Review Article

A Decrease in the Activity of Inflammatory and Fibrogenic Pathways in Non-alcoholic Fatty Liver Disease as a Potential Defensive Strategy: A Mini-review

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Abstract

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver diseases, from steatosis to cirrhosis. Simple steatosis remains stable over time, and its development into non-alcoholic steatohepatitis (NASH) takes several years. Several pathways are involved in the progression of NAFLD, including the transforming growth factor-β (TGF-β), Hedgehog (Hh), Hippo, and AMP-activated protein kinase (AMPK) signaling pathways. These pathways have a complex interplay, and each pathway can either activate or inhibit other signaling pathways. This review summarizes the evidence implicating that a decline in the activity of the inflammatory and fibrogenic pathways decreases NAFLD. Considering the spectrum of NAFLD and fibrogenic signaling pathways and their cross-talks, it was expected that the activity of the pathways would increase through the occurrence of simple steatosis. However, the expression and activity of fibrogenic signaling pathways decreased in simple steatosis. The levels of Hh ligands, TGF-β1 gene expression, Smad2/3 and P-smad2/3 protein expression, and *Taz* gene and protein expression decreased in simple steatosis. In addition, the expression of Fos proto-oncogene, AP-1 transcription factor subunit (FOS), MYC, interleukin-1β, early growth response factor 1, and AMPK decreased in simple steatosis. Probably, hepatocyte metabolism is changed in simple steatosis to decrease the risk of production of different oxidative stress molecules, cellular proliferation, and apoptosis, as well as inflammatory and fibrogenic responses, as a potential defensive strategy. However, it should be noted that some changes in the liver tissue metabolism, including AMPK reduction, could be the consequence of high-energy balances in simple steatosis.

Keywords: Non-alcoholic fatty liver disease, Liver cirrhosis, Fibrosis, AMP-activated protein kinases, Transforming growth factor beta1, Hippo signaling pathway

Background

Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent chronic liver diseases, and its prevalence is globally increasing (1). The worldwide prevalence of NAFLD in adults is around 25% (2). The disease encompasses a spectrum of liver diseases, including simple steatosis, non-alcoholic steatohepatitis (NASH), a condition with hepatocyte inflammation and a small amount of fibrosis, different degrees of fibrosis whose final stage is cirrhosis, and finally, hepatocellular carcinoma (3). Fibrosis occurs in all stages of NAFLD, except for simple steatosis. Fortunately, the progression from simple steatosis to the stages of fibrosis occurs in a few patients and is typically stable over time. Most patients with simple steatosis do not experience significant clinical consequences (4). However, a larger proportion of patients with NASH, approximately 9%–20%, are at risk of developing fibrosis and cirrhosis within 5–10 years (4). Therefore, the clinical condition of patients with NASH or fibrosis should be assessed regularly.

Inflammatory and Fibrogenic Pathways

Although frequent studies in the literature have attempted to explain the precise mechanisms of steatosis and fibrosis, unfortunately, most of them have not fully elucidated the pathophysiology of these diseases. However, these studies have suggested several signaling pathways and metabolic intermediates that play a role in the pathogenesis of steatosis and fibrosis (5). The transforming growth factor-β (TGF-β) signaling pathway is one of the major pathways that have a

role in fibrogenesis (6). TGF-β affects fibrosis progression through its downstream mediators, including SMAD proteins. Fibroblasts are the major targets of the signaling pathway. In addition, the TGF-β signaling pathway can affect macrophages as well as vascular and epithelial cells (6). The Hedgehog (Hh) signaling pathway also plays an important role in liver regeneration and fibrosis (7). This signaling pathway has three different Hh ligands in mammals, including Indian (Ihh), Sonic (Shh), and Desert (Dhh) hedgehogs (8). The three ligands stimulate the Hh pathway in Hh-target cells in the same way. However, Hh ligand expression is regulated differently. Ihh and Shh are widely expressed, but Dhh is expressed mainly in the testis and nervous system (9). The pathway can induce liver fibrosis through its key components, including Gli1 and Gli2, which are transcription effectors (7). It has been shown that Hh pathway deficiency in hepatocytes dysregulates lipid metabolism and promotes hepatic lipotoxicity and metabolic consequences, including insulin resistance. Further, this condition induces inflammatory responses and fibrogenesis (10). In recent studies, it has been suggested that the Hippo signaling pathway is a player in NAFLD pathogenesis. The pathway has an essential role in many aspects of liver biology, including liver size regulation, stem cell self-renewal, and liver regeneration (11). In the mammalian Hippo pathway, transcriptional co-activators, Yes-associated protein (YAP1), and transcriptional co-activators with a PDZ-binding motif (TAZ) are regulated by mammalian large tumor suppressor kinases (LATS1/2) and Ste20-like kinases (MST1/2) (12). It has been indicated that while YAP1 generally contributes to cellular proliferation and fibrogenesis, TAZ induction is not universally associated with liver disease, and its function is limited to conditions involving steatosis and inflammation (13).

Crosstalk Between Inflammatory and Fibrogenic Pathways

Complex interplay among fibrogenic signaling pathways in different liver diseases, including NAFLD, has been reported in several studies. It has been confirmed through ChIP experiments that transcriptional co-activator Taz interacts with an enhancer/promoter region in the *Ihh* gene. Furthermore, Taz silencing prevents the overexpression of Ihh and its target gene *osteopontin* in a NASH mouse model (14). These findings approve the interplay between Hippo and Hh pathways. The Hippo pathway can affect TGF-β pathway responses. In other words, the pathway is the cardinal modulator of the TGF-β pathway and tissue remodeling (15). Taz nuclear localization is required for the nuclear accumulation of the Smad2/3-Smad4 complex. Therefore, the inhibition of Taz expression and its activity decreases the nuclear accumulation of the Smad2/3-Smad4 complex and thus inhibits TGF-β signaling pathway activity (16). Moreover, there are significant cross-talks between the Hh and TGF-β pathways. TGF-β and Hh signaling pathways can

directly regulate the key components of each other (17). Shh can induce TGF-β ligand and receptor expression (18). On the other hand, Smads can also increase *Gli2* gene expression, which in turn increases Gli1 expression. Therefore, TGF-β pathway deficiency could decrease Gli-mediated Hh signaling (19).

Decreased Level of Inflammatory and Fibrogenic Pathway Component

Considering the spectrum of NAFLD and fibrogenic signaling pathways and their cross-talks, it was expected that the activity of the pathways would increase through the occurrence of simple steatosis. However, there are two critical issues with simple steatosis. First, unlike the other forms of the spectrum of NAFLD, the progression of simple steatosis to NASH takes a long time and usually takes many years. This delay in the progress of the disease has led to various hypotheses and explanations, so that it has been hypothesized that simple steatosis and NASH are distinct conditions (3). Second, the expression and activity of fibrogenic signaling pathways decrease in simple steatosis, contrary to what was expected. It has been reported that the gene and protein expression of Ihh and Shh ligands, TGF-β1 gene expression, Smad2/3 and P-smad2/3 protein expression, and *Taz* gene and protein expression have decreased in simple steatosis. Furthermore, there were significant correlations between the components of all of these fibrogenic signaling pathways in simple steatosis (20). Interestingly, a decrease in the expression of components of different signaling pathways is not limited to those of fibrogenic pathways. A recent study using bioinformatics analysis and then verification by quantitative real-time polymerase chain reaction has reported that the hub genes of simple steatosis are likely Fos proto-oncogene, AP-1 transcription factor subunit (FOS), MYC, interleukin-1β (IL-1β), and early growth response factor 1 (EGR1), and the expression of these genes significantly decreases in simple steatosis (21).

FOS is a cellular protooncogene that contributes to TGF-β1 production (22). These findings are in line with the decrease in the activity of the TGF-β pathway in simple steatosis. MYC is a transcription factor that has been implicated in the induction of cellular proliferation or apoptosis (23). IL-1β, a pro-inflammatory cytokine, is involved in cell proliferation, apoptosis, and differentiation (24). It has been shown that IL-1β has an important effect on the progression of NAFLD (21). EGR1, a transcription factor, is typically involved in the processes of tissue injury, immune responses, and fibrosis (25). It has been reported that EGR1 has a negative correlation with the degree of steatosis. However, it is unclear why the expression of a factor that plays a role in fibrosis has a negative correlation with simple steatosis. As discussed, parallel to fibrogenic signaling pathways, the activities of the pathways that are involved in cellular proliferation and apoptosis are decreased in simple steatosis. Epidermal growth factor receptor (EGFR) deficiency is another finding that has

been reported in various models of mouse liver steatosis. The condition results in a decrease in the expression of two kinases, including Wee1 and Myt1 kinases. Because of the function of the kinases in liver regeneration, the steatosis models have liver regeneration defects (26). AMP-activated protein kinase (AMPK) is usually known as an energy sensor that is involved in cellular metabolism regulation (27). Similar to MYC, AMPK has dual functions in the pathogenesis of cancer. While AMPK deficiency has been shown in several tumor types, its activation can also increase tumor cell growth (28). Similar to other discussed pathways, AMPK expression is downregulated in both NAFLD and AFLD (29,30).

Expert Opinion
Simple steatosis, as the initial phase of NAFLD, is strongly linked to metabolic imbalances, particularly in high-energy conditions. In this condition, some fuels, including triglycerides, are accumulated in the liver tissue, and some increase in circulation, including lipids and carbohydrates. In theory, the accumulation of lipids in hepatocytes can induce oxidative stress and thus stimulate different pathologic conditions. Simultaneous activation of inflammatory and fibrogenic pathways with lipid-induced oxidative stress leads to the rapid progression of simple steatosis to NASH and cirrhosis. Hence, it is not out of mind that the hepatocyte metabolism is changed to decrease the risk of production of different oxidative stress molecules, to decrease cellular proliferation and apoptosis, and to decrease inflammatory and fibrogenic responses as a defensive strategy. However, it should be noted that some changes in liver tissue metabolism, including AMPK reduction, are consequences of high-energy balances in simple steatosis.

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