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Original contribution

Low frequency of Helicobacter DNA in benign and malignant liver tissues from Baltimore, United States

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Summary Helicobacter DNA has been reported in hepatocellular carcinoma tissues in several studies from varying geographic locations, raising the possibility that Helicobacter infection may contribute to the pathogenesis of hepatocellular carcinoma. Other known risk factors for hepatocellular carcinoma show significant geographic variability, but whether the same holds for Helicobacter is unknown. We studied the prevalence of Helicobacter DNA in a US cohort of hepatocellular carcinoma, where the prevalence of Helicobacter infection is low in the general population. Liver tissues from 57 individuals were examined. Thirty-five individuals had paired tumor/nontumor samples, including 21 cases of hepatocellular carcinoma, for a total of 92 samples studied. Both Helicobacter genus and Helicobacter pylori species-specific polymerase chain reaction was performed. Helicobacter DNA was detected in 5 (9%) of 57 cases, all in nonneoplastic cirrhotic liver tissues from individuals with hepatitis C infection (n = 4) or alcohol liver disease (n = 1). Tissues from 22 hepatocellular carcinomas and 10 cholangiocarcinomas were all negative as were tissues from 8 benign primary hepatic tumors. In conclusion, Helicobacter DNA was detectable in 9% of liver tissues in this cohort but was not found in primary benign or malignant liver tumors. These findings indicate that Helicobacter infection is unlikely to be etiologically associated with hepatocellular carcinoma in this cohort. If Helicobacter infection does contribute to the development of hepatocellular carcinoma in general, then significant regional variability must exist.

1. Introduction

Helicobacter species were reported to colonize the livers of mice and cause hepatitis and hepatocellular carcinoma (HCC) in 1994 [1-3]. A subsequent report demonstrated the presence of the Helicobacter DNA in the human biliary tree [4]. Since that time, a growing body of literature has suggested a role for Helicobacter in human HCC. Specifically, individuals with HCC are more likely to have serologic evidence for past Helicobacter infection [5-8], and Helicobacter DNA can be found in HCC tissues more commonly than in controls [9-16]. In addition to HCC, Helicobacter DNA has also been reported in intrahepatic cholangiocarcinomas [9,12]. The potential role of Helicobacter infection in the development of HCC and intrahepatic cholangiocarcinomas has not been studied in the United States, where the prevalence of Helicobacter is low. Thus, the aims of this study were to determine the frequency of Helicobacter DNA in benign liver tissues, primary HCC, and intrahepatic cholangiocarcinoma, to better understand the role of Helicobacter infection, if any, in this region.

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2. Materials and methods

Liver tissues from the Johns Hopkins Hospital, Baltimore, MD, were retrospectively studied. All tissues were from individuals who underwent liver transplantation (n = 40) or surgery for a hepatic mass (n = 17). Tissues were harvested at the time of surgery and stored at −80°C before use.

2.1. Helicobacter DNA amplification

DNA was extracted from 20 to 25 mg of liver tissue using the QIAmp DNA mini kit (Qiagen, Valencia, CA). At least 100 ng of liver DNA was used for each 25-μL polymerase chain reaction (PCR), using primers listed in Table 1. To aid in comparing results from other studies, single-round PCR was performed using primers reported by Rocha et al [15]. For Helicobacter single-round PCR assays using genus- and species-specific primers, 45 cycles of amplification were performed to enhance sensitivity. Hot start Taq was used, and conditions were 95°C for 10 minutes followed by 45 cycles of 95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 45 seconds. A nested PCR assay was also performed on all liver samples (Table 1), with a first round of 50 cycles of 95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 45 seconds. Using 1 μL of the first-round product as template, a second round of PCR was performed for 45 cycles using the conditions of 95°C for 20 seconds, 55°C for 30 seconds, and 72°C for 45 seconds. In all samples, primers to the beta-catenin gene (CTNNB1) were used to ensure that extracted DNA was amplifiable.

2.2. Positive controls

Three gastric biopsies (unrelated to the liver samples) with active chronic Helicobacter pylori infection were microdissected from formalin-fixed, paraffin-embedded tissues. The DNA was extracted and used as a control to ensure that the Helicobacter primers worked adequately. The amplicons from 1 of the positive controls were then cloned (Invitrogen, Carlsbad, CA) and PCR performed on 10-fold serial dilutions to determine the sensitivity of the PCR assays. In addition, for each PCR assay, 10, 100, and 1000 copies of cloned Helicobacter DNA were spiked into pooled liver DNA from 5 randomly selected liver samples and each PCR performed to investigate the performance of the primers in the setting of abundant liver genomic DNA.

3. Results

All sets of Helicobacter primers amplified all positive control stomach biopsies. For the single-round PCR assay, serial dilutions of the cloned amplicons demonstrated sensitivity down to 10 copies per reaction for primers C-97/C-98 and HPYS/HPYA and 100 copies per reaction for HS-1/HS-2 (Fig. 1). Spiking the cloned Helicobacter DNA into liver genomic DNA did not interfere with the assay's sensitivity (Fig. 1).

Liver tissues from 57 individuals were examined. Thirty-five individuals had paired tumor/nontumor samples, including 21 cases of HCC, for a total of 92 samples studied.

Table 1 Primers used in PCR amplification

Primer	Sequence, 5′ to 3′	Target	Amplicon size (bp)
Helicobacter genus			
HS-1 [9]	AACGATGAAGCTTCTAGCTTGCTAG	16S rDNA	400
HS-2	GTGCTTATTCGTTAGATACCGTCAT		
C97 [4]	GCTATGACGGGTATCC	16S rDNA	400
C98 [4]	GATTTTACCCCTACACCA		
Helicobacter pylori			
HPY S [17]	AGGTTAAGAGGATGCGTCAGTC	23S rDNA	267
HPY A [17]	CGCATGATATTCCCATTAGCAGT		
Heminested PCR			
Helicobacter genus			
Outer primers [18]			
HG-1	TATGACGGGTATCCGGC	163 rDNA	375
HG-2	ATTCCACCTACCTCTCCCA		
Inner primers			
HG-3	CTGAGACACGGTCCAGACTC		293
HG-2	CAAATGCAGTTCTRYRGTTAAGC		
CTNNB1			
For	ATGGAACCAGACAGAAAAGC	Beta-catenin	200
Rev	GCTACTTGTTCTTGAGTGAAG		

The nonnested primers were selected from those used by Rocha et al [15]. The original citation is also indicated in the table. rDNA indicates ribosomal DNA.