Tumoral presence of human cytomegalovirus is associated with shorter disease-free survival in elderly patients with colorectal cancer and higher levels of intratumoral interleukin-17

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Abstract

Infectious diseases are closely related to cancer. Human cytomegalovirus (HCMV) has been implicated in the promotion of tumour growth, and is present in the tumour specimens of colorectal cancer (CRC). This study aimed to investigate whether tumoral presence of HCMV is associated with a different clinical outcome in elderly patients with CRC. We analysed archived tumour specimens from 95 CRC patients aged ≥65 years. HCMV was detected by PCR. Clinical, pathological, disease-free and overall survival data were compared between patients with HCMV-positive and HCMV-negative tumours. A quantitative RT-PCR array was used to evaluate the expression levels of cytokines genes of T-helper subpopulations in tumours. In the Kaplan–Meier analysis of the 81 patients who underwent curative surgery, 39 patients with HCMV-positive tumours had a lower disease-free survival rate (p 0.024). For patients with stage II or stage III tumours, tumoral HCMV status correlated with disease-free survival more closely than the traditional histopathological staging methods. In a multivariate Cox proportional hazard model, tumoral presence of HCMV independently predicted tumour recurrence in 5 years (hazard ratio 4.42; 95% CI 1.54–12.69, p 0.006). The qRT-PCR analysis of ten stage II tumours showed that the gene expression levels of interleukin-17—the signature cytokine of T-helper 17 cells—and its receptor, interleukin-17 receptor C, were higher in the five HCMV-positive tumours. Our results suggest that the presence of HCMV in CRC is associated with poorer outcome in elderly patients. How the virus interacts with the tumour microenvironment should be further investigated.

Keywords: Colorectal cancer, human cytomegalovirus, malignancy, survival, T-cell
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Introduction

Infectious diseases are closely related to human cancers. The WHO estimates that >15% of new cancer cases are attributable to infectious diseases [1]. Infectious agents can participate in the oncogenic process in diverse ways. Persistent infection arouses inflammatory cascades that damage the genetic

content of the host. Viruses such as human papillomaviruses and hepatitis B virus directly invade cells, and stimulate cell proliferation or inactivate tumour suppressor genes [2,3]. Microorganisms may also be involved in the oncogenic process by modulating the host immune system, as in the case of human immunodeficiency virus [4].

Human cytomegalovirus (HCMV), a herpesvirus that infects 60–90% of the general population, has been regarded as an oncomodulatory virus because of its effects on cell-cycle progression, mutagenesis, angiogenesis, and immune evasion [5–7]. Genetic components or gene products of HCMV have been detected in a number of human cancers [8–12]. Several studies have identified HCMV in the tumour tissues of colorectal cancer (CRC) [13–15]. We recently demonstrated that >40% of CRC specimens were positive for HCMV. Viral DNA was detected preferentially in tumoral specimens over the adjacent non-neoplastic tissue [15]. Nevertheless, whether tumoral infection with HCMV is associated with a different clinical outcome is not clear. This study aimed to clarify whether the tumoral presence of HCMV is associated with a different clinical outcome. Here, we show that the tumoral presence of HCMV is associated with shorter disease-free survival after curative surgery in elderly CRC patients, and is associated with higher levels of intratumoral interleukin (IL)-17, the signature cytokine of the T-helper (T_H)17 pathway, which was recently found to be closely related to the prognosis of CRC.

Materials and Methods

Study population and data collection

The study was approved by the Institutional Review Board of Taipei Veterans General Hospital (VGHTPE). For HCMV detection, tumour specimens from patients with CRC who underwent primary resection between 2000 and 2010 were randomly retrieved from the bank of residual surgical tissues at the Division of Colorectal Surgery, VGHTPE. No restriction was placed on gender, ethnicity, or disease staging. Demographic, clinical and pathological data were extracted from the CRC database of the Division of Colorectal Surgery, VGHTPE. These data had been recorded prospectively with regular updates, and included patient demographic information, underlying medical problems, details of surgery, characteristics of the tumour, important pathological prognostic features, and disease condition and survival at the last follow-up.

For tumour staging, the tumour-node-metastasis (TNM) classification was used [16]. Early metastasis was defined by the presence of vascular emboli, lymphatic invasion, and perineural invasion, singly or in combination. The observation time was the interval between the diagnosis and the last follow-up or death. Data were censored at the last follow-up for patients who had not shown relapse and for those who had died. Disease-free survival was defined as the period from the date of surgery to the date of confirmed tumour relapse for patients with relapse, or from the date of surgery to the date of the last follow-up for patients without detectable tumour.

Detection of HCMV in tumour specimens

Before undergoing primary resection, the patients had provided written informed consent. Intraoperatively, the tumour mass was identified by the surgeon, and a small part (approximately 500 mg) of the tumour tissue was resected and stored at −70°C at the bank of residual surgical tissues at the Division of Colorectal Surgery, VGHTPE. In this study, we used 20–25 mg of tumour tissue obtained from these specimens. Genomic DNA was extracted with a commercial kit (QIAamp DNA mini kit; Qiagen, Germantown, MD, USA), according to the manufacturer's instructions. The resulting DNA extracts were stored at −80°C until use.

To detect HCMV, PCR was carried out with primers targeting the viral genes *UL55*, *UL73*, and *UL144*, as previously described [15]. The reaction was carried out over 35 cycles (95°C for 1.5 min, 55°C for 2 min, and 72°C for 1 min, with an initial temperature of 95°C for 5 min, and an additional 10 min at 72°C in the last cycle) in a thermal cycler (GeneAmp PCR system 9600; Life Technologies, Carlsbad, CA, USA). The products were then gel-electrophoresed, stained, and photographed. The presence of HCMV was defined as positive PCR results for any of three viral genes tested.

Analysis of T_H cytokines and pathways

After homogenization of the tumour specimens, total RNA was extracted with a QIAamp mini kit (Qiagen), and reverse-transcribed with an ABI High Capacity cDNA reverse transcription kit (Life Technologies). The resulting cDNA was analysed with a 96-well plate quantitative PCR array (Human T_H17 Response RT^2 Profiler PCR array; Qiagen), according to the manufacturer's instructions. The array includes 84 key genes of cytokines and signal pathways of major $CD4^+$ T-cell subsets (Table S1). Expression levels of genes were quantified on the basis of intercalation of SYBR Green on an ABI 7000 real-time PCR system (Life Technologies), and analysed with the vendor's web-based software module. The relative level of mRNA expression for each gene was normalized to the expression level of the reference gene β-actin.

Statistical analysis

Categorical variables were compared by use of the chi-squared test with Fisher's exact test. Continuous variables were