European Review for Medical and Pharmacological Sciences 2015; 19: 2246-2253

Predicting transcription factors in human alcoholic hepatitis from gene regulatory network

A. MOHAMMADNIA[1], M. YAQUBI[1], H. FALLAHI[2,3]

[1]National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
[2]Department of Biology, School of Science, Razi University, Kermanshah, Iran
[3]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abdulshakour Mohammadnia and *Moein Yaqubi* contributed equally to this work

Abstract. – OBJECTIVE: Alcoholic hepatitis (AH) is a type of alcoholic liver disorder caused by overconsumption of alcohol. The involvement of several transcription factors (TF), as the main regulators of disease related gene expression has been documented previously. However, despite the importance of analysis of gene regulatory network for understanding the molecular basis in any disease, so far, there is no report on construction of such network for AH in human.

MATERIALS AND METHODS: Here, we used microarray analysis to construct a rather complete gene regulatory network and used it to predict TFs and pathways that affected by this disease.

RESULTS: Ten TFs were shown to undergo significant alteration in AH. These TFs are AR, EGR1, MYC, TCF4, ATF3, JUN, FOXO3, STAT1, HIF1A and EOMES, where ATF3, TCF4 and MYC are the new TFs with a role in AH. Comparisons of gene expression profile of patients with those of healthy persons indicates 820 differentially expressed (DE) genes. Network analysis indicates that, these ten TFs regulate expression of 516 DE genes (out of 820 genes), by 1057 interactions. Furthermore, we report pathways that significantly affected by these ten TFs.

CONCLUSIONS: These results may contribute to our limited understanding of the molecular basis of AH.

Key Words:
Alcohol consumption, Gene expression profile, Microarray analysis, Network, Regulatory interaction.

Introduction

Alcoholic liver diseases (ALD) is a chronic disease in which liver gradually losses its function. ALD composed of different liver abnormalities including steatosis, fibrosis and cirrhosis. Alcoholic hepatitis is one of the severe form of ALD[1], originate from overconsumption of alcohol for a long time. Based on World Health Organization (WHO), annually 2.5 million death occurs due to heavy drinking of alcohol[2]. Because of its importance and impacts on human health, drinking and all disease related to it attract attentions to evaluate cellular and molecular mechanism involved in alcoholic hepatitis.

One aspect of molecular mechanism involved in any disease is analyzing gene expression pattern in patients and comparing it with healthy people. Gene expression analysis of AH patients were reported previously. For example, Seth et al[3] analyzed gene expression profile in AH, alcoholic steatosis (AS) and normal liver donors. They found 211 differentially expressed genes between AH patients and normal liver donors[3]. Colmenero et al[4] demonstrated that overexpressed genes in AH patients mainly have a role in hepatic fibrogenesis, inflammatory response and oxidative stress. In addition to all these limited gene analysis studies, several researchers tried to put all available gene expression data into gene regulatory network to gain an overview of this disease. Huang et al[5] investigated protein-protein network of hepatitis C viruses-human, to predict biomarkers that can be used for diagnosing hepatocellular carcinoma and liver cirrhosis[5]. Wu et al[6] analyzed interaction of Hepatitis B virus with cellular proteins and relationship between HBV and progression of hepatocellular carcinoma.

Based on previous studies, some TFs have been identified in alcoholic hepatitis. Mandrekar et al[7] showed that NFKB, EGR1, STAT1 and AP-1 are differentially expressed TFs in alcoholic liver injury. Roman et al[8] reported that NFKB and AP-1 TF activate when hepatocytes treat with acetaldehyde. Nagy[9] states that NFKB TF activates in exposure to ethanol. Gao[10] demonstrated that in response to alcohol consumption

Corresponding Author: Hossein Fallahi, Ph.D; e-mail: h.fallahi@razi.ac.ir

Kuppfer cell produce anti hepatoprotective interleukin 6 (IL-6). IL-6 subsequently activate STAT3 TF that can protect liver from damage.

However, the majority of the previously reported networks in liver disease, were focused on different types of hepatitis that caused by viruses and none contained the gene regulatory network involved in alcoholic liver disease itself and especially alcoholic hepatitis. The present study conducted a gene regulatory network analysis for AH using available microarray data to identify new TFs that may have a role in this disorder.

Materials and Methods

Microarray Availability and Analysis

Gene expression profile were obtained from the GEO dataset with GSE accession number 28619[11]. Raw data were normalized using Robust Multichip Averaging (RMA) algorithm[12] in R package[13]. Identification of differentially expressed (DE) genes has been conducted using t-test algorithm (two sample student t-test) in Flexarray software v 1.6.2[14]. Fold changes at 2 and a *p-value* of 0.05 were set as threshold for finding DE genes, except for identification of transcription factors that fold change 1.5 was used. HG-U133_Plus_2.na33.annot file was used for transformation of transcript ID to gene symbol. This file was obtained from Affymetrix website (www.affymetrix.com).

Functional Annotation and Clustering of DE Genes

We have used DAVID (The Database for Annotation, Visualization and Integrated Discovery) v.6.7 database to annotate DE genes[15,16]. DAVID categorizes genes in specific clusters, where a unique enrichment score would dedicate to each cluster. Clusters with enrichment scores higher than 1.3 would contain the most significantly altered genes.

Transcription Factor Binding Sites and Construction of Gene Regulatory Network

ChEA is a database in which interactions of proteins with DNA have been deposited[17] and includes data obtained from ChIP-chip, ChIP-seq, ChIP-PET and DamID techniques. Browsing ChEA database for our DE genes resulted in identification of TFs that regulate these genes. A *p-value* of less than 0.05 applied. Protein-protein interactions were obtained from BioGRID database, a database that contains valid interactions of proteins[18]. Combined interactions of proteins-DNA and proteins-proteins were loaded into Cytoscape v 2.8.3 to construct and visualize network[19].

Identification of Cellular Process Affected by Transcription Factors in Gene Regulatory Network

Ontology of network that lead to identification of the affected process in alcoholic hepatitis done using ClueGO[20] and Clupedia[21] plug-ins in Cytoscape v 3.0.1.

Identification of Central Genes and Active Modules in Gene Regulatory Network

Central genes in main network were found using CentiScaPe plug-in in Cytoscape v 2.8.3[22]. CentiScaPe computes centrality parameters for each network's node such as Eccentricity, Betweenness, Closeness, Degree and Stress, to detect nodes that have a central role in network. JActiveModules plug-in was used to extract active modules from main network[23].

Results

Gene Expression has Been Altered in AH Patients Liver Cells

Comparisons of microarray data obtained from healthy liver with those reported for alcoholic hepatitis samples, resulted in identification of 820 DE genes. Among these 820 genes, 375 genes are down regulated while 445 genes exhibit up regulation. The extent of down regulation ranges from 2-fold changes (*TIPARP* gene) to 43-fold changes (*CNN2* gene). While from 2-fold changes (*LAMC1* gene) up to 183-fold changes (*AKR1B10*) were observed for up regulated genes.

As expected, the expressions of TFs are much lower than those observed for other genes. In TFs down regulated gene list, EOMES, MYC and FOXO3 show lower than 2-fold decreases in their expression. While, *TCF4, HIF1A* and *STAT1* are up regulated by less than 2-fold.

AH Affected Many Important Cellular Processes in Liver Cells

In order to understand the function of DE genes, we used DAVID database. Results indicate that oxidation-reduction and electron carrier activities are the most affected pathways in this disease. In addition, secreted proteins, signal,

signal peptide, extracellular region part, disulfide bond and glycoproteins are classified in the second group of cellular processes containing several DE genes. Yet, genes that are involved in response to different stimulus such as response to hormone stimulus, response to corticosteroid and glucocorticoid hormones make the third most important groups of genes. Table I presents three, five and five terms that are present in the first, second and third clusters, respectively.

Ten TFs Are Involved in Regulation of Differentially Expressed Genes

To predict TFs that regulate expression of DE genes, ChEA database was used. The results show that ten TFs expressed differentially which can be potential regulator of our DE genes. *AR, MYC, EGR1, JUN, ATF3, TCF4, FOXO3, STAT1, HIF1A* and *EOMES* are TFs that identified in this database. Interestingly, three of them (*MYC, TCF4* and *ATF3*) are not previously reported and therefore can be considered as new TFs with a role in alcoholic hepatitis.

TFs Involvement in Affected Process and Signaling Pathways in AH

To study the pathways that might be affected in alcoholic hepatitis, we have constructed gene regulatory network. Interactions for 516 genes, out of 820 DE genes, were found in the databases, which were used for construction of gene regulatory network (Figure 1). Biological process analysis of the components of this network revealed specific pathways and processes that altered in response to alcoholic hepatitis. In our assessment, pathways with 20 % affected genes were considered for analysis. The results show that based on the number of genes that are involved in each pathway, DNA biosynthetic linked processes rank as the first affected pathway. *MYC* TF directly has a role in this pathway. DE genes in this process mainly regulate by *EGR1, TCF4, ATF3, JUN, MYC, AR* and *EOMES* TFs. Negative regulation of stress-activated MAPK cascade locates in the second rank. All TFs except *STAT1* and *HIF1A* have a role in expression regulation of DE genes of this group. In other ranks there are the cellular response to calcium ion, organic cation transport, defense response to Gram-negative bacterium, fatty-acyl-CoA metabolic process and triglyceride homeostasis (Table II). Interestingly, *JUN* and its associated TF, *FOS* directly take part in the cellular response to calcium ion, although we have not detected *FOS* in our analysis. The results demonstrate eight out of ten transcription factors (except *STAT1* and *HIF1A*) have a major role in expression regulation of pathways harboring 20% or more affected genes.

Among signaling pathways that expression of 4 percent of their members altered, cell surface receptor signaling pathway with 97 DE genes, ranked as the first one. Interestingly, TFs that in network analysis assigned a high score, predominantly control expression of these 97 DE genes. AR, MYC, EGR1 and ATF3 TFs show the most number of target genes among members of this signaling pathways.

Table I. Collaboration of DE genes in affected process of alcoholic hepatitis based on DAVID database analysis.

Cluster	GO term	Enrichment score	*p*-value	Number of genes
	Oxidoreductase	11.08	3.18E-15	71
1	Oxidation reduction	11.08	2.93E-11	74
	Electron carrier activity	11.08	5.87E-09	34
	Signal	10.00	1.24E-14	231
	Glycoprotein	10.00	2.07E-09	264
2	Extracellular region part	10.00	1.40E-11	99
	Extracellular space	10.00	6.75E-09	72
	Disulfide bond	10.00	5.78E-08	187
	Response to steroid hormone stimulus	6.88	1.95E-09	33
	Response to hormone stimulus	6.88	3.22E-09	48
3	Response to endogenous stimulus	6.88	9.77E-09	50
	Response to corticosteroid stimulus	6.88	4.34E-06	17
	Response to glucocorticoid stimulus	6.88	1.32E-06	17

In this table part of results of DAVID clustering annotation presented. For the full list of clusters and terms see Supplementary Table III.

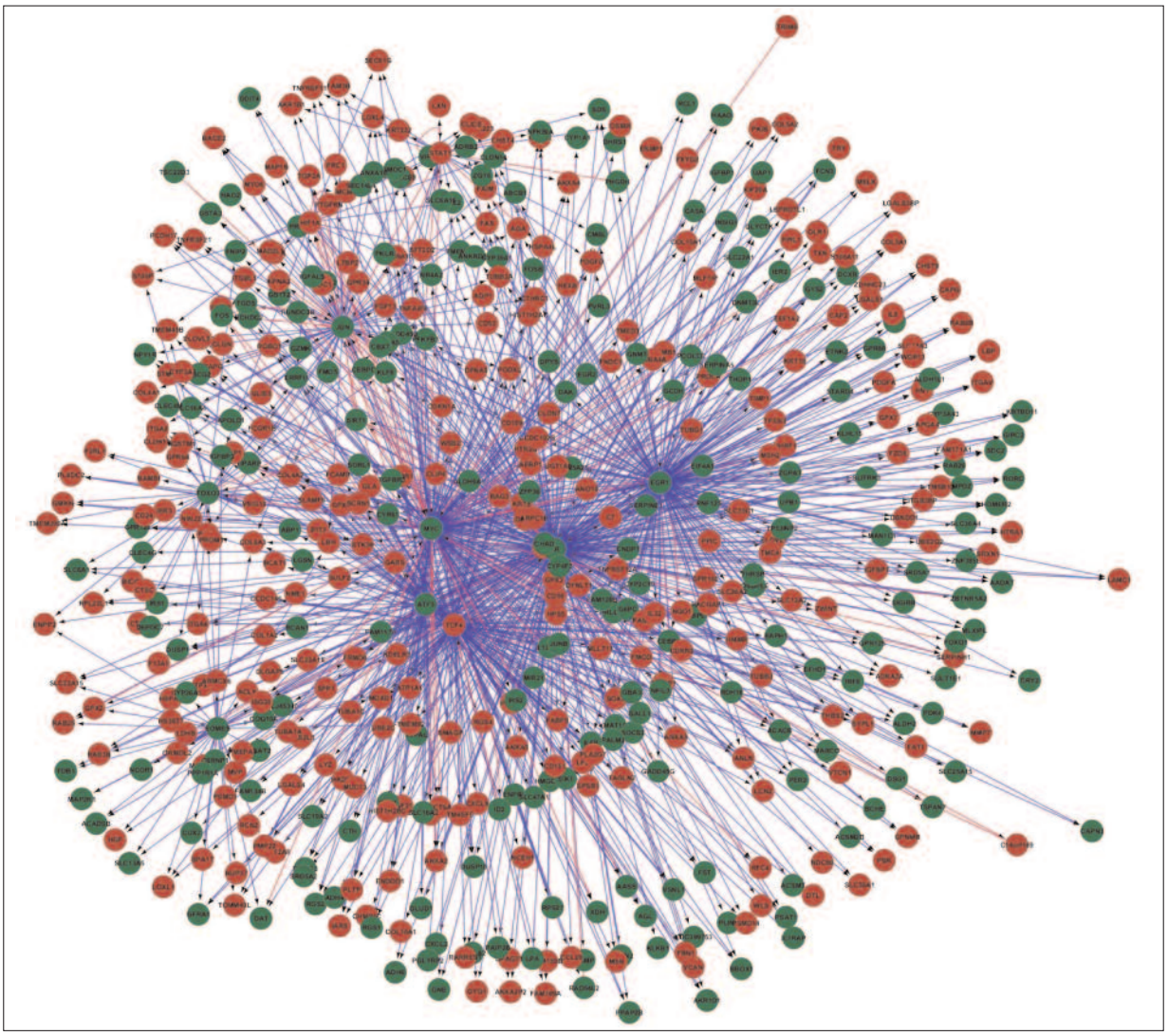

Figure 1. Gene regulatory network in alcoholic hepatitis. Protein-DNA interactions and Protein-Protein interactions are shown using blue and red edges, respectively. Arrows indicate the direction of protein-DNA and Protein-Protein interactions.

Table II. The most affected process in alcoholic hepatitis during gene regulatory network analysis.

GO term*	% of DE genes	*p*-value	Genes
DNA biosynthetic process	20.58	7.98E-05	CDKN1A, DTL, HGF, MYC, PCNA, PDGFA, SIRT1
Negative regulation of stress-activated MAPK cascade	24	1.05E-04	DUSP10, F2RL1, FOXO1, GSTP1, MYC, NCOR1
Cellular response to calcium ion	24	1.05E-04	CAPN3, FOS, FOSB, HTR2B, JUN, JUNB
Organic cation transport	20	3.09E-04	ACACB, AQP1, SLC12A2, SLC22A1, SLC22A18, SLC47A1
Defense response to Gram-negative bacterium	23.80	4.23E-04	F2RL1, IL6R, LBP, MMP7, SERPINE1
Fatty-acyl-CoA metabolic process	22.72	5.33E-04	ACLY, DGAT2, ELOVL1, ELOVL7, GCDH
Triglyceride homeostasis	21.73	6.64E-04	APOA5, DGAT2, LPL, MLXIPL, SIRT1

*Terms ordered based on number of genes that take part in each term.

Central Role of Some TFs in Alcoholic Hepatitis Gene Regulatory Network

Network analysis without considering biological function is able to highlight the important genes among our list of detected DE genes. To this end, centrality parameter analysis was conducted for the components of the constructed gene regulatory network. Centrality parameters including Betweenness, Closeness, Eccentricity, Degree, Centroid and Stress applied to all genes. Centrality parameters analysis results in detection of some genes as central components and key regulators of the main network, however our main goal was ranking TFs. Results obtained for each centrality parameter analysis presented in Table III. Betweenness algorithm, rank *AR, MYC, JUN, EGR1, ATF3, TCF4, FOXO3, STAT1, HIF1A* and *EOMES* as the first to tenth most important genes in network, respectively. Here, *AR* has shown the highest value compared to all other TFs studied. However, other centrality algorithms, classified TFs in relatively different orders. Taking an average from results of ranking of all six algorithms, results in the overall ranking of TFs in the network (Table III).

EGR1 Appears to be the Most Important TF in Alcoholic Hepatitis Gene Regulatory Network

Biological networks usually made from small modules, therefore, analysis of these modules can simplify overall understanding of any network. In this study, five modules, showing highest score, were extracted from the gene regulatory network. The first and second modules contain two nodes. In these modules, EGR1 exhibits alteration in alcoholic hepatitis (Figure 2). While, the third module contains 83 nodes and 143 interactions, where AR, EGR1, TCF4, ATF3, FOXO3 and STAT1 TFs are involved. The fourth and fifth modules contain only two nodes, where EGR1 is present in these modules (Figure 2).

Discussion

In the current study, gene expression profile as well as gene regulatory network of alcoholic hepatitis patients were analyzed, to find new DE TFs in alcoholic hepatitis.

We have identified ten transcription factors with differential expression pattern. Our results are in good agreement with those reported by Mandrekar et al[7], where they found *NFKB, AP-1, EGR1* and *STAT1* TFs as DE TFs in alcoholic liver disease. Our list of DE TFs contains *EGR1*, *STAT1* and *JUN* (part of *AP-1* complex). However, we could not detect NFKB as DE TFs. The role of these TFs in cellular processed related to development of AH have been discussed here.

Androgen receptor (*AR*), is a TF involved in many hormone related cancers, including lung, kidney and liver cancer. In liver cancer, *AR* promotes proliferation of hepatocellular carcinoma cell, while at the same time suppresses apoptosis in tumor cells[24]. *AR* knockout mice showed increased rates of developing hepatic steatosis, in part because of decreases in fatty acid, beta oxidation and increases in *de novo* lipid synthesis[25]. In this study, we found that expression of *AR* down regulated, while, in accordance with earlier reports in alcoholic hepatitis patients, metabolism of fatty acids have been affected.

Kuppfer cells in the liver produce inflammatory compound such as tumor necrosis factor in response to long-term exposure to ethanol. Interest-

Table III. Ranking of ten transcription factors based on centrality analysis.

Gene symbol	Betweenness	Closeness	Eccentricity	Centroid	Out degree	Stress	Score*	Overall rank
AR	1	1	1	2	1	1	1.16	1
MYC	2	3	2	2	3	2	2.33	2
EGR1	4	2	4	2	2	5	3.16	3
JUN	3	5	3	2	6	3	3.66	4
ATF3	5	6	5	2	5	4	4.5	5
TCF4	6	4	6	2	4	6	4.66	6
FOXO3	7	10	7	2	8	8	7	7
STAT1	8	8	8	2	9	7	7	7
HIF1A	9	7	9	2	10	9	7.66	8
EOMES	10	9	10	1	7	10	7.83	9

*Score of each gene obtained by taking an average from results of all six algorithms.

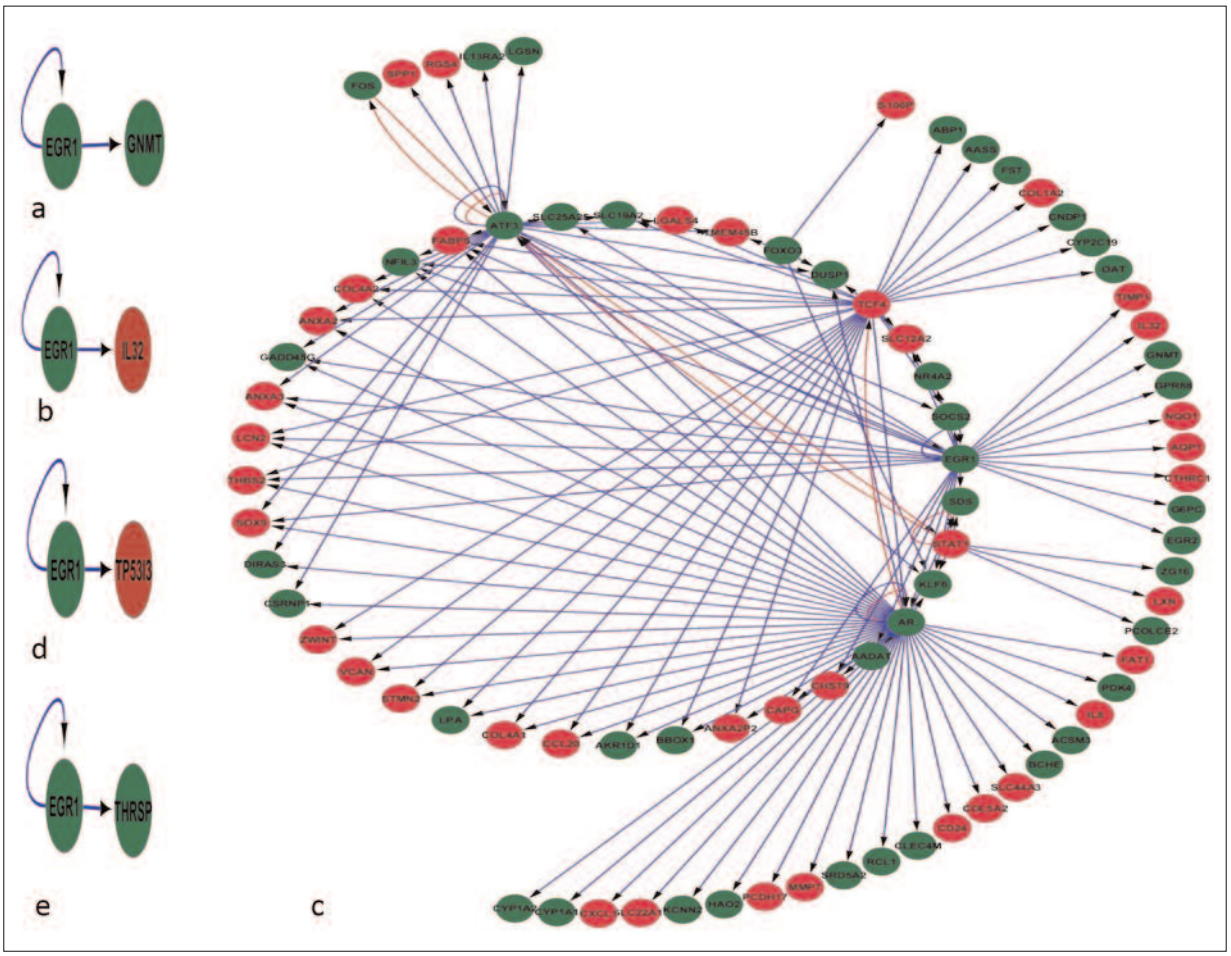

Figure 2. The network of extracted modules from main network. Protein-DNA and Protein-Protein interactions represented by blue and red edges, respectively. Arrows indicate the direction of protein-DNA and Protein-Protein interactions.

ingly, it has been shown that EGR1 transcription factor can regulate production of such compounds[26]. Unexpectedly, we found expression of EGR1 has been decreased in alcoholic hepatitis. In two separate studies, Nath et al[27] and Nishiyama et al[28] showed that in mice, chronic alcohol consumption causes accumulation of triglyceride plus hypoxia stress in the liver and subsequently up regulation of hypoxia-inducible factor 1 alpha (*HIF1A*) that may regulate Lipo-regulatory genes in the liver. Here, we showed that expression of this gene was up regulated in alcoholic hepatitis condition, indicting a good consistency with aforementioned reports.

FOXO3 TF is a member of FoxO family genes that have a role in multiple cell process, including cell death, cell cycle, DNA damage repair and especially the oxidative stress response. Tikhanovich et al[29] and Tumurbaatar et al[30] reported that alcohol consumption in mice increases the expression level of *FOXO3* gene. Up regulation of FOXO3 expression prevents liver from injury by activating antioxidant responses. However, Ni et al[31], confirming previous results, found that an increase in FOXO3 expression, correspond to rather acute alcohol consumption. They showed that in mice with FOXO3 knockdown, alcohol administration results in appearance of liver injury. Whereas, it seems continues intake of alcohol decreases the expression of *FOXO3*, and subsequently, increases the chance for developing liver injuries such as alcoholic hepatitis.

The *EOMES* TF takes part in maturation of natural killer cell in combination with T-bet gene[32]. In alcoholic hepatitis patients, we found that the expression of *EOMES* decreased, where this decrease may suggest an incomplete maturation of natural killer cells, increasing the risk of exposure to different pathogens.

The most interesting contribution of the current study is identification of three new TFs, *ATF3, TCF4* and *MYC*, which have not been described previously. Among these three TFs, MYC directly has a role in the pathways that expression of their member altered 20 percent; e.g., in regulation of MAPK cascade. But TCF4 and ATF3, by regulating expression of other genes, indirectly have a role in these cascades, so it seems MYC has a more important function than the other two TFS.

Combined results obtained from the network analysis (centrality parameters and modules) highlight importance of the *AR*, *EGR1* and *ATF3*. Centrality parameters ranked *AR, MYC, EGR1, JUN*, and *ATF3* as five most important TFs in the main network. Consequently, modules analysis revealed the presence of AR, EGR1 and ATF3 proteins in the 5 top modules. Importantly, EGR1 is present in all five modules, whereas AR and ATF3 present only in the third module. Surprisingly, although *STAT1, FOXO3* and *TCF4* ranked as sixth and seventh important TFs, they are only present in third module.

Negative regulation of the stress activated MAPK cascade was identified as one of the pathways which significantly altered in alcoholic hepatitis. There are many reports that show the effects of alcohol consumption on the function of MAPK cascade in different cell types[33,34]. Here we show that all of TFs (except *STAT1* and *HIF1A*) that predicted in alcoholic hepatitis have a role in regulation of member of this signaling cascade. Alcohol abuse increases entrance rate of gut bacterial to hepatic portal, so lipopolysaccharide of the outer cell wall of Gram negative bacteria can motivate defense response to these bacteria and activates Toll like receptor 4 and its derivatives and MAPK cascade[35].

Conclusions

In the current study, we have studied gene expression profiles of alcoholic hepatitis patients and constructed transcriptional regulatory networks for the DE genes. Analysis of gene regulatory network results in identification of ten transcription factors that may have a role in progressing alcoholic hepatitis. *AR, EGR1*, *JUN, FOXO3, STAT1, EOMES, HIF1A* directly have a role in alcoholic liver disease and alcohol metabolism. However, here we showed that these TFs may be important in progressing alcoholic hepatitis. But we do not find any report that demonstrates a role for *ATF3, TCF4* and *MYC* in alcoholic hepatitis. In this study, we unravel the potential role of ATF3, TCF4 and MYC in alcoholic hepatitis.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Lucey MR, Mathrin P, Morgan TR. Alcoholic hepatitis. N Engl J Med 2009; 360: 2758-2769.
2) World Health Organization. Global strategy to reduce the harmful use of alcohol. World Health Organization, 2010.
3) Seth D, Gorrell MD, Cordoba S, McCaughan GW, Haber PS. Intrahepatic gene expression in human alcoholic hepatitis. J Hepatol 2006; 45: 306-320.
4) Colmenero J, Bataller R, Sancho-Bru P, Bellot P, Miquel R, Moreno M, Jares P, Bosch J, Arroyo V, Caballería J, Ginès P. Hepatic expression of candidate genes in pateints with alcoholic hepatitis: Correlation with disease severity. Gastroenterology 2007; 132: 687-697.
5) Huang T, Wang J, Cai YD, Yu H, Chou KC. Hepatitis C virus network based classification of hepatocellular cirrhosis and carcinoma. PLoS One 2012; 7: e34460.
6) Wu ZJ, Zhu Y, Huang DR, Wang ZQ. Constructing the HBV-human protein interaction network to understand the relationship between HBV and hepatocellular carcinoma. J Exp Clin Canc Res 2010; 29: 146
7) Mandrekar P, Szabo G. Signalling pathways in alcohol-induced liver inflammation. J Hepatol 2009; 50: 1258-1266.
8) Roman J, Gimenez A, Lluis JM, Gasso M, Rubio M, Caballeria J, Pares A, Rodes J, Fernandez-Checa JC. Enhanced DNA binding and activation of transcription factors NFKB and AP-1 by acetaldehyde in HEPG2 cells. J Biol Chem 2000; 275: 14684-14690.
9) Nagy LE. Molecular aspects of alcohol metabolism: transcription factors in early ethanol-induced liver injury. Annu Rev Nutr 2004; 24: 55-78.
10) Gao B. Hepatoprotective and anti-inflammatory cytokines in alcoholic liver disease. J Gastroenterol Hepatol 2012; 27: 89-93.
11) Affo S, Dominguez M, Lozano JJ, Sancho-Bru P, Rodrigo-Torres D, Morales-Ibanez O, Moreno M, Millan C, Loaeza-del-Castillo A, Altamirano J, García-Pagán JC, Arroyo V, Ginès P, Caballería J, Schwabe RF, Bataller R. Transcriptome analysis identifies TNF superfamily receptors as potential therapeutic targets in alcoholic hepatitis. Gut 2012; 62: 452-460.

12) Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003; 4: 249-264.

13) Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004; 5: R80.

14) Blazejczyk M, Miron M, Nadon R. A statistical data analysis software for gene expression microarray. Genome Quebec, Montreal, Canada, 2007.

15) Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009; 37: 1-13.

16) Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.

17) Lachmann A, Xu H, Krishnan J, Berger SI, Mazloom AR, Ma'ayan A. ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. Bioinformatics 2010; 26: 2438-2444.

18) Stark C, Breitkreutz BJ, Reguly T, Boucher L, Breitkreutz A, Tyers M. BioGRID: a general repository for interaction datasets. Nucleic Acids Res 2006; 34: D535-D539.

19) Saito R, Smoot ME, Ono K, Ruscheinski J, Wang PL, Lotia S, Pico AR, Bader GD, Ideker T. A travel guide to Cytoscape plugins. Nat Methods 2012; 9: 1069-1076.

20) Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pagès F, Trajanoski Z, Galon J. ClueGO: a Cytoscape plugin to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 2009; 25: 1091-1093.

21) Bindea G, Galon J, Mlecnik B. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. Bioinformatics 2013; 29: 661-663.

22) Scardoni G, Petterlini M, Luadanna C. Analyzing biological network parameters with CentiScaPe. Bioinformatics 2009; 25: 2857-2859.

23) Ideker T, Ozier O, Schwikowski B, Seigel AF. Discovering regulatory and signalling circuits in molecular interaction networks. Bioinformatics 2002; 18: 233-240.

24) Chang C, Lee SO, Yeh S, Chang TM. Androgen receptor (AR) differential roles in hormone-related tumors including prostate, bladder, kidney, lung, breast and liver. Oncogene 2013 Jul 22. [Epub ahead of print].

25) Ma WL, Jeng LB, Yeh CC, Chang C. Androgen and androgen receptor signals jamming monocyte/macrophage functions in premalignant phase of livers. Biomedicine 2012; 2: 155-159.

26) Thakur V, McMullen MR, Pritchard MT, Nagy LE. Regulation of macrophage activation in alcoholic liver disease. J Gastroenterol Hepatol 2006; 22: S53-S56.

27) Nath B, Levin I, Csak T, Petrasek J, Mueller C, Kodys K, Catalano D, Mandrekar P, Szabo G. Hepatocyte-specific Hypoxia Inducible Factor-1α is a determinant of lipid accumulation and liver injury in alcoholinduced steatosis in mice. Hepatology 2011; 53: 1526-1537.

28) Nishiyama Y, Goda N, Kanai M, Niwa D, Osanai K, Yamamoto Y, Senoo-Matsuda N, Johnson RS, Miura S, Kabe Y, Suematsu M. HIF-1a induction suppresses excessive lipid accumulation in alcoholic fatty liver in mice. J Hepatol 2012; 56: 441-447.

29) Tikhanovich I, Kuravi S, Campbell RV, Kharbanda KK, Artiques A, Villar MT, Weinman SA. Regulation of Foxo3 by phosphorylation and methylation in hepatitis C virus infection and alcohol exposure. Hepatology 2014; 59: 58-70.

30) Tumurbaatar B, Tikhanovich I, Li Z, Ren J, Ralston R, Kuravi S, Campbell R, Chaturvedi G, Huang TT, Zhao J, Hao J, O'Neil M, Weinman SA. Hepatitis C and alcohol exacerbate liver injury by suppression of FOXO3. Am J Pathol 2013; 183: 1803-1814.

31) Ni HM, Du K, You M, Ding WX. Critical role of FOXO3A in alcohol-induced autophagy and hepatotoxicity. Am J Pathol 2013; 183: 1815-1825.

32) Gordon SM, Chaix J, Rupp LJ, Wu J, Madera S, Sun JC, Lindsten T, Reiner SL. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. Immunity 2012; 36: 55-67.

33) Aroor AR, Custer GW, Weng YI, Lee YJ, Shukla SD. Phosphatidylethanol mimics ethanol modulation of p42/44 mitogen activated protein kinase signaling hepatocytes. Alcohol Alcohol 2002; 37: 534-539.

34) Aroor AR, Shukla SD. MAP kinase signaling in diverse effects of ethanol. Life Sci 2004; 74: 2339-2364.

35) Soares JB, Pimentel-Nunes P, Roncon-Albuquerqu R, Leite-Moreira A. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. Hepatol Int 2010; 4: 659-672.