

Potential candidate treatment agents for targeting of cholangiocarcinoma identified by gene expression profile analysis

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Abstract. Cholangiocarcinoma (CCA) remains to be a major health problem in several Asian countries including Thailand. The molecular mechanism of CCA is poorly understood. Early diagnosis is difficult, and at present, no effective therapeutic drug is available. The present study aimed to identify the molecular mechanism of CCA by gene expression profile analysis and to search for current approved drugs which may interact with the upregulated genes in CCA. Gene Expression Omnibus (GEO) was used to analyze the gene expression profiles of CCA patients and normal subjects. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG), gene ontology enrichment analysis was also performed, with the KEGG pathway analysis indicating that pancreatic secretion, protein digestion and absorption, fat digestion and absorption, and glycerolipid metabolism may serve important roles in CCA oncogenesis. The drug signature database (DsigDB) was used to search for US Food and Drug Administration (FDA)-approved drugs potentially capable of reversing the effects of the upregulated gene expression in CCA. A total of 61 antineoplastic and 86 non-antineoplastic drugs were identified. Checkpoint kinase 1 was the most interacting with drug signatures. Many of the targeted protein inhibitors that were identified have been approved by the US-FDA as therapeutic agents for non-antineoplastic diseases, including cimetidine, valproic acid and lovastatin. The current study demonstrated an application for bioinformatics analysis in assessing the

potential efficacy of currently approved drugs for novel use. The present results suggest novel indications regarding existing drugs useful for CCA treatment. However, further *in vitro* and *in vivo* studies are required to support the current predictions.

Introduction

Cholangiocarcinoma (CCA) is the second most common hepatobiliary cancer after hepatocellular carcinoma and accounts for 10-15% of all liver carcinomas (1,2). It arises in the biliary tract epithelium (cholangiocyte) and is categorized into three groups: Intrahepatic, perihilar and distal hepatic carcinoma. Previous studies in the US and worldwide have demonstrated that the associated mortality and incidence rates have increased since 2002 (3,4). Clinically, CCA is a challenge to treat as patients often do not exhibit clear symptoms, making early diagnose difficult (5). At present, there is no specific molecular targeting treatment for CCA. Conventional chemotherapy is still used but is generally ineffective in cases of CCA associated with different genetic variants (6). A greater understanding of the biology of CCA may improve treatment and diagnosis.

Recent research has focused on target identification and selective inhibitors of several molecular signaling pathways (7). In earlier reports, genetic alterations were identified to be involved in CCA carcinogenesis, including point mutations in p53, B-Raf proto-oncogene serine/threonine kinase and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (8,9). Liver-specific downregulation of Smad family member 4 and phosphatase and tensin homolog, as established tumor suppressor genes, may also induce CCA (10,11). In addition, increased expression of cyclooxygenase-2, receptor tyrosine-protein kinase c-erbB-2 and interleukin 6 are considered to be involved in early carcinogenesis in the biliary tract epithelium (12,13).

Although a variety of anticancer protein inhibitors, including Notch, Wnt, growth factor receptor and histone deacetylase inhibitors, are being developed to suppress oncoproteins, an average time of 7 years is typically required for drug approval

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following clinical trial evaluation (14). However, it has been demonstrated that certain drugs already approved by the US Food and Drug Administration (FDA) may be used to suppress cancer. For example, inhibitors of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, including lovastatin, atorvastatin and simvastatin, and of cyclooxygenase-2 (COX-2), including celecoxib, have potential preventative effects on CCA carcinogenesis (15,16). Identifying novel uses for the approved drugs also minimizes the need for toxicity studies, particularly with regard to non-antineoplastic drugs.

The study of cancer genomes, and CCA mechanisms in particular, is still in its infancy. Single gene studies are no longer feasible for this complex disease. Improved and more powerful tools for analysis by high-throughput array are required to provide greater accuracy, precision and cost-effectiveness. Gene Expression Omnibus (GEO) is a public functional genomics data repository created to aid the identification and analysis of gene regulatory and cancer signaling pathways. By also serving as a gene-drug interaction database, this may further be used to determine novel therapeutic drugs and targets that may be crucial to the identification of novel protein inhibitors.

In the present study, gene expression profiles from the GSE26566 dataset were collected to analyze differential gene expression, and currently approved drugs associated with the upregulated genes were searched in drug interaction databases. The greater availability of gene expression analytical tools and improved access to information on currently approved treatments may be useful in developing novel indications for CCA drug treatment.

Materials and methods

Data collection. The gene expression dataset of normal subjects and intrahepatic CCA (iHCCA) patients [Gene Expression Omnibus (GEO) accession no. GSE26566 (17)] were obtained from the National Center for Biotechnology Information GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). A total of 104 cholangiocarcinoma and 6 normal intrahepatic bile duct gene expression data profiles were collected. This microarray platform (GPL6104) was collected from one type of platform: Illumina humanRef-8 v2.0 expression beadchip.

Gene expression analysis. The microarray database GPL6104 platform was analyzed using GEO2R (version 2.14.1) for the collected datasets. The data was normalized by log 2 transformation in GEO2R along with the Benjamini-Hochberg procedure for false discovery rate correction. Samples were assigned to normal and cholangiocarcinoma subject groups. Following analysis by GEO2R, the dataset was displayed in terms of adjusted P-values, P-values, log fold changes, gene symbols and gene names. The top 15 most up- and downregulated genes according to most adjusted P-value (from Student's t-test, $P < 0.05$) were selected as differentially expressed genes (DEGs).

Gene ontology (GO) analysis. To investigate the DEGs at a functional level, the Database for Annotation, Visualization and Integrated Discovery (DAVID; <https://david.ncifcrf.gov/>) was used to establish gene clustering following GO analysis, as described previously (18). The GO was categorized into

cellular component, biological process and molecular function following standard GO analysis as detailed previously (19).

Pathway analysis. The Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/>) was accessed for assistance in investigating the dysregulated signaling pathways in cholangiocarcinoma as detailed previously (20). The DEGs were searched in the database for investigation of biochemistry pathways that may be involved in the tumorigenesis and development of cholangiocarcinoma. The up- and downregulated genes were analyzed by DAVID. The significant categories were predicted by an Expression Analysis Systematic Explorer score < 0.01 and the minimum number of genes for the corresponding term > 2 were considered.

Protein-protein interaction (PPI) analysis. PPI network analysis was conducted by using the search tool for the retrieval of interacting genes/proteins (STRING; <http://string-db.org/>) as in previous study (21). The threshold protein interaction reliability score was 0.400, which represents a medium level of confidence (22).

Validation by The Cancer Genome Atlas (TCGA) Kaplan-Meier-plotter analysis. To confirm the analysis, an overall survival (OS) Kaplan-Meier computation was performed by using the top 30 DEGs (15 most upregulated and 15 most downregulated). Expression and OS data of the iHCCA cohort in the TCGA database was assessed and P-values obtained by log-rank test using the cBioPortal for Cancer Genomics (<http://www.cbioportal.org/>) (23-25).

Gene-drug interaction analysis. The drug signatures of upregulated genes and their interacting genes were identified by searching the drug signatures database for gene set analysis (DSigDB) (26). The signatures of the database are grouped into four categories: i) 1,202 FDA-approved drugs retrieved for bioactivity assay results from PubChem and ChEMBL; ii) 1,220 kinase inhibitors from human kinome profiling databases (Medical Research Council Kinase Inhibitor database and Harvard Medical School Library of Integrated Network-based Cellular Signatures database); iii) 1,309 perturbagens identified from DEGs in the three cell lines PC3 (prostate cancer), HL60 (leukemia) and SKMEL5 (melanoma) (with > 2 -fold change compared with controls); and iv) drug candidates identified by manual curation and text mining from the Therapeutics Targets Database and the Comparative Toxicogenomics Database (27-29). The drugs were further categorized according to the US-FDA database (<https://www.accessdata.fda.gov/scripts/cder/daf/>) as FDA-approved non-antineoplastic and neoplastic drugs.

Results

Gene expression analysis. The dataset retrieved from GEO26566 included 104 iHCCA and 6 normal profiles. The top 15 most significantly up- and downregulated genes, respectively, determined by integrative analysis in GEO2R, are listed in Table I. FYVE, RhoGEF and PH domain-containing 6 (*FGD6*) and chymotrypsinogen B2 (*CTRB2*) were the most significantly up- and downregulated genes, respectively (Table I).

Table I. Top 30 most significantly up- and downregulated differentially expressed genes.

A, Upregulated genes				
Gene probe ID	Gene symbol	Description	P-value	Log fold change
154240685	<i>FGD6</i>	FYVE, RhoGEF and PH domain-containing 6	9.58E-09	3.86
349501059	<i>CHEK1</i>	Checkpoint kinase 1	3.22E-07	4.34
61743966	<i>CTBP1</i>	C-terminal-binding protein 1	3.29E-07	3.63
168229167	<i>BUB1B</i>	BUB1 mitotic checkpoint serine/threonine kinase B	6.76E-07	5.38
154800452	<i>TROAP</i>	Trophinin-associated protein	7.45E-07	5.41
157419137	<i>LAMC2</i>	Laminin subunit gamma 2	1.57E-06	4.8
19882252	<i>CST2</i>	Cystatin-SA	1.77E-06	4.71
748821156	<i>CKAP2L</i>	Cytoskeleton-associated protein 2-like	2.44E-06	3.93
300794717	<i>WDHD1</i>	WD repeat and HMG-box DNA-binding protein 1	4.42E-06	4.05
938148819	<i>MMP11</i>	Matrix metalloproteinase 11	1.23E-05	6.10
444189305	<i>FAM83B</i>	Family with sequence similarity 83 member B	3.77E-05	5.82
54607054	<i>GJB3</i>	Gap junction protein beta 3	1.36E-04	4.90
148806907	<i>FNDC1</i>	Fibronectin type III-domain containing 1	1.45E-04	4.41
379056370	<i>CDCA8</i>	Cell division cycle-associated 8	1.66E-04	4.01
748983174	<i>CNBD2</i>	Cyclic nucleotide-binding domain-containing 2	2.68E-04	6.61
B, Downregulated genes				
Gene probe ID	Gene symbol	Description	P-value	Log fold change
118498349	<i>CTRB2</i>	Chymotrypsinogen B2	8.80E-34	-10.15
236459772	<i>CELA2A</i>	Chymotrypsin-like elastase family member 2A	3.21E-29	-9.71
54607079	<i>CPB1</i>	Carboxypeptidase B1	9.23E-29	-10.98
38016927	<i>PLA2G1B</i>	Phospholipase A2 group IB	8.53E-25	-10.78
747165370	<i>CPA1</i>	Carboxypeptidase A1	1.09E-21	-11.95
58331210	<i>CELA2B</i>	Chymotrypsin-like elastase family member 2B	3.17E-20	-8.90
440309868	<i>CEL</i>	Carboxyl ester lipase	5.85E-18	-9.68
734703929	<i>PNLIPRP1</i>	Pancreatic lipase-related protein 1	1.14E-14	-8.37
236460049	<i>CELA3A</i>	Chymotrypsin-like elastase family member 3A	2.42E-14	-10.80
357588512	<i>CLPS</i>	Colipase	1.88E-12	-12.09
310923201	<i>PRSS1</i>	Protease, serine 1	2.48E-10	-11.52
217416389	<i>CPA2</i>	Carboxypeptidase A2	3.24E-10	-11.27
62526042	<i>CTRC</i>	Chymotrypsin 2	7.93E-09	-10.82
1010226556	<i>CELA3B</i>	Chymotrypsin-like elastase family member 3B	8.45E-07	-11.10
1042779786	<i>PNLIP</i>	Pancreatic lipase	1.22E-06	-12.04

Functional enrichment analysis. GO enrichment analysis of DEGs was performed for the top 30 most significant DEG signatures (15 most upregulated and 15 most downregulated). The DEGs were classified into the three GO categories of biological process, cellular component and molecular function. It was identified that the significantly enriched GO terms for biological process included proteolysis and lipid catabolic processes, while those for cellular component included extracellular space and extracellular region; the significantly enriched GO terms for molecular function included serine-type endopeptidase activity and triglyceride lipase activity (Table II).

Pathway enrichment analysis based on the KEGG database demonstrated that the DEGs were significantly enriched in

4 terms (Table III). The most significant pathway was pancreatic secretion ($P=1.6E-17$).

PPI network analysis. To investigate the associations between the DEGs and signaling pathways, a comprehensive analysis of protein interactions of the top 15 up- and downregulated genes, respectively, was conducted using the STRING database. As illustrated in Fig. 1, direct or indirect interactions were identified with the exception of 12 genes [Forkhead box p1, ADAM metalloproteinase domain 15, FGD6, sentrin-specific-peptidase 3, fermitin family member 2, cystatin-SA, pinin, Snail family transcriptional repressor 2, fibronectin type III domain containing 1 (*FNDC1*), family with sequence similarity 83 member B (*FAM83B*), DIS3-like 3'-5' exonuclease 2 and gap junction

Table II. Enriched GO categories of differentially expressed genes.

A, Biological process clustering				
GO term	Biological process	Gene count	Genes	P-value
GO:0006508	Proteolysis	11	CPA1, CPA2, CPB1, CTRC, CELA2A, CELA2B, CELA3A, CELA3B, CTRB2, MMP11, PRSS1	1.7E-09
GO:0007586	Digestion	4	CELA3A, CTRB2, CLPS, PRSS1	1.2E-04
GO:0044241	Lipid digestion	3	CEL, CLPS, PNLIP	1.8E-04
GO:0022617	Extracellular matrix	4	CTRB2, LAMC2, MMP11, PRSS1	2.1E-04
GO:0016042	Lipid catabolic process	4	CLPS, PNLIPRP1, PNLIP, PLA2G1B	3.0E-04
GO:0009235	Cobalamin metabolic process	3	CTRC, CTRB2, PRSS1	4.8E-04
GO:0030299	Intestinal cholesterol absorption	2	CEL, PNLIP	1.4E-02
GO:0006629	Lipid metabolic process	3	CEL, CLPS, PNLIPRP1	2.4E-02
GO:0007093	Mitotic cell cycle checkpoint	2	BUB1B, CHEK1	4.8E-02
GO:0001523	Retinoid metabolic process	2	CLPS, PNLIP	9.0E-02
B, Cellular component				
GO term	Biological process	Gene count	Genes	P-value
GO:0005615	Extracellular space	14	CEL, CPA1, CPA2, CPB1, CHEK1, CELA2A, CELA2B, CELA3A, CELA3B, CTRB2, CST2, LAMC2, PNLIPRP1, PLA2G1B	4.1E-08
GO:0005576	Extracellular region	13	CEL, CPA2, CTRC, CELA2B, CTRB2, CLPS, FNDC1, LAMC2, MMP11, PNLIPRP1, PNLIP, PLA2G1B, PRSS1	2.7E-06
GO:0051233	Spindle midzone	2	BUB1B, CDCA8	3.0E-02
C, Molecular function				
GO term	Biological process	Gene count	Genes	P-value
GO:0004252	Serine-type endopeptidase activity	8	CTRC, CELA2A, CELA2B, CELA3A, CELA3B, CTRB2, MMP11, PRSS1	1.1E-07
GO:0004806	Triglyceride lipase activity	3	CEL, PNLIPRP1, PNLIP	3.3E-04
GO:0004181	Metal carboxypeptidase activity	3	CPA1, CPA2, CPB1	8.4E-04
GO:0016298	Lipase activity	2	CEL, PNLIPRP1	1.4E-02
GO:0004180	Carboxypeptidase activity	2	CPA2, CPB1	2.7E-02
GO:0008236	Serine-type peptidase activity	2	CTRB2, PRSS1	9.6E-02
GO, gene ontology.				

protein beta 3 (*GJB3*)). The associations between pathways and gene expression were categorized into 5 pathways, namely pancreatic secretion, protein digestion and absorption, lipid digestion and absorption, cell cycle and p53 signaling pathway.

Validation by TCGA Kaplan-Meier-plotter analysis. For validation of altered gene signatures using clinical data, CCA data was retrieved from the TCGA database (n=36). Cases were divided into those with alterations in the previously identified DEGs (n=20) and those without (13). The Kaplan-Meier curve demonstrated that the altered genes group was associated with a significantly lower survival rate ($P=0.0947$; Fig. 2).

Assessment of gene-drug interaction. Drug signatures from drug-upregulated gene interactions in DsigDB were classified, based on known FDA-approved targeted agents including neoplastic and non-neoplastic drugs. A total of 86 non-neoplastic and 61 neoplastic drugs were identified. Among the top upregulated genes, *CHEK1* was the most interacting with drug signatures. No drug signatures were identified for *CST2*, *MMP11* or cyclic nucleotide binding domain-containing 2 (Table IV). The interacting anticancer drugs belonged to 6 categories in the FDA database, namely alkylating agent, hormone agent, cytotoxic alkaloid, antitumor antibiotic, antimetabolite and protein inhibitor (Table V).

Table III. Enriched Kyoto Encyclopedia of Genes and Genomes pathways of differentially expressed genes.

Term	Pathway	P-value	Gene count	Genes
Hsa:04972	Pancreatic secretion	1.6E-17	12	<i>CEL, CPA1, CPA2, CPB1, CELA2A, CELA2B, CELA3A, CELA3B, PNLIPRP1, PNLIP, PLA2G1B, PRSS1</i>
Hsa:04972	Protein digestion and absorption	7.5E-10	8	<i>CPA1, CPA2, CPB1, CELA2A, CELA2B, CELA3A, CELA3B, PRSS1</i>
Hsa:04975	Fat digestion and absorption	2.0E-06	5	<i>CEL, CLPS, PNLIPRP1, PNLIP, PLA2G1B</i>
Hsa:00561	Glycerolipid metabolism	8.7E-03	3	<i>CEL, PNLIPRP1, PNLIP</i>

Hsa, *Homo sapiens*.

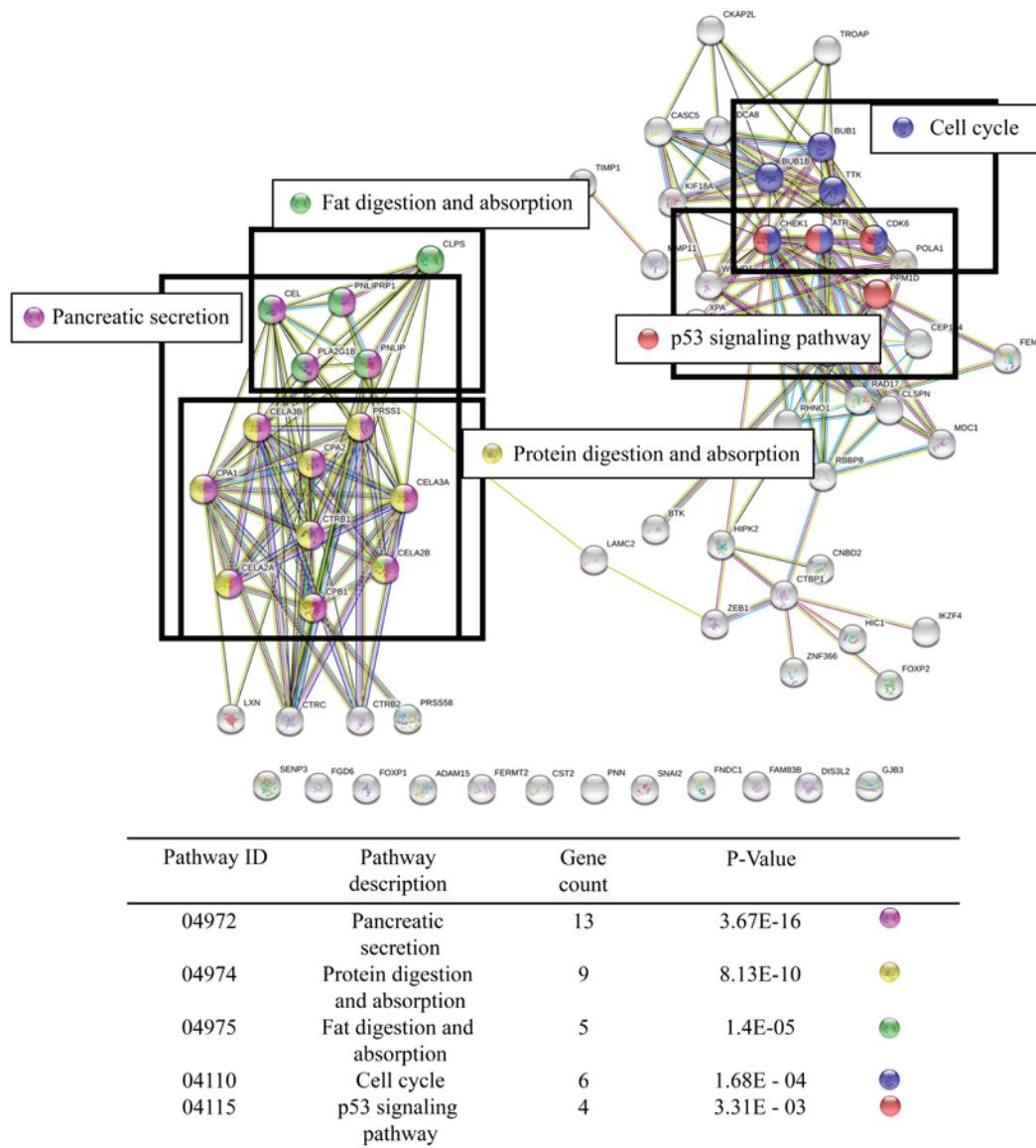


Figure 1. Protein-protein interaction network for differentially expressed genes. The genes with no direct interaction with any other partner are the co-expression genes of the genes of interest.

Discussion

Although previous study has suggested numerous biomarkers

and therapeutic targets for CCA (30), there remains to be few treatments and drugs available to overcome the disease. In addition, the molecular mechanisms of CCA are not fully

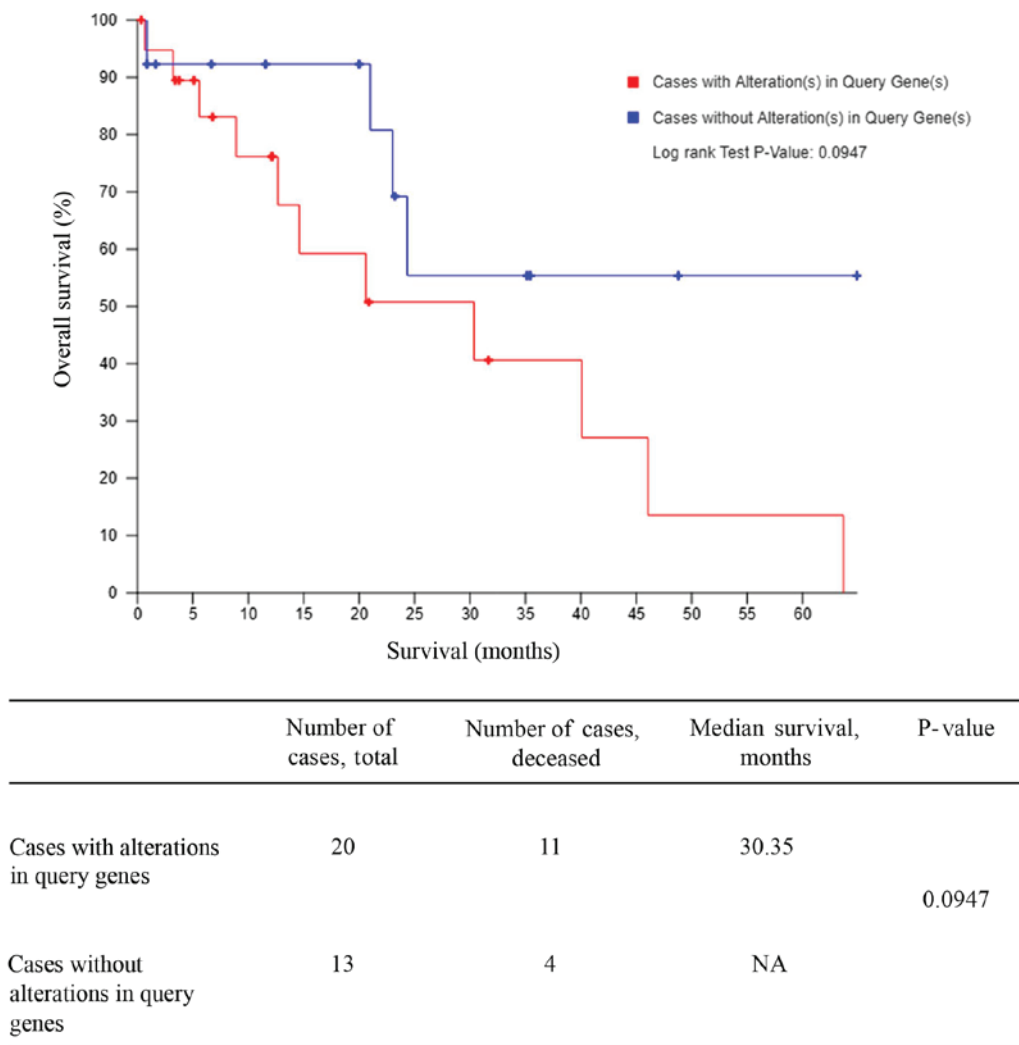


Figure 2. Kaplan-Meier survival curves for the association between the gene alteration of differentially expressed genes and the overall survival time of patients with cholangiocarcinoma.

understood. In the present study, gene expression profiles and dysregulated pathways of CCA were analyzed, along with gene-drug interactions for drug repositioning.

In the present study, gene expression analysis was performed on a CCA patient dataset. The analysis firstly determined the top 30 DEGs that may be important for CCA carcinogenesis. Checkpoint kinase 1 (CHEK1) is required for DNA replication and cell survival in cancer (31). BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) and cell division cycle-associated 8 (CDCA8) have been identified to be involved in cell division and the cell spindle check-point. Increased expression in progressive cholangiocarcinoma has also been identified (32-34). A previous study observed that matrix metalloproteinase 11 (MMP11) was upregulated in CCA patients with poor prognosis. Therefore, MMP11 may be useful as a prognostic marker in CCA (35-36).

GO and KEGG pathway analysis demonstrated that the main biological processes involved in CCA were extracellular matrix and protein and lipid metabolism. A previous study of bile acid identified downregulated genes including CTRB, colipase, chymotrypsin-like elastase family member 3A (*CELA3A*) and *CELA3B*, phospholipase A2, chymotrypsin 2, carboxypepti-

dase B (*CPB*) and CPA, similar to the present gene expression analysis. The profiles of these genes also differ markedly between CCA patients and normal subjects, and they may be protein markers for differentiating malignant from benign tumors (37). Integrative analysis of five microarrays in the GSE26566 dataset indicated that *MMP11* and *FGD6* were upregulated while *CELA2A* and *CTRB2* were downregulated. These genes were associated with the extracellular matrix, cell division processes, bile acid secretion and protein and lipid metabolism, with these biological processes implicated in various pathways in CCA progression (38). These results of DEG pathway analysis may be used to investigate and target the mechanism of disease initiation, to ultimately treat CCA, and to clarify the association between metabolic dysregulation and CCA progression.

Previous PPI analysis revealed an association of ataxia telangiectasia with Rad3-related protein/casein kinase 1 signaling in p53 activation, which serves an important role in DNA repair, cell division and the cell cycle (39-40). CTRB, a member of a family of serine proteases, is secreted into the gastrointestinal tract and activated through proteolytic cleavage by protease, serine 1. A previous study observed low-level expression of CTRB in pancreatic cancer (41). The

Table IV. Non-neoplastic and neoplastic drugs relating to upregulated genes in cholangiocarcinoma.

Gene symbol	Total drugs	Non-neoplastic drugs	Neoplastic drugs
<i>FGD6</i>	8	Zalcitabine, digoxin, raloxifene, retinoic acid	Vorinostat, tamoxifen, 5-fluorouracil, melphalan
<i>CHEK1</i>	44	Chlorzoxazone, dirithromycin, medrysone, latamoxef, clindamycin, ipratropium bromide, danazol, thymidine, caffeine, mucosolvan, lovastatin, troglitazone, rimonabant hydrochloride, menadione, retinoic acid, diclofenac, carbamazepine, valproic acid, folic acid, auraptan, zidovudine	Etoposide, bosutinib, sunitinib, daunorubicin, irinotecan, bortezomib, melphalan, mitomycin vorinostat, hydroxyurea, olaparib, 5-fluorouracil gemcitabine, temozolomide, doxorubicin, decitabine, carmustine, bortezomib, fludarabine, irinotecan, clofarabine, busulfan
<i>CTBP1</i>	18	Nifedipine, clofazimine, chlortetracycline, chlozoxazone, baclofen, levonorgestrel, cimetidine, ambroxol, medrysone, valproic acid, dorzolamide, clindamycin, ipratropium bromide, danazole, niclozamide, vitamin E, acetaminophen	Vorinostat
<i>BUB1B</i>	20	Ciclopirox, trifluridine, pyriminium, clindamycin, colchicine, piroxicam, acetaminophen, valproic acid	Daunorubicin, etoposide, azacitidine, irinotecan, fulvestrant, docetaxel, dasatinib, decitabine, doxorubicin, 5-fluorouracil, paclitaxel, bicalutamide
<i>TROAP</i>	11	Ciclopirox, trifluridine, pergolide, acetaminophen, valproic acid	Etoposide, azacitidine, methotrexate, doxorubicin, irinotecan, 5-fluorouracil
<i>LAMC2</i>	4	Valproic acid, mifepristone	Mechlorethamine, cyclophosphamide
<i>CST2</i>	0	-	-
<i>CKAP2L</i>	3	Retinoic acid, acetaminophen	Dasatinib
<i>WDHD1</i>	10	Zalcitabine, primaquine, doxycycline, latamoxef, cianidanol, retinoic acid, acetaminophen, valproic acid	Dasatinib, vorinostat
<i>MMP11</i>	0	-	-
<i>FAM83B</i>	1	Valproic acid	-
<i>JGB3</i>	5	Retigabine	Vorinostat, azacitidine, doxorubicin, decitabine
<i>FNDC1</i>	1	Valproic acid	-
<i>CDC48</i>	22	Chlortetracycline, chlorzoxazone, ciclopirox, trifluridine, cimetidine, medrysone, pyriminium, latamoxef, glibenclamide, metronidazole, monobenzene, piroxicam, retinoic acid, acetaminophen, zidovudine	Azacitidine, methotrexate, doxorubicin, irinotecan, 5-fluorouracil, vinblastine, cytarabine
<i>CNBD2</i>	0	-	-
Total	147	86	61

current analysis of CCA patient profiles revealed similar results. As concluded previously (41), this suggests that *CTRB* may be a genetic marker for CCA. Furthermore, since genes including carboxyl ester lipase, pancreatic lipase-related protein 1, pancreatic lipase and phospholipase A2 group IB associated with pancreatic secretion and fat digestion and absorption exhibited significant interaction, the tumorigenesis and progression of CCA may lead to the dysfunction of liver metabolism and disruption of pancreatic function.

According to the survival analysis, patients with CCA in the altered gene group had a lower rate of OS compared with those patients with no alterations in the DEGs of interest. Genetic alterations in cell metabolism may contribute to poor prognosis in human cancers (42). Lipid metabolism is now considered to have a key role in cancer progression; it has been

proposed that increased metabolic flux may serve as substrate source for phospholipid synthesis in the rapid growth stages of cancer (43).

The use of molecular signatures in cancer to determine potential drug therapies is a widespread technique (44). Potential drugs that target molecular aberrations in various CCA pathways have been proposed (45). In the present study, the genetic signatures of upregulated genes from microarray datasets were analyzed for gene-drug interactions, and US-FDA-approved drugs were grouped according to their FDA classification as antineoplastic and non-antineoplastic agents. The present study demonstrated the potential of various FDA approved antineoplastic drugs for use in CCA treatment. For example, vorinostat is a histone deacetylase inhibitor which has been approved by the FDA for the treatment of cutaneous

Table V. Categories of the US Food and Drug Administration-approved drugs associated with upregulated genes.

Category	Drug name
Alkylating agent	Melphalan, temozolomide, busulfan, carmustine, mechlorethamine, cyclophosphamide, busulfan
Hormone agent	Tamoxifen, fulvestrant, bicalutamide
Cytotoxic alkaloid	Etoposide, irinotecan, vinblastine, docetaxel, paclitaxel
Antitumor antibiotic	Doxorubicin, daunorubicin, mitomycin,
Antimetabolite	Methotrexate, 5-fluorouracil, clofarabine, gemcitabine, cytarabine, azacitidine, fludarabine, decitabine, hydroxyurea
Protein inhibitor	Vorinostat, bosutinib, sunitinib, bortezomib, olaparib

manifestations of cutaneous T-cell lymphoma; olaparib is a poly-ADP-ribose polymerase inhibitor approved for ovarian and breast cancer treatment; and sunitinib, a multitargeted receptor tyrosine kinase inhibitor, has been approved by the FDA for the treatment of renal cell carcinoma (46-48). Also dasatinib, a kinase inhibitor approved for use in patients with chronic myelogenous leukemia, may be used to treat CCA by targeting the *FGD6*, *CHEK1*, C-terminal binding protein 1 (*CTBPI*), cytoskeleton-associated protein 2-like and WD repeat and HMG-box DNA-binding protein 1 (*WDHDI*) genes. Sunitinib has been reported to have possible use in iHCCA chemotherapies, where resistance is an issue in advanced stage patients (49-51).

Several chemotherapeutic drugs may be used to treat bile duct cancer, for example, 5-fluorouracil, gemcitabine, cisplatin, capecitabine and oxaliplatin. The current standard treatment drugs for CCA are 5-fluorouracil, gemcitabine, or their combinations with cisplatin. In certain cases, two or more of these drugs may be combined. However, their treatment efficacy is unsatisfactory with low clinical response rate (52). A previous study observed survival of patients treated with cisplatin plus gemcitabine, there may be little benefit to patients because of the debilitating side effects, depending on the amount and frequency of the dosage (53,54). Nonetheless, CCA is a heterogeneous cancer involving multifactorial risks (55,56). The present results demonstrate that the other antineoplastic drugs, including protein inhibitors such as vorinostat, sunitinib and olaparib, and standard chemotherapy drugs such as melphalan, etoposide, and mitomycin, may be suitable candidates for drug repositioning for CCA and should be studied further.

The approach of the current study was to investigate established drugs to identify novel indications for cancer treatment. The results indicated that non-antineoplastic agents interacted with certain genes implicated in CCA. Cimetidine, a histamine type 2 antagonist, is used to treat heartburn and peptic ulcer; however, data also indicates that cimetidine may contribute to growth inhibition and apoptosis induction in CCA *in vitro* and *in vivo* (57). Valproic acid is used to treat bipolar disorders and prevent migraine headaches; previous study identified that it may inhibit the growth of CCA cell lines (TFK-1, QBC939 and CCLP1) by inducing cell cycle arrest and promoting cell differentiation via induction of dendrite-like structures (58). The effects of each drug may be mediated by several molecular mechanisms. For

example, valproic acid, an anti-epileptic drug, appears to act primarily by histone deacetylase inhibition, and not solely by inhibition of *CHEK1*, *CTBPI*, *BUB1B*, trophinin-associated protein, laminin subunit gamma 2, *WDHDI*, *FAM83B* and *FNDC1*. For each of these drugs, further extensive studies, both *in vitro* and *in vivo*, are needed to confirm their effectiveness, molecular mechanisms and toxicity in the treatment of CCA.

Previous study (59) and the present data have demonstrated that lipid metabolism pathways may be significantly altered, and that lovastatin interacts with the dysregulated gene signatures. The lipid metabolism pathway may serve important roles in CCA growth and progression by downregulating the farnesoid x receptor and lipid metabolism pathways (59). Lovastatin is a medication used for lipid metabolic disorder, and blocks HMG-CoA reductase enzyme in cholesterol synthesis. This drug has been studied *in vitro* and *in vivo* and has been demonstrated to exert anticancer effects in CCA and enhancement of gefitinib-induced antiproliferation in resistant CCA (60-62).

Clofazimine is a drug used in the treatment of leprosy. It also has an anticancer effect and may be used for hepatocellular carcinoma (63). Certain antimalarial primaquines have been shown to block amino acid uptake in *in vivo* studies (64). From the present gene-drug interaction results, these drugs may have certain therapeutic roles in the context of CCA treatment; however further study is required.

COX-2 also serves an important role in CCA carcinogenesis, and is expressed at high levels in CCA tissue compared with non-tumorous adjacent tissue (65). Inhibition of COX-2 by nonsteroidal anti-inflammatory drugs, including peroxicam in combination with cisplatin, may trigger cell cycle regulation and apoptosis in different mesothelioma cell lines (MSTO-211H and NCI-H2452) (66).

The present results also revealed retinoic acid and caffeine to interact with gene signatures of the top most DEGs. Retinoic acid is categorized as a retinoid and has been studied for its therapeutic efficacy in numerous cancers. In CCA, cell migration and tumor invasion may be blocked by all trans-retinoic acid-incorporated glycol chitosan nanoparticles (67). Caffeine is a methylxanthine alkaloid related to the adenine and guanine bases. Previous reports demonstrated that caffeinated coffee consumption was associated with reduced risk of HCC and CCA (68,69). However, since a molecular mechanism

underlying the potential anticancer activity of caffeine is yet to be elucidated, it is probably best utilized as a supplement in daily diet. The present research is limited as the application of molecular data based on bioinformatics analysis may not be sufficient to predict the efficacy of potential targeted agents or protein inhibitors. Therefore, validation of the current data with case studies and additional functional downstream approach experiments is necessary.

In conclusion, the present study performed gene expression analysis, and based on the findings, assessed molecular pathways and drug interactions associated with DEG signatures in CCA. The present study may be developed to further examine drug repurposing and to search for additional anticancer activities among known pharmaceuticals. The results may provide a novel hypothesis regarding the mechanisms of non-antineoplastic drugs in cancer treatment. The data may be a useful resource for preclinical and clinical research on existing FDA-approved drugs into their potential use in CCA treatment.

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Availability of data and materials

All data used and analyzed during this study are included in this published article.

Authors' contributions

SC performed data acquisition and data analysis and contributed to the drafting of the manuscript. TS was responsible for initiation, conception, experimental design and execution of the project and for critical revision of the manuscript. TU supervised the collection of anti-cancer drug information and contributed to the revision of the manuscript. YP supervised data analysis and contributed to the revision of the manuscript. JS supervised experimental design and critical revision of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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