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Review Article

Signaling in Simple Steatosis and Non-alcoholic Steatohepatitis Cirrhosis: TGF-β1, YAP/TAZ, and Hedgehog Pathway Activity

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Abstract

Non-alcoholic fatty liver disease (NAFLD) refers to the accumulation of fat in the liver tissue that is usually associated with metabolic disorders. Traditionally, the disease is regarded as a spectrum of pathological conditions ranging from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH) and hepatic fibrosis with progression to cirrhosis. However, so far, there is no available explanation for the disease progression. Several signaling pathways such as transforming growth factor (TGF)-β, hedgehog (HH), and yes-associated protein 1 (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) signaling are attributed to the NAFLD pathogenesis. TGF-β1 pathway component expression aligns with HH pathway ligands expression elevate in NASH cirrhosis while they decrease in SS. YAP and TAZ are two transcriptional co-activators from the Hippo signaling pathway. Similarly, the TAZ level (but not YAP1) is higher in NASH cirrhosis compared to SS. In addition, these three signaling pathways have little molecular similarity but their changes are totally similar in SS and NASH cirrhosis. The present review discusses the main changes in the expression of TGF-β, HH, and YAP/ TAZ pathway components in SS and NASH cirrhosis. It is hoped that these data provide a better understanding of the mechanisms that underlie the pathophysiology of NAFLD.
Keywords: Amphiregulin, Hedgehog, Hippo signaling pathway, Liver cirrhosis, Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis

Background

Currently, fatty liver is considered as one of the most important causes of liver disease and fatty liver disorders are generally divided into alcoholic fatty liver disease and non-alcoholic fatty liver disease (NAFLD). NAFLD refers to the aggregation of fat in the liver tissue, which typically related to metabolic disorders (1). The prevalence of NAFLD is increasing due to the epidemy of obesity so that 25% of the world population is infected with NAFLD. During the last decade, NAFLD has been described as the most prevalent cause of chronic liver disease in the world. In other words, NAFLD is considerably progressed in developed countries, and it is predicted that until 2020, NASH becomes the main cause of liver transplantation in the United States (2). Similar to many chronic liver diseases, most people with NAFLD have no clinical symptoms. According to Lim et al (3), NAFLD includes a range of pathological conditions that range from benign to acute form, including simple steatosis (SS), non-alcoholic steatohepatitis (NASH), progressive inflammation, fibrosis, cirrhosis, and eventually, hepatocellular carcinoma (HCC). NASH is a NAFLD case in which the accumulation of lipids within hepatocytes is associated with inflammation and varying degrees of fibrosis. In 12 to 40% of cases, SS progresses to NASH, and in 10 to 15 years, 15% of these patients develop advanced liver fibrosis (4). Although the exact mechanism of the progress of this disorder is still unknown, it has recently been suggested that, for the most part, SS cannot progress to NASH, and they are described as 2 different diseases. Therefore, the accurate and effective identification of the conditions leading to the creation of NASH is essential for proper therapeutic interventions. Accordingly, the present study reviewed and discussed the most important factors associated with signaling pathways involved in the development of this disorder in 3 groups including control liver tissue (histologically normal liver tissue), liver tissue with SS and the cirrhosis of the liver tissue derived from NASH.

Recent studies have challenged the progress of NAFLD of SS to NASH. It is shown that in the steatosis stage, the major fat accumulated in hepatocytes is triglyceride which is not hepatotoxic (5). In this regard, it is observed that

most of these patients have a steady state over time and rarely progress to the NASH stage (6). On the contrary, in the NASH stage, an accumulation of hepatotoxic lipids is observed, including diacylglycerol, saturated fatty acids, cholesterol, and phospholipids in the liver cells (7).

Hedgehog Signaling Pathway

Hedgehog (HH) is a morphogenic signaling pathway that helps the regulation of fibrotic responses in the liver (8). Our experiments showed that the expression of HH signaling ligands (i.e., sonic hedgehog: SHH and Indian hedgehog: IHH) was significantly lower in liver samples with SS in comparison to NASH-related cirrhosis and the control liver tissue (9). The presence of damaged hepatocytes in the liver tissue of NASH is one of the most important aspects of the distinction between NASH and SS. These hepatocytes are important sources of HH ligands (10) since the high amounts of HH pathway ligands is observed in human and animal NASH specimens. In addition, there is a direct relationship between the severity of liver disease and the activity of the HH pathway in the NAFLD from the NASH phase (11). Further investigations indicated that the expression of HH ligands in the liver tissue of patients with NASH cirrhosis was significantly higher than their expression in the liver tissue with SS (9). Although the mechanisms leading to the low expression of HH ligands in the liver tissue with SS have not been identified, the lack of the progression of SS to NASH has been reported recently (10). Our experiments are consistent with this theory of differentiation between SS and NASH. It is found that increasing the activity of the HH pathways results in the loss of white adipose tissue and the prevention of the formation of brown adipose tissue (12). Further, the activation of the HH pathway is confirmed to lead to the inhibition of lipogenesis in mammals (13). Contrary, it is reported that the mutation of the Smo protein leads to an increase in the expression of the enzymes involved in fatty acids, cholesterol, and triglyceride synthesis, resulting in high levels of adipogenesis and ultimately steatosis (14). It is worth mentioning that this protein is one of the mediators of the HH signaling pathway and its mutation causes impairment in the HH signaling pathway. These findings indicate an inverse relationship between lipogenesis and HH pathway activity. Therefore, the low activity of the HH pathway can be one of the causes of making SS in the person.

Regarding the inhibitory effect of HH pathway on lipogenesis, this question arises that how the activity of this pathway and the accumulation of fat in the liver lead to liver fibrosis. Various studies investigated the relationship between the activity of the HH pathway and the creation of SS. For example, it was revealed that an increase in the activity of the HH ligands in hepatocytes with steatosis reduces lipid accumulation and modulates its metabolism (14). Furthermore, the exposure of NASH mouse model to SHH ligand in a short time leads to fibrosis and then cirrhosis and HCC (15). On the other hand, it is observed that the prevalence of steatosis increases in patients with a defect in the SHH pathway. This finding is confirmed by animal studies as well (16). In addition, the mutation in the mouse liver Gli2 protein (one of the mediators of HH pathway that plays a role in expressing various genes and its mutation leads to the impairment of HH pathway), and then its exposure to high-fat diet causes reduced inflammation and fibrosis. These results are consistent with the finding of a previous study indicating that individuals with a mutated Smo gene were resistant to the progress of NAFLD (17). It is noteworthy that the exposure of normal hepatocytes with saturated fatty acids and lysophospholipids results in an increased expression of HH signaling pathway ligands (11). Therefore, there is a little chance that the expression of HH ligand in individuals with steatosis would be reduced due to exposure to lipid accumulation. Based on previous evidence, the basic activity of the HH pathway is necessary to prevent steatosis. Contrarily, steatosis development is likely due to a change in the mechanisms involved in the regulation of HH ligand expression. In other words, the reduction of the expression of the HH ligand leads to steatosis, but it prevents its progress toward NASH and NASH-related fibrosis. However, this issue requires more studies to indicate the low level of HH ligand expression in individuals with liver steatosis.

YAP/TAZ Signaling Pathway

Recent research on NAFLD has shifted to a new signaling pathway called yes-linked protein1 (YAP1)/PDZ-binding motif (TAZ). TAZ and YAP proteins are the ultimate goal of the Hippo pathway (18). Interestingly, in our recent study, TAZ expression in the liver tissue with SS, consistent with changing the level of HH ligands, was significantly lower than that of the control group (9). In some previous studies, it was suggested that the induction of TAZ expression is not a common feature of chronic liver disorders, and its expression is largely limited to those that are associated with steatosis, inflammation, and fibrosis (19, 20). In this regard, it was reported that inhibiting TAZ expression in hepatocytes leads to a reduction in the histological characteristics of NASH, along with a reduction in the expression of fibrogenic and inflammatory genes. Conversely, the excessive expression of TAZ in hepatocytes causes an increase in histological characteristics of NASH (21). Further investigation revealed that there was a direct and significant relationship between TAZ expression and HH ligands in the liver tissue (9). The results of our recent study are in line with those of other studies that highlighted the effect of TAZ activity on the expression of HH ligands expression. These studies initially confirmed the interaction between TAZ and the regulatory regions of

the IHH gene. Subsequently, they showed that inhibiting and depleting the TAZ gene resulted in inhibiting and reducing the expression of IHH in the mouse NASH model. More importantly, the increased expression of IHH in the mouse hepatocytes of NASH model results in the loss of positive effects of TAZ silence on these models. In other words, if we increase the expression of IHH in mouse hepatocytes of NASH, the TAZ shutdown is no longer able to reduce the histological characteristics of NASH, as well as the expression of fibrogenic and inflammatory genes (21). In summary, this evidence confirms the effect of TAZ on the regulation of HH expression.

In contrast to the remarkable role of TAZ, our recent experiments showed that YAP1 expression does not differ between liver tissue with steatosis and the control liver tissue (22). However, in a study in NASH mouse model, the results represented that YAP1 nuclear expression in the liver tissue is directly related to the severity of fibrosis and the accumulation of the cells with positive α-smooth muscle actin (23). The interpretation of this result is a bit complicated. Based on the evidence, the main role of YAP1 is to primarily interfere with liver cell renewal during hepatic injury and has a negligible role in inducing the liver fibrosis (22,24). It was reported that the overexpression and nuclear localization of YAP1 could result in hepatomegaly (23) and HCC (25). Further, it was shown that overexpression of YAP1 cannot induce the liver fibrosis in murine models (26). However, the YAP1 pathway needs more studies during NAFLD progress.

TGF-β1 Signaling Pathway

The significance of the TGF-β1 pathway is identified in the progression of fibrosis. Furthermore, TGF-β1 and HH pathways control various aspects of the process such as embryo development and cancer progression. Several studies suggested that these pathways are overlapping in their functions (23, 27). It is shown that HH pathway induces the expression of TGF-β1 family members in gastric cancer cells (28) while the pharmacologic inhibitors of the HH pathway in the lung cancer cells inhibit the activity of TGF-β1 pathway (29). TGF-β1 is one of the main pathways for the regulation of myofibroblastic cell function and communication and its activity increases in fibrotic tissues, leading to the accumulation of R-Smads (Smad2 + Smad3) with an increase in the expression of the target genes of this pathway (18). Based on the findings of another previous study, the expression of the TGF-β1 gene in the liver tissue with steatosis was significantly lower than that of the control liver tissue (22). Moreover, the expression of connective tissue growth factor (CTGF) and amphiregulin (AREG) genes (that contribute to fibrosis as the target genes of the TGF-β1 pathway) is significantly lower in the liver tissue with steatosis compared to NASH-related cirrhosis (22). Another important finding was the significant reduction in the levels of Smad2/3 and P-smad2/3 proteins in liver tissues with steatosis compared to the control liver tissue (9). These proteins are considered as the main intermediaries of the TGF-β1 pathway, and their reduction reflects a decrease in the activity of TGF-β1 signaling pathway. Investigating the relationship between TGF-β1 and HH signaling pathways showed that the expression of SHH ligand is directly related to the expression of the TGF-β1 gene, as well as Smad2/3 and P-Smad2/3 proteins (9, 22).

Additionally, the HH pathway involves in differentiating hepatic stellate cells (HSCs) into myofibroblasts (11). Similarly, SHH is one of the important ligands of the HH signaling pathway, which is synthesized and secreted in adults mainly by damaged hepatocytes instead of the normal hepatocytes. In addition, the expression of this ligand increases with the binding of lipids, especially fatty acids (30,31). Similarly, SHH ligand increases the expression of TGF-β1, CTGF, and AREG genes that play a significant role in fibrosis of hepatic stellate cells (7). Further, these genes mainly contribute to converting HSCs into myofibroblasts and thus creating fibrosis. Therefore, it is likely that the low expression of the fibrogenic genes in steatosis patients is due to the low activity of the HH pathway (Figure 1). Overall, the finding suggests that

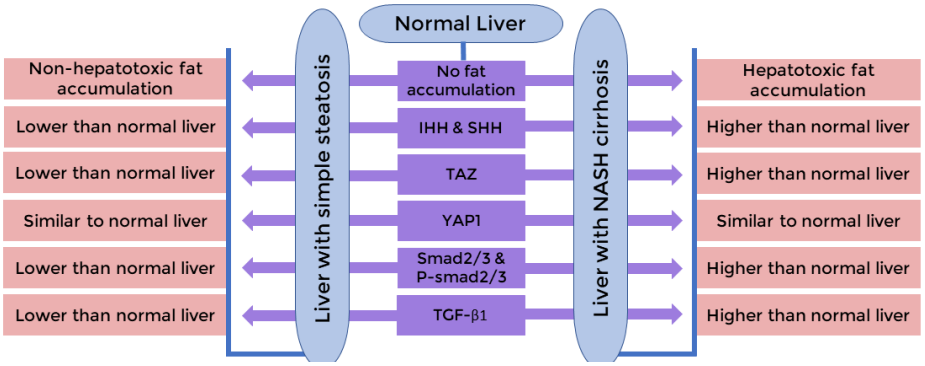

Figure 1. Differences in the Nature of Accumulated Fat and Different Expression of TGF-β1, YAP/TAZ, and HH Pathway Components in Simple Steatosis and NASH Cirrhosis
Note. SHH: Sonic hedgehog; IHH: Indian hedgehog; TGF-β: Transforming growth factor-β; YAP1: Yes-associated protein1; NASH: Non-alcoholic steatohepatitis.

mechanisms that reduce the level of HH ligands in the liver tissue with steatosis can also affect the activity of TGF-β1 pathway. In this regard, we investigated the relationship between TGF-β1 and TAZ pathways and found that the expression of TAZ was directly related to the expression of the Smad2/3 and P-Smad2/3 proteins (9). This result is in line with the findings of studies which suggested that TAZ is related to the TGFβ-Smad pathway during the fibrosis of many tissues (32,33).

Conclusion

Based on the findings, a significant reduction in the expression of HH ligands is regarded as one of the major causes of SS and this change may simultaneously prevent the development of this disorder to NASH. It is also possible that the mechanisms that reduce the level of HH ligands in liver tissues with steatosis can affect the activity of the TGF-β1 pathway as well. Finally, the results showed that TAZ may be an essential regulator for expressing HH ligands and TGF-β1 pathway activity during liver injury due to NAFLD.

Authors' Contributions

SM contributed to the drafting the manuscript and approval of the final version of the manuscript. ZK participated in the drafting the manuscript and approval of the final version of the manuscript; Heidar Tayebinia, contributed to the conception of the work, revising the manuscript, and approval of the final version of the manuscript.

Conflict of Interest Disclosures

None.

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