Dysregulation of autophagy and Nrf2 antioxidant responses contributes to accumulation of oxidative damage in Biliary Atresia

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ABSTRACT

Objective: Biliary Atresia (BA) is a cholestatic liver disease characterized by severe portal inflammation that progresses to fibrosis/cirrhosis. Effective therapies are lacking; 10-year transplant-free mortality rates are <40%. In children, biliary atresia accounts for approximately 40% of all liver transplants stressing the need for better understanding these diseases and developing innovative therapies. The objective of this study was to determine the status of autophagic and antioxidant responses in human biliary atresia. Methods: Using hepatic tissue and whole cell extracts isolated from healthy humans and patients diagnosed with end stage BA, overall fibrosis, ductal proliferation, oxidative stress, and induction of autophagy and Nrf2 antioxidant responses were assessed by immunohistochemical and immunoblotting methods. Results: From picrosirius red staining, extensive fibrosis was evident in human BA. Western blotting revealed a significant upregulation of expression of autophagic proteins but surprisingly, targets of the Nrf2 antioxidant response were suppressed. Increased autophagic staining colocalized in parenchymal cells with the accumulation of proteins that were post-translationally modified by reactive aldehydes as a consequence of increased oxidative stress. Conclusions: These data indicate that both oxidative stress/protein damage and autophagy are present in parenchymal cells in the perportal region in Biliary Atresia. This work was funded by a GALIIP award (CTS) and NIH U01DK062453 (R.J.S.) and NHL BI1 TH002535 (R.J.S.)

BACKGROUND

With the advent of effective therapeutics to treat common hepatic diseases such as Hepatitis C, there is increased interest in understanding rarer hepatic diseases, such as childhood and adult cholangiopathies. Cholangiopathies such as Biliary Atresia (BA) and are characterized by extensive biliary inflammation with destruction of the bile ducts. Alongside, these diseases accounted for ~16% of all liver transplants and 40% of pediatric transplants (Biliary Atresia) that were performed in the US from 1988-2014. No effective long-term therapies are currently available for Biliary Atresia patients. With only poor therapeutic options available, there is an urgent need for an improved mechanistic understanding of these diseases so that new strategies can be developed.

Autophagy is a regulated homeostatic mechanism in cells that recycles cellular components. Under normal conditions, these components can be proteins as well as lipids. In disease, dysregulation of autophagy contributes to the accumulation of damaged proteins/aggregates. During chronic inflammatory hepatic diseases, increased inflammation results in the abnormal production of oxidative radicals that can result in lipid peroxidation of cellular membranes forming highly reactive lipid aldehydes that can modify proteins on critical cytosine, lysine and histidine residues impairing function and impairing cellular processes. Recent evidence has linked oxidative injury with the initiation of autophagy in murine models of NASH. The objective of this study was to examine the localization of oxidative injury and autophagy in hepatic tissue procured from pediatric patients with Biliary Atresia.

Table 1. Demographics and laboratory tests of Biliary Atresia patients used in this study

<table>
<thead>
<tr>
<th>Age</th>
<th>MELD</th>
<th>Total Bilirubin</th>
<th>Creatinine</th>
<th>AST</th>
<th>ALT</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.95±1.27</td>
<td>20.00±6.35</td>
<td>6.95±4.17</td>
<td>0.31±0.03</td>
<td>162.00±35.12</td>
<td>487.25±110.19</td>
<td>286.25±39.9</td>
</tr>
</tbody>
</table>

RESULTS and CONCLUSIONS

1. Global Expression of Autophagic pathway members is upregulated in Biliary Atresia (Figure 2).

2. Global Expression of Nrf2 pathway members is suppressed in Biliary Atresia (Figure 3).

3. Periportal Nrf2 nuclear localization is increased in Biliary Atresia (Figure 4).

4. Increased expression of p62 is present in the perportal region during Biliary Atresia (Figure 5).

5. Increased expression of p62 occurs in the perportal region during Biliary Atresia (Figure 6).

6. Chronic cholestasis induces an increase in oxidative stress as demonstrated by increased MDA staining in parenchymal cells immediately adjacent to damaged perportal regions during Biliary Atresia (Figure 7).

7. Periportal accumulation of lipid aldehyde modified proteins occurs colocalizes in the same cell as the autophagic protein p62 during Biliary Atresia (Figure 8).

8. These results indicate that autophagy and oxidative damage are co-occurring in parenchymal cells immediately adjacent to the damaged perportal region during Biliary Atresia.

Conclusion: These data indicate that both oxidative stress/protein damage and autophagy are co-occurring in the parenchymal cells in the perportal region in pediatric Biliary Atresia. Future research will determine the cellular mechanisms and cross-talk between these 2 pathways.