Feeding the Hedgehog: a new meaning for JNK signalling in liver regeneration

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Keywords: Hedgehog, JNK, IHH, ALPPS, liver regeneration

Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding conflicts of interest respect to this manuscript.

Acknowledgments

The authors acknowledge the research funding from Brunel Research Initiative & Enterprise Fund, Brunel University of London, Kay Kendall Leukemia Fund (KKL443) (C.B.), 250 Great Minds Fellowship, University of Leeds (S.P.), AMMF Cholangiocarcinoma Charity (S.P.) and Bloodwise (17014) (S.P. and C.B.). Figures were created modifying illustrations provided by Servier (http://smart.servier.com) under Creative Commons Attribution 3.0 Unported License.

Authors' contributions

S.P. and C.B. both contributed equally to the data review and interpretation for this editorial, the manuscript writing and editing.

An unmatched characteristic of the liver is the ability to rebuild its lost mass either after toxic tissue damage or liver resection.[1] The latter is a well-established surgical technique, known as partial hepatectomy in which up to 70% of the entire liver can be removed.[1,2] Partial hepatectomy is used clinically as a treatment strategy for primary and selected metastatic liver cancers, and experimentally as a stimulus to study liver regeneration.[3] The main drawback of partial hepatectomy, however, is that not all patients with liver disease are candidates for liver resection. Indeed, in many clinical scenarios liver regeneration is not sufficient to circumvent loss of a large volume of hepatic tissue, with detrimental effects on the survival of the recovering patient.[3,4] It is now appreciated that liver regeneration involves compensatory growth followed by proliferation of all adult cells that compose the liver including hepatocytes, sinusoidal endothelial cells, biliary epithelial cells, Kupffer cells and hepatic stellate cells (HSCs).[1,2] Although the mechanisms behind the failure of liver regeneration are not entirely clear, research in the field has aided the development of a new surgical procedure to promote an accelerated liver regeneration,[5,6] opening up the benefits of liver resection to the wider populations of patients undergoing extensive liver resection. Indeed, a two-stage hepatectomy – also known as associating liver partition and portal vein ligation (PVL) in staged hepatectomy (ALPPS) – is routinely adopted in the clinic to accelerate liver regeneration.[5,6] While the ALPPS-mediated hepatic regeneration process is histologically well described,[5,6] the signalling pathways that orchestrate the acceleration of compensatory liver growth have been only partially characterized. Recent studies have suggested that the Hedgehog pathway is a crucial mediator of accelerated liver regeneration triggered by ALPPS.[7] Hedgehog is a secreted protein that regulates cell fate of different cell targets in a concentration- and duration-dependent manner.[8] Mammals synthesise three different Hedgehog ligands: Sonic (SHH), Indian (IHH) and Desert (DHH) hedgehog. While SHH and IHH proteins are ubiquitously expressed, DHH expression is restricted to brain and testis.[8] Growing evidence indicates that Hedgehog pathway is a critical regulator of adult liver repair and regeneration.[7,8] Animal models of liver regeneration present transient activation of Hedgehog pathway. Moreover, administration of recombinant IHH accelerates liver regeneration and improves outcome. Notably, both patients and animal rodents subjected to ALPPS also show an increase in the circulating levels of IHH that associates with accelerated compensatory liver growth.[7,8] The fundamental question is: what does support elevated and, more important, sustained levels of Hedgehog ligand during liver regeneration?

In this issue of Journal of Hepatology, Langiewicz and colleagues [9] presented a very intuitive study addressing the complexity in the molecular mechanism of Hedgehog signal production in regenerating livers followed by ALPPS. By performing differentiated gene

expression analyses in ALPPS mouse livers the authors demonstrated that ALPPS livers had elevated mRNA expression of *Mapk8* (JNK1) compared to appropriate surgery controls.[9] Importantly, similar to Hedgehog protein, both JNK1 (not-phosphorylated) and phosphorylated-JNK (active-JNK) levels were also highly detected in hepatic lysates post-ALPPS.[9] These similarities led the authors to investigate a putative link between JNK and Hedgehog pathways. The c-Jun N-terminal kinase (JNK), an evolutionarily conserved mitogen-activated protein kinase (MAPK), is one of the crucial signalling pathways that regulate cell proliferation, survival and cell death.[10-12] Notably, phospho-active JNK co-localised with IHH in activated hepatic stellate cells, specialised cells that exist in the space between parenchymal cells and sinusoidal endothelial cells of the hepatic lobule (**Figure 1**). This co-localisation was also associated with IHH downstream effector GLI1 and its proliferative target cyclin D1, adding support to a putative link between cell proliferation and JNK-IHH axis (**Figure 1**).[9]

To establish a direct link between JNK, IHH and accelerated hepatic regeneration following ALPPS, Langiewicz *et al.* pre-treated mice with SP600125 JNK inhibitor[13] and analysed liver regeneration following ALPPS hepatic resection. Compared to JNK inhibited livers, control mouse livers regenerated more rapidly following ALPPS, suggesting that JNK is crucial for ALPPS-mediated acceleration of liver regeneration. Remarkably, SP600125-trated mice showed diminished levels of GLI1, cyclin D1 and other tissue proliferative markers.[9] Altogether these data represent the first step linking JNK activation to Hedgehog signalling in regenerating livers (**Figure 1**), although genetic validation to strength this link in regenerating livers would be necessary. As the authors discussed, the use of pharmacological inhibition of JNK is a limitation to the study. Above all, SP600125 can bind to a broad range of protein kinases (including other MAPKs such as p38-MAPK and ERK-MAPK) and inhibit some of them with similar or greater potency than JNK.[14,15] In addition, SP600125 does not discriminate between JNK1 and JNK2, the two ubiquitously expressed JNK proteins coded by two distinct JNK genes: *Mapk8* (*Jnk1*) and *Mapk9* (*Jnk2*).[13] Indeed, recent studies have demonstrated that the JNK proteins differ in function especially in liver regeneration. Mice deficient in *Jnk1* display impaired liver regeneration following two-thirds partial hepatectomy.[10,16] Less clear is, however, the role of JNK2 in liver regeneration. While one study demonstrated that the loss of JNK2 accelerated liver regeneration,[17] another more recent study reported no role for JNK2 in liver regeneration.[18] Moreover, mice deficient in growth arrest and DNA-damage-inducible gene 45β (GADD45β) have reduced hepatocyte proliferation and increased apoptosis associated with sustained activation of JNK following two-thirds partial hepatectomy.[19] Importantly, disruption of *Jnk2* increased liver regeneration in Gadd45β-/- mice, indicating that the magnitude and duration of JNK activation is relevant

in regenerating livers.[19] Therefore, the use of genetically engineered JNK1 (and/or JNK2) mice, along with *in-vivo* analyses of SP600125 treated livers, would have given a direct evidence of the involvement of JNK signalling in the acceleration of hepatic regeneration following ALPSS hepatic resection.

What's intriguing is that restoring IHH signal reinstated the regenerative response in SP600125-treated mice after ALPPS hepatic resection. With a clinically relevant approach, Langiewicz and colleagues [9] have indeed showed that administration of recombinant IHH ligand (rIHH) compensates for the inhibition of JNK in SP600125-treated, ALPPS resected mice. Importantly, rIHH administration restored the hepatic levels of GLI1 and cyclin D1 (lost after SP600125 treatment) and, consequently, livers had more Ki67-postive hepatocytes and other mitotic figures than control groups. Surprisingly, rIHH treatment not only restored liver regeneration but also increased the nuclear levels of both JNK and phospho-JNK suggesting that a positive feedback loop exists for IHH in regulating levels of JNK in ALPPS regenerating livers. Mechanistically, the authors presented a very plausible explanation in which IHH enhances JNK levels that favours sustained JNK activation needed to maintain elevated levels of IHH during liver regeneration (**Figure 1**)[9].

Although the described feed forward mechanism would need further evidence, a similar mechanism has been suggested in a model of injury-stimulated Hedgehog signalling in the regeneration of the *Drosophila* midgut.[20] In this latter study, the authors hypothesise a similar feed forward mechanism whereby midgut injury favours JNK activation which drives Hedgehog-mediated proliferation of intestinal stem cells.[20] Moreover, the ectopic supplementation of Hedgehog in the *Drosophila* midgut leads to JNK pathway activation with, yet, unclear mechanisms.[20] It will be interesting to explore the mechanisms behind the complex relationship between JNK and Hedgehog signalling pathways during tissue regeneration.

To summarise, Langiewicz and colleagues' work documents a functional link between JNK activation and Hedgehog signal pathways during liver regeneration after ALPPS and suggests that compounds that stimulate the JNK1-IHH pathway may enhance the outgrowth of liver cell populations (including progenitor cells) and trigger tissue remodelling and promote accelerated liver regeneration.

Reference:

1. Michalopoulos GK. Liver regeneration. J Cell Physiol. 2007; 213: 286-300.
2. Fausto N. Liver regeneration. J Hepatol. 2000; 32: 19-31.
3. van Mierlo KM, Schaap FG, Dejong CH, Olde Damink SW. Liver resection for cancer: New developments in prediction, prevention and management of postresectional liver failure. J Hepatol. 2016; 65: 1217-1231.
4. Troja A, Khatib-Chahidi K, El-Sourani N, Antolovic D, Raab HR. ALPPS and similar resection procedures in treating extensive hepatic metastases: our own experiences and critical discussion. Int J Surg. 2014; 12:1020-1022.
5. Vennarecci G, Laurenzi A, Levi Sandri GB, Busi Rizzi E, Cristofaro M, Montalbano M, Piselli P, Andreoli A, D'Offizi G, Ettorre GM. The ALPPS procedure for hepatocellular carcinoma. Eur J Surg Oncol. 2014; 40: 982-988.
6. Shi H, Yang G, Zheng T, Wang J, Li L, Liang Y, Xie C, Yin D, Sun B, Sun J, Wang H, Pan S, Jiang H, Lau W, Liu L. A preliminary study of ALPPS procedure in a rat model. Sci Rep. 2015; 5: 17567.
7. Langiewicz M, Schlegel A, Saponara E, Linecker M, Borger P, Graf R, Humar B, Clavien PA. Hedgehog pathway mediates early acceleration of liver regeneration induced by a novel two-staged hepatectomy in mice. J Hepatol. 2017; 66: 560-570.
8. Machado MV, Diehl AM. Hedgehog signalling in liver pathophysiology. J Hepatol. 2018; 68: 550-562.
9. Langiewicz M, Graf R, Humar B, Clavien PA. JNK1 induces hedgehog signaling from stellate cells to accelerate liver regeneration in mice. J Hepatol. 2018. pii: S0168-8278(18)32028-2.
10. Seki E, Brenner DA, Karin M. A liver full of JNK: signaling in regulation of cell function and disease pathogenesis, and clinical approaches. Gastroenterology. 2012; 143: 307-320.
11. Bubici C, Papa S. JNK signalling in cancer: in need of new, smarter therapeutic targets. Br J Pharmacol. 2014; 171: 24-37.
12. Iansante V, Choy PM, Fung SW, Liu Y, Chai JG, Dyson J, Del Rio A, D'Santos C, Williams R, Chokshi S, Anders RA, Bubici C, Papa S. PARP14 promotes the Warburg effect in hepatocellular carcinoma by inhibiting JNK1-dependent PKM2 phosphorylation and activation. Nat Commun. 2015; 6: 7882.
13. Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, Leisten JC, Motiwala A, Pierce S, Satoh Y, Bhagwat SS, Manning AM, Anderson DW. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. Proc Natl Acad Sci U S A. 2001; 98: 13681-13686.
14. Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, Klevernic I, Arthur JS, Alessi DR, Cohen P. The selectivity of protein kinase inhibitors: a further update Biochem J. 2007; 408: 297-315.
15. Zhang T, Inesta-Vaquera F, Niepel M, Zhang J, Ficarro SB, Machleidt T, Xie T, Marto JA, Kim N, Sim T, Laughlin JD, Park H, LoGrasso PV, Patricelli M, Nomanbhoy TK, Sorger PK, Alessi DR, Gray NS. Discovery of potent and selective covalent inhibitors of JNK. Chem Biol. 2012; 19: 140-154.
16. Hui L, Zatloukal K, Scheuch H, Stepniak E, Wagner EF. Proliferation of human HCC cells and chemically induced mouse liver cancers requires JNK1-dependent p21 downregulation. J Clin Invest. 2008;118: 3943-3953.

17. Sabapathy K, Wagner EF. JNK2: a negative regulator of cellular proliferation. Cell Cycle. 2004; 3: 1520-1523.

18. Das M, Garlick DS, Greiner DL, Davis RJ. The role of JNK in the development of hepatocellular carcinoma. Genes Dev. 2011; 25: 634-645.

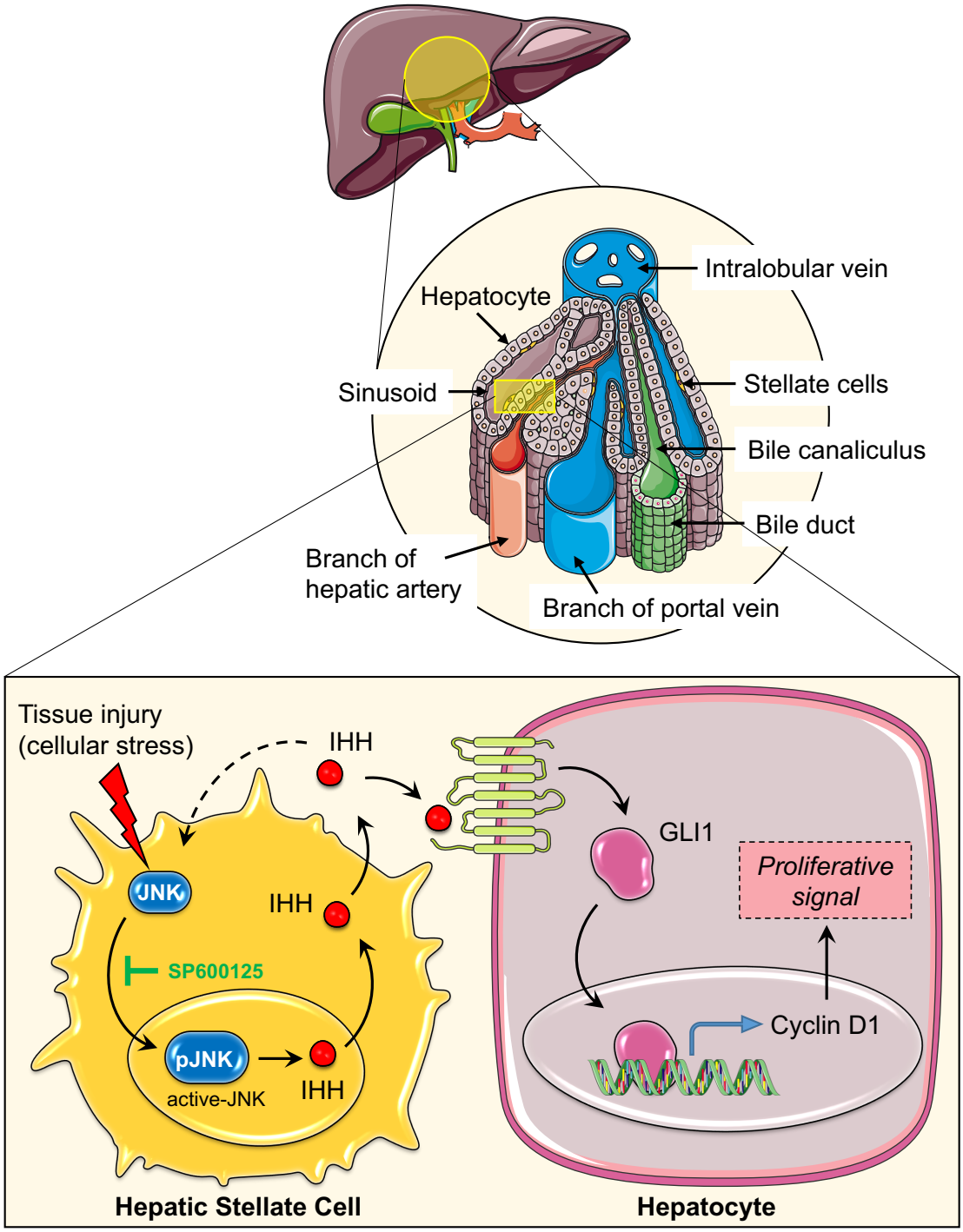
19. Papa S, Zazzeroni F, Fu YX, Bubici C, Alvarez K, Dean K, Christiansen PA, Anders RA, Franzoso G. Gadd45beta promotes hepatocyte survival during liver regeneration in mice by modulating JNK signaling. J Clin Invest. 2008; 118: 1911-23.

20. Tian A, Shi Q, Jiang A, Li S, Wang B, Jiang J. Injury-stimulated Hedgehog signaling promotes regenerative proliferation of Drosophila intestinal stem cells. J Cell Biol. 2015; 208: 807-819

Figure Legends

Figure 1. The simplified representation of the crosstalk between the Hedgehog and JNK signalling pathways. Schematic drawing of the mechanisms of accelerated liver regeneration triggered by ALPPS hepatic resection. At the microscopic level, the liver consists of functional units called lobules, which are made mostly of hepatocytes (the most common type of liver cell), sinusoidal endothelial cells, biliary epithelial cells, Kupffer cells and hepatic stellate cells. Hepatic stellate cells and Kupffer cells closely interact with hepatocytes supporting cytokines and growth factors production needed for hepatocyte growth and survival. Following an injury or resection, the liver responds to reinstate the original mass by stimulating cellular proliferation. In the model described by Langiewicz and colleagues' study [9], the authors propose a model whereby ALPPS-mediated liver regeneration is mediated by the accumulation of JNK in hepatic stellate cells. Nuclear active-JNK is then associated with an increase in of IHH ligand expression levels, which can be released by the hepatic stellate cells in the hepatic sinusoidal space. Circulating IHH ligand is intercepted by receptors exposed on the surface of hepatocytes and stimulates hepatocyte proliferation through the transcriptional activation of the IHH downstream transcription factor GLI1, which regulates the gene expression of the cell cycle regulator cyclin D1. Administration of SP600125 JNK inhibitor blocks activity of JNK with consequential reduction of IHH levels and hepatic regeneration. Surprisingly, administration of recombinant IHH ligand restores the nuclear levels of both JNK and active-JNK in SP600125-treated livers, suggesting that a positive feedback loop (dotted lines) exists for IHH in regulating levels of JNK in ALPPS regenerating livers. The mechanisms underlying the proposed model are, however, unclear.

Figure 1


Intralobular vein
Hepatocyte
Sinusoid
Stellate cells
Bile canaliculus
Bile duct
Branch of
hepatic artery
Branch of portal vein
Tissue injury
(cellular stress)
IHH
JNK
IHH
SP600125
pJNK
active-JNK
IHH
GLI1
Proliferative
signal
Cyclin D1
Hepatic Stellate Cell
Hepatocyte