Biochemical and Biophysical Research Communications 436 (2013) 730–735

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Sexual dimorphisms in zonal gene expression in mouse liver

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ARTICLE INFO

Article history:
Received 5 June 2013
Available online 18 June 2013

Keywords:
Hepatic zonation
Sexual dimorphism
Laser capture microdissection (LCM)

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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http://dx.doi.org/10.1016/j.bbrc.2013.06.025

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

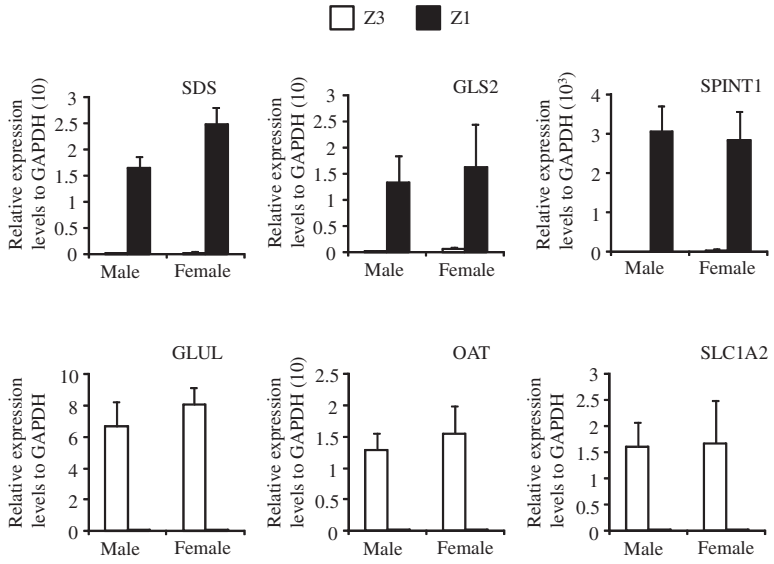
To verify the accurate separation of zone 1 and zone 3 samples by LCM, the expression levels of zone 1 specific markers SDS, GLS2 and SPINT1 and zone 3 specific markers GLUL, OAT and SLC1A2 were measured in zone 1 and zone 3 samples. The mRNA levels of SDS, GLS and SPINT1 were high exclusively in zone 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-

A. Q-PCR



B. microarray

genes	Male	Female
SDS (Z1/Z3)	17.88	9.41
GLS2 (Z1/Z3)	45.70	35.73
SPINT1 (Z1/Z3)	36.78	37.23
GLUL (Z1/Z3)	-37.67	-59.19
OAT (Z1/Z3)	-85.79	-64.45
SLC1A2 (Z1/Z3)	-7.63	-100

Fig. 1. Quality control of separation of zone 1 and zone 3 tissue. (A) q-PCR assay was performed to determine mRNA expression levels of SDS, GLS2, SPINT1, GLUL, OAT and SLC1A2 in each sample of male and female, zone 1 and zone 3 tissue isolated by LCM. mRNA levels were normalized to GAPDH. (B) Data of SDS, GLS2, SPINT1, GLUL, OAT and SLC1A ratio between zone 1 and zone 3, male and female from microarray analysis. Values determined by intensity of signal and expressed as log(zone 1 – zone 3).

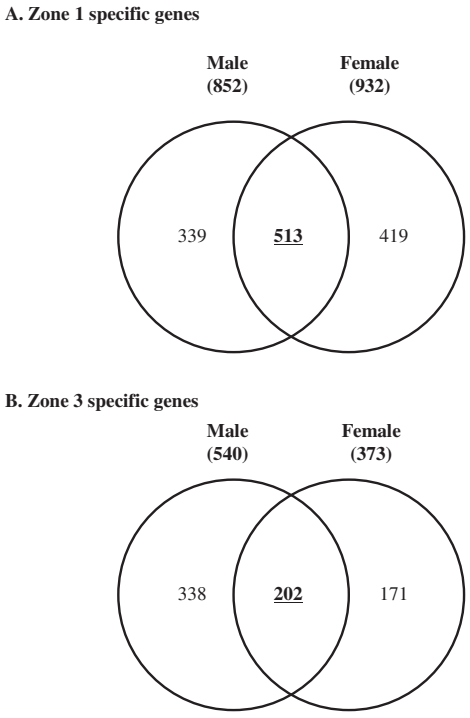


Fig. 2. Venn diagram. The number of IPA mapped zone 1 specific genes (A) and zone 3 specific genes (B) in male and female are shown.

sion using the digitonin-collagenase perfusion method [20]. In this method, the liver is first perfused in one direction with digitonin to kill the cells in one zone and then perfused in the opposite direction with collagenase to isolate the live cells from the other zone. It is likely that hepatocytes isolated by this method are subject to stress; moreover the purification of zone-specific hepatocytes by this method is not absolute. On the other hand, LCM capture of zone 1 and zone 3 would not produce any stress to the liver and would absolutely separate the two zones, improving the identification of zone-specific genes. In addition, we identified a higher number of zone 1 specific genes than zone 3 specific genes in both male and female liver, while the study of Braeuning et al. [20] identified a similar number of zone 1 and zone 3 specific genes. It has been proposed that non-parenchymal cells have also zone-specific properties [4]. Thus, differences in the distribution of non-parenchymal cells might contribute to the higher number of zone 1 specific genes in our study.

3.3. Zone-specific canonical pathways in male and female liver

To address the phenotypic outcomes of zone-specific gene expression, we analyzed canonical pathways using Ingenuity Pathway Analysis to find those that were associated with our lists of zone specific genes. The top canonical pathways are listed in Table 1 (zone 1) and Table 2 (zone 3). In zone 1, 8 canonical pathways were commonly highlighted in both male and female; these were: Urea Cycle, Histidine Degradation III, Citrulline Biosynthesis, Histidine Degradation VI, Arginine Biosynthesis IV (ranked

Table 1
Top 10 canonical pathways significantly associated with genes differently expressed in zone 1 of male and female liver.

Male		Female	
Canonical pathways	*P*-value	Canonical pathways	*P*-value
Urea cycle	7.94E−05	Histidine Degradation III	6.61E−07
Histidine Degradation III	3.47E−04	Histidine Degradation VI	8.91E−04
Citrulline Biosynthesis	3.47E−04	Urea cycle	2.81E−03
Histidine Degradation VI	6.03E−04	Citrulline Biosynthesis	7.41E−03
Arginine Biosynthesis IV	1.10E−03	Trehalose Degradation II	8.51E−03
Glycolysis I	3.72E−03	PRPP Biosynthesis	8.51E−03
Gluconeogenesis I	4.57E−03	Phospholipases	9.33E−03
Trehalose Degradation II	6.92E−03	Folate Transformations I	1.05E−02
D-myo-inositol-Triphosphate Biosynthesis	7.94E−03	Bupropion Degradation	1.58E−02
		Myo-inositol Biosynthesis	1.66E−02
D-myo-inositol-5-Phosphate Metabolism	1.12E−02	(Arginine biosynthesis IV)	2.63E−02
(phospholipases)	1.74E−02	(D-myo-inositol-Triphosphate Biosynthesis)	5.00E−02

P-values are calculated by Ingenuity Pathway Analysis using lists of zone 1 specific genes. Pathways specific to either male or female only are underlined.

Table 2
Top 10 canonical pathways significantly associated with genes differently expressed in zone 3 of male and female liver.

Male		Female	
Canonical pathways	*P*-value	Canonical pathways	*P*-value
Nicotine Degradation II	2.51E−11	Nicotine Degradation II	5.62E−09
Melatonin Degradation I	3.16E−11	Nicotine Degradation III	1.29E−08
Nicotine degradation III	3.98E−10	Melatonin Degradation I	1.29E−08
Bupropion Degradation	4.67E−09	Estrogen Biosynthesis	5.25E−07
Acetone Degradation I	6.31E−09	Bupropion Degradation	6.45E−07
Estrogen Biosynthesis	9.77E−09	Acetone Degradation I	7.94E−07
Glutathione-mediated	3.01E−06	Bile Acid Biosynthesis	2.09E−06
Detoxification	3.63E−06	Serotonin Degradation	2.51E−06
Serotonin Degradation	1.14E−05	Glutathione-mediated	5.75E−06
Bile Acid Biosynthesis	2.76E−04	Detoxification	3.63E−05
Retinoate Biosynthesis I (Dopamine degradation)	2.09E−03	Dopamine Degradation (Retinoate biosynthesis I)	3.55E−03

P-values are calculated by Ingenuity Pathway Analysis using lists of zone 3 specific genes.

top10 only in male), Trehalose Degradation II, D-myo-inositol-Triphosphate Biosynthesis (ranked top10 only in male), and Phospholipases (ranked top 10 only in female). On the other hand, Glycolysis I, Gluconeogenesis I and D-myo-inositol-5-Phosphate Metabolism were highlighted only in male liver, while PRPP Biosynthesis, Folate Transformations I, Bupropion Degradation and Myo-inositol Biosynthesis were highlighted only in female liver. In zone 3, all of the top 10 ranked canonical pathways were common between male and female liver. These pathways were: Nicotine Degradation II, Melatonin Degradation I, Nicotine Degradation III, Bupropion Degradation, Acetone Degradation I, Estrogen Biosynthesis, Glutathione-mediated Detoxification, Serotonin Degradation, Bile Acid Biosynthesis, Retinoate Biosynthesis I (ranked top 10 only in male), Dopamine Degradation (ranked top 10 only in female).

Thus, zone 1 had some sexual dimorphism in canonical pathways, while canonical pathways in zone 3 were not sexually dimorphic. However, both zone 1 and zone 3 had some sexual dimorphism in gene expression. Thus, genes that play a central role in zone specific functions were commonly regulated in zone 3 of male and female liver.

The highlighted canonical pathways in the present study are consistent with previous reports. 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]. In zone 1, Urea Cycle, Citrulline Biosynthesis and Arginine Biosynthesis IV are related to urea synthesis. Histidine Degradation III and Histidine Degradation VI are related to amino acid metabolism. Trehalose degradation II, Glycolysis I and Gluconeogenesis I are related to gluconeogenesis, although Glycolysis I and Gluconeogenesis I are highlighted only in the male zone 1. It has been reported that hepatic glucose output was much higher in males than females at 4 h fasting, although blood insulin level was much higher in male than female [21]. Thus, our observation of a male predominance of canonical pathways related to gluconeogenesis may explain the sexual dimorphism in glucose output in the liver. D-myo-inositol-Triphosphate Biosynthesis, D-myo-inositol-5-Phosphate Metabolism, Phospholipases and Myo-inositol Biosynthesis are related to immune response; this suggests that these canonical functions are associated with the high content of non-parenchymal cells in zone 1 of the liver. PRPP Biosynthesis and Folate Transformation are related to nucleic acid synthesis, suggesting that these canonical functions are associated with hepatic progenitor cells. The female specific observation of these canonical functions associated with hepatic progenitor cells is in agreement with a previous finding that female sex is associated with better hepatic regeneration [22,23].

In zone 3, Nicotine Degradation II, Melatonin Degradation I, Nicotine Degradation III, Bupropion Degradation, Estrogen Biosynthesis, Glutathione-mediated Detoxification, Serotonin Degradation and Dopamine Degradation are related to endobiotic and xenobiotic metabolism. Bile Acid Biosynthesis is also a zone 3 specific function along with Acetone Degradation I, which is related to glycolysis. In addition to canonical pathways associated with previously characterized zone specific functions, our study points out a new zone 3 specific function, Retinoate Biosynthesis I, which is related to vitamin A synthesis and storage. It has been reported that vitamin A is synthesized and stored in the liver [24] and this appears to be a zone 3 specific function.

3.4. Zone-specific upstream transcriptional regulators in male and female liver

Next, to understand the zone-specific regulation of gene expression, we analyzed the zone-specific gene lists in male and female liver using Ingenuity Pathway Analysis to identify significant associations with upstream regulators. Zone-specific upstream transcriptional regulators were identified using the criteria of more than 2 or −2 *z*-score and $P < 0.01$. Overall, 5 transcriptional regulators, TP53, AR, SRF, CEBPA and TAF4B were identified as zone 1 specific in both male and female liver, with one additional transcriptional regulator, SREBF1, specific to male (Table 3). HTT was characterized as a zone-specific upstream regulator in zone 1 of female liver, but was also identified in zone 1 of male liver (*z*-score = 3.357 and $P = 0.0229$). In zone 3, four transcription regulators, AHR, NR1I3, CTNNB1 and TAF4, were identified in both male and female liver (Table 4), with TRIM24 specific only to female. N-cor was also specific to zone 3 of female liver but was also weighted in zone 3 of male liver (*z*-score = −2.449 and $P = 0.0144$).

Table 3
Upstream transcriptional regulators significantly weighted in zone 1 of male and female liver.

Male			Female		
Upstream regulator	Activation *z*-score	*P*-value	Upstream regulator	Activation *z*-score	*P*-value
TP53	2.916	1.30E−07	HTT	4.000	7.65E−03
SRF	2.888	3.31E−04	TP53	3.135	1.53E−10
AR	2.720	2.97E−04	AR	2.985	1.11E−03
SREBF1	2.433	4.22E−03	SRF	2.426	6.60E−07
CEBPA	2.428	6.21E−04	TAF4B	2.236	5.20E−04
TAF4B	2.236	6.54E−04	CEBPA	2.080	9.14E−04
HTT	3.357	2.29E−02			

Activation z-scores and P-values are calculated by Ingenuity Pathway Analysis using microarray data of the zone 1/ zone 3 ratio of gene expression. Sex specific transcriptional regulators are underlined.

Table 4
Upstream transcriptional regulators significantly weighted in zone 3 of the male and female liver.

Male			Female		
Upstream regulator	Activation *z*-score	*P*-value	Upstream regulator	Activation *z*-score	*P*-value
CTNNB1	−3.483	1.58E−05	AHR	−4.263	2.59E−17
AHR	−3.413	1.58E−22	TRIM24	−3.051	7.15E−03
TAF4	−2.383	3.67E−05	TAF4	−2.733	7.52E−05
NR1I3	−2.141	1.34E−07	CTNNB1	−2.467	2.40E−06
(N-cor)	−2.449	1.44E−02	N-cor	−2.449	2.68E−03
			NR1I3	−2.308	1.28E−08

Activation *z*-scores and *P*-values are calculated by Ingenuity Pathway Analysis using microarray data of the zone 1/zone 3 ratio of gene expression. Sex specific transcriptional regulators are underlined.

Thus, although more than 40% of gene expressions are sex-specific, only 1 out of 10 upstream regulators are sex-specific in male liver, with a similar finding in female liver. These results suggest that the majority of upstream signals are common between males and females. In the present study, a major sex-specific transcription factor, STAT5B, was not zone-specific in either male or female. This suggests that zone-specific upstream regulators coordinate with sex-specific transcription factors, such as STAT5B, to drive sexually dimorphic gene expression in different zones of the liver.

Our study addressed upstream transcriptional regulators of hepatic zonation; such studies of zone-specific transcriptional regulators are very limited. Recently, CTNNB1 was characterized as a zone-specific transcriptional regulator directing liver metabolic zonation [6,25,26]. In the present study, we have characterized TP53, AR, SRF, CEBPA and TAF4B as zone 1 specific upstream transcriptional regulators and AHR, NR1I3, CTNNB1 and TAF4 as zone 3 specific upstream transcriptional regulators in both male and female liver. This global analysis of zone-specific upstream transcriptional regulators expands our understanding of zone-specific gene regulation in the liver.

3.5. Sex-specific upstream transcriptional regulators in zone 1 and 3

Among zone-specific upstream transcriptional regulators that we characterized, SREBF1 and TRIM24 were specific for male zone 1 and female zone 3, respectively. To delineate the role of these upstream transcriptional regulators, networks were generated using SREBF1 and TRIM24 and their target genes within the zone-specific gene lists. Seven genes regulated by SREBF1 were weighted in zone 1, namely ACACA, ACLY, FADS2, FASN, PNPLA3, PTGDS and THRSP, while no genes were weighted in zone 3 (Fig. 3A). Within biological functions, the synthesis of lipid overlapped with the SREBF1 network (8 out of 8 molecules are involved and $P = 1.01E{-}10$). On

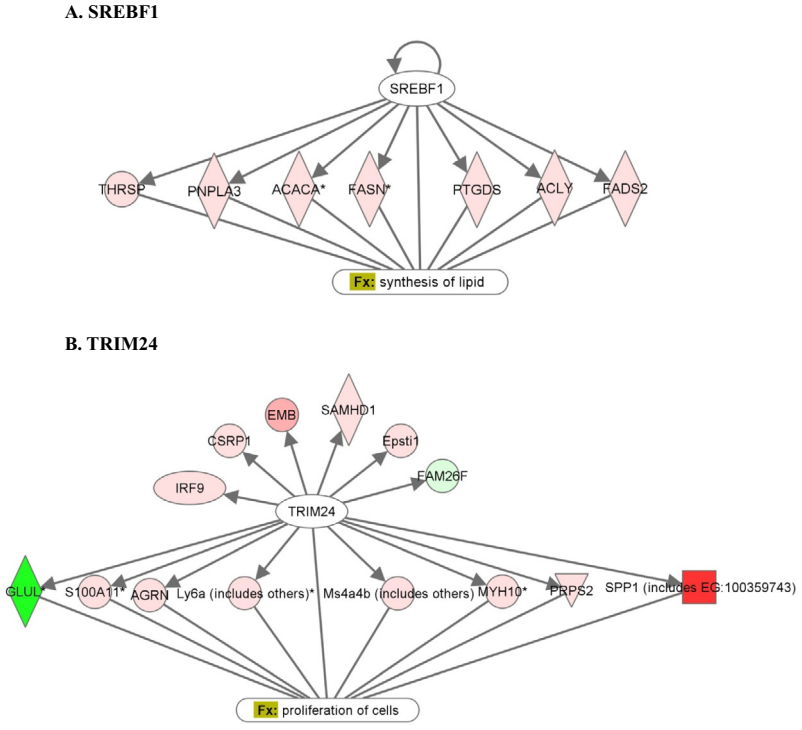


Fig. 3. Network analysis for SREBF1 and TRIM24. Network analysis for genes regulated by (A) SREBF1 and (B) TRIM24. Genes significantly expressed in zone 1 by more than 2-fold are in red and in zone 3 by more than 2-fold are in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the other hand, 12 genes regulated by TRIM24 were weighted in zone 1, while 2 genes were weighted in zone 3 (Fig. 3B). Those genes are AGRN, CSRP1, EMB, Epsti1, IRF9, Ly6a (includes others), Ms4a4b (includes others), MYH10, PRPS2, S100A11, SAMHD1 and SPP1 (includes EG:100359743) for zone 1, and FAM26F and GLUL for zone 3. Within the biological functions, the proliferation of cells overlapped with the TRIM24 network (9 out of 15 molecules are involved and $P = 1.76E{-}03$).

SREBF1 is a transcription factor regulating lipid synthesis [27,28] and we have characterized SREBF1 as a male-specific upstream transcriptional regulator weighted in zone 1. This observation is consistent with previous findings that lipid synthesis is predominant in zone 1 [5]. However, there was no significant difference in gene regulation by SREBF1 between zone 1 and zone 3 of female liver. It has been reported that lipid content in the liver is much higher in female than male [29]. Therefore, SREBF1 may be active in both zone 1 and zone 3 of female liver to increase hepatic lipid synthesis.

TRIM24 is a transcriptional coactivator for ESR, RAR and VDR and has been reported to suppress development of hepatocellular carcinoma [30–32]. We have identified TRIM24 as a female-specific upstream transcriptional regulator weighted in zone 3; its downstream biological function is proliferation of cells. Since 12 out of 14 TRIM24 target genes on the microarray were expressed specifically in zone 1, TRIM24 may suppress negative target genes regulating proliferation of cells in zone 3 of female liver. Proliferation of cells is associated with development of hepatocellular carcinoma and female mice are more resistant for development of hepatocellular carcinoma than male mice and this is regulated by estrogen [33,34]. Although the frequency of incidence of hepatocellular carcinoma within different hepatic zones remains to be elucidated, TRIM24 activation in zone 3 may contribute to the decreased incidence of hepatocellular carcinoma in females.

In summary, we have described the zone-specific and sexual dimorphic expression of a variety of genes and metabolic processes in mouse liver. This should contribute to a better understanding of liver metabolism.

Acknowledgments

The authors thank Rick Moore (NIEHS) for preparing liver sections; Pat Stockton, Julie Foley and Dr. Yuichi Yamazaki (NIEHS) for technical support of laser capture microdissections and Dr. Kevin Gerrish (NIEHS) for performing microarray and data analysis. This research was supported by the Intramural Research Program of National Institute of Environmental Health Sciences, Z01ES1005-01.

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