

# Update on Hepatobiliary Plasticity

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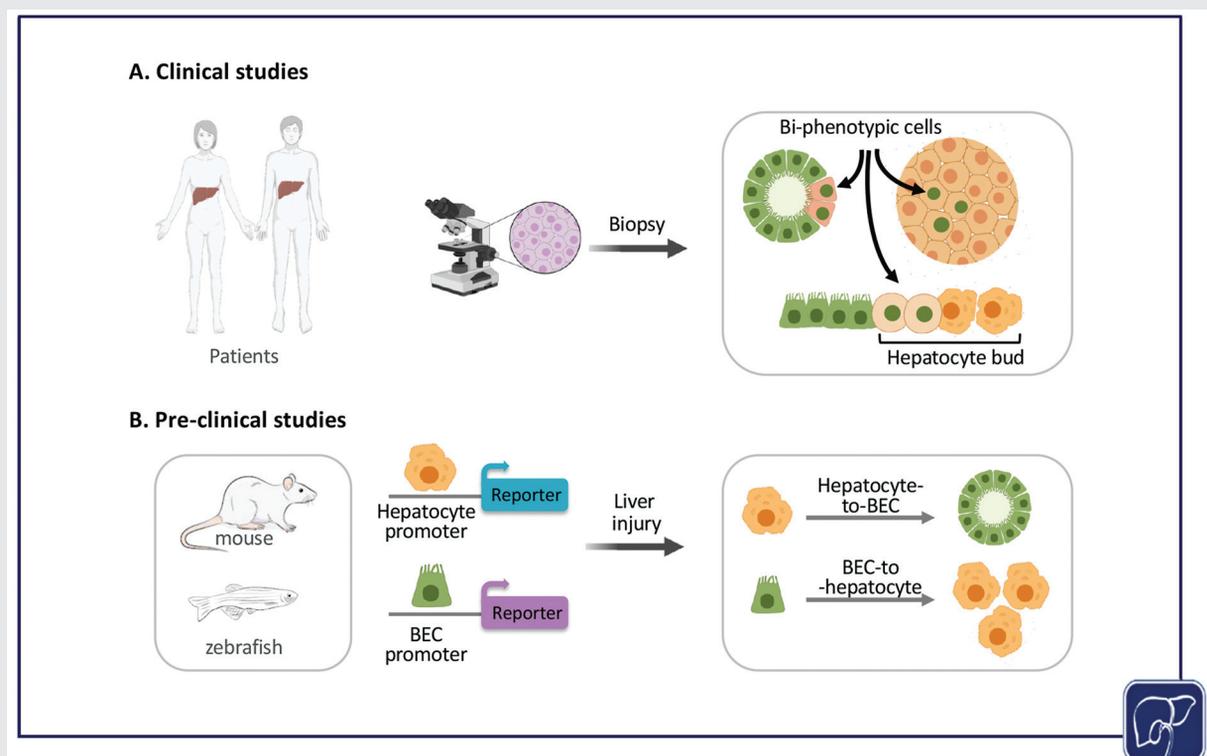
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## Graphical Abstract



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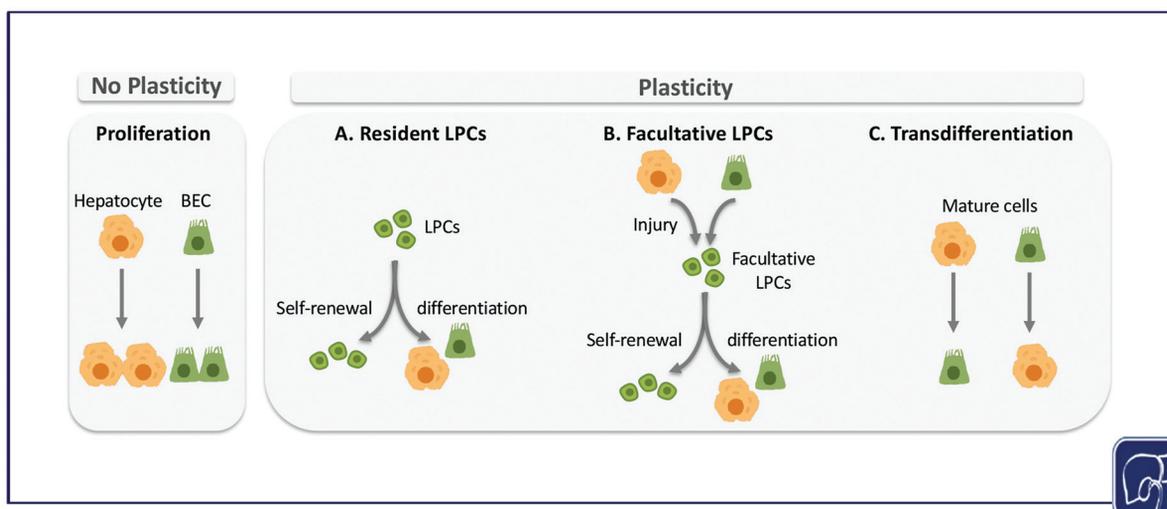
**Abstract****Keywords**

- ▶ hepatocyte plasticity
- ▶ cholangiocyte plasticity
- ▶ biliary epithelial cell plasticity
- ▶ liver progenitor cells
- ▶ liver regeneration

The liver field has been debating for decades the contribution of the plasticity of the two epithelial compartments in the liver, hepatocytes and biliary epithelial cells (BECs), to derive each other as a repair mechanism. The hepatobiliary plasticity has been first observed in diseased human livers by the presence of biphenotypic cells expressing hepatocyte and BEC markers within bile ducts and regenerative nodules or budding from strings of proliferative BECs in septa. These observations are not surprising as hepatocytes and BECs derive from a common fetal progenitor, the hepatoblast, and, as such, they are expected to compensate for each other's loss in adults. To investigate the cell origin of regenerated cell compartments and associated molecular mechanisms, numerous murine and zebrafish models with ability to trace cell fates have been extensively developed. This short review summarizes the clinical and preclinical studies illustrating the hepatobiliary plasticity and its potential therapeutic application.

During the last three decades, the liver field has been questioning “liver progenitor cell (LPC)” features and the plasticity of the two liver epithelial cell compartments, hepatocytes and biliary epithelial cells (BECs), for their therapeutic ability to regenerate the liver. The term “LPC” is used here to describe cells that have the potential to differentiate into hepatocytes and BECs and that usually express markers from both epithelial compartments. Early developmental studies undoubtedly support the existence of a common fetal precursor of BECs and hepatocytes, the hepatoblast. Although there is no evidence for a direct relationship between fetal hepatoblasts and adult LPCs, a growing literature reports some functional and phenotypic similarities between them.<sup>1</sup> Both can self-renew and differentiate into hepatocytes and BECs, and they both share cell surface markers.<sup>2-11</sup> Given this critical question, it is not surprising to notice that studies on BEC-to-hepatocyte or hepatocyte-to-BEC conversion have garnered increased at-

tention in the liver field. Yet, outstanding questions remain unanswered such as the following (▶**Fig. 1**): (1) Are there some resident LPCs (▶**Fig. 1A**) that have a potential to proliferate and differentiate into both BECs and hepatocytes? If so, as identified in intrahepatic small hepatic bile ductules (hepatic stem/progenitor cells, HpSCs) or large intra- and extra-hepatic bile ducts (biliary tree stem/progenitor cells, BTSCs),<sup>12</sup> are they phenotypically distinct from BECs and can we identify them with specific markers? (2) LPCs may be instead facultative (▶**Fig. 1B**) as they emerge only after liver injury. In this scenario, mature hepatocytes or BECs dedifferentiate into LPCs, reminiscent of fetal hepatoblasts. This process is succeeded by the proliferation of facultative LPCs and differentiation into the mature cell type in demand, hepatocytes when the hepatocyte pool is damaged or lost, or BECs in cholangiopathies. Yet, again, one can question whether all hepatocytes or BECs possess this capability. (3) Another possibility is that the mature parenchymal cells,



**Fig. 1** Mechanisms of hepatobiliary plasticity. Mature hepatocytes and BECs are known to divide to overcome minor injuries (no plasticity, left panel); however, when injuries are chronic or more severe, plasticity of the hepatobiliary compartment is observed (plasticity, right panel). In this case, various options have been proposed: (A) Resident LPCs self-renew and differentiate into both hepatocytes and BECs, (B) Facultative LPCs emerge following liver injury, self-renew and differentiate as the resident LPCs do, and (C) mature hepatocytes and BECs transdifferentiate into each other without transitioning through a LPC stage. BECs, biliary epithelial cells; LPC, liver progenitor cell.

hepatocytes and BECs, exhibit cell plasticity to directly give rise to each other by transdifferentiation (► Fig. 1C) without transitioning into an intermediate LPC stage. Studies investigating adult liver regeneration in animal models as well as evidence from analyses of human diseased liver specimens may support one theory or the other; however, a careful assessment of the literature as well as continuous investigations will help reach some consensus. In this short review, we have specifically focused our attention on the facultative LPCs, summarized clinical and preclinical studies to identify potential explanations of regenerative mechanisms in adult livers, and recognized gaps in the field that are limiting the therapeutic application of hepatobiliary plasticity to treat liver diseases. In an attempt to address some of these questions, we focus on the evidence of cell plasticity of hepatocytes and BECs in human liver diseases in the first section and compare them to animal study findings in the second and third sections to finally discuss the possible modulations of hepatobiliary plasticity as a therapeutic intervention and their limitations in the last two sections.

## Evidence of Hepatobiliary Plasticity in Humans

The process of ductular reactions (DRs), which involves the expansion of BECs, is a hallmark of all chronic and acute human liver diseases.<sup>13–23</sup> This suggests an alternative BEC-driven liver regeneration to overcome an exhausted hepatocyte-driven repair, by which BECs proliferate and contribute to hepatocytes. The observation of hepatocytes expressing the BEC marker EpCAM within highly proliferative DR areas in advanced human cirrhotic livers<sup>24</sup> and that of hepatocyte-like cells expressing the central vein hepatocytic marker glutamine synthetase budding from BECs within the DRs<sup>25–29</sup> could represent the contribution of BECs to de novo hepatocytes. Further evidence is illustrated by the detection of “bi-phenotypic cells”<sup>30</sup> or “ductular hepatocytes”<sup>31</sup> that express both the hepatocyte marker HNF4a<sup>30</sup> or HepPar1<sup>31</sup> and the BEC marker KRT19. Human cirrhotic liver samples frequently harbor intermediate hepatocyte-like cells with morphology and size intermediate between hepatocytes and BECs.<sup>32</sup> Specifically, in human cirrhotic livers, quantification of immature hepatocytes with glutamine synthetase positivity budding from KRT19<sup>+</sup> BECs demonstrated that they represented up to 70% of hepatocytes within the septa.<sup>25</sup> It has been suggested that glutamine synthetase re-expression away from the central vein areas is linked to a repair process, as a recent study demonstrated that aberrant glutamine synthetase positivity adjacent to portal tracts is associated with regressed cirrhosis in humans.<sup>33</sup> Lin and colleagues attempted to lineage trace LPCs among the DRs in human cirrhotic liver<sup>34,35</sup> using mutational analysis in mitochondrial DNA encoding cytochrome c oxidase enzyme, demonstrating that hepatocytes within monoclonal regenerative nodules descend from adjacent LPC-associated DRs. This study supported the differentiation potential of BECs in humans, suggesting the clinical application of LPC-derived hepatocytes in resolving human cirrhosis.

As in disorders of hepatocyte degeneration, biliary degenerative diseases are also associated with prominent DRs along with occurrence of intermediate hepatobiliary cells (IHBCs).<sup>12</sup> Cholangiopathies are associated with genetic- or immune-mediated damage to the intrahepatic or extrahepatic biliary tree, fibrotic response, and subsequent liver damage. The need to replace deteriorating BECs that are impaired in their proliferative capabilities by chronic damage elicits an alternative regenerative mechanism facilitated by hepatocyte plasticity. Many histopathological examinations have reported the expression of the BEC marker KRT7 in hepatocytes during cholangiopathies.<sup>36–42</sup> In cases of Alagille syndrome as well as biliary atresia, the number of IHBCs co-expressing the BEC markers KRT7 or HNF6 and the hepatocyte markers LKM-1, BSEP, or HNF4a is significantly increased.<sup>43</sup> In both primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), the appearance of IHBCs increases with the stage of fibrotic damage to the tissue.<sup>39,44,45</sup> A significant number of hepatocytes express the BEC transcription factor FOXA2 in the late stage of PBC and biliary obstruction.<sup>46</sup> However, the DR phenotype in PSC differs from that in PBC.<sup>44</sup> In cirrhotic PSC, there are lesser reactive ductules due to their lower proliferative index, but more EpCAM+/Hep-Par1+ newly derived hepatocytes.<sup>44</sup> It seems, since BECs in biliary cholangiopathies often show senescent phenotypes<sup>47–52</sup> and lower proliferative index,<sup>44,46</sup> the transition of hepatocytes into BEC-like cells or IHBCs could be a way to compensate for deteriorating biliary function. The prominent expression of OV6, known to characterize ductal plates, bile ducts, and ductules in fetal tissue, in periseptal hepatocytes in the liver of biliary atresia patients,<sup>53</sup> as observed in the liver of PBC and PSC patients,<sup>54</sup> further supports hepatocyte metaplasia,<sup>55</sup> transitioning from a mature cell to an immature LPC stage in cholangiopathies.

The clinical studies illustrate both cell plasticity processes: hepatocytes give rise to BECs when the BEC compartment is compromised, and BECs give rise to hepatocytes when hepatocyte proliferation is exhausted. However, given the lack of lineage tracing strategy in humans, the question of the true identity of the origin of the progeny still stands. Recent studies using lineage tracing animal models have proven to be instrumental to specifically identify the contribution of hepatocytes and BECs to each other's compartments during various liver diseases.

## BEC-to-Hepatocyte Conversion in Animal Models

### Rodents

BEC-to-hepatocyte conversion was first examined in rats followed by mice and zebrafish. In the Solt-Farber protocol, 2-acetylaminofluorene (2-AAF) is given to rats 1 week before partial hepatectomy (PHx); PHx stimulates liver regeneration and 2-AAF suppresses hepatocyte proliferation, thereby permitting BECs to contribute to hepatocytes.<sup>56,57</sup> Following the PHx, LPCs expressing BEC markers and the fetal marker AFP appeared in the portal regions and expanded.

Subsequently, they lost BEC features and acquired hepatocyte features,<sup>56</sup> suggesting BEC-to-hepatocyte conversion. Given the lack of lineage tracing tools in rats, mice have been extensively used to prove BEC-to-hepatocyte conversion. Using both BEC- and hepatocyte-specific lineage tracing approaches (→ **Table 1**), it was initially reported that in mice, BECs barely contribute to hepatocytes during homeostasis and even in diverse liver injury models, including CCl<sub>4</sub>, DDC, and CDE.<sup>58–66</sup> This minimal contribution of BECs to hepatocytes raised skepticism about the significance of BEC-to-hepatocyte conversion in liver regeneration. Given the difference in the proliferation capacity of hepatocytes between the rat Solt-Farber model, in which hepatocyte proliferation was blocked, and the commonly used mouse liver injury models, novel mouse models were established in which hepatocyte proliferation was suppressed either by deleting *Mdm2*,<sup>67</sup>  $\beta$ 1-integrin,<sup>68</sup> or  $\beta$ -catenin<sup>69</sup> or by overexpressing p21<sup>68</sup> in hepatocytes. These improved models revealed a significant contribution of BECs to hepatocytes ranging from 15 to 70%, depending on the use of either direct BEC- or indirect hepatocyte-lineage tracing mouse models. In addition to these genetic blocks of hepatocyte proliferation, long-term liver injury with 6- or 12-month administrations of DDC or thioacetamide (TAA), leading to natural impairment of hepatocyte proliferation, also induced a significant BEC-to-hepatocyte conversion.<sup>30</sup> These lineage tracing studies have been instrumental to demonstrate the potential of BECs to generate healthy de novo hepatocytes when hepatocyte proliferation is compromised, reflecting most human chronic liver diseases.<sup>32,70–75</sup> However, very few studies have started to elucidate the molecular mechanisms underlying BEC-to-hepatocyte conversion. The Notch-IGF1 axis<sup>76</sup> and Tet1<sup>77</sup> have been reported to control LPC proliferation during the conversion, thereby affecting the number of BEC-derived hepatocytes. We recently showed that vascular endothelial growth factor A (VEGFA) delivered to the liver via nucleoside-modified mRNA encapsulated into lipid nanoparticles<sup>78</sup> induced a fivefold increase in BEC-to-hepatocyte conversion using tamoxifen-inducible KRT19 and VEGFA receptor KDR lineage mouse models during acute and chronic liver injuries (unpublished data, Rizvi and Gouon-Evans, in preparation). VEGFA-mediated cell conversion may be mediated through the activation of a VEGFA receptor KDR expressed on a subset of BECs after liver injury (unpublished data, Rizvi and Gouon-Evans, in preparation). Further investigation is needed to understand the molecular mechanisms driving the cell conversion and to leverage them for therapeutic intervention.

### Zebrafish

Given the strengths of zebrafish as a vertebrate model organism, including (1) rapid and external embryogenesis, (2) an easy drug administration, (3) a large number of progenies, and (4) a low maintenance cost, and similar cellular compositions in the liver between zebrafish and mammals albeit a difference between the two in the mode of connection between hepatocytes and bile ductules,<sup>79,80</sup> zebrafish have been widely used for investigating liver dis-

eases and for liver toxicology tests.<sup>81–95</sup> A decade ago, three groups independently developed a hepatocyte ablation model by generating the *Tg(fabp10a:NTR)* fish lines that express bacterial nitroreductase (NTR) specifically in hepatocytes.<sup>91,96,97</sup> Since NTR converts metronidazole (MTZ) into a cytotoxic drug, MTZ treatment ablates nearly all hepatocytes in *Tg(fabp10a:NTR)* fish. Following MTZ washout, the liver robustly and synchronously among animals regenerates through BEC-to-hepatocyte conversion. This liver regeneration occurs through four steps: (1) BEC-to-LPC dedifferentiation, (2) LPC proliferation, (3) LPC-to-hepatocyte differentiation, and (4) hepatocyte proliferation and maturation.<sup>96,97</sup> In this model, LPCs are distinguished from BECs based on cell and nuclear shape and the expression of hepatocyte markers. Given the synchrony and robustness of BEC-driven liver regeneration in this ablation model combined with the general strengths of zebrafish as a vertebrate model organism, the ablation model has been actively used to reveal the molecular mechanisms underlying BEC-to-hepatocyte conversion. It was revealed using the zebrafish model that Notch signaling,<sup>96</sup> bromodomain and extraterminal (BET) proteins,<sup>98</sup> Dnmt1,<sup>99</sup> and mTORC1<sup>100</sup> regulate the first step of the conversion process, BEC-to-LPC dedifferentiation. It was also revealed that the second step, LPC proliferation, is positively regulated by BET proteins,<sup>98</sup> Tel2,<sup>101</sup> and Stat3<sup>102</sup> and negatively regulated by Notch<sup>91</sup> and FXR<sup>103</sup> signaling. Specifically, FXR activation did not only suppress LPC proliferation but also induced its death.<sup>103</sup> Given the correlation between LPC number and the severity of human liver diseases<sup>21,104</sup> and the potential of regenerative therapy to promote LPC-to-hepatocyte differentiation in the diseased livers, the third step, LPC-to-hepatocyte differentiation, was more extensively investigated using the zebrafish model than the other steps. It was revealed that BMP signaling<sup>105</sup> and Tel2<sup>101</sup> positively regulate the third step through Tbx2b and Hhex, respectively, and that Notch signaling negatively regulates the step.<sup>91,96</sup> Epigenetic regulators, Hdac1<sup>106</sup> and Dnmt1,<sup>99</sup> also positively control LPC-to-hepatocyte differentiation by repressing *sox9b* and *tp53* expression, respectively. p53 inhibits the differentiation by suppressing BMP signaling.<sup>99</sup> It was also reported that FXR activation suppresses LPC-to-hepatocyte differentiation via the FXR-PTEN-PI3K-AKT-mTORC1 axis.<sup>103</sup> Conversely, another group reported using a similar ablation model that FXR is required for LPC-to-hepatocyte differentiation by regulating ERK1,<sup>107</sup> suggesting that FXR can play a dual role in this process. Regarding the last step of the conversion process, hepatocyte proliferation, and maturation, it was reported that BET and Wnt2bb regulate the hepatocyte proliferation<sup>97,98</sup> and that Stat3 regulates its maturation.<sup>102</sup>

In addition to the hepatocyte ablation model, an oncogene-induced hepatocyte damage model has been used to study BEC-to-hepatocyte conversion, particularly LPC-to-hepatocyte differentiation. In *Tg(fabp10a:pt- $\beta$ -catenin)* fish, hepatocyte-specific overexpression of the stable form of  $\beta$ -catenin triggers oncogene-induced senescence and apoptosis in hepatocytes, thereby inducing BEC-driven liver

**Table 1** Summary of mouse experiments showing liver parenchymal cell plasticity

	Cre lines used for lineage tracing	Injury	Genetic modulation	Contribution (◦) and limitations (•)
BEC-to-HC	<i>Foxl1-Cre</i>	BDL, <sup>66</sup> DDC, <sup>61,63,66</sup> CDE <sup>61</sup>	–	◦ ~ 29% of HCs derived from Foxl1-Cre+ cells after CDE diet <sup>61</sup> (5% of HCs were labeled during CDE injury <sup>61</sup> ) • Noninducible Cre line
	<i>Hnf1b-CreER</i>	CDE <sup>140</sup>	–	◦ 0.22% of HCs derived from BECs • Rare contribution of BECs to HCs
	<i>Sox9-CreERT2</i>	CCl <sub>4</sub> , <sup>141</sup> BDL, <sup>141</sup> MCDE, <sup>141</sup> DDC, <sup>65,141</sup> APAP <sup>141</sup>	–	◦ 1% of HCs derived from BECs <sup>65</sup> • Sox9 is also expressed in a subset of periportal hepatocytes in a normal condition <sup>60,142</sup>
	<i>OPN-CreERT2</i>	CDE, <sup>62</sup> CCl <sub>4</sub> , <sup>59</sup> MCD <sup>76</sup>	–	◦ 2.45% of HCs derived from BECs <sup>62</sup> ◦ ~ 13% of HCs derived from BECs <sup>59</sup> • OPN is also expressed in other cell types <sup>143–145</sup>
	<i>CK19-CreERT</i>	DDC, <sup>30,68</sup> MCD, <sup>68</sup> TAA, <sup>30,68</sup> CDE <sup>69</sup>	$\Delta\beta 1$ -integrin <sup>68</sup> p21 overexpression <sup>68</sup> Ctnnb1-siRNA <sup>69</sup>	◦ 9.1–10% of HCs derived from BECs <sup>30</sup> ◦ ~ 6.12% of HCs derived from BECs <sup>68,69</sup> • Low labeling efficiency <sup>143,146</sup>
HC-to-BEC	AAV8-TBG-Cre or AAV8-CMV-Cre	No injury	<i>R26-LSL-NICD1</i> <sup>113</sup>	◦ 23% of BECs derived from HCs assessed by KRT19 and BEC apical markers (PAR6, PKC $\zeta$ , and Ac-tub)
		DDC, <sup>113</sup> BDL <sup>113</sup>	–	◦ 4.4–14.3% of BECs derived from HCs assessed by KRT19 and BEC apical markers (PAR6, PKC $\zeta$ , and Ac-tub)
	<i>Alb-Cre</i>	Retrorsine + PHx + DDC or CCl <sub>4</sub> <sup>112</sup>	–	• Transplanted HCs converted to BECs assessed by KRT19
	<i>Mx1-Cre</i> (induced by poly(I:C))	DDC, <sup>112</sup> DAPM, <sup>112</sup> BDL, <sup>112</sup> TAA, <sup>112</sup> CCl <sub>4</sub> <sup>112</sup>	–	◦ 1.9–20.6% of BECs derived from HCs assessed by KRT19
	<i>Alb-CreER</i> or <i>Alb-CreERT2</i>	DDC, <sup>60</sup> BDL, <sup>60</sup> TAA <sup>147</sup>	–	◦ 10–11.31% of BECs derived from HCs assessed by KRT19 <sup>60</sup>
		DDC, <sup>114</sup> CCl <sub>4</sub> <sup>114</sup>	–	–
		DDC <sup>114</sup>	<i>R26R-LSL-NICD1</i> <i>Hes1</i> <sup>fl/fl</sup>	BEC differentiation is assessed by KRT19, EpCAM, and keratin
	AAV-TBG-Cre; R26-LSL-rtTA	No injury	<i>TetO-YAP</i> <sup>S127A126</sup> <i>TetO-YAP</i> <sup>S127A</sup> ; <i>Rbpj</i> <sup>fl/fl126</sup>	BEC differentiation is assessed by KRT19 and pan-CK
	AAV8-TTR-Flp	<i>Alb-Cre</i> ; <i>Rbpj</i> <sup>fl/fl</sup> ; <i>Hnf6</i> <sup>fl/fl127</sup>	–	◦ ~ 100% of BECs derived from HCs assessed by KRT19 and wide-spectrum CK
	<i>Hnf4a-DreERT2</i> ; <i>Sox9-CreERT2</i>	DDC, <sup>60</sup> BDL <sup>60</sup>	–	◦ ~ 3.63% of BECs derived from HCs assessed by KRT19
–	No injury	HDTVI of <i>CAGGS-GFP-IRES-SOX9</i> <sup>148</sup>	◦ ~ 18% of BECs derived from HCs assessed by KRT19	

Abbreviations: HC, hepatocyte; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; CDE, choline-deficient, ethionine-supplemented; CCl<sub>4</sub>, carbon tetrachloride; PHx, 70% partial hepatectomy; MCD, methionine- and choline-deficient; MCDE, methionine- and choline-deficient, 0.15% ethionine-supplemented; APAP, acetaminophen; BDL, bile duct ligation; DAPM, methylene dianiline; TAA, thioacetamide; LSL, *loxP-Stop-loxP* cassette; HDTVI, hydrodynamic tail vein injection; Ac-tub, acetylated tubulin.

regeneration.<sup>108</sup> Indeed, in this model, both BECs and surviving hepatocytes dedifferentiate into LPCs, and later, the LPCs differentiate into hepatocytes. Using this model, it was revealed that suppressing EGFR signaling promotes LPC-to-

hepatocyte differentiation via the EGFR-ERK-SOX9 axis.<sup>108</sup> Importantly, this study suggests EGFR inhibitors as a potential regenerative therapeutic drug to promote LPC-to-hepatocyte differentiation in diseased livers.

## Hepatocyte-to-BEC Conversion in Animal Models

### Rodents

Hepatocyte-to-BEC conversion was first examined in rats<sup>109,110</sup> followed by mice. Rats subjected to bile duct ligation (BDL) with pretreatment with the biliary toxin 4,4'-methyleneedianiline (MDA) exhibited hepatocyte-to-BEC conversion.<sup>109</sup> In this model, BDL induces biliary damage and subsequent regeneration and MDA blocks BEC proliferation, which is in symmetry with the rat Solt-Farber model, in which PHx induces liver regeneration and 2-AAF blocks hepatocyte proliferation. In addition to the BDL-MDA combination, repeated administrations of MDA alone induced chronic biliary damage and hepatocyte-to-BEC conversion.<sup>111</sup> In these rat models, hepatocyte-derived BECs were identified based on dipeptidyl dipeptidase IV (DDP-IV) expression using DDP-IV-negative rats having hepatocytes from DDP-IV-positive donors.

Using the Cre/loxP system for hepatocyte-lineage tracing, hepatocyte-to-BEC conversion was validated in mice with multiple liver injury models, including DDC, CCl<sub>4</sub>, MDA, and BDL<sup>112-114</sup> (►Table 1). Compared with BEC-lineage tracing, hepatocyte-lineage tracing is much more robust and unquestionable owing to the availability and tracing efficiency of hepatocyte-specific Cre lines that are not activated in any other cell types. Given the essential role of Notch signaling in biliary formation during development,<sup>115-117</sup> it has been reported that in mice, Notch signaling controls hepatocyte-to-BEC conversion.<sup>113,114,118,119</sup> Hepatocyte-specific deletion of *Rbpj* (the principal mediator of Notch signaling)<sup>113</sup> or *Hes1* (a key effector of Notch signaling)<sup>114</sup> reduced the number of hepatocyte-derived BECs in mice fed a DDC diet, while hepatocyte-specific overexpression of Notch intracellular domain (NICD) induced hepatocyte-to-BEC conversion.<sup>113,118,119</sup> In addition to Notch signaling, Yap signaling plays a crucial role in both biliary development<sup>117,120,121</sup> and hepatocyte-to-BEC conversion.<sup>122-126</sup> Hepatocyte-specific deletion of *Yap1* greatly reduced the number of DRs,<sup>122,123</sup> while hepatocyte-specific overexpression of constitutive-active YAP1 induced hepatocyte-to-BEC conversion<sup>124-126</sup> through the induction of NOTCH2 and SOX9.<sup>126</sup>

Contrary to the prevailing thought that Notch signaling is indispensable for hepatocyte-to-BEC conversion, a Notch-independent mechanism for the conversion was recently identified.<sup>147</sup> In a mouse model that mimics Alagille syndrome, *Alb-Cre; Rbpj<sup>fl/fl</sup>; Hnf1b<sup>fl/fl</sup>*, intrahepatic peripheral bile ducts do not develop initially, but later, the bile ducts form via hepatocyte-to-BEC conversion. Additional deletion of *Tgfb2* blocked the bile duct recovery, whereas hepatocyte-specific overexpression of constitutive-active TGFBR1 promoted it, indicating the crucial role of TGFβ signaling in hepatocyte-to-BEC conversion in the absence of Notch signaling.<sup>147</sup>

Cholangiocarcinoma models in which hepatocyte-specific overexpression of certain oncogenes induces cholangiocarcinoma are also useful to study the molecular mechanisms of hepatocyte-to-BEC conversion, because the conversion is a

prerequisite for the cancer formation. Using these models, it has been reported that not only Notch<sup>119,128-130</sup> and Yap<sup>118,124</sup> but also Dnmt1<sup>118</sup> play key roles in hepatocyte-to-BEC conversion. Particularly, NICD overexpression in hepatocytes induces the conversion through the NICD-YAP1-DNMT1 axis.<sup>118</sup>

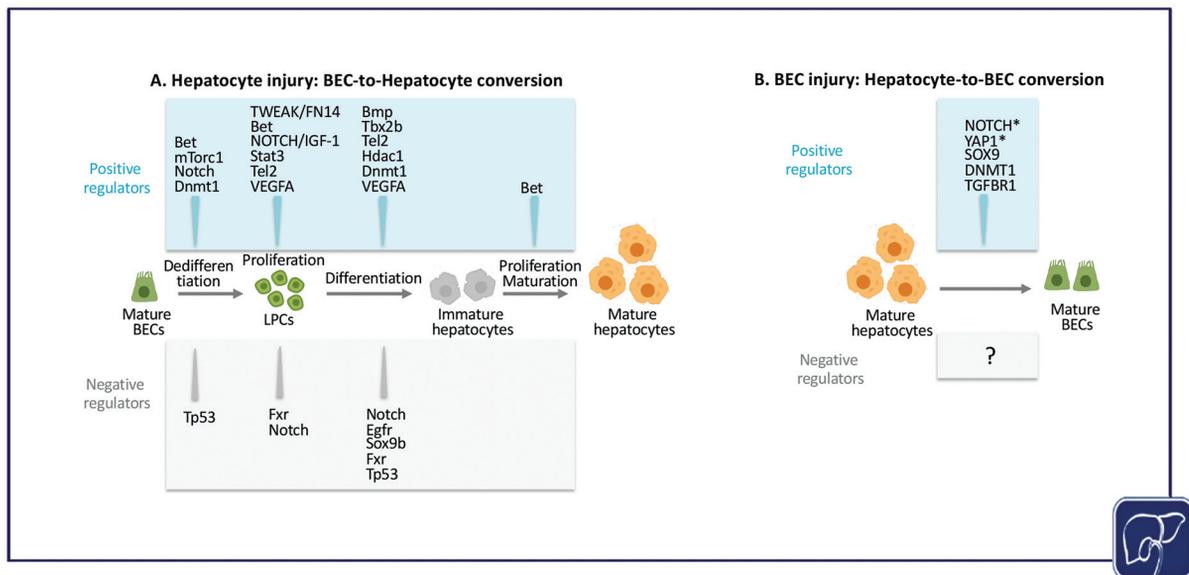
### Zebrafish

Several biliary injury models with genetic modifications causing BEC paucity were developed in zebrafish; however, hepatocyte-to-BEC conversion has not been investigated in these models.<sup>81,131</sup> Given that severe liver injury is required for plasticity-mediated liver regeneration, severe biliary injury models may be needed to study hepatocyte-to-BEC conversion in zebrafish. We have recently developed a zebrafish model for the conversion, in which all regenerating BECs originate from hepatocytes.<sup>132</sup> Temporal Notch inhibition during BEC-driven liver regeneration triggered by hepatocyte ablation generates zebrafish that completely lack BECs in the liver. Subsequent removal of Notch inhibition permits a subset of hepatocytes to give rise to BECs. In this new zebrafish model, both Notch and Yap signaling control hepatocyte-to-BEC conversion,<sup>132</sup> consistent with the findings in mice.<sup>114,121,126</sup> Given the strengths of zebrafish as a vertebrate model organism, particularly chemical screening, we expect that our novel model as well as other zebrafish models to be developed will significantly contribute to a better understanding of the molecular mechanisms underlying hepatocyte-to-BEC conversion.

## Regulating Hepatobiliary Plasticity as a Therapeutic Intervention

Orthotopic liver transplantation is a main curative approach for end-stage liver diseases. However, the shortage of organ donors results in many patients dying while waiting for transplantation. For such patients, there is a need for discovery of alternative therapies that could act as a bridge to support them until availability of liver donors. The development of effective cell replacement therapy could provide such a bridge and represent a promising approach to the treatment of liver diseases. Another alternative to liver transplant is leveraging the innate liver repair by harnessing mechanisms of cell plasticity.

Studies using zebrafish models of hepatocyte ablation have demonstrated key pathways that can be manipulated to promote LPC-to-hepatocyte differentiation (►Fig. 2). Sox9b repression is important for this process<sup>106</sup>; hence, use of Notch inhibitor LY411575 that represses Sox9b demonstrated the enhanced induction of Hnf4a in LPCs.<sup>133</sup> Furthermore, pharmacological inhibition of EGFR or MEK/ERK promoted LPC-to-hepatocyte differentiation, demonstrating the prospects of the epidermal growth factor receptor (EGFR) signaling pathway as a candidate therapeutic target.<sup>108</sup> Manipulation of the BEC niche is shown to facilitate BEC-to-hepatocyte differentiation during chronic injury in mice.<sup>62</sup> Inhibition of laminin deposition using Iloprost, a synthetic analog of prostaglandin I<sub>2</sub> known to



**Fig. 2** Molecular mechanisms driving hepatobiliary plasticity. Studies in rodents and zebrafish together with observations in human specimens have revealed positive and negative molecular regulators implicated in BEC-to-hepatocytes conversion following hepatocyte injury (A) and in hepatocyte-to-BEC conversion following BEC injury (B). Factors written with capital and small letters are related to mouse and zebrafish studies, respectively. Factors with an asterisk (\*) are related to both mouse and zebrafish studies. BEC, biliary epithelial cell.

block TGF $\beta$ 1-mediated fibrogenesis, enhanced the presence of differentiated hepatocytes after 3 weeks of CDE-induced liver injury in mice. However, the low efficiency of the process still posed a question about its clinical significance. We have recently demonstrated that VEGFA significantly enhanced BEC-to-hepatocyte conversion with a factor 5 to produce healthy hepatocytes in acute as well as chronically injured livers (unpublished data, Rizvi and Gouon-Evans, in preparation). Importantly, the results provide evidence of the therapeutic potential of VEGFA to harness BEC-driven liver regeneration with the use of nucleoside-modified mRNA-LNP, a tool that we have validated to express regenerative factors for preclinical therapeutic interventions in various murine liver diseases.<sup>78,134</sup> Some studies have also explored pathway modulation to promote BEC differentiation to benefit biliary diseases. YAP activation in hepatocytes is required for hepatocyte-to-BEC conversion after DDC-induced liver injury.<sup>135</sup> YAP-mediated hepatocyte transdifferentiation was further confirmed in mouse models of alcohol exposure.<sup>136</sup>

### Limitations and Concluding Remarks

The findings from the juxtaposition of both rodent and zebrafish liver injury models have indisputably revealed the ability of hepatocytes and BECs to generate each other when needed, a fact that is not that surprising as hepatocytes and BECs come from a common fetal progenitor, the hepatoblast. Even though this review largely focuses on the contribution of facultative LPCs to liver regeneration, the role of resident LPCs in this process is very likely. A critical limitation of the current lineage tracing models is that they do not discriminate between LPCs and BECs. Indeed, we are yet to discover markers specific to LPCs that are not expressed in BECs. Furthermore, with respect to cell plasticity

in rodents, it is not clear whether the dedifferentiation of mature cells into facultative LPCs always precedes their conversion to different cell fates. Additional lineage tracing studies using yet-to-be discovered unique markers for LPCs, in combination with isolated LPC fate mapping investigation in ex vivo clonal cultures or following transplantation in vivo, will be instrumental to further reveal the true potential of LPCs in regenerating a damaged liver.

Interestingly, although animal studies indicate that LPC-driven liver regeneration restores liver parenchyma in liver diseases, it does not appear to benefit patients with advanced liver disease. In fact, the clinical benefits of LPCs may be questionable as the presence of DRs has been associated with poor prognosis in advanced human chronic liver diseases.<sup>21,32</sup> However, a correlation between LPC numbers and disease severity in patients with chronic liver diseases may also imply that while LPCs are activated, their differentiation into hepatocytes may be ineffective. Indeed, persistent LPCs release profibrogenic factors that may induce inflammation and subsequent fibrosis, and instead aggravate the chronic liver disease.<sup>137,138</sup> Yet, a recent study demonstrated a positive clinical outcome from BEC-derived hepatocytes in resolving human cirrhosis,<sup>33</sup> indicating that aberrant glutamine synthetase positivity in portal hepatocytes is significantly associated with regressed cirrhosis in humans. Moreover, in cases of severe intoxication with drugs such as acetaminophen, BEC-to-hepatocyte conversion is observed and associated with decreased DRs in patients.<sup>139</sup> However, this study concluded that the expansion and differentiation of BECs into hepatocyte-like cells take longer than required to prevent urgent liver transplantation. Hence, to become a viable and effective treatment, particularly for acute liver injuries, the cell conversion must be accelerated. Stimulating the cell plasticity is an attractive therapeutic

option for patients with advanced liver disease. The ability to reliably identify a true progenitor population and to, thus, define druggable pathways that would accelerate their plasticity and differentiation into functional hepatocytes would immensely facilitate the therapeutic potential of BECs within the naturally occurring DRs in the vast majority of human liver diseases.

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#### Conflict of interest

None declared.

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