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REVIEW

Mechanisms of human cytomegalovirus infection with a focus on epidermal growth factor receptor interactions

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Summary

Human cytomegalovirus (HCMV) is a widespread opportunistic herpesvirus that causes severe diseases in immunocompromised individuals. It has a high prevalence worldwide that is linked with socioeconomic factors. Similar to other herpesviruses, HCMV has the ability to establish life-long persistence and latent infection following primary exposure. HCMV infects a broad range of cell types. This broad tropism suggests that it may use multiple receptors for host cell entry. The identification of receptors used by HCMV is essential for understanding viral pathogenesis, because these receptors mediate the early events necessary for infection. Many cell surface components have been identified as virus receptors, such as epidermal growth factor receptor (EGFR), which is characterized by tyrosine kinase activity and plays a crucial role in the control of key cellular transduction pathways. EGFR is essential for HCMV binding, signaling, and host cell entry. This review focuses on HCMV infection via EGFR on different cell types and its implications for the cellular environment, viral persistence, and infection.

KEYWORDS

cell surface receptor, cytomegalovirus, DNA virus infections, epidermal growth factor receptor, signal transduction

1 | INTRODUCTION

Virus entry pathways are largely defined by interactions between virus particles and their receptors at the cell surface, where these molecules have functions that are essential for productive infection. In simple situations, receptors can efficiently target viruses for endocytosis and can activate specific signaling pathways that facilitate entry or directly drive fusion/penetration events at the cell surface or within endocytic compartments by inducing conformational changes in virus surface structures.[1]

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein that regulates cellular homeostatic processes. Increasing evidence indicates that many viruses usurp EGFR endocytosis or EGFR-mediated signaling for entry, replication, inflammation, and antagonism of host antiviral responses. EGFR is a coreceptor for the entry of human cytomegalovirus (HCMV), a betaherpesvirus that causes severe complications in immunocompromised individuals.[2]

This review summarizes the current understanding of the interactions between HCMV and EGFR on various cell types and its implications for the cellular environment, viral persistence, and infection.

List of abbreviations: AREG, amphiregulin; COX-2, cyclooxygenase-2; CVDs, cardiovascular diseases; EC, endothelial cell; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; gB, glycoprotein B; HAART, highly active antiretroviral therapy; HB-EGF, heparin-binding EGF; HCMV, human cytomegalovirus; MCMV, mouse cytomegalovirus; MEC, mucoepidermoid carcinoma; miRNA, microRNA; PDGFR, platelet-derived growth factor receptor; pp65, phosphoprotein 65; SOT, solid organ transplant; TGF-α, transforming growth factor-α; UL83, unique long 83; WT1, Wilms' tumor 1

2 | HUMAN CYTOMEGALOVIRUS

Goodpasture and Talbert in 1921 were the first to suggest that the "cytomegalia" could be due to a viral agent. HCMV, or human herpesvirus 5 (HHV-5), is a betaherpesvirus that was first noticed in 1881 by a German scientist who thought that the intranuclear inclusions typical of HCMV infection were caused by protozoan infections. After the development of methods for virus growth in cell cultures, Thomas Weller, Margaret Smith, and Wallace Rowe independently isolated and grew CMV from humans and mice in 1956 to 1957.[3]

Among human herpesviruses, HCMV has the largest genome of nearly 236 kb that encodes 173 genes (Table 1). The virion consists of a double-stranded linear DNA core in an icosahedral nucleocapsid, surrounded by a tegument that contains the majority of the virion proteins. The most abundant protein is the lower matrix phosphoprotein 65 (pp65), also termed unique long 83 (UL83) because it is encoded by the 83rd gene in the unique long region of the genome. These components are enclosed in a lipid bilayer envelope that contains a number of viral glycoproteins involved in cell attachment and penetration.[4]

The HCMV encodes numerous proteins and microRNAs (miRNAs) that function to evade the immune response.[5] During lytic infection, HCMV encodes genes that can interfere with both MHC class I and II–restricted antigen processing and presentation. Proteins encoded within the US2-11 gene cluster target MHC class I and II molecules for retention within the endoplasmic reticulum, redirect MHC for degradation, and inhibit the normal loading of peptides onto MHC class I molecules.[6,7] In addition, UL82 (pp71) and UL83 (pp65) proteins introduced into cells immediately upon infection interfere with the endoplasmic reticulum egression of MHC class I molecules to the Golgi apparatus and with viral peptide generation, respectively.[8] The miRNAs encoded by the virus potentially provide an ideal mechanism to mediate immune evasion in latently infected cells. Several miRNAs have been shown to target components of the immune system during HCMV lytic infection such as miR-UL112.1,[9] miR-US4.1,[10] and miR-UL148D.[11]

The HCMV is a widespread opportunistic pathogen that causes birth defects in newborns. The clinical presentation of congenital HCMV infection varies widely, from asymptomatic infection (85-90%) to fetal hydrops, abortion, and postnatal death (0.5%).[12,13] The clinical signs and symptoms in between these extremes range from subclinical sensorineural losses to the growth of mental and motor retardation, to central nervous system damage (microcephaly), with possible involvement of the inner ear, retina, bone marrow, and internal organs.[14,15]

It is also a serious opportunistic infection in immunocompromised individuals with an impaired adaptive immune system, such as HIV/AIDS patients and transplant patients on immunosuppressive medication.[4] Before highly active antiretroviral therapy (HAART) became available, HCMV was a major cause of opportunistic infection in HIV-infected patients and was associated with accelerated progression to AIDS and death. The detection of HCMV in blood continues to identify patients with a poor prognosis, even in the era of HAART.[16,17]

Despite improved treatment and surveillance, HCMV is still regarded as being the most significant infectious pathogen in solid organ transplant (SOT) recipients, and it continues to cause morbidity and mortality after transplantation and is associated with diminished graft survival.[4] The highest risk for infection from transplantation is the combination of a serologically negative patient receiving an organ from a serologically positive donor.[18,19] In contrast with SOTs, HCMV infections following bone marrow transplantation are more frequent because of the reactivation of the latent virus present in the seropositive recipient as opposed to primary infection.[20] In addition to the donor and recipient serostatus, the risk of HCMV infection is also influenced by the patient age, source of donor stem cells, degree of human leukocyte antigen disparity between the donor and recipient, use of T-cell-depleted grafts or anti-T-cell antibodies, post-transplant immunosuppression, time to engraftment, and prophylaxis of acute graft-versus-host disease.[21,22]

IgG antibodies representing past infection are found in approximately 60% of adults in developed countries and 100% of adults in developing countries. In general, people born into poorer socio-economic circumstances acquire HCMV earlier.[23] It can be transmitted via saliva, urine, blood, sexual contact, placental transfer, breastfeeding, blood transfusion, or SOT.[4,24]

The HCMV infects a broad range of cell types during natural infection. Epithelial cells might be the first cell type to become infected and likely transfer the virus to monocytes that are thought to be particularly important for the dissemination of HCMV throughout the body.[25] Additionally, the virus can infect endothelial cells (ECs), fibroblasts, hepatocytes, salivary glands, neutrophils, macrophages, lymphocytes, and sometimes smooth muscle cells and neural cells.[25-29]

The broad tropism of HCMV infection suggests that it either has a receptor that is found in most cell types or that it utilizes multiple receptors. The identification of HCMV receptors is essential for understanding viral pathogenesis, because these receptors are involved in mediating the immediate early events necessary for infection.[27] Many cell surface components have been identified as virus receptors, such as platelet-derived growth factor receptor (PDGFR), and integrins.[30,31] EGFR has been identified as a receptor for HCMV and is essential for virus binding, signaling, and entry.[27]

TABLE 1 Classification of human herpesviruses

Family	Subfamily	Genus	Species	Size (kb)	Gene
Herpesviridae	*Alphaherpesvirinae*	*Simplexvirus*	Herpes simplex virus type 1 (HSV-1/ HHV-1)	152.22	79
			Herpes simplex virus type 2 (HSV-2/ HHV-2)	154.68	79
		Varicellovirus	Varicella-zoster virus (VZV/ HHV-3)	124.88	73
	Betaherpesvirinae	*Cytomegalovirus*	Human cytomegalovirus (HCMV/ HHV-5)	235.65	173
		Roseolovirus	Human herpesvirus 6A (HHV-6A)	159.32	88
			Human herpesvirus 6B (HHV-6B)	162.11	103
			Human herpesvirus 7 (HHV-7)	153.08	86
	Gammaherpesvirinae	*Lymphocryptovirus*	Epstein-Barr virus (EBV/HHV-4)	172.76	82
		Rhadinovirus	Human herpesvirus 8 (HHV-8)	137.97	96

3 | EPIDERMAL GROWTH FACTOR RECEPTOR

The EGFR is a cell membrane growth factor receptor characterized by tyrosine kinase activity that plays a crucial role in the control of key cellular transduction pathways.[32] It is widely distributed in epithelial, mesenchymal, and neuronal tissue and plays an important role in tissue homeostasis and the differentiation and morphogenesis of many organs.[33]

The EGFR belongs to a family of 4 closely related receptor tyrosine kinases: EGFR (ErbB1/HER-1), ErbB2 (HER-2/Neu), ErbB3 (HER-3), and ErbB4 (HER-4). Each receptor consists of a ligand-binding extracellular domain, a transmembrane domain, and an intracellular tyrosine kinase domain.[34] The activating ligands can be divided into 3 groups: epidermal growth factor (EGF), amphiregulin (AREG), and transforming growth factor-α (TGF-α) that bind specifically to EGFR; betacellulin, heparin-binding EGF (HB-EGF), and epiregulin that exhibit dual specificity for EGFR and ErbB4; and neuregulins that bind to ErbB3 and ErbB4 or only ErbB4.[34]

Ligand binding induces receptor homo- or heterodimerization, which is essential for the activation of tyrosine kinases and the phosphorylation of multiple tyrosine residues that serve as docking sites for several proteins containing Src homology 2 domains, leading to the activation of several intracellular signaling pathways that include the PI3K/Akt, JAK/STAT, NF-κB, PLCγ/PKC, and Ras/MAPK/ERK pathways. All the intracellular signaling pathways activated through EGFR converge in the nucleus where cell cycle regulators (cyclin D1) and transcription factors (c-Fos, c-Jun, c-Myc, STAT, NF-kB, and zinc finger transcription factors) control the biological outcome of EGFR activation. As a result, a variety of biochemical changes take place within the cell, including protein synthesis, cytoskeleton changes, apoptosis inhibition, angiogenesis, and cell motility (Figure 1).[35-37]

The misregulation of these pathways is implicated in many cases of cancer, where the EGFR is often perpetually stimulated because of the sustained production of EGFR ligands in the tumor microenvironment or as a result of a mutation in EGFR itself that locks the receptor in a state of continual activation.[37] Posttranslational modifications, epigenetic influences, and miRNA often lead to aberrant EGFR compartmentalization, aberrant EGFR trafficking, and increased EGFR signaling because of transactivation. These molecular events are commonly associated with tumor formation in patient samples, most likely because the resulting constitutive activation of the EGFR pathway leads to increased proliferation, increased invasiveness, increased motility, and decreased adhesion.[38]

Signaling through EGFR is frequently misregulated in solid tumors, leading to the abnormal activation of pro-proliferative and antiapoptotic pathways, notably the PI3K/Akt, RAS/RAF/MEK/ERK, and the JAK/STAT pathways.[39,40] In non-small cell lung cancers, constitutively activating mutations within the EGFR kinase domain also decrease responsiveness to apoptotic agents and increase cell survival through the selective activation of the Akt and STAT signaling pathways.[41]

One of the most important aspects of EGFR is its potential as a target in anticancer therapy for EGFR-positive tumors.[32,42] The role of the EGFR network in the life cycle of viral infections, its availability for extracellular manipulation, and the detailed understanding of the underlying biochemistry have made the EGFR pathway an attractive target for antiviral therapy.[2]

4 | EGFR AS A RECEPTOR FOR HCMV

Several glycoprotein complexes in the envelope of HCMV particles have been described to contribute to the entry of HCMV into target

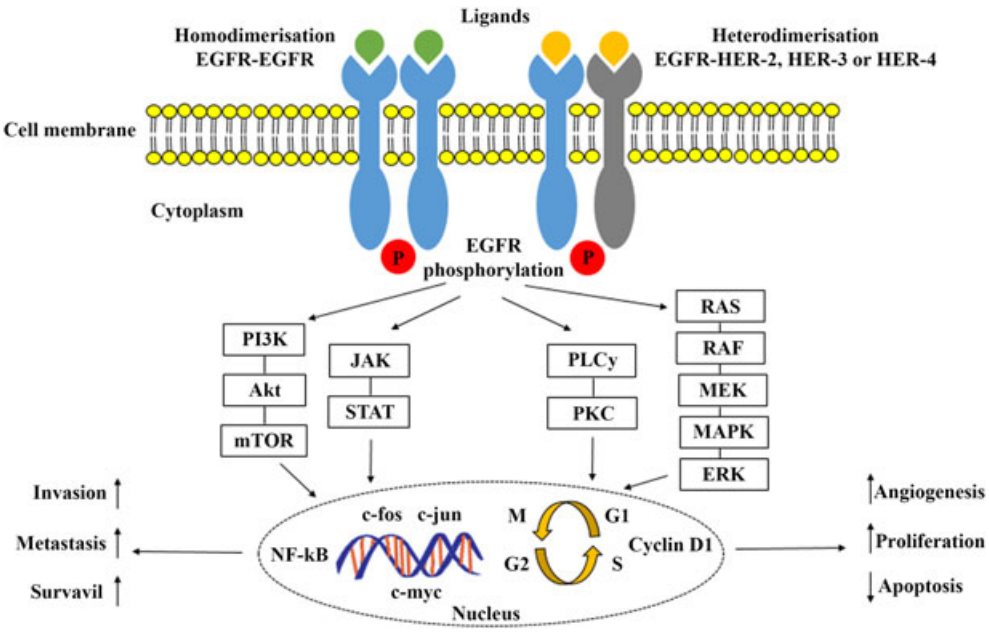

FIGURE 1 Signal transduction pathway mediated by EGFR. Ligand binding induces receptor homo- or heterodimerization that is essential for the activation of Tyr kinases and the phosphorylation of multiple Tyr residues

cells. On the cellular side, numerous proteins have been controversially proposed as entry receptors for HCMV, such as EGFR,[27] PDGFR-α,[43] and integrins.[44]

The patterns of EGFR downregulation and phosphorylation during HCMV infection are similar to those observed in human embryonic lung cells treated with EGF. When cells were treated with an EGFR neutralizing antibody or with AG1478 (an EGFR tyrosine kinase inhibitor), the viral yield decreased by 97% and 95%, respectively, suggesting that HCMV interacts with EGFR. In addition, the principal HCMV envelope protein glycoprotein B (gB) was shown to be responsible for binding to EGFR to initiate viral entry and signaling.[27]

Wang et al[30] demonstrated that both EGFR and integrin αvβ3 are required for HCMV infection. The virus simultaneously and independently binds to EGFR (via gB) and αvβ3 (via gH) and induces both EGFR-dependent PI3K and αvβ3-dependent Src signaling. The disruption of the EGFR-αvβ3 interaction and specific inhibition of the coordination between PI3K and Src inhibit viral entry. Furthermore, coordination between PI3K and Src is needed for HCMV-induced RhoA (GTP-RhoA) downregulation that is required for the disruption of actin stress fibers and viral nuclear translocation (Figure 2).

According to Feire et al,[44] α2β1, α6β1, and αvβ3 integrin heterodimers are critical determinants of HCMV entry and infection, and the gB of HCMV contains a sequence very similar to the integrin-binding disintegrin-like domain that can mediate viral entry. The interaction between the gB disintegrin-like domain and the β1 integrin ectodomain has significant consequences for the viral life cycle, where a soluble version of the gB disintegrin module was shown to efficiently block HCMV infection at a post-attachment step during entry into multiple in vivo cell targets.[43]

Wang et al[30] proposed that gH could function as a ligand for αvβ3 integrin. However, the precise molecular mechanisms involved in αvβ3 engagement by gH is unknown because this glycoprotein lacks any previously identified integrin recognition motif. The data presented by Feire et al[43,44] combined with the report that αvβ3 integrin interacts with gH[30] suggest that HCMV interacts with both β1 and β3 integrin heterodimers during the entry pathway to mediate HCMV entry to permissive human fibroblasts.

Additional to gB, Soroceanu et al[45] demonstrated that HCMV gB directly interacts with PDGFR-α resulting in receptor tyrosine phosphorylation, indicating that PDGFR-α is a more critical receptor required for HCMV entry than EGFR. Wu et al[46] reported that the HCMV glycoprotein complex gH/gL/gO binds to PDGFR-α on the surface of fibroblasts and that gH/gL/gO either directly or indirectly recruits gB to this complex. Additionally, PDGFR-α functions as an entry receptor for HCMV expressing gH/gL/gO, but not for HCMV mutants lacking the gH/gL/gO complex.

The EGFR and PDGFR have been shown to perform a similar function and trigger the PI3K/Akt pathway upon virus entry. The relative contribution of signaling and virus-host cell attachment for each of these growth factor receptors remains to be further characterized. Vanarsdall et al[47] investigated whether PDGFR-α and EGFR could promote entry of the wild type HCMV strain TR. They found no evidence that EGFR could promote entry of HCMV strain TR. In a panel of PDGFRα-specific antibodies, PDGF ligand and silencing of PDGFRα did not inhibit HCMV entry, suggesting that PDGFRα is not normally required for virus entry. Instead, increased expression of PDGFRα alters cells, facilitating virus entry by an abnormal pathway that promotes a different pathway of endocytosis and pH-independent fusion.

Stegmann et al[48] also investigated the mechanisms involved in EGFR and PDGFRα entry into fibroblasts and ECs. The authors showed that soluble derivatives of PDGFRα bind to HCMV virions only if they contain the gH/gL/gO trimer, thereby inhibiting entry into these cells types. In contrast, soluble EGFR did not inhibit infection in either cell type, arguing against a role for EGFR as a binding receptor

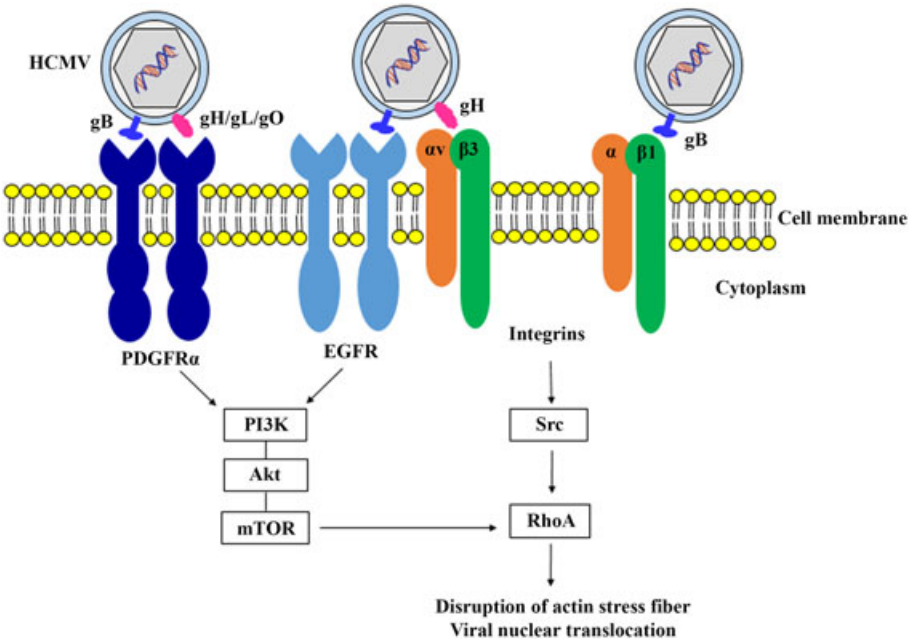

FIGURE 2 EGFR-mediated signaling facilitates HCMV entry. gB and gH of HCMV binds to EGFR and αvβ3 integrins, respectively, and the induced EGFR-dependent PI3K and αvβ3-dependent Src signaling leads to RhoA downregulation, disruption of actin stress fibers, and viral nuclear translocation. Other receptors have also been identified: PDGFRα and αβ1 integrin

on the cell surface, although a more indirect contribution of this molecule during HCMV entry cannot be ruled out.

According to Isaacson et al,[49] EGFR does not play a significant role in HCMV entry or signaling, because high levels of EGFR expression by a human breast cancer cell line failed to enhance HCMV infection compared with an EGFR-negative cell line, indicating that EGFR alone is not able to mediate HCMV entry. EGFR phosphorylation was undetectable in fibroblasts upon stimulation with HCMV, even at high multiplicities of infection, indicating that EGFR kinase is not stimulated by HCMV.

Cobbs et al[50] also demonstrated that EGFR is not required for the cellular expression of HCMV-essential genes or for virus-induced signaling. The stimulation of glioma and fibroblast cell lines overexpressing EGFR with HCMV or soluble gB did not induce tyrosine phosphorylation of the receptor as did the genuine ligand, EGF. HCMV caused phosphorylation of an approximately 180-kDa protein that is distinct from EGFR and could be coimmunoprecipitated with the p85 regulatory subunit of PI3K.

5 | PATHOGENESIS

The HCMV is an important cause of congenital viral infection. LaMarca et al[51] examined the regulation of EGFR by HCMV in a first trimester extravillous cytotrophoblast cell line. Both HCMV and EGF activated EGFR by inducing tyrosine phosphorylation and promigration signaling events. However, HCMV inhibits migration/invasion, while EGF stimulates these processes, which represents a paradox. In support of this, HCMV was found to induce the phosphorylation of EGFR at tyrosine residue 1068 only, while tyrosine residues 845, 992, and 1068 were activated in response to EGF. HCMV infection preferentially induces EGFR homodimers or EGFR-ErbB3 heterodimers. In contrast, EGF treatment traditionally induces EGFR homodimers and EGFR-ErbB2 heterodimers, rather than the activation of ErbB3.[27] The induction of EGFR-ErbB3 heterodimers by HCMV gB presents an interesting and feasible explanation for some of the differential effects of HCMV and EGF.[51]

A positive expression of pp65 was found in patients with atherosclerosis, suggesting that local HCMV infection might be associated with the pathogenesis of this disease.[52] This inflammatory artery disease that is characterized by alterations in the levels of lipids and other metabolites is the most important cause of cardiovascular diseases (CVDs). A recent meta-analysis showed that HCMV infection was also an important risk factor for atherosclerosis.[53] ECs, leukocytes, and intimal smooth muscle cells are the major players in the development of this disease.[54] As such, HCMV is also a candidate in the etiology of CVDs. The virus produces a proangiogenic response required for hematogenous dissemination after primary infection, the seeding of the virus in peripheral organs, and the establishment of viral persistence. The cells associated with CVDs are primary in vivo cellular targets for HCMV, altering EC functions such as proliferation, migration, and morphogenesis.[55]

Vascular endothelial growth factor receptors and hepatocyte growth factor receptors are key cellular receptors that trigger angiogenesis through the activation of the PI3K and MAPK pathways. However, there is no evidence of viral binding to these receptors in HCMV-mediated angiogenesis. EGFR is required for the HCMV-induced angiogenic response, whereas the Src family of tyrosine kinases is only partially required.[52,53] Furthermore, EGFR-mediated and integrin-mediated signaling (via Src) is rapidly activated in ECs after infection, suggesting that the engagement of such signaling after viral binding is intimately associated with functional changes in ECs.[55]

Monocytes are other primary in vivo targets for HCMV and are believed to be responsible for the hematogenous dissemination of HCMV to multiple organ systems. On the surface of these cells, EGFR is rapidly activated after infection with 1 of 2 HCMV strains (Towne/E or TB40/E), followed by the subsequent phosphorylation of downstream PI3K/Akt. Pretreatment of monocytes with AG1478 or a neutralizing EGFR antibody inhibits the internalization of the viral capsid, clustering of viral particles at the cell surface, and the viral entry decreases by 59% and 41%, respectively, indicating that EGFR mediates HCMV entry into monocytes. Furthermore, HCMV-activated EGFR induces lamellipodium and tail formation (hallmarks of cell motility) in monocytes and also promotes transendothelial migration. These events are required for monocyte extravasation into peripheral tissues and viral spread.[56]

The EGFR and PI3K signaling is required for efficient HCMV entry into CD34+ cells, and EGFR kinase activity was also required for HCMV DNA to reach the nucleus, suggesting a role for continued signaling in viral trafficking following entry. In addition, inhibition of EGFR signaling after viral entry and nuclear translocation leads to the increased expression of lytic IE1 and IE2 transcripts and decreased expression of the latency-associated UL138 transcript, indicating that EGFR signaling plays a role in the early steps of establishment of latency in CD34+ cells. HCMV-induced EGFR signaling also curbs the expression of the cellular IL-12 cytokine transcript, an important regulator of hematopoiesis. Thus, EGFR-dependent viral-induced regulation of this cellular factor may reflect a mechanism by which HCMV infection also shapes the differentiation outcome of infected bone marrow and blood cells to promote viral persistence.[57]

Recently, Buehler et al[58] demonstrated that HCMV coordinates the expression of 2 genes, *UL135* and *UL138*, with opposing roles in regulating states of latency and replication. UL135 promotes virus replication and reactivation from latency, in part, by overcoming the suppressive effects of UL138. Curiously, pUL135 and pUL138 regulate the viral cycle by targeting EGFR. While pUL138 potentiates EGFR signaling by enhancing cell surface levels and activity, pUL135 diminishes EGFR signaling by promoting its turnover or impedes the recycling of EGFR back to the cell surface. This opposing dual targeting of EGFR suggests that modulation of trafficking and signaling underlies, at least in part, the transition of the virus into and out of latency. Heightened EGFR levels at the cell surface may contribute to the establishment of latency by modulating cell survival, differentiation, or innate signaling. These data suggest that HCMV insulates active EGFR in the cell and that pUL135 and pUL138 function to fine-tune EGFR levels at the cell surface to allow the infected cell to respond to extracellular cues.

Some activities downstream of EGFR are required for replication and reactivation. It is possible that HCMV suppresses EGFR for reactivation while selectively maintaining the activity of specific downstream pathways. Within productively infected cells, EGFR is

associated with endocytic vesicles localized to the viral assembly compartment that may also function as a viral signaling compartment in addition to roles in virus maturation. This compartment may allow HCMV to insulate host signaling from extracellular up- or downregulation, or be a means by which the virus senses and responds to host cues. However, more work is required to understand how HCMV regulates the specific activities of EGFR and its downstream pathways and how this regulation influences latency and reactivation. Furthermore, pUL138-mediated stimulation of surface expression in latently infected cells raises the possibility that the viral regulation of EGFR contributes to the oncomodulatory properties of HCMV. Natural oscillations between states of latency and subclinical reactivation in the host might result in heightened or sustained EGFR expression.[58]

A time-course analysis of HCMV-infected human foreskin fibroblasts showed that EGFR mRNA was downregulated as early as 24 hours postinfection, and this downregulation was sustained until 48 hours postinfection. The ability of HCMV to decrease EGFR expression results, at least in part, from the virus-mediated induction of a known transcriptional repressor of EGFR expression, Wilms' tumor 1 (WT1), a pleiotropic regulator of mitogenesis, apoptosis, and differentiation. HCMV infection results in the profound upregulation of WT1 and increased binding of WT1 to the EGFR promoter. When WT1 is inhibited in human foreskin fibroblast-infected cells, EGFR downregulation is abolished. These HCMV effects on WT1 may not be limited to the regulation of cellular EGFR but might be reflected in multiple downstream cellular processes and perhaps viral WT1 targets.[59]

According to Fairley et al,[60] HCMV infection prevents external signaling by disrupting the function of EGFR. The virus decreases the cell-surface expression of EGFR in primary lung fibroblast cells because of an overall reduction in the steady-state levels of EGFR, and HCMV-infected cells showed no EGF-mediated autophosphorylation. The treatment of cells with phosphonoformate that prevents viral DNA replication and hence late viral gene expression still resulted in receptor downregulation. Consequently, HCMV early gene products are likely to be responsible for this virus-mediated EGFR perturbation. It is possible that the downregulation of EGFR function by HCMV may be required in order for the virus to mediate cell cycle advance in resting cells and to prevent the cell from responding to host EGF signaling and may drive the cell into a state that conflicts with optimal virus production.

6 | POTENTIAL LINKS WITH CARCINOGENESIS

Viruses have long been described as etiologic factors in carcinogenesis, and the oncogenic potential of HCMV has been implicated in malignancies of the breast,[61,62] brain,[63-66] lung,[67] colon,[68] and prostate.[69] Cobbs et al[70] were the first to show that HCMV nucleic acids and proteins are present in a high percentage of low- and high-grade malignant gliomas, the most common primary brain tumor in adults, and that the expression of early and delayed HCMV gene products occurs in these tumors. In glioblastoma cells, the HCMV IE72 protein directly interacts with the telomerase reverse transcriptase (TERT) promoter at SP1 binding sites to induce telomerase activity and telomere lengthening. Enhanced telomerase activity is necessary for tumor cells to divide indefinitely and is commonly induced by oncogenic viruses.[71]

HCMV, both active and latent, has a particular tropism for salivary glands, suggesting that it might play a role in the pathogenesis of mucoepidermoid carcinoma (MEC), a common malignant salivary gland neoplasm.[72] The cell-specific localization of HCMV was observed in 97% of human MECs of salivary glands. Protein markers for active HCMV (IE1 and pp65) were expressed in tumor cells, but not in non-tumor cells, and were positively correlated with the severity of malignancy. HCMV infection in human MEC cells is correlated and colocalized with a clear upregulation and activation of an established oncogenic signaling pathway involving cyclooxygenase 2 (COX-2), AREG, EGFR, and extracellular signal-regulated kinase (ERK).[73]

Using mouse models, Melnick et al[72] established that mouse cytomegalovirus (MCMV)-induced tumorigenesis in salivary glands displayed histological and molecular characteristics similar to those found with HCMV. Tumorigenesis was associated with the overexpression of activated EGFR and the significant concomitant upregulation of phosphorylated family members, ErbB2 and ErbB3, a relationship that has been observed in a variety of human malignancies. In addition, inhibition of the MEK-mediated phosphorylation of ERK results in complete rescue of MCMV-induced pathology, suggesting that the upregulation of ERK phosphorylation is necessary for initial MCMV-induced salivary gland pathogenesis.

Although the precise mechanisms involved in HCMV-related salivary gland tumorigenesis are unknown, a model of carcinogenesis has been proposed. First, HCMV directly initiates the malignant transformation of salivary gland progenitor cells, and the subsequent oncomodulation of these cells and/or HCMV promotes the oncomodulation of already precancerous salivary gland stem cells.[73] Melnick et al[74] demonstrated that MCMV infection of submandibular salivary glands upregulates host cell NF-κB activation, which in turn upregulates the COX-2/PGE2/EP4 pathway. PGE2/EP4 can induce AREG and thereby activate EGFR signaling. Later, the authors demonstrated that MCMV-induced COX-2 overexpression was coincident with the overexpression of AREG, activated EGFR, and ERK1/2 (Figure 3).[72] EGFR and AREG have been shown to be co-overexpressed in breast cancer, and the AREG/EGFR autocrine loop is required for human breast cancer cell proliferation, motility, and invasion.[75] Willmarth et al[76] proposed that AREG alters EGFR internalization and degradation in a way that favors accumulation of EGFR at the cell surface and ultimately leads to changes in EGFR signaling.

Mouse submandibular salivary gland tumors exhibit an acquired resistance to EGFR/ERK pathway kinase inhibitors. MCMV-dependent kinase inhibitor resistance was associated with ectopic overexpression of fibronectin and IL-6 in abnormal stromal cells. The IL-6/IL-6 receptor and fibronectin/integrin binding initiate multifunctional signaling (PI3K/Akt and MEK/ERK), thereby mediating cell growth, differentiation, and survival in the development and progression of tumorigenesis. It would appear that in abnormal stromal cells, MCMV-dependent fibronectin and IL-6 overexpression increases the activation of the targeted kinase ERK and likely induces alternative signaling pathways such as PI3K/Akt. Thus, in the presence of active MCMV, even the highest nontoxic dose of MEK/ERK inhibitor is unable to preclude

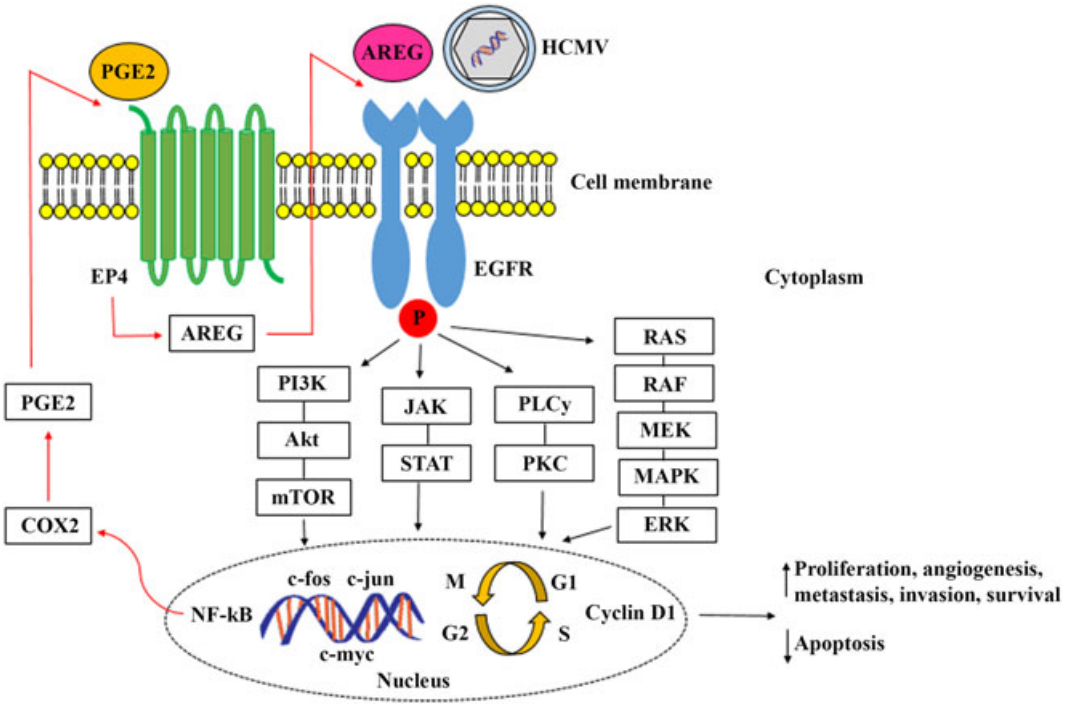

FIGURE 3 Oncogenic signaling pathway. Model of pathogenesis for HCMV-induced tumors of salivary glands

progressive tumorigenesis, nor is the highest dose of EGFR inhibitor or combination of inhibitors.[77]

Uncontrolled stimulation of the ERK pathway leads to cellular transformation and oncogenesis.[78] Rodems and Spector[79] found that HCMV infection resulted in the maintenance of previously activated ERK1/2 by a mechanism that appears to involve the inhibition of cellular phosphatase activity. ERK phosphorylation and activity were sustained for at least 8 hours after infection, and inhibition of the ERK pathway with a MEK inhibitor resulted in decreased expression from the UL112-113 promoter. This indicates that the ERK pathway is involved in regulating HCMV early gene expression and that specific virus-host cell interactions alter the regulation of the pathway to benefit viral replication. It would be advantageous for the virus to maintain the activity of specific regulatory kinases, such as ERK1/2, to ensure that the viral and cellular transcription factors involved in stimulating early viral genes remain in their active form. As such, keeping the ERKs active during infection may lead to higher levels of cellular transcription factors, resulting in enhanced activation of both viral and cellular promoters.

Several viruses constitutively regulate EGFR signaling by expressing viral proteins that transcriptionally regulate *EGFR* gene expression, or act as active components of EGFR signaling, or interfere with the EGFR degradation pathway. All of these chronic stimulations of EGFR signaling or expression may be involved in the development of cancer or viral pathogenesis. Therefore, EGFR phosphorylation and downstream signaling are highly relevant targets for drug therapy.[2]

7 | PERSPECTIVES

Increasing evidence has shown that many viruses, including HCMV, can interact with and modulate EGFR activity. However, some studies have shown that EGFR does not mediate HCMV entry or signaling and that PDGFR, as opposed to EGFR, is required for viral entry. These conflicting results remain unresolved. Upon infection, EGFR phosphorylation occurs, and downstream signaling proteins are activated with efficiencies and kinetic properties resembling those of EGFR's natural ligand, EGF. HCMV also downregulates EGFR expression to isolate infected cells from host-specific signals, forcing cells to respond solely to viral signals, thereby optimizing the cellular environment for productive infection. Heightened EGFR levels at the cell surface may contribute to the establishment of HCMV latency by modulating cell survival, differentiation, or innate signaling. Furthermore, the chronic stimulation of EGFR signaling or expression might be involved in the development of cancer. Thus, the central role of the EGFR network in the life cycle of viral infections suggests that it is a relevant target for antiviral therapy. Therefore, the identification of EGFR as an HCMV receptor may assist in the development of agents that target viral entry.

CONFLICT OF INTEREST

The authors have no competing interest.

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