世界华人消化杂志®
wcjd@wjgnet.com
世界华人消化杂志 2007年7月18日; 15(20): 2186-2193
ISSN 1009-3079 CN 14-1260/R

基础研究 BASIC RESEARCH

CEBPs及其调节的基因与大鼠肝再生相关性分析

王 望, 陈晓光, 徐存拴

■背景资料
肝脏具有很强的再生能力, 大鼠部分肝切除模型被广泛用于研究肝再生. 肝再生涉及细胞激活、去分化、增殖及调控、再分化和组织结构功能重建等生理生化过程, 受到包括转录因子在内的多种因素调控.

王望, 徐存拴, 河南师范大学生命科学学院 河南省新乡市 453007
陈晓光, 河南省科技部共建细胞分化调控重点实验室 河南省新乡市 453007
王望, 河南师范大学生命科学学院细胞生物学专业在读硕士研究生, 主要从事肝再生分子机制的研究.
通讯作者: 徐存拴, 453007, 河南省新乡市建设东路46号, 河南师范大学生命科学学院. xucs@x263.net
电话: 0373-3326001
收稿日期: 2007-04-24 接受日期: 2007-05-10

Correlation analysis of CCAAT/enhancer-binding proteins and their regulated genes with rat liver regeneration

Wang Wang, Xiao-Guang Chen, Cun-Shuang Xu

Wang Wang, Cun-Shuang Xu, Life Science College of He'nan Normal University, Xinxiang 453007, He'nan Province, China
Xiao-Guang Chen, Key Laboratory of Cell Differentiation and Regulation Co-construction from Science and Technology Department of He'nan Province, Xinxiang 453007, He'nan Province, China
Correspondence to: Cun-Shuang Xu, Life Science College of He'nan Normal University, 46 Jianshe East Road, Xinxiang 453007, He'nan Province, China. xucs@ x263.net
Received: 2007-04-24 Accepted: 2007-05-10

Abstract

AIM: To explore the effects on transcription levels of CCAAT/enhancer-binding proteins (CEBP) genes and genes regulated in liver regeneration (LR).

METHODS: CEBP family transcription factors correlated with LR were input into the websites of NCBI (www.ncbi.nlm.nih.gov) and RGD (rgd.mcw.edu). Documents related to the transcription functions of CEBP family transcription factors were located and the CEBP family downstream target genes in human, rat and mice obtained. The human and mice non-repeated genes in rat genes were screened out; the results were confirmed by comparison by Rat Genome 230 2.0 chip examination. Twice up-regulated and down-regulated genes were regarded as significantly changed rat homologous genes. The Rat Genome 230 2.0 chips were used to detect the expressions of the above genes in rat RL. Genes correlated with liver regeneration were confirmed by comparison in a true and false operation.

RESULTS: Twenty-seven genes correlated with LR, 11 of which were up-regulated, 6 down-regulated, and 10 that were up-regulated at certain time points but down-regulated at others. The up-regulated range was 2 to 128 fold of the comparison group, while the down-regulated range was 2 to 16 fold. The number of genes expressed in the LR initiation [0.5-4 hours after partial hepatectomy (PH)], G_0/G_1 transition (4-6 hours after PH), cell proliferation (6-66 hours after PH) and cell differentiation and tissue structure reconstruction (72-168 hours after PH) stages were 18, 3, 8 and 1, respectively. Total expression times were 18, 11, 25 and 16, respectively, showing that the correlated genes were primarily expressed in the LR initiation stage. Genes were up-regulated 126 fold and down-regulated 76 fold, indicating that more genes were up-regulated than down-regulated in LR.

CONCLUSION: CEBPs and their regulated genes are highly correlated with cell proliferation, cell differentiation, cell apoptosis, inflammation, stress response, lipids metabolism and changes in the extracellular matrix.

Key Words: Partial hepatectomy; Rat genome 230 2.0 chip; CCAAT/enhancer-binding proteins; Liver regeneration correlated genes

Wang W, Chen XG, Xu CS. Correlation analysis of CCAAT/enhancer-binding proteins and their regulated genes with rat hepatic regeneration. Shijie Huaren Xiaohua Zazhi 2007;15(20):2186-2193

摘要

目的: 探讨基因转录水平CCAAT增强子结合蛋白(CEBPs)及其调节基因在肝再生(LR)中的作用.

方法: 将与肝再生相关的CEBPs家族转录因子输入NCBI(www.ncbi.nlm.nih.gov)和RGD(rgd.mcw.edu)等网站查找与其转录功能相关的文献, 从中得出大鼠、小鼠和人的

CEBPs家族下游基因. 然后将人和小鼠基因与大鼠比对, 筛选出与大鼠不重复的人和小鼠基因. 再将他们与Rat Genome 230 2.0芯片的检测结果进行比对确认, 把其中表达上调或下调2倍以上的基因视为有意义变化的大鼠同源基因. 用Rat Genome 230 2.0 芯片检测他们在大鼠再生肝中的表达情况, 用真、假手术比较方法确定肝再生相关基因.

结果: 27个基因与肝再生相关, 其中11个基因表达上调, 6个基因表达下调, 10个基因在有的时点表达上调, 有的时点表达下调(简称上/下调表达). 他们的上调范围为对照的2-128倍, 下调范围为对照的2-16倍. 肝再生启动[部分肝切除(PH)后0.5-4 h]、G_0/G_1过渡(PH后4-6 h)、细胞增殖(PH后6-66 h)、细胞分化和组织结构功能重建(PH后72-168 h)等4个阶段起始表达的基因数分别为18, 3, 8和1; 基因的总表达次数分别为18, 11, 25和16. 表明相关基因主要在肝再生启动阶段起始表达, 在不同阶段发挥作用. 他们共表达上调126次, 下调76次. 表明肝再生中表达上调基因多于表达下调基因.

结论: CEBPs及其调节的基因与肝再生中细胞增殖、细胞分化、细胞凋亡、炎症反应、应激反应、脂类代谢和细胞外基质变化等密切相关.

关键词: **部分肝切除; 大鼠基因组230 2.0芯片; CCAAT增强子结合蛋白; 肝再生相关基因**

王望, 陈晓光, 徐存拴. CEBPs及其调节的基因与大鼠肝再生相关性分析. 世界华人消化杂志 2007;15(20):2186-2193
http://www.wjgnet.com/1009-3079/15/2186.asp

0 引言

部分肝切除(partial hepatectomy, PH)[1]或肝损伤后, 残肝细胞通过细胞增殖(细胞数目增加)和肥大(细胞体积增加)由基本不生长状态转变为快速生长状态以补偿丢失、损伤的肝组织[2-3], 这个过程称为肝再生(liver regeneration, LR)[2]. 同时, 机体可精确感知再生肝的大小, 适时停止再生[4]. 通常, 根据细胞的生理活动将肝再生分为启动(PH后0.5-4 h)、G_0/G_1过渡(PH后4-6 h)、细胞增殖(PH后6-66 h)、细胞分化和组织结构功能重建(PH后72-168 h)等4个阶段[5]. 根据时间进程分为早期(PH后0.5-4 h)、前期(PH后6-12 h)、中期(PH后16-66 h)、后期(PH后72-168 h)等4个时期[6]. 涉及细胞激活、去分化、增殖及调控、再分化、组织结构和功能重建等生理生化过程[7], 受到包括转录因子在内的多种因素调控[8]. 研究表明, CCAAT增强子结合蛋白(CCAAT/enhancer-binding proteins, CEBPs)家族转录因子CEBPα, CEBPβ, CEBPγ, CEBPδ和CEBPε均含碱性亮氨酸拉链结构域, 在C端有DNA结合元件和异源或同源亮氨酸拉链元件. CEBPα通过调节scd1, cd36和cds1[9], CEBPβ通过调节afp[10], a2m[11], gsta2[12], cat[13], il6, col1a2和col2a1[14], CEBPγ通过调节ercc5和xrcc1[15], CEBPδ通过调节ccl2[16], CEBPε通过调节il6, ccl2, ccl4, csf1r[17], itgam, bcl2l1, bcl2, ccna2, ccnd2, ccne1和cdk4[18], 从而在细胞增殖、细胞分化、细胞凋亡、炎症反应、应激反应、脂类代谢和细胞外基质变化等方面发挥重要作用. 为在基因转录水平[19-20]了解CEBPs及其调节基因与肝再生的相关性, 我们用含42个CEBPs及其调节基因的Rat Genome 230 2.0芯片[21]检测了大鼠2/3肝切除后再生肝基因表达情况, 并初步分析了他们在肝再生中的表达方式与肝再生的相关性和作用[22].

■研究前沿
再生生物学和再生医学已成为当今生物学和医学的研究热点. 其中, 肝再生的功能基因组学、蛋白质组学、细胞组学等研究正在广泛深入地进行.

1 材料和方法

1.1 *材料* SD纯系大鼠由河南师范大学实验动物中心提供, 体质量200-250 g, 将大鼠随机分组, 每组6只, 雌雄各半. 对照组材料为正常成年大鼠肝. 按Higgins *et al*[1]方法切除大鼠肝左叶和中叶后恢复0.5, 1, 2, 4, 6, 8, 12, 16, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 96, 120, 144和168 h时再生肝. 于相应恢复时间颈椎脱臼处死动物, 切取再生肝置4℃ PBS中涮洗3次, 从右叶再生肝中部取100-200 mg组织, 将每组的6个样品混合后放到一起(总肝量为0.1-0.2 g×6 = 1-2 g), 于-80℃保存备用. 假手术(sham-operation, SO)组除不切除肝叶外, 其他同部分肝切除组. 实验中严格遵循中国动物保护法. 总RNA提取按Invitrogen公司的TRIzol试剂盒操作程序进行[23]. 总RNA纯化按Qiagen公司的RNeasy mini试剂盒操作程序进行[24]. 用琼脂糖凝胶电泳(180 V, 0.5 h)检测总RNA的28S和18S比例; 其亮度约为2∶1; 在260/280 nm波长下测定总RNA浓度和纯度[25].

1.2 *方法* cDNA, cRNA合成按Affymetrix公司方法进行[26]. 合成cDNA时, 模板量为1-8 μg总RNA. 合成生物素标记的cRNA时, 取12 μL上述cDNA溶液作模板. cDNA, cRNA纯化按基因芯片分析样品纯化操作程序进行[27]. 两者的浓度、纯度和质量检测方法同上[25]. 1 g/L的cRNA 15 μL, 5×片段化缓冲液6 μL, 无RNA酶水9 μL

■相关报道
研究表明, CEBPs家族转录因子对细胞增殖和细胞分化等相关基因有调控作用, 肝再生亦涉及CEBPs对细胞增殖和细胞分化等相关基因的调节.

混匀, 94℃温浴35 min, 得到长度为35-200 bp的cRNA片段. 按Affymetrix公司提供的配方配制杂交液, 然后将杂交液加至经预杂交处理的Rat Genome 230 2.0芯片中, 于45℃ 60 r/min杂交16 h, 吸去杂交液, 用GeneChip全自动洗涤工作站450(Affymetrix公司, USA)洗涤和染色芯片. 用高分辨芯片扫描仪3000(Affymetrix公司, USA)扫描芯片, 获得基因表达信号值[21]. 用GCOS1.2软件读取、处理信号值数据, 获得归一化后的信号值、信号检出(P, A, M)以及实验和对照组的比值[21]. 为减少芯片分析误差, 用Rat Genome 230 2.0芯片对每个时点再生肝重复检测3次. 把3次检测中总比值最大的那次结果视为最接近真实(R^m), 同时, 也将3次检测中3个持家基因(β-actin, hexokinase和glyseraldehyde-3-phosphate dehydrogenase)平均值最接近1的那次结果视作最接近真实(R^h). 将前者比后者, 得到矫正系数, 用矫正系数乘以后者每个时点每个基因对应的比值, 得到用于分析基因表达模式和作用的基因相对表达丰度. 为克服芯片分析误差导致的基因不合常规和/或不合逻辑的表达变化, 又根据肝再生中肝实质细胞的细胞周期进程, 用合理化分析软件(RAP)整理部分肝切除后0-4 h, 6-12 h, 12-24 h的基因表达变化值使之更具科学性. 然后用GeneMath, GeneSpring, Microsoft Excel等分析软件对各组数据进行统计和聚类分析[21,27-28].

将查出的与肝再生相关的CEBPs家族转录因子输入NCBI(www.ncbi.nlm.nih.gov)和RGD(rgd.mcw.edu)等网站查找与其转录功能相关的文献, 从中得出大鼠、小鼠和人的CEBPs家族下游基因. 然后将人和小鼠基因与大鼠比对, 筛选出与大鼠不重复的人和小鼠基因. 再将他们与Rat Genome 230 2.0芯片的检测结果进行比对确认, 把其中表达上调或下调2倍以上的基因视为有意义变化[28]的大鼠同源基因. 用上述芯片对再生肝进行检测, 将3次检验结果相同或相似, 至少在部分肝切除后一个时点表达上调或下调2倍以上, 部分肝切除组与假手术组差异显著($P<0.05$)或极显著($P\leqslant 0.01$)的基因视为肝再生相关基因.

2 结果

2.1 肝再生中CEBPs及其调节基因的表达 查NCBI, RGD网站资料表明, 46个基因受CEBPs调节; 查Rat Genome 230 2.0芯片资料表明, 该芯片含上述42个基因, 其中27个基因至少在部分肝切除(partial hepatectomy, PH)后一个时点发生了有意义表达变化, 部分肝切除组与SO组有显著或极显著差异, 3次Rat Genome 230 2.0芯片检测结果具有可重复性. 因此, 初步确认这些基因与LR相关. 其中, 11个基因表达上调, 6个基因表达下调, 10个基因在有的时点表达上调, 有的时点表达下调(简称上/下调表达). 他们的上调范围为对照的2-128倍, 下调范围为对照的2-16倍(表1).

分析表明, 肝再生各时点起始上调和下调及总表达的基因数为: 0.5 h时均为6和3; 1 h时3和1, 7和2; 2 h时2和0, 7和1; 4 h时1和2, 6和3; 6 h时0和0, 7和4; 8 h时0和1, 5和5; 12 h时0和0, 4和5; 16 h时1和0, 6和5; 18 h时0和2, 7和7; 24 h时1和0, 6和6; 30 h时1和0, 4和2; 36 h时0和0, 5和5; 42 h时1和0, 5和1; 48 h时0和0, 8和5; 54 h时0和0, 7和4; 60 h时0和1, 7和5; 66 h时0和0, 7和2; 72 h时1和0, 7和4; 96 h时0和0, 4和3; 120 h时0和0, 6和1; 144 h时0和0, 1和2; 168 h时0和0, 3和1. 肝再生中基因起始表达的总体情况是: 17个基因起始上调, 10个基因起始下调. 其中, 在启动阶段(PH后0.5-4 h)12个基因起始上调, 6个基因起始下调; G_0/G_1过渡阶段(PH后4-6 h)1个基因起始上调, 2个基因起始下调; 细胞增殖阶段(PH后6-66 h)4个基因起始上调, 4个基因起始下调; 细胞再分化和组织结构功能重建阶段(PH后72-168 h)1个基因起始上调. 肝再生中基因表达的总体情况是: 共表达上调126次, 下调76次. 其中, 肝再生启动阶段(PH后0.5-4 h)基因上调26次, 下调9次; G_0/G_1过渡阶段(PH后4-6 h)基因上调13次, 下调7次; 细胞增殖阶段(PH后6-66 h)基因上调79次, 下调56次; 细胞再分化和组织结构功能重建阶段(PH后72-168 h)基因上调21次, 下调11次(图1).

2.2 CEBPs及其调节的基因与肝再生相关性 根据功能和表达变化将CEBPs及其调节的22个基因分为7组: (1)细胞增殖相关基因. 其中, cebpα在4-66 h表达下调, 其调节的scd1在1-6 h表达上调, 48和66 h表达下调. cebpβ在0.5-8 h表达上调, 他调节的a2m在0.5-24 h表达上调, afp在6-12 h表达下调, 54-72 h表达上调. cebpε在16-120 h表达下调, 其调节的ccna2在18-72 h表达上调, ccnd2在42 h表达上调, ccne1在8-72 h表达上调, cdk4在24和66 h表达上调(图2A); (2)细胞分化相关基因. 其中, cebpα及其调节的scd1表达同上. cebpε调节的il6在2-8, 18, 48, 60, 96 h表达上调, 在36

表 1 CEBPs及其调节的22个基因在肝再生中表达丰度

名称		Abbr.	Fold difference
CEBPs转录因子			
	CCAAT/enhancer binding protein alpha	Cebpα	0.06
	CCAAT/enhancer binding protein beta	Cebpβ	3.07
	CCAAT/enhancer binding protein gamma	Cebpγ	0.44
	CCAAT/enhancer binding protein delta	Cebpδ	6.27, 0.40
	CCAAT/enhancer binding protein epsilon	Cebpε	0.14
细胞增殖			
Cebpα	stearoyl–Coenzyme A desaturase 1	Scd1	3.48, 0.29
Cebpβ	alpha–2–macroglobulin	A2m	46.16, 0.40
	alpha–fetoprotein	Afp	3.72, 0.14
Cebpε	cyclin A2	Ccna2	45.07
	cyclin E	Ccne1	18.47
	cyclin–dependent kinase 4	Cdk4	2.47
	cyclin D2	Ccnd2	2.23
细胞分化			
Cebpα	stearoyl–Coenzyme A desaturase 1	Scd1	3.48, 0.29
Cebpε	integrin alpha M	Itgam	3.41
Cebpβ/Cebpε	interleukin 6	Il6	6.06, 0.28
凋亡			
Cebpα	stearoyl–Coenzyme A desaturase 1	Scd1	3.48, 0.29
Cebpβ	glutathione–S–transferase	Gsta2	6.96, 0.22
Cebpγ	excision repair cross–complementing rodent repair deficiency complementation group 5	Ercc5	2.38
Cebpβ/Cebpδ/Cebpε	hemokine C–C motif ligand 2	Ccl2	128.00
	B–cell leukemia/lymphoma 2	Bcl2	0.32
	bcl2–like 1	Bcl2l1	3.00, 0.44
炎性反应			
Cebpβ	alpha–2–macroglobulin	A2m	46.16, 0.40
Cebpβ/Cebpδ/Cebpε	chemokine C–C motif ligand 2	Ccl2	128.00
	colony stimulating factor 1 receptor	Csf1r	13.00
	integrin alpha M	Itgam	3.41
	chemokine C–C motif ligand 4	Ccl4	4.00, 0.22
应激反应			
Cebpβ	catalase	Cat	5.00
	glutathione –S–transferase	Gsta2	6.96, 0.22
Cebpβ/Cebpε	interleukin 6	Il6	6.06, 0.28
Cebpγ	excision repair cross–complementing rodent repair deficiency complementation group 5	Ercc5	2.38
	x–ray repair cross–complementing group 1 protein	Xrcc1	5.00, 0.31
脂质代谢			
Cebpα	CDP–diacylglycerol synthase 1	Cds1	0.29
	CD36 antigen	Cd36	0.10
	stearoyl–Coenzyme A desaturase 1	Scd1	3.48, 0.29
细胞外基质			
Cebpβ	rocollagen, type I alpha 2	Col1a2	7.00
	procollagen, type II alpha 1	Col2a1	7.00, 0.30

黑体字: 转录因子; 非黑体字: 转录因子调节的基因.

■创新盘点

用高通量Rat Genome 230 2.0基因表达谱芯片分析了大鼠2/3肝切除后CEBPs及其调节的基因在肝再生中表达情况, 发现上述基因中有27个基因与肝再生相关.

■应用要点
根据结果进一步研究CEBPs及其调控的基因在肝再生中的作用和机制.

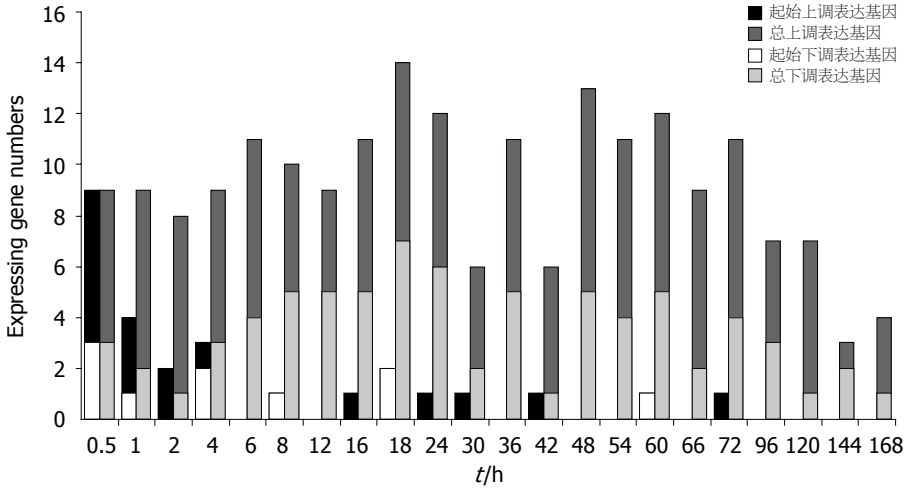

图 1 CEBPs及其调节的22个基因在肝再生各时点起始表达及总表达情况.

和72 h表达下调, itgam在72和120 h表达上调(图2B); (3)细胞凋亡相关基因. 其中, cebpα及其调节的scd1表达同上. cebpβ调节的gsta2在8-24 h表达下调, 在30, 42, 96 h表达上调. cebpγ在60 h表达下调, 其调节的ercc5在30 h表达上调. cebpδ在1-72 h表达上调, 其调节的ccl2在12-120 h表达上调. cebpε调节的bcl2在18-72 h表达下调, bcl2l1在0.5 h表达下调, 在6 h表达上调(图2C); (4)炎症反应相关基因. 其中, cebpβ及其调节的a2m, cebpδ及其调节的ccl2, cebpε及其调节的ccl2和itgam表达同上. cebpε调节的ccl4在18, 54, 96 h表达下调, csf1r在16, 30, 42, 96 h表达上调(图2D); (5)应激反应相关基因. cebpβ及其调节的gsta2和il6表达同上, cat在0.5 h表达上调. cebpγ及其调节的ercc5表达同上, xrcc1在36, 48, 60 h表达上调, 在1和144-168 h表达下调(图2E); (6)脂类代谢相关基因. cebpα及其调节的scd1表达同上, cd36在0.5-72 h表达下调, cds1在4-18 h表达下调(图2F); (7)细胞外基质变化相关基因. cebpβ调节的col1a2在16-24 h和66-168 h表达上调, col2a1在2和54 h表达上调, 在24 h表达下调(图2G).

3 讨论

我们研究了CEBP家族转录因子CEBPα, CEBPβ, CEBPγ, CEBPδ和CEBPε及其调节的基因与肝再生相关性. 其中, cebpα通过促进cds1, cd36和scd1转录促进脂类合成[9,29]. cds1在肝再生的4-18 h, cd36在0.5-72 h、scd1在48和66 h表达下调, 可能受cebpα在4-66 h表达下调影响, 并推测肝再生早期、前期和中期的脂类合成受抑制. 此外, scd1还能通过改变细胞膜流动性和影响信号转导抑制细胞凋亡、促进细胞增殖和促进细胞分化[30]. cebpα在4-66 h及scd1在48和66 h表达下调可能与肝再生中期的细胞增殖和分化减弱、细胞凋亡起始有关. 一般认为, cebpβ通过抑制cat转录促进氧化应激反应[13]. cebpβ在0.5-8 h表达上调, cat在0.5 h表达上调, cat表达可能还受其他因素促进, 并在抑制肝再生早期的氧化应激反应中起重要作用. cebpβ能促进col1a2转录和抑制col2a1转录[14]. 后两者均促进胶原蛋白基因表达. 推测3者的转录平衡与再生肝的胞外基质构建调控密切相关. cebpβ还是促细胞增殖基因afp的主要促进因子[10,31]. afp在54-72 h表达上调, 与cebpβ共同促进肝再生中期和后期的细胞增殖. cebpβ通过促进a2m转录抑制蛋白酶活性促进细胞增殖[11,32]. a2m在0.5-24 h表达上调, 在8 h达到高峰, 是对照的46倍. 可能他在肝再生的细胞激活、细胞周期进程和缓解炎症反应中起重要作用. cebpβ通过促进gsta2转录抑制细胞凋亡, 参与药物反应来防止化学物诱导的细胞癌变[12,33-34]. gsta2在8-24 h表达下调, 在30, 42和96 h表达上调. 推测前期和中期再生肝细胞的抗癌变能力降低, 中期和后期细胞生存能力提高.

研究表明, cebpγ促进DNA损伤修复基因xrcc1表达[15,35]. 前者在PH后60 h表达下调, 后者在144-168 h表达下调. 推测肝再生后期的DNA损伤修复活动减少. 此外, cebpγ通过促进ercc5转录促进DNA氧损伤修复和抑制细胞凋亡[15,36]. ercc5在30 h表达上调, 可能与肝再生中期的DNA修复活动加强有关. 研究表明, cebpε通过抑制ccnd2, ccne1和cdk4抑制细胞增殖[18,37-39], 前者在肝再生的16-120 h表达下调, 后3者在8-72 h表达上调, 推测促进相应时期的细胞增殖. cebpε通过抑制bcl2l1促进细胞凋亡[18]. 后者在6 h表达上调, 可能与肝再生前期的细胞抗凋亡能力提高有关. cebpε还能通过促进csf1r和ccl4转录促进炎

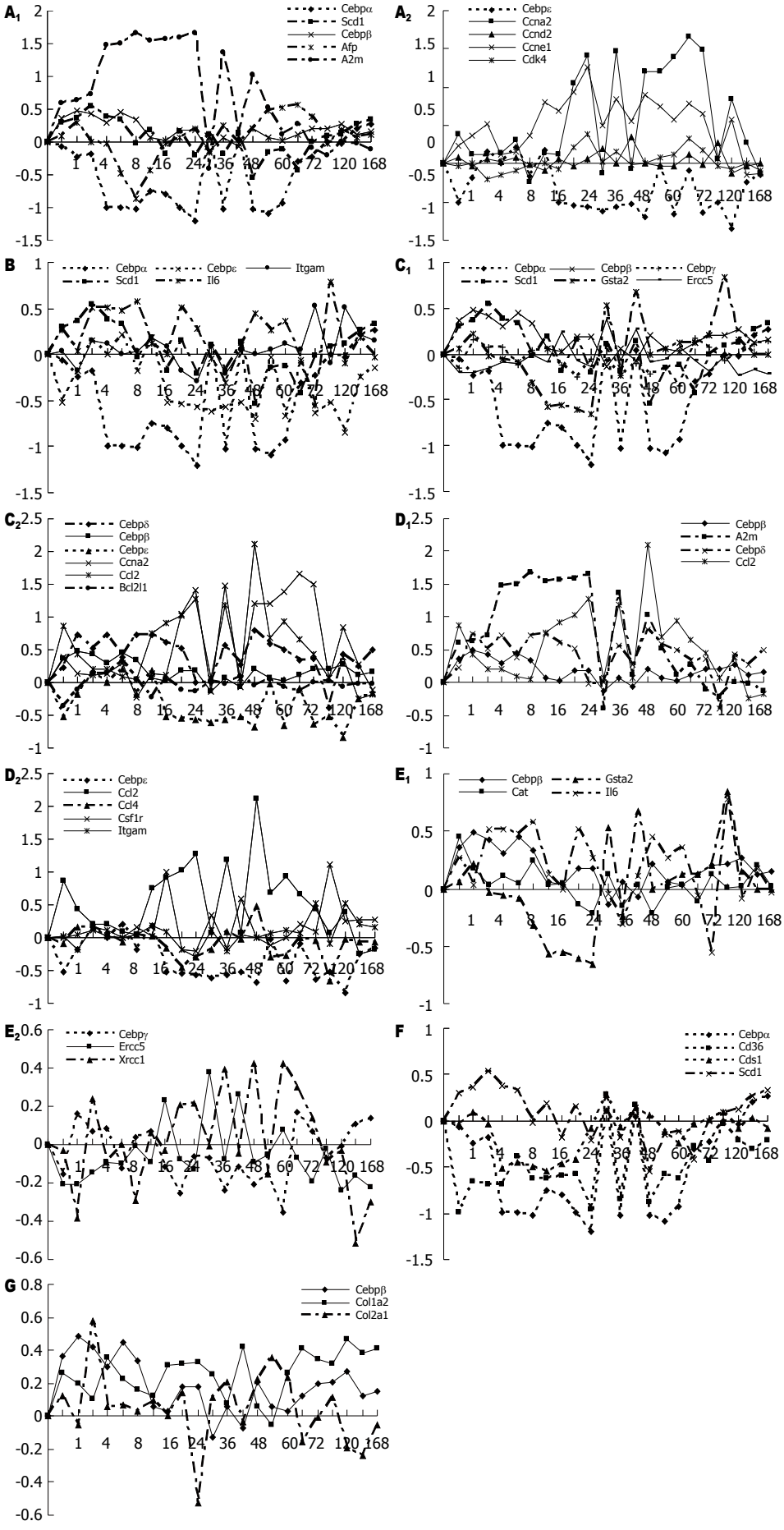

图 2 CEBPs及其调节的22个基因在肝再生中表达变化. A: 细胞增殖相关基因; B: 细胞分化相关基因; C: 细胞凋亡相关基因; D: 炎症反应相关基因; E: 应激反应相关基因; F: 脂类代谢相关基因; G: 细胞外基质变化相关基因. 实线示基因表达上调, 虚线示基因表达下调; 实-虚线示上/下调表达基因. X轴为部分肝切除后恢复时间(h), Y轴为各时点基因表达产物信号值与对照比值的对数.

■名词解释

部分肝切除(PH): 是Higgins和Anderson于1931年建立的通过外科手术切除大鼠2/3肝脏的方法. 部分肝切除诱导的肝再生(LR)是指用外科手术切除大鼠2/3肝脏后, 残肝细胞通过细胞增殖(细胞数目增加)和肥大(细胞体积增加)由基本不生长状态转变为快速生长状态以补偿丢失的肝组织.

■同行评价
本文系统观察了肝损伤后不同时相CEBPs及其调控的基因的表达变化, 文章设计合理, 结果分析准确, 条理清晰, 结果对探讨创伤性肝损伤后肝再生的基因调控机制有重要参考意义.

症反应[18,40-41]. ccl4在肝再生中表达下调, csf1r表达上调. 推测炎症反应受3者的平衡调控. cebpε通过抑制bcl2转录促进细胞凋亡[18,42]. 后者在18-72 h表达下调, 可能还受其他因素抑制. cebpε还能通过抑制ccna2转录抑制细胞增殖[18,43]. 后者几乎在整个肝再生中表达上调, 在66 h达到高峰, 是对照的45倍. 可能在肝再生的细胞增殖中起重要作用. cebpε通过促进itgam表达促进细胞分化和炎症反应[18,44-45]. 后者在72和120 h表达上调, 推测尚存在促进itgam表达的其他因素. 研究表明, ccl2有抑制细胞凋亡和促进炎症反应的功能[46-47]. 其表达除受cebpβ和cebpε促进外, 还受cebpδ促进[16]. cebpδ在1-72 h表达上调, ccl2在12-120 h表达上调, 并在48 h达到高峰, 是对照的128倍. 根据其表达趋势与cebpβ和cebpδ更为一致推测, 肝再生中ccl2表达主要受cebpβ和cebpδ促进, 并在抑制再生肝细胞凋亡中起重要作用. cebpβ和cebpε促进il6的转录[17]. il6是体温调节因子, 能促进细胞分化, 还与出血导致的肝脏功能紊乱有关[48-50]. 他在2-8 h表达上调, 在16 h后的有些时点上调, 有些时点下调. 3者在肝再生中的作用和相互关系有待进一步研究.

总之, 我们用高通量基因表达谱芯片分析了大鼠部分肝切除后CEBPs及其调节的基因在肝再生中表达情况, 初步证实他们与再生肝的细胞增殖、细胞凋亡、细胞分化、炎症反应、应激反应、脂类代谢、细胞外基质建成等有关. 然而, 从基因→mRNA→蛋白质→功能等受包括蛋白互作在内的多种因素影响, 以及同一基因受多个转录因子调控等情况今后我们将进一步用Northern印迹、蛋白质芯片、RNA干扰、蛋白互作等技术验证上述结果.

4 参考文献

1 Higgins GM, Anderson RM. Experimental pathology of the liver: restoration of the liver of the white rat following partial surgical removal. *J Arch Pathol* 1931; 12: 186–222
2 Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006; 43: S45-53
3 徐存拴, 赵利峰, 杨柯金, 章静波. 肝干细胞的来源与作用. 实验生物学报 2004; 37: 72-77
4 Kountouras J, Boura P, Lygidakis NJ. Liver regeneration after hepatectomy. *Hepatogastroenterology* 2001; 48: 556-562
5 Michalopoulos GK, DeFrances M. Liver regeneration. *Adv Biochem Eng Biotechnol* 2005; 93: 101-134
6 Taub R. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004; 5: 836-847
7 Pahlavan PS, Feldmann RE Jr, Zavos C, Kountouras J. Prometheus' challenge: molecular, cellular and systemic aspects of liver regeneration. *J Surg Res* 2006; 134: 238-251
8 Chang YJ. Mitochondrial calcium ion and oxidative phosphorylation in regenerating rat liver. *J Formos Med Assoc* 2002; 101: 189-194
9 Zhang F, Pan T, Nielsen LD, Mason RJ. Lipogenesis in fetal rat lung: importance of C/EBPalpha, SREBP-1c, and stearoyl-CoA desaturase. *Am J Respir Cell Mol Biol* 2004; 30: 174-183
10 Thomassin H, Hamel D, Bernier D, Guertin M, Belanger L. Molecular cloning of two C/EBP-related proteins that bind to the promoter and the enhancer of the alpha 1-fetoprotein gene. Further analysis of C/EBP beta and C/EBP gamma. *Nucleic Acids Res* 1992; 20: 3091-3098
11 Milosavljevic TS, Petrovic MV, Cvetkovic ID, Grigorov II. DNA binding activity of C/EBPbeta and C/EBPdelta for the rat alpha2-macroglobulin gene promoter is regulated in an acute-phase dependent manner. *Biochemistry (Mosc)* 2002; 67: 918-926
12 Ki SH, Cho IJ, Choi DW, Kim SG. Glucocorticoid receptor (GR)-associated SMRT binding to C/EBPbeta TAD and Nrf2 Neh4/5: role of SMRT recruited to GR in GSTA2 gene repression. *Mol Cell Biol* 2005; 25: 4150-4165
13 Taniguchi M, Hashimoto M, Hori N, Sato K. CCAAT/enhancer binding protein-beta (C/EBP-beta), a pivotal regulator of the TATA-less promoter in the rat catalase gene. *FEBS Lett* 2005; 579: 5785-5790
14 Imamura T, Imamura C, Iwamoto Y, Sandell LJ. Transcriptional Co-activators CREB-binding protein/p300 increase chondrocyte Cd-rap gene expression by multiple mechanisms including sequestration of the repressor CCAAT/enhancer-binding protein. *J Biol Chem* 2005; 280: 16625-16634
15 Mullins DN, Crawford EL, Khuder SA, Hernandez DA, Yoon Y, Willey JC. CEBPG transcription factor correlates with antioxidant and DNA repair genes in normal bronchial epithelial cells but not in individuals with bronchogenic carcinoma. *BMC Cancer* 2005; 5: 141
16 Sekine O, Nishio Y, Egawa K, Nakamura T, Maegawa H, Kashiwagi A. Insulin activates CCAAT/enhancer binding proteins and proinflammatory gene expression through the phosphatidylinositol 3-kinase pathway in vascular smooth muscle cells. *J Biol Chem* 2002; 277: 36631-36639
17 Williams SC, Du Y, Schwartz RC, Weiler SR, Ortiz M, Keller JR, Johnson PF. C/EBPepsilon is a myeloid-specific activator of cytokine, chemokine, and macrophage-colony-stimulating factor receptor genes. *J Biol Chem* 1998; 273: 13493-13501
18 Nakajima H, Watanabe N, Shibata F, Kitamura T, Ikeda Y, Handa M. N-terminal region of CCAAT/enhancer-binding protein epsilon is critical for cell cycle arrest, apoptosis, and functional maturation during myeloid differentiation. *J Biol Chem* 2006; 281: 14494-14502
19 Xu CS, Chang CF, Yuan JY, Li WQ, Han HP, Yang KJ, Zhao LF, Li YC, Zhang HY, Rahman S, Zhang JB. Expressed genes in regenerating rat liver after partial hepatectomy. *World J Gastroenterol* 2005; 11: 2932-2940
20 Dransfeld O, Gehrmann T, Kohrer K, Kircheis G, Holneicher C, Haussinger D, Wettstein M. Oligonucleotide microarray analysis of differential transporter regulation in the regenerating rat liver. *Liver Int* 2005; 25: 1243-1258
21 Collins JF. Gene chip analyses reveal differential

genetic responses to iron deficiency in rat duodenum and jejunum. *Biol Res* 2006; 39: 25-37
22 Yue H, Eastman PS, Wang BB, Minor J, Doctolero MH, Nuttall RL, Stack R, Becker JW, Montgomery JR, Vainer M, Johnston R. An evaluation of the performance of cDNA microarrays for detecting changes in global mRNA expression. *Nucleic Acids Res* 2001; 29: E41-41
23 Knepp JH, Geahr MA, Forman MS, Valsamakis A. Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. *J Clin Microbiol* 2003; 41: 3532-3536
24 Nuyts S, Van Mellaert L, Lambin P, Anne J. Efficient isolation of total RNA from Clostridium without DNA contamination. *J Microbiol Methods* 2001; 44: 235-238
25 Arkin A, Ross J, McAdams HH. Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected Escherichia coli cells. *Genetics* 1998; 149: 1633-1648
26 Li L, Roden J, Shapiro BE, Wold BJ, Bhatia S, Forman SJ, Bhatia R. Reproducibility, fidelity, and discriminant validity of mRNA amplification for microarray analysis from primary hematopoietic cells. *J Mol Diagn* 2005; 7: 48-56
27 Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A* 1998; 95: 14863-14868
28 Werner T. Cluster analysis and promoter modelling as bioinformatics tools for the identification of target genes from expression array data. *Pharmacogenomics* 2001; 2: 25-36
29 Weeks R, Dowhan W, Shen H, Balantac N, Meengs B, Nudelman E, Leung DW. Isolation and expression of an isoform of human CDP-diacylglycerol synthase cDNA. *DNA Cell Biol* 1997; 16: 281-289
30 Kim YC, Ntambi JM. Regulation of stearoyl-CoA desaturase genes: role in cellular metabolism and preadipocyte differentiation. *Biochem Biophys Res Commun* 1999; 266: 1-4
31 Li MS, Li PF, Yang FY, He SP, Du GG, Li G. The intracellular mechanism of alpha-fetoprotein promoting the proliferation of NIH 3T3 cells. *Cell Res* 2002; 12: 151-156
32 Rajan S, Radhakrishnan J, Rajamanickam C. Direct injection and expression in vivo of full-length cDNA of the cardiac isoform of alpha-2 macroglobulin induces cardiac hypertrophy in the rat heart. *Basic Res Cardiol* 2003; 98: 39-49
33 Xie J, Shults K, Flye L, Jiang F, Head DR, Briggs RC. Overexpression of GSTA2 protects against cell cycle arrest and apoptosis induced by the DNA inter-strand crosslinking nitrogen mustard, mechlorethamine. *J Cell Biochem* 2005; 95: 339-351
34 Kang KW, Cho IJ, Lee CH, Kim SG. Essential role of phosphatidylinositol 3-kinase-dependent CCAAT/enhancer binding protein beta activation in the induction of glutathione S-transferase by oltipraz. *J Natl Cancer Inst* 2003; 95: 53-66
35 Audebert M, Salles B, Weinfeld M, Calsou P. Involvement of polynucleotide kinase in a poly (ADP-ribose) polymerase-1-dependent DNA double-strand breaks rejoining pathway. *J Mol Biol* 2006; 356: 257-265
36 Clement V, Dunand-Sauthier I, Clarkson SG. Suppression of UV-induced apoptosis by the human DNA repair protein XPG. *Cell Death Differ* 2006; 13: 478-488
37 Kukoski R, Blonigen B, Macri E, Renshaw AA, Hoffman M, Loda M, Datta MW. p27 and cyclin E/D2 associations in testicular germ cell tumors: implications for tumorigenesis. *Appl Immunohistochem Mol Morphol* 2003; 11: 138-143
38 Matsumoto Y, Maller JL. A centrosomal localization signal in cyclin E required for Cdk2-independent S phase entry. *Science* 2004; 306: 885-888
39 Berthet C, Klarmann KD, Hilton MB, Suh HC, Keller JR, Kiyokawa H, Kaldis P. Combined loss of Cdk2 and Cdk4 results in embryonic lethality and Rb hypophosphorylation. *Dev Cell* 2006; 10: 563-573
40 Le Meur Y, Tesch GH, Hill PA, Mu W, Foti R, Nikolic-Paterson DJ, Atkins RC. Macrophage accumulation at a site of renal inflammation is dependent on the M-CSF/c-fms pathway. *J Leukoc Biol* 2002; 72: 530-537
41 Zipfel PF, Balke J, Irving SG, Kelly K, Siebenlist U. Mitogenic activation of human T cells induces two closely related genes which share structural similarities with a new family of secreted factors. *J Immunol* 1989; 142: 1582-1590
42 Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* 1997; 275: 1129-1132
43 Shen WH, Jackson ST, Broussard SR, McCusker RH, Strle K, Freund GG, Johnson RW, Dantzer R, Kelley KW. IL-1beta suppresses prolonged Akt activation and expression of E2F-1 and cyclin A in breast cancer cells. *J Immunol* 2004; 172: 7272-7281
44 Wu H, Rodgers JR, Perrard XY, Perrard JL, Prince JE, Abe Y, Davis BK, Dietsch G, Smith CW, Ballantyne CM. Deficiency of CD11b or CD11d results in reduced staphylococcal enterotoxin-induced T cell response and T cell phenotypic changes. *J Immunol* 2004; 173: 297-306
45 Pilione MR, Agosto LM, Kennett MJ, Harvill ET. CD11b is required for the resolution of inflammation induced by Bordetella bronchiseptica respiratory infection. *Cell Microbiol* 2006; 8: 758-768
46 Eugenin EA, D'Aversa TG, Lopez L, Calderon TM, Berman JW. MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. *J Neurochem* 2003; 85: 1299-1311
47 Mirzadegan T, Diehl F, Ebi B, Bhakta S, Polsky I, McCarley D, Mulkins M, Weatherhead GS, Lapierre JM, Dankwardt J, Morgans D Jr, Wilhelm R, Jarnagin K. Identification of the binding site for a novel class of CCR2b chemokine receptor antagonists: binding to a common chemokine receptor motif within the helical bundle. *J Biol Chem* 2000; 275: 25562-25571
48 De Jongh RF, Vissers KC, Booij LH, De Jongh KL, Vincken P, Meert TF. Interleukin-6 and perioperative thermoregulation and HPA-axis activation. *Cytokine* 2003; 21: 248-256
49 Takanaga H, Yoshitake T, Hara S, Yamasaki C, Kunimoto M. cAMP-induced astrocytic differentiation of C6 glioma cells is mediated by autocrine interleukin-6. *J Biol Chem* 2004; 279: 15441-15447
50 Toth B, Yokoyama Y, Schwacha MG, George RL, Rue LW 3rd, Bland KI, Chaudry IH. Insights into the role of interleukin-6 in the induction of hepatic injury after trauma-hemorrhagic shock. *J Appl Physiol* 2004; 97: 2184-2189

编辑 程剑侠 电编 张敏