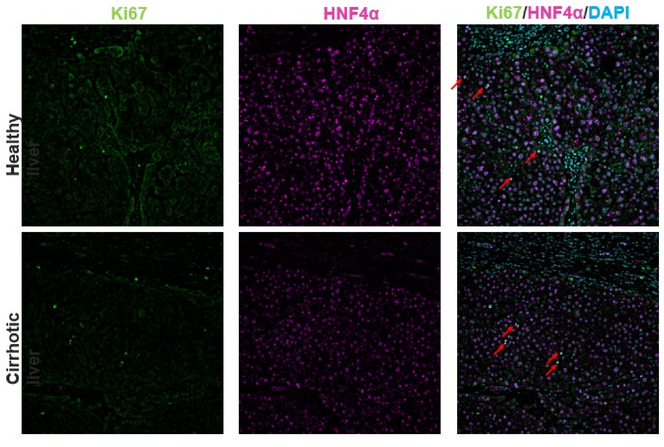
Wnt Ligands were strongly elevated in diseased livers



WNT2B, *WNT4*, *WNT7B and WNT9A* were significantly elevated in alcoholic cirrhosis livers as shown by qPCR.

Mean +/- SEM, * $p < 0.05$, ** $p < 0.01$, *** $p<0.001$, **** $p<0.0001$

Rare Ki67⁺HNF4a⁺ Dual + Cells are Observed in Healthy and Cirrhotic Livers



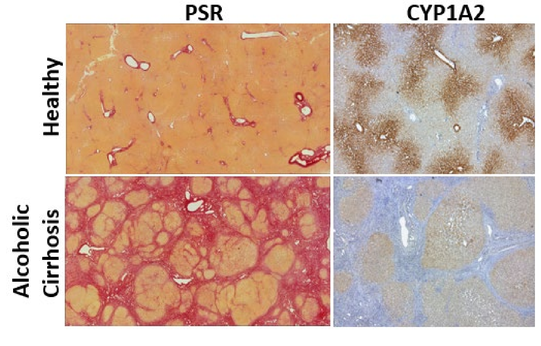
In spite of elevated Wnt ligands expression, there was no obvious difference in the number of proliferating hepatocytes (red arrows), as shown by double immuno-fluorescence with the proliferation marker Ki67 and the hepatocyte-specific marker HNF4A. These results were consistent with the expression of the proliferation markers *CCND1* and *MKI67*, as observed by bulk RNAseq analysis.

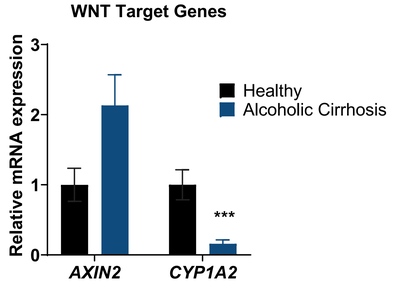
Genes	Fold Change	p	FDR	Significance
Proliferation Markers				
CCND1	-1.2	0.218	0.283	≠
MKI67	1.3	0.349	0.429	≠
Pericentral Markers				
CYP1A2	-55.4	0.000	0.001	↓↓↓
CYP2E1	-6.1	0.000	0.002	↓↓
OAT	-5.1	0.000	0.004	↓↓
PCK1	-2.6	0.030	0.07	≠
FGN	-2.0	0.002	0.015	↓
GLUL	-1.8	0.001	0.006	↓
Periportal Markers				
RNASE4	-1.2	0.460	0.544	≠
CYP2F1	-1.4	0.093	0.131	≠
ASS1	-1.1	0.521	0.602	≠
ASL	1.0	0.967	0.975	≠
HAL	1.3	0.326	0.403	≠
PIGR	1.9	0.007	0.039	↑

Table shows the fold change in proliferation, pericentral and periportal markers in diseased livers when compared to healthy livers, and their statistical significance, false discovery rate (FDR) < 0.05, as measured by bulk RNAseq.

Results (continued)

The pericentrally expressed and Wnt target gene, CYP1A2 was strongly downregulated in diseased livers (see immunohistochemistry and qPCR results, right). Interestingly, another Wnt target gene, Axin2 was not significantly differentially expressed. These results were consistent with the downregulation of several additional pericentral markers (table). In contrast the expression of periportal markers were little affected, in spite of bridging portal fibrosis as seen by Picrosirius Red staining.





Conclusions

In patients with alcohol-associated liver disease. We observed,

- **Several Wnt ligands are elevated.**
- **A loss of pericentral gene expression, including enzymes involved in the metabolism of xenobiotics such as *CYP1A2* and *CYP2E1*, and**
- **Impaired hepatocyte proliferation**

These results suggest that a molecule which induces pericentral gene expression and hepatocyte proliferation, such as SZN-043, a bispecific fusion protein and hepatocyte-specific R-spondin mimetic, could be beneficial for the treatment of alcohol-associated liver disease.

Presenting author: Helene Baribault
Surrozen Inc., South San Francisco, CA94080
Email: Helene@surrozen.com
www.surrozen.com

SURROZEN