International Journal of
Cancer and Clinical Research

REVIEW ARTICLE

The Role of Notch Signaling in Liver Diseases: Contribution to Development and Cancer

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Abstract

Liver tissue consists of several types of cells such as hepatocytes, endothelial cells, stellate cells, cholangiocytes, and immune cells, all with essential functions. The liver plays an important role in metabolism because its cells act on a variety of substances, transforming them into metabolic products essential for life and the production of waste products. One interaction between different adjacent cells is known as Notch signal transduction, and abnormalities in this signaling pathway can cause serious liver diseases including inherited diseases and cancer that sometimes exhibit aggressive phenotypes and have poor prognoses. Moreover, over activation of Notch signaling is associated with tumorigenesis and linked to the development of cancer stem cells. Here, we review the association between liver diseases and Notch signaling abnormalities related to signal transduction in the liver and the development of hepatocytes or cholangiocytes.

Keywords

Notch, Jagged, DLL, HES, NICD

Introduction

Liver diseases have a diverse range of etiologies including infections, such as hepatitis A to E, cytomegalovirus, and Epstein-Barr virus, non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver diseases (NAFLD), and alcoholic liver injury or fibrosis. In addition, primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH) are immune-mediated, while Wilson's disease and Alagille syndrome, which exhibit severe liver dysfunction, are inherited. Furthermore, drug-induced liver injury is a common cause of abnormal elevation of transaminase. These liver diseases are detected by abnormal pathological findings in the liver tissue, for example, advanced liver disease may show severe fibrosis and inflammation in a specific area, and extra hepatic abnormalities such as portal hypertension, systemic edema, ascites and jaundice. Lymphocyte infiltration can be seen in the portal triad area in viral hepatitis, and around the central vein area in a congestive liver. These infiltrating cells consist of hepatocytes and in the case of cancer, hepatoma cells and occasionally cancer stem cells and non-liver parenchymal cells. These include lymphocytes, stellate cells, endothelial cells, macrophages, and cholangiocytes. These cells each have essential functions and there may be functional and signaling interactions between adjacent cells, regardless of cell type. Thus, to clarify the mechanisms of liver diseases, it is crucial to observe both the functions of the individual cell types and how the different types of cells interact, for example, the interactions between hepatocytes and stellate cells, hepatocytes and endothelial cells, and hepatocytes and lymphocytes. These interactions are associated with specific signal transductions such as Wnt/β-catenin signaling, and MAPK and IFN signaling. Notch signaling is reportedly associated with signal transduction in these cells and in the development of liver cells. Furthermore, abnormalities in a ligand in the Notch signaling pathway, known as the Jagged1 gene, leads to Alagille syndrome, which is a well-known liver disease. This syndrome displays hypoplasia of cholangiocytes and systemic abnormalities in other organs, such as the lungs and bodies of the vertebrae. However, recent research has revealed Notch-related abnormalities in other liver diseases such as liver cancer and steatosis. Therefore, Notch signaling is closely associated with liver diseases as well as the physiological functions of

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the liver. Here, we introduce the association between Notch signaling and various liver diseases with reference to the literature.

Notch Signaling and the Functions of Component Molecules

Notch signaling is mainly associated with cell-cell signal transduction to adjacent cells, and is not a form of communication between remote cells. There are several Notch transduction ligands, such as Jagged1, Jagged2, DLL1, DLL3, and DLL4, and receptors such as Notch1, Notch2, Notch3, and Notch4 [1]. In 1913, Notch signaling was discovered by observing a notch-like defect in the wings of *Drosophila* and the phenotype was named "Notch" [2]. In the 1930s, a homozygote Notch mutation was found not in the epidermidis but in nerve tissues, which suggested that Notch expression is related to the formation of neurogenic factors [3]. Notch-related genes were cloned and sequenced for the first time in 1985, revealing that Notch is a transmembrane receptor with extracellular and intracellular domains [4,5]. The Notch receptor and ligand were also found later in *Drosophila*. However, although many Notch-related factors including ligands and receptors have been discovered, their exact functions have not been clarified [1]. Research conducted on *C. elegans* discovered a Notch homolog and found that Presenilin1 (PSEN1) is an essential factor for Notch activation [6-8]. Thereafter, it was discovered that the function of PSEN1 is to cut the transmembrane protein, and gamma secretase was found to act as catalyst at the transmembrane region of Notch. Kopan, et al. found that the Notch intracellular domain (NICD) translocates to the nucleus and activates the expression of the target gene, while removal of the signal results in loss of the gene's function [9]. This discovery was associated with Notch signaling in that the receptor, such as Notch1, was activated by contacting the ligand of adjacent cells such as Jagged1, cut by gamma secretase, and NICD was transported to the nucleus directory to regulate downstream gene transcription. Furthermore, NICD is transported to the nucleus and binds to Su(H)/RBPj and p300 as a histone accessory factor. HES or HEY family genes are activated as downstream genes [10,11]. After these genes are transcribed, NICD undergoes proteasomal degradation with phosphorylation by Cdk8, poly-ubiquitination by Fbxw7, resulting in the termination of signal activation [12]. Glycosylation is another phenomenon of Notch signaling, which is important for the modulation of signaling [13,14]. Notch is glycosylated by rumi or POGLUT1 and fucosylated by O-FUT1 or POFUT1. The glycosylic chain of the fucosylated Notch is elongated at the Golgi apparatus by Fringe, LFNG, MFNG, and RFNG. These enzymes are glycosyl chain elongation enzymes in addition to fucose and GLcNAc. Fringe-modified Notch is affected by D1/DLL but not Ser/JAG, and the non-fringe modified form is affected by Ser/JAG but not D1/DLL. Therefore, Notch activation differs depending on the type of ligand and receptor, and these indicate whether there is fringe or not [15,16].

Whether there are sufficient adjacent interactions between Notch ligands and receptors is also important, and is has been found in *Drosophila* studies that receiving cells such as Notch receptors rely on dynamin to perform endocytosis [16]. This is adjusted by ubiquitin E3 ligase, which codes mind bomb 1 (mib1), resulting in an association with amino acid that is sensitive to ADAM protease. Notch receptor at the transmembrane is first processed by ADAM protease and that changes to NEXT (Notch extracellular truncation), before processing to NICD (S3 cleavage) by gamma secretase complex [17]. Therefore, Notch activation-related factor is not a ligand or receptor as described above, and its signaling does not have an amplifying effect in receiving cells; adistinct mechanism from other types of signal transduction. This indicates that the intensity of the Notch signal between cells is affected by the amount of Notch receptor transcription, accessory factor patterns, amount of expression and binding, amount of endocytosis of the ligand-receptor complex, and level of S2 and S3 cleavage [18], instead of simply by signal amplification a single cell.

The role of Notch signaling is associated with the generation of more than two types of cells from uniform cells types. There are several mechanisms that can achieve this, such as lateral inhibition, asymmetric cell division, lineage decisions, and induced signaling [19,20]. Lateral inhibition by Notch activation results in a decrease in Notch ligand expression [21]. This generates two types of Notch-off cells and Notch-on cells patterns. When observing asymmetric cell division, a cell duplicates, and in one duplicates Notch is activated, and in the other it is not, leading to different cell fates, and is also asymmetrically distributed by the interaction of the cell polarization molecule and endoplasmic reticulum transportation in cells [22]. The induction of Notch signaling causes activation by one-sided signaling of ligand-expressing cells and signaling is dependent on the type of Notch receptor and the Fringe.

Notch Signaling Abnormalities and Diseases Related to Development

Molecular research into Notch signaling has developed through the analysis of *Drosophila* and *C. elegans*; however, this is insufficient for the analysis and discovery of Notch signaling related molecules in mammalian cells. On the other hand, this research has resulted in the discovery of Notch-related essential molecules in mammalian cells in mice and humans. Neural development-related studies using a mouse model have led to rapid progression in the field [23,24]. Alagille syndrome is a well-known autosomal dominant Notch-related inherited disease in humans [25-27], and causes cholestasis by decreasing the numbers and functions of the bile duct cells and causing chronic liver injury. Fur-

thermore, abnormalities of the heart and blood vessels, vertebrae, eyes, and specific facial characteristics may occur. The disease was discovered in 1969 by Alagille, and patients with all five of the characteristic abnormalities are known as "complete types", while those with four are known as "incomplete types" [28-30]. In 1997, the discovery of a Jagged1 genomic abnormality at 20q was reported [27,31-33]. In 2006, a Notch2 genomic abnormality was also reported [34]. The population frequency of Alagille syndrome is 1 per 30,000 to 70,000 and ulsodeoxy-acid, and cholestyramine is used to control bile acid metabolism in cholestasis patients. However, if liver cirrhosis occurs, transplantation may be considered. Nutritional therapy with lipid soluble vitamins and medium chain fatty acids may also be considered [35,36].

It has been reported that a Notch1 mutation is associated with inherited heart diseases [37]. This mutation is especially linked to the bicuspid aortic valve and calcification of the aorta [38,39]. Moreover, it is associated with the tetralogy of Fallot, which encompasses pulmonary artery stenosis, ventricular septal defect (VSD), over-riding aorta, and right ventricular hypertrophy. Mutations of Jagged1 and Notch2 gene cause Alagille syndrome and tetralogy of Fallot [40,41]. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominant inherited disease that causes cerebral infarction and angiogenic dementia [42-45]. This disease is reported to be associated with a Notch3 mutation which results in the extracellular domain of Notch3 accumulating in the cerebral micro vessels.

The relationship between Notch signaling and heart vessels reportedly includes stages of development from bone marrow cells to endothelial cells during fetal life, and Notch signaling facilitates inflammation and induces senescence of endothelial cells, resulting in atherosclerosis [46,47]. These reactions are reported to involve macrophages [48]. For example, antigen-presenting cells that express DLL1 or DLL4 cause accelerated differentiation to Th1 cells. Th1 and Th1 cytokines accelerate inflammation of the heart vessels. However, Jagged1accelerates differentiation to Th2 cells, which suppresses inflammation in the heart vessels [49,50]. Dll4-Notch signaling enhances plaques and fibroblast collagen and calcification in vessels, upregulates insulin resistance, increases the amount of fat cells, and promotes fatty liver by the deposition of fat in the liver, resulting in fatty liver diseases including NAFLD or NASH [51,52]. DLL4 antibody is reported to suppress M1 macrophages [53] and experiments have shown that it induces inflammation via mediators such as iNOS, which is atypical M1 macrophage mediator [48]. The study also showed that M1 macrophage polarization related to DLL4 antibodies could be a candidate for various diseases related to inflammation, including in the heart vessels and organs related to metabolism.

Differentiation of Hepatocytes or Cholangiocytes by Notch Signaling and Liver Diseases

Alagille syndrome has characteristic ductal loss that indicates hypoplasticity in the bile ductal cells. This is because of a loss of Notch transduction function caused by a Jagged1 mutation. However, Notch signaling is involved only in the bile ducts in this disease. A previous report has shown that Notch contributes to the formation of intrahepatic cholangiocarcinoma (ICC) arising from the conversion of hepatocytes rather than cholangiocytes activated by Notch [54]. This indicates that Notch signaling activates malignant characteristics in hepatomas and ICC. Liver cancer has been reported especially frequently, even among other hepatomas. Reports suggest that RUNX3 is associated with the suppression of liver cancer, and that Jagged1 gene and RUNX3 are associated [55,56]. We previously analyzed clinical samples and showed that Jagged1 genomic amplification and over expression in AFP-producing cells was associated with liver cancer, as well as the malignant characteristics of cancer and overall survival [57]. Jagged1 is reportedly associated with upstream YAP and Hippo signaling. Moreover, gamma secretase inhibitors (GSIs) are effective angiogenetic factors of liver cancer [58-60]. Liver tumorigenesis caused by hepatitis B virus (HBV) is associated with the HBV-x genes directory or disease progression [61-65], and the HCV core protein is regulated by gamma secretase, which regulates Notch signaling [66,67]. Liver cancer is mainly associated with stemness and Notch regulation is associated with stem cell differentiation [68], and POGLUT 1 copy number variations [69,70]. Furthermore, DLL-Notch pathway is associated with liver fibrosis and M2 macrophage activation [51,71,72]. Conversely, M1 macrophages are associated with alcoholic liver injury via Notch signaling [73,74]. Some liver cancers are associated with angiogenesis, which is regulated by Ephrin [75] and in turn regulated by GSIs [76], which decrease hepatoma cells, especially α-protein (AFP)-upregulated cells. Blocking of notch signaling components such as Jagged1 shRNA results in an effective decrease of AFP-upregulating hepatoma cells [57]. Thus, gamma secretase may be a target for liver cancer therapy, although the phenotype might be restricted. Fibrosis-related Notch signaling abnormalities in liver diseases are important for liver cancer research because many liver cancers have a background of progressive fibrosis and inflammation, and these microenvironments are closely associated with tumorigenesis cancer formation and more aggressive carcinogenic characteristics. This phenomenon can result in Notch transduction abnormalities between adjacent but different types of cells.

Function of Notch Signaling Related Molecules and the Liver

The association between Notch signaling and liver tissue is introduced in relation to the development of he-

Table 1: Roles of notch ligands and receptors associated with liver diseases, especially liver cancer.

		Related fucntions with liver diseases	Ref.	Association with liver cancer	Ref.
Ligands	Jagged1	mutation: Alagille syndrome	[31]	upregulation: cancer, poor prognosis	[65]
				copy number gain: cancer, poor prognosis	[57]
	Jagged2	NR		NR	
	DLL1	NR		NR	
	DLL3	NR		NR	
	DLL4	fibrosis and NASH pathogenesis	[52]	HBV-x relation	[90]
Receptors	Notch1	biliary differentiation from HPCs by autophagy	[77]	upregulation: cancer, poor prognosis	[78]
				HBV-x relation	[90]
	Notch2	bile duct development	[80]	aggressiveness of liver cancer	[79]
		relation with hepatoblastoma	[82]	HPCs: dedifferentiated liver cancer	[81]
		genomic abnormality: Alagille syndrome	[34]		
	Notch3	diffentiation and progression of cholangiocarcinoma	[83]	HBV-x relation	[90]
		modulating the stemness of tumor cells	[84]		
	Notch4	hepatocytic-liniage commitment of HPCs	[87]	upregulation: cancer, poor prognosis	[88]
				HBV-x relation	[90]

NR: not definitely reported; HPCs: hepatocyte progenitor cells; NASH: nonalcoholic steatohepatitis.

patocytes and cholangiocytes, including progenitor cells that include cancer-related cells. We have introduced the functions of Notch ligand and receptors, showing that each molecule has a different function, and the different functions lead to different types of diseases (Table 1). Notch1 is associated with the tumorigenicity of hepatocytes, and biliary differentiation from HPCs is controlled by autophagy [77,78]. Notch2 is associated with developmental retardation and bile duct development, since this defect results in hypogenesis of cholangiocytes. Moreover, Notch2 is associated with the aggressiveness of liver cancer and hepatoblastoma [79-82]. Notch3 drives the differentiation and progression of cholangiocarcinoma [83]. However, the relationship between its activation levels and liver cancer has not been defined, although it is reported to modulate the stemness of tumor cells [84]. Notch4 induces reversible arteriovenous malformation and this deficiency results in angiogenesis, vascular remodeling, and hepatocyte-lineage-HPCs, resulting in tumorigenesis [85-88].

Yang, et al. reported Notch1, Notch3, and Notch4 upregulation is associated with liver cancer involving HBV-x protein [61,86]. As Notch ligands, Jagged1 mutations or defects constitute the pathogenesis of Alagille syndrome, while Jagged2 is not usually related to hepatocytes or liver tissue. Though DLL1 defects stimulate neuronal differentiation and severe somite patterning defects, reports on their relationship to the liver are scarce. Moreover, while DLL3 defects produce abnormalities in somitogenesis and autosomal recessive spondylocostal dysostosis, they show little association with liver diseases [89]. DLL4 defects exhibit arteriovenous shunting and severe vascular remodeling defects, and the molecule has been reported many times in relation to liver diseases such as liver cancer, NASH, and HBV-x related tumorigenesis [76,90,91]. These results indicate that Notch-related ligands and receptors produce loss of organ or tissue formation, especially in vascular tissue, bile ducts, and neurons.

Notch activation is associated with liver cancer and this triggers epithelial-mesenchymal transformation promoting the self-renewal of cancer stem-like cell niches in primary and monastic tumors. In chronic HBV infection, the repression of Notch receptors in chronic hepatitis B (CH-B) patients is suggested to repress immune regulation, which results in the inhibition of differentiation and the proliferation of effect or cells, consequently leading to further pathogenesis [92]. The pro-oncogene function of mastermind2 (MAML2) is to target genetic alterations in various types of cancer, and it is associated with Notch activation even in hepatobiliary neoplasms [93-95]. Genetic analysis has shown that mice over expressing NICD represent a cluster of liver cancers [96]. Notch2 over expression causes HPCs to spontaneously develop into dedifferentiated liver cancer cells [97] and Notch-induced malignant hepatocyte transformation is associated with down regulation of hepatocyte-associated genes, including Sox9 [98]. In the development of cholangiocarcinoma NICDs associated with protein kinase B signaling stimulates the malignant differentiation of hepatocytes [99].

Notch Inhibition is Effective for Liver Cancer Treatment

Most reports showed that Notch signaling is enhanced in mouse cancer models and inhibition results in a reduction in tumor size. Moreover, Notch activation is reported to result in a more malignant phenotype, and from our report of clinical samples, Notch upregulation was associated with poor outcome even after initial therapy such as surgery [57]. Both mouse models and human clinical samples show that notch promotion results in poor survival prognoses; thus, it would be a useful biomarker for aggressive types of liver cancer. Experimentally, GSIs are useful for tumor suppression and prolong survival in mouse liver tumor models [100-102]. GSIs have already been investigated in clinical trials for Alzheimer's disease; however, the trials were terminated because

of gastrointestinal toxicity [103,104]. Several types of GSIs are in ongoing investigations and some exhibit less toxicity, therefore they may be useful as anticancer therapies [105]. The pharmacological characteristics show that there are some differences in the catalytic positions of gamma secretase at the transmembrane region and several types of GSIs have been introduced [106-108]. One GSI shows less gastrointestinal toxicity and is in ongoing clinical trials [103,109]. Besides reports of GSIs treatments for liver cancer, there are some Notch targeting therapies using other clinically relevant drugs. The combination therapy reports the effects of IL-17 antibody Secukinumab and with IL-35 that shows blockade of Notch signaling pathway [110]. Akt inhibitor effect for suppressing hepatoma cells proliferation by modulating PI3-K/Akt and Notch pathway is also reported [111]. Thymoquinone is reported Notch inhibiting effect with cell cycle suppression that related with NICD expression [112]. Moreover, this drug may be useful in combination with other anticancer therapies. Anticancer therapies for liver cancer do not always need to be systemic, since localized therapy such as radio frequency ablation (RFA) or transcatheter arterial chemoembolization (TACE) may be useful. It may be more effective to administer GSIs locally along with these therapies to reduce the adverse effects associated with GSIs.

Notch Signaling and Liver Immunity

Burghardt, et al. reported that immune regulation in the liver was associated with Notch activation in T cells, especially in the regeneration of livers of Con-A treated mice via an induced regulatory phenotype in naïve CD4+T cells [113,114]. These T regulatory cells release IL-10 and produce IFN-γ associated with the activation of Notch signaling [115]. ConA-pretreated mice show increased Jagged1 expression with high receptor density of Notch1 in CD4+T cells. Th1 cell Notch upregulation, particularly Jagged1 production, indicates liver inflammation, which is associated with liver regeneration after Th-1-mediated hepatitis. Another report has shown that liver sinusoidal endothelial cells (LSECs) induce immunosuppressive IL-10-producing Th1 cells via Notch signaling [115]. The group studied the capacity of LSECs in the regulation of T-cell induced liver regeneration. They found that DLL and JAG family Notch ligands induced activation of downstream HES-1 and deltex-1 in Th1 cells and this was associated with liver inflammation. Other reports have hypothesized that DLL4-Notch signaling is only associated with inflammatory responses, and these immune responses appear in adipose tissue, arteries, vein grafts, and the liver via macrophage activation associated with IL-1b, IL-6, CCL2/MCP-1, iNOS, NF-kB, DLL4, and MMP-9 and MMP-13 [52,116,117]. These inflammation processes related with Notch signaling are very close with not only liver regeneration after acute or chronic liver injury but also key immune molecules for cancer therapy for malignancies and immunity against infections [118].

Notch Signaling is Closely Associated with Liver Diseases and Useful Key Molecules for Liver Cancer Therapy

Reports show that liver diseases are associated with abnormal Notch signal transduction because disease arises from abnormalities in the signaling between adjacent cells-whether the cells are of the same type or not-and Notch signaling is closely associated with the development of many types of cells, including cancer cells and immune cells. The mechanism applies to liver diseases related to infection with hepatitis viruses, including HBV and HCV. Such viruses contribute to advanced liver disease via Notch signaling abnormalities and, based on clinical data, exhibit aggressive cancer phenotypes and poor prognoses. HBV-x contributes to Notch activation and promotes liver carcinogenesis, and HCV core protein is also associated with gamma secretase catalysis at the transmembrane region. These mechanisms are associated with cell-to-cell contact, and closely linked to signal transduction. Liver immune function may be associated with Notch signaling in cases of regeneration after liver injury. Anticancer therapies related with Notch signaling for liver cancer are ongoing in various basic and clinical studies, and from many recent reports indicate that modulating Notch signaling is useful therapies to the extent of targeting cancer stem cells, highly malignant and short prognosis types of cancer cells and holding immune modulating effect. These characteristics will result in improving prognosis in combination with existing therapies.

Conclusion

Notch signaling abnormalities are present in various liver diseases and affected by changes in the tissue microenvironment in the liver. These changes originate from hepatocytes and non-hepatocyte cells, including lymphoid, endothelial, stellate, and cholangiocyte cells. Notch inhibition related anti-cancer therapy is useful for hepato-biliary malignancies and may be more effective in combination with existing anticancer drugs.

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