Human cytomegalovirus replication cycle is regulated by hepatocyte growth factor/c-met pathway

Walid Basha[1]

[1]Faculty of Medicine, An-Najah National University, P.O.Box 7, Nablus, Palestine

Introduction

Human cytomegalovirus (HCMV) infections are generally subclinical in immunocompetent hosts; the virus can cause severe morbidity and mortality in immunocompromised patients, such as transplant patients and AIDS patients (Cunningham, ET. et al 1998; Jabs, DA. et al 1998; Nussenblatt, RB.et al 1998). In graft-versus-host disease (GVHD), hepatocyte growth factor (HGF) up-regulation is a common occurrence with HCMV infection (Aharinejad, S. et al. 2004). HGF exerts mitogenic, morphogenic and motogenic activities in various types of cells. All these physiological activities are initially mediated by c-Met tyrosine kinase, the receptor for HGF. Most of c-Met downstream signaling is common with HCMV replication cycle regulators, such as PI3-kinase and NF‐B (Crljen, V. et al. 2002; Naldini, L. et al. 1991). These signaling pathways are also activated by many other extra-cellular factors such as epidermal growth factor and interleukins. In hypothesis HCMV infection activates the HGF/ c-Met pathway, which is a crucial upstream regulator pathway for HCMV replication.

Materials and Methods

Human embryonic lung (HEL) cells were infected with HCMV at a multiplicity of infection (MOI) of 0.01 – 1 PFU. Viral and cellular protein syntheses were examined by western blot analysis and double immunofluorescence staining. HEL cells were treated with the recombinant HGF or HGF activator or inhibitor compounds before and after HCMV infection and virus growth cycle was monitored using western blot and plaque assay techniques.

Results

HCMV replication was enhanced by treatment of HEL cells with recombinant HGF (rHGF) [3 ng/ml] or activation of HGF by treating cells with heparin [0.5 ‐ g/ml] and serine/threonine phosphatase inhibitors calyculin A (CA) [1 nM] and okadaic acid (OA) [10 nM]. At 5 days p.i. rHGF, heparin, CA and OA increased HCMV yields by 2-, 3-, 7-, and 5-fold respectively. In contrast, neutralization of HGF by mouse monoclonal anti-HGF [4ng/ml] down regulated HCMV replication by 2 folds. Treating cells with HGF/c-Met pathway inhibitors such as aspirin [2 mM] and geldanamycin (GA) [180 nM] markedly inhibited virus replication. At 5 days p.i., GA treatment reduced the virus yield to an undetectable level.

Conclusions

These results suggest several possible options for inhibiting HCMV replication. This work could lead to development of therapeutic candidates for treatment of GVHD.

References

Aharinejad, S. et al. (2004) Prediction of lung-transplant rejection by hepatocyte growth factor. Lancet , 363 : 1503-1508.

Cunningham, ET. et al (1998) Ocular manifestations of HIV infection. N. Engl. J. Med. 339 : 236-244.

Crljen, V. et al. (2002) Hepatocyte growth factor activates phosphoinositide 3-kinase C2β in renal brush-border plasma membranes. Biochem. J. , 365 : 791-799.

Jabs, DA. et al (1998) Discontinuing anti-cytomegalovirus therapy in patients with immune reconstitution after combination antiretroviral therapy. Am. J. Ophthalmol 126, 817-822.

Lane, H.C. et al (1998) Human immunodeficiency virus disease: changing patterns of intraocular inflammation. Am. J. Ophthalmol 125 , 374-382.

Naldini, L. et al. (1991) Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-Met. Oncogene , 6 : 501-504.