 doi:10.20944/preprints201809.0351.v1

Organogenesis: A New Theme to Study Genetic Expression in Adult Liver Regeneration After Partial Hepatectomy

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Abstract

Researchers in different disciplines studied liver's genetic expression of organogenesis in embryogenesis; however, organogenesis has not been studied as an independent and a complementary process during adult liver regeneration. This paper reviewed studies and extracted information related to organogenesis in adult liver regeneration because of organogenesis' important role in cancer and tissue regeneration.

Key words: organogenesis, adult liver, translational, regeneration, homeobox, hox, Wnts, growth factors, theme, gene expression, cancer, hepatectomy, three-dimensional, organoid

Introduction

The liver has a unique ability to regenerate and to retain a specific three-dimensional shape after partial hepatectomy; researchers investigated the liver's abilities because of the multiple factors involved and the complexities of the processes. The focus of this review is organogenesis, and because it is part of the regeneration process, and it overlaps and intermingles with proliferation, both processes were reviewed. Most previous studies focused on the proliferation and differentiation of liver cells (for citations, please refer to Table 1, 2, and 3). In addition, this paper will discuss a theme in which the organogenesis process would be recognized as a separate yet parallel and complementary process in all phases of liver regeneration to help identify factors associated with each process.

Studies investigated adult liver organogenesis, and factors associated with it were scarce (Lokmane et al., 2008[1]; Wang et al., 2015[2]). Signaling and genes expression that control three-dimensional modeling of liver are important for many reasons, including the identification of termination factors that may help in controlling both proliferation and organogenesis in tumor tissue, and the identification of signaling factors that may help in initiating organogenesis of regenerative tissue for organ donation. Researchers also proposed phases to describe these processes (Xu et al., 2005[3]; Zimmermann, 2002[4]). There is gap in current knowledge because few studies identified causality of specific factors in the processes, especially morphogens. Furthermore, some factors associated with triggering and termination of organogenesis are still unknown (see Table 3), and most importantly, there is a lack of controlled studies to identify factors that control how the liver achieves its final three-dimensional shape. Some studies experimented with models of three-dimensional cultures to study a variety of effects (Li et al.,

2018[5]; Tegge et al., 2018[6]). In addition, similarities (or a lack thereof) between adults and embryonic genetic expression of organogenesis process need to be investigated (Weiss et al., 2016[7]).

Phosphorylation and mRNA

Emergence of new discoveries related to phosphorylation and mRNA directed the focus of researchers toward the application of these concepts to explore their association with liver regeneration. Studies found that high phosphorylation activities are associated with cell proliferation; however, these studies did not state whether or not an association with organogenesis exists (see Table 1). Table 1 reveals important studies in regeneration and their lack of indication to organogenesis in regeneration process. An exception to these studies is Shu et al.'s (2009)[8] contribution of an important observation associated with STAT3 phosphorylation (see orange highlight in Table 1): their team observed that defective regeneration correlates with steatosis, this observation extended to the group with adiponectin-deficiency, but this study did not describe the pattern in which the defective liver regenerates.

Hormones

Hormonal effects were studied to explore whether hormones have an enhancing or inhibitory effect on the regeneration process, but studies were not specific as to whether or not hormones have any effect on organogenesis (Table 2). Proliferation could give rise to mass, but organogenesis is the process that determines how the liver forms its three-dimensional figure, lobar shapes, and chirality.

Factors Associated with Regeneration Process Pace

While many studies investigated different factors involved in liver regeneration, only a few researched factors involved in organogenesis (Table 3). Lokmane et al. (2008)[1] studied hepatocyte nuclear factor (vHnf1), which is a transcription factor, and concluded that a lack of vHNF1 leads to formation of a defective hepatic bud and an abnormal gut regionalization. Wang et al. (2015)[2] observed that the absence of FGF signaling associated with halting of only anterior murine liver bud; FGF signaling was specific for anterior hepatic bud and it has no effect on the posterior portion. The posterior hepatic bud might be controlled by another signaling pathway. Based on their experiment on HNF4A, Taniguchi et al. (2018)[9] indicated that a mutation that reduced HNF4A transcription led to tumor growth; and noted that it acts as both a major regulator of organogenesis and a tumor suppressor to hepatocyte growth. Meng (2017)[10] found

that PROX1 and LYVE1 correlate with liver regeneration and can be used as biomarkers to identify formation of new liver sinusoidal endothelial cells. The abovementioned studies found factors correlated with organogenesis (see orange highlight in Table 3). In addition, these four studies demonstrate the importance of organogenesis in liver regeneration. The rest of the studies in Table 3 showed association of factors with regeneration process; however, they did not indicate the association with organogenesis.

Genetics

Insufficient studies identified genes involved in organogenesis (including signaling factors or inhibitors associated with gene activities). Arai et al. (2003)[11] conducted extensive gene expression analysis during liver regeneration of rats and found 496 out of 2,304 genes showed expression, with up- and down-regulation in 317 genes of at least 2 folds. Nevertheless, Arai et al. (2003)[11] did not indicate how these genes are associated with the organogenesis of the liver after partial hepatectomy. Youn et al. (2003)[12] reported high expression of *abdB hox* genes during liver regeneration of murine rats; however, *hoxa1* and *b* had the same level pre- and post-organogenesis process, indicating both *hoxa1* and *b* are not involved in this organogenesis process. Group IX and X—including *hoxad10 and 10*—of *hox* genes are regeneration specific. Matz-Soja (2016)[13] reviewed signaling pathways of morphogenesis in adult liver mathematical model (e.g., Hedgehog and *Wnt/β-Catenin* pathways*)*. These signaling pathways were lagging yet had similarities with those that occur in embryogenesis. Matz-Soja (2016)[13] further emphasized the importance of signaling pathways of morphogenesis in initiating and orchestrating liver regeneration. *Homeobox containing 1* could prevent liver tumor progression; its action might be attributed to different mechanisms, including promoting autophagy, inhibiting CSC phenotype, or increasing tumor cells' sensitivity to natural killer cell cytolysis (Zhao et al., 2018)[14]. The Wingless/Integrated "*Wnts"* signaling pathway was confirmed to have an important role in hepatic progenitor cells' fate (Boulter et al., 2012)[15]; however, it was not demonstrated how its deficiency would affect organogenesis. Godoy et al. (2013)[16] reviewed morphogens—particularly Wnts (p. 1327) and their team identified canonical Wnts *'* signaling pathway as a major determinant in zonation of the parenchyma. *β-Catenin* is important in the expression of enzymes that metabolize drugs and in localizing liver reactions to xenobiotic inducers (Godoy et al., 2013)[16]. Aging is associated with a decrease in liver ability to regenerate and an increase in transcription of inflammatory and immune response; this aging effect on liver regeneration

remains ambiguous, but it might be contributed to a depressed level of extracellular factors and hormones (Pibiri et al., 2015)[17]. Mizuta et al. (1996)[18] used competitive reverse-transcriptase polymerase chain reaction to clone and sequence *homebox* genes at different phases after partial hepatectomy of rat liver, high levels of caudal related gene (RCdx-3) at one hour, while low levels of *hox* gene (RHoxB5) was noticed; however, other identified *hox* genes [RHoxA1, B2, B3, A4, and A5] that were expressed before and after hepatectomy. The focus of the majority of previous studies was liver regeneration, but the study of *Homeobox containing 1* demonstrated the importance of organogenesis factors in halting tumors; in addition, some of *hox* genes are associated with regeneration; however, their role in organogenesis needs to be determined. Furthermore, there is lack of studies that compare genes and their expression of organogenesis in adults versus embryos.

Three-Dimensional Modeling of Adult Liver Organogenesis

Multiple studies were conducted to investigate three-dimensional cultures of the liver. A study found that hepatocytes' size increases dramatically 18 hours after hepatectomy; it was followed by a noticeable increment in volume which happens 24 hours after partial hepatectomy (Vizzotto et al.,1989)[19]. Hepatocyte stellate cells play a mediator role in communication between hepatocytes and endothelial cells in terms of endothelial cells morphogenesis (Kasuya et al., 2011)[20]. Parsons-Wingerter & Saltzman (1993)[21] reported that aggregate cultures retained two folds of functional parameter compared to singlet cultures; in addition, functional recovery starts when growth is completed. However, the abovementioned studies did not indicate how the volume cells orient, regionalize, or take specific shape. Three-dimensional organotypic liver culture was used to understand liver sinusoidal endothelial cells' (LSECs) and Kupfer cells' (KCs) behavior in three-dimensional cultures and found that LSECs, accompanied by KCs, demonstrate a decrement in focal adhesion kinase's (FAK) signaling expression, which is a pathway linked to liver sinusoidal endothelial cells dedifferentiation (Tegge et al., 2018)[6]. A functional three-dimensional spheroid was produced, and a hepatic bud was developed from naive mesenchymal stem cells (MSCs), MSC-derived hepatocytes, hepatic stellate cells (HSCs), HSC- and LSEC-like cells (Li et al., 2018)[5]; however, specific mechanisms and pathways of organogenesis in this study is still unraveled and needs to be determined.

Three or Six Phases and Two Processes

Most of previous research conducted on liver regeneration concentrated on the tissue's competency to proliferate and the proliferation process. Three distinct and sequential stages were described during liver regeneration: (1) initiation stage involves replicating competency; (2) proliferation stage includes the expansion of cells population; and (3) termination stage: here the cellular growth is halted to terminate regeneration at a set point; however, organogenesis is still vague process (Zimmermann, 2002)[4]. In their review, Pahlavan et al. (2006)[22] used staging to discuss factors associated with regeneration; the team also stated the process is ambiguous, but they predicted the presence of inhibitors to control the size of regeneration. A three-stages model was diagramed with factors and pathways associated with these factors in the regeneration (e.g., TNF-alpha and IL6 activate TNFR, STAT3, NF-kB and AP1 in priming [initiation] stage) (Pagano et al., 2012)[23]. Xu et al. (2005)[3] recognized six temporal phases based on gene expression: 1) immediate early; 2) early; 3) intermediate; 4) early-late; 5) late; and 6) terminal.

A networks triad was suggested to explain the mechanism of liver regeneration: cytokine, growth factor, and metabolic (Fausto et al., 2006)[24]. There was no indication on how are these networks are correlated with organogenesis. Furchtgott et al. (2009)[25] proposed a model of liver regeneration based on interaction of multiple factors, the model divided regeneration process into three phases: 1) quiescent; 2) primed; and 3) replicating. This model lacks a termination phase as a separate phase, and the model did not discuss organogenesis in liver regeneration.

There was a difference in the staging timelines and phase counts between liver regeneration models in the previously mentioned studies mainly because the phases were established according to the timing of the samples' retrieval, and these models did not reflect the actual timeline of regeneration events. Another factor was the technology that was used in analyzing the data and the kind of data were analyzed. However, these staging models will aid in the classification and assigning of different factors to their temporal window in future studies.

Stimulators of signaling pathways', enhancers', and inhibitors' roles in organogenesis are not well identified. But, the independency of organogenesis process in liver regeneration is supported by ongoing evidences. A study found that liver growth defect associated with Bone morphogenic protein-7 (BMP-7) deficiency (Sugimoto et al., 2006)[26]. Adult hepatocytes play a major role in organogenesis (Utoh et al., 2016)[27]. Lokmane et al. (2008)[1] reported that the lack of vHNF1 leads to formation of a defective hepatic bud and an abnormal gut regionalization. Organogenesis requires Fibroblast Growth Factor (FGF) signaling for anterior but not posterior

specification of the murine liver bud (Wang et al., 2015)[2]. These studies demonstrated that some areas and regions in the liver are controlled by different factors during organogenesis; which makes the process more unique.

The paper proposes a theme (Figure 1) in which organogenesis process would be identified as independent, yet parallel and complementary process to proliferation in all phases of liver regeneration, this theme will help identify factors associated with each process. In this theme, organogenesis starts at prime stage, and continue to direct the expansion of three-dimensional growth and ends the process in termination stage. Factors that affect organogenesis are different from those associated with proliferation because of specificity of these factors to three-dimensional spatial control other than random mass expansion (mass can be achieved by proliferation alone).

Summary

Organogenesis is a distinct process that encompasses multiple factors that need to be studied during proliferation. Signaling pathway, cell-cell interactions, extracellular matrix and multiple factors are associated and required to achieve the organogenesis process. This paper discussed a theme to recognize organogenesis in adult's liver as separate, yet parallel and complementary process to proliferation in order to achieve liver regeneration. The theme will help explain organogenesis's factors rather than proliferation alone in regeneration.

Based on retrieved studies, four important observations were concluded after review stratification: (a) Organogenesis is an overlooked process in adult liver regeneration. (b) The factors associated with organogenesis lack causality relationship and mechanisms. (c) Multiple factors are probably associated with organogenesis; most are unknown. (d) Structural architecture and heterogeneity of cells and tissues (including neovascularization and sinusoidal endothelial integration) to achieve final liver function are controlled by organogenesis. Further studies are warranted to determine each factor and its relationship to its pathway. Three conclusions were reached: (a) Phases of liver regeneration fall under two parallel processes: organogenesis and cell proliferation. (b) Current regeneration staging classification depends on timing of tissue retrieval from experimental groups for testing and does not reflect actual system biology of liver regeneration. And (c) Time recording is important to identify factors in each phase; however, causality of factors in their phase is more important. Further studies are warranted to determine each factor and its relationship to its pathway.

 doi:10.20944/preprints201809.0351.v1

Table 1
Summary of Phosphorylation Processes in Liver Regeneration and Organogenesis

Process	Observation during proliferation	Observation during organogenesis
Phosphorylation of Lysine-Rich Histone (Balhorn et al., 1971)[28]	High rate	not stated
Nuclear proteins phosphorylation pattern (Ballal et al., 1975)[29]	High rate, except for two protein (All and A24) were markedly decreased.	not stated
Acidic nonhiston proteins phosphorylation of nuclear matrix (Allen et al., 1977)[30]	High rate	not stated
Phosphorylation of H4 histidine (Chen et al., 1977)[31]	High rate	not stated
Polyadenylated mRNA formation (Atryzek & Fausto, 1979)[32]	High level	not stated
Mitochondrial oxidative phosphorylation and mitochondrial glutathione concentration (Vendemiale et al., 1995)[33]	Low level in prereplective phase, but recovered in second phase	not stated
STAT3 phosphorylation (Shu et al., 2009)[8]	Fat excess in hepatocyte harm regeneration process	Steatosis correlates with a defective regeneration. Same observation in adiponectin-deficiency
Tyrosine phosphorylation (Pagano et al., 2012)[23]	Signaling initiation	not stated

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Table 2
Hormonal Effect on Liver Regeneration and Organogenesis

Hormone	Effect on proliferation	Effect on organogenesis
Parathormone (Rixon et al., 1971)[34]	It is important to maintain parenchymal cells competency to proliferate	not stated
Testosterone (Vic et al., 1982)[35]	Enhancer	not stated
Estrogen (Biondo-Simões Mde et al., 2009)[36]	Enhancer	not stated
Growth hormone (Asakawa et al., 1989[37]; Pennisi et al., 2004[38])	Enhancer	not stated
Steroid (Glanemann et al., 2004)[39]	no effect	not stated
Prolactin (Moreno-Carranza et al., 2011)[40]	Enhancer	not stated
Thyroid hormone (Alisi et al., 2004)[41]	Enhancer	not stated

Table 3

Factors Associated with Liver Regeneration and Organogenesis

Factors	Observation during proliferation	Observation during organogenesis
DNA polym+A4:D13 (Fry et al., 1985)[42]	There was an increase of 13 folds of DNA polymerase alpha stimulating protein during liver regeneration	not stated
Albumin and alpha feto-protein (Panduro et al., 1986)[43]	A reduction in Albumin level and increase level of Fetoprotein was observed during liver regeneration	not stated
Acidic nonhiston proteins phosphorylation of nuclear matrix (Allen et al., 1977)[30]	High rate	not stated
Hepatocytes' number and size, surface and volume fractions, their nuclei, cytoplasm, and their relation to the sinusoidal bed (Vizzotto et al., 1989)[19]	An increase in the number of nuclei and the nucleus/cytoplasm ratio associated with regenerating liver	not stated
Transcription factors (c-jun, jun B, c-fos fos B, fra 1, zifl268, nurl77) (Dubois, 1990)[44]	High level of transcription factors is associated with liver regeneration	not stated
Hepatocyte Growth Factor (HGF) mRNA and DNA synthesis (Zarnegart et al., 1991)[45]	High level of Hepatocyte Growth Factor (HGF) and an increase of DNA synthesis associated with liver regeneration process, researchers direct toward that HGF is a signaling factor	not stated
DNA Polymerase delta, alpha and proliferating cell nuclear antigen activities (Yang et al., 1991)[46]	High levels of DNA Polymerase delta, alpha and proliferating cell nuclear antigen activities observed in association with regenerating rat liver	not stated
LDH, uric acid, albumin, DNA, laminin and collagen IV (Parsons-Wingerter &Saltzman, 1993)[21]	High levels of uric acids and albumin associated with proliferation compared to low level in hepatocytes monolayer, also high level of laminin and collagen IV associated with proliferation compared to low level with mature cells	not stated
STAT3 (signal transducer and activator of transcription 3), NF-kB (nuclear factor kappa B), OVA1, OVA2 and OVA3 (Sanchez et al., 2004)[47]	OV1 used as a marker for identification and sorting of oval cells, researchers established that both NF-kB and STAT3 were highly activated in the OV1+ cell population.	not stated
Tissue inhibitors of metalloproteinases (TIMP-1) (Mohammed et al., 2005)[48]	TIMP-1 inhibits proliferation process.	not stated
Myeloid differentiation factor (MyD) 88 (Seki et al., 2005)[49]	It is important in signaling pathway for proliferation	not stated
Hepatocyte nuclear factor (vHnf1) (Lokmane et al., 2008)[1]	vHnf1 deficiency associated with abnormal growth	Absence of vHNF1 forms defective hepatic bud and leads abnormal gut regionalization
Fibroblast Growth Factor (FGF) (Wang et al., 2015)[2]	Absence of FGF signaling associated with halting of anterior murine liver bud	FGF signaling is required for anterior but not posterior specification of the murine liver bud
Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) and prospero homeobox protein 1 (PROX1) (Meng, 2017)[10]	PROX1 and LYVE1 correlate with regeneration process and can be used as biomarkers to identify formation of new liver sinusoidal endothelial cells.	not stated
Tumor Necrosis Factor-alpha (TNF-α) and Inteleukin-6 (IL-6), Transforming Growth Factor Beta (TGF-β) and related TGF-β family members (Tao et al., 2017)[50]	TNF-α and IL-6 act as complete mitogen, (TGF-β) and related TGF-β family members associated with termination of liver regeneration	not stated
Hepatocyte nuclear factors (HNF4A) (Taniguchi et al., 2018)[9]	A mutation that reduced HNF4A transcription led to tumor growth	Major regulator of organogenesis

 doi:10.20944/preprints201809.0351.v1

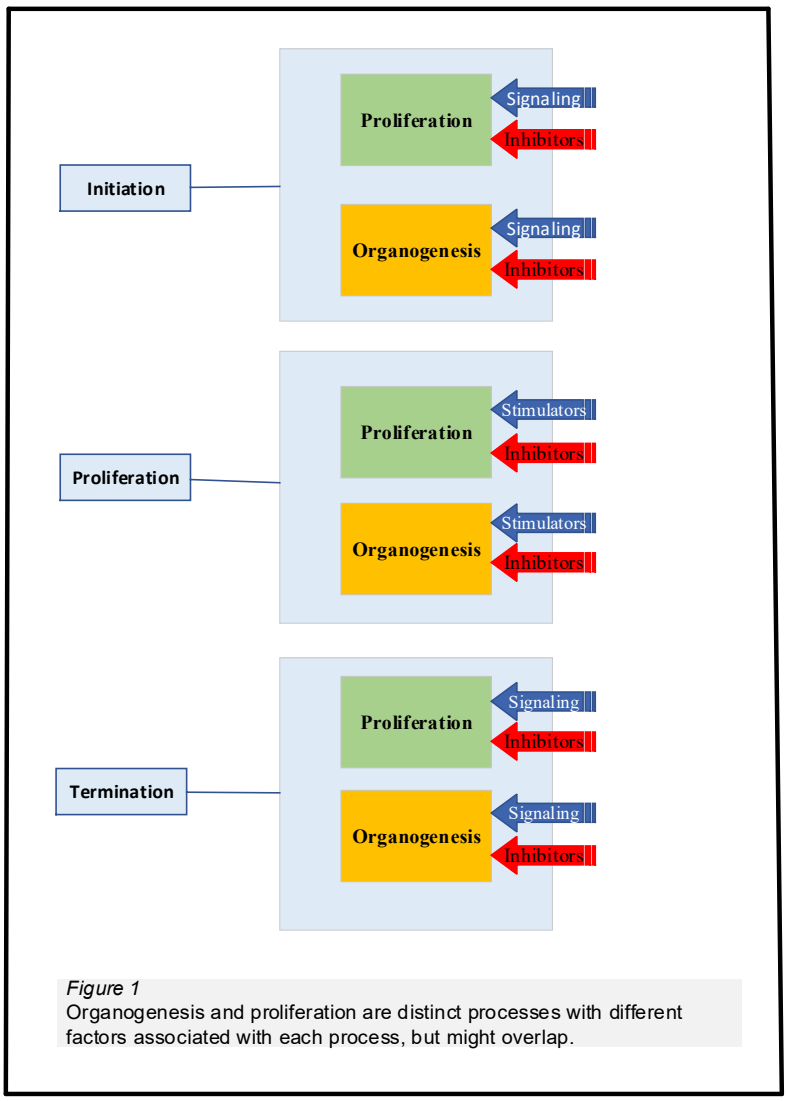

Figure 1
Organogenesis and proliferation are distinct processes with different factors associated with each process, but might overlap.

Acknowledgement

I'd like to thank Dr. Veronica Ardi for her help and national university library team.

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