Gene 739 (2020) 144508

Contents lists available at ScienceDirect

Gene

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

Research paper

Identification of common key regulators in rat hepatocyte cell lines under exposure of different pesticides

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ARTICLE INFO

Keywords:
Functional genomics
Gene regulatory networks
Pesticides toxicity
Regulator genes
System biology
Transcriptome

ABSTRACT

Pesticides exposure can have harmful effects on human health. The liver is the most common organ of pesticides toxicity due to its major metabolic activity. The molecular mechanism of pesticides effect is complex and is controlled by gene regulatory networks. All components of regulatory networks are controlled by transcription factors and other regulatory elements. Therefore, identification of key regulators through system biology approaches and high-throughput techniques can help to provide comprehensive insights into molecular mechanisms of the pesticide effect. In the current study, a microarray data-set was used to potentially identify molecular mechanisms that regulate gene expression profile of rat hepatocyte cell lines in response to pesticides exposure. Results showed that the number of differentially expressed genes (DEGs) and differentially expressed transcription factors (DE-TFs) were dramatically different among pesticides tested. Results also revealed 205 common DEGs and 11 DE-TFs among pesticides tested. Additionally, we found that six DE-TFs (CREB1, CTNNB1, PPARG, SP1, SRF and STAT3) had the highest number of interactions with other DEGs and acted as the key regulatory genes. The results of this study revealed regulator genes that have the key functions in response to pesticides toxicity in rat liver, which can provide the basis for future studies. Furthermore, these regulatory genes can be used as toxicity biomarkers to improve diagnosis and prognosis.

1. Introduction

The environmental occurrence of dangerous chemicals such as pesticides has raised a wide-spread disputation recently (Bao et al., 2015). Protecting human health from pesticides, conservative substances added into esculents, drinking water and toxic air pollutants is a global mission (Damalas and Koutroubas, 2016). Contaminants are propagating in the environment and human organs, leading to long-term diseases just after their first identification (Gavrilescu et al., 2015). The accumulation of pesticides in the organisms and the long-term and severe effects of them, is a well-studied phenomenon, though, given the wide range of the variables of interest, it is complicated to define the severe impacts and disease outcomes (Iida and Takemoto, 2018).

The real challenge, here, is the accurate evaluation of the dangerous impacts which pesticides and other chemicals may pose to the human body and other organisms (Collins et al., 2008; Judson et al., 2009; Wei et al., 2014). Traditional methods (experimental methods) for evaluation of chemicals are often time-consuming and very inefficient. Accordingly, along with experimental methods, other available methods should be considered (Brown, 2003; Wei et al., 2014). Experimental evaluation of all available chemicals is hard and the majority of them are not yet tested. Thus, developing fast, high-performance and efficient methods is necessary to predict the potential risks of chemicals.

The availability of omics data (genomics, transcriptomics, proteomics, metabolomics) as well as the development of modern tools and

Abbreviations: CREB1, CAMP Responsive Element Binding Protein 1; CTD, Comparative Toxicogenomics Database; CTNNB1, Catenin (Cadherin-Associated Protein), Beta 1; DEGs, Differentially Expressed Genes; DE-TFs, Differentially Expressed Transcription Factors; GEO, Gene Expression Omnibus; KEGG, Kyoto Encyclopedia of Genes and Genomes; NCBI, National Center for Biotechnology Information; PPARG, Peroxisome Proliferative Activated Receptor, Gamma; RPKM, Reads Per Kilobase of Transcript; SP1, Specificity Protein 1; SRF, Serum Response Factor; STAT3, Signal Transducer And Activator Of Transcription 3; TFs, Transcription Factors

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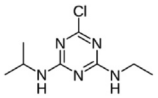
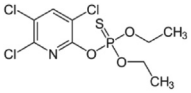
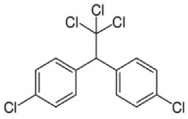
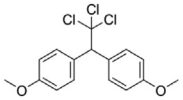
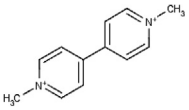
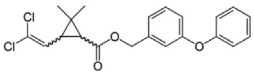
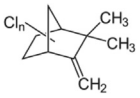
https://doi.org/10.1016/j.gene.2020.144508
Received 9 November 2019; Received in revised form 15 December 2019; Accepted 21 February 2020
Available online 22 February 2020
0378-1119/

Table 1
The brief information of studied pesticides.

Name	CAS Registry Number	MeSH® ID	Chemical Structure	Chemical class	Pesticide type
Atrazine	1912-24-9	D001280		Triazine	Herbicide
Chlorpyrifos	2921-88-2	D004390		Organophosphate	Insecticide Wormicide
DDT	50-29-3	D003634		Organochloride	Insecticide
Methoxychlor	72-43-5	D008731		Organochloride	Insecticide
Paraquat	4685-14-7	D010269		Bipyridyl	Herbicide
Permethrin	52645-53-1	D026023		Pyrethrin	Insecticide
Toxaphene	8001-35-2	D014112		Organochloride	Insecticide

annotation techniques has leveraged the gene expression analyses to clarify how biological systems are affected by the toxicity mechanism of the chemical compounds (Hasan et al., 2019). These technologies are providing the new field “toxicogenomics”, which is a combination of toxicology and genomics (Aardema and MacGregor, 2002). Toxicogenomics endeavors to elucidate the involvement of the genome in response to environmental toxicants (Aardema and MacGregor, 2002; Hasan et al., 2019; Ulrich and Friend, 2002). Toxicants induce pathological changes in the relevant organs and subsequently in the expression of genes, protein synthesis and metabolism. Detecting the changes made by toxicants is possible by evaluating changes in gene expression profile (Hasan et al., 2019; Uehara et al., 2008; Woods et al., 2007). Recent advances in molecular genetics and genomics have facilitated the identification of multiple genes that may regulate the response to the chemical exposures directly or indirectly. Another knowledge gap in the chemical toxicology is related to regulatory roles of transcription factors (TFs), which form regulatory networks and the global transcription response (Zhernovkov et al., 2019). TFs are one of the most important protein groups in response to chemical exposure (Corton et al., 2019; Iida and Takemoto, 2018; Schüttler et al., 2017). TF genes are found throughout the genome of human and other organisms and playing a significant role in the gene regulatory networks (Ignatieva et al., 2015).

Toxicogenomics provides the ability of a complete analysis of the changes occurred in the related organs and tissues by the external stimulus (Hasan et al., 2019; Igarashi et al., 2014). The toxicogenomic experiments such as microarray and RNA-seq lead to a huge gene expression data. Analysis of such big data is a very sophisticated task and sometimes captures unreliable results that are not robust to the identification of toxicity-responsive genes. Therefore, the analysis of pathway or molecular network-based gene expression data enhances the predictive power and captures more reliable results (Hardt et al., 2016; Hasan et al., 2018; Kim, 2017). Gene regulatory network analysis has provided special computational tools for interpreting huge volumes of data e.g., from disease-gene associations and promoted our understandings of the interactions that take place in biological processes (Goh et al., 2007; Reyes-Palomares et al., 2013). Indeed, the gene regulatory network approach is used to capture complete information about the interactions between contaminants and biological functions (Iida and Takemoto, 2018). Previous studies have demonstrated the power of system biology approaches to find interactions between contaminants and biological functions. Such approaches were used to identify biomarkers in cancer and other diseases (Chen et al., 2014). Darabos et al. (2015) studied the relationship between biological pathways and environmental pollutants. They aimed to identify the biological pathways that may be affected or even disrupted while exposing to environmental contaminants (Darabos et al., 2015). Adverse outcome pathways for chemicals were interpreted from high-throughput transcriptomic data-sets in two separate studies (Perkins et al., 2011; Villeneuve et al., 2014). In the other study, key transcription regulators associated with nanomaterial induced toxicity were identified through computational methods (Zhernovkov et al., 2019).

The aim of this study is, firstly, to global investigation of transcriptome alterations in response to different pesticides, as well as, to identify genes that respond directly to pesticides. A second aim is to identification of common key TFs that regulate responses to pesticides. We compared gene expression profile of rat hepatocyte cell lines under seven pesticide exposure, including Atrazine, Chlorpyrifos, DDT, Methoxychlor, Paraquat, Permethrin and Toxaphene to evaluate transcriptome alterations and identify common genes among all samples. Our approach is as follows: first, each sample is re-analyzed separately; in this manner, we could identify the differentially expressed genes (DEGs). Then we determine the DEGs-overlap among all samples. Our focus in the second approach is on the identification of core gene sets that may regulate the pesticides exposure responses. The characterization of DEGs is done through functional enrichment analysis of metabolic pathways and TF families. Additionally, to identify hub genes or key TFs, we performed a system biology analysis and provided more accurate insight into the associated mechanisms of the toxic responses.

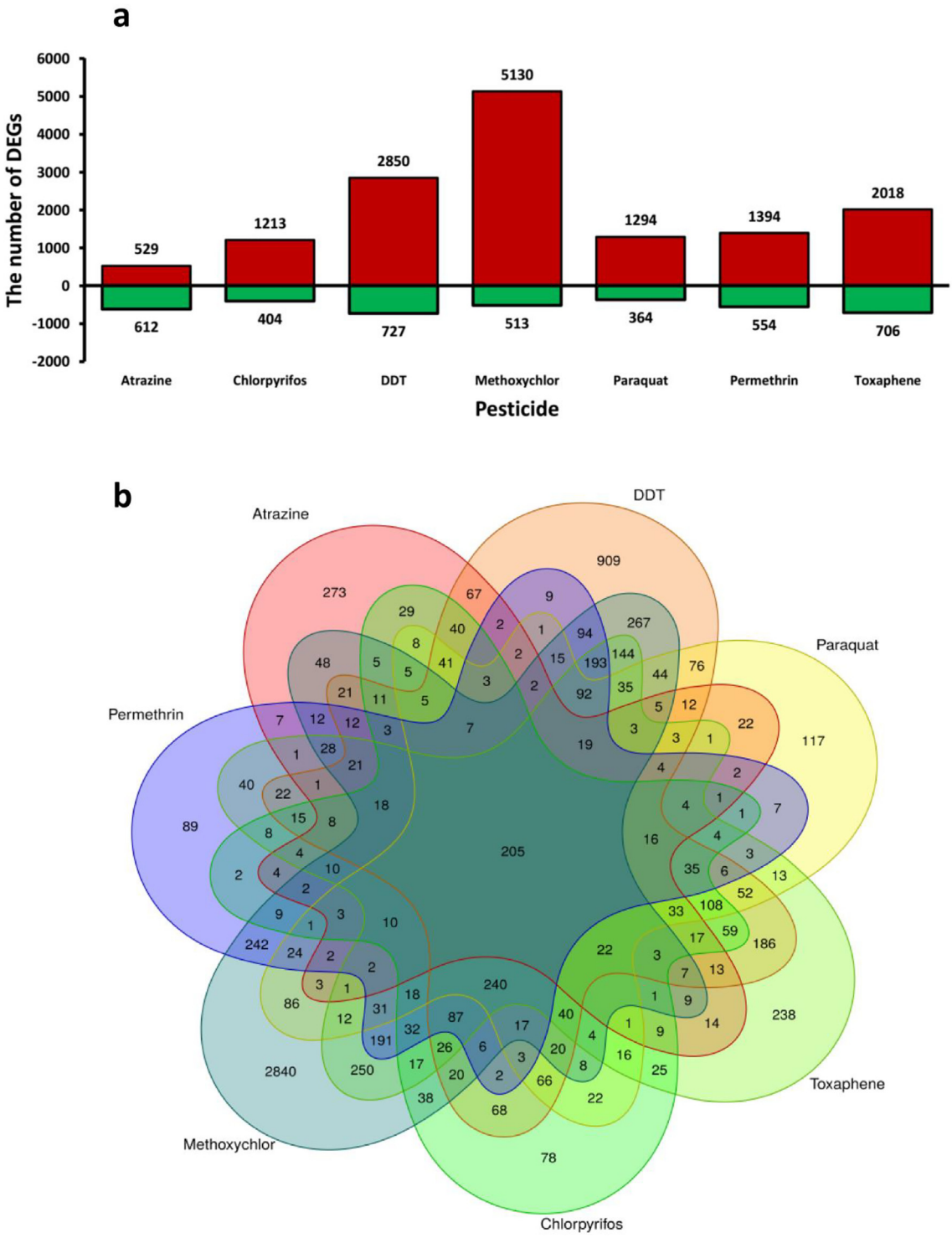


Fig. 1. Gene expression analysis and identification of DEGs under pesticide treatments. (a) DEGs were detected under pesticide treatments. The numbers on top of each bar show the number of up- (red color) or down-regulated (green color). (b) Venn diagram of overlapping DEGs among pesticide treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

2.1. Data collection and expression analysis

In this study, a microarray data-set (GEO accession number GSE19662), including 531 samples of chemical treated rat hepatocyte cell lines and vehicle control, was selected for expression analysis. This data-set obtained from a genome-wide expression analysis of rat hepatocyte cell lines under chemical exposure (Deng et al., 2010). Gene expression analysis has been done on Agilent-014879 Whole Rat Genome Microarray platform (GPL4135) (Agilent Technologies, Palo Alto, USA). We analyzed the gene expression profile of rat hepatocyte cell lines under seven pesticide treatments, including Atrazine, Chlorpyrifos, DDT, Methoxychlor, Paraquat, Permethrin and Toxaphene. Pesticides information is summarized in Table 1.

The NCBI GEO2R tool was used to analyze the data-set (Barrett

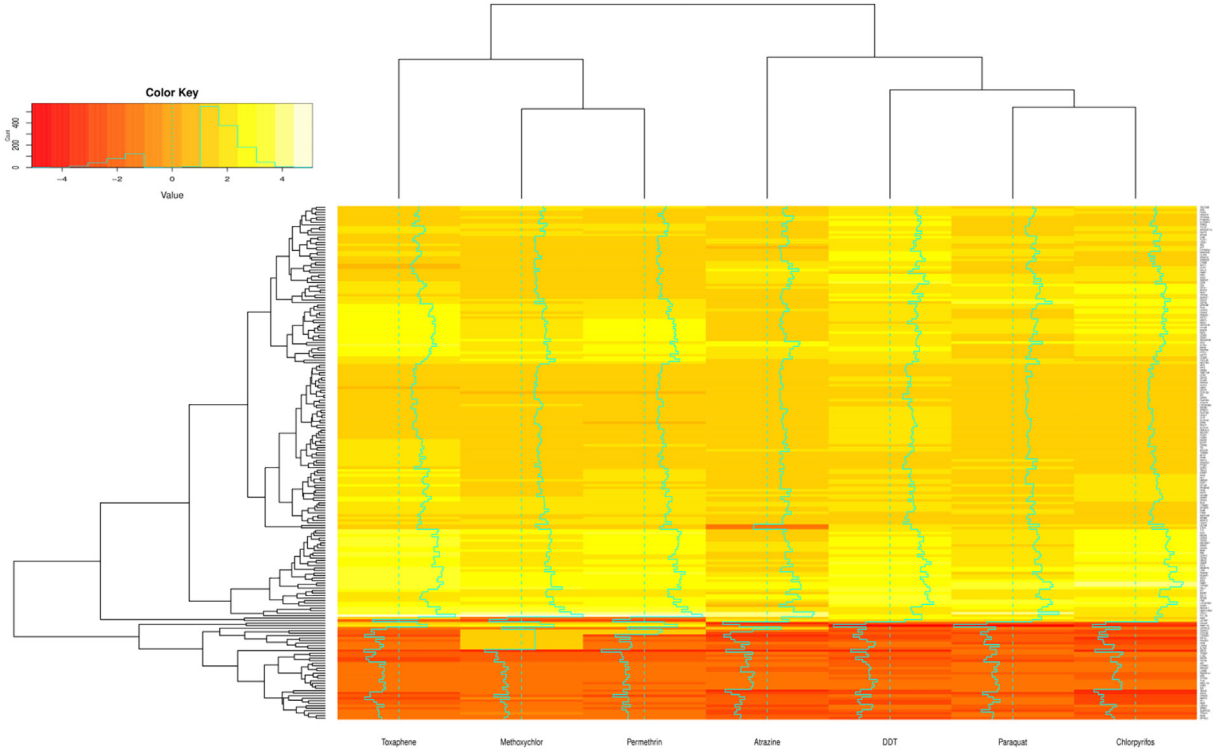


Fig. 2. Heat map of hierarchical clustering of common DEGs among pesticide treatments. The range of log 2 fold change values is from −5 to 5 to enhance visualization.

et al., 2012). GEO2R utilizes R packages to analyze normalized samples and identifies DEGs using statistical tests. We have compared pesticide-treated samples versus control samples to detect DEGs. After comparison, we have filtered results based on significance (p-value $<$ 0.01) and absolute |Log 2 (fold change)| $\geq$ 1. The resulted genes were considered to be significantly differentially expressed under the pesticide treatments. The duplicate values, ambiguous names and missing gene symbols were manually removed from DEGs. By comparing the final unique lists of DEGs, common DEGs among pesticides were identified and visualized in a Venn diagram. The log 2 FC values were used to cluster of DEGs in all samples. The heatmap3 package of R was used to cluster and visualize DEGs (Zhao et al., 2014).

2.2. Identification of pesticide-related TFs in the list of DEGs

TFs regulate sets of genes in the cells under different conditions and it is possible that different gene sets share common TFs. Therefore, Tfcheckpoint server was used to identify TFs in DEG lists of different pesticides (Chawla et al., 2013). The TFs in TFcheckpoint server are manually evaluated for experimental evidence supporting their role in regulation of RNA polymerase II and DNA binding activity (Chawla et al., 2013). Only TFs with these two criteria were considered for further analysis. Furthermore, using the Enrichr tool, the differential expressed TFs (DE-TFs) that are involved in the regulation of the DEGs were identified (Kuleshov et al., 2016; Lachmann et al., 2010). The DEGs list was imported to the Enrichr tool and predicted TFs and their target genes were obtained. The results with a p-value $<$ 0.01 were selected and their presence in the DEGs list was determined. This analysis created a list of DE-TFs and their target genes for each pesticide.

2.3. Ontology analysis, pathway and disease enrichment of DEGs

Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2016) and Comparative Toxicogenomics Database (CTD) (Davis et al., 2018) were used to classify the DEGs among different biological processes, molecular functions, cellular components, pathways and diseases. Results with p-value $<$ 0.01 were considered significant and selected for the analysis.

2.4. Gene regulatory network analysis

The results of Enrichr tool (TFs and their target genes) were used to construct gene regulatory network. Networks were constructed based on the interaction between TFs and target genes. Individual networks for each pesticide were constructed and analyzed by Cytoscape 3.7.1 (Franz et al., 2015). Cytohubba plug-in of Cytoscape was used to detect important genes in the networks (Chin et al., 2014). Cytohubba plug-in uses specific methods such as betweenness, bottleneck, closeness, clustering coefficient, degree, dmnc, eccentricity, epc, mcc, mnc, radiality and stress to rank nodes in networks (Chin et al., 2014). Based on ranking by 12 different methods, common hub genes for each pesticide were detected. Common regulatory network among pesticides was constructed using results of Cytohubba ranking.

2.5. Organ-specific expression analysis of identified hub genes

Information on identified hub genes was obtained from rat genes database of NCBI. The RPKM values of hub genes in rat adrenal, brain, heart, kidney, liver, lung, muscle, spleen, testis, thymus and uterus organs were retrieved from rat genes database of NCBI. The mean of RPKM values in each organ was considered as organ-specific expression.

2.6. Validation of the results

To validate our findings, we have used of batch query tool in CTD database (Davis et al., 2018). The batch query tool uses a text mining algorithm to provide search results. Identified hub genes were submitted to the batch query tool and interacted pesticides were selected.

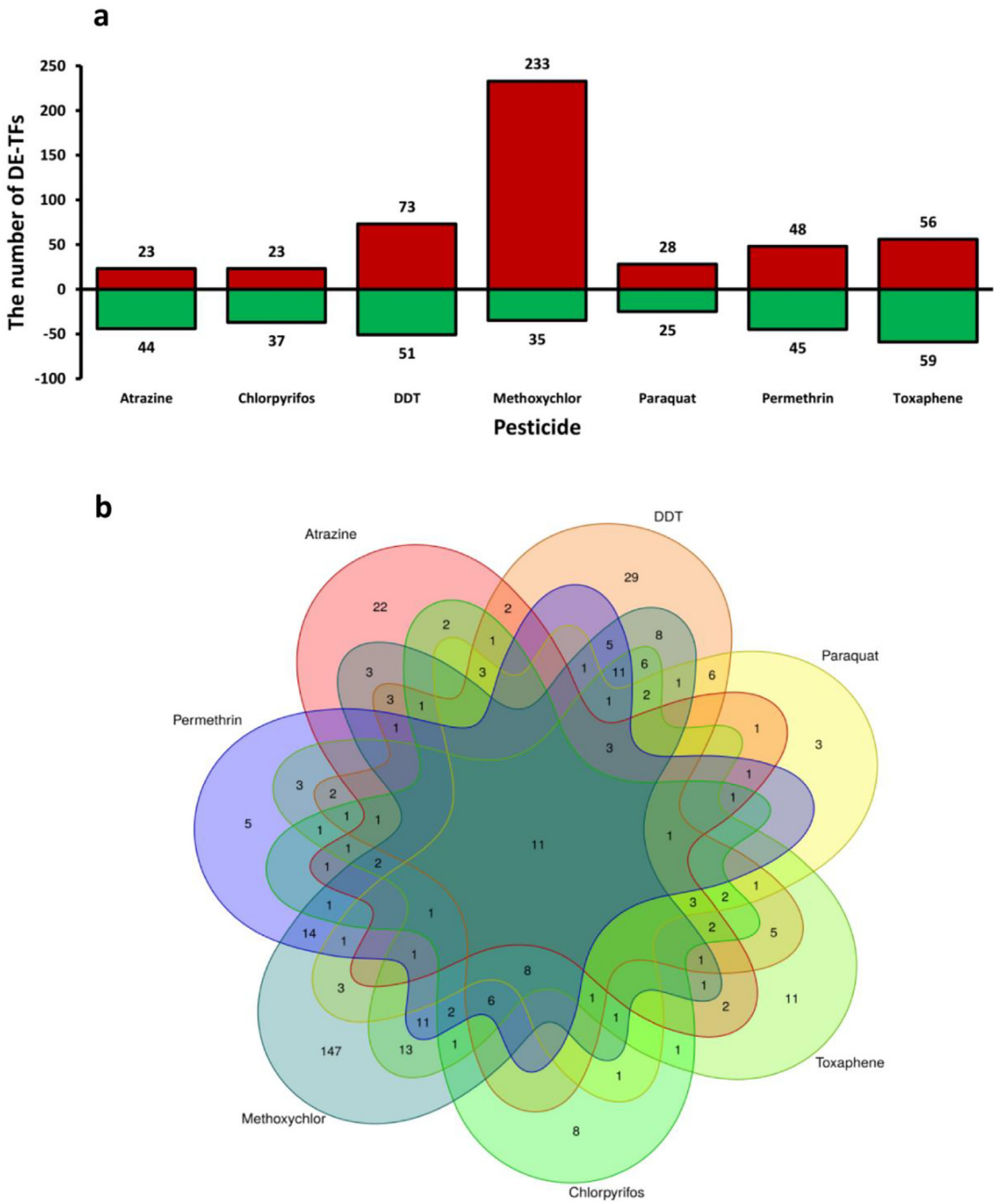


Fig. 3. Identification of DE-TFs under pesticide treatments. (a) DE-TFs were detected under pesticide treatments. The numbers on top of each bar show the number of up- (red color) or down-regulated (green color). (b) Venn diagram of overlapping DE-TFs among pesticide treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Then, expression trend (up- and down-regulation) of hub genes were determined under treatment of selected pesticides.

3. Results

3.1. Identification of DEGs

The gene expression pattern alterations were observed for many genes in rat hepatocyte cell lines under chemical treatment (Fig. 1a). In Atrazine treatment, 1,141 DEGs were identified. For Chlorpyrifos treatment, 1,617 DEGs were observed. DDT treatment showed 3,588 DEGs. Methoxychlor treatment resulted in 5,643 DEGs. In Paraquat treatment, 1,658 DEGs were observed. Permethrin treatment induced 1,948 DEGs and Toxaphene treatment resulted in 2,724 DEGs (Fig. 1a). The highest and the lowest number of DEGs were observed for Methoxychlor (5,643 DEGs) and Atrazine (1,141 DEGs), respectively (Fig. 1a). Interestingly, up-regulated DEGs were dominant in all pesticide treatments, except for the Atrazine. The highest and the lowest number of up-regulated DEGs were observed for Methoxychlor (5,130 DEGs) and Atrazine (529 DEGs), respectively (Fig. 1a). DDT showed the highest number of down-regulated DEGs and Paraquat had the lowest number of down-regulated DEGs (Fig. 1a). Venn diagram was used to determine common and specific DEGs among different pesticides (Fig. 1b). Venn diagram showed 205 common DEGs among pesticides.

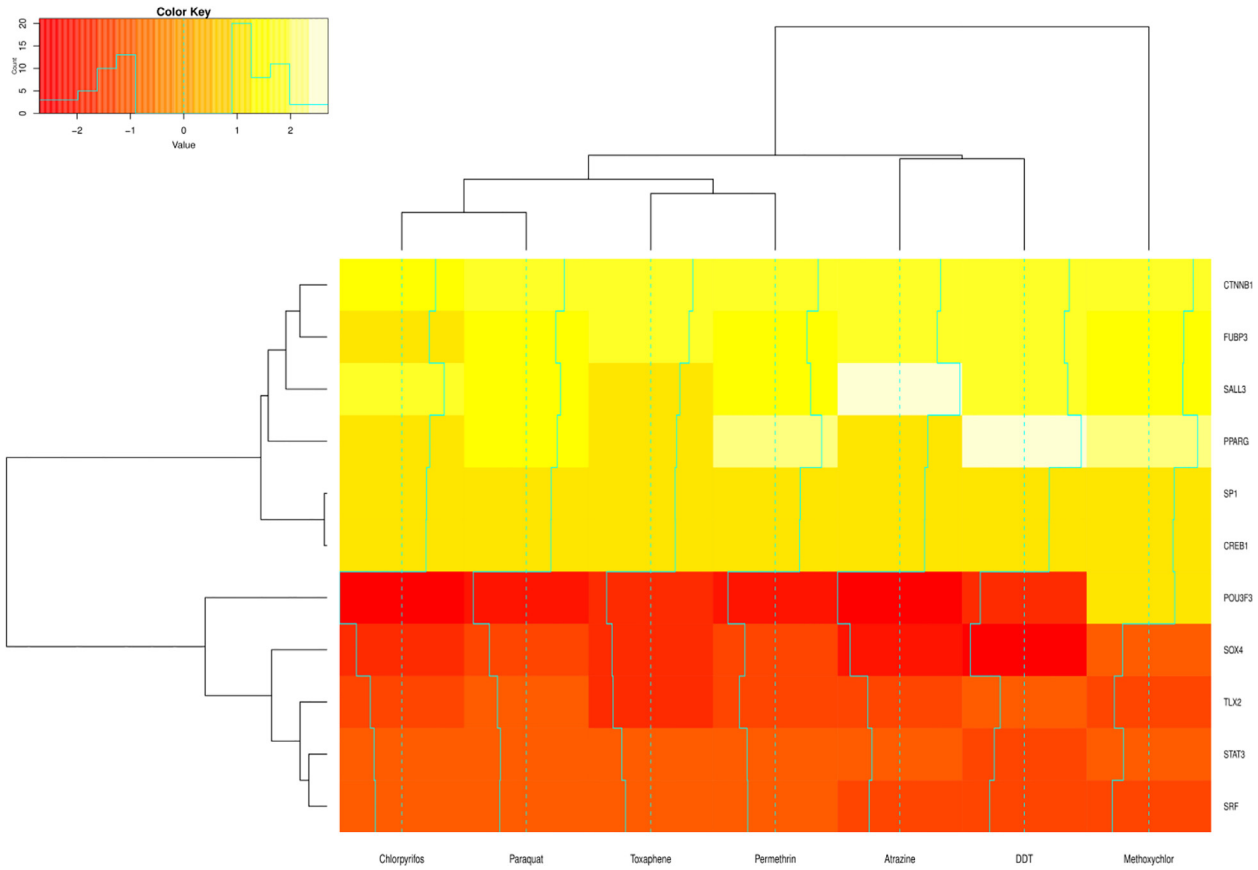


Fig. 4. Heat map of hierarchical clustering of common DE-TFs among pesticide treatments. The range of log 2 fold change values is from −3 to 3 to enhance visualization.

Methoxychlor exhibited the highest number of specific DEGs (2,840 DEGs), while Chlorpyrifos had the lowest number of specific DEGs (78 DEGs) (Fig. 1b).

Hierarchical clustering of common DEGs showed two distinct groups of pesticides (Fig. 2). Toxaphene, Methoxychlor and Permethrin was clustered in one group and Atrazine, DDT, Paraquat and Chlorpyrifos clustered in other group. Two herbicides (Atrazine and Paraquat) were clustered together in one group (Fig. 2).

3.2. Identification of TFs and DE-TFs

Search for TFs in the DEG list of pesticides showed a different number of TFs (Fig. 3a). In Atrazine treatment, 67 DE-TFs were identified. For Chlorpyrifos treatment, 60 DE-TFs were observed. DDT treatment showed 124 DE-TFs. Methoxychlor treatment resulted in 268 DE-TFs. In Paraquat treatment, 53 DE-TFs were observed. Permethrin treatment induced 93 DE-TFs and Toxaphene treatment resulted in 115 DE-TFs (Fig. 3a). The highest and the lowest number of DE-TFs were observed for Methoxychlor (268 DE-TFs) and Paraquat (53 DE-TFs), respectively (Fig. 3a). The up-regulated DE-TFs were dominant in DDT, Methoxychlor, Paraquat and permethrin treatments, while down-regulated DE-TFs were dominant in Atrazine, Chlorpyrifos, Permethrin and Toxaphene treatments (Fig. 3a). The highest and the lowest number of up-regulated DE-TFs were observed for Methoxychlor (233 DE-TFs) and Atrazine and Chlorpyrifos together (23 DE-TFs), respectively (Fig. 3a). Toxaphene showed the highest number of down-regulated DE-TFs and Paraquat had the lowest number of down-regulated DE-TFs (Fig. 3a). Venn diagram was used to determine common and specific DE-TFs among different pesticides (Fig. 3b). Venn diagram showed 11 common DE-TFs among pesticides. Methoxychlor exhibited the highest number of specific DE-TFs (147 DEGs), while Paraquat had the lowest number of specific DE-TFs (3 DEGs) (Fig. 3b). Hierarchical clustering of common DE-TFs showed four distinct groups of pesticides (Fig. 4). The Chlorpyrifos and Paraquat, Toxaphene and Permethrin, Atrazine and DDT were clustered in three distinct groups, while Methoxychlor was separately clustered in one group. Two herbicides (Atrazine and Paraquat) were clustered in different group (Fig. 4).

3.3. Ontology analysis, pathway and disease enrichment of DEGs

Gene ontology analysis classified common DEGs into three groups of biological processes, cellular components and molecular functions (Fig. 5). In biological processes group, most of the DEGs were categorized in organelle organization, cell cycle, cell cycle process, mitotic cell cycle and regulation of cell cycle. The dominant terms in cellular components group were cytosol, chromosome, chromosomal part, microtubule cytoskeleton and chromosomal region while in molecular functions group, catalytic activity, ion binding and enzyme regulator activity were the top enriched terms (Fig. 5). The KEGG pathway analysis showed that the DEGs were significantly enriched in the metabolic pathways and pathways in cancer (Fig. 6). The disease enrichment analysis showed that cancers, digestive system diseases, urogenital diseases, genetic diseases, endocrine system diseases, musculoskeletal diseases and skin diseases are the most related diseases with chemical treatments (Table 2).

3.4. Gene regulatory network analysis

Gene regulatory network analysis was performed on DEGs, to explore common regulatory network among studied pesticides. Based on ranking results in Cytohubba plug-in, six common hub genes were identified among pesticides. These six hub genes (CREB1, CTNNB1,

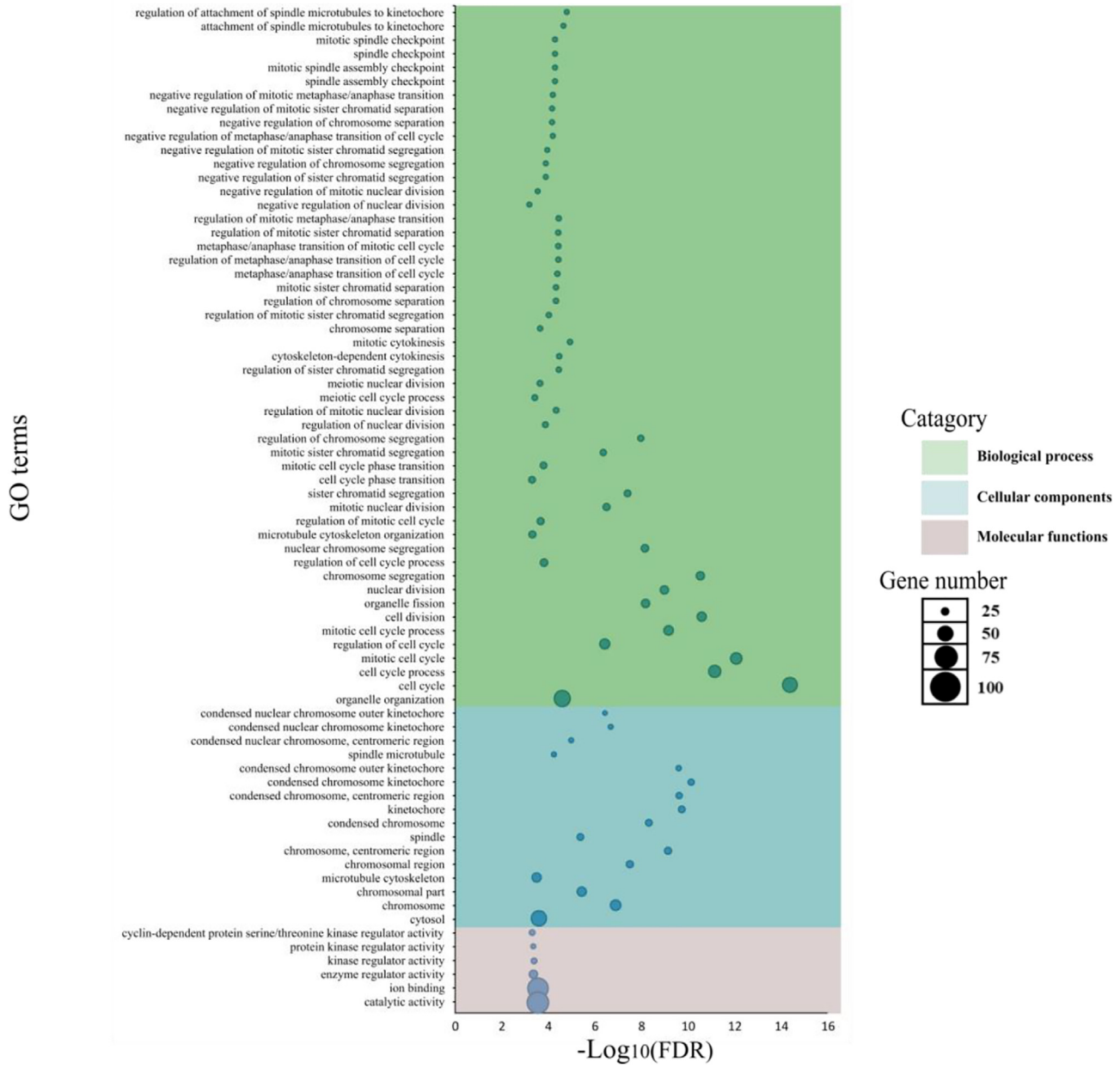


Fig. 5. GO enrichment analysis of common DEGs. The Y-axis indicates the enriched GO-terms and the X-axis indicates the significance level based on -log (FDR). The green, blue, and pink color ranges indicate biological process, cellular components, and molecular function GO-terms, respectively. Circles' size shows the number of enriched genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PPARG, SP1, SRF and STAT3) had the highest number of interactions with other DEGs (Fig. 7). Further analyses showed that all identified hub genes were DE-TFs. Two of six hub genes (SRF and STAT3) were down-regulated, while four hub genes (CREB1, CTNNB1, PPARG and SP1) were up-regulated (Fig. 4). Interestingly, all hub genes showed the same expression trend among pesticide treatments (Fig. 4).

3.5. Organ-specific expression analysis of identified hub genes

The expression analysis of hub genes showed differential expression among rat organs. Results showed that CTNNB1 had the highest and PPARG had the lowest expression level among analyzed tissues. Information and organ-specific expression of six identified hub genes (CREB1, CTNNB1, PPARG, SP1, SRF and STAT3) are shown in Table 3 and Fig. 8.

3.6. Validation of the results by different pesticides

To validate the role of six identified hub genes under pesticide treatments, we have used of batch query tool in CTD database (Davis et al., 2018). Fig. 9 contains the results obtained from microarray and text mining studies alongside each other. Interestingly, a relatively similar trend of gene expression was observed for the hub genes under pesticide treatment. These results could approve the specific role of these hub genes.

4. Discussion

In the present study, microarray data-sets of rat hepatocyte cell lines under seven pesticide treatments, including Atrazine, Chlorpyrifos, DDT, Methoxychlor, Paraquat, Permethrin and Toxaphene were analyzed through system biology methods. At first, microarray data-sets of

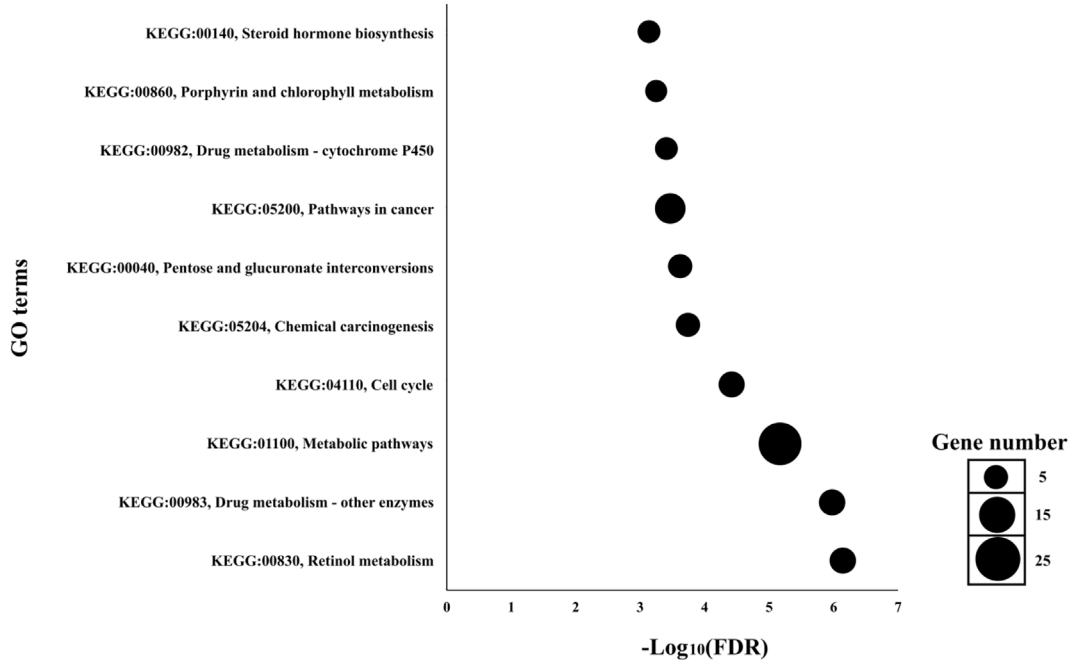


Fig. 6. Metabolic pathway analysis of common DEGs. The Y-axis represents the enriched KEGG pathway names, and the X-axis represents the significance level based on -log (FDR). Circles' size shows the number of enriched genes.

Table 2
Disease enrichment analysis of common DEGs.

Disease	Enriched DEGs	FDR
Cancer	80	5.28E − 07
Digestive system disease	63	5.85E − 07
Urogenital disease	30	1.88E − 05
Genetic disease	26	3.51E − 03
Endocrine system disease	22	1.65E − 05
Musculoskeletal disease	22	8.82E − 04
Skin disease	20	1.16E − 03

each pesticide were individually analyzed and common DEGs and DE-TFs among seven pesticides were selected. Then, gene regulatory network was constructed and common hub genes were identified. Functional annotation was performed to determine the likely roles of hub genes in diseases.

Interestingly, the number of identified DEGs were dramatically different among the pesticides. The highest and the lowest number of DEGs were observed for Methoxychlor and Atrazine pesticides, respectively. Due to more changes in the transcriptome profile, Methoxychlor and DDT were more toxic than other studied pesticides. In contrast, Atrazine induced a little change in the transcriptome profile and had the least toxicity (Fig. 1a). In general, insecticides induced more DEGs than the herbicides in the rat hepatocyte cell lines. Among insecticides, organochloride insecticides changed more DEGs than other insecticides (Fig. 1a). A similar trend was observed for DE-TFs in the rat hepatocyte cell lines exposed to pesticides (Fig. 3a). These findings highlight different mode of action and the nature of these pesticides. It has been well documented that the toxicity of pesticides is varied due to their chemical structures and their mode of actions (Cao et al., 2018; Hamadache et al., 2016; Kaushik and Kaushik, 2007; Mesnage et al., 2018; Zahouily et al., 2002).

We found 205 overlapping genes among DEGs obtained from each pesticide treatment that reflects the overlap between affected processes by these pesticides (Fig. 1b). Gene ontology analysis of the common DEGs revealed that organelle organization and cell cycle processes comprised the highest number of genes. This indicates the genotoxicity effects of pesticides on cell cycle processes. Results of a genotoxicity study showed that cypermethrin and chlorpyrifos insecticides cause cell cycle perturbations and apoptosis in mouse bone marrow cells (Chauhan et al., 2016). In the study conducted by Marc et al., glyphosate-based pesticides affect cell cycle regulation and induced cell cycle dysfunction in sea urchin (*Sphaerechinus granularis*) eggs (Marc et al., 2004).

Functional annotation of common DEGs showed that these genes are notably involved in cancers and digestive system diseases (Table 2). Metabolic pathway analysis using KEGG database confirmed role of common DEGs in cancers (Fig. 6). Relation between pesticides exposure and cancers have been reported in many studies. Glyphosate herbicide act as an estrogen agonist in human breast cancer and can stimulate the growth of cancer cell lines at the same concentrations to estrogen (Hokanson et al., 2007; Mesnage et al., 2015; Thongprakaisang et al., 2013). Results of a long-term study revealed stimulating effects of four organocholride (1,3-dichloropropene, captafol, pentachloronitrobenzene and dieldrin) insecticides on pancreatic cancer (Clary and Ritz, 2003). Exposure to endosulfan pesticide induces genes that are related to human diseases, including liver cancer, prostate cancer and leukemia (Xu et al., 2016).

We have detected the six hub genes (CREB1, CTNNB1, PPARG, SP1, SRF and STAT3) through network analysis that were common in all studied pesticides. All of these hub genes were DE-TFs and had the highest number of interactions with other DEGs. It seems that these hub genes form a key regulatory network to regulate pesticide induced genes (Fig. 7). The text mining showed a relatively similar trend of gene expression for the hub genes under other pesticide treatment. These results could approve the specific role of these hub genes (Fig. 9).

The first hub gene, CREB1 transcription factor, is a member of the CREB family of leucine zipper (bZIP) transcription factors (Lonze and Ginty, 2002). The rat CREB1 has a complex gene structure with 13 exons that result in 10 isoforms with different regulatory activity (Ortega-Martínez, 2015).

The gene expression analysis showed relatively low expression level for CREB1 gene among different rat organs. The highest expression level of CREB1 was in thymus, while the lowest expression level was found in the liver (Fig. 8). Like other bZIP transcription factors, CREB1 contains a leucine zipper domain that helps dimerization and a C-

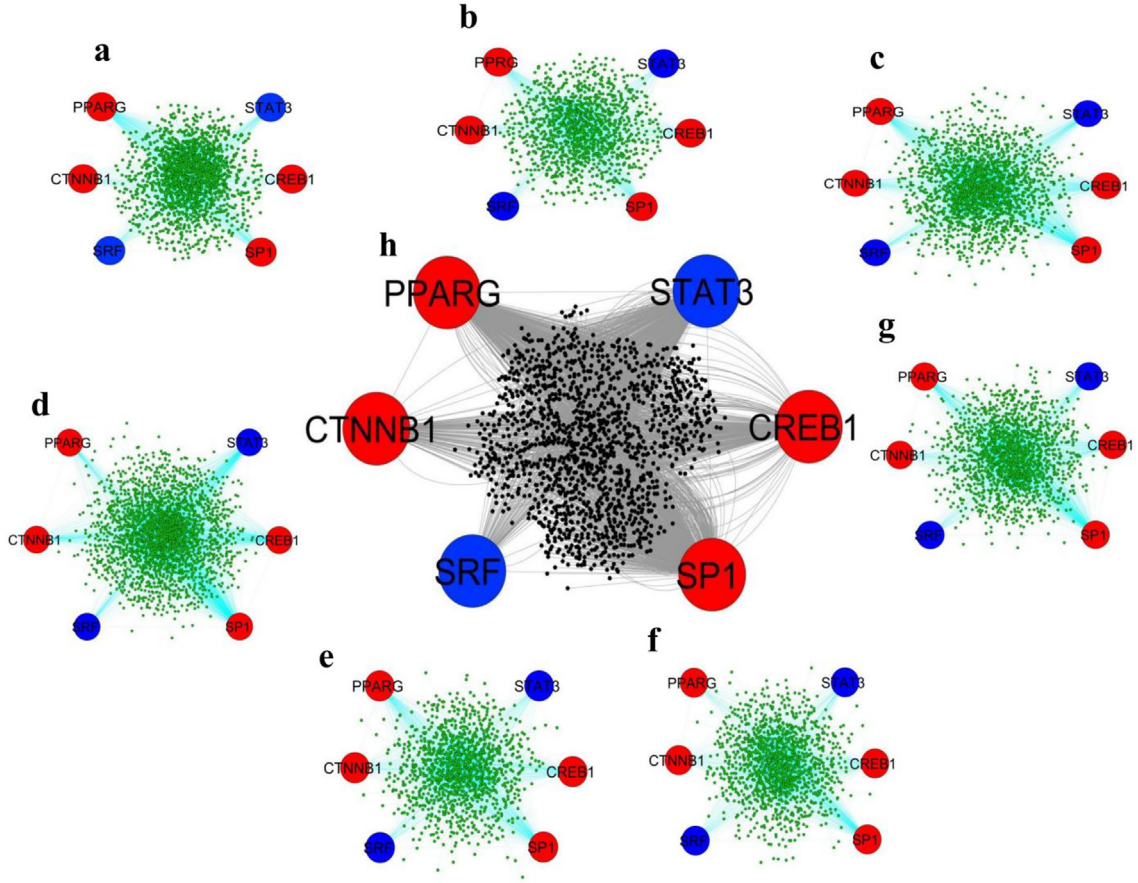


Fig. 7. Gene regulatory networks under pesticide treatments. (a) Atrazine treatment. (b) Chlorpyrifos treatment. (c) DDT treatment. (d) Methoxycholor treatment. (e) Paraquat treatment. (f) Permethrin treatment. (g) Toxaphene treatment. (h) Common gene regulatory network. Red circles are up-regulated and blue circles are down-regulated hub DE-TF genes. Green and black circles are other DEGs in gene regulatory networks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Information of six identified hub DE-TF genes.

Gene symbol	Gene ID	Full name	Functional group	Cellular localization
CREB1	81646	CAMP responsive element binding protein 1	Transcription factor	Nuclear
CTNNB1	84353	Catenin (cadherin associated protein) beta 1	Transcription factor/signalling	Cytoplasmic/Nuclear
PPARG	25664	Peroxisome proliferator activated receptor gamma	Transcription factor	Nuclear
SP1	24790	Specificity protein 1	Transcription factor	Nuclear
SRF	501099	Serum response factor	Transcription factor	Nuclear
STAT3	25125	Signal transducer and activator of transcription 3	Transcription factor	Cytoplasmic/Nuclear

terminal basic domain for DNA binding. The CREB1forms a homodimer and binds to the cAMP-responsive element after phosphorylation at a conserved serine (Ser-133). This protein acts through transcription of genes in response to hormonal stimulation of the cAMP pathway (Borrelli et al., 1992; Lonze and Ginty, 2002; Mayr and Montminy, 2001; Zhang et al., 2005). The CREB1 is activated in response to a wide range of environmental and physiological stimuli. The CREB1 regulates proliferation, survival and differentiation of cells, inflammatory activities, protecting against oxidative-stress mediated cell death and anti-apoptotic activities (Lee et al., 2009; Sadamoto et al., 2010; Wen et al., 2010).

CTNNB1, also known as β-catenin, is another identified hub gene belongs to the catenin family. The CTNNB1 is a dual function protein involved in the regulation of transcription and cell–cell adhesion process. Like CREB1, rat CTNNB1 has a complex gene structure with 18 exons and and only 1 isoform. The CTNNB1 is widely expressed in rat organs (Fig. 8). The CTNNB1 has broad functions in intracellular signal transducer in the Wnt signaling pathway and cadherin-mediated cell–cell adhesion (Abe and Takeichi, 2008; MacDonald et al., 2009; McCrea and Gu, 2010). The CTNNB1, as a component of adherens junctions, can regulate adhesion between cells and cell growth (Brembeck et al., 2006). The CTNNB1 also can directly induces by diffusible extracellular substances and changes the transcription of specific genes (Brembeck et al., 2006; Monga, 2015). Due to its broad functions, CTNNB1 plays a vital role in directing several developmental processes and in regulation of physiological regeneration processes (Brembeck et al., 2006; Haegel et al., 1995; Monga, 2015; Schaefer and Peifer, 2019). Alterations in the localization or expression level and lack of function of CTNNB1 are related to many diseases such as cancers and various forms of heart disorders (Morin, 1999).

PPARG, also called peroxisome proliferator-activated receptor gamma, another identified hub gene belongs to the family of peroxisome proliferation-activated receptors that are involved in a wide variety of regulatory functions (Desvergne and Wahli, 1999). The rat PPARG has a complex gene structure with 10 exons that result in 2 isoforms. The gene expression analysis showed relatively low

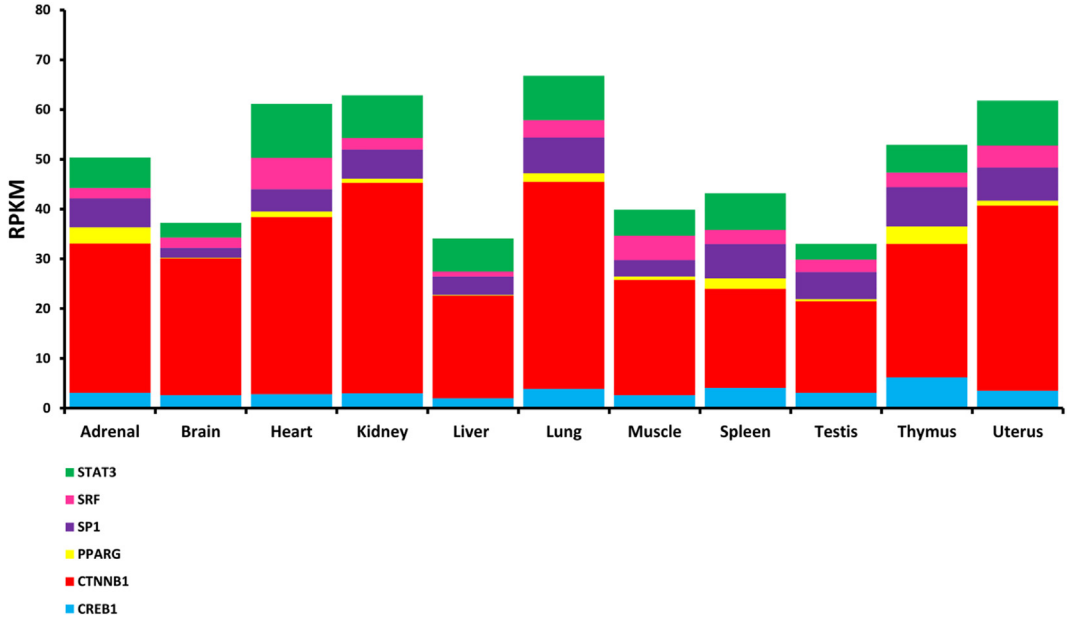


Fig. 8. Organ-specific expression analysis of six identified hub DE-TF genes. The Y-axis indicates the gene expression levels calculated by the RPKM method in different rat tissues (X-axis). In each column, the height of blue, red, yellow, purple, pink, and green colors respectively indicates the expression levels of CREB1, CTNNB1, PPARG, SP1, SRF and STAT3 genes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

expression level for PPARG gene among different rat organs. The highest expression level of PPARG was in thymus and adrenal, while the lowest expression level was found in the liver and brain (Fig. 8). The PPARG is a macronutrient metabolism regulator and regulates processes such as glucose metabolism, lipid metabolism, fatty acid storage, adipogenesis, adipose tissue formation, cell proliferation and apoptosis (Braissant et al., 1996; Chinetti et al., 2000; Grygiel-Górniak, 2014; Janani and Kumari 2015). PPARG protects non-adipose tissues (such as liver and skeletal muscle) against excessive lipid overload and maintains their normal function (Kintscher and Law, 2005). Apart from metabolism regulatory activity, PPARG is also important in inflammation and regulates targets relevant to inflammation in endothelial cells (Marx et al., 1999).

SP1, also known as specificity protein 1, another identified hub gene, is a member of the SP transcription factor family. Members of this family belong to zinc finger transcription factors (Black et al., 2001; Safe and Abdelrahim, 2005; Suske et al., 2005). The rat SP1 has six exons and only one isoform. The highest expression level of SP1 was observed in the thymus, while the lowest expression level was found in the brain (Fig. 8). The SP1 undergoes various post-translational modifications such as glycosylation and phosphorylation that significantly modulate its activity. These modifications determine the activatory or inhibitory properties of the SP1 transcription factor (Chang and Hung, 2012; Waby et al., 2008). The SP1 transcription factor binds to GC-rich motifs of many gene promoters. Investigations have revealed that SP1 is involved in many cellular processes, including cell proliferation and differentiation, apoptosis, chromatin remodeling, DNA damage and immune responses (Beishline and Azizkhan-Clifford, 2015; Black et al., 2001; Chang and Hung, 2012; Vizcaíno et al., 2015).

SRF, also known as serum response factor, another identified hub gene, is a member of the MADS-box transcription factors superfamily (Shore and Sharrocks, 1995). The rat SRF has 8 exons and 2 isoforms. The highest expression level of SRF was observed in heart, while the lowest expression level was found in the liver (Fig. 8). The SRF binds to the serum response element and induces expression of target genes (Treisman, 1992). This transcription factor regulates many immediate early genes and it is involved in cell proliferation and differentiation, cell cycle regulation and apoptosis (Chai and Tarnawski, 2002; Miano, 2003; Miano et al., 2007; Treisman, 1992). The SRF is involved in many pathways such as the mitogen-activated protein kinase pathway (MAPK) and plays a vital role in development of the embryo and growth of skeletal muscle (Chai and Tarnawski, 2002; Miano, 2003; Miano et al., 2007; Treisman, 1992).

The last identified hub gene, STAT3, also called signal transducer and activator of transcription 3, is a member of STAT transcription factors family. The rat STAT3 has a complex gene structure with 24 exons that result in 3 isoforms. The STAT3 is widely expressed in rat organs. The highest expression level of STAT3 was in the heart, while the lowest expression level was found in brain and testis (Fig. 8). Member of STAT transcription factors family relay signals from plasma membrane receptors to the nucleus and regulate transcription of target genes. In response to cytokines and growth factors, STAT3 is phosphorylated and regulates the expression of genes involved in many vital processes, including cell proliferation and differentiation, apoptosis, immune responses and tumor angiogenesis and metastasis (Cheng et al., 2003; Jing and Tweardy, 2005; Johnston and Grandis, 2011; Levy and Lee, 2002).

As discussed previously, all of the key regulator genes introduced by this study were important regulatory genes and played pivotal roles in various cellular processes. Six identified key genes regulate responses, including response to environmental and physiological stimuli, response to oxidative-stress, anti-apoptotic responses, response to inflammation, DNA damage and immune responses. Identified genes also regulate processes such as cell proliferation, cell survival, cell differentiation, cell cycle, apoptosis, cadherin-mediated cell-cell adhesion, glucose metabolism, lipid metabolism, fatty acid storage, adipogenesis, adipose tissue formation, tumor angiogenesis and metastasis. These key regulatory genes also play vital role in signaling pathways such as the Wnt signaling pathway and MAPK pathway.

In the current study for the first time, we reported six key regulatory genes associated with pesticide exposure responses. The key regulatory genes identified in this study could be used for diagnostics or therapeutic aims in further studies. Furthermore, these genes can be used as toxicity biomarkers to improve diagnosis and prognosis (Lewis, 2011; Mohammadi et al., 2011).

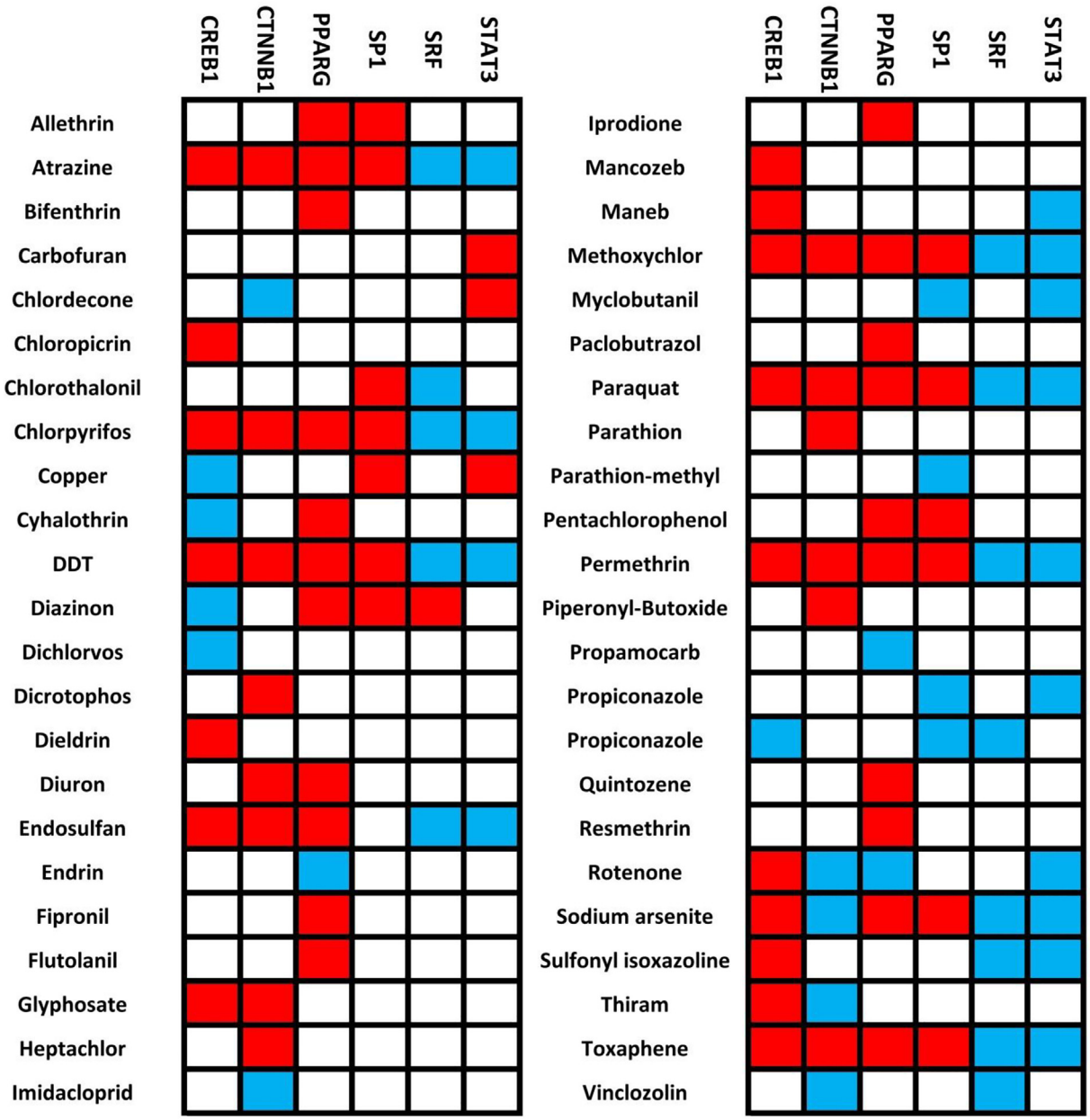


Fig. 9. Results of data validation. Red and blue colors show the up- and down-regulated hub DE-TF genes, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5. Conclusion

In the present study, we used system biology approaches to identify the key regulatory genes by investigating data from rat hepatocyte cell lines under pesticide treatment. We found 205 common DEGs among pesticides treatment that involved in organelle organization, cell cycle processes, regulation of cell cycle, catalytic activity, ion binding and enzyme regulatory activity. These common DEGs also related to cancers, digestive system diseases, urogenital diseases, genetic diseases, endocrine system diseases, musculoskeletal diseases and skin diseases. Interestingly, six key genes introduced by this study were important regulatory genes and played pivotal roles in various cellular processes. The reliable source of data, such as gene regulatory networks, assists to better understand the function of genes. Identification and characterization of key regulatory genes is an opportunity to provide comprehensive insights into the relationship between pesticide exposure and diseases. Although the results of this study provide new information in assessing the effect of pesticides on diseases, it is also important to understand the limitations of the methodology. We analyzed transcriptome data of rat hepatocyte cell lines under pesticide treatment to find common key regulatory genes. Therefore, other rat tissue and organ have not been investigated and it is difficult to find a comprehensive insight. For this reason, it is suggested that transcriptome of different tissue and organ of rat under pesticide treatment to be examined. By functional genomics approaches, a comprehensive insight into relationship between pesticide exposure and diseases and the main common regulatory network underlying the pesticide exposure will be available.

6. Compliance with ethical standards

The present research does not involve human participants and/or animals.

Author contributions

Seyed Sajad Sohrabi and Seyyed Mohsen Sohrabi conceived and designed the experiments. Seyed Sajad Sohrabi, Seyyed Mohsen Sohrabi, Marzieh Rashidipour, Mohsen Mohammadi, Javad Khalili Fard and Hossein Mirzaei Najafgholi prepared the data. Seyed Sajad Sohrabi and Seyyed Mohsen Sohrabi analyzed the data. Seyed Sajad Sohrabi, Seyyed Mohsen Sohrabi, Marzieh Rashidipour and Mohsen Mohammadi wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aardema, M.J., MacGregor, J.T., 2002. Toxicology and genetic toxicology in the new era of "toxicogenomics": Impact of "-omics" technologies. Mutation Res./Fundam. Mol. Mech. Mutagenesis 499 (1), 13–25.

Abe, K., Takeichi, M., 2008. Eplin mediates linkage of the cadherin–catenin complex to f-actin and stabilizes the circumferential actin belt. Proc. Natl. Acad. Sci. 105 (1), 13–19.

Bao, L.-J., Wei, Y.-L., Yao, Y., Ruan, Q.-Q., Zeng, E.Y., 2015. Global trends of research on emerging contaminants in the environment and humans: a literature assimilation. Environ. Sci. Pollut. Res. 22 (3), 1635–1643.

Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M., Marshall, K.A., Phillippy, K.H., Sherman, P.M., Holko, M., 2012. Ncbi geo: archive for functional genomics data sets—update. Nucl. Acids Res. 41 (D1), D991–D995.

Beishline, K., Azizkhan-Clifford, J., 2015. Sp1 and the 'hallmarks of cancer'. FEBS J. 282 (2), 224–258.

Black, A.R., Black, J.D., Azizkhan-Clifford, J., 2001. Sp1 and krüppel-like factor family of transcription factors in cell growth regulation and cancer. J. Cell. Physiol. 188 (2), 143–160.

Borrelli, E., Montmayeur, J., Foulkes, N., Sassone-Corsi, P., 1992. Signal transduction and gene control: the camp pathway. Crit. Rev. Oncog. 3 (4), 321–338.

Braissant, O., Foufelle, F., Scotto, C., Dauça, M., Wahli, W., 1996. Differential expression of peroxisome proliferator-activated receptors (ppars): tissue distribution of ppar-alpha,-beta, and-gamma in the adult rat. Endocrinology 137 (1), 354–366.

Brembeck, F.H., Rosário, M., Birchmeier, W., 2006. Balancing cell adhesion and wnt signaling, the key role of β-catenin. Curr. Opin. Genet. Dev. 16 (1), 51–59.

Brown, V.J., 2003. Reaching for chemical safety. Environ Health Perspect. 111 (14), A766–A769.

Cao, F., Souders II, C.L., Perez-Rodriguez, V., Martyniuk, C.J., 2018. Elucidating conserved transcriptional networks underlying pesticide exposure and Parkinson's disease: a focus on chemicals of epidemiological relevance. Front. Genetics 9.

Chai, J., Tarnawski, A., 2002. Serum response factor: discovery, biochemistry, biological roles and implications for tissue injury healing.

Chang, W.-C., Hung, J.-J., 2012. Functional role of post-translational modifications of sp1 in tumorigenesis. J. Biomed. Sci. 19 (1), 94.

Chauhan, L.K., Varshney, M., Pandey, V., Sharma, P., Verma, V.K., Kumar, P., Goel, S.K., 2016. Ros-dependent genotoxicity, cell cycle perturbations and apoptosis in mouse bone marrow cells exposed to formulated mixture of cypermethrin and chlorpyrifos. Mutagenesis 31 (6), 635–642.

Chawla, K., Tripathi, S., Thommesen, L., Lægreid, A., Kuiper, M., 2013. Tfcheckpoint: a curated compendium of specific DNA-binding rna polymerase ii transcription factors. Bioinformatics 29 (19), 2519–2520.

Chen, J.C., Alvarez, M.J., Talos, F., Dhruv, H., Rieckhof, G.E., Iyer, A., Diefes, K.L., Aldape, K., Berens, M., Shen, M.M., 2014. Identification of causal genetic drivers of human disease through systems-level analysis of regulatory networks. Cell 159 (2), 402–414.

Cheng, F., Wang, H.-W., Cuenca, A., Huang, M., Ghansah, T., Brayer, J., Kerr, W.G., Takeda, K., Akira, S., Schoenberger, S.P., 2003. A critical role for stat3 signaling in immune tolerance. Immunity 19 (3), 425–436.

Chin, C.-H., Chen, S.-H., Wu, H.-H., Ho, C.-W., Ko, M.-T., Lin, C.-Y., 2014. Cytohubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst. Biol. 8 (4), S11.

Chinetti, G., Fruchart, J.-C., Staels, B., 2000. Peroxisome proliferator-activated receptors (ppars): Nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm. Res. 49 (10), 497–505.

Clary, T., Ritz, B., 2003. Pancreatic cancer mortality and organochlorine pesticide exposure in california, 1989–1996. Am. J. Ind. Med. 43 (3), 306–313.

Collins, F.S., Gray, G.M., Bucher, J.R., 2008. Toxicology. Transforming environmental health protection. Science 319 (5865), 906–907.

Corton, J.C., Kleinstreuer, N.C., Judson, R.S., 2019. Identification of potential endocrine disrupting chemicals using gene expression biomarkers. Toxicol. Appl. Pharmacol. 380, 114683.

Damalas, C.A., Koutroubas, S.D., 2016. Farmers' exposure to pesticides: toxicity types and ways of prevention. Toxics 4 (1), 1.

Davis, A.P., Grondin, C.J., Johnson, R.J., Sciaky, D., McMorran, R., Wiegers, J., Wiegers, T.C., Mattingly, C.J., 2018. The comparative toxicogenomics database: update 2019. Nucl. Acids Res. 47 (D1), D948–D954.

Deng, Y., Johnson, D.R., Guan, X., Ang, C.Y., Ai, J., Perkins, E.J., 2010. In vitro gene regulatory networks predict in vivo function of liver. BMC Syst. Biol. 4 (1), 153.

Desvergne, B., Wahli, W., 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr. Rev. 20 (5), 649–688.

Franz, M., Lopes, C.T., Huck, G., Dong, Y., Sumer, O., Bader, G.D., 2015. Cytoscape. Js: a graph theory library for visualisation and analysis. Bioinformatics 32 (2), 309–311.

Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S., Fava, F., 2015. Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. New Biotechnol. 32 (1), 147–156.

Goh, K.-I., Cusick, M.E., Valle, D., Childs, B., Vidal, M., Barabási, A.-L., 2007. The human disease network. Proc. Natl. Acad. Sci. 104 (21), 8685.

Grygiel-Górniak, B., 2014. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications-a review. Nutr. J. 13 (1), 17.

Haegel, H., Larue, L., Ohsugi, M., Fedorov, L., Herrenknecht, K., Kemler, R., 1995. Lack of beta-catenin affects mouse development at gastrulation. Development 121 (11), 3529–3537.

Hamadache, M., Benkortbi, O., Hanini, S., Amrane, A., Khaouane, L., Moussa, C.S., 2016. A quantitative structure activity relationship for acute oral toxicity of pesticides on rats: validation, domain of application and prediction. J. Hazard. Mater. 303, 28–40.

Hardt, C., Beber, M.E., Rasche, A., Kamburov, A., Hebels, D.G., Kleinjans, J.C., Herwig, R., 2016. Toxdb: Pathway-level interpretation of drug-treatment data. Database. 2016.

Hasan, M.N., Malek, M.B., Begum, A.A., Rahman, M., Mollah, M.N.H., 2019. Assessment of drugs toxicity and associated biomarker genes using hierarchical clustering. Medicina (Kaunas) 55 (8), 451.

Hasan, M.N., Rana, M.M., Begum, A.A., Rahman, M., Mollah, M.N.H., 2018. Robust co-clustering to discover toxicogenomic biomarkers and their regulatory doses of chemical compounds using logistic probabilistic hidden variable model. Front. Genet. 9, 516.

Hokanson, R., Fudge, R., Chowdhary, R., Busbee, D., 2007. Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Hum. Exp. Toxicol. 26 (9), 747–752.

Igarashi, Y., Nakatsu, N., Yamashita, T., Ono, A., Ohno, Y., Urushidani, T., Yamada, H., 2014. Open tg-gates: a large-scale toxicogenomics database. Nucl. Acids Res. 43 (D1), D921–D927.

Ignatieva, E.V., Levitsky, V.G., Kolchanov, N.A., 2015. Human genes encoding transcription factors and chromatin-modifying proteins have low levels of promoter polymorphism: a study of 1000 genomes project data. Int. J. Genomics 2015, 260159.

Iida, M., Takemoto, K., 2018. A network biology-based approach to evaluating the effect of environmental contaminants on human interactome and diseases. Ecotoxicol. Environ. Saf. 160, 316–327.

Janani, C., Kumari, B.R., 2015. Ppar gamma gene–a review. Diabetes & Metabolic Syndrome. Clin. Res. Rev. 9 (1), 46–50.

Jing, N., Tweardy, D.J., 2005. Targeting stat3 in cancer therapy. Anticancer Drugs 16 (6), 601–607.

Johnston, P.A., Grandis, J.R., 2011. Stat3 signaling: anticancer strategies and challenges. Mol. Interventions 11 (1), 18.

Judson, R., Richard, A., Dix, D.J., Houck, K., Martin, M., Kavlock, R., Dellarco, V., Henry, T., Holderman, T., Sayre, P., et al., 2009. The toxicity data landscape for environmental chemicals. Environ. Health Perspect. 117 (5), 685–695.

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K., 2016. Kegg: new perspectives on genomes, pathways, diseases and drugs. Nucl. Acids Res. 45 (D1), D353–D361.

Kaushik, P., Kaushik, G., 2007. An assessment of structure and toxicity correlation in organochlorine pesticides. J. Hazard. Mater. 143 (1–2), 102–111.

Kim, S., 2017. Identifying dynamic pathway interactions based on clinical information. Comput. Biol. Chem. 68, 260–265.

Kintscher, U., Law, R.E., 2005. Pparγ-mediated insulin sensitization: the importance of fat versus muscle. Am. J. Physiol. Endocrinol. Metabolism 288 (2), E287–E291.

Kuleshov, M.V., Jones, M.R., Rouillard, A.D., Fernandez, N.F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S.L., Jagodnik, K.M., Lachmann, A., 2016. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucl. Acids Res. 44 (W1), W90–W97.

Lachmann, A., Xu, H., Krishnan, J., Berger, S.I., Mazloom, A.R., Ma'ayan, A., 2010. Chea: Transcription factor regulation inferred from integrating genome-wide chip-x experiments. Bioinformatics 26 (19), 2438–2444.

Lee, B., Cao, R., Choi, Y.S., Cho, H.Y., Rhee, A.D., Hah, C.K., Hoyt, K.R., Obrietan, K., 2009. The creb/cre transcriptional pathway: protection against oxidative stress-mediated neuronal cell death. J. Neurochem. 108 (5), 1251–1265.

Levy, D.E., Lee, C-k, 2002. What does stat3 do? J. Clin. Investig. 109 (9), 1143–1148.

Lewis, J.D., 2011. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology 140 (6) 1817–1826. e1812.

Lonze, B.E., Ginty, D.D., 2002. Function and regulation of creb family transcription factors in the nervous system. Neuron 35 (4), 605–623.

MacDonald, B.T., Tamai, K., He, X., 2009. Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev. Cell 17 (1), 9–26.

Marc, J., Mulner-Lorillon, O., Bellé, R., 2004. Glyphosate-based pesticides affect cell cycle regulation. Biol. Cell 96 (3), 245–249.

Marx, N., Bourcier, T., Sukhova, G.K., Libby, P., Plutzky, J., 1999. Pparγ activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: Pparγ as a potential mediator in vascular disease. Arterioscler. Thromb. Vasc. Biol. 19 (3), 546–551.

Mayr, B., Montminy, M., 2001. Transcriptional regulation by the phosphorylation-

dependent factor creb. Nat. Rev. Mol. Cell Biol. 2 (8), 599.
McCrea, P.D., Gu, D., 2010. The catenin family at a glance. J. Cell Sci. 123 (5), 637–642.
Mesnage, R., Arno, M., Costanzo, M., Malatesta, M., Séralini, G.-E., Antoniou, M.N., 2015. Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose roundup exposure. Environ. Health 14 (1), 70.
Mesnage, R., Biserni, M., Wozniak, E., Xenakis, T., Mein, C.A., Antoniou, M.N., 2018. Comparison of transcriptome responses to glyphosate, isoxaflutole, quizalofop-p-ethyl and mesotrione in the heparg cell line. Toxicol. Rep. 5, 819–826.
Miano, J.M., 2003. Serum response factor: toggling between disparate programs of gene expression. J. Mol. Cell. Cardiol. 35 (6), 577–593.
Miano, J.M., Long, X., Fujiwara, K., 2007. Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus. Am. J. Physiol.-Cell Physiol. 292 (1), C70–C81.
Mohammadi, A., Saraee, M.H., Salehi, M., 2011. Identification of disease-causing genes using microarray data mining and gene ontology. BMC Med. Genomics 4 (1), 12.
Monga, S.P., 2015. B-catenin signaling and roles in liver homeostasis, injury, and tumorigenesis. Gastroenterology 148 (7), 1294–1310.
Morin, P.J., 1999. B-catenin signaling and cancer. BioEssays 21 (12), 1021–1030.
Ortega-Martínez, S., 2015. A new perspective on the role of the creb family of transcription factors in memory consolidation via adult hippocampal neurogenesis. Front. Mol. Neurosci. 8, 46.
Perkins, E.J., Chipman, J.K., Edwards, S., Habib, T., Falciani, F., Taylor, R., Van Aggelen, G., Vulpe, C., Antczak, P., Loguinov, A., 2011. Reverse engineering adverse outcome pathways. Environ. Toxicol. Chem. 30 (1), 22–38.
Reyes-Palomares, A., Rodríguez-López, R., Ranea, J.A.G., Jiménez, F.S., Medina, M.A., 2013. Global analysis of the human pathophenotypic similarity gene network merges disease module components. PLoS ONE 8 (2), e56653.
Sadamoto, H., Kitahashi, T., Fujito, Y., Ito, E., 2010. Learning-dependent gene expression of creb1 isoforms in the molluscan brain. Front. Behav. Neurosci. 4, 25.
Safe, S., Abdelrahim, M., 2005. Sp transcription factor family and its role in cancer. Eur. J. Cancer 41 (16), 2438–2448.
Schaefer, K.N., Peifer, M., 2019. Wnt/beta-catenin signaling regulation and a role for biomolecular condensates. Dev. Cell 48 (4), 429–444.
Schüttler, A., Reiche, K., Altenburger, R., Busch, W., 2017. The transcriptome of the zebrafish embryo after chemical exposure: a meta-analysis. Toxicol Sci. 157 (2), 291–304.
Shore, P., Sharrocks, A.D., 1995. The mads-box family of transcription factors. Eur. J. Biochem. 229 (1), 1–13.
Suske, G., Bruford, E., Philipsen, S., 2005. Mammalian sp/klf transcription factors: Bring in the family. Genomics 85 (5), 551–556.
Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T., Satayavivad, J., 2013. Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem. Toxicol. 59, 129–136.
Treisman, R., 1992. The serum response element. Trends Biochem. Sci. 17 (10), 423–426.
Uehara, T., Hirode, M., Ono, A., Kiyosawa, N., Omura, K., Shimizu, T., Mizukawa, Y., Miyagishima, T., Nagao, T., Urushidani, T., 2008. A toxicogenomics approach for early assessment of potential non-genotoxic hepatocarcinogenicity of chemicals in rats. Toxicology 250 (1), 15–26.
Ulrich, R., Friend, S.H., 2002. Toxicogenomics and drug discovery: Will new technologies help us produce better drugs? Nat. Rev. Drug Discovery 1 (1), 84–88.
Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C.A., Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., 2014. Adverse outcome pathway (aop) development i: Strategies and principles. Toxicol. Sci. 142 (2), 312–320.
Vizcaíno, C., Mansilla, S., Portugal, J., 2015. Sp1 transcription factor: a long-standing target in cancer chemotherapy. Pharmacol. Ther. 152, 111–124.
Waby, J., Bingle, C., Corfe, B., 2008. Post-translational control of sp-family transcription factors. Curr. Genomics 9 (5), 301–311.
Wei, X., Ai, J., Deng, Y., Guan, X., Johnson, D.R., Ang, C.Y., Zhang, C., Perkins, E.J., 2014. Identification of biomarkers that distinguish chemical contaminants based on gene expression profiles. BMC Genomics 15, 248.
Wen, A.Y., Sakamoto, K.M., Miller, L.S., 2010. The role of the transcription factor creb in immune function. J. Immunol. 185 (11), 6413–6419.
Woods, C.G., Vanden Heuvel, J.P., Rusyn, I., 2007. Genomic profiling in nuclear receptor-mediated toxicity. Toxicol. Pathol. 35 (4), 474–494.
Xu, D., Li, S., Lin, L., Qi, F., Hang, X., Sun, Y., 2016. Gene expression profiling to identify the toxicities and potentially relevant disease outcomes due to endosulfan exposure. Toxicol. Res. 5 (2), 621–632.
Zahouily, M., Rhihil, A., Bazoui, H., Sebti, S., Zakarya, D., 2002. Structure-toxicity relationships study of a series of organophosphorus insecticides. Mol. Modeling Annual 8 (5), 168–172.
Zhang, X., Odom, D.T., Koo, S.-H., Conkright, M.D., Canettieri, G., Best, J., Chen, H., Jenner, R., Herbolsheimer, E., Jacobsen, E., 2005. Genome-wide analysis of camp-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc. Natl. Acad. Sci. 102 (12), 4459–4464.
Zhao, S., Guo, Y., Sheng, Q., Shyr, Y., 2014. Advanced heat map and clustering analysis using heatmap3. BioMed Res. Int.
Zhernovkov, V., Santra, T., Cassidy, H., Rukhlenko, O., Matallanas, D., Krstic, A., Kolch, W., Lobaskin, V., Kholodenko, B.N., 2019. An integrative computational approach for a prioritization of key transcription regulators associated with nanomaterial-induced toxicity. Toxicol. Sci. 171 (2), 303–314.