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Review

Cytokine regulation of liver development

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Abstract

Liver development is a sequential array of distinct biological events. Each step of differentiation is regulated by intrinsically programmed mechanisms as well as by extracellular signals. The establishment of cell culture systems that recapitulate each stage of liver development has led to the identification of several extracellular signals that affect hepatocytic differentiation. Furthermore, studies on genetically engineered animals, especially knockout and transgenic mice, have highlighted a number of molecules essential for liver development. By applying primary culture techniques to analyses of mutant mice, it is now possible to link extracellular signals to intracellular pathways that provoke cellular responses of differentiation. Improvement in gene transfer technology utilizing viral vectors has further expanded the molecular analysis of liver development. In this review article, we summarize recent advances and attempt to describe the molecular basis of liver development from beginning to end as a sequential event.
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Keywords: Cytokine; Liver; Development

1. Introduction

The process of liver development can be divided into several distinct stages based on molecular and functional properties (Fig. 1). The initial event in the development is commitment (also referred to as specification) of the foregut endoderm to the hepatic lineage [1,2]. This is characterized by the expression of two of the liver-specific markers, albumin (Alb) and alpha feto-protein (AFP), which are detected as early as embryonic day 8–9 (E8–9; 6–8 somites stage) in the mouse system. Hepatic cells at this stage possess the potential to differentiate into both parenchymal hepatocytes and bile duct epithelial cells [3]. A small number of such hepatic progenitors or hepatic stem cells are believed to remain undifferentiated throughout development and reside even in the adult liver [4], although their precise characteristics and physiological significance have not been clearly documented.

At around E10–11, hematopoietic stem cells originating from the extrahepatic organ (aorta–gonads–mesonephros region; AGM region) colonize the fetal liver and expand their mass and lineage diversity [5,6]. Hepatic progenitors participate in creating the hematopoietic microenvironment in concert with other stromal cells to promote embryonic hematopoiesis [7]. Along with the maturation of bone marrow and spleen around birth, hematopoiesis in the liver declines and hematopoietic stem cells migrate from the liver to these organs responsible for adult-type hematopoiesis. On the other hand, hepatic cells up-regulate the expression of numerous genes relating to the functions of mature liver in order to achieve their own metabolism after birth [8,9]. Therefore, liver development at around birth is more like a functional switch from a hematopoietic microenvironment to a metabolic organ than a simple maturation process.

Throughout the process of development, hepatic cells continuously proliferate partly, if not entirely, through an autocrine mechanism [10]. However, the growth of cells gradually slows and is eventually arrested during postnatal development. Thereafter, hepatocytes no longer proliferate autonomously and their proliferation requires exogenously provided growth factors such as epidermal growth factor (EGF) and hepatocyte growth factor (HGF). Concurrently, a set of genes, including those involved in amino acid

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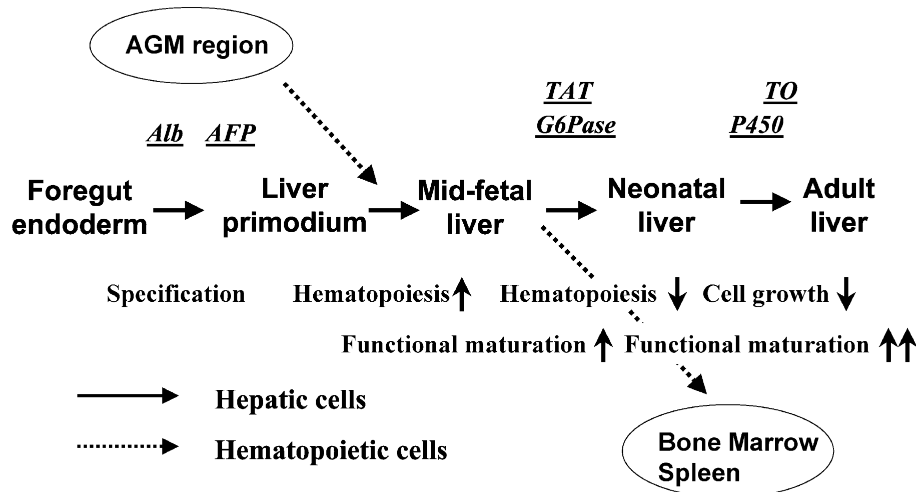

Fig. 1. Process of liver development. Hepatic cells arise from foregut endodermal cells in response to signals from neighboring mesenchymal cells. In time, hematopoietic stem cells from the extrahepatic organ (the AGM region) colonize the fetal liver and expand their mass and lineage diversity. Along with maturation of the liver as a metabolic organ at around birth, hematopoietic cells relocate to the bone marrow or spleen. Hepatocytes proceed to further round of maturation process during postnatal development that accompanies termination of cell proliferation. Letters in italic are developmental marker genes often used in the developmental biology of the liver. TAT, tyrosine amino-transferase; G6Pase, glucose-6-phosphatase (peri/postnatal hepatocyte markers); TO, tryptophan oxygenase; *P*450, cytochrome *P*450 species (terminal differentiation markers).

metabolism and detoxification, are up-regulated to constitute fully differentiated adult hepatocytes (terminal differentiation) [8]. In addition to the differentiation at the single cell level, hepatocytes and several other nonparenchymal cells start creating the sinusoidal structure and major hepatic vessels as well as bile ducts to organize a liver lobule that is a basic unit of the liver tissue.

Several cell culture systems briefly described below were shown to reproduce partial process of liver development. Besides these in vitro systems, there is an increasing number of knockout (KO) mice that display abnormalities in the liver at different developmental stages. Studies have now provided the outline of the process of liver development from beginning to end. In this review, we summarize recent progress in this field, primarily focusing upon the roles of extracellular factors and their possible link with downstream pathways.

2. Specification of the hepatocytic lineage from the foregut endoderm

Parenchymal hepatocytes and bile duct epithelial cells originate from foregut endodermal epithelial cells. LeDouarin's classic experiment utilizing chicken embryos demonstrated that transplantation of an endodermal tissue at the 4–6-somite stage into the precardiac mesoderm gave rise to a tissue that morphologically resembled the hepatocytic organ [11]. This was the first demonstration that interaction between endodermal and mesodermal tissues is important for hepatic development. Zaret and his colleagues adapted this idea and established a tissue explant assay in a microwell format using mouse embryos and investigated the molecule that mediates the endoderm–mesoderm interaction [12]. They found that while the inductive signal from the cardiac organ was not dependent on direct contact with the endoderm, it still required close proximity to the endoderm [13]. In addition, the inductive signal was apparently not mediated by a soluble factor secreted into culture media. Based on these observations, they searched for locally acting factors expressed in the cardiac mesoderm at this stage and identified fibroblast growth factor (FGF) family proteins (FGF1, 2 and 8) as potential candidates. (Though FGFs are secreted proteins, they often retain locally in association with extracellular matrices such as heparin.) In contrast, FGFR-1 and FGFR-4, receptors for these FGF species, were found to be expressed on endodermal cells [14], suggesting that the FGF–FGFR system is operative between endoderm and cardiac mesoderm. They therefore stimulated endodermal cells with either of these FGFs in the absence of the cardiac organ and found that both FGF-1 and FGF-2 were capable of inducing the expression of albumin mRNA at physiological concentrations. Moreover, neutralizing antibodies against FGFR-1 and FGFR-4 strongly inhibited the expression of albumin mRNA induced by the cardiac organ. While FGF8 was unable to up-regulate albumin mRNA expression, it seemed to have a positive effect on the outgrowth of hepatic cells after specification. Based on these observations, it was proposed that the FGF–FGFR system is critical for the initial process of liver development (Fig. 2) [14].

A more recent study from the Zaret laboratory further extended our understanding of the initial step of hepato-

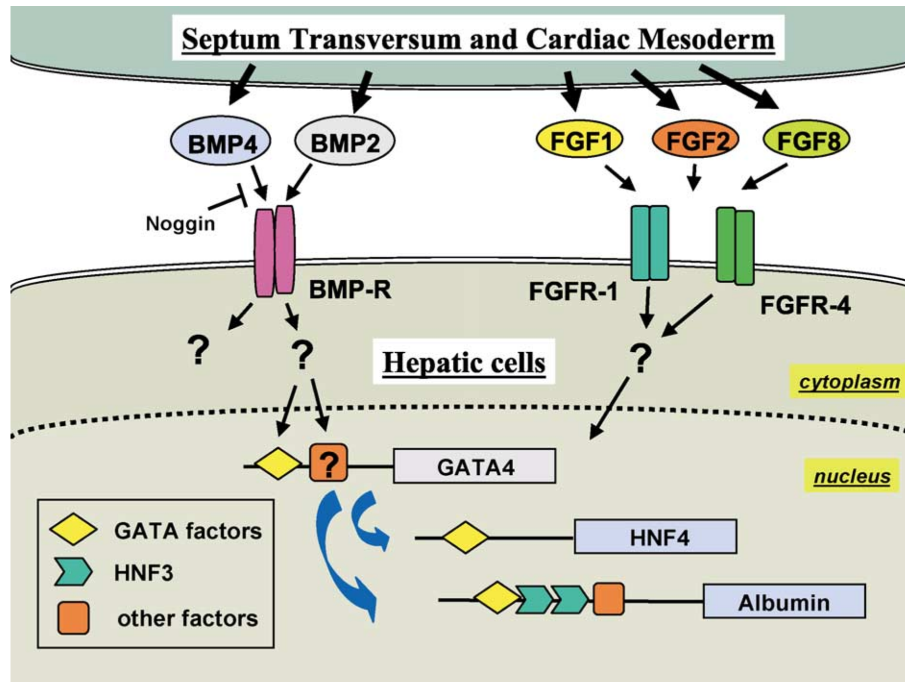

Fig. 2. A possible model for the mechanism of early hepatic specification. Generation of the hepatic primordium from the foregut endoderm requires at least two different signals, i.e., BMP and FGF family proteins. These proteins are produced by mesenchymal cells in septum transversum and cardiac organ, respectively. Although intracellular signaling mechanisms are yet unclear, several lines of evidence indicated that GATA and HNF3 transcription factors play critical roles in inducing liver-specific or enriched proteins including albumin and HNF4.

genesis [15]. Several preceding studies have suggested that an additional signal from the septum transversum mesenchyme, originating from the lateral plate mesoderm, contributes to the initial stage of hepatic induction as well [11,16,17]. It was also known that a member of the transforming growth factor beta (TGFβ)/bone morphogenic protein (BMP) family plays a critical role in tissue organogenesis, such as the lung and tooth, and often acts synergistically with FGF signals [18–20]. To examine the involvement of BMP proteins in the early stage of hepatogenesis, several distinct approaches were utilized as follows. First, a mouse model was used in which the *LacZ* reporter gene was inserted into the BMP4 locus [15]. LacZ reconstituted the normal BMP4 expression pattern and was co-localized with a marker gene (*Mrg1*) [21] specific for the septum transversum. In the BMP4-deficient embryos, the liver bud failed to enlarge and albumin was not expressed. In agreement with these observations, suppression of BMP signaling by a natural antagonist, noggin (Xnoggin) [22], inhibited albumin induction in the explant assay. Moreover, the FGF-induced albumin expression in endodermal cells was also inhibited by Xnoggin, suggesting that a BMP signal is essential for FGF-mediated induction of liver development. It was also shown that a signal from BMPs is insufficient for induction of hepatic differentiation. The expression patterns of TGFβ/BMP proteins during development and the activities of these proteins to restore albumin expression in the presence of Xnoggin indicated that BMP4 and BMP2 are likely to be responsible for hepatic induction in vivo (Fig. 2). Interestingly, cells, which are inhibited by Xnoggin to become the hepatic lineage cells, express a marker gene for the pancreas (*Pdx*) that also originates from the foregut endoderm. Thus, the systems of FGF and BMP appear to cooperatively control the bi-directional differentiation of endodermal cells [15].

The downstream pathway(s) of FGFs and BMPs responsible for induction of hepatic differentiation is unclear. While the FGF family generates inductive signals for many solid tissues [23,24], they are potent growth factors for many cell types as well [25]. As both cell proliferation and differentiation signals are transmitted through the same FGF receptor, the specificity of differentiation signals could be generated through the BMP receptors. The albumin gene enhancer has binding sites for liver-enriched transcription factors such as hepatic nuclear factor (HNF) 3, GATA4 and CAAT-enhancer binding proteins (C/EBP) [26–28], and GATA4 expression was found to be reduced in liver explants derived from BMP4-deficient mice [15]. Thus, BMP4-regulated GATA4 expression could be a key determinant to become hepatic cells or to proliferate in response to FGF. GATA factors are known to regulate the transcription of other liver-enriched factors such as HNF4α in hepatic cells [29]. Therefore, it is likely that the network of these transcription factors regulated by BMPs contributes to endodermal patterning by allowing liver-specific genes to be expressed in response to FGFs, and consequently prevents induction of the pancreas.

3. From the fetal to neonatal stage

After endodermal cells commit to the hepatic cell lineage, they undergo maturation programs during which they acquire various specific functions necessary for the metabolism after birth. Along with this process, hepatic progenitors differentiate into either hepatocytes or bile duct cells. Embryonic hepatic cells should not simply be considered as immature nonfunctional cells, because fetal liver is the major hematopoietic tissue in the late gestation to neonatal stage [30]. Therefore, liver development from the fetus to neonate is a functional switch rather than a simple maturation, although the precise mechanism regulating this process had not been characterized until recently.

Extensive studies by Darlington and colleagues on C/EBPα KO mice have provided a molecular basis of liver development at this stage [31–33]. C/EBPα is a transcription factor expressed in the liver that binds to the CAAT/enhancer sequences of the 5′-upstream region and activates target genes in the liver [26]. C/EBPα KO mice die immediately after birth, partly if not entirely, because of hypoglycemia, and administration of glucose extends their survival for several days [31]. Although the structure of the liver tissue was apparently normal in C/EBPα KO mice, the storage levels of hepatic glycogen and lipids were extremely low in comparison with those in control mice, indicating that C/EBPα is essential for functional maturation of hepatocytes. It has also been shown that C/EBPα is involved in the down-regulation of cell growth [34]. In C/EBPα KO mice, the expression of growth-related genes (*PCNA*, c-*jun* and c-*myc*) was up-regulated, while molecules involved in cell cycle arrest such as p21 were down-regulated [32]. Consistent with these observations, the number of cells remaining in a growth phase increased in KO livers at the perinatal stage. Thus, C/EBPα plays dual roles during liver development, i.e., in hepatic maturation and cell cycle regulation.

We previously established a primary culture system for murine fetal hepatocytes derived from the liver at the mid-embryonic stage (E14.5) [35,36]. The major cell population in this culture is parenchymal hepatocytes, judging from their epithelial morphology, expression of liver-specific marker proteins (albumin and AFP) and autonomous proliferation. In addition, cells do not yet express marker proteins in neonatal and adult livers. We used this system to screen extracellular factors that promote hepatocytic development and identified Oncostatin M (OSM), an interleukin (IL) 6-related cytokine, and glucocorticoid as powerful inducers of liver development [35]. OSM not only stimulates expression of hepatic differentiation markers but also induces morphological changes, up-regulation of multiple liver-specific functions, ammonia clearance, lipid synthesis, glycogen synthesis, detoxification, [35,37] and enhancement of homophilic cell adhesion [38], all of which are characteristics of postnatal liver cells. This was not a consequence of the inhibition of cell proliferation, because TGFβ, a potent inhibitor of fetal hepatocyte proliferation, did not up-regulate the expression of differentiation markers [35]. Stimulation with glucocorticoid alone was capable of inducing most of the cellular responses of differentiation, however, to a far lesser extent than the combination with OSM. In contrast, OSM alone failed to induce differentiated phenotypes of the liver. This implies that glucocorticoid is an essential trigger for hepatic maturation, while OSM enhances the effect of glucocorticoid. In the developing liver, OSM is expressed in $CD45^{+}$ hematopoietic cells, but not in hepatocytes [35]. These results suggest that OSM is a paracrine mediator of liver development, although more detailed analysis including those on OSM and OSMR KO mice is necessary to further define the role for OSM in vivo. Shen et al. [39] demonstrated that embryonic pancreatic islet cells transdifferentiated into cells of the hepatocytic lineage in vitro when stimulated with glucocorticoid. Interestingly, OSM strongly potentiated the expression of albumin induced by glucocorticoid in these cells. This suggests that OSM acts on multiple types of cells and enhances cell differentiation toward the hepatocytic lineage, although its physiological relevance in vivo remains to be addressed.

OSM manifests its biological activity by activating downstream intracellular signaling pathways through a specific receptor complex (OSM receptor; OSMR) composed of the OSM-specific subunit (OSMR β chain) and a common signal transducer, gp130 [40,41]. Two major pathways are activated through OSMR, the signal transducer and activator of transcription (STAT) 3 and Ras pathways, and each of them was shown to be involved in many different biological responses in a cell type-dependent manner [42,43]. Studies using a retrovirus-mediated gene transfer technique revealed that the STAT3 pathway is essential for the expression of hepatic differentiation markers induced by OSM [44]. Since 5′-regulatory sequences of hepatic differentiation marker genes often contain STAT3-binding sequences [45,46], it is likely that STAT3 directly regulates transcription. On the contrary, however, Leu et al. [46] demonstrated that regulation by STAT3 of HNF1-dependent transcription in hepatic cells does not depend on the DNA-binding domain of STAT3. Therefore, it is also possible that STAT3 regulates gene expression indirectly through regulation of other transcription factors presumably through protein–protein interaction. On the other hand, the Ras pathway appears to have an opposing effect on gene activation, since the blockade of this pathway either by dominant negative Ras or by an inhibitor for its downstream target, MEK, augmented expression of hepatic differentiation markers.

Our group recently highlighted the specific role for K-Ras in regulation of homophilic adhesion during OSM-induced hepatic development [38]. In response to OSM, fetal hepatic cells in culture form cadherin-dependent adherence junction. Since this effect was blocked by dominant-negative Ras, we examined cells from KO mice deficient for K-Ras, H-Ras and/or N-Ras allele. Interestingly, only K-Ras deficiency abolished OSM-induced for-

mation of adherence junction without an apparent effect on expression of hepatic differentiation markers. Moreover, ectopic expression of K-Ras cDNA restored the signal; therefore, it is likely that K-Ras mediates the signal from OSMR that leads to homophilic adhesion. Since K-Ras KO mice die at E14 prior to the formation of adherence junctions in vivo [47,48], it is impossible to address at this moment how much this function of K-Ras is required for liver development. However, our data provide a good example that a primary culture system can be utilized to analyze the consequence of gene knockout even if embryos die in utero.

4. Possible factors involved in hepatic maturation

OSM is not the only factor that induces hepatocytic development at this stage. HGF is known to be involved in regeneration of the liver after hepatic injury. Disruption of the HGF allele resulted in liver abnormality and embryonic lethality at around E14 [49,50]. Importantly, in utero injection of HGF ameliorated the liver defects and rescued the embryos [51], suggesting that HGF is also involved in liver development. Consistent with these results, stimulation of fetal hepatic cells in primary culture with a physiological concentration of HGF up-regulated the expression of hepatic differentiation markers in the presence of glucocorticoid, although to a lesser extent in comparison with OSM [52]. Interestingly, the induction of differentiation markers by HGF was not inhibited by dominant negative STAT3, even though STAT3 is activated by HGF in these cells. This suggests that there is an alternative intracellular pathway regulating hepatocytic development at least in vitro. However, it is again controversial how this growth-promoting factor transduces differentiation signals through the same receptor complex.

TGFβ is also a possible soluble regulator of liver development, although TGFβ failed to induce differentiation markers in fetal hepatic cells in vitro. However, hepatic cells at a later stage (E18-19) were induced to express these genes upon TGFβ stimulation, implicating TGFβ in hepatic development as well [53]. Since TGFβ (or its family members such as BMPs and activins) inhibits hepatocyte proliferation that occurs late-fetal to postnatal development, TGFβ may take more important role in cell cycle inhibition than in maturation at this stage.

Another inductive signal of hepatic development is apparently generated through cell–cell adhesion, since hepatocytes are induced to exhibit many characteristics of the differentiated liver, when they are cultured at high cell densities in the presence of glucocorticoid [37]. Since hepatocytes are able to differentiate to some extent in gp130-deficient mice in high-density cultures [37], it is not due to endogenously produced OSM or other IL-6-related factors. Thus far, a molecular basis of gp130-independent differentiation remains unclear; however, gene expression induced by OSM or by high-density culture was abrogated by an inhibitor of protein tyrosine phosphatases (PTPs) (Nakano and Miyajima, unpublished). It is therefore possible that a certain PTP molecule plays an essential role in hepatic development.

5. Fetal liver hematopoiesis

Transient hematopoiesis occurs in the fetal liver during embryogenesis and has a close interaction with liver cell development [54]. While hepatic cells are believed to participate in creating the hematopoietic microenvironment, mechanisms of embryonic hematopoiesis have been studied solely by researchers in the hematology field. Such studies include the establishment of a number of cell lines from fetal livers capable of supporting hematopoiesis [30,55]. While most of these stromal cells are believed to originate from mesenchymal cells, several lines of evidence indicate that parenchymal hepatocytes participate in the regulation of hematopoiesis as well [7,56,57]. We utilized primary fetal hepatic cells as hematopoietic stroma and investigated how embryonic hematopoiesis is regulated along with the liver development [36,58]. Co-culture of hematopoietic stem cells over the primary culture of fetal hepatic cells allowed expansion of both hematopoietic stem cells and of lineage-committed cells including myeloid, erythroid and lymphoid cells. Of particular interest, when stromal cells (fetal hepatic cells) were induced to differentiate by OSM and glucocorticoid, hematopoiesis was apparently suppressed [36,59]. Since OSM does not directly act on hematopoietic cells except for megakaryocyte [60], OSM appeared to have suppressed hematopoiesis by inhibiting the activity of fetal hepatic cells to support hematopoiesis. These results thus suggest that promotion of hepatic differentiation by OSM not only induces maturation as a metabolic organ but also down-regulates embryonic hematopoiesis. It is, however, noteworthy that a fraction of hematopoietic progenitor cells (including stem cells) generated during co-culture was not influenced by differentiation signals. Moreover, those immature cells were even amplified in the presence of OSM when AGM cells were used as a source of hematopoietic stem cells [58]. It is therefore likely that induction of hepatic differentiation preferentially suppresses expansion of lineage-committed cells. As reported by Wineman et al. [55], distinct types of stromal cells differentially contribute to regulation of lineage-committed hematopoietic cells and stem cells. Probably, a subpopulation of stromal cells insensitive to OSM regulates expansion and self-renewal of hematopoietic stem cells.

6. Multiple signals are required for terminal differentiation

The final step of hepatic differentiation takes place several days after birth in rodents (terminal differentia-

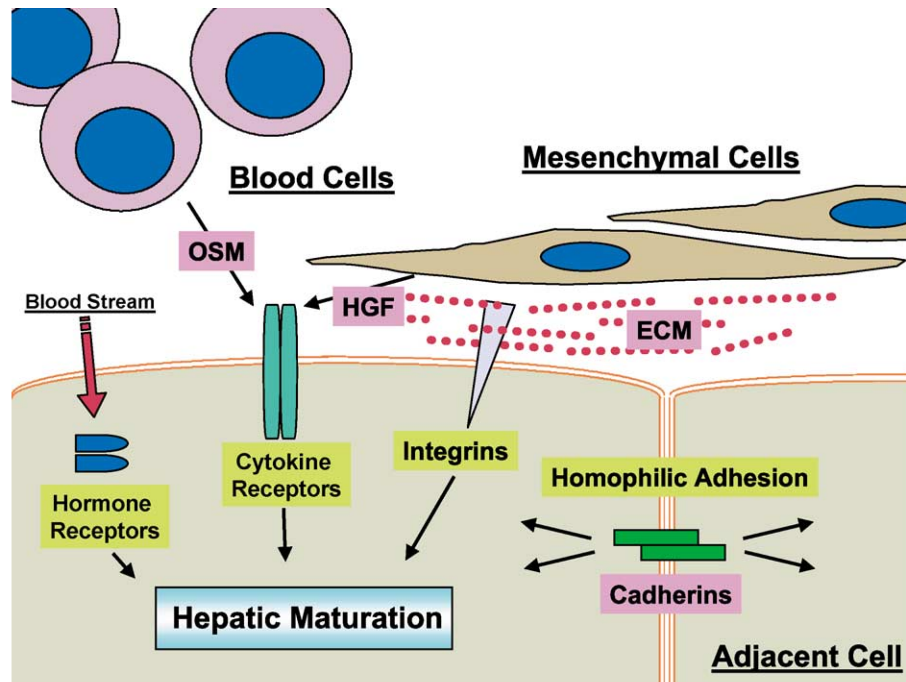

Fig. 3. Multiple distinct signals are required to promote the late stage of liver development. While development from late-fetal to neonatal stage can be stimulated with OSM and glucocorticoid hormone, terminal differentiation of hepatocytes apparently requires signals from extracellular matrix as well as homophilic interaction. Note that many different types of cells are involved in production of such signals.

tion). This process includes induction of another set of genes relating to functions of the adult liver, such as tryptophan oxygenase (TO) [61] and several *P*450 species [62]. In addition, cellular growth and expression of growth-related genes were either terminated or down-regulated during terminal differentiation. Terminal differentiation of hepatocytes in primary culture evidenced by TO mRNA expression was not induced by OSM or by high-density culture and required an additional signal(s) generated through an extracellular matrix (ECM) to be induced [63]. Importantly, terminal differentiation does not occur in the absence of either one of these signals, i.e., OSM, glucocorticoid, high-density culture and ECM. Thus, there appears to be a highly complicated cross-talk between intracellular signaling pathways generated by these extracellular stimuli (Fig. 3). Since transformed hepatomas or hepatocarcinomas often lose expression of TO mRNA [64], analysis on regulatory mechanism of TO gene activation is an important issue to be addressed.

7. Growth control of hepatocytes revealed by gene targeting

A number of KO mice have been generated in the past decade and there is a significant number of mutant mice that show developmental defects in the liver. Fig. 4 summarizes genes whose knockout caused abnormalities in hepatocytes during development. For example, disruption of the c-*jun* allele (c-*jun* KO) gave rise to embryonic lethality at around E12-14 [65]. In the liver of c-*jun* KO embryos, severe growth retardation and apoptosis of hepatocytes were observed in the entire liver tissue. Moreover, analysis on chimeric mice consisting of c-*jun* KO and wild-type cells revealed that ES cells lacking the c-*jun* allele were unable to contribute to the liver, further confirming that c-*jun* is essential for hepatocytic development. More recently, Behrens et al. [66] reported that c-*jun* deficiency also perturbs postnatal growth as well as liver regeneration, implicating c-*jun* in growth regulation of hepatocytes throughout development and tissue-repairing process. The importance of the c-Jun signaling pathway was further supported by KO mice deficient for SEK1/MKK4, an upstream regulator for c-Jun [67]. The phenotypes of *SEK1* KO share many similarities with those of c-*jun*, for example, lethality at around 12–14 and massive apoptosis of hepatocytes. In both KO lines, no apparent defect was found in remaining hematopoietic cells and they were able to reconstitute the full hematopoietic system in lethally irradiated adult mice. Accordingly, both genes are likely to be implicated specifically in hepatocytic development at this stage. A possible candidate for the extracellular signal that triggers this pathway is HGF, since there are certain similarities between the phenotypes of these KO mice [49,50]. In addition, HGF can activate the c-Jun pathway in hepatocytes [68,69], supporting the possibility that HGF regulates c-Jun and SEK1 in the developing liver.

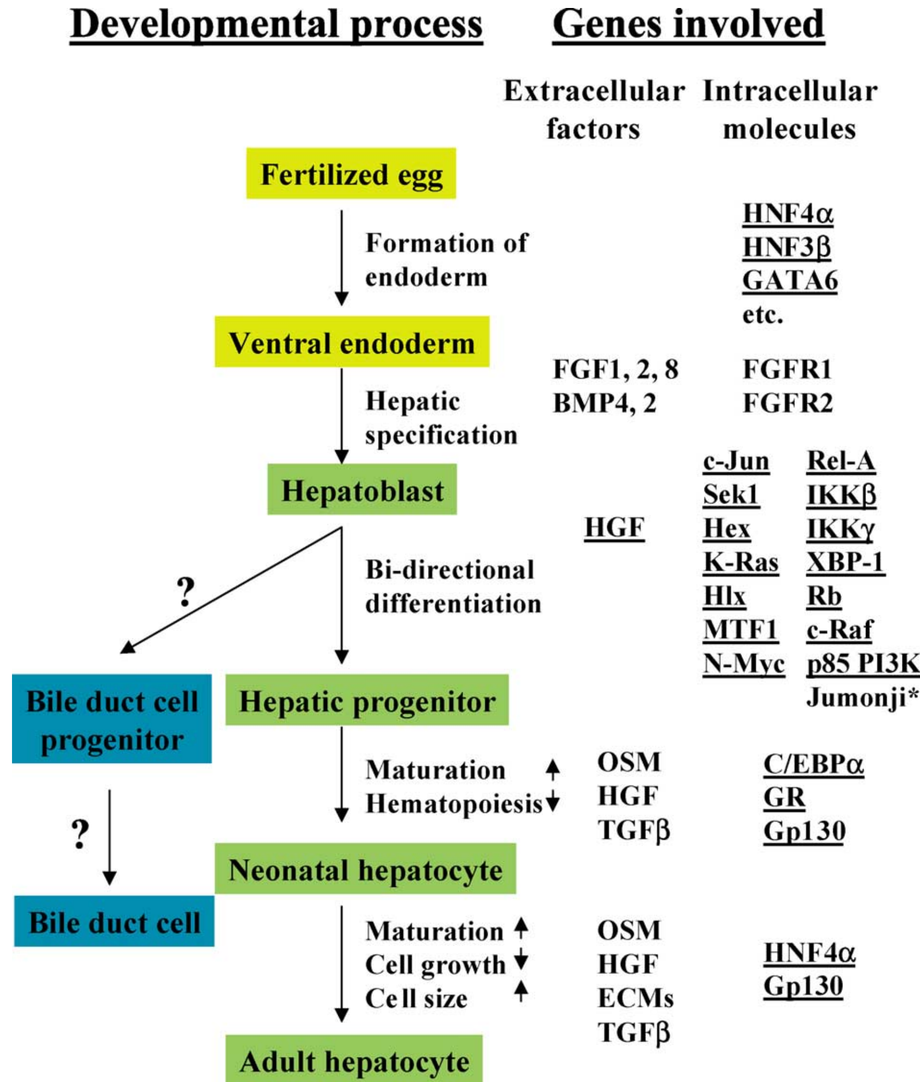

Fig. 4. Extra- and intracellular molecules involved in hepatic development. Genes or proteins involved in various stages of liver development are shown. Molecules with underline were identified from studies of KO mice. *The KO model that has been generated by a gene-trap technology.

Another important intracellular player identified from KO studies is NFκB, a transcription factor that stays in the cytosol as an inactive complex with an inhibitory protein, IκB, in the absence of stimuli [70]. Upon stimulation with cytokines such as tumor necrosis factor alpha (TNF-α) and IL-1, IκB is phosphorylated and released from the NFκB–IκB complex. Free NFκB translocates to the nucleus and stimulates transcription of target genes. It is also known that a specific protein complex in cytosol is responsible for stimuli-dependent IκB phosphorylation (IκB kinase, IKK) that leads to ubiquitin-dependent degradation of IκB [71]. Knockout of a subunit of NFκB (Rel-A) or IKK (IKK-β, NEMO/IKK-γ) resulted in reduced activation of NFκB in response to TNF-α- and of NFκB-dependent transcription [72–74]. During embryonic development, fetal hepatocytes suffer from massive apoptosis and subsequent loss of tissue, leading to the lethality at around E14. Since activation of NFκB is known to be necessary for suppression of the TNF-α-triggered apoptotic program [75,76], it is conceivable that the lethality caused by KO of NFκB or its regulators was resulted from reduced expression of genes indispensable for hepatocyte survival at this stage. These observations also raise a possibility that TNF-α is a principal regulator for survival of hepatocytes during embryonic development; however, knockout of RIP, a signal transducer for the TNF-α receptor [77], did not cause apparent liver abnormality. Therefore, another molecule that activates the NFκB pathway appears to be involved in liver development in addition to TNF-α.

Above two signaling pathways are known to be stimulated during regenerative responses of the adult liver following various hepatic injuries [78]. Thus, there seem to be regulatory mechanisms of hepatocyte proliferation

common to both liver development and liver regeneration. Embryonic lethality of KO mice has often been an obstacle to analyze the function of a gene of interest in later stages of development or in adult. To avoid this problem, a conditional targeting strategy is becoming popular in various fields. Analysis of 5′-regulatory regions of hepatic differentiation marker genes, such as TAT and TO (see Fig. 1, legend), will help us not only to understand the molecular basis of their regulation but also to generate conditional KO mice using a regulatory region of these genes.

8. Liver stem cells

Stem cells are defined as the cells with the self-renewing activity and capability to generate multiple types of lineage-committed cells. As for the hepatocytic lineage, stem cells have to possess the ability to generate both parenchymal hepatocytes and bile duct epithelial cells [79]. In the mouse system, it is known that differentiation of liver stem-like cells into these lineages takes place from around E12. Liver stem cells or progenitors have been characterized based on expression of albumin and a subset of cytokeratins [3]. On the other hand, increasing evidence indicates the presence of stem-like cells in the adult liver, namely oval cells [3]. Characterization of these stem-like cells has revealed many common features between immature embryonic hepatic cells and oval cells. Since transplantation of stem cells (or equivalents) is a promising therapeutic strategy for several liver diseases, purification procedures of them need to be established.

Numerous hematopoietic cell surface antigens have been identified and extensively used as lineage markers in hematology. These antigens were successfully used to identify hematopoietic stem cells as well [80]. Likewise, identification of cell surface antigen in liver cells is beneficial for characterization of cell types as well as purification of a cell population by cell sorting. In fact, it was reported that the cell population with $CD45^{-}TER119^{-}c\text{-}Kit^{-}CD49^{+}$ contains hepatic stem cells [81]. Unexpectedly, cell surface antigens such as c-Kit and CD34, expressed in hematopoietic stem cells, are also found in immature hepatic cells [82]. In 1999, Petersen et al. [83] demonstrated that bone-marrow transplants injected into the spleen of mice with liver damage migrated to the injured liver and expressed albumin. These results indicate that bone-marrow cells harbor the potential to become cells of the hepatocytic lineage. Furthermore, it was shown that a highly enriched fraction of hematopoietic stem cells ($Lin^{-}c\text{-}Kit^{high}Thy\text{-}1^{low}Sca\text{-}1^{+}$) was shown to give rise to albumin-positive cells [84]. Hematopoietic stem cells were also shown to become vascular endothelial, muscle, nerve and epithelial cells [85]. The plasticity of hematopoietic stem cells is one of the hottest topics in the current biology, because it may provide a means to develop novel cell therapies for these organs including the liver [86]. On the other hand, it remains to be investigated how much this cell population contributes to developmental process or regenerative responses of liver cells under physiological circumstances.

9. Concluding remarks

During the last several years, understanding of liver development at the molecular level has been significantly advanced. While genetic manipulations of mice has been a major technique for molecular dissection of solid tissue development, in this article we would like to suggest the use of primary culture systems for detailed molecular analysis of genetically engineered animals. A major advantage of primary culture derived from mutant animals is that it avoids unexpected secondary effects or lethality owing to defects in the liver itself or in another organs. This advantage allows us to trace the consequences of gene inactivation in a simple system. Furthermore, by applying gene transfer techniques, it is possible to examine functions of a gene of interest, e.g., expression of a missing gene or its downstream targets. We believe that a combination of these new technologies will further extend our understanding on liver development at the molecular level and contribute to the advancement of "Molecular Hepatology" in the next few years.

Besides such molecule-oriented approaches, liver stem cells became one of major subjects for basic hepatology as well as regenerative medicine. We presume that it does not take a long time to characterize the cell with stem-like activity inside a liver tissue, because of the preceding strategy that has been successfully used for characterization of hematopoietic stem cells. Combination of cell sorting techniques and differentiation assays would undoubtedly help to uncover the characteristics of "liver stem cells" as well. Yet a rather controversial issue is the emerging concept of the cellular plasticity. It is raising several difficult questions; for example, do all stem cells in each tissue come from the bone marrow through blood circulation? Or, do they have different origins in spite of possessing similar capabilities? From the point of clinical application of stem cells, it does not really matter where they come from as long as they possess multi-potency. The presence of stem-like cells in the bone marrow can be even beneficial, since it avoids many of the political and ethical barriers of stem cell therapy. However, as Terada et al. [87] and Ying et al. [88] recently suggested, cellular plasticity shown by transplantation assays could have been a consequence of cell fusion between transplanted and host cells rather than trans-differentiation. In conclusion, the field is still immature and awaits further extensive studies from many different points of view. It is necessary to establish in vivo assays to trace the fate of stem/progenitor cells from different origins including the bone marrow and fetal liver.

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