Genetic Factors in the Progression of Alcoholic Steatohepatitis

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Abstract:
Alcoholic liver disease (ALD) is the leading cause of illness and death from liver complications in the United States. Specifically, alcoholic steatohepatitis (ASH) is a type of liver disease characterized by the accumulation of fat in liver (steatosis) in conjunction with liver inflammation (hepatitis). ASH is also a precursor to severe liver complications such as fibrosis and cirrhosis. Previous studies have shown that the development of ASH varies greatly in different genetic populations. Recently, we have identified a target gene (Nr4c4) that may play an important role in ASH progression. We have seen that Nr4c4 expression levels vary greatly between different strains of mice, and that Nr4c4 expression is regulated by transcription factor Cdx1. In this project we examine the role of Nr4c4 in the progression of ASH, and the effect of a single nucleotide deletion at the Cdx1 binding site of Nr4c4 on the gene's expression levels in the liver. We found that Nr4c4 is involved in the inflammatory response. Elevated levels of Nr4c4 resulted in increased cell apoptosis. It was also seen that a single nucleotide deletion at the Cdx1 binding site of Nr4c4 resulted in down regulated Nr4c4 expression in hepatocytes and upregulated Nr4c4 expression in liver macrophage cells. We believe that this deletion mutation has a positive effect by reducing hepatocyte death in conjunction with reduced inflammatory marker release from liver macrophages.

Introduction:
The development of liver disease is variable. It has been seen, both in humans and in mice, that some populations are resistant to the development of steatohepatitis and the progression from steatohepatitis to fibrosis. Specifically, in previous work, we have noted that the gene Nr4c4 may play a significant role in the progression of liver disease in mice. The Nr4c4 gene is located on the mouse chromosome 17. We were able to produce mouse models that contained a mostly B6 wild-type genetic background with a chromosome 17 from the A/J background. We were also able to divide chromosome 17, resulting in a mouse with just a small section of A/J chromosome 17.

Mouse Strains:

<table>
<thead>
<tr>
<th>Strain</th>
<th>B6</th>
<th>CSS-17</th>
<th>B6 x CSS-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion</td>
<td>12.8±0.2</td>
<td>12.7±0.3</td>
<td>12.8±0.2</td>
</tr>
<tr>
<td>WT</td>
<td>12.7±0.2</td>
<td>12.8±0.2</td>
<td>12.7±0.2</td>
</tr>
</tbody>
</table>

Liver injury from alcohol:

Figure 1. Generation of chromosome substitution strains. The panel of chromosome substitution strains (CSS) was constructed by crossing B6 and A/J mice to generate F1 progeny. The F1 progeny were backcrossed to B6. The non-recombinant A/J chromosome was selected by genotyping for further backcrosses. At least 10 backcross generations, mice which inherited the same non-recombinant A/J chromosome were selected and intercrossed to make the A/J chromosome homozygous.

Figure 2. Liver histology and tricholester content in B6, A/J, and CSS-17 strains after chronic ethanol feeding. Mice were fed 5% Lieber-DeCarli ethanol-containing diet (ECD) or pair fed diet (PF) for four weeks. A) Oil Red O staining of liver sections were performed to determine the degree of steatosis after ethanol feeding.

Chronic treatment with Lieber DeCarli ethanol containing diet:

Cdx1 - Over expression in hepatocytes (HepG2 Cells):

Figure 3. Consequences binding sites near single bp deletion 331bp upstream in Cdx1 promoter region. Sequencing of Cdx1 promoter region resulted in identification of a single nucleotide deletion located 331bp upstream of first exon. In addition to affecting several conserved binding sites near this location, this site due to base pair deletion may affect numerous binding sites in <300bp region downstream of this mutation. This deletion may result in significant changes of Cdx1 function in A/J and B6 mice.

Cdx1 - Over expression in Kupffer Cells (RAW 264.7):

Table 1. Food intake, Hepatic triglycerides and ALT/AST levels in B6/CSS-17 and congenic mice. Mice were chronically fed ethanol-containing diet (ECD) or pair fed (PF) for 4 weeks. Liver triglycerides were measured biochemically with Triglyceride (GPO). ALT and AST plasma levels were measured enzymatically in mice using a commercially available kit. Statistics determined by ANOVA analyses. All mice on ethanol diet had increased triglycerides and elevated ALT/AST levels, however, the 17C-6 strain had lowered triglyceride and ALT levels in both ethanol and pair fed diet when compared to B6 controls. This provides evidence of increased resistance to alcohol-induced injury in the 2760 strain of A/J in 17C-6 mice.

Figure 5. Consequences binding sites near single bp deletion 331bp upstream in Cdx1 promoter region. Sequencing of Cdx1 promoter region resulted in identification of a single nucleotide deletion located 331bp upstream of first exon. In addition to affecting several conserved binding sites near this location, this site due to base pair deletion may affect numerous binding sites in <300bp region downstream of this mutation. This deletion may result in significant changes of Cdx1 function in A/J and B6 mice.

Figure 6. In vitro Cdx1-over expression in hepatocytes and Kupffer Cells. B6 and A/J sequences of the Cdx1 binding site were inserted into liver cells. The Cdx1 region was over expressed, and downstream gene expression was determined by measuring fluorescence levels. It was seen that expression levels were reduced in hepatocytes and increased in Kupffer cells in the strain with the A/J deletion mutation.

Conclusions:
Liver disease is an extremely important topic in the current field of human health and medicine. With high rates of both alcohol and non-alcohol related liver complications, elucidating the pathways behind this disease has the potential to significantly improve the quality of treatment for liver disease. While the details of the molecular pathway is still not well understood, previous work on steatohepatitis has provided us with a strong foundation for continued research in this disease. Evidence from past work has led us to believe that Nr4c4 may have a significant role in the development of steatohepatitis. Using both mouse models and cell cultures, we have developed a better understanding of the role of Nr4c4 in liver disease. A mutation at the Cdx1 binding site appears to alter the downstream expression of Nr4c4 which may play a role in a resistance to liver damage. As we proceed, we will need to relate this mutation in the mouse gene to the human gene. We also need to look into Cdx1 and examine what role it might play in the development of liver disease. Evidence and data from our experiments will provide valuable insights into the pathogenesis of the disease and liver disease, and will be a key step in locating an effective target for improved treatment of liver disease.

References:

Figure 7. Nr4c4 gene has a significant role in the progression of liver disease. Nr4c4 is involved with the inflammatory response. We saw that a mutation in the Cdx1 binding site resulted in significant changes in Nr4c4 expression levels in different liver cells. Reduced Nr4c4 in hepatocytes with increased expression in Kupffer cells may result in a reduction of markers that activate hepatic stellate cells (HSC). HSC are the main cell involved in fibrosis development, a reduction in activation of HSC may result in the resistance to development of severe liver damage.

Figure 8. Nr4c4 gene has a significant role in the progression of liver disease. Nr4c4 is involved with the inflammatory response. We saw that a mutation in the Cdx1 binding site resulted in significant changes in Nr4c4 expression levels in different liver cells. Reduced Nr4c4 in hepatocytes with increased expression in Kupffer cells may result in a reduction of markers that activate hepatic stellate cells (HSC). HSC are the main cell involved in fibrosis development, a reduction in activation of HSC may result in the resistance to development of severe liver damage.

Figure 9. Nr4c4 gene has a significant role in the progression of liver disease. Nr4c4 is involved with the inflammatory response. We saw that a mutation in the Cdx1 binding site resulted in significant changes in Nr4c4 expression levels in different liver cells. Reduced Nr4c4 in hepatocytes with increased expression in Kupffer cells may result in a reduction of markers that activate hepatic stellate cells (HSC). HSC are the main cell involved in fibrosis development, a reduction in activation of HSC may result in the resistance to development of severe liver damage.