Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

p.Q511L mutation of HNF1α in hepatocellular carcinoma suppresses the transcriptional activity and the anti-tumor effect of HNF1α

Chen-Hong Ding [a, 1], Long-Fei Deng [a, 1], Fei Chen [a], Kai Ding [a], Wan-Sheng Chen [b], Wei-Fen Xie [a], Xin Zhang [a, b, *]

[a] Department of Gastroenterology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[b] Department of Pharmacy, Changzheng Hospital, Second Military Medical University, 415 Fengyang Road, Shanghai 200003, China

ARTICLE INFO

Article history:
Received 17 October 2017
Accepted 31 October 2017
Available online xxx

Keywords:
HNF1α
HCC
Mutation
Transcription activity
Subcellular localization
Malignancy

ABSTRACT

Hepatocyte nuclear factor 1α (HNF1α) is a liver-enriched transcription factor that regulates many aspects of hepatocyte functions. Our previous studies have demonstrated that HNF1α has potent therapeutic effects on hepatocellular carcinoma (HCC). Mutations in HNF1α gene are frequently associated with maturity-onset diabetes of the young type 3 (MODY3) and hepatocellular adenomas. However, the association of HNF1α mutation and HCC remains elusive. In this study, the point mutation of HNF1α gene with c.A1532 > T/p.Q511L was identified in an HCC patient by exon-capture high-throughput sequencing. Mutation of c.A1532 > T/p.Q511L in HNF1α gene was only detected in the tumor tissue but not in the adjacent non-tumorous liver tissue of the patient. Luciferase reporter assay and real-time PCR revealed that mutation of p.Q511L reduced the transcriptional activity of HNF1α. Immunofluorescence staining and subcellular fraction analysis revealed that mutation of p.Q511L disturbed the intracellular localization of HNF1α in HCC cells. Moreover, the inhibitory effect of HNF1α on the proliferation, migration and invasion in HCC cells was also partially abolished by the mutation of p.Q511L. Our data suggested that the missense mutation of HNF1α (p.Q511L) may associate with the progression of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and the second leading cause of cancer death globally. The family of hepatocyte nuclear factors (HNFs) consists of a series of liver-enriched transcription factors, including HNF1, HNF3, HNF4, HNF6, and CCAAT/enhancer binding proteins (CEBPs). They play crucial roles in the development and function maintenance of the liver [1–3]. In addition, the depressed expression level of HNFs is associated with the EMT [4,5] and dedifferentiation status of HCC [6].

Hepatocyte nuclear factor 1α (HNF1α), a member of HNF family, is a Pit-Oct-Unc-homeodomain-containing transcription factor and is predominantly expressed in liver. HNF1α binds to the *cis*-acting elements of at least 200 genes in human hepatocytes and contributes to many aspects of the hepatocyte functions, such as carbohydrate synthesis and storage, lipid metabolism, detoxification, and synthesis of serum proteins [7,8]. We have previously reported that the enforced expression of HNF1α re-established the expression of certain liver-specific genes in HCC cells and impedes the growth of HCC xenografts in mice by inducing the differentiation of hepatoma cells into hepatocytes [9]. Our recent study further demonstrated that hepatocyte-specific Hnf1α knockout mice spontaneously develop HCC from fatty liver [10]. In addition, it has been reported that HNF1α inhibits Wnt and NF-κB signalling during hepatocarcinogenesis and HCC metastasis [11,12]. These studies suggest that dysregulation of HNF1α plays an important role during hepatocarcinogenesis and HCC progression.

Over the past years, substantial evidences have revealed the association of the missense mutations in HNF1α gene with hepatocellular adenomas and maturity-onset diabetes of the young type 3 (MODY3) [13,14]. However, whether mutations in HNF1α gene affects HCC development remains largely unknown. In this study, we screened the genetic variations in promoter, exons and flanking introns of HNF1α gene in HCC tissues from 16 patients by using exon-capture high-throughput sequencing. A somatic mutation of

* Corresponding author. Department of Gastroenterology, Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China.
E-mail address: zhang68@hotmail.com (X. Zhang).
[1] These authors contribute equally to this work.

https://doi.org/10.1016/j.bbrc.2017.10.174
0006-291X/

HNF1α was identified in the HCC tissue from a patient with malignant tumor family history and its effect on the function of HNF1α was further investigated.

2. Materials and methods

2.1. Patients and tissue samples

Human liver tissues were obtained from HCC patients undergoing surgical resection at the Eastern Hepatobiliary Surgery Hospital (Shanghai, China), and all patients provided written informed consent. HCC tissues with typical macroscopic features were collected from tumor nodules and were examined with hematoxylin and eosin (H&E) staining to confirm the diagnosis. The adjacent non-tumorous tissues without histopathologically identified tumor cells were collected from at least 5 cm away from the tumor border. All human experiments were approved by the ethics committee of the Second Military Medical University (Shanghai, China).

2.2. Exome-capture high-throughput sequencing and mutation detection

Genomic DNA of 16 HCC tissues was isolated by QIAamp DNA Mini Kit. The primers used for amplifying the fragment of the promoter, exons and flanking introns of HNF1α were designed by Ion AmpliSeq Designer (http://ampliseq.com). Amplified libraries were submitted to emulsion PCR using the Ion OneTouch™ 2 system with the Ion PGM™ Template OT2 200 Kit (Life Technologies, USA) according to the manufacturer's instructions. The data from the PGM runs were processed initially using the standard Torrent Mapping Alignment Program (Torrent Suite v.4.0) to generate sequence reads, trim adapter sequences, filter, and remove poor signal-profile reads. Then, reads were aligned against the hg19 reference genome with Torrent Mapping Alignment Program (TMAP), using the default alignment settings. After variant detection, variants were annotated to determine overlapping information with the genetic database ANNOVAR. Alternative allele frequency values were taken from the 1000 Genomes project, reappearing mutation were annotated with COSMIC and dbSNP137. The sequencing and analysis were performed by Shanghai Biotechnology Corporation (Shanghai, China). To validate the potential mutation of HNF1α, the fragment containing the HNF1α mutant site was amplified by PCR from the genomic DNA and cDNA of the patient and subjected to Sanger sequencing. The primers used for genomic DNA: forward primer 5′-CAGCCCTCTACAGCCACAAG-3′ and reverse primer 5′-CTGGACCTTACCTGCTTGGT-3′). The primers used for cDNA: forward primer 5′-CAGAGCCATGTGACCCAGA-3′ and reverse primer 5′-AGGTGAAGACCTGCTTGGTG-3′.

2.3. Plasmid construction and lentivirus preparation

Full-length cDNA of HNF1α and the mutation of HNF1α^{Q511L} generated by overlapping PCR were sub-cloned into the pCDH-CMV-MCS-EF1-copGFP vector (System Biosciences) and pFlag-CMV-2 vector (Sigma). For luciferase reporter plasmid pGL3-HNF4α-P2, the DNA fragment of HNF4α promoter region containing HNF1α response element (HNF1α RE, GGTTACTCTTTAAC) was inserted into pGL3-promoter (Promega). All constructs were verified by DNA sequencing. The primer sequences are listed in Supplementary Table 1.

To generate the lentiviruses for the overexpression of HNF1α and HNF1α^{Q511L}, the lentiviral vectors were transfected into subconfluent HEK293T cells together with the packaging plasmid psPAX2 and envelope plasmid pMD2.G (Addgene) using FuGENE 6 transfection reagent (Promega) to produce lentiviral particles. The lentiviruses in the medium were collected 48 h later and concentrated by ultracentrifugation.

2.4. Cell culture

The human HCC cell lines Huh-7 and Hep3B were obtained from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). 293T cells were purchased from American Type Culture Collection. Huh-7 and 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. Hep3B cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% FBS and 1×nonessential amino acid (NEAA).

2.5. Luciferase reporter assay

HCC cells plated in 24-well plates were transfected with pFlag-CMV2, pFlag-HNF1α or pFlag-HNF1α^{Q511L} together with pGL3-HNF4α-P2 and the control pRL-SV40 vector (E2261, Promega). Luciferase activity was measured using the Dual-Glo Luciferase Assay System (E2920, Promega) 24 or 48 h post-transfection.

2.6. RNA isolation, cDNA generation, and real-time PCR

Total RNA was isolated using RNAiso Plus kit (TaKaRa) according to the manufacturers' protocol, cDNA was generated by reverse PCR, and the real-time polymerase chain reaction (real-time PCR) was performed with the SYBR Green PCR Kit (Applied Biosystems, Foster City, CA). Primers for real-time PCR are listed in Supplementary Table 1.

2.7. Immunofluorescent staining

Huh-7 cells plated on the cover slides were transfected with pFlag-CMV2, pFlag-HNF1α or pFlag-HNF1α^{Q511L} for 48 h. The cells were fixed with 4% paraformaldehyde for 20 min at room temperature, permeabilized with 0.3% Triton X-100 for 5 min, blocked with 5% donkey serum for 30 min and incubated with anti-Flag antibody (Sigma) at 4 °C overnight. Alexa Fluor 488 conjugated donkey anti-mouse secondary antibody (Thermo Fisher) was further incubated at room temperature for 30 min and the slides were mounted with Mounting Medium (Sigma) containing 1 μg/ml DAPI. The images were acquired with a Leica SPE confocal microscope.

2.8. Subcellular fraction and western blot analysis

Nuclear and cytoplasmic fractions of Huh7 cells transfected with pFlag-CMV2, pFlag-HNF1α or pFlag-HNF1α^{Q511L} were prepared using Minute™ Cytoplasmic and Nuclear Extraction Kit (Invent Biotechnologies, Inc.). Proteins were subjected to SDS-PAGE electrophoresis and then transferred onto nitrocellulose membrane. The membrane was blocked in PBST (0.1% Tween-20) containing 5% skim milk, then incubated in anti-HNF1α antibody (1:2000, rabbit, Santa Cruz), anti-Flag antibody (1:3000, mouse, Sigma) or anti-GAPDH antibody (1:3000, mouse, KangChen Bio-tech) at 4 °C overnight. After 2 h of incubation with secondary antibody (donkey anti-mouse or donkey anti-rabbit, IRDye 700 or IRDye 800), signals were detected using an Odyssey Infrared Imaging System (LI-COR) at 700 or 800 nm.

2.9. Cell proliferation and colony formation ability assay

To test the effect of mutation p.Q511L of HNF1α on the proliferation of HCC cells, Huh-7 and Hep3B cells pre-infected with

Please cite this article in press as: C.-H. Ding, et al., p.Q511L mutation of HNF1α in hepatocellular carcinoma suppresses the transcriptional activity and the anti-tumor effect of HNF1α, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.10.174