Upstream regulators of hepatic Wnt/β-catenin activity control liver metabolic zonation, development and regeneration

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In a brilliant paper, Planas-Paz and colleagues elaborated a complex study to unravel the implication of each component of the RSPO-LGR4/5-ZNRF3/RNF43 module as upstream spatiotemporal regulators of the hepatic Wnt/β-catenin activity mediating liver metabolic zonation, development and regeneration (Planas-Paz *et al.* The RSPO-LGR4/5-ZNRF3/RNF43 module controls liver zonation and size. **Nature Cell Biology** 2016; 18 (5) 467-81)**.**

Wnt/β-catenin pathway has been shown to be a major regulator of hepatic zonation, development and regeneration. β-catenin deletion impairs metabolic liver zonation and hepatic proliferation, whereas β-catenin overexpression leads to ungoverned expression of metabolic zonated proteins and caused uncontrolled liver growth in mice (1, 2). However, the upstream effectors dictating the time and zone-specific regulation of Wnt/β-catenin activity are still unidentified. It has been shown in gut, kidney and skin that the module consisting of the R-spondin (RSPO) ligands, its LGR4/5 membrane receptors and the ZNRF3 and RNF43 E3 ubiquitin ligases plays an essential role in regulating stem cell compartments via the Wnt/β-catenin signaling (3, 4, 5). Briefly, ZNRF3 and RNF43 ubiquitin ligases target Wnt receptor for degradation, impairing the activation of the Wnt/β-catenin pathway. RSPO binding to its LGR4/5 membrane receptors clears ZNRF3 and RNF43 from the membrane preventing Wnt receptor degradation and thereby leading to increased Wnt signaling inducing β-catenin target gene expression.

To assess the contribution of each component of the RSPO-LGR4/5-ZNRF3/RNF43 module to the Wnt/β-catenin signaling gradient and liver metabolic zonation, Planas-Paz and colleagues analyzed the expression of Wnt target genes, such as Axin2, Glutamine synthetase (GS) and Cyp2e1 after modulation of each component. In adult wild-type liver, Wnt target genes were expressed in hepatocyte layers close to central veins, and cells in these proximal layers co-expressed LGR5 and RNF43 while LGR4 and ZNRF3 were detected in all lobular hepatocytes (Figure 1A). The authors showed that when LGR4 receptor alone or both LGR4/5 receptors were knocked out (KO) in mouse livers (but not LGR5 alone) or when inducible ubiquitous extracellular domain of the ZNRF3 ubiquitin ligase (ZNRF3-ECD) was overexpressed, Wnt/β-catenin pathway activation was impaired as assessed by a severe reduction of the pericentrally expressed metabolic genes (Figure 1B). Conversely, when both ZNRF3/RNF43 ubiquitin ligases were deleted or when recombinant RSPO1 ligand was administrated in mice with functional hepatic LGR4/5 receptors, Wnt signaling was increased in the entire liver lobule as Wnt genes expression was upregulated and gradually expanded from pericentral to periportal hepatocytes (Figure 1C). With these data, the authors

demonstrate that the Wnt machinery is present throughout the liver lobule and that all hepatocytes are competent to respond to Wnt pathway activation. The authors also show that the RSPO-LGR4/5-ZNFR3/RNF43 module does not only potentiate but is also essential for the spatial regulation of the Wnt/β-catenin signaling gradient and metabolic zonation in the adult liver. An essential question however remains: what are the signals restricting Wnt pathway activation to pericentral hepatocytes? A centro-portal gradient in RSPO ligands may tune the response to Wnt ligands and direct spatial organization. In line with this idea, a recent paper showed that RSPO3 is specifically expressed in central vein endothelial cells and is required to maintain hepatic metabolic zonation (6). Moreover, co-expression of both LGR4 and LGR5 and/or both ZNRF3 and RNF43 may be needed to increase the responsiveness of hepatocytes to the RSPO-LGR4/5-ZNRF3/RNF43 module leading to stronger Wnt activation in the pericentral zone where both receptors and both ubiquitin ligases are expressed. This moves the question to the signals restricting expression of LGR5 to central hepatocytes. LGR4 could act as an upstream regulator of LGR5 in the liver (as LGR5 mRNA was not detected anymore in LGR4 KO liver), but why and how would LGR4 regulate LGR5 expression only in pericentral hepatocytes? Other, yet unknown, mediators may be at play. This is meat on the bone for further studies.

The liver of LGR4/5 KO mice was found hypoplastic due to lower hepatocyte proliferation, while hepatocyte and biliary cell differentiation normally occurred during liver development. The authors therefore explored the roles of LGR4 and LGR5 receptors in liver mass maintenance by lineage tracing experiments. In undamaged liver, they showed that LGR4+ hepatocytes present in the entire liver lobule are quantitatively the main contributors to liver mass homeostasis. The proliferation rates of hepatocytes were similar whether being LGR4/5+ and Wnt activated in the pericentral zone, belonging to the largest LGR4+ mid-zonal area or located in the periportal zone suspected to contain a progenitor compartment. These data contrast with the actively cycling LGR5+ cells found in stem cells compartments in gut or skin (5) and contradict the findings of a recent study showing that Wnt activated and Axin2+ pericentral hepatocytes fuel the liver with new hepatocytes for maintenance of the liver mass (7). However, in the light of many other reports showing no zonal domination during liver homeostasis (8, 9), the data presented here reinforce the message that any hepatocyte, irrespectively of its location in the lobule, possesses the same proliferative potential and that a preferential zonal proliferative response, when observed, could rather depend on the nature or site of injury. Finally, Huch *et al.* identified small LGR5+ cells near bile ducts only upon liver injury (10). In their model however, Planas-Paz *et al.* did not detect LGR5+ cells in the periportal area whether during liver homeostasis or during physiological regeneration. It would be interesting to analyze liver regeneration in LGR5 KO mice following hepatic damage inducing proliferation of the liver progenitor cells compartment.

The authors further investigated the RSPO-LGR4/5 signaling in liver regeneration after partial hepatectomy. Noteworthy, the activation of the Wnt pathway extends to periportal hepatocytes during regeneration. Liver-specific deletion of LGR4 decreased the proliferation

of hepatocytes post partial hepatectomy in the entire lobule. On the contrary, deletion of LGR5 receptor only impeded proliferation of pericentral hepatocytes. Delayed liver mass regeneration is therefore observed when most of the hepatocytes are prevented from proliferation (in case of LGR4 deletion) while liver mass recovery is normal when only a small number of LGR5+ pericentral hepatocytes are subtracted from the process. Activation of the Wnt pathway amplified by LGR4 receptor signaling thus appears necessary for optimal regeneration. However, the nature of the stimuli initiating or commanding liver regeneration remains an enigma. Planas-Paz and colleagues propose here that signals modulating the Wnt pathway are at play and if so, stimulation of the pathway should enhance regeneration. Indeed, they showed that administration of the LGR4/5 ligand RSPO1 amplified Wnt signal in the entire liver and accelerated physiological regeneration, a finding of valuable interest for regenerative therapies.

LEGEND to the figure:

Figure 1. Implication of the RSPO-LGR4/5-ZNRF3/RNF43 module as upstream spatial regulator of the hepatic Wnt/β-catenin gradient mediating liver metabolic zonation. **(A)** In adult wild-type mouse liver, Wnt target gene expression is restricted to hepatocytes around the central veins where Wnt signaling is high. **(B)** When LGR4/5 receptors are deleted in the liver or upon overexpression of the extracellular domain of the ZNRF3 ubiquitin ligase (ZNRF3-ECD), hepatic Wnt/β-catenin activity was impaired leading to a severe reduction of Wnt target gene expression. **(C)** Conversely, upon ZNRF3/RNF43 deletions or after injection of recombinant RSPO1 ligand, Wnt/β-catenin activity was increased leading to target gene expression progressively expanded from pericentral to periportal hepatocytes. CV=central vein, PT=portal tract.

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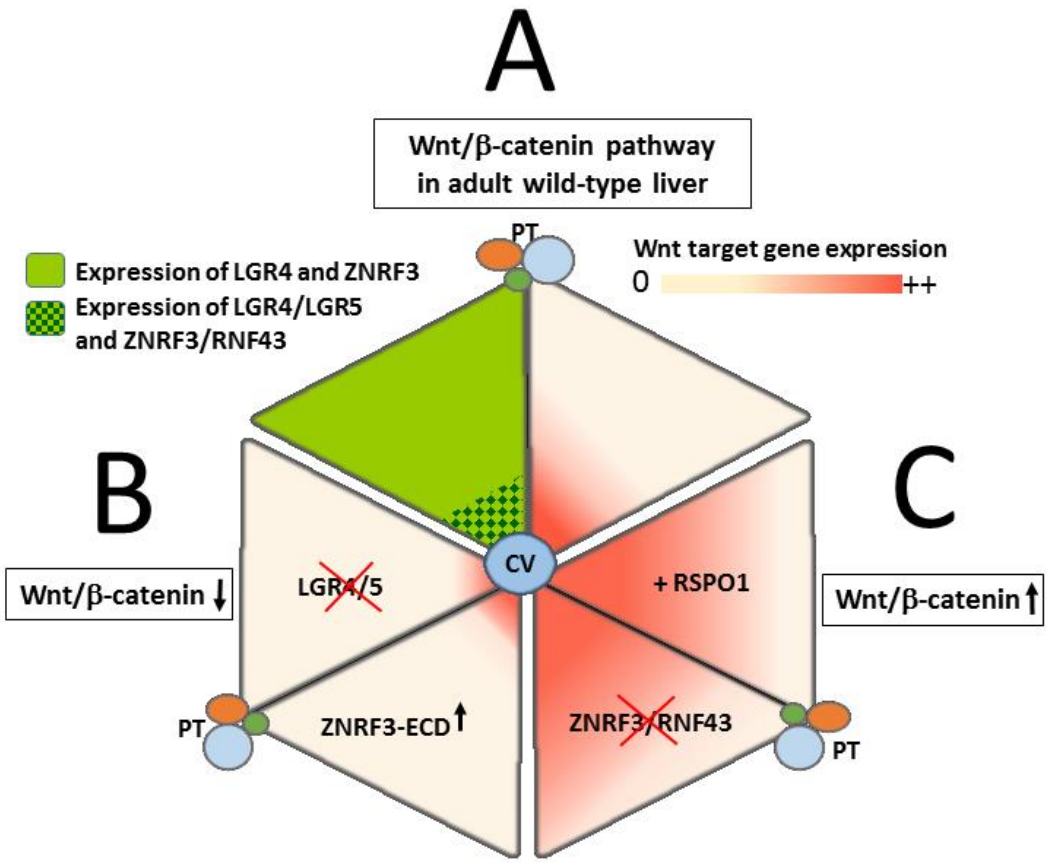
Figure 1



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