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Unique genetic alterations and clinicopathological features of hepatocellular adenoma in Chinese population

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ABSTRACT

Hepatocellular adenoma (HCA) is a benign hepatocyte-derived tumor commonly seen in reproductive-aged women with long-term use of oral contraceptives (OCs) in European and North American countries. Accordingly, HCA is currently classified into four molecular subtypes as adopted by the World Health Organization. The present study was firstly to characterize and determine the genetic alterations and clinicopathological features of the largest series of HCAs in China. We reviewed 189 patients with HCA who underwent hepatectomies at our liver center from January 1984 to January 2012, among which 36 HCAs were randomly selected for the sequencing of HNF1α, β-catenin and gp130 genes, and 60 HCAs were randomly selected for detecting microsatellite instability (MSI). Compared with Western studies, our data showed distinctive findings including male (69.8%) and overweight/obese (50.3%) predominance. Only 3.5% of female patients had a documented history of OCs use for 2–4 years. All 36 sequenced HCAs showed HNF1α mutations (72% missense, 28% synonymous), 2 hotspot polymorphisms of HNF1α (I27L: rs1169288 and S487N: rs2464196) were seen in 17 (47%) and 10 (27.8%) cases, respectively, and a novel single nucleotide polymorphism site (rs1169304) in intron 9 of HNF1α was detected in 32 (88%) cases, but no β-catenin or gp130 gene mutation was detected, and no nuclear β-catenin staining was detected by immunohistochemistry. The frequency of MSI was 75% for D12S1398 (HNF1α inactivated pathway) and 78.5% for D6S1064 (HIPPO signaling pathway) in 34 overweight/obese patients with HCA. Our results firstly indicate that patients with HCA in China frequently occur in male overweigh and obese adult population, lack an association with OCs use and exhibit unique genetic alterations. Taken together, these observations suggest that alternative pathogenetic pathways involve in HCA tumorigenesis in Chinese patients.

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1. Introduction

According to the studies from European and North American countries, hepatocellular adenoma (HCA) is commonly associated with the use of oral contraceptives (OCs) in reproductive-aged women (~80%), anabolic steroid abuse, and glycogen storage disease (GSD) type Ia [1–3]. As HCA tends to bleed and occasionally undergo malignant transformation, it needs either periodic radiographic monitoring or surgical resection [4].

Recent researches in hepatic molecular pathology have greatly advanced our understanding of the pathogenesis of HCA development [5–8]. One example is that in 2002, Bluteau et al. identified distinctive point mutations in hepatocyte nuclear factor 1-alpha (HNF1α) gene using microsatellite genotyping in a subset of HCAs [9]. Several years later, they found mutations in exon 3 of the β-catenin gene in 12.5% of HCAs that had a higher propensity to undergo malignant transformation to hepatocellular carcinoma

Abbreviations: AFP, alpha-fetoprotein; BMI, body-mass index; CEA, cancer embryo antigen; CK1, casein kinase 1; GSD, glycogen storage disease; GSK3β, glycogen synthase kinase; HBsAg, Hepatitis B surface antigen; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; H-HCA, HNF1α-inactivated HCA; HNF1α, hepatocyte nuclear factor 1-alpha; I-HCA, inflammatory HCA; LATS1, large tumor suppressor homolog 1; LOH, loss of heterozygosity; MM, missense mutations; MSI, microsatellite instability; OCs, oral contraceptives; PCR, polymerase chain reaction; SM, synonymous mutations; SNP, single nucleotide polymorphism; WHO, World Health Organization.

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(HCC) [10]. Further genotype-phenotype correlation studies have led to an immunohistochemistry-consistent molecular classification of HCA [11], which, as subsequently adopted by the World Health Organization (WHO), included HNF1α-inactivated HCA (H-HCA), β-catenin-activated HCA, inflammatory HCA (I-HCA) and unclassified HCA [12].

Through a systematic review of 191 Chinese patients and 104 Europe patients, Lin et al. found that the male-to-female ratio was 1.61:1 and 1:11.6, respectively, and 4.2% and 71.2% of patients had been taking OCs for a period of 3–20 years, respectively [13]. Similar features of HCA in Chinese patients also have been reported in our early observation [14]. Given such marked gender difference, it is interesting to know whether there are differences in the genetic alterations and clinicopathological features of HCA between China and European/North American countries.

In the present study, we performed a clinicopathological study on the largest series of 189 Chinese patients with HCA who underwent hepatectomies in our hospital; sequenced HNF1α, β-catenin and gp130 genes in 36 randomly selected HCAs; and detected microsatellite instability (MSI) and loss of heterozygosity (LOH) in 60 randomly selected HCAs. Our results indicate that HCA in Chinese patients may develop along unique pathogenetic pathways that are different from those seen in European/North American patients.

2. Materials and methods

2.1. Patients

Between January 1984 and January 2012, 189 patients were pathologically diagnosed with HCA after surgical resection at Eastern Hepatobiliary Surgery Hospital. Informed consent was obtained from each patient, and the protocol was approved by the Research Ethics Committee of the hospital. According to the Chinese body-mass index (BMI), we defined a $BMI < 18.5\,m^2$ as lean, $18.5 \leq BMI < 24\,m^2$ as normal, $24 \leq BMI < 28\,m^2$ as overweight, and $BMI \geq 28\,kg/m^2$ as obese [15]. Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin.

2.2. Pathological review

Paraffin tissue sections were stained with hematein–eosin (HE). HCA diagnostic criteria are based on the WHO classification. The routine immunohistochemical staining was performed for glypican-3, CD34, cytokeratin 18 and cytokeratin 19 to differentiate HCA from focal nodule hyperplasia and HCC. Two experienced hepatic pathologists (Dr. Cong WM and Dr. Dong H) reanalyzed all cases for consistency. For each case, a set of variables was systematically recorded, including serological markers of Hepatitis B surface antigen (HBsAg), alpha-fetoprotein (AFP) and cancer embryo antigen (CEA), the macroscopic characteristics of the tumors such as tumor size and numbers, and microscopic features such as the presence of peliosis, steatosis, inflammatory infiltration, pseudoglands, and cytological abnormalities (large and irregular nuclei, high nucleo/cytoplasmic ratio). The diagnostic criteria of HCA were based on the WHO classification [12].

2.3. DNA extraction

Tumor-rich areas were carefully scraped from 10 to 16 μm unstained serial sections based on confirmation with paired HE stained sections. Genomic DNA was extracted from HCAs and non-neoplastic liver tissues using the genomic DNA isolation kit (Foregene, Chengdu, China) according to the manufacturer's instructions. The concentration of all DNA samples was quantified by a NanoDrop ND-1000 spectrophotometer (Nanodrop, Wilmington, DE, USA).

2.4. Mutation screening

Thirty-six HCA cases were randomly selected for mutation analysis. Genomic DNA was analyzed by polymerase chain reaction (PCR) using Takara Taq™ kit (Takara Bio Inc., Dalian, China). All HNF1α exons, mutational hot spots within exon 3 of β-catenin and exon 6 of gp130 were PCR-amplified using conditions and primers as described previously [10]. The PCR products were purified with the QIAquick PCR Purification Kit (250) (Qiagen), then directly sequenced with the use of Big Dye Terminator Chemistry (Applied Biosystems, Carlsbad, CA, USA) with an Applied Biosystems 3700 sequencer. The sequence data were analyzed using Factura and Autoassembler software (PE Biosystems, Carlsbad, CA, USA). Mutations that resulted in an amino acid substitution were defined as missense mutations (MM), and those with no amino acid substitution were defined as synonymous mutations (SM).

2.5. Microsatellite analyses

Sixty HCAs (including 24 cases of overweight and 10 cases of obesity) were randomly selected for microsatellite MSI and LOH analyses. Evaluation of 5 metabolism-related genes by detection of MSI and LOH frequencies on 12 microsatellite markers were: HNF1α (D12S1398, D12S2073, D12S2088); insulin-like growth factor 2 receptor (D6S2075, D6S2151, D6S1581); mitochondrial uncoupling protein 2 (D11S3588, D11S4207); large tumor suppressor homolog 1(LATS1) (D6S1064, D6S2184); cytochrome P450 1B1 (CYP1B1) (D2S1346, D2S2726). The primers were searched from PubMed. As we described previously [16,17], MSI and LOH were detected by PCR simple sequence length polymorphism method. The existence of MSI and LOH was determined by comparing tumor tissues with corresponding non-tumor tissues. When two amplified bands per locus were detected in non-tumor tissue samples, the case was defined as informative. MSI was defined as band shift or the presence of one or more novel bands in one of the two alleles in tumor DNA. LOH was identified when the band intensity of tumor DNA was reduced more than 50% compared with normal DNA bands. MSI and LOH were only detected in informative cases. MSI-low (MSI-L) and MSI-high (MSI-H) were defined as the total frequency of MSI $<30\%$ and $\geq 30\%$ in a microsatellite marker, respectively [18]. The assays for LOH and MSI detection were performed at least in duplicate.

2.6. Immunohistochemical analyses

Besides above-mentioned routine immunohistochemical markers, a mouse monoclonal anti-β-catenin antibody (BD Biosciences) diluted at 1:200 was also performed immunohistochemically on paraffin sections of HCA tissue blocks containing both tumor and adjacent non-tumor tissues. Antigen retrieval was achieved using citrate buffer, and immunoreactivity was amplified by the Dako Envision system (Dako Cytomation, Glostrup, Denmark). Only staining within the cell nucleus was considered to be positive [11,12].

2.7. Statistical analyses

The correlation between genetic alternations and clinicopathological features was analyzed using SPSS 19.0 software. For quantitative variables, data were shown as means ± standard error. All reported p-values were two-tailed; and a p-value of less than 0.05 was considered statistically significant.

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