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Sexual dimorphisms in zonal gene expression in mouse liver

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ABSTRACT

Many of the metabolic functions of the liver are localized either in the pericentral region (zone 3) or in the periportal region (zone 1). However, a systematic analysis of the heterogeneity and sexual dimorphism of gene expression in the liver is lacking. Our objective was to obtain sections of intact tissue from zone 1 and zone 3 from both male and female mouse liver, and to measure the patterns of gene expression in these sections. Zone 1 and zone 3 areas were isolated by laser capture microdissection of liver sections, total RNA was isolated and microarray analysis was conducted using Agilent Whole Mouse Genome oligo arrays. To investigate functional characteristics as well as upstream regulators of specific gene lists, we used Ingenuity Pathway Analysis. We identified more than 925 genes in zone 1 and more than 450 genes in zone 3 of both male and female mice. Sexual dimorphism in metabolic functions was present in zone 1 but not zone 3. In zone 1, canonical pathways related to gluconeogenesis were male predominant, while canonical pathways related to hepatic progenitor cells were female predominant. In addition, we also analyzed the upstream regulators of zone-specific genes. SREBF1 was male-specific in zone 1, while TRIM24 was female-specific in zone 3. These results demonstrate the heterogeneity and sexually dimorphic differences in gene expression in the liver.

1. Introduction

The liver plays a pivotal role in the maintenance of energy supply, in catalyzing biosynthetic and biodegradative processes, and in the excretion of final metabolic products. Many of the functions of the liver occur in specific zones, being localized either in the periportal hepatocytes surrounding the portal triad (portal vein, hepatic artery and bile duct; zone 1) or the pericentral hepatocytes surrounding centrilobular vein (zone 3). These hepatic functions are at variance with the apparent morphological homogeneity of hepatocytes [1,2]. Oxidative energy metabolism, gluconeogenesis and urea synthesis occur mainly in zone 1, while glycolysis, glutamine synthesis and xenobiotic metabolism are more predominant in zone 3 [3–6]. The majority of studies on the heterogeneity of expression of hepatic genes have been done with rat liver or isolated rat hepatocytes, and usually target specific individual genes. The mouse is an equally important model species for studying gene expression, so estimates of the heterogeneity of gene expression in mouse liver are also needed. In addition, while the metabolic zonation of liver functions has been extensively reviewed, few global studies using methods such as microarray analysis to measure the heterogeneity of gene expression patterns in the liver have been reported.

Hepatic functions also vary with different sexes [7,8] and the sexually dimorphic expression of a number of hepatic genes has been described. Plasma and urinary proteins, cytochrome P450 and other enzymes involved in the metabolism of steroids and xenobiotic compounds, various receptors and signaling molecules exhibit sexually dimorphic expression. Sexual dimorphisms in liver gene expression are dictated by the temporal patterns of circulating growth hormone (GH), which is sex dependent and under gonadal control [9–11]. Under a male-specific pulsatile GH pattern, signal transducer and activator of transcription (STAT) 5 is intermittently activated. Activated STAT5 primarily or secondarily targets male- and female-specific genes [12–14]. STAT5 has been reported to coordinate the transcriptional regulation of sex-specific genes with other non-sex specific hepatic transcription factors such as hepatocyte nuclear factor 4 alpha (HNF4) [15,16]. However, it remains unclear whether or not zonal gene expression patterns in the liver are also sexually dimorphic.

Laser-capture microdissection (LCM) can be used to dissect and capture a select region from an intact tissue that has been fixed to a microscope slide, and this method has been used to collect tissue

Abbreviations: LCM, laser capture microdissection; GH, growth hormone; STAT, signal transducer and activator of transcription; HNF4, hepatocyte nuclear factor 4 alpha; RIN, RNA integrity number.

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from zone 1 and zone 3 from intact liver sections [17,18]. The tissue obtained using the LCM method can be used to isolate RNAs, which can then be used to measure gene expression by methods such as quantitative real-time PCR or microarray analysis. Therefore, in the present study, we used LCM to separately collect intact tissue from zone 1 and zone 3 of male and female mouse liver. We characterized a total of 1982 genes as being zone-specific and 715 genes are common in male and female mouse liver. Within these genes, about 51% and 55% of them are common in male and female, respectively. Moreover, we also highlighted canonical pathways and up-stream regulators of these zone-specific genes. Our present study improves the knowledge of sexually dimorphic regulation of genes in the liver.

2. Materials and methods

2.1. Animals

Three C3H/HeNCrlBR (C3H) male and 3 female mice were purchased from Charles River Laboratories, Inc. (Wilmington, NC). Mice were housed in a room maintained at 22 °C with a 12:12-h light/dark cycle (7:00 AM–7:00 PM) and fed control chow and water ad libitum. The animal procedures were approved by the Animal Ethics Committee, NIEHS, National Institute of Health (Research Triangle Park, NC).

2.2. Preparation of slides and LCM

Liver tissues were frozen in Tissue-Tech O.C.T. Compound (Sakura Finetek USA, Torrance, CA) and sectioned on a clean, RNase-free cryostat. Section staining and laser capture microdissection were performed as described previously [17]. Forty-five to 50 individual areas from either zone 1 or zone 3 were dissected from an individual slide and captured on the microcentrifuge tube cap. Regions of zone 1 and zone 3 were identified as descried previously [18]. For each individual mouse, 5 slides were used to capture zone 1 and 5 slides were used to capture zone 3 areas for RNA extraction. RNAs were prepared using the Arcturus PicoPure Kit (Life Technologies, Carlsbad, CA) and the RNA integrity number (RIN) was determined by RNA bioanalyzer. All RNA samples had a RIN of 6 or better; RIN 6 is usual for the total RNAs prepared by stained LCM and can be applied to microarray analysis [19].

2.3. Real-time PCR

cDNAs were synthesized from the RNAs extracted from the LCM samples using the High-Capacity Reverse Transcription Kit with random primers (Life Technologies) and were subjected to real-time quantitative PCR. Real-time PCR was performed with a 7900HT fast real-time PCR System (Life technologies) with the following probes of Taq-Man gene expression assays (Life Technologies): serine dehydratase (SDS, Mm00455126_m1*), glutaminase 2 (GLS2, Mm01164862_m1), Kunitz-type protease inhibitor 1 (SPINT1, Mm00444186_m1), glutamate–ammonia ligase (GLUL, Mm00725701_s1*), ornithine aminotransferase (OAT, Mm00497544_m1) and solute carrier family 1 member 2 (SLC1A2, Mm00441457_m1). Mouse GAPDH (GAPDH) Endogenous Control (Life Technologies) was used as an internal control and to normalize expression levels of all other genes.

2.4. Microarray analysis and data analysis

Microarray analysis of gene expression was conducted using Agilent Whole Mouse Genome 4×44 multiplex format oligo arrays (014868) (Agilent Technologies). Fifty ng of total RNA was amplified and labeled as directed in the NuGEN Ovation Pico WTA System protocol for Agilent microarrays. For each sample, 3 μg of Cy3 labeled cRNAs were fragmented and hybridized for 17 h in a rotating hybridization oven. Slides were washed and then scanned with an Agilent Scanner. Data was obtained using the Agilent Feature Extraction software (v9.5), using the 1-color defaults for all parameters. The Agilent Feature Extraction Software performed error modeling, adjusting for additive and multiplicative noise. The resulting data were processed using the Rosetta Resolver® system (version 7.2) (Rosetta Biosoftware, Kirkland, WA). Significant differences in expression of gene tags were estimated by ANOVA analysis. Gene tags were identified as zone specific genes using the criteria of more than 2.0-fold differences in expression between zone 3 and zone 1 and P value <0.01. To investigate functional characteristics as well as upstream regulators of specific gene lists, we used Ingenuity Pathway Analysis (IPA) (Ingenuity Systems; www.ingenuity.com). We used zone specific gene lists to map and consolidate overlapped genes and calculate P values (using right-tailed Fisher's exact test) in canonical pathway analysis and to calculate z-scores and P values in upstream regulator analysis. In canonical pathway analysis, we focused on metabolic pathways and a P value <0.05 was considered significant. The Activation z-score predicts the activation state of the upstream regulator, using the gene expression patterns of the genes downstream of that regulator. An upstream regulator is considered activated if the z-score is $\geqslant 2$ and inhibited if the z-score $\leqslant -2$ with a P value <0.01 considered significant. Networks of genes regulated by the upstream regulators were developed to graphically illustrate the molecular relationships and functional relationships to phenotypic outcomes.

3. Results and discussion

3.1. Quality control

To verify the accurate separation of zone 1 and zone 3 samples by LCM, the expression levels of zone 1 specific markers SDS, GLS2 and SPINT1 and zone 3 specific markers GLUL, OAT and SLC1A2 were measured in zone 1 and zone 3 samples. The mRNA levels of SDS, GLS and SPINT1 were high exclusively in zone 1, while those of GLUL, OAT and SLC1A2 were high exclusively in zone 3 in both male and female mouse liver (Fig. 1A). Given the verification of the separation of zone 1 and zone 3 samples by LCM, the samples were subjected to microarray analysis. Consistent with real-time PCR, the microarray signal of SDS had a positive zone 1/zone 3 ratio, while that of GLUL had a negative zone 1/zone 3 ratio in both male and female (Fig. 1B). These results verified the accurate separation of zone 1 and zone 3 tissue by LCM.

3.2. Overall profiles of gene expression in zone 1 and zone 3 in male and female liver

We next examined the patterns of zone-specific gene expression in both male and female mouse livers. Genes were identified as zone specific genes using the criteria of more than a 2.0-fold differences in expression between zone 3 and zone 1, and $P < 0.01$. Overall, 852 and 932 genes were mapped and characterized as zone 1 specific genes in male and female liver, respectively, with 513 of these genes in common between males and females (Fig. 2). On the other hand, 540 and 373 genes were mapped and characterized as zone 3 specific genes in male and female, respectively, with 202 genes in common between the two sexes.

In total, we identified 1392 and 1305 genes with zone specific expression in male and female, respectively, which are 6.5-fold higher than the previous report of zonation of hepatic gene expres-