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REVIEW

Phosphorylation events during viral infections provide potential therapeutic targets

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SUMMARY

For many medically relevant viruses, there is now considerable evidence that both viral and cellular kinases play important roles in viral infection. Ultimately, these kinases, and the cellular signaling pathways that they exploit, may serve as therapeutic targets for treating patients. Currently, small molecule inhibitors of kinases are under investigation as therapy for herpes viral infections. Additionally, a number of cellular or host-directed tyrosine kinase inhibitors that have been previously FDA approved for cancer treatment are under study in animal models and clinical trials, as they have shown promise for the treatment of various viral infections as well. This review will highlight the wide range of viral proteins phosphorylated by viral and cellular kinases, and the potential for variability of kinase recognition sites within viral substrates to impact phosphorylation and kinase prediction. Research studying kinase-targeting prophylactic and therapeutic treatments for a number of viral infections will also be discussed. Copyright © 2011 John Wiley & Sons, Ltd.

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INTRODUCTION

The phosphorylation of viral and cellular proteins can have major impacts on viral infection, replication, and cytotoxicity in a host cell. The phosphorylation of proteins is a reversible post-translational modification. The addition of a negatively charged phosphate group by kinases (and potential removal of the phosphate group by phosphatases) can regulate a viral protein's stability, activity, and interactions with other cellular and viral proteins [1]. Up to 30% of all human proteins may be modified by kinase action [2,3] and clearly a number of viral proteins in human infections are also phosphorylated, but this has not yet been cataloged or studied systematically.

Phosphorylation events are ways in which infectious agents can exploit cellular signaling pathways for their own replication and propagation benefits [4]. Upon a viral infection, a number of cellular signaling pathways (including the MAPK and Jak/STAT pathways) utilize cellular phosphorylation events stimulated by viral proteins [5,6]. For example, WNV infection of microglial cells is associated with an increase in p38 MAPK, ERK, and JNK phosphorylation [7]. This phosphorylation and activation of the p38 MAPK and ERK pathways may induce chemokine and cytokine production in WNV-infected microglial cells [7]. EBV LMP1 activates the PI3K/Akt pathway, as LMP1 expression induces the phosphorylation of Akt; this signaling pathway is involved in the actin cytoskeleton reorganization of EBV-infected cells [8]. There are many ways that viral infections can induce and/or inhibit cellular signaling pathways, but it is clear that one common mechanism is through the phosphorylation of cellular and viral proteins.

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Abbreviations used
CDK, cyclin-dependent kinase; CKI, casein kinase I; CKII, casein kinase II; CSK, c-terminal Src kinase; DENV, dengue virus; DYRK1A, dual-specificity tyrosine-phosphorylation-regulated kinase; ERK1/2, extracellular signal-regulated kinase; FDA, Food and Drug Administration; Flk-1/KDR, fetal liver kinase 1/kinase insert domain receptor; GAPD-PK, glyceraldehyde-3-phosphate dehydrogenase protein kinase; GSK, glycogen synthase kinase; HBx, hepatitis B X protein; HCMV, human cytomegalovirus; HPV, human papillomavirus; Jak/STAT, Janus kinase/signal transducer and activator of transcription; JNK, c-Jun N-terminal kinase; LMP1, latent membrane protein 1; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NS2, NS5(A), nsP, non-structural proteins; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PI3K, phosphatidylinositol 3-kinase; PLK1, polo-like kinase 1; PRK2, protein kinase C-related kinase 2; Ser, serine; SRPK1, serine–arginine-specific protein kinase; Thr, threonine; WNV, West Nile virus; YFV, Yellow Fever virus.

This review will focus on medically relevant animal viruses. Phosphorylation of plant viral proteins is covered in various recent articles and reviews [9,10]. Here, we summarize various topics relating to phosphorylation during the course of medically relevant viral infections. The variation in kinase recognition motifs in viral proteins, the phosphorylation of non-protein small molecules in viral therapies, and the study of kinase inhibitors for use as treatments for poxviruses and herpesviruses will be highlighted.

Viral proteins are phosphorylated by a variety of cellular and viral kinases

Table 1 lists kinases that have been demonstrated to phosphorylate viral proteins in a number of medically relevant viruses. The table includes viruses encoding proteins that were phosphorylated in an isolated experimental system by a single, identified kinase. Although evidence such as phosphorylation/kinase prediction data and kinase inhibitor data is also very useful, this table includes only viral protein substrates that were experimentally phosphorylated by purified and/or isolated kinases. For those viruses listed as having proteins phosphorylated by an "unknown kinase" in Table 1, the viral proteins were shown to be phosphorylated, but biochemical data have not yet identified the kinase responsible. The table is representative of kinases that have been found to phosphorylate viral proteins and demonstrates the extensive role that phosphorylation plays in medically relevant viral infection, if not 100% inclusive of the published literature. Viral phosphorylation events in Table 1 were found through PubMed literature searches by using the search terms "(specific virus) + kinase" and/or "(specific virus) + phosphorylation."

Once specific phosphorylation sites of viral proteins are identified, mutational analyses are necessary to determine any potential phenotypic effects of a specific phosphorylation. For example, the HIV-1 protein p6, which contains the late domain involved in virus budding, was determined to be a phosphoprotein phosphorylated by multiple kinases [11]. Further analysis identified a specific phosphosite (Thr23) that was phosphorylated *in vitro* and *in vivo* by the MAPK, ERK-2 [12]. When Thr23 was mutated to an alanine (thus blocking phosphorylation of the site) within the virus, the mutant showed reduced infectivity as well as defective viral particle maturation and budding [12]. Further work is necessary to determine more precisely how exactly the Thr23 phosphorylation is leading to the observed effects on the virus, but this example highlights the ability of phosphorylation at a specific site on a viral protein to impact a viral life cycle. As a caveat, when performing mutational analyses, it is important to ensure that the non-phosphorylatable mutant protein is stably expressed to similar levels as the wild-type protein to more clearly associate phenotypic effects to the absence of a specific phosphorylation.

In a number of cases, multiple kinases are able to phosphorylate the same viral protein (for example, multiple cyclin-dependent kinases phosphorylate human adenovirus E1A [13] and the HIV-1 Rev protein is able to be phosphorylated by CKII, MAPK, and CDK1 *in vitro* [14]). A number of kinases, including CKI and CKII, phosphorylate the HCV NS5A protein and contribute to NS5A's hyperphosphorylated form [15,16], although in this specific case, CKI and CKII phosphorylate different sites within NS5A. Although both of these phosphorylations may be involved in transitioning the viral protein from genome replication to particle assembly, the sites are distinct [16]. Although the aforementioned *in vitro* data are helpful in the preliminary identification of kinases, *in vivo* experiments must also be performed to determine if multiple kinases are in fact phosphorylating a viral protein in an infected cell. By utilizing multiple kinases to phosphorylate a viral protein, a virus could have the ability to expand its host and cellular tropism and infect different species and cell types with varying kinase profiles. Additionally, kinase redundancy provides multiple opportunities for a viral protein to be phosphorylated, ensuring the chance for the phosphorylated protein to induce pathogenic effects on the cell. Examples of kinase redundancy exist for a number of viruses, including poxviruses [17–19] and Ebola virus [20]. Vaccinia virus is phosphorylated by members of both the Src and Abl kinase families [21], and these kinases are involved in viral particle release [17]. Inhibiting either the Src or Abl family does not block viral release, but inhibiting both kinase families strongly inhibits the release of viral particles in cell culture [17,19]. This example of kinase redundancy illustrates how the presence of one kinase or family of kinases may be sufficient to induce a virus' pathogenic effect, even in the absence of the other kinases utilized by the virus. Kinase

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Table 1. Medically relevant viruses are phosphorylated by cellular and viral kinases

Viral genus	Virus	Cellular kinase (viral substrate)	Viral kinase (viral substrate)
Single-strand (+) RNA			
Flavivirus	WNV	PKG (NS5 [61]), PKC (capsid [115])	
	DENV	PKG (NS5 [60]), CKII (NS5 [116])	
	YFV	CKI (NS5 [117])	
	Tick-borne encephalitis virus	Kinase unk. (NS5 [118])	
Hepacivirus	HCV	CKIα (NS5A [15]), PKA (core [119], NS5A [120]), PKC (core [119]), CKII (NS5A [16,121], NS2 [122]), PRK2 (NS5B [123]), PLK1 (NS5A [124]), MAPK (NS5A [125]), Akt (NS5A [125]), p70S6K (NS5A [125])	
Coronavirus	Severe acute respiratory syndrome coronavirus	ERK1/2 (nucleocapsid (N) [126]), cyclin-CDK (N [126]), SRPK1 (N [127]), GSK-3 (N [128])	
Alphavirus	Sindbis	Kinase unk. (nsP3 [129,130])	
	Semliki forest	Kinase unk. (nsP3 [131])	
Rubivirus	Rubella	Kinase unk. (capsid [22])	
Aphthovirus	Aphthovirus	Kinase unk. (VP3, VP4 [132])	
Picornavirus	Encephalomyocarditis virus	Kinase unk. (leader [133])	
Hepevirus	Hepatitis E	MAPK (ORF3 [134])	
Single-strand (−) RNA			
Rhabdovirus	Vesicular stomatitis virus	CKII (phosphoprotein (P) [135,136])	
	Rabies	PKCγ (P [137])	
Paramyxovirus	Human parainfluenza	PKCζ (P [138]), PLK1 (P [139])	
	Sendai	PKCζ (P [140])	
	Human respiratory syncytial virus	CKII (P [141]), CKI (P [142])	
	Measles (Morbillivirus)	CKII (P [143]), c-src (P [144])	
Orthomyxovirus	Influenza A	PKC (M1 [145], PB1 and NS1 [146], PB1-F2 [147]), CKII (PA [148])	
Filovirus	Ebola	Kinase unk.(VP30 [149])	

Continues

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Table 1. (Continued)

Viral genus	Virus	Cellular kinase (viral substrate)	Viral kinase (viral substrate)
	Marburg	Kinase unk. (nucleoprotein [150],VP30 [151])	
Double-strand RNA			
Reovirus	Rotavirus A	CKI (NSP5 [59]), CKII (NSP5 [152])	NSP5 (NSP5 [45])
Retrovirus			
Lentivirus	HIV	Cdk2 (Tat [153]), PKC (Gag [154], Nef [155]), CKII (Rev [14]), MAPK (Rev [14]), Cdk1 (Rev [14])	
Double-strand DNA			
Poxvirus	Vaccinia virus	Src, Fyn, Yes, Abl, Arg (A36R [21])	F10 (A17 [26])
Papillomavirus	HPV	CKII (E7 [156]), SRPK1 (HPV1 E1^E4 [157]), PKN (HPV16 E6 [158]), PKA (HPV1 E4 [159], HPV11 E1^E4 [160]), PKC (HPV6 E7 [161]), MAPK (HPV11 E1^E4 [160]), DYRK1A (HPV16 E7 [162])	
Adenovirus	Human adenovirus	CKII (E1A [163]), MAPK (E1A [164]), Cdk4 (E1A [13]), Cdk2 (E1A [13]), Cdk1 (E1A [13])	
Herpesvirus	Varicella zoster virus	CKII (gpI [165]), Cdk1 (IE63 [166]), CKI (gpI [165])	ORF47 (ORF62 [167,168], gE [169], ORF32 [170], ORF63 [168], ORF47 [30,167]), ORF66 (IE62 [171,172])
	HSV-1	PKA (VP13/14 and VP22 [173], ICP27 [174]), CKII (VP1/2 and VP13/14 [173],VP22 [173,175], ICP27 [174]), PKC (VP13/14 and VP22 [173], ICP27 [174]), Lck (VP11/12) [176]	Us3 (UL31, ICP22, Us9 [177]), UL13 (US3 [178] gE/gI [179], VP22 [173], ICP22 [180], ICP0 [181], UL13 [30,48])
	Human cytomegalovirus	ERK2 (IE2 [182]), CKII (IE2/IEP86 [183]), PKA (IE2/IEP86 [183]), PKC (IE2/IEP86 [183]), JNK1 (IE2/IEP86 [183]), Cdk1 (IE2/IEP86 [183])	UL97 (UL97 [46])
	EBV	CKII (EB2 [184], LMP1 [185]), Cdk1 (EBNA-LP [33],EBNA2 [186]), MAPK (LMP2 [187]), CSK (LMP2 [188]), PKC (BZLF1 [189]), lck (LMP2A [190]), lyn (LMP2A [190]), fyn (LMP2A [190])	BGLF4 (EA-D [32], BZLF1 [31], BGLF4 [47])
	Kaposi's sarcoma-associated herpesvirus	Cdk1 (K-bZIP [191]), Cdk2 (K-bZIP [191]), p38MAPK (kaposin B [192]), CKII (ORF57 [193])	ORF36 (ORF36 [194])
Hepadnavirus	HBV	PKA (core [195,196]), PKC (HBx [197], core [198]), ERK1/2 (HBx [199]), SRPK1 (core [200]), SRPK2 (core [200]), MAPK (HBx [197]), GAPD-PK (core [201])	

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redundancy must also be considered when designing and using kinase inhibitors, because inhibitors are not necessarily entirely specific for one kinase or kinase family. For example, Sprycel acts on both Src and Abl cellular kinase families and has a more profound effect in cell culture than an inhibitor that only affects Abl. In the mouse though, this less specific inhibition may not be helpful, as will be discussed later [17].

A number of viral proteins have been found to have phosphorylated forms, but the kinases responsible are yet to be identified. However, the importance of phosphorylation in the viral life cycle can be investigated even without the identity of kinase(s). The Rubella virus capsid protein, for instance, is phosphorylated at various sites by an unknown kinase(s), and these phosphorylations are necessary for optimal viral replication [22,23].

CKII and cyclin-dependent kinases phosphorylate viral protein from diverse viral families

A number of cellular kinases appear repeatedly in Table 1, phosphorylating proteins from many different viruses. For example, CKII has been shown to phosphorylate proteins from nearly 50% of the viruses listed. CDKs are also utilized by a number of viruses. The use of CDKs by viruses is understandable, as kinases regulating the state of cellular growth and replication would seem to be an obvious target for viral manipulation. Table 1, however, is not based on tissue data. Variable expression of kinases in different tissues could affect which kinase(s) phosphorylate proteins from different viruses, depending on which tissue(s) are infected by a particular virus. There have been no systematic evaluations of which cellular kinases are most central to different viral life cycles. The preponderance of CKII, for example, could be due to the ease of demonstrating phosphorylation of viral proteins by CKII as opposed to CKII being more important in viral life cycles than other cellular kinases.

Virally encoded kinases are able to phosphorylate viral and cellular substrates and be autophosphorylated

In addition to cellular kinases phosphorylating viral proteins, some viruses encode their own kinases (as first described by Bishop and Varmus) [24,25]. Virally encoded kinases may phosphorylate cellular substrates [26–28], which may impact these cellular proteins' function and activity [29]. Viral kinases may also phosphorylate other viral proteins and/or be autophosphorylated [30], potentially affecting viral replication or production within the cell. The EBV-encoded protein kinase BGLF4 is able to phosphorylate a number of viral proteins, including Epstein Barr nuclear antigen (EBNA-2) [31–34]. EBNA-2, a transcriptional regulator, is hyperphosphorylated during mitosis by cdk1 during the virus' latent phase [35]. The viral kinase BGLF4 also phosphorylates EBNA-2, in a manner similar to the cellular kinase cdk1, during the lytic phase [34]. This hyperphosphorylation of EBNA-2 inhibits EBNA-2's normal ability to transactivate the EBV LMP1 promoter; the regulation of the LMP1 promoter via BGLF4's EBNA-2 phosphorylation may induce the continuation of EBV's lytic replication cycle [34]. Although not all phosphorylations may have clear-cut effects on viral life cycles, it is of use to investigate the role of specific phosphorylations to more fully understand the role of that viral protein in an infected cell.

Viral kinases may also be autophosphorylated. Autophosphorylation of cellular kinases can occur intermolecularly [36] or intramolecularly [37]. Autophosphorylation can positively [38,39] or negatively [40] regulate a cellular kinase's catalytic activity, potentially by altering the enzyme's conformation [41]. Phosphorylation of a cellular kinase can also influence its interaction with other proteins — for example, tyrosine phosphorylation of specific residues within Src-family kinases is required for Src's interaction with proteins' SH2 domains [42–44]. In Table 1, rotavirus NSP5 [45], HCMV UL97 [46], EBV BGLF4 [47], and HSV-1 UL13 [48] have all displayed an ability to autophosphorylate, although the full effects of these autophosphorylations on protein activity remain under investigation. As reviewed in Michel and Mertens [49], HCMV UL97 is autophosphorylated, but there are conflicting data regarding the role of this autophosphorylation in UL97's ability to phosphorylate and interact with other proteins. It is not definitively known whether autophosphorylation of viral kinases will induce the same effect(s), such as regulating catalytic activity and recruiting proteins for interaction, as autophosphorylation of cellular kinases. Evidence comparing the autophosphorylation of a specific site between

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homologous cellular and viral (Rous sarcoma virus) Src kinases suggests that the effects of a specific autophosphorylation could differ between even related cellular and viral kinases [39,50]. Further studies are necessary to determine how autophosphorylations of viral kinases affect these proteins' activity, structure, and function.

Viral proteins may be phosphorylated at "non-canonical" kinase recognition motifs

Many kinases have characterized recognition motifs — substrate sequences that are phosphorylated most efficiently by a particular kinase. A number of motif-recognition programs, such as NetPhosK [51] and Scansite [52], have been developed to predict phosphorylation sites and kinases involved on the basis of substrate sequences. Although very useful, these search engines cannot account for some factors such as a cellular localization signal distant from a kinase substrate site that might sequester an otherwise perfectly good substrate away from a kinase. Some, but not all, of the research cataloged in Table 1 was aided by bioinformatic queries to try to identify specific phosphorylation sites within viral proteins, but for all of the references listed in Table 1, there are experimental data supporting that phosphorylation of viral proteins occurred as well. Because a great deal of research has been performed in identifying cellular substrates of kinases [53–56], it is worth noting any variation in the amino acid sequence of kinase recognition sites between cellular and viral substrates that still allows for phosphorylation of the viral substrate.

A number of viral substrates are phosphorylated at sites that match known kinase recognition motifs. For example, the hepatitis C virus phosphoprotein NS5A is phosphorylated *in vitro* by CKI at its Ser2204 site, and in a peptide corresponding to this region of NS5A, Ser2204 is phosphorylated most efficiently when Ser2201 has previously been phosphorylated [15]. The CKI phosphorylation of Ser2204 fits with a canonical CKI recognition site that expects a phosphorylated residue in the −3 position [57].

Variations in the CKI recognition site have been noted in the literature and may depend on tertiary structure as well as primary amino acid sequence [58]. However, viral amino acid sequences that differ from canonical kinase recognition sites may impact the ability of current programs to predict phosphorylation and specific kinase recognition sites. Eichwald *et al*. [59] determined that CKI is capable of phosphorylating rotavirus NSP5 at its Ser67 residue. Ser67 lacks a phosphorylated residue at the −3 position and a string of three to four acidic residues, so the Ser67 phosphorylation site deviates from the ideal CKI recognition site [57]. As such, neither NetPhosK nor Scansite programs recognize Ser67 as a potential phosphorylation site, let alone a site able to be phosphorylated by CKI. Likewise, the vaccinia virus A36R protein is able to be phosphorylated at Tyr112 by both Src and Abl tyrosine kinases [21]. However, the Tyr112 site does not sit within a canonical Abl kinase recognition site, as it lacks a proline or phenylalanine at the +3 position. Both NetPhosK and Scansite predicted that only Src would phosphorylate A36R Tyr112. In another example of kinase recognition site variation among related viruses, protein kinase G (PKG) phosphorylates DENV NS5 protein [60], YFV NS5, and WNV NS5 [61] at two sites that both differ substantially from the canonical PKG substrate sequence, R-K-R-K-S/T [62]. The flaviviral PKG sites additionally differ from each other, with no clear motif to be identified from these viral substrates (Figure 1). Curiously, while DENV Thr449 and YFV Ser450 sites are both phosphorylated by PKG within the same C-X-T/S-C motif [60], NetPhosK analysis does not predict any Thr449 phosphorylation in DENV NS5 but correctly predicts a PKG phosphorylation of Ser450 in YFV NS5. It is possible that kinases may bind substrates in sites distant from the recognition motif where phosphorylation occurs. These distant binding sites may be

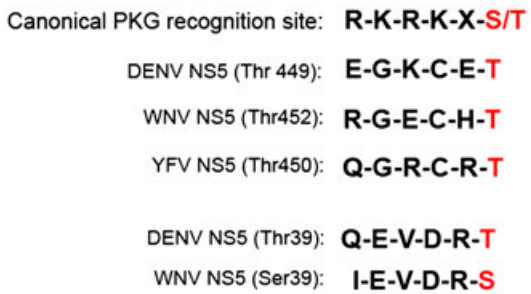

Figure 1. The PKG kinase recognition sites vary in flaviviral substrates. The canonical PKG recognition site was identified in cellular substrates [62]. However, PKG phosphorylation sites (highlighted) that exist in non-canonical PKG recognition sites have been experimentally identified in mosquito-borne flaviviruses [60,61]. Although there is some sequence conservation between viruses at each site, the sequences surrounding each individual site (449/452/450 versus 38) differ from each other, as well as from the canonical PKG recognition motif. An "X" within the sequence denotes any amino acid

important for phosphorylation of a substrate by a specific kinase.

Whereas Scansite's recognition of potential kinase recognition motifs is based on experiments identifying optimal sequences surrounding phosphorylation sites within a peptide substrate library [63], NetPhosK is a neural network-based program. Thus, NetPhosK's criteria for identifying a phosphorylation site and the kinase responsible for the phosphorylation are not easily defined by the programmers or users. Despite the variations from known recognition sequences and/or phosphorylation prediction analyses, biochemical and cell culture data suggest that all of the sites discussed earlier can be phosphorylated by the kinases in question. Thus, although programs such as these provide a good starting point for studying phosphorylation of a viral protein, one must use caution when predicting phosphorylation patterns and the kinases involved in phosphorylating viral substrates as there can be substantial differences between actual and predicted phosphorylations/kinases. Detection of specific phosphorylations has become easier in recent years because of advancements in phosphopeptide enrichment chromatography that is compatible with mass spectrometry [64]. Overall, various other experimental methods, including phosphospecific antibodies and mass spectrometry, are necessary for more rigorous identification of *in vivo* viral (and cellular) site-specific protein phosphorylations [65,66].

Nucleoside analogues are phosphorylated by viral kinases

Nucleosides are a class of non-protein small molecules that includes both drugs and nucleotide precursors for DNA and RNA. Nucleosides frequently contain phosphorylatable hydroxyl groups. Typically, phosphorylation is required for nucleic acid synthesis or blockage of synthesis through the activation of a nucleoside analogue drug. Many herpesviruses, including HSV strains and varicella zoster virus, contain thymidine kinases that are capable of phosphorylating nucleoside analogues [67,68]. HCMV, does not encode its own viral thymidine kinase but has a viral kinase (UL97) that is capable of phosphorylating nucleoside analogues [69].

A number of nucleoside analogues have been developed to exploit these viral kinases for therapeutic purposes. Ganciclovir and acyclovir are guanosine analogues currently used in the treatment of herpesviruses [70,71]. These nucleoside analogues are first phosphorylated by viral kinases [67,69] and subsequently phosphorylated by cellular kinases [72] to form nucleoside triphosphates. The nucleoside triphosphates are incorporated by viral DNA polymerases into the nascent DNA strands, leading to chain termination in the case of acyclovir [73] and internucleotide incorporation in the case of ganciclovir [74]. Overall, the nucleoside analogues inhibit viral DNA replication and thus decrease herpesviral replication [71,75]. Cidofovir, another nucleoside analogue that inhibits herpesviral replication, is only phosphorylated by cellular kinases before being incorporated into viral DNA and terminating the DNA chain [76,77]. Different nucleoside analogue drugs have varying efficacies in inhibiting specific herpesviruses. For example, ganciclovir is more effective than acyclovir in inhibiting HCMV replication, because of the increased accumulation of ganciclovir triphosphate (as compared with acyclovir triphosphate) in HCMV-infected cells [70,78].

As with many drugs used in treatments for viral infections, use of nucleoside analogues in patients can lead to the development of resistance. Mutations in viral thymidine kinases and/or kinases with nucleoside analogue substrates (such as HCMV's UL97) can reduce or eliminate the kinase's ability to phosphorylate nucleoside analogues and lead to viral resistance to the drugs [70,71,79]. On the other hand, because cidofovir is not phosphorylated by viral kinases, mutations in these kinases do not affect cidofovir's efficacy. Additionally, mutations in DNA polymerases can alter the nucleoside analogue's inhibition of DNA synthesis, inducing resistance to the nucleoside analogue treatment [80–82]. Whereas acyclovir and its prodrugs are very well tolerated, the considerable toxicities of ganciclovir (particularly bone marrow suppression) and cidofovir (nephrotoxicity) make strategies such as UL97 inhibition (maribavir and others) and lipidated cidofovir subjects of ongoing research. Overall, however, nucleoside analogues that require viral and cellular kinases have become a mainstay in the treatment of herpesviral infections.

Although nucleoside analogues have been traditional antivirals, it turns out that a specific benzimidazole riboside, maribavir [83], inhibits the HCMV viral kinase UL97 rather than the viral polymerase, and maribavir-resistant mutants map

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to UL97 [84,85]. Phase II studies showed treatment efficacy in HIV, HCMV-coinfected patients, as well as a role in prophylaxis in stem cell transplant patients [86], but a phase III study was unable to show superiority of a maribavir prophylaxis strategy over a pre-emptive therapy with valganciclovir or ganciclovir [87]. Retrospectively, problems with study design may explain this disappointing result [88,89], but regardless, inhibition of UL97 kinase remains an avenue of investigation for antiviral therapy [90].

Tyrosine kinase inhibitors as chemotherapies for poxvirus infection

Poxviral proteins are phosphorylated by a number of cellular tyrosine kinases (Table 1). Src-family and Abl-family tyrosine kinases phosphorylate the vaccinia viral membrane protein A36R [21,91]. The phosphorylation of A36R in cell-associated enveloped virions induces actin tail formation [91,92], allowing viral motility toward the cell surface. Abl-family tyrosine kinases are involved in the detachment of cell-associated enveloped virions from actin tails, leading to the formation of extracellular enveloped virus [17]. Extracellular enveloped viruses are hypothesized to be involved in viral dissemination throughout an infected organism [93]. These effects of cellular tyrosine kinases on viral motility and release are conserved in variola and monkeypox viruses as well [17,19,93].

Because of the importance of tyrosine kinases in poxvirus replication, ongoing studies are examining the ability of tyrosine kinase inhibitors to serve as treatments for poxviruses. Since the tyrosine kinase inhibitor Gleevec (STI-571, imatinib mesylate) first transformed treatment of chronic myeloid leukemia over a decade ago [94,95], a number of tyrosine kinase inhibitors are in use as therapies for cancers [96–99]. In cells infected with poxviruses (vaccinia [17,21], variola, and monkeypox [19]), Gleevec inhibits the Abl family of tyrosine kinases [100] and reduces poxviral extracellular enveloped virus release. Prophylactic treatment of vaccinia-infected mice reduced viral loads in ovaries at 4 days post-infection and increased survival among lethally challenged mice [17]. Therapeutic treatment with Gleevec at 24 and 48 h post-infection likewise increased survival among vaccinia-infected mice (although efficacy decreased as the time between infection and treatment increased) [19]. Additionally, treatment with Gleevec reduced viral dissemination to distal tissues in mice infected intranasally with vaccinia [19]. Sprycel, an inhibitor of both Src-family and Abl-family kinases, strongly inhibited extracellular enveloped virus formation and release in cell culture. However, unlike Gleevec, Sprycel (dasatinib) had minimal effect on mouse survival and *in vivo* viral load, possibly because of effects on the spleen and/or bone marrow caused by the drug's Src inhibition [19].

Poxviruses encode epidermal growth factor (EGF)-like growth factors, which interact with the EGF receptor (EGFR, ErbB-1) [101]. The virally encoded EGF-like growth factors activate ErbB-1's tyrosine kinase activity to induce downstream signaling cascades that promote viral replication [102]. An FDA-approved small molecule inhibitor of ErbB-1 tyrosine kinase activity, IRESSA (gefitinib), decreased viral-induced ErbB-1 and ERK1/2 phosphorylation and activation [102]. IRESSA's effect on ErbB-1 activation and its downstream effects on ERK1/2 were correlated with a decrease in *in vitro* vaccinia viral infection [103] and viral spread, indicated by a dose-dependent decrease in vaccinia plaque number and size [102]. IRESSA is FDA-approved, but its efficacy in inhibiting poxviral replication has yet to be demonstrated in a mammalian system.

Other inhibitors of ErbB-1's kinase activity, while not yet FDA approved, have been studied in mice. Such inhibitors include the 4-anilinoquinazoline family, which includes the small molecule inhibitor Canertinib (CI-1033) [104]. Canertinib reduced variola and vaccinia extracellular enveloped virus formation and/or release in cell culture [104]. *In vivo*, prophylactic treatment with canertinib showed modest effects in increasing vaccinia-infected mouse survival, reducing viral titers in the lung, and augmenting the immune response (increasing levels of IL-1β, IL-1Ra, and IFN-γ) [104]. Combining canertinib chemotherapy with immunotherapy (anti-L1R (Vaccinia protein) antibody treatment) enhanced these effects, especially in post-infection treatments [104].

Adsorption of a rabbit poxvirus (myxoma virus) induces tyrosine phosphorylation of the cellular CCR5 receptor and the tyrosine kinases Jak1 and Jak2 [105]. Reducing the tyrosine phosphorylation of Jak2 was also associated with a decrease in myxoma viral replication [105], suggesting a role in poxviral replication for these tyrosine kinases as well. Peptide

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mimetics of the suppressor of cytokine signaling 1 (SOCS-1) inhibit the tyrosine kinases Jak2 and ErbB-1 [106]. These peptides significantly decreased phosphorylation of Jak2 (and downstream STATs) and ErbB-1 and decreased vaccinia virus replication in cell culture [106]. *In vivo*, the mimetic peptides (SOCS-1-KIR and Tkip) improved survival among vaccinia lethally infected mice when administered both prophylactically and therapeutically [106]. Distal tissues had no detectable levels of virus at 6 days after an intranasal infection in Tkip-treated mice [106].

Some of these tyrosine kinase inhibitors have now been FDA approved for use in cancer for several years, and therefore, considerable knowledge has been accumulated on their side effect profile and risk benefit ration for specific cancers. For example, since Gleevec was FDA approved in 2001, some form of tyrosine kinase inhibition remains mainstay of treatment for chronic myeloid leukemia [107]. The cancer indications for IRESSA have been controversial, but it was first FDA approved in 2003 for salvage use in non-small cell lung cancer, and the decision to use it or other tyrosine kinase inhibitors of the epidermal growth factor receptor as opposed to other chemotherapy with distinct modes of action has more to do with efficacy and cost than toxicity (IRESSA's generally mild toxic effects, such as acne and dermatologic conditions, respond to treatment interruptions) [108]. The efficacy of tyrosine kinase inhibitors against poxviruses in animal models is promising but has yet to be used in patients to our knowledge. Treatments, including cidofovir [109], for poxviruses are currently limited and may have toxic effects; thus, the potential development of tyrosine kinase inhibitors as safe and effective prophylactic and therapeutic treatments for poxviruses is a point of interest in current research.

Kinase inhibitors approved for transplant immunosuppression may also effectively treat viral infections

One use of kinase inhibitor(s) that has already become a reality is in the treatment of virally infected patients, including the treatment of Kaposi's sarcoma (KS) post-transplant by sirolimus/everolimus (rapamycin) [110]. Sirolimus and everolimus are serine/threonine kinase inhibitors of the cellular mTOR kinase. KS-associated herpesvirus (also known as human herpesvirus 8) can cause neoplastic hypervascular lesions in both immunocompromised and, rarely, immunocompetent patients infected by the virus. This is particularly common in patients with HIV (20 000× the rate of the general population) or after a solid organ or bone marrow transplant patient (500× the rate of the general population). Although no single approach to treating KS has become universally accepted, before 2004 it was common practice in transplant patients who developed KS to limit the use of immunosuppressants, leading to the resolution of some, but not all, KS-related disease. A complete stop in immunosuppression, though, left patients vulnerable to graft rejection. In 2004/5, two groups [110,111] reported a series of patient as well as accompanying animal and tissue data suggesting that switching a solid organ transplant patient's post-transplant immunosuppressive cocktail from a cyclosporine-based regimen to one that included sirolimus (rapamycin) resulted in a complete resolution of their biopsy-proven KS lesion. Subsequent reports (reviewed by Stallone *et al*. [112]) suggest that other mTOR inhibitors (everolimus) have the same effect and that mTOR inhibition plays an important role in the resolution of KS, although the degree to which the mechanism is less immunosuppression versus blockage of phosphorylation event(s) remains a subject of research. Mechanistic data included in these reports though do show that endothelial cells from KS tumors have upregulated vascular endothelial growth factor (VEGF) receptor FLK-1/KDR and that mTOR inhibitors block the interaction of VEGF with FLK-1/KDR and limit the cellular response to VEGF.

An ever-widening array of kinase inhibitors is becoming approved for cancer and other therapy. Patients with virally associated cancers such as HCV-related and HBV-related hepatocellular cancer, as well as HPV-related cervical and head and neck cancer, may receive these kinase inhibitor-based therapies on the basis of their cancer diagnosis [113,114]. Some of these kinase inhibitors have cost and toxicity issues that must be taken into account if research into their utility as antivirals is to be undertaken. Nevertheless, as the efficacy of sirolimus and everolimus has demonstrated, kinase inhibitors could have a role to play in combating viral infections.

CONCLUSION

A wide variety of both cellular and viral kinases impact viral replication. These kinases phosphorylate viral protein substrates and small non-protein

molecules and promote the initiation and continuation of cellular signaling pathways. Many medically relevant viruses have evolved to exploit the activity of these kinases to promote viral replication. Thus, these cellular and viral kinases may serve as targets for prophylactic and therapeutic treatments of viral infections. Kinase-inhibitory compounds have previously been successful in treating various cancers, and research is ongoing to determine these drugs' efficacies in treating viral infections.

CONFLICT OF INTEREST

The authors have no competing interest.

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