GATA binding protein 4 promotes the expression and transcription of hepatitis B virus by facilitating hepatocyte nuclear factor 4 alpha in vitro

Xiaoqin Lv1, Xia Xiang1, Yue Wu2, Yang Liu3, Ruqing Xu1, Qin Xiang4 and Guoqi Lai1*

Abstract

Background: GATA binding protein 4 (GATA4) has been reported as a potential target of gene therapy for hepatocellular carcinoma (HCC). It is well known that the main cause of HCC is the chronic infection of hepatitis B virus (HBV). However, whether the effect of GATA4 on HBV has not yet been reported.

Methods: In this study, the regulation of GATA4 on HBV was analyzed in vitro. In turn, the effect of HBV on GATA4 was also observed in vitro, in vivo, and clinical HCC patients. Subsequently, we analyzed whether the effect of GATA4 on HBV was related to hepatocyte nuclear factor 4 alpha (HNF4α) in vitro.

Results: The results showed that GATA4 significantly promoted the secretion of HBV surface antigen (HBsAg) and HBV e antigen in the cell culture medium, improved the replication of HBV genomic DNA, and increased the level of HBV 3.5 kb pre-genomic RNA and HBV total RNA (P < 0.05). Moreover, it was showed that HBV had no significant effect on GATA4 in vitro and in vivo (P > 0.05). At the same time, GATA4 expression was decreased in 78.9% (15/19) of HCC patients regardless of the HBV and HBsAg status. Among them, there were 76.9% (10/13) in HBV-associated patients with HCC (HBV-HCC), and 83.3% (5/6) in non-HBV-HCC patients. In addition, the expression of HNF4α was also up-regulated or down-regulated accordingly when stimulating or interfering with the expression of GATA4. Furthermore, stimulating the expression of HNF4α could only alleviate the HBsAg level and HBV transcription levels, but had no significant effect on GATA4.

Conclusions: In summary, this study found that GATA4 has a positive effect on HBV, and the potential pathway may be related to another transcription factor HNF4α that regulates HBV.

Keywords: Hepatitis B virus, GATA binding protein 4, Hepatocyte nuclear factor 4 alpha, Hepatocellular carcinoma, HBV transcription

Introduction

It is reported that the global incidence and mortality of hepatocellular carcinoma (HCC) in 2018 are 4.7% and 8.2% respectively, which is the sixth most common cancer and the fourth leading cause of death for cancer [1]. Treatment methods for HCC include surgical resection, radiotherapy, and chemotherapy, each of with its shortcomings [2]. Gene therapy is considered to be a potential treatment for effectively suppressing HCC with the in-depth research on tumor suppressor genes [3]. GATA binding protein 4 (GATA4), one of the members of the GATA transcription family, is a key transcription
factor that regulates cell differentiation and tissue development [4, 5]. Studies have reported that GATA4 can inhibit the development of a variety of cancers [6–9]. Recently, GATA4 has also been found to be an important tumor suppressor gene for HCC [10, 11]. Previously, we found that GATA4 induces mesenchymal-to-epithelial transition and cellular senescence through the NF-κB pathway in hepatocellular carcinoma. GATA4 plays a crucial tumor-suppressive role in HCC [12].

HCC is a primary liver cancer, and its main pathogenic factors include alcoholism, drug stimulation, obesity, virus (hepatitis B virus and hepatitis C virus), and so on [13]. Chronic hepatitis B (CHB) is the most common pathogenic factor leading to HCC [14–16]. HBV chronic infection accounts for at least 50% of HCC patients in the worldwide [14], and even more than 90% in some regions [17]. Transcription factors play an important role in chronic HBV infection [18]. Interestingly, studies have reported that GATA4 can regulate retrovirus infection [19–21]. However, the relationship between GATA4 and HBV is unclear.

Hepatocyte nuclear factor 4 alpha (HNF4α) is a necessary factor that regulates HBV transcriptional activity in hepatocytes. It can bind to the basic core promoter region of HBV cccDNA to promote synthesis of the 3.5 kb pgRNA [22, 23]. The continuous amplification of HBV in host cells relies on the reverse transcription process of HBV using 3.5 kb pgRNA as a template [24]. More importantly, both GATA4 and HNF4α are involved in the regulation of tissue development pathways, and GATA4 is considered to be the potential upstream of HNF4α, regulating different stages of liver development [25]. Whether there is an association between GATA4 and HNF4α in the regulation of HBV remains to be further observed.

In this study, we discovered for the first time that GATA4 may play a positive regulatory role on HBV in vitro. Meanwhile, HBV status was found to have no significant effect on GATA4. Further analysis showed that HNF4α may be located downstream of GATA4 and regulate the level of HBV transcription.

**Materials and methods**

**Retrospective analysis of GATA4 expression and HBV carrying status in clinical HCC patients**

19 HCC clinical patient information involved in the previous study was obtained from the Molecular Oncology and Epigenetics Laboratory of the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) [12]. This study retrospectively analyzed the relationship between GATA4 expression and HBV carrier status in these patients.

**Cell lines and HBV cccDNA preparation**

The normal liver cell line used in this study was L02 cells, the hepatoma or hepatoblastoma cell lines were SMMC7721, HepG2 and HepG2.2.15 cells. These cell lines were purchased from the ATCC cell bank (American Type Culture Collection, Manassas, VA, USA), and all cell lines have been tested for mycoplasma according to the manufacturer’s protocol (Solarbio, Beijing, China). The synthesis of HBV cccDNA was performed as described in a previous study [26]. Briefly, the HBV genome fragment was amplified and synthesized using the phusion high-fidelity DNA polymerase (F530L, Thermo scientific, Lithuania) and the HBV-pEASY plasmid as a template, then subjected to restriction digestion, circularization, and purification to obtain HBV cccDNA.

**Cell culture and transfection**

L02 cells and SMMC7721 cells were maintained in RPMI 1640 medium (Thermo Fisher Scientific, Suzhou, China) supplemented with 10% fetal bovine serum (Biological Industries, Beit HaEmek, Israel). HepG2 cells and HepG2.2.15 cells were maintained in Dulbecco’s modified Eagle’s medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum. All cell lines were treated with 1% penicillin and streptomycin and grown...

| Gene                  | Forward sequence (5’–3’)                | Reverse sequence (5’–3’)               |
|-----------------------|-----------------------------------------|----------------------------------------|
| β-actin               | GTCTTCCCTCCATCCTGTC                | AGGGTGAGGATGCGCTCTTCT                |
| GATA4                 | CCAATCCACGAAATGATGAGAGC             | CCCTTTCCGATGGCTGAAAGA               |
| HNF4α                 | CTAAACAGGTGCCTCCCTCAC              | GCAGGATCTGTTAGGATTCTAG               |
| HBV 3.5 kb pgRNA      | GCCCTTAAACGTTCCTGAGCA             | GAGGGATTTCTCTCTCTAGG               |
| HBV total RNA         | ACGACCTTGGAGCCATCTTT              | GCTACAGCCCTCTAGTACA               |
| HBV DNA               | TTCCTCGGCTCGTGTACC               | GCTTTTCTGTCGCCGTC                  |
| HDAC2                 | TGGACTTCTTGGAGTTTGTCA             | CATCACATGATGAAATTCTAT               |
in 5% CO₂ at 37 °C. L02 cells and SMMC7721 cells were plated and cultured overnight, then co-transfected with stable expression plasmid of GATA4 (represented as adGATA4) and HBV cccDNA for 48 h (h). HepG2 cells were plated overnight and treated with a concentration of 10 nM GATA4 small interfering RNA (siGATA4) for 24 h, and then transfected with HBV cccDNA for 48 h. HepG2.2.15 cells were only treated with siGATA4 for 48 h. Lipofectamine™ 8000 reagent (Beyotime, Shanghai, China) was used in all transfection processes according to the manufacturer’s protocol. The adGATA4 and siGATA4 are the same as in our previous study [12]. In addition, HepG2.2.15 cells were treated with a specific stimulator of HNF4α, benfluorex hydrochloride (JP-992 hydrochloride; MedChem Express, Monmouth Junction, NJ, USA) for 48 h after siGATA4 for 24 h, the concentration of benfluorex hydrochloride was the same as reported previously [27]. Each experiment was repeated independently at least three times.

Reverse transcription polymerase chain reaction (RT-PCR) and reverse transcription quantitative PCR (RT-qPCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA (1 µg) was reverse transcribed at 42 °C for 1 h into cDNA using GoScript™ Reverse Transcriptase (A5002, Promega, Beijing, China). HBV Genomic DNA was extracted from cells and liver tissues with a TIANamp Genomic DNA kit (TIANGEN, Beijing, China). All operations were performed using the manufacturers’ protocols. The mRNA levels of GATA4, HNF4α, HBV 3.5 kb pgRNA, and HBV total RNA were detected by RT-PCR or RT-qPCR, and β-actin was used as the internal control. Copies of HBV DNA from genomic DNA were determined by RT-qPCR with a serial dilution of known copies of HBV standards. The RT-qPCR was conducted using the Bio-Rad CFX connect Real-Time system with iTaq Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA), referring to the manufacturer’s instructions. The 2−∆∆Ct method was used to calculate the Cq data of the samples obtained by RT-qPCR. At least three independent repeat experiments were performed. All primers used are listed in Table 1.

Enzyme-linked immunosorbent assays (ELISA)
The level of HBsAg and HBeAg in cell culture medium were detected by using ELISA kits according to the manufacturer’s instructions (Kehua Bioengineering, Shanghai, China). The results were judged to be effective or not in advance referencing the instructions. Subsequently, the HBsAg and HBeAg levels in each sample were calculated according to the standard curve established with HBsAg and HBeAg standard (Kangchesitan, Beijing, China).

Western blotting analysis

Total cellular proteins were extracted using radioimmunoprecipitation assay lysis solution (strong; Beyotime), a protease/phosphatase inhibitor mix (Universal Type, 50×; Beyotime), and ultrasonic disruption. After centrifugation at 13,400 × g for 15 min at 4 °C, the concentration of protein in the supernatant was detected using a bicinchoninic acid protein assay kit (Solarbio, Beijing, China). Then, 20% volume of 6× protein loading buffer (Beyotime) was added to the supernatant, all
Fig. 2 (See legend on previous page.)
operations were followed the manufacturer’s agreement. The protein was then placed at 100 °C for 5–8 min for denaturation. Subsequently, the protein samples were electrophoretically separated on a 7.5% sodium dodecyl sulfate polyacrylamide gel, then transferred onto a 0.45 μm polyvinylidene fluoride membrane (Merck KGaA, Darmstadt, Germany) in an ice bath. The membrane was blocked with QuickBlock™ Western Blocking Solution (Beyotime) for 15 min, washed three times with tris-buffered saline containing 0.1% Tween 20 for 15 min each time, and the incubated with the primary antibody at 4 °C overnight. The primary antibodies used were as follows: rabbit anti-HNF4α antibody (bs-3828R, BIOSS, Beijing, China; 1:1000), mouse anti-human GATA4 monoclonal antibody (sc-25310, Santa Cruz Biotechnology, Shanghai, China; 1:1000), and mouse anti-tubulin antibody (sc-5274, Santa Cruz Biotechnology, Shanghai, China; 1:1000) as the internal control. After incubation with the primary antibodies, the membrane was washed with tris-buffered saline containing 0.1% Tween 20 and incubated with the anti-rabbit or anti-mouse secondary antibody for 1 h. Proteins were visualized using an ultra-high sensitivity enhanced chemiluminescence kit (HY-K1005, MedChem Express). All assays were performed three times independently.

Immunofluorescence analysis
Cells were seeded into 24-well plates with microcoverslips overnight and then transfected with a GATA4 plasmid and cultured for 48 h. Cells were fixed with 4% paraformaldehyde for 30 min, treated with rupture working solution (PN00014) for 10 min, and blocked with serum for 30 min. And then the slides were incubated with primary antibodies at 4 °C overnight. The primary antibodies used were as follows: GATA4 mouse monoclonal antibody, Clone OT19F9 (TA500091S, OriGene, Beijing, China; 1:1000), HNF4α (C11F12) Rabbit monoclonal antibody (3113S, Cell Signaling Technology, Boston, USA, 1:1000), and mouse anti-tubulin antibody (sc-5274, Santa Cruz Biotechnology, Shanghai, China; 1:1000) as the internal control. After incubation with the primary antibodies, the membrane was washed with tris-buffered saline containing 0.1% Tween 20 and incubated with the anti-rabbit or anti-mouse secondary antibody for 1 h. Proteins were visualized using an ultra-high sensitivity enhanced chemiluminescence kit (HY-K1005, MedChem Express). All assays were performed three times independently.

Statistical analysis
Data were analyzed using SPSS 22.0 software (IBM, Chicago, IL, USA) and ImageJ v1.8.0 (NIH, Bethesda, Maryland, USA). Continuous variables are reported as means± standard deviations calculated by Prism Software version 5.0 (GraphPad Software, La Jolla, CA, USA). Data with a normal distribution were compared with a two-tailed Student’s t-test. All experiments were repeated independently at least three times. Significance was set at P < 0.05 (non-significance at $P \geq 0.05$).

Results
Expression of GATA4 in cells
We first verified the expression of GATA4 in different normal liver cell lines or HCC cell lines via RT-PCR and western blotting (WB) to determine the appropriate cell models. The mRNA levels (Fig. 1a) and protein levels (Fig. 1b) results of these cells showed that GATA4 was expressed at low levels in the L02 cells and SMMC7721 cells, but at highly levels in the HepG2 cells and HepG2.2.15 cells. The expression level of GATA4 in different cells was the basis for the selection of cell models. Therefore, in this study, plasmid adGATA4 was treated in L02 cells and SMMC7721 cells, and siGATA4 was interfered in HepG2 cells and HepG2.2.15 cells.

GATA4 overexpression enhances the levels of HBV DNA, HBsAg and HBeAg, 3.5 kb pgRNA and total RNA
To verify whether GATA4 has a regulatory effect on HBV, adGATA4 with different mass concentrations and equal amounts of HBV cccDNA were co-transfected into L02 cells and SMMC7721 cells, respectively. The results showed that the expression of GATA4 increased with the increasing adGATA4 concentrations (all $P < 0.01$, Fig. 2a). The levels of HBV DNA copies in the cells increased significantly in the treatment group with high concentration of adGATA4 (Fig. 2b). The levels of HBsAg and HBeAg secreted into the culture medium were consistent with GATA4 expression, the increase of HBsAg was particularly significant (all $P < 0.05$, Fig. 2c).
Fig. 3 (See legend on previous page.)
Then, the levels of 3.5 kb pgRNA and total RNA of HBV were further observed after transfection with adGATA4 with a final concentration of 400 ng/mL. The results showed that the levels of 3.5 kb pgRNA and total RNA of HBV were increased compared with the normal group (Fig. 2d), although the increase in the level of HBV 3.5 kb pgRNA in L02 cells was not significant (P > 0.05). GATA4 protein was increased after transfection with adGATA4 (Fig. 2e). General results revealed that GATA4 may be involved in promoting the expression and replication of HBV.

Table 2 Retrospective analysis of GATA4 expression and HBV carrying status in clinical HCC patients

| HBV and HBsAg in HCC patients | Positive | Negative |
|-------------------------------|----------|----------|
| 68.4% (13/19)                 | 31.6% (6/19) |
| GATA4 expression              | High     | Low      |
| 68.4% (13/19)                 | 31.6% (6/19) |
| Total number                  | 3        | 10       |
| Ratio                         | 23.1% (3/13) | 76.9% (10/13) |
|                               | 16.7% (1/6) | 83.3% (5/6) |
siGATA4 negatively regulates HBV
To further verify the effect of GATA4 on HBV, we silenced GATA4 expression in HepG2 cells and HepG2.2.15 cells using siGATA4. The expression of GATA4 was effectively suppressed in the siGATA4 group (all $P<0.001$, Fig. 3a). The levels of HBV DNA and HBsAg were reduced in cells (Fig. 3b, c). Moreover, the levels of 3.5 kb pgRNA and total RNA of HBV were significantly decreased after siGATA4 (all $P<0.01$, Fig. 3d). GATA4 protein was also decreased after transfection with siGATA4 (Fig. 3e). The above results converse to prove the promoting effect of GATA4 on HBV.

GATA4 may be an upstream transcription factor regulating HBV
In order to observe whether HBV affected the expression of GATA4, we used HBV cccDNA cell model to observe the mRNA level of GATA4, and the results showed there was no clear difference in GATA4 expression in HepG2 cells before and after transfection with HBV ($P=0.46$, Fig. 4a). Subsequently, the copies of HBV DNA and the mRNA level of GATA4 were analyzed in liver tissue samples of the HBV mouse models at 4 and 24 weeks [26]. The results showed that the average copy numbers of HBV DNA are $4.2 \times 10^4$ copies and $5.7 \times 10^4$ copies in the liver tissue of the HBV CBA/CaJ mice (Fig. 4b). The expression of GATA4 did not significantly change in HBV models group compared with the control mice ($P=0.91$ or $P=0.26$, Fig. 4c). The results demonstrated that HBV had no significant effect on GATA4 in HBV cccDNA cell models and mouse models.

GATA4 reduced expression in HCC patients regardless of the HBV and HBsAg status of patients
The levels of GATA4 in clinical patients ($n=19$) were based on the results of the previous study [12]. The results showed that 68.4% of the patients were HBV and HBsAg positive, and 31.6% were HBV and HBsAg negative. Among the 19 HCC patients, compared with adjacent tissues, high or low expression of GATA4 in cancer tissues accounted for 23.1% and 76.9%, 16.7% and 83.3% in HBV-HCC patients and in non-HBV-HCC patients, respectively. The results showed that regardless of the patient's HBV and HBsAg status, the expression level of GATA4 decreased in liver cancer tissues of most patients (Table 2).

GATA4 may be associated with the host factor HNF4α that regulates HBV transcription
To observe whether HNF4α is related to the regulation of HBV by GATA4, HNF4α was detected in adGATA4 or siGATA4 cell models using RT-qPCR or western blotting (WB) assay. The results suggested that the mRNA and protein levels of HNF4α were concomitant with the expression of GATA4 (Fig. 5a, b). The results of immunofluorescence analysis of GATA4 and HNF4α in cells showed that, compared with the control group, the expression of HNF4α was increased or decreased in the cells transfected with adGATA4 or siGATA4 (Fig. 5c). Collectively, these results indicate that GATA4 may be associated with the host factor HNF4α.

Stimulating HNF4α alleviates the inhibitory effect of siGATA4 on HBV in HepG2.2.15 cells
To further confirm the role of HNF4α in HBV regulation by GATA4, GATA4 and HNF4α were observed in HepG2.2.15 cells. The results displayed that HNF4α levels could be specifically upregulated by benfluorex hydrochloride but had no significant effect on GATA4 in HepG2.2.15 cells (Fig. 6a, b). Meanwhile, HBsAg and HBV RNAs were significantly reversed by benfluorex hydrochloride in siGATA4 HepG2.2.15 cells (Fig. 6c, d). The upregulation of HNF4α attenuated the inhibitory effect of siGATA4 on HBV. Taken together, the overall results revealed that GATA4 may play a role in the upstream of HNF4α in HBV-related pathways (Fig. 7).

Discussion
HBV is one of the smallest hepatotropic DNA viruses among all identified to date, and HBV infection is one of the most difficult to be eliminated completely [28, 29]. The possibility of HBV being reactivated is still extremely high after receiving anti-tumor treatment, even in HCC patients whose HBV has turned negative [30–32]. HBV transcription, which uses HBV covalently closed circular DNA (cccDNA) as a template to synthesize 3.5 kb pgRNA and multiple messenger RNAs, is an important process for HBV persistent infection [33, 34]. This process depends on the activities of various transcription factors and chromatin-modifying enzymes [35, 36]. Therefore, exploring the effect of GATA4 on HBV has significant research value for the treatment of HBV-HCC.
Fig. 5 (See legend on previous page.)
The synthesis of various antigens in HBV utilizes different messenger RNA as the template, among which the HBsAg is the main serum marker of HBV [37]. The levels of HBsAg and HBV DNA are important indicators for judging the active status of HBV replication [38]. 3.5 kb pgRNA is the template for reverse transcription synthesis of HBV genome relaxes circular DNA (rcDNA), so it is a key intermediate to ensure
HBV continuous replication [34]. The most important finding of this study was that GATA4 had a positive regulatory effect on HBV. GATA4 overexpression promoted the secretion of HBsAg, increased the copies of HBV DNA, and enhanced the synthesis of HBV 3.5 kb pgRNA and total RNA. In contrast, the levels of HBsAg, HBV DNA and HBV RNAs were downregulated by siGATA4. Although the present study found that GATA4 could promote HBV, it has not yet been verified by southern blotting or northern blotting.

HNF4α is widely regarded as one of the key factors in the activation of HBV cccDNA and the synthesis of pgRNA [23, 39–41]. Previous studies found that HNF4α binds to the HBV genome enhancer, IEnh1/Xp [23, 42]. Moreover, HNF4α participates in the formation of cccDNA chromatin microsomes by binding to the core promoter group region of cccDNA, initiating HBV replication and transcription [22, 43]. HNF4α is also a potential target for the treatment of HBV [44–48]. In this study, the results demonstrated that HNF4α was increased simultaneously after the up-regulation of GATA4. In contrast, up-regulation of HNF4α could only increase HBV markers, but had no effect on GATA4. These results suggest that GATA4 may be a potential upstream regulator of HNF4α, and a stimulus of HBV replication and transcription. Remarkably, the direct interaction between GATA4 and HNF4α in this study had not been identified using a luciferase reporter assay and an immunoprecipitation assay. Therefore, the underlying mechanism requires further investigation.

Conclusion

Previous studies have found that GATA4 is a potential anti-HCC therapeutic target, the current study found that the expression of GATA4 was also decreased in cancer tissues of the most HBV-HCC patients. Furthermore, we innovatively found that GATA4 can promote HBV replication and transcription, and the regulation effect of GATA4 on HBV may be related to HNF4α. The overall results imply that the combination of anti-HBV agents should be considered in HBV-HCC patients when GATA4 is used as the target of antitumor gene therapy.

Abbreviations
HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBV DNA: Hepatitis B virus DNA; cccDNA: Covalently closed circular DNA; pgRNA: Pre-genomic RNA; HBV RNA: Hepatitis B virus RNA; HCC: Hepatocellular carcinoma; GATA4: GATA binding protein 4; HNF4α: Hepatocyte nuclear factor 4 alpha; adGATA4: Expression plasmid of GATA4; siGATA4: GATA4 small interfering RNA.

Acknowledgements
Not applicable.

Authors’ contributions
GQL and XQL designed the research methods and analyzed the data. XQL, XX, YW and RQX were involved in the in vitro cell experiments and drafting the manuscript. YL provided samples and data analysis obtained from in vivo experiments. QX collected the information of 19 HCC clinical patients, and provided experimental materials, such as GATA4 expression plasmid (adGATA4) and small interfering RNA (siGATA4). All authors read and approved the final manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China [Grant No. 81570541], the Min-Sheng Project of Chongqing Science and Technology [Grant No. cstc2017jshmsA130097], and the Chongqing Postgraduate Innovation Project in Scientific Research [Grant No. CYS19219].

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable. The analysis of clinical sample information in this study is a retrospective analysis of published research content. The samples and data of the mouse model in this study are derived from the remaining parts of the published research content.

Consent for publication
Not applicable.

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

Author details
1 Laboratory Animal Center of Chongqing Medical University, No. 1, Yixueyuan Road, Yuwhong District, Chongqing 400016, China. 2 The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China. 3 LuXian No. 2 High School, Schuan 646100, China. 4 Molecular Oncology and Epigenetics Laboratory of the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China.

Received: 18 June 2021   Accepted: 21 September 2021
Published online: 28 September 2021

References
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
2. De Mattia E, Cecchin E, Guardascione M, Foltran L, Di Raimo T, Angelini F, Dandrea M, Toffoli G. Pharmacogenetics of the systemic treatment in advanced hepatocellular carcinoma. World J Gastroenterol. 2019;25:3870–96.
3. Liu YX, Li QZ, Cao YN, Zhang LQ. Identification of key genes and important histone modifications in hepatocellular carcinoma. Comput Struct Biotechnol J. 2020;18:2657–69.
4. Holtzinger A, Evans T. Gata4 regulates the formation of multiple organs. Development. 2005;132:4005–14.
5. Watt AJ, Zhao R, Li J, Duncan SA. Development of the mammalian liver and ventral pancreas is dependent on Gata4. BMC Dev Biol. 2007;7:37.
6. Gao L, Hu Y, Tian Y, Fan Z, Wang K, Li H, Zhou Q, Zeng G, Hu X, Yu L, Zhou S, Tong X, Huang H, Chen H, Liu Q, Liu W, Zhang G, Zeng M, Zhou G, He...
Q. Ji H, Chen L. Lung cancer deficient in the tumor suppressor GATA4 is sensitive to TGFβR1 inhibition. Nat Commun. 2019;10:1665.

7. Gong Y, Zhang L, Zhang A, Chen X, Gao P, Zeng Q. GATA4 inhibits cell differentiation and proliferation in pancreatic cancer. PLoS ONE. 2018;13:e0204499.

8. Ma X, Ji L. Effect of GATA4 gene methylation on proliferation and apoptosis of SGC-7901 gastric cancer cells. Neoplasma. 2020;67:1193–203.

9. Han X, Tang J, Chen T, Ren G. Restoration of GATA4 expression impedes breast cancer progression by transcriptional repression of RelA and inhibition of NF-κappaB signaling. J Cell Biochem. 2019;120:917–27.

10. Liu Y, Zhao ZH, Lv XQ, Tang YW, Cao M, Xiang Q, Wu Y, Zhang HT, Lai GQ. The zinc finger protein GATA4 induces mesenchymal-to-epithelial transition and cellular senescence through the nuclear factor-kappaB pathway in hepatocellular carcinoma. J Gastroenterol Hepatol. 2019;34:2196–205.

11. Marengo A, Rossì C, Bugianesi E. Liver cancer: connections with obesity, fatty liver, and cirrhosis. Ann Conv Med. 2016;67:103–17.

12. Xie Y. Hepatitis B Virus-associated hepatocellular carcinoma. Adv Exp Med Biol. 2017;1018:11–21.

13. Sayiner M, Golabi P, Younossi ZM. Disease burden of hepatocellular carcinoma: a global perspective. Dig Dis Sci. 2019;64:910–7.

14. Jia L, Gao Y, He Y, Hooper JD, Yang P. HBV-induced hepatocellular carcinoma and related potential immunotherapy. Pharmacol Res. 2020;159:104992.

15. Wong MCS, Huang JW, George J, Huang J, Leung C, Eslam M, Chan HLY, Ng SC. The changing epidemiology of liver diseases in the Asia-Pacific region. Nat Rev Gastroenterol Hepatol. 2019;16:57–73.

16. Oropeza CE, Tarnow G, Sridhar A, Taha TY, Shalaby RE, McLachlan A. Precise analysis of the effect of basal core promoter/precore mutations on the main phenotype of chronic hepatitis B in mouse models. J Med Virol. 2020;92:1538–47.

17. Schadler S, Hildt E. HBV life cycle: entry and morphogenesis. Viruses. 2009;1:185–209.

18. Zhang Y, Mao R, Yan R, Cai D, Zhang Y, Zhu H, Kang Y, Liu H, Wang J, Qin Y, Huang Y, Guo H, Zhang J. Transcription of hepatitis B virus covalently closed circular DNA is regulated by CpG methylation during chronic infection. PLoS ONE. 2014;9:e10442.

19. Liu F, Campagna M, Qi Y, Zhao X, Guo F, Xu C, Li S, Li W, Block TM, Chang J, Guo J. Alpha-interferon suppresses hepatitis B virus transcription by altering epigenetic modification of cccDNA minichromosomes. PLoS Pathog. 2013;9:e1003613.

20. Kao JH. Diagnosis of hepatitis B virus infection through serological and virological markers. Expert Rev Gastroenterol Hepatol. 2008;2:553–62.

21. Gish RG, Given BD, Lai CL, Locarnini SA, Lau JY, Lewis DL, Schleupf T. Chronic hepatitis B virology: natural history, current management and a glimpse at future opportunities. Antiviral Res. 2015;117:45–58.

22. Zheng Y, Li J, Ou JH. Regulation of hepatitis B virus core promoter by transcription factors HNF1 and HNF4 and the viral X protein. J Virol. 2004;78:6908–14.

23. Leng XH, Chen EQ, Du LY, Bai L, Gong DY, Cheng X, Huang J, Tang H. Biological characteristics of the A1762T/G1764A mutant strain of hepatitis B virus in vivo. Mol Med Rep. 2015;12:1411–2.

24. Oropeza CE, Li L, Mclachlan A. Differential inhibition of nuclear hormone receptor-dependent hepatitis B virus replication by the small heterodimer partner. J Virol. 2008;82:3814–21.

25. Bock CT, Malek NP, Tillmann HL, Manns MP, Trautwein C. The enhancer I core region contributes to the replication level of hepatitis B virus in vivo and in vitro. J Virol. 2000;74:1919–202.

26. Chen EQ, Sun H, Feng P, Gong DY, Liu C, Bai L, Yang WR, Lei XZ, Chen LY, Huang FJ, Tang H. Study of the expression levels of Hepatocyte nuclear factor 4alpha and 4beta in 3 beta in patients with different outcome of HBV infection. J Virol. 2012;9:293.

27. Tseng YP, Wu YC, LeeYL, Yeh SF, Chou CK. Scutellariae radix suppresses hepatitis B virus production in human hepatoma cells. Front Biosci (Elite Ed). 2012:13:38–47.

28. Tseng YP, Kuo YH, Hu CP, Jeng KS, Jammanichi D, Lin CH, Chou CK, Yeh SF. The role of heloexanin in inhibiting human hepatitis B virus replication and gene expression by interfering with the host transcriptional machinery of viral promoters. Antivir Res. 2008;77:206–14.

29. Bai L, Nong Y, Shi Y, Liu M, Yan L, Shang J, Huang F, Lin Y, Tang H. Luteolin inhibits hepatitis B virus replication through extracellular signal-regulated kinase-mediated down-regulation of hepatitis B viral factor 4alpha expression. Mol Pharm. 2016;13:568–77.

30. Huang H, Zhou W, Zhu H, Zhou P, Shi X. Baicalin benefits the anti-HBV therapy via inhibiting HBV viral RNAs. Toxicol Appl Pharmacol. 2017;323:153–63.

31. Dai XQ, Cai WT, Wu X, Chen Y, Han FM. Proteostatic acid inhibits hepatitis B virus replication by activating E3泛素/2 pathway and down-regulating HNF4α and HNF1α in vitro. Life Sci. 2017;180:674–74.