the actual blood flow as in the brain. Therefore, we could not measure the alterations of the HTBF for an extended time period during this study. However, we could compare the flow in the same animal before and after hepatectomy using arbitrary units. The initial flow reduction affected liver regeneration and function following hepatectomy. We do not know exactly the duration of the flow change after hepatectomy in this model. A method for continuous measurement of the HTBF should be developed to study the flow effect on liver regeneration in the future.

CT-1 in Liver Regeneration

CT-1 is a member of the IL-6 family of cytokines. The mRNA expression of CT-1 is upregulated during liver regeneration, and it is produced actively by stressed hepatocytes acting in an autocrine manner to activate survival signals and maintain cell viability [6]. As we have shown in this study, CT-1 was upregulated 1 day after hepatectomy in the PH model, consistent with results of a previous report [6]. In the PHPL model, the expression of CT-1 mRNA was delayed and weak during the experiment, indicating that the cell stress in the PHPL model might be milder than that in the PH model, so hepatocytes did not need to express CT-1 to survive. Therefore, elevation of the HTBF generated shear stress which could be a cell stress to induce CT-1 expression. This observation suggests that reduction of the HTBF diminished cell stress and regulated gene expression during liver regeneration.

Cyclins in Liver Regeneration

Liver regeneration is tightly regulated by cell-cycle-associated proteins [2, 5, 25–27]. In the quiescent state, most hepatocytes are in the G_0 phase, where no cyclins are expressed in the nucleus at all. Once the stimulus for liver regeneration triggers the cell cycle, the hepatocytes transit into the G_1 phase, where cyclin D is expressed and promotes replication of cellular DNA [26, 28]. Cyclin E plays an important role beyond the G_1/S checkpoint (restriction point), where the cell starts to synthesize DNA and is committed to completion of the cell cycle [26]. Subsequently, cyclin B promotes progress of the cell cycle in the G_2 phase to mitosis [29]. Therefore, the pattern of expression of each gene represents the status of hepatocytes in the cell cycle during liver regeneration.

As the results showed, cyclin expression in the PH model was synchronized after hepatectomy, and the peak of the cell cycle could be on day 1, when the hepatocytes proliferated vigorously. On the other hand, in the PHPL model, the peak of cyclin expression was delayed to day 3, and the level of expression was lower than in the PH model. The reason for the reduction of cyclins in the PHPL group could be lack of initiation of regenerative signals due to reduction of the HTBF. The gene expression was completely consistent with the results of BrdU immunohistochemistry and LI. These results support the idea that reduction of the HTBF diminished hepatocyte proliferation and slowed liver regeneration.

C/EBP Regulation in Liver Regeneration

Liver regeneration is also regulated by transcription factors in hepatocytes, such as hepatocyte nuclear factors and C/EBPs [3]. All these transcription factors play important roles in the regulation of hepatocyte proliferation and differentiation (or function). During the initial 24 h after PH, the levels of C/EBPα mRNA and protein decline transiently [26], as we have shown. On the other hand, the C/EBPβ mRNA and protein levels increase during the initial period [30]. C/EBPα regulates many genes associated with liver functions such as lipid metabolism, gluconeogenesis, and detoxification of ammonia and bilirubin [31]. The absence of C/EBPα increases DNA synthesis and immortalizes hepatocytes [32]. Therefore, reduction of the liver function after PH could explain the decrement of C/EBPα. On the other hand, C/EBPβ promotes hepatocyte proliferation during liver regeneration [33]. Furthermore, phosphorylation of C/EBPβ on Ser239 inhibits albumin gene expression [34]. Translational control of the reciprocal expression of C/EBPα and C/EBPβ plays an important role in the control of cell proliferation and differentiation [35]. Therefore, the functions of hepatocytes should decrease, when they proliferate, and the balance between C/EBPα and C/EBPβ expression regulates hepatocyte proliferation and differentiation (or function).

Our results show that the increment of C/EBPβ expression was suppressed and that the decrement of C/EBPα expression was prevented when HTBF was reduced after hepatectomy. Partial maintenance of the C/EBPα expression enabled hepatocytes to preserve their function. In fact, we found that the CYP protein level was maintained in the PHPL group, whereas it dropped significantly in the PH group. These results support the idea that a reduction of the HTBF could reduce regenerative stimulation, but could maintain liver function.

Conclusions

We found that the activity of liver regeneration can be determined by the portal blood flow, especially the initial stimulation. The portal blood flow after PH affects the gene expression associated with cell cycle regulators. In addition, reciprocal gene expression and levels of C/EBPα and C/EBPβ are consistent with the activity of liver regeneration. Furthermore, the CYP reduction during liver regeneration was minimal after portal flow control. Therefore, reduction of the portal blood flow can reduce cell proliferation and maintain liver function. This may be an optional strategy to treat liver failure resulting from extended liver resection or small-for-size graft syndrome. Further study is necessary to determine the conditions and strategies for clinical use.

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