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Research article

Down-regulation of solute carrier family 10 member 1 is associated with early recurrence and poorer prognosis of hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most frequent malignancies and the fourth-leading cancer-related death worldwide. Most patients with HCC are diagnosed at a late stage in which curable therapies are limited. Thus, identifying biomarkers for early diagnosis and prognosis of HCC is essential for improving the treatment effectiveness in patients with HCC. In this paper, the *SLC10A1* expression levels in the cells and the tissues and their correlation with HCC were analyzed using bioinformatics tools. Clinical information data and gene expression profiles were retrieved from the Gene Expression Omnibus and The Cancer Genome Atlas. Chi-square tests, log-rank tests, and Kaplan-Meier curves were performed using R packages. In all statistical analyses, a *p*-value of less than 0.05 was considered significant. We found that SLC10A1 primarily expresses in the liver, especially on the plasma membrane. The expression levels of *SLC10A1* in tumors were consistently lower than that in normal tissue. Down-regulation of *SLC10A1* was correlated with a poor survival outcome [p = 4.50e-05] and recurrence-free survival [p = 8.0e-04] in patients with HCC. In addition, multivariate analysis indicated that the expression of *SLC10A1* was an independent predictor for survival outcome [p = 2.17e-05] and recurrence-free survival [p = 1.63e-04]. We concluded that SLC10A1 is a potential biomarker for the early diagnosis and prognosis of HCC in the era of personalized medicine.

1. Introduction

Hepatocellular carcinoma is the most common cause of primary liver cancer. It represents more than 85% of liver cancers, and it is the fourth-leading cause of death among all types of cancer worldwide [1, 2]. Very few HCC patients are diagnosed at the early stage of the most effective treatment phase [3]. Early diagnosis of HCC is essential because it can improve clinical outcomes [4]. Currently, HCC diagnostics rely on methods, such as magnetic resonance imaging, and computed tomography. However, these methods are very costly and difficult to detect small tumors [5, 6]. Having biomarkers that can early detect HCC may lead to effective treatment. Thus, there is a need to identify sensitive biomarkers for the early diagnosis and prognosis of HCC.

Recently, several serums and tissue biomarkers have been studied for the early diagnosis of HCC. Alpha-fetoprotein (AFP), a 70 kD glycoprotein, is the most common biomarker for HCC surveillance, but it has limited sensitivity [7]. Des-γ-carboxyprothrombin (DCP) was suggested as a promising biomarker in the diagnosis of early-stage HCC. But its sensitivity in early-stage patients from a large multicenter case-control study of DCP was only 56% [8]. Glypican-3 (GPC3) has the potential as a biomarker for the diagnosis of HCC at the early stage [9]. Other biomarkers, such as osteopontin, Golgi protein-73, squamous cell carcinoma antigen, annexin A2, soluble urokinase plasminogen activator receptor, and thioredoxins, were also investigated [10, 11, 12, 13, 14]. But they have not been widely accepted in clinical practice.

SLC10A1 expresses primarily in the liver. It encodes the Na + -taurocholate co-transporting polypeptide (NTCP) [15, 16, 17]. NTCP is also known as the sodium/bile acid cotransporter or liver bile acid transporter [15, 16, 17]. Bile salts are the main components of bile. They play an important role in the digestion of fats and the absorption of fat-soluble vitamins [16, 18]. NTCP transporter has a critical role in the regulation of the transport of bile salts [19]. NTCP can mediate the transport of additional substrates, such as thyroid hormones, drugs, and toxins [16, 20]. Recently, several studies have found that NTCP is a receptor for the hepatitis B virus and hepatitis D virus [21, 22]. This suggests an application of NTCP in virology [23]. Yan et al. reported that when the

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SLC10A1 gene was inactivated in human or mouse liver cells, the hepatitis B virus infection was significantly reduced [24]. The expression of NTCP is reduced in patients with cirrhosis [25] and is increased in patients with nonalcoholic fatty liver or early stage of liver transplantation [26]. SLC10A1 has been extensively studied, but its role in HCC remains unclear. To learn more about the relationship between SLC10A1 and HCC, we analyzed the gene expression, and distribution of SLC10A1 in cells, and the correlation with survival and recurrence in HCC patients.

2. Methods

2.1. Subcellular localization and expression of SLC10A1 among normal and cancer tissues

Subcellular localization of SLC10A1 was predicted and visualized by using the COMPARTMENTS, a subcellular localization database (http://compartments.jensenlab.org) [27]. The gene type from *Homo sapiens* was selected for further steps.

To investigate the gene expression levels of SLC10A1 across human tissues, RNA-sequencing analyses were conducted via the GTEx consortium (http://www.gtexportal.org) [28]. All data were browsed and searched by gene symbol.

mRNA expression levels of SLC10A1 across all cancers and paired normal tissues were analyzed by GEPIA [29]. In the Single Gene Analysis tab, the gene symbol was used to search for interesting information.

2.2. The protein expression of SLC10A1 by immunohistochemistry

Protein expression levels of SLC10A1 were identified using the Tissue Atlas and the Pathology Atlas from Human Protein Atlas database (https://www.proteinatlas.org/) [30]. The protein expression data from 44 normal human tissue types and 17 different forms of human cancer is derived from antibody-based protein profiling using immunohistochemistry. Gene symbol was used to search for information from the database. The IHC staining of normal liver tissues from tissue atlas and HCC tissues from pathology atlas were collected and analyzed.

2.3. Protein-protein interaction analysis

Protein-protein interactions were conducted using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (http://www.string-db.org/) [31]. Search for a single protein by name was performed in the *Homo sapiens* database. Protein networks were linked based on the following six criteria: experimental evidence and existing databases, neighborhood, gene fusion, co-occurrence, and co-expression.

2.4. Patients and gene expression profiles

Gene expression profiles and clinical information of HCC patients were downloaded from TCGA (https://www.cbioportal.org/) and the National Center for Biotechnology Information Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/). The robust multi-array method (RMA) was used to adjust the raw data to the median via Cluster 3.0. The dataset GSE14520 [32] has been selected as the main data for the construction of our experiment. 242 patients with available clinical information were used for this study. The probe set identifiers have been transferred to gene symbols. Patients were classified into low or high expression groups. Patients with SLC10A1 expression level higher than the median were in the high expression group, and patients with SLC10A1 expression level less or equal to than median were in the low expression group. These groups were used for further study.

2.5. SLC10A1 expression and its correlation with clinical information

607 patients were selected from GSE14520 [32] and TCGA [33] datasets to observe the mRNA expression levels of *SLC10A1* between normal and tumor tissues. The Kaplan-Meier method was used to compare the survival of two patient groups based on mRNA expression. Chi-square and log-rank tests were used to assess survival risk and recurrence time. Cox univariate and multivariate proportional regression analyzes were performed to evaluate independent prognostic factors associated with survival and recurrence time.

2.6. Statistical methods

The Kaplan-Meier curves, a two-sample t-test, Chi-squared tests, and log-rank tests were performed using the R language environment (www.r-project.org). The Wilcoxon signed-rank test was used to compare two groups of clustered data, and the Kruskal-Wallis test was applied to examine more than two independent clustered data and displayed by a boxplot. In the tests, a p-value less than 0.05 was considered statistically significant.

3. Results

3.1. SLC10A1 subcellular localization and expression among normal and cancer tissues

According to COMPARTMENTS, a subcellular localization database considering multiple information sources regarding different cell types, the *SLC10A1* protein product was identified with the highest confidence (confidence level 5) in the plasma membrane. Low confidence (confidence level 2) was in the extracellular, mitochondrion, peroxisome nucleus, endoplasmic reticulum, endosome, and cytosol. And the lowest confidence (confidence level 1) was in the cytoskeleton, lysosome, and Golgi apparatus (Figure 1).

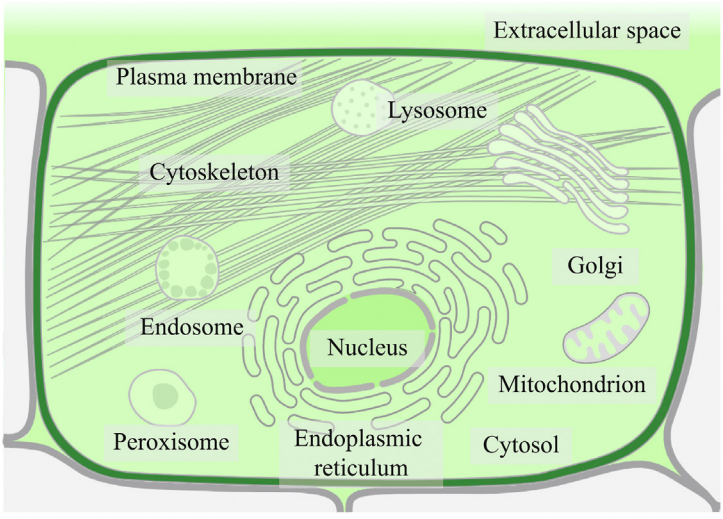
According to the GTEx database, the expression of *SLC10A1* was very high in the liver (median transcripts per million (TPM) is 94.29), low in whole blood (median TPM is 0,10), and very low or no expression in other tissues (Figure 2A). In tissue from HCC patients, the median expression of *SLC10A1* was 33.82 (TPM). This expression level was lower than that in normal tissues (69.57 TPM) (Figure 2B). *SLC10A1* is mainly expressed in the liver. It shows very low or no expression of other tissues (Figure 2C). In both females and males, protein expression of *SLC10A1* through immunohistochemistry differed between HCC tissues and normal liver tissues (Figure 3). The expression level of *SLC10A1* was higher in normal liver tissues than in hepatocellular carcinoma tissue. *SLC10A1* has only one variant that was ENSG00000100652.4 primarily expressing in the liver (Figure 4).

3.2. Protein-protein interaction analysis

To study the relationship of SLC10A1 with other proteins, we performed a molecular network by introducing SLC10A1 into the STRING database. SLC10A1 was closely related to other proteins, such as SLC10A7, SLCO1A2, SLCO1B3, SLCO1B1, NR0B2, NR1H4, CYP7A1, ABCB11, and ALB (Figure 5).

3.3. SLC10A1 expression and its correlation with clinical information

To investigate the association between the *SLC10A1* expression level and clinicopathological characteristics, including gender, age at diagnosis, and BCLC stage, we performed Chi-square ($\chi 2$) test. As shown in Table 1, Gender and age were not significantly correlated with *SLC10A1* expression. BCLC stage, survival and recurrence were significantly associated with *SLC10A1* expression (BCLC, $p = 1.61e\text{-}03$; OS, $p = 9.72e\text{-}04$; RFS, $p = 2.74e\text{-}02$). To compare the prognostic value of *SLC10A1* with other prognostic variables, such as age and gender, we performed univariate and multivariate Cox regression analysis by using the GSE14520 dataset (Table 2). In univariate analysis, age was not significant in both OS and RFS but gender was significant with RFS (HR 2.40; 95% CI 1.20–4.5; $p = 0.01$). In multivariate analysis, age was not



Compartment	Confidence
Plasma membrane	5
Extracellular	2
Cytoskeleton	2
Mitochondrion	2
Peroxisome	2
Nucleus	2
Endoplasmic reticulum	2
Endosome	2
Cytosol	2
Lysosome	1
Golgi apparatus	1

Figure 1. The expression of *SCL10A1* at the cellular level.

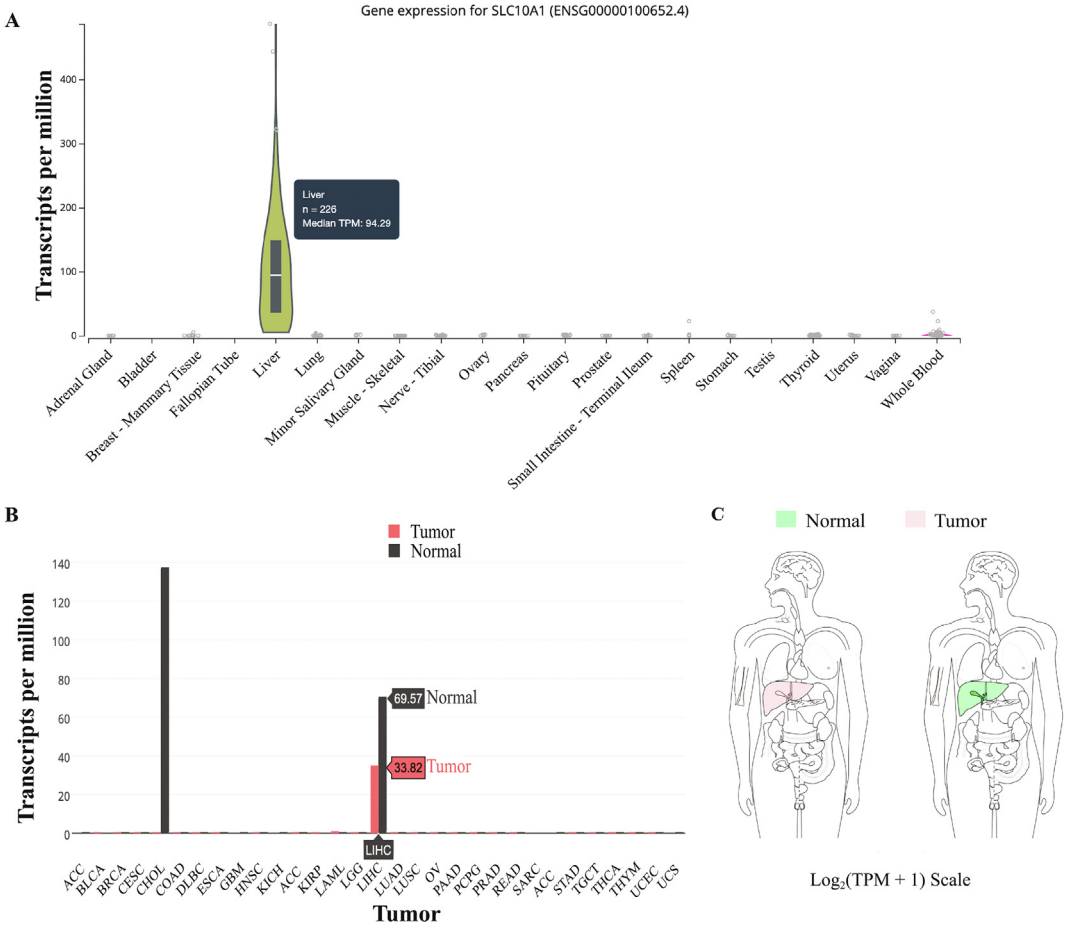


Figure 2. Gene expression for *SLC10A1*. (A) Gene expression for *SLC10A1* (ENSG00000100652.4) among different tissues. (B) The median expression of tumor and normal samples in the body map. (C) The gene expression profile of *SLC10A1* across all tumor tissue and paired normal tissues. (LIHC, liver hepatocellular carcinoma; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangio carcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe, KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia, LGG, brain lower grade glioma; LUAD, lung adenocarcinoma, LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma).

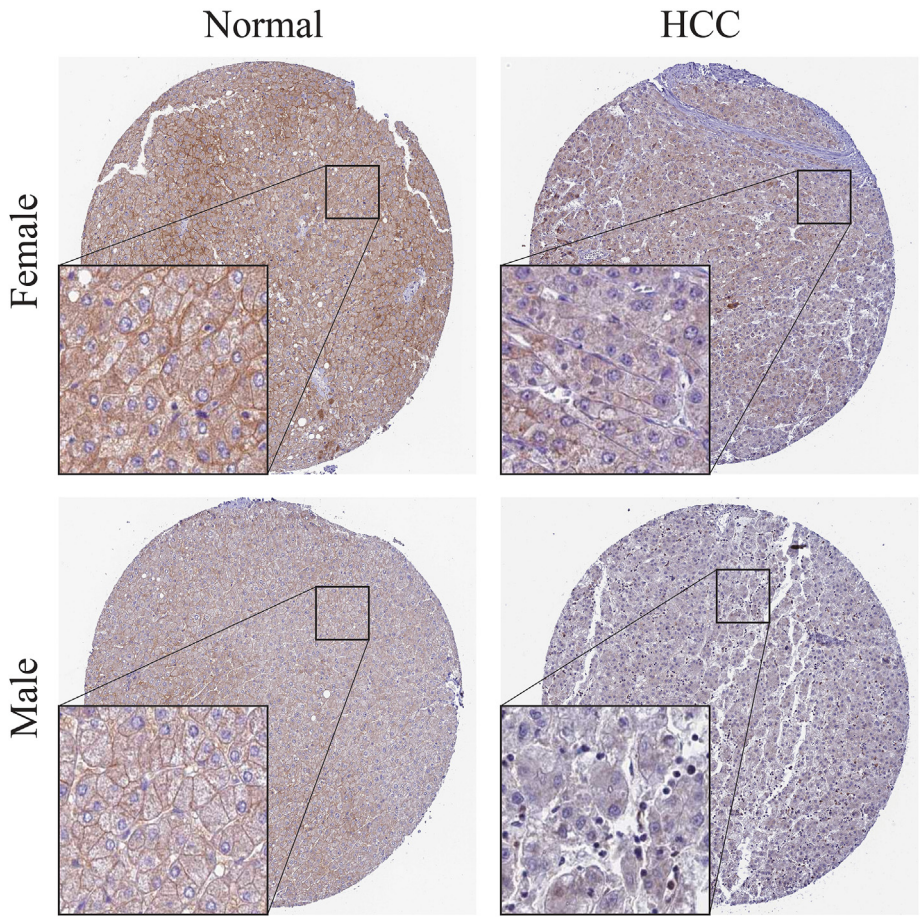


Figure 3. Protein expression of *SLC10A1* in normal and HCC tissues. Expression of SLC10A1 in hepatocellular carcinoma tissues and normal tissues were analyzed by immunohistochemistry. Data were obtained from the Human Protein Atlas.

significant for both OS and RFS but gender was significant with RFS (HR 2.63; 95% CI 1.38–5.03; p = 3.36e-03).

GSE14520 (n = 242) and TCGA (n = 365) were used to analyze the association between SLC10A1 expression and survival and recurrence outcomes. Patients in GSE14520 dataset were classified into high expression group (n = 121) and low expression group (n = 121). The Kaplan-Meier method was performed and showed that *SLC10A1* expression level was significantly associated with survival outcome in patients with HCC (p = 2.61e-05; Figure 6A). Patients with low *SLC10A1* expression had a shorter survival time than patients with high SLC10A1 expression. The patients in the TCGA dataset were classified into a high expression group (n = 181) or a low expression group (n = 184). Patients with high *SLC10A1* expression experiences a longer survival time than patients with low SLC10A1 expression (p = 1.03e-03; Figure 6B).

We further analyzed the association of SLC10A1 with recurrence in the GSE14520 dataset. We found that patients with a low expression level of *SLC10A1* had a higher recurrence rate than patients with a high expression level of *SLC10A1* patients (p = 6.75e-04, Figure 6C).

4. Discussion

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver, and the number of HCC-related death per year has increased worldwide [1, 34]. Many major risk factors for hepatocellular carcinoma have been identified, such as chronic cirrhosis, viral hepatitis, nonalcoholic fatty liver, alcohol use, genetic disease, and exposure to hepatotoxicity (aflatoxin) [35]. The diagnosis of patients with HCC remains challenging, especially in the early stages of the disease. If the patient is diagnosed early and correctly with HCC, the 5-year survival rate is >70% [34]. However, there are no single biomarkers with high specificity and sensitivity for the accurate detection of HCC, especially in the early stages of HCC to increase survival for the patients [27].

In this study, we found that the *SLC10A1* encodes only for NTCP, and it has only one variant. Furthermore, NTCP was expressed specifically in the liver with primary function in bile salt metabolism to transport extracellular bile salts into hepatocytes. It accounts for greater than 80% of the hepatic uptake [36, 37]. NTCP was recently shown to serve as an entry receptor for hepatitis B and D viruses [21]. Increased or decreased SLC10A1 expression level is associated with cirrhosis or fatty liver [25, 26], which is one of the risk factors for HCC. The median expression level of *SLC10A1* in HCC tissues was significantly lower than its level in normal liver tissues. The significance of *SLC10A1* was further confirmed by analysis of immunohistochemistry from the protein atlas database. The expression levels of SLC10A1 were significantly higher in normal liver tissues and significantly lower in tumor tissues from both male and female. This finding suggests that SLC10A1 expression levels may be used as a biomarker for assessing the risk of HCC development.

Univariate Cox analysis suggests that SLC10A1 can predict patients' survival and disease recurrence. It predicts better after adjustment with other clinical variables such as age, and sex in multivariate Cox analysis. This suggests that SLC10A1 is an independent biomarker for survival and recurrence outcomes of patients with HCC. The better performance of SLC10A1 in multivariate analysis suggests that it may be more useful for the detection of HCC in clinical practice.

Long-term survival has been achieved in HCC patients after hepatic resection of HCC. But, it significantly reduces since the development of recurrence with the rate of 70% at 5 years after resection of HCC [34, 38].

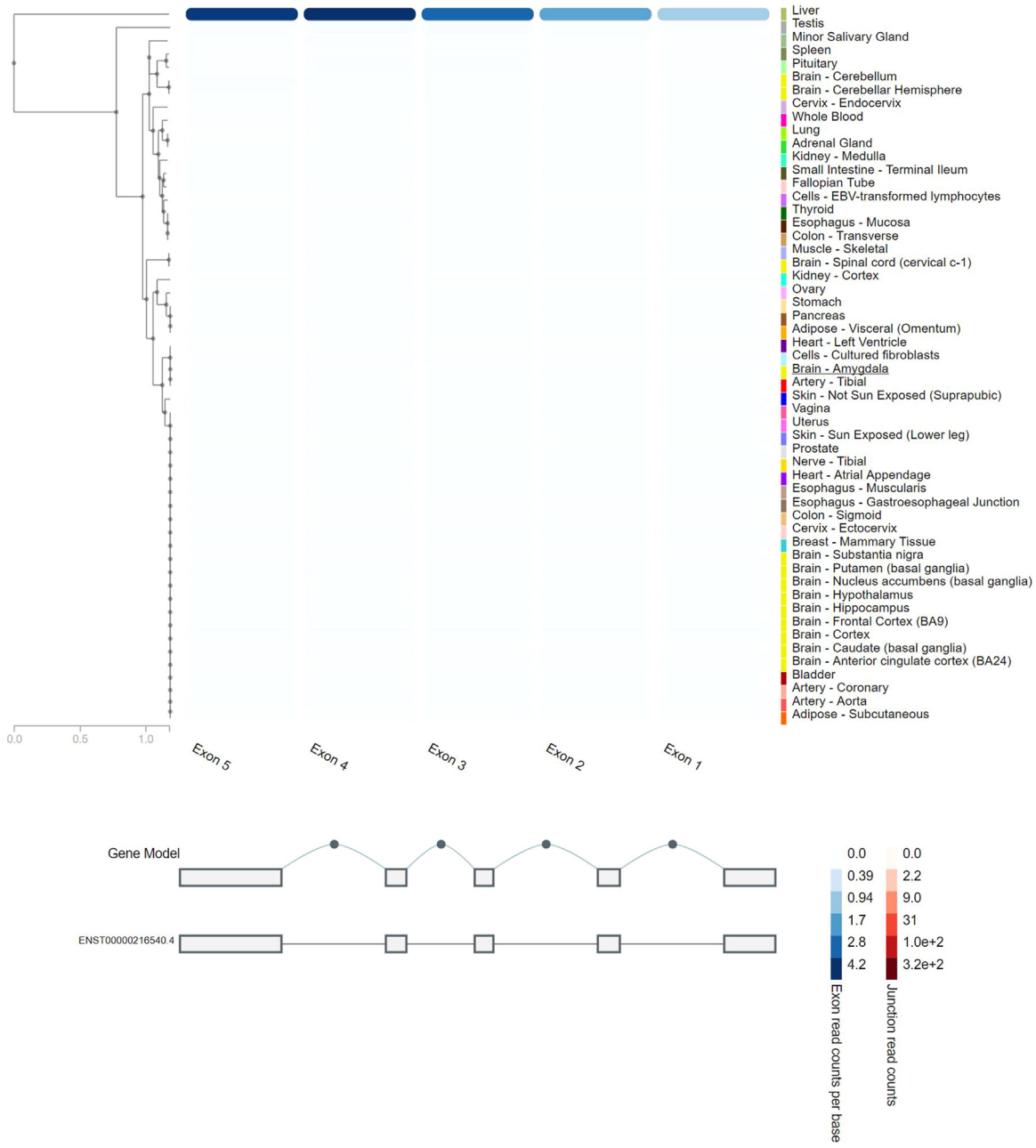


Figure 4. Exon expression of SLC10A1 (ENSG00000100652.4).

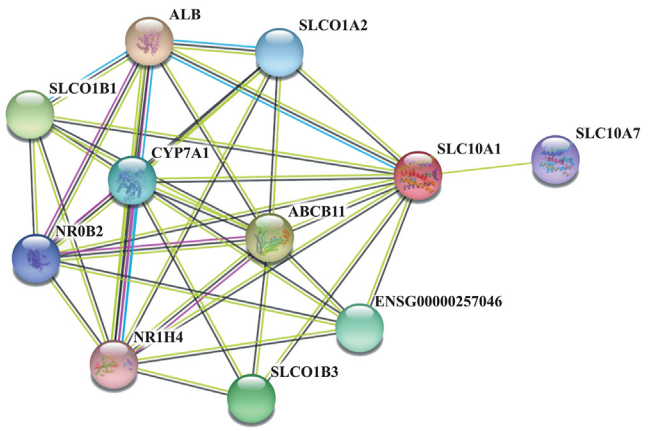


Figure 5. *SLC10A1* protein interactive network.

Therefore, biomarkers useful for the prediction of tumor recurrence still need to be investigated. We show that *SLC10A1* expression levels were correlated with survival and recurrence outcome. HCC patients with low expression of *SLC10A1* were associated with significantly lower survival and recurrence rate than HCC patients with high expression of *SLC10A1*. This finding suggests that the upregulation of *SLC10A1* can serve as a biomarker for predicting recurrence in early-stage HCC.

SLC10A1 interacts with other proteins, such as NR1H4, ABCB11, and CYP7A1. It is primarily known for its involvement in the bile salt reabsorption transport pathway, through NTCP. Expression of NTCP is regulated by many transcription factors, such as farnesoid X receptor (FXR or NR1H4), small heterodimer partner (SHP or NR0B2), bile salt export pump (BSEP or ABCB11), and cholesterol 7a-hydroxylase (CYP7A1) [37]. FXR does not interact directly with the NTCP promoter but it induces the expression of other factors that indirectly suppress NTCP expression. An analysis based on the TCGA dataset indicated that NR1H4 was downregulated in liver cancer. This

Table 1. Clinicopathological features of *SLC10A1* in two expression group of GSE14520 data set.

Variables	Total	*SLC10A1* expression		*p*-value
		Low	High	
Number of patients	242	121 (50.0%)	121 (50.0%)	
Gender				0.19
Female	31	18 (14.9%)	13 (10.7%)	
Male	211	103 (85.1%)	108 (89.3%)	
Age				0.44
>50	117	53 (43.8%)	64 (52.9%)	
≤50	125	68 (56.2%)	57 (47.1%)	
BCLC				1.61e-03
0	20	12 (09.9%)	8 (06.61%)	
A	152	61 (50.4%)	91 (75.2%)	
B	24	16 (13.2%)	8 (6.61%)	
C	29	21 (17.4%)	8 (06.6%)	
NA	17	11 (09.1%)	6 (05.0%)	
OS				9.72e-04
0	146	60 (49.6%)	86 (71.1%)	
1	96	61 (50.4%)	35 (28.9%)	
RFS				2.74e-02
0	106	44 (36.4%)	62 (51.2%)	
1	136	77 (63.6%)	59 (48.8%)	

BCLC, Barcelona Clinic Liver Cancer; OS, overall survival; RSF, recurrence free survival; *p* -values were obtained from the χ^2 -test.

Table 2. Univariate and multivariate Cox proportional hazard regression analyses of clinical variables in the GES14520 dataset.

	Variable	Univariate			Multivariate		
		HR	95% CI	*p* Value	HR	95% CI	*p* value
OS	Gender	1.90	0.62–1.40	0.69	2.09	1.01–4.32	0.04
	Age	0.92	0.90–3.80	0.093	1.07	0.71–1.60	0.76
	Expression	2.40	1.60–3.60	4.50e-05	2.49	1.63–3.79	2.17e-05
RSF	Gender	2.40	1.20–4.50	0.01	2.63	1.38–5.03	3.36e-03
	Age	1.10	0.77–1.50	0.66	1.25	0.89–1.77	0.19
	Expression	1.80	1.30–2.50	8.0e-04	1.94	1.37–2.74	1.63e-04

OS, overall survival; RSF, recurrence-free survival; HR, hazard ratio; CI, Confidence Interval; *p* -values were obtained from the χ2 -test.

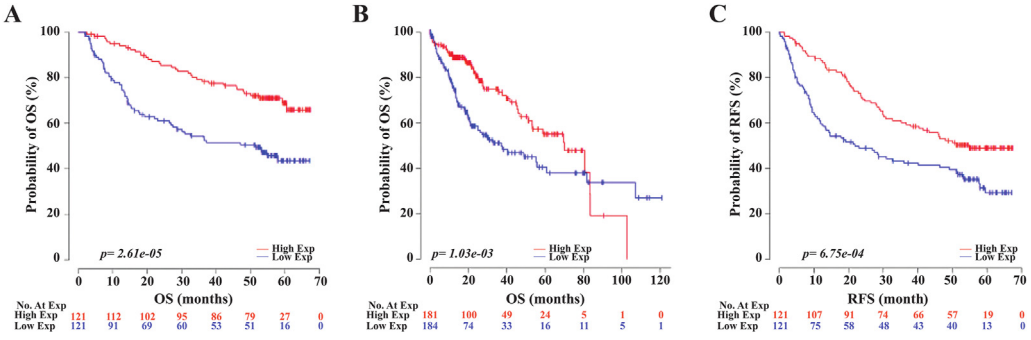


Figure 6. Kaplan Meier plots of *SLC10A1* expression with survival and recurrence. (A) *SLC10A1* expression with the survival of the GSE14520 dataset. (B) *SLC10A1* expression with the survival of the TCGA dataset. (C) SLC10A1 expression with the recurrence of the GSE14520 dataset.

suggests that NR1H4 may play an important role in tumorigenesis [39]. If bile acid levels increase, FXR activates the expression of SHP and then SHP inhibits transcription of NTCP [40]. NR0B2 involved in regulatory processes in HCC, which acts as a tumor suppressor through inhibition of cell growth and activation of apoptosis in this tumor entity [41]. The activation of FXR reduces the expression level of CYP7A1, an important enzyme involved in bile acid biosynthesis [17, 37]. CYP7A1 is the rate-limiting enzyme in the classic pathway of bile acid synthesis. Overexpression of an exogenous CYP7A1 gene impaired liver regeneration after 70% partial hepatectomy. This was accompanied by increased hepatocyte apoptosis and liver injury [42]. FXR upregulates the bile salt export pump (BSEP) to prevent the intracellular accumulation of cytotoxic bile salts [43]. BSEP has a vital role in maintaining bile acid homeostasis. A lack of BSEP leads to severe

cholestasis and hepatocellular carcinoma [44]. Thus, SLC10A1 interacts with proteins, which are major risk factors of HCC.

This study showed the correlation between *SLC10A1* expression and hepatocellular carcinoma. *SLC10A1* is expressed mainly in liver cells. The mRNA expression of *SLC10A1* in hepatocellular carcinoma tissue was lower than its expression in normal tissue. *SLC10A1* is an independent biological indicator that can be used in the diagnosis and prognosis of HCC. Low expression of *SLC10A1* was related to worse prognosis and recurrence in patients with HCC. We conclude that *SLC10A1* is a potential biomarker for the early diagnosis and prognosis of hepatocellular carcinoma.

Declarations

Author contribution statement

Quynh Hoa Tran: Conceived and designed the experiments; Wrote the paper.

Van Gio Nguyen: Analyzed and interpreted the data; Wrote the paper.

Cong Manh Tran: Analyzed and interpreted the data.

Minh Nam Nguyen: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] Freddie Bray, Jacques Ferlay, Isabelle Soerjomataram, Rebecca L. Siegel, Lindsey A. Torre, Ahmedin Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca Cancer J Clin 68 (2018) 394–424.

[2] Monica Marra, Ignazio M. Sordelli, Angela Lombardi, Monica Lamberti, Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview, J. Transl. Med. 9 (2011) 1–14.

[3] Jessica Zucman-Rossi, Augusto Villanueva, Jean-Charles Nault, Josep M. Llovet, Genetic landscape and biomarkers of hepatocellular carcinoma, Gastroenterology 149 (2015) 1226–1239.

[4] Richard Todd Stravitz, Douglas M. Heuman, Nisha Chand, Richard K. Sterling, Mitchell L. Shiffman, Velimir A. Luketic, Arun J. Sanyal, Habib Adil, Anastasios A. Mihas, Ho-Chong S. Giles, Surveillance for hepatocellular carcinoma in patients with cirrhosis improves outcome, Am. J. Med. 212 (2008) 119–126.

[5] Idad S. Bialecki, M. Adrian, Di Bisceglie, Diagnosis of hepatocellular carcinoma, HPB 7 (2005) 26–34.

[6] Jiahui Qi, Jin Wang, Hiroshi Katayama, Subrata Sen, Song-mei Liu, Circulating microRNAs (cmiRNAs) as novel potential biomarkers for hepatocellular carcinoma, Neoplasma 60 (2013) 135–142.

[7] Lin Zhou, Liu Jia, Feng Luo, Serum tumor markers for detection of hepatocellular carcinoma, World J. Gastroenterol. 12 (2006) 1175–1181.

[8] A.S. Lok, R.K. Sterling, J.E. Everhart, E.C. Wright, J.C. Hoefs, A.M. Di Bisceglie, T.R. Morgan, H.Y. Kim, W.M. Lee, H.L. Bonkovsky, J.L. Dienstag, H.-C.T. Group, Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma, Gastroenterology 138 (2010) 493–502.

[9] N. Shafizadeh, L.D. Ferrell, S. Kakar, Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum, Mod. Pathol. 21 (2008) 1011–1018.

[10] H.G. Wan, H. Xu, Y.M. Gu, H. Wang, W. Xu, M.H. Zu, Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis, Clin Res Hepatol Gastroenterol 38 (2014) 706–714.

[11] Y. Sun, G. Gao, J. Cai, Y. Wang, X. Qu, L. He, F. Liu, Y. Zhang, K. Lin, S. Ma, X. Yang, X. Qian, X. Zhao, Annexin A2 is a discriminative serological candidate in early hepatocellular carcinoma, Carcinogenesis 34 (2013) 595–604.

[12] A. Chounta, C. Ellinas, V. Tzanetakou, F. Pliarhopoulou, V. Mplani, A. Oikonomou, K. Leventogiannis, E.J. Giamarellos-Bourboulis, Serum soluble urokinase plasminogen activator receptor as a screening test for the early diagnosis of hepatocellular carcinoma, Liver Int. 35 (2015) 601–607.

[13] J. Li, Z.J. Cheng, Y. Liu, Z.L. Yan, K. Wang, D. Wu, X.Y. Wan, Y. Xia, W.Y. Lau, M.C. Wu, F. Shen, Serum thioredoxin is a diagnostic marker for hepatocellular carcinoma, Oncotarget 6 (2015) 9551–9563.

[14] G. Giannelli, E. Fransvea, P. Trerotoli, M. Beaugrand, F. Marinosci, L. Lupo, G. Nkontchou, P. Dentico, S. Antonaci, Clinical validation of combined serological biomarkers for improved hepatocellular carcinoma diagnosis in 961 patients, Clin. Chim. Acta 383 (2007) 147–152.

[15] Barbara Döring, Thomas Lütteke, Joachim Geyer, Ernst Petzinger, The SLC10 carrier family: transport functions and molecular structure, Curr. Top. Membr. 70 (2012) 105–168.

[16] D.W. Russell, Fifty years of advances in bile acid synthesis and metabolism, J. Lipid Res. (2009) S120–S125.

[17] Tatiana Claro da Silva, James E. Polli, Peter W. Swaan, The solute carrier family 10 (SLC10): beyond bile acid transport, Mol. Aspect. Med. 34 (2013) 252–269.

[18] A.F. Hofmann, The enterohepatic circulation of bile acids in mammals: form and functions, Front. Biosci. 14 (2009) 2584–2598.

[19] Peter J. Meier, B. Stieger, Bile salt transporters, Annu. Rev. Physiol. 64 (2002) 635–661.

[20] B. Stieger, The role of the sodium-taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (BSEP) in physiology and pathophysiology of bile formation, Handb. Exp. Pharmacol. 201 (2011) 205–259.

[21] Huan Yan, Zhenchao Gao, Mei Song, Xiaofeng Feng, Guocai Zhong, Yi Huang, Pan Chen, Guangwei Xu, Yonghe Qi, Jianhua Sui, Wenqing Gao, Wenhui Li, Bo Peng, Bijie Ren, Wenhui He, Zhiyi Jing, Haimin Wang, Yinyan Sun, Tao Cai, Liran Fu, Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus, eLife (2012), e00049.

[22] Yi Ni, A. Florian, Lempp, Stefan Mehrle, Shirin Nkongolo, Christina Kaufman, Maria Fälth, Jan Stindt, Christian Königer, Michael Nassal, Ralf Kubitz, Holger Sültmann, Stephan Urban, Hepatitis B and D viruses exploit sodium taurocholate co-transporting polypeptide for species-specific entry into hepatocytes, Gastroenterology 146 (2014) 1070–1083.

[23] Davor Slijepcevic, F. Stan, J. van de Graaf, Bile acid uptake transporters as targets for therapy, Dig. Dis. 35 (2017) 251–258.

[24] Huan Yan, Bo Peng, Wenhui He, Guocai Zhong, Yonghe Qi, Bijie Ren, Zhenchao Gao, Zhiyi Jing, Mei Song, Guangwei Xu, Jianhua Sui, Wenhui Lia, Molecular determinants of hepatitis B and D virus entry restriction in mouse sodium taurocholate cotransporting polypeptide, J. Virol. 87 (2013) 7977–7991.

[25] Verena Keitel, Burdelski Martin, Warskulat Ulrich, Thomas Kühlkamp, Dietrich Keppler, Dieter Haussinger, Ralf Kubitz, Expression and localization of Hepatobiliary transport proteins in progressive Familial intrahepatic cholestasis, Hepatology 41 (2005) 1160–1172.

[26] Erwin Geuken, Dorien Visser, Folkert Kuipers, Hans Blokzijl, G. Henri, D. Leuvenink, Koert P. de Jong, M. Paul, J.G. Peeters, Peter L.M. Jansen, Maarten J.H. Slooff, Annette S.H. Gouw, Robert J. Porte, Rapid increase of bile salt secretion is associated with bile duct injury after human liver transplantation, J. Hepatol. 41 (2014) 1017–1025.

[27] Janos X. Binder, Sune Pletscher-Frankild, Kalliopi Tsafou, Christian Stolte, Seán I. O'Donoghue, Reinhard Schneider, Lars Juhl Jensen, COMPARTMENTS: unification and visualization of protein subcellular localization evidence, Database 2014 (2014) 1–9.

[28] Marta Melé, Pedro G. Ferreira, Ferran Reverter, David S. DeLuca, Monlong Jean, Michael Sammeth, Taylor R. Young, Jakob M. Goldmann, Dmitri D. Pervouchine, Timothy J. Sullivan, Rory Johnson, Ayellet V. Segrè, Sarah Djebali, Anastasia Niarchou, The GTEx Consortium, Fred A. Wright, Tuuli Lappalainen, G.G. Miquel Calvo, Emmanouil T. Dermitzakis, Kristin G. Ardlie, Roderic Guigó, The human transcriptome across tissues and individuals, Research 348 (2015) 660–665.

[29] Zefang Tang, Chenwei Li, Boxi Kang, Ge Gao, GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res. (2017) 1–5.

[30] Mathias Uhlén, Linn Fagerberg, Björn M. Hallström, Cecilia Lindskog, Per Oksvold, Adil Mardinoglu, Åsa Sivertsson, Caroline Kampf, Evelina Sjöstedt, Anna Asplund, IngMarie Olsson, Karolina Edlund, Emma Lundberg, Sanjay Navani, Cristina Al-Khalili Szigyarto, Jacob Odeberg, Dijana Djureinovic, Jenny Ottosson Takanen, Sophia Hober, Tove Alm, Per-Henrik Edqvist, Holger Berling, Hanna Tegel, Jan Mulder, Johan Rockberg, Peter Nilsson, Jochen M. Schwenk, Marica Hamsten, Kalle von Feilitzen, Mattias Forsberg, Lukas Persson, Fredric Johansson, Martin Zwahlen, Gunnar von Heijne, Jens Nielsen, Fredrik Pontén, Tissue-based map of the human proteome, Science 347 (2015) 12604191–12604199.

[31] Robert Hoffmann, Alfonso Valencia, A gene network for navigating the literature, Nat. Genet. 36 (2004) 664.

[32] Xin Chen, Siu Tim Cheung, John Higgins, Matt van de Rijn, Samuel So, Sheung Tat Fan, Kin-Man Lai, Jiafu Ji, David Botstein, Patrick O. Brown, Gene expression patterns in human liver cancers, Mol. Biol. Cell 13 (2002) 1929–1939.

[33] The Cancer Genome Atlas Research Network, John N. Weinstein, Eric A. Collisson, Gordon B. Mills, Kenna R Mills Shaw, Brad A. Ozenberger, Kyle Ellrott, Ilya Shmulevich, Chris Sander, Joshua M. Stuart, The cancer Genome atlas pan-cancer analysis project, Nat. Genet. 45 (2013) 1113–1120.
[34] Nobuhiro Tsuchiya, Sawada Yu, Itaru Endo, Keigo Saito, Yasushi Uemura, Tetsuya Nakatsura, Biomarkers for the early diagnosis of hepatocellular carcinoma, World J. Gastroenterol. 21 (2015) 10573–10583.
[35] D. Kremsdorf, P. Soussan, P. Paterlini-Brechot, C. Brechot, Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis, Oncogene 25 (2006) 3823–3833.
[36] Richard H. Ho, Brenda F. Leake, Richard L. Roberts, Wooin Lee, Richard B. Kim, Ethnicity-dependent polymorphism in Na+-taurocholate cotransporting polypeptide (SLC10A1) reveals a domain critical for bile acid substrate recognitio, J. Biol. Chem. 279 (2004) 7213–7222.
[37] Senko Tsukuda, Masashi Iwamoto, Koichi Watashi, NTCP (Sodium taurocholate cotransporting polypeptide), Encyclop. Signal. Molec. (2017) 1–8.
[38] P. Tabrizian, G. Jibara, B. Shrager, M. Schwartz, S. Roayaie, Recurrence of hepatocellular cancer after resection: patterns, treatments, and prognosis, Ann. Surg. 261 (2015) 947–955.
[39] Qiuhang Song, Mingyue Li, Cong Fan, Yucui Liu, Lihua Zheng, Yongli Bao, Luguo Sun, Chunlei Yu, Zhenbo Song, Ying Sun, Guannan Wang, Yanxin Huang, Yuxin Li, A novel benzamine lead compound of histone deacetylase inhibitor ZINC24469384 can suppresses HepG2 cells proliferation by upregulating NR1H4, Sci. Rep. 9 (2019).
[40] Paul A. Dawson, Lan Tian, Rao Anuradha, Bile acid transporters, JLR (J. Lipid Res.) 50 (2009) 2340–2357.
[41] Katharina Prestin, Maria Olbert, Janine Hussner, Tamara L. Isenegger, Daniel G. Gliesche, Kerstin Böttcher, Uwe Zimmermann, E. Henriette, Meyer zu Schwabedissen, Modulation of expression of the nuclear receptor NR0B2 (small heterodimer partner 1) and its impact on proliferation of renal carcinoma cells, OncoTargets. Terapy 9 (2016) 4867–4878.
[42] Lisheng Zhang, Xiongfei Huang, Zhipeng Meng, Bingning Dong, Steven Shiah, David D. Moore, Wendong Huang, Significance and mechanism of CYP7a1 gene regulation during the acute phase of liver regeneration, Mol. Endocrinol. 23 (2009) 137–145.
[43] M. Ananthanarayanan, N. Balasubramanian, Makoto Makishima, David J. Mangelsdorf, Frederick J. Suchy, Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor, J. Biol. 36 (2001) 28857–28865.
[44] Yuan Chen, Xiulong Song, Leila Valanejad, Alexander Vasilenko, Vijay More1, Qiu2 Xi, Weikang Chen, Yurong Lai, Angela Slitt, Matthew Stoner, Bingfang Yan, Ruitang Deng, Bile salt export pump is dysregulated with altered farnesoid X receptor isoform expression in patients with Hepatocellular Carcinoma, Hepatology 57 (2013) 1530–1541.