Diagnostic Microbiology and Infectious Disease 88 (2017) 62–68

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Human cytomegalovirus detection in gastric cancer and its possible association with lymphatic metastasis

Liang Zhang [a,1], Gangqiang Guo [b,1], Jianfeng Xu [a], Xiangwei Sun [a], Wenjing Chen [a], Jinji Jin [a], Changyuan Hu [a], Peichen Zhang [a], Xian Shen [a,c,*], Xiangyang Xue [b,**]

[a] Department of Gastrointestinal Surgery, the First Affiliated Hospital of Wenzhou Medical University, Ouhai District, 325000, Wenzhou, Zhe Jiang, P.R.China
[b] Department of Microbiology and Immunology, Institute of Molecular Virology and Immunology, Institute of tropical medicine, Wenzhou Medical University, Ouhai District, 325000, Wenzhou, Zhe Jiang, P.R.China
[c] Department of Gastrointestinal Surgery, the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Luchen District, 325027, Wenzhou, Zhe Jiang, P.R.China

ARTICLE INFO

Article history:
Received 18 July 2016
Received in revised form 21 December 2016
Accepted 1 February 2017
Available online 8 February 2017

Keywords:
Quantitative real-time PCR
Immunohistochemistry
Virus and cancer
Metastasis

ABSTRACT

Increasing evidence suggests that human cytomegalovirus (HCMV) is associated with many human malignancies. However, its prevalence in gastric cancer (GC) and clinical association remain unknown. HCMV IgG and IgM antibodies in the sera of 80 GC patients and 80 healthy controls were detected using a microparticle enzyme immunoassay. The prevalence of HCMV UL47, UL55, UL56, and UL77 genes among 102 GC tumor tissues and adjacent normal specimens was measured by polymerase chain reaction (PCR) or nested PCR. Quantitative real-time PCR (Q-PCR) was used to determine viral load. Virus localization in neoplastic tissues was determined by immunohistochemistry. No significant difference of HCMV IgG and IgM seropositivity was found between GC patients and the healthy group. However, the overall HCMV DNA positivity rate was significantly higher in GC cancerous tissue compared with in paired normal tissue ($P < 0.01$). HCMV infection was mainly localized in the tumorous epithelium. Q-PCR in HCMV-positive specimens indicated that the viral copy number was notably higher in GC tissues than in adjacent normal specimens ($P < 0.001$). Clinical statistical analysis indicated that HCMV load in GC tumor tissue was positively associated with lymphatic metastasis ($P = 0.043$), the area under the receiver operating characteristic (ROC) curve was 0.6638. Our data clearly provide the prevalence of HCMV in GC patients. We conclude that HCMV infection in malignant tissues might be associated with carcinogenesis or progression of GC and possibly relates to lymphatic metastasis.
© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Gastric cancer (GC) is the fourth most common malignancy worldwide and a leading cause of cancer mortality second only to lung cancer (Torre et al., 2015). It is generally accepted that gastric carcinogenesis is a multistep and multifactorial process with various etiologies, genetic changes, and phenotypes (Hunt et al., 2015). Among them, infections such as with *Helicobacter pylori* or Epstein–Barr virus are important risk factors leading to GC (Abreu and Peek, 2014; Chen et al., 2015).

Human cytomegalovirus (HCMV) is a large member of the human herpesvirus family with a double-stranded DNA genome of over 230 kb (Dunn et al., 2003). HCMV affects 30–100% of normal adults and can cause a life-long latent infection (Ho, 2008). Recent data have suggested that HCMV is associated with several cancers such as glioblastoma, colorectal cancer, breast cancer, prostate cancer, and leukemia (Bishop et al., 2015; Chen et al., 2012; Geder et al., 1977; Harkins et al., 2002; Kim and Ahn, 2015; Rahbar et al., 2013). Additionally, HCMV can induce disorders in the gastrointestinal tract, including ulcerations, erosions, and mucosal hemorrhage (Goodgame, 1993). However, there has been little research on the relationship between HCMV and GC other than our previous study that identified viral RNA in GC tissues (Jin et al., 2014). In this present study, we used a variety of methods to detect the prevalence of HCMV in GC, and to examine the possible significant association between HCMV infection and GC clinical features.

2. Materials and methods

2.1. Study population and specimens

This study was approved by the Review Board of the First Affiliated Hospital of Wenzhou Medical University (Zhejiang Province, China) and written informed consent was obtained from all participants. One hundred and two patients (84 male and 18 female, mean age and standard deviation, 65.29 ± 11.6 years), who underwent surgical resection for GC between May 2014 and December 2014, were included in this prospective cohort study. Gastric adenocarcinoma and paired adjacent normal tissues (at least 10 cm from the negative reception margin)

* Corresponding author. Tel.: +86-13968888872; fax: +86-577-88832693.
** Corresponding author. Tel.: +86-15058788169; fax: +86-577-86689961.
E-mail addresses: 13968888872@163.com (X. Shen), wzxxy001@163.com (X. Xue).
[1] The first two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.diagmicrobio.2017.02.001
0732-8893/© 2017 Elsevier Inc. All rights reserved.

were collected. Both gastric tumorous and adjacent normal specimens were confirmed by histopathologic diagnosis. Once specimens were separated, they were rinsed twice in phosphate-buffered saline (PBS) to wash off external fluid before being deposited into freezing tubes and stored in liquid nitrogen at the tissue bank of the Division of Gastrointestinal Surgery, First Affiliated Hospital of Wenzhou Medical University. The remnant excision parts were fixed with formalin for immunohistochemistry (IHC).

Eighty sera samples were obtained from GC patients (53 male and 27 female, mean age and standard deviation, 64.32 ± 9.70 years) before surgery and without chemoradiotherapy or immunotherapy. Eighty age- and sex-matched samples collected from healthy donors (48 male, 32 female, mean ages and standard deviation, 62.82 ± 8.99 years) were used as controls. All serum samples were stored at −20 °C.

2.2. Testing of HCMV-specific serum IgG and IgM antibodies

Serum IgG and IgM antibodies against HCMV were detected by chemiluminescence immunoassay according to the manufacturer's instructions (Cobas 8000 Analysis System and Auxiliary Kit; Roche, IN, USA). The kit includes a blank control, negative control, and positive control.

2.3. Genomic DNA extraction

Total genomic DNA from 102 paired tumor and normal tissue samples was extracted using a QIAamp DNA mini kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocol. The concentration and purity of the extracted DNA samples were quantified using a NanoDrop 1000 spectrophotometer (Thermo, MA, USA) and 1% agarose gel electrophoresis. Samples were stored at −20 °C until use.

2.4. Polymerase chain reaction detection of UL47, UL55, UL56, and UL77

To avoid polymerase chain reaction (PCR) contamination, experiments were conducted as described previously (Harkins et al., 2002). Primer information is shown in Table 1. DNA from a clinically isolated HCMV strain or AD169 (ATCC, VA, USA) was used as a positive control, and sterile double-distilled water was used as a negative control. PCR amplification was performed in T100TM Thermal Cycler (Bio-Rad, CA, USA). PCR reactions contained 200 ng DNA temple, 1× Taq MasterMix (Tiangen Biotech Co., Ltd.), and 0.2 μM of each specific forward and reverse primer. The total volume was adjusted to 20 μL with double-distilled water. After initial denaturation at 95 °C for 5 min, 35 cycles of DNA amplification were performed (95 °C for 30 s, annealing for 30 s, 72 °C for 30 s), followed by terminal extension at 72° for 10 min. UL55-based nested-PCR detection (Table 1) followed a previously reported method (Alkhawaja et al., 2012). Finally, 5 μL of PCR product were electrophoresed on 1.2% agarose gels stained with ethidium bromide. We regarded samples as HCMV positive if a band could be seen in the correct position on the agarose gel. The PCR products were further subjected to gene sequencing to confirm specific amplification of HCMV genes.

2.5. Quantitative real-time PCR (Q-PCR)

HCMV viral loads in tumorous tissues and paired adjacent normal tissues from GC patients were further tested by UL56 Q-PCR detection. DNA from a clinically isolated HCMV strain or AD169 was used as a positive control, and sterile double-distilled water was used as a negative control. The human *GAPDH* gene was chosen as an internal control. The reaction (20 μL) contained 200 ng DNA, 1× FastStart Essential DNA Green Mastermix (Roche), and 0.2 μM UL56- or *GAPDH*-specific forward and reverse primer. The PCR program was 95 °C for 5 min; then 40 cycles of 94 °C for 30 s, 67 °C for 30 s, and 72 °C for 30 s. Q-PCR was performed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). Melting curves were generated for each reaction to verify their specificity and reactions were performed in duplicate. The comparative viral copy (C_{virus}) was normalized to the amount of *GAPDH* using the $2^{-\Delta Ct}$ method. $\Delta CT = CT_{UL56} - CT_{GAPDH}$.

2.6. Immunohistochemistry

IHC detection in paraffin-embedded pathological tumor and paired normal specimens was performed according to a previously described method (Chen et al., 2016). Briefly, formalin-fixed specimens embedded in paraffin were sectioned into 5-μm samples and processed with 0.1% poly-L-lysine. The sections were dewaxed using dimethyl benzene followed by immersion in distilled water. Endogenous peroxidase activity was inhibited by incubation in a 0.3% hydrogen peroxide bath for 10 min. After washing three times with 0.01 M PBS (pH 7.4), the slides were immersed in citrate antigen retrieval buffer (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 1.5 min. After blocking in sheep serum for 2 h, the sections were incubated in a mouse anti-HCMV immediate early (IE) antibody (MAB810R; Millipore, MA, USA) diluted 1:200, in a humidified chamber at 4 °C overnight. A PBS-only negative control was also performed. After washing three times with PBS, the sections were incubated in horseradish peroxidase-conjugated secondary goat anti-mouse antibodies (MaiXin Bio, Fuzhou, China) at 37 °C for 30 min. The color reaction was developed using a 3,3′-diaminobenzidine kit (Zhongshan Golden Bridge Biotechnology) according to the manufacturer's instructions. Slides were then counterstained using hematoxylin, dehydrated, and sealed with neutral gum.

2.7. Statistical analysis

The differences in the prevalence of HCMV infection in gastric tumor and adjacent normal tissue, as well as the prevalence of sera HCMV-specific IgG and IgM antibodies in GC patients and healthy controls, were analyzed using the chi-squared test, the R × 2 test with Yates' correction, or Fisher's exact test. Because the HCMV viral loads fit the Gaussian distribution, the Mann–Whitney test or Kruskal–Wallis H test was used to evaluate the differences in viral loads between groups. The SPSS 19.0 package (SPSS Institute, Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA) were used for statistical analyses and scientific graphing. $P < 0.05$ was considered statistically significant.

Table 1
Primer information.

Gene[a]	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Target Size	Anneal temperature (°C)
UL47	GTACAGCCCCACGTTCCTG	CCCGATACAGGTACTCGCGCT	212 bp	58
UL56	TCCTCCACGTCCTCCCCGTA	AGGCGCTGAGGGAGTACAAC	200 bp	65
UL77	GCACTTTTGATCGTCACGTGCT	ACGCAGATATTGCTGTTCGTGC	215 bp	65
UL55-outer	GAGGACAACGAAATCCTGTTGGGCA	GTCGACGGTGGAGATACTGCTGAGG	150 bp	58
UL55-inner	ACCACCGCACTGAGGAATGTCAG	TCAATCATGCGTTTGAAGAGGTA	100 bp	58
GAPDH	CAGGGCTGCTTTTAACTCTGGTAA	GGGTGGAATCATATTGGAACATGT	101 bp	58

[a] UL55 primers were as previous reported[16].

Download English Version:

https://daneshyari.com/en/article/5665939

Download Persian Version:

https://daneshyari.com/article/5665939

Daneshyari.com