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New Cell-Signaling Pathways for Controlling Cytomegalovirus Replication

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Abstract

Cytomegalovirus (CMV) is increasingly recognized as an accomplished modulator of cell-signaling pathways, both directly via interaction between viral and cellular proteins, and indirectly by activating metabolic/energy states of infected cells. Viral genes, as well as captured cellular genes, enable CMV to modify these pathways upon binding to cellular receptors, up until generation of virus progeny. Deregulation of cell-signaling pathways appears to be a well-developed tightly balanced virus strategy to achieve the desired consequences in each infected cell type. Importantly and perhaps surprisingly, identification of new signaling pathways in cancer cells positioned CMV as a sophisticated user and abuser of many such pathways, creating opportunities to develop novel therapeutic strategies for inhibiting CMV replication (in addition to standard of care CMV DNA polymerase inhibitors). Advances in genomics and proteomics allow the identification of CMV products interacting with the cellular machinery. Ultimately, clinical implementation of candidate drugs capable of disrupting the delicate balance between CMV and cell-signaling will depend on the specificity and selectivity index of newly identified targets.

Keywords

AMPK; ATM; CMV; mTOR; signal transduction; Wnt

Introduction

Infection with human cytomegalovirus (CMV), a member of the herpesvirus family, is common in humans. Seroprevalence rates increase with age, reaching 90% in individuals older than 80 years. The virus establishes lifelong persistence in infected individuals, without causing apparent sequelae. In immunocompromised hosts, transplant recipients and patients with AIDS, CMV infection is associated with significant morbidity and mortality (1).CMV is the most common congenital infection causing mental retardation and deafness in infected children. While in normal hosts latency does not cause overt damage, in populations at risk for CMV disease, the additional stress imposed by chemotherapy,

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radiation or organ development allows CMV to disrupt the fine stalemate with the cell, reactivate and cause disease.

Other syndromes have been associated with detection of CMV in endothelial cells, including coronary artery disease, stroke, atherosclerosis in transplanted hearts and rates of graft rejection (1). CMV reactivation may also affect the outcome of sepsis and pulmonary complications in patients in intensive care units. Thus, virus reactivation may have a wider role in health outcomes.

To accomplish infection of multiple cell types, during its evolution with the host CMV developed multiple tactics to either cause lytic replication, or remain latent depending on the target cell. *In vitro* studies of lytic replication are usually performed in human fibroblasts, while latency is studied in endothelial/epithelial cells and monocytes. Although CMV-glycoprotein B (gB) is abundant in all virus strains and induces cell-signaling pathways, during adaptation to tissue culture, the laboratory-adapted strains (AD169, Towne) lost certain genetic regions. These include 19 genes in the UL/b′ boundary (encoding for cytokine and chemokine homologs) and the gH/gL/UL128–131 complex (required for virus endocytic entry [or endocytosis] into endothelial/epithelial cells)—which allows clinical isolates (TR, TB40/E and others) to enter endothelial/epithelial cells (2,3). These genetic changes could result in differential modulation of cell-signaling pathways.

This review provides an update on newly identified human cell-signaling pathways modulated by CMV and their potential relevance to CMV therapeutics. Previously reported pathways are briefly reviewed. Table 1 summarizes CMV-associated cell-signaling, virus facilitators and potential therapeutics.

Signaling pathways operate at the protein level, beginning with an extracellular signal triggering a receptor, leading to a chain of events whereby specific proteins transduce the signal to the nucleus, activating or deactivating transcription of specific genes. CMV binding to cellular receptors initiates the first wave of signal modulation, followed rapidly by effects imposed by components of the virion and subsequently to effects of viral gene products.

Platelet-Derived Growth Factor Receptor

Platelet-derived growth factor receptors (PDGFRs) are tyrosine kinase receptors, minimally expressed in normal tissues, but over-expressed in multiple malignancies. There are two types of PDGFR, α and β, and their ligands, PDGFs A and B, are important for cell migration and proliferation. Physiologically, they participate in wound healing, inflammation and angiogenesis. Blocking PDGFR-α with an anti-PDGFR-α antibody or imatinib mesylate (Gleevec), an inhibitor of several tyrosine kinases used for treatment of chronic myeloid leukemia and solid gastrointestinal tumors, inhibited entry of several CMV strains and viral gene expression in fibroblasts and epithelial/endothelial cells (Figure 1). Neutralizing antibodies against CMV-gB prevented PDGFR-α activation, suggesting a potential interaction between gB and PDGFR-α (4). However, in another study (5) CMV entry was not blocked by anti-PDGFR-α antibody or by short hairpin silencing of PDGFR-α in epithelial cells. Since PDGFR-α transduction enhanced entry of TR (a clinical isolate)

and AD169 (a laboratory-adapted strain) into epithelial/endothelial cells, it was suggested that CMV may not interact with PDGFR-α, but rather PDGFR-α induces a novel entry pathway for CMV (5). Additional studies are required to elucidate how PDGFR-α promotes CMV entry, which could lead to identification of specific inhibitors of CMV entry.

AMP-Activated Protein Kinase

AMP-activated protein kinase (AMPK) consists of three subunits (α, β, γ) and plays a central role in numerous metabolic processes. Through adenylate kinase action, low adenosine triphosphate (ATP) levels result in increased concentrations of adenosine monophosphate (AMP), which, in turn, induce AMP binding to AMPK and subsequent stimulation of AMPK activity, primarily through liver kinase B1 or Ca^{2+}-calmodulin-dependent kinase kinase (CaMKK)-dependent phosphorylation. Upon activation, AMPK restores ATP pools by activating ATP-producing pathways and inhibiting ATP-consuming pathways.

RNA inhibition screen identified the AMPK pathway as a key kinase in CMV replication (6,7). An AMPK inhibitor (compound C) reduced CMV-induced metabolic changes and infectious progeny (6,7). AMPK inhibition attenuated early and late CMV gene expression and viral DNA synthesis, but had no impact on CMV immediate early (IE) gene expression, suggesting the effects of AMPK occur at the IE to early transition of viral gene expression. Inhibition of CaMKK with a small-molecule ST0–609 blocked CMV-mediated AMPK activation and virus production. Interestingly, specific levels of AMPK activity are required to create a favorable environment for CMV replication, since both AICAR (an AMPK activator) and compound C abrogated CMV replication (Figure 2). The fine, tightly regulated balance of this pathway is also supported by: (a) Akt activation reduces AMPK activity (8). Since CMV induces Akt early during infection (4), it must activate AMPK using alternative pathways; (b) AMPK activates tuberous sclerosis complex 1/2 (TSC1/2) to decrease mammalian target of rapamycin (mTOR) signaling, which would not be beneficial to CMV. However, CMV-encoded UL38 binds to TSC1 and prevents it from responding to AMPK phosphorylation (9). The net effect of protein function changes during infection, resulting in productive or abortive replication.

A novel role of AMPK was discovered in cancer cells, sensing genomic stress and activating the DNA damage response (DDR) pathway. Chemotherapy and radiation activated AMPK to mediate signal transduction downstream of ataxia telangiectasia mutated (ATM) (10). Similar interactions in CMV-infected cells have not been studied.

Mammalian Target of Rapamycin

mTOR, a member of the phosphatidylinositol 3-kinase (PI3K) related kinase superfamily, is found in two complexes that have distinct functions and different sensitivities to rapamycin. mTORC 1 (consisting of mTOR, raptor and mLST8) regulates translation and cell growth via phosphorylation of S6 kinase (S6K) and eukaryotic initiation factor eIF4E binding protein (4E-BP), and is inhibited by rapamycin. mTORC2 (consisting of mTOR, rapamycin-insensitive rictor and mSIN1) regulates a diverse set of substrates, including AKT S473, glucocorticoid-regulated kinase, and protein kinase C-α, and is resistant to acute (but not

chronic) administration of rapamycin; chronic administration inhibits both mTORC1 and mTORC2 (Figure 2).

Clinical and basic studies demonstrate the importance of this pathway in CMV replication. Retrospective and prospective studies identified the benefit of mTOR inhibitors, sirolimus (Rapamune) and everolimus (Afinitor), in reducing the risk of CMV infection compared to other immunosuppressive regimens (11). The underlying mechanisms for decreased episodes of CMV are not well understood, but may involve an improvement in CMV-specific $CD8^+/CD4^+$ T cell responses (12). Everolimus add-on therapy against CMV disease in comparison to valganciclovir/ganciclovir alone is in phase II clinical trial for CMV disease in renal transplant recipients (ClinicalTrials.gov NCT00828503). These studies are important for directing immunosuppressive therapy in CMV-positive solid organ recipients and in supporting implementation of anti-CMV agents that target cell-signaling pathways (rather than directly inhibiting a virus protein).

In vitro studies have elegantly shown a complex and dynamic relationship between CMV and components of mTOR, leading to its activation at different time points during infection and altering its expected sensitivity to rapamycin. CMV IE proteins activate PI3K/Akt, resulting in mTOR activation and maintaining cap-dependent translation (13,14). Although this pathway is inhibited by rapamycin, the function of eIF4F is maintained in CMV-infected cells (13). CMV also activates mTORC2 via increased phosphorylation of Akt S473. mTORC2 is important in CMV replication, since CMV inhibition by rapamycin is rictor-, not mTORC1-, dependent, and both raptor- and rictor-containing complexes mediate the phosphorylation of 4E-BP and S6K (13). Use of Torin1, which inhibits protein synthesis by disrupting the formation of the eIF4F complex, revealed that rapamycin-resistant mTORC1 activity is required for CMV DNA accumulation (Figure 2) (14). Torin1 activities were mTORC2-independent because they occurred in cells lacking rictor. Thus, inhibition of eIF4F-dependent translation by Torin1 may result in decreased expression of cellular protein(s) necessary for viral DNA replication.

CMV induction of mTOR indicates that it overcomes AMPK inhibition of mTOR, the latter mediated through phosphorylation of TSC1/2. CMV-encoded UL38 binds to TSC1 and prevents it from responding to AMPK phosphorylation (9). While early AMPK activation (by AICAR) inhibited CMV-induced phosphorylation of 4E-BP and S6K, likely secondary to virus inhibition (15), AICAR treatment at 12 h post-infection did not inhibit CMV or the activation of 4E-BP/S6K. Taken together, CMV controls components of mTOR and its upstream effectors. While both AMPK activation and inhibition constrain CMV replication, these activities occur at different stages of virus replication resulting in differential effects on other signaling pathways (6).

Ataxia Telangiectasia Mutated and the DNA Damage Response

ATM is a central protein kinase in DDR. It is activated in response to DNA double-strand breaks and phosphorylates downstream proteins to initiate the DNA damage checkpoint, leading to cell cycle arrest and DNA repair, or, if damage is too severe, to apoptosis. A human protein microarray identified approximately 100 shared substrates of all herpesvirus-

conserved kinases (16). DDR proteins were enriched, and the histone acetyltransferase TIP60 (an upstream regulator of DDR) was required for replication of all tested herpesviruses. Knockdown of TIP60 in CMV-infected cells reduced extracellular viral progeny.

CMV deregulates the DDR pathway, initially by activating ATM and ataxia telangiectasia and rad-3 related kinases (ATR) (17–19) followed by blockage at the checkpoint kinase 2 (Chk2) (19).ATM and Chk2, which normally localize to the nucleus, instead migrate later during infection to the cytoplasm where they colocalize with virion structural proteins. Despite localizing to viral replication centers, proteins required for nonhomologous end joining, which might rejoin viral replicating DNA ends, are excluded from the replication centers (17). Thus, the host DDR (targeted toward viral inhibition) seems to become dysfunctional (17,19).

The exact requirement of ATM for CMV replication is debated: originally, CMV was reported to replicate in cells lacking ATM (17), but a recent report suggests inhibition of virus replication in these cells (20). The difference in these findings may not be adequately explained by strain differences, but could originate from the use of cells at different stages of cell cycle. The E2F1 transcription factor may play a role in mediating DDR and CMV replication (20). Since ATM and ATR control multiple pathways, additional studies are required to elucidate how CMV targets the DDR, and which specific components are regulated by CMV. The CMV protein kinase, UL97, phosphorylates and inactivates Rb tumor suppressor gene (21), releasing E2F1, which regulates transcription of many genes, including those required for S-phase progression and DNA repair. AMPK may therefore control DDR through Rb in CMV-infected cells.

Cyclin-Dependent Kinases

Cyclin-dependent kinases (CDKs) control cell cycle progression. The human genome encodes for 13 putative CDKs and 25 cyclins. Many viruses modify CDK activity to regulate their own replication cycle and synchronize it to the host cell so as to avail of the required enzymes and biomolecules. The host cell in turn, has evolved multiple checkpoints that prevent a cell from replicating when damaged and/or infected. CMV infection modifies cell cycle regulation and CDKs. Several steps of CMV replication require CDK1, CDK2, CDK7 and CDK9 along with their respective cyclins (reviewed in (22)). Roscovitine (Seliciclib), a CDK2 inhibitor used in clinical trials for B cell malignancies and lung cancer, disrupts IE gene expression and halts virus replication (23).

CDKs are induced at a very early stage of CMV infection (24). During the S/G2 phase of the cell cycle, CMV cannot initiate IE gene expression. When cellular CDK is inhibited by roscovitine, CMV overcomes this limitation and S/G2 cells become fully permissive for CMV. Moreover, in undifferentiated teratocarcinoma cells, in which CMV establishes latency, CDK inhibition relieves the block of IE gene expression and reactivates CMV, suggesting a broader role for CDK activity in generating a nonpermissive host environment for viral gene expression (24). Thus, the mobilization of CDKs and progression of CMV

replication are also delicately balanced, dependent not only on the relative levels of host and viral proteins, but also on the stage of viral replication relative to the host cell cycle.

Phosphatidylinositol-3-Kinase

Binding of CMV envelope glycoproteins to host cell receptors causes an immediate activation of mitogenic cell-signaling pathways required for virus replication, such as the cellular PI3K (Figure 1). LY294002, a specific PI3K inhibitor, reduced viral titers in fibroblasts; it did not inhibit CMV entry, but down-regulated viral IE gene expression (25). Adding downstream molecules of the PI3K pathway restored virus replication, confirming the specificity of LY294002 for PI3K (25).

Phosphorylation of PI3K in CMV-infected cells results in subsequent activation of Akt, p70 S6K and nuclear factor kappa beta (NF-κB). Activation of these targets suppresses apoptosis, yet activates host gene expression and protein synthesis (25,26). The following main pathways downstream of the signaling pathways mentioned above are dysregulated by CMV.

Nuclear factor kappa beta

This transcription factor family is a central hub of signaling events and is rapidly activated by multiple triggers, including viruses, many of which harbor binding sites for NF-κB in their promoter elements to ensure a strong and programmed cellular response. CMV-gB and gH interact with cellular receptors (epidermal growth factor receptor, integrins, Toll-like receptor 2), resulting in activation of NF-κB and specificity protein 1; (Figure 1) (27–30). Signaling activation through gB/gH was reported in fibroblasts and monocytes, indicating this to be an important strategy for CMV. In monocytes NF-κB maintains the balance between latency and reactivation (31).

Following virus entry, NF-κB activates viral IE genes, mainly through binding to the CMV major IE promoter (MIEP) (32). Since NF-κB activation, albeit an important strategy for efficient CMV replication (27,33), will ultimately trigger the synthesis of anti-viral cytokines, CMV must balance these deleterious effects. This is corroborated by the lack of effect of NF-κB binding site deletion in MIEP on CMV replication (34,35) and IE86-mediated suppression of NF-κB-dependent cytokine/chemokine gene expression (36). Although NF-κB inhibitors (aspirin and MG-132) blocked CMV MIEP activation (37), off-target effects of these compounds should be considered.

Specific viral components that modulate NF-κB activity were recently identified. UL76 (a highly conserved virion-associated protein) induced IL-8 expression through the NF-κB pathway (38) and UL144 (a truncated tumor necrosis factor (TNF)α-receptor homolog), present in clinical isolates but deleted in laboratory-adapted strains, up-regulated NF-κB activity via TNF receptor-associated factor 6 (35).

NF-κB activation, an integral response of both host and virus, is central to pro- and anti-viral responses. The resolution of the apparently conflicting outcomes would require the separation of these two contrary facets of NF-κB activation. Given the complex effects of

CMV on NF-κB signaling, there is no focal point at which the NF-κB network can be specifically targeted to contain viral infection or improve host response. Compounds such as artesunate were reported to inhibit CMV replication (in addition to their anti-malarial activities) and down-regulate NF-κB (39). However, their mechanism of CMV inhibition is currently unknown and NF-κB inhibition likely represents a final readout of upstream effects.

RAS/RAF/ERK pathway

Mitogen-activated protein kinase (MAPK) pathways are kinase modules that coordinate cellular response to extracellular signals resulting in growth, proliferation, differentiation, migration and apoptosis. Five different groups of MAPKs have been identified in mammals. Of these, CMV activates ERK1/2 and p38 MAPK pathways for its productive replication (Figure 1). The kinase inhibitor, sorafenib (Nexavar, used for treatment of solid tumors), inhibited replication of different CMV strains. IE expression was decreased through inhibition of Raf, and depletion of Raf reduced IE expression (40). MEK1/2 inhibitors, U0126 and PD98059, inhibited IE72 and IE86 phosphorylation in infected cells without altering their protein expression (41,42). At the transcriptional level, the UL4 promoter is MAPK-responsive. MEK1/2 inhibitor, U0126, and the p38 MAPK inhibitor, FHPI, reduced UL4 promoter activity *in vitro*. However, endogenous UL4 RNA expression was not as low, suggesting an MEK/MAPK independent component to UL4 activation (43).

Recently identified pathways with or without requirement for protein kinases are as follows.

Unfolded Protein Response and Ubiquitin-Mediated Protein Degradation

The proteasome is indispensable for selective degradation of cellular proteins. Its major function is to prevent accumulation of damaged, misfolded and mutant proteins. Proteins degradation is an ATP-dependent multistage process that requires ubiquitination of the target protein prior to its degradation by the 26S proteasome. Ubiquitination is achieved via an enzymatic cascade involving E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin ligase) enzymes. Generally, proteasome activity increases during the course of CMV infection, and proteasome inhibition have negative effects on CMV replication (44,45), including reduction of expression of early and late CMV proteins (46). The 19S and other proteasome subunits relocalize to the periphery of replication centers. The viral tegument protein, pp71, promotes ubiquitin-independent, proteasome-mediated degradation of nuclear domain 10-associated Daxx, Rb, p107 and p130 proteins in favor of virus replication (47,48).

Unfolded protein response (UPR), functionally related to the proteasome, is a mechanism that counters endoplasmic reticulum (ER) stress resulting from unfolded polypeptide chains exceeding the protein folding capacity of the ER. CMV modulates multiple ER stress response pathways to its advantage, since prolonged ER stress (leading to apoptosis) would be detrimental to virus replication (Figure 3).

Under normal conditions, ER chaperone BiP is bound to inositol-requiring enzyme 1 (IRE-1) and PKR-like ER kinase (PERK), rendering them inactive. In response to ER stress,

BiP is sequestered away to bind to unfolded proteins, releasing and activating PERK and IRE-1. PERK phosphorylates initiation factor 2 (eIF2) leading to a general attenuation of translation (inhibitory to viral growth). However, it increases translation of activating transcription factor 4 (ATF4), which, in turn, transcribes genes encoding metabolism and redox regulatory factors to help the cell recover from ER stress. CMV activates the UPR to benefit viral replication. Regulation of PERK, ATF6, and IRE-1 by CMV results in a replication-favorable environment. Although CMV infection activated eIF2, the phosphorylation of eIF2 was limited such that global translation was barely inhibited. Thus, the positive effects of ATF4 activation were maintained while virus damage from PERK activation was limited (49). CMV-UL38 modulates ER stress and prevents ER stress-induced cell death independent of mTORC1 activation (50). UL38-deficient CMV fails to up-regulate PERK and eIF2 phosphorylation resulting in loss of robust ATF4 accumulation (Figure 3). Although ATF6 activation was suppressed in CMV-infected cells, genes that are normally activated by ATF6 were activated via an ATF6-independent mechanism. CMV infection also activated the IRE-1 pathway, indicated by splicing of Xbp-1 mRNA. However, transcriptional activation of the XBP-1 target gene was inhibited.

Another marker of CMV-induced stress response is the accumulation of BiP. A BiP-specific subtilase cytotoxin SubAB blocks CMV assembly. CMV proteins US2 and US11 bind to BiP to degrade MHC class I proteins, preventing viral recognition by the host immune response (51,52). Induction of ER stress and terminal UPR as a strategy for virus control was tested using Clotrimazole, which induces ER stress by disrupting ER calcium homeostasis. Clotrimazole inhibited production of CMV virions *in vitro* primarily because of an inhibition in global translation (53). Protease inhibitors induce ER stress and cause accumulation of misfolded proteins. Although the HIV-protease inhibitor, Nelfinavir, was reported to inhibit herpesviruses including CMV, whether this inhibition is UPR-dependent is unknown (54).

Wnt Signaling

Signaling by the Wnt family of secreted glycoproteins is a fundamental mechanism directing cell proliferation, cell polarity and cell fate determination during embryonic development and tissue homeostasis. The most studied Wnt pathway is the canonical Wnt signaling, which regulates the amount of the transcriptional co-activator bcatenin and controls key developmental gene expression programs. It is a tightly regulated pathway and deregulated frequently in cancers. Because of the role Wnt signaling plays in embryonic development, a time in which CMV infects multiple cells and causes injury to major organs, it is not surprising that CMV would control this pathway. Recent reports from cancer chemotherapy suggest that cancer cure depends on targeting stem cells within the tumor environment that are resistant to available chemotherapeutic agents (55). Compounds that inhibit cancer stem cell growth via modulation of Wnt were recently reported (56). One of these compounds, salinomycin, was toxic to cancer cells but had no toxic effects on normal cells. While Wnt signaling is targeted and induced by γ-herpesviruses, EBV and KSHV, the interaction of CMV with this pathway appears to be unique and finely balanced. CMV infection of human fibroblasts and extravillous cytotrophoblasts results in degradation of β-catenin and a decrease in its transcriptional activity in response to Wnt ligand stimulation (57).We

investigated the effects of CMV infection on several components of the Wnt pathway (58). CMV infection resulted in significant decrease in the expression of Wnt 5a/b and β-catenin, an effect that was augmented as infection proceeded. Phosphorylated and total lipoprotein receptor related protein 6 levels were also reduced significantly in CMV-infected cells. CMV infection resulted in enhanced and sustained expression of the negative Wnt regulator, axin 1. Interestingly, Wnt modulating agents, monensin, nigericin and salinomycin, inhibited CMV replication, resulting in decreased expression of axin 1 and β-catenin, demonstrating again the concept of "right amount at the best timing" for efficient CMV replication, and supporting further research to disrupt this fine balance. Wnt modulators were suggested as potential biomarkers for CMV disease (59).

Summary

CMV uses multiple cell-signaling pathways to achieve efficient replication. There is growing interest in identifying compounds that can modulate cellular pathways used by CMV. Such "anti-viral anti-cellular" agents may not be under direct viral control and therefore are less likely to select for resistant mutants. Compounds with specific effects on cellular pathways have been reported to inhibit CMV replication. Broadly, these can be grouped into early inhibitors of CMV replication and others active at multiple steps of virus replication. An ongoing concern with these compounds is their potential cellular toxicity especially during prolonged course of therapy. Although host protein kinases and the downstream pathways they control play critical roles in CMV replication, studies are needed to identify kinases that have a good selectivity against CMV replication; that is the therapeutic window between cellular toxicity and virus inhibition must be demonstrated. Recently discovered anti-CMV agents that modulate cell-signaling pathways have shown differential toxicity; that is they were toxic to cancer cells while nontoxic to primary cells. Dissection of CMV interaction with specific components of cell-signaling pathways could pave the way to developing novel anti-viral agents.

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Abbreviations

4E-BP	eukaryotic initiation factor eIF4E binding protein
AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
ATF4	activating transcription factor 4
ATM	ataxia telangiectasia mutated
ATP	adenosine triphosphate
CaMKK	$Ca2^{+}$-calmodulin-dependent kinase kinase

CDK	cyclin-dependent kinase
Chk2	checkpoint kinase 2
CMV	cytomegalovirus
DDR	DNA damage response
ER	endoplasmic reticulum
ERK	extracellular signal-related kinase
gB	glycoprotein B
IE	immediate early
IRE-1	inositol-requiring enzyme 1
MAPK	mitogen-activated protein kinase
MIEP	major IE promoter
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa beta
PDGFR	platelet-derived growth factor receptor
PERK	PKR-like ER kinase
PI3K	phosphatidylinositol-3-kinase
S6K	S6 kinase
TNF	tumor necrosis factor
TSC 1/2	tuberous sclerosis complex 1/2
UPR	unfolded protein response

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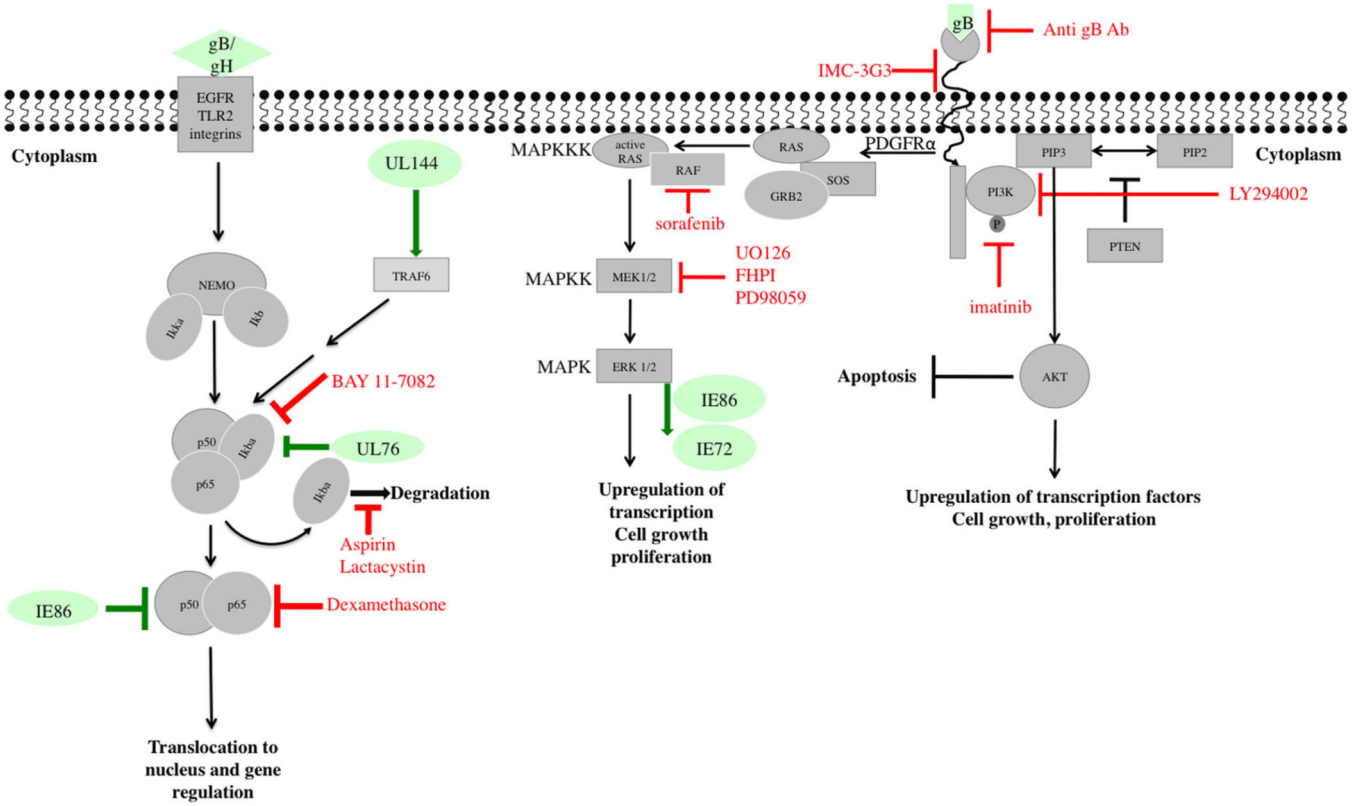

Figure 1. Modulation of PDGFR, MAPK and NF-κB pathways by CMV

Depicted are schematics of PDGFR-α, MAPK and NF-κB signaling pathways as well as downstream effects and resulting cell activities. Viral proteins that modulate or interact with these pathways at various stages are shown in green. Compounds or antibodies that were reported to inhibit CMV replication by targeting specific steps in a given pathway are shown in red. Cellular proteins appear in gray. CMV, cytomegalovirus; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa bet; PDGFR, platelet-derived growth factor receptor.

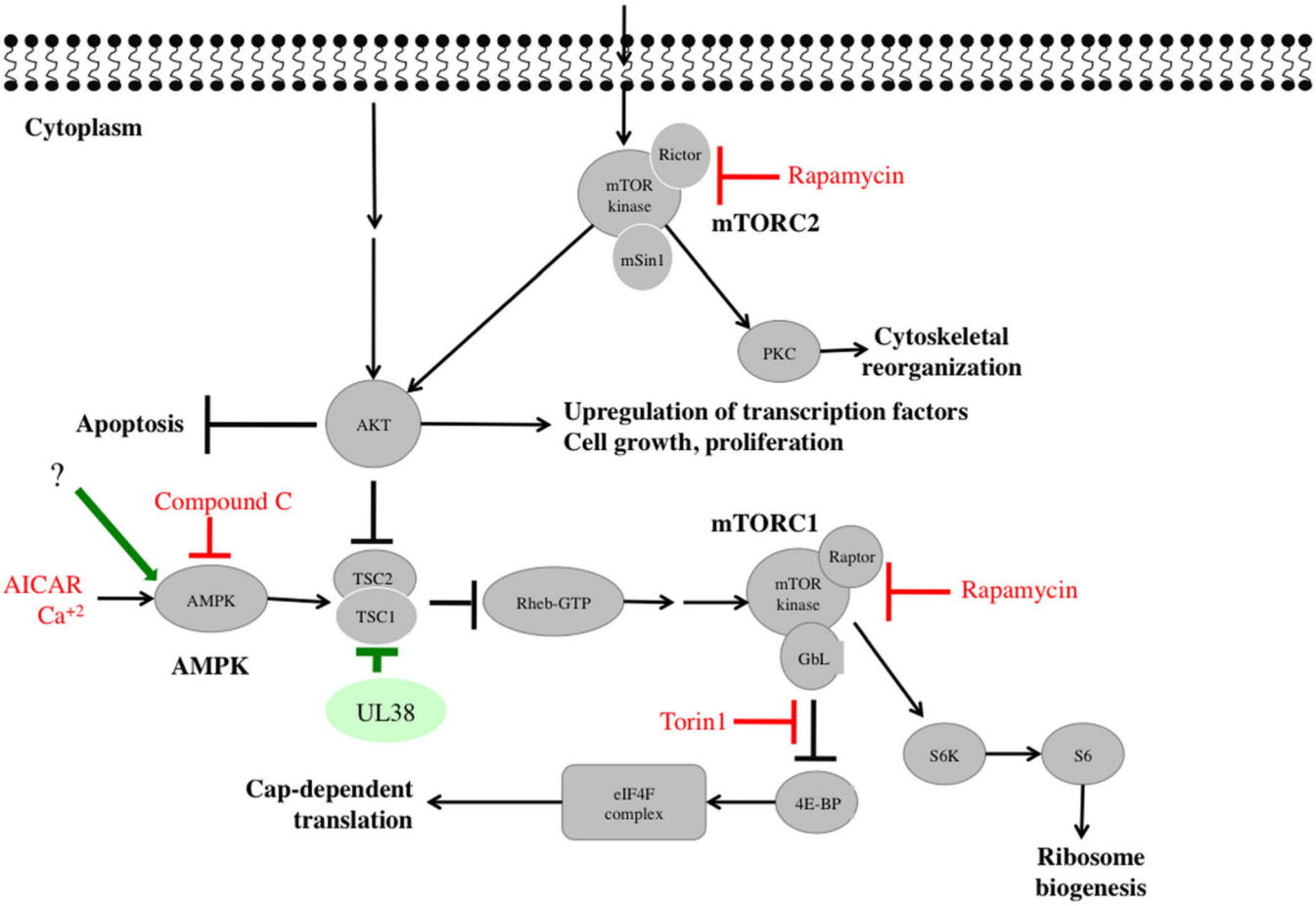

Figure 2. Modulation of AMPK and mTOR pathways by CMV

The AMPK and mTOR pathways with viral effectors are shown. Extracellular signals and upstream proteins resulting in the activation of Akt are shown in greater details in Figure 1. Viral proteins that modulate or interact with these pathways at various stages are shown in green. Compounds or antibodies that were reported to inhibit CMV replication by targeting specific steps in a given pathway are shown in red. Cellular proteins appear in gray. AMPK, AMP-activated protein kinase; CMV, cytomegalovirus; mTOR, mammalian target of rapamycin.

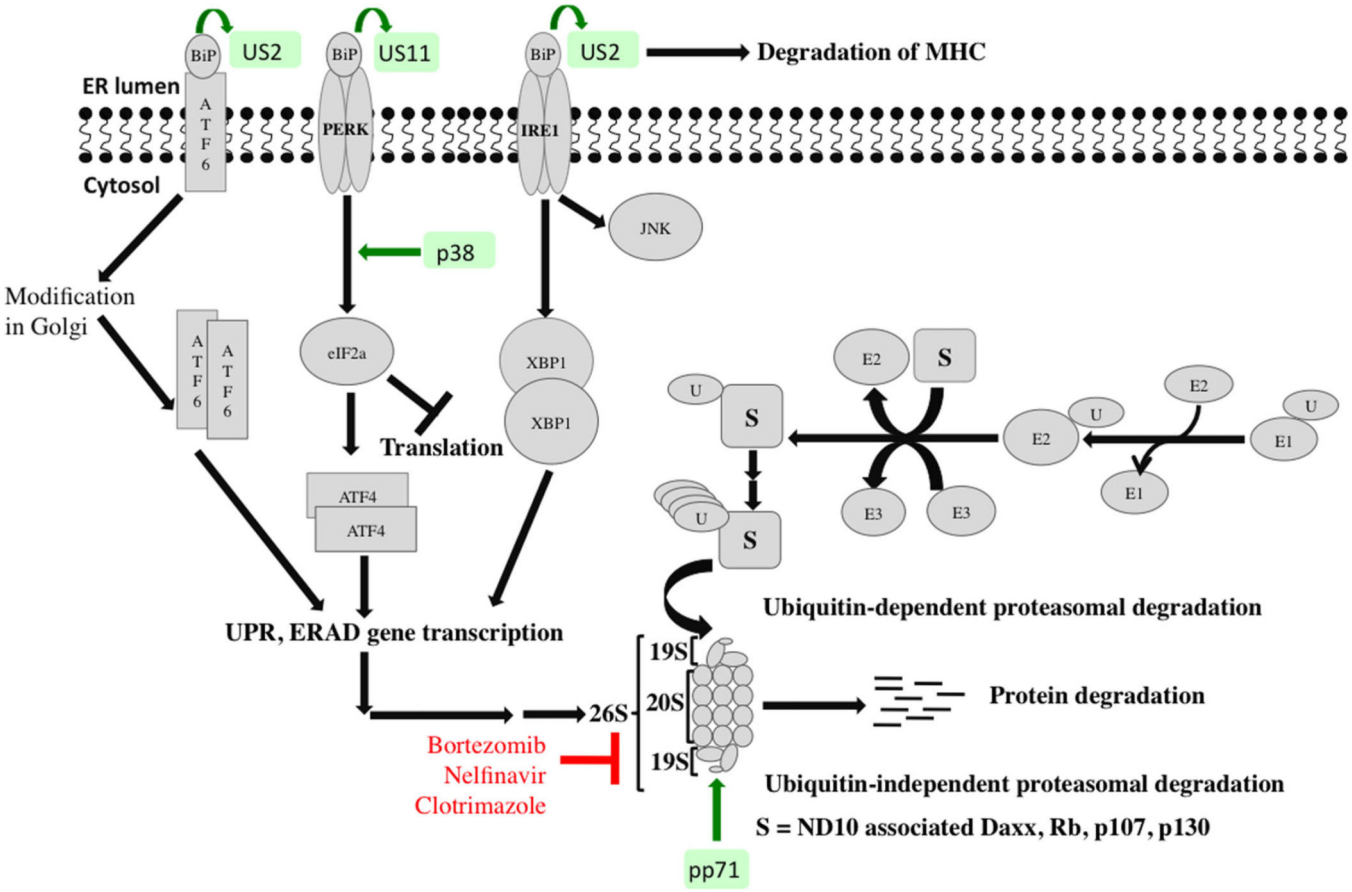

Figure 3. Modulation of UPR and proteasome-mediated degradation by CMV

Simplified schematic of the crosstalk between UPR and proteasome-mediated protein degradation is shown. “S” represents substrate proteins being targeted to the 26S proteasome. Viral proteins that modulate or interact with these pathways at various stages are shown in green. Compounds or antibodies that were reported to inhibit CMV replication by targeting specific steps in a given pathway are shown in red. Cellular proteins appear in gray. CMV, cytomegalovirus; UPR, unfolded protein response.

Table 1

Cell-signaling pathways controlled by cytomegalovirus

Primary pathway	Virus-derived facilitator (s)	Drug	Reference
AMPK	Unknown	Compound C, AICAR, ST0–609	(4,5)
mTOR	IE72, IE86, UL38	Sirolimus, everolimus, rapamycin, torin	(9–12)
CDKs	Unknown	Roscovitine	(21)
PDGFR-α	gB (?)	Imatinib mesylate, IMC-3G3,	(2)
PI3K	gB	LY294002	(23)
NF-κB	gB/gH, UL76, UL144, IE86	BAY-11–7082, dexamethasone, lactacystin	(25–28,35,36)
RAS/RAF/MEK1/2	IE86, IE72	Sorafenib, U0126, PD98059, FHPI	(38–41)
UPR/proteasome degradation	US2, US11, pp71, UL38	Clotrimazole	(51)
WNT	Unknown	Monensin, nigericin, salinomycin	(56)

AMPK, AMP-activated protein kinase; CDKs, cyclin-dependent kinases; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa beta; PDGFR-α, platelet-derived growth factor receptor-α; PI3K, phosphatidylinositol-3-kinase; UPR, unfolded protein response.