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Viral Product Trafficking to Mitochondria, Mechanisms and Roles in Pathogenesis

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Abstract

A wide variety of viruses cause significant morbidity and mortality in humans. However, targeted antiviral therapies have been developed for only a subset of these viruses, with the majority of currently licensed antiviral drugs targeting viral entry, replication or exit steps during the viral life cycle. Due to increasing emergence of antiviral drug resistant viruses, the isolation of multiple viral subtypes, and toxicities of existing therapies, there remains an urgent need for the timely development of novel antiviral agents, including those targeting host factors essential for viral replication. This review summarizes viral mitochondrial products and their effects on common mitochondria regulated pathways. These viral products and the mitochondrial pathways affected by them provide potential novel targets for the rational design of antiviral drugs. Viral products alter oxidative balance, mitochondrial permeability transition pore, mitochondrial membrane potential, electron transport and energy production. Moreover, viruses may cause the Warburg Effect, in which metabolism is reprogrammed to aerobic glycolysis as the main source of energy. Finally, viral products affect proapoptotic and antiapoptotic signaling, as well as mitochondrial innate immune signaling. Because of their importance for the generation of metabolic intermediates and energy as well as cell survival, mitochondrial pathways are targeted by multiple independent viral products. Structural modifications of existing drugs targeted to mitochondrial pathways may lead to the development of novel antiviral drugs with improved efficacy and reduced toxicity.

Keywords

Antiviral drugs; calcium signaling; endoplasmic reticulum; human viruses; metabolism; mitochondria; mitochondria-associated membranes; reactive oxygen species

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INTRODUCTION

Viruses of multiple families cause significant clinical pathology in both immunocompetent and immunocompromised individuals. Although viral infection may result in minor self-limited disease, such as upper respiratory tract and gastrointestinal illnesses, in the general population, the economic impact to society in terms of disrupted work and school attendance is nonetheless significant. Viral infection may also result in severe life-threatening disease such as meningoencephalitis, myocarditis, fulminant hepatitis, lower respiratory tract disease, and disseminated multiorgan disease, with resultant significant morbidity and mortality. Individuals at both extremes of age, as a function of their relatively impaired immune responses, are at higher risk of severe disease following infection with multiple viruses (such as influenza in the elderly and herpes simplex virus, HSV, in neonates). As stem cell and organ transplantation have become more prevalent, the use of accompanying immunomodulating agents has been associated with increased risk for a variety of life-threatening viral infections (including HSV, human cytomegalovirus, CMV, and adenovirus infection) in these hosts. Similarly, advancements in the treatment of a variety of autoimmune disorders using potent immunomodulatory agents (such as inhibitors of tumor necrosis factor alpha, TNFα, and other biologics) have resulted in additional patient populations at high risk for severe viral infection.

In addition to the need for specific antiviral agents to treat these patients in the setting of clinical disease, development of nontoxic agents for prophylaxis in high-risk populations is also a high priority. Advances in quantitative viral molecular detection techniques have introduced the possibility of routine screening and preemptive therapy for multiple viruses (such as CMV, HSV, and adenovirus), prior to the development of clinical disease. However, in addition to the paucity of effective virus-specific therapies, the toxicities of available drugs (such as bone marrow suppression, renal and hepatic toxicity) in these already vulnerable populations, as well as the emergence of viruses resistant to current antiviral drugs, have provided significant barriers to their effective clinical use. Thus, development of novel and specific antiviral therapies with limited toxicity is a high clinical priority.

Viruses depend upon cellular metabolism for the production of macromolecular substrates and energy during replication. Many viral products target mitochondria and alter metabolic pathways during viral growth. Some of these viral products increase viral pathogenicity or virulence. This review focuses on potential new viral targets as well as cellular targets of mitochondrial regulated pathways that are altered by human viral infection for the development of new antiviral drugs.

Mitochondria, as sites of pyruvate and fatty acid oxidation, the tricarboxylic acid (TCA) cycle and electron transport system, are powerhouses for cellular energy production and provide sites for the synthesis of macromolecular precursors for virus production as well as integration of proapoptotic and antiapoptotic signaling and mitochondrial innate immune signaling [1,2]. Viruses whose replication is prolonged and inherently dependent on continued cell survival for the production of progeny viruses have, in turn, evolved

countermeasures to enable their replication by targeting cellular defense mechanisms [3]. Further, mitochondria provide high capacity, low affinity calcium (Ca^{2+}) stores and actively participate in Ca^{2+} signaling, which affects ATP production, reactive oxygen species (ROS) production and can alter apoptotic signaling [4]. Recent studies [5] implicate the induction of the Warburg Effect, a metabolic reprogramming observed in tumor cells [6] from mitochondrial oxidative phosphorylation to aerobic glycolysis, as the main source of energy production for the maintenance of Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus type 8 (HHV-8) latency and for human cytomegalovirus (CMV) permissive infection.

Herein, we review the targeting of viral products to mitochondria and of mitochondrial pathways altered by human virus infection. We focus on medically important viruses for which there are urgent unmet medical needs to provide new targets for antiviral drug design. Whenever possible, we cite current reviews on partially overlapping fields. This review is not meant to be exhaustive and cannot fully encompass the work of all investigators due to space limitations.

TRAFFICKING OF HUMAN VIRUS PROTEINS TO MITOCHONDRIA

A large number of viral products localize in mitochondria and interact with cellular mitochondrial proteins. Some of these traffic directly from the cytosol to mitochondria similarly to most cellular mitochondrial proteins, wherein they affect mitochondrial functions such as maintaining oxidative balance, altering the mitochondrial permeability transition pore (PTP) and mitochondrial membrane potential ($\Delta\Psi_m$), electron transport and energy production. In contrast, other viral proteins traffic sequentially from the endoplasmic reticulum (ER) to mitochondria through the mitochondria-associated membrane (MAM) sub-compartment of the ER. The MAM can regulate key mitochondrial functions by Ca^{2+} signaling [7]. Viruses can furthermore directly target components of mitochondrial innate immune signaling and apoptotic signaling to enhance their own replication programs.

Roughly ninety-nine percent of the approximately 1,000 cellular proteins which constitute mitochondrial organelles are encoded by the nuclear genome and must be transported to mitochondria, rather than being nascently translated within the mitochondria by mitoribosomes [8]. Kept unfolded by interactions with soluble cytosolic chaperones, such as the ATP-dependent mitochondrial import stimulation factor, Hsc70, the 90 kilodalton (kDa) heat shock protein (Hsp90), and several 40 kDa heat shock protein-like J-domain proteins [9-11], most nascent mitochondrial proteins are translocated through the OMM and subsequently traffic to one of the four discrete submitochondrial destinations [12-15]. The double-membraned structure of mitochondria divides the organelle into four distinct destinations for incoming proteins: the outer mitochondrial membrane (OMM), intermembrane space (IMS), inner mitochondrial membrane (IMM), and the mitochondrial matrix. Each of these trafficking pathways utilizes the common translocon complex within the OMM, called the translocase of the outer membrane (TOM). This multi-subunit TOM complex consists of a central pore protein, Tom40; receptor proteins, Tom20 and Tom70; a Tom22 subunit involved in complex stability; and three small accessory proteins, Tom5, Tom6, and Tom7 [16-22].

Best characterized is the matrix-targeting pathway utilized by precursor proteins containing a cleavable, NH_2-terminal presequence comprised of a positively-charged amphipathic helix. These matrix-targeted preproteins interact with Tom20 and Tom22 on the OMM [23]. Upon binding to Tom20 and Tom22, precursor proteins are threaded through the OMM pore, Tom40, to the IMS. As they emerge into the IMS, a subunit of the translocase of the inner mitochondrial membrane (TIM), Tim50, stimulates the interaction of the nascent protein with a tail domain of Tom22. Subsequently, a Tim21 subunit binds the Tom22 tail within the IMS, physically linking the TOM and TIM complexes. The preprotein is then threaded through the central pore of the TIM complex, known as Tim23, to the mitochondrial matrix. Translocation into the matrix requires a functional $\Delta\Psi_m$ across the IMM, as well as the cooperation of a presequence translocase-associated motor complex. Within the matrix, mitochondrial processing peptidase cleaves the presequence and allows the remaining protein to be folded into its mature form.

However, many mitochondrial proteins do not contain cleavable presequences. Such proteins can be targeted to the mitochondria through internal targeting signals or hydrophobic domains. These nascent polypeptides are directed to the Tom70 receptor of the OMM, and through the Tom40 pore subunit [24,25]. From here, noncleavable precursor proteins can be directed to three separate pathways. Proteins of the IMS subsequently interact with the mitochondrial intermembrane space assembly system, which facilitates importation into the IMS and oxidizes cysteine motifs [26-29]. Polytopic proteins of the IMM interact with a soluble hexameric Tim9/Tim10 complex within the IMS. This Tim9/Tim10 complex transfers the nascent proteins to the IMM metabolite carrier translocase, Tim22, which mediates proper membrane insertion and folding [30-32]. Likewise, OMM β-barrel proteins are imported into the IMS through Tom40, interact with the soluble Tim9/Tim10 complex, and subsequently, are transferred to the sorting and assembly complex of the OMM. This complex directs membrane insertion of these β-barrel proteins into the OMM [33,34].

Mitochondrial proteins of the outer membrane also do not contain cleavable presequences, and most of them span the OMM once, or several times, with α-helical hydrophobic transmembrane domains (TMDs) [35-37]. Even though α-helix anchored proteins represent the majority of proteins within the OMM, very little is known about how they are imported [38]. A hydrophobic TMD along with downstream flanking residues often are sufficient for targeting proteins to the OMM. This signaling information can closely resemble the signal recognition particle-dependent NH_2-terminal signal sequences utilized in ER translocation. In fact, studies suggest that for traditional ER-targeting signal sequences of moderate hydrophobicity (defined as a hydropathy of 1.97-2.16 on the Kyte-Doolittle scale), the existence of one or more basic amino acids within five residues from the end of the TMD α-helix is sufficient to direct the protein to be imported into the OMM [38,39]. Alternatively, studies with the OMM proteins, Tom5 and Tom20, demonstrated that low TMD hydrophobicity can also mark the escape from ER-targeting machinery and allow mitochondrial OMM importation [35,38]. Mutations, which increased the hydrophobicity of these sequences, caused mislocalization to the ER.

In contrast to nascent protein import into mitochondria, which relies heavily on the ability of chaperones to keep a newly translated protein in an unfolded, immature conformation; mature, folded proteins are also known to target mitochondria for import. The most recognized mechanism involves conformational changes in response to discrete intracellular signals, exposing hidden domains that then facilitate peripheral membrane association or integral membrane insertion into the OMM. One example is the proapoptotic Bax protein, which upon activation in the cytosol, exposes an NH_2-terminal α-helical TMD [40]. Once exposed, this domain can directly interact with Tom22, leading to mitochondrial importation of Bax monomers through the TOM complex of the OMM, or can facilitate a less efficient direct membrane insertion of oligomerized Bax from the cytosol into the OMM in a TOM-independent manner [41].

Less well-understood mechanisms of sequential trafficking of mature proteins from ER to mitochondria have also been described. First reported in rat liver cells, a purified mitochondrial protein was discovered to originate within the ER due to its modification with endoglycosidase H sensitive, *N*-glycosyl oligosaccharides [42]. While this protein was never identified, its presence in mitochondria, which could be inhibited with tunicamycin treatment, established the existence of sequential ER-to-mitochondria trafficking of cellular proteins. Further evidence of sequential ER-to-mitochondria trafficking of mature proteins comes from HCMV UL37 proteins [43-46]. A highly conserved leader sequence encoded by exon 1 of the HCMV UL37 gene drives ER translocation of these proteins, MAM association, and subsequent trafficking to the OMM [47]. The UL37 exon 1 protein (pUL37x1), also known as viral mitochondrial-localized inhibitor of apoptosis (vMIA), is the most abundant UL37 protein isoform detected throughout HCMV infection [45] and can elicit separate functions in the ER and mitochondria [48-53], consistent with its mature conformation in both sub-compartments. The core protein of hepatitis C virus (HCV) was also found to move sequentially from the ER, where it is cleaved to its mature form by the signal peptidase enzyme, to the OMM [54-56]. This sequential trafficking was suggested to be mediated by the MAM, which comes into direct physical contact with mitochondria organelles, as well as lipid droplets, which bud from the MAM membrane [56].

Lastly, viral RNAs have also been found to target mitochondria. HCMV β2.7 mRNA can target mitochondrial complex I (reduced nicotinamide adenine dinucleotide-ubiquinone oxido-reductase) in the IMM [57]. mRNA targeting to the surface of mitochondria is well documented, first described to occur in yeast where nearly 47% of all mitochondria-targeted proteins demonstrated discrete accumulations of their mRNAs on the OMM. Later studies revealed the role of the 3′ untranslated region (UTR) in targeting mRNAs to the OMM, for the purpose of increasing efficiency of mitochondria import of encoded proteins [58,59]. The HCMV β2.7 mRNA is unique, however, in that it is functional without translation, and traffics to the IMM to play a role in protecting cells from apoptosis.

Viruses may employ any of these pathways to target their encoded products to mitochondria. It is likely that the ability of viral proteins to manipulate mitochondrial functions may depend on their trafficking route, as the cellular host proteins with which they are likely to interface can differ between various pathways of mitochondrial importation. Indeed, the study of mitochondria-targeted viral proteins helps us not only better characterize

mechanisms by which mitochondria interact with the rest of the cell, but also may provide unique antiviral targets for future therapeutics.

CALCIUM FLUX BETWEEN THE ENDOPLASMIC RETICULUM AND MITOCHONDRIA

Communication between the ER and mitochondria is important for cellular bioenergetics and cell survival [60]. The MAM, transient contact sites between the ER and mitochondria [7,61], provides enriched Ca^{2+} microdomains for mitochondrial signaling, regulation of mitochondria innate immune signaling, apoptotic signaling and activation of Ca^{2+}-dependent metabolic enzymes [62]. Under physiological conditions, ER chaperones, including BiP, store ER Ca^{2+} [63]. Ca^{2+} handling proteins, such as inositol 1,4,5-triphosphate receptor type 3 (IP3R3), are highly compartmentalized in the MAM [64]. IP3R3, cytosolic glucose responsive protein 75 (GRP75), and the voltage dependent anion channel 1 (VDAC1) form a macromolecular complex at the ER-mitochondrial interface [65] that functionally controls Ca^{2+} efflux from ER stores through the cytosol into mitochondria. The MAM contacts allow a microdomain where Ca^{2+} can reach high concentrations and thereby enable the function of the mitochondrial uniporter [66]. Thus, the ER can transmit Ca^{2+} signals to mitochondria without increasing bulk cytosolic Ca^{2+} concentrations above physiological thresholds. IP3R-mediated Ca^{2+} release can activate apoptosis by inducing cytochrome *c* release from mitochondria [67]. Increased mitochondrial Ca^{2+} has been associated with increasing electron transport, increased ROS production, and potentially PTP opening [68].

OXIDATIVE BALANCE

Mitochondria generate ATP through the process of oxidative phosphorylation. As high energy electrons derived from metabolic intermediates, including glucose, amino acids and lipids, are passed through the mitochondrial electron transport chain (ETC), their energy is used to transfer protons through the inner membrane into the IMS, generating a gradient known as the mitochondrial membrane potential. The electrons reduce oxygen to form water. The protons flow down their gradient driving the formation of ATP through ATP synthase.

ROS are highly reactive molecular generated by partial reduction of thee unpaired electrons of oxygen [69]. Mitochondria are the primary site of ROS production in the cell. The ETC is a major source of ROS production by complex I and III. Complex III catalyzed transfer of an electron to oxygen generates superoxide, which is converted to peroxide by superoxide dismutase and is subsequently detoxified by thioredoxine reductase or glutathione peroxidase. Excessive ROS can result in lipid peroxidation, protein and DNA oxidation, a shift in thiol/disulfide Redox state, and cell death by apoptosis or necrosis. ROS can also trigger release of Ca^{2+} from mitochondria, which can affect Ca^{2+} signaling pathways.

ENERGY PRODUCTION AND METABOLIC REPROGRAMMING (THE WARBURG EFFECT)

Viral replication requires energy and macromolecular precursors derived from cellular metabolism. Rather than simply activating cellular growth pathways, some human viruses such as CMV reprogram cells to a metabolic state similar to that observed in tumor cells, known as the Warburg Effect or aerobic glycolysis [6,70,71]. Glucose plays a key role in ATP production through glycolysis and the TCA cycle. Growth of malignant cells depends upon their ability to adopt an altered metabolic profile which shifts from oxidative phosphorylation to aerobic glycolysis as the main source of energy production. Surprisingly, use of glucose for aerobic glycolysis limits energy production to 2 ATPs per glucose molecule. To overcome this limitation, tumor cells replenish the TCA cycle anaplerotically using glutamine [72,73]. This allows tumor cells to use glucose biosynthetically rather than breaking it down for energy and to instead use glutamine for energy production.

Viruses can analogously increase aerobic glycolysis and use glucose biosynthetically [70,74]. Further, they can induce a modified Warburg Effect replenishing TCA cycle intermediates using α-ketoglutarate derived from glutamine to continue energy production [71]. The switch to aerobic glycolysis may benefit enveloped virus production by increasing the available pool of nucleotides, fatty acids, and lipids for progeny production [75]. Other viruses that increase glycolysis include Rous sarcoma virus and feline leukemia virus [76,77].

MITOCHONDRIA AND APOPTOSIS

Programmed cell death is a critical tool to control not only growth and development, but also to maintain homeostasis in multi-cellular organisms. Mitochondria are situated at an important regulatory nexus for cell death signaling. Apoptosis can be initiated through extrinsic activation of cellular death receptors such as Fas, TNFR-1, or TRAIL receptors -1 and -2 [78,79], which cleave effector caspase-3 and -7 through activation of initiator caspases-8 and -9. Extrinsic triggered apoptosis, while not directly mediated by mitochondria, can be modulated by feedback signaling from the mitochondria. More often however, apoptosis is induced by intracellular signals such as ER stress, Ca^{2+} dysregulation, DNA damage, lysosomal stress, or increased ROS [78], with caspase-9 and -3 activation directly reliant upon the integrity of the OMM and the inner $\Delta\Psi_m$. While viruses have evolved complex abilities to enhance or subvert cell death signaling to their reproductive advantage [79,80], their targeted manipulation of mitochondria often contributes heavily to clinical pathogenesis.

To commandeer apoptotic control of their host cells, viruses may encode death receptor decoys, regulators of endogenous death receptor expression, direct caspase inhibitors, modulators of Bcl-2 family proteins, or their own viral homologues of cellular Bcl-2 family members [80]. Bcl-2 family proteins are modulators of mitochondrial permeability and function, and thus act as gatekeepers of intracellular apoptotic activation. These proteins are broadly defined by the presence of at least one of four Bcl-2 homology (BH) domains. Multidomain Bcl-2 family proteins are grouped into either proapoptotic (e.g., Bax, Bak) or

antiapoptotic (e.g., Bcl-2, Bcl-X_L) members. There is a further subgroup of proapoptotic Bcl-2 family proteins containing only a single 9-12 amino acid BH3-like domain. Multidomain Bcl-2 family members, both proapoptotic and antiapoptotic, predominantly carry a short C-terminal hydrophobic TMD, which is used to target these proteins to the OMM [81-85]. The C-terminal tail anchors utilized to target these apoptotic regulators to the OMM do not frequently retain exact sequence homology, but rather possess a stretch of hydrophobic amino acids predicted to fold into an alpha-helix, often with charged flanking residues. Variations in hydrophobicity and the flanking sequence contribute to mitochondrial targeting specificity [86-88].

Antiapoptotic viral Bcl-2 (vBcl-2) homologues are commonly employed by viruses that must persist within host cells for long periods of time. To date, all sequenced gammaherpesviruses encode at least one homologue of Bcl-2 [80]. Other notable viral Bcl-2 homologues include the KsBcl-2 and K7 proteins of HHV-8, E1B-19K of Adenovirus, as well as BHRF1 and BALF1 of Epstein-Barr virus. Each of these vBcl-2 proteins transfers antiapoptotic capacities to their encoding viruses.

MITOCHONDRIAL INNATE IMMUNE SIGNALING

Mitochondria have recently been described as scaffolds for signaling molecules involved in host cell innate immune recognition of invading pathogens [89,90]. Besides the toll-like receptor proteins expressed on plasma membranes and internalized endosomes, which recognize extracellular pathogens [91], pattern-recognition proteins (PRPs) of the cytosol can also aid host cell detection of invading pathogens. The activity of cytosolic, RIG-I-like helicase (RLH) pathway, in particular, was described as mediated by mitochondria. The RLH pathway is composed of three RNA helicases, which recognize viral nucleic acids: the retinoic acid inducible gene-I (RIG-I), melanoma differentiation-associated gene-5 (MDA-5), and laboratory of genetics and physiology 2 (LGP2). RIG-I and MDA-5 both contain tandem N-terminal caspase activation and recruitment domains (CARDs), as well as a C-terminal RNA helicase domain required for binding to viral substrates [92]. Viral RNA binding to the C-terminal helicase domain of RIG-I and MDA-5 stimulates a conformational change in these proteins, exposing the two N-terminal CARDs and facilitating their interaction with the CARDs of mitochondrial antiviral signaling protein (MAVS) [3,90,93]. LGP2 contains a helicase domain but lacks a CARD and functions as a regulator of RLH signaling [94,95].

MAVS activation, through interaction with RIG-I or MDA-5, causes signaling through downstream kinases and the induction of cytokine production, such as type I IFN [3,90,93,96]. Cellular depletion of endogenous MAVS protects cells from apoptosis induced through innate immune anti-viral responses [97]. MAVS is targeted to the OMM by its C-terminal TMD and, at least in part, is found at MAM domains of ER-mitochondrial contact. This is supported by the interaction of the MAVS protein with mitofusins 1 (Mfn1) and 2 (Mfn2) [98,99], which are known to help tether ER and mitochondrial membranes as well as function in mitochondrial elongation/fusion [100]. As well, mitochondria-localized MAVS also interacts with the ER resident protein, STING (also called MITA or ERIS), to assist cellular recognition of non-self RNA and double stranded DNA within the cytosol and

downstream IFN induction [101]. Importantly, the OMM localization of MAVS is required for its signaling activity [90]. This places mitochondria and MAM at critical positions for regulating early host cell responses to viral pathogens.

Many viruses have evolved to usurp host cell innate immune responses, and direct proteins to mitochondria to inhibit the function of MAVS, and block RLH pathway signaling. HCV encodes a chymotrypsin-like serine protease within the N-terminal region of the non-structural protein 3 (NS3). NS3 binds to its activator, the non-structural protein 4A (NS4A), to initiate proteolytic processing of the HCV polyprotein. The NS3/4A protein also blocks RLH signaling by directly cleaving MAVS at Cys-508, displacing it from the OMM [3,102,103]. Notably, current screenings for new HCV therapeutics are targeting this mitochondria-localized phenomenon of MAVS cleavage, and the virus' ability to overcome innate immune recognition from host cells [104].

Hepatitis A virus (HAV) accomplishes MAVS proteolysis by directing its 3ABC protein precursor of the 3C(pro) cysteine protease to the mitochondria, with an internal TMD (within the 3A domain) [105]. HBV uses the non-structural HBx to promote the degradation of MAVS through Lys136 ubiquitination and of Mcl-1, an antiapoptotic member of the Bcl-2 family [106,107]. HBx is targeted to the OMM by its C-terminus [108]. HBx has also been described as capable of interacting with VDAC3 on the OMM, and modulating $\Delta\Psi_m$ [109,110].

IAV targets the PB2 protein subunit of its viral RNA polymerase to the mitochondria, as well as the nucleus. In the mitochondria, PB2 interacts with MAVS and inhibits MAVS signaling and downstream IFN-β expression. Intriguingly, PB2 of seasonal human influenza viruses localized to mitochondria, while PB2 proteins of avian influenza viruses remained nonmitochondrial. In animal model experiments, the nonmitochondrial PB2 proteins displayed virulence attenuation through innate immune signaling and IFN-β production [111].

Human viruses of medical importance with unmet medical needs for antiviral drug discovery and their mitochondrially targeted products are summarized below. Notable features of their mitochondrial products are highlighted in Figure 1 and in Table 1.

ADENOVIRUS

More than 50 serotypes of adenoviruses have been implicated in a variety of clinical syndromes including upper and lower (potentially severe) respiratory tract illness, keratoconjunctivitis, gastroenteritis, hepatitis, myocarditis, and meningoencephalitis in immunocompetent hosts. Infection of stem-cell and solid-organ transplant recipients is typically severe and frequently fatal. There is currently no approved antiviral agent for treatment of adenovirus infections. Cidofovir has good *in vitro* activity against adenoviruses and has been used in immunocompromised adults and children with reported clinical improvement (case reports), although with significant associated nephrotoxicity. A lipophilic preparation with reduced nephrotoxicity has recently been developed and has been used on a compassionate use basis in stem cell transplant patients. Infusion of adenovirus-

specific donor T cells in pediatric stem cell transplant patients, as well as intravenous immunoglobulin have also been reported in case series [112].

Adenovirus E1B

E1B-19K, one of the oncogenes of adenovirus, counteracts E1A induced apoptosis during adenovirus infection [113,114]. E1B-19 is localized to mitochondria during early and late times of adenovirus infection [115]. E1B-19K was the first viral homologue of Bcl-2 to have been discovered [116]. It possesses BH1, BH2, and BH3 domains and inhibits apoptosis induced via adenovirus E1A-triggered p53 activation, TNFα and Fas stimulation, TGF-β induction, ultraviolet radiation, and DNA damaging agents [117-127]. Similar to cellular Bcl-2, E1B-19K can interact with Bak and Bax and prevents their co-oligomerization and from forming pores in the OMM [114,128-132]. E1B-19K also interacts with Bik [133,134], BNip [135] and p53 [115]. E1B-19K blocks the ability of Bik to activate apoptosis through inhibition of protein synthesis by disrupting Bak-Mcl-1 and Bak-Bcl-XL complexes [136,137]. In addition to its predominant nuclear location, p53 is targeted to mitochondria early during adenovirus infection [115]. E1B-19K can interact with p53 and suppresses the mitochondrial mediated apoptosis induced by p53. By dual interaction with p53 and Bak, E1B-19K may prevent Bak activation as well as Bak-dependent activation [115].

ENTEROVIRUSES

The enterovirus family comprises greater than 70 species affecting humans, including polioviruses (PVs), coxsackieviruses (CVs), echoviruses and numbered enteroviruses. In addition to paralytic poliomyelitis due to PVs and enterovirus 71, the non-polio enteroviruses are amongst the most common etiologies of seasonal upper respiratory and gastrointestinal illness, aseptic meningitis, and fulminant myocarditis in immunocompetent hosts. In addition, enterovirus infection may lead to overwhelming sepsis in neonates, and chronic meningoencephalitis in hypogammaglobulinemic individuals. Currently, there is no approved agent available for treatment of enterovirus disease. Pleconaril, which was molecularly engineered to block enterovirus binding to host cells, and was studied in the setting of upper respiratory disease, aseptic meningitis and neonatal sepsis, is not yet commercially available [138].

Non-structural protein 2B

Coxsackievirus B (CVB) encodes a non-structural protein 2B, which is antiapoptotic [139,140]. CVB 2B forms channels in the ER membrane, decreasing Ca^{2+} levels in the ER, decreasing Ca^{2+} flux into mitochondria and thus blocking Ca^{2+} induced proapoptotic signaling [140]. ER Ca^{2+} release induced by CVB 2B is not taken up by mitochondria [139,140]. Intriguingly, 2B from the closely-related PV has been reported to be proapoptotic activities in BHK-21 cells [141]. PV infection causes mitochondrial dysfunction in various cell lines [142,143]. Ca^{2+} flux between the ER, mediated partly by IP3R, and mitochondria contribute to PV-induced apoptosis, which is required for release of PV progeny viruses [142,144]. ER Ca^{2+} release and mitochondrial Ca^{2+} uptake during PV infection results in mitochondrial dysfunction as evidenced by cytochrome *c* release [144]. Thus, the role of

Ca^{2+} in regulating PV-induced apoptosis is complex and likely to result from multiple pathways dictating the balance between proapoptotic and antiapoptotic signaling.

HEPATITIS B VIRUS (HBV)

An estimated 350 million people worldwide live with chronic infection with human hepatitis B virus (HBV). More than 90% of perinatally infected infants and 2-6% of adults develop chronic HBV infection. Chronic HBV infection is a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC) [145]. No specific therapy for HBV infection is available and all currently utilized treatments have limited short and long term efficacies. Treatment with IFN-α-2b, pegylated IFN-α-2a and lamivudine are FDA approved for treatment of children and adults, although response rates are poor (approximately 25-30% overall). Multiple nucleoside analogues (lamivudine, entecavir, and telbivudine) and a nucleotide analogue (adefovir) are FDA-approved for treatment of adults, and emtricitabine has also been utilized (although not FDA-approved), but safety and efficacy in children have not been established. Development of resistance to all therapies is a major limitation. Renal and musculoskeletal toxicities are reported [146,147].

HBx protein

HBV encodes HBx, a multifunctional protein that can regulate cellular transcription, protein degradation, Ca^{2+} signaling, cell proliferation and apoptotic pathways (recently reviewed in [148]). Most HCCs retain and express HBx, suggesting an important role during transformation [149]. HBx enhances HBV replication in HepG2 cells, a human hepatoblastoma cell line used in the field to study HBx-dependent HBV replication [150]. HBx localizes to mitochondria where it interacts with VDAC3, a component of mitochondrial PTP [109,110,151]. Mitochondrial association of HBx during HBV replication in HepG2 cells modulates PTP and elevates cytosolic Ca^{2+} [152]. HBV replication in primary rat hepatocytes requires HBx regulation of Ca^{2+} signaling and PTP activity, which allow hepatocytes to exit G_0 and stall in the G_1 phase of the cell cycle [153]. As HBx appears to be important for HBV-caused HCC, drugs designed to inhibit HBx functions may also be useful to treat active HBV infections as well as the development of HCC.

HEPATITIS C VIRUS (HCV)

HCV chronically infects about 2% of the world's population and is the leading cause for liver transplantation in the US, due to resultant chronic hepatitis, cirrhosis and HCCs [154-156]. The majority of transmission occurs as a result of parenteral exposure; however, up to a third of reported cases have no identifiable risk factor. Up to 85% of infected individuals develop chronic infection. No specific drug therapy is available for treatment of HCV. Interferon-α (IFN-α) or pegylated IFN-α alone are approved by FDA for treatment of chronic HCV infection in adults; pegylated IFN-α in combination with ribavarin is approved for treatment of adults and children. However, response to therapy, ranging from 50-80%, is highly variable dependent upon the genotype of infecting virus. IFN therapy is expensive and associated with significant adverse reactions such as influenza-like symptoms,

hematologic abnormalities and neuropsychiatric symptoms. Thus, specific anti-HCV drugs are an unmet medical need [146,157].

Hepatocytes infected with HCV respond by generating ROS [158,159]. Over-expression of multiple HCV proteins enhances the production of ROS and other mitochondrial dysregulation [160,161]. Concerted expression of HCV proteins increased mitochondria Ca^{2+} uptake, resulting in inhibition of ETC complex I, dissipation of $\Delta\Psi_m$, decreased oxidative phosphorylation and increased generation of ROS and reactive nitrogen species [162,163]. More recently mitochondrial function and apoptosis have been examined in the hepatoma cells stably replicating genome length HCV replicons [164]. Surprisingly, despite HCV-induced mitochondrial dysfunction the cells preserve full bioenergetic competence if not forced to rely exclusively on oxidative phosphorylation [163]. HCV induces an increase in glycolysis by stabilizing hypoxia induced factor (HIF) through its transcriptional control of glycolytic enzyme genes [163].

Chronic alcohol consumption has been identified as a significant cofactor in exacerbation of HCV-related liver disease [165]. Because of a lack of a small animal model for HCV pathogenesis and of hepatocyte derived cell lines expressing alcohol dehydrogenase and cytochrome P450 2E1, the relationship between HCV infection and alcohol use have been inconclusive. To overcome these limitations, a cell line, constitutively expressing cytochrome P450 2E1 and harboring HCV replicons, has been generated. Using these cells, it was found that physiological concentrations of alcohol increased HCV replication [166]. The increase could be blocked by the antioxidant *N*-acetylcysteine, suggesting that oxidative stress plays a central role in HCV replication. Although others [167] have found that high levels of oxidative stress, induced by hydrogen peroxide treatment, can decrease HCV replication, these studies suggest that antioxidant therapy might play a supplemental role in standard IFN based anti-HCV therapy [166].

HCV Core Protein

Oxidative stress and mitochondrial dysfunction have been widely observed in liver specimens from patients with HCV [168,169]. HCV is an enveloped virus that contains a single-stranded, positive-sense RNA genome of ~9.6 kb. The HCV genome encodes a large open reading frame that is flanked by structured 5′ and 3′ UTRs. There are at least 10 mature proteins that are contained in the polyprotein, which is post-translationally processed. Although synthesis and maturation of HCV proteins occur in the ER, HCV core protein and NS3/4a protease localize partially at the OMM [56,170,171]. Of these, HCV core protein is best characterized in its regulation of mitochondrial functions. The HCV core protein, the first 191 amino acids of the viral precursor polypeptide protein, is cleaved to a mature 21 kDa species that lacks the signal peptide. The HCV core protein associates with the ER and mitochondria and traffics sequentially from the ER, through the MAM, to mitochondria [56,170]. A C-terminal tail anchoring domain is necessary and sufficient for directing its ER and mitochondrial localizations [170]. Core protein induces ER Ca^{2+} leakage and ER stress in liver cells but it is not dependent upon IP3R function [172-174].

In mitochondria, the HCV core protein increases Ca^{2+} uptake via the mitochondrial Ca^{2+} uniporter and thereby increases ROS [175,176]. Because of its localization in the MAM, it is

possible the HCV core protein interacts with components of the MAM IP3R-GRP75-VDAC complex affecting both ER Ca^{2+} efflux and mitochondrial Ca^{2+} uptake [175]. In addition, HCV core protein increases NADPH oxidase (Nox) 4 production in a transforming growth factor beta (TGF β)-dependent manner, contributing to HCV-induced oxidative stress [177]. The alteration of Ca^{2+} signaling by HCV core protein may provide a desirable target to inhibit HCV growth.

HERPES SIMPLEX VIRUSES

Herpes simplex virus (HSV) type 1 (HSV-1) and type 2 (HSV-2) cause a wide variety of clinical manifestations in humans, ranging from mild mucocutaneous and genital infections and keratitis, to severe life-threatening neurological disease in neonates and adults. For non-neurologic disease, antiviral agents such as acyclovir, famciclovir and valacyclovir are effective. For HSV encephalitis and neonatal disease, intravenous acyclovir is the drug of choice, although foscarnet and cidofovir can be utilized in the case of infection with acyclovir resistant viruses due to deficient thymidine kinase activity. Although intravenous acyclovir has dramatically improved the mortality associated with neuroinvasive HSV infection, the proportion of patients with subsequent permanent neurologic deficits remains high. Additional antiviral agents and combination therapies are needed to improve outcomes in these patients [178].

Herpesvirus infection perturbs the oxidative balance within cells. Protein carbonylation, an irreversible modification that alters conformation of proteins, and usually results in degradation by the proteasome, is an indicator of oxidative stress of cells. HSV infection triggers oxidative imbalance by depleting glutathione upon entry [179]. Preliminary studies indicate that key cellular proteins are targeted for carbonylation during HSV infection, predictably enhancing its ability to overtake the cell [180].

HSV US3, UL12.5 and UL7

HSV causes oxidative stress and Ca^{2+} release as well as cytochrome *c* release from mitochondria [181,182]. HSV suppresses cellular respiration by inhibiting electron transfer [183]. HSV US3 is required and sufficient to affect mitochondrial respiration profoundly. US3 inhibits electron transfer between complexes II and III. Furthermore, the HSV UL12.5 nuclease localizes to mitochondria and therein degrades mitochondrial DNA [182,184]. Mitochondrial DNA encodes 13 proteins involved in oxidative phosphorylation and the RNA components of mitochondrial translational machinery. However, the biological significance of mitochondrial DNA loss during HSV growth is unclear. HSV UL7 traffics to mitochondria and interacts with ANT2 [185]. Further, the biological significance of the UL7-ANT2 interaction and the UL12.5 catalyzed loss of mitochondrial DNA for HSV growth is unclear at this time.

HUMAN CYTOMEGALOVIRUS (CMV)

CMV is a herpesvirus, which is the leading viral etiology of congenital birth defects (including sensorineuronal hearing loss and developmental delay) in developed countries. CMV is also a major cause of morbidity and mortality in immunocompromised individuals

[186-188]. Severe manifestations of CMV include interstitial pneumonia, hepatitis, meningoencephalitis, gastrointestinal disease, myocarditis, bone marrow suppression, and retinitis. FDA-approved therapies that inhibit the viral DNA polymerase include ganciclovir (and valganciclovir), foscarnet and cidofovir. Additional agents with potential efficacy in the setting of retinitis include valganciclovir and formivirsen (antisense inhibitor of CMV). Maribavir is currently under study for treatment of CMV in immunocompromised hosts. Development of resistance and cross-resistance to all currently licensed therapies is commonly due to selection of mutations in the UL97 protein kinase and DNA polymerase genes. Significant toxicities of current therapies include nephrotoxicity, electrolyte abnormalities, and bone marrow suppression. The availability of nontoxic, effective therapies for prophylaxis and pre-emptive treatment of CMV infection in transplant populations is of high importance [189].

CMV infection places a large energetic and biosynthetic demand on cells to complete its protracted life cycle. From very early times of CMV infection, expression of cellular glucose transporter (GLUT) 1, which is abundantly expressed in permissive fibroblasts, decreases; whereas, the expression of GLUT4, normally expressed in adipose tissue, skeletal and cardiac muscle, increases dramatically [190]. CMV infection circumvents the negative regulation of GLUT4 by high glucose levels and allows for glucose to be transported into the infected cell at levels 3-fold higher than achievable by GLUT1. Thus, glucose is readily transported into the infected cell. Notably, treatment of CMV-infected cells with indinavir, an inhibitor of human immunodeficiency virus (HIV) protease, which also selectively inhibits glucose uptake by GLUT4 [191,192], inhibited CMV progeny production [193]. However, it is not clear whether indinavir reduces CMV progeny production by inhibiting GLUT4 or by inhibiting CMV proteases required for its progeny production. Because of the peculiarities of its specific metabolic program, CMV-induced changes in glucose transport required for its increased rate of aerobic glycolysis and the production of progeny virions during infection may provide potential targets for novel anti-CMV drugs.

To drive the utilization of glucose for biosynthetic pathways, CMV-infected cells rely on glutamine, which becomes the major anaplerotic substrate to replenish intermediates of the TCA cycle [71]. Consistent with this major role is the finding that glutamine is necessary for ATP production in CMV-infected cells and its uptake is increased during infection. During infection, glutamine is efficiently converted into α-ketoglutarate for anaplerotic use in the TCA cycle. Thus, similar to many tumor cells, glutamine is used in CMV-infected fibroblasts to maintain the TCA cycle for energy production and for biosynthetic intermediates, allowing glucose to be used biosynthetically [71]. These dramatic metabolic changes induced during CMV infection suggest that inhibition of aerobic glycolysis, glutamine uptake, or glutaminolysis may provide novel and effective targets for anti-CMV drugs.

By the middle of its life cycle, CMV infection effectively disrupts cellular metabolic homeostasis and institutes its own metabolic program [70]. CMV infection causes a global metabolic up-regulation of central carbon metabolic flux [70,74]. In particular, CMV infection induces increased glucose and glutamine consumption during permissive infection of human fibroblasts [70,71,74]. Increased aerobic glycolysis allows CMV to use glucose

biosynthetically, wherein most of the acetyl CoA supports fatty acid synthesis needed for membrane formation for progeny viruses.

CMV infection increases glycolysis and influx through TCA cycle and its efflux to fatty acid biosynthetic pathway [74]. The importance of the increased fatty acid biosynthetic pathway was underscored by reduction of CMV growth by inhibition of acetyl-CoA carboxylase, using 5-tetradecyloxy-2-fuoic acid, and inhibition of fatty acid synthase, using C75 (trans-4-caroxy-5-octyl-3-methylene-butyrolactone). Because the expression of CMV immediate early (IE), early and true late genes were unaffected by these inhibitors, these results suggest a role of fatty acid synthesis subsequent to viral DNA synthesis, possibly for the envelopment of progeny virions. Importantly, treatment with these inhibitors did not cause toxicity or apoptosis of the uninfected cells. Pharmacological reversal of the Warburg Effect has shown to cause selective apoptosis of tumor cells, presumably by stimulating mitochondrial respiratory chain activity and ROS production, which in turn results in caspase-induced cell death. Thus, alterations of metabolism have identified metabolic targets for potential anti-CMV drug design.

CMV UL37 pUL37x1/vMIA

CMV gene expression is temporally regulated and the first class of encoded viral proteins is comprised of IE proteins. Of these, the CMV genome encodes multiple products from the UL37 IE gene locus [45,194-196]. CMV pUL37x1/vMIA is essential for growth of clinical CMV strains and has potent antiapoptotic activity at the OMM, where it binds to Bax, blocking its proapoptotic activities [48-50,197-199]. CMV UL37 proteins traffic sequentially from the ER to the MAM and into the OMM, directed by an NH_2-terminal signal sequence [46,200,201]. A triply membrane-anchored UL37 glycoprotein cleavage site mutant is first ER translocated, where it is *N*-glycosylated, and then imported into the OMM [44,46]. Targeting of the MAM, common to all studied UL37 isoforms [45,46], suggests important functions during CMV infection.

CMV pUL37x1 causes Ca^{2+} efflux from the ER and thereby F-actin disruption, as well as early and late cytopathology [51,53,197,202,203]. Defects in its ER to mitochondrial trafficking result in defective CMV pUL37x1/vMIA antiapoptotic function [50,200]. Further, pUL37x1/vMIA controls a mitochondrial serine protease HtrA2/Omi, an initiator of caspase independent cell death at late times of CMV infection [52]. Importantly, potent UL37 protein antiapoptotic activity is downstream of ER translocation and MAM importation. CMV mutants defective in pUL37x1 antiapoptotic functions are also defective in the growth of most CMV strains [51,204]. Notably, targeting of intron-exon boundary of CMV UL37 and UL36 transcripts by antisense oligonucleotides provided potent antiviral effect and was active against ganciclovir resistant strains [205]. The successful inhibition of CMV growth by targeting essential UL37 gene products suggests the feasibility of rational anti-CMV pUL37x1 drug design.

CMV beta 2.7 (β2.7) RNA

A CMV early transcript, β2.7 RNA, protects CMV-infected cells from rotenone-induced cell death [57]. CMV β2.7 RNA is abundantly expressed at early times of infection, does not

encode a protein, and is localized in mitochondria [206-208]. Rotenone is an inhibitor of mitochondrial complex I [209]. β2.7 RNA interacts with the genes associated with retinoid/IFN-induced mortality (GRIM)-19 subunit of complex I, which prevents its relocalization from the IMM to discrete perinuclear sites [57]. In addition, pUL37x1/vMIA has been reported inhibit mitochondrial ATP synthesis by decreasing the activity of the mitochondrial phosphate carrier in transfected cells [53].

HUMAN HERPESVIRUS TYPE 8 (HHV-8) OR KAPOSI'S SARCOMA HERPESVIRUS (KSHV)

HHV-8, also termed KSHV, an oncogenic human herpesvirus, has been identified in all types of Kaposi's sarcoma (KS), including acquired immunodeficiency syndrome (AIDS)-associated KS, African (endemic) KS, and transplant-associated KS. HHV-8 has also been associated with rare lymphoproliferative diseases including Castleman's disease and body cavity lymphoma in HIV patients. No specific antiviral therapy is currently available for treatment of HHV-8 infections, although ganciclovir and foscarnet have been shown to have *in vitro* activity against HHV-8. Treatment of KS relies upon highly active antiretroviral therapy (HAART) for concomitant HIV infection, local radiotherapy, systemic antineoplastic agents, and IFN-α [210].

HHV-8 increases aerobic glycolysis and inhibits oxidative phosphorylation in latently infected endothelial cells, the relevant KS tumor cell type, but not in human fibroblasts. Moreover, blockage of glycolysis by oxamate treatment induced apoptotic cell death of the HHV-8-infected endothelial cells. Thus, induction of aerobic glycolysis by HHV-8 may adapt infected endothelial cells for growth in low oxygen tumor microenvironment tumor formation [5]. Additionally, the Warburg Effect may circumvent apoptosis induction caused by HHV-8 latency. Inhibitors of glycolysis may provide previously unexplored treatments for latent HHV-8 infection in endothelial cells [5]. If effective, these anti-HHV-8 drugs may provide the first drugs against latent herpesvirus infections. Intriguingly, it has been recently found that ROS levels and cellular antioxidant systems regulate HHV-8 reactivation and survival of HHV-8-infected tumor cells [211]. These findings may allow for novel approaches to treating HHV-8 associated malignancies.

KsBcl-2 RNA is expressed upon activation of the HHV-8 lytic cycle in cultured cells, and the protein blocks apoptosis induced by several stimuli [212-214]. It shares limited overall sequence identity with other Bcl-2 family members, but significant amino acid conservation is observed within the BH1 and BH2 domains [212]. The KsBcl-2 protein is similar in structure to Bcl-2 and Bcl-XL, but cannot homodimerize or heterodimerize with other Bcl-2 family members, which may affect it regulation by host cell proapoptotic Bcl-2 family proteins [212,215,216].

HHV-8 K7 protein

K7 is a 16 kDa antiapoptotic HHV-8 glycoprotein which shows structural homology to the survivin-DeltaEx3 protein, but limited sequence homology to Bcl-2 [217]. K7 contains an NH_2-terminal mitochondrial targeting sequence, can be detected within ER, mitochondria,

and nuclear membrane, and inhibits Bax-induced as well as extrinsic-initiated apoptosis. K7 did not directly bind Bax, but was found to tether Bcl-2 and active caspase-3 to inhibit caspase activity. K7 binding to mitochondrial VDAC3 was also observed [217].

HHV-8 K7 localizes to ER, mitochondria and nucleus [217,218]. K7 interacts with Ca^{2+} modulating cyclophilin ligand (CAML), an ER protein that controls Ca^{2+} homeostasis and attenuates ER Ca^{2+} release induced by thapsigargin, an inhibitor of SERCA [218].

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

The human retrovirus HIV was first recognized as the cause of AIDS in 1984. As of 2009, the World Health Organization reported 33.3 million adults and children worldwide living with HIV infection, with 2.6 million new infections and 1.8 million deaths due to AIDS reported in 2009 [219]. Multiple classes of antiretroviral agents have been developed including nucleoside, non-nucleoside, and nucleotide reverse transcriptase inhibitors, protease inhibitors, entry inhibitors and integrase strand transfer inhibitors [220]. Despite the availability of greater than 30 antiretroviral drugs and combinations, none has resulted in complete eradication of viral burden in HIV-infected patients. Development of antiviral resistance, even in the setting of combination antiviral therapy, remains a significant challenge in this population. Toxicities of these therapies, including gastrointestinal, hematologic, metabolic and cardiovascular, are a barrier to adherence to therapy, as well as long term outcome [221,222].

HIV viral protein R (Vpr)

HIV Vpr has emerged as a major proapoptotic viral product [223]. Vpr is synthesized late in HIV infection and causes many cellular dysfunctions including induction of caspase-dependent apoptosis [224]. FAT-10 is a mediator of Vpr-induced apoptosis in renal tubular epithelial cells [225]. It is a matter of debate how it does so. Vpr can localize to mitochondria and induces mitochondrial membrane permeabilization (MMP) [226,227]. MMP is a key event in the release of proapoptotic activators and dissipation of the inner mitochondrial $\Delta\Psi_m$. ANT and VDAC are major components of the PTP [228]. Vpr primarily affects IMM MMP as it interacts with ANT. This interaction causes ANT to become a nonspecific channel and triggers inner MMP. The antiapoptotic cellular protein Bcl-2 prevents Vpr binding to ANT and its induced MMP. A PTP inhibitor (bongkrekic acid) also reduced Vpr binding to ANT. While these studies suggest that HIV Vpr induces apoptosis by interacting with ANT, more recent studies suggest that ANT binding is not required for HIV-induced apoptosis [229]. Rather, Bax (instead of ANT) is required and HIV-induced apoptosis is linked to Vpr's ability to arrest cells in the G_2 phase of the cell cycle [229]. Nonetheless, mitochondrial PTP is a major target of viral proteins and may be useful for pharmacological intervention for the rational design of antiviral drugs.

HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1)

HTLV-1 is a human retrovirus endemic in Japan, the Caribbean, parts of South and Central America, and Africa. HTLV-1 is associated with development of malignant neoplasms and progressive neurologic disorders in adults, including adult T cell leukemia/lymphoma

(ATLL) [230], and HTLV-associated myelopathy, also termed tropical spastic paraparesis. To date, no specific antiviral therapies have been shown to be clinically effective for treatment of HTLV-1-associated disease, although nucleoside analogues (zidovudine and lamivudine) have shown activity against HTLV-1 *in vitro*. Trials of IFN-α in combination with nucleoside analogues have been utilized in some patients with ATLL, in combination with standard antitumor chemotherapy [231].

HTLV-1 p13 and p12 proteins

HTLV-1 p13 protein is targeted to the IMM and appears to cause a rapid flux of Ca^{2+} across the IMM [232]. However, this does not seem to be a direct effect on mitochondrial Ca^{2+} permeability [233]. p13 specifically inhibits mitochondrial Ca^{2+} uptake, predictably by its ability to induce mitochondrial K^+ influx and depolarization. p13 is inserted into the IMM, triggers an inward potassium (K^+) current, depolarization and thereby enhances respiratory chain activity [234]. These changes were accompanied by increased ROS production and may trigger cell death. Consistent with these *in vitro* studies using purified mitochondria, expression of HTLV-1 p13 in HeLa and Jurkat T cells is associated with increased sensitivity to ceramide-induced apoptosis [235]. Further, the ability of HTLV-1 p13 to reduce $\Delta\Psi_m$ in a dose dependent manner was verified in living cells [233]. HTLV-1 p12 accumulates in the ER and Golgi and interacts with calnexin and calreticulin, two Ca^{2+} chaperones in the ER [236]. This interaction increases release of Ca^{2+} from the ER and may explain why p12 is required for viral infectivity in primary cells [237]. Thus, HTLV-1 expresses two proteins that affect Ca^{2+} signaling and may provide tractable targets for novel antiviral drugs.

INFLUENZA VIRUSES

Influenza viruses are the cause of annual epidemics of febrile respiratory disease worldwide, with significant resultant morbidity and mortality; in the US alone, over 30,000 people die of influenza annually. Highly pathogenic influenza A viruses (IAVs) have the potential to cause worldwide pandemics as occurred in 1918 (Spanish) with 21 million deaths, and subsequently in 1957 (Asian), 1968 (Hong Kong) and most recently, 2009. The avian influenza H5N1 virus poses a continued global health hazard as it continues to emerge in the human population. A comprehensive review of anti-influenza therapy has been recently published [238]. Four antiviral agents in two classes have been approved for prevention and treatment of influenza including the M2 inhibitors, amantadine and rimantadine, as well as the neuraminidase inhibitors, zanamivir and oseltamivir. Resistance to these agents emerges frequently during treatment and remains a clinical challenge. The need for intravenous preparations for critically ill and immunocompromised patients is high. Intravenous zanamivir, peramivir and oseltamivir are currently being studied in clinical trials [239].

IAV PB1-F2 proteins

IAVs encode 11 viral proteins, one of which, PB1-F2 protein, is an alternative translation product of the PB1 gene [240]. PR8 PB1-F2 and H5N1 PB1-F2 are important regulators of IAV virulence [241]. The size of PB1-F2 ranges from 57 to 101 residues [242]. A point mutation in PB1-F2 from N66 to S66 converts a moderately pathogenic strain (H5N1) to a

highly pathogenic virus [243]. This mutation was found in PB1-F2 of the 1918 pandemic strain and is within the mitochondrial targeting signal [244]. Although PB1-F2 is not essential for influenza growth [245], a new function ascribed to PB1-F2 N66S is its ability to modulate type I IFN responses leading to increased disease severity [246]. PR8 H1N1 PB1-F2 traffics to the IMM and OMM and enhances apoptosis, which is more pronounced in cells of immune origin [240,244]. In contrast, H5N1 PB1-F2, lacks a specific mitochondrial targeting signal, and does not enhance apoptosis [247]. Mitochondrial-mediated apoptosis is important for influenza growth as cells over-expressing of Bcl-2 are less permissive for influenza virus replication than their parental lines [248,249].

PB1-F2 dissipates $\Delta\Psi_m$ [240]. It is thought that apoptosis induction occurs by direct interaction with mitochondrial proteins or by destabilization of mitochondrial membranes by pore formation [250]. PB1-F2 interacts with ANT3 and VDAC1, disrupts mitochondrial morphology, and increases tBid-induced cytochrome *c* release [250]. ANT3 and VDAC1 are believed to interact and form the PTP, which leads to dissipation of $\Delta\Psi_m$ and release of apoptotic mediators from the IMS [251,252]. Nonetheless, it was recently found that PB1-F2 itself can generate channel activity [253]. PB1-F2 sequences from different pathogenic IAV strains differ. Nonetheless, these proteins all share the ability to generate ion channel activity [253]. Therefore, the biological activities of PB1-F2 appear to result from mitochondrial targeting or an association with other proteins [253].

SUMMARY AND PERSPECTIVE

Many currently licensed antiviral drugs inhibit viral enzymes required for their replication or assembly of virions, while others target entry or exit mechanisms. Despite targeting these critical pathways with antiviral drugs, there remain many unmet medical needs for novel antiviral drugs, and the potential for increasing therapeutic efficacy and lowering harmful side effects of current therapies by expanding the clinical repertoire of antiviral drugs (see Table 1). This review provides a summary of the rapidly-evolving field of mitochondria-targeted viral products, which enhance viral growth by priming multiple signaling pathways to elicit virus-favorable host conditions, as well as fine-tuning cellular energetic output and metabolite availability to meet viral replicative needs. Mitochondria-targeted viral products and the mitochondrial pathways affected by them provide potential novel targets for the rational design of antiviral drugs. Viral products alter oxidative balance, mitochondrial PTP, $\Delta\Psi_m$, electron transport, and ATP production. Moreover, viruses can cause the Warburg Effect, known to occur in tumor cells, in which metabolism is reprogrammed to aerobic glycolysis as the main source of energy. The finding that blocking of these functions inhibits viral growth in many systems suggests that antiviral drugs designed to affect viral mitochondrial products or their targets will be effective in inhibiting the targeted virus. Understanding the mechanisms underlying the effects of viral mitochondrial products and their targeted pathways will enable rapid and efficacious drug design.

The design of new antiviral drugs targeted to viral mitochondrial products or to altered mitochondrial pathways may lead to the development of novel antiviral drugs with improved activity and reduced toxicity. Because targeting mitochondrial organelles occurs through defined pathways, it is conceivable to inhibit mitochondrial targeting of viral products and

the critical alterations to cellular homeostasis they induce, without actually affecting normal mitochondrial function. Patterned alterations of mitochondrial dynamics represent another crucial step in productive infection shared by many viruses, and give researcher a new tool for the control or eradication of these clinically important pathogens. Tellingly, multiple, independent viral mitochondrial products target the same mitochondrial pathways suggesting that inhibiting these targeted pathways may inhibit those viruses which depend upon the altered mitochondrial pathways.

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ABBREVIATIONS

$\Delta\Psi_m$	mitochondrial membrane potential
AIDS	acquired immunodeficiency syndrome
ANT	adenine nucleotide translocator
ATLL	adult T-cell leukemia/lymphoma
Ca^{2+}	calcium
CAML	Ca^{2+} modulating cyclophilin ligand
CARD	caspase activation and recruitment domain
CMV	human cytomegalovirus
CNS	central nervous system
CV	coxsackievirus
CVB	coxsackievirus B
ER	endoplasmic reticulum
ETC	electron transport chain;
GLUT	glucose transporter
GRIM	retinoid/interferon-induced mortality
GRP	glucose responsive protein
HAART	highly active antiretroviral therapy
HAM	HTLV-associated myelopathy
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HHV-8	human herpesvirus type 8

HIV	human immunodeficiency virus
HSV	herpes simplex virus
HTLV-1	human T-cell lymphotropic virus type 1
IAV	influenza A virus
IE	immediate early
IFN-α	interferon alpha
IMM	inner mitochondrial membrane
IMS	intermembrane space
IP3R	inositol 1,4,5-triphosphate receptors
IV	intravenous
kDa	kilodalton
KS	Kaposi's sarcoma
KSHV	Kaposi's sarcoma-associated herpesvirus
LGP2	laboratory of genetics and physiology 2
MAM	mitochondria-associated membranes
MAVS	mitochondrial antiviral signaling
MDA-5	melanoma differentaition associated gene-5
Mfn	mitofusin
NNRTI	non-nucleoside reverse transcriptase inhibitor Nox
NADPH oxidase	NRTI
nucleoside reverse transcriptase inhibitor	OMM
outer mitochondrial membrane	PTP
permeability transition pore	PV
poliovirus pUL37x1	UL37 exon 1 protein
RIG-I	retinoic acid-inducible gene I
RLR	RIG-I-like receptor
ROS	reactive oxygen species
TCA	tricarboxylic acid cycle
TGF-β	transforming growth factor β
TMD	transmembrane domain
TNFα	tumor necrosis factor alpha

TOM	translocase of the outer mitochondrial membrane
UTR	untranslated region
vBcl-2	viral Bcl-2
VDAC	voltage dependent anion channel
vMIA	viral mitochondria-localized inhibitor of apoptosis

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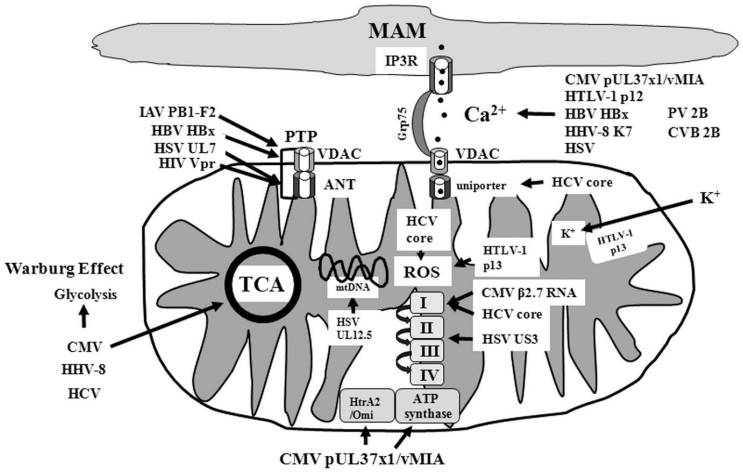

Figure (1). Targeting of mitochondrial regulated pathways by viral mitochondrial products

Shown is the viral targeting of Ca^{2+} efflux from the ER, ER to mitochondrial Ca^{2+} signaling and mitochondrial Ca^{2+} uptake. Also represented are the effects of viral products on the mitochondrial respiratory chain (complexes I-IV), ATP synthase, HtrA2/Omi, and the production of reactive oxygen species (ROS). Viral products additionally alter the permeability transition pore (PTP), including VDAC and ANT, can induce the Warburg Effect increasing aerobic glycolysis and altering the TCA cycle, and degrade mitochondrial DNA (mtDNA). See the text for details.

Table 1

Summary of Potential Mitochondrial Targets for Antiviral Drug Development.

Virus	Medical Need for Novel Therapy	Current Antiviral Therapies	Limitations of Current Therapies	Potential Target	Known Target or Partner	Metabolic or Mitochondrial Function	References
Adenovirus	HCST and solid organ transplant patients: Pulmonary, Gastrointestinal and Disseminated disease	*Cidofovir *Lipophilic Cidofovir	Toxicities: Bone marrow suppression Nephrotoxicity.	E1B-19K	Bax, Bak, Bik, BNip p53	Antiapoptosis	[115,129,134, 135]
Enteroviruses (Nonpolio)	Neonatal sepsis Myocarditis Aseptic meningitis Meningoencephalitis Upper Respiratory Infections	None *Pleconaril	N/A	Nonstructural protein 2B	Viroporin	Antiapoptosis Increased ER Ca^{2+} efflux, decreased mitochondrial Ca^{2+} uptake	[139]
Hepatitis B Virus (HBV)	Chronic Hepatitis	Interferon-α Pegylated IFN-α Lamivudine Adefovir Entecavir, Telbivudine *Emtricitabine	Variable clinical response Toxicities: Flu-like symptoms Nephrotoxicity Musculoskeletal Antiviral Resistance	HBx	VDAC3	Disrupts $\Delta\Psi_m$ Proapoptosis	[109]
Hepatitis C Virus (HCV)	Chronic Hepatitis	Interferon-α Pegylated IFN-α Ribavarin	Variable clinical response Toxicities: Flu –like symptoms Hematologic Neuropsychiatric	Core protein	MOMP opening	ROS generation Inhibition of ETC complex I Increase Ca^{2+} from ER to mitochondria	[164,168]
Herpes Simplex Virus (HSV)	Neonatal CNS and Disseminated Disease Meningoencephalitis Genital Disease Keratitis	CNS: Acyclovir Foscarnet Cidofovir Non CNS: Acyclovir Famciclovir V alacyclovir Ophthalmic : Trifluridine Idoxuridine Vidarabine	CNS Disease: High morbidity Toxicities: Bone marrow suppression Hematologic Nephrotoxicity Electrolyte Imbalance	UL7 UL12.5 US3	ANT2 Mitochondrial DNA ETC	Degradation of mitochondrial DNA Inhibits mitochondrial ETC, between complexes II and III	[185] [184] [183]

Virus	Medical Need for Novel Therapy	Current Antiviral Therapies	Limitations of Current Therapies	Potential Target	Known Target or Partner	Metabolic or Mitochondrial Function	References
Human Cytomegalovirus (CMV)	Congenital Infection Pulmonary, Gastrointestinal, Hepatic, Retinal and Disseminated disease in immunocompromised hosts	Ganciclovir Valganciclovir Cidofovir Foscarnet *Maribavir Ophthalmic: Valganciclovir Formivirsen	Antiviral resistance Toxicities: Bone marrow suppression, Hematologic Nephrotoxicity Electrolyte imbalance	pUL37x1/vMIA β2.7 RNA Warburg Effect TCA cycle	Bax GRIM-19 Complex	Antiapoptosis ER Ca^{2+} efflux Regulates mitochondrial HtraA2/Omi Inhibits ATP synthase Antiapoptosis	[49] [51] [52] [53] [57] [71] [70,74]
Human Herpesvirus type 8 (HHV-8; KSHV)	Kaposi Sarcoma Lymphoproliferative disease in HIV co-infected patients	None	N/A	Warburg Effect K7 K15 KSBcl2	Bcl-2, active caspase 3 HAX1	Required for Latency	[5] [217]
Human Immunodeficiency Virus (HIV)	AIDS	>30 NRTI, NNRTI, Protease inhibitors, Integrase inhibitors	Failure to eradicate infection Antiviral resistance Adherence Toxicities: Gastrointestinal Hematologic Metabolic Cardiovascular	Vpr	VDAC, ANT3	Promotes PTP opening $\Delta\Psi_m$ loss	[220,226]
Human T-cell Lymphotrophic Virus (HTLV-1)	ATLL Spastic Paraparesis (HAM)	*Interferon-α *Nucleoside analogues		p13		Rapid mitochondrial K^+ influx, depolarization, alteration of mitochondrial Ca^{2+} uptake	[232]
Influenza	Upper and Lower Respiratory Tract Infection Sepsis	Amantadine, Rimantidine, Oseltamivir, Zanamivir, *IV Peramivir *IV Oseltamivir *IV Zanamivir	Antiviral resistance Need for IV formulations for severe disease	PB1-F2	VDAC1, ANT3 Nonselective ion channel	ΔΨm dissipation PTP opening proapoptotic	[245,250] [253]

*Indicates investigational or not FDA-approved

Abbreviations:

ATLL: Adult T-cell Leukemia/Lymphoma
CNS: Central Nervous System
HAART: Highly active antiretroviral therapy
HAM: HTLV-Associated Myelopathy
IV: Intravenous
NRTI: Nucleoside Reverse Transcriptase Inhibitor
NNRTI: Non-nucleoside Reverse Transcriptase Inhibitor