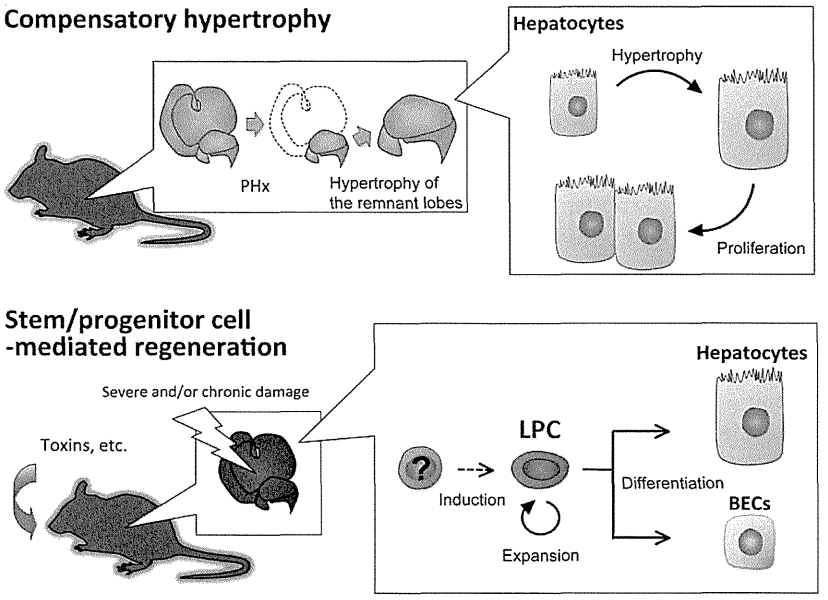

Fig. 1. Two modes of liver regeneration. Upon surgical removal of a part of the liver (PHx), the remaining tissue enlarges to recover the original mass, a process called compensatory hypertrophy. This type of regeneration is achieved by mature hepatocytes in the remaining tissue, whose primary response is an increase in size (hypertrophy), followed by entry into cell cycle. When the proliferative capacity of hepatocytes is impaired as a result of severe or chronic liver injury, immature adult LPCs are activated and contribute to the regeneration process by differentiating to hepatocytes and BECs.

their DNA synthesis peaks at approximately 40-48 hours.[2,3]

By contrast, upon severe or chronic liver injury, the proliferative capacity of hepatocytes is impaired, so that the liver requires another mechanism to regenerate and recover its function. Immature adult liver stem/progenitor cells (LPCs), also often referred to as oval cells in rodents, are activated and assumed to repair the liver damages by differentiating to mature cells.

Compensatory Hypertrophy: The Revised Mechanism

The multilobular structure of rodent liver allows the surgical resection of a lobe of choice to achieve different degrees of liver mass loss by PHx. By cutting out a particular lobe at its pedicel after ligating vessels and ducts, a defined proportion of the organ (up to 90%) can be removed without bleeding or any direct damage to the remaining tissue. The most commonly used protocol is two-thirds (approximately 70%) PHx, initially described by Higgins and Anderson in 1931 using the rat system. Since then, this protocol has been the most generally and extensively used model and serves as the paradigm to study the mechanisms of liver regeneration. It should be noted that, upon PHx, the liver does not regrow the lost lobe(s), but the remaining ones simply increase their size. This process is obviously different from "epimorphic regeneration," such as limb or heart regeneration in newts,[4] and the term "compensatory hypertrophy" (or compensatory growth) can be used to describe this phenomenon in a more accurate way. In the long history of studies using the PHx model in rodents, extensive analyses have been made on various aspects of cellular response of hepatocytes and the underlying molecular mechanisms in the course of the regenerative process (reviewed previously[4-7]), including those involving extracellular growth-regulatory signals (reviewed previously[8]). Here, we briefly introduce recent topics on the process of compensatory hypertrophy in the liver.

Studies in the 1960s have already shown that almost all hepatocytes incorporated radiolabeled nucleotides during the regeneration process after 70% PHx,[2,9-11] indicating that they entered into the cell cycle, more specifically, into S phase. This was interpreted as that all hepatocytes *proliferate*, which led to establishing the long-standing model that hepatocytes simply proliferate to recover the original mass of the liver. That is, all remnant hepatocytes duplicate once or twice to recover the original mass.[5,12,13] However, entering into S phase does not necessarily indicate that the cells undergo a subsequent M phase to execute cell division.

Using a newly established method based on the genetic lineage-tracing approach, the exact number of cell division, rather than cell-cycle entry, of hepatocytes was examined in the 70% PHx model in mice.[14] Statistical analysis revealed that the average number of hepatocyte divisions during the course of regeneration was only 0.7 times (1.5-fold increase in cell number), far below the level anticipated from the traditional

model. Measurement of cell size revealed that hepatocytes actually enlarged (approximately 1.5-fold) after 70% PHx. Thus, proliferation and hypertrophy of hepatocytes equally contribute to regeneration. Time-course analyses demonstrated that, after 70% PHx, the primary response of hepatocytes was an increase in size, followed by entry into cell cycle. Only approximately one half of them complete cytokinesis to increase the cell number. Consistently, in the case of 30% PHx, hepatocytes increased the size, but did not enter into cell cycle, indicating that hepatocyte hypertrophy is sufficient for recovery. Thus, hepatocyte hypertrophy is the primary response to surgical loss of liver mass, and cell-cycle entry follows depending on the mass of lost tissue.

Although this revised model is based on the results obtained in the mouse system, this may also be applicable to the rat system. Indeed, Higgins and Anderson commented, in their 1931 report, that "there was a certain amount of nuclear and cytoplasmic hypertrophy during this early period [= the first 24 hours], which with the slight mitotic activity no doubt accounts for the increment [of the liver parenchyma]," although no exact data regarding hepatocyte hypertrophy were presented. Subsequent studies consistently provided quantitative data showing that both nuclear and cell volume in hepatocytes increased in the regenerating liver in the rat system[15,16] (reviewed previously[17]). Notably, the increase in hepatocyte size was shown to be transient, peaking at approximately 24 hours after operation and declining thereafter. This seems to be consistent with the results of more recent studies, showing that cellular hypertrophy in hepatocytes were not observed at 5 or 28 days after PHx in the rat system.[18,19] Thus, it is highly likely that hypertrophy of hepatocytes upon PHx is the primary cellular response conserved both in mice and rats, whereas that the extent of their contribution to the eventual achievement of liver regeneration could vary among species.

Binucleation is an interesting feature of adult hepatocytes, and, interestingly, reduction of nuclear number in regeneration has been known to occur.[20-25] Genetic lineage tracing in the 70% PHx model indeed showed that binuclear hepatocytes preferentially become mononuclear cells.[14] Thus, it is tempting to speculate that these binucleated hepatocytes in the adult liver are prepared to achieve prompt, efficient regenerative response. Recent studies using knockout (KO) mice for the atypical repressors, E2Fs, E2F7, and E2F8, addressed the role of these molecules in inducing endoreplication,[26,27] which is required for binucleation as well as ploidy increase. Genetic loss of both E2F7 and E2F8 affected endoreplication and reduced the extent of polyploidy and binucleation in hepatocytes, yet liver functions in the double KO mice did not seem to be significantly affected. Surprisingly, the double KO livers regenerated just as well as wild-type livers after PHx, suggesting that hepatocyte binucleation or polyploidy is not necessarily required for this process. Thus, it still remains unclear whether hepatocytes with different nuclear numbers have different functions.

Liver Stem Cells

Hepatocytes and BECs are the two epithelial-type cell populations in the liver and topologically connected in the tissue. The junctional structure connecting and lined asymmetrically by hepatocytes and BECs is called the canal of Hering. During the course of liver development at fetal stages, hepatoblasts emerging from the foregut endoderm give rise to hepatocytes and BECs. Because of their bidirectional differentiation potential, hepatoblasts are considered as the fetal liver stem cell population.[28-30] In contrast, the presence and nature of such a "liver stem cell" population in adulthood still remains obscure. Under the normal, uninjured condition, immature cells with characteristics similar to fetal hepatoblasts are not apparently observed in the adult liver.

Based upon marker gene expression and flow cytometric (FCM) cell purification, many groups have actually succeeded in isolating cell populations from the adult liver, which are clonogenic with high growth potential and can be induced to differentiate to both hepatocytes and BECs under certain culture conditions.[31-34] Although these can be considered as potential liver stem cells that can be defined in culture, it remains unclear whether and where they exist *in situ* in living organisms and how they behave under physiological conditions. It should be noted that those potential liver stem cell populations are often identified as those that are positive for BEC markers, including epithelial cell adhesion molecule (EpCAM), CD133 (also known as prominin 1), and the MIC1-1C3 antigen, implicating a possible role of the biliary system as a compartment harboring resident liver stem cells, if any exist. Notably, $EpCAM^+$ cells isolated from human postnatal livers, as well as fetal livers, have also been found to contain hepatic stem cells (HpSCs) that can be defined *in vitro*.[35] Moreover, the presence of multipotential stem/progenitor cells that can give rise to hepatocytes and BECs, and also to

pancreatic islets, has been suggested in peribiliary glands of extrahepatic biliary trees in humans.[36]

Stem cells in adult organs are typically defined based on their involvement in tissue turnover. In tissues with relatively rapid turnover, such as the hematopoietic system and intestinal epithelia, the presence and importance of continuous supply of newly differentiated cells from the resident stem cell compartment is evident. In contrast, the normal turnover of hepatocytes occurs over a period of more than several months, making it difficult to find the stem cell compartment. Perhaps it is a more widely and favorably accepted view that maintenance of hepatic tissue under normal physiological conditions is achieved by the proliferation of mature hepatocytes that occurs at random throughout liver parenchyma. It would be interesting to examine whether hepatocyte hypertrophy is also involved in the maintenance process. At the same time, several lines of evidence also support the so-called streaming liver hypothesis,[37,38] which claims that hepatocytes are newly produced in the periportal area and flow along the hepatic cord toward the pericentral region to continuously replenish tissue.

Evidence supporting this hypothesis, as well as the aforementioned potential of the biliary system as the HpSC compartment, has been provided by a genetic lineage-tracing study using SRY (sex-determining region Y)-box 9 (Sox9)-CreERT2 knock-in (KI) mice.[39] Whereas tamoxifen-induced, lineage-labeled cells were initially confined to BECs, they gradually spread out to hepatocytes from the periportal toward the pericentral regions and eventually occupied the whole parenchyma nearly completely. The labeled cells also remained present in bile ducts, thereby indicating that Sox9-expressing biliary cells can continuously supply mature hepatocytes for normal tissue turnover while possessing self-renewing activity as well. Another study using a unique "*in vivo* chronometer" system based on Alb-Cre–mediated lineage tracing also revealed that 0.076% of all hepatocytes in steady-state adult mouse livers were newly born within the previous 4 days from Alb-Cre–negative cell populations, which may contribute to homeostatic maintenance of liver parenchyma under normal conditions.[40] However, genetic lineage-tracing studies in mice by other groups, using a different Sox9-CreERT2 strain and osteopontin-CreERT2 to label biliary cells or a Cre-expressing adeno-associated viral vector to specifically label hepatocytes, have, together, provided rather conflicting results with the above-cited study and thus strongly argue against the streaming liver hypothesis.[41-43] Further studies are needed to solve the discrepancy and elucidate the exact nature and the underlying mechanisms for physiological maintenance of the liver.

Stem/Progenitor Cell-Mediated Liver Regeneration

Upon PHx, there is basically no "injury" to the remnant hepatic tissue, so that it is considered to provide a "clean" model to study the mechanisms of regeneration. However, the model does not recapitulate pathological situations in human liver diseases, which often involve hepatocyte death and concomitant induction of inflammatory and fibrogenic responses. Under certain pathological conditions, such as chronic viral hepatitis, alcoholic liver disease, and nonalcoholic fatty liver disease, unique immature epithelial cell populations with bilineage differentiation potential are found to emerge and expand, a phenomenon also referred to as the "ductular reaction" from the standpoint of histological alterations.[44-47] Such progenitor cell populations are often termed as "hepatic progenitor cells" or "intermediate hepatobiliary cells" in humans. Importantly, the degree of their expansion and ductular reaction is known to correlate with severity of liver disease,[48] implying its relevant role in liver pathogenesis and regeneration.

Some researchers consider such disease-activated bipotential progenitor cell populations as "liver stem cells." The term "facultative liver stem cells" seems to be more accurate, because these cells can be identified only under a limited condition in a transient manner.[49,50] On the other hand, some also use the terminology "liver stem cells" to denote the cell of origin in the normal liver for those disease-activated progenitors in the injured liver. As will be discussed below, the origin of progenitor cells activated under diseased conditions still remains obscure, and it is not clear whether and how resident liver stem cells for normal tissue turnover, facultative liver stem cells in injured liver, the cell of origin for these cells, as well as the potential liver stem cells defined *in vitro* are the same or different. In addition, other criteria can also be used, in some cases, to evaluate and describe stem cells in the liver, such as the capacity to repopulate the liver upon transplantation or long-term label-retaining activity. To avoid possible misunderstanding and misleading, we use, in the following sections, the term LPC (for adult liver progenitor cells) to describe the epithelial progenitor cell population, typically characterized by staining for cytokeratin-19 (CK19) or pan-CK markers, that are activated and observed under liver injury conditions.

Characterization of LPCs

The prototype of stem/progenitor cell populations specifically induced in the injured liver is oval cells, which were initially described by Farber using a rat model of liver carcinogenesis.[51] Since then, many studies have further characterized these cells and have established them as facultative LPCs that are likely to be capable of differentiating into two hepatic epithelial lineages (i.e., hepatocyte and BEC). The most popular model to induce oval cells is the 2-acetylaminofluorene (2-AAF)/PHx system in rats, where hepatocyte proliferation is blocked by 2-AAF before PHx. However, this model is not applicable to induce oval cells in mice. Among various kinds of liver injury models, currently, the administration of a 3,5-diethoxycarbonyl-1,4-dihidro-collidine (DDC)-containing diet[52] or a choline-deficient, ethionine-supplemented (CDE) diet[53] are the most extensively used to induce "oval cells" in mice. However, it should be emphasized that etiologies of those mouse models are different from that of the 2-AAF/PHx model in the rat. DDC-induced injury is considered to be targeted primarily in the biliary compartment and serves as a model for sclerosing cholangitis and biliary fibrosis,[54] whereas the CDE protocol induces fatty liver and is sometimes used as a model for nonalcoholic steatohepatitis. The phenotypic and mechanistic differences among these, as well as other, protocols have become increasingly recognized, leading to the notion that "oval cells" induced therein are not exactly the same. For example, in line with that, the immature hepatocyte marker, alpha-fetoprotein, is known to be expressed in oval cells in rats, but not in LPCs in mice.[55] Similarly, expression of the hepatoblast marker, Dlk1, has been shown in a subpopulation of rat oval cells, but is not found in the mouse counterpart.[55-57] Thus, applying the same terminology in mouse injury models seems to have caused substantial confusion. This also prompted us to use the term LPCs, rather than oval cells, to denote such progenitor cell populations, particularly in the mouse system.

Much effort has been made in recent years to explore cell-surface molecules that can be used to identify and isolate LPCs for their characterization. This has led to the identification and establishment of EpCAM and CD133 as LPC markers in both mice and rats.[33,34,58,59] The oncofetal protein, glypican-3, has also been documented as a rat oval cell marker.[60] However, notably, these molecules, as well as most of the other known LPC markers, including CK19, Sox9, and the MIC1-1C3 antigen, are also all expressed in BECs in the normal liver. This fact strongly implies a close relationship between BECs and LPCs, as mentioned earlier, with the former possibly being an origin of the latter.

Trop2 (Tacstd2), a transmembrane molecule that is structurally related to, and a paralog of, EpCAM, has been found to be expressed exclusively in LPCs under the DDC-induced injury condition, but not in BECs in the normal liver.[33] Thus, Trop2 may serve as a genuine "LPC-specific marker" and would be advantageous for further characterization of LPCs. It would be of significant interest to examine whether this molecule is functionally involved in the transition of BECs to LPCs. Another molecule that can potentially distinguish BECs and LPCs is vascular cell adhesion molecule.[54] However, its expression is not specifically restricted to LPCs, but also seems to be induced in other cells, including endothelial cells. The transcription factor, forkhead box protein L1 (Foxl1), has been identified as another potential LPC–specific marker.[61] Although this molecule is not a cell-surface antigen, a transgenic (Tg) mouse line expressing the Cre recombinase under the control of the Foxl1 promoter has been made and proven to be useful. Thus, a lineage-tracing study using this Foxl1-Cre Tg mouse model demonstrated that both hepatocytes and BECs were found as descendants of Foxl1$^+$ LPCs upon bile duct ligation, and, possibly, in the DDC injury model as well. This does not necessarily indicate that single LPCs can clonally differentiate into these two lineages, but strongly supports the notion that LPCs are bipotential progenitors for hepatocytes and BECs. Another study using osteopontin-CreERT2 to label BECs and track their fate also demonstrated that LPCs derived from BECs showed significant contribution to new hepatocytes in the course of recovery from CDE-induced liver injury.[42] More recently, leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5), a well-established marker for stem cells in the intestine as well as in several other tissues and organs, has also been demonstrated to be expressed specifically in damage-induced LPCs in the liver using *Lgr5-LacZ* and *Lgr5-CreERT2* KI mice.[62] A major drawback for Foxl1 and Lgr5 is that expression of the endogenous genes and proteins in LPCs has not been clearly demonstrated thus far, so that the assay should totally rely on the use of those particular Tg and KI mouse lines.

A panel of surface-reactive monoclonal antibodies (mAbs), including MIC1-1C3, has been established that can each detect different populations of ductal and periductal cells in mouse LPC response.[63] Intriguingly, some of them seem to label cell populations that are

apparently enriched or reside specifically in LPC-induced livers, with little or no reactivity shown in the normal liver. Identification of the corresponding antigen molecules, as well as further characterization of these cell populations, should expedite our understanding of the mechanisms of LPC response at cellular and molecular levels. Using FCM-based cell separation methods in combination with the aforementioned cell-surface markers, LPCs can be viably isolated and subjected to *in vitro* culture to evaluate their proliferation and differentiation potentials. Studies based on the expression of EpCAM, CD133, MIC1-1C3, *Foxl1-Cre*–mediated fluorescent reporter, or *Lgr5*-driven LacZ have consistently demonstrated that LPCs isolated from injured livers proliferate to form colonies *in vitro* in the presence of certain combinations of growth factors. Moreover, the clonally expanded cells are capable of differentiating into both hepatocyte and BEC lineages under appropriate culture conditions.[31,33,34,62,64] These results suggest that LPCs indeed possess clonal bilineage differentiation potential, at least *in vitro*, a notion that needs to be evaluated using *in vivo* experimental systems as well.

Origin of LPCs

Whereas LPCs are well known to emerge almost always from the periportal area, the cellular origin of LPCs is still not clarified. Ever since their initial characterization, phenotypic resemblance between LPCs and BECs has suggested that they presumably originate from the biliary tree. The fact that most of the molecular markers for LPCs are also expressed in BECs supports this notion. However, It is not clear whether most, if not all, BECs can equally or similarly behave as precursors for LPCs, or whether there is a certain type of specialized "cell of origin for LPC" subpopulation located somewhere among BECs. Potentially lying on an extension of the latter possibility is the model in which the canal of Hering is the origin of LPCs.[65] Given its anatomical location in between BECs and hepatocytes, it appears reasonable to assume that this structure may serve as a niche for putative stem cells for these two cell lineages. Unfortunately, a direct proof for this model is hampered by lack of any specific marker for cells constituting the canal of Hering. Identification of such a molecule and a subsequent genetic lineage-tracing study should help clarifying this issue. A possible contribution of hepatocytes as an origin of LPCs can also be considered. Though a study using mice with chimeric livers has suggested that this is not likely the case,[66] more recent studies employing the Cre-loxP system-mediated, lineage-tracing approach have demonstrated that hepatocytes can be reprogrammed to LPCs under certain liver injury conditions.[67] It remains to be elucidated whether most, if not all, mature hepatocytes indeed possess such plasticity or whether there is some specific subpopulation of hepatocytes that can be converted to LPCs.

Notably, an intriguing possibility that hepatic stellate cells (HSCs) are capable of serving as "LPCs" and contributing to parenchymal regeneration by mesenchymal-to-epithelial transition has also been suggested and is under continuous debate. A very recent study employing glial fibrillary acidic protein-CreER and alpha-smooth muscle actin (α-SMA)-CreER mice for lineage tracing provided evidence that myofibroblasts (MFs) derived from HSCs upon liver injury produced hepatocytes and BECs.[68] Unfortunately, it remains uncertain of which types of cell populations were exactly pulse labeled and tracked in their experimental settings, and the possibility does not seem to be ruled out that not only HSCs and HSC-derived MFs, but also BECs and/or hepatocytes were marked *ab initio*. Further studies with a more definite labeling and tracing system should clarify whether or not HSCs, MFs, as well as other mesenchynal cell lineages have epithelial progenitor activities *in vivo*.

Regulatory Mechanisms for LPC Response

Whereas characterization of LPCs and their origin(s) is still on the way, understanding of the underlying mechanisms for their induction and regulation has made significant progress. Emergence and expansion of LPCs upon liver injury is not an autonomous process within these cells, but involves various other types of cells, which interact either directly or indirectly with LPCs and/or their putative precursor cells. In general, tissue stem/progenitor cells are supported and regulated by their surrounding microenvironment, the so-called "stem cell niche." Recent studies elucidating the cellular and molecular frameworks for LPC regulation have determined the specific cell populations supporting their activities as well as the responsible signals mediating their interaction, thereby substantiating the concept of the niche for LPCs in the regenerating liver.

Fibroblast Growth Factor 7. Mesenchymal cells, such as stellate cells, have long been suggested to physically interact with LPCs and exert some signals on them.[65] It has also been shown that a population of mesenchymal cells expressing thymus cell antigen 1 (Thy1; also known as CD90), which is distinct from stellate cells or MFs, resides in close proximity to oval

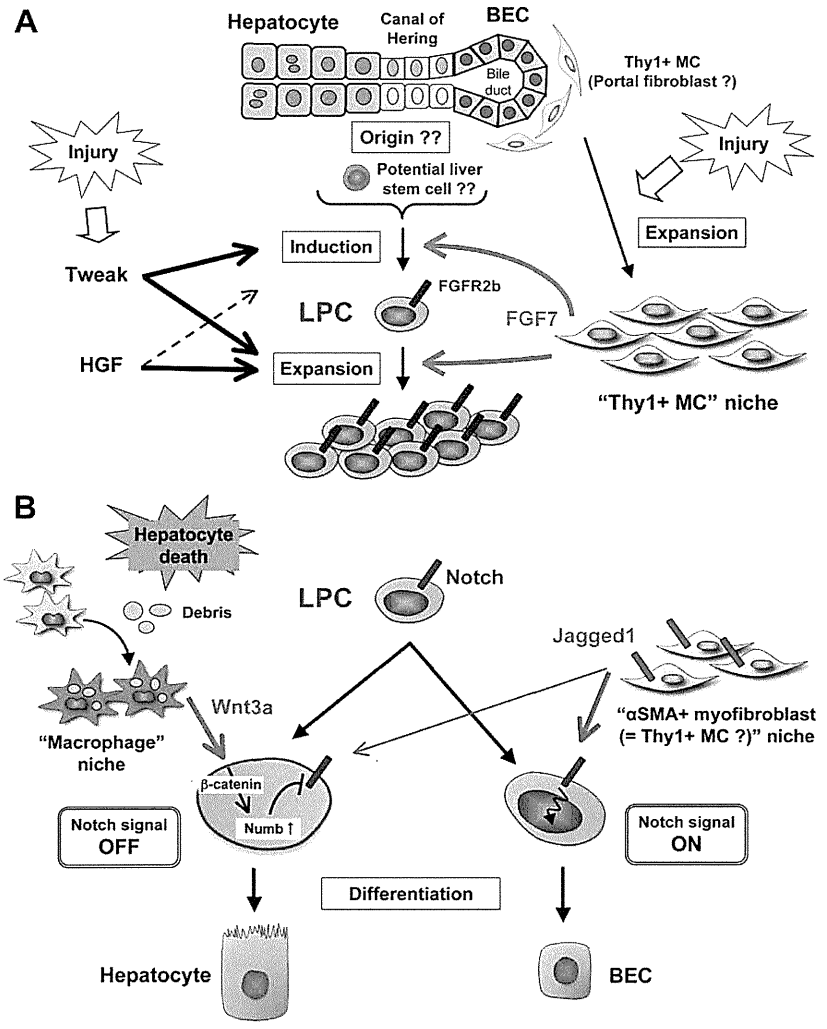

Fig. 2. LPC-niche interactions and signaling pathways regulating stem/progenitor cell-mediated liver regeneration. (A) Upon severe or chronic liver injury, LPCs are induced and expand in the periportal region from an, as yet, unidentified origin. It is possible that the cell of origin for LPCs is not uniformly defined, but may vary depending on the nature of injury. Thy1$^+$ mesenchymal cells (Thy1$^+$ MC) play a critical role in the induction and expansion of LPCs by providing FGF7. TWEAK and HGF are also known to be important for LPC induction and/or expansion. (B) Bidirectional differentiation of LPCs is controlled by the balance between distinct niche activities. α-SMA$^+$ myofibroblasts, which is likely to be equivalent to, or overlapped with, the Thy1$^+$ MC population, provide the Notch ligand, Jagged 1, thereby inducing a biliary differentiation program in LPCs. When hepatocytes are damaged, their corpses are engulfed by macrophages and Wnt3a production is induced. Wnt3a activates the canonical β-catenin pathway in LPCs to inhibit the Notch pathway by Numb expression, thereby skewing the cellular differentiation program toward hepatocytes.

cells in rat liver.[69] A recent study in mice has revealed that the Thy1-expressing mesenchymal cell population indeed plays a critical role as a "niche" to stimulate LPC activation.[70] Upon DDC-induced liver injury as well as in several other liver disease models, Thy1$^+$ cells were found to expand in the periportal region along with, and in close proximity to, LPCs. Searching for candidate signaling molecules involved in their interaction identified a member of the fibroblast growth factor (FGF) family ligand, FGF7, as a signal emanating from Thy1$^+$ cells. Notably, this FGF family ligand in general is produced by mesenchymal cells and, in turn, acts on epithelial cells.[71] Accordingly, expression of its cognate receptor, FGFR2b, on LPCs was confirmed. Genetic loss-of-function and gain-of-function experiments using KO and Tg mice, respectively, revealed that FGF7 is both necessary and sufficient to induce LPC response in the adult mouse liver. Intriguingly, overexpression of this growth factor in the course of DDC-induced injury significantly ameliorated hepatocyte injury and cholestatic disorders, suggesting its role in both hepatocyte and biliary regeneration by activation of bipotential LPCs. Thus, a novel Thy1$^+$ mesenchymal cell niche for LPC induction and expansion that depends on FGF7 has been established (Fig. 2A).

TNF-Related WEAK Inducer of Apoptosis. Chronic liver injury conditions, where LPCs are activated, usually accompany provocation of inflammatory responses, and the role of lymphocytes and other inflammatory and immune cells have also been suggested.[72,73] Accordingly, involvement of several inflammatory cytokines, such as tumor necrosis factor (TNF)-alpha, interleukin-6, and interferon-gamma, for induction and regulation of LPCs has been reported on,[74-76] although their modes of action remain not

fully clarified. One of the best established cytokine among these is TNF-related WEAK inducer of apoptosis (TWEAK; Fig. 2A). Forced expression of this cytokine in the mouse liver by using a Tg model or adenoviral gene transfer led to induction of LPCs.[77] Conversely, in mice lacking the TWEAK receptor, Fn14, as well as in those treated with a neutralizing anti-TWEAK mAb, LPC response upon the DDC or CDE regimen was significantly suppressed.[77,78] However, this suppression was only in a partial or transient manner, implicating a role of some other signals for LPC activation. It has been shown that transplantation of bone marrow (BM)-derived cells can have some beneficial effects on liver injury, including resolution of fibrosis and improvement of liver function, possibly through stimulating tissue progenitor cell activation and subsequent regeneration.[79,80] A recent study in mice revealed that the macrophage fraction in BM cells were responsible for LPC activation in the engrafted liver by producing TWEAK.[81] The study further demonstrated that administration of recombinant TWEAK was sufficient to induce LPC activation or ductular reaction, thereby implicating its potential therapeutic use.

Hepatocyte Growth Factor/c-Met. Another key regulatory mechanism for LPC response is hepatocyte growth factor (HGF)/c-Met signaling. Though its paramount role as a primary mitogen during the process of compensatory hypertrophy had long been known, it remained obscure whether this growth factor also played a mandatory role in regulating LPCs. A study using conditional KO mice for c-Met demonstrated that LPC response was significantly suppressed in these mice upon DDC-induced liver injury.[82] Although it has been shown that *in vivo* administration of HGF can augment the extent of LPC response preinduced by liver injury, it is not clear whether it is also capable of stimulating *de novo* induction of the response as TWEAK or FGF7 does (Fig. 2A).

Wnt/β-catenin. Wnt/β-catenin signal, a well-known pathway playing critical roles in regulating stem/progenitor cells in many tissues and organs, has also been reported, by several groups, to be involved in LPC regulation in the liver. Under various liver injury conditions with LPC/oval cell response in rats, mice, and humans, expression of several Wnt family genes has been observed.[83-86] Concomitant activation of the downstream β-catenin pathway is induced in LPCs/oval cells. There are 19 members in the Wnt ligand family, and it is not consistent among those articles which of the ligands are expressed and may play a relevant role. More recently, a role of Wnt/beta-catenin signaling in regulating differentiation of LPCs has been proposed[87] (Fig. 2B). In chronically injured liver, periportal myofibroblasts expressing α-SMA, which may correspond to the $Thy1^+$ cells mentioned above, provide the Notch ligand, Jagged1. This acts on the Notch receptor expressed on LPCs to activate the downstream signaling pathway, leading to differentiation of LPCs to BECs. Notably, the Notch signal is well known to induce differentiation of fetal hepatoblasts to BEC lineages. This Notch-dependent signal for biliary differentiation from the $\alpha\text{-SMA}^+$ myofibloblast niche is dominant when the liver is suffering from biliary injury, as is the case with the DDC protocol. When hepatocyte death was induced in other injury models, such as the CDE regimen, cellular debris derived from injured hepatocytes were engulfed by macrophages, which leads to activation of the macrophage and stimulates production of Wnt3a. This canonical Wnt molecule acts on LPCs to induce beta-catenin signaling and expression of a Notch signal inhibitor, Numb. This eventually results in suppression of the default Notch signaling for biliary differentiation in LPCs and, in turn, stimulates their differentiation to hepatocytes. Thus, the balance between two distinct niche structures with different signals is critical to shape the outcome of activated LPCs to induce proper regenerative response according to the nature of liver injury.

Concluding Remarks

As has been discussed, the liver can employ different modes of regeneration according to type and extent of injury. Hepatocytes can switch their behavior from hypertrophy to proliferation in the process of regeneration upon PHx and, under certain injury conditions, may also be able to adapt their cellular program to LPCs and BECs. Such a flexible nature should underlie the robust capacity of the liver to regenerate. Fundamental questions remain as to how the liver, hepatocytes, as well as other liver component cells sense a particular condition of injury or functional failure and make decisions to select and coordinate the balance of appropriate regenerative responses. Collapse of such mechanisms may constitute the basis for various liver diseases and should eventually lead to tumorigenesis. Conversely, thorough understanding of the cellular and molecular mechanisms for liver regeneration, in particular, development of means to control endogenous stem/progenitor cell activity, should pave the way for establishing effective therapeutic strategies to treat patients with liver failure.

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