4. Neutralization of hepatocyte growth factor inhibits human cytomegalovirus replication in human lung fibroblast

Walid Basha¹ and Junji Tanaka²

¹Department of Medical Lab. Science, Faculty of Allied Medical Sciences, Arab American University-Jenin, Jenin, Palestine, ²Department of Laboratory Science, School of Health Sciences, Faculty of Medicine, Kanazawa University, Kanazawa, Japan

Background: Human cytomegalovirus (HCMV) infections generally are subclinical in immunocompetent hosts; the virus can cause severe morbidity and mortality in immunocompromised patients, such as transplant patients and AIDS patients. In graft-versus-host disease (GVHD) hepatocyte growth factor (HGF) is a common occurrence with HCMV infection. 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, and these signaling are activated by many other extra-cellular factors.

Hypothesis: HCMV infection activates HGF/c-Met pathway, which is a crucial upstream regulator pathway for HCMV replication.

Methods: Human embryonic lung (HEL) cells 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: Up-regulation of the HGF/c-Met pathway is a common occurrence during the early stages of HCMV infection in HEL fibroblasts, which is a complete permissive cell for HCMV. Much as, treatment of HEL cells with recombinant HGF [3 ng/ml] or activation of HGF by treating cells with other chemicals such as heparin [0.5 µg/ml] and serine/threonine phosphatase inhibitors calyculin A (CA) [1 nM] and okadaic acid (OA) [10 nM] enhanced HCMV replication. HCMV yields at 5 days p.i. were 3- and 7 fold increased by pre or post treatment with CA. Alternatively, the neutralization of HGF by mouse monoclonal anti-HGF [4ng/ml] down regulates HCMV replication. Treating cells with known HGF/c-Met pathway inheritors such as aspirin [2 mM] and geldanamycin (GA) [180 nM] although markedly inhibits virus replication. At 5 days p.i., GA treatment reduced the virus yield to undetectable level.

Conclusions: These results and further studies on this subject may be able to open a possibility to use this phenomenon as a target for future therapeutic candidates for GVHD.