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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)

HCMV-positive glioblastoma patients receiving anti-viral therapy [27]. The 2-year survival rate increased from 18% to 70% (n = 40) with 6 months of valganciclovir treatment and to 90% (n = 25) with continuous treatment ($P < .0001$). The median OS increased from 13.5 months to 30.1 months and 56.4 months, respectively ($P < .0001$). Thus, targeting HCMV in virus-positive tumors may offer new therapeutic options [27].

Recently, we demonstrated the presence of HCMV proteins and nucleic acids in 94% of sentinel lymph node metastases of breast cancer [23]. HCMV protein expression was mainly confined to tumor cells in both primary tumors and sentinel lymph node metastases. In this study, we determined the prevalence of HCMV infection in BMs from colorectal and breast cancers and evaluated the level of HCMV protein expression in relation to patient survival.

Materials and Methods

Clinical Samples

We collected archived paraffin-embedded tissue samples of all available BMs in patients diagnosed with breast or colorectal cancer who underwent neurosurgical removal of the tumor at Karolinska University Hospital (Stockholm, Sweden) during 1990 to 2009. Patients were selected from the pathology registry based on their primary diagnosis and localization of a central nervous system tumor that was surgically removed. Seventy-eight patients were identified; 41 had primary breast cancer and 37 had primary colorectal cancer (Table 1). Paraffin-embedded tissue sections from corresponding primary tumors were available from 13 of the patients with breast cancer and 12 with colorectal cancer (Table 1). In addition, fresh breast tumor tissues were obtained from surgical specimens of 15 patients who underwent surgery at Akershus University Hospital (Oslo, Norway) during 2014. We performed a review of medical records to collect the following information: age and calendar year of primary cancer diagnosis, clinical cancer characteristics (ER, PR, and HER2 status and colon cancer stage according to Dukes), and date of death or date of last clinical follow-up. The Regional Human Ethics Committees in Sweden and Norway (Nos 2008/628-31 and 577-06-04148, 06118) approved the study. The tumor diagnosis was determined at the Pathology Department at Karolinska University Hospital and confirmed by a pathologist (P.R.) and at Akershus University Hospital by Professor Torill Sauer.

Immunohistochemical Analyses

Paraffin sections (4 μm) were cut, dewaxed in xylene, and rehydrated in decreasing concentrations of ethanol (Apoteket Farmaci, Stockholm, Sweden). HCMV-IE and late proteins were detected in the tumor tissues by using an immunohistochemical (IHC) technique as previously described [23,28]. In negative control sections, the primary antibody was omitted. Cytokeratin and β-catenin served as staining controls. The following antibodies were used: anti–HCMV-IE and HCMV-LA antibodies (both from Chemicon, Temecula, CA), anti-cytokeratin antibody (DakoCytomation, Glostrup, Denmark), and anti–β-catenin antibody [BD Biosciences (Pharmingen), Stockholm, Sweden]. HCMV-infected lung tissue sections from a patient with human immunodeficiency virus (HIV) were used as a positive control for staining.

The number of HCMV protein–expressing cells in the tissue sections was estimated in one tissue section of the tumor specimen for each antibody (in serial sections). The sections were graded on the basis of the estimated percentages of IE- and LA-positive cells: negative (0% positive cells), low-grade infection (<50% positive cells), or high-grade infection (≥50% positive cells). IHC staining was evaluated and graded by two investigators (A.R. and C.T.); neither had access to the clinical records of the patients. The results were confirmed by a pathologist (PR).

TaqMan Polymerase Chain Reaction

To confirm that HCMV nucleic acids were present in the pathologic sections, DNA was extracted from 12 tissue samples with the PicoPure DNA Extraction Kit (Applied Biosystems, Branchburg, NJ) according to the manufacturer's instructions. Paraffin-embedded tissue sections used to generate DNA were adjacent to the sections used for IHC and protein extraction for Western blot analyses, and RNA was extracted from the same piece of frozen tissue used. In brief, BM tissue sections on slides were dewaxed in xylene, treated with 99.9% ethanol, and air dried. The sections were then scraped with a sterilized blade into a tube and digested with proteinase K in DNA extraction buffer for 24 hours at 65°C. Samples were heated to 95°C to deactivate the proteinase K. Total RNA and protein were isolated from 15 frozen breast tumor tissues by using TRIzol LS protocol, according to the manufacturer's application manual (Life Technologies, Stockholm, Sweden). cDNA was synthesized by using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Stockholm, Sweden). Both DNA and cDNA were analyzed by TaqMan polymerase chain reaction (PCR), performed with a 7900HT fast real-time PCR system (Applied Biosystems) using primers and probes for the *HCMV-IE* gene (IE DNA primers: forward primer sequence, GTGACCCATGTGCTTATGACTCTAT; reverse primer sequence, CTCAACATAGTCTGCAGGAACGT; probes, reporter TTGGTCACGGGTGTCTC quencher; IE cDNA primers: forward primer sequence, TGACGAGGGCCCTTCCT; reverse primer sequence, CCTTGGTCACGGGTGTCT; probes, reporter AAGGTGC CACGGCCCG quencher). RNase P and β_2-microglobulin served as the internal control (Applied Biosystems) as previously described [29].

For quantification and standardization purposes, the viral DNA copy number in the examined samples was estimated using the first WHO International Standard for HCMV for nucleic acid amplification techniques developed by the National Institute for Biological Standards and Control (code 09/162; Table S1).

Western Blot Assay

TRIzol LS protocol was used for extraction of total protein from fresh frozen breast cancer tissues (Life Technologies). Western blot assay was performed by preparing protein samples in Laemmli buffer containing 5% β-mercaptoethanol, boiled, and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis with 4% to 12% gels and transferred onto a polyvinylidene difluoride membrane. The membrane was blocked with 5% non-fat dry milk dissolved in Tris-buffered saline supplemented with 0.1% Tween 20 and probed with mouse monoclonal anti–HCMV-IE proteins (11-003; Argene (Verniolle, France) or MAB810R; Millipore, Stockholm, Sweden, both at 1:1000) or mouse monoclonal anti–β-actin (NB600-501, 1:3000). After washing in Tris-buffered saline with 0.1% Tween 20, the membrane was incubated with anti-mouse IRDye 680RD (1:15000; LI-COR Biosciences, Hamburg, Germany). Bound antibodies were detected with ODISSEY CLx Infrared Imaging System (LI-COR Biosciences) and quantification was performed using the Image Studio Lite Software (LI-COR Biosciences). All the values below 0.1 were undetectable and were considered as negative.

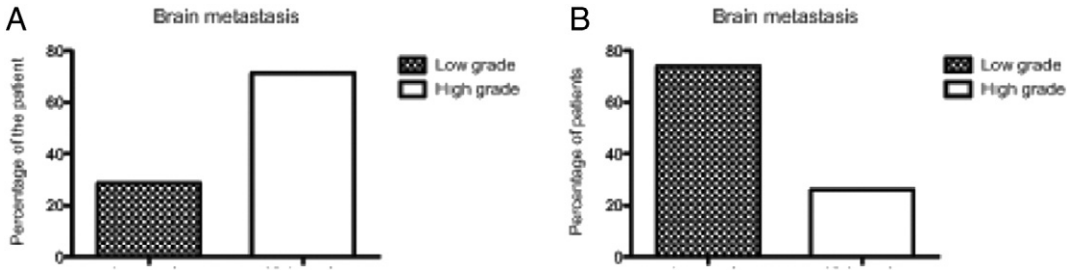

Figure 1. Summarizing results for all IHC staining on brain tumor tissues. Results from IHC staining from all brain tumor specimens including both IE and LA HCMV proteins were plotted in A and B, respectively.

Statistical Analysis

OS data are presented in graphs as Kaplan-Meier estimates, calculated from the date of primary tumor diagnosis and BM diagnosis to the date of death or end of follow-up. Kaplan-Meier curves were tested for significance with the log-rank test using the GraphPad Prism program. All statistical hypotheses were two-sided, and $P < .05$ was considered significant.

Results

High Prevalence of HCMV Proteins and Nucleic Acids in BMs and Corresponding Primary Tumor Samples of Breast and Colorectal Cancer Patients

HCMV-IE proteins were detected in 77 of 78 (99%) archived metastatic brain tumor specimens, and LA antigens in 73 of 74 (99%; Figures 1, 2, *A* and *B*, and 3, *A* and *B*). Both HCMV-IE and HCMV-LA proteins were also detected in 12 of 13 (92%) primary breast cancer specimens (Figure 2, *G–J*, *M*, and *N*) and in all primary colorectal cancer specimens available for analyses (12 of 12 for IE and 11 of 11 for LA; Figure 3, *G–J*, *M*, and *N*). HCMV positive control tissue analysed by IHC was positive for HCMV (S3). Western blot detected an HCMV-IE reactive protein in 8 of 15 (53%) frozen breast tumor tissue samples (Table S1). HCMV-IE DNA and transcripts were detected by TaqMan PCR in 11 of 12 (92%) and in 12 of 15 (80%) examined tissue samples, respectively. In contrast to the evident high prevalence of HCMV protein expression in examined tissue specimens, low copy numbers of CMV DNA and transcripts were detected in tumor specimens, except in two patients with low C_T values corresponding to high levels of HCMV transcripts (Table S1). These observations confirm our previous observations of a discrepancy of the HCMV DNA and protein detection levels in tumors [20].

HCMV Infection Level in BMs Affects Patient Outcome

We earlier observed that the HCMV infection level was predictive for outcome in glioblastoma patients [28]. To determine whether the level of HCMV infection was associated with time to tumor progression and OS for BM patients, we graded the tissue specimens for HCMV-IE and HCMV-LA protein expression into low-grade (< 50% positive cells) and high-grade infection (≥ 50% positive cells) based on the estimated percentage of HCMV protein–positive cells in the tumor tissue section (Table 2). One patient was HCMV-IE negative (not included in the analysis), 28% (22 of 78) of patients had low-grade infections, and 71% (55 of 78) had high-grade infections (Figure 1*A* and Table 2). One patient was negative for HCMV-LA protein expression; 73% (54 of 74) of patients had low-grade infections, and 26% (19 of 74) had high-grade infections (Figure 1*B* and Table 2). The IHC staining results for all of the BM specimens are summarized in Table 2.

For all 78 patients, the median OS after diagnosis of BMs was 8.6 months (range, 0.4-177.8; Table 1). Patients with low-grade HCMV infection tended to have longer median OS than those with high-grade infection (13.5 *vs* 6.9 months; Hazard Ratio (HR), 1.58; 95% Confidence Interval (CI), 0.97-2.56; $P = .064$; Figure 4*A* and Table 2). Breast cancer patients ($n = 41$) survived longer than colorectal cancer patients ($n = 37$) with BM: OS 15.4 months (range, 0.7-177.8) *versus* 6.0 months (range, 0.4-20.1; HR, 3.71; 95% CI, 2.13-6.46; $P < .0001$).

In 25 paired available specimens of primary breast cancer ($n = 13$) and colorectal cancer ($n = 12$), HCMV-IE proteins were detected in 92% of breast cancer specimens and in all primary colon cancer specimens; high-grade infection was observed in 39% and 75% of those specimens, respectively (Figures 2*M* and 3*M*). Patients with high-grade HCMV infection in their primary tumors had shorter median time to BM diagnosis than those with low-grade HCMV infection (30 *vs* 65.1 months; HR, 2.93; 95% CI, 1.21-7.05; $P = .016$; Figure 4*B*). These patients also had shorter median OS than those with low-grade HCMV infection (median, 37.8 *vs* 81.5 months; HR, 3.57; 95% CI, 1.44-8.87; $P = .006$; Figure 4*C*).

Hormone Receptor Expression in Breast Cancer Cells Correlates with HCMV Staining

Hormonal receptor expression (ERα and PR) and HER2 in primary and metastatic breast cancers are well-known prognostic markers [30,31]. We obtained staining results for ERα ($n = 29$) and PR ($n = 21$) expression in primary breast cancer from clinical records; ERα expression was available for 15 BMs, and PR expression was available for 14 (Table 1); we performed survival analysis for these well-known prognostic markers and compared these with results for HCMV grading.

As expected, both tumor aggressiveness and survival data differed significantly between receptor-positive and receptor-negative breast cancer cells in specimens of primary tumors and BMs (Figures S1 and S2). The time to BM was shorter in patients whose tumors had no ERα expression (median, 30 *vs* 73.3 months, $P = .002$) or no PR expression (median, 30 vs 70.4 months, $P = .01$) in comparison with patients whose tumors expressed those receptors. Compared with receptor-positive patients, receptor-negative patients also had shorter survival after primary tumor diagnosis (median: 44.9 *vs* 97.3 months for ERα, $P = .0165$, and 37.0 *vs* 88.1 months for PR, respectively, $P = .003$; Figures S1 and S2).

ERα and PR staining results from BMs also showed shorter survival after diagnosis of the metastasis in patients with ERα- and PR-negative tumors than in those with receptor-positive tumors (median: 12 *vs* 34.8 months for ERα, $P = .02$, and 12.7 *vs* 57.5 months for PR, $P = .04$).

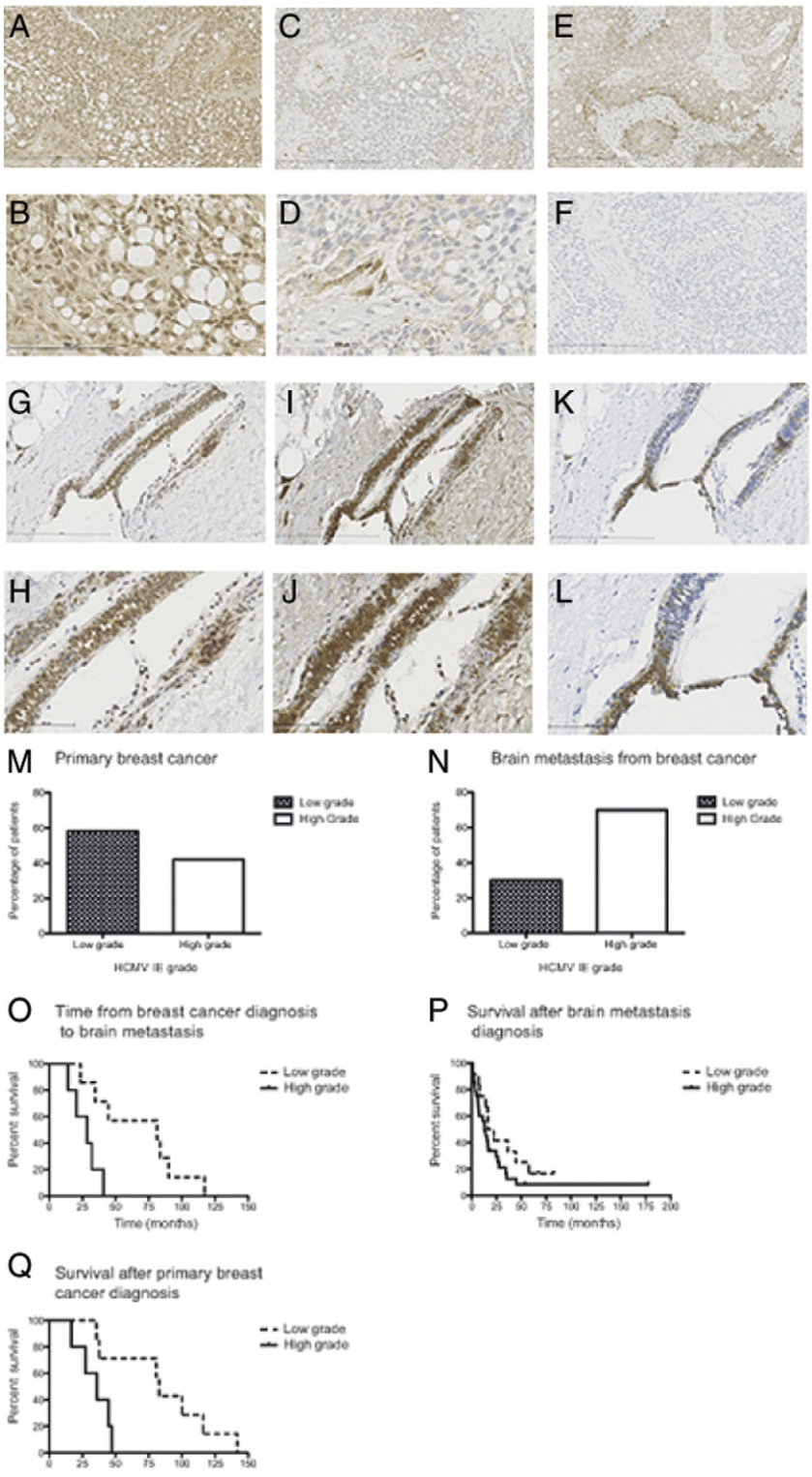

Figure 2. IHC analysis of BMs from breast and primary breast cancers. HCMV proteins were detected in tissue sections of BMs (A–D) and in primary breast cancer tissues (G, J). HCMV-IE expression is confined to tumor cells in brain tumor specimens (A and B and G and H). Serial sections of the same tumor show immunoreactivity to HCMV-LA protein in the same tumor area (C and D and I and J). Cytokeratin was used as an epithelial marker and positive control for tumor cells (E) and β-catenin served as a staining control (K–L). Primary antibody was omitted as a negative control (F). Scale bars: (A, C, E, F, G, I, and K) 300 μm and (B, D, H, J, and L) 100 μm. Results of IHC staining for primary breast cancer specimens and BMs were plotted in M and N, respectively. HCMV-IE grade of primary tumor correlates with time to BM (O) and survival after primary tumor diagnosis (P). HCMV grade of BMs from breast cancers correlates with survival from diagnosis of BM to death (Q).

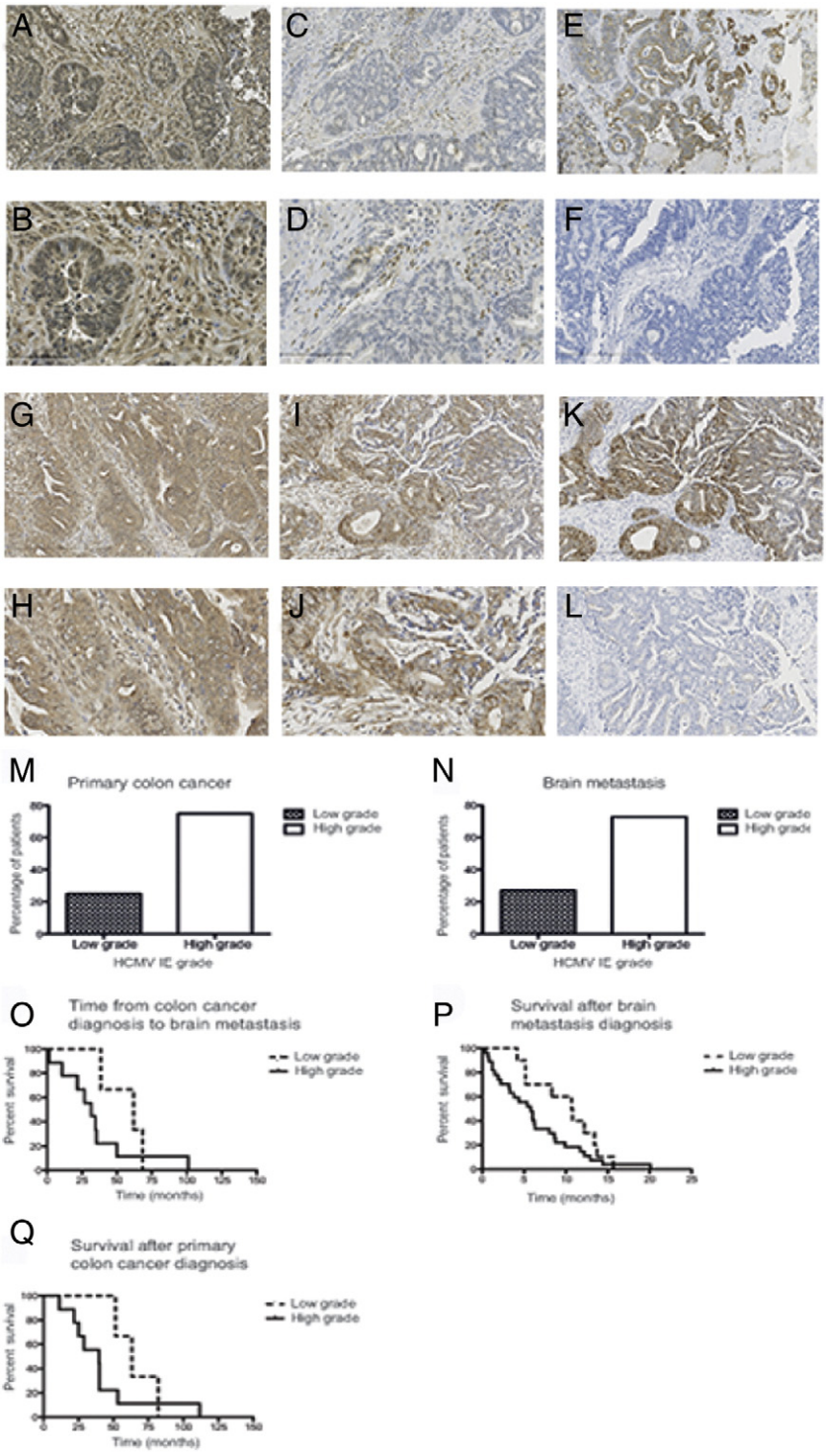

Figure 3. IHC analysis of BMs from colon cancer and primary colon cancer. HCMV-IE expression is confined to tumor cells in BMs specimens (A and B). Serial sections show immunoreactivity to HCMV-LA protein in the same tumor areas (for BMs: C-D and for primary colon cancer tissues: I-J). Cytokeratin was used as an epithelial marker and positive control (E) and β-catenin served as a staining control (K). Primary antibody was omitted as a negative control (F and L). (A, C, E, F, G, I, K and L) 300 μm; (B, D, H and J) 100 μm. HCMV-IE graded as low, <50% positive cells and high, ≥50% positive cells. Results of IHC staining for primary breast cancer specimens and BMs were plotted in M and N, respectively. Time to BM (O) as well as survival after primary tumor diagnosis (P) and diagnosis of the BM (Q) were correlated with HCMV-IE infection grade.

Table 2. Risk of Death Associated with HCMV Expression in BMs and Primary Tumors

HCMV Expression Grade	Positive Cells, *n* (%)
BM	
IE protein (*n* = 78)	
Negative	1 (1.3)
Low grade	22 (28.2)
High grade	55 (70.5)
LA protein (*n* = 74)	
Negative	1 (1.4)
Low grade	54 (72.9)
High grade	19 (25.7)
Colon cancer (primary) (*n* = 12)	
IE protein	
Negative	0 (0)
Low grade	3 (25)
High grade	9 (75)
Colon cancer (BM) (*n* = 37)	
IE protein	
Negative	0 (0)
Low grade	10 (27)
High grade	27 (73)
Breast cancer (primary) (*n* = 13)	
IE protein	
Negative	1 (7.7)
Low grade	7 (53.8)
High grade	5 (38.5)
Breast cancer (BM) (*n* = 41)	
IE protein	
Negative	1 (2.4)
Low grade	12 (29.3)
High grade	28 (68.3)

Adjusted for age (as a continuous variable) and primary tumor type at diagnosis of BM.

Among primary breast cancer patients with available ERα staining and PR staining results, tissue specimens were available for 10 patients with ERα staining and for 6 patients with PR staining. We found that the HCMV infection grade correlated with receptor expression status for both ERα and PR (Figures S1 and S2). Unfortunately, HER2 staining was only available from very few patients, which did not allow further analysis.

Discussion

HCMV proteins and nucleic acids are highly prevalent in several different malignancies. This study shows, for the first time, a very high prevalence of HCMV infection in BMs from both breast and colorectal cancers using well-established techniques for HCMV detection in tumor specimens [23,28,32]. HCMV proteins were detected in 99% (*n* = 78) of BM samples and also in 92% to 100% (*n* = 25) of corresponding primary breast and colorectal cancer specimens. Western blot detected an HCMV-IE reactive protein in 53% (8 of 15) of frozen breast tumor tissue samples, whereas quantitative TaqMan PCR confirmed the presence of HCMV nucleic acids in 80% to 92% of breast tumor samples; the reason for this discrepancy may be differences in sampling handling, limitations in the sensitivity of the Western blot assay, and the fact that the HCMV-positive tumor tissue part is not clearly defined in the frozen samples that were used for Western blot analysis in this study. Interestingly, viral protein expression was mainly confined to tumor cells, although some endothelial cells and inflammatory cells in the tumors were also HCMV positive.

We further observed that patients who had low-grade HCMV infection in the primary tumor or the BM had longer time to tumor progression and longer survival. Patients with low-grade HCMV infection tended to have longer median OS from BM diagnosis, 13.5 months in patients with low-grade HCMV infection compared to 6.9 months in those with high-grade infection. Time to tumor progression was longer in patients with low-grade HCMV infection (65.1 *vs* 30 months; HR, 2.93; 95% CI, 1.21-7.05; *P* = .016), as was survival after primary diagnosis (81.5 *vs* 37.8 months; HR, 3.57; 95% CI, 1.44-8.87; *P* = .006; Figure 4, *B* and *C*). Patients with high-grade infections were also more likely to have low or absent ER and PR expression, and HCMV hence had similar prognostic value for breast cancer patients as the well-known prognostic factors ER and PR for breast cancer [11,12]. These observations imply that HCMV may represent a prognostic factor for metastatic breast and colorectal cancers, which merit further evaluation in larger patient cohorts. Importantly, the presence of HCMV in BMs may also open for new therapy options for these patients who face a very poor prognosis with

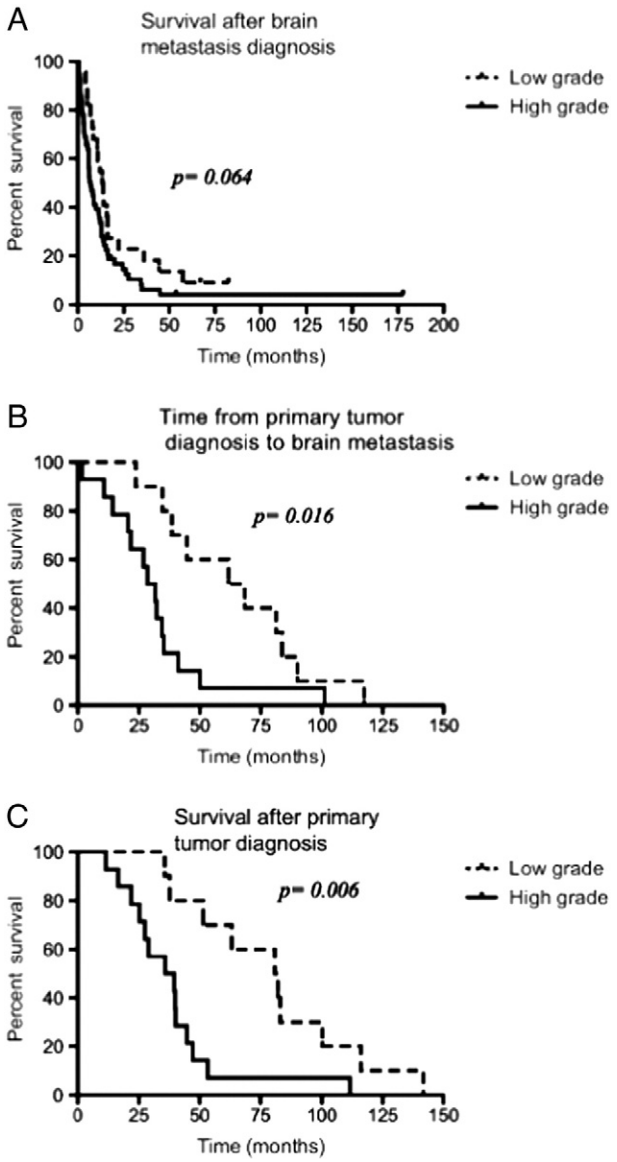

Figure 4. Correlation of survival time with HCMV-IE grade. (A) Patients with low-grade HCMV-IE infection in BM samples have improved survival time from diagnosis of BM to death. Patients with low-grade HCMV-IE infection in primary colon and breast cancers have significantly longer time to BM (B) and survival time from diagnosis of the primary tumor (C).

limited treatment options. This statement is supported by our recent findings that a low-grade HCMV infection in glioblastoma is associated with improved survival [28,33] and that anti-viral therapy against HCMV prevents tumor growth of HCMV-positive xenograft tumors in animal models [20,21] and indicates strongly improved survival among glioblastoma patients with HCMV-positive tumors [27,33].

In further support of the hypothesis that HCMV may be a driving factor of metastatic cancer, we observed that the grade of HCMV infection in the primary and metastatic tumors was associated with patient outcome. Specifically, all patients with low-grade HCMV infection tended to have higher median OS from BM diagnosis, 13.5 months in patients with low-grade HCMV infection compared to 6.9 months in those with high-grade infection in the BM. The HCMV infection grade also correlated with time to tumor progression and survival after primary diagnosis. Time to tumor progression was longer in patients with low-grade HCMV infection (65.1 *vs* 30 months; HR, 2.93; 95% CI, 1.21-7.05; $P = .016$), as was survival after primary diagnosis (81.5 *vs* 37.8 months; HR, 3.57; 95% CI, 1.44-8.87; $P = .006$; Figure 4, *B* and *C*). Patients with high-grade infections were also more likely to have low or absent ER and PR expression, and HCMV hence had similar prognostic value for breast cancer patients as the well-known prognostic factors ER and PR for breast cancer.

The fact that 94% of sentinel lymph node metastases of breast cancer [23] and 99% of BMs of colorectal and breast cancers are HCMV protein positive, but virus positivity is nearly absent in healthy surrounding tissue, also implies a viral presence in metastasis-initiating cells. Such scenario may affect tumor biology and metastatic processes of breast and colorectal cancers and suggests a biologic role of HCMV rather than being an epiphenomenon in metastatic cancer [23]. Viral factors that could affect epithelial-to-mesenchymal transition pathways known to be important in metastatic cancer are therefore under investigation in our laboratory.

In conclusion, we found that HCMV infection is highly prevalent in BMs of breast and colorectal cancer and in paired primary cancer specimens, which identifies a novel potential driver of metastatic cancer development in the brain. Further studies in larger patient cohorts are needed to further evaluate HCMV as a prognostic marker for breast and colorectal cancers, to assess the possible role of HCMV infection in metastatic cancer, and to determine whether HCMV-targeted therapies have a place in the treatment of metastatic brain cancer.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tranon.2014.09.008.

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