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(2/2)

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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 (2/2)

ANALYZING THE REGULATING GENES OF LIVER REGENERATION IN CHANGING DEGREE, PATTERN, TIMING AND VERIFYING THE ROLES OF SPECIFIC AND CLUSTER GENES BY cDNA MICROARRY (2/2)

計畫編號：NSC 93-2314-B-002-056

執行期限：93 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。

本計畫第一年以大鼠切除 70%肝臟模式研究發現：(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。進一步以切除較少(40%)肝臟比較其程度差異，以及比對各種基因之重要性，則於第二年進行。

本計劃第二年仍以重約 200 克之 Wistar 雄性大鼠做實驗，測定肝細胞再生過程中，超過 20000 種基因種別之基因表現，並分析及比較上一年 70%切肝後，其變遷程度、型態、時程及各基因族群之相關性。所有大鼠均接受約百分之四十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，仍測定：(1)剩餘肝臟之重量比值；(2)剩餘肝臟之有絲分裂指標；(3)以基因微陣列高密度晶片、肝細胞 mRNA 標號、hybridization 及影像分析等方法，測定 20,500 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、型態及時程與 70%切肝時，變化之差異性。結果發現：(1)剩餘肝臟重量比值，切肝 40%後恢復速度較慢，但至術後 72 小時，40%與 70%兩組幾乎到達同樣重量；(2)有絲

分裂切肝 40%後 48 小時也出現，至 72 小時逐漸減少，但程度上比 70%切肝後明顯較少；(3)基因表現變遷之種類與型態，與 70%切肝組類似，亦為 72 種型態，部分基因變化程度雖較低，但變化型態相近；(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)免疫相關基因(如 IL-6 和 IL-10)及血管新生相關基因(如 Angiotensiongen, VEGF, 及 VEGF receptor 2)之變化 40%與 70%切肝後相近於重量比值變化，可能在肝細胞再生之調控，扮演較重要的角色。

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.

We used 70% portal hepatectomized rat model for studying genes variation after partial hepatectomy, and found that: (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 72h 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. Study with 40% partial hepatectomy was performed in the second year.

Male Wistar rats around 200g will be used as subject. Partial hepatectomy around 40% 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 chip of 20,500 identified cDNA clones, 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 recovered slower, but can reach 90% in 72h after partial hepatectomy; (2) the mitosis of hepatocytes also increased markedly at 48h although not so high as 70% group rats, and also decreased at 72h after partial hepatectomy; (3) analyzing the gene expression of microarray chips, the variation of 40% group rats could also be classified into 72 patterns just like 70% group, with some variation degree were not so marked as 70% group rats; (4) gene clusters of immune, hormone, growth factor, enzyme and angiogenesis have changed markedly; (5) The changes of gene expression in immune related genes (such as IL-6 and IL-10 control genes) and angiogenesis related genes (such as Angiotensiongen, VEGF, and

VEGF-receptor 2) were more similar to the remnant liver weight variations. It may indicate that these genes play more important roles in the control mechanism 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 G_0-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.

We used 70% portal hepatectomized rat model for studying genes variation after partial hepatectomy, and found that: (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 72h 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. Study with 40% partial hepatectomy was performed in the second year.

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 inceased 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 compare the variation patterns of 20,500 regulating genes expressions by cDNA microarray chip

during liver regeneration after 40% vs 70% 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 40% 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 left lateral lobes (around 40%) according to the procedure of Higgins and Anderson.[9] The removed liver sample was immediately weighed. Laparotomies with manipulation of liver were done in the sham operated rats. All of the surgeries were performed between 8 am and 11 am to reduce the influence of diurnal variation.

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 hisopathological examination at certain postoperative period will be fixed in 10% neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin for microscpic observation. The mitotic index will be determined by counting the number of parenchymal cells undergoing mitosis in 50 randomly-selected fields under magnification ×400. The results will be expressed as the mitotic index (the total number of mitoses per 50 different fields examined).

(3)Genomic survey of remnant liver by cDNA microarray chips.

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.

Five micrograms of mRNA was annealed with 6 uM random hexamer in a total volume of 50 ul. The cDNA synthesis was performed in a 100 ul mixture containing 0.5 mM each dATP, dCTP, dGTP: 40 uM dTTP, 40 uM biotin-16-dUTP (Boehringer Mannheim), 10 mM DTT; 0.5 units/ul Human Placental Ribonuclease Inhibitor (HT Biotechnology Ltd., UK), and 200 units of Superscript RT II (GIBCO-BRL, Gaithersburg, MD). The mixture was incubated for 90 min at 42ºC and terminated by heating at 90ºC for 5 min. The RNA was degraded by addition of 5.5 ul of 3N NaOH and incubated at 50ºC for 30min. The mixture was neutralized by addition of 5.5 ul of 3M acetic acid and precipitated by addition of 50 ul of 7.5M ammonium acetate, 20 ug of linear polyacrylamide, and 1125 ul ethanol. The pellet was suspended in 36 ul deionized water.

b. Hybridization and image analysis of microarray

The chip containing 20,500 spots cDNA was pre-hybridized in 5 ml 1×hybridization

buffer containing 5×SSC, 0.1% SDS, 1% BM blocking buffer (Boehringer Mannheim), and 10 ug/ml denatured salmon sperm DNA, at 60ºC 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×hybridization buffer to a final volume of 80 ul, followed by denaturation of the probe mixture at 95ºC for 5 min and then cooling on ice. The membrane was annealed with the probe mixture in a hybridization chamber, incubated at 95ºC for 5 min, and then at 58ºC for 12-16h. The membrane was washed twice with 5 ml of 2×SSC, 0.1%SDS for 5 min at room temperature, followed by three washes for 15 min each with 5 ml of 0.1×SSC, 0.1% SDS at 58ºC. The membrane was blocked with 5 ml of 1% BM blocking reagent containing 2% dextran sulfate at room temperature for 1 h, followed by incubation with 5 ml mixture containing 700×diluted Streptavidin-β-galactosidase (1.38 U/ml, enzyme activity) (Gibco BRL), 4% polyethylene glycol 8000 (Sigma), and 0.3% BSA in TBS buffer (10 mM Tris, pH 7.4, 150 mM NaCl) at room temperature for 1 hour. 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 $MgCl_2$, 3 mM $K_3Fe(CN)_6$, 3 mM $K_4Fe(CN)_6$ in TBS buffer for 45 min at 37ºC 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. The pre-treated data will be then clustered by hierarchical method or self-organizing maps. 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. However, the growing speed is not so fast as 70% group rats when 40% partial hepatectomy was done (Fig. 1). The mitosis index showed that the mitosis of hepatocytes in the remnant liver increased to 64±8.8 at 48h, then decreased to 20±4.6 at 72h after 40% partial hepatectomy. The elevation was also not as high as that in 70% group rats (Table 1).

When the microarray chip was analyzed by a flatted scanner and the GenePix in each time sequence, the variations of all the 20,500 proto-oncogenes expression could be classified into 72 different patterns as previous report. The various patterns of the genes expressions include the pattern containing a single peak which occurred at 2h, 4h, 6h, 12h, 48h, 72h, 5d, and 7d. In addition, typical double-peaks patterns occurred at different time sequence such as 12h, and 5d after partial hepatectomy. Moreover, patterns showing increasing trend since 4h or patterns illustrating a decreasing trend since 4h were also noticed in some proto-oncogenes expressions. Beyond that, the protruding types of patterns from 12h to 24h and from 6h to 72h, or the excavated types of patterns from 4h to 5d and 6h to 5d were found. Mixed types of time-dependent curves were detected as other unclassified variation patterns of genes expressions. The changing patterns are about the same when compared 40% vs 70% groups rats, however, the changing degrees was not so high in 40% partial hepatectomized rats. The name, NCBI number and features of the chosen representative genes which were contained in the 5 categories (including immune related, hormone related, growth factor related, enzyme related, and angiogenesis related) of genes expressions were listed in Table 2. Each category of gene expression pattern contained 8 to 13 identified proto-oncogenes.

Among the immune related genes, the

changing degrees in 40% group rats were not so marked but more stable when compared with 70% group rats (Fig. 2). Histocompatibility locus class II region was highly elevated at 3d after 40% partial hepatectomy that was high at 6h and 7d, but not 3d after 70% partial hepatectomy. That means this gene might be not directed related to the control mechanism of liver regeneration. As for IL-6 and IL-10 genes, the time sequence changes and changing patters are similar in 40% vs 70% groups rats, with a milder degree in 40% group rats.

Hormone related genes showed an almost flat changing curve in 40% group rats (Fig. 3). Although the changes of these genes were markedly variable in 70% group rats, they may not be considered as contral genes because that the patter was not similar between 40% and 70% group rats.

Growth factor related genes as shown at Fig. 4. Although growth factor independent 1, fibroblast growth factor and transforming growth factor had specific changes respectively after 70%, there were no change after 40% partial hepatectomy.

Enzyme related genes had wider ranging flat changing curves after 40% portial hepatectomy (Fig. 5). Ubiquitin elevated a little, and glutathione peroxidase dedreased a little during liver regeneration period. However, the elevated peaks which appeared at 70% group rats had never seen in 40% group rats.

Angiogenesis related genes had changing curves with single peak elevated occurred on angiotension gene both after 40% and 70% partial hepatectomy (Fig. 6). VEGF and VEGF receptor 2 genes also had similar elevated peak after 40% and 70% partial hepatectomy. Zinc finger protein gene demonstrated a wide based elevation after 40% but not 70% partial hepatectomy.

DISCUSSION

For the gene expressions during liver regeneration, several proto-oncogenes with some chemical agents involved in mechanism during liver regeneration were reported[22-24]. Arora et al reported that c-Myc antisense limits rat liver regeneration by regulating cytochrome p-450 3A activity[22]. Ozeki and Tsukamoto found that retinoic acid can repress c-fos and c-jun expression and induce apoptosis in regenerating liver[25]. Some proto-oncogenes about angiogenesis reaction such as insulin-like growth factor II [26], and TNP-470 angiogenesis inhibitor[27] were also detected to be involved in the regulation during the process of liver regeneration. Liver regeneration might be regulate by expression of angiogenesis related proto-oncogenes concerned about VEGF changes[9]. However, mass survey and more detailed analysis about immune related genes by microarry method, that will be very important, are still not reported.

In our preliminary study of gene expression during liver regeneration, we monitored the variation of regulating genes by selected liver-related genes cDNA microarry nylon membrane. The results showed that there were fifty-nine regulating proto-oncogenes expression increased markedly and nineteen regulating proto-oncogenes expression decreased significantly during liver regeneration after partial hepatectomy in rats. However, the roles of these regulating genes can't be verified because the changing pattern and timing were varied a lot. We repeated the study by cDNA microarry membrane carrying 6144 proto-oncogenes identified and PCR-amplified by cDNA clones, the results showed many more genes had changed significantly during liver regeneration. The changing patterns could be categorized in seventy-two patterns.

There were twenty-two immune related genes were detected with different variations during the time sequence after partial hepatectomy. Among these 22 genes, ten of them had marked changes in six different kinds of patterns in changing curve with

quantitative hybridized proto-oncogenes expression with different degree and timing. We have selected γIFN receptor 2 (γIFN-R2) for trying verification by RT-PCR and Western Blot, and the results were shown in Fig. 6,7,8 and 9. The preliminary data showed that γIFN-R2 transcripted mRNA was strongly enhanced when compared with the index by accounting the relative quantitation of γIFN-R2 against GAPDH expression as a controlled base. The melting peak of γIFN-R2 was also showed a unique peak which demonstrated a definite amplification product of the detected cDNA, and can be a good indicator for further PT-PCR analysis according time sequence and Western Blot analysis.

As for angiogenesis vs liver regeneration, we have proved that there were thirty-two angiogenesis related genes detected with different variations during the time sequence after partial hepatectomy. Among these 32 genes, eleven of them had marked changes and could be classified into six different kinds of patterns in changing curve with quantitative hybridized proto-oncogenes expression with different degree and timing. We also detected some enhanced expressed angiogenesis related genes, such as ①insulin-like growth factor (ILGF) binding protein 1, ②fibroblast growth factor (FGF) binding protein, ③metalloproteinase 2, ④ephrin A, ⑤laminin-beta, ⑥transforming growth factor (TGF)β-2, ⑦transforming growth factor (TGF)β-2 receptor, ⑧TGF regulated gene, ⑨Leukotriene C4 synthase, ⑩placenta growth factor, ⑪TGFB inducible protein, ⑫ VEGF. To acknowledge whether the changes of these angiogenesis related genes are truly effective, further verification by real time PCR (RT-PCR) for RNA expression, and Western Blot for protein products variation is necessary to find out the compatible reactions of these angiogenesis related genes which really involved in the mechanism of liver regeneration.

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Table 1. Mitotic Index after 40% and 70% PH

Group \ Time	6H	24H	48H	72H
40% PH	0	Rare	64±8.8	20.6±4.6
70% PH	0	Rare	104.1±12.2*	24.2±6.2
C	0	Rare	0	0

Mean±SD; Sum of 50 randomized field under 400x; C: control; H: hour; PH: Partial hepatectomy; *: vs 40%PH, $p<0.05$

Table 2. Gene Expressions in Time Sequence after 40% vs 70% Partial Hepatectomy

Genes\Time	40%2h / 70%2h	40%4h / 70%4h	40%6h / 70%6h	40%12h / 70%12h	40%24h / 70%24h	40%3d / 70%3d	40%7d / 70%7d
Immune related							
histocompatibility locus class II region	1.177 / 0.875	0.893 / 0.910	1.262 / 0.921	1.194 / 0.910	1.260 / 1.122	1.677 / 1.447	1.398 / 1.279
early B-cell factor	0.954 / 1.109	1.043 / 1.058	0.772 / 0.801	1.033 / 1.028	0.964 / 0.883	1.172 / 0.994	1.032 / 0.927
B-cell receptor-associated protein 29	1.438 / 1.506	1.033 / 1.377	1.244 / 1.283	1.339 / 1.626	1.186 / 1.838	1.134 / 1.146	1.118 / 1.064
macrophage migration inhibitory factor	0.991 / 0.996	1.067 / 1.015	1.341 / 1.103	1.020 / 1.144	1.311 / 1.508	1.316 / 1.111	1.125 / 1.137
histocompatibility antigen H13 isoform 1	0.808 / 0.871	1.000 / 0.974	1.117 / 1.084	1.040 / 1.034	1.081 / 0.875	1.067 / 1.064	1.109 / 1.070
immunoglobulin J chain homologPartial	1.085 / 0.909	1.042 / 1.224	1.484 / 0.955	0.937 / 1.011	1.131 / 0.837	1.232 / 1.053	1.090 / 0.911
histocompatibility class II antigen-associated	0.956 / 0.980	0.961 / 1.018	0.886 / 0.834	0.979 / 0.800	1.017 / 0.789	1.198 / 1.059	0.982 / 1.054
histocompatibility locus class II,O region	1.177 / 0.875	0.893 / 0.910	1.262 / 0.921	1.194 / 0.910	1.260 / 1.122	1.677 / 1.447	1.398 / 1.279
mast cell growth factor	1.318 / 1.301	1.231 / 1.278	1.026 / 1.158	1.055 / 1.098	1.025 / 1.681	1.146 / 1.073	0.976 / 1.081
interferon gamma receptor	0.780 / 0.923	0.929 / 1.062	1.362 / 1.174	0.885 / 1.171	0.950 / 0.906	1.050 / 1.241	1.246 / 1.278
Hormone related							
huntingtin-associated protein interacting protein	1.138 / 1.101	1.341 / 1.136	1.153 / 0.927	1.183 / 1.086	1.097 / 0.743	1.199 / 1.211	1.293 / 0.918
Somatostatin	0.614 / 0.586	0.840 / 0.769	1.133 / 1.250	1.144 / 1.036	1.105 / 0.966	1.080 / 1.181	1.115 / 1.211
Cholecystokinin B receptor (Cckbr), mRNA.	0.958 / 0.896	1.059 / 0.930	0.933 / 1.017	0.983 / 0.907	0.976 / 0.772	0.930 / 0.927	1.003 / 0.984
Prepronociceptin (neuropeptide nociceptin) (N23K) (Pnoc)	1.046 / 1.148	1.095 / 1.108	1.089 / 1.125	1.019 / 1.069	1.055 / 0.949	1.013 / 1.095	1.000 / 1.024
Proteolipid protein(Plp1), mRNA.	1.076 / 0.875	1.042 / 1.033	0.876 / 0.767	0.761 / 0.794	0.645 / 0.630	0.789 / 0.765	0.928 / 0.882
GATA-binding protein 1 (globin transcription factor 1)	0.895 / 0.857	0.865 / 0.948	1.144 / 1.064	0.881 / 0.851	1.062 / 0.891	0.933 / 0.900	1.051 / 1.040
growth hormone releasing hormone (Ghrh), mRNA.	1.076 / 1.062	1.069 / 0.992	0.980 / 0.954	0.878 / 0.848	0.984 / 0.685	0.977 / 0.993	1.016 / 1.036
Secretogranin II (Scg2), mRNA.	0.876 / 1.017	0.912 / 1.226	1.118 / 1.622	1.059 / 0.844	1.083 / 1.296	1.587 / 0.824	0.922 / 1.286
Growth factor related							
Growth factor independent-1 (Gfi1), mRNA.	0.861 / 0.895	1.056 / 0.968	1.008 / 0.838	1.103 / 0.960	1.116 / 1.016	1.124 / 0.999	1.024 / 1.002
epidermal growth factor receptor related protein (Errp)	1.012 / 1.018	1.351 / 0.985	0.948 / 1.019	1.066 / 1.063	1.236 / 1.214	0.859 / 1.041	0.963 / 0.860
Insulin-like growth factor II (Igf2), mRNA.	1.013 / 0.993	0.986 / 0.961	0.988 / 0.890	1.087 / 1.038	1.091 / 0.780	0.998 / 1.053	1.101 / 1.140
fibroblast growth factor 1 (Fgf1), mRNA.	0.712 / 0.843	0.794 / 0.771	0.921 / 0.826	0.788 / 0.759	0.802 / 0.566	0.848 / 0.740	0.956 / 1.013
fibroblast growth factor 12 (Fgf12), mRNA.	0.898 / 0.866	1.026 / 0.913	0.803 / 0.836	0.774 / 0.757	0.935 / 0.665	0.872 / 0.807	0.883 / 0.946
fibroblast growth factor receptor 3 (Fgfr3), mRNA.	1.101 / 1.100	1.109 / 1.165	1.179 / 1.246	1.088 / 1.066	1.076 / 1.060	1.111 / 1.050	1.139 / 1.145
fibroblast growth factor receptor 1 beta-isoform	1.359 / 1.297	0.970 / 1.248	1.041 / 0.942	0.961 / 1.034	0.953 / 0.754	1.113 / 1.387	1.146 / 1.200
transforming growth factor, beta receptor 1 (Tgfbr1)	0.760 / 1.059	0.961 / 1.227	0.799 / 0.894	1.046 / 1.177	0.908 / 1.079	0.964 / 1.080	1.079 / 0.914
Enzyme related							
ubiquitin C-terminal hydrolase UCH37 (UCH37) mRNA	1.306 / 1.184	1.430 / 1.368	1.471 / 1.449	1.376 / 1.695	1.212 / 2.140	1.200 / 1.119	1.130 / 1.131
glycolipid-anchored form of acetylcholinesterase(R)	0.901 / 0.986	0.914 / 0.917	1.244 / 0.981	1.013 / 0.943	1.065 / 0.908	0.937 / 0.799	1.111 / 1.228
glutathione synthetase (Gss), mRNA.	0.739 / 0.931	1.121 / 1.131	1.475 / 1.558	1.186 / 2.264	0.682 / 1.790	1.208 / 1.188	1.120 / 1.016
glutathione peroxidase (GSH-PO) mRNA	0.470 / 0.589	0.477 / 0.498	0.612 / 0.725	0.504 / 0.510	0.636 / 0.600	0.964 / 0.838	0.654 / 0.734
carnitine palmitoyltransferase 1 (Cpt1a), mRNA.	0.447 / 0.689	0.696 / 0.469	0.800 / 0.517	0.668 / 0.449	0.688 / 0.593	0.414 / 0.355	0.698 / 1.030
Protein kinase, cAMP dependent, regulatory, type 1 (Prkar1a)	1.132 / 1.070	1.303 / 1.307	1.131 / 1.199	0.988 / 1.607	1.085 / 1.180	1.179 / 1.117	1.093 / 0.856
Deoxyribonuclease I (Dnase1), mRNA.	1.139 / 1.065	1.112 / 0.801	0.890 / 0.868	0.781 / 0.953	1.010 / 0.811	1.054 / 0.946	0.883 / 0.918
membrane amine oxidase	0.850 / 0.868	0.984 / 1.062	0.970 / 1.047	1.012 / 0.983	1.137 / 0.948	0.977 / 1.025	0.984 / 0.959
pyruvate dehydrogenase 2 (Pdk2), mRNA.	0.562 / 0.789	0.656 / 0.635	0.906 / 0.803	0.737 / 0.691	0.901 / 0.645	1.066 / 1.157	1.138 / 1.333
cysteine proteinase inhibitor cystatin C.	0.827 / 0.948	0.793 / 0.896	0.988 / 0.921	0.955 / 0.904	0.912 / 0.749	0.900 / 1.012	1.077 / 1.047
RNA polymerase II subuunit RPB14	1.125 / 1.081	1.036 / 0.940	1.069 / 1.013	1.160 / 1.043	0.892 / 1.048	1.058 / 1.123	1.040 / 1.147
arginine-glutamic acid dipeptide (RE) repeats (Rere)	0.902 / 0.916	0.722 / 1.127	0.935 / 1.170	1.001 / 1.061	1.061 / 1.366	1.068 / 1.250	1.076 / 0.905
dipeptidase 1 (Dpep1), mRNA.	1.175 / 1.412	1.208 / 0.960	0.934 / 1.467	0.931 / 1.214	0.984 / 0.695	0.871 / 0.815	0.992 / 1.091
Angiogenesis							
cardiovascular heat shock protein	1.497 / 1.153	1.267 / 1.385	1.303 / 1.042	1.345 / 1.110	1.320 / 1.123	1.268 / 1.093	0.989 / 1.046
cadherin 16	0.946 / 1.010	1.330 / 1.284	1.039 / 1.171	0.827 / 1.025	0.716 / 1.445	1.028 / 1.325	0.848 / 0.899
zinc finger protein 46	0.981 / 0.927	1.027 / 1.020	0.987 / 1.138	1.013 / 1.139	1.044 / 0.964	1.028 / 1.142	1.120 / 0.880
Rattus norvegicus angiotensinogen (Agt), mRNA.	0.667 / 1.597	0.991 / 1.594	2.492 / 3.618	1.348 / 2.439	0.867 / 1.226	0.779 / 0.771	0.945 / 1.219
transforming growth factor beta binding protein 1 (Ltbp1)	1.346 / 1.431	0.917 / 1.242	0.907 / 0.958	1.073 / 1.051	1.071 / 0.787	1.043 / 1.126	1.035 / 1.001
cadherin 22 (Cdh22), mRNA.	1.028 / 1.101	1.063 / 1.045	0.994 / 1.080	1.038 / 0.953	1.096 / 0.984	1.032 / 1.024	1.089 / 1.040
thrombospondin 1	0.974 / 1.009	0.837 / 0.953	0.850 / 0.799	0.939 / 0.878	0.924 / 0.852	0.851 / 0.919	0.880 / 0.978
metalloproteinase 14, membrane-inserted (Mmp14), mRNA.	0.444 / 0.656	0.641 / 0.616	1.155 / 1.284	1.229 / 1.441	0.756 / 0.813	0.689 / 0.827	0.814 / 0.509
fibroblast growth factor 1 (Fgf1), mRNA.	0.712 / 0.843	0.794 / 0.771	0.921 / 0.826	0.788 / 0.759	0.802 / 0.566	0.848 / 0.740	0.956 / 1.013
integrin beta 2 alpha subunit mRNA	1.163 / 0.958	1.478 / 2.389	1.781 / 1.310	1.721 / 1.148	1.565 / 1.464	1.232 / 1.389	1.692 / 1.425
calreticulin (Calr), mRNA.	0.461 / 0.571	0.517 / 0.544	1.214 / 1.225	1.549 / 1.401	1.527 / 1.537	1.076 / 1.369	1.331 / 1.357
Fibronectin 1 (Fn1), mRNA.	1.239 / 1.153	2.018 / 1.884	2.288 / 2.717	2.366 / 2.098	1.474 / 1.431	1.187 / 1.206	1.141 / 1.254

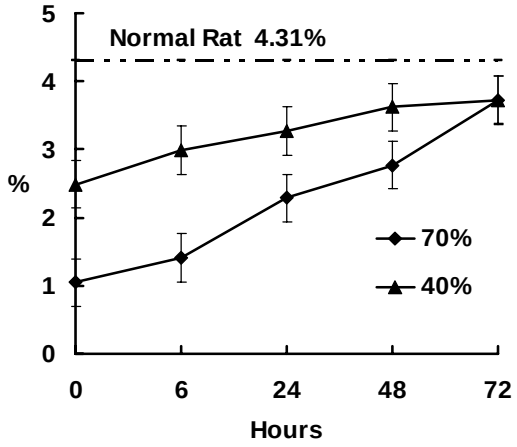

Fig 1. Remnant Liver Weight/Body Weight Ratio after 40% and 70% partial Hepatectomy.

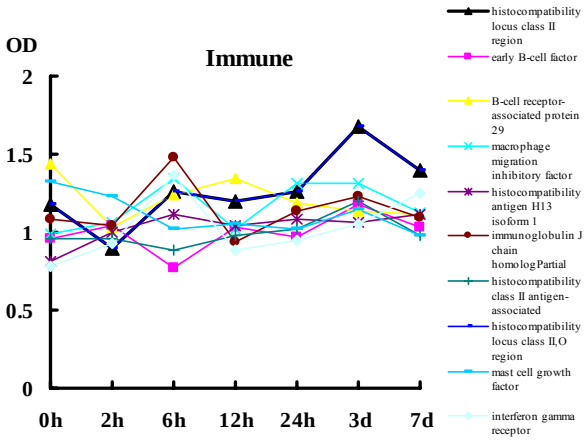

Fig 2. The names and their changing patterns of immune related genes.

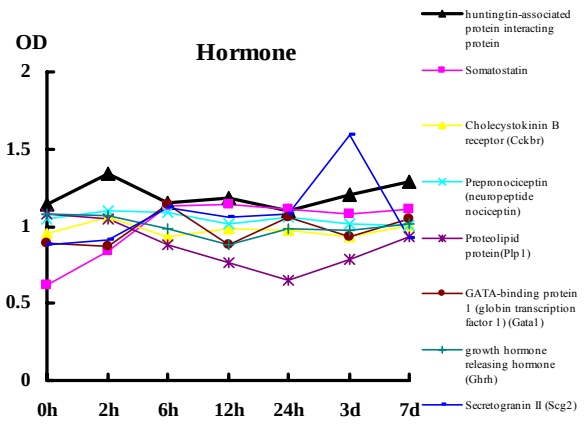

Fig 3. The names and their changing patterns of hormone related genes.

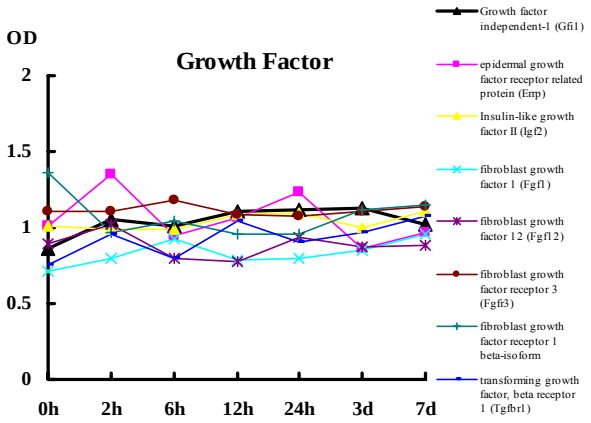

Fig 4. The names and their changing patterns of growth factor related genes.

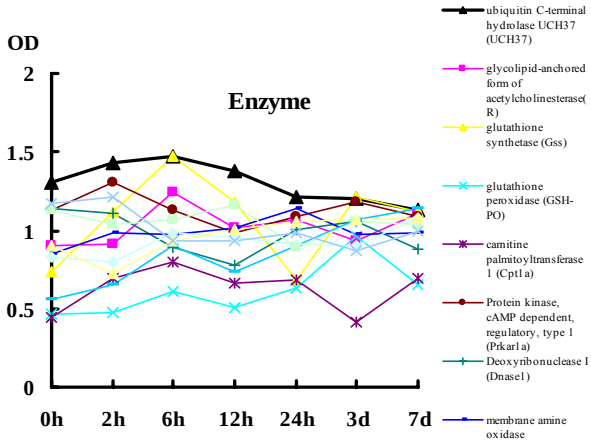

Fig 5. The names and their changing patterns of enzyme related genes.

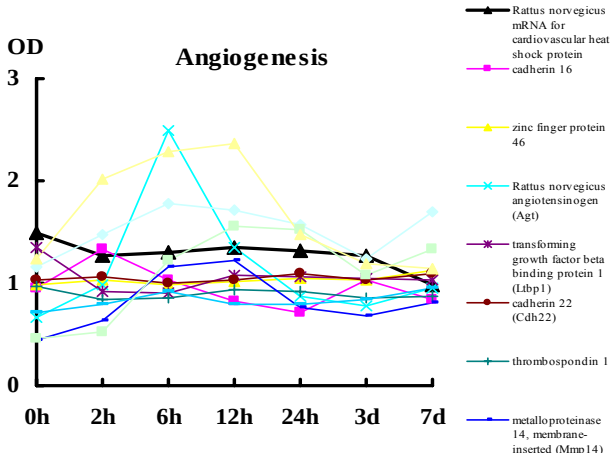

Fig 6. The names and their changing patterns of angiogenesis related genes