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Expression profiles of genes associated with mitochondria-mediated apoptosis and their roles in liver regeneration

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ABSTRACT. Mitochondria are closely associated with cell survival, and it is of interest to determine whether apoptosis pathways, which are mediated by mitochondria, are involved in liver regeneration (LR). To identify the mechanisms underlying mitochondria-mediated apoptosis during rat LR, we used the Rat Genome 230 2.0 Array to investigate changes in gene expression. Next, we searched the GO and NCBI databases for genes associated with apoptosis mediated by mitochondria, and QIAGEN and KEGG databases for any related signaling pathways. The expression profile function (Et) was then used to calculate the activity level of known signaling pathways associated with apoptosis. The results revealed the expression of 436 genes associated with apoptosis signaling pathways, among which 152 were confirmed to be primarily related to LR. Overall, 99, 136, 95, and 91 genes were first expressed during the initiation [0.5-4 h after partial

hepatectomy (PH)], G0/G1 transition (4-6 h after PH), cell proliferation (6-66 h after PH), and redifferentiation and structural reconstruction (66-144 h after PH) phases, demonstrating that LR-related genes were primarily induced in the initiation phase, and were then expressed across multiple phases. Analysis using the gene synergy formula (Et) showed that caspase-dependent and DNA fragment-related/unrelated pathways induced apoptosis in the early and late periods of LR, and the caspase-independent and DNA fragment-related/unrelated pathways almost in the whole process. Therefore, these results show that several apoptosis pathways regulate LR in rat.

Key words: Mitochondria; Liver regeneration; Microarray; Gene expression; Gene synergetic effect; Apoptosis

INTRODUCTION

Liver regeneration (LR) is an important process that allows the organ to recover from various pathological insults, as well as from surgery or transplantation. Hepatocytes are parenchymal cells that are major contributors to LR. Under physiological conditions, less than 0.01% of hepatocytes in adult rat livers undergo mitosis (Fausto, 2000; Koniaris et al., 2003; Taub, 2004; Markiewski et al., 2006). However, when the liver is injured by toxicants, side effects of medication, and/or partial hepatectomy (PH), the remaining hepatocytes rapidly enter the cell cycle (Fausto, 2000). Together with other types of liver cells, hepatocytes then compensate for the lost tissue and restore liver function in a process referred to as LR (Fausto and Campbell, 2003). LR is usually divided into four stages: initiation (0.5-4 h after PH), G0/G1 transition (4-6 h after PH), cell proliferation (6-66 h after PH), and redifferentiation and structural reconstruction (66-144 h after PH) (Xu et al., 2005). Numerous cellular events are involved in LR, including activation, de-differentiation, proliferation, re-differentiation, and remodeling of tissue structures (Michalopoulos and DeFrances, 1997).

Apoptosis, or programmed cell death (Green and Kroemer, 2004), requires energy and can be induced by a variety of factors. Apoptosis plays an important role in normal cell development and tissue homeostasis (Joza et al., 2001; Danial and Korsmeyer, 2004), and dysregulated apoptosis is associated with many pathological conditions (Lawen, 2003), such as Alzheimer's disease (Yuan and Yankner, 2000; LeBlanc, 2005), Parkinson's disease (Wang et al., 2016), and cancer (Oltersdorf et al., 2005). Mitochondria promote apoptosis by releasing Cytochrome (CYTC), Diablo homolog (DIABLO) (Mastrangelo et al., 2015), AIF (Li et al., 2003), ENDOG (Dupont-Versteegden et al., 2006), and other bioactive substances. Mitochondrial apoptosis pathways are either caspase-dependent or -independent (Pinkoski et al., 2006). Caspase-dependent pathways may involve apoptosis caused by DNA fragmentation, or by other factors, whereas caspase-independent pathways involve apoptosis caused by ENDOG or AIF.

A total of 180 genes are associated with mitochondrial apoptosis pathways, and multiple interactions exist between genes and proteins. To understand the transcriptional mechanisms underlying mitochondria-mediated apoptosis during rat LR, we used the Rat Genome 230 2.0 Array, to investigate 175 genes associated with mitochondrial apoptosis pathways. Changes

in gene expression were determined at 0.5, l, 2, 4, 6, 8, 12, 16, 24, 30, 36, 42, 54, 66, 96, and 144 h post PH, and 60 genes related to LR were identified. We also employed the gene synergy formula (Et) (Xu et al., 2012) to analyze gene interactions in an effort to determine the role of genes related to mitochondrial apoptosis pathways in rat LR.

MATERIAL AND METHODS

Rat partial hepatectomy model

Adult (6-week-old) male Sprague Dawley rats weighing 230 ± 20 g were obtained from Henan Normal University's animal center. The rats were kept at 21 ± 2°C, with relative humidity of 60 ± 10%, 12 h of light per day (8:00-20:00), and free access to food and water. A total of 114 rats were randomly divided into 19 groups with six rats per group: nine groups underwent a two-thirds hepatectomy (PH groups), nine groups underwent a "sham" operation (SO groups), and one group underwent no procedure (control group). Rats in the PH groups were operated on according to the method described by Higgins and Anderson (1931) to remove the left and median lateral liver lobes. Rats in the SO groups underwent the same operation without removal of the liver lobes. Rats were sacrificed 0, 2, 6, 12, 24, 30, 36, 72, 120, and 168 h after the operations. All study procedures complied with Chinese animal protection laws.

Rat genome 230 2.0 microarray detection and analysis

Total RNA was extracted and purified following the same protocol as previously described (Xu et al., 2005). Briefly, biotin-labeled cRNA was prepared using the GeneChip IVT kit according to the manufacturer instructions. Hybridization was conducted automatically using a GeneChip Fluidics Station 450 (Affymetrix Inc). The results were scanned and converted into signal values using a GeneChip scanner 3000 (Affymetrix Inc), and signal values were normalized according to the manufacturer instructions.

P values were determined using the Affymetrix GCOS 2.0 software. At $P < 0.05$, the gene was defined as present (P); $P < 0.065$ was defined as marginal (M), and $P > 0.065$ was defined as absent (A). Changes in the expression gene were considered significant if the ratio of its PH value to that of the control was ≥ 3 or ≤ 0.33.

The significance of differences in gene expression between PH and SO groups was analyzed with an F-test. Genes found to change significantly at ≥1 time point during LR ($P < 0.05$) were defined as being associated with LR. To minimize errors due to experimental operation and the microarray, three repetitions were performed at each time point and the average value of the three independent assays was used for subsequent statistical analyses.

Gene synergy (Et) analysis

Xu et al. (2012) established the mathematical model E(t) to describe how physiological activities are governed by gene synergy according to the expression levels of genes detected by the Rat Genome 230 2.0 Microarray. Based on these methods of multivariate statistics and time series analysis, and the fact that physical activity is regulated by the synergy of some genes with others, the following spectrum function E(t) was employed:

$$E_t = \frac{\sum_{i=1}^{n}\sum_{k=1}^{n}\left[\left(X_i^{(t)} + X_k^{(t)}\right)\times r_{ik}\right]}{n(n+1)} \quad \text{(Equation 1)}$$

where the gene correlation coefficient (rik) is defined by the Pearson correlation coefficient:

$$r_{ik} = \frac{m\left(\sum_{t=1}^{m} X_i^{(t)} X_k^{(t)}\right) - \left(\sum_{t=1}^{m} X_i^{(t)}\right)\left(\sum_{t=1}^{m} X_k^{(t)}\right)}{\sqrt{\left[m\sum_{t=1}^{m} X_i^{(t)^2} - \left(\sum_{t=1}^{m} X_i^{(t)^2}\right)\right]\left[m\sum_{t=1}^{m} X_k^{(t)^2} - \left(\sum_{t=1}^{m} X_k^{(t)^2}\right)\right]}} \quad \text{(Equation 2)}$$

where *n* is the number of all genes participating in a physiological activity at time point *t*. The spectral function E(t) describes the effectiveness of the gene synergy dominating a physiological activity at a single point. By comparison with a control, the strength of the physiological activities at a certain time can be predicted. At *t*, assuming that the reference value is E(0), the corresponding physical activity is stronger than the control when $E(t) - E(0) \geq E(0)$, weaker when $E(t) - E(0) < 0$, and similar when $E(0) < E(t) < 2E(0)$.

RESULTS

Gene profiles related to mitochondria-mediated apoptosis-signaling pathways

According to data obtained from NCBI and Gene Ontology (GO), and from biological pathway maps in QIAGEN and Kyoto Encyclopedia of Genes and Genomes (KEGG), 180 genes are involved in mitochondria-mediated apoptosis signaling pathways. Of these, 175 genes are present on the Rat Genome 230 2.0 Microarray. Our array analysis showed that 60 genes were significantly changed in LR, which were identified as LR-related genes by comparing the differential gene expression in the PH and SO groups. Of these genes, 34 were upregulated, 23 were downregulated, and 3 were upregulated at certain time points and downregulated at others (referred to as "up/downregulated") during rat LR (Table 1).

Genes upregulated 230 times in total, and downregulated 132 times (Figure 1A). Throughout the rat LR process, 19 genes were upregulated and 12 genes were downregulated at the initiation phase (0.5-4 h after PH), 17 genes were upregulated and 10 genes were downregulated at the G0/G1 transition (4-6 h after PH), 31 genes were upregulated, 24 genes were downregulated, and 1 gene was up/downregulated during cell proliferation (6-66 h after PH), and 28 genes were upregulated, 10 genes were downregulated, and 1 gene was up/downregulated at the redifferentiation and structural reconstruction phase (66-144 h after PH) (Figure 1B).

Table 1. Expression profiles of 60 genes related to the mitochondria-mediated apoptosis signaling pathway.

Abbr.	Pathway	Recovery time (h) after partial hepatectomy (PH)																
		0	0.5	1	2	4	6	8	12	16	24	30	36	42	54	66	96	144
Cidea	1	1.00	1.23	1.87	1.23	1.87	1.41	2.64	1.32	1.62	1.23	0.93	1.62	0.93	1.23	1.52	1.23	2.46
Birc5	1, 2	1.00	0.23	0.35	0.33	0.71	0.22	1.00	0.87	0.33	0.93	0.57	0.33	1.15	0.81	0.29	0.22	0.31
Casp2	1, 2	1.00	0.91	0.39	1.09	0.73	0.56	1.01	0.88	0.68	1.02	0.72	0.74	1.29	0.42	1.14	0.95	0.50
Casp3	1, 2	1.00	0.82	0.88	0.62	0.83	0.58	0.32	0.89	0.75	0.97	0.63	0.73	0.92	0.85	0.82	0.74	0.97
Cyct	1, 2	1.00	0.84	1.07	0.95	1.49	1.16	3.46	0.76	1.42	1.21	0.79	1.20	0.92	0.79	1.83	1.18	1.57
Diablo	1, 2	1.00	0.57	0.14	0.76	2.00	1.52	2.64	2.30	1.62	1.62	2.30	1.87	1.52	2.64	2.14	1.52	2.00
Mtch1	1, 2	1.00	0.93	1.00	3.73	1.15	3.03	5.28	6.96	4.59	1.07	1.62	2.83	2.83	0.71	1.07	1.00	1.23
Rnf7	1, 2	1.00	0.99	2.31	2.15	1.89	1.60	1.69	0.84	1.87	1.65	0.58	1.89	0.74	1.61	1.77	2.24	3.50
Smpd1	1, 2	1.00	1.00	0.41	0.66	0.27	0.71	1.00	0.62	0.81	0.76	0.71	0.47	1.07	0.54	0.76	0.31	0.81
Abl1	1, 2, 3, 4	1.00	1.07	1.07	1.07	1.32	1.41	1.52	1.07	1.41	1.32	1.00	1.32	0.87	0.87	1.74	1.32	5.66
Adm	1, 2, 3, 4	1.00	1.23	0.76	0.76	0.54	1.62	1.74	1.87	0.57	0.93	1.15	0.50	1.07	0.76	0.71	1.00	0.93
Aes	1, 2, 3, 4	1.00	1.94	1.96	4.47	5.11	1.78	2.75	1.78	1.08	1.24	1.63	1.28	1.11	0.90	0.96	1.05	1.12
Anxa7	1, 2, 3, 4	1.00	0.54	1.95	3.81	3.66	4.97	3.16	2.77	3.89	2.68	2.05	3.14	3.30	3.26	3.28	6.79	6.39
Bad	1, 2, 3, 4	1.00	0.87	1.00	0.81	1.07	0.81	0.87	0.76	1.74	4.00	4.92	3.25	3.73	1.62	1.62	1.62	1.62
Bak1	1, 2, 3, 4	1.00	3.48	8.00	3.25	2.00	6.06	5.28	1.15	4.29	3.03	3.48	4.29	1.87	3.03	6.96	4.00	4.92
Bcl2a1	1, 2, 3, 4	1.00	1.15	2.00	0.93	0.93	1.32	1.23	0.93	0.93	0.93	1.41	1.00	0.81	0.81	0.81	1.00	1.15
Bcl2l11	1, 2, 3, 4	1.00	1.19	1.06	5.68	2.28	4.50	7.72	5.59	4.49	5.17	4.81	2.24	1.70	3.23	3.01	1.21	1.22
Bdnf	1, 2, 3, 4	1.00	1.04	2.77	2.80	1.32	2.38	1.51	1.28	2.09	3.54	1.89	3.10	1.20	2.03	3.75	2.56	3.00
Bid3	1, 2, 3, 4	1.00	2.83	1.87	3.73	4.92	6.06	4.92	1.62	3.73	2.30	1.52	1.00	0.81	0.81	2.64	2.83	3.73
Capn1	1, 2, 3, 4	1.00	0.41	0.25	0.62	0.81	0.33	0.87	0.44	0.23	0.27	0.50	0.25	0.16	0.25	0.31	0.33	0.71
Casp8	1, 2, 3, 4	1.00	0.57	1.74	0.93	0.54	0.44	1.15	1.15	1.52	1.52	1.15	0.54	1.32	1.41	1.62	1.15	1.23
Casp9	1, 2, 3, 4	1.00	1.00	1.41	0.33	0.63	0.81	1.23	0.81	0.64	0.67	0.49	0.22	0.57	0.38	0.59	0.46	0.73
Dap3	1, 2, 3, 4	1.00	4.92	1.52	2.14	5.28	3.73	5.28	4.59	3.25	3.73	3.25	3.73	4.59	1.00	2.83	3.73	3.48
Dnaja3	1, 2, 3, 4	1.00	1.07	1.00	0.76	0.93	0.54	0.50	0.62	0.57	1.41	1.00	1.15	1.15	1.15	0.93	1.07	0.81
Egr2	1, 2, 3, 4	1.00	1.21	1.33	3.64	9.28	5.16	6.51	5.16	3.28	4.24	2.24	3.74	3.04	3.14	4.80	5.99	6.01
Fdxr	1, 2, 3, 4	1.00	0.29	0.50	0.19	0.20	0.33	0.10	0.19	0.15	0.13	0.16	0.20	0.23	0.10	0.25	0.16	0.12
Fhit	1, 2, 3, 4	1.00	2.83	1.07	6.96	4.00	3.03	6.50	4.92	3.73	3.73	6.96	6.96	4.59	2.46	8.00	4.00	9.85
Frap1	1, 2, 3, 4	1.00	0.31	0.76	3.25	1.52	2.00	2.46	1.00	1.52	3.25	1.41	1.32	1.23	1.52	2.46	2.14	2.30
Gimap5	1, 2, 3, 4	1.00	1.23	1.07	0.81	0.87	1.15	1.52	1.07	1.87	1.32	2.14	1.87	1.52	2.83	1.74	1.52	2.14
Hdac7a	1, 2, 3, 4	1.00	1.21	9.61	1.29	0.85	0.77	1.55	1.52	1.15	1.02	0.59	1.16	0.87	0.62	2.47	4.32	2.29
Hmox1	1, 2, 3, 4	1.00	0.71	1.15	1.23	1.41	1.15	1.32	0.50	0.81	0.71	0.62	0.81	0.54	0.93	0.81	1.00	1.52
Il10	1, 2, 3, 4	1.00	1.32	1.23	0.87	1.07	0.71	0.57	0.41	0.54	0.66	0.66	0.66	0.71	0.76	0.87	0.87	0.81
Lck	1, 2, 3, 4	1.00	1.15	0.15	1.07	1.23	1.62	0.20	1.87	0.41	0.81	0.47	0.81	1.07	1.23	1.32	1.87	2.14
Mcl1	1, 2, 3, 4	1.00	1.55	1.86	1.06	3.16	3.04	2.31	4.54	1.75	1.67	0.85	1.80	2.23	5.37	2.72	4.31	2.98
Mrpl37	1, 2, 3, 4	1.00	1.63	0.96	0.92	2.57	0.98	1.39	1.00	1.04	1.04	0.86	1.28	0.98	0.99	1.63	2.34	1.60
Mrpl41	1, 2, 3, 4	1.00	0.87	1.15	2.30	1.74	0.76	1.07	0.87	0.81	0.81	0.20	0.66	0.25	0.27	2.00	0.36	1.62
Nr3c1	1, 2, 3, 4	1.00	3.00	2.39	2.31	2.69	3.78	3.18	2.32	2.41	2.28	1.69	2.63	1.89	1.66	2.87	2.72	4.39
Opa1	1, 2, 3, 4	1.00	0.85	0.89	2.61	1.73	2.58	1.75	2.30	1.28	3.48	0.73	2.13	1.07	1.93	3.14	2.62	3.61
Paox	1, 2, 3, 4	1.00	0.81	0.71	0.33	0.62	0.44	0.66	0.81	0.54	0.57	0.54	0.50	0.47	0.41	0.54	0.66	0.62
Plscr3	1, 2, 3, 4	1.00	0.87	1.15	0.35	0.38	0.35	0.54	0.93	1.41	0.29	0.38	0.38	0.31	0.47	0.76	0.81	0.54
Prdx3	1, 2, 3, 4	1.00	0.47	0.65	0.50	0.62	0.64	0.74	0.70	0.50	0.60	0.68	0.71	0.71	0.57	0.81	0.77	0.74
Prkca	1, 2, 3, 4	1.00	0.54	1.74	2.00	1.74	2.64	3.48	1.23	3.48	1.41	2.64	3.03	3.73	2.46	6.96	4.59	5.28
Rela	1, 2, 3, 4	1.00	0.54	0.50	1.15	0.71	0.93	1.74	1.23	1.41	1.23	0.57	0.87	0.81	0.54	1.62	1.07	1.41
Siva	1, 2, 3, 4	1.00	0.19	0.14	0.31	0.25	0.29	0.29	0.44	0.27	0.25	0.47	0.50	0.50	0.76	0.23	0.33	0.38
Slc25a4	1, 2, 3, 4	1.00	0.33	0.71	0.66	0.50	1.23	1.74	1.07	1.00	1.23	0.29	1.07	1.00	0.50	1.07	0.81	1.74
Sod2	1, 2, 3, 4	1.00	1.07	1.47	1.20	1.22	1.54	1.11	1.20	1.99	2.08	0.95	0.70	1.14	1.17	2.06	1.55	1.54
Tp53	1, 2, 3, 4	1.00	0.89	0.93	1.09	0.95	0.91	3.55	1.24	0.91	1.05	0.76	0.98	0.83	0.85	4.44	2.01	3.68
Amid	4	1.00	1.46	1.22	2.03	0.95	1.59	1.94	1.67	3.79	6.89	4.33	5.09	5.07	3.51	5.14	1.82	4.57
Cul7	r	1.00	2.30	2.30	0.62	1.87	0.81	0.35	1.07	0.87	1.00	0.35	0.31	0.62	0.23	1.07	0.47	0.81
Hspa5bp1	r	1.00	1.07	0.81	0.66	0.93	1.07	1.00	1.52	1.23	1.87	2.00	1.41	1.52	1.23	1.15	1.07	1.15
Hspb1	r	1.00	0.38	1.41	1.32	1.87	0.38	0.76	1.52	3.48	11.31	9.85	11.31	8.00	3.25	5.66	3.73	5.66
Hspd1	r	1.00	0.87	1.07	1.07	0.76	1.23	1.52	2.00	1.07	1.23	1.07	1.00	0.81	0.57	0.81	0.87	0.93
Il3	r	1.00	0.25	1.00	0.71	0.57	0.93	0.44	0.57	0.19	0.18	0.09	0.93	0.50	0.29	0.71	0.35	0.62
Mapk1	r	1.00	1.96	1.71	1.71	1.55	1.62	2.43	1.22	2.45	1.81	1.03	2.25	2.26	1.19	2.17	1.23	1.85
Mapt	r	1.00	0.93	1.44	1.33	1.49	1.29	1.50	1.12	1.58	1.25	0.84	1.27	0.97	0.76	2.36	1.93	1.86
Nfkb1	r	1.00	0.47	1.07	0.44	0.66	0.23	0.41	0.71	0.50	0.66	0.38	0.44	0.50	0.71	0.47	0.35	0.38
Nfkbia	r	1.00	4.59	1.41	2.64	1.23	1.74	1.23	2.64	1.74	1.41	6.06	1.07	1.74	1.00	1.23	0.87	1.41
Ogg1	r	1.00	0.79	0.99	0.77	0.88	1.68	0.80	0.49	0.54	0.64	0.64	0.41	0.58	0.56	0.73	0.95	0.88
Serpinb5	r	1.00	1.07	0.54	0.71	0.81	0.25	1.15	0.50	1.41	0.81	0.44	1.00	1.23	1.07	0.76	0.76	1.62
Tert	r	1.00	0.54	0.81	0.71	0.54	0.76	0.81	0.57	1.15	0.71	0.76	0.66	0.62	0.23	1.07	0.76	1.07

r = genes related to mitochondria-mediated apoptosis signaling pathways. 1 = Caspase-dependent and DNA fragmentation-related pathways. 2 = Caspase-dependent and DNA fragmentation-unrelated pathways. 3 = Caspase-independent and DNA fragmentation-related pathways (ENDOG). 4 = Caspase-independent and DNA fragmentation-irrelevant pathways (AIF). Light gray backgrounds represent gene up-expression, and dark grey backgrounds represent gene down-expression.

Initiation of expression

The array data show that at each time point during LR, the number of upregulated and downregulated genes initially expressed, and the number of total genes upregulated and downregulated were as Table 2 and Figure 2.

Similarity and temporal correlations between genes

Based on similarities in the expression of LR-related genes, the 60 genes described here are divided into three categories: all-upregulated, all-downregulated, and dominant-downregulated, involving 34, 23, and 3 genes, respectively (Figure 3). Based on the temporal

expression of genes during LR, these 60 genes can be divided into seven groups: 0.5 and 1 h, 2-6 h, 8 and 12 h, 16 and 24 h, 30 and 42 h, 36 and 54 h, and 66 and 144 h, in which the number of upregulated and downregulated genes were 11 and 9, 19 and 11, 21 and 13, 18 and 8, 17 and 13, 18 and 17, and 27 and 10, respectively (Figure 3).

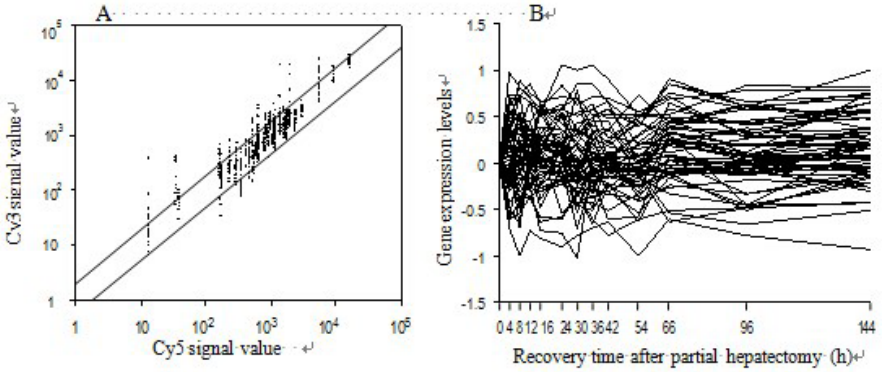

Figure 1. Expression profiles of 60 genes related to the mitochondria-mediated apoptosis signaling pathway during rat liver regeneration. **A.** Frequency and abundance of gene expression. Each point shown represents a gene product signal value at the appropriate point in time. Points above the slash represent upregulated genes, points below represent downregulated genes, and points within the shaded area indicate no significant changes in gene expression. The farther away from the diagonal, the greater the multiple changes of gene expression; **B.** Expression changes of genes related to LR.

Table 2. Initial expression and total expression of 60 mitochondrial apoptotic-pathway-related LR genes at several time points.

	0.5	1	2	4	6	8	12	16	24	30	36	42	54	66	96	144
Initial up	8	11	7	2	0	3	1	1	1	2	0	0	0	2	1	2
Initial down	6	8	3	0	2	3	3	1	0	3	0	0	2	0	0	0
Total up	8	11	17	16	16	19	12	14	15	14	15	11	12	22	17	22
Total down	6	8	8	7	9	9	7	7	5	12	12	8	11	5	10	5

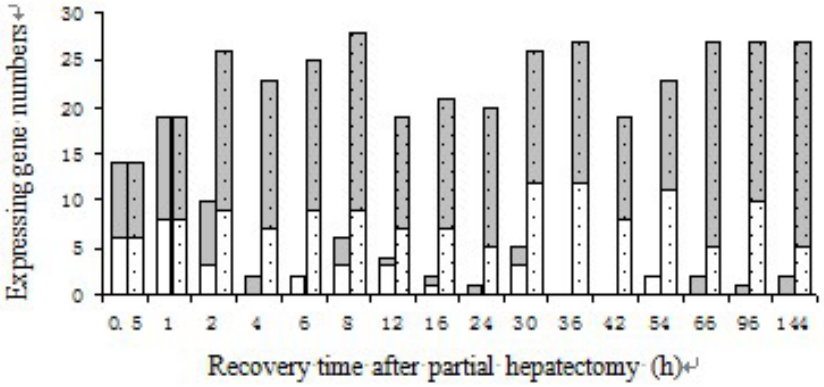

Figure 2. Starting expression and total expression of 60 mitochondrial apoptotic-pathway-related LR genes at several time points. Non-dotted columns: initial number of expressed genes. Dotted columns: total number of expressed genes. Gray columns: number of upregulated genes. White columns: number of downregulated genes.

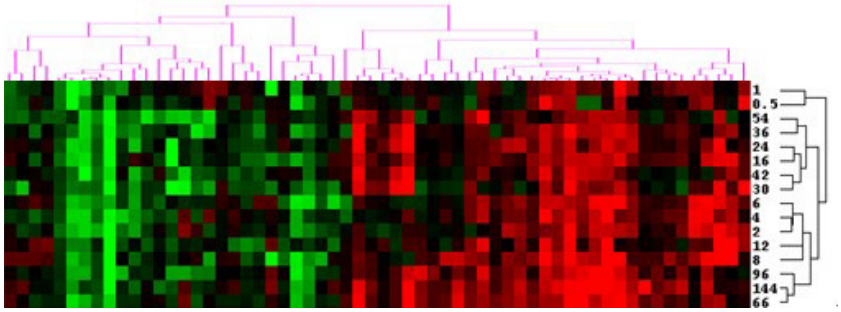

Figure 3. Similarity clustering and temporal correlation of 60 gene expressions related to mitochondrial-mediated apoptosis-signaling pathway during LR. Reds represent upregulated genes, greens represent downregulated genes, and black indicates no significant expression changes. Pink tree shows similarity clustering and black tree shows temporal correlation clustering.

Gene expression patterns

The expression patterns of the 60 genes related to LR can be divided into the following 19 categories (Table 3 and Figure 4).

Table 3. Nineteen expression patterns of 60 genes related to mitochondrial-mediated apoptosis-signaling pathways during liver regeneration.

Category		Number of genes	Figure	Category		Number of genes	Figure
Upregulation	1 point	6	4A	Downregulation	1 point	8	4E
	2 points	3	4A		2 points	1	4F
	3 points	1	4A		3 points	3	4F
	1 period	3	4B		1 period	1	4F
	2 periods	3	4B		2 periods	2	4F
	3 periods	1	4B		1 period/2 points	1	4F
	1 period/1 point	4	4C		2 periods/1 point	1	4F
	1 period/2 points	1	4C		Many periods	6	4G
	2 periods/1 point	3	4C	Up/downregulation		6	4H
	Many periods	6	4D				

Figure 4. Expression patterns of 60 genes related to mitochondrial-mediated apoptosis-signaling pathways during LR. See the 19 categories referenced above. X-axis represents recovery time after partial hepatectomy (h), y-axis represents logarithm of product gene expression signal ratio between each time point and the control.

Correlations between genes

To analyze correlations between genes related to mitochondria-mediated apoptosis and LR, the spectrum function E(t) was used to analyze the gene synergetic effects. The E(t) values of the target genes were significantly higher than those of the control at 6, 8, 66-144 h (Figure 5A). The E(t) values of the genes related to caspase-dependent/DNA fragmentation-related pathways were significantly higher than the control at 2-8, 66-144 h (Figure 5B). The E(t) values of the genes related to caspase-dependent/DNA fragment-unrelated pathways were significantly higher than the control at 4-8, 66-144 h (Figure 5C). The E(t) values of the genes related to caspase-independent/DNA fragment-related pathways were significantly higher than the control at 2-8, 16-24, 36, 66-144 h (Figure 5D). The E(t) values of the genes related to caspase-independent/DNA fragment-unrelated pathways were significantly higher than the control at 2-8,16-24, 36, 66-144 h (Figure 5E).

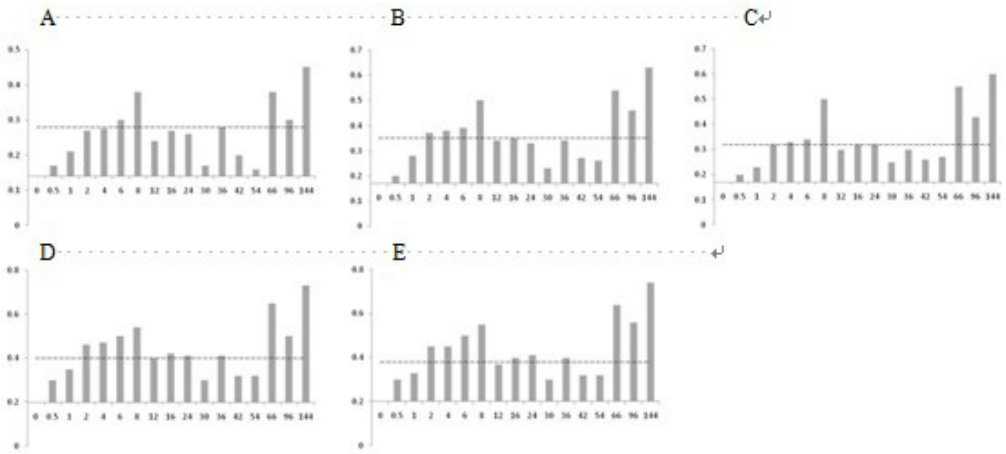

Figure 5. Analysis of correlation between genes related to mitochondria-mediated apoptosis-signaling pathways and LR. **A. B.** Mitochondrial apoptosis signaling pathways; **C. D.** caspase-dependent/DNA fragment- related pathways; **E. F.** caspase-dependent/DNA fragment-irrelevant pathways; **G. H.** caspase- independent/DNA fragment-related pathways (ENDOG); **I. J.** caspase-independent/DNA fragment-irrelevant pathways (AIF). Blank columns represent pathway-related genes; dotted columns represent LR-related genes. X-axis is recovery time after partial hepatectomy (h), y-axis is spectrum function E(t).

DISCUSSION

The primary focus of this study was to investigate the roles and changes in expression of 60 genes related to mitochondria-mediated apoptosis signaling pathways during rat LR. Among them, B-cell leukemia/lymphoma 2-antagonist/killer 1 (BAK1), B-cell leukemia/lymphoma 2-like 11 (BCL2L11), and Death-associated protein 3 (DAP3) are known to promote cell apoptosis (Harada et al., 2010; Gu et al., 2014; Dai and Grant, 2015), and all were upregulated during the entire period of rat LR, indicating that they may regulate apoptosis in tandem. Adrenomedullin (ADM) and 8-oxoguanine DNA-glycosylase 1 (OGG1) can inhibit apoptosis, and both were downregulated at 36 h post-PH, therefore promoting apoptosis.

Mitochondrial carrier homolog 1 (MTCH1), polyamine oxidase (PAOX), and phospholipid scramblase 3 (PLSCR3) are all associated with changes in mitochondrial membrane structure and the release of cytochrome C; *mtch1* was upregulated at 2-6 and 36-54 h after PH, while *paox* and *plscr3* were downregulated. CYTC, caspase 3 (CASP3), and heat shock protein 40 homolog subfamily A member 3 (DNAJA3) can promote apoptosis (Pu et al., 2015); *cyct* was upregulated at 8 h, while *casp3* and *dnaja3* were downregulated. AIF-like mitochondrion-associated inducer-of-death (AMID) can promote apoptosis, and the 27-kDa heat shock protein 1 (HSPB1) can inhibit apoptosis (Elguindy and Nakamaru-Ogiso, 2015), and both were upregulated at 16-66 and 144 h post HR. Calpain 1 (CAPN1) promotes apoptosis, CD27 binding protein (SIVA) inhibits apoptosis (Zheng et al., 2015), and both were downregulated during the entire LR process. All 15 of these genes may interact to regulate LR.

Caspases, a group of cysteine proteases that have similar amino acid sequences and secondary structure, are closely associated with apoptosis, cytokine maturation, and cell growth and differentiation. Among them, caspase 8 (CASP8) may alter membrane permeability to cause mitochondria to release CYTC. Caspase 9 (CASP9) can activate CASP3 to promote apoptosis (Ni et al., 2011). Caspase 2 (CASP2) can degrade BID and release CYTC, and can also directly induce CYTC, Apoptosis inducing factor (AIF), diablo, IAP-binding mitochondrial protein (SMAC), and other mediators to promote cell apoptosis. These were downregulated at 6 h; 30, 36, 54, and 96 h; and 54 and 144 h, respectively, after PH, suggesting that they inhibit apoptosis at different stages of LR.

Abelson murine leukemia viral (v-abl) oncogene homolog 1 (ABL1) is a tyrosine kinase that is involved in cell differentiation, apoptosis, cell adhesion, cell cycling, and mismatch repair; it was upregulated at 144 h after PH, possibly due to cellular differentiation, as well as tissue, structure, and function reconstruction. Amino-terminal enhancer-of-split (AES) is involved in cell differentiation and organ development, and was upregulated at 2, 4, and 8 h after PH, possibly related to protein modifications and cell differentiation during rat LR. Annexin 7, which causes aggregation of membranes in a Ca^{2+}-dependent manner and has been suggested to promote membrane fusion during exocytosis of lung surfactant, catecholamines, and insulin (Caohuy and Pollard, 2002), was upregulated after PH and is likely involved in the secretion of bioactive molecules and in the regulation of the intracellular environment. The B-cell leukemia/lymphoma 2 (BCL2) families, which include BAD, BAK1, BCL2A1, BCL2L11, BID3, and MCL1, can form homo/heterodimers, function during embryonic development, and exert anti-apoptotic or pro-apoptotic effects during tumor formation. In a concentration-dependent manner, B-cell leukemia/lymphoma 2-associated death-promoter (BAD) replaces the location of BAX in the heterodimer of BCL2-XL/BAX or BCL2/BAX, then the free BAX promotes cell apoptosis; *bad* was upregulated 24-42 h after PH, and may have promoted defective and/or excessive apoptosis of new cells. B-cell leukemia/lymphoma 2-related protein A1 (BCL2A1) can inhibit apoptosis (Dai and Grant, 2015) and prevents CYT release from mitochondria; it was upregulated 1 h after PH, and possibly inhibited residual liver cell apoptosis. BH3 domain interacting with BCL 2 family genes (BID3) are antagonists of apoptosis and interact with BCL-XL and BCL2. BID3 was upregulated at 0.5-8, 16, 24, and 66-144 h after PH, and may have played an important role in preventing apoptosis and in regulating liver volume. Brain-derived neurotrophic factor (BDNF), which promotes nerve development, was upregulated at 1-6, 16, 24, 36, and 54-144 h post-PH, and was possibly related to nerve reconstruction. Cell death-inducing DNA fragmentation factor,

alpha subunit-like effector A (CIDEA), promotes DNA fragmentation and apoptosis (Omae et al., 2012); it was upregulated 8 h after PH and likely promoted apoptosis during the initial phase. It was also upregulated 144 h after PH and may have been related to defective and/or excessive apoptosis of new cells and changes in liver volume. DIABLO promotes apoptosis (Amar et al., 2015) and was upregulated at multiple time points, indicating its functionality throughout the entire rat LR process. Ferredoxin reductase (FDXR) is a component of the cytochrome P-450 mitochondrial electron transport chain, and was downregulated during the entire LR process, indicating changes in mitochondrial biochemical activity and function. FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) can bind with checkpoint proteins, ATM, ATR, FRP1, and DNA protein kinase, and inhibit cell cycle progression; it was upregulated at 2-8, 24, and 66-144 h after PH, and may have been involved in regulating cell cycle progression. GTPase immunity-associated protein family member 5 (GIMAP5), located in the external membrane, can control membrane voltage; it was upregulated at 30, 54, and 144 h after PH and was likely related to mitochondrial development and function. Histone deacetylase 7 (HDAC7A) inhibits transcription and activates apoptosis; it was upregulated at 66-144 h after PH, during which it likely inhibited hepatocyte proliferation while regulating liver volume. Mitochondrial ribosomal protein L37 (MRPL37) and programmed cell death factor 9 (PDCD9) share high homology, were upregulated during LR, and were probably involved in the regulation of liver volume. Mitochondrial ribosomal protein L41 (MRPL41) is an antagonist of BCL2; it was upregulated at 2 and 66 h post-PH, probably related to nascent cell protein synthesis. It also was downregulated at 30, 42, 54, and 96 h post-PH, which may have promoted apoptosis. V-rel reticuloendotheliosis viral oncogene homolog A (RELA) may associate with NFκB to form active NFκB complexes, both of which are associated with a variety of cellular activities. It was downregulated 1 h after PH, and was likely involved in cellular activation and differentiation. Nuclear receptor subfamily 3 group C member 1(NR3C1) reduces inflammation by inactivating NFκB and AP-1; it was upregulated almost entirely throughout LR, indicating that inhibition of the inflammatory response was related to LR. Solute carrier family 25 member 4 (SLC25A4) can promote NFκB complex enter mitochondria and inhibit anti-apoptosis proteins such as BCL-XL, which may enhance the sensitivity of cells to apoptosis; it was downregulated at 30 and 54 h post-PH, preventing cells from undergoing apoptosis. Upon phosphorylation, ring finger protein 7 (RNF7) promotes G1/S phase transition; it was upregulated at 1, 2, 96, and 144 h post-PH, possibly promoting cell cycle progression at the appropriate time points. Optic atrophy 1 homolog (OPA1) promotes CYTC release and mitochondrial lysis (Zhao et al., 2013), and was upregulated in early and late phases of LR, probably inducing damaged and excess apoptosis in the corresponding periods. Peroxiredoxin 3 (PRDX3) has antioxidant effects, and was downregulated 0.5-2 h after PH, most likely relative to cell differentiation. Protein kinase C alpha (PRKCA) is a mitochondrial protein that promotes cell proliferation, differentiation, and has antioxidant function; it was upregulated at 2-8, 16, and 30-144 h post-PH, suggesting that it played an important role in LR. Acid lysosomal sphingomyelin phosphodiesterase 1 (SMPD1) is related to bone marrow cell differentiation; it was downregulated at 1-4, 36, and 96 h after PH, suggesting that it also relates to the regeneration of liver tissue. Mitochondrial superoxide dismutase 2 (SOD2) inhibits proline oxidase-induced apoptosis by reducing the release of CYTC; it was upregulated 24 and 66 h after PH, and likely inhibited apoptosis during these two periods.

Cullin 7 (CUL7) is a component of chromosome segregation complex that is related to homologous chromosome separation, selective degradation of proteins, and other processes, and plays an important role in normal development and tumorigenesis. CUL7 was upregulated at 0.5 and 1 h post-PH, possibly related to the degradation of specific proteins and to cell differentiation. It was downregulated 8, 30, 36, 54, and 96 h after PH, suggesting that it was also involved in physiological activities during these periods. The 70-kDa heat shock protein 5 binding protein 1 (HSPA5BP1) can enhance the function of HSP70, and was upregulated at 30 h post-PH, during which it likely had a protective effect on remnant liver cells. Interleukin-3 (IL-3) can promote the maturation of T-cells and proliferation and differentiation of bone marrow pluripotent hematopoietic stem cells; it was downregulated at 16-30, 42, 54, and 96 h after PH, and may have inhibited cell differentiation during LR. Microtubule-associated protein tau (MAPT) can inhibit the expression of inhibitor of apoptosis proteins (IAPs) and activate the expression of the pro-apoptotic gene *casp3*, causing the cell cycle to arrest in the G2/M phase; it was upregulated during LR, likely inhibiting hepatocyte proliferation and regulating liver volume. Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor alpha (NFκBIA) was upregulated in early stages of LR and may have played a role in eliminating NFκB present in liver cells. Serine or cysteine proteinase inhibitor clade B member 5 (SERPINB5) can penetrate the outer membrane of mitochondria, decrease transmembrane potential, and reduce the anti-apoptosis ability of cells; it was downregulated 6, 12, and 30 after PH, likely inhibiting cell apoptosis.

To analyze correlations between genes related to mitochondria-mediated apoptosis during rat LR, the spectrum function E(t) was used to analyze the gene synergetic effects. We found that the E(t) values of the caspase-dependent and DNA fragment-related pathways were higher than that of the control at 2-8, 66-144 h, indicating that this branch of pathways promotes apoptosis during the early and late periods of LR process. The E(t) values of the caspase-dependent and DNA fragment-unrelated branch were higher than that of the control at 4-8, 66-144 h, suggesting that these pathways promote apoptosis during the early and late periods of LR process. The E(t) values of the caspase-independent and DNA fragment-related branch (ENDOG) were higher than that of the control at 2-8, 16-24, 36, 66-144 h, indicating that this branch promotes apoptosis almost during the entire LR process. The E(t) values of the caspase-independent and DNA fragment-unrelated branch (AIF) were higher than that of the control at 2-8, 16-24, 36, 66-144 h, indicating that this branch promotes apoptosis almost during the entire LR process.

In summary, the four branches of the mitochondrial apoptosis signaling pathways were found to promote apoptosis of damaged, defective, diseased, and excess cells throughout the LR process in rats, and co-operated to regulate the quality and quantity of cells in the regenerating liver. In the future, we will further verify these results at the cellular level by applying a variety of molecular biology techniques.

Conflicts of interest

The authors declare no conflict of interest.

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