Case Report

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Exome analysis of the evolutionary path of hepatocellular adenoma-carcinoma transition, vascular invasion and brain dissemination

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Summary

Hepatocellular adenoma (HCA) is a rare benign liver tumor, predominantly seen in young women. Its major complications are malignant transformation, spontaneous hemorrhage, and rupture. We describe a case of a young female with no underlying liver disease who presented with acute abdominal pain and was found to have a 17 cm heterogeneous mass in the left lobe of the liver. She underwent left hepatectomy and pathology revealed a 14 cm moderately differentiated hepatocellular carcinoma (HCC) arising in a shell of a HCA. At that time, vascular invasion was already present. She rapidly developed recurrent multifocal hepatic lesions and subsequent spread to the brain, leading to her death 18 months after surgery. To investigate the underlying genetic events occurring during hepatocellular adenoma-carcinoma transition and extra-hepatic dissemination, we performed whole exome sequencing of DNA isolated from peripheral blood leucocytes, HCA, HCC, tumor thrombus and brain metastasis. Our data show a step-wise addition of somatic mutations and copy number variations with disease progression, suggesting a linear tumor evolution, which is supported by clonality analysis. Specifically, using a model based clustering of somatic mutations, one single founding clone arising in the HCA, which included catenin beta 1 (*CTNNB1*) and *IL6ST* driver mutations, was identified and displayed an increasing clonality rate in HCC, tumor thrombus and brain metastasis. Our data highlight the feasibility of performing whole exome capture, sequencing and analysis using formalin-fixed paraffin-embedded (FFPE) samples, and we describe the first genomic longitudinal study of hepatocellular adenoma-carcinoma transition, vascular invasion and brain metastasis with detailed clinico-pathologic annotation.

Introduction

Hepatocellular adenoma (HCA) is a rare benign liver tumor, predominantly seen in young females. HCAs are known to be associated with prolonged exposure to estrogens (*i.e.* oral contraceptives, OCPs), anabolic androgens, and rare genetic disorders, namely maturity-onset diabetes of the young type 3 (MODY 3, caused by mutation in the hepatocyte nuclear factor 1-alpha gene, *HNF1A*), glycogen storage disease type 1a, and McCune-Albright syndrome. The most severe complications of HCAs are malignant transformation and spontaneous hemorrhage and rupture, both associated more often with large adenomas measuring $\geqslant$5 cm in maximum diameter. Additionally, β-catenin activation is known to be an early event in HCA transformation[1] and exon 3 β-catenin mutated HCAs have a higher risk of malignant transformation.[2] The application of massively parallel sequencing technologies to study tumors, including HCC, has refocused the field of cancer genetics from hypothesis-driven to hypothesis-generating, overcoming the limitations and biases of candidate gene approaches. Genomic profiling of HCAs has recently identified genetic and epigenetic events associated with adenoma-carcinoma transition, such as recurrent genetic alterations in catenin beta 1 (*CTNNB1*), interleukin 6 signal transducer (*IL6ST*), and telomerase reverse transcriptase (*TERT*) promoter; increased occurrence of copy number variation (CNVs); and hypomethylation of liver-specific genes.[1] Currently, HCAs are classified into five major molecular subgroups; (i) *HNF1A*-mutated HCAs;[3]

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(ii) inflammatory HCAs characterized by Janus kinase and two signal transducer and activator of transcription (JAK/STAT) pathway activation;[1,4–6] (iii) exon 3 *CTNNB1*-mutated HCAs (half of them also have an inflammatory phenotype/genotype);[2] (iv) exon 7 or 8 *CTNNB1*-mutated HCAs, with a lower risk of malignant transformation as compared to exon 3 *CTNNB1*-mutated tumors;[1,7] and (v) HCAs characterized by sonic hedgehog pathway activation via fusion of the inhibin beta E subunit (*INHBE*) and glioma-associated oncogene family zinc finger 1 (*GLI1*) genes.[8] Less than 10% of HCAs remain molecularly unclassified. However, the prognosis for those afflicted with HCA(s) is not predictable, and it remains clinically challenging to manage and counsel these patients. Furthermore, the incidence of HCAs has been increasing in the last several decades, likely due to the introduction and rising use of OCPs, and improvements in diagnostic imaging leading to ever increasing incidental findings in patients undergoing abdominal imaging studies for evaluation of unrelated or nonspecific abdominal symptoms. Identification of reliable predictive biomarkers of malignant transformation is necessary to determine optimal therapy (medical *vs.* surgical).

Here, we describe a rare case of a 33-year-old female with no significant past medical history who presented with acute abdominal pain and was found to have a 17 cm left hepatic lesion for which she underwent left hepatectomy. Pathology revealed a 14 cm moderately differentiated HCC in a background of HCA. Vascular invasion was present. The patient rapidly developed recurrent multifocal hepatic lesions and subsequent spread to the brain, culminating with her death 18 months after surgery. We hypothesized that a comprehensive longitudinal genomic and pathological analysis of the patient's HCA, HCC, tumor thrombus and brain metastasis would inform the evolutionary path of this atypical HCC presentation.

Materials and methods

Blood and tumor samples

The study was performed in accordance with the Yale Institutional Review Board standards. DNA was extracted from a sample of the patient's peripheral leucocytes, as well as from formalin-fixed paraffin-embedded (FFPE) samples of her typical HCA, atypical steatotic HCA, HCC, tumor thrombus and brain metastasis.

Whole exome capture and sequencing

We performed whole exome sequencing (WES) of DNA isolated from typical HCA, atypical steatotic HCA, HCC, tumor thrombus, brain metastases, and matching peripheral blood leucocytes with mean target coverage of 205×, 235×, 223×, 194×, 163× and 78×, respectively. The quality control on raw reads, alignment, marking PCR duplicates, local realignment and base quality score was performed as detailed previously.[9]

Exome sequencing analysis

Somatic variant calling
We used the haplotype caller implemented in GATK (version 2.5)[10] for all five tumor stages and matching blood. We identified the somatic variants as previously described.[9]

Somatic copy number variation analysis
CNV segments were calculated using the segments called by the ExomeCNV R package, and depth of coverage log ratio (between tumor sample and blood) was calculated using the GATK-depth of coverage tool. The called segments were corrected by calculating minor allele frequencies (BAF) in each CNV segment. The admixture rate, *i.e.* the rate of normal tissue contamination in the tumor samples, was calculated using the BAF shift of germ line heterozygous mutations in the regions of copy number loss using qpure R package.[11] The calculated admixture rates for the vascular invasion and the brain metastasis were 0.5 and 0.2, respectively. However, for the samples with no loss of heterozygosity (LOH), *i.e.* HCA, atypical HCA and HCC, the admixture rate could not be calculated, and it was estimated to be closer to the brain metastasis (a solid tumor), and a default admixture rate of 0.3 was used.

Clonality analysis
We performed clonality analysis by integrating the CNV information, admixture rate and non-reference variant allele frequency in the somatic variant data as previously described.[12] Clonality rate corresponded to the percentage of tumor cells harboring the identified somatic mutation. Mclust package in R was used to cluster the unique 37 protein altering somatic mutations in four tumors based on their clonality rate distributions. Bayesian information criteria was used to find the model with the optimal number of clusters (http://www.stat.washington.edu/mclust/).

Mutational signature analysis
Mutational signature analysis comprised all somatic single nucleotide polymorphisms, including protein altering, synonymous and (captured) non-coding variants.

Sanger sequencing of genomic DNA

Sanger sequencing of genomic DNA isolated from HCA and HCC FFPE samples for recurrent *TERT* promoter mutations in two "hot spots" (chr5:1,295,228, G>A/T, and chr5:1,295,250, G>A) was performed by PCR amplification, using forward primer: 5′-CAGCCCTGGGGCCCCAGGCGCCGCACG-3′, and reverse primer: 5′-CTG GGAGCCCGGCCTGGCCCCGACAG-3′ for mutation at chr5:1,295,228; and forward primer: 5′-CTGGGGCCCCAGGCGCCGCACGAACG-3′, and reverse primer: 5′-GCTG CTCCGGGCGGACCCGGGGGTC-3′ for mutation at chr5:1,295,250.

Droplet digital PCR (ddPCR)

Bio-Rad IL6ST deletion ddPCR assay (forward primer: 5′-ATTCTCTGCTTCTACC CAG-3′; reverse primer: 5′-CCTCATGCACTGTTGATTAT-3′; deletion probe: cagacttcaatGacagtagaa; wild-type (WT) probe: caatGTTGACAAAATACacagt) was performed using genomic DNA isolated from peripheral leucocytes, HCA, HCC and brain metastasis.

RNA isolation and quantitative RT-PCR

RNA was isolated from background normal liver parenchyma, HCA and HCC FFPE samples and cDNA was synthetized. Expression was assessed by quantitative RT-PCR using Taqman (Applied Biosystems) gene expression assay (TERT, Hs00972656_m1). Taqman gene expression assay for RNA ribosomal 18 S (Hs99999901_s1) was used as internal control. HCT116 colon cancer cell line was used as a positive control for TERT gene expression assay.

Liver immunohistochemistry

Immunohistochemistry was performed in FFPE liver tissues with the following antibodies: anti-c-reactive protein (CRP) (monoclonal rabbit, 1/400 dilution; Abcam), anti-liver fatty acid binding protein (LFABP) (polyclonal rabbit, 1/100 dilution; Abcam), anti-β-catenin (monoclonal mouse, 1/200 dilution; BD Biosciences); anti-glutamine synthetase (GS) (monoclonal mouse, 1/400 dilution; BD Biosciences), and anti-serum amyloid A (SAA) (monoclonal mouse, 1/100 dilution; Dako).

For further details regarding the materials used, please refer to the CTAT table.

Case report

A 33-year-old female with no underlying liver disease or other significant co-morbidities presented with acute abdominal pain and was found to have a 17 cm left hepatic mass. She underwent