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HCMV modulation of cellular PI3K/AKT/mTOR signaling: New opportunities for therapeutic intervention?

Aaron M. Altman[a], Jamil Mahmud[a], Zaneta Nikolovska-Coleska[b], Gary Chan[a,*]

[a] Department of Microbiology & Immunology, SUNY Upstate Medical University, Syracuse, NY 13210, USA
[b] Department of Pathology, University of Michigan, Ann Arbor, MI 48109, USA

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ABSTRACT

Human cytomegalovirus (HCMV) remains a major public health burden domestically and abroad. Current approved therapies, including ganciclovir, are only moderately efficacious, with many transplant patients suffering from a variety of side effects. A major impediment to the efficacy of current anti-HCMV drugs is their antiviral effects are restricted to the lytic stage of viral replication. Consequently, the non-lytic stages of the viral lifecycle remain major sources of HCMV infection associated with transplant recipients and ultimately the cause of morbidity and mortality. While work continues on new antivirals that block lytic replication, the dormant stages of HCMV's unique lifecycle need to be concurrently assessed for new therapeutic interventions. In this review, we will examine the role that the PI3K/Akt/mTOR signaling axis plays during the different stages of HCMV's lifecycle, and describe the advantages of targeting this cellular pathway as an antiviral strategy. In particular, we focus on the potential of exploiting the unique modifications HCMV imparts on the PI3K/Akt/mTOR pathway during quiescent infection of monocytes, which serve an essential role in the dissemination strategy of the virus.

1. Introduction

Human cytomegalovirus (HCMV) is a member of the betaherpesvirus family and a major worldwide public health burden. While primary infections in immunocompetent individuals are generally self-limiting, infections are associated with severe morbidity and mortality in immunocompromised individuals such as transplant and chemotherapy patients (Bissinger et al., 2002; Lawlor and Moss, 2010; Nerheim et al., 2004; Reinke et al., 1999; Staras et al., 2006). HCMV is also the most common infectious cause of newborn malformations in developed countries (Bristow et al., 2011; Dollard et al., 2007; Kenneson and Cannon, 2007; Manicklal et al., 2013). Despite the huge public health burden of HCMV, limited options for prophylactic or fulminant therapy exist today (Ahmed, 2011). Historically, replication inhibitors targeting viral proteins has been the first choice in developing new antiviral therapeutics as viral proteins mediate distinct evolutionary conserved functions during the virus lifecycle. Such therapies approved for HCMV are ganciclovir, valganciclovir, cidofovir, and foscarnet, which are synthetic nucleotide analogues that inhibit the viral polymerase UL97 to halt replication (Griffiths and Boeckh, 2007; Prichard and Kern, 2011). However, there are several drawbacks to this class of anti-HCMV drugs, including: 1) The side effects from these inhibitors can be severe, particularly for transplant patients on long-term regimens; 2) The emergence of antiviral resistant strains; 3) The low efficacies of these drugs with up to 30% of transplant patients on ganciclovir therapy eventually developing disease and a significant proportion experiencing transplant rejection (Ahmed, 2011; Echenique et al., 2017; Griffiths and Boeckh, 2007). Overall, the poor efficacy of

Abbreviations: HCMV, human cytomegalovirus; IE, immediate early; E, early; L, late; MCMV, murine cytomegalovirus; gB, glycoprotein B; gH, glycoprotein H; gL, glycoprotein L; gO, glycoprotein O; EGFR, epidermal growth factor receptor; PDGFR-α, platelet-derived growth factor receptor α; NRP1, neuropilin 1; BST2, bone marrow stromal antigen 2 (tetherin); PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; PI(4,5)-P_2, phosphatidylinositol (4,5)-bisphosphate; PI(3,4,5)-P_3, phosphatidylinositol (3,4,5)-triphosphate; PH, pleckstrin homology; PDK1, phosphoinositide-dependent kinase 1; mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 2; PTEN, phosphatase and tensin analog; SHIP1, SH-2 containing inositol 5′ phosphatase 1; UV, ultraviolet; EGF, epidermal growth factor; αvβ3, integrin αvβ3; M-CSF, macrophage colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; AMPK, adenosine monophosphate kinase; TSC, tuberous sclerosis complex; eIF4F, eukaryotic initiation factor 4F; S6K, S6 kinase; eIF4E, eukaryotic initiation factor 4E; 4E-BP1, eIF4E-binding protein 1; mTOR, mammalian target of rapamycin; KAP1, KRAB-associated protein-1; HSF1, heat shock factor 1; IRES, internal ribosomal entry site; Mcl-1, myeloid cell leukemia-1; HSP27, heat shock protein 27; XIAP, X-linked inhibitor of apoptosis protein

* Corresponding author. Department of Microbiology and Immunology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210, USA.
E-mail address: chang@upstate.edu (G. Chan).

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replication inhibitors is in large part due to the inability of these drugs to exert any effect on HCMV during the dormant (non-lytic replication) periods of the virus life cycle. Thus, current antivirals are inadequate to address the needs of patients, and new therapeutic strategies must consider the other stages of HCMV's unique life cycle.

2. HCMV life cycle

HCMV infection can be divided into three stages: lytic, latent, and quiescent (Chan et al., 2010; Jean Beltran and Cristea, 2014; Sinzger and Jahn, 1996; Smith et al., 2004a; Yurochko and Huang, 1999). During a lytic infection HCMV expresses three temporal classes of viral genes: immediate early (IE), early (E), and late (L) genes (Crough and Khanna, 2009). Lytic gene expression allows for replication of the viral genome, packaging of nascent virions, and release from the host cell (Hamirally et al., 2009; Jean Beltran and Cristea, 2014; Milbradt et al., 2007, 2014). In contrast, during a latent infection HCMV transiently expresses a subset of viral latent genes in the absence of a fully productive viral infection (Cheng et al., 2017; Gatherer et al., 2011; Shnayder et al., 2018). Undifferentiated hematopoietic progenitor cells in the bone marrow are generally thought to be the major site of latent HCMV infection (Goodrum et al., 2012; Hahn et al., 1998; Mendelson et al., 1996; Movassagh et al., 1996; Taylor-Wiedeman et al., 1993; Taylor-Wiedeman et al., 1991; von Laer et al., 1995). An external stimulus is required to differentiate these cells and reactivate lytic replication (Ibanez et al., 1991; Soderberg-Naucler et al., 1997; Söderberg-Nauclér et al., 2001; Taylor-Wiedeman et al., 1994). A “quiescent infection” is a state recently defined by our and the Yurochko group to occur within monocytes (Chan et al., 2012b; Smith et al., 2004b; Stevenson et al., 2014). While our understanding of a quiescent infection is largely incomplete, there is lack of lytic gene expression similar to latent infection (Ibanez et al., 1991; Sinclair and Sissons, 1996; Smith et al., 2004a). However, a quiescent infection appears to differ from a latent infection by being able to “reactivate” and begin lytic replication without the need of an external activation signal (Chan et al., 2008; Smith et al., 2004a). In quiescently infected monocytes, spontaneous expression of lytic viral gene products and productive replication occurs 2–3 weeks after the initial infection (Chan et al., 2008; Smith et al., 2004a). However, while latency in $CD34^+$ or other hematopoietic cell types and quiescence in monocytes are referred to as being separate and distinct in this review, the line differentiating these two types of HCMV infections is likely blurred with overlapping biological traits. Indeed, monocytes harboring episomal viral genome can be induced to reactivate with growth factor treatment prior to 2 weeks post infection (Sinclair and Sissons, 1996, 2006; Soderberg-Naucler et al., 1997, 2001; Streblow and Nelson, 2003; Taylor-Wiedeman et al., 1991, 1994). Consequently, HCMV-infected monocytes have been widely used as a model of latency. Nonetheless, HCMV utilizes quiescent infections in monocytes as a key cog in its dissemination strategy during a primary infection in order to bridge the initial transient lytic infection with the establishment of a persistent latent infection.

3. HCMV dissemination

HCMV was first detected in peripheral blood monocytes, which are the primary cell type in circulation harboring the viral genome, suggesting these blood sentinels may be important for the early dissemination of the virus despite not being permissive for lytic replication (Schrier et al., 1985; Taylor-Wiedeman et al., 1991; von Laer et al., 1995). In support, leukocyte depletion eliminates HCMV transmission through blood donations, monocytes are carriers of the virus following organ transplantation, and monocyte-derived macrophages are the first cells to express viral antigen within infected organs (Adler et al, 1983; Gnann et al., 1988; Larsson et al., 1998; Lipson et al., 2001; Mazeron, 2000; Sinzger et al., 1996). Ex vivo studies from naturally HCMV infected monocytes showed HCMV reactivation from monocytes that have been matured into macrophages using differentiation factors, supporting a role for monocytes in the HCMV dissemination strategy (Poole et al., 2015; Soderberg-Naucler et al., 1997; Taylor-Wiedeman et al., 1994). The importance of myelomononuclear cells during virus dissemination was further corroborated by *in vivo* murine CMV (MCMV) studies showing monocytes were the predominant cell type responsible for spread during an acute infection (Bale and O'Neil, 1989; Collins et al., 1993; Collins et al., 1994; Daley-Bauer et al., 2014; Stoddart et al., 1994). A recently developed humanized mouse model of HCMV infection that supported latent viral infection and dissemination also found the source of HCMV in the peripheral organs was from human macrophages derived from peripheral blood monocytes (Smith et al., 2010). In addition to facilitating spread to peripheral organs, HCMV-infected monocytes can travel into the bone marrow and transmit the virus to $CD34^+$ bone marrow stem cells, the major site of HCMV latency. In these ways, monocytes represent a key link between acute and persistent infections as an initial lytic infection cannot propagate to distant organs, nor establish a latent infection within the bone marrow without monocyte-mediated dissemination. While effective therapies targeting all three stages of the viral lifecycle would be ideal, quiescently infected monocytes present new opportunities for specific interventions at a crucial stage linking both lytic and/or latent infections.

As current therapies are virally focused, resistance has rapidly developed through selective pressure. Rather than targeting viral replication and proteins, cellular pathways represent highly conserved and regulated processes. Signaling pathways are differentially regulated during HCMV infection in a multitude of cell types across all three life stages. Viral infection tends to usurp the activities of select cellular kinases important to the viral life cycle (Brinkmann and Schulz, 2006; Cheeran et al., 2005; Dawson et al., 2003; Herbein et al., 2010). Targeting these pathways may be key to not only suppressing virus replication, but also eliminating latent and/or quiescent viral reservoirs, which are highly dependent on cellular kinases to effect cellular change due to the limited expression of viral gene products. In particular, a number of prosurvival pathways have been recently identified to be specifically upregulated in HCMV-infected monocytes but not uninfected cells (Chan et al., 2010; Cojohari et al., 2016; Collins-McMillen et al., 2015; Peppenelli et al., 2016; Peppenelli et al., 2018; Stevenson et al., 2014). These pathways represent a unique opportunity for the design of new antivirals that target the virally infected cell rather than the virus itself. This review summarizes the current research on these signaling pathways during HCMV infection with a particular focus on the quiescent stage of the viral life cycle.

4. Cellular receptors and HCMV

The first step in modifying cellular signaling pathways comes during viral entry. HCMV enters cells through the interaction of a number of its surface viral glycoproteins with a panoply of cell surface receptors. In monocytes, these key glycoproteins include gB, gH, gL, gO, and UL128-131 (Chan et al., 2009; Isaacson and Compton, 2009; Nogalski et al., 2011, 2013; Smith et al., 2004b; Yurochko and Huang, 1999; Yurochko et al., 1997). Glycoprotein gB forms a trimer linked by disulfide bonds (Sharma et al., 2013). Glycoproteins gH and gL form a complex through disulfide bond interactions at the viral envelope, which then forms two separate complexes, the trimeric gH/gL/gO or the pentameric gH/gL/UL128-131, either through disulfide or covalent interactions (Huber and Compton, 1998, 1999; Yurochko et al., 1997). The trimeric complex is required for viral entry into fibroblasts, while the pentameric complex is essential for entry into endothelial, epithelial, monocytic, and dendritic cells (Adler et al., 2006; Liu et al., 2018; Straschewski et al., 2011; Wang and Shenk, 2005; Wille et al., 2013).

As HCMV has tropism for a wide range of cell types, there is a significant body of research into discovering entry receptors. Huang et al. identified epidermal growth factor receptor (EGFR) bound with

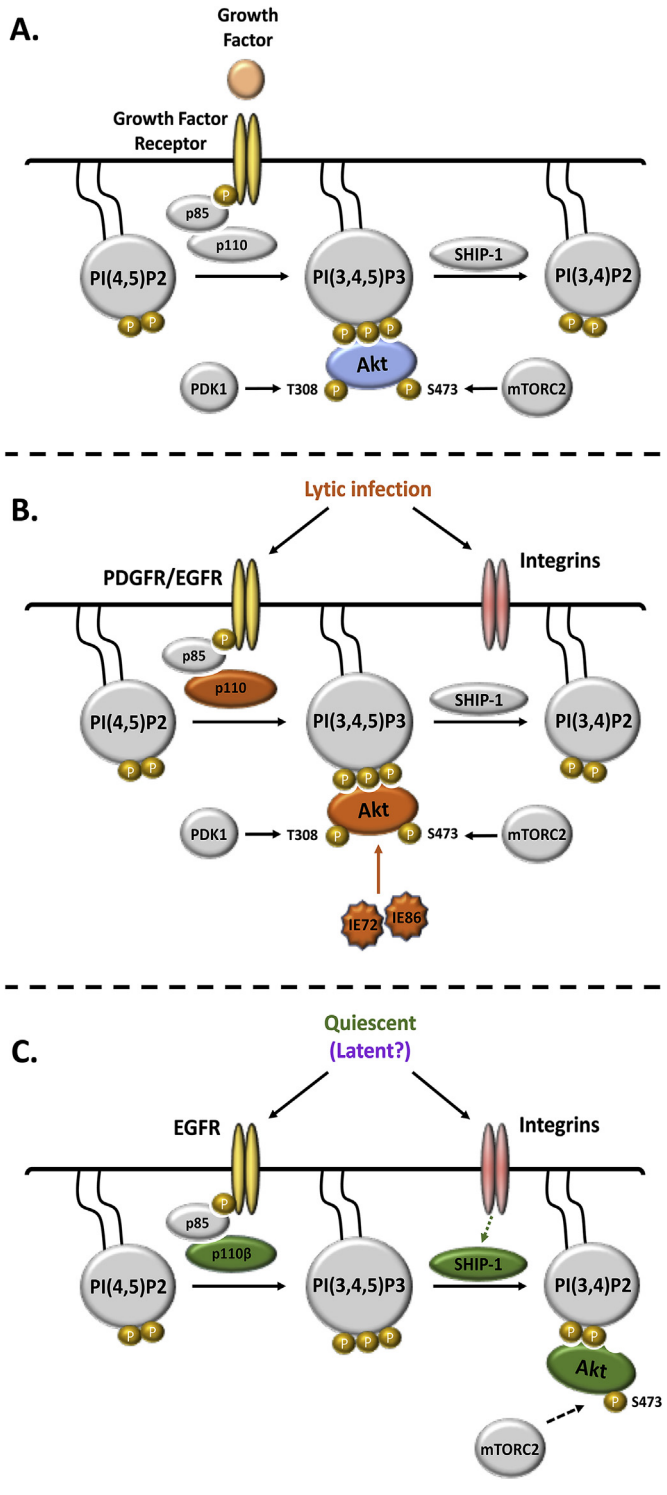
gB in order to mediate viral binding and entry in a number of cells (Wang et al., 2003). However, conflicting reports indicated that EGFR was not the receptor for gB and has no role in mediating viral entry (Isaacson et al., 2007). The relationship between gB and EGFR during viral entry remains unsettled across a variety of cell types, but in monocytes, gB was required for EGFR activation and subsequent entry into monocytes (Chan et al., 2009). Recently, the interaction of gB with PDGFR-α was described (Cobbs et al., 2014; Kabanova et al., 2016; Soroceanu et al., 2008; Wu et al., 2017); however, like with EGFR, there are conflicting reports about the importance of PDGFR-α with regard to both its ability to bind gB as well as function as a bona fide entry receptor (Vanarsdall et al., 2012). Moreover, monocytes do not express PDGFR-α as a surface receptor (Chan et al., 2009; Inaba et al., 1993; Krettek et al., 2001). Integrins were also found to be important in facilitating viral entry, with different integrins mediating entry in different cell types. In monocytes, β1 and β3 integrins bind glycoprotein complexes gH/gL/gO and gH/gL/UL128-131 in order to mediate viral entry into these cells (Feire et al., 2004; Nogalski et al., 2013; Wang et al., 2005). Additional HCMV receptors, including Nrp1, CD147, CD90, and BST2 have emerged as potentially having key roles in mediating entry as well (Li et al., 2015, 2016; Martinez-Martin et al., 2018; Vanarsdall et al., 2018; Viswanathan et al., 2011). Given the complexity of HCMV entry, targeting this step of infection as an antiviral strategy will be challenging as finding a “magic bullet” to prevent entry and signaling in all the different cell types infected by HCMV is doubtful. Moreover, cellular receptors such as EGFR are also involved in stages of the viral life cycle other than viral entry. Early inhibition of EGFR prevents viral entry and viral gene expression, which is likely due to decreased viral entry, while inhibition of EGFR post entry promotes viral replication, suggesting that late EGFR activity lowers virus yields in fibroblasts (Buehler et al., 2016). These time dependent opposing effects on HCMV infection decrease the likelihood of targeting HCMV entry receptors as a viable antiviral strategy.

5. Phosphatidyl inositol 3 kinase/protein kinase B (PI3K/Akt) pathway

Despite the multitude of receptors engaged by HCMV, a constant feature among several of the receptors is the activation of the same downstream targets critical to viral infection. In particular, EGFR, PDGFR-α, and integrins independently activate the PI3K/Akt signaling axis highlighting the possibility of intervention at this central signaling hub within the HCMV-induced signalsome (Alessi et al., 1996; Lemmon and Schlessinger, 2010; Liu et al., 2009; Soroceanu et al., 2008). Canonical Akt signaling involves an initial recruitment and activation of class 1 PI3Ks (compromised of a p85 regulatory and a p110 catalytic subunit) by receptor tyrosine kinases, such as EGFR, to mediate the phosphorylation of PI(4,5)-P2 into PI(3,4,5)-P3 (Manning and Cantley, 2007; Martini et al., 2014) (Fig. 1A). Isoforms of the p110 catalytic subunit include p110α, p110β, and p110δ, the latter of which is the predominant isoform found in monocytes (Papakonstanti et al., 2008). Production of PI(3,4,5)-P3 allows for the recruitment of Akt to the membrane through the binding of pleckstrin homology (PH) domain to PI(3,4,5)-P3. Akt is then phosphorylated by the combined actions of phosphoinositide-dependent kinase-1 (PDK1) at T308 and mammalian target of rapamycin complex 2 (mTORC2) at S473 (Scheid et al., 2002a, 2002b). The production of PI(3,4,5)-P3, and thus Akt activation, is negatively regulated by the actions of phosphatase and tensin homolog (PTEN), which converts PI(3,4,5)-P3 back to PI(4,5)-P2, and SH-2 containing inositol 5' polyphosphate (SHIP1), which dephosphorylates PI(3,4,5)-P3 into PI(3,4)-P2 (Manning and Cantley, 2007; Martini et al., 2014).

5.1. PI3K/Akt during HCMV infection

The PI3K/Akt pathway is temporally and differentially regulated by



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HCMV during all three stages of infection. During lytic infection of fibroblasts, a biphasic activation of PI3K/Akt occurs with an early transient activation triggered by receptor signaling and a later sustained activation mediated by viral immediate early proteins IE72 and IE86 (Cobbs et al., 2008; Yu and Alwine, 2002) (Fig. 1B). The early activation of PI3K is required for efficient viral entry while the later sustained activation is needed for optimal viral gene expression and viral DNA replication (Cobbs et al., 2008; Johnson et al., 2001; McFarlane et al., 2011). However, the late activation of EGFR and PI3K represses lytic replication in fibroblasts, hinting at the possibility that EGFR and PI3K

Fig. 1. Model of differential activation of PI3K/Akt signaling by growth factors and HCMV infection. (A) Growth factors binding to their cognate receptors activate canonical PI3K signaling, leading to activation of Akt through phosphorylation at T308 and S473 by PDK1 and mTORC2, respectively. (B) During lytic infection, a biphasic activation of PI3K/Akt occurs with an early transient burst of activation mediated by PDGFR, EGFR, and/or integrin engagement followed by a sustained secondary activation mediated by viral proteins IE72 and IE86. Both T308 and S473 appear to be phosphorylated during lytic infection. (C) The atypical activation of PI3K/Akt during quiescent infection of monocytes is initiated by HCMV binding to EGFR and integrins. The coordinated action of PI3K's catalytic isoform p110β, as opposed to the predominant p110δ isoform found in monocytes, and SHIP-1 leads to preferential phosphorylation of Akt at S473 by a yet to be identified mechanism. While not much is known about the PI3K activity during latency, EGFR is believed to be activated during HCMV entry in $CD34^+$ cells and PI3K has been observed to be rapidly upregulated during early latent infection.

promote entry into and/or maintenance of latency (Buehler et al., 2016). In support, pUL138 promotes latency by sustaining EGFR activity in $CD34^+$ stem cells (Buehler et al., 2016). Regardless, the early activation of PI3K during lytic replication underscores the importance of the PI3K/Akt signaling pathway to a productive HCMV infection. PI3K is also rapidly activated during the initial establishment of latency within $CD34^+$ stem cells in order to promote viral entry and alter cellular gene expression that favors latency (Kim et al., 2017). Whether elevated PI3K activity is maintained throughout latency is unknown; however, the inhibition of PI3K enhances reactivation from latently infected $CD34^+$ cells, suggesting a basal level of PI3K is at least required for the maintenance of latency (Buehler et al., 2016). As with lytic and latent infection, recent reports have also demonstrated the importance of PI3K/Akt signaling during quiescent infection of monocytes. HCMV induces PI3K/Akt within 15 min post infection in monocytes, which remains elevated through 48 h (Chan et al., 2010; Cojohari et al., 2016; Smith et al., 2004b, 2007). Maintenance of PI3K/Akt signaling is critical to the long-term survival of HCMV-infected monocytes. Pharmacological targeting of the Akt pathway prevents quiescently infected monocytes from surviving through a 48-h viability checkpoint when monocytes must differentiate towards macrophages or undergo cell death. Moreover, because of their increased dependency on Akt, infected monocytes had increased sensitivity to the effects of Akt inhibition when compared to uninfected monocytes. These data indicate the prosurvival function of PI3K/Akt signaling is essential to the progression of a quiescent infection by bridging the gap between initial infection and the expression of viral antiapoptotic proteins at 2–3 weeks post infection (Cline et al., 1978; Smith et al., 2004a; Whitelaw, 1966, 1972). Thus, deciphering the mechanism by which HCMV sustains the PI3K/Akt signaling during quiescent infection may provide critical insight to new therapeutic targets aimed at selectively eliminating infected monocytes.

HCMV triggers PI3K/Akt signaling in an EGFR-dependent manner during entry into monocytes (Chan et al., 2008, 2009, 2012a; Cojohari et al., 2016; Smith et al., 2004b, 2007). Yet, HCMV's persistent induction is in contrast to EGF's transient activation of PI3K, suggesting a virus-specific regulatory mechanism controlling PI3K/Akt signaling (Chan et al., 2010; Cojohari et al., 2016; Smith et al., 2004b). UV-inactivated virus stimulates a chronic PI3K/Akt activation similar to “live” virus, indicating that sustained PI3K activity is not mediated by de novo IE gene products as it is with lytic infection (Smith et al., 2004a; Yurochko and Huang, 1999). HCMV utilizes a multitude of glycoprotein complexes and putative cellular receptors during viral entry. Thus, while stimulation of EGFR alone with gB may lead to the canonical activation of PI3K, co-signaling from other glycoprotein and receptor interactions may be responsible for the persistent nature of PI3K/Akt signaling within infected monocytes. Indeed, binding of the virus particle to fibroblasts brings EGFR and integrins into close proximity leading to receptor clustering within lipid rafts and crosstalk between the two signaling cascades (Chan et al., 2012a; Kim et al., 2017; Wang et al., 2005). Signaling by gB/EGFR and gH/integrins needs to occur within 5 min of each other in order to facilitate virus entry (Wang et al., 2005). In monocytes crosstalk between the gB/EGFR and gH/αvβ3 axes is required for full Akt signal strength (Chan et al., 2012a). Together, these data indicate that the unique spatial and temporal kinetics of glycoprotein-initiated signaling lead to the formation of a HCMV-specific PI3K/Akt signalsome.

Monocytes express all class 1A PI3Ks including the p110α, p110β, and p110δ isoforms (Martini et al., 2014). Although highly homologous, PI3K isoforms have divergent, non-redundant biological functions, as well as differential effects on Akt activity (Thorpe et al., 2015). PI3K p110δ is the major isoform found in uninfected monocytes and is induced following M-CSF treatment to promote long-term survival (Cojohari et al., 2016; Voss et al., 2005). However, HCMV entry induces a switch from p110δ to p110β as the central PI3K isoform regulating the survival of infected monocytes and Akt activity (Cojohari et al., 2016) (Fig. 1C). Biologically, we speculate that the preferential usage of p110β is due to its lack of negative self-regulatory activity and decreased antiviral activity (Guo et al., 2008; Vanhaesebroeck et al., 1999). Simultaneous to the activation of PI3K, HCMV entry into monocytes modifies the activities of two major Akt negative regulators, PTEN and SHIP1 (Cojohari et al., 2016). PTEN directly reverses PI3K activity but is rapidly shutdown during HCMV infection allowing for maximum Akt activity (Cojohari et al., 2016). Alternatively, SHIP1 antagonizes Akt activation under homeostatic conditions via the conversion of PI(3,4,5)-P_3 into PI(3,4)-P_2 (Kerr, 2011). Yet, HCMV rapidly upregulates SHIP1 expression in contrast to normal myeloid growth factors. Inhibition of SHIP1 also reduces, rather than enhances, Akt activity (Cojohari et al., 2016). Accordingly, loss of SHIP1 activity prevents HCMV-infected monocytes from acquiring a prosurvival state (Cojohari et al., 2016). Interestingly, leukemia cells also overexpress SHIP1 and PI(3,4)-P2 to promote Akt-dependent cell survival (Kerr, 2011). With PI3K isoform specific inhibitors and SHIP1 inhibitors available, these data hint at the possibility of selectively eliminating infected monocytes by targeting the highly virus-specific changes made to the PI3K signaling pathway. Accordingly, inhibition of p110β and SHIP1 stimulates the death of infected monocytes, while having minimal effects on the viability of uninfected cells (Cojohari et al., 2016).

The functional output of PI3K/Akt signaling is largely governed by two main regulatory sites on Akt, T308 and S473, both of which are believed to be required for full Akt activity (Alessi et al., 1996). However, the diverse cellular effects of Akt appear to be dependent on the specific combination of targets activated and/or deactivated by Akt-mediated phosphorylation. Recent studies showed the ratio of S473 to T308 phosphorylation to modulate Akt target specificity (Yung et al., 2011). HCMV infection of monocytes induces a site-specific phosphorylation of Akt at S473 (Cojohari et al., 2016). In contrast, GM-CSF and M-CSF treatments stimulate both S473 and T308 phosphorylation (Baran et al., 2003; Goyal et al., 2002), indicating that the growth factor and HCMV-initiated PI signaling have distinct functional outputs. Indeed, global analysis revealed differential phosphorylation of downstream targets between HCMV- and growth factor-activated Akt (Peppenelli et al., 2018). One major downstream target differentially regulated was mTOR (Peppenelli et al., 2018), which is responsible for controlling protein translation. HCMV rapidly phosphorylated mTOR in an Akt-dependent manner following HCMV infection, while GM-CSF and M-CSF treatment had no effect on mTOR activity despite also activating Akt, suggesting that downstream targets may provide increased selectivity in terms of antivirals (Peppenelli et al., 2016).

6. mTOR pathway

MTor is found in two complexes, mTORC1 and mTORC2 (Helliwell et al., 1994; Loewith et al., 2002). MTORC2 plays a central role in

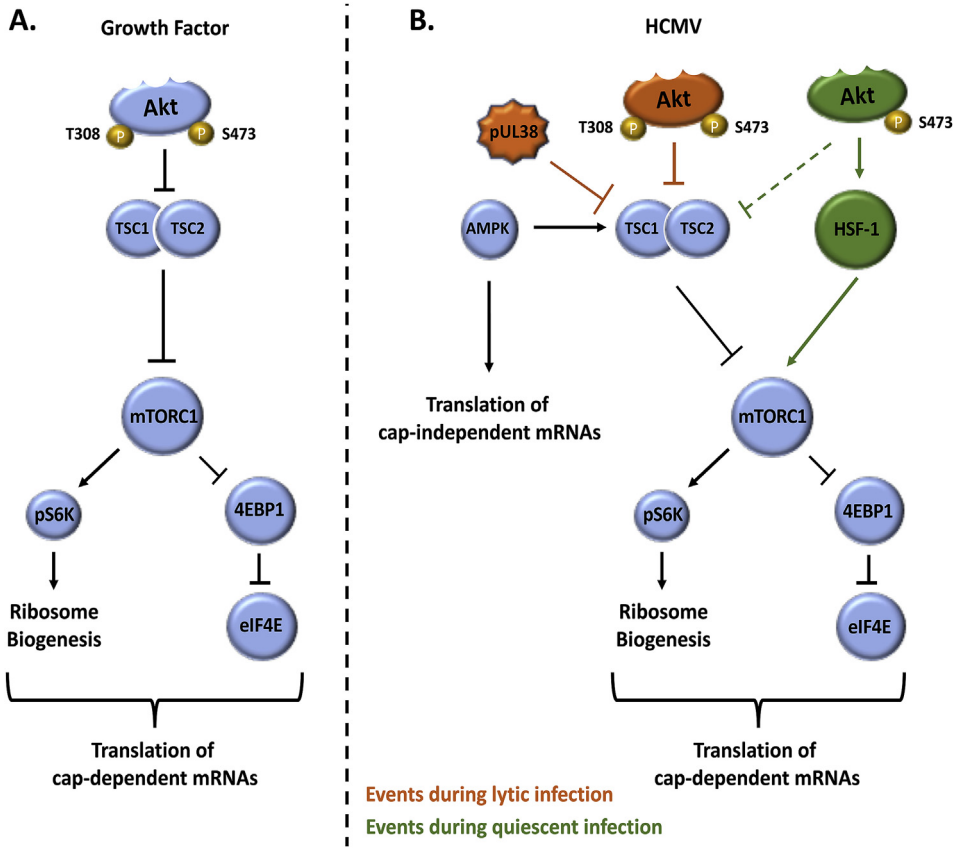

Fig. 2. Model of regulation of cellular translation by growth factors and HCMV infection. (A) Growth factor activation of Akt leads to the inhibitory phosphorylation of TSC2, thereby relieving the suppressive effect of the TSC complex on mTORC1. Activation of mTORC1, in turn, stimulates translation of cap-dependent mRNAs through S6K and eIF4E. (B) MTORC1 is activated through distinct mechanisms during lytic and quiescent infections. Activated during times of cellular stress, such as HCMV infection, AMPK stabilizes the TSC complex through activating phosphorylation of TSC2, thereby inhibiting mTORC1 activation, even in the presence of activated Akt. Simultaneously, AMPK activation has also been associated with an upregulation of IRES-dependent, cap-independent translation. However, during lytic infection, TSC complex inhibition is achieved despite AMPK activation through a yet uncharacterized interaction with pUL38 in order to sustain cap-dependent translation. In contrast, the atypical activation of Akt that occurs during quiescent infection maintains mTORC1 activity and cap-dependent translation by circumventing the TSC complex via HSF1. Overall, HCMV infection leads to the simultaneous translation of cap- and IRES-dependent proteins during both quiescent and lytic infections.

phosphorylating and activating Akt, as detailed previously. MTORC1's major function is the control of protein synthesis through the regulation of protein translation (Kim et al., 2002; Thoreen, 2013) (Fig. 2A). MTORC1 is repressed by AMP-regulated protein kinase (AMPK) and the tuberous sclerosis complex (TSC) in times of nutrient stress (Gao and Pan, 2001; Inoki et al., 2003a). When resources are plentiful AMPK is not activated, while Akt phosphorylates and inhibits the TSC, leading to activation of mTORC1 and increased cap-dependent translation (Hardie et al., 2016; Inoki et al., 2003b; Manning et al., 2002). In times of stress, such as during viral infection, AMPK becomes activated and increases cap-independent translation through initiation of IRES-dependent translation (Mizrachy-Schwartz et al., 2011). MTORC1 regulates translation initiation through phosphorylation of the eIF4F complex and S6 kinase (S6K) (Faller et al., 2015; Shahbazian et al., 2006; Wang et al., 2001). EIF4E is normally bound in the hypophosphorylated state to eIF4E-binding protein 1 (4E-BP1), which prevents the formation of a functional translation initiation complex (Pause et al., 1994). MTORC1 phosphorylates 4E-BP1, reducing its affinity for eIF4E and allowing for eIF4F complex formation and increasing translation through direction of the ribosome to the 5′ cap on mRNA (Fadden et al., 1997). Overall levels eIF4F directly relate to levels of protein synthesis within a cell (Pestova et al., 2001; Vincent et al., 2016). Phosphorylation of S6K leads to activation of several other translation factors, which increase translational scanning (ze et al., 2011), repress translational inhibitors (Faller et al., 2015; Wang et al., 2001), and stimulate the addition of amino acids to nascent peptide chains (Redpath et al., 1996; Wang et al., 2000). Overall, mTORC1 serves as a master regulator of translation in the presence of growth factors and/or plentiful resources.

6.1. mTOR during HCMV infection

Multiple studies have established the importance of mTOR during lytic infection. Typically, during times of cellular stress, such as during a viral infection, mTOR activity is decreased. Although AMPK serves as an inhibitor of mTOR activation and is upregulated during HCMV infection, this relationship appears to be uncoupled in infected cells (Kudchodkar et al., 2007; McArdle et al., 2012; Terry et al., 2012). HCMV pUL38 disrupts the negative regulatory effects of AMPK by binding to TSC1/2, a mechanism distinct from Akt-mediated inhibition through phosphorylation (Moorman et al., 2008) (Fig. 2B). Consequently, increased phosphorylation and levels of eIF4E are maintained during lytic infection allowing for continued cap-dependent protein translation (Clippinger et al., 2011a, b; Vincent et al., 2016). Blocking eIF4F complex formation reduces viral replication and progeny production, underpinning the critical need to maintain mTOR activity during a productive HCMV infection (Kudchodkar et al., 2004; Lenarcic et al., 2014; Moorman and Shenk, 2010). There is little known about the role of mTOR during latency. MTOR has been found to phosphorylate KAP1, a transcriptional co-repressor that can force HCMV out of latency when phosphorylated, suggesting that suppression of mTOR may be required for the maintenance of latency (Rauwel et al., 2015). Other results suggest that mTOR does not play a role in reactivation from latency (Glover et al., 2014). During a quiescent infection of monocytes, HCMV stimulates mTOR activity in a PI3K/Akt dependent manner (Peppenelli et al., 2018). Despite increasing mTOR activity, lytic replication is not initiated indicating either a threshold level is needed to drive replication or additional factors are required in combination with mTOR to drive replication. Nonetheless, similarly to lytic infection, cellular stress appears to be uncoupled to decreasing mTOR activity; however, in the absence of pUL38 during a quiescent infection, the mechanism employed by HCMV to uncouple mTOR and stress is unclear.

Activated during times of cellular stress, heat shock factor 1 (HSF1) transcription factor responsible for the expression of stress-associated proteins, which are generally independent of cap-mediated translation (Calderwood et al., 2010; Wu, 1995). HCMV rapidly phosphorylates

HSF1 in an Akt-dependent fashion while myeloid growth factors have little effect on HSF1 activity (Peppenelli et al., 2018). A positive feedback loop from HSF1 to mTOR was found to exist in HCMV-infected monocytes where inhibition of HSF1 activity decreased mTOR activity (Fig. 2B). Thus, HCMV-activated Akt specificity towards HSF1 provides a mechanism by which HCMV is able to activate mTOR during times of stress. Biologically, although generally a switch from cap-dependent to IRES-mediated translation occurs during cellular stress, HCMV appears to simultaneously drive the translation of both cap-dependent and independent survival proteins within infected monocytes in part due to the substrate specificity of Akt for HSF1.

The unique interplay between mTOR and HSF1 during HCMV infection of monocytes stimulates the synthesis of a unique milieu of prosurvival proteins, including myeloid leukemia cell differentiation protein 1 (Mcl-1), X linked inhibitor of apoptosis (XIAP), and heat shock protein 27 (HSP27), that were not or marginally induced in growth factor-treated cells (Collins-McMillen et al., 2015; Peppenelli et al., 2016; Peppenelli et al., 2018). Consistent with our previous studies, we found that inhibition of Mcl-1 with C10 (a selective Mcl-1 small-molecule inhibitor (Abulwerdi et al., 2014)) led to significant induction of apoptosis and death of infected monocytes (6.6% in untreated versus 46.9 in treated) (Burrer et al., 2017; Chan et al., 2010; Peppenelli et al., 2018). Although uninfected cells are naturally programmed to undergo apoptosis, we now show that the loss of Mcl-1 has minimal effect on accelerating this process (57.9% in untreated versus 67.1% in treated), suggesting the possibility of selectively eliminating HCMV-infected monocytes while allowing uninfected monocytes to maintain normal immune surveillance functions (Fig. 3). Inhibition of XIAP with small molecule inhibitors also induces death of infected monocytes but had minimal effect on uninfected cells (Burrer et al., 2017; Chan et al., 2010; Peppenelli et al., 2018). Thus, this select pool of upregulated prosurvival proteins represents novel cellular antiviral targets aimed at selectively eliminating HCMV-infected monocytes while permitting uninfected monocytes to maintain their normal function. Interestingly, latently infected CD34 $^{+}$ stem cells are also highly dependent on Mcl-1 for viability (Reeves et al., 2012). Consequently, Mcl-1 inhibitors have the potential to eliminate quiescently and latently infected cells.

7. Conclusions

The use of antiviral agents as prophylaxis to limit virus replication has significantly reduced the incidence of early infection to ≤10% in transplant patients (Fishman et al., 2007a; Sagedal et al., 2004). However, prophylactic treatment appears to have simply shifted the kinetics of HCMV infection to later after transplantation (≥100 days), and thus HCMV still remains a serious post-transplantation problem (Fishman et al., 2007b; Rubin and Colvin, 1986; Sagedal et al., 2004). Since therapies against HCMV are designed to block specific steps along the virus replication cycle, the delayed onset of disease indicates that current antivirals are ineffective at preventing viral spread mediated by quiescently infected monocytes. We advocate that the suppression of HCMV replication with current prophylactic treatments must be done in combination with drugs capable of directly eliminating infected monocytes. Inhibiting cellular factors crucial to the survival of HCMV infected cells provides an alternative strategy to the development of replication inhibitors as targeting host proteins has the advantage of also affecting the dormant phases of the viral life cycle. Moreover, targeting cellular factors would decrease the likelihood of drug resistance as host factors are highly conserved.

The PI3K/Akt/mTOR pathway has critical function during all three stages of HCMV infection by 1) ensuring metabolic requirement are met for optimal virus production during lytic infection, 2) altering cellular transcription that favors the establishment of latency, and 3) maintaining the survival of quiescently infected monocytes. However, PI3K plays a critical role to normal cellular function, thus inhibition may have significant bystander effects. HCMV-infected cells display increased dependency on this pathway often significantly altering the IC50 of the kinases within the signaling cascade. Ultimately, these

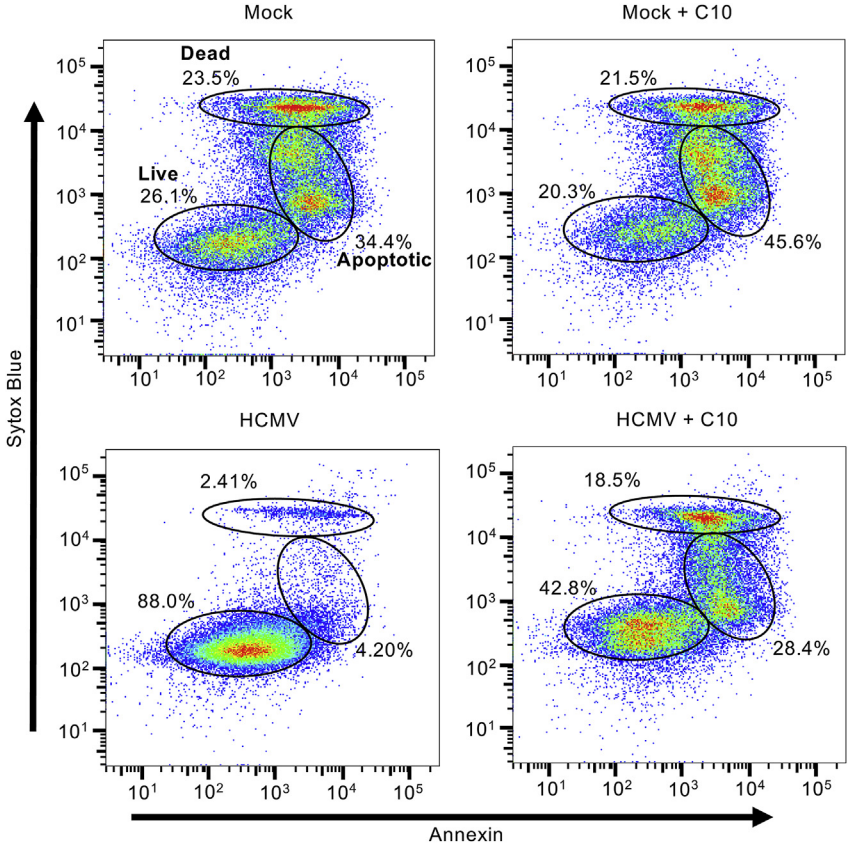

Fig. 3. HCMV utilizes Mcl-1 to prevent apoptosis in infected monocytes. Primary human monocytes were mock or HCMV infected for 24 h (h), after which infected cells were treated with Compound 10 (C10), a Mcl-1 inhibitor, for an additional 24 h. Viability was measured by flow cytometry using Sytox Blue (live/dead stain) and Annexin V (early apoptotic marker) staining. Gates represent live cells (Sytox Blue and Annexin V negative), apoptotic cells (Sytox Blue low and Annexin V high), and late apoptotic or dead cells (Sytox Blue and Annexin V positive).

virus-induced changes may provide a therapeutic window for the use of PI3K/Akt inhibitors for the treatment of HCMV infection. In addition, HCMV makes highly specific alterations to kinase activities during both lytic and quiescent infection. Targeting these unique changes may provide increased selectivity in eliminating the virus-infected cell populations. As a proof-of-concept, several drugs targeting the components of the PIK/Akt cascade led to death of infected monocytes while having little effect on uninfected cells (Burrer et al., 2017; Cojohari et al., 2016; Peppenelli et al., 2018). Consequently, inhibition of the PI3K/Akt signaling pathway is positioned to play a dual role in inhibiting HCMV replication and eliminating reservoirs of persistently infected cells, which is critical to the long-term prognosis of transplant patients.

Conflicts of interest

All authors declare that they have no conflicts of interest with the contents of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.01.009.

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