Global gene expression changes in liver following hepatocyte nuclear factor 4 alpha deletion in adult mice

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ABSTRACT

Hepatocyte nuclear factor 4 alpha (HNF4α) is known as the master regulator of hepatic differentiation, which regulates over 60% of the hepatocyte specific genes. Recent studies including this (Walesky et al. Am J Physiol Gastrointest Liver Physiol. 304:G26-37, 2013) demonstrated that HNF4α also inhibits hepatocyte proliferation via repression of pro-mitogenic genes. In this study hepatocyte specific HNF4α knockout mice were generated using 2–3 month old HNF4α-floxed mice treated with Cre recombinase under Major Urinary Protein promoter delivered in AAV8 vector (MUP-iCre-AAV8). Control mice were treated with MUP-EGFP-AAV8. Livers were isolated from control and KO mice one week after AAV8 administration and used for gene array analysis. These data revealed several new negative target genes of HNF4α, majority of which are pro-mitogenic genes inhibited by HNF4α in adult hepatocytes.

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35782.

2. Experimental design, materials and methods.

2.1. Microarray analysis.

The global differences in genes expression between HNF4αfl/fl mice treated with the MUP-iCre-AAV8 vector and those treated with the MUP-EGFP-AAV8 control vector were measured using Affymetrix’s GeneChip Mouse Genome 430 2.0 arrays. These arrays consist of over 45,000 probe sets representing over 34,000 well characterized mouse genes. Each probe set consists of 11 pairs of probes with each pair consisting of a perfect matched and mismatched 25-mer oligonucleotide interrogating the 3' end of a transcript. The mismatch probe differs from the perfect match probe by a single base substitution at the center of the probe and is used to determine the level of nonspecific hybridization. The Affymetrix chip definition file (CDF) used in the analysis was Mouse430_2.cdf and the annotation file used was Mouse430_2_na27.annot.csv.

2.2. Array normalization

We used a single knock-out sample (mice treated with MUP-iCre-AAV8 vector) and two control (mice treated with MUP-EGFP-AAV8 control vector) biological replicate samples generated from pooled (100 mg per mouse) livers of three individual mice each for microarray analysis. The microarrays were background-corrected using the robust multi-genomic data.
array average (RMA) background correction model [1]. This model assumes that the observed intensity \( z \) is the sum of the true intensity \( x \) distributed exponentially, and random noise distributed normally \( y \), \( z = x + y \). The background-corrected intensity values were quantile normalized across all chips, making their probe intensity distributions the same. The resulting intensity values were log (base 2) transformed. Probesets were then summarized using the median polish method [2]. A boxplot of the background-corrected, normalized and summarized intensity values is shown in Fig. 1.

2.3. Differential expression analysis

The principal components analysis (PCA) plot shown in Fig. 2 demonstrates significant global differences in gene expression between the knock-out and control samples. For each transcript, the log (base 2) ratio of the difference in expression between knock-out and control was calculated by subtracting the average log control transcript intensity from the knock-out transcript intensity. This value was transformed to a linear scale by taking two to the power of the value giving the ratio \( r = 2^{K_i - C_i} \) where \( K_i \) is the log signal intensity of the knock-out transcript \( i \) and \( C_i \), the average log signal intensity of the control transcript \( i \). This ratio was converted to a fold change using the formula:

\[
FC(r) = \begin{cases} 
-1/r, & r < 0 \\
1/r, & r \geq 0
\end{cases}
\]

The statistical significance of the observed fold change was calculated by fitting a 1-way ANOVA model by using the method of moments [3]. The model is described by the equation \( Y_{ij} = \mu + \text{CATEGORY}_i + E_{ij} \) where \( Y_{ij} \) represents the \( j \)th observation on the \( i \)th category. The

Fig. 1. Boxplot of the background-corrected, normalized and summarized intensity values.

Fig. 2. The principal components analysis (PCA) plot of the control and knockout samples.

Fig. 3. Scatterplot of significantly (\( p \)-value \( \leq 0.05 \)) differentially expressed genes.
intercept, $\mu$, models the common effect for the whole experiment. CATEGORY is a categorical variable representing the knock-out and control transcript and $\epsilon_{ij}$ represents the random error present in the $j$th observation of the $i$th category. The errors $\epsilon_{ij}$ are assumed to be normally and independently distributed with mean 0 and a fixed standard deviation, $\sigma$, for all measurements. A scatterplot showing the significantly ($p$-value $\leq 0.05$) differentially expressed genes are shown in Fig. 3.

2.4. Chromatin immunoprecipitation-sequencing data analysis.

In order to distinguish those genes specifically regulated by Hnf4a among the significantly perturbed genes obtained from microarray, we analyzed previously published chromatin immunoprecipitation (ChiP)-sequencing (ChiP-Seq) data for Hnf4a targets obtained from the National Center for Biotechnology Information Short Read Archive study SRA008281 [4]. The following experiments from this study were used in our analysis; Hnf4a: SRX003308, INPUT: SRX020706 and SRX020707. The raw reads were mapped to the mouse reference genome (NCBI37/mm9) using bowtie-0.12.3 [5]. The mapping statistics for the Hnf4a ChiP and Input samples are shown in Table 1. Peak detection was performed using the Model-based Analysis of ChiP-Seq (MACS) algorithm [6] with the peak detection $p$-value cutoff set at $1e-5$ (default). These resulting binding sites were filtered for significant sites based on a false discovery rate cutoff set at 10%. We searched for the Hnf4a consensus sequence within a 250 bp region from either side of the called peaks using a weight-matrix match with at least 80% similarity. The Hnf4a weight matrix was obtained from the JASPAR database [7]. Binding sites were annotated by PeakAnalyzer [8] using the nearest TSS option. Significantly differentially expressed genes with an Hnf4a binding target within 10 kb of its transcriptional start site were identified as putative Hnf4a target genes.

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