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Review Article

# Growth Factors and Transcription Factors in Liver Regeneration

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**Submitted:** 13 November 2013

**Accepted:** 15 November 2013

**Published:** 18 November 2013



**Keywords**
- Growth factor
- Transcription factor
- Partial hepatectomy
- Oval cell
- Small hepatocyte
- Bone marrow cell

**Abstract**

Liver recapitulates the same size as before partial hepatectomy (PH). Once hepatectomized, tumor necrosis factor (TNF) α and interleukin (IL)-6 are secreted from non-parenchymal cells. The cytokines promotes hepatocyte proliferation via signal transducer and activator of transcription 3. Hepatocyte growth factor and epidermal growth factor are potent mitogen of hepatocytes. CCAAT/enhancer binding protein (C/EBP) α is down-regulated and C/EBPβ is up-regulated after PH. C/EBPα suppresses proliferation of hepatocytes. C/EBPβ is up-regulated by TNFα and IL-6, and induces secretion of HGF. Oval cells and small hepatocytes are postulated to be hepatic stem/progenitor cells. Transplanted bone marrow cells home to liver and produce hepatocytes. Mature and fetal hepatocytes are transplanted to patients. Although hepatic encephalopathy improves, survival benefit is not obvious. Autologous bone marrow cells are transplanted to patients with acute hepatic failure.

## INTRODUCTION

Liver has a capacity to regenerate after damage. Hepatocytes proliferate and size of liver enlarges after 70 % partial hepatectomy (PH). PH is fatal when hepatocytes lose proliferative potential with irradiation [1]. The resection changes secretion of growth factors and expression patterns of transcription factors. Molecular mechanism of liver regeneration has been studied with growth factors and transcription factors focusing on hepatocyte proliferation and differentiation.

### Growth factors

Growth factors are secreted from non-parenchymal cells of liver and affect hepatocytes in regenerating liver. Rodent PH has been used as a model of liver regeneration. After PH, lipopolysaccharide triggers the expression of nuclear factor (NF)-κB in non-parenchymal cells such as Kupffer cells [2]. The activated non-parenchymal cells secrete tumor necrosis factor (TNF) α and interleukin (IL)-6 [3,4]. IL-6 binds to its receptor of hepatocytes and activates signal transducer and activator of transcription (STAT) 3. The activated STAT3 translocates to the nucleus and regulates expression of genes.

Hepatocyte growth factor (HGF) and epidermal growth factor (EGF) are secreted from non-parenchymal cells after PH. HGF binds c-Met and EGF receptors, respectively [5,6]. The receptors transmit stimulation of HGF and EGF to progress cell cycle of hepatocytes. HGF and EGF stimulates DNA synthesis of cultured hepatocytes [7,8].

### Transcription factors

Transcription factors directly regulate expression of genes. Naturally, transcription factors are involved in liver regeneration. CCAAT/enhancer binding protein (C/EBP) α is a basic leucin zipper transcription factor. C/EBPα is enriched in liver governing expression of liver specific genes and down-regulated after partial hepatectomy [9,10]. Hepatocytes of mice deficient with C/EBPα have increased proliferative potential [11]. Expression of C/EBPα is down-regulated in both hepatocellular carcinoma and hepatoblastoma and correlated with poor prognosis[12,13]. These facts suggest that C/EBPα is a tumor-suppressor gene for hepatocytes. Introduction of the gene successfully suppresses proliferation of hepatocellular carcinoma cells [12].

C/EBPβ, another basic leucin zipper transcription factor, is

up-regulated after PH [10]. DNA synthesis of mice deficient in C/EBPβ is decreased to 25 % of mice [14]. TNFα and IL-6 up-reglate C/EBPβ in stellate cells, leading to expression of HGF [15]. These facts indicate that C/EBPα and C/EBPβ are major player in liver regeneration. Hepatocytes of mice deficient in C/EBPα show pseudoglandular structure. Unexpectedly, hepatocytes lining the structure have potential of both hepatocytes and bile duct epithelial cells suggesting that C/EBPα promotes differentiation of hepatoblasts to mature hepatocytes [16].

## Stem cells involved in liver regeneration

Small cells appear in portal are of remnant liver treated with 2-acetylaminofluorence followed by 70 % PH (Solt-Farber protocol). The small hepatocytes are called oval cells after their shape. Oval cells proliferate and expand into parenchyma. They have not only proliferative potential but also characteristics of both hepatocytes and bile epithelial cells because they express albumin as well as cytokeratin 19 [17]. They are, therefore, thought to be stem/progenitor cells of liver. Interestingly, transgenic mice of TNF-like weak inducer of apoptosis show oval cell proliferation in liver through its receptor Fn14 [18]. TNF-like weak inducers of apoptosis are involved in oval cell proliferation during liver regeneration. Exact origin of oval cells is not clear. Two origins are postulated with them; Canals of Hering duct connecting bile canaliculus and biliary tree and bone marrow cells [19,20].

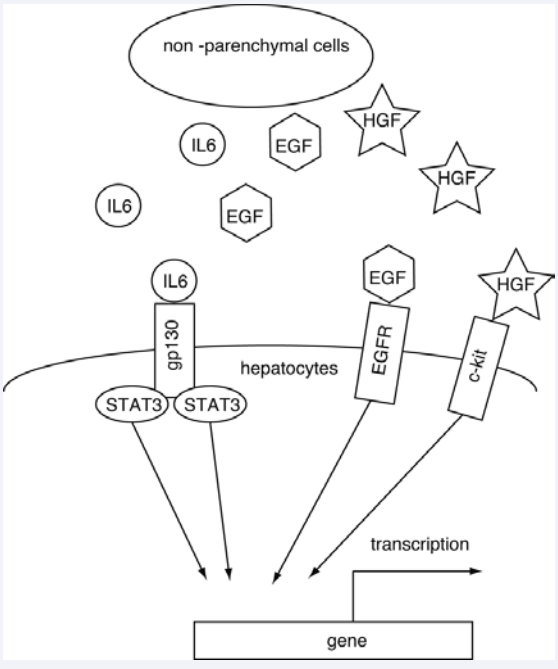


**Figure 1** Growth factors secreted from non-parenchymal cells. In rodents with partial hepatectomy, non-parenchymal cells secret interleukin (IL)-6, epidermal growth factor (EGF), and hepatocyte growth factor (HGF). IL-6, EGF, and HGF bind gp130, epidermal growth factor receptor and c-kit., respectively. Once cytokine or growth factor binds its receptor, downstream pathway is activated, and transcription of target gene is initiated.

**Abbreviations:** IL-6: Interleukin-6, EGF: Epidermal Growth Factor, HGF: Hepatocyte Growth Factor, STAT3: Signal Transducer and Activator of Transcription 3.

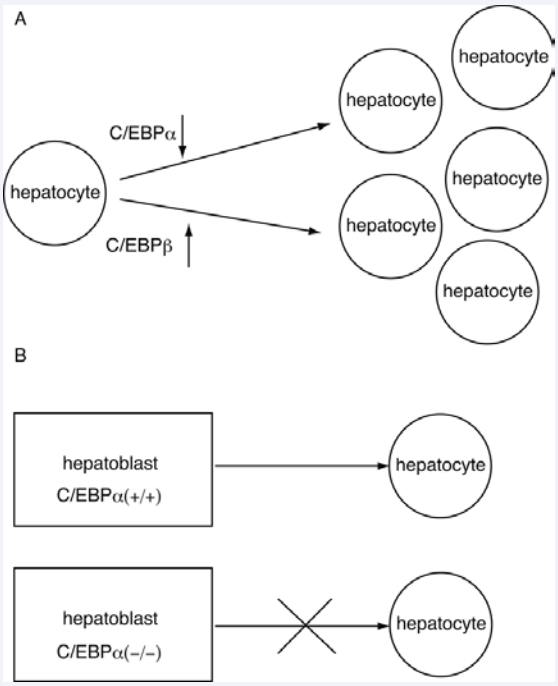


**Figure 2** CCAAT/enhancer binding protein α and β in hepatocyte proliferation and differentiation.When hepatocytes proliferate, CCAAT/enhancer binding protein (C/EBP) α is down-regulated and C/EBPβ up-regulated (A). Wild type hepatoblasts (C/EBPα(+/+)) mature to hepatocytes (B). Hepatoblasts deficient in C/EBPα (C/EBPα(-/-)), they fail to differentiate to hepaotcytes.

Small hepatocytes are thought to be immature hepatocytes with proliferative potential. They appear in rat primary hepatocytes cultured in serum-free media with 10 mmol/L nicotinamide and 10 ng/ml epidermal growth factor [21]. The small cells have single nuclei and a higher nucleus/cytoplasm ratio than surrounding hepatocytes. They are positive with albumin and transferrin. Small hepatocytes enlarge after culture with non-parenchymal cells in the medium with feral bovine serum, nicotinamid, epidermal growth factor, and tumor growth factor-α [22]. CD44 is discovered as a specific antigen to small hepatocytes with microarray analysis between small hepatocytes and mature hepatocytes [23]. Now a method is established to isolate oval cells and small hepatocytes with cell sorting with Thy1(+) and CD44 as markers, respectively [24] .

Bone marrow stem cells have the capacity to self-renewal, differentiation, and homing. Bone marrow stem cells, purified from a donor, home to recipient [25]. Surprisingly, these bone marrow cells with CD34 and SCA-1 expression differentiate to epithelial cells of the liver [26]. Transplanted bone marrow cells differentiated to hepatocyte in humans with autopsy analysis [27]. Interestingly, these transplanted cells reduce fibrosis of liver [28].

## Clinical application to liver failure

The facts above mentioned suggest that bone marrow transplantation would be a novel therapy to liver failure. CD133(+) bone marrow stem cells are transplanted via portal vein to patients following portal vein embolization of right lobe to expand left lobe [29]. Volume of left liver enlarges 2.5 times

as compared with untransplanted control Autologous bone marrow cell infustion from peripheral vein or portal vein has been performed for patients with liver cirrhosis [30]. Child-Pugh scores were significantly improved with no adverse effects.

25 patients with acute liver failure have been transplanted with mature hepatocytes [31]. Fetal hepatocytes have been transplanted [32]. Hepatic encephalopathy improves but there is no survival benefit. The reason is speculated that quantity of transplanted cells is not enough [33].

### Conclusion and future remarks

Liver regeneration constitutes of proliferation and differentiation of stem cells triggered by growth factors and transcription factors. Molecular knowledge on liver regeneration would be applied to pluripotent cell lines to promote human embryonic stem cells and induced pluripotent stem cells to hepatocytes [34,35].

## ACKNOWLEDGEMENTS

This work was in part supported by Grant-in-Aid for Encouragement of Scientists (Grant No. 22931047) and Research Grant-in-Aid for Scientific Research (C) (Grant No. 23591002), both from Japan Society for the Promotion of Science (JSPS).

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**Cite this article**

*Tomizawa M, Shinozaki F, Motoyoshi Y, Sugiyama T, Yamamoto S, et al. (2013) Growth Factors and Transcription Factors in Liver Regeneration. JSM Gastroenterol Hepatol 2(1): 1006.*