Original Article
Regulation of gene expression in HBV- and HCV-related hepatocellular carcinoma: integrated GWRS and GWGS analyses

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Abstract: Objectives: To explore the molecular mechanism of hepatitis B virus-related and hepatitis C virus-related hepatocellular carcinoma, samples from hepatitis B virus and hepatitis C virus infected patients and the normal were compared, respectively. Methods: In both experiments, genes with high value were selected based on a genome-wide relative significance and genome-wide global significance model. Co-expression network of the selected genes was constructed, and transcription factors in the network were identified. Molecular complex detection algorithm was used to obtain sub-networks. Results: Based on the new model, the top 300 genes were selected. Co-expression network was constructed and transcription factors were identified. We obtained two common genes FCN2 and CXCL14, and two common transcription factors RFX5 and EZH2. In hepatitis B virus experiment, cluster 1 and 3 had the higher value. In cluster 1, ten of the 17 genes and one transcription factor were all reported associated with hepatocellular carcinoma. In cluster 3, transcription factor ESR1 was reported related with hepatocellular carcinoma. In hepatitis C virus experiment, the value of cluster 3 and 4 was higher. In cluster 3, nine genes were reported to play a key role in hepatocellular carcinoma. In cluster 4, there were 5 genes in the 34 genes. To compare the relevance of a node in holding together communicating nodes, centralities based analysis was performed and we obtained some genes with high stress value. Conclusion: The analysis above helped us to understand the pathogenesis of hepatitis B virus and hepatitis C virus associated hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma, hepatitis B virus, hepatitis C virus, transcription factor

Introduction

Primary liver cancer is the sixth most common cancer in the world and the third most common cause of cancer mortality [1]. In liver cancer, hepatocellular carcinoma (HCC) is the main form [2]. In HCC, hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most important etiological factors which accounting for more than 70% of cases worldwide [3]. HBV is a small DNA virus with a 3.2 kb genome. Virus particles have a partially double-stranded genome with a cohesive overlap [4]. HCV is a positive, single-stranded RNA virus that encodes a large polyprotein of about 3,000 amino acids from a single open reading frame [5]. Viral hepatitis B and C infections have been common diseases during recent decades [6]. These diseases take place via similar routes of transmission, and population affected most are intravenous drug

users (IVDU), hemophilia, dialysis patients, and health care workers [7].

HBV- and HCV-induced HCC develops in an environment of inflammation and regeneration that results from chronic liver damage [8]. HBV- and HCV-encoded proteins can alter host gene expression and cellular phenotypes that are identified as hallmarks of cancer, which promote growth factor-independent proliferation, resistance to growth inhibition and tissue invasion [9]. For HBV, virus DNA exists as an episome in the form of a minichromosome in the nuclei of cells. Hepatitis B x (HBx) sometimes truncated S and pre-S sequences can be integrated into many sites in the host DNA [10]. The contribution of HBV to HCC involves the expression of HBx and possibly carboxy terminally truncated pre-S or S polypeptides [11]. In HBV infection, HBx binds and stabilizes HIF1α and

Table 1. Samples and platform information of the selected microarray expression profiles

Class	Samples	Platform information
E-GEOD-14520	Gene expression patterns in healthy donor liver as well as tumor and paired non-tumor tissue of HCC patients	Affymetrix Human Genome U133A 2.0. Affymetrix HT Human Genome U133A
E-GEOD-41804	Hepatic gene expression of HCV-related HCC and non-cancerous tissue	Affymetrix Human Genome U133 Plus 2.0
E-MTAB-950	Human normal liver, HBV, HCV and hemochromatosis background cirrhotic and HCC liver samples	GeneChip Human Genome U133 Plus 2.0
E-GEOD-19665	HBV-related HCC and HCV-related HCC and their matched noncancerous tissues on genome- wide scale	Affymetrix Human Genome U133 Plus 2.0

stimulates HIF1α transcription, which promote angiogenesis and cell 'stemness' [12]. HBx can promote angiogenesis by stimulating the ERK, MAPK, which upregulates the pro-angiogenic growth factor angiopoietin 2 [13]. It can alter the DNA binding specificity of CREB and ATF-2 by protein-protein interactions [14]. HBx can also regulate the epigenetic control of cDNA function in HBV replicating cells [15]. For HCV, the core protein, and non-structural (NS) proteins NS3 and NS5A contribute to oncogenic transformation [16]. HCV persistence does not include integration and maintains itself as an endoplasmic reticulum (ER)-associated episome [17]. HCV core, E1, NS3 and NS5A can up-regulate hypoxia inducible factor 1α (HIF1α) under hypoxic conditions, resulting in increased vascular endothelial growth factor, cyclooxygenase 2, ANG2 and several matrix metalloproteinases [18]. Both viruses upregulate DNA methyltransferases, which decrease the expression of genes involved in DNA repair [19, 20]. MicroRNA-221 can be upregulated in HBV- and HCV-related HCC, which block the expression of the cyclin-dependent kinase inhibitor p27 and promote tumour growth and progression by activation of the PI3K-AKT-mTOR pathway [21, 22]. Although there were a large number of studies reported the pathogenic mechanisms of HBV- and HCV-associated HCC, few reported the relationship of gene expression pattern between HBV- and HCV-associated HCC.

In this study, we aimed to analyze the relationship between HBV- and HCV-related HCC in molecular level. Genes with high rank in HBV and HCV were selected based on a genome-wide relative significance (GWRS) and genome-wide global significance (GWGS) model, respectively. Co-expression network of the selected genes was constructed using Empirical Bayesian approach and differential coexpression (DC) gene pairs were identified. Transcription factors (TFs) in the network were identified and compared in HBV and HCV experiment. To obtain sub-networks with densely connected

genes in the co-expression network, molecular complex detection (MCODE) algorithm was used. Centralities based analysis for the co-expression network was performed to analyze the relevance of a gene in functionally holding together communicating nodes. This study provides a basis for researchers to understand the similarity and difference between HBV- and HCV-related HCC.

Material and methods

Samples

In this study, we conducted two groups of experiment, one was about HBV, and the other was about HCV. In HBV experiment, three microarray expression profiles of E-GEOD-14520 [23], E-MTAB-950 [24] and E-GEOD-19665 [25] were used. In HCV experiment, we used three microarray expression profiles of E-GEOD-41804 [26], E-MTAB-950 [24] and E-GEOD-19665 [25]. All of them were downloaded from Arraypress database. The samples and platform information of them were shown in **Table 1**.

GWRS and GWGS for integrated analysis of cross-laboratory microarray data

In this study, a new approach based on measuring the GWRS and GWGS of expressed genes was used. We measured the GWRS of a gene using its ranking position on a genome-wide scale (*r* value) based on a differential expression measure, which can be the fold change, *t*-test *P*-value, SAM (Significance Analysis of Microarray data) *P*-value etc. [27]. Compared to most existing meta-analysis methods, this new model enables the integrative analysis of microarray datasets produced by different platforms and protocols [28]. It not only applies multiple different methods for measuring the degree of differential expression of a gene (e.g. fold change, *t*-test, ANOVA or SAM *P*-values), but also uses a ranking *r* value instead of the test

statistic (i.e., fold change, or *P*-value) to avoid the influence of high variation test statistics.

In this study, the primary comparison of samples from HCC patients to samples from the normal was conducted by Linear Models for Microarray Data (LIMMA) package [29]. Moreover, the GWRS of a gene was measured based on *P*-value. In both HBV and HCV experiment, first, we converted the probe-level data in CEL files into expression measures. Then, background correction and quartile data normalization were performed. The file from different expression profile was used to map the relationship between the probes and gene symbols. A probe would be filtered if the probe did not have corresponding gene symbol. Finally, we obtained the average value of gene symbol with multiple probes. And a list of unique genes (G) from the datasets was retrieved. In HBV analysis experiment, E-GEOD-14250, E-MTAB-950 and E-GEOD-19665 were used. In HCV analysis experiment, E-GEOD-41804, E-MTAB-950 and E-GEOD-19665 were used.

Measuring the GWRS of genes in each single microarray database

For each gene in the list of unique genes, a rank number (in descending order starting from 1 to m) was given based on their corresponding degree of differential expression. For example, a gene with a high degree of differential expression was ranked more highly and so with a smaller ranking number. The GWRS of the *i*-th gene in the *j*-th dataset was measured by:

$$S_{ij} = -2log\left(\frac{r_{ij}}{m}\right)$$

Where r_{ij}, *i* = 1-m; *j* = 1-n, is the rank number of the *i*-th gene in the *j*-th study; n is the number of datasets; m is the number of unique genes across n datasets; the range of GWRS value (s_{ij}) is between 0 and -2log (1/m). For a gene with 'NA' value the s_{ij} is set to be 'NA', The value 'NA' was applied in cases where a gene is absent from an individual study.

Measuring the GWGS of a gene across multiple microarray datasets

We estimated the GWGS (S_t^r) of a gene based on its corresponding GWRS across *n* datasets, by: $$S_t^r = \sum_{j=1}^{n} \omega_i s_{ij}$$

Where ω_j represents the relative weight of the *j*-th dataset, and $\sum_{j=1}^{n} \omega_i = 1$. The value of weight ($\omega_j$) can be assigned based on the data quality of the *j*-th datasets. In this study, we treated all the dataset equally, thus the weight of each datasets was set equally to be 1/n for j = 1-n.

The genes were ranked based on the GWGS value and the top 300 genes were selected for further analysis.

Functional enrichment and pathway enrichment analysis

Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for the 300 genes were performed by the online tool Database for Annotation, Visualization, and Integrated Discovery (DAVID) [30]. GO project provides ontologies to describe attributes of gene products in three non-overlapping domains of molecular biology: Molecular Function (MF), Biological Process (BP) and Cellular Component (CC) [31]. KEGG is a knowledge base for systematic analysis of gene functions, linking genomic information with higher order functional information [32]. GO terms and KEGG pathways with *P* value less than 0.01 were selected based on expression analysis systematic explorer (EASE) test implemented in DAVID. The principle of EASE was shown in formula.

$$p = \frac{\begin{bmatrix} a+b \\ a \end{bmatrix}\begin{bmatrix} c+d \\ c \end{bmatrix}}{\begin{bmatrix} n \\ a+c \end{bmatrix}}$$

Where n is the number of background genes; a' is the gene number of one gene set in the gene lists; a' + b is the number of genes in the gene list including at least one gene set; a' + c is the gene number of one gene list in the background genes; a' is replaced with a = a' - 1.

Empirical bayesian approach for identifying differential co-expression

The co-expression network of the top 300 ranked genes was constructed using Empirical Bayesian approach. Empirical Bayesian approach provides an FDR controlled list of significant DC gene pairs without sacrificing power. It

is applicable within a single study as well as across multiple studies [33]. Empirical Bayesian approach outperforms the current differentially expressed (DE) methods in far less computational time may be a useful complement to a traditional DE analysis. Details of the approach are given in Dawson and Kendziorski [34]. We presume a user has normalized expression levels profiled from m genes in n subjects. The subjects are partitioned into K conditions. Then we try to identify gene pairs for which the true latent correlation within condition varies across conditions. The latent correlation in condition k was denoted by λ^k. When $K = 2$ conditions are being considered, a gene pair may be equivalently co-expressed (EC; $\lambda^1 = \lambda^2$) or differentially co-expressed (DC; $\lambda^1 \neq \lambda^2$). A gene pair is identified as DC under a hard thresholding mechanism if the posterior probability of DC > 0.95 or EC ≤ 0.05. This threshold conservatively controls the posterior expected FDR at 5%. Then meta-analysis was performed to explain the flow of execution. Meta-analysis assumes that each study has its own study-specific parameters. Thus, those parameters should be estimated using a single-study DC function on each study, and then saving the resulting study-specific parameters. The details were shown by Dawson and Kendziorski [34].

Molecular complex detection for sub-network analysis

To detect the modules in the co-expression network, MCODE algorithm was used. MCODE could recognize the modules with specific functions by selecting the clusters of densely connected nodes from the network [35]. In both HBV and HCV experiments, we set Degree Cutoff = 2, K-core = 3, Max. depth = 100 as the parameters in MCODE for the detection of clusters in co-expression network of the top 300 ranked genes.

Centralities based analysis for co-expression network

TFs are key cellular components that control gene expression: their activities determine how cells function and respond to the environment. In the identification of TFs in this study, a census of TFs was mapped to the co-expression network [36]. If a DCG is a TF, we can predict its related differential co-expression may be resulted from the change of its regulation relationships with its targets. This DCG is considered to be a differential regulation gene (DRG). To study the effect of DCG and DRG on the co-expression network, Centralities Based Analysis was conducted.

In any network structure, the role of a node depends, not only on the features of the node itself, but also on the topological structure of the network and on the other nodes features. We used network centralities interference to identify those nodes that are more sensitive to deletion or adding of a particular node in the network [37]. In this study, we used stress, one of the classical network centralities. Stress is a node centrality index and can be calculated by determining the number of shortest paths passing through the node. The stress of a node in a biological network can point out the relevance of the node in holding together communicating nodes. The higher the value, the higher the relevance of the node in connecting regulatory molecules [37]. A "stressed" node is a node being gone across by a high number of shortest paths. However, a node with high stress values does not mean the node is critical to keep the connection between nodes whose paths are passing through it. Therefore, the high or low stress values are more significant when compared to the average stress value of a graph G which is calculated by averaging the stress values of all nodes in the graph. The stress was calculated by:

$$C_{str}(\upsilon) := \sum_{s \neq \upsilon \epsilon N} \sum_{t \neq \upsilon \epsilon N} \sigma_{st}(\upsilon)$$

Nodes with threshold < 1901.8601 (average score) were filtered.

Results

The selection of genes

We used GWRS and GWGS for integrated analysis of the microarray data. In HBV experiment, the top 300 ranked genes were selected and the top 5 were CRHBP, FCN3, FCN2, PLAC8 and CXCL14. In HCV experiment, the top 5 ranked genes were FCN2, CLEC4G, CLEC1B, ECM1 and CXCL14.

GO enrichment analysis

In the GO enrichment analysis of gens obtained in HBV experiment, we can concluded that biological process GO term ($P < 0.01$) was related with regulation of cell cycle ($P = 3.37E-006$),
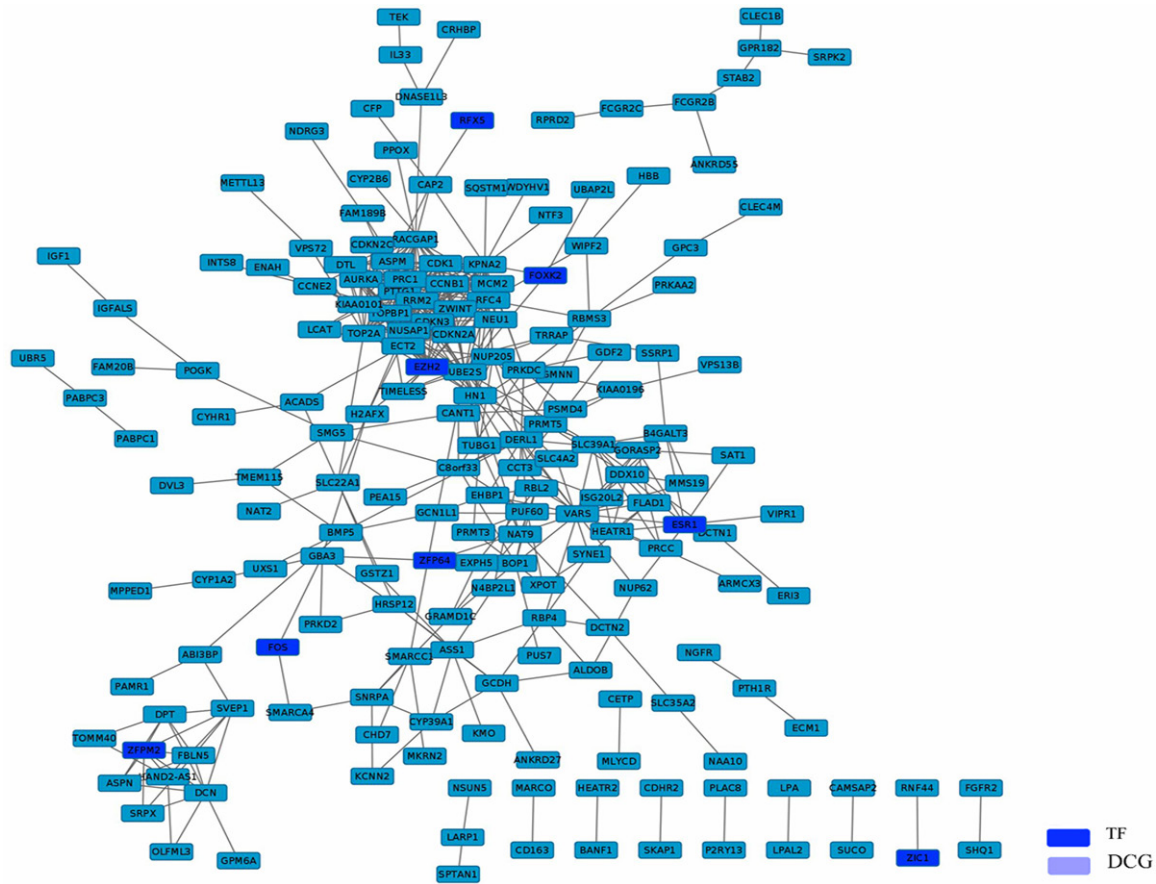
Co-expression network of hepatocellular carcinoma

Figure 1. Co-expression network of the top 300 ranked genes in HBV experiment. TF is the abbreviation of transcription factor.

cell cycle phase (P = 7.50E-005) and mitotic cell cycle (P = 1.80E-004). Cellular component GO term (P < 0.01) was related with chromosome (p = 2.81E-005), nucleoplasm (P = 604E-005) and cytosol (P = 0.0012). Molecular function GO term (P < 0.01) was related with chromatin binding (P = 0.0044), protein N-terminus binding (P = 0.0088), enzyme binding (P = 0.0088) and protein phosphatase 2A binding (p = 0.0098).

In the GO enrichment analysis of gens obtained in HCV experiment, we can concluded that biological process GO term (P < 0.01) was related with cell cycle phase (P = 4.73E-009), mitotic cell cycle (P = 1.04E-008) and mitosis (P = 2.39E-008). Cellular component GO term (P < 0.01) was related with membrane-enclosed lumen (P = 3.11E-006), organelle lumen (P = 3.65E-006) and nuclear lumen (P = 6.14E-006). Molecular function GO term (P < 0.01) was related with carbohydrate binding (P = 0.0015), hyaluronic acid binding (P = 0.0031) and protein C-terminus binding (P = 0.0070).

Pathway enrichment analysis

EASE was used in this study to select KEGG pathways. EASE contains many factors such as the statistical significance of the set of differentially expressed genes in the pathway, the topology of the signaling pathway and their interactions. The pathway enrichment analysis of genes obtained in HBV experiment yield many significant pathways contained p53 signaling pathway (P = 0.0016) and cell cycle (P = 0.0023). Genes CCNE2, CCNB1, CDK1, CDKN2A, RRM2, IGF1 and IGFBP3 were included in the p53 signaling pathway. In cell cycle, there were genes CCNE2, CCNB1, CDK1, CDKN2A, RBL2, CDKN2C, PRKDC, MCM2 and PTTG1. Similarly, in HCV experiment, many significant pathways were obtained and they contained cell cycle (P = 1.07E-004), oocyte meiosis (P = 2.48E-004) and p53 signaling pathway (P = 0.0037). Genes such as CCNE2, CCNB1, CDK1, CDKN2A, CCNB2, CDKN2C, ANAPC7, PTTG1, MCM4 and MCM6 were involved in the cell cycle pathway.
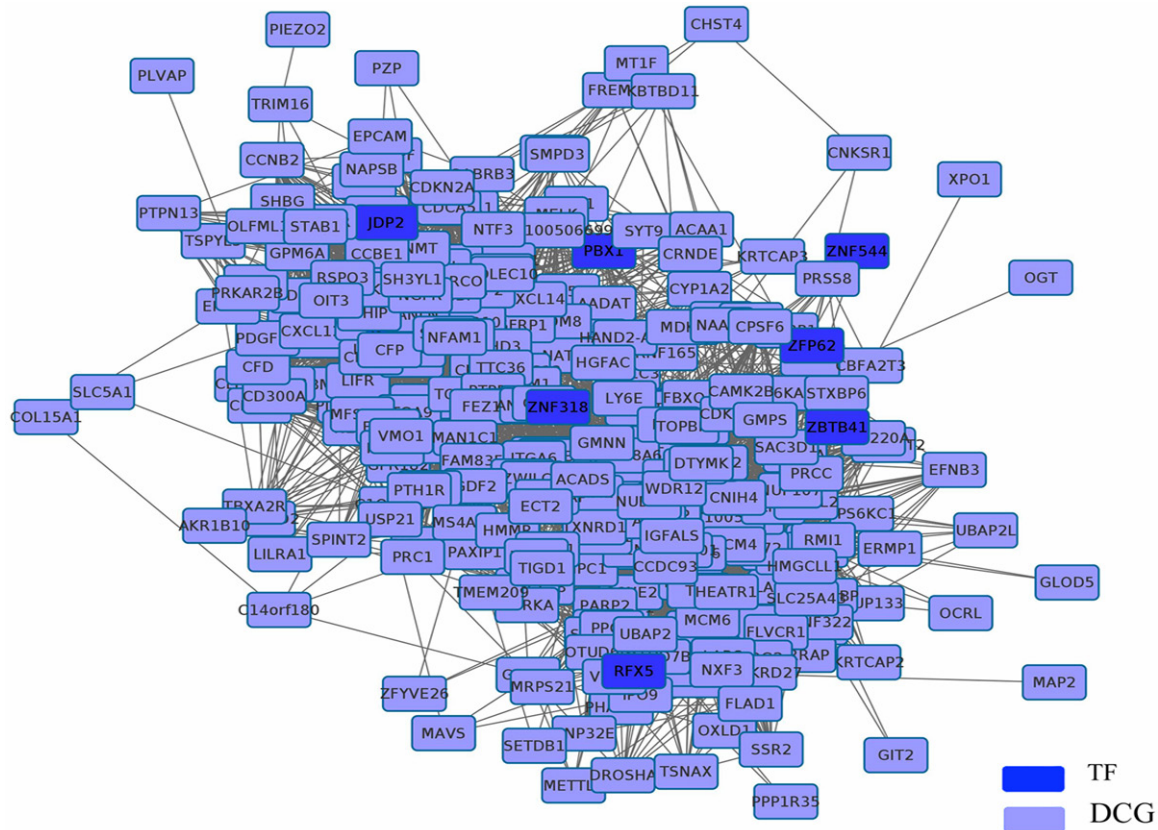
Co-expression network of hepatocellular carcinoma

Figure 2. Co-expression network of the top 300 ranked genes in HCV experiment. TF is the abbreviation of transcription factor.

Figure 3. Sub-network in the co-expression network of the top 300 ranked genes in HBV experiment. A: Cluster 1; B: Cluster 2; C: Cluster 3. TF is the abbreviation of transcription factor.

Identification of TFs

The co-expression networks of the top 300 ranked genes in HBV and HCV experiment were constructed and shown in **Figures 1** and **2**, respectively. To identify the TFs, TED was used. In HBV experiment, DCGs of 187 were obtained and 8 of them were TFs. The 8 TFs were ZIC1, ZFPM2, RFX5, FOXK2, FOS, EZH2, ESR1 and ZFP64. In HCV experiment, DCGs of 295 were obtained and 9 of them were TFs. The 9 TFs

were RFX5, PBX1, EZH2, DACH1, ZNF544, ZNF318, ZFP62, ZBTB41 and JDP2.

Sub-network analysis using molecular complex detection

To identify the clusters in the co-expression network, MCODE algorithm was used. When we set Degree Cutoff = 2, K-core = 3, Max. depth = 100, we got 3 clusters in HBV experiment and they were shown in **Figure 3**. The GWGS value

Table 2. GWGS value and genes of the clusters in HBV experiment

Style	Average of GWGS value	Number of the genes	TFs
Cluster 1	8.550	18	EZH2
Cluster 2	6.892	6	ZFPM2
Cluster 3	8.140	7	ESR1

of every cluster was shown in **Table 2**. From **Table 2**, we can conclude cluster 1 had the highest GWGS and it included many genes such as ASPM, ECT2, NUSAP1, PTTG1, KIAA0101 and AURKA. In HCV experiment, 5 clusters were obtained and shown in **Figure 4.** The GWGS value of every cluster was shown in **Table 3.** From **Table 3**, cluster 1 was seen have the highest GWGS. Genes ASPN, SVEP1, DPT, FBLN5, DCN and ZFPM2 were included in it.

Centralities based analysis of co-expression network

Centralities based analysis of the co-expression network was performed. Genes with high stress value were selected. In HBV experiment, the top 5 genes and their stress value were shown in **Table 4**. In HCV experiment, the top 5 genes and their stress value were shown in **Table 5**.

Discussion

In this study, we explored the gene regulation of HCC infected with HBV and HCV, and compared the similarity and difference between HBV and HCV in molecular level. First, samples from HCC patients with HBV infection and the healthy person were compared using LIMMA package. Then GWGS values of the genes were estimated. In the two experiments, two of the top 5 ranked genes were the common genes. They were FCN2 and CXCL14. We speculated they may play a key role in HCC and took a further analysis for them.

FCN2 gene is a soluble pattern-recognition molecule and 80% homologous at the amino acid level [38-40]. It locates on chromosome 9q34 and is highly polymorphic, presenting variation in both structural as well as regulatory region [41]. FCN2 can regulate both the expression as well as the function of Ficolin-2 [42]. Study showed FCN2 Genotypes was associated with chronic Chagas disease [43]. Moreover, it was

also related with HCC. Study showed ficolin-2 levels and FCN2 haplotypes contributed to hepatitis B infection outcome [44].

As for CXCL14, there were many reports on it. CXCL14 is a CXC chemokine constitutively expressed at the mRNA level in certain normal tissues but absent from many established tumor cell lines and human cancers [45]. It was up-regulated in pancreatic cancer tissues compared to chronic pancreatitis and normal pancreas [46]. CXCL14 was also found up-regulated in the sciatic nerve of the mouse with charcot-marie-tooth disease type 1A [47]. A negative correlation was found between CXCL14 expression and methylation status in the 20 primary HCCs [48]. At present, there have been many studies on the relationship between CXCL14 and HCC. Research showed an intronic polymorphism rs2237062 in the CXCL14 gene can influence HBV-related HCC progression [49]. From above all, we can conclude both FCN2 and CXCL14 were associated HCC infected with HBV and HCV.

To extract biological meaning from the selected top 300 ranked genes, GO functional enrichment analysis and KEGG pathway enrichment analysis were performed. In HBV experiment, we found GO terms were related with regulation of cell cycle, chromosome and chromatin binding. There produced many significant pathways and the top two ranked were p53 signaling pathway and cell cycle. In HCV experiment, GO terms were related with cell cycle phase, membrane-enclosed lumen and carbohydrate binding. In the produced pathways, the top 3 ranked were cell cycle, oocyte meiosis and p53 signaling pathway. In the result above, we can conclude cell cycle and p53 signaling pathway were all significant pathways in both HBV and HCV infected HCC.

After that, Empirical Bayesian approach was used for co-expression construction and TFs were identified. We obtained 8 TFs in HBV experiment and 9 TFs in HCV experiment. In the two groups, two TFs were the same and they were RFX5 and EZH2.

RFX5 is a member of the RFX family of DNA binding proteins [50]. The DNA binding proteins can function as regulatory factors in many systems, such as control of immune response in mammals and promotion of infection by human

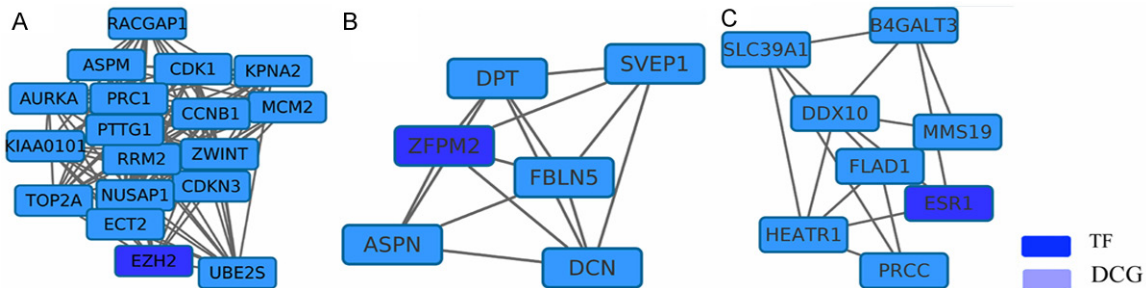**Figure 4.** Sub-network in the co-expression network of the top 300 ranked genes in HCV experiment. A: Cluster 1; B: Cluster 2; C: Cluster 3; D: Cluster 4; E: Cluster 5. TF is the abbreviation of transcription factor.

Table 3. GWGS value and genes of the clusters in HCV experiment

Style	Average of GWGS value	Number of the genes	TFs
Cluster 1	6.885	24	-
Cluster 2	7.035	13	-
Cluster 3	8.510	50	ZBTB41
Cluster 4	8.403	34	EZH2
Cluster 5	7.635	37	JDP2, DACH1, ZFP62

Table 4. Top 5 genes and their stress value in HBV experiment

Gene	UBE2S	VARS	BMP5	GCN1L1	ABI3BP
Stress	29602	19554	18938	16428	15718

Table 5. Top 5 genes and their stress value in HCV experiment

Gene	ECM1	FAM65C	ECT2	ADAMTS13	VIPR1
Stress	63176	61646	47518	43684	38758

HBV [51]. RFX5 plays a pivotal regulatory role in the immune system and an important role in regulating the expression of the major histocompatibility complex class II genes [52, 53]. In Chlamydia-infected cells, RFX5 was found up-regulated [54]. RFX5 was found be related with gastrointestinal cancer, it can control the IFN-γ-induced HLA-DR expression [55]. Defects in genes encoding RFX proteins, such as RFXANK,

RFX5 and RFXAP, can lead to the development of bare lymphocyte syndrome which is a severe autosomal recessive immuno-deficiency disease [56]. All these reported showed RFX5 played an important role in regulation and we speculated it may be related with HCC.

EZH2 is a mammalian histone methyltransferase and located at 7q36. 1 [57]. It plays an important role in many biological processes through its ability to trimethylated lysine 27 in histone H3 [58]. Study showed B cell development can be controlled by EZH2 through histone H3 methylation and IgH rearrangement [59]. In a variety of diseases, EZH2 showed different forms of regulation. It was reported up-regulated in proliferating, cultured human mantle cell lymphoma, but down-regulated in estrogen receptor-negative invasive breast carcinoma [60]. EZH2 was related with cancers and played a role in regulation. It can regulate the survival and metastasis of cancer cells [61]. It was a marker of aggressive breast cancer and could promote the neoplastic transformation of breast epithelial cells [62]. It has a role in Ewing tumors pathology by shaping the oncogenicity and stem cell phenotype of this tumor [63]. It also plays a role in epigenetic regulation of signaling pathways in cancer [64]. Moreover, EZH2 was associated with HCC. EZH2 mRNA expression was up-regulated in human HCC and played a key role in tumour

progression, especially by facilitating portal vein invasion [65]. EZH2-mediated epigenetic silencing contributes to constitutive activation of Wnt/β-catenin signaling and consequential proliferation of HCC cells [66]. In HCC, EZH2 tightly regulates the self-renewal and differentiation of murine hepatic stem/progenitor cells [67].

Except for the same TFs, there were also many specific TFs in HBV and HCV affected HCC. DACH1 was a TF identified in HBV experiment. It was found to be expressed in breast cancer cell lines and can inhibit transforming growth factor-beta signaling [68]. It can also inhibit oncogene-mediated breast oncogenesis, blocking breast cancer epithelial cell DNA synthesis and colony formation [69]. TF ESR1 was identified in HCV experiment. As reported, genetic polymorphism in ESR1 may play a role in mediating susceptibility to HCC in Chinese HBV carriers [70].

To conduct sub-network analysis, MCODE algorithm was used. To identify the relevance of a node in holding together communicating nodes, Centralities Based Analysis was adopted and stress of genes was calculated. In Centralities Based Analysis, genes with the higher stress value were identified. In sub-network analysis, we obtained 3 and 5 clusters in HBV and HCV experiment, respectively.

In HBV experiment, cluster 1 and 3 have the higher GWGS value and were selected for analysis. In cluster 1, there were 18 genes and one TF named EZH2. Among the 18 genes, ten were reported associated with HCC. They were MCM2, NUSAP1, CCNB1, RACGAP1, TOP2A, CDKN3, ASPM, PTTG1, KIAA0101 and CDK1. For example, MCM2 was up-regulated in HCC and overexpression of MCM2 protein related to poor-differentiation in HCC [71, 72]. NUSAP1 was associated with HCC and played an important role in clinical diagnosis of HCC [73, 74]. The mRNA expressions of CCNB1was significantly lower in HCC patients [75]. Research demonstrated the upregulation of RACGAP1 is significantly associated with the early recurrence of human HCC [76]. In the rest 8 genes, UBE2S was reported a potential PVHL-HIF pathway-dependent role in cancer development [77]. AURKA was reported one of the downstream targets of MAPK1/EPK2 in pancreatic cancer [78]. PRC1 was found up-regulated in the development of breast cancer [79]. The

overexpression of CDKN3 was found in human breast and prostate cancer [80]. Therefore, we speculated they may be related with HCC. EZH2 has an important role in HCC as reported above. In cluster 3, there were 7 genes and one TF named ESR1. As for ESR1, its polymorphisms were related to HCC risk among chronic HBV carriers [81]. However, at present, there has no direct report on the role of those 7 genes in cluster 3 shown in **Figure 3** on HCC. Only MMS19 was reported related with histology of non-small cell lung cancer [82]. Therefore, we speculated Therefore, we speculated they may be novel genes to the field of HBV related HCC.

In HCV experiment, the GWGS value of cluster 3 and 4 were higher. In cluster 3, among the 50 genes, 9 were reported associated with HCC. They were RRM2, PTTG1, ASPM, CDKN2A, CDKN3, ADAMTS13, ECM1, CXCL12 and CDK1. Among them, EMC1 was also found one of the top 5 ranked genes in Centralities Based Analysis. In the rest 41 genes, many were reported be related with cancer. HHIP was underexpressed in pancreatic cancer [83]. CCNE2 can be taken as independent prognostic markers for lymph node-negative breast cancer patients [84]. In cluster 4, among the 34 genes, 5 were reported associated with HCC. They were CENPF, FCN2, MKI67, LAMC1 and LYVE1. In the rest 29 genes, MKI67 is important prognostic factor in premenopausal women with node-negative breast cancer [85]. Those genes lack the reports on the role in HCC, represent the novel genes in HCV related HCC and need further research.

Conclusion

This study was conducted to understand the molecular mechanism of HCC infected with HBV or HCV. The similarity and difference between them were also investigated. The genes in HBV and HCV experiment were ranked based on GWGS and the top 300 ranked were selected. Among the top 5 ranked genes, there were two genes named FCN2 and CXCL14 were the same. As reported by previous studies, they were related with HCC. Co-expression network of the top 300 ranked genes was constructed and TFs in the network were identified. Among the two groups of TFs obtained in HBV and HCV experiment, two TFs named RFX5 and EZH2 were the same. They have also been reported

associated with HCC. Sub-network analysis of the co-expression network was conducted. We obtained 3 and 5 clusters in HBV and HCV associated HCC, respectively. In HBV experiment, cluster 1 and 3 had higher GWGS value. In HCV experiment, clusters with higher GWGS value were 3 and 4. In these clusters with higher GWGS value, many genes were found related with HCC. Therefore, the molecular mechanism of HCC infected with HBV and HCV may be partly understood by this study. We can also conclude the similarity and difference between HBV and HCV associated HCC in molecular level.

Acknowledgements

Disclosure of conflict of interest

None.

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