The Role of Notch Signaling Pathway in Non-Alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, and progressive NAFLD can develop into non-alcoholic steatohepatitis (NASH), liver cirrhosis, or hepatocellular carcinoma (HCC). NAFLD is a kind of metabolic disordered disease, which is commonly associated with lipid metabolism, insulin resistance, oxidative stress, inflammation, and fibrogenesis, as well as autophagy. Growing studies have shown Notch signaling pathway plays a pivotal role in the regulation of NAFLD progression. Here, we review the profile of the Notch signaling pathway, new evidence of Notch signaling involvement in NAFLD, and describe the potential of Notch as a biomarker and therapeutic target for NAFLD treatment.

Keywords: Notch signaling pathway, non-alcoholic fatty liver disease (NAFLD), steatohepatitis, lipid metabolism, insulin resistance (IR), fibrogenesis, autophagy

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), affecting over a quarter of the global population, has emerged as the highest prevalent type of chronic liver disease (Younossi et al., 2016). NAFLD encompasses a spectrum of progressive liver diseases including simple steatosis (SS), non-alcoholic fatty steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (D. Q. Huang et al., 2021; Powell et al., 2021). NAFLD is defined by the presence of steatosis in >5% of hepatocytes in histological analysis and exclusion of excessive alcohol consumption daily (≥30 g for men and ≥20 g for women) (EASL et al., 2016). Evidence suggests that NAFLD is related to liver manifestations of metabolic syndrome such as obesity, diabetes, insulin resistance (IR), and dyslipidemia (Younossi et al., 2018; Jarvis et al., 2020).

The individual clinical outcomes of patients with NAFLD are highly variable. For the majority of patients with simple steatosis, their liver disease is in non- or slow-progression. A prospective cohort study reported in a three-year period, over 20% of patients with simple steatosis developed into NASH (Wong et al., 2010), a more severe stage in which fatty liver is accompanied by necroinflammatory changes like hepatocyte ballooning and lobular inflammation (Vernon et al., 2011). In the final stages, collagen deposition and subsequent vascular remodeling result in fibrosis and cirrhosis (EASL et al., 2016). Thus far, there is no accurate non-invasive diagnostic biomarker and effective treatment toward NAFLD (Francque and Vonghia, 2019; Younossi, 2019), and current therapy is mainly focused on lifestyle changes (EASL et al., 2016; Chalasani et al., 2018).

Studies have shown that NAFLD is mainly characterized by hepatocyte inflammation and steatosis in the early stage and fibrosis and/or cirrhosis in the late stage (Wang et al., 2020). However, the pathogenesis of NAFLD has not been fully understood. In 1998, scientists first proposed the “two-hit” hypothesis to explain that steatosis (the first “hit”) and other factors associated with free radicals (the second “hit”) are necessary for NASH progression (Day and James, 1998). In recent years, based on animal models and descriptive clinical trials, the “multiple hits” hypothesis is widely accepted (Tilg and Moschen, 2010; Buzzetti et al., 2016; Tilg et al., 2021). The primary hit is the infiltration and pro-inflammatory state of macrophages in the visceral adipose
tissue, resulting in IR. Meanwhile, the abnormal lipolysis increases the delivery of fatty acids to the liver, and along with steatosis, aggravates the lipid metabolic burden. The imbalance results in the formation of lipotoxic lipids that generate a series of multiple hits, including oxidative and/or endoplasmic reticulum (ER) stress, inflammasome activation, and apoptotic damage, followed by inflammation, tissue regeneration, and fibrogenesis (Tilg and Moschen, 2010; Bessone et al., 2019; Sanyal, 2019). Besides, mitochondrial dysfunctions, lifestyle, and epigenetic and genetic factors also jointly affect the occurrence and progression of the NAFLD (Loomba et al., 2021) (Figure 1).

Notch signaling pathway plays a crucial role in cell differentiation (Amsen et al., 2009; Amsen et al., 2015), proliferation (Bartolome et al., 2019), and apoptosis (Guruharsha et al., 2012). Recently, it has also been demonstrated that Notch is involved in liver development, homeostasis, and metabolism (Bi and Kuang, 2015; Geisler and Strazzabosco, 2015; Adams and Jafar-Nejad, 2019). However, the association of the Notch signaling with NAFLD has rarely been reported. Here we review the recent advances in Notch signaling in liver pathophysiology and analyze the Notch signaling pathway as a potential target to prevent and treat NAFLD.

OVERVIEW OF NOTCH SIGNALING PATHWAY

Notch signaling is a juxtracrine signal transduction mechanism that enables cell-cell communication directly (Artavanis-Tsakonas et al., 1999). In mammals, four receptors (Notch1-4) and five ligands [Jagged (JAG) 1-2, Delta-like ligand (DLL) 1, 3, and 4] have been identified in canonical Notch signaling (D’Souza et al., 2010). In the liver of adults, four Notch receptors are expressed, while only two Notch ligands (JAG1 and DLL4) are expressed (Y. Chen et al., 2012). The ligand-receptor interaction is the initiation of Notch signaling pathway, making various cellular regulations more precise and orderly (Bray, 2016).

The core signaling pathway most commonly used to describe Notch-dependent processes is named the canonical Notch signaling pathway (Andersson et al., 2011; Guruharsha et al., 2012). The ligand presented by the Notch signal sending cell binds to the receptor on the signal-receiving cell. The endocytosis of the ligand leads to a conformational change of the Notch receptor, exposing the cleavage site of the ADAM10. Subsequent cleavage of the γ-secretase complex releases the Notch intracellular domain (NICD) (Kopan and Ilagan, 2009). NICD then migrates to the nucleus, binds to the transcription factor RBP-Jκ (also called CSL) (Koval and Ilagan, 2009), and recruits the co-activator Mastermind-like (MAML) to initiate downstream gene transcription, including the hairy enhancer of split (HES) and HES-related (HEY) family genes (Nam et al., 2006; Wilson and Koval, 2006; Bolos et al., 2007; Guruharsha et al., 2012) (Figure 2).

Different from other classical signal transduction processes, the canonical Notch signaling pathway is characterized by the lack of cascade amplification in the transduction process, and only NICD is generated after a Notch receptor is consumed. Therefore, its signal intensity is crucial for generating the corresponding cellular response, and any deviation in the expression level of any molecular component in the Notch
signaling pathway may have a vital impact (Andersson et al., 2011). For example, Alagille syndrome (AGS) is caused by mutations in the gene for the Notch ligand JAG1 and NOTCH2 receptor (McDaniell et al., 2006). Currently, various Notch signaling pathway modulation approaches have been explored, including inhibition of the ligand-receptor interaction and interference with the proteolytic process of the receptor (Groth and Fortini, 2012; Shao et al., 2012; Andersson and Lendahl, 2014). Several Notch inhibitors are demonstrated effects in the NAFLD (Table 1).

**NOTCH IN LIPID METABOLISM**

As the central hub of lipid homeostasis, the liver is responsible for coordinating the whole process of lipid circulation, including the synthesis, export, redistribution, and utilization of free fatty acids (Nguyen et al., 2008). The main pathways that constitute hepatic lipid homeostasis, including uptake of circulating lipids, de novo lipogenesis (DNL), fatty acid oxidation (FAO), and export as very-low-density lipoprotein (VLDL) particles (Gluchowski et al., 2017; Ipsen et al., 2018). Hallmarked by hepatic steatosis, NAFLD is connected with lipid metabolism. When lipid acquisition exceeds lipid disposal in the liver, that is, the uptake of fatty acids and DNL covering oxidation and output of fatty acids, hepatic steatosis occurred (Ipsen et al., 2018). Feng et al. (2017) proposed that no significant differences between free fatty acids (FFAs) in lean or obese patients with NAFLD were observed, and the value of serum FFAs in early diagnosis of NAFLD. The studies suggested that nutrition-induced activation of mammalian target of rapamycin (mTOR) may cause an increase in liver lipid content, which also increases the activity of basal serine/threonine kinases, leading to a self-perpetuating

![Diagram of Notch signaling pathway](image)

**TABLE 1** Major Notch signaling inhibitors in NAFLD

| Inhibitor                        | Target         | Function                                      | Object | References                  |
|----------------------------------|----------------|-----------------------------------------------|--------|-----------------------------|
| Peroxiredoxin 6 (PRDX6)          | Notch1         | Improve lipid accumulation through induction of mitophagy | Mice   | Lee et al. (2019)           |
| Notch1 decoy                     | Notch1         | Decrease hepatic glucose production           | Mice   | Funahashi et al. (2008)     |
| N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) | Notch1         | Alleviate lipid accumulation and hepatocyte injury | Mice   | Zhang et al. (2021)         |
| Delta-like1 homolog (Dlk1)       | Notch1         | Reduce hepatic steatosis and improve glucose and insulin tolerance | Mice   | Lee et al. (2016)           |
| Triptolide (TP)                  | Jag1/JAG1      | Initiate oxidative stress in hepatocyte       | Mice/   | Shen et al. (2019)          |
| Hepatocyte Toll-like receptor 4 (TLR4) | Jag1/JAG1     | Reduce NASH related liver fibrosis            | Human  | Yu et al. (2021)            |
| Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) | NICD           | Ameliorate hepatic lipogenesis dystipidemia and insulin resistance | Mice   | Chartoumpekis et al. (2018) |
| γ-secretase inhibitor (GSI)       | γ-secretase    | Improve glucose metabolism and ameliorate liver fibrosis | Mice   | Richter et al. (2020)       |
| Liver-specific Rbp-jk knockout (L-RBP-Jk) | RBP-Jk         | Protect from obesity-induced insulin resistance | Mice   | Pajvani et al. (2011)       |
| Silybin (SIL)                    | NOTCH1         | Hepatoprotective and antitumorigenic effect in HCC cells | Human  | Zhang et al. (2013)         |
| Delta-tocotrienol (δ-T)          | NOTCH1         | Reduce biochemical markers of hepatocellular injury and steatosis | Human  | Pervez et al. (2020)        |
lipogenic cycle (Lamming and Sabatini, 2013; Caron et al., 2015; Han and Wang, 2018). Pajvani et al. (Pajvani et al., 2013) demonstrated that inhibition of Notch signaling prevented hepatic steatosis by blocking mTOR complex 1 (mTORC1) activity, which could be reversed by rapamycin treatment. They also showed that Notch signaling augmented mTORC1 function and SREBP1c-mediated lipogenesis and that inhibition of hepatic Notch signaling protects from the fatty liver by reducing DNL.

Although the specific pathogenesis of lipid metabolism disorder in NAFLD patients is still not completely clear, studies have shown it may be associated with Notch pathway regulation (Li et al., 2019). Ding et al. (2020) investigated the dynamic role of Notch gene expression in the development of NAFLD in vitro and in vivo. They used palmitic acid (PA) and methionine-choline-deficient (MCD) models to assess notch signaling genes expression changes at different time points. Based on the characteristics of Notch mRNA expression levels, they evaluated that expression of Notch3 mRNA has been dynamically changed significantly in the development of hepatic steatosis during NAFLD (Ding et al., 2020). Furthermore, Auguet et al. (2020) explored the association between the Notch transcriptional repressor and hepatic expression of lipid metabolism-related genes in a cohort of women with NAFLD. They found a negative relationship between hepatic HEY2 expression and low-density lipoprotein (LDL) cholesterol (Auguet et al., 2020).

**NOTCH IN INSULIN RESISTANCE**

It is generally recognized that IR is pivotal in the pathogenesis and progression of NAFLD (Lomonaco et al., 2012). IR is essentially a decrease in the sensitivity of whole-body, liver, and adipose tissue to insulin, which is involved in the development of hepatic steatosis (E. Bugianesi, 2010). In NAFLD patients, increases in circulating glucose and insulin associated with IR promote the abnormal activation of Notch signaling pathway and IR are closely linked. It is recognized factor forhead box protein O1 (FOXO1) has a beneficial effect on insulin-mediated glucose homeostasis (Matsumoto et al., 2007; O-Sullivan et al., 2015). Notch signal mainly affects hepatic glucose via the synergistic effect of NICD and FoxO1 transcription. Glucose-6-phosphatase catalytic subunit (G6PC) and phosphoenolpyruvate carboxykinase (PCK1) are both rate-limiting enzymes of hepatic glycogenolysis and gluconeogenesis, which would be correlated with Notch activation (Valenti et al., 2013; Dongiovanni et al., 2016). Pajvani et al. (2011) reported that combined haploinsufficiency of FoxO1 and Notch1 notably improves insulin sensitivity in diet-induced IR. Hepatic overexpression of Notch1 regulates hepatic gluconeogenesis by inducing G6PC in a FoxO1-dependent mode, in turn, aggravates insulin resistance (Pajvani et al., 2011; Bernsmeier et al., 2016). Additionally, the reduction of metabolic activity in brown adipose tissue (BAT) has been found connected with IR in human (Stanford et al., 2013; Mottillo et al., 2016). Bi et al. (2014) revealed mice in which Notch1 or Rbp-jk selectively deleted in adipocytes show upregulated expression of BAT-specific genes and improvement in glucose tolerance and insulin sensitivity.

Based on the close relation between IR and Notch, several possible pharmacological targets of NAFLD are identified. Blocking the abnormal expression of Notch at the gene level can inhibit the accumulation of liver gluconeogenesis and triglycerides (TGs), thereby reducing the risk of NAFLD. The cleavage of NICD by γ-secretase inhibitor (GSI) exhibited an improvement of glucose homeostasis and insulin sensitivity in diet-induced obese (DIO) mice (Pajvani et al., 2011). Lee et al. (2016) demonstrated that Delta-like 1 homolog (DLK1), an inhibitory regulator of Notch signaling, would reduce hepatic steatosis and hyperglycemia via exogenous administration. Chartoumpakis et al. (2018) showed nuclear factor (erythroid-derived 2)-like 2 (Nrf2) could profoundly ameliorate hepatic lipogenesis and IR by repressing NICD. Besides, researchers also found plant extracts (such as curcumin) have been shown to suppress NOTCH1, which could ameliorate fatty liver and enhance insulin sensitivity in the high-fat diet (HFD) model (Zhao et al., 2017; Saadati et al., 2019; El et al., 2021).

**NOTCH IN OXIDATIVE STRESS**

Oxidative stress (OS) is a concept used to describe an imbalance between pro-oxidants and antioxidants, leading to cellular damage and tissue injury (Sies, 2015). The chronic high-calorie diet causes lipid accumulation in hepatocytes and excessive generation of reactive oxygen species (ROS) (Sahini and Borlak, 2014). Meanwhile, affected by lipotoxicity from high levels of lipid metabolites, OS inhibits insulin sensitivity and facilitates DNL (Gehrke and Schattenberg, 2020).

In the pathophysiological process of NAFLD, OS is considered a pivotal mediator of the inflammatory response (Koek et al., 2011). Notch signaling has been reported to be associated with steatosis and OS. It has been proposed that ROS like H2O2 regulates the expression of Notch (Marinho et al., 2014). Notch1 regulates the expression of lipid oxidation genes and exhibited an obvious lipid accumulation reduction in Notch1 deficient antisense transgenic (NAS) mice (Song et al., 2016). Similarly, Notch1 inhibitor reduces ethanol-induced OS and lipid accumulation in HepG2 cells (Wang et al., 2014).

Among the multiple mechanisms that accelerate the progression of NAFLD to NASH, mitochondrial dysfunction is the prime one (Caldwell et al., 1999). Mitochondrial abnormalities disrupt the balance between pro-oxidants and antioxidants, leading to an increase of FFAs (Begriche et al., 2013). Peroxisiredoxin 6 (PRDX6) is a mitochondrial antioxidant enzyme and is highly expressed in the liver (Fisher, 2011; Arriga et al., 2013).
et al., 2019). Lee et al. (2019) demonstrated that PRDX6 induces effects of maintaining mitochondrial integrity and inhibits OS-induced Notch signaling, thereby reducing ROS production and lipid accumulation. They pointed out that PRDX6 mitophagy-mediated mechanisms offer endogenous protection against NAFLD (Lee et al., 2019).

Moreover, triptolide (TP) is the main ingredient of the medicinal herb Tripterygium wilfordii Hook f (TWHF) (Ziaei and Halaby, 2016). TP caused hepatotoxicity through initiating OS. Shen et al. (2019) investigated TP inhibited the protein expression of Notch1 and NICD, and the activation of Notch signaling has the potential to protect against TP-induced liver injury. Interestingly, Huang et al. (2021) demonstrated that dose-related TP as an allosteric AMPK agonist alleviates NAFLD. Combined, the regulation of Notch signaling pathway may better enable TP to play a protective role in NAFLD.

**NOTCH IN INFLAMMATION AND FIBROGENESIS**

Liver fibrosis is a decisive factor of liver disease progression, particularly as it is associated with adverse prognosis and mortality in patients with NASH (Vilar-Gomez et al., 2018; Powell et al., 2021). Even in the early stage of fibrosis, it is shown a series of adverse liver-related events are gradually increasing (Angulo et al., 2015; Dulai et al., 2017; Hagstrom et al., 2017). In advanced NASH, hepatocytes are partially replaced by fibrotic scar tissue, the severe pathological change makes it difficult to treat NASH by correcting the underlying metabolic abnormality. Therefore, anti-fibrosis has become the focus of NASH therapy.

Notch activity is almost absent in healthy adult hepatocytes, mildly elevated in simple steatosis, and significantly increased in NASH (Valenti et al., 2013; Zhu et al., 2018). In various mouse models of fibrosis, over 80% of collagenous myofibroblasts are caused by hepatic stellate cell (HSC) (Mederacke et al., 2013). Notch-activated hepatocytes facilitate liver fibrogenic in NASH by both osteopontin (Opn) secretion mediated HSC activation in vitro and in vivo (Zhu et al., 2018), leading to a continuous extracellular matrix (ECM) accumulation and liver parenchyma gradually replaced by fibrous tissue (Mederacke et al., 2013). Conversely, in Notch loss-of-function mouse models, hepatocyte-specific liver inflammation and fibrosis are reduced, suggesting maladaptive hepatic Notch response to NASH-associated liver fibrosis (Zhu et al., 2018).

Sawitza et al. (2009) explored Jag1 as one of the cell surface ligands in Notch signaling activates HSC to stimulate α-SMA and collagen production. Yu et al. (2021) proved increasing Jag1 is responsible for fibrosis-inducing Notch reactivation. Also, other hepatic non-parenchymal cells could activate the Notch pathway to promote NASH latently through various mechanisms. Duan et al. (2018) investigated Notch activation in liver sinusoids endothelial cell (LSEC), which leads to HSC activation and the subsequent hepatic fibrosis, by downregulating eNOS-sGC signaling. Besides, researchers found that inhibitors inactivate M1 polarization of macrophage by regulating Notch signaling could reduce the secretion of inflammatory cytokine and fibrogenesis in CCl4-induced liver injury mice (Bansal et al., 2015; Xu et al., 2015; Sheng et al., 2020). Additionally, γ-secretase inhibitor (Chen et al., 2012) and Notch3 siRNA (Y. X. Chen et al., 2012) suppressed the myofibroblastic gene expression of rat HSC line by blocking Notch signaling. Therefore, selective interruption of these Notch-related targets may provide more anti-fibrosis strategies for NAFLD (Romeo, 2019).

**NOTCH IN AUTOPHAGY**

Autophagy is a process in which cells degrade and metabolize their own damaged organelles or protein aggregation (Wang et al., 2019), which plays a vital role in regulating multiple liver functions and maintaining hepatic homeostasis (Ueno and Komatsu, 2017). Accumulating evidence suggests autophagy regulates liver-mediated systemic glucose and lipid metabolism (Singh et al., 2009; Galluzzi et al., 2014). Meanwhile, the liver is surrounded by exogenous substances from the portal vein circulation, including potential inflammatory mediators, in which autophagy has major cell-protective and anti-inflammatory effects (Deretic et al., 2013; Deretic and Levine, 2018; Hazari et al., 2020). All of the above suggests autophagy is associated with the occurrence and development of various liver diseases such as NAFLD.

The lipid droplets (LDs) are specialized cytosolic organelles in which some organs including the liver store neutral lipids (such as TGs) to protect from lipotoxicity (Gross and Silver, 2014). The progression of LDs degradation is regarded as a specific form of autophagy, also known as lipophagy (Garcia et al., 2018). Recent studies have revealed that disturbances in lipophagy have been linked to hepatic lipid accumulation, the process of lipophagy could be regarded as a new way of controlling NAFLD development (Greffhorst et al., 2021).

Because autophagy can remove damaged organelles, autophagy may alleviate hepatocellular injury during NASH. The protective effects of carbamazepine-induced autophagy could reduce steatosis and improve IR in the NAFLD model (Lin et al., 2013). Indeed, modulating autophagy may prevent the progression of NAFLD. Zhang et al. (2021) investigated that Notch1 is an activated intensity of autophagy in FFA-treated HepG2 cells, and decreased Notch1 levels may alleviate hepatocyte damage by enhancing autophagy, which could be reversed by autophagy inhibitor chloroquine. Niture et al. (2018) demonstrated that inhibition of Notch reduced the expression of autophagy biomarker and serotonin-mediated liver cell steatosis. These findings provide helpful clues for the strategy of Notch signaling pathway to regulate autophagy and thereby remit the progression of NAFLD.

**NOTCH IN NAFLD-RELATED HCC**

HCC is the fourth leading cause of cancer-related deaths worldwide and occurs in patients with various chronic liver diseases (Bray et al., 2018; Llovet et al., 2021). Although hepatitis B virus (HBV) infection
has been the prominent risk factor of HCC, NAFLD has become the most rapidly growing driver of HCC in many countries (Younossi et al., 2019; Hester et al., 2020). The incidence in patients with NAFLD-related HCC increases with the histological stage, which is highest in patients with NAFLD-cirrhosis (Ioannou, 2021).

Thus far, the exact pathogenesis underlying NAFLD-induced HCC is only incompletely understood but mainly focuses on the effects of DNA damage response, inflammation, autophagy, and intestinal microbiota (Anstee et al., 2019; Behary et al., 2021). In addition, the chronic activation of metabolic pathways seems to play a critical role (Baffy et al., 2012). These pathways may provoke infinite hepatocyte proliferation and genomic instability, and on the other hand, provide a microenvironment conducive to malignant transformation and tumor growth.

Recent studies suggest that Notch signaling pathway is frequently associated with tumorigenesis (Nowell and Radtke, 2017). Selective blocking of Notch1 inhibits cancer cell growth and deregulates angiogenesis (Wu et al., 2010). By performing RNA sequencing of hepatocyte populations HFD-fed reporter mice, Zhu et al. (2021) illustrated that Notch-active hepatocytes showed transcriptional enrichment of ECM-related genes, which may represent a mechanism that persists in the tumorigenic process. Furthermore, they found HFD-diet mice with Notch-active mutation spontaneously formed fully developed liver tumors (Zhu et al., 2021). Therefore, it can be inferred that the continuous activation of Notch signaling pathway promotes the occurrence of NAFLD-related HCC.

CONCLUSION AND PERSPECTIVE

NAFLD is a manifestation of metabolic syndrome in the liver. With the changes in lifestyle and dietary habits, the incidence of NAFLD is rising rapidly. The previous studies have revealed the significance of the Notch signaling pathway in metabolism. The abnormal expression of Notch may lead to several metabolic disorders, thus inducing NAFLD. Although the relation between NAFLD and Notch signaling has been observed both in vitro and in vivo, most of the research findings are based on phenotypic studies and the underlying mechanisms and potential associations between different Notch molecules, and require further in-depth research.

The development of liver-specific Notch inhibitors is pivotal for the treatment of NAFLD-related hepatic lipid accumulation, IR, OS, fibrogenesis, and autophagy progression (Figure 3). But...
until now, most intervention studies are conducted in animal models (especially mice), the potential role of Notch regulators in human NAFLD needs to be explored extensively. Recently, the rising field of “hepatokines” biology would help reveal the complex molecular regulation in NAFLD (Watt et al., 2019). If so, it would promote the development of more non-invasive diagnostic tests to improve early diagnosis rates.

There is no specific and effective pharmacotherapy toward NAFLD, however, some drugs have shown therapeutic potential by regulating a Notch signal pathway. Vitamin E (α-tocopherol) is a dietary antioxidant recommended as a treatment for NASH (Yakaryilmaz et al., 2007; Chalasani et al., 2018). Recent clinical research supports vitamin E use brought obvious histological benefits and improved prognosis in patients with NASH (Sato et al., 2015; Brunt et al., 2019; Vilar-Gomez et al., 2020). δ-tocotrienol, an isomer of vitamin E, has been explored to inhibit tumor invasion and metastasis via downregulating the NOTCH1 signaling pathway (Rajasinghe et al., 2018). Notably, Pervez et al. (2020) launched a randomized, double-blind, placebo-controlled trial of 71 patients with NAFLD. Compared with placebo, δ-tocotrienol significantly reduced biochemical markers of hepatocellular injury and steatosis in patients (Pervez et al., 2020). Silybin (SIL), a hepatoprotective drug, could be an inhibitor targeting the NICD, RBP-Jκ, and Hes1 proteins in HCC cells and exert antitumorigenic effects (Zhang et al., 2013).

The precise drug delivery without toxicity brings a wide application prospect for the treatment of NAFLD. A nanoparticle-mediated delivery system to target GSI in the liver (GSI NPs) has been developed (Richter et al., 2020), which avoids goblet cell metaplasia caused by intestinal Notch inhibition (van Es et al., 2005). Based on similar studies above would advance clinical therapy research, thereby optimizing therapies for various NAFLD subtypes to increase the cure rate while complications can be decreased. In a word, findings on Notch signaling pathway research could bring NAFLD patients a hopeful future with ever more promising targets for prevention and treatment.

**AUTHOR CONTRIBUTIONS**

HX designed the outline of the review and drafted the manuscript. LW contributed his scientific advice and revision of the manuscript. All authors read and approved the submitted version.

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GLOSSARY

AGS  Alagille syndrome
DIO  diet-induced obese
DLK1  Delta-like 1 homolog
DNL  de novo lipogenesis
ECM  extracellular matrix
ER  endoplasmic reticulum
FAO  fatty acid oxidation
FFAs  free fatty acids
FOXO1  factor forkhead box protein O1
G6PC  glucose-6-phosphatase catalytic subunit
GSI  γ-secretase inhibitor
HCC  hepatocellular carcinoma
HFD  high-fat diet
HSC  hepatic stellate cell
IR  insulin resistance
IR  insulin resistance
LDs  lipid droplets
LSEC  liver sinusoids endothelial cell
MCD  methionine-choline-deficient

mTOR  mammalian target of rapamycin
mTORC1  mTOR complex 1
NAFL  non-alcoholic fatty liver
NAFLD  non-alcoholic fatty liver disease
NASH  non-alcoholic fatty steatohepatitis
NAS  Notch1 deficient antisense transgenic
NICD  Notch intracellular domain
Nrf2  nuclear factor (erythroid-derived 2)-like 2
Opn  osteopontin
OS  oxidative stress
PA  palmitic acid
PCK1  phosphoenolpyruvate carboxy kinase
PRDX6  Peroxiredoxin 6
ROS  reactive oxygen species
SIL  Silybin; Δ-T(Delta-tocotrienol)
SS  simple steatosis
TGs  triglycerides
TP  triptolide
TWHF  tripterygium wilfordii Hook f
VLDL  very-low-density lipoprotein