Changes in Expression of Cell Cycle Regulators and Their Hepatic Lobular Distribution in Partial Hepatectomy-induced Regenerating Rat Liver

Partial hepatectomy (PH) endorses quiescent hepatocytes to reenter the cell cycle. The regenerating liver returns to its preresection weight after 7 days, following one or two cell division and maintains nearly its original volume after then. We focused on the inhibition of further hepatocyte proliferation, hypothesizing possible involvement of cell cycle upregulators and inhibitors. We studied protein levels in expression of cyclins, cyclin dependent kinases (CDKs) and CDK inhibitors (CKIs), and their in situ hepatic lobular distributions in partial hepatectomized rat liver. Cyclin E was expressed in the same levels in normal liver and after PH. Expression of cyclin A, not detected in normal liver, increased in following times after PH and reached a maximum at 7 day. CDK2 and 4 showed increased expression toward terminal period. Contradictory findings of cyclin A and these CDKs might play an important role in the inhibition of further cell division, although still unclear. Constitutively expressed CDK6 decreased after 1 day. p18 showed peak expression within 1 day, and p16, p21, p27 and p57 were stronger at terminal periods. During the expected period of their activity, intranuclear translocations were observed in cyclin E, p18 and p16. There was no evidence of regional distribution in hepatic lobular architecture, instead, diffuse in situ expression, corroborating synchronous event, was found.

Key Words : Hepatectomy; Liver regeneration; Cyclins; Cyclin-dependent kinases; Enzyme inhibitors

Jin Sook Jeong, Jeong Hee Lee, Hyeong In Kim, Joo In Park*

Departments of Pathology, Biochemistry*, Institute of Life Science, Dong-A University College of Medicine, Pusan, Korea

Received : 22 June 1999
Accepted : 8 August 1999

Address for correspondence
Jin Sook Jeong, M.D.
Department of Pathology, Institute of Life Science, Dong-A University College of Medicine, 3-1, Dongdaeshin-dong, Soe-gu, Pusan 602-103, Korea
Tel : +82.51-240-5352, Fax : +82.51-247-2903
E-mail : jsjung1@daunet.donga.ac.kr

*This research was supported by the Dong-A University Research Fund in 1997.

INTRODUCTION

In normal adult animals, hepatocytes are highly differentiated, and can remain for a long period in a quiescent G0 state (1). However, hepatocytes have the potential to proliferate after chemical or physical partial hepatectomy (PH) (2). Normal liver regeneration following PH serves as an excellent model to study cell proliferation and cell cycle control in differentiated and nontransformed cells (3). After removal of the liver, the remaining hepatocytes and nonparenchymal cells synchronously enter the cell cycle, progress through G1 phase, and reach maximum ^{3}H-thymidine uptake at 24 hr with subsequent mitosis at 32 hr (1, 4, 5). Seven days after PH, all hepatocytes divided once or twice and the liver recovered its original mass (1, 6).

The eukaryotic cell cycle is regulated by signal transduction pathways mediated by complexes of cyclin dependent kinases (CDKs) and their partner cyclins (7, 8).

The catalytic activity of CDK is dependent on tyrosine and threonine phosphorylation by CDK activating kinases, binding to cyclins and interaction with CDK inhibitors (CKIs) (7, 9-14). CDK2, 4 and 6 in conjunction with cyclin C, D and E have been implicated in the passage through G1; while CDK1 and 2 complexed with cyclin A and B seem to have critical importance in the S and G2/M phases (7, 9-14). CDK inhibitors are negative regulators of cell cycle. Two families of CKIs have been described; the Ink4 family and the Cip/Kip family (15-17). The Ink4 members include p16, p15, p18, and p19. They bind specifically to CDK4 and its homologue, CDK6. The Cip/Kip family includes p21, p27 and p57, and they bind to and inhibit the activity of a wide range of cyclin-CDK complexes, including cyclin D-CDK4/6, cyclin E-CDK2 and cyclin A-CDK2.

Recently, it has been reported that most of the proteins of cell cycle regulators are already present in regenerating rat liver cells and most of them are related to

transcriptional and translational expression levels within 24-36 hr. They focus on cell cycle upregulation, although the detailed mechanisms are obscure. Intracellular localization with translocation of some of these cell cycle regulators was reported this year (18), but the overall distribution tendency of protein expression within hepatic lobular architecture was not tried. This experiment was designed to decipher the consecutive timing throughout 7 day post-surgery and in situ hepatic lobular distribution of cell cycle mediators (upregulators & inhibitors) expression during liver regeneration induced by PH.

MATERIALS AND METHODS

Experimental animals and partial hepatectomy

Two to three-month-old male Sprague Dawley rats (200-250 g) were used for all our experiments. The animals were fed with labchow and water ad libitum, and maintained with a 12-hr light/12-hr darkness schedule. PH were performed, according to Higgins and Anderson (2), in which approximately 70% of the liver mass was removed under ether anesthesia. Hepatectomized rats were sacrificed at 6 hr, 12 hr, 16 hr, 1 day, 2 day, 3 day, 4 day, and 7 day post-surgery. Sham operated rats were used as controls.

Liver sample preparations

Rat liver was perfused with normal saline through the portal vein to remove RBCs. Removed livers were flash-frozen in liquid nitrogen and stored at -80℃. For the histologic studies, perfusion with normal saline was performed. Then, perfusion with 10% neutral buffered for-

malin was continued for about 1 hr and removed livers were sliced and fixed in 10% neutral buffered formalin for 24 hr.

Protein extraction

Using a Teflon-glass homogenizer, 0.5 g of liver tissue was homogenized in 1 mL of homogenization buffer (Tris-HCl, pH 7.4, 20 mM; NaCl, 100 mM; NP-40, 1%; sodium deoxycholate, 0.5%; $MgCl_2$, 5 mM; pepstatin, 0.1 mM; antipain, 0.1 mM; chymostatin, 0.1 mM; leupeptin, 0.2 mM; aprotinin, 0.4 mM; soybean trypsin inhibitor, 0.5 mg/mL). Tissue homogenates were centrifuged at 15,000 g for 5 min and the supernatant fraction was collected. After measuring the protein concentration, aliquots of samples stored at -80℃. All procedures were done at 4℃.

Antibodies

Antibodies used in this study are listed in Table 1.

SDS polyacrylamide gel electrophoresis and immuno-blotting

Proteins were fractionated on 12% SDS-polyacrylamide slab gels, according to Laemmli (19). The gels were transferred into nitrocellulose membrane for 2 hr at 60 V. The membranes were preincubated in Tris buffered saline (TBS) including 10% defatted milk powder for 1 hr at room temperature. Then, adequately diluted antibodies were added, and incubated for 1 hr. After washing with TBS-0.1% Tween 20, membranes were incubated with alkaline-phosphate-conjugated secondary antibodies for 30 min. After washing with TBS-0.1% Tween 20 and

Table 1. Antibodies to cell-cycle associated proteins*

| Target protein	Host	Specificity	Application	Cat. #
Cyclin E	h	P	WB, IP, IHC	SC-198
Cyclin A	m	M	WB, IP, IHC	SC-239
CDK2	h	M	WB, IP, IHC	SC-6248
CDK4	m	P	WB, IP, IHC (F/P)	SC-260
CDk6	h	P	WB, IP, IHC	SC-177
p15	h	P	WB, IHC	SC-613
p16	m	M	WB, IHC (F/P)	SC-1661
p18	m	P	WB, IP, IHC	SC-12078
p19	m	P	WB, IP, IHC	SC-1063
p21	m	M	WB, IP, IHC	SC-6246
p27	m	M	WB, IP, IHC (F/P)	SC-1641
p57	m	P	WB, IHC	SC-1039

h, human; m, mouse; P, polyclonal; M, monoclonal; WB, Western Blotting; IP, Immunoprecipitation; IHC, Immunohistochemistry; F/P, formalin fixed, paraffin embedded
*All antibodies are purchased from Santa-Cruz Biotechnology, INC., Santa-Cruz, CA, U.S.A.)

TBS, the reaction was visualized with nitro blue tetrazolium/S-bromo-4-chloro-3-indoyl-1-phosphate.

Immunohistochemistry

Processed and paraffin embedded tissue blocks were cut into 4-6 μm thickness onto Probe-on slides. They were deparaffinized with xylene and hydrated with decreasing concentrations of ethanol and washed with phosphate buffered saline (PBS). For the retrieval of nuclear antigen, they were placed in Coplin jars, immersed in citrate buffer and put in a microwave for 5 min. LSAB detection system was used for immunostaining as follows: Pretreated slides were incubated in normal goat serum for 30 min to block nonspecific bindings. The primary antibodies were incubated overnight at 4℃. Twice washing with PBS for 10 min followed incubation with ABC-linked peroxidase solution, and rinsed with PBS for 10 min, two times. For colorization, AEC, 0.01% hydrogen peroxide, Tris buffer 50 mmol/L, pH 7.2 was applied until positive controls were detected. After the PBS wash, the slides were mounted and microscopic examination followed. Negative control slices were processed in the same manner, but the primary antibody treatment was omitted. For general inspection of liver morphology, slices were stained with conventional hematoxylin-eosin.
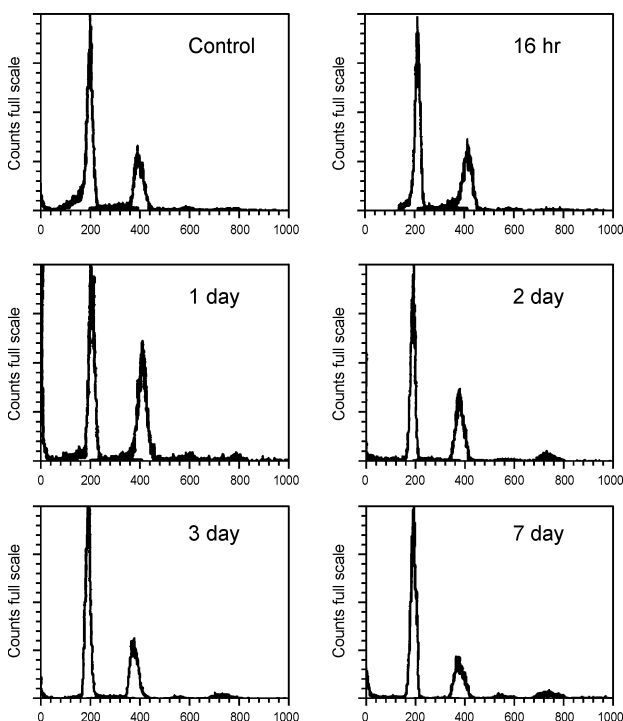
Fig. 1. DNA content analysis in normal and post-partial hepatectomized rat liver. Fraction of S phase increases at 16 hr, and fraction of G2/M phase peaks at 1 day. Note population of G0/G1 is higher at 7 day, than control.

Flow cytometry

After perfusion with normal saline through portal vein, the liver was removed, teased, filtered and trypsinized for nuclear isolation. Isolated nuclei were stained with propidium iodide and analyzed in a FACSort-flow cytometer (Beckton Dickinson).

RESULTS

## DNA content analysis by flow cytometry

S phase fraction for DNA synthesis increased 16 hr after PH (9.2%), when compared with sham-operated control (8.7%). G2/M fraction increased from 16 hr till 2 day after PH, showing a peak in 1 day after PH (43.9%). Resting hepatocytes corresponding to G0/G1 phase were more abundant in 7 day after PH (70.6%) than in control (61.2%) (Fig. 1).

Changes in expression of cyclins

The expression of cyclin E was not changed throughout control and sequential timing after PH, representing constitutive expression (Fig. 2). In situ protein expression showed no evidence of regional distribution in hepatic lobular architecture and control liver showed diffuse intracytoplasmic staining (data not shown). One noteworthy observation was earlier partial translocation (6 hr after PH) of cyclin E, showing both intracytoplasmic and intranuclear localization (Fig. 3A, B). One day after PH, the nuclear protein shifted to the cytoplasms (Fig. 3C, D). The immunostaining density was relatively constant, corresponding to the data of immunoblot. On the contrary, cyclin A was not detected in control and increased after 12 hr to 7 day after PH (Fig. 2). Cyclin A was not detected in liver tissue (data not shown).
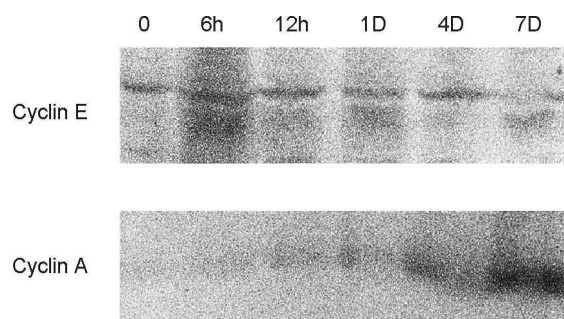
Fig. 2. Immunoblot for cyclins. Upper panel shows constitutive expression of cyclin E, and lower panel shows increasing expression of cyclin A, according to sequential timing after PH with a peak at 7 day.
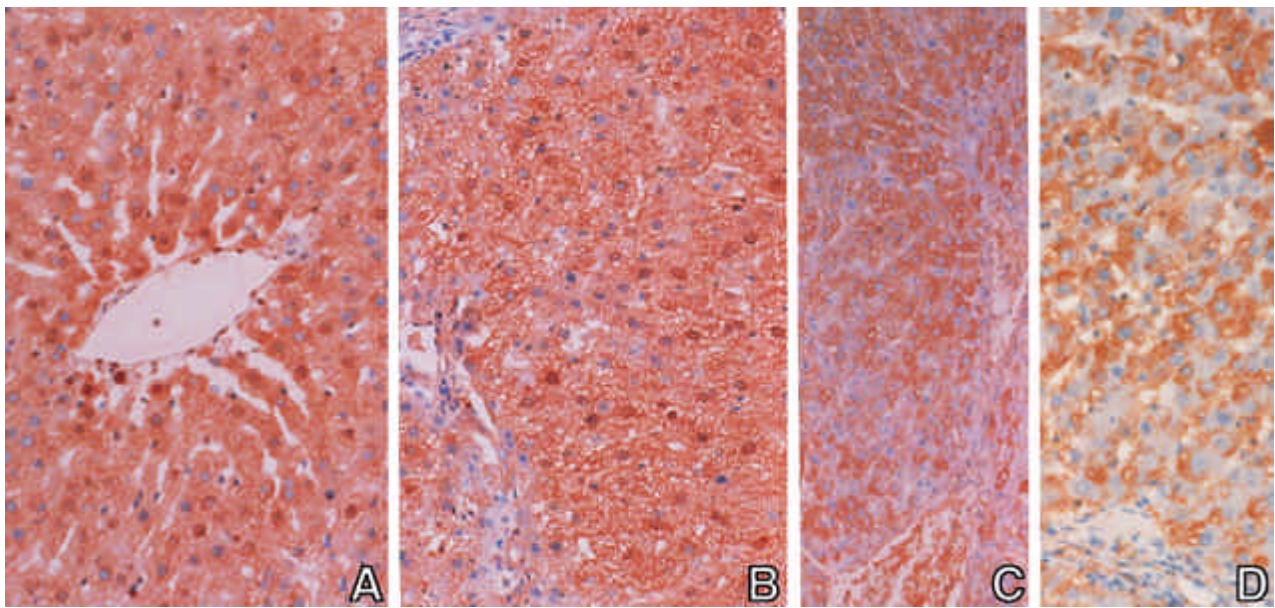
Fig. 3. In situ expression of cyclin E (A: 6 hr, ×200, B: 6 hr, ×200, C: 1 day, ×100, D: 4 day, ×200). The immunostaining densities are constant. At 6 hr after PH, cyclin E shows both cytoplasmic and nuclear localization. One day after PH, the nuclear protein returns to cytoplasms.

Changes in expressions of CDKs

CDK2, CDK4 and CDK6 were all expressed in sham operated liver, although CDK2 and CDK4 were low in signal. The expression of CDK2 and CDK4 increased along with progression in time after PH. On the contrary, CDK6 showed similar expression in the earlier period after PH with control, and at 1 day after PH, the expression decreased remarkably (Fig. 4). In situ expression pattern of CDK6 showed similar immunodensities with immunoblot in control, 6 hr and 12 hr after PH, mainly in cytoplasms and specific regional distribution in hepatic lobular architecture was not observed (Fig. 5). With immunohistochemistry, CDK2 and CDK4 were not detected (data not shown).

Changes in expression of CKIs (Ink family)

Expression of p16 was lower in the earlier time (6 hr, 12 hr and 1 day) after PH than control and elevated after 4 day, reaching a maximum at 7 day (Fig. 6). In situ expression was similar to its immunodensities, that is, lower at 12 hr and higher at 7 day (Fig. 7). Intranuclear localization was noted at 7 day. There was no regional tendency in distribution as well. p18 expression was low in control, and showed two peaks after PH, of which the first one was 6 hr after PH and in 12 hr, the protein level was slightly decreased, and the second stronger peak followed 1 day after PH (Fig. 6). From 4 day after PH, the expression decreased. In situ expression of p18 showed both intranuclear and intra-

cytoplasmic immunoreaction at 6 hr, and at 1 day after PH, most of the immunoreaction was located in cytoplasms (Fig. 8). Seven day after PH showed remarkably decreased immunoreaction which was similar to control liver. No evidence of regional hepatic distribution was noted. p15 and p19 were not detected in both the immunoblot and immunohistochemistry (data not shown).

Changes in expression of CKIs (Cip/Kip family)

p21 was expressed low, p27 was obscure and p57 was not detected in control liver. p21, p27 and p57 all show-
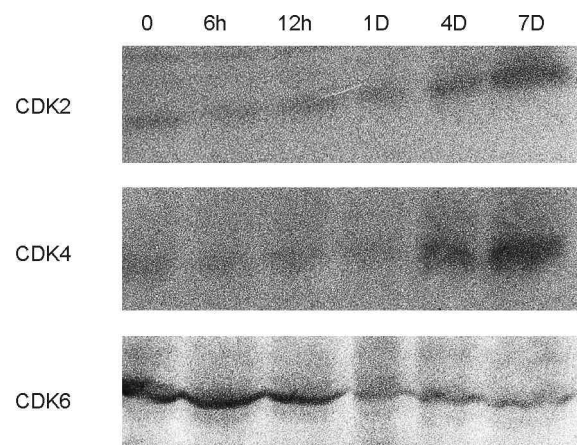

Fig. 4. Immunoblot for CDKs. CDK2 and CDK4 (upper and middle panels) show increasing expressions toward later period after PH. CDK6 (lower panel) shows similar expressions in control and early period, and soon decreases after 1 day.
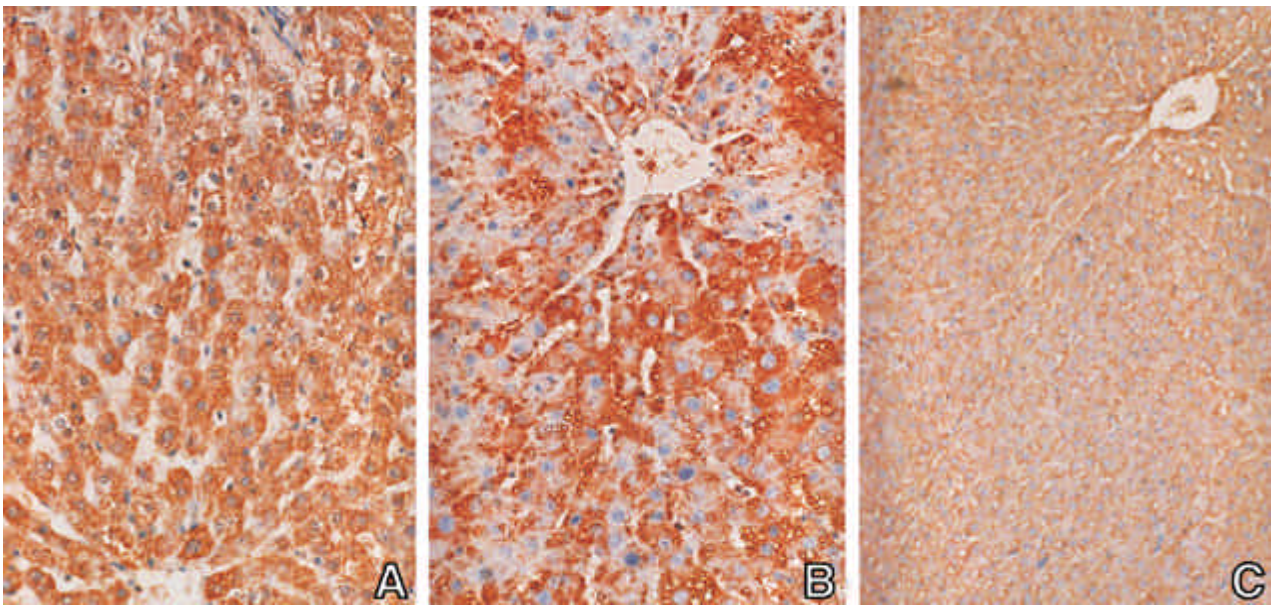
Fig. 5. In situ expression of CDK6 (A: 6 hr, ×200, B: 12 hr, ×200, C: 1 day, ×100). Diffuse cytoplasmic detection of CDK6 in earlier stages after PH soon decreases, well corresponding to the data of immunoblot.
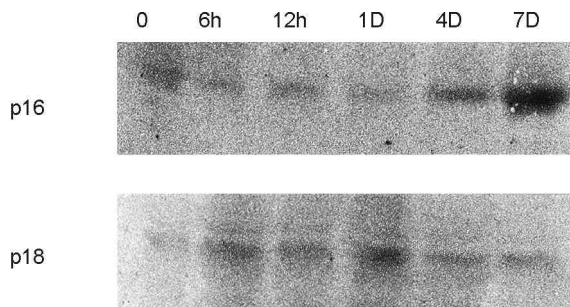

Fig. 6. Immunoblot for CKIs (p16 and p18). Expression of p16 (upper panel) increases from 4 day and peaks in 7 day. p18 (lower panel) shows prompt increased expression from 6 hr and second peak in 1 day, and decreases after 4 day.

ed increasing patterns of their expression, toward the late periods, especially at 4 day and 7 day (Fig. 9). Using immunohistochemistry, they were unable to stain.

DISCUSSION

Even though significant insight has been gained into factors that promote hepatocyte proliferation, little is known about mechanisms that regulate cell cycle progression and inhibition in reconstruction after PH in vivo. This comprehensive study of cell cycle parameters following PH corroborates the findings of several other research groups (4, 18, 20-23) and further expands our knowledge base by examining cell cycle control proteins, especially CKIs. In addition, we tried in situ distribution of cell cycle regulators expression to understand zonal tendency
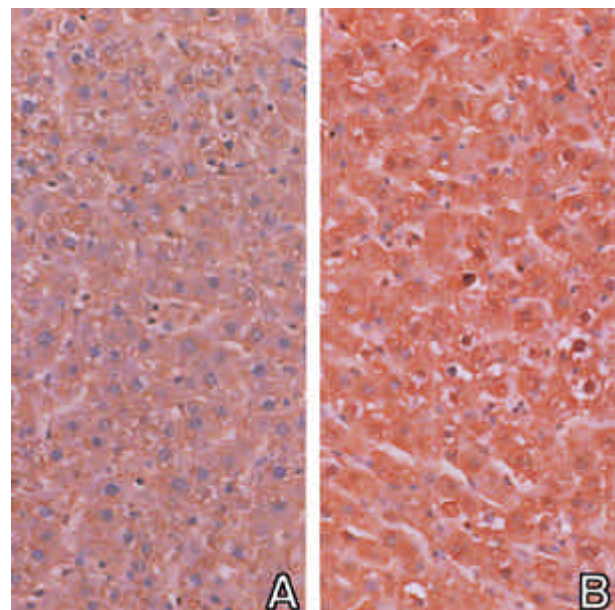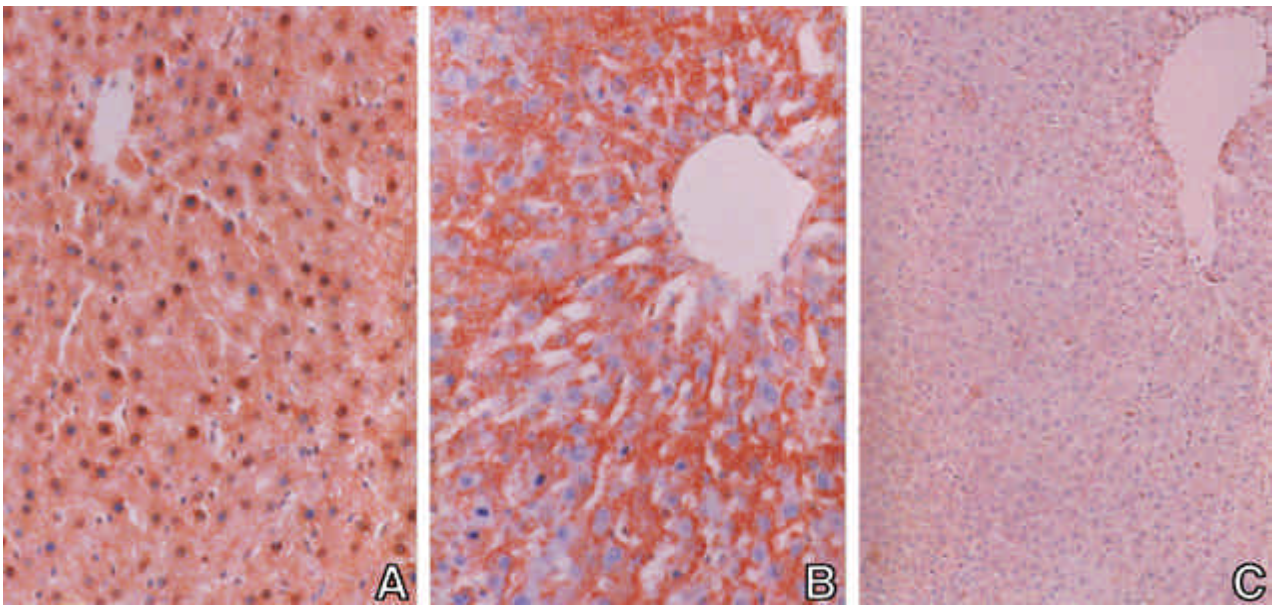

Fig. 7. In situ expression of p16 (A: 12 hr, ×200, B: 7 day, ×200). Weakly cytoplasmic reaction is noted at 12 hr, and increased expression both in cytoplasmic and nuclear locations is noted in 7 day.

along with consecutive sequence of timing.

The cellular proliferation induced by PH serves as a normal physiologic cellular proliferation from wound, and the inhibition of further cell division to return to the previous liver weight mimics healing process after wound. Proliferation stimulation is taken via interactions between growth factor and growth factor receptors at the cell surface, resulting in DNA synthesis from 16 hr and

Fig. 8. In situ expression of p18 (A: 6 hr, ×200, B: 1 day, ×200, C: 7 day, ×100). p18 expression is detected from 6 hr after PH, in both the cytoplasm and nuclei, and peak is at 1 day after PH, chiefly in cytoplasm and remarkably decreases at following periods.
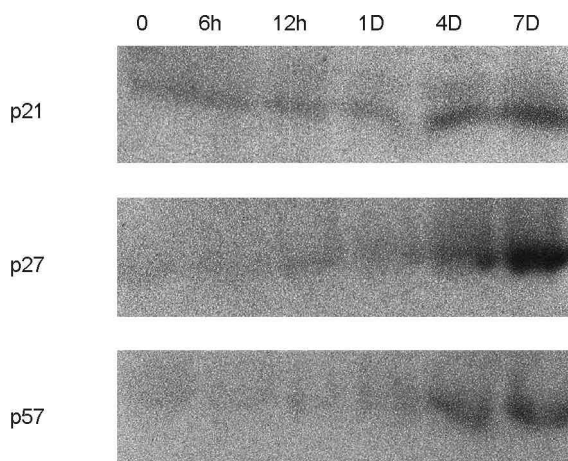
Fig. 9. Immunoblot for CKIs (p21, p27, p57). These proteins show increasing expression levels, toward late stage, especially at 4 day and 7 day after PH.

peaking at 24 hr (1, 4, 5). DNA analysis shown in Fig. 1 depicted increased S and G2/M phase fractions at 16 hr and 1 day, respectively, corroborating previous studies.

The key proteins involved in the regulation of CDK4, its homologue CDK6 and CDK2 were present in quiescent liver cells and immediately after PH; however both CDK4 and CDK2 were inactive. The activity of both enzymes started to increase after 13 hr and reached a maximum at 24 hr, coinciding with PCNA expression (18). The semiquantitative expression levels in our data showed similar patterns till 1 day. A few reports treating expressions after 2 day were reviewed, with Ehrenfried et al. (23) reporting that the expression of CDK2 and 4

decreased after 1-2 day, which contradicts with increased expression at 4 day and 7 day in our investigation. Although the expression is controversial and should be clarified, increased expression of CDK4 and 2 at nearly reconstructed period might suggest another role at a later period, in addition to active kinase at first cell division, or simple accumulation of inactive protein. In addition, CDK4 has been implicated as a biosensor for stimulatory and inhibitory growth signaling, as well (24). There have been several studies on the changes of regulatory mechanisms involving G1 phase cyclins, cyclin D and cyclin E during liver regeneration. The expression of mRNAs of cyclin D1 and E was reported to increase after PH (25, 26). On the contrary, the protein levels of cyclin D1 and cyclin E were noted to be constant after PH (4, 26), and the association of CDK4 and cyclin D1 was the same as well (26). Sometimes contradictory results to the unchanged cyclin D1 and E were reported (21). Cyclin A, an important S and G2/M phase regulatory protein, is associated with CDK2 and CDC2 kinases in each phase (27-29). Recently, cyclin A has been implicated in liver cell transformation (30), but its role or regulation during liver regeneration has been rarely investigated. We did not screen the cyclin D family. Cyclin E protein levels after PH was similar to previous studies (4, 26), and cyclin A was induced after PH and gradually increased, reaching a maximum at 7 day, although quiescent liver cell did not express cyclin A. The increased expression of cyclin A and CDK2 (Fig. 2, 4) coincided, suggesting an important biologic role in the inhibition of cell division, which is contradictory to a recent paper (30). Fur-

ther approaches for unknown substrates and kinase activity determination should be followed, so we will continue.

For CKIs, a few reports talked about p21 and p27, which have shown controversial expressions, but constantly coincided with cyclins or CDKs. Cho et al. (21) reported p21 was not expressed in normal liver, but recent studies (18, 23) revealed its expression in normal liver, which corroborates in our results (Fig. 9). p57, a tight-binding CKI of several G1 cyclin/CDK complexes, is a strong G1 arrest regulator when overexpressed, and not regulated by p53 (31). p57 was not detected in normal liver, and p21, p27 and p57 all showed increased expression, especially in the terminal period during liver regeneration. These findings reveal that these inhibitors all contribute to reconstruction and holding. Ink families (p15, p16, p18, p19) were investigated and showed that p15 and p19 were not detected in normal liver and not induced by PH, and p16 and p18 were detected in normal liver and induced by PH. A recent report about p16 during liver regeneration showed unchanged expression both in normal liver and after PH till 28 hr (18). Our result showed rather decreased expression up till 1 day after PH, but revealed remarkable increase at 4 day and a maximum at 7 day. On the contrary, p18 which was lowly expressed in normal liver, showed two peaks, at 6 hr and 1 day after PH, corresponding to the first stage of cell division during liver regeneration, and decreased tendency at late period. These findings suggest that in the same family CKIs, p16 and p18 might have different roles at different periods, but may be important in the inhibition regulation.

There were little studies on cell cycle regulators expression in in situ liver tissue, but recently, intranuclear rearrangements of cyclin-CDK complexes during rat liver regeneration was reported (18). We could not find any hepatic zonal trends in in situ distribution of cyclins, CDKs and CKIs and they showed diffuse intranuclear and/or intracytoplasmic expression, with similar results to data from immunoblots. Noteworthy findings were intranuclear translocation of cyclin E at 6 hr after PH, which returned to cytoplasm at 1 day after PH (Fig. 3), even though the protein levels were unchanged and same intranuclear translocation was noted in p18 (Fig. 8). On the contrary, p16 showed nuclear translocation at 7 day when the protein expression was at its peak. These findings suggest that the roles of intracellular proteins need specific intracellular location and compartment for their functional activities.

In summary, we observed different expression levels and their in situ distribution with intracellular localization of cyclins, CDKs and CKIs during liver regeneration after PH, using immunoblot and immunohistochemistry.

We did not study kinase activity. Cyclin E was constitutively expressed and protein levels were not changed, but intranulcear translocation, immediately after PH was noted, indicating a possible role in nuclei. Cyclin A, not detected in normal liver, increased in the following periods after PH and reached a maximum at 7 day. CDK2 and 4 showed increased expression toward terminal period. Contradictory findings of cyclin A and these CDKs might play an important role in the inhibition of further cell division. Constitutively expressed CDK6 decreased at 1 day after PH. p16, p18, p21 and p27 were detected in normal liver, p18 showed peak expression within 1 day, and p16, p21, p27 and p57 were stronger at terminal periods. These findings suggest that individual CKI would like to act at different points in time in normal liver and during liver regeneration. During the expected period of their activity, intranuclear localization was observed in cyclin E, p18 and p16. There was no evidence of regional distribution in hepatic lobular architecture, instead, diffuse an in situ expression similar to results of immunoblot was found. This corroborates liver regeneration as synchronous events of nearly all hepatocytes, instead of stem cell proliferation and differentiation.

REFERENCES

1. Steer CJ. *Liver regeneration. FASEB J 1995; 9: 1396-400.*
2. 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.*
3. Fausto N, Webber EM. *Liver regeneration. In The Liver, Biology and Pathobiology. New York: Raven press, 1994: 1059-84.*
4. Loyer P, Glaise D, Carious S, Baffet G, Meijer L, Gaguen-Guillouzo C. *Expression and activation of CDKs (1 and 2) and cyclins in the cell progression during liver regeneration. J Biol Chem 1994; 269: 2491-500.*
5. Goldworthy TL, Goldworthy SM, Sprankle CS, Butterworth BE. *Expression of myc, fos and Ha-ras associated with chemically induced cell proliferation in rat liver. Cell Prolif 1994; 27: 269-78.*
6. Michalopoulos GK, DeFrances MC. *Liver regeneration. Science 1997; 276: 60-6.*
7. Pines J. *The cell cycle kinases. Sem Cancer Biol 1994; 5: 305-13.*
8. Van den Heuval S, Harlow E. *Distinct roles for cyclin-dependent kinases in cell cycle control. Science 1993; 262: 2050-4.*
9. Draetta G. *Mammalian G1 cyclins. Curr Opin Cell Biol 1994; 6: 842-6.*
10. King RW, Jackson PK, Kirschner MW. *Mitosis in transition. Cell 1994; 79: 563-71.*
11. Nurse P. *Ordering S phase and M phase in the cell cycle. Cell 1994; 79: 547-50.*

12. Sherr CJ. *G1 progression; Cycling on cue. Cell* 1994; 79: 551-5.

13. Heichman KA, Roberts JM. *Rules to replicate by. Cell* 1994; 79: 557-62.

14. Hunter T, Pines J. *Cyclins and cancer II: Cyclin D and CDK inhibitors come of age. Cell* 1994; 79: 573-82.

15. Sherr C, Roberts JM. *Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev* 1995; 9: 1149-63.

16. Peter M, Herkowitz I. *Joining the complex; Cyclin-dependent kinase inhibitory proteins and the cell cycle. Cell* 1994; 79: 181-4.

17. Grana X, Reddy EP. *Cell cycle control in mammalian cells: Role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). Oncogene* 1995; 11: 211-9.

18. Jaumot M, Estanyol JM, Serratosa J, Agel N, Bach O. *Activation of Cdk4 and Cdk2 during rat liver regeneration is associated with intranuclear rearrangements of cyclin-cdk complexes. Hepatology* 1999; 29; 385-95.

19. Laemmli UK. *Cleavage of structural protein during the assembly of the head of bacteriphage T4. Nature* 1970; 227: 680-3.

20. Loyer P, Caribou S, Glaise D, Bilodeau M, Baffet G, Guguen-Guillouzo C. *Growth factor dependence of progression through G1 and S phase of adult rat hepatocytes in vitro. J Biol Chem* 1996; 271: 11484-92.

21. Cho H, Lim IK, Lee JH. *Changes in the expression of cell cycle regulators during rat liver regeneration after partial hepatectomy. Exp Mol Med* 1996; 28: 187-91.

22. Rininger JA, Goldsworthy TL, Babish JG. *Time course comparison of cell-cycle protein expression following partial hepatectomy and WY14,643-induced hepatic cell proliferation in F344 rats. Carcinogenesis* 1997; 18: 935-41.

23. Ehrenfried JA, Ko TC, Thompson A, Evers BM. *Cell cycle-mediated regulation of hepatic regeneration. Surgery* 1997; 122: 927-35.

24. Ando K, Griffin JD. *CDK4 integrates growth stimulatory and inhibitory signals during G1 phase of hematopoietic cells. Oncogene* 1995; 10: 751-5.

25. Lu XP, Koch KS, Lew DJ, Dulic V, Pines J, Reed SI, Hunter T, Leffert HL. *Induction of cyclin mRNA and cyclin associated histone HI kinase during liver regeneration. J Biol Chem* 1992; 267: 2841-4.

26. Akbrecht JH, Hu MY, Cerra FB. *Distinct patterns of cyclin D1 regulation in models of liver regeneration and human liver. Biophys Res Commun* 1995; 209: 648-55.

27. Walker DH, Maller JL. *Role of the cyclin A in the dependence of mitosis on completion of DNA replication. Nature* 1991; 345: 314-7.

28. Pagano M, Repperkok R, Verde F, Ansorge W, Dreaetta G. *Cyclin A is required at two points in the human cell cycle. EMBO J* 1992; 11: 961-71.

29. Rosenblatt J, Gu Y, Morgan DO. *Human cyclin-dependent kinase 2 is activated during the S and G2 phases of the cell cycle and associates with cyclin A. Proc Natl Acad Sci USA* 1992; 89: 2824-8.

30. Chao Y, Shih YL, Chiu JH, Chau GY, Lui WY, Yang WK, Lee SD, Huang TS. *Overexpression of cyclin A but not skp 2 correlates with the tumor relapse of human hepatocellular carcinoma. Cancer Res* 1998; 58: 985-90.

31. Hengst L, Dulic V, Slingerland JM, Lees E, Reed SI. *A cell cycle-related inhibitor of cyclin-dependent kinases. Proc Natl Acad Sci USA* 1994; 91: 5291-5.