Is Cytomegalovirus a Therapeutic Target in Glioblastoma?

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Several investigators have now demonstrated the expression of genes unique to cytomegalovirus (CMV) in malignant gliomas. Many of these genes promote oncogenesis, alter tumor microenvironment, and serve as immunologic targets. Is the level of CMV infection within tumor cells sufficient to drive important oncogenic or immunosuppressive processes? Can CMV serve as a target for therapeutic intervention? Clin Cancer Res; 17(14); 4619–21. ©2011 AACR.

In this issue of Clinical Cancer Research, Dziurzynski and colleagues (1) report that CD133+ glioma stem cells (gCSC) express human CMV proteins and secrete CMV interleukin (IL)-10, a viral cytokine homolog of IL-10 that is capable of converting the widespread monocytes and microglia in glioblastomas (GBM) to an immunosuppressive tumor-supportive phenotype.

CMV is an endemic β-herpesvirus that does not usually cause significant clinical disease. However, CMV can cause fetal encephalitis and significant problems in immunocompromised adults. Herpesviruses have also been implicated in a number of human malignancies, including lymphoma, nasopharyngeal cancer, cervical cancer, and Kaposi sarcoma. Recent studies have reported the expression of proteins unique to CMV in a large proportion of malignant tumors, including colorectal carcinoma, prostate cancer, and gliomas (2, 3).

CMV induces numerous molecular changes within infected cells that could contribute to an oncogenic phenotype (3). For example, CMV has been shown to increase production of angiogenic factors, create a chronic inflammatory environment, and mediate numerous immunosuppressive changes in host cells. Furthermore, CMV-infected cells exhibit increased cellular motility and invasion, reduced p53 and retinoblastoma (Rb) function, elevated levels of telomerase, and increased resistance to chemotherapy-induced apoptosis. Although viral infection has not been shown to directly result in tumor formation, the CMV genome encodes several proteins that have been shown to possess direct transforming capability when stably expressed in normal cells.

Cobbs and colleagues (2) first identified CMV antigen expression in GBM. Using a series of optimized protocols, these authors showed the expression of CMV proteins (IE1, pp65, and late antigens) by immunohistochemistry in 27 out of 27 specimens, but not within surrounding normal brain or other brain pathology specimens. They detected CMV nucleic acids within these tumors using in situ hybridization and nested PCR confirmed by DNA sequencing. Our group (4) elaborated on this work and confirmed expression of IE1 and pp65 in >90% of GBM specimens by immunohistochemistry, detection of early and late viral proteins (IE1, pp65, and gB) in GBM primary cultures, and detection of nucleic acids using PCR (gB, pp65, and IE1) coupled with confirmatory DNA sequencing. Independent analyses by other groups (5, 6) also confirmed CMV antigen expression in these tumors, including the expression of US28, a viral gene that was recently implicated in tumorigenesis in a transgenic mouse model of colorectal cancer (7, 8).

Dziurzynski and colleagues (1) investigated whether CMV infection can contribute to the immunosuppressive microenvironment that is characteristic of GBM. Tumor-associated macrophages (TAM) exposed to factors such as IL-10 can acquire M2 properties that suppress immune responses and support proliferative ones, thereby enhancing malignant growth. Herein, Dziurzynski and colleagues demonstrate that TAMs derived from single-cell GBM digests express CMV antigens. Additionally, their examination of established CD133+ gCSCs revealed that CD133ghigh gCSCs preferentially express CMV antigens and secrete viral IL-10. Thus, neural precursor cells, cells with self-renewal capacity, and monocytes are permissive to CMV infection and could function as reservoirs for CMV reactivation within these tumors. In vitro exposure of CD14+ monocytes to viral IL-10 downregulates expression of major histocompatibility complex (MHC) II and costimulatory molecule CD86, upregulates expression of the immune inhibitory molecule B7-H1, and increases intracellular TGF-β, VEGF, and phosphorylated STAT3. In return, viral IL-10–exposed monocytes increase gCSC migration. Therefore, in vivo exposure to viral IL-10 secreted by gCSCs could induce gCSC migration and an immunosuppressive milieu (Fig. 1).

Although the presence of CMV in gliomas has been reported by several independent laboratories, other groups...
have reported a failure to detect the presence of CMV within these tumors (9, 10). This discrepancy is likely attributable to methodologic differences, as we and others have demonstrated that sensitive and optimized protocols are required to detect the very low levels of CMV likely present within these tumors. This observation raises the following questions: (i) is the level of CMV infection within tumor cells sufficient to drive important oncogenic or immunosuppressive processes, and (ii) is CMV a clinically relevant target within these tumors for therapeutic intervention?

Koch’s postulates are a stringent set of criteria that are used to determine whether an infectious agent causes a disease. However, these strict criteria are not often met in cases of virally induced cancers (11). The long latency between infection and cancer diagnosis, and the fact that only a small minority of individuals exposed to a cancer-promoting virus (i.e., human papillomavirus or hepatitis B) ever develop a malignancy associated with the viral infection, clearly implicates multiple factors in addition to the infectious insult in the oncogenic process. In the majority of cancers caused by viral infections, the viral DNA is present in very small copy number, usually less than 1 DNA copy per 10 tumor cells. Furthermore, it is now clear that chronic infections can facilitate oncogenesis through indirect mechanisms without coding for transforming genes themselves, such as Helicobacter pylori promotion of gastric cancer and gastric lymphoma, providing several mechanisms by which infections may promote cancer formation. Finally, microbial genomes within cancer cells are often altered such that viable infectious pathogens are not recoverable from tumor cells and thus fail to fulfill the major criteria for Koch’s postulates. Future experiments that can demonstrate true modulation of the oncogenic phenotype via physiologic levels of infection with CMV-associated tumors will be pivotal for assessing any direct or indirect causality between CMV and oncogenesis.
The immunologic impact of CMV as reported by Dziurzynski and colleagues (1) raises a question: Would the levels of CMV viral IL-10 produced by in vivo gCSCs be sufficient to drive the immunosuppressive changes described within TAMs? If so, treatments targeting viral IL-10 could constitute novel and highly relevant therapeutic modalities. Similarly, evidence already exists that low levels of CMV viral gene expression may be sufficient for immunologic targeting (5), and it has also been demonstrated that killing by cytotoxic T lymphocytes can occur with presentation of as few as 3 antigenic peptides upon the surface of the target cell (12). Furthermore, if gCSCs do preferentially express CMV antigens in vivo, CMV-targeted therapies may profoundly inhibit malignant growth through eradication of this tumor-propagating population. The work of Dziurzynski and colleagues exemplifies that the continued characterization of biologically relevant levels of CMV infection as related to malignant progression and examination of CMV antigens as immune-mediated targets represent important and potentially promising areas of future research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

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Received May 4, 2011; accepted May 12, 2011; published OnlineFirst June 1, 2011.

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