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Hepatic zonation now on hormones!

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The liver plays important roles in maintaining systemic metabolic homeostasis, but our understanding of how the liver coordinates such a wide range of functions is still evolving. A recent study led by Dr. Gromada advanced the knowledge of the regulators for liver zonation with the discovery of a novel role for glucagon as a liver metabolic zonation regulator utilizing *Glucagon (gcg)* KO mice combined with RNA sequencing and in situ hybridization (ISH) (1). The established mechanism of metabolic liver zonation is determined by the architecture of the liver, which is composed of millions of hepatic lobules the basic functional units within each of the lobes of the liver. Within each lobule, blood rich in nutrients and hormones enters at the portal triad through the portal vein (PV), mixes with oxygen rich blood from the hepatic artery (HA), courses through the sinusoids and eventually exits the lobule through the central vein (CV) (2). As the blood flows through the sinusoids which are lined by hepatocytes separated from the blood by sinusoidal endothelial cells that are highly fenestrated without any basement membrane, there is a free exchange of substrates, nutrients and metabolites between blood and hepatocytes. Such exchange is key to maintaining systemic homeostasis. To perform more efficient metabolic function, functional variance is observed in hepatocytes based on their location along the portal-central axis by virtue of distinct gene expression based on their location within the lobule. Based on this organization and heterogeneity of hepatocytes, each hexagonal liver lobule has three geographical areas or zones: periportal or zone 1 (adjacent to portal triad), perivenous or zone 3 (surrounding the CV) and midzonal or zone 2 (between periportal and perivenous). Such compartmentalization not only allows for functional division of labor but also limits many toxicant based injuries to specific zones, thereby allowing hepatocytes from other zones to replace the dead cells thus restoring structure and function.

A little more than a decade ago, Wnt/ β -catenin signaling pathway was shown to govern metabolic liver zonation (3). It was shown that Wnt/ β -catenin signaling is most active in zone 3 hepatocytes as demonstrated by restricted expression of Wnt-dependent metabolic

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genes including (glutamine synthetase) GS and Cytochrome P450 Family 2 Subfamily E Member 1 (Cyp2e1), which was verified in conditional β -catenin knockout (KO) mice. At the same time, high expression of Adenomatous Polyposis Coli (APC) was shown in normally zone 1 and 2, which allowed for repression of β -catenin in these areas (3). Optimal β -catenin activation in zone 3 was further shown to depend on Wnt signaling as well as RSPO-LGR4/5-ZNRF3/RNF43 axis (4, 5). In addition to high expression of APC, several signaling pathways involved in metabolic liver zonation have been postulated to counteract the β -catenin activation in zone 1, such as the Ras/Erk, (yes-associated protein 1) Yap, the hepatocyte nuclear factor 4- α (Hnf4 α), and the hedgehog pathways (Figure 1). Technical advances have now allowed our understanding of the basis of around 50% of zoned gene expression in the liver lobules (6). Additionally, metabolic liver zonation can be dynamically regulated by various pathophysiologic factors like nutrient/hormonal conditions, hepatitis C virus infection, circadian rhythm and systemic metabolic disturbances including advanced non-alcoholic steatohepatitis (NASH) (2). Indeed, liver is subjected to various systemic hormones in the blood stream, which can directly regulate metabolic functions of the liver. Given the explosive increase in the prevalence of metabolic diseases that affect liver functions and are accompanied by perturbations in systemic hormonal homeostasis, fundamental questions addressing the systemic metabolic status and effect on metabolic liver zonation and related signaling pathways are highly relevant.

As one of the essential metabolic hormones, glucagon is secreted by the pancreatic α cells and antagonizes the actions of insulin by enhancing gluconeogenesis and hepatic glucose secretion (1, 2). The study led by Dr. Gromada found that lack of glucagon in plasma, induced by the genetic deletion of *gcg* exon-3, perturbed metabolic zonation in the liver. By RNA-seq, ISH and immunohistochemistry, the authors discovered that glucagon deficiency enhanced the expression of Wnt/ β -catenin-dependent pericentral expression programs at the expense of periportal gene expression evidenced by an expansion of the GS+ hepatocyte layers and diminution of the glutaminase (Gls)+ region, respectively. Similar results were also observed during the hyper-activation of β -catenin pathways in the Yap KO, APC KO or Zinc and Ring Finger 3 (ZNRF3)/Ring Finger Protein 43 (RNF43) deletion in mice. Despite the defective expression of ammonia/urea detoxifying genes in glucagon-deficient animals, there was no defect in serum ammonia and urea levels, similar to the APC KO mice, suggesting low abundance genes can sufficiently maintain ammonia homeostasis as in the *gcg* KO mice. Intriguingly, glucagon infusion, in a dose-dependent manner, restored defective liver zonation following *gcg* ablation and simultaneously decreased β -catenin-dependent GS expression in WT mice although it was unable to deplete GS in the most pericentral hepatocytes. Eventually, the authors supported their claims that glucagon regulated zonation by counteracting Wnt/ β -catenin signaling through a rescue study using hydrodynamic delivery of a dominant-negative form of Lgr5, which is known to accentuate Wnt/ β catenin signaling (4) and demonstrating normalized GS expression in *gcg* KO mice.

While the study has exciting implications, certain observations need further validation. The authors showed an even distribution of glucagon and insulin receptors across the liver lobule, suggesting that the effect of glucagon on liver zonation is likely dependent on its concentration. However, previous studies in rats reported glucagon receptors to be predominantly in the periportal region and insulin receptors to be abundantly expressed in

the pericentral region (7). Thus, it would be important to examine the distribution of glucagon and insulin receptors in healthy and diseased human livers. The finding that these receptors and pathway components such as Prkaca (PKA), Gnas and Map2k2 were evenly expressed across the different metabolic zonation suggests that detailed analysis of glucagon and insulin level need to be further investigated in respective zones to strengthen the importance of glucagon concentration for shifting hepatic zonation. Future studies will need to address the molecular mechanisms of how glucagon represses Wnt/ β -catenin signaling. Specifically, relevant may be the effects on glycogen synthase kinase-3 β (GSK3 β) which regulates glycogen synthesis but also regulates β -catenin degradation. Furthermore, the effect of fasting and feeding and of plasma ratios of glucagon to insulin, and plasma glucose levels on dynamic liver zonation remains to be further investigated. Indeed, fasting serum levels of insulin and glucagon are increased in patients with various metabolic disorders including type 2 diabetes and NASH (8). Their hepatic concentrations are much higher than in the periphery since pancreas directly releases insulin and glucagon into portal vein to the liver. While insulin and glucagon production, secretion and response to the dietary glucose and lipids are perturbed in various metabolic diseases (8). Given the similarity between the mouse and human liver zonation, comprehensive investigation of gastrointestinal hormone levels and their effect on metabolic liver zonation, using various mouse metabolic disease models, would be pertinent studies in this field.

In conclusion, Cheng et al found that gain or loss of systemically circulating glucagon dynamically regulates metabolic zonation within the hepatic lobule through interaction with the Wnt/ β -catenin pathway and likely other signaling pathways (1). Fundamentally, this discovery extends the basis of dynamic liver zonation to systemic hormones. Undoubtedly such research will lead to discovery of novel regulators of metabolism and hepatic zonation and may not only explain the zonal bias evident in certain hepatic pathologies but may also help identify new treatments for various metabolic diseases.

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Figure 1.

Regulation of metabolic liver zonation orchestrated by multiple signaling pathways including glucagon and Wnt/β-catenin pathway. PV: portal vein, HA: hepatic artery, CV: central vein, BD: bile duct.