Electronic Supplementary Material (ESI) for Metallomics.

S5 Supplementary RNA-seq analysis

RNAseq DIFFERENTIAL EXPRESSION AND VALIDATION

Validation of the RNAseq differential expression data was accomplished by comparison with published transcriptome analysis of the *Atp7b*$^{-/-}$ mouse liver at the same age.[1] For example, genes encoding metal homeostasis proteins metallothioneins 1 and 2 (*Mt1*, *Mt2*) and oxidative stress responses including glutathione S-transferases (*Gst1*, *Gst2*) were up-regulated in *Atp7b*$^{-/-}$ liver. Differential expression of transcripts involved in lipid metabolism were expected and included *Lpl, Acot1, Acot2, Lrp11, Lrp2,* and *Ppargc1b*. *Ppar*α was down-regulated in the previous array, while the current RNAseq study identified down-regulation of lipid-activated *Ppar*γ*c1b targets Acot1* and *Acot2.* We previously reported changes in DNA repair protein abundance in the hepatocyte nuclei of this mouse model,[2] and here observed differential expression of DNA repair-associated transcripts including *Fancb, Fanci, Mad2, Mgmt, MutS.*

Zfp750, Zfp473 and Zfp101, which encode Zn-finger proteins annotated with DNA binding were identified with differential expression. Their increase in transcript abundance suggests that some Zn-dependent targets are upregulated at the mRNA level, perhaps in response to the changes in cellular Zn distribution.

TRANSCRIPTIONAL NETWORK ANALYSIS

The network contains a total of 246 nodes (15 clusters of transcription factors and 231 gene targets) connected by 918 putative common *cis*-regulatory elements, encompassing more than the 78% of the total differentially expressed genes. Topological analysis indicates that the *Atp7b*$^{-/-}$ network has a power law distribution in which the network shows innate, error-tolerant organization similar to those of non-biological systems, as defined in classical biochemical networks.[3] The connectivity number for each node enables identification of two types of transcription factors: master and global regulators. Master regulators control the expression of several different, but functionally similar genes within a functional module of the network, whereas global regulators control the expression of a larger sub-set of genes that span across multiple functional modules. In the network, these factors act as global regulators with high interconnectivity. When the *Atp7b*$^{-/-}$ transcriptional response is compared to other models, the *Sco1* and *Zfp36l2* mutants can be classified as a local effect; whereas Cu accumulation in the livers of *Atp7b*$^{-/-}$ mice induces a systemic transcriptional adjustment (global effect).

GENE ONTOLOGY ANALYSIS

Gene ontology (GO) enrichment analysis to identify over-represented cellular functions activated in the *Atp7b*$^{-/-}$ mouse model revealed two major over-represented groups (Supplementary Figure S6). The major branch corresponds to genes encoding for processes involved in cell cycle, including mitotic division and cell division regulation, consistent with prior work in this mouse model.[1] The second branch corresponds to processes directly involved in metal homeostasis. Genes involved in both Zn and Fe metabolism were overrepresented, including the expected *Mt1* and *Mt2* transcripts, but also *Scara5*, a ferritin receptor that controls cellular delivery of non-transferrin-bound iron.[4] There was also noted enrichment of numerous members of the cytochrome p450 family, proteins that contain a heme-iron center and function to metabolize various substrates, especially those found in the liver and considered to be toxic. Gene expression changes also indicate disruption of Fe targets in addition to Zn proteins, as revealed by an increase in hemoglobin alpha and beta in *Atp7b*$^{-/-}$ protein fractions.

References cited

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