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mRNA chip-based analysis on transcription factor regulatory network central nodes of protection targets of Deproteinized Extract of Calf Blood on acute liver injury in mice

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ABSTRACT

Our previous study found that Deproteinized Extract of Calf Blood (DECB) could protect the acute liver injury induced by carbon tetrachloride in mice, but the target-related transcription factors and their regulatory networks were not comprehensively studied. Based on the mRNA expression microarray data obtained in the previous study, the mRNA transcription factor regulatory networks were constructed by screening the transcription factors of differentially expressed genes and their corresponding target proteins, and the analysis on the functions and pathways of the regulatory network central nodes was performed. Eight genes Ltf, Tnf, Il6, Jun, Il12b, Stat3, Rel and Crem could regulate the inflammatory factors, and TNF signaling pathway and Jak-STAT signaling pathway might play an important role in the mechanism through which DECB protected the liver of mice. DECB can not only inhibit the apoptosis of hepatocytes, but also inhibit the inflammatory cytokines.

1. Introduction

DECB (Deproteinated Extract of Calf Blood) is a bioactive substance that the serum or whole blood of well-developed calf at ages from 1 to 6 months is deproporonized, filtered, concentrated and purified by physical or chemical methods, with less toxic and side effects. The DECB's organic substances are in account for 30%, such as low molecular peptides, amino acids, lipids and sugars, and lipid metabolic intermediates. Small molecular peptides are one of the active ingredients in the DECB. Capillary zone electrophoresis and RP-HPLC are used for the fingerprint analysis of small peptides in DECB, and small peptides in DECB are qualitatively analyzed by LC-MS. Our previous study found that DECB could protect the acute liver injury induced by CCl_4 in mice, but the key targets and related pathways related to its mechanisms were only investigated, and the target-related transcription factors and their regulatory networks were not comprehensively and deeply explored in the study [1].

The regulatory networks here refer to those formed in the interaction of genes, RNA and proteins in cells [2]. There are some central coupling sites in gene regulatory networks, and the central nodes can couple some other key regulatory factors to play an important regulatory role in the occurrence and development of diseases [3,4]. Therefore, the mechanism of a drug can be understood through deducing the regulatory system of various metabolites (DNA, RNA and protein) and their related biochemical reaction pathways to establish a cause-and-effect relationship network linking multiple biochemical pathways closely related to diseases, and analyzing the network central coupling sites and their regulatory genes.

In this study, based on the data obtained by the mRNA expression microarray in the previous study, mRNA transcription factor-target protein regulatory networks were constructed, and functions and signal pathways of the central coupling nodes in the regulatory networks were analyzed, to supplement and improve the targets of protection of DECB on acute liver injury in mice and the regulatory mechanisms of its related transcription factors, which was expected to provide an important theoretical basis for the further research and development of DECB.

2. Experimental method

2.1. Microarray data

The microarray data of this study were derived from the previous experimental mRNA data, which have been uploaded to the National Biotechnology Information Center (NCBI) database GEO DataSets, and GES accession is GSE82771 [1].

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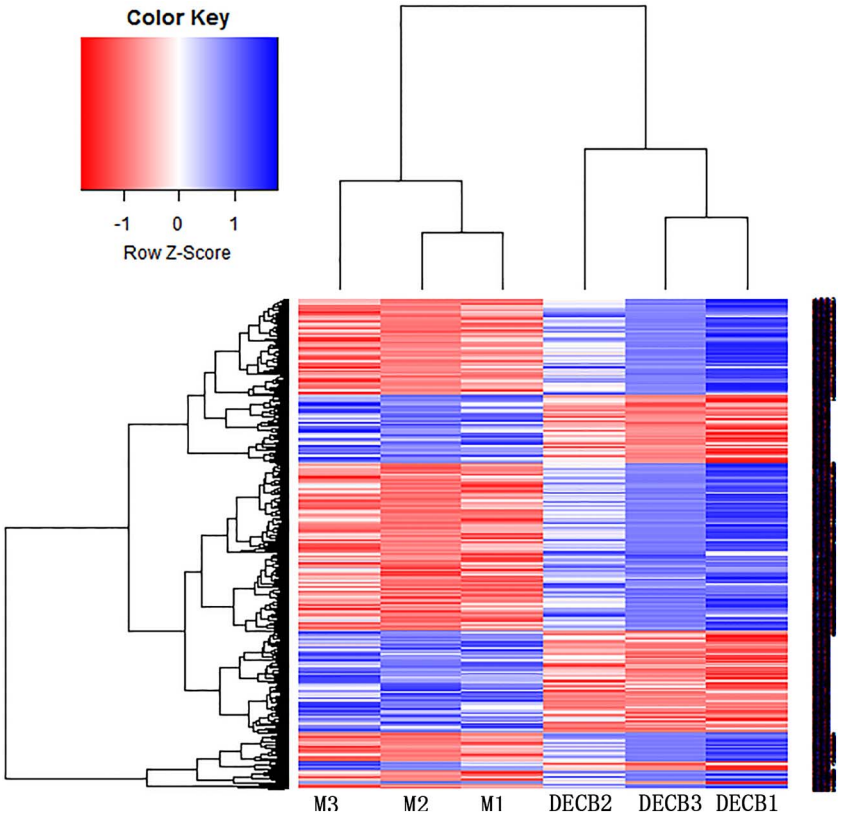

Fig. 1. Results by mRNA expression profile microarray.
(M, model group; DECB, Deproteinized Extract of Calf Blood group.)

Table 1
Twelve transcription factors and their corresponding target genes.

TF	Target gene no.	Gene ID	Description
Ltf	236	17002	Lactotransferrin
Crp	28	12944	C-reactive protein, pentraxin-related
Stat3	25	20848	Signal transducer and activator of transcription 3, transcript variant 1
Atf2	13	11909	Activating transcription factor 2 transcript variant 1
Junb	11	16477	Jun-B oncogene
Crem	11	12916	cAMP responsive element modulator, transcript variant 3
Egr2	9	13654	Early growth response 2
Rel	8	19696	Reticuloendotheliosis oncogene
Cebpe	4	110794	CCAAT/enhancer binding protein, epsilon
Bcl3	3	12051	B cell leukemia/lymphoma 3
Arnt	2	11863	Aryl hydrocarbon receptor nuclear translocator, transcript variant 1
Tes	2	21753	Testis derived transcript

2.2. Screening of transcription factors and their target genes

On the http://rulai.cshl.edu/TRED website, the option "Search TF Target Genes" was selected. After entering the next page, the Factor Name in the options of Type of search key was selected and Gene symbol name was input, then the *Mus musculus* in Target Gene Organism was selected, "all" in the Promoter Quality and Binding Quality was selected, and finally "search" was clicked to carry out the search to obtain the corresponding target genes [5].

2.3. Neural regulatory network diagram

Twelve transcription factors (TF) and 352 target genes were predicted to combine a total of 352 TF-to-target pairs. The relation obtained from the analysis on the differential co-expression was mapped to the *Mus musculus* transcription factors and target gene pairs to obtain transcription regulation pairs. Finally, Cytoscape software was used for plotting [6].

2.4. Analysis of GO functional annotation

On the website http://david.abcc.ncifcrf.gov/, DAVID database was opened, 1355 genes were submitted as the gene sets for the further analysis, and the corresponding gene identifier (the gene identifier corresponding to the gene name was OFFICIAL_GENE_SYMBOL) was selected at the same time; the *Mus musculus* whole genome was checked as the background gene, and then "Functional Annotation Tool" was selected as the analysis tool, to get the results of the GO enrichment analysis and biological pathway enrichment analysis of differentially expressed genes [7].

2.5. Analysis of differentially expressed gene functions

Based on the NCBI Gene Ontology database, the GO annotation of these genes was performed to obtain all GOs that genes were involved. Fisher exact test and X^2 test were used to calculate the significance level and misdiagnosis rate of each GO, and the *P* value was calibrated with the misdiagnosis rate, thereby screening out the GO significance reflected by the differentially expressed genes ($P < 0.05$). Using the European Bioinformatics Institute (EBI) database, the experimental results were manually analyzed [8].

3. Experimental results

3.1. Results mRNA expression microarray data

The mRNA microarray results showed that 1355 differentially expressed genes were screened out, of which the expression of 507 genes (30.49%) was significantly up-regulated and that of 848 genes (69.51%) was significantly down-regulated (Fig. 1).