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Multi-stage analysis of gene expression and transcription regulation in C57/B6 mouse liver development

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ABSTRACT

The liver performs a number of essential functions for life. The development of such a complex organ relies on finely regulated gene expression profiles which change over time in the development and determine the phenotype and function of the liver. We used high-density oligonucleotide microarrays to study the gene expression and transcription regulation at 14 time points across the C57/B6 mouse liver development, which include E11.5 (embryonic day 11.5), E12.5, E13.5, E14.5, E15.5, E16.5, E17.5, E18.5, Day0 (the day of birth), Day3, Day7, Day14, Day21, and normal adult liver. With these data, we made a comprehensive analysis on gene expression patterns, functional preferences and transcriptional regulations during the liver development. A group of uncharacterized genes which might be involved in the fetal hematopoiesis were detected.

Background

The liver is one of the largest organs in the body and performs a number of complex functions essential to life. Functions of the liver range from converting glucose to glycogen, producing bile acids, to forming plasma proteins and filtering harmful substances from the blood.

In mouse, liver ontogeny begins around E9 (embryonic day 9). At E10.5 to E12.5, liver becomes a major site of fetal hematopoiesis [1]. Around E14.5, both hepatocytes and bile-duct epithelial cells develop from the bipotential hepatoblast [1]. As these hepatoblasts gradually become mature hepatocytes the main function of liver switches from hematopoiesis to metabolism [1,2]. During liver development, gene expression profiles change over time and determine the phenotypes and functions of liver [3]. Elucidating molecular regulations in this developmental process is important for understanding liver functions and also useful for exploring liver diseases. High-throughput gene expression profiling techniques are well suited to reveal the expression changes in the developmental process.

We used high-density oligonucleotide microarrays to investigate gene expression profiles and studied the transcriptional regulation in mouse liver development. The gene expression data in this study were sampled from fetal mouse liver tissue at E11.5, E12.5, E13.5, E14.5, E15.5, E16.5, E17.5, E18.5, Day0 (the day of birth), Day3, Day7, Day14, Day21, and from a normal adult liver tissue. Hierarchical clustering on genes whose expressions changed by more than 1.5-fold during liver development uncovered two groups of genes with distinct temporal expression patterns. Genes in the first group have high expression mainly in the late stage of liver development and genes in the other group are mostly activated in the early stage. GO biological function analyses on the elevated and inhibited genes at each time point reveal that in early embryo development, cell-cycle-related genes are highly expressed; around the birth, defence-related genes are activated; and liver-function-related TFs and genes are highly activated in the later stage of development. Transcriptional regulation which controls the gene expression in a tissue-specific and quantitative manner is a major regulatory mechanism in embryonic developmental processes. In order to identify transcriptional mechanisms involved in these development stages, a computational approach was used to identify the regulatory motifs that associated with the elevated and inhibited

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gene expression at each time point. With these identified TFs, regulatory networks during mouse liver development were constructed.

Results and discussion

Differential expression pattern in mouse liver development

Embryonic liver development goes through progressive series of steps starting from the gastrula stage of embryogenesis [2]. Transcriptional activities of genes are expected to be enhanced or inhibited to regulate the abundance of gene products responsible for their concerted functions during development. Transcription profiles at different time points provide us novel insights into groups of genes important to mouse liver development. To filter genes which do not show significant change in expression during liver development, we select genes with expression values at any time point at least higher than 1.5-fold or lower than 1/1.5-fold compared to the average (the mean expression value of all 14 time points) for further analyses. This gives 8640 genes in total, the list of which can be found in Supplementary Table S1. To verify the microarray profiling, semi-quantitative Reverse Transcription PCR (RT-PCR) is performed to analyze the expression of four genes, alpha-fetoprotein (Afp), glucose-6-phosphatase (G6pc), tyrosine aminotransferase (Tat), and albumin (Alb) in different stages of mouse liver development. Semi-quantitative RT-PCR results show that the expression of Afp starts in early fetal liver (at least from E11.5) and nearly can't be detected in adult liver. The expression of G6pc and Tat start in late fetal liver (E17.5). The expression of Alb ranges from E11.5 to adult liver, which covers all developmental stages of mouse liver. Expression profiles got by microarray and Semi-quantitative RT-PCR are shown in Supplementary Fig. S1. From the figure we can find that these two expression profiles accord with each other very well. They are also very consistent with current knowledge on these genes, suggesting that our microarray data are appropriate for further transcriptomic analysis. With these expression data, we can look into how some genes known to be involved in liver development such as Hnf4a, Nfkb1, Cebpa, Xbp1 and Foxa1 are regulated temporally and involved in the function switch from fetal to postnatal liver development (Supplementary Fig. S2). The scientific community who is interested in liver development and function can also find the developmental expression patterns of their interested genes from this work (Supplementary Table S1).

To overview gene expression profiles across mouse liver development, two-dimensional hierarchical clustering analysis is performed on 8640 selected genes (Fig. 1A) by Cluster 3.0 [4] and visualized by TreeView 1.60 (Michael Eisen, Stanford University. http://rana.lbl.gov/EisenSoftware.htm). Expression values are adjusted by log transform and mean center genes before clustering. The clustering result (Fig. 1A) on the time dimension shows that all time points remain in their original order. It is reasonable, since the development is a gradually changed process and neighbouring time points have similar expression snapshots. The first bifurcating hierarchy of the cluster divides 14 time points into two developmental stages, the embryonic and the postnatal stage, delineated by the dividing point between E17.5 and E18.5. It would be natural to expect that time points before and after birth (around E18.5 and Day0) should be separated into two clades. But the clustering result indicates that the expression profile of E18.5 is more similar to those of time points after birth. It was reported that premature mice by caesarean section could not live if their gestational ages were less than 19 days [5]. While caesarean birth mice can survive and their weight handicaps in organs can be overcome in postnatal days [6]. Results here may provide transcription-level explanation to this anatomic observation.

The sample dendrogram is further divided into two sub-stages (Stages I and II) corresponding to transiting from E14.5 to E15.5. We observed that expression levels of each gene at the first sub-stage are more homogenous compared with the second sub-stage. Previous experiments indicated that E14.5 is a transition point of mouse liver development around which hepatocytes and bile-duct epithelial cells occurs [1]. Consistent with this result, hierarchical clustering reveal two sub-stages of time points during embryonic liver development. Data points of the postnatal stage are also divided into two clusters (Stages III and IV). Expression profiles of the stage IV (ranging from Day7 to NL) exhibited less time-invariant property suggesting the liver has become near to the maturation.

Genes in Fig. 1A are also clearly split into two distinctive groups. The first group (Group A) includes 3703 genes, majority of which have high expression levels in the postnatal stage of liver development. While the other group (Group B) includes 4937 genes, most of which are activated in the embryonic stage. Opposite trends of these two groups suggest their different functions. We searched over-represented and under-represented GO terms for these two groups of genes separately. Bonferroni corrected hypergeometric p-values are calculated to determine significant enriched/depleted terms in each group compared to the whole set of genes on the microarray (enriched/depleted categories are listed in Fig. 1B for group A and Fig. 1C for group B, with lengths of pink bars equal to minus log of corrected p-values). We find that liver-related functional terms (e.g. lipid, cholesterol, fatty acid metabolism, and steroid biosynthesis) are enriched in group A; while cell cycle related terms (e.g. replication, transcription, mRNA processing and translation) are enriched in group B.

Visual inspection of group A identified a small clade of genes with consistently high expression level at the postnatal stage (the pink part of gene dendrogram in Fig. 1A with 414 genes). GO analysis indicates the enrichment of “blood coagulation” biological process term in these 414 genes (Bonferroni corrected p-value 5.66e−16, blue bars in Fig. 1B). Among those forty genes which are annotated with the term “blood coagulation” within the whole set (Fig. 1D), twenty two of them are included in the group A and seventeen are included in these 414 genes, which indicates the specific expression pattern of these blood coagulation related genes during mouse liver development.

In group B, majority of genes start high expression values from E11.5 and become inactivated from E15.5. But a small subgroup of genes (blue part in the gene dendrogram of Fig. 1A) distinguishes themselves with augmented expression from E12.5 to E17.5. This small group is comprised of 861 genes. GO analysis shows that the “heme biosynthetic process” and “porphyrin biosynthetic process” terms are significantly enriched in this group (Bonferroni corrected p-values equal to 6.48e−07 and 8.29e−07 respectively, blue bars in Fig. 1C), consistent with the hematopoiesis function in the early stage of liver development. After a closer examination of enriched genes, we found that eight of total eleven genes annotated with “heme biosynthetic process” in GO are included in the 861-gene clade of group B (Fig. 1E). Genes labelled both with “porphyrin biosynthetic process” and “heme biosynthetic process” terms in annotation file are all encompassed in the 861-gene clade, which forms the major contribution to the enrichment of the related GO terms. It has been learned that around E10.5 to E12.5, liver becomes a major site of fetal hematopoiesis [1]. Accordingly, the expression activities of these genes come to a climax from E12.5 to E17.5 and then become to wane from E18.5 on. The turning point in gene expression thus reveals a functional switch from hematopoiesis to metabolism [1,2]. This finding also suggests that the uncharacterized genes included in the 861-gene clade might be associated with the fetal hematopoiesis.

The liver is an essential organ for mammals and hepatocellular carcinoma is among the top most common, deadly cancer world-wide. It is interesting to find the relation between hepatocellular carcinoma (HCC) and liver development. We compared microarray data in this study to a published array profiling dataset with 49 pairs of HCCs and