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High Prevalence of Human Cytomegalovirus in Brain Metastases of Patients with Primary Breast and Colorectal Cancers[1,2,3]

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Abstract

BACKGROUND: Brain metastases (BMs) develop by largely unknown mechanisms and cause major morbidity and mortality in patients with solid tumors. Human cytomegalovirus (HCMV) is frequently detected in tumor tissue from patients with different cancers. Here, we aimed to determine the prevalence and potential prognostic role of HCMV in BMs. *METHODS:* We obtained archived samples of BMs from 41 patients with breast cancer and 37 with colorectal cancer and paired primary tumor tissues from 13 and 12 patients in each respective group. In addition, primary breast cancer tissues from 15 patients were included. HCMV proteins were detected with an immunohistochemical technique and Western blot. HCMV nucleic acids were detected with TaqMan polymerase chain reaction (PCR) assay. *RESULTS:* HCMV proteins were abundantly expressed in 99% of BM specimens, and in 12 of 13 (92%) paired primary breast cancer specimens. All 12 paired colon cancer samples were positive for HCMV proteins. Protein staining was mainly confined to neoplastic cells. Western blot analysis detected an HCMV-IE reactive protein in 53% of breast cancer specimens, and PCR detected the presence of HCMV DNA and transcripts in 92% and 80% of samples, respectively. Patients with high-level expression of HCMV-IE proteins in their tumors had a shorter time to tumor progression and shorter overall survival. *CONCLUSIONS:* The prevalence of HCMV proteins and nucleic acids is very

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high in primary and metastatic tumors and may drive the development of metastatic brain tumors; therefore, this virus may represent a potential therapeutic target in metastatic cancer.

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Introduction

Brain metastases (BMs) are the most common intracranial neoplasms in adults and cause major morbidity and mortality in patients with solid tumors, as the prognosis for these patients is very poor [1]. BMs develop in approximately 10% to 48% of patients with solid tumors, but their prevalence is likely higher, as regular screening is not routine for patients with cancer [1,2]. BMs have been reported in 48% of patients with lung cancer, 15% of patients with breast cancer, 10% to 15% of patients with testicular cancer, 6% to 10% of patients with malignant melanoma, and 4% of patients with colorectal cancer [3,4]. The incidence of BMs also appears to be increasing [5,6], probably as a result of improved overall survival (OS) in patients with cancer [7] and earlier and more accurate detection with modern neuroimaging modalities [8]. We recently showed that the incidence of BM in a Swedish population–based cohort of patients with cancer (National Patient Registry) doubled between 1987 and 2006 [5]. The incidence of BM was 9% in men and 7% in women with colorectal cancer and 33% in women with breast cancer [5] in 2006. Median survival after first admission for BM was 3.2 months in patients with breast cancer and 2.6 months in those with colorectal cancer. The proportion of patients surviving 1 year was higher among patients with breast cancer than among those with colorectal cancer (19% *vs* 6.7%) [5]. The increased incidence of BMs may also be explained by insufficient delivery of drugs across the blood-brain barrier, limiting the efficiency of systemic chemotherapy for BMs [8]. As patients who are diagnosed with BMs have a median OS of 4.2 months [9], new treatment strategies are highly warranted.

The exact mechanism by which BMs develop is unknown [10]. Several risk factors are associated with BMs. These include human epidermal growth factor receptor 2 (HER2)–positive breast cancer and triple-negative breast cancer [negative for estrogen receptor α (ERα), progesterone receptor (PR), and HER2] [11,12], COX-2 expression [13], as well as enhanced expression of integrin $\alpha_v\beta_3$ [14], CXCR4/SDF-1 [15], and CD44 [16]. COX-2 expression is thought to mediate impaired blood-brain barrier functions [13], while CXCR4/SDF-1, CD44, and integrin $\alpha_v\beta_3$ are thought to mediate increased metastatic potential to the brain and promote angiogenesis [13–16], which may contribute to the development of BM.

Human cytomegalovirus (HCMV) is a β-herpes virus that infects and establishes latency in most of the world's populations [17]. Emerging evidence demonstrates that HCMV proteins and nucleic acids are frequently detected in tissue specimens in very high prevalence in patients with cancers of different origin, including colon, breast, prostate mucoepidermoid salivary gland tumors, medulloblastomas, neuroblastoma, glioblastoma, and rhabdomyosarcoma [18–23]. Because of its high prevalence in cancer, HCMV may play an important but not yet well-defined role in the establishment of several cancer forms. HCMV proteins are known to interfere with cellular and immunologic functions that may affect tumor biology [18] in a complex manner. This virus encodes more than 750 proteins, of which only about 45 to 57 are estimated to be essential for viral replication [24,25]. While the functions of many of these proteins are unknown and HCMV's direct oncogenic properties are still under debate, this virus clearly has numerous oncomodulatory and oncogenic mechanisms [18,26].

However, regardless of its potential role in tumor development, the presence of HCMV in tumors of different origin may offer new therapeutic strategies. In support of this statement, we recently demonstrated that anti-viral treatment against HCMV significantly reduced neuroblastoma growth in an animal model, and combined treatment with valganciclovir and celecoxib (both acting against HCMV) reduced tumor growth by 72% in a xenograft model [20]. Furthermore, we observed a remarkably increased survival rate among

Table 1. Characteristics of the Study Participants

Characteristic	All (*n* = 78)	Breast Cancer (*n* = 41)	Colorectal Cancer (*n* = 37)
Age at BM diagnosis, *n* (%)			
≤60	54 (69)	36 (88)	18 (49)
>60	24 (31)	5 (12)	19 (51)
Median (range), years	58 (29-80)	52.5 (30-72)	62.5 (29-80)
Sex, *n* (%)			
Women	58 (74)	41 (100)	17 (46)
Men	20 (26)	0 (0)	20 (54)
Calendar year of primary diagnosis, *n* (%)			
1985 to 1999	40 (51)	19 (46)	21 (57)
2000 to 2009	31 (40)	28 (44)	13 (35)
Missing	7 (9)	4 (10)	3 (8)
Calendar year of BM diagnosis, *n* (%)			
1990 to 1999	19 (24)	8 (19)	11 (30)
2000 to 2004	29 (37)	13 (32)	16 (43)
2005 to 2009	30 (38)	20 (49)	10 (27)
Died during follow-up, *n* (%)			
Yes	73 (94)	36 (88)	37 (100)
No	5 (6)	5 (12)	0
Time from primary diagnosis to BM surgery, months			
Median (range)	35.4 (0-170)	44.7 (4.1-170)	34.5 (0-101)
Time from BM surgery to death, months			
Median (range)	8.6 (0.4-178)	15.4 (0.7-178)	6.0 (0.4-20.4)
Primary breast cancer, *n* (%)			
ER+		13 (32)	
ER−		16 (39)	
PR+		10 (24)	
PR−		11 (27)	
HER2+		8 (20)	
HER2−		5 (12)	
BM, *n* (%)			
ER+		9 (22)	
ER−		6 (15)	
PR+		3 (7)	
PR−		11 (27)	
HER2+		3 (7)	
HER2−		3 (7)	
Primary colorectal cancer, *n* (%)			
Dukes A			0 (0)
Dukes B			4 (11)
Dukes C			14 (38)
Dukes D			5 (14)