Hepatology Snapshot

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Liver regeneration

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In this '*Snapshot*' article, we take a fresh look at the fundamentals of liver regeneration in light of recent technical innovations. While the mammalian response to partial hepatectomy (PH) has been studied for decades [1,2], in recent years the use of new experimental techniques has not only lead to a re-affirmation of basic principles of regeneration initially described by Nancy Bucher and others [3]; it also highlights remaining questions. PH leads to tightly synchronous rounds of replication, and given that the remaining liver is uninjured, it offers a physiological way to study both proliferative pathways in normal hepatocytes and abnormal regeneration in genetically modified mice. Liver regeneration after PH has provided a deeper understanding of mammalian cell proliferation *in vivo*, and may provide molecular insights into the interminable self-renewal of mature cells, a property often ascribed exclusively to stem cells. Hepatocytes appear to be cell autonomous in deciding their replication fate. This concept is based on an amalgam of work from many laboratories, and has been well illustrated by Weglarz and Sandgren [4]. These investigators transplanted mouse hepatocytes into a rat liver and then performed 2/3 PH. The mouse hepatocytes divided at 40 h, in contrast to the surrounding rat hepatocytes, which divided at 24 h. Each hepatocyte maintained its own proliferative cadence despite similar endocrine and paracrine influences. These findings are complemented by a recent study by Wu *et al.*, in which sequential injections of differently labeled nucleotide analogs were used to determine the time course of hepatocytes' proliferation in different zones of the liver lobule [5]. They demonstrated that there are three peaks of DNA synthesis after PH, initially in zone 1, then in the mid-lobule (Fig. 1A). Interestingly, 15% of pre-existing hepatocytes never divide after PH, while 11% divide at least three times. Whether this phenomenon is due to proximity to portal nutrients, contact with specific non-parenchymal cells (NPCs), altered hemodynamics, or purely intracellular events is yet unknown. How does a hepatocyte that just has divided choose to exit the cell cycle, while its sister proceeds through another round of proliferation?

Whether new hepatocytes in the regenerating liver are derived from adult hepatocytes, intrahepatic stem cells, or circulating stem cells is an ongoing controversy. Early experiments suggested the former case, but in the current era of stem cell biology, many authors have favored the hypothesis of an expansion of a progenitor cell population during regeneration and normal liver homeostasis, the so-called 'streaming liver hypothesis' [6]. Willenbring and colleagues have recently addressed this question by developing a means of stably expressing enhanced yellow fluorescent protein (EYFP) in adult hepatocytes [7]. This system allowed them to 'fate map' these cells over time, and to confirm that adult hepatocytes are the source of new cells during normal liver homeostasis. Further, they found that after PH, the vast majority (~99%) of new hepatocytes came from pre-existing adult hepatocytes, confirming that progenitor cells do not play an important role in normal liver homeostasis and regeneration. The use of these innovative techniques, in conjunction with experimental models of inflamed, fibrotic, or fatty livers undergoing resection, will further increase our understanding of compromised regeneration, as progenitor cells may play a more prominent role in restitution of hepatocyte mass during liver injury [8].

In addition to hepatocyte-autonomous signals (Fig. 1B), endocrine and paracrine factors are critical to normal regeneration, and extensive work has focused on the role of the liver microenvironment, i.e. NPCs and the extra-cellular matrix (ECM), in liver homeostasis and regeneration [1,2,9] and (Fig. 1C). Recent studies from Shahin Rafii's laboratory have focused on the role of endothelial cells in supporting normal hepatocyte proliferation and, more predictably, in restoring functional vasculature to the regenerating liver [10]. Other NPCs, such as Kupffer cells, stellate cells, and intrahepatic lymphocytes also provide critical signals to hepatocytes during regeneration [8], (Fig. 1B); we predict that these intercellular interactions would be even more crucial during regeneration in livers with an altered microenvironment [9,11]. For example, do steatosis, inflammation and fibrosis shorten the self-renewal capacity of hepatocytes? Would paracrine signaling pathways override the hepatocyte cell kinetics or the indefatigable capacity to replicate? In elegant experiments aimed at addressing the role of the abnormal microenvironment in affecting hepatocyte function and proliferation, Liu *et al.* isolated primary hepatocytes from normal rats and from rats with compensated or decompensated cirrhosis [12]. They then performed repopulation experiments with those cells, and found that hepatocytes from normal rats or compensated cirrhotics were immediately able to engraft and proliferate in the normal microenvironment of the recipient liver. Interestingly, though the hepatocytes from rats with decompensated cirrhosis initially did not expand (or produce albumin), after two months in the recipient, their function was re-established. It would be fascinating to know how these cells would respond to a proliferative

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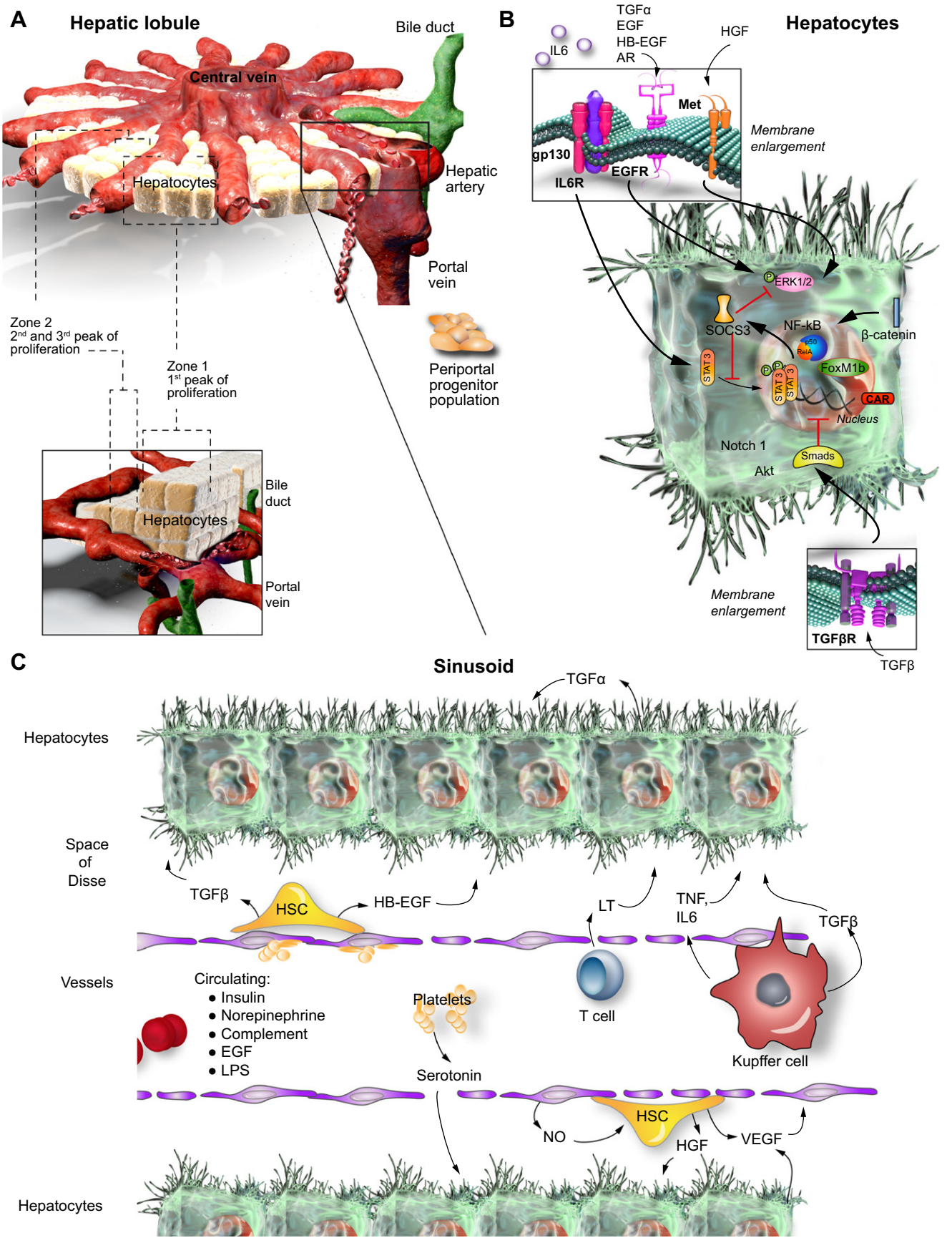
A
Hepatic lobule
Bile duct
Central vein
Hepatocytes
Hepatic artery
Portal vein
Periportal progenitor population
Zone 2
2nd and 3rd peak of proliferation
Zone 1
1st peak of proliferation
Hepatocytes
Bile duct
Portal vein
B
Hepatocytes
TGFα
EGF
HB-EGF
AR
HGF
IL6
Met
gp130
EGFR
IL6R
Membrane enlargement
ERK1/2
SOCS3
NF-kB
β-catenin
STAT 3
FoxM1b
CAR
Nucleus
Notch 1
Smads
Akt
Membrane enlargement
TGFβR
TGFβ
C
Sinusoid
TGFα
Hepatocytes
Space of Disse
TGFβ
HSC
HB-EGF
LT
TNF, IL6
TGFβ
Vessels
Circulating:
• Insulin
• Norepinephrine
• Complement
• EGF
• LPS
Platelets
T cell
Kupffer cell
Serotonin
NO
HSC
HGF
VEGF
Hepatocytes

stimulus at this later time point. Our hope and anticipation is that the use of innovative approaches, such as those outlined above, will lead to a deeper understanding of liver regeneration, so that therapies will soon be available for patients with acute and chronic liver failure, and will facilitate liver resection in patients who would not otherwise have been considered as surgical candidates [11].

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Fig. 1. Liver regeneration from three different perspectives. (A) Hepatocyte proliferation after partial hepatectomy (PH). Depicted is the structure of the liver lobule, showing that zone 1 (periportal) hepatocytes divide during the first peak of DNA synthesis, whereas zone 2 hepatocytes comprise the second and third peaks of hepatocyte proliferation. (B) Key intracellular signaling pathways in hepatocytes after PH. (C) Hematogenous factors and intercellular interactions during liver regeneration, depicting signals between hepatocytes, Kupffer cells, T cells, hepatic stellate cells (HSC), liver sinusoidal endothelial cells (LSECs), and platelets. TGFα, transforming growth factor alpha; EGF, epidermal growth factor; HB-EGF, heparin binding EGF-like growth factor; AR, amphiregulin; EGFR, epidermal growth factor receptor; gp130, glycoprotein 130; HGF, hepatocyte growth factor; Met, receptor for hepatocyte growth factor; IL6, interleukin 6; IL6R, interleukin 6 receptor; ERK, extracellular related kinase; CAR, constitutive androgen receptor; NF-κB, nuclear factor kappa B; STAT3, signal transducer and activator of transcription 3; SOCS3, suppressor of cytokine signaling 3; TGFβ, transforming growth factor beta; TGFβR, transforming growth factor beta receptor; TGFα, transforming growth factor alpha; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; NO, nitric oxide; LT, lymphotoxin; LPS, lipopolysaccaride.