

行政院國家科學委員會專題研究計畫成果報告

以基因微陣列分析肝細胞再生時，特種基因及基因族群之基因表現在程度、型態、時程的變遷，並鑑別其扮演之角色

ANALYZING THE REGULATING GENES OF LIVER REGENERATION IN CHANGING DEGREE, PATTERN, TIMING AND VERIFYING THE ROLES OF SPECIFIC AND CLUSTER GENES BY cDNA MICROARRAY

計畫編號：NSC 92-2314-B-002-276

執行期限：92年8月1日至94年7月31日

主持人：賴鴻緒 台大醫學院 外科

中文摘要

有關肝細胞在肝損傷後可以再生，雖已是公認的事實，許多研究也證實多種營養素、賀爾蒙、生長因子、藥劑等，可直接或間接影響肝細胞再生，但肝細胞再生基因控制之詳細機轉，則仍不明瞭。

任何細胞之分裂與再生，必然與細胞核內 proto-oncogene 之表現有相當大的關聯，肝細胞再生也不例外。在多項以 mRNA 定量的研究報告中指出，肝臟經部分切除後，剩餘肝臟內某些基因，如 c-fos、c-myc、p53、p21、gas-6 及 ras 家族基因等的表現的確有增加現象。Arora 等人發現抗 c-myc 物質可經由 p-450 3A 活性之調控可抑制肝細胞再生；Ozeki 及 Tsukamoto 發現 retinoic acid 可抑制 c-fos 及 c-jun 之表現，促成肝細胞凋亡而使再生程度減少；而其他基因如 p21 及 gas-6 等之表現也曾被提出與肝細胞再生有關，但大量搜尋相關基因變化之研究，則尚未有報告。吾人初步以 384 點肝臟相關之 cDNA 基因微陣列 (microarray) 研究，發現肝細胞再生過程中，有 59 種基因表現明顯增加，19 種明顯降低，但因其變化程度、型態、時程與基因種別、基因

族群均十分複雜，無法清楚分析，其扮演之角色也尚難了解。更大量的 cDNA 基因微陣列搜尋，以進一步分析及了解基因管控之機轉仍屬必要。

本計劃以重約 200 克之 Wistar 雄性大鼠做實驗，測定肝細胞再生過程中，超過 6000 種確定基因種別之基因表現，並分析其變遷程度、型態、時程及各基因族群之相關性，以了解其在肝細胞再生中確實扮演之角色。所有大鼠均接受約百分之七十之肝臟部分切除手術，各於術前及術後 2、4、6、12、24、72 小時及 5、7、10 天後犧牲取樣，測定：(1)剩餘肝臟之重量比值；(2)剩餘肝臟之有絲分裂指標；(3)以基因微陣列尼龍膜(6144 identified cDNA clones, Wittech Co, Taipei, Taiwan) 肝細胞 mRNA 標號、hybridization 及影像分析等方法，測定 6144 種基因表現之變遷程度、型態及時程；(4)將基因表現變化大者，依特性分為免疫、賀爾蒙、生長因素、酵素、及血管新生因子等基因族群，並比較其程度、型態及時程之差異性。結果發現：(1)剩餘肝臟重量比值，於切肝術後 72 小時即恢復 90%以上；(2)有絲分裂於術後 48 小時大量出現，術後 72 小時逐漸減少；(3)所有基因

表現變遷之型態及時程共分為 72 種，包括第 2、6、12、24、72 小時及、7 天單一尖峰型、雙尖峰、遞增型、遞減型、突出型、凹陷型和混和型等，每種型態包括 40 至 218 種基因；(4)包括免疫、賀爾蒙、生長因子、酵素及血管新生因子等基因族群，均有明顯的變遷；(5) fas-associating protein with death domain, carnitine palmitoyltransferase 1, fas death domain-associating protein, 及 steroid O-acyltransferase 1 等早期變化基因可能與肝再生之啟動有關；(6) transforming growth factor beta 2 及 beta receptor 等中期變化基因可能與肝再生之分化有關；(7) TGF- β regulated gene 3 及 small inducible cytokine A2 等晚期變化基因可能與肝再生之終止有關。

關鍵字：肝細胞再生、部分肝臟切除術、proto-oncogene、基因微陣列、基因表現型態、基因族群

ABSTRACT

Although there are much controversy on the initiation, regulation, metabolic changes, and termination of liver regeneration after partial hepatectomy that well initiate proliferation of the remaining hepatocytes, several factors, such as hormones, growth factors, nutritional components, and pharmacological agents, have been demonstrated to directly or indirectly affect liver regeneration. However, the regenerative mechanism and genetic control of liver after major tissue loss is still not clear.

The regenerating liver is a system in which the relationships between proto-oncogene expression and cell replication should be examined during a physiologic growth response. Proto-oncogene expression after partial hepatectomy should be specific, sequential, and highly regulated. As measured by levels of mRNAs, the changes have been detected in the expression of c-fos, c-myc, p53, p21,

gas-6 and the ras gene family (c-Ha-ras, c-Ki-ras, and N-ras). In contrast, expression of c-src and c-abl does not change after partial hepatectomy while c-mos transcripts cannot be detected in normal or regenerating liver. Arora et al reported that c-Myc antisense limits rat liver regeneration by regulating cytochrome p-450 3A activity. Ozeki and Tsukamoto found that retinoic acid can repress c-fos and c-jun expression and induce apoptosis in regenerating liver. Our previous study monitored the variation of regulating genes by 384 liver-related gene cDNA microarray nylon membrane, and found that there are 59 proto-oncogenes expression increased markedly and 19 decreased significantly during liver regeneration. However, the changing degree, patterns, timing and gene grouping were very sophisticated and not clear. Mass survey and more detailed analysis by more cDNA microarray method should be very important.

Male Wistar rats around 200g will be used as subject. Partial hepatectomy around 70% were performed. They were sacrificed before and 2, 4, 6, 12, 24, 72 hours and 5, 7, 10 days after hepatectomy. We have measured: (1)weight of remnant liver; (2)mitotic index; (3)genomic survey of the gene expression by microarray of 6144 identified cDNA clones on nylon membrane (Witech Co., Taipei, Taiwan), labeling of liver mRNA hybridization and image analysis; and (4)Grouping of genes expression into immune, nutrition, hormone, growth factor, enzyme, oncologic and embryonic subgroups, and compare the expression degree, changing pattern and specific timing.

The results were: (1) the remnant liver weight increased to 90% in 72h after partial hepatectomy; (2) the mitosis of hepatocytes increased marked at 48h then decreased at 72 after partial hepatectomy; (3) analyzing the gene expression of microarray chips, the variation could be classified into 72 different patterns in cluding the patterns with a single peak at 2, 4, 6, 12, 24, 72h and 5, 7d after

partial hepatectomy; (4) gene clusters of immune, hormone, growth factor, enzyme and angiogenesis have changed markedly; (5) early stage changed genes including fas-associating protein with death domain, carnitine palmitoyltransferase 1, fas death domain-associating protein, and steroid O-acyltransferase 1 could be related to the initiation of liver regeneration; (6) intermediate stage changed genes including transforming growth factor beta 2 and beta receptor could be related to the differentiation of liver regeneration; (7) late stage changed genes including TGF- β regulated gene 3 and small inducible cytokine A2 could be related to the termination of liver regeneration.

Key words: liver regeneration, partial hepatectomy, proto-oncogene, microarray, genetic changing pattern, gene cluster

INTRODUCTION

Hepatocytes have a quiescent and highly differentiated phenotype. They rarely divide in adult humans or animals while remaining in the G₀-phase of the cell cycle. However, their capacity to replicate is not lost and is readily activated after liver resection or after injury induced by chemicals or drugs. Therefore, hepatocytes constitute a conditional renewal cell system that may proliferate in vivo under well-defined conditions.¹⁻³ It seems that liver "knows" when to start and when to stop growing, and thereby accurately regulates its mass.^{4,5} Partial hepatectomy triggers hepatocyte proliferation whereas excessive liver mass is regulated by apoptosis. The process of initiation and the control of the final size of the regenerated liver have been the subject of research for many years.⁶⁻⁹ Genetic regulation should have played an important role during the liver regeneration, however, the knowledge on the genetic mechanism is still limited.

It is reported that proto-oncogene expression after partial hepatectomy is

specific, sequential, and highly regulated.^{10,11} Changes have been detected in the expression of c-fos, c-myc, p53 and the ras gene family (c-Ha-ras, c-Ki-ras, and N-ras).¹²⁻¹⁴ In contrast, expression of c-src and c-abl does not change after partial hepatectomy while c-mos transcripts cannot be detected in normal or regenerating liver.^{15,16} Recently, p21 cyclin-dependent kinase (CDK) inhibitor, Fas, interleukin (IL)-18, and several caspases which increased apoptosis, and Bcl-2, heat shock proteins, glutathione-S-transferase genes which down regulated cell proliferation were noted to be involved in liver regeneration.¹⁷ D6.1A gene was proved relate to stimulation of cell proliferation and differentiation.¹⁸ Fox MIB transcription factor was proved contribute to the decline in liver regeneration in the aging process.¹⁹ Insulin like growth factor binding protein 1 (IGFBP-1), HURP mRNA were also noted involve in the process of liver regeneration.^{20,21} The mass survey about the variation of all the regulating proto-oncogenes expression according time sequence, which is not reported yet, should be very important for investigating the genetic mechanism of liver regeneration.

This study was conducted to find out the variation patterns of more than 6,000 regulating genes expressions by cDNA microarray during liver regeneration after partial hepatectomy in rats.

MATERIALS AND METHODS

Experimental Protocol

Sixty male Wistar rats (purchased from Charles River, Osaka, Japan) weighing approximately 200g were used as subjects. All of them received partial hepatectomy and they were sacrificed before and 2, 4, 6, 8, 12, 24, 48, 72 hours and 5, 7 days after hepatectomy. Six were sacrificed each time and the remnant livers were removed immediately for further tests.

Surgical Procedures

All rats are anesthetized by intraperitoneal pentobarbital (10mg/kg) injection. A midline laparotomy was performed. Partial hepatectomy was then carried out by means of aseptic extirpation of the median and left lateral lobes (around 70%) according to the procedure of Higgins and Anderson.⁹

Measurements

(1) Evaluation of the remnant liver

Observation of the liver surface and color. Then weighing the liver immediately after sacrifice, and the ratio of remnant liver weight/body weight will be calculated.

(2) Mitotic index of remnant liver

The small pieces of liver tissue for histopathological examination at certain postoperative period will be fixed in 10% neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin for microscopic observation. The mitotic index will be determined by counting the number of parenchymal cells undergoing mitosis in 50 randomly-selected fields under magnification $\times 400$.

(3) Genomic survey of remnant liver by cDNA microarray

a. Non-isotopic labeling of liver mRNA

Total RNA was extracted from remnant livers of sacrificed rats in each postoperative time sequence. The tissue was homogenized in 3 ml of solution A containing 4M guanidine thiocyanate, 0.5% sarcosyl, 25 mM sodium citrate, and 0.1M betamercaptoethanol at pH 7.0, followed by phenol extraction, isopropanol precipitation, and ethanol precipitation. Quality of RNA was examined by agarose gel electrophoresis. Messenger RNA was purified using Qiagen Oligotex extraction Kit.

b. Hybridization and image analysis of

microarray

The membrane containing 384 spots cDNA was pre-hybridized in 5 ml 1 \times hybridization buffer containing 5 \times SSC, 0.1% SDS, 1% BM blocking buffer (Boehringer Mannheim), and 10 ug/ml denatured salmon sperm DNA, at 60°C for 1 h. The probe was mixed with 2 ul of 10 ug/ul poly d(A) 10 and 2 ul of 10 ug/ul human Cot-1 DNA (Gibco BRL) and 40 ul of 2 \times hybridization buffer to a final volume of 80 ul, followed by denaturation of the probe mixture at 95°C for 5 min and then cooling on ice. The membrane was then washed with TBS buffer three times for 5 min each. The membrane was then treated with 5 ml X-gal substrate containing 1.2 mM X-gal, 1 mM MgCl₂, 3 mM K₃Fe(CN)₆, 3 mM K₄Fe(CN)₆ in TBS buffer for 45 min at 37°C with gentle shaking, followed by mini-Q water wash and air dry. Color image was generated using UMAX PowerLook 3000 flatbed scanner at a resolution 3048 dpi and processed by ScanAlyze.

Data analysis

The images captured by a scanner could be digitized by a commercial analysis software such as GenePix 3.0 (Axon instruments) or the program written in-house. These softwares are free and available from Stanford university and Massachusetts Institute of Technology once got the license permission for non-profit use.

RESULTS

The rats were living well during experimental stage. After sacrifice, the remnant liver weight increased to 90% in 72h after partial hepatectomy. The mitosis index showed that the mitosis of hepatocytes in the remnant liver increased to 104 \pm 12 at 48h, then decreased to 24 \pm 6 at 72h after partial hepatectomy.

The mRNA extracted from the remnant liver was collected at the indicated intervals

(before and 2, 4, 6, 12, 24, 48, 72 hours and 5, 7 days after partial hepatectomy) and analyzed by Northern blot hybridization as shown in Fig 1. The mRNA examined by agarose gel electrophoresis were well qualified in the 28s/18s ratio of 2, and the 260nm/280nm OD ratio of 2 by spectrophotometry.

The colorimetric image of cDNA microarray hybridization with 6,144 putative genes on the nylon membrane of the remnant liver before and 6, 24, 72 hours after partial hepatectomy were shown in Fig 2. The scattering of the scanned spots were not eventually even and the deep-colored spots were not much in the cDNA microarray chip from the normal liver tissue before hepatectomy. The spots increased in number and in the density on the chips at 6, 24, 72, hours after partial hepatectomy. The variation was uneven and not in a regular pattern.

When the microarray chip was analyzed by a flatted scanner and the GenePix in each time sequence, the variations of all the 6,144 proto-oncogenes expression could be classified into 72 different patterns (Fig.3). The various patterns of the genes expressions include the pattern containing a single peak which occurred at 2h (c29, 86 genes), 4h (c7, 80 genes), 6h (c0, 178 genes), 12h (c24, 117 genes), 48h (c64, 71 genes), 72h (c40, 73 genes), 5d (c69, 141 genes), and 7d (c44, 93 genes). In addition, typical double-peaks patterns occurred at different time sequence such as 12h, 5d (c16, 58 genes) and 6h, 5d (c68, 70 genes). Moreover, patterns showing increasing trend since 4h (c61, 65 genes) or patterns illustrating a decreasing trend since 4h (c21, 67 genes) were also noticed in some proto-oncogenes expressions. Beyond that, the protruding types of patterns from 12h to 24h (c25, 48 genes) and from 6h to 72h (c33, 56 genes), or the excavated types of patterns from 4h to 5d (c38, 98 genes) and 6h to 5d (c47, 101 genes; c55, 65 genes) were found. Mixed types of time-dependent curves were detected as other unclassified variation

patterns of genes expressions. The name, NCBI number and features of the chosen representative genes which were contained in the 72 patterns of genes expressions were listed in Table 1. Each category of gene expression pattern contained 40 to 218 identified proto-oncogenes.

The genes with a single peak at 2h (e.g. fas-associating protein with death domain, NCBI# NM_152937), 4h (e.g. carnitine palmitoyltransferase 1, NCBI# NM_031559), 6h (e.g. fas death domain-associating protein, NCBI# NM_007829), 12h (e.g. steroid O-acyltransferase 1, NCBI# NM_009230), and the genes with an enhancing trend (e.g. protein kinase, DNA activated catalytic polypeptide, NCBI# NM_011159), a protruding increasing curve (e.g. killer cell lectin-like receptor, NCBI# NM_012745), occurred before 24h after partial hepatectomy might be considered as the genes closely related G₀ to G₁ phase. The genes with a diminished trend (e.g. fibroblast growth factor 1, NCBI# NM_010197) or an excavated curve (e.g. glutathione-S-transferase alpha, NCBI# NM_017013) which occurred before 24h are also considered to be the down-regulation genes in this phase. These genes changed markedly before 24h might be considered as the initiation related genes of liver regeneration. Further verification by real time polymerase chain reaction (RT-PCR) or Western blot of these genes should be needed. As for the further process of cell cycles into S, G₂ and M periods, the triggered hepatocytes might just go on in a nature course. However, further study is needed to evaluate whether other genes involved in the whole cell cycles or not.

As for subgrouping the genes into clusters of immune, hormone, growth factor, enzyme and angiogenesis, many genes in these clusters were found to have markedly changed in different patterns. The name and changing pattern of these genes clusters were shown in Fig. 4-8.

DISCUSSION

The multistep process of liver regeneration constitutes at least 2 critical phases: the transition of the quiescent hepatocyte into the cell cycle (priming) and the progression beyond the restriction point in the G₁ phase of the cycle.² Four transcription factors, NFκB, STAT3 (both are strongly induced by TNF), AP-1, and C/EBP β are activated after partial hepatectomy and they may play important roles in the initiation of liver regeneration.²³⁻²⁶ Harber et al. proved that 70 genes were induced with relation to liver regeneration during 9 days after partial hepatectomy.²⁷ Since the initiation and termination of liver regeneration is a self-regulating growth process, the progression phase must be dependent on proto-oncogene regulatory mechanisms. Thereafter, mass survey and analysis of genes expression, although not reported yet, should be very important in the investigation of the genetic mechanism of liver regeneration.

Analysis of genes expression by cDNA microarray technology led to the identification of many regeneration-related genes expressions during liver regeneration stage after partial hepatectomy. We analyzed 6,144 genes according the time sequency after partial hepatectomy by PCR-amplified cDNA fragments and arraying machine, and found that a lot of genes changed there expressions in totally 72 patterns of changing curves. The extraction of mRNA in the regenerated liver was well qualified by both Northern blot hybridization and colorimetric image of microarray chips as shown in Fig.1 and Fig.2. The quantative analysis of time-dependent patterns of the genes expression by the computer showed the variation of patterns including a single peak at early, intermediate, late phase, or two separated peaks, a protruding curve, an excavated curve, ascending or descending trends with a different number of genes. It's hard to conclude how many or which genes

are involved in the regenerating mechanism. However, many of these genes play very important roles at some specific timing during the liver regeneration. The effects may be direct or indirect, enhanced or diminished.

Quantitative gene expression profiles as shown in this study proved that a lot of proto-oncogenes in the remnant liver were found to have their expressions changed and were classified into 72 categories of patterns according to the time sequence during liver regeneration from 2 hours to 7 days after partial hepatectomy. It should be an important implication for the further investigation about the genetic mechanisms and, furthermore, the gene therapy for enhancing the liver regeneration.

REFERENCES

1. George K, Marie C. Liver regeneration. *Science* 1997;276:60-6.
2. Kountouras J, Boura P, Lygidakis NJ. Liver regeneration after hepatectomy. *Hepato-Gastroenterology* 2001;48:556-62.
3. Sato Y, Koyama S, Tsukada K, Hatakeyama K. Acute portal hypertension reflecting shear stress as a trigger of liver regeneration following partial hepatectomy. *Surg Today* 1997;27:518-26.
4. Drixler TA, Vogten MJ, Ritchie ED, van Vroonhoven TJ, Gebbink MF, Voest EE, et al. Liver regeneration is an angiogenesis-associated phenomenon. *Ann Surg* 2002;236:703-11.
5. Gaglio PJ, Liu H, Dash S. Liver regeneration investigated in a non-human primate model (*Macaca mulatta*). *J Hepatol* 2002;37: 625-32.
6. Higgins GM, Anderson RM. Experimental pathology of the liver; I. Restoration of the liver by the white rat following partial surgical removal. *Arch Pathol* 1931;12:186-202.
7. Lai HS, Chen Y, Chen WJ. Carnitine contents in remnant liver, kidney and

- skeletal muscle after partial hepatectomy in rats: randomized trial. *World J Surg* 1998;22:42-7.
8. Court FG, Wemyss-Holden SA, Dennison AR, Maddern GJ. The mystery of liver regeneration. *Br J Surg* 2002;89:1089-95.
 9. Mitsue S, Hamanoue M, Tanabe G, Ogura Y, Yoshidome S, Aikou T, et al. Expression of HGF and TGF-beta 1 mRNA after partial hepatectomy in rats with liver cirrhosis. *Surg Today* 1995;25:237-43.
 10. Fausto N, Shank PR. Oncogene expression in liver regeneration and hepatocarcinogenesis. *Hepatology* 1983;3:1016-23.
 11. Fausto N, Mead JE, Braun L, Thompson NL, Panzica M, Goyette M, et al. Proto-oncogene expression and growth factors during liver regeneration. *Symp Fundam Cancer Res* 1986;39:69-86.
 12. Thompson NL, Mead JE, Braun L, Goyette M, Shank PR, Fausto N. Sequential protooncogene expression during rat liver regeneration. *Cancer Res* 1986;46:3111-7.
 13. Corral M, Paris B, Guguen-Guillouzo C, Corcos D, Kruh J, Defer N. Increased expression of the N-myc gene during normal and neoplastic rat liver growth. *Exp Cell Res* 1988;174:107-15.
 14. Fausto N. Hepatic regeneration. In: Zakim D, Boyer TD eds. *Hepatology*. Philadelphia, W.B. Saunders Co. 1990:Pp 49-65
 15. Fausto N, Shank PR. Analysis of proto-oncogene expression during liver regeneration and hepatic carcinogenesis. In: Okuda K, Ishak KG, eds. *Neoplasms of the liver*. New York, Springer-Verlag, 1987:Pp57-61.
 16. Goyette M, Petropoulos CJ, Shank PR, Fausto N. Regulated transcription of c-Ki-ras and c-myc during compensatory growth of rat liver. *Mol Cell Biol* 1984;4:1493-8.
 17. Morita T, Togo S, Kubota T, Kamimukai N, Nishizuka I, Kobayashi T, et al. Mechanism of postoperative liver failure after excessive hepatectomy investigated using a cDNA microarray. *Hepat Biliar Pancr Surg* 2002;9:352-9.
 18. Tanaka F, Hori N, Sato K. Identification of differentially expressed genes in rat hepatoma cell lines using subtraction and microarray. *J Biochem* 2002;131:39-44.
 19. Wang X, Quail E, Hung NJ, Tan Y, Ye H, Costa RH. Increased levels of forkhead box M1B transcription factor in transgenic mouse hepatocytes prevent age-related proliferation defects in regenerating liver. *Proceed Nat Aca Sci USA* 2001;98:11468-73.
 20. Leu JI, Crissey MA, Craig LE, Taub R. Impaired hepatocyte DNA synthetic response posthepatectomy in insulin-like growth factor binding protein 1-deficient mice with defects in C/EBP beta and mitogen-activated protein kinase/extracellular signal-regulated kinase regulation. *Mol Cell Bio* 2003;23:1251-59.
 21. Tsou AP, Yang CW, Huang CY, Yu RC, Lee YC, Chang CW, et al. Identification of a novel cell cycle regulated gene, HURP, overexpressed in human hepatocellular carcinoma. *Oncogene* 2003;22:298-307.
 22. Chen JJ, Peck K, Hong TM, Yang SC, Sher YP, Shih JY, et al. Global analysis of gene expression in invasion by a lung cancer model. *Cancer Res* 2001;61:5223-30.
 23. FitzGerald M, Webber E, Donovan J, Fausto N. Rapid DNA binding by nuclear factor κ B in hepatocytes at the start of liver regeneration. *Cell Growth Diff* 1995;6:417-27.
 24. Cressman DE, Diamond RH, Taub R. Rapid activation of the Stat3 transcription complex in liver regeneration. *Hepatology* 1995;21:1443-1449.
 25. Greenbaum LE, Li W, Cressman DE, Peng Y, Ciliberto G, Poli V, et al. CCAAT enhancer-binding protein beta is required for normal hepatocyte proliferation in mice after partial

- hepatectomy. *J Clin Invest* 1998;102:996-1007.
26. Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signaling through the gp130/Jak/STAT pathway. *Biochem J* 1998;334:297-314.
27. Haber BA, Mohn KL, Diamond RH, Taub R. Induction patterns of 70 genes during nine days after hepatectomy define the temporal course of liver regeneration. *J Clin Invest* 1993;91:1319-2

Table 1. Representative genes contained in the 72 patterns of genes expressions*

Pattern	Name	NCBI number	Features		
			Source	Gene	CDS
0	fas death domain-associated protein	NM_007829	Mus musculus (house mouse)	1..2360	25..2244
1	myelocytomatosis oncogene	BC006728	Mus musculus (house mouse)	1..1799	101..1420
2	MEK binding partner 1 (Mp1) mRNA, complete cd	AF082526	Mus musculus (house mouse)	1..1315	147..521
3	myeloid cell leukemia sequence 1	NM_008562	Mus musculus (house mouse)	1..3464	76..1071
4	Bcl2-like 1	NM_009743	Mus musculus (house mouse)	1..1466	103..804
5	epidermal growth factor receptor	NM_031507	Rattus norvegicus (Norway)	1..4161	154..3783
6	IL2-inducible T-cell kinase	NM_010583	Mus musculus	1..4294	93..1970
7	carnitine palmitoyltransferase 1	NM_031559	Rattus norvegicus (Norway)	1..4377	103..2424
8	RAS p21 protein activator 1	NM_145452	Mus musculus	1..2626	1..2442
9	oncprotein induced transcript	NM_146050	Mus musculus (house mouse)	1..1299	257..928
10	small inducible cytokine A1	AF065928	Mus musculus (house mouse)	1..>276	48..>276
11	clusterin	BC061534	Rattus norvegicus (Norway)	1..1678	77..1420
12	apoptosis inhibitor	BC062055	Rattus norvegicus (Norway)	1..2907	643..2412
13	insulin-like growth factor 1	NM_184052	Mus musculus (house mouse)	1..2170	1344..184
14	pyruvate dehydrogenase	BC002188	Mus musculus (house mouse)	1..1311	1..964
15	interleukin 1 receptor accessory protein	NM_134103	Mus musculus (house mouse)	1..1916	133..1215
16	v-src suppressed transcript 5	XM_356456	Mus musculus (house mouse)	1..552	1..552
17	cadherin	NM_199470	Mus musculus (house mouse)	1..2783	216..2561
18	glutamic acid decarboxylase	NM_008077	Mus musculus (house mouse)	1..3198	185..1966
19	cytochrome P450, 2f	NM_019303	Rattus norvegicus (Norway)	1..1768	13..1488
20	fibroblast growth factor receptor 1	BC033447	Mus musculus (house mouse)	1..2877	29..2224
21	fibroblast growth factor 1	NM_010197	Mus musculus (house mouse)	1..3404	189..656
22	fos-like antigen	NM_008037	Mus musculus (house mouse)	1..5825	171..1151
23	cell death-inducing DNA fragmentation factor,	NM_009894	Mus musculus (house mouse)	1..1167	121..780
24	sterol O-acyltransferase 1	NM_009230	Mus musculus (house mouse)	1..3697	810..2432
25	killer cell lectin-like receptor, subfamily D, member	NM_012745	Rattus norvegicus (Norway)	1..1118	263..802
26	histocompatibility 2, Q region locus 1	NM_010390	Mus musculus (house mouse)	1..1092	1..1092
27	sterol carrier protein 2, liver	BC018384	Mus musculus (house mouse)	1..2626	56..1699
28	integrin alpha 2	BC065139	Mus musculus (house mouse)	1..4235	109..3645
29	fas-associating protein with death domain	NM_152937	Rattus norvegicus (Norway)	1..1556	64..690
30	Fyn proto-oncogene	NM_012755	Rattus norvegicus (Norway)	1..1844	231..1844
31	leukotriene A4 hydrolas	NM_008517	Mus musculus (house mouse)	1..2039	81..1916
32	ribosomal protein S2	BC002186	Mus musculus (house mouse)	1..954	19..900
33	tumor necrosis factor induced protein	NM_182950	Rattus norvegicus (Norway)	1..2123	172..1122
34	interleukin 4 receptor, alpha	NM_010557	Mus musculus (house mouse)	1..3697	237..929
35	glycerol kinase	NM_024381	Rattus norvegicus (Norway)	1..2989	100..1674
36	insulin-like growth factor binding protein 4	XM_340897	Rattus norvegicus (Norway)	1..1390	245..1009
37	cytochrome P450, 2d9	NM_010000	Mus musculus (house mouse)	1..1877	26..1501

38	glutathione-S-transferase, alpha	NM_017013	Rattus norvegicus (Norway)	1..831	64..732
39	histocompatibility 2, L regio	BC023409	Mus musculus (house mouse)	<1..151	<1..1001
40	transforming growth factor, beta receptor	NM_031132	Rattus norvegicus (Norway)	1..2080	252..1955
41	oncostatin M receptor	NM_011019	Mus musculus (house mouse)	1..4792	96..3011
42	ubiquitin-conjugating enzyme E2H	NM_009459	Mus musculus (house mouse)	1..2828	242..793
43	histocompatibility 2, class II, locus DM	NM_010386	Mus musculus (house mouse)	1..1135	44..829
44	small inducible cytokine A2	NM_031530	Rattus norvegicus (Norway)	1..780	76..522
45	galactosidase, alph	NM_013463	Mus musculus (house mouse)	1..2801	25..1284
46	RAS, guanyl releasing protein	AF060819	Rattus norvegicus (Norway)	1..2883	7..2394
47	fumarylacetoacetate hydrolase	NM_017181	Rattus norvegicus (Norway)	1..1386	23..1282
48	TNF-related apoptosis inducing ligan	AY115578	Rattus norvegicus (Norway)	-	10..873
49	lipase, hepatic (Lipc)	NM_008280	Mus musculus (house mouse)	1..1934	249..1781
50	RNA polymerase 1-3 (16 kDa subunit)	NM_181730	Mus musculus (house mouse)	1..914	102..482
51	platelet derived growth factor receptor, alpha	NM_011058	Mus musculus (house mouse)	1..6576	190..3459
52	interferon regulatory factor 3	NM_016849	Mus musculus (house mouse)	1..1915	132..1391
53	Jun oncogene	NM_010591	Mus musculus (house mouse)	1..3135	917..1921
54	CD4 antigen	BC039137	Mus musculus (house mouse)	1..3082	149..1522
55	CD36 antigen (collagen type I receptor,	NM_054001	Rattus norvegicus (Norway)	1..1938	231..1667
56	interleukin 7 receptor	NM_008372	Mus musculus (house mouse)	1..3116	48..1427
57	peptidase 4	NM_008820	Mus musculus (house mouse)	1..1876	55..1536
58	C-src tyrosine kinase	NM_007783	Mus musculus (house mouse)	1..2292	249..1601
59	Large multifunctional protease 7	NM_010724	Mus musculus (house mouse)	1..828	1..828
60	interleukin 15 receptor, alpha chain	NM_133836	Mus musculus (house mouse)	1..1465	367..738
61	protein kinase, DNA activated catalytic polypeptide	NM_011159	Mus musculus (house mouse)	1..1265	1..12387
62	carbonyl reductase 4	NM_182672	Rattus norvegicus (Norway)	1..1188	106..816
63	FK506 binding protein 1a	NM_008019	Mus musculus (house mouse)	1..1556	98..424
64	transforming growth factor, beta 2	BC011170	Mus musculus (house mouse)	1..1741	127..1371
65	xanthine dehydrogenas	NM_017154	Rattus norvegicus (Norway)	1..4198	27..4022
66	tumor susceptibility gene 101	NM_181628	Rattus norvegicus (Norway)	1..1176	1..1176
67	guanine nucleotide binding protein (G protein),	NM_025278	Mus musculus (house mouse)	1..1151	235..453
68	squalene epoxidase	NM_009270	Mus musculus (house mouse)	1..2611	620..2338
69	transforming growth factor beta regulated gene 3	NM_178871	Mus musculus (house mouse)	1..2822	244..660
70	casein kinase II, alpha 2, polypeptide	NM_009974	Mus musculus (house mouse)	1..1877	344..1396
71	macrophage activation 2	NM_008620	Mus musculus (house mouse)	1..3295	90..1961

NCBI, national Center for Biotechnology Information; *, only one representative was listed in each pattern which may contained 40 to 218 identified proto-oncogenes

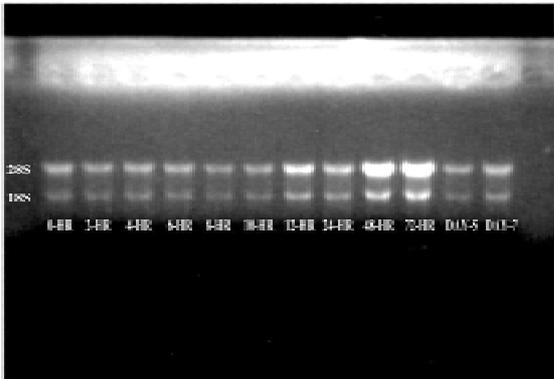


Fig 1. The mRNA extracted from the remnant liver showed a qualified picture by agarose gel electrophoresis.

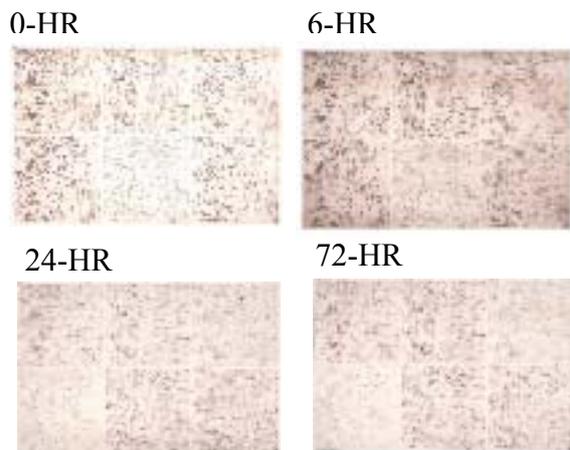


Fig 2. The colorimetric image of cDNA microarray hybridization chips with 6,144 genes showed uneven changed patterns before (0-HR), and 6, 24, 72 hours after partial hepatectomy.

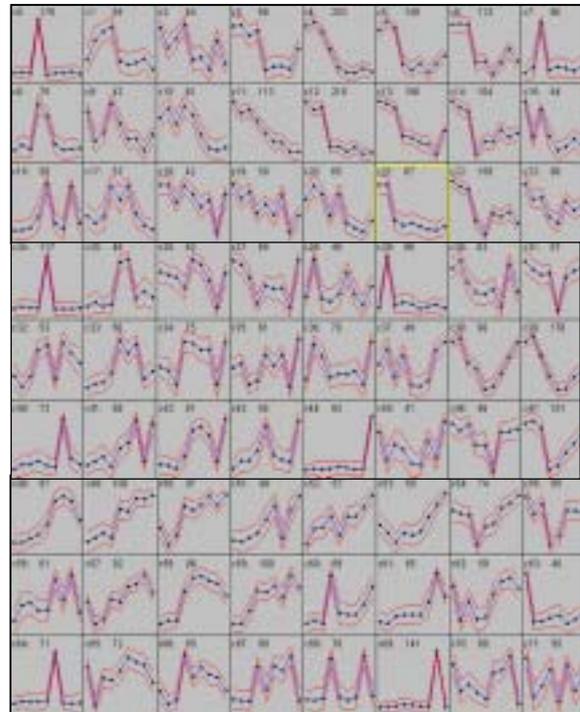


Fig 3. Seventy-two different patterns of genes expressions in the remnant liver during liver regeneration according the time sequence of 0, 2, 4, 6, 12, 48, 72 hours and 5, 7 days after partial hepatectomy (C= sequence number of categories; the following number= numbers of genes included in this category; the longitudinal line is the intensity of gene expression; the longitudinal line is the intensity of gene expression).

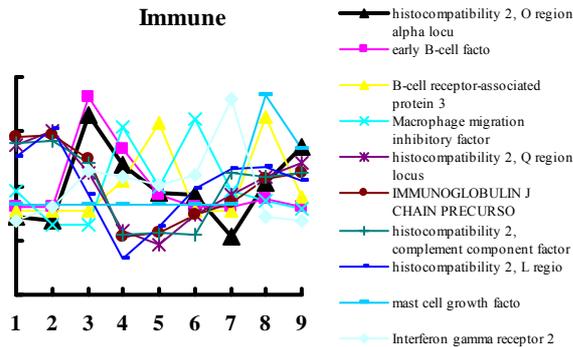


Fig 4. The names and their changing patterns of immune related genes.

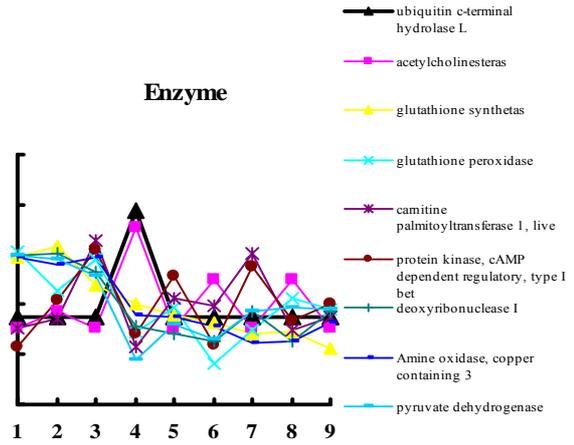


Fig 7. The names and their changing patterns of enzyme related genes.

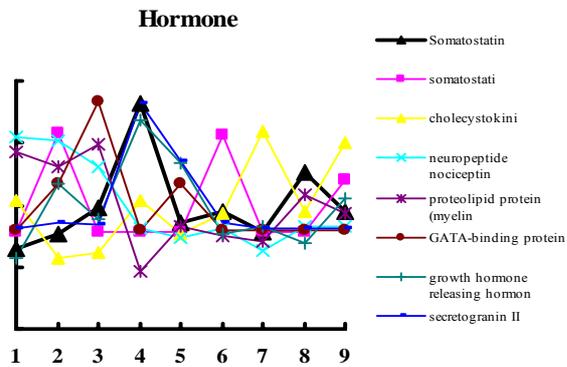


Fig 5. The names and their changing patterns of hormone related genes.

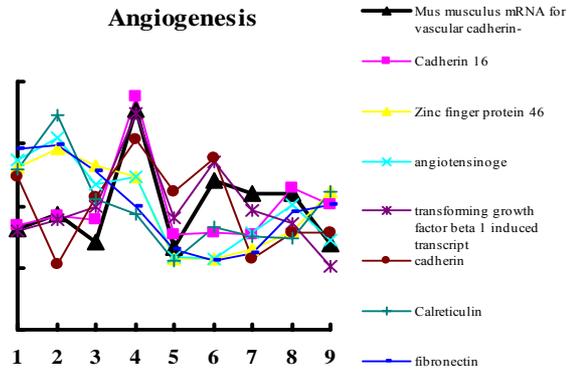


Fig 8. The names and their changing patterns of angiogenesis related genes

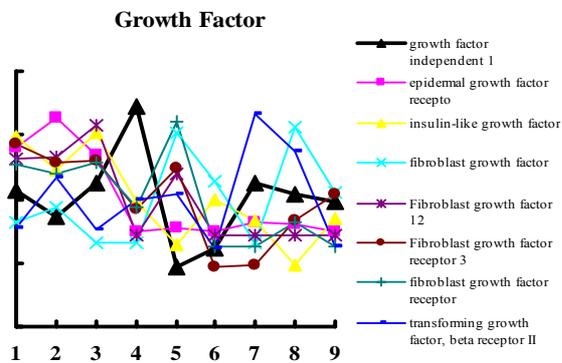


Fig 6. The names and their changing patterns of growth factor related genes.

