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Clinical Study

Human cytomegalovirus viral load in tumor and peripheral blood samples of patients with malignant gliomas

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ABSTRACT

Malignant gliomas are the most common primary brain tumors in adults. The disease has no known etiology, progresses rapidly, and is fatal despite current therapies. Human cytomegalovirus (HCMV) is a beta herpes virus that is trophic for glial cells and infects 50% to 90% of the adult human population. HCMV-mediated disease in immunosuppressed patients has highlighted the possible role of this virus in the development of other diseases, particularly inflammatory diseases such as vascular diseases, autoimmune diseases, and certain malignancies. Sensitive detection of viral DNA, mRNA, and antigens in tumor tissues, as well as seroepidemiologic evidence, suggest a link between HCMV and several human malignancies. HCMV gene products are proposed to dysregulate multiple cellular pathways involved in oncogenesis, such as cell cycle regulation, apoptosis, migration, and angiogenesis. These theories, currently being researched, suggest that HCMV acts as an oncomodulator in malignancies. We investigated the association between HCMV infection and reactivation, and malignant gliomas. An open, matched case-control, parallel group pilot study was performed in a tertiary referral center. The HCMV viral load in peripheral blood and tumor samples of 19 patients newly diagnosed with glioblastoma multiforme was compared with a matched control cohort comprising 19 patients newly diagnosed with non-malignant brain tumors. There was no significant correlation between peripheral blood and tumor tissue HCMV viral load in patients with glioblastoma multiforme compared to the control cohort. The findings of the present study did not support an oncomodulatory role for HCMV in malignant gliomas.

1. Background

Malignant gliomas, such as glioblastoma multiforme (GBM), pose a significant challenge for treatment. Despite broad research in this area, increasing progress in oncologic knowledge, and integrated treatment consisting of aggressive surgical resections (while using novel neuroimaging modalities pre- and intra-operatively), radiotherapy, and chemotherapy as adjuvant therapies, the prognosis for GBM remains grave. Median patient survival is approximately 14 months after diagnosis [1] with 5% of patients surviving more than 2 years. To date, the etiology of this disease remains unknown. Numerous research studies are aimed at better understanding the positive prognostic factors, determining which patients survive longer, and finding novel methods to treat patients and extend their life expectancy with good quality of life.

Human cytomegalovirus (HCMV) is a beta herpes virus carried by most of the world's population [2]. The prevalence of asymptomatic individuals carrying the virus has been extensively researched, including in remote aboriginal tribes, and indeed HCMV is carried in all populations studied. The prevalence of seropositivity for antigens specific for HCMV increases with age. One epidemiologic study reported a prevalence of 47% in those aged between 10–12 years, 68% in those aged 15–35 years, and 81% in those aged 36–60 years [3]. For many years it was widely accepted that HCMV is not a clinically important pathogen in the healthy human, and reactivation of the virus, if manifested clinically, presents as an infectious mononucleosis-like disease; in contrast, in immunocompromised hosts, the virus can cause complicated and sometimes deadly diseases. Reactivation of HCMV in these patients may present with high fever, leukopenia, viral hepatitis, interstitial pneumonia, gastrointestinal disease, and acute retinitis. A large percentage (50–90%) of bone marrow transplant and solid organ transplant recipients experience an active HCMV infection after

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transplant. In acquired immunodeficiency syndrome patients, the prevalence of HCMV infection nears 100% [2]. These immunocompromised patients are the largest risk group for contacting a clinically significant infection with HCMV [4,5].

Herpes viruses have recently been linked with the development of certain malignancies. It is an accepted hypothesis that viruses are responsible for at least 15% of human malignancies. There is evidence for herpesviridae involvement in lymphoma, nasopharyngeal carcinoma, Kaposi sarcoma, and cervical carcinoma [6]. New studies link the presence of genetic products of HCMV with miscellaneous malignancies, such as colorectal carcinoma [7], GBM [8], non-Epstein–Barr virus-related Hodgkin's lymphoma [9], and prostate carcinoma [10]. These studies and others reveal active HCMV in tumor cells, and in contrast its absence in non-malignant cells in the tumor's periphery, although it is currently unclear why the presence of lytic phase HCMV in tumor cells does not infect non-tumor cells. These findings are impossible to ignore, though the question of whether reactivation of the virus constitutes a part of the pathogenesis for tumor development or whether it is a result of tumor development does not have an unequivocal answer [2]. The role of HCMV as an oncogenic virus has been extensively studied in recent decades. It is currently accepted that cells infected with HCMV do not develop malignant transformations, because cells expressing HCMV viral proteins actively stop dividing and eventually die. Therefore, HCMV is not considered an oncogenic virus. A recent suggestion, based on the fact that HCMV infects cells and changes their characteristics in ways that do not consist of malignant transformations, is that HCMV is an oncomodulatory virus. Under this premise, a malignant transformation activates the immune system, which recognizes the tumor as anomalous and initiates a local inflammatory response; HCMV is carried by macrophages to the inflamed area and reactivates. HCMV infection of cells would then change certain characteristics of the cell, thus influencing the aggression and resistance of the tumor [11].

Reviews by Michaelis [12], Soderberg-Naucler [5], and Cinatl [13] present several mechanisms by which HCMV contributes to tumor development, as described briefly below.

1.1. Influence of HCMV on cancer cell apoptosis mechanisms

Resistance to apoptosis is a common feature of cancer cells. Not only do apoptotic mechanisms become ineffective due to the pathogenesis of the malignancy, but also ineffective apoptosis mechanisms protect the tumor cells from chemotherapy, and contribute to treatment resistance. It has been proposed that HCMV activates anti-apoptotic mechanisms on fibroblasts in tumor cells by numerous mechanisms, such as by inhibiting caspases and protecting against p53-mediated apoptosis.

1.2. Influence of HCMV on tumor cell adhesion to the endothelium, tumor invasion, and migration

These factors play a crucial role in the evolution of metastases. HCMV downregulates neural cell adhesion molecule receptors, thus increasing tumor cell adhesion to the endothelium, and invasion through the endothelium. This effect on neural cell adhesion molecules also weakens the connection between adjacent tumor cells, thus increasing the risk for developing metastasis.

1.3. Influence of HCMV on angiogenesis

Recruiting blood vessels to redirect blood to tumor cells is a crucial stage of tumor genesis. HCMV overexpresses interleukin-8 and encodes the human cytomegalovirus US28 protein, thus increasing proangiogenic factors within the host. Moreover, HCMV suppresses the expression of angiogenesis inhibitors such as thrombospondins 1 and 2. Cyclooxygenase-2 (COX-2) contributes to angiogenesis in tumors to such an extent that treatment with COX inhibitors (non-steroidal anti-inflammatory drugs [NSAID]) is a proposed strategy for tumor prevention and even as an adjuvant therapy in some malignancies [14]. Infection with HCMV leads to overexpression of COX-2 in the human body, and thus contributes to angiogenesis.

To summarize, reactivation of HCMV promotes angiogenesis by more than one mechanism, thus contributing to tumor growth and survival.

1.4. Influence of HCMV on tumor immunogenicity

When reactivated, HCMV encodes a number of proteins (US2, US3, US6, US11) which decreases the cell surface expression of major histocompatibility complex I and major histocompatibility complex II on infected cells, thus interfering with the recognition of these tumor cells by the adaptive immune system. Moreover, HCMV stimulates the production of transforming growth factor beta 1, a cytokine that suppresses the cellular immune response, in some tumors. Michaelis proposed that transforming growth factor beta 1 is the most prominent glioblastoma immunosuppressant [12]. If the virus assists in reducing tumor immunogenicity, it is a critical factor for tumor survival inside the human body, and may be a therapeutic challenge and target.

1.5. Influence of HCMV on chromosomal stability

HCMV encourages chromosomal damage and genetic instability, which might contribute to the development of malignancies. These effects become even more pronounced when cytotoxic treatment is initiated. Under the influence of HCMV, specific chromosomal strand breaks develop at positions 1q21 and 1q42. In these areas, deletion mutations are correlated with the development of malignant gliomas.

1.6. Influence of HCMV on telomerase activation

Telomeres are nucleotide sequences at the ends of chromosomes, which become shorter with every cell cycle. When telomeres become too short, the cell cycle stops in an irreversible way, a process known as senescence. The physiologic mechanism by which telomere shortening is prevented and senescence is delayed is the action of telomerase-dependent and RNA-dependent DNA polymerase, which lengthens telomere DNA. When telomerases are redundant, they bestow "eternal life" upon the cell, and thus malignant properties. In 2009, Straat found that infecting cells with HCMV activates telomerases, thereby conferring malignant properties to the cell [15]. This is yet another mechanism by which HCMV changes the characteristics of cells and improves their survival. These findings represent advances in determining the cause *versus* the effect of virus infection and malignancy development.

1.7. Recent discoveries

Although it was reported approximately 30 years ago that HCMV infects malignant cells in patients with carcinomas of the colon and prostate [16,17], later pathologic studies contradict these findings [18]. The development of new, highly sensitive laboratory techniques enables the identification of minute amounts of viral nucleic acids, proteins, and antigens inside different tumors. These advances have reignited the discussion of the role of HCMV in the development of malignancies. Indeed, there have been some impressive findings in this area, as discussed below. In 2002, Cobbs et al. [9] used immunohistochemistry and *in situ* hybridization to identify HCMV nucleic acids and viral proteins in gliomas, and