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MINIREVIEW

Characteristics and functions of human cytomegalovirus UL128 gene/protein

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Summary. – Human cytomegalovirus (HCMV) ORF UL128 protein is highly conserved among viral field isolates and functions in two different molecular forms, monomeric UL128 protein and in a complex with glycoproteins gH, gL, UL130, and UL131A protein. Monomeric UL128 protein works as soluble chemokine analogue to attract peripheral blood mononuclear cells (PBMCs) and selectively induces expression of interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) in PBMCs. The gH/gL/UL128/UL130/UL131A complex is indispensable for entry into both endothelial and epithelial cells. In conclusion, UL128 plays an important role in HCMV infection.

Keywords: human cytomegalovirus; UL128 gene/protein; chemokine

Contents:

1. Introduction

HCMV is a widespread herpesvirus, and the seroprevalence in people varies from 45% to 100% (Cannon *et al.*, 2010). After the primary infection, it usually persists for the whole life of an individual in a latent form. Although HCMV does not cause severe diseases in immunocompetent people, congenital infection is the leading viral cause of defects at birth, including wide range of neurodevelopmental disabilities and hearing loss. In immunocompromised patients with AIDS and after allogeneic bone marrow or organ transplants, HCMV infections often lead to life-threatening diseases (Vanarsdall *et al.*, 2008).

HCMV usually establishes primary infection in two ways. The first is via epithelial cells (EpCs) of the rhinopharynx or the genital tract if the route of transmission is oral or sexual contact. The second way is via endothelial cells (EnCs) of the vascular tree, if the route of transmission is blood transfusion. PBMCs are also important sites for HCMV infection. They are recruited at the sites of primary infection, carrying virus and viral products, thus initiating haematogenous dissemination and contribute to the reactivation by cell differentiation (Reeves and Sinclair, 2008). During this process, HCMV expresses a number of proteins to achieve cell tropism, transmission, reactivation, and immune evasion (Revello and Gerna, 2010; Avdic *et al.*, 2011; Montag *et al.*, 2011).

Recent studies have revealed that the product of HCMV ORF UL128 can interact with many different cell types including EnCs, EpCs, and PBMCs. It may play a part in virus infection through the interference with these cells. In this review, we describe the current knowledge about the characteristics of the UL128 gene and protein and func-

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Abbreviations: EnC(s) = endothelial cell(s); EpC(s) = epithelial cell(s); ERK = extracellular regulated protein kinase; gB = glycoprotein B; gH = glycoprotein H; gL = glycoprotein L; HCMV = human cytomegalovirus; IL-6 = interleukin 6; MAPK = mitogen-activated protein kinase; PBMC (s) = peripheral blood mononuclear cell(s); RCMV = rat cytomegalovirus; TNF-α = tumor necrosis factor α

tions of two different forms of the protein, monomeric and bound to the gH/gL/UL130/UL131A complex, in infection of different cell types.

2. Characteristics of UL128 gene and protein

2.1 UL128 gene

UL128 gene is found to be highly conserved among 34 wild virus isolates in pregnant women with primary HCMV infection, their fetuses or newborns, as well as in solid organ transplant recipients and patients with AIDS (Baldanti *et al.*, 2006). However, it exhibits high variation between different strains: the Merlin strain has a C to T transition in UL128 exon 3, which introduces a stop codon and leads to premature translational termination; the 3157 strain has a G to C transversion in the GT dinucleotide of the splice donor site at the end of UL128 exon 1 which disrupts splicing; the Toledo strain has an inversion of a substantial region, which results in disruption of UL128 by introducing UL148A in place of UL128 exon 3 (Dolan *et al.*, 2004). In addition, UL128 gene is demonstrated to be suppressed when HCMV strains are extensively passaged in human fibroblasts (Dolan *et al.*, 2004; Dargan *et al.*, 2010). These strains also loose their ability to grow in epithelial and endothelial cells, indicating that UL128 gene may play an important role in HCMV infection.

Sequencing of clinical strains showed that UL128 gene has two sets of transcription patterns, one consisted of three 519 nt long exons, and the other consisted of three exons with the first 642 nt long intron (Sun *et al.*, 2010). The respective roles of two patterns of UL128 transcript in HCMV infection still requires further exploration.

2.2 UL128 protein

UL128 proteins of the HCMV, chimpanzee cytomegalovirus (CCMV) and simian cytomegalovirus (SCMV) commence with a predicted signal peptide, suggesting they may be secreted and work as a soluble molecule. Moreover, UL128 proteins share four conserved cysteine residues near their N termini which is the structural characteristic of β- (or CC-) chemokines (Akter *et al.*, 2003). These findings indicate that monomeric UL128 protein may be secreted and may act as a β-chemokine analogue in HCMV infection, just as HCMV ORF UL146 encoding a homologue of α-chemokine vCXC-1 (Penfold *et al.*, 1999).

UL128 protein was also proved to act as a part of the gH/gL/UL128/UL130/UL131A complex in the process of entry into EnCs and EpCs (Hahn *et al.*, 2004; Ryckman *et al.*, 2008a; Ryckman *et al.*, 2006; Wang and Shenk, 2005). The protein-protein interactions in gH/gL/UL128/UL130/UL131A complex are confirmed to operate as follows: gH

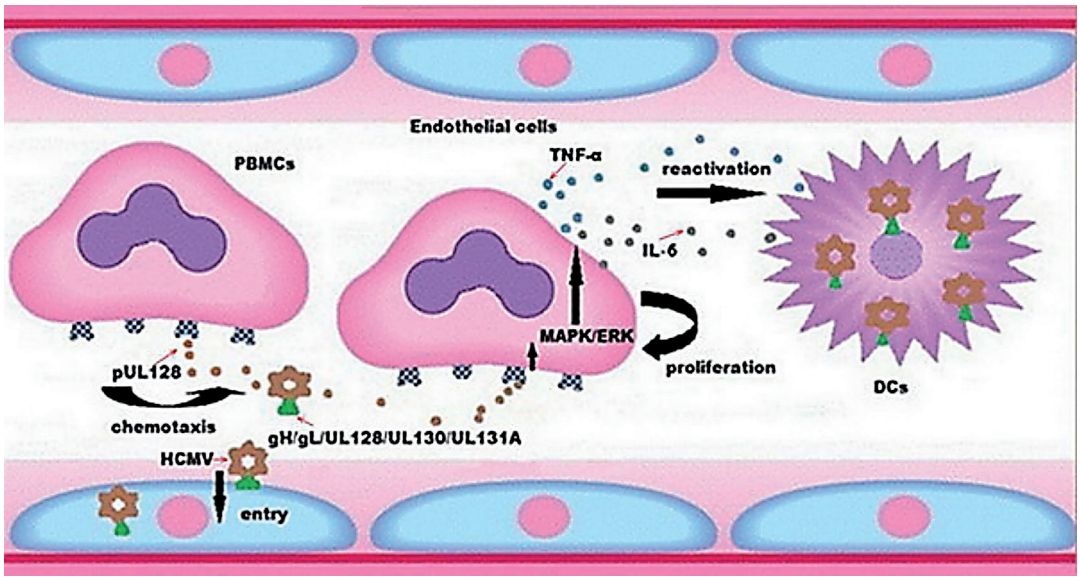

Fig. 1
Functions of UL128 protein
The figure illustrates the main functions of UL128 protein. Monomeric pUL128 protein attracts PBMCs to infected area, promotes PBMCs to proliferate by activating the MAPK/ERK signaling pathway, induces IL-6 and TNF-α expression in PBMCs and contributes to reactivation of HCMV. The complexed UL128 is indispensable for HCMV entry into EnCs and EpCs.

protein together with gL and also UL130 with UL131 interact through disulfide linkage; UL128 interacts with both UL130 and gL through noncovalent interactions and a surface created by both gH and gL is required for UL131 binding. The whole pentamer is predicted to be inserted in to the virion envelope through gH (Ryckman *et al*., 2008b).

Since UL128 protein has two kinds of molecular forms in HCMV infection, it may work in different ways to contribute to the pathogenesis of the virus.

3. Role of UL128 protein in HCMV infection

In vivo, HCMV can infect a broad range of cell types including fibroblasts, EnCs, EpCs, monocytes, macrophages, and smooth muscle cells (Scrivano *et al*., 2011). Two molecular forms of UL128 protein, namely monomeric UL128 protein and complexed UL128 protein, act by different ways to influence various cells involved in HCMV infection.

3.1 Functions of monomeric UL128 protein

There are several reports on the relationship between monomeric UL128 protein and PBMCs. For example, monocytes as an important part of PBMCs have been described as shelters for HCMV to avoid clearance, vehicles for HCMV dissemination and reservoirs for HCMV latency (Frascaroli *et al*., 2006; Chan *et al*., 2012; McCormick *et al*., 2010). Monocytes originating from $CD34^+$ progenitors in the bone marrow circulate in the bloodstream for 1 to 3 days and then move into peripheral tissues to differentiate toward macrophages and dendritic cells. They will encounter HCMV in the bone marrow, the bloodstream, and the peripheral tissues during or after transendothelial migration from the bloodstream (Straschewski *et al*., 2011). Monocyte movements are tightly controlled by members of chemokines and their receptors to ensure the movements occur in the proper spatial and temporal fashion during immune responses (Lira and Furtado, 2012).

Since UL128 protein may be secreted and works as soluble chemokine analogue, we have synthesized recombinant UL128 protein and proved it could attract PBMCs with potency equal to that of MIP-1α *in vitro* by chemotaxis assay (Gao *et al*., 2012). This result is in line with the previous study that the r129 protein encoded by rat cytomegalovirus (RCMV) also as a CC chemokine analogue was demonstrated to be released into culture supernatants of RCMV infected cells and induced migration of lymphocytes isolated from rat (Vomaske *et al*., 2012). In our previous report, UL128 protein was also proved to promote PBMCs proliferation by activating the MAPK/ERK signaling pathway (Zheng *et al*., 2012). On the contrary, Straschewski *et al.* (2011) demonstrated that soluble recombinant UL128 protein could block chemokine-driven migration of monocytes by down-regulation of CCR1, CCR2, and CCR5 chemokine receptors. The possible reason for the controversy may be that monomeric UL128 protein interacts with more chemokine receptors than CCR1, CCR2, and CCR5 to influence the migration of monocytes. That means, UL128 may work as a viral protein to interfere with chemokine-driven motility or as a β-chemokine homologue to attract monocytes depending on the chemokine receptors it recognizes. In the case of UL128 impairing chemotaxis of monocytes towards inflammatory chemokines CCL2 and CCL5 by down-regulation of chemokine receptors CCR1, CCR2, and CCR5 (Straschewski *et al*., 2011), it indicates that strict chemokine-dependent directional controls are absent in these monocytes, and the immune response of the host may be paralyzed. On the other hand, it is possible that UL128 docks the virions to a specific chemokine receptor of monocytes, such as CCR8 (Tiffany *et al*., 1997), activating the MAPK/ERK signaling pathway to promote proliferation of monocytes, recruiting uninfected monocytes to the infected tissue, and eventually increasing viral targets and promoting viral spread. In brief, UL128 protein would be able to facilitate HCMV infection by hindering host immunity or promoting viral transmission based on its bidirectional regulation of chemokine motility.

In addition, we demonstrated that UL128 protein could selectively induce cytokine expression in PBMCs, such as IL-6 and TNF-α, which are involved in HCMV reactivation and virus spread (Zheng *et al*., 2012). The increased serum levels of TNF-α in the patients with sepsis and atopic dermatitis were related to the activation of latent HCMV (Kutza *et al*., 1998; Docke *et al*., 2003). Expression of IL-6 was involved in dendritic cells differentiation to influence HCMV reactivation (Huang *et al*., 2012; Reeves and Compton, 2011). Moreover, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) are related to HCMV-mediated angiogenesis, which contributes to virus spread and the development of HCMV-associated vascular diseases (Botto *et al*., 2011; Fiorentini *et al*., 2011). These findings suggest that monomeric UL128 protein may interfere with certain inflammatory cytokines to increase HCMV replication and dissemination.

3.2 Functions of complexed UL128 protein

HCMV replication in EnCs and EpCs appears to be important in virus persistence and spread. EnCs and EpCs are the primary targets for HCMV infection in immunocompromised patients (Revello and Gerna, 2010). Some EnCs originating from $CD34^+$ stem cells act as the reservoirs of HCMV in the host and also the sites of virus latency (Quirici *et al*., 2001; Cheung *et al*., 2006). Microvascular EnCs of

capillaries and venules in various organs, including the entire gastrointestinal tract, lungs, kidneys, liver, salivary glands and brain are susceptible to HCMV, indicating that HCMV-infected EnCs contribute heavily to dissemination of HCMV infection (Revello and Gerna, 2010; Bissinger, *et al*., 2002). EpCs are also the cell type that is mostly involved in HCMV infection *in vivo*, and are predominantly infected cell population in the gastrointestinal tract, secretory glands and kidneys (Sinzger *et al*., 2008).

The previous researchers have opened the door to the discovery of UL128 protein as an important part of gH/gL/UL128/UL130/UL131A complex. The complex has been characterized as structural component of the viral envelop (Ryckman *et al*., 2008a; Wang and Shenk, 2005; Adler *et al*., 2006; Patrone *et al*., 2005), which is indispensable for infecting both EnCs and EpCs (Hahn *et al*., 2004; Ryckman *et al*., 2006, 2008a; Wang and Shenk, 2005). The significance of gH/gL/UL128/UL130/UL131A complex in viral entry is based on several facts. Firstly, HCMV entry into EnCs depending on particular strains suggests that specific viral genes are required for efficient replication in this cell type. HCMV strains which were extensively passaged in human fibroblasts such as AD169 lose their ability to grow in EnCs and EpCs (Grazia *et al*., 2001). The HCMV strains extensively passaged in fibroblasts could gain loss-function mutations of UL128, UL131A or UL130 (Spear and Longnecker, 2003). Secondly, recent experiments demonstrated that antibodies against pUL128, pUL130 or pUL131A potently neutralize the entry into EnCs and EpCs but have no effect on fibroblasts (Wang and Shenk, 2005; Adler *et al*., 2006; Gerna *et al*., 2008; Macagno *et al*., 2010). Thirdly, introduction of targeted deletions into the UL128/UL130/UL131A locus affects the ability of HCMV growing in EnCs or transmitting to leukocytes, and gain of function is associated with reversal of mutations within UL128/UL130/UL131A or trans-complementation with individual UL131A, UL130 or UL128 genes by retrovirus-mediated transduction (Hahn *et al*., 2004).

The definite mechanism of gH/gL/UL128/UL130/UL131A complex participating in the cellular tropism of HCMV is poorly understood. Ryckman *et al.* indicated that gH/gL/UL128/UL130/UL131A complex contributed to HCMV adsorption, penetration, and entry to EnCs and EpCs by endocytosis and the process was dependent on low pH (Ryckman *et al*., 2006). Once HCMV entry was initiated, gH/gL/UL128/UL130/UL131A complex would attach to a certain receptor involved in HCMV entry or signaling transduction (Ryckman *et al*., 2008a). This receptor contributed to gB transition from a protease-resistant to protease-sensitive form so that gB could interact with gH (Patrone *et al*., 2007). Glycoprotein homologues, gB, gH, and gL were essential for cell fusion and the interaction between gB and gH/gL were sufficient for HCMV fusion execution (Vanarsdall *et al*., 2008; Gerna *et al*., 2008; Patrone *et al*., 2007). The above hypothesis is in line with the process of HCMV entry into fibroblast cells, which promotes a transient association between the gB receptor as epidermal growth factor receptor and the gB/gH receptor integrin αV3 (Wang *et al*., 2005). But up to now, the detailed roles of UL128, UL130 and UL131A in the pentamer, and which of the proteins is most important, still remains unknown and requires further research. For example, UL131A had been proved to be important in virus entry into EnCs, however, it could not eliminate the possibility that UL128 protein played a more crucial role in virus entry (Adler *et al*., 2006).

4. Conclusions

HCMV ORF UL128 gene is highly conserved among viral field isolates and has two different molecular forms, monomeric UL128 protein and complexed UL128 protein as a part of gH/gL/UL128/UL130/UL131A complex. Monomeric UL128 protein is produced and works as a soluble chemokine analogue to attract PBMCs and selectively induces expression of IL-6 and TNF-α in PBMCs. The gH/gL/UL128/UL130/UL131A complex is indispensable for HCMV entry into both EnCs and EpCs. UL128 protein plays an important role in HCMV infection. It is necessary to make deeper studies to explore the relationship between UL128 gene and HCMV infection.

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