ORIGINAL ARTICLE

Human cytomegalovirus infection contributes to glioma disease progression via upregulating endocan expression

YAN XING, YISONG WANG, SHIJIE WANG, XIN WANG, DONGYING FAN, DABIAO ZHOU, and JING AN

BEIJING, CHINA

The etiology of malignant glioma remains unclear. To examine the association between glioma and human cytomegalovirus (HCMV) infection and the possible mechanism through which HCMV contributes to malignant glioma, we investigated the expression of HCMV components and an angiogenesis marker, endocan, in 79 glioma specimens and 8 control brain samples. HCMV pp65 protein and DNA were detected in 65.8% (52 of 79) and 54.4% (43 of 79) of glioma specimens, respectively. The positive rate and expression levels of pp65 were significantly correlated with the glioma grades. The endocan expression was detected in 78.5% (62 of 79) of glioma specimens, and elevated endocan immunoreactivity was also significantly associated with high-grade glioma. The pp65 was predominantly detected and colocalized with endocan in the cytoplasm of tumor cells. Importantly, there was a significant positive correlation in detection rates between those 2 proteins. In control samples, neither HCMV pp65 nor endocan expression was detected. Moreover, the serum endocan levels in glioma patients were markedly higher than that in healthy subjects. In in vitro study, HCMV infection induced the expression of interleukin 6 and tumor necrosis factor-α in human glioblastoma U87 MG (U87) cells and human umbilical vein endothelial cells (HUVECs). Furthermore, elevated endocan levels were also observed in HCMV-infected U87 cells and HUVECs and antiviral treatment with ganciclovir reduced the endocan expression. These results suggest HCMV infection leads to glioma progression through an upregulation of endocan and the secretion of inflammatory cytokines. Thus, anti-HCMV treatment may represent a potentially novel therapeutic strategy for glioma. (Translational Research 2016;■:1–14)

Abbreviations: HCMV = human cytomegalovirus; U87 = human glioblastoma U87 MG cells; HUVECs = human umbilical vein endothelial cells; GBM = glioblastoma multiforme; VEGF = vascular endothelial growth factor; VECs = vascular endothelial cells; GCV = ganciclovir; FBS

From the Department of Microbiology and Parasitology, School of Basic Medical Sciences, Capital Medical University, Beijing, China; Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University, Beijing, China; Center of Epilepsy, Beijing Institute for Brain Disorders, Beijing, China.

Yan Xing and Yisong Wang contributed equally to this work.

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Reprint requests: Jing An, Department of Microbiology and Parasitology, Center of Epilepsy, Beijing Institute for Brain Disorders, No. 10 You An Men Wai Xi Tou Tiao, Beijing 100069, China; e-mail: anjing@ccmu.edu.cn or Dabiao Zhou, No.6 Tiantan Xili Dongcheng District, Beijing 100050, P.R. China; e-mail: dabiaozhou@163.com.

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= fetal bovine serum; IL-6 = interleukin-6; TNF-α = tumor necrosis factor (TNF)-alpha; MTT = 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide; IHC = immunohistochemistry; ISH = in situ hybridization; ELISA = enzyme-linked immunosorbent assay; HGG = high-grade glioma; LGG = low-grade glioma; dpi = days post infection; HGF/SF = hepatocyte growth factor/scatter factor; IFN-γ = interferon gamma; STAT3 = signal transducers and activators of transcription 3; NF-κB = nuclear factor κB

AT A GLANCE COMMENTARY

Xing Y, et al.

Background

Etiology of glioma is unclear. Viruses are the second most important risk factor for cancer development. Previously, human cytomegalovirus (HCMV) gene products had been found in glioma tissue. However, how HCMV infection leads to glioma is controversial. Because glioma progression is dependent on angiogenesis, and endocan, a novel endothelial cell-specific molecule that regulates angiogenesis, was upregulated in glioma tissues, we hypothesize that HCMV influences glioma progression through modulating endocan.

Translational Significance

We demonstrated a possible mechanism for HCMV to affect glioma progression through upregulating endocan. Our results provide insight into a potential new therapeutic target for glioma treatment.

INTRODUCTION

Glioma is the most common and aggressive primary brain tumor that cannot be completely resected surgically. Among them, malignant glioma is associated with high recurrence and high mortality rates. Patients with glioblastoma multiforme (GBM), a form of malignant glioma, currently have a median survival time of less than 15 months. The etiology and pathogenesis of glioma remain largely unclear. Genetic alterations due to intrinsic or environmental factors are thought to be involved in the initiation of glioma and its progression.[1] Epidemiologic data have shown that viruses are the second most important risk factor for cancer development in humans.[2] Several cancer types are known to have a viral etiology and some viruses have been established as cancer causing agents. For example, close associations between hepatitis B and C viruses and hepatic cell carcinoma have been established; and certain types of human papillomavirus have been demonstrated to cause cervical cancer. However, there are other viruses whose roles in oncogenesis are more controversial. For instance, what is the role of the human cytomegalovirus (HCMV) in the initiation and progression of glioma[3]?

HCMV is a double-stranded DNA virus that belongs to the herpesviridae family. It carries a large genome which encodes approximately 200 proteins, including immediate-early and pp65 proteins. pp65 is the most abundant virion protein and a major component of the dense bodies of noninfectious viral particles. Thus, the expression levels of pp65 are often used as an important indicator of HCMV infection. HCMV is prevalent in up to 70%–90% of the general population, and it persists for life long after primary infection. During initial infection or viral reactivation, HCMV can cause serious and even fatal complications in fetuses or immunocompromised individuals.

The role of HCMV in glioma was first noted in 2002 when Cobbs et al[4] reported the presence of HCMV gene products in 100% of the tested GBM samples compared with none in the normal control tissues. This was confirmed by several other groups recently.[5,6] In addition, anti-HCMV therapy with valganciclovir has achieved promising results in GBM patients.[7] In an experimental model, perinatal cytomegalovirus infection promotes glioma progression in transgenic mice.[8] However, conflict results had also been reported. In some studies, no HCMV genes or proteins were detected in glioma samples.[9,10] These controversial results raise uncertainty about the role of HCMV in the pathogenesis of glioma. Is it a true pathogenic element or simply a bystander factor?

Glioma growth has been demonstrated to be strongly dependent on angiogenesis.[11] The main factors indicative of angiogenesis have been well established, and all of which may have therapeutic implications. Early antiangiogenesis therapy was considered a promising tool for the treatment of glioma. Bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF), is an important agent in the treatment of recurrent glioma.[12] However, treatment based on this target has shown limited therapeutic effect on glioma, mainly due to the drug resistance. In fact, bevacizumab treatment for GBM only achieved 20%–40% efficacy, and the 6-month progression-free survival rate is only 30%–50%.[13] Therefore, additional therapeutic targets need to be identified.

Many angiogenesis-related genes are potentially involved in tumor growth. In our preliminary experiments, levels of angiogenesis-related genes including VEGF, angiogenin-1, angiogenin-2, and endocan in HCMV-infected glioblastoma U87 MG (U87) cells