Eexpression Pattern of CyclinD1, PCNA and HSP70 in Regenerating Liver of Rat

Kexue Ma[1], Xingzi Xi[2], Keshi Ma[3]

[1.] College of Life Sciences, Henan Normal University, Xinxiang, Henan 453007, China
[2.] Department of Education Sciences, Xinxiang University, Xinxiang, Henan 453003, China
[3.] College of Life Sciences, Zhoukou Normal University, Zhoukou, Henan 466000, China
xingzixi2003@sina.com

Abstract: Adult mammalian liver is best known for its exceptional regeneration ability, in which the remaining liver tissue can recover its original size in a week after 2/3 liver resection. Therefore, the investigation of the molecular mechanism underlying the liver regeneration is of vital importance in the treatment of many liver diseases. In this paper, western blot was employed to examine the changes of cyclin D1, PCNA and HSP70 during liver regeneration. Blot bands were assessed by Bio-Rad software and all data were assessed by SPASS software. The results showed that cyclin D1 was induced at 12h, appeared two peaks at 36h and 96h respectively after partial hepatectomy; Expression of PCNA increased dramatically at 12h, appeared three peaks at 16h, 36h and 96h respectively during liver regeneration; The up-expression of HSP70 occurred at 16h-24h after PH, at which hepatocytes enter S phase of cell cycle. The results indicated that cyclin D1, PCNA and HP70 could interact each other to mediate the cell cycle progression in liver regeneration.
[Kexue Ma, Xingzi Xi, Keshi Ma. **Eexpression Pattern of CyclinD1, PCNA and HSP70 in Regenerating Liver of Rat.** *Life Sci J* 2013; 10(3): 63-66]. (ISSN: 1097-8135). http://www.lifesciencesite.com 11

Keywords: liver regeneration, cyclin D1, PCNA, HSP70

1. Introduction

Liver, one of the most important organs of the organisms, takes on more than 5000 kinds of physiological functions and displays a remarkable ability to regenerate in response to the injury of the liver (Higgins and Anderson 1931; Carnovale and Ronco, 2012). Several studies have validated that the remaining hepatocytes rapidly enter cell cycle and undergo cell division after partial hepatectomy (PH). Several days after PH, all liver cells have divided once or twice and the liver has recovered its original mass.

In pluricelluar organisms, progression through the cell cycle is regulated by the activity of protein kinase complexes consisting cyclins and cyclin-dependent kinase (CDK). Specific cyclin-CDK complexes are activated at distinct stage, which are the engines that drive the events of cell cycles (Gallorini et al, 2012). During the G1 phase, the activation of cyclin D-CDK4 complex is responsible for the phosphorylation of retinoblastoma protein and the other members of the pocket family (p107 and p130), which leads to the release of E2Fs and triggers the cell cycle progression from G1 to the S phase (Mayol and Grana 1997). Proliferating cell nuclear antigen (PCNA), a cofactor of DNA polymeraseδ, is involved in the DNA replication.

Heat shock protein 70 (HSP70), the major stress-induced protein, is often related to cell resistance, cell metabolism, selective degradation of protein and embryogenesis (Mjahed et al, 2012). Many recent works are reported that HSP70 is involved in cell cycle progression. In *Hela* cells, the expression of HSP70 is restricted to the G1/S boundry (Milarski et al, 1989). It is assumed that HSP70 may affect the cell cycle progression by mediated those cell cycle-related proteins (Shi et al, 2007), but the detail mechanism remains unknown.

For the past years, numerous studies have provided good information about cyclin D1, PCNA and HSP70 involved in liver regeneration. However, there has been no reported in the published literature, concerning the interaction of cyclin D1, PCNA and HSP70 during rat liver regeneration. For this reason, the changes of cyclin D1, PCNA and HSP70 throughout the regenerating liver were investigated in this paper.

2. Material and Methods

2.1 Animals and materials

Sprague-Dawley rats (200g-250g) were provided by the experimental animal house of Collge of life Science of Henan Normal University. Chemicals and reagents were of anaylytical grades, mouse monoclonal antibody to cyclin D1, HSP70 and β-Actin (Santa Cruz), mouse monoclonal antibody to PCNA (Wuhan Boster), horse anti-mouse IgG-AP (Beijing Zhongshan).

2.2 Partial hepatectomy

Partial hepatectomy was performed according to the mathod of Higgins and Andersion (1931). As a control sham operations (laparatomy and liver manipulation without tissue removal) were also performed. Animal were offered food and water

ad libitum post-operatively.

2.3 Preparation the samples and determination of protein concentration

At different time after PH, the rats were killed and bled by taking off the eyeballs. Liver was washed until it became white through coronary vein perfusion, then put into the culture dish with ice physiological salt solution , and sheered into pieces for homogenating in the buffer (40 mmol/L NaCl, 20 mmol/L Tris-HCl, 1 mmol/L PMSF, 10 μg/ml leupeptin, 0.5μg/ml aprotinin, pH 7.5) at 4℃, and centrifuged at 10 000 g for 15min. The supernatant was aliquoted and stored at -85℃.Protein concentration was determined according to Neugoff method (Neuhoff et al, 1979).

2.4 Gel electrophosis and western-blotting

Protein were separated electrophoretically in sodium dodecyl sulfate-polyacrylamide gels as described by Laemmli (1970). The gels were transferred onto Immobilon-p membranes (Millipore) for 2 hours at 60 V. Subsequently, the membranes were blocked with TBS containing 3% bovine serum albumin for 1 h at room temperature. The membranes were then incubated in primary antibody (cyclin D1, 1:500 dilution; PCNA, 1:1000 dilution; HSP70, 1:1000 dilution; β-Actin, 1:1000 dilution;) overnight at 4℃. After incubation, the membranes were exposed to secondary antibody (1:5000 dilution) and developed by NBT/BCIP.

2.5 Image analysis and statistical method

The blot bands were assessed by Bio-Rad software, all data values were subjected to one-way analysis of variance (one-way ANOVA). Significance was concluded at $P < 0.05$. Statistical analysis was performed using SPASS 10.0 for windows.

3. Results

3.1 Change of cyclin D1 expression

To study expression of cyclin D1 during the liver regeneration, we performed western blot analysis throughout the 144-h detailed kinetic synchrony (Fig.1). In the normal liver tissue, the expression of cyclin D1 was very low. The up-regulated expression cyclin D1 occurred at 12 h (1.2-foled), appeared two peaks at 36 h (2.2-fold) and 96 h (1.5-fold) respectively and returned to the normal level at 144 h.

3.2 Change of PCNA expression

PCNA can be detected as a faint band in normal liver. There was a significant induction at 16h (2.5-fold) after PH, then the level of protein descended at 24h but still much higher than the control. At 36h, the expression of PCNA reached a maxium (3.2-fold), then remained a high level for the remain period of study(Fig.2).

3.3 Change of HSP70 expression

The dynamic change of HSP70 following partial hepatectomy was examined in this study. HSP70, whose protein level was very low, but was clearly detected in quiescent cell (Fig. 3). its expression increased dramatically at 16-24h (1.2-fold and 0.88-fold, respectively), then decreased gradually to the normal level.

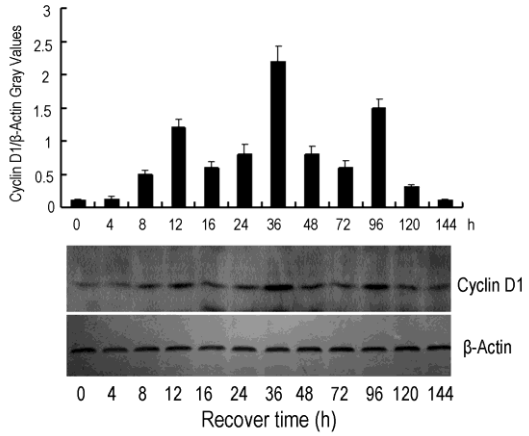

Figure 1 Change of cyclin D1 during liver regeneration. Ninety micrograms of total proteins/lane was loaded on the gel. β-Actin was used as the control to normalized the amount of total proteins. Values are expressed as mean ± S.D. of three independent experiments. Asterisk indicates significant differences comparing to that of control samples（$P < 0.05$). The same is as the follows.

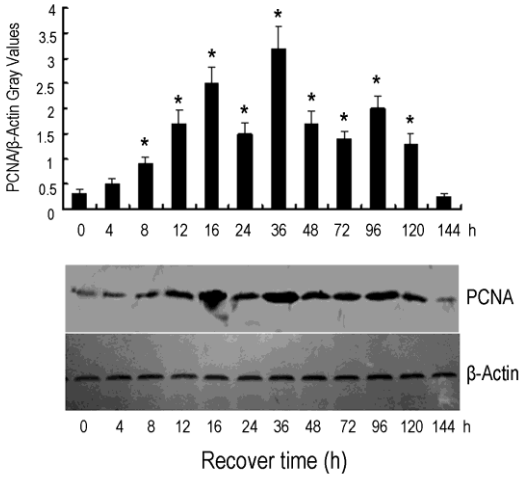

Figure 2 Change of PCNA during liver regeneration. Ninety micrograms of total proteins/lane was loaded on the gel. β-Actin was used as the control to normalized the amount of total proteins. Values are expressed as mean ± S.D. of three independent experiments. Asterisk indicates significant differences comparing to that of control

samples（$P < 0.05$).

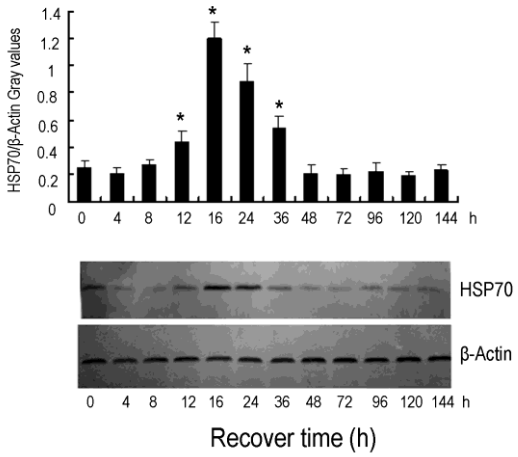

Figure 3 Change of HSP70 during liver regeneration. Ninety micrograms of total proteins/lane was loaded on the gel. β-Actin was used as the control to normalized the amount of total proteins. Values are expressed as mean ± S.D. of three independent experiments. Asterisk indicates significant differences comparing to that of control samples（$P < 0.05$).

4. Discussions

In adult liver, hepatocytes are highly differentiated and predominantly in G0 state of cell cycle, but they can undergo rapid proliferation following partial hepatectomy. The proliferation of hepatocytes after PH occurs in response to the action of a group of extracellular growth factors, which generate intracellular signals that activate the cell-cycle regulatory machinery and trigger the hepatocytes into cell cyeles. Although significant insight has been gained into the regulation of cell cycle, very little is known about mechanisms that regulated cell-cycle progression in the hepatocytes proliferating in *vivo* after PH. During the last few years, a number of articles reported new information about the action of cyclin D, PCNA and HSP70 during liver regeneration. However, many points still remain unclear.

The first aspect for discussion concerns the role of cyclin D in liver regeneration. Several previous works had provided obvious evidences that cyclin D is the key regulator during the G1/S cell cycle transition, and perhaps the most important checkpoint in the mammalian cell cycle (Albrecht et al, 1995). However, there has been insufficient study of the expression of the cyclin D in regenerating liver cell. In present study, we detected the level of cyclin D1 following partial hepatectomy in rats. The results showed that the expression of cyclin D1 increased at 12 h, and two peaks appeared at 36 h and 96h respectively. Other studies reported the mRNA of cyclin D1 is induced rapidly after 8 h post-PH, and it reached the maximum between 12 h-15 h (Kato et al, 1998). The up-regulated expression of cyclin D1 performs two functions during G1. One is phosphorylation of Rb, whose phosphorylation is required for entry into the S phase. The other is sequestration of CDK inhibitors such as p27, p21, which decreases the inhibitory threshold imposed by the pool of free CDK inhibitors in the cell. This result is in agreement with the hypothesis that cyclin D1 plays a critical role in cell progression in late G1, and contributes to the so-called cell cycle clock by ensuring the commitment of cells to enter S phase (Bravo et al, 1987).

The PCNA protein has been proven to be an auxiliary protein of DNA polymeraseδ, and in eukaryotic cells it appears essential for full DNA replication (Bravo et al, 1987). PCNA had been widely used as a marker of cell proliferation in various normal and neoplastic tissues to provide insight into cell growth, transformation, and tissue repair after injury (Theocharis et al, 1994). In this study, Western blot has been used for examining the kinetics of PCNA protein expression during the liver regeneration. Bio-Rad softwar was employed to analyze the blot band quantitatively, the results showed that the expression pattern of PCNA displayed a good correlation with that of cyclin D1 throughout liver regeneration. PCNA is an acidic protein weighted 36 kD located in nucleus, and it appears late in the G1 phase and early in the S phase. Many of these properties are shared by cyclin D1 (molecular weight 35 kD). Therefore, PCNA is also named “cyclin” in previous works (Bravo and Macdonated-Bravo, 1987). It is assumed that cyclin D1 and its associated CDK form a quaternary complexes with PCNA, as well as p21 throughout the cell cycle (Ng and Song, 2012). The strong correlation and interaction mechanism between cyclin D1 and PCNA may reflect the key role of cyclin D1 and PCNA in G1/S transition and S phase DNA synthesis, respectively.

The most important aspect for discussion is the role of HSP70 involved in hepatocyte cell cycle. Our results demonstrated that the expression of HSP70 increased at 12h, 16h and 24h after PH, at which hepatocytes are in late-G1 phase and S phase. This finding was supported by other studies. It is reported that HSP70 can forms a complex with P53 in rat cell (Inoue et al, 1995). Because P53 and HSP70 are both nuclear antigen, an interaction between these proteins may have an important for the G1/S-phase transition of the cell cycle. It has been postulated the activation of cyclin D-CDK4 complexes could be related to the differential

association of the inhibitors, p21 and p27 during liver regeneration (Jaumot et al, 1999). Furthermore, these proteins were supposed to translocation from cytoplasm into nucleus, and form protein-protein interacting complexes. Recent data indicated that the association, separation and translocation of these complexes could be mediated by a 70 kD chaperone protein (Jaumot et al, 1999). The coincidence that cyclin D1, PCNA and HSP70 are induced at the time of the maximal liver regenerative activity can be suggested that HSP70 could participate in these events.

To establish the mechanisms involving the translocation of cyclin D, PCNA, CDKs and CDK-inhibitors from cytoplasm to the nucleus during liver regeneration, and those responsible for the intranuclear movements of these proteins are vital goals for the understanding of how the cell-cycle regulatory machinery is activated after PH. Experiments to shed light on these mechanism are currently under way in our laboratory.

Acknowledgements:

This work was supported by the Natural Science Research Program of Education Department of Henan Province (No.2011B180031).

Corresponding Author:
Xingzi Xi
Department of Education Sciences
Xinxiang University
East Jinsui Road, Xinxiang 453003, China
E-mail: xingzixi2003@sina.com

References

[1] Albrecht JH, Hu MY, Cerra FB. Distinct patterns of cyclin D1 regulation in models of liver regeneration and human liver. Biochem Biophys Res Commun 1995, 209: 648-655.
[2] Bravo R, Frank R, Macdonald-Bravo H. Cyclin/ PCNA is the auxiliary protein of DNA polymerase-delta. Nature 1987, 327: 515-517.
[3] Bravo R, Macdonated-Bravo H. Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: Association with DNA replication sites. J Cell Biol 1987, 105:1549-1554.
[4] Carnovale CE, Ronco MT. Role of nitric oxide in liver regeneration. Ann Hepatol 2012, 11:636-647.
[5] Gallorini M, Cataldi A. di Giacomo V. Cyclin-dependent kinase modulators and cancer therapy. BioDrugs 2012, 26:377-391.
[6] Higgins GM, Anderson RM. Experimental pathology of the liver, I. Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931, 12:186-202.
[7] Inoue A, Torigoe T, Sogahata K et al. 70 kD a heat shock protein cognate protein interacts directly with the N-terminal region of retinoblastom a gene product pRb. J Biol Chem 1995, 270: 22571-22576.
[8] Jaumot M, Estanyol JM, Agell N, Bachs O. Activation of CDK4 and CDK2 during rat liver regeneration is associated with intranuclear rearrangements of cyclin-CDK complex. Hepatology 1999, 29: 385-395.
[9] Kato A, Ota S, Bamba H,et al. Regulation of cyclin D-dependent kinase activity in rat liver regeneration. Biochem Biophys Res Commun 1998, 245:70-74.
[10] Laemmli UK. Cleavage of structural proteins in the assembly of the head of bacteriophase T4. Nature 1970, 227: 680-685.
[11] Mayol X, Grana X. pRB, p107 and p130 as transcriptional regulators: role in cell growth and differentiation. Prog Cell Cycle Res 1997, 3:157-169.
[12] Milarski KL, Welchm W, Morimoto RI. Cell cycle-dependant association of HSP70 with specific cellular proteins. J Cell Biol 1989, 108: 413-423.
[13] Mjahed H, Girodon F, Fontenay M, Garrido C. Heat shock proteins in hematopoietic malignancies. Exp Cell Res 2012, 318:1946-1958.
[14] Neuhoff V, Philipp K, Zimmer HG, Mesecke S. A simple, versatile, sensitive and volume independent method for quantitative protein determination which is independent of other external influences. Hoppe Seylers Z Physiol Chem 1979, 360:1657-1670.
[15] Ng R, Song G. A microRNA-21 surge facilitates rapid cyclin D1 translation and cell cycle progression in mouse liver regeneration. J Clin Invest 2012, 122:1097-1108.
[16] Shi Q, Dong Z, Wei H. The involvement of heat shock proteins in murine liver regeneration. Cell Mol Immunol 2007, l4: 53-57.
[17] Theocharis SE, Skopelitou AS, Margeli AP, et al. (1994) Proliferating cell nuclear antigen (PCNA) expression in regenerating rat liver after partial hepatectomy. Dig Dis Sci 1994, 39: 245-252.

7/5/2013