Identification of Core Genes Related with Trophinin-Associated Protein (TROAP) Expression in Liver Cancer

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Research article

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Abstract

**Background:** Liver cancer is one of the malignant tumors with the highest incidence in the world. Trophinin-associated protein (TROAP) was related with prognosis in liver cancer. However, the core genes associated with TROAP have not been identified yet.

**Methods:** In this study, we performed *in vitro* cell experiments and bioinformatics analysis including screening of differentially expressed genes (DEGs), gene ontology (GO) enrichment analysis, and Kyoto Encyclopedia of Gene and Genome (KEGG) enrichment analysis. Hub genes with high degree of connectivity was picked out by establishing protein-protein interaction (PPI) network.

**Results:** Our *in vitro* cell experiments suggested that down regulation of TROAP inhibited the proliferation and migration of liver cancer cells. A total of 20530 genes were analyzed and 953 differential expressed genes including 529 up-regulated DEGs and 424 down-regulated DEGs were detected. 10 hub genes with higher degree of connectivity including BUB1B, TOP2A, KIF23, UBE2C, KIF15, CDC20, PLK1, HJURP, BUB1, and DLGAP5 were selected.

**Conclusions:** Our study may provide some evidence for the future genomic individualized treatment of liver cancer.

Introduction

Liver cancer is one of the malignant tumors with the highest incidence in the world[1, 2]. Although there are various treatments available, the mortality rate of liver cancer is still high, and it is the second leading cause of cancer-related deaths in the world[2–4]. The morbidity and mortality rates of liver cancer are particularly high in China[4]. The poor prognosis of liver cancer may be related to many factors, such as lack of reliable tumor biomarkers, less understanding of tumor genetic and epigenetic changes, and insufficient research on the mechanism[5–7]. Thus, we focus on the identification of core genes in liver cancer using bioinformatics analysis.

Trophinin-associated protein (TROAP) also known as tastin is a cytoplasmic protein, involved in cell adhesion with association with bystin and trophinin[8]. Besides, TROAP plays a role in embryo implantation and regulation of proper spindle assembly during mitosis[9]. In structure, TROAP is rich of proline containing three domains with 778 amino acid residues[10, 11]. TROAP gene, located in chromosome 12q13.12, encodes the TROAP protein[12].

TROAP was involved in the progression of different cancers, including ovarian carcinoma[13], gastric cancer[14], and colorectal cancer[15]. Our previous study and other studies have found the TROAP was related with prognosis in liver cancer[12, 16]. However, the core genes associated with TROAP have not been identified yet. In this study, we performed *in vitro* cell experiments and bioinformatics analysis including screening of differentially expressed genes, GO enrichment analysis, and KEGG enrichment analysis. Then, hub genes with high degree of connectivity was picked out by establishing PPI network.
Methods

Microarray data

RNA-sequencing (RNA-Seq) expression data was downloaded from TCGA database using R package[17].

Cell culture

HepG2 cell line was purchased from ATCC. Dulbecco's modified Eagle's medium (DMEM) medium with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin Solution was used for cell culture.

RT-qPCR

Total RNA was extracted from HepG2 cell line, and then was reverse transcripted into cDNA. The cDNA was amplified by PCR using PCR Master Mix kit (Tiangen, Beijing, China), and quantitative real-time PCR was performed using SYBR Green kit (Applied Biosystems, CA, USA). The relative mRNA expression level of TROAP were analyzed using the $2^{-\Delta\Delta Ct}$ method[18]. The primer sequences were as follows:

TROAP:
Forward: 5′-GGTCAGGAGAAAAGCGGAGGAAG-3′
Reverse: 5′-AGGCGTGCGTTTCTGAGAGC-3′

β-actin:
Forward: 5′-GTGGACATCCGCAAAGAC-3′
Reverse: 5′-AAAGGGTGTAACGCAACTA-3′

Cell proliferation assay

The cells were treated with plasmids and cultured for 24 h. Then, 10 µL of CCK-8 reagent was added and cultured for 20 min. A microplate reader was used to measure the absorbance at 490 nm. The cell viability was calculated relative to the untreated control.

Colony formation assay

The HepG2 cells were seeded at the density of 1 × 10^6 cells per well, and cultured for 24 h. After treatment, the cells were incubated for 96 h. Then, the cells were stained by Giemsa reagent and photographed.

Wound healing assay

Wound-healing assays were performed as previously described[19]. The migration of cells toward the wound was photographed under a Nikon fluorescence microscope.

GO analysis and KEGG pathway analysis
To annotate genes and identify characteristic biological attributes, Gene ontology (GO) analysis was carried out[20].

To deal with genomes, biological pathways, diseases, drugs, and chemical substances, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was carried out[21].

**PPI network and module analysis**

To evaluate the protein–protein interaction (PPI) information, an online tool called Search Tool for the Retrieval of Interacting Genes (STRING) was used[22]. Moreover, 10 hub genes were mapped.

**Statistical Analysis**

Data were analyzed using independent two-tailed t-test, and all *in vitro* cell experiments were performed in triplicate and the data was presented as mean ± standard deviation. Values of $P < 0.05$ were considered statistically significant.

**Results**

**Down regulation of TROAP inhibits the proliferation and migration of liver cancer cells**

As shown in Fig. 1A, the liver cancer cells (HepG2) treated with siTROAP1, siTROAP2 and siTROAP3 showed decreased mRNA expression of TROAP. Of note, the siTROAP3 showed the best effect and was used (as siTROAP) for the subsequent cell experiments. As shown in Fig. 1B, the cells treated with siTROAP showed lower proliferation than control and NC ($P < 0.05$). As shown in Fig. 1C and 1D, the result of colony formation was in consistent with the proliferation. Furthermore, the migration of cells treated with siTROAP was inhibited in comparison with control and NC (Fig. 1E and 1F; $P < 0.05$). Therefore, our *in vitro* cell experiments suggested that down regulation of TROAP inhibited the proliferation and migration of liver cancer cells.

**Identification of differential expressed genes**

A total of 20530 genes were analyzed and 953 differential expressed genes were detected (Table 1). As shown in Fig. 2A, the number of up gene was 529 and the number of down gene was 424 with the cutoff logFC set as 1.315. Based on the result of differential expressed genes (DEGs) analysis, top 50 DEGs were selected shown in Fig. 2B.

**GO function analysis**

To better understand the selected DEGs in depth, GO function analysis were performed. As shown in Fig. 3A, all DEGs (up and down regulated DEGs) were enriched in biological processes (BP), including mitotic cell cycle phase transition, organelle fission, nuclear division, chromosome segregation, mitotic nuclear division, positive regulation of cell cycle process, nuclear chromosome segregation, sister chromatid segregation, mitotic sister chromatid segregation, and regulation of chromosome segregation.
As shown in Fig. 3B, all DEGs were enriched in cell component (CC), including chromosomal region, spindle, chromosome centromeric region, condensed chromosome, kinetochore, condensed chromosome centromeric region, condensed chromosome kinetochore, blood microparticle, high-density lipoprotein particle, and condensed chromosome outer kinetochore. As shown in Fig. 3C, all DEGs were enriched in molecular function (MF), including cofactor binding, iron ion binding, monooxygenase activity, oxidoreductase activity, heme binding, tetrapyrrole binding, DNA-dependent ATPase activity, and steroid hydroxylase activity. Moreover, the up regulated DEGs enriched in BP, CC and MF were shown in Fig. 4A, 4B and 4C respectively. The down regulated DEGs enriched in BP, CC and MF were shown in Fig. 4D, 4E and 4F respectively.

**KEGG pathway enrichment analysis**

To further understand the selected DEGs in depth, KEGG pathway enrichment analysis was performed. As shown in Fig. 5A, the most significantly enriched KEGG pathway of the up-regulated and down-regulated DEGs were picked out. The up-regulated DEGs were enriched in cell cycle, oocyte meiosis, Fanconi anemia pathway, DNA replication, MicroRNAs in cancer, p53 signaling pathway, homologous recombination, progesterone-mediated oocyte maturation, cellular senescence, etc. The down-regulated DEGs were enriched in retinal metabolism, drug metabolism, chemical carcinogenesis, metabolism of xenobiotics, complement and coagulation cascades, bile secretion, etc.

Furthermore, KEGG pathway enrichment analysis together with Gene Set Enrichment Analysis (GSEA) was carried out and the result was shown in Fig. 5B. The up-regulated DEGs were enriched in Herpes simplex virus 1 infection, cellular senescence, MicroRNAs in cancer, Ribosome, oocyte meiosis, human T-cell leukemia virus 1 infection, viral carcinogenesis, RNA transport, and alcoholism. The down-regulated DEGs were enriched in AMPK signaling pathway, vascular smooth muscle contraction, JAK-STAT signaling pathway, cytokine-cytokine receptor interaction, insulin signaling pathway, fluid shear stress and atherosclerosis, cGMP-PKG signaling pathway, osteoclast differentiation, non-alcoholic fatty liver disease (NAFLD), cell adhesion molecules (CAMs), and FoxO signaling pathway.

**Hub genes and module screening from PPI network**

As shown in Fig. 6A and 6B, the information in the STRING protein query was gathered from public database. Furthermore, the PPI network of the top 10 hub genes with higher degree of connectivity was made (Fig. 6C). We selected BUB1B, TOP2A, KIF23, UBE2C, KIF15, CDC20, PLK1, HJURP, BUB1, and DLGAP5 as core genes.

**Discussion**

TROAP is a significantly high expression molecule in liver cancer. Our previous study demonstrated that high TROAP expression is an independent predictor of poor survival in liver cancer and related to T stage and clinical stage in HCC[16]. The molecular functions of TROAP include small G protein, nucleotide transferase, non-receptor serine/threonine protein activating enzyme, protein-related calcineurin, KRAB
box transcription factor. The TROAP is involved in the biological processes including DNA repair, mRNA transcription regulation, tRNA metabolism, cyclic nucleotide metabolism, signal transduction, evolutionary process and cell structure (DAVID)[10]. However, the construction of the TROAP network in liver cancer remains to be studied.

We found that down regulation of TROAP inhibited the proliferation and migration of liver cancer cells by \textit{in vitro} cell experiments. To further study the mechanism of how TROAP is related with prognosis of liver cancer, we performed a series of bioinformatics analysis. After screening of differentially expressed genes, we found 529 up-regulated genes and 424 down-regulated genes. For a more in-depth understanding of these DEGs, we performed GO function and KEGG pathway analysis of these DEGs. It was shown that up-regulated genes were mainly involved in mitotic cell cycle phase transition, chromosomal region, and ATPase activity, and down-regulated DEGs were involved in small molecule catabolic process, extracellular matrix, and cofactor binding. Furthermore, the KEGG pathways of up-regulated DEGs included Herpes simplex virus 1 infection, cellular senescence, MicroRNAs in cancer, Ribosome, oocyte meiosis, human T-cell leukemia virus 1 infection, viral carcinogenesis, RNA transport, and alcoholism. Meanwhile, the down-regulated DEGs were enriched in AMPK signaling pathway, vascular smooth muscle contraction, JAK-STAT signaling pathway, cytokine-cytokine receptor interaction, insulin signaling pathway, fluid shear stress and atherosclerosis, cGMP-PKG signaling pathway, osteoclast differentiation, non-alcoholic fatty liver disease (NAFLD), cell adhesion molecules (CAMs), and FoxO signaling pathway. Among these DEGs, 10 hub genes with high degree of connectivity were selected by establishing PPI network.

BUB1B has been reported to play a role in checking proper chromosome segregation and prevent separation of the duplicated chromosomes in normal cells[23]. High expression of BUB1B is associated with progression of liver cancer and poor survival of in hepatocellular carcinoma patients[24]. High level of TOP2A is mainly detected in G2/M cell cycle of rapidly proliferated cells[25]. Overexpression of TOP2A may lead to aberrant cell proliferation, aneuploidy, an aggressive tumor phenotype, advanced disease stage, tumor recurrence, and decreased overall survival[25, 26]. CDC20 plays an important role in chromosome segregation and mitotic exit, and regulates APC/C ubiquitin activity for degradation of specific substrates[27]. CDC20 is regarded as a promising therapeutic target for combating human cancer contributing to its critical function in cell cycle progression, apoptosis, and ciliary disassembly[28]. The kinesin superfamily, including KIF15 and KIF23, was involved in several essential cellular processes including mitosis, meiosis, and the transport of macromolecules, thus being a novel therapeutic target for treatment of liver cancer [29, 30]. PLK1 acts as a pivotal regulator of mitosis and cytokinesis in eukaryotes[31, 32]. Besides, PLK1 regulates the activity of BUB1 and its pseudokinase paralog BUBR1 to coordinate spindle checkpoint activation and inactivation[31]. DLGAP5 as a mitotic spindle protein promotes the formation of tubulin polymers[33]. Liao et al. reported the up-regulation of DLGAP5 contributes to hepatocellular carcinoma tumorigenesis by promoting cell proliferation[34].

\textbf{Conclusions}
In conclusion, this study identified DEGs related to TROAP expression in liver cancer by bioinformatics analysis. 953 DEGs and 10 hub genes were selected. BUB1B, CDC20, KIF15, KIF23, PLK1 and etc. may be the core genes of liver cancer. Our study may provide some evidence for the future genomic individualized treatment of liver cancer.

**Abbreviations**

TROAP: Trophinin-associated protein  
DEGs: Differentially expressed genes  
GO: gene ontology  
KEGG: Kyoto Encyclopedia of Gene and Genome  
PPI: protein-protein interaction  
GSEA: Gene set enrichment analysis  
TCGA: The Cancer Genome Atlas

**Declarations**

**Ethics approval and consent to participate**  
The data of this study are from public database, and do not involve animal experiments and human specimens, no ethics-related issues.

**Consent for publication**  
All authors support publishing.

**Availability of data and materials**  
The data of this study are from TCGA database.

**Competing interests**  
The authors declare that they have no competing interests.

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Authors’ contributions

BJ and YL contributed to the conception of the study. HC and YJ performed the data analyses. YJ contributed significantly to process data. BJ and HC wrote the manuscript. All of the authors read and approved the final manuscript.

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### Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

### Figures
Down regulation of TROAP inhibits the proliferation and migration of liver cancer cells. (A) the liver cancer cells (HepG2) treated with siTROAP1, siTROAP2 and siTROAP3 showed decreased mRNA expression of TROAP. (B) The cells treated with siTROAP showed lower proliferation than control and NC (P < 0.05). (C) Colony formation assay and (D) quantification. (E) The migration of cells treated with siTROAP was inhibited in comparison with control and NC, and (F) quantification. The experiments were repeated for three times. NC, negative control; NS, no significance; * P < 0.05.
Figure 2

Identification of differential expressed genes. (A) The number of up gene was 529 and the number of down gene was 424 with the cutoff logFC set as 1.315. (B) Top 50 DEGs were selected.
Figure 3

GO function analysis. All DEGs including up and down regulated DEGs were enriched in (A) biological processes (BP), (B) cell component (CC), and (C) molecular function (MF).
Figure 4

GO function analysis. The up regulated DEGs were enriched in (A) biological processes, (B) cell component, and (C) molecular function. The down regulated DEGs were enriched in (D) biological processes, (E) cell component, and (F) molecular function.

Figure 5

Pathway Enrichment

Pathway names

Herpes simplex virus 1 infection
Cytokine-cytokine receptor interaction
CAMP-PKG signaling pathway
Oxidative phosphorylation
Cytokine-cytokine receptor interaction
JAK-STAT signaling pathway
KEGG pathway enrichment analysis. (A) The most significantly enriched KEGG pathway of the up-regulated and down-regulated DEGs. (B) KEGG pathway enrichment analysis together with Gene Set Enrichment Analysis (GSEA) of the up-regulated and down-regulated DEGs.

**Figure 6**

Hub genes and module screening from PPI network. (A) and (B) The information in the STRING protein query was gathered from public database. (C) The PPI network of the top 10 hub genes with higher degree of connectivity.

**Supplementary Files**

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- Table1.csv