

Activation of Telomerase in Glioma Cells by Human Cytomegalovirus: Another Piece of the Puzzle

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In this issue of the Journal, Strååt et al. (1) show that human cytomegalovirus (HCMV) infection induces telomerase activation in human malignant glioma cells and fibroblasts. These results reveal a novel mechanism that may be relevant for the potential of HCMV to promote oncogenesis and thus provide another piece of the puzzle that has drawn the attention of oncologists and virologists for almost four decades.

A possible relationship between HCMV and cancer has been considered since the beginning of the 1970s when Fuccillo et al. (2) found increased anti-HCMV antibody titers in cervix carcinoma patients compared with healthy individuals. Although many subsequent studies compared HCMV antibody titers between cancer patients and healthy people or investigated the presence of HCMV proteins, DNA, or RNA in tumor tissues from different cancer entities, the role of HCMV in cancer is still unclear (3).

HCMV is a ubiquitous herpesvirus that after (subclinical) primary infection persists for the life of its host. The virus is believed to persist mainly in myeloid cells and may be reactivated in immunocompromised patients with accompanying signs of HCMV disease and infection in cells of many different tissues. In immunocompetent hosts, subclinical virus reactivations may occur (4). Several recent investigations suggest that in cancer patients, clinically manifest or subclinical HCMV reactivations occur more frequently than previously supposed. Reactivations may be induced by stimuli such as cancer-related immunosuppression, conventional chemotherapy, gamma radiation, or inflammation (3,5–7).

Findings from the 1970s suggested that HCMV could transform human embryonal fibroblasts (8,9). However, these studies failed to detect HCMV nucleic acids or proteins after long-term subculture of the transformed cells. Moreover, HCMV infection did not induce malignant transformation of normal human cells in most other studies. Although various attempts were made to explain the role of HCMV in oncogenesis (eg, hit-and-run hypothesis), the potential of the virus to initiate transformation is regarded with skepticism, and HCMV is not generally considered an oncogenic virus (3). We hypothesized a process of HCMV oncomodulation in the 1990s to explain a possible contribution of HCMV to tumor progression (10). Oncomodulation means that HCMV infects established tumor cells and increases tumor malignancy without necessarily being oncogenic (3,10). Support for this idea is mainly based on experimental findings indicating that HCMV regulatory proteins and noncoding RNAs may influence properties of tumor cells, including cell proliferation, survival, invasion, immunogenicity, tumor angiogenesis, and chromosomal stability, as well as inflammatory processes (3,4). Conceivably, HCMV infection of nontransformed cells in tumor stroma such as fibroblasts or infiltrating immune cells may also influence tumor behavior. According to the concept of oncomodulation, tumor cells provide a genetic environment, characterized by disturbances in intracellular signaling pathways, transcriptional control, and

tumor suppressor proteins, that enables HCMV to manifest its oncomodulatory potential in cancer cells but not in normal cells.

The cancer that has been most frequently investigated for its association with HCMV is glioma. HCMV infection was for the first time correlated with a tumor-promoting activity in glioma cells when the HCMV 72 kDa immediate early (IE) 1 protein was found to decrease the expression of the endogenous angiogenesis inhibitor thrombospondin-1 independently of the tumor suppressor protein p53 (11,12). Other cell culture experiments demonstrated that HCMV infection of glioma cells may stimulate tumor cell cycle progression and invasiveness (13–15). Animal studies demonstrated that a single HCMV protein increases the malignancy of glioma cells. Human glioblastoma cells expressing US28, a chemokine receptor of HCMV, exhibited increased malignancy after injection in nude mice. In contrast, US28 induced apoptosis in nontumorigenic human cells, suggesting that US28 oncomodulatory properties are limited to tumorigenic cells (16).

Histological studies using highly sensitive techniques for virus detection found genomic sequence and antigens of HCMV in tumor cells but not in adjacent normal tissue in a large fraction of glioma patients (3,4,7,17,18). Groups that used less sensitive histological methods established for the detection of active (high-level) HCMV infection failed to detect low-level HCMV infection in glioma cells (3,19–21). Clearly, HCMV infection of glioma tissue(s) does not prove that the virus contributes to tumor progression or increases tumor malignancy. However, additional data suggest that low-grade HCMV infection of cancer cells is sufficient to influence the severity of cancer diseases. In support of a role for the virus in tumor progression, increased numbers of infected cancer cells were associated with a more unfavorable outcome in glioma patients (4). It has also been reported that the fraction of HCMV-infected tumors was higher in glioblastoma multiforme (79%) than in lower grade tumors (48%) (18).

The elegant experiments of Strååt et al. describe a novel molecular mechanism that may turn out to be critical to the relationship between HCMV and cancer (1). The authors show for the first time that infection with several different strains of HCMV results in the induction of human telomerase reverse transcriptase (hTERT) expression and increases telomerase activity in normally telomerase-silent human diploid fibroblasts. The authors also

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found that telomerase induction correlated with increased hTERT promoter activity following infection in both primary fibroblasts and malignant glioblastoma cell lines.

Strååt et al. have further demonstrated that HCMV-dependent telomerase induction depends on active viral gene expression. In fact, ectopic expression of only one viral protein (IE1), out of the roughly 200 different HCMV gene products, was sufficient to recapitulate (at least partly) the viral effects on hTERT promoter activation. This finding may not come as a big surprise for many HCMV researchers because IE1 has long been known to be a promiscuous transcriptional activator of numerous viral and host cell genes. This protein reportedly interacts with several common cellular transcription factors including CTF1, E2Fs, and Sp1 (22). We have shown that IE1 also targets histone deacetylases (HDACs) to promote histone acetylation (23). Strååt et al. demonstrate that some IE1 are physically associated with hTERT promoter sequences during HCMV infection of fibroblasts. They also provide evidence that the viral protein activates transcription from the hTERT promoter via mechanisms that involve both Sp1 recruitment and HDAC sequestration, followed by increased histone H3 acetylation. These findings are additional confirmation that epigenetic modifications are of fundamental importance for infection and pathogenesis.

Several groups have previously reported the presence of the IE1 protein in HCMV-positive tumor cells [reviewed in (3)], and intriguingly, the authors demonstrate a striking correlation between IE1 and hTERT protein levels in histological glioblastoma samples. This is the first time that expression of a protein known to play an important part in cancer formation and/or progression has been directly linked to HCMV infection of tumor cells.

Telomerase activation is also caused by infection with other prominent tumor viruses including Epstein-Barr virus, Kaposi sarcoma-associated herpesvirus, human papillomavirus, hepatitis B virus, hepatitis C virus, and human T-cell leukemia virus-1 (24). In fact, telomerase is commonly activated in cancer cells of both viral and nonviral origin (25), and hTERT activation is sufficient to immortalize normal diploid cells (26). Besides elongation of telomeres, telomerase may favor tumor cell survival and proliferation by several other mechanisms. The enzyme may inhibit cancer cell apoptosis, promote cancer cell growth, favor emergence of cancer stem cells, and enhance DNA repair (25,27,28). Therefore, HCMV-induced telomerase activation represents a mechanism that is of possible relevance for both (initiation of) malignant transformation and oncomodulation by HCMV infection.

General antiviral therapeutic strategies (29) or inhibition of HCMV IE gene expression (30) was shown to revert HCMV-induced malignant changes in experimental models. Such strategies may also be effective to suppress HCMV-induced telomerase activation, thus possibly preventing tumor progression. Moreover, antiviral treatment may reverse HCMV-induced chemoresistance of tumor cells as already demonstrated in experimental models (3). The first clinical trial in which the anti-HCMV drug valganciclovir was used to treat glioma patients has been recently completed at the Karolinska Institutet (31), and information about the results is eagerly awaited.

Naturally, the report of Strååt et al. also leaves some open questions. First, it is difficult to really judge the role of HCMV-induced telomerase activation in a possible HCMV-induced oncogenic trans-

formation process because no transformation was shown. The cell types used were either subject to lytic infection and therefore destroyed by HCMV (human diploid fibroblasts) or already transformed cancer (glioblastoma) cells. Moreover, the oncomodulatory effects that might be exerted by HCMV-induced telomerase activation remain to be defined in functional assays. Nevertheless, the work of Strååt et al. represents a very important step toward the elucidation of the complicated relationship between HCMV and cancer.

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