Identification of potential biomarkers in hepatocellular carcinoma: a network-based approach

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the leading causes of death worldwide. Identification of potential therapeutic and diagnostic biomarkers can be helpful to screen cancer progress. This study implemented with the aim of discovering potential biomarkers for HCC within a network-based approach integrated with microarray data.

Methods: Through downloading a gene expression profile GSE62232 differentially expressed genes (DEGs) were identified. Gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis for DEGs were performed utilizing enrichr server. Following reconstruction of protein-protein interaction network of DEGs with STRING, network visualization, analyses, and clustering into structural modules carried out using Cytoscape. Considering degree centrality, 15 hub genes were selected as early biomarker candidates for final validation. In order to validate hub genes, GEPIA server was used to perform overall survival (OS) and disease-free survival (DFS).
Results: In our approach 1996 DEGs were identified including 995 up-regulated genes and 1001 down-regulated genes. KEGG pathway enrichment analysis shown that DEGs are associated with Chemical carcinogenesis, and Cell cycle. GO term enrichment analysis indicated the relation of DEGs with epoxygenase P450 pathway, arachidonic acid monooxygenase activity, and secretory granule lumen. Following analysis of protein-protein interaction network of DEGs top three structural modules and 15 early hub genes were selected. Validation of hub genes performed using GEPIA. Consequently, CDK1, CCNB1, CCNA2, CDC20, AURKA, MAD2L1, TOP2A, KIF11, BUB1B, TYMS, EZH2, and BUB1 were considered as our final proposed biomarkers.

Conclusion: using an integrated network-based approach with microarray data our results revealed 12 final candidates with potential to considered as biomarkers in hepatocellular carcinoma.

Keywords
Network-based, biomarkers, hepatocellular carcinoma (HCC), hub genes

Introduction
Hepatocellular carcinoma (HCC), a predominant primary liver cancer, is the sixth leading causes of death by cancer (1). A multi-step mechanism consists of an accumulation of gene alterations that is resulted in various molecular and cellular modifications is the main cause of HCC pathogenesis (2). Recent studies, have indicated various genes such as epidermal growth factor receptor (EGFR) (3), transforming growth factor-beta 1 (TGF-β1) (4), c-myc (5), to name but few involve in tumorigenesis and progress of HCC. However, there is still a lack of a specific biomarker for precise diagnostic of HCC (6). Therefore, finding an accurate biomarker for screening HCC at different stages with high specificity and sensitivity is an urgent need.
Data obtained from microarray technology in combination with bioinformatics approaches provides a prominent strategy at molecular level for an extensive studying of dysregulation in gene expression between cancer and normal samples from patients. A large-scale microarray data has been published in different databases through recent years and utilizing these data to perform integrated analysis is valuable strategy to follow up the cancer progression in the patients. Despite all efforts in finding different gene alterations in HCC, the molecular mechanisms of HCC progression remain unclear. Consequently, analyzing microarray data to obtain differentially expressed genes (DEGs) between tumor and normal samples is a promising method to discover hub genes and key pathways, which results in HCC tumorigenesis.

In this study, expression profile of hepatocellular carcinoma was obtained from the gene expression omnibus (GEO) database for identification of hub genes in HCC. Following the DEGs identification, they were utilized for gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis, and protein-protein interaction (PPI) network reconstruction studies. Ultimately, survival analyses were performed using GEPIA to evaluate the prognostic value of each hub genes. CDK1, CCNB1, CCNA2, CDC20, AURKA, MAD2L1, TOP2A, KIF11, BUB1B, TYMS, EZH2, and BUB1 were considered as our suggested biomarkers.

**Methods**

**Data selection and preliminary analysis**

A gene expression profile of hepatocellular carcinoma (GSE62232) was obtained from gene expression omnibus (GEO) which is a repository of transcriptional and expression data (7). Our selected dataset contained samples form patients with age from 21 to 82 years old and different etiological factors. 14 tumor samples were from female patients while 67 belonged to male;
moreover, 10 normal samples were used as control. Normalization and expression calculation of downloaded CELL files were implemented with transcriptome analysis console (TAC) through summarization using RMA method additionally eBayes was applied as ANOVA method. To determine differentially expressed genes (DEGs) a threshold value of fold change $> 2$ or $-2 < -$ and $p$-value $< 0.05$ were chosen.

**Gene ontology (GO) term and KEGG pathway enrichment analysis of DEGs**

Following DEGs identification, gene ontology (GO) term and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis were performed utilizing Enrichr (8, 9) which is available at [https://amp.pharm.mssm.edu/Enrichr](https://amp.pharm.mssm.edu/Enrichr). GO (10, 11) is capable of determine details about three functional features of a genes set, including biological process (BP), molecular function (MF), and cellular component (CC). KEGG (12) is a part of Japanese GenomeNet service and is available at [https://www.genome.jp/kegg/](https://www.genome.jp/kegg/). It can reveal the relationships between biological pathways related to disease and drugs. $P < 0.05$ is considered cutoff criteria to obtain significant result for GO and KEGG enrichment analysis.

**PPI-network of DEGs and module analysis**

To discover the connection between differentially expressed genes, a protein-protein interaction (PPI) network was reconstructed utilizing STRING v11.0 (13) at [https://string-db.org/](https://string-db.org/). Cytoscape 3.6.0 (14) was used for network visualization and analysis. Clustering network into structural modules was performed utilizing MCODE app (15) based on degree cutoff $= 2$, node score cutoff $= 0.2$, K-core $= 2$, and max-depth $= 100$. GO term and KEGG pathway enrichment analysis of top three modules were carried out with Enrichr software.

**Hub genes PPI network and enrichment analysis**
Based on highest degree centrality, top 15 nodes from PPI-network of DEGs were considered as hub genes. Selected hub genes were inputted into STRING to analysis their relationships. Furthermore, GO term and KEGG pathway enrichment analysis was also performed for hub genes.

**Survival analysis of hub genes**

In order to validate the selected hub genes, gene expression profiling interactive analysis (GEPIA) which is available at [http://gepia.cancerpku.cn](http://gepia.cancerpku.cn) were employed to investigate their potential to choose as biomarkers. Therefore, in patients with liver hepatocellular carcinoma (LIHC) overall survival (OS) and disease-free survival (DFS) related to hub genes were examined.

**Results**

**Differentially expressed genes in hepatocellular carcinoma**

After downloading a microarray dataset of HCC (array type: HG-U133_Plus_2) containing 81 carcinoma and 10 normal samples, the sample normalization and comparison analysis was performed between two groups (shown in figure 1), resulted in identifying 1996 DEGs. Among identified DEGs, 995, and 1001 genes have been up-regulated and down-regulated, respectively; moreover, volcano plot and scatter plot were applied for visualization of DEGs between carcinoma and normal groups (shown in figure 2). Each of top 20 up- and down-regulated DEGS are listed in table 1, additionally expression pattern of DEGs were revealed by performing hierarchical clustering analysis (shown in figure 3).

Figure 1. Principal components analysis (PCA) of samples. Blue circles are cancer samples and red circles are normal samples.
Figure 2. A) Volcano plot and b) scatter plot for DEGs. In both pictures, green and red circles represent down-regulated, and up-regulated DEGs, respectively.
Figure 3. Hierarchical clustering of DEGs. The x axis is belonging to samples and the y axis represent the genes. Blue and red color indicates cancer and normal condition, respectively.
Table 1. Top 20 up-regulated and down-regulated DEGs.

| Rank | Up-regulated DEGs | Description | Down-regulated DEGs | Description |
|------|-------------------|-------------|---------------------|-------------|
| 1    | AKR1B10 | Aldo-keto reductase family 1, member B10 (aldose reductase) | ASCL1 | Achaete-scute family bHLH transcription factor 1 |
| 2    | SPINK1 | Serine peptidase inhibitor, Kazal type 1 | CNDP1 | Carnosine dipeptidase 1 (metallopeptidase M20 family) |
| 3    | RPS4Y1 | Ribosomal protein S4, Y-linked 1 | HAMP | Hepcidin antimicrobial peptide |
| 4    | DDX3Y | DEAD (Asp-Glu-Ala-Asp) box helicase 3, Y-linked | CYP26A1 | Cytochrome P450, family 26, subfamily A, polypeptide 1 |
| 5    | GABBR1 | Gamma-aminobutyric acid (GABA) B receptor, 1; ubiquitin D | MT1M | Metallothionein 1M |
| 6    | GPC3 | Glypican 3 | ASCL1 | Achaete-scute family bHLH transcription factor 1 |
| 7    | EIF1AY | Eukaryotic translation initiation factor 1A, Y-linked | CXCL14 | Chemokine (C-X-C motif) ligand 14 |
| 8    | COL15A1 | Collagen, type XV, alpha 1 | KCNN2 | Potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 2 |
| 9    | ACSL4 | Acyl-CoA synthetase long-chain family member 4 | FCN2 | Ficolin (collagen/fibrinogen domain containing lectin) 2 |
| 10   | CCL20 | Chemokine (C-C motif) ligand 20 | LINC01093 | Long intergenic non-protein coding RNA 1093 |
| 11   | ASPM | Abnormal spindle microtubule assembly | SLCO1B3 | Solute carrier organic anion transporter family, member 1B3 |
| 12   | IL32 | Interleukin 32 | FCN3 | Ficolin (collagen/fibrinogen domain containing) 3 |
| 13   | RRM2 | Ribonucleotide reductase M2 | CYP2C19 | Cytochrome P450, family 2, subfamily C, polypeptide 19 |
| 14   | TMEM45B | Transmembrane protein 45B | CYP1A2 | Cytochrome P450, family 1, subfamily A, polypeptide 2 |
| 15   | CRNDE | Colorectal neoplasia differentially expressed (non-protein coding) | CYP1A2 | Cytochrome P450, family 1, subfamily A, polypeptide 2 |
| 16   | TOP2A | Topoisomerase (DNA) II alpha | MT1F | Metallothionein 1F |
| 17   | RRM2 | Ribonucleotide reductase M2 | OIT3 | Oncoprotein induced transcript 3 |
| 18   | LYZ | Lysozyme | CLEC1B | C-type lectin domain family 1, member B |
| 19   | CAP2 | CAP, adenylate cyclase-associated protein, 2 (yeast) | AVPR1A | Arginine vasopressin receptor 1A |
| 20   | SPP1 | Secreted phosphoprotein 1 | CRHBP | Corticotropin releasing hormone binding protein |
Gene ontology (GO) term and KEGG pathway enrichment analysis of DEGs

The enrichr GO term and KEGG pathway enrichment analysis were performed for identified DEGs. In regard to GO biological process results, DEGs are related to ‘epoxygenase P450 pathway’, ‘alpha-amino acid catabolic process’, ‘arachidonic acid metabolic process’, ‘steroid metabolic process’, ‘monocarboxylic acid metabolic process’, ‘organic cyclic compound catabolic process’, ‘exogenous drug catabolic process’, ‘cellular amino acid biosynthetic process’, ‘monocarboxylic acid biosynthetic process’, and ‘drug catabolic process’.

GO molecular function showed DEGs are associated with ‘arachidonic acid monooxygenase activity’, ‘arachidonic acid epoxygenase activity’, ‘steroid hydroxylase activity’, ‘oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor’, ‘protein homodimerization activity’, ‘oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen’, ‘heme binding’, ‘transaminase activity’, ‘oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen’, and ‘oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor’.

GO cellular component in regard to enrichment analysis were related to ‘secretory granule lumen’, ‘membrane attack complex’, ‘spindle’, ‘condensed nuclear chromosome kinetochore’, ‘condensed nuclear chromosome, centromeric region’, ‘chromosome, centromeric region’, ‘cytoplasmic vesicle lumen’, ‘endoplasmic reticulum lumen’, ‘intercalated disc’, ‘mitotic spindle’. (Table 2, Figure 4a, 4b, 4c).
KEGG pathway enrichment analysis of DEGs are associated with ‘Chemical carcinogenesis’, ‘Cell cycle’, ‘Drug metabolism’, ‘Retinol metabolism’, ‘Glycine, serine and threonine metabolism’, ‘Metabolism of xenobiotics by cytochrome P450’, ‘p53 signaling pathway’, ‘Fatty acid degradation’, ‘PPAR signaling pathway’, ‘Tryptophan metabolism’. (Table 3, figure 4d)

Table 2. GO term enrichment analysis of DEGs.

| Term                                                | Biological process                                                                 | P-value     | Adjusted P-value | Combined Score | Genes                                                                 |
|------------------------------------------------------|------------------------------------------------------------------------------------|-------------|------------------|----------------|----------------------------------------------------------------------|
| Epoxigenase P450 pathway (GO:0019373)                |                                                                                    | 2.18E-10    | 1.11E-06         | 186.2269       | EPHX2; CYP4A11; CYP2C19; CYP4F12; CYP2C9; CYP2A7; CYP2A6; CYP2C8; CYP2B6; CYP2A13; CYP1A2; CYP1A1; CYP2E1 |
| Alpha-amino acid catabolic process (GO:1901606)      |                                                                                    | 5.44E-09    | 1.39E-05         | 105.573        | AADAT; TAT; HAO; HOGA1; KMO; FTCD; ACSMD; ALDH6A1; HAL; TDO2; UROC1; CBS; AMDHD1; KNYU; SARDH; IDO2 |
| Arachidonic acid metabolic process (GO:0019369)      |                                                                                    | 1.04E-08    | 1.78E-05         | 87.70913       | PTGIS; EPHX2; CYP4A11; PLA2G4C; AKR1C3; CYP2C19; PTGS2; CYP4F12; CYP2A7; CYP2C9; CYP2A6; CYP2C8; CYP2B6; CYP2A13; CYP1A2; CYP1A1; CYP2E1; MGLL |
| Steroid metabolic process (GO:0008202)               |                                                                                    | 3.77E-08    | 4.81E-05         | 58.37231       | AKR1D1; NR1D2; HSD17B14; CYP2C19; CYP3A4; HSD11B1; CYP2B6; SULT1E1; CYP4V2; ACA1A; SRD5A2; SRD5A1; UGT2B15; AKR1C3; UGT2B17; CYP8B1; DHRS2; CYP39A1; CYP2C9; CYP26A1; CYP2C8; CYP2A6; CYP1A2; CYP1A1; CYP2E1 |
| Monocarboxylic acid metabolic process (GO:0032787)   |                                                                                    | 5.81E-08    | 5.93E-05         | 54.23803       | GLYAT; GHR; MTHFD1L; KNYU; BSG; ME1; BCO2; ACA1A; PCK1; PCK2; GGT3; AGXT2; CYP4A11; ACOT12; HOGA1; IGF1; PRODH2; GNPAT; CYP2C9; GRHPR; CYP26A1; ALDH1A3; VNN1; CYP1A2; AGXT; ALDH8A1 |
| Organic cyclic compound catabolic process (GO:1901361) |                                                                                    | 1.18E-07    | 1.01E-04         | 156.857        | HSD11B1; AOC1; EPHX2; CYP1A2; HSD17B14; CYP3A4; ACA1A; PRODH2 |
| Exogenous drug catabolic process (GO:0042738)         |                                                                                    | 2.05E-07    | 1.50E-04         | 99.59451       | CYP2C9; CYP2A7; CYP2A6; CYP2C8; CYP2B6; CYP2A13; NR1H2; CYP1A2; CYP2E1; CYP3A4; CYP2C19 |
| Cellular amino acid biosynthetic process (GO:0008652) |                                                                                    | 2.55E-07    | 1.63E-04         | 87.98716       | OAT; FOLH1; GLS2; FOLH1B; PAH; ASPG; ASNS; ALDH18A1; BCAT1; CDO1; ASPA; GLS |
| Monocarboxylic acid biosynthetic process (GO:0072330) |                                                                                    | 2.91E-07    | 1.65E-04         | 56.86805       | SCS; OSBPL6; ACS3M; OSBPL3; AKR1D1; BGN; LPL; ASNS; ACSM5; HOGA1; CYP8B1; DSCN; ACSMD; CYP39A1; ACLY; ALDH1A3; DSEL; DSE; SLC27A5 |
| Drug catabolic process (GO:0042737)                   |                                                                                    | 3.53E-07    | 1.80E-04         | 92.07408       | CYP2C9; CYP2A7; CYP2A6; CYP2C8; CYP2B6; CYP2A13; NR1H2; CYP1A2; CYP2E1; CYP3A4; CYP2C19 |
| Arachidonic acid monoxygenase activity (GO:0008391)   |                                                                                    | 5.70E-08    | 6.56E-05         | 132.6786       | CYP2C9; CYP2A7; CYP2A6; CYP2C8; CYP2B6; CYP2A13; CYP4A11; CYP2E1; CYP2C19; CYP4F12 |
| Biological Process | gene IDs | p-value | fold-change |
|--------------------|----------|---------|-------------|
| Arachidonic acid epoxygenase activity (GO:0008392) | | 5.70E-08 | 3.28E-05 | 132.6786 |
| Steroid hydroxylase activity (GO:0008395) | | 4.30E-06 | 0.001649 | 57.2952 |
| Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor (GO:0016616) | | 4.98E-06 | 0.001432 | 37.52801 |
| Protein homodimerization activity (GO:0042803) | | 5.49E-06 | 0.001264 | 19.98055 |
| Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen (GO:0016712) | | 8.38E-06 | 0.001608 | 67.74414 |
| Heme binding (GO:0020037) | | 1.47E-05 | 0.002416 | 35.53057 |
| Transaminase activity (GO:0008483) | | 2.57E-05 | 0.003703 | 71.45018 |
| Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen (GO:0016709) | | 1.39E-04 | 0.017727 | 32.23068 |
| Oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor (GO:0016620) | | 3.04E-04 | 0.034934 | 35.05117 |
| Cellular component | | | | |
| Secretory granule lumen (GO:0034774) | | 1.74E-08 | 7.75E-06 | 40.3977 |

| | | | |
|-----------------|---------------|-----------------|-----------------|
| Protein | CYP2C9; CYP2A7; CYP2A6; CYP2C8; CYP2B6; CYP2A13; CYP4A11; CYP2E1; CYP2C19; CYP4F12 | | |
| Steroid hydroxylase activity | CYP39A1; CYP2C9; CYP2A7; CYP2A6; CYP2C8; CYP2B6; CYP2A13; CYP4A11; CYP2E1; CYP3A4; CYP2C19; CYP8B1 | | |
| Oxidoreductase activity | ABCC4; ADH1C; HPGD; CYP4A22; AKR1D1; AKR1C3; DHR52; ADH6; ADH4; GRHPR; AKR7A3; Bdh2; Fam213B; AKR1B10; Gpd1; Me1; Rdh16; Rdh5; PhgdH; Glyr1 | | |
| Protein homodimerization activity | TOP2A; CDA; Tenm1; Hsp90ab1; Hip1; Uxs1; Hexb; Hspb1; Pth1r; Eprs; Jchain; Gys2; Ghr; Kynu; Map3k9; Hif1an; Pspih; Pdgfra; Sds; Tpm4; Vwf; Tpm1; Tapi; Adami10; APOA4; Hoga1; Muf2; Dck; Foxp2; Tox3; Aldh1a3; Msh2; Cdhi1; Cat; Pecam1; Masp1; Plcb1; Agxt; Atf3; Mgl; Abcg2; Cdh19; Camk2b; Poni; Hpgd; Shmt1; Poni; Pdgfa; Adb2r; Tmem27; Nppf5; Mthfd1l; Irak1; Erbb3; Cbs; Tpr; Kcnn2; Ect2; Xdh; S100a10; Ugt1a6; Bard1; Aoc1; Gchi1; Stat1; Aadd1; Ephx2; Asns; Gust21; Nra4a2; Grhpr; Cenpf; Cd4; Tp53i3;Nr4a3;Gsta4; Acpo2; Bax; Glα; Cd200; Mad2l1 | | |
| Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen | CYP2C9; CYP2A7; CYP2A6; CYP2C8; CYP2B6; CYP2A13; CYP1A2; CYP2E1; CYP2C19 | | |
| Heme binding | Ptgis; Cbssl; CYP2C19; Ptgs2; Cyp8b1; CYP2A7; CYP26A1; CYP2C9; CYP2A6; CYP2C8; CYP2B6; Tdo2; Cyp2A13; CBS; Cat; Cyp1A2; Cyp2E1 | | |
| Transaminase activity | Oat; Aadata; Agxt2; Psat1; Bcat1; Agxt; Etnppl | | |
| Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen | Sqle; Cyp26a1; Fadxc2; Akrid1; Cyp4a22; Cyp4a11; Akrid3; Cyp2e1; Kmo; Cyp8b1; Cyp4f12 | | |
| Oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor | Adh4; Aldh1a3; Aldh6a1; Akrid10; Aldh1b1; Akrid3; Aldh18a1; Aldh8a1 | | |
| Term                                                                 | P-value          | Adjusted P-value | Combined Score | Genes                                                                 |
|----------------------------------------------------------------------|-----------------|------------------|----------------|----------------------------------------------------------------------|
| **Membrane attack complex (GO:0005579)**                              | 1.24E-05        | 0.00276          | 127.336        | C6; C7; C9; C8B; C8A                                                  |
| **Spindle (GO:0005819)**                                              | 4.23E-05        | 0.006282         | 21.96729       | KIF14; BUB1B; CAPG; TTK; KIF11; AURKA; CDC20; CCNB1; RACGAP1; TPR; NUSAP1; SPDL1; ECT2; CDK5RAP2; RMDN2; CDC7; CDC6; TUBG1; CKAP5; MZT1; TPX2; CENPF; NCO1; KIF2A; PRC1; KIF4A; CDK1; KIF20A; MAD1L1; MAD2L1 |
| **Condensed nuclear chromosome kinetochore (GO:0000778)**             | 5.41E-05        | 0.006028         | 72.47135       | CCNB1; NUF2; BUB1B; CENPA; BUB1; NDC80                                |
| **Condensed nuclear chromosome, centromeric region (GO:0000780)**     | 1.01E-04        | 0.009041         | 62.18329       | NUF2; BUB1B; CENPA; BUB1; NDC80; AURKA                               |
| **Chromosome, centromeric region (GO:0000775)**                       | 1.80E-04        | 0.013408         | 28.54812       | HELLs; CENPF; CENPW; MIS18A; H2AFY; RAD21; NUF2; BIRC5; NAPCPD2; SMC3; CENPA; NDC80 |
| **Cytoplasmic vesicle lumen (GO:0060205)**                            | 1.88E-04        | 0.011995         | 19.78179       | CFD; CDA; CSTB; SERPINB1; HSP90A1B; GSN; PSMD14; MVP; AGL; NME2; HBB; ARPC5; DBH; HBA1; PSMA5; PNP; PKM; CRISPLD2; CAT; PMG2; HYOU1; S100A8 |
| **Endoplasmic reticulum lumen (GO:0005788)**                          | 2.04E-04        | 0.011395         | 15.74304       | COL15A1; PROZ; PDGFA; LAMC1; CFP; PTGS2; THBS1; ADAMTS13; GPC3; SPP1; ARSD; APOL1; ARSE; CTSC; ERAP2; GOLM1; IGBP3; P3H2; ADAM10; APOA4; PDAIA6; APOA5; COL1A1; COL1A2; F9; COL4A2; COL4A1; P4HA2; CANX; DNAJB11; DNAJC10; ALB; CALU; SPARCL1; HYOU1; PPB |
| **Intercalated disc (GO:0014704)**                                    | 2.92E-04        | 0.014493         | 31.94614       | GJC1; GJA1; TMEM65; FXYD1; CTNN3A; ANK3; FGF13; VAMP5; SLC8A1 |
| **Mitotic spindle (GO:0072686)**                                       | 3.86E-04        | 0.017203         | 20.24638       | CAPG; CDC7; RMDN2; KIF11; SMC3; AURKA; ASPM; TPX2; RACGAP1; TPR; NUSAP1; CDK1; ECT2; FAM83D; MAD1L1; MAD2L1 |

Table 3. The top 10 KEGG pathway enrichment analysis of DEGs.
Table 4. Top 3 structural modules.

| Modules          | Score  | Nodes | Edges | Genes                                                                 |
|------------------|--------|-------|-------|----------------------------------------------------------------------|
| 1                | 70.263 | 77    | 2670  | DTL, CEP55, BUB1B, Ckap2, ANLN, MCM4, MCM6, ECT2, RACGAP1, KIF20A, FOXM1, BUB1, CASC5, MAD2L1, NEK2, MCM2, PTTG1, CDC7, NUSAP1, NDC80, NUF2, CCNB2, PBK, CDC20, PRC1, TOP2A, EZH2, HMMR, GTSE1, UBE2C, KIAA0101, FANC1, UBE2T, CDC6, CDC43, RRM2, ATAD2, ASPM, NCPAPD2, MELK, TTK, NCAH, CENPA, MCM5, CFS2, TRIP13, KIF14, GMNN, MCM3, UHRF1, BIRC5, FEN1, GINS1, CDKN3, RFC4, CENPF, POLE2, KPNA2, AURKA, TPX2, HELLS, CENPU, KIF11, |
Figure 4. Top 10 GO term and KEGG pathways enrichment analysis,

a) GO enrichment analysis – Biological process

- epoxygenase P450 pathway (GO:0019373)
- alpha-amino acid catabolic process (GO:1901606)
- arachidonic acid metabolic process (GO:0019359)
- steroid metabolic process (GO:0008202)
- monocarboxylic acid metabolic process (GO:0032787)
- organic cyclic compound catabolic process (GO:1901361)
- exogenous drug catabolic process (GO:0042738)
- cellular amino acid biosynthetic process (GO:0008652)
- monocarboxylic acid biosynthetic process (GO:0072330)
- drug catabolic process (GO:0042737)

b) GO enrichment analysis – Molecular function

- arachidonic acid monooxygenase activity (GO:0008391)
- arachidonic acid epoxide hydrolase activity (GO:0008392)
- steroid hydroxylase activity (GO:0008396)
- oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor (GO:0016616)
- protein homodimerization activity (GO:0042803)
- oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as acceptor (GO:0016618)
- heme binding (GO:0020037)
- transaminase activity (GO:0008483)
- oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and inorganic ferrous ion as one acceptor (GO:0016620)

- oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor (GO:0016620)

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d) KEGG enrichment analysis for DEGs

Figure 5. PPI network of DEGs and top 3 modules. A) PPI network of DEGs. B) module 1. C) module 2. D) module 3.

A)
Table 5. GO enrichment analysis of top 3 modules.

| module | node | edges | **GO enrichment analysis** | Term | P-value | Adjusted P-value | Combined Score | Genes |
|--------|------|-------|---------------------------|------|---------|-----------------|----------------|-------|
| 1      | 77   | 2670  | Biological process        | 8.78E-21 | 4.48E-17 | 1031.256 | UBE2C; CDC7; CDC6; FOXM1; AURKA; CCNA2; CCNB2; CCNB1; MELK; CCNE2; POLE2; MCM3; CDK1; MCM4; NEK2; MCM5; MCM6; MCM2; CDKN3 |
|        |      |       | Mitotic cell cycle phase transition (GO:0044772) | 6.82E-18 | 1.74E-14 | 621.2265 | UBE2A; HELLS; FEN1; RFC4; PCNA; UHRF1; CDC7; CDC6; TYMS; RAD51AP1; UBE2T; POLE2; MCM3; CDK1; |

DNA metabolic process (GO:0006259)
| Molecular function | Pathway | G1/S transition of mitotic cell cycle (GO:0000082) | Regulation of mitotic cell cycle phase transition (GO:1901990) | Mitotic sister chromatid segregation (GO:0000070) | Kinase binding (GO:0019900) | Protein kinase binding (GO:0019901) | Microtubule binding (GO:0008017) | Histone kinase activity (GO:0035173) | Tubulin binding (GO:0015631) | Spindle (GO:00005819) | Nuclear chromosome part (GO:0044454) | Mitotic spindle (GO:0072686) | Microtubule cytoskeleton (GO:0015630) | MCM4; MCM5; MCM6; KPN2; MCM2 | PCNA; RRM2; CDC7; CDC6; TYMS; CCNE2; POLE2; MCM3; MCM4; MCM5; MCM6; MCM2; CDKN3 | UBE2C; KIF14; BUB1B; HMMR; CDC6; AURKA; CDC20; ANLN; TPX2; CCNB1; CENPF; CDK1; NEK2; MAD2L1 | CCNB1; PRC1; KIF14; NUSAP1; NCAPG; NCAPD2; SMC4; DLGAP5; NDC80; ZWINT; SMC2 | KIF14; KIF11; CDC6; FOXM1; AURKA; CCNA2; TPX2; CCNB1; RACGAP1; PRC1; CKS2; KIF20A; FAM83D | TOP2A; KIF14; KIF11; FOXM1; AURKA; CCNA2; TPX2; CCNB1; RACGAP1; PRC1; CKS2; KIF20A; FAM83D | RACGAP1; KIF4A; KIF14; NUSAP1; BIRC5; KIF11; KIF20A; FAM83D | MELK; CDK1; AURKA | KIF14; BUB1B; TTK; CDC7; KIF11; CDC6; AURKA; CDC20; TPX2; CCNB1; CENPF; RACGAP1; PRC1; KIF4A; NUSAP1; CDK1; KIF20A; ECT2; MAD2L1 | GINS1; FEN1; PCNA; UHRF1; BUB1B; CENPA; NDC80; AURKA; RAD51AP1; CCNB1; POLE2; NUF2; MCM3; CDK1; MCM4; MCM5; NCAPD2; MCM6; BUB1; MCM2; EZH2 | ASPM; TPX2; RACGAP1; NUSAP1; CDK1; CDC7; KIF11; ECT2; FAM83D; AURKA; MAD2L1 | CENPU; KIF14; CKAP2; TTK; KIF11; HMMR; AURKA; CDC20; CCNB2; TPX2; CENPF; PRC1; KIF4A; KIF20A; CEP55; DLGAP5; MCM2 |
| 2 | 13 | 78 | Biological process | Condensed nuclear chromosome kinetochore (GO:0000778) | 1.21E-12 | 1.08E-10 | 3887.177 | CCNB1; NUF2; BUB1B; CENPA; BUB1; NDC80 |
| 8 | 13 | 78 | Biological process | Neutrophil degranulation (GO:0043312) | 7.26E-22 | 3.70E-18 | 2032.348 | CTSA; ANXA2; HEXB; GGH; LYZ; GNS; PLAC8; ACLY; FABP5; GM2A; AGA; GLA; CTSC |
| 8 | 13 | 78 | Biological process | Neutrophil activation involved in immune response (GO:0002283) | 8.10E-22 | 2.07E-18 | 2010.983 | CTSA; ANXA2; HEXB; GGH; LYZ; GNS; PLAC8; ACLY; FABP5; GM2A; AGA; GLA; CTSC |
| 8 | 13 | 78 | Biological process | Neutrophil mediated immunity (GO:0002446) | 9.03E-22 | 1.54E-18 | 1990.007 | CTSA; ANXA2; HEXB; GGH; LYZ; GNS; PLAC8; ACLY; FABP5; GM2A; AGA; GLA; CTSC |
| 8 | 13 | 78 | Biological process | Glycosphingolipid metabolic process (GO:0006687) | 3.33E-08 | 4.25E-05 | 1962.072 | CTSA; GM2A; HEXB; GLA |
| 8 | 13 | 78 | Biological process | Glycolipid metabolic process (GO:0006664) | 7.10E-08 | 7.25E-05 | 1558.338 | CTSA; GM2A; HEXB; GLA |
| 8 | 13 | 78 | Biological process | Exopeptidase activity (GO:0008238) | 3.80E-04 | 0.437457 | 538.4726 | CTSA; GGH |
| 8 | 13 | 78 | Biological process | Exo-alpha-sialidase activity (GO:0004308) | 0.003894 | 1 | 1422.639 | CTSA |
| 8 | 13 | 78 | Biological process | Alpha-sialidase activity (GO:0016997) | 0.003894 | 1 | 1422.639 | CTSA |
| 8 | 13 | 78 | Biological process | Omega peptidase activity (GO:0008242) | 0.004542 | 1 | 1185.592 | GGH |
| 8 | 13 | 78 | Biological process | Galactosidase activity (GO:0015925) | 0.005189 | 1 | 1011.771 | GLA |
| 8 | 13 | 78 | Biological process | Azurophil granule lumen (GO:0035578) | 1.25E-31 | 5.58E-29 | 15812.48 | CTSA; ANXA2; HEXB; GGH; LYZ; GNS; PLAC8; ACLY; FABP5; GM2A; AGA; GLA; CTSC |
| 8 | 13 | 78 | Biological process | Azurophil granule (GO:0042582) | 1.99E-28 | 4.44E-26 | 8283.607 | CTSA; ANXA2; HEXB; GGH; LYZ; GNS; PLAC8; ACLY; FABP5; GM2A; AGA; GLA; CTSC |
| 8 | 13 | 78 | Biological process | Vacuolar lumen (GO:0005775) | 3.63E-28 | 5.40E-26 | 7848.771 | CTSA; ANXA2; HEXB; GGH; LYZ; GNS; PLAC8; ACLY; FABP5; GM2A; AGA; GLA; CTSC |
| 8 | 13 | 78 | Biological process | Secretory granule lumen (GO:0034774) | 3.11E-24 | 3.47E-22 | 3414.919 | CTSA; ANXA2; HEXB; GGH; LYZ; GNS; PLAC8; ACLY; FABP5; GM2A; AGA; GLA; CTSC |
| 8 | 13 | 78 | Biological process | Lysosome (GO:0005764) | 4.32E-11 | 3.85E-09 | 696.0632 | CTSA; ANXA2; GM2A; HEXB; AGA; GNS; GLA; CTSC |
| 3 | 29 | 136 | Biological process | Post-translational protein modification (GO:0043687) | 3.44E-14 | 1.75E-10 | 718.6612 | PSMD4; GOLM1; IGFBP3; GPC3; CALU; ADAM10; SPARCL1; LAMC1; |
| Biological Process                                      | p-value    | AAF   | APO1; APOA5; PDIA6; CKAP4          |
|---------------------------------------------------------|------------|-------|-----------------------------------|
| Cellular protein metabolic process (GO:0044267)         | 3.97E-14   | 1.01E-10 | PRKDC; GOLM1; IGFBP3; ADAM10; APOA4; LAMC1; APOA5; PDIA6; CKAP4; GPC3; CALU; SPARCL1; APO1 |
| Cellular protein modification process (GO:0006464)      | 2.17E-11   | 3.69E-08 | 236.8335                          |
| Regulation of fibrinolysis (GO:0051917)                 | 2.52E-09   | 3.22E-06 | 4200.982                          |
| Extracellular matrix organization (GO:0030198)          | 8.04E-07   | 8.21E-04 | 253.5776                          |
| Molecular function                                      |            |       |                                    |
| Phosphatidylcholine-O-acyltransferase activator activity (GO:00660228) | 3.03E-05   | 0.034922 | 2391.501                          |
| Cholesterol transporter activity (GO:0017127)           | 2.72E-04   | 0.156756 | 665.988                           |
| Sterol transporter activity (GO:0015248)                 | 7.50E-04   | 0.287603 | 354.4801                          |
| Peptidase inhibitor activity (GO:0030414)               | 0.00124    | 0.356931 | 256.4102                          |
| Endopeptidase activity (GO:0004175)                     | 0.001615   | 0.371838 | 50.09349                          |
| Cellular Components                                     |            |       |                                    |
| Endoplasmic reticulum lumen (GO:0005788)                | 2.07E-17   | 9.24E-15 | 1275.603                          |
| Very-low-density lipoprotein particle (GO:0034361)      | 1.23E-06   | 2.75E-04 | 1876.755                          |
| Membrane attack complex (GO:0005579)                    | 3.03E-05   | 0.004511 | 2391.501                          |
| Rough endoplasmic reticulum (GO:0005791)                | 7.50E-04   | 0.083582 | 354.4801                          |
| Platelet alpha granule lumen (GO:0031093)               | 0.004234   | 0.377665 | 112.4988                          |

**Molecular Function:**
- **Phosphatidylcholine-O-acyltransferase activator activity**
- **Cholesterol transporter activity**
- **Sterol transporter activity**
- **Peptidase inhibitor activity**
- **Endopeptidase activity**

**Cellular Components:**
- **Endoplasmic reticulum lumen**
- **Very-low-density lipoprotein particle**
- **Membrane attack complex**
- **Rough endoplasmic reticulum**
- **Platelet alpha granule lumen**
Table 6. KEGG enrichment analysis of top 3 modules.

| module | node | edges | Term                                      | P-value   | Adjusted P-value | Combined Score | Genes                                                                 |
|--------|------|-------|-------------------------------------------|-----------|------------------|----------------|----------------------------------------------------------------------|
| 1      | 77   | 2670  | Cell cycle                                | 1.02E-25  | 3.13E-23         | 2290.387       | PCNA; BUB1B; TTK; CDC7; CDC6; CDC20; CCNA2; CCNB2; CCNB1; PTTG1; CCNE2; MCM3; CDK1; MCM4; MCM5; MCM6; BUB1; MCM2; MAD2L1 |
| DNA replication | 9.92E-15 | 1.53E-12 | 2093.77 |                                          |                |                  | FEN1; PCNA; RFC4; POLE2; MCM3; MCM4; MCM5; MCM6; MCM2               |
| Oocyte meiosis | 1.23E-09 | 1.26E-07 | 383.732 |                                          |                |                  | CDC20; CCNB2; CCNB1; PTTG1; CCNE2; CDK1; BUB1; AURKA; MAD2L1        |
| Progesterone-mediated oocyte maturation | 1.06E-07 | 8.19E-06 | 294.8769 |                                          |                |                  | CCNA2; CCNB2; CCNB1; CDK1; BUB1; AURKA; MAD2L1                    |
| p53 signaling pathway | 3.41E-07 | 2.10E-05 | 322.3209 |                                          |                |                  | CCNB2; CCNB1; RRM2; CCNE2; CDK1; GTSE1                             |
| 2      | 13   | 78    | Lysosome                                   | 4.66E-13  | 1.43E-10         | 2486.122       | CTSA; GM2A; HEXB; AGA; GNS; GLA; CTSC                               |
| Other glycan degradation | 5.93E-05 | 0.009135 | 1663.683 |                                          |                |                  | HEXB; AGA                                                           |
| Glycosaminoglycan degradation | 6.63E-05 | 0.006804 | 1558.168 |                                          |                |                  | HEXB; GNS                                                           |
| Glycosphingolipid biosynthesis | 3.80E-04 | 0.029265 | 538.4726 |                                          |                |                  | HEXB; GLA                                                           |
| Renin-angiotensin system | 0.014851 | 0.914857 | 281.5819 |                                          |                |                  | CTSA                                                                |
| 3      | 29   | 136   | Complement and coagulation cascades        | 9.32E-08  | 2.87E-05         | 706.5978       | C6; SERPINE1; F11; C8A; KLKB1                                      |
| p53 signaling pathway | 3.42E-06 | 5.27E-04 | 482.1627 |                                          |                |                  | CDKN2A; IGFBP3; SERPINE1; THBS1                                    |
| Prion diseases | 1.74E-05 | 0.001785 | 647.869 |                                          |                |                  | C6; LAMC1; C8A                                                      |
| Systemic lupus erythematosus | 3.90E-05 | 0.003004 | 210.5579 |                                          |                |                  | C6; HIST1H2BK; C8A; HIST1H2AC                                     |
| Cellular senescence | 0.001575 | 0.097074 | 83.44321 |                                          |                |                  | CDKN2A; IGFBP3; SERPINE1                                           |
Table 7. Top 15 hub genes in regard to their related degree.

| Gene  | Degree |
|-------|--------|
| ALB   | 187    |
| CDK1  | 156    |
| CCNB1 | 136    |
| CCNA2 | 128    |
| CDC20 | 124    |
| AURKA | 122    |
| MAD2L1| 118    |
| TOP2A | 117    |
| KIF11 | 116    |
| CCNB2 | 115    |
| BUB1B | 115    |
| TYMS  | 110    |
| EZH2  | 109    |
| BUB1  | 108    |
| ESR1  | 107    |

Hub genes PPI network and enrichment analysis

Top 15 hub genes considering their degree are ALB (albumin), CDK1 (Cyclin Dependent Kinase 1), CCNB1 (Cyclin B1), CCNA2 (Cyclin A2), CDC20 (Cell Division Cycle 20), AURKA (Aurora Kinase A), MAD2L1 (Mitotic Arrest Deficient 2 Like 1), TOP2A (DNA Topoisomerase II Alpha), KIF11 (Kinesin Family Member 11), CCNB2 (Cyclin B2), BUB1B (BUB1B Mitotic Checkpoint Serine/Threonine Kinase B), TYMS (Thymidylate Synthetase), EZH2 (Enhance Of Zeste 2 Polycomb Repressive Complex 2 Subunit), BUB1 (BUB1 Mitotic Checkpoint Serine/Threonine Kinase), ESR1 (Estrogen Receptor 1). (table 7)

PPI network of 15 hug genes reconstructed using STRING (Figure 6). Additionally, GO term (Table 8) and KEGG pathway (Table 9) enrichment analysis for 15 hub genes performed by enrichr server.
Table 8. Top 5 GO term enrichment analysis of 15 Hub genes.

| GO enrichment analysis | Term                                                                 | P-value     | Adjusted P-value | Combined Score | Genes                      |
|------------------------|----------------------------------------------------------------------|-------------|------------------|----------------|---------------------------|
| Biological process     | anaphase-promoting complex-dependent catabolic process (GO:0031145) | 1.52E-11    | 7.77E-08         | 2522.3532      | CDC20; CCNB1; CDK1; BUB1B; AURKA; MAD2L1 |
|                        | negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle (GO:0051436) | 1.43E-09    | 3.64E-06         | 1912.4721      | CDC20; CCNB1; CDK1; BUB1B; MAD2L1 |
|                        | regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle (GO:0051439) | 1.53E-09    | 2.61E-06         | 1879.2843      | CDC20; CCNB1; CDK1; BUB1B; MAD2L1 |
|                        | positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition (GO:0051437) | 2.02E-09    | 2.58E-06         | 1756.1385      | CDC20; CCNB1; CDK1; BUB1B; MAD2L1 |
|                        | negative regulation of ubiquitin protein ligase activity (GO:1904667) | 2.16E-09    | 2.20E-06         | 1727.5547      | CDC20; CCNB1; CDK1; BUB1B; MAD2L1 |
| Molecular function     | cyclin-dependent protein kinase activity (GO:0097472) | 9.37E-09    | 1.08E-05         | 2899.6898      | CCNA2; CCNB2; CCNB1; CDK1 |
|                        | protein kinase binding (GO:0019901) | 9.23E-07    | 5.31E-04         | 224.57388      | TOP2A; CCNA2; CCNB1; KIF11; ESR1; AURKA |
Table 9. top 5 KEGG pathway enrichment analysis of 15 Hub genes

| Term                                | P-value     | Adjusted P-value | Combined Score | Genes                                      |
|-------------------------------------|-------------|------------------|----------------|--------------------------------------------|
| Cell cycle                          | 1.08E-14    | 3.32E-12         | 2766.618       | CCNA2; CDC20; CCNB2; CCNB1; CDK1; BUB1B; BUB1; MAD2L1 |
| Progesterone-mediated oocyte maturation | 3.65E-13  | 5.63E-11         | 2699.841       | CCNA2; CCNB2; CCNB1; CDK1; BUB1; AURKA; MAD2L1 |
| Oocyte meiosis                      | 1.94E-12    | 1.99E-10         | 2013.641       | CDC20; CCNB2; CCNB1; CDK1; BUB1; AURKA; MAD2L1 |
| Human T-cell leukemia virus 1 infection | 4.13E-07  | 3.18E-05         | 447.4824       | CCNA2; CDC20; CCNB2; BUB1B; MAD2L1          |
| Cellular senescence                | 5.03E-06    | 3.10E-04         | 406.6895       | CCNA2; CCNB2; CCNB1; CDK1                  |

**Survival analysis of hub genes**

Each of hub genes was submitted to GEPIA in order to obtain survival plots.

Figure 7. The overall survival of 15 hub genes in LIHC patients with median cutoff. (a) ALB, (b) CDK1, (c) CCNB1, (d) CCNA2, (e) CDC20, (f) AURKA, (g) MAD2L1, (h) TOP2A, (i) KIF11, (j) CCNB2, (k) BUB1B, (l) TYMS, (m) EZH2, (n) BUB1, (o) ESR1.
Figure 8. Disease-free Survival (DFS) of 15 hub genes in LIHC patients with median cutoff. (a) ALB, (b) CDK1, (c) CCNB1, (d) CCNA2, (e) CDC20, (f) AURKA, (g) MAD2L1, (h) TOP2A, (i) KIF11, (j) CCNB2, (k) BUB1B, (l) TYMS, (m) EZH2, (n) BUB1, (o) ESR1.
Discussion

Hepatocellular carcinoma (HCC) is one of the leading causes of death by cancer (16). Due to the important role of biomarkers in diagnosis of HCC, various biomarkers have been introduced in the recent years (17). Despite all efforts in developing diagnosis methods and treatment strategy for HCC patients, there is still shortcomings in this area (18). Consequently, biomarkers with high sensitivity and specificity need in precise detection of HCC.

Integration of network-based approach with microarray has been resulted in the emergence of a robust strategy to find potential biomarkers in various cancers specially HCC (19). In our study, firstly, a gene expression profile from gene expression omnibus (GEO) with accession number GSE62232 was downloaded. The downloaded profile consisted of 81 HCC samples and 10 normal samples. Following normalization of CELL files with Transcriptome Analysis Console (TAC) software, 1996 DEGs were identified including 995 up-regulated and 1001 down-regulated genes.
GO term and KEGG pathway enrichment analysis were also performed to achieve more information about DEGs. Afterward PPI network reconstruction and determining structural modules with Cytoscape, based on highest degree, 15 hub genes were selected as the HCC candidates’ biomarkers. Ultimately, OS and DFS plots using GEPIA were applied to validate hub genes.

Comparing enrichment analysis of DEGs and top 3 modules showed that module 1 is more important than other modules considering its two common pathways with top 10 KEGG pathways of DEGs (cell cycle, and p53 signaling pathway). There was no common GO biological process and GO molecular function between top 5 GO bp and GO mf of the selected modules and top 10 GO bp and GO mf of DEGs. However, module 1 based on GO cellular component results showed to be more important in compare to other modules (common GO cellular components are spindle, mitotic spindle, condensed nuclear chromosome kinetochore).

Final hub genes based on the highest degree are ALB, CDK1, CCNB1, CCNA2, CDC20, AURKA, MAD2L1, TOP2A, KIF11, CCNB2, BUB1B, TYMS, EZH2, BUB1, ESR1. Interestingly, all hub genes are present in module 1 except ALB and ESR1. Common GO terms between hub genes and DEGs are: I) ‘cell cycle’ in KEGG pathway; II) ‘spindle’, ‘condensed nuclear chromosome kinetochore’, ‘condensed nuclear chromosome, centromeric region’, and ‘mitotic spindle’ in GO cellular components. No common in GO bp and GO mf was seen.

Overall survival (OS) and disease-free survival (DFS) studies were performed using GEPIA in order to validate final hub genes. Common genes between OS and DFS which their up-regulation is related to poor prognosis of HCC were considered as final biomarkers. These genes are CDK1, CCNB1, CCNA2, CDC20, AURKA, MAD2L1, TOP2A, KIF11, BUB1B, TYMS, EZH2, BUB1.
Therefore, a literature review was done to obtain more details about these genes in different cancers.

CDK1 (Cyclin Dependent Kinase 1) gene belongs to serine/threonine protein kinase family (20). CCNB1 encodes G2/mitotic-specific cyclin-B1 and based on our result its up-regulation is related with low survival in HCC patients. The potential role of CDK1 and CCNB1 inhibition in increasing the efficacy of HCC treatment have also been investigated (21, 22). CCNA2 encodes Cyclin A2 and the regulation of CCNA2 using RNA interference has been shown recently (23). A protein produced from CDC20 (Cell Division Cycle 20 interacts with anaphase-promoting complex/cyclosome (APC/C) in the cell cycle. Up-regulation of CDC20 has been reported in various cancers, including oral squamous cell carcinoma (24), and gastric cancer (25). Additionally, the effects of CDC20 up-regulation in the progression of HCC have been examined (26). Overexpression of BUB1B (also termed BUBR1) which encodes mitotic checkpoint serine/threonine-protein kinase BUB1 beta are associated with poor prognosis in HCC (27). BUB1 encodes mitotic checkpoint serine/threonine-protein kinase and recently its role to maintain breast cancer stem cell has been studied (28). MAD2L1 encodes mitotic spindle assembly checkpoint protein MAD2A, its suppression using RNA interferences has been shown to control the proliferation and metastasis in HCC (29). CDK1, CCNB1, CCNA2, CDC20, MAD2L1, BUB1B, and BUB1 are enriching cell cycle. Interestingly, cell cycle is among top KEGG pathways which is enriched with both module 1 and DEGs.

AURKA encodes Aurora Kinase A protein and due to our findings, AURKA enriches progesterone-mediated oocyte maturation and oocyte meiosis in KEGG pathways. A study suggests that variation in AURKA gene is important to predict early-stage of HCC, and it is a reliable biomarker for HCC (30). DNA topoisomerase 2-alpha is a protein produced from TOP2A;
it has been suggested that TOP2A overexpression in HCC patients is a potential candidate for therapeutic purposes (31) and based on GO molecular function it is related to protein kinase binding. KIF11 encodes Kinesin-like protein KIF11 and due to results of GO cellular component it is related to spindle and mitotic spindle. Recently, a study demonstrated that kinesin family members, including KIF11 are potential markers to predict poor prognosis and cell proliferation in HCC (32). TYMS encode thymidylate synthetase protein and through a study it was shown that TYMS related polymorphisms are valuable factors in predicting clinical outcomes of HCC (33). EZH2 encode Histone-lysine N-methyltransferase protein and considering GO cellular component enrichment analysis, it is related to nuclear chromosome part. It has shown that EZH2 through modulation of miR-22/galectin-9 axis can cause progression of HCC (34).

**Conclusion**

In conclusion, our findings alongside recent studies validate the importance of CDK1, CCNB1, CCNA2, CDC20, AURKA, MAD2L1, TOP2A, KIF11, BUB1B, TYMS, EZH2, BUB1 to be considered as potential biomarkers in hepatocellular carcinoma. Based on our findings, we demonstrate that network-based approach integrated with microarray data is a promising method to obtain potential therapeutic and diagnostic biomarkers in various cancers, in our case, hepatocellular carcinoma. However, further experimental analysis is needed to confirm our findings.
**Abbreviations**

HCC: Hepatocellular carcinoma

EGFR: Epidermal growth factor receptor

TGF-β: Transforming growth factor-beta 1

DEGs: Differentially expressed genes

GEO: Gene expression omnibus

GO: Gene ontology

KEGG: Kyoto encyclopedia of genes and genomes

PPI-network: Protein-protein interaction network

TAC: Transcriptome analysis console

BP: Biological process

MF: Molecular function

CC: Cellular component

GEPIA: Gene expression profiling interactive analysis

LIHC: Liver hepatocellular carcinoma

OS: Overall survival

DFS: Disease-free survival

PCA: Principal components analysis
ALB: albumin

CDK1: Cyclin Dependent Kinase 1

CCNB1: Cyclin B1

CCNA2: Cyclin A2

CDC20: Cell Division Cycle 20

AURKA: Aurora Kinase A

MAD2L1: Mitotic Arrest Deficient 2 Like 1

TOP2A: DNA Topoisomerase II Alpha

KIF11: Kinesin Family Member 11

CCNB2: Cyclin B2

BUB1B: BUB1B Mitotic Checkpoint Serine/Threonine Kinase B

TYMS: Thymidylate Synthetase

EZH2: Enhance of Zeste 2 Polycomb Repressive Complex 2 Subunit

BUB1: BUB1 Mitotic Checkpoint Serine/Threonine Kinase

ESR1: Estrogen Receptor 1
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Mehrdad Ameri, Haniye Salimi, Sedigheh Eskandari, Navid Nezafat participated in data analysis and writing the manuscript, discussing data and supervised the study; and all authors performed data analysis and interpretation and read and approved the final manuscript.

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Ethics approval and consent to participate

Availability of data and materials

The datasets analyzed during the current study are available in the GEO repository, (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62232).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
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