Journal of Bacteriology and Virology 2016. Vol. 46, No. 4 p.326 – 329
http://dx.doi.org/10.4167/jbv.2016.46.4.326

Research Update (Minireview)

Functions of Herpesvirus-Encoded Homologs of the Cellular Ribonucleotide Reductase Large Subunit

Ki Mun Kwon and Jin-Hyun Ahn*

Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea

Deoxyribonucleotides (dNTPs) are important for the efficient growth of DNA viruses. Therefore, many DNA viruses have strategies for the upregulation of cellular dNTP levels. Both α- and γ-herpesviruses encode functional homologs of cellular dNTP anabolic enzymes, including the class I ribonucleotide reductase (RNR) large (R1) and small (R2) subunits, whereas β-herpesviruses modulate host cells to induce genes that increase dNTP levels. Interestingly, β-herpesviruses still express the nonfunctional RNR R1 subunit. However, it is not clear why β-herpesviruses still carry inactive R1 homologs. Recently, the R1 homologs of herpesviruses have been shown to inhibit innate immune signaling pathways. In particular, both functional and nonfunctional R1 homologs target receptor-interacting protein kinase 1 (RIP1) and inhibit RIP1-mediated signaling pathways to promote viral replication. Here, we summarize recent findings on the activity of herpesviral R1 homologs and discuss their roles in the regulation of innate immune signaling pathways.

Key Words: Herpesvirus, Ribonucleotide reductase large subunit, Immune signaling

INTRODUCTION

Human herpesviruses consist of three subfamilies: α-, β-, and γ-herpesviruses. Human α-herpesviruses include herpes simplex virus type-1 (HSV-1), HSV-2, and Varicella-Zoster virus (VZV). Human β-herpesviruses include human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6), and HHV-7. Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) belong to the human γ-herpesviruses. All herpesviruses share common characteristics, including similar virion structures and the ability to establish persistent and latent infection.

Ribonucleotide reductases (RNRs) catalyze a rate-limiting step in *de novo* deoxyribonucleotide (dNTP) synthesis and are essential for DNA replication and the repair of both eukaryotic cells and DNA viruses (1, 2). All RNRs share a common catalytic mechanism and are grouped into three classes (3). Class I reductases comprise a large R1 subunit and a small R2 subunit. The large R1 subunit contains both the catalytic site for ribonucleotide reduction and allosteric sites for substrate specificity. The small R2 subunit contains an iron center-derived tyrosyl free radical, which is required for substrate activation. The substrate activation reaction requires oxygen and an external electron donor. Class II reductases contain a single subunit and use thioredoxin or

Received: November 16, 2016/ Revised: November 17, 2016/ Accepted: November 17, 2016
*Corresponding author: Jin-Hyun Ahn. Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, 2066 Seoburo, Jangangu, Suwon, Gyeonggido 16419, Korea.
Phone: +82-31-299-6222, Fax: +82-31-299-6239, e-mail: jahn@skku.edu
**This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1A2B4011848).

A

Subfamily	Virus	RNR		Thymidine kinase	dUTPase
		R1	R2		
α	HSV-1	+	+	+	+
	HSV-2	+	+	+	+
	VZV	+	+	+	+
β	HCMV	NF	-	-	NF
	HHV-6	NF	-	-	NF
	HHV-7	NF	-	-	NF
γ	EBV	+	+	+	+
	KSHV	+	+	+	+

B

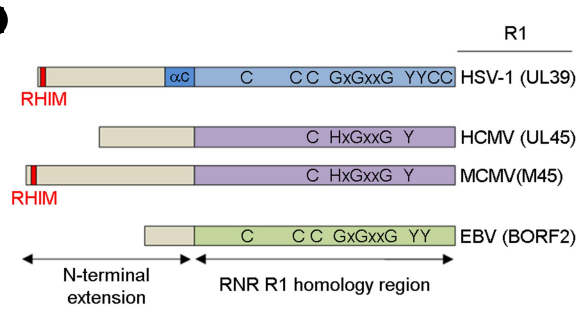

Figure 1. Distribution and organization of herpesvirus R1 proteins. (A) Unlike human α- and γ-herpesviruses, human β-herpesviruses lack genes encoding the RNR R2 subunit and thymidine kinase and encode a nonfunctional RNR R1 subunit and dUTPase. (B) The C-terminal region of β-herpesvirus R1 proteins shares homology with other viral counterparts, but lacks most of the residues known to have a direct catalytic role. The proposed nucleotide-binding site (GxGxxG) and the catalytically active sites, i.e., five cysteines and two tyrosines (2), are shown. NF: nonfunctional.

glutaredoxin as electron donors. Like class I reductases, class III enzymes consist of two dimeric proteins. However, class III reductases are anaerobic enzymes and use formate as an electron donor.

Since dNTP supplementation is important for the efficient growth of DNA viruses, herpesviruses have developed strategies for the upregulation of cellular dNTP levels. Both α- and γ-herpesviruses encode functional homologs of cellular dNTP anabolic enzymes, including thymidine kinase, deoxyuridine triphosphate pyrophosphatase (dUTPase), and class I RNRs, which are essential for virus replication (4~7) (Fig. 1A). β-herpesviruses, such as HCMV, murine cytomegalovirus (MCMV), HHV-6, and HHV-7, have developed ways to modulate host cells to induce genes required to increase dNTP levels. Therefore, they do not carry most genes encoding enzymes involved in dNTP synthesis, such as the genes for thymidine kinase and the RNR R2 subunit. Intriguingly, however, β-herpesviruses still encode nonfunctional homologs of dUTPase and the RNR R1 subunit (Fig. 1B). Why β-herpesviruses still encode these nonfunctional homologs, devoid of enzymatic activity, is a long-standing question. Here, we summarize recent findings on the function of herpesviral RNR R1 homologs in the regulating regulation of immune signaling pathways.

MCMV M45

In MCMV infection, M45, an inactive R1 homolog, is expressed at late stages of infection and is associated with virus particles. The M45-deleted mutant viruses do not replicate in endothelial cells and grow poorly on macrophages, but exhibit nearly normal growth in fibroblast cells (8). Recently, several reports have shown that M45 interacts with mouse receptor-interacting protein kinase 1 (RIP1). Both RIP1 and M45 have the RIP homotypic interaction motif (RHIM). However, the requirement for the RHIM in M45-RIP1 binding is controversial (9, 10). Several studies analyzing M45-RIP1 interactions have indicated that M45 functions as an RIP1 inhibitor. RIP1 is an attractive target for the inhibition of viral infection, since it mediates various signaling pathways. M45 inhibits the activation of NF-κB after the stimulation of tumor necrosis factor (TNF) receptor 1 (TNFR1) and Toll-like receptor 3 (TLR3) by inhibiting the K63-linked poly-ubiquitination of RIP1, a process that is required for the recruitment of the IKK complex (10). Furthermore, M45 inhibits NF-κB activation mediated by the cytosolic DNA sensor DAI and RIP1 via RHIM-dependent interactions (11). In addition, M45 inhibits RIP1-independent NF-κB activation and cytokine production after IL-1R and TLR stimulation and binds to and induces lysosomal degradation of NEMO by targeting it to autophagosomes (12). Interestingly, Krause *et al*. recently showed that the virion-associated M45 protein mediates rapid NF-κB activation immediately after viral entry (13). Meanwhile, M45 acts as a suppressor of virus-induced or TNFα-induced cell death

by targeting RIP1 (9, 10). M45 also inhibits necroptosis, a caspase-independent form of programmed necrosis, which requires the adaptor kinase RIP3 and DAI (14, 15). Taken together, M45 functions as an inhibitor of RIP1-mediated signaling pathways during virus infection.

HSV-1 ICP6 and HSV-2 ICP10

In the case of human α-herpesviruses, the HSV-1 ICP6 and HSV-2 ICP10 RNR R1 proteins target both RIP1 and RIP3 in a RHIM-dependent manner and inhibit TNFα-induced necroptosis (16). Although these R1 proteins of α-herpesviruses have catalytic activity for ribonucleotide reduction, they also act as suppressors of cellular immune signaling. Unlike MCMV M45, HSV-2 ICP10 does not affect TNFα-induced NF-κB activation, although it has the RHIM and interacts with RIP1 in a RHIM-dependent manner (17). The effect of HSV-1 ICP6 on TNFα-induced NF-κB activation is not yet understood.

HCMV UL45

In HCMV, UL45 encodes a nonfunctional cellular R1 homolog that lacks some residues required for catalytic activity. The function of UL45 remains largely unknown. The UL45-null mutant is defective in the accumulation of virus particles and growth in human fibroblasts at low multiplicities of infection (MOI) (18). Both the wild-type virus and UL45-null mutant induce the synthesis of cellular RNR subunits and upregulate dNTP levels with similar efficiency, indicating that they are indeed unrelated to dNTP production. Fas-induced apoptosis is minimally increased in UL45-null mutant virus-infected fibroblasts compared to that in wild-type virus-infected cells (18). Whether UL45 targets RIP1 and RIP3 has not been extensively investigated, since UL45 lacks an apparent RHIM.

UL45 interacts with UL48, the largest tegument protein that exhibits deubiquitinase activity (19, 20). Both UL45 and UL48 are viral tegument proteins that are present within virions and delivered into host cells upon viral entry. Virion-associated tegument proteins control viral entry, gene expression, and innate immune responses immediately after infection. Therefore, UL48 and UL45 may also regulate host cell signaling immediately after viral entry. UL45 appears to be associated with the capsid by binding to UL48. This capsid-associated activity of UL45 may also play a role in the nuclear egress of the capsid or virion maturation in the cytoplasm, but this awaits further investigation.

CLOSING REMARKS

Efficient supplementation of dNTPs is critical for DNA virus replication. Thus, many DNA viruses have strategies for increasing cellular dNTP biosynthesis. Both α- and γ-herpesviruses encode functional equivalents of cellular nucleotide anabolic enzymes to increase dNTPs levels. β-herpesviruses do not carry these genes, but instead up-regulate cellular nucleotide biosynthesis machinery genes. It has been unclear why β-herpesviruses encode nonfunctional R1 homologs. However, accumulating evidence indicates that the herpesvirus-encoded R1 homologs can regulate innate immune signaling pathways. HSV-1 ICP6, HSV-2 ICP10, and MCMV M45 target RIP1 in a manner dependent on the RHIM, and downregulate RIP1-mediated signaling. MCMV M45 also binds to NEMO and inhibits NF-κB activation and cytokine production independent of RIP1 via the lysosomal degradation of NEMO. Future studies should address whether the regulation of innate immune signaling by R1 is common to all herpesviruses. Additional analyses of R1 mutant in the different viral replication cycles are necessary to reveal their exact functions in virus infection.

REFERENCES

1) Jordan A, Reichard P. Ribonucleotide reductases. Annu Rev Biochem 1998;67:71-98.
2) Lembo D, Brune W. Tinkering with a viral ribonucleotide reductase. Trends Biochem Sci 2009;34:25-32.
3) Nordlund P, Reichard P. Ribonucleotide reductases. Annu Rev Biochem 2006;75:681-706.
4) Jacobson JG, Leib DA, Goldstein DJ, Bogard CL, Schaffer PA, Weller SK, *et al*. A herpes simplex virus ribonucleotide reductase deletion mutant is defective for productive acute and reactivatable latent infections of mice and for replication in mouse cells. Virology

1989;173:276-83.
5) Idowu AD, Fraser-Smith EB, Poffenberger KL, Herman RC. Deletion of the herpes simplex virus type 1 ribonucleotide reductase gene alters virulence and latency *in vivo*. Antiviral Res 1992;17:145-56.
6) Heineman TC, Cohen JI. Deletion of the varicella-zoster virus large subunit of ribonucleotide reductase impairs growth of virus *in vitro*. J Virol 1994;68:3317-23.
7) Aurelian L. Herpes simplex virus type 2: unique biological properties include neoplastic potential mediated by the PK domain of the large subunit of ribonucleotide reductase. Front Biosci 1998;3:d237-49.
8) Brune W, Menard C, Heesemann J, Koszinowski UH. A ribonucleotide reductase homolog of cytomegalovirus and endothelial cell tropism. Science 2001;291:303-5.
9) Upton JW, Kaiser WJ, Mocarski ES. Cytomegalovirus M45 cell death suppression requires receptor-interacting protein (RIP) homotypic interaction motif (RHIM)-dependent interaction with RIP1. J Biol Chem 2008; 283:16966-70.
10) Mack C, Sickmann A, Lembo D, Brune W. Inhibition of proinflammatory and innate immune signaling pathways by a cytomegalovirus RIP1-interacting protein. Proc Natl Acad Sci U S A 2008;105:3094-9.
11) Rebsamen M, Heinz LX, Meylan E, Michallet MC, Schroder K, Hofmann K, *et al*. DAI/ZBP1 recruits RIP1 and RIP3 through RIP homotypic interaction motifs to activate NF-kappaB. EMBO Rep 2009;10:916-22.
12) Fliss PM, Jowers TP, Brinkmann MM, Holstermann B, Mack C, Dickinson P, *et al*. Viral mediated redirection of NEMO/IKKgamma to autophagosomes curtails the inflammatory cascade. PLoS Pathog 2012;8:e1002517.
13) Krause E, de Graaf M, Fliss PM, Dolken L, Brune W. Murine cytomegalovirus virion-associated protein M45 mediates rapid NF-kappaB activation after infection. J Virol 2014;88:9963-75.
14) Upton JW, Kaiser WJ, Mocarski ES. Virus inhibition of RIP3-dependent necrosis. Cell Host Microbe 2010;7: 302-13.
15) Upton JW, Kaiser WJ, Mocarski ES. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. Cell Host Microbe 2012;11:290-7.
16) Guo H, Omoto S, Harris PA, Finger JN, Bertin J, Gough PJ, *et al*. Herpes simplex virus suppresses necroptosis in human cells. Cell Host Microbe 2015;17:243-51.
17) Dufour F, Sasseville AM, Chabaud S, Massie B, Siegel RM, Langelier Y. The ribonucleotide reductase R1 subunits of herpes simplex virus types 1 and 2 protect cells against TNFalpha- and FasL-induced apoptosis by interacting with caspase-8. Apoptosis 2011;16:256-71.
18) Patrone M, Percivalle E, Secchi M, Fiorina L, Pedrali-Noy G, Zoppe M, *et al*. The human cytomegalovirus UL45 gene product is a late, virion-associated protein and influences virus growth at low multiplicities of infection. J Gen Virol 2003;84:3359-70.
19) Phillips SL, Bresnahan WA. Identification of binary interactions between human cytomegalovirus virion proteins. J Virol 2011;85:440-7.
20) Kim YE, Oh SE, Kwon KM, Lee CH, Ahn JH. Involvement of the N-Terminal Deubiquitinating Protease Domain of Human Cytomegalovirus UL48 Tegument Protein in Autoubiquitination, Virion Stability, and Virus Entry. J Virol 2016;90:3229-42.