



COMMENTARY

PXR and Hepatocyte Proliferation

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The liver has a unique ability to regenerate. The hepatocytes in the livers of healthy adults are usually quiescent (G_0 phase) in contrast to their cycling counterparts in the fetal and neonatal livers. However, the quiescent hepatocytes undergo a significant proliferation in regenerating livers after the loss of functional tissue resulting from the injuries caused by various physiologic, pathologic, and environmental factors. The mechanisms responsible for liver regeneration have often been experimentally investigated using the mouse model of partial hepatectomy (i.e., a surgical procedure to trigger liver regeneration by removing up to two-third of the liver mass).¹ In addition to the *in vivo* models, primary hepatocytes and hepatoma cell lines have also been used to study liver regeneration. Many signaling pathways, including those mediated by retinoic acid, are involved in regulating hepatocyte proliferation during liver regeneration. In their review, Bushue and Wan² summarized the retinoic X receptor α -dependent activation of nuclear receptors (NRs) in the induction of hepatocyte proliferation.

Liver regeneration is achieved primarily by driving quiescent hepatocytes to reenter the cell cycle.^{3,4} The activation of cell cycle in hepatocytes occurs in two phases:¹ the early priming phase (G_0 – G_1 transition) and the delayed progression or proliferative phase (G_1 – S transition). The priming is induced by cytokines such as the tumor necrosis factor- α , interleukin-1, and interleukin-6, whereas the progression is induced by growth factors such as hepatocyte growth factor (HGF) and augmenters of liver regeneration (ALR).^{5,6} Cytokines mediate priming through nuclear factor- κ B and signal transducer and activator of transcription 3,^{5,6} whereas growth factors mediate proliferation through various signaling pathways, including phosphatidylinositol 3'-kinase (PI3K)–protein kinase B or Akt (PI3K–Akt), cyclin-dependent kinase 2 (CDK2), and 70-kDa form of ribosomal protein S6 kinase (p70 S6K) signaling.^{7–11}

Many NRs, including the pregnane X receptor (PXR), have been implicated in hepatocyte proliferative response in the regenerating liver. Bushue and Wan² discussed the roles of PXR, constitutive androstane receptor, peroxisome proliferator-activated receptor α , and farnesoid X receptor in inducing hepatocyte proliferation in response to retinoic acid stimulation. Strikingly, lack of PXR was shown to impair normal progression of liver regeneration.¹² Dai et al¹² performed partial hepatectomy on wild-type and PXR-null mice to determine the role of PXR in liver regeneration after hepatectomy. They observed a considerable reduction of hepatocyte proliferation in mice lacking PXR, leading to 17% less liver mass at the end of liver regeneration, demonstrating that PXR is required for normal progression of liver regeneration by regulating hepatocyte proliferation. However, how PXR affects the normal progression of liver regeneration, and the activity level of PXR that is required for such a normal progression, remain to be defined.

CDK2 is a member of the family of CDKs that drive the cell cycle through each phase (G_1 , S, G_2 , and M). CDK2 is a key regulator of cell cycle progression, and its activity fluctuates during the cell cycle, peaking at both the G_1 /S checkpoint and during S phase. Lin et al⁷ showed that CDK2 phosphorylates and attenuates the function of PXR in proliferating hepatocytes, although it is unknown whether reduced activity of the CDK2-phosphorylated PXR is sufficient for hepatocyte proliferation during the normal progression of liver regeneration. Protein phosphatase type 2C isoform beta long (PP2C β l; also known as PP2C β 2 or PP2C β x) has been shown to inactivate CDK2^{13,14} and arrest cell growth.^{15–17} Pondugula et al¹⁸ reported that PP2C β l is essential for PXR function in proliferating hepatocytes. While upregulation of PP2C β l counteracts the inhibitory effect of CDK2 signaling on PXR function, downregulation of PP2C β l dramatically attenuates PXR function and significantly enriches proliferating HepG2 cells in the S phase of the cell cycle as well as promotes cell proliferation.

During liver regeneration, the levels of growth factors such as HGF and ALR are upregulated. These growth factors play important roles in liver regeneration by activating the proliferation of

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hepatocytes. Both HGF and ALR, through the PI3K-Akt pathway, induce hepatocyte proliferation and repress the genomic function of PXR.^{19–21} It has been shown that PI3K-Akt signaling is critical for hepatocyte proliferation in the partial hepatectomy mouse model of liver regeneration,⁸ and that the expression and activity of p70 S6K are upregulated in human liver tumors,^{9,10} indicating that these kinase pathways might affect PXR activity in hepatocyte proliferation response. Indeed, Kodma et al²² and Pondugula et al¹¹ have reported that both Akt and p70 S6K negatively regulate PXR function in actively dividing HepG2 liver carcinoma cells. However, whether the effect of PI3K-Akt and p70 S6K on PXR is limited to cells in the active cell cycle is unknown. Forkhead in rhabdomyosarcoma (or forkhead box protein O1) belongs to the family of FOXO factors, which are growth factor-sensitive transcription factors that influence the function of NRs including PXR.^{22,23} Forkhead in rhabdomyosarcoma is negatively regulated by PI3K-Akt and induces apoptosis and augments PXR function.^{22,23}

The study by Dai et al¹² also showed that PXR mediates hepatocyte proliferation during the early stages of liver regeneration. Cytokines trigger the early regeneration events by priming the hepatocyte proliferation mainly through nuclear factor-kappa B, which negatively regulates PXR function.^{24–26}

Although the regulation of PXR by growth factors, cytokines, and other signaling pathways activated in proliferating hepatocytes suggests that PXR activity is affected by the cell cycle status of the hepatocytes, it is unclear how PXR signaling regulates hepatocyte proliferation in regenerating livers. Future studies using proliferating primary hepatocytes or *in vivo* liver regeneration animal models are necessary to improve our understanding of the role of PXR-mediated mechanisms in normal progression of liver regeneration.

It is intriguing to note that the signaling mechanisms that initiate both the early and delayed events during liver regeneration also negatively regulate the function of PXR, although PXR was shown to be required for liver regeneration. Therefore, it remains to be understood whether the reduction of PXR activity by signaling pathways activated in regenerating liver cells is required and/or sufficient for normal progression of liver regeneration.

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