



REVIEW ARTICLE

Regeneration and activation of liver progenitor cells in liver cirrhosis

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Abstract Cirrhosis is characterized as the progress of regenerative nodules surrounded by fibrous bands in response to chronic hepatic injury and causes portal hypertension and end-stage hepatic disease. Following liver injury, liver progenitor cells (LPCs) can be activated and differentiate into hepatocytes in order to awaken liver regeneration and reach homeostasis. Recent research has uncovered some new sources of LPCs. Here, we update the mechanisms of LPCs-mediated liver regeneration in cirrhosis by introducing the origin of LPCs and LPCs' niche with a discussion of the influence of LPC-related cells. This article analyzes the mechanism of regeneration and activation of LPCs in cirrhosis in recent years aiming to provide help for clinical application.

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Introduction

Liver is an important organ which detoxifies various metabolites, synthesizes proteins and produces biochemicals

necessary for digestion and growth. Liver, moreover, has a strong regenerative and compensatory capacity. Hepatocytes are produced by the proliferation and division of hepatocytes and the differentiation of liver progenitor cells (LPCs). However, the ability of liver cells to divide and proliferate is weakened under pathological conditions such as cirrhosis, in which, thereupon, progenitor cells are activated to differentiate into hepatocytes and bile duct cells to maintain liver homeostasis. The proliferation of mature hepatocytes and the expansion of LPCs are two ways of liver regeneration. After partial hepatectomy or acute liver injury, liver regeneration occurs mainly through proliferation of liver cells. In severe or chronic liver injury,

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the proliferation of liver cells is impaired and LPCs are activated, which could differentiate toward hepatocytes and cholangiocytes, and repopulate the injured epithelial and promote the recovery of liver function.

Origin and activation of LPCs

In adult liver and biliary tree, there are two distinct populations of progenitor cell, namely Liver Progenitor Cells (LPCs), located in the smallest branches of the biliary tree (canals of Hering and bile ductules), and Biliary Tree Stem Cells (BTSCs), found in the peribiliary glands (PBGs) of large intrahepatic and extrahepatic bile ducts.^{1,2} In chronic and severe injury, liver progenitor cells (LPCs) are first activated and then differentiate into hepatocytes and bile duct cells to maintain liver homeostasis.

Actually all new hepatocytes do not come from facultative stem cells, also known as "oval cells" or "atypical ductal cells". These conclusions were confirmed by studies based on genes and nucleoside analogues that marks and tracks the origin and contribution of various cell populations during liver regeneration.³ A study to determine the origin of cells using the method of genetic lineage tracing found that hepatocytes, rather than cholangiocytes, are the major source of cells for primitive ductules formed in response to chronic liver damage, and in which Notch-hes1 signal plays an important role in this process.⁴ In addition, other studies found that the loss of 1-integrin in hepatocytes induced liver injury triggers a ductular reactions (DRs) on the origin of cholangiocytes, about 25% of which are from non-hepatocyte origin. β 1-integrin knockdown or overexpression of p21 could inhibit hepatocytes proliferation, which led to the proliferation of

hepatocytes derived from cholangiocyte.⁵ This suggests that cholangiocytes are also a source of progenitor cells. HSC expresses the stem cell/progenitor marker CD133 and can differentiate into hepatocytes after dealing with a variety of cytokines such as VEGF164, bFGF, EPO and IL-6.⁶ After 7 days of culture of CD133+HSC on a medium containing PDGF-BB, they were transformed into myofibroblast like cells.⁶ *In vivo* lineage tracing studies which uses an injury mouse model induced by bile duct ligation and Choline-Deficient, Ethionine Supplemented (CDE) diet have shown that HSC is a source of myofibroblasts and progenitor cells.⁷ The stationary hepatic stellate cells transform into myofibroblast promoted by Hedgehog (Hh) pathway, and some of which become regenerative progenitor cells.⁸ Hence we could draw conclusion that multiple cell sources have the ability to convert into LPC (Fig. 1).

Hepatocytes contributing to liver regeneration can be differentiated from LPCs activation following liver injury. Hepatocyte-mediated regeneration predominates as normal liver undergoes partial hepatectomy or acute injury, while in chronic and severe injury, however, ductular reactions (DRs) of activated biliary epithelial cells containing LPCs appear in the periportal regions.⁹ DRs refer to epithelial components and their related inflammatory niche. The proliferation of the hepatocytes can be inhibited by disrupting Ikk-NF- κ B pathway, however this can also stimulate the production of Hh ligands which emits viability signals to progenitors and myofibroblasts resulting in the accumulation of these cell types and the cause of fibrogenesis.¹⁰ Investigation showed autophagy and the Wnt/ β -catenin pathway are activated during the differentiation of LPCs. Autophagy was inhibited by down-regulation of Atg5 gene expression, which attenuated the differentiation of LPC and inhibited the Wnt/ β -catenin pathway. In addition,

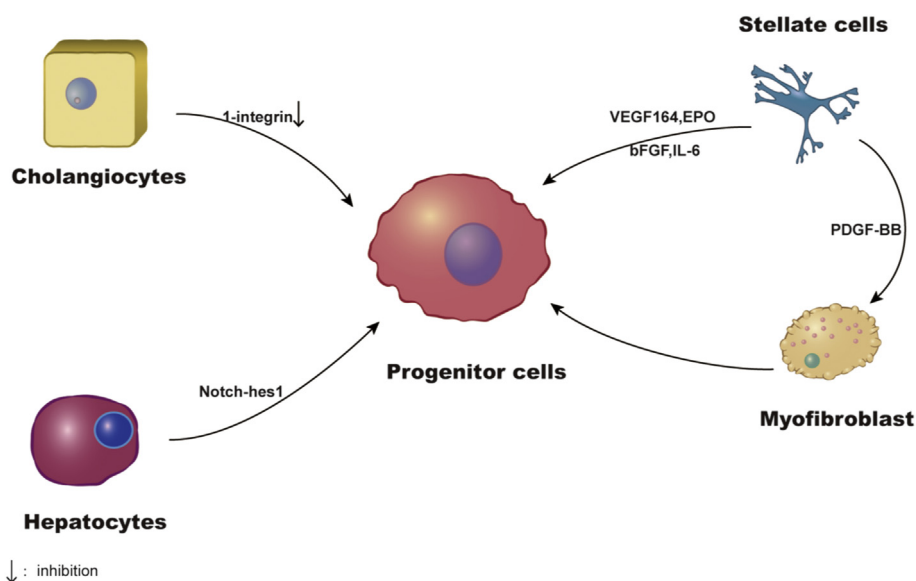


Figure 1 The origin of liver progenitor cells. LPCs come from a variety of cells such as cholangiocytes, hepatocytes, HSCs and myofibroblasts.

the expression of β -catenin gene down-regulated without inhibiting autophagy also hindered the differentiation of LPCs.¹¹ Autophagy regulates the signal of Wnt/ β -catenin promoting LPC differentiation after bile duct ligation, the Notch signaling pathway increased, and DAPT (a Notch pathway inhibitor) treatment reduced the expression of CK19, OV6, Sox9, and EpCAM, blocking bile duct cell proliferation and cholestatic liver fibrosis (CLF).¹² This study shows that in CLF, LPC differentiating into bile duct cells requires activation of Notch signals. Monocytes produce TNF-like weak inducer of apoptosis (TWEAK)¹³ and Wnt3a¹⁴ to stimulate oval cell proliferation.

LPC niche

Stem cells are maintained and controlled in their unique niches which enable their behavior to ensure tissue homeostasis and regeneration throughout life. LPCs niche is a special microenvironment which contains of different cell types. Extracellular matrix (ECM), growth factors, and cytokines released from niche cells help control the characteristics of LPCs and balance their expansion and differentiation. Liver progenitor cells, namely human hepatic progenitor cells and rodents oval cells, function when hepatocyte proliferation is prolongedly or severely impaired in liver injury. The oval cell, named for its morphological characteristics, is a dual-energy precursor cell that can be used to study the formation of bile ducts and regeneration of liver parenchyma. Both supportive and non-supportive stromal lines secrete extracellular vesicles (EVs), and EVs also play an important role in liver fibrosis, such as, connective tissue growth factor 2 (CCN2) is overexpressed in liver fibrosis and can contribute to promote fibrosis through HSCs.¹⁵ HSCs can transfer mir-214 to adjacent cells through exosomes and regulate expression of CCN2. During chronic liver injury, secretion of mir-214 is decreased, leading to the upregulation of CCN2 and promoting the development of liver fibrosis. In addition, secretion of exosome Twist1 was inhibited in liver fibrosis, which reduced the expression of mir-214 and promoted the process of liver fibrosis.¹⁶ Activated HSCs can transport exosome CCN2 to other HSCs, which may lead to the amplification of fibrogenic signaling.

Related cells

Hepatic stellate cells (HSCs)

HSCs are located in the Disse space between hepatic epithelial cells and sinusoidal endothelial cells. Producing extracellular matrix after chronic liver injury, HSCs are the main cells involved in liver fibrosis. It was found that LPCs were co-cultured in transwell plates with an immortalized human HSC line to induce LPCs to differentiate into hepatocytes.¹⁷ HSC releases many cytokines and growth factors that affect the survival, proliferation, migration, and differentiation of LPC as listed in Table 1.^{18–22} LPCs lead to

Table 1 The effect of related cells on LPCs.

Cell	Cytokines/ pathway	Function	References
HSC	Lymphotoxin-beta IL-6	LPCs proliferation	18
		LPCs proliferation	6
	The fibroblast growth factor 7 Hedgehog (Hh) pathway	LPCs expansion	15
		LPCs proliferation and differentiation	5
	Notch	LPCs differentiation	14
Macrophage	TWEAK Wnt pathway	LPCs proliferation and differentiation	22
		LPCs expansion	13,27
		LPCs differentiation; promote the catheter response	14
Hepatocyte	Hedgehog (Hh) pathway	LPCs proliferation and differentiation	10

the accumulation of HSCs through the TGF β signaling pathway in the ductular reaction, resulting in enhanced fibrosis and disease progression.²³

Macrophage

Hepatic macrophages consist of two subgroups: tissue-resident Kupffer cells (KCs) and MDMs infiltrating peripheral blood. In damaged liver tissues, macrophages are clustered in areas where LPC response is evident, and also in tissues other than the response sites of precursor cells.²⁴ In 2-AAF/PH-treated rats liposomes coated with clodronate reduced macrophages, inhibited LPCs expansion, and reduced residual liver regeneration.²⁵ Resident and recruited macrophages play a key role in response to tissue damage. KC depletion reduced LPC proliferation in PBS liposome treated mice fed with CDE.²⁶ These data suggest that KCs are necessary for early recruitment of monocytes to liver during CDE diet-induced injury and regeneration.²⁶ Hence, KC may have effects on LPC proliferation by recruitment of monocyte.

Cytokine TNF-like weak apoptosis inducer (TWEAK), a member of the pro-inflammatory TNF family is produced by macrophages, monocytes and T lymphocytes. TWEAK can stimulate oval cell proliferation in mouse liver and cell culture models.^{13,27} TWEAK directly stimulates LPC mitosis

in an Fn14-dependent and NF κ B-dependent manner, and signals sent by this pathway mediate LPC response to CDE induced damage and regeneration.²⁸ Notch and Wnt signaling directly promote the catheter response of LPCs through interactions with activated myofibroblasts or macrophages in human diseased liver and mouse models. Macrophage phagocytic hepatocyte fragment induces the expression of Wnt3a, which leads to the typical Wnt signal in the adjacent oval cells to express the Notch pathway inhibitor Numb, blocks the differentiation of LPCs into cholangiocytes, and turns into hepatocytes.¹⁴

Extracellular matrix

Recent studies have shown that ECM and LPCs interact in liver injury. LPC-ECM interaction mechanism consists of the following points²⁹:

1. Molecules on the LPC surface act as receptors for the matrix ligands, and the activation of the matrix ligands can initiate the downstream intracellular signaling pathway in the LPC.
2. The matrix sequesters and presents growth factors, cytokines and chemokines, which are regulators of LPC behavior.
3. LPC itself influences matrix composition and remodeling by producing matrix proteins or matrix degrading enzymes, so they can contribute to homeostasis.
4. Intracellular signaling pathways are involved in LPC-ECM interactions.

Laminin can be detected around the LPCs and heavily correlates to LPC responses in many models of chronic liver injury and LPC activation in rodents and is closely related to LPC response, such as chronic fibrotic carbon tetrachloride (CCl₄) injury in C57BL/6 Wt mice and collagen I α 1 (r/r) mice.³⁰ In addition to the models mentioned above, other models include the pyrrolizidine alkaloid- and retrorsine-induced LPC proliferation model in transgenic mice with hepatitis B surface antigen gene, CDE diet-treated C57/B6 mice.²⁴ Similarly, laminin in humans has been detected associated with LPCs in hepatitis B/C virus-related chronic hepatitis, and following fibrosing cholestatic hepatitis that occurred in a patient who received a liver transplant for hepatitis C.²⁴ Study using the collagen I α 1 (r/r) mouse (r/r) to test the relationship between collagen degradation, laminin deposition and LPC response, found that r/r mice had a markedly attenuated LPC response compared to wild-type, which correlated with persistence of collagen type I and a failure to deposit laminin, both in the model of chronic CCl₄ injury and CDE-induced LPC activation.³⁰ This suggests that degradation of the collagen matrix is required to induce LPC response and that degradation of collagen matrix enables the formation of a laminin rich progenitor cell niche.

It was reported that zone 1-like matrix proteins, collagen types III and IV and laminin, enabled attachment and expansion of LPCs independent of feeders, whereas zone 3-like matrix proteins, collagen type I and fibronectin,

elicited growth arrest and differentiation and inhibited attachment.³¹ Moreover, liver cells cultured on laminin for more than 1 month have a strong proliferation capacity, while the cells in type I collagen differentiate into liver cells and bile duct cells.³² Pluripotent progenitor cells cultured on a matrix containing a mixture of laminin and type IV collagen could differentiate into liver cells, while stem cells cultured on type I collagen were similar to biliary tract cells.³³

Besides, α 6 β 1 and α 5 β 1³² integrins as well as CD44³⁴ on the surface of LPCs, and their related downstream signals, are important mediators of interactions between LPCs and the ECM.

Study suggests that matrix hardness has no major effect on hepatocyte differentiation, but may affect cell survival or adhesion during differentiation. In addition, the ECM components, especially type IV collagen, have a significant effect on hepatocyte differentiation of liver progenitor cells.³⁵ Both the ECM protein and the stiffness properties affect differentiation of cholangiocyte. In particular, rigidity-mediated differentiation of cholangiocyte is observed in cells cultured on fibronectin, whereas type IV collagen-induced differentiation is independent of matrix stiffness.³⁵ The related effect factors are listed in the Table 2.

Table 2 The effect of microenvironment on LPCs.

Factors	Source	Function	References
EVs	stromal lines	maintains the survival and clonogenic potential of HSPCs	15
Cytokines	Niche cells	LPCs activation, proliferation and differentiation	16–19,31
Laminin	Extracellular matrix	LPCs proliferation and differentiation	24
collagen type I	Extracellular matrix	LPCs proliferation and differentiation	23,30,31
collagen types III	Extracellular matrix	LPCs proliferation	31
collagen types IV	Extracellular matrix	LPCs proliferation	31
1-like matrix proteins	Extracellular matrix	LPCs proliferation	31
3-like matrix proteins	Extracellular matrix	LPCs differentiation;	31
Matrix stiffness	Extracellular matrix	LPCs survival or adhesion	35

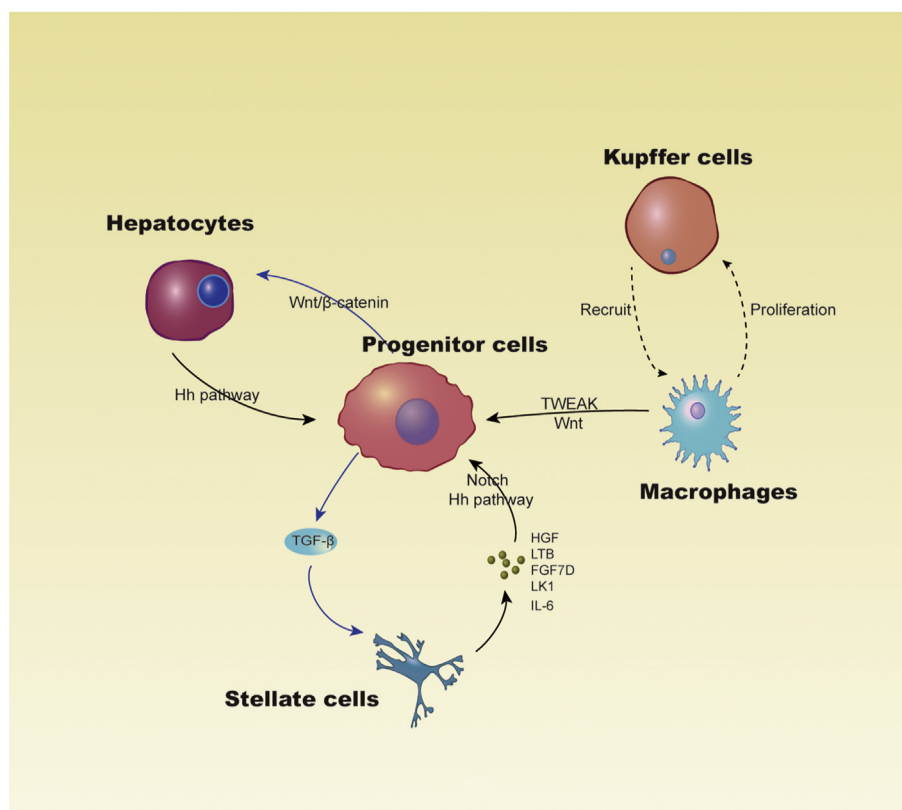


Figure 2 LPC-related cells in liver cirrhosis. The proliferation and activation of LPCs are regulated by niche cells secreting cytokines.

Conclusion

LPC plays an important role in the process of liver cirrhosis repair. LPC has more than one source of cells, such as hepatocytes, cholangiocytes, HSCs, and fibroblasts. The niche has important influence on the proliferation and differentiation of LPC as well. Various components in ECM, in particular, matrix hardness, have an impact on the proliferation of LPC. Other cells in liver, such as macrophages, also function by secreting cytokines and various signaling pathways (Fig. 2).

Although many advances have been made in the field of LPC-mediated regeneration, there are still many problems to be studied. It is still unknown that the proportion of various cell sources in disease conditions. Therefore, further research is needed to find the main source of LPC, so as to determine the following research direction. The relevant cellular and molecular mechanisms need to be further refined, so as to promote the transformation of LPC into hepatocytes to achieve liver function compensation from bench to bedside.

Conflict of Interests

The authors declare no conflict of interest.

Research involving human and/or animal rights

This Mini Review does not contain any studies with human or animal subjects.

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Abbreviations

BTSCs	Biliary Tree Stem Cells
CCN2	connective tissue growth factor 2
CDE	Choline-Deficient, Ethionine
CLF	cholestatic liver fibrosis
DRs	ductular reactions
ECM	Extracellular matrix
EVs	Extracellular vesicles
Hh	Hedgehog

HSPCs	hematopoietic stem and progenitor cells
HSCs	Hepatic stellate cells
KO	knockout
KCs	Kupffer cells
LPCs	liver progenitor cells
PBGs	peribiliary glands
TWEAK	TNF-like weak inducer of apoptosis

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