

HCMV Infection Promotes the High Expression of BST-2 in Glioma Cells

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Abstract: To investigate the effect of human cytomegalovirus (HCMV) on proliferation and migration of glioma cells and the role of BST-2 in this process. U251 cells were infected by HCMV and the effect of HCMV infection on U251 migration were examined by wound healing assay. The effect of HCMV infection on the expression of BST-2 protein was detected by Western blot. The influence on cell proliferation and migration after down-regulation of BST-2 in HCMV infected U251 cells were detected by CCK-8, cell wound healing assay method. Results reveals that HCMV infection can promote the migration of U251 cells and increase the expression of BST-2. Silencing BST-2 can inhibit the proliferation and migration induced by HCMV infection. These results confirm that HCMV infection can promote the proliferation and migration of U251 glioma cells, and BST-2 participates in the proliferation and migration of malignant glioma cells caused by HCMV infection

Keywords: Glioma, BST-2, HCMV

1. Introduction

Human cytomegalovirus (HCMV) is a double-stranded DNA virus of the herpes virus subfamily beta, and its population infection rate in China is 70%-90%. Immune-sufficient humans are often asymptomatic after being infected with HCMV, but the virus may remain latent for life. The long-term coexistence of this virus and host cells may have a series of effects on the biological behaviors such as tumor differentiation, migration, and invasion. HCMV is not recognized as a tumorigenic virus, but studies have shown that HCMV protein products and genomes can be detected in a variety of tumor tissues, such as gliomas^[1-3], breast cancer, colon cancer, and prostate cancer. Gliomas are the most common primary intracranial tumors. Most of them exhibit aggressive growth, strong invasiveness,

high degree of malignancy, high recurrence rate of surgical treatment, poor prognosis, and high mortality rate. Recent studies have suggested that HCMV infection is closely related to the degree of malignancy of gliomas^[1-3].

Bone Marrow Stromal cell antigen 2 (BST-2, also known as Tetherin/CD317/HN1.24) is a type II transmembrane protein with a molecular weight of 30-60 Kd and consists of 181 amino acids. The structure is divided into N-terminal cytoplasmic region (CT), transmembrane region (TM), extracellular region (EC), and C-terminal glycosylphosphatidylinositol (GPI) anchors, in which both TM and GPI regions are membrane anchored. Structure [5]. BST-2 is considered to be a natural antiviral immune protein and has a

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significant inhibitory effect on the release of many types of virus [6-8], but BST-2 is not able to inhibit the release of HCMV, and even studies have confirmed that BST-2 It can promote the entry of HCMV into host cells, down-regulation of BST-2 can effectively reduce HCMV replication [9]. In recent years, studies have shown that BST-2 expression is also closely related to the degree of malignancy of the tumor.

In previous studies, we confirmed that HCMV infection can induce human primary astrocytes and U251 glioma cells to up-regulate BST-2 expression, which can be up-regulated by 250-fold compared with the control group. We speculate that BST-2 may be a key target for the malignant progression of gliomas after HCMV infection. The relationship between BST-2 and HCMV infection and gliomas deserves further investigation and study.

2. Materials and methods

Cells

Two cell lines derived from human glioblastoma were cultured in modified Eagle's medium (MEM, HyClone) containing 10% fetal bovine serum (Gibco, NY, USA) in a humidified atmosphere with 5% CO₂ at 37°C.

Viruses and infection

HCMV AD169 (kindly provided by France Pasteur Laboratory) was expanded in human embryonic lung fibroblast (HELFL) cells (stored in our laboratory) and was tittered by plaque titration as the number of plaque-forming units (PFU) per milliliter. Viruses stored at -86°C. U251MG cells were infected at a multiplicity of infection (MOI) of 0.1 or 1. In order to remove the possibility of BST-2 affecting the HCMV

entry into host cells, all the HCMV groups were infected with the viruses 4 hours before RNA interference.

Cell proliferation assay

In order to determine cell proliferation capacity, Cell Count Kit-8 (CCK-8, Hanbio, Shanghai, China) was monitored. Four hours after HCMV infection or BST-2 RNAi, cells were seeded into 96 well plates at a density of 1×10^4 cells/well. After 24 hours of incubation, the medium in each well was replaced with fresh medium. At different time points (0, 12, 24 and 36 hours), 10 μ L of CCK-8 reagent was subsequently added to each well and the cells were incubated at 37°C for an additional 2 hours according to the manufacturer's instructions. The optical density (OD) at 450 nm in each well was measured with a microplate reader. Three independent experiments were performed and the mean and standard error of each experiment were calculated.

Wound healing assay

After HCMV infection and RNAi, U251MG and U87MG cells were seeded into 6-well plates (5×10^5 per well) and grown to 90%-95% confluence. A similar size wound was introduced into the confluent monolayer using a sterile toothpick. After washing with MEM several times to remove cell debris, fresh medium was replaced with 2% FBS and then allowed to migrate at 37°C for 24 hours. The wound closure was photographed using a phase contrast microscope. The migratory capacity was determined as the percent of wound closure and the initial wound width was defined as 100%. Statistical significance of the results was analyzed using the Student's t test. Differences were considered significant at $p < 0.05$.

3.Results

3.1 HCMV infection enhances proliferation and migration of glioblastoma cells

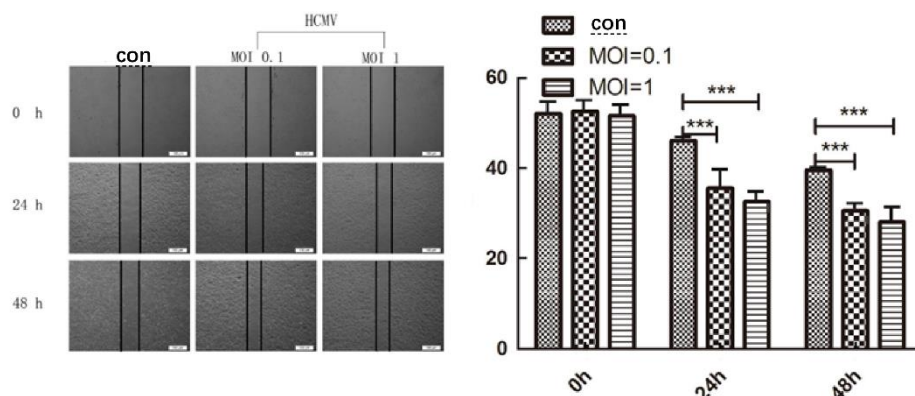


Fig.1 U251 cells infected with different concentrations of HCMV changes with time of cell migration.

*** : compared with control group, $p < 0.01$

The results of the scratch healing experiment showed that the scratch distance was approximately the same at 0 h in the control group, the HCMV MOI=0.1 group and the HCMV MOI=1 group. After 24 hours, the scratch distance of infected HCMV cells was shortened. Compared with the control group, the cell migration

increased significantly ($P < 0.01$), and the high MOI value (MOI=1) group was lower than the MOI value (MOI=0.1.) The group cells are more able to migrate. The 48 h experiment results are similar to 24 h (Figure 1).

3.2 HCMV infection promotes high expression of BST-2 in U251 cells

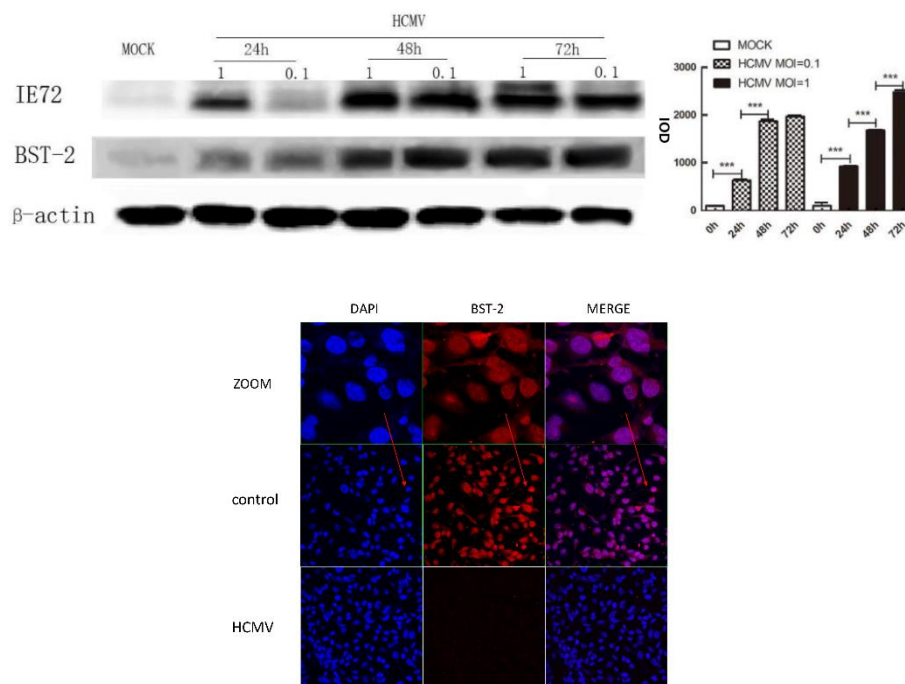


Fig.2 BST-2 protein expression of U251 cells infected HCMV

*** : compared with shNC group, $p < 0.01$

Western blot analysis of U251 cells infected with HCMV with different MOI values showed that U251 cells (MOCK) that were not infected with HCMV did not express the marker protein IE of HCMV and low expression of BST-2. With the extension of the infection time, IE expression appeared, confirming the establishment of a viral infection. The expression of

BST-2 protein was time-dependent within 24-72 h after HCMV infection. However, there was no significant difference in the expression of BST-2 within the same time period after HCMV infection with different MOI values (Figure 2). The same result was also found in immunofluorescence experiments.

3.3 Down-regulation of BST-2 expression after HCMV infection affects migration of glioma cells

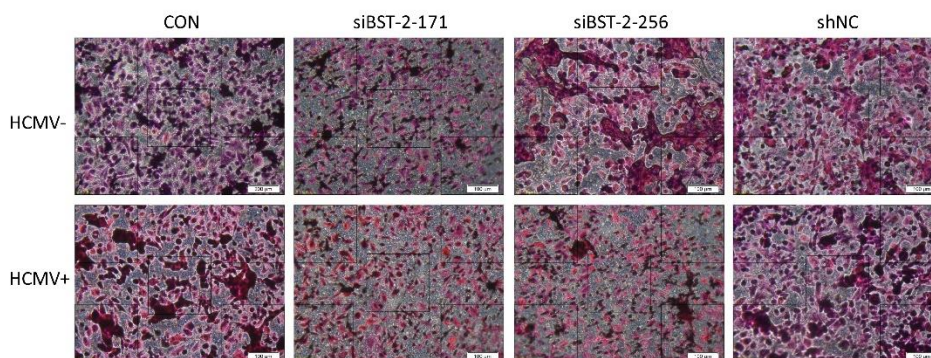


Fig.3 The influence on cell migration after down regulation BST-2 in HCMV infected U251 cells

In order to detect BST-2 as a key protein for the promotion of glioma cell migration induced by HCMV infection, we verified the cell scratch and tanswell method, respectively. After U251 cells were not infected or infected with HCMV (MOI=1), they were transfected with shBST-2-171, shBST-2-256, shNG or not treated. The cells were scratched for 24 h and observed under an inverted microscope. Similar results were obtained with the Tanswell migration test (Figure 3). Compared with the uninfected group, the number of transmembrane in the HCMV-infected group increased, and the down-regulation of BST-2 significantly decreased the ability of transmembrane migration .

4. Discussion

HCMV infection is closely related to malignant glioma cells. Its role and mechanism in the development of glioma has become a hot topic in recent years. In 2002, Cobbs et al [1] first reported that HCMV genes and proteins could be detected in grade II-IV glioma tissue. Subsequently, studies have shown that more than 90% of glioblastomas (grade IV gliomas) can detect HCMV genome and protein, 80% of patients have detected

HCMV markers in serological samples [2], HCMV infection It is closely related to the degree of malignancy of gliomas [3]. However, the mechanism by which HCMV infection participates in the occurrence of gliomas so far is still not clear. Our results further confirm that HCMV can establish infection in U251 cells and can promote the proliferation and migration of glioma cells, indicating that it can play an important role in the development and progression of glioma.

Although BST-2 is thought to antagonize the release of host cells from enveloped viruses, recent studies have shown that BST-2 is highly expressed in many types of tumors, such as head and neck tumors [7], lung cancer [8], and breast cancer. [6, 9], cervical cancer [10], myeloma, endometrial cancer [11], etc., suggesting that BST-2 has a carcinogenic effect. This experiment confirmed that HCMV infection can promote the expression of BST-2 and show a time-dependent relationship within a certain period of time. Given the close relationship between BST-2 and tumorigenesis, we speculate that BST-2 may play an important role in promoting the malignant development of glioma in HCMV. The effect

may be a contributing factor to the development of cancer. We then screened for interfering targets and effectively blocked up-regulation of BST-2 levels caused by HCMV infection. The effects of down-regulation of BST-2 on the proliferation and migration of U251 cells after HCMV infection were tested by CCK-8 cell proliferation assay, cell scratch healing, and transwell assays, confirming that HCMV infects glioma U251 cells and promotes the proliferation of U251 cells. Migration, but inhibition of BST-2 can effectively inhibit its proliferation and migration ability. The results of this experiment to a certain extent explain the HCMV infection to promote the development of glioma a possible mechanism, BST-2 may become a new target for the treatment of glioma cancer.

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