A CYTOPLASMIC-REPLICATING RNA VIRUS SENSITIZES CANCER CELLS TO DNA MODIFYING AGENTS

by

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ABSTRACT

The Parainfluenza virus 5 (PIV5) mutant P/V-CPI- is restricted for spread in normal cells but not in cancer cells in vitro and is effective at reducing tumor burden in mouse model systems. Here we show that P/V-CPI- infection of human laryngeal cancer HEp-2 cells resulted in the majority of the cells dying, but unexpectedly, a population of cells emerged as P/V-CPI- persistently infected (PI) cells. P/V-CPI- PI cells had elevated levels of basal caspase activation, and viability was highly dependent on activity of cellular inhibitors of apoptosis, such as Survivin. In challenge experiments with external inducers of apoptosis, the PI cells were highly sensitive to cisplatin-induced DNA damage and cell death. This increased cisplatin sensitivity correlated with defects in the phosphorylation cascade controlling DNA damage signaling pathways, as well as translocation of damage-specific DNA binding protein 1 (DDB1) to the nucleus. Similar sensitivity to cisplatin was seen with cells during acute infection with P/V-CPI-, as well as acute infections with WT PIV5. Based on this finding, we tested the hypothesis that histone deacetylase (HDAC) inhibitors would also act with P/V-CPI- infection to enhance cancer cell killing. Using human lung and laryngeal cancer cell lines, 10 HDAC inhibitors were tested for their effect on viability of P/V-CPI- infected cells. HDAC inhibitors such as scriptaid enhanced caspase-3/7, -8 and -9 activity induced by P/V-CPI- and overall cell toxicity. Scriptaid treatment also enhanced the spread of P/V-CPI- through a population of cancer cells and suppressed interferon-beta induction through blocking phosphorylation and nuclear translocation of Interferon Regulatory Factor 3 (IRF-3). These results support a therapeutic approach of combining parainfluenza infection and chemotherapy, but also raise questions on the mechanism by which a cytoplasmic-replicating RNA virus can alter cellular DNA damage responses.

Keywords: parainfluenza virus, oncolytic virus, DNA damage

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LIST OF ABBREVIATIONS

AP-1……...Activator protein-1

ATF6.....Activating transcription factor 6

ATM.....Ataxia telangiectasia mutated

ATR.....Ataxia telangiectasia and rad3-related

BIP.....Immunoglobin binding protein

BSA……....Bovine serum albumin

C…….Paramyxovirus C protein

cDNA…….Complementary DNA

c-FLIP.....Cellular FLICE-like inhibitory protein

CHK.....Checkpoint kinase

CHOP.....C/EBP homologous protein

CPE……Cytopathic effect

CPI…….Canine parainfluenza

CYTO.....Cytoplasmic

DAPI……..4',6-diamidino-2-phenylindole

DDB.....Damage specific DNA binding protein

DDR.....DNA damage response

DMEM…..……..Dulbecco's modified Eagle's medium

DMSO……Dimethyl sulfoxide

dsRNA……Double-stranded RNA

ECL…...…….Enhanced chemiluminescence

EGFR..... Epidermal growth factor receptor

eIF2α….…….Eukaryotic initiation factor-2 alpha

ELISA……Enzyme-linked immunosorbent assay

ER……..Endoplasmic reticulum

F…….Fusion protein

FADD……Fas-associated protein with death domain

FasL……..…Fas ligand

FBS……Fetal bovine serum

FMDV.....Foot and mouth disease virus

GFP……..…Green fluorescent protein

GG-NER.....Global genome-NER

HDAC.....Histone deacetylase

HI.....Heat inactivated

HN…….Hemagglutinin-neuraminidase protein

HPI……Hours post infection

HPIV……..…Human parainfluenza virus type

HRP……...Horseradish peroxidase

I…….I protein

IAP.....Inhibitor of apoptosis

IFN……Interferon

IL……...Interleukin

IRE1.....Inositol requiring 1

IRF…….Interferon regulatory factor

ISG……Interferon stimulated gene

ISGF……..Interferon stimulated factor

ISRE……..Interferon sensitive response element

JAK…….Janus activated kinase

JNK…….....c-Jun NH_2-terminal kinase

L…….Large protein

LACV.....La Crosse virus

Le……Leader

M…….Matrix protein

Mda-5…….Melanoma differentiation-associated gene-5

MDBK……Madin-Darby bovine kidney cells

MeV……Measles virus

MOI……Multiplicity of infection

mRNA.....Messenger RNA

MTT……[3–5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt]

MuV……..Mumps virus

NER.....Nucleotide excision repair

NDV……..Newcastle disease virus

NF-κB……....Nuclear factor κB

NK……..Natural killer cell

NP……...Nucleocapsid protein

NP^0……..……..Soluble Nucleocapsid protein

NS1…….Nonstructural protein 1 of influenza virus

NSCLC.....Non-small cell lung cancer

NUC.....Nuclear

OV….…….Oncolytic virus

P……..Phosphoprotein

PAGE……..Polyacrylamide gel electrophoresis

PARP.....Poly ADP ribose polymerase

PBS…….Phosphate buffered saline

PD-1.....Programmed death-1

PDI.....Protein disulfide isomerase

PD-L1..... Programmed death-1 ligand

PERK.....PKR-like ER kinase

PFA…….Paraformaldehyde

PFU…….....Plaque forming unit

PI……..…Persistent infection

PIV5…….……...Parainfluenza virus type 5

PKR…….……Protein kinase R

PUMA…….p53 upregulated mediator of apoptosis

RANTES…….Regulated on activation, normally T cell expressed and secreted

RIG-I……...…Retinoic acid inducible gene product-I

rPIV5……...Recombinant parainfluenza virus 5

RPMI 1640.....Roswell Park Memorial Institute medium

RSV…….Respiratory syncytial virus

RT-PCR……..…Reverse transcription polymerase chain reaction

SAHA.....Suberoylanilide hydroxamic acid

SBHA.....Sodium 4 Phenylbutuyrate, Suberoyl bis-hydroxamic acid

SDS……...…….....Sodium dodecyl sulfate

SeV……..…Sendai virus

SH…….....Small hydrophobic protein

SSPE.....Subacute sclerosing panencephalitis

ssRNA……..…Single stranded RNA

STAT……...….....Signal transducer and activator of transcription

SV40……Simian virus 40

TBS…….....Tris buffered saline

TC-NER.....Transcription-coupled NER

TFIIH.....Transcription factor II H

TLR……..……...…Toll-like receptor

TNF….……Tumor necrosis factor

Tr…….……Trailer

TRADD……...……TNF receptor-associated death domain

TRAIL…….…TNF related apoptosis inducing ligand

TUNEL……..……..Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling

T-VEC..... Modified herpes simplex virus type 1 designated talimogene laherparepvec

UPR.....Unfolded protein response

V……..……V protein

VDC…..……..V-dependent degradation complex

vRdRp…....……Viral RNA-dependent RNA polymerase

VSV…….Vesicular stomatitis virus

WIP1.....Wild-type p53 inducible protein 1

WF-PIV……...…Wake Forest parainfluenza virus

WT……..….Wild-type

XIAP.....X-linked inhibitor of apoptosis

XP.....Xeroderma pigmentosum

ZIKV.....Zika virus

CHAPTER ONE: INTRODUCTION

Oncolytic Viruses

Oncolytic viruses (OVs) are viruses that can selectively infect and replicate in cancer cells as opposed to normal healthy cells. OVs can be naturally occurring or engineered to have oncolytic properties. The selectivity of OV for cancers cells can be determined by a number of factors, including: OV receptors may be overexpressed in cancer cells, viral replication can be enhanced in tumor cells, enhanced viral mediated cell-to-cell fusion may occur in cancer cells, immune responses can be increased due to viral antigen expression (as reviewed by Mateeva et al., 2015).

The production of type I interferon (IFN) and subsequent IFN signaling can be one such limiting factor in tumor selectivity. Most cancer cells are defective for IFN induction and/or IFN responses (Haus, 2000). OVs can capitalize on this characteristic and have unrestricted replication in cancer cells as opposed to normal cells with intact IFN pathways. This property of OVs is beneficial because it can act as a layer of selectivity to OVs.

There is intense interest in developing oncolytic viral vectors with increased potency against cancer cells. As examples, a modified herpes simplex virus type 1 designated talimogene laherparepvec (T-VEC, IMLYGIC™; Amgen) is the first oncolytic virus approved for human use by the food and drug administration (Kaufman et al., 2015), and there has been a large increase in clinical trials for new viruses for tumor therapy (Fukuhara et al., 2016; Russell et al., 2012). While many virus types have been explored as potential OV, a number of paramyxoviruses have shown promise as oncolytic vectors based in part on their inherent cytopathic properties, including measles virus (MeV), Newcastle disease virus (NDV), Sendai

virus (SeV), and mumps virus (MuV; Elankumaran et al., 2006; Kinoh et al., 2004; Lorence et al., 1994; Myers et al., 2005; Peng et al., 2003; Yu et al., 2015; Matveeva et al., 2015). This thesis is based on the proposal of utilizing a mutant version of Parainfluenza virus type 5 (PIV5) as an oncolytic vector.

Parainfluenza Virus Type 5 (PIV5)

PIV5 belongs to rubulavirus genus within the *Paramyxoviridae* family, comprised of nonsegmented negative strand RNA virus (Lamb and Parks, 2007). The *Paramyxoviridae* family includes many human pathogens such as mumps virus (MuV), measles virus (MeV), respiratory syncytial virus (RSV), and the human parainfluenza viruses (hPIV 1-4). Members of the *Paramyxovirus family are o*ne of the most common causes of acute respiratory tract and systemic infections in humans.

The PIV5 genome is a negative stranded ssRNA of 15,246 bases and encodes seven genes; 3'-NP, P/V, M, F, SH, HN, L-5' (Paterson et al., 1984). RNA synthesis is controlled in part by viral genomic and antigenomic promoters that are composed of non-coding sequences located at both the 3' end, leader (le), and the 5' end, trailer (tr). These act as single-entry promoters which drive sequential transcription or alternatively, genome and antigenomic replication.

The PIV5 virion is pleomorphic in shape and when budding, obtains its lipid bilayer envelope from the host plasma membrane. Hemagglutinin-neuraminidase (HN) and fusion (F) proteins are membrane glycoproteins that are associated with the virion and aid in attachment and fusion of virion with the host membrane. The nucleocapsid protein (NP) encapsidates the viral genome to form a helical structure that is stable and protects the viral genome from degradation. The phosphoprotein (P) is an essential non-catalytic subunit of the viral RNA-

dependent RNA polymerase (vRdRp, Lamb and Parks, 2007). The viral polymerase is also composed of the large (L) protein, which is believed to contain the polymerase catalytic sites and is involved in nucleotide polymerization, 5' end guanylylation and methylation, and 3' end polyadenylation (Grdzelishvili et al., 2005; Hercyk et al., 1988; Ogino et al., 2005). The matrix (M) protein is involved in virus assembly and budding. The small hydrophobic (SH) protein is an integral membrane protein and functions to block apoptosis (He et al., 2001; Wilson et al., 2006).

The V protein is an accessory protein expressed from the P/V gene through a mechanism of "RNA editing". The V protein is multifunctional, acting to slow the cell cycle (Lin and Lamb, 2000), regulate viral RNA synthesis (Lin et al., 2005), inhibit type I IFN synthesis and signaling (Didcock et al., 1999; Poole et al., 2002), and block apoptotic pathways (Sun et al., 2004).

PIV5 Lifecycle

Extracellular PIV5 virions bind to sialic acid on the cell surface through the action of the protein HN (Fig. 1). Once HN attaches to the receptor, F undergoes a conformational change that allows for the viral lipid envelope to fuse with the host cell membrane. The viral nucleocapsid is then released into the cytoplasm and the vRdRp initiates viral RNAs and proteins synthesis. Primary transcription occurs when the viral polymerase produces low levels of viral mRNAs from the incoming virion. Gene junctions located between genes consist of a gene start sequence, an intergenic region, and a gene end sequence that have unique lengths and sequences. These sequences direct viral polymerase functions, resulting in the 5' end of the viral mRNA being methylated and capped, while the 3' end is polyadenylated.

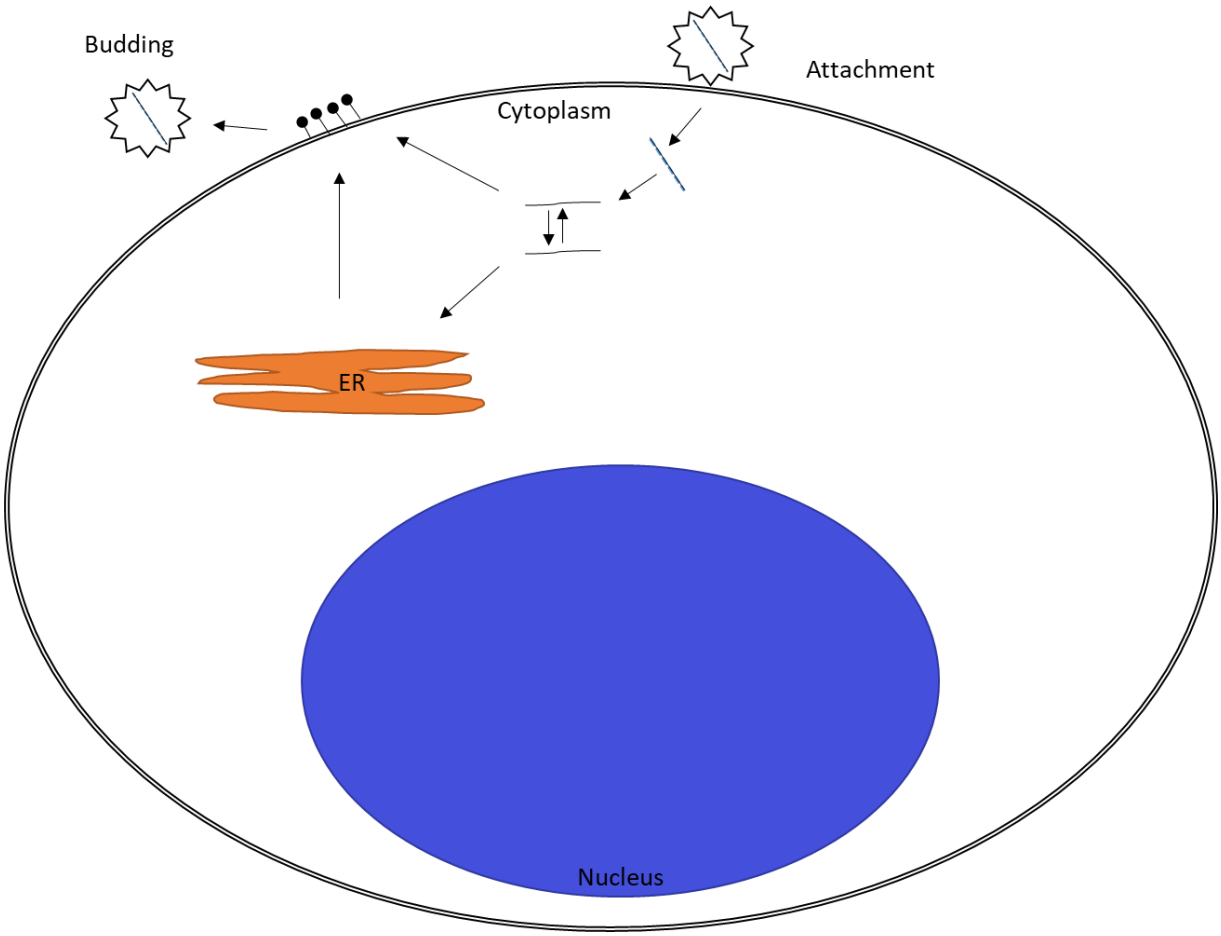

Figure 1: Parainfluenza Type 5 life cycle.

Once viral attachment and membrane fusion occurs, primary transcription of the input viral genome produces the initial viral mRNAs and proteins. Replication of the input genome produces anti-genomes, which are then used to make new progeny genomes. Secondary transcription amplifies the levels of viral mRNAs and proteins. Assembly of the progeny genomes occurs at the cell membrane and buds out of the host cell to produce progeny virions.

A “start-stop” mechanism produces a decreasing gradient of monocistronic mRNAs. The most promoter-proximal 3’ end gene, NP, is transcribed to the highest level while the most

promoter-distal gene, L, is transcribed to the lowest level. The newly synthesized viral mRNAs are then translated into viral proteins. 4-8 hours post infection, a switch occurs from transcription to replication and results in the synthesis of positive sense antigenome, which serve as templates to produce new genomes. This replication switch is thought to depend on the levels of viral NP proteins. It has been proposed that once an enough NP is made, it will bind the nascent RNA as it emerges from the polymerase. Encapsidation of the nascent RNA is required for productive replication of the viral RNA, generating full length genome and antigenomes (Blumberg et al., 1981; Vidal and Kolakofsky, 1989). It is thought that the same vRdRp that transcribes the genome is used for replication as well, but now gene junctions are ignored, and an exact complimentary copy of the template is generated. The new genomes can have either of two fates; they can traffic to the cell surface to assemble and bud out of the cell as a new progeny virion, or can serve as a template for another round of transcription, termed secondary transcription. Secondary transcription results in an amplification of viral mRNA and proteins.

Transcription of the PIV5 P/V gene differs from the other genes in that it produces two mRNAs through a process termed RNA editing or co-transcriptional editing (Thomas et al., 1988). The P/V gene contains an editing site in the genomic RNA. When the polymerase reaches the editing site, it occasionally stutters and inserts 2 extra G residues. This results in a shift in the translational reading frame producing different polypeptides. For PIV5, accurate transcription of the P/V gene produces the V mRNA. The P mRNA is synthesized with the addition of two extra G residues at the editing site, where the polymerase stutters and produces P mRNA approximately 50% of the time it transcribes the gene (Thomas et al., 1988). P and V proteins have identical N-terminal 164 amino acids (P/V region), but have unique C-terminal domains.

Both the P and V proteins play a role in paramyxovirus RNA synthesis. The P protein is a subunit of the viral polymerase along with the L protein and thus, is required for viral gene expression. The P protein can also bind NP and is believed to act as a chaperone during NP encapsidation of the nascent viral RNA strand during RNA replication (Curran et al., 1995). The SeV V protein is thought to play an inhibitory role in RNA synthesis by binding to NP and preventing its encapsidation of nascent RNA (Curran et al., 1991, Horikami et al., 1996). PIV5 V protein has also been shown to negatively regulate replication and transcription, but the mechanism has yet to be elucidated (Lin et al., 2005).

PIV5 Immunity Evasion

Host cell responses to viral infection are important factors that can contribute to viral pathogenesis and to tropism for specific tissues or cells. These antiviral responses can include the secretion of proinflammatory cytokines, such as interleukin-6 (IL-6) and IL-8, stimulation of type 1 interferon (IFN) pathways, and induction of apoptosis. IFNs are cytokines in the innate immune response and can control cell proliferation, directly limit viral replication and spread, and activate the adaptive immune response (reviewed in Stark et al., 1998; Biron and Sen, 2007). IFNs are synthesized primarily in response to stimuli, such as viral infection. Type 1 IFNs, IFN-α and IFN-β, are produced by most cell types following viral infection and lead to the synthesis of hundreds of antiviral genes (reviewed in Stark et al., 1998). The induction and amplification of type 1 IFN responses to viral infection has been thought to follow a three-step model (reviewed in Levy et al., 2002).

Viral RNA synthesis yields byproducts, such as double stranded RNA (dsRNA) and uncapped 5'-triphosphate RNA, which are detected by host cell sensor proteins that can lead to the initial induction of the IFN pathway (Hornung et al., 2006; Jacobs and Langland, 1996;

Pichlmair et al., 2006). Other viral components can also activate the IFN pathway, for example the MeV nucleocapsid protein (tenOever et al., 2002). Host cell sensors that can recognize these pathogen-associated molecular patterns include protein kinase R (PKR; Robertson and Mathews, 1996), retinoic acid-inducible gene-I (RIG-I; Andrejeva et al., 2004; Hornung et al., 2006; Pichlmair et al., 2006), melanoma differentiation-associated gene 5 (mda-5; Yoneyama et al., 2005), and toll-like receptor 3 (TLR3; Alexopoulou et al., 2001). Downstream signaling by these sensors can lead to the activation of latent transcription factors such as the IFN regulatory factors (IRFs; Taniguchi et al., 2001) and nuclear factor kappa B (NF-κB; Karin and Ben-Neriah, 2000), which are necessary for IFN-β synthesis. IRF-3, NF-κB, and AP-1 translocate to the nucleus and activate transcription from the IFN-β promoter.

In the second phase following synthesis and secretion, IFN-β then binds to its cognate type 1 IFN receptor in an autocrine or paracrine fashion to initiate the IFN pathway signaling phase. Once activated, the receptor associates with members of the Janus activated kinase (JAK) family leading to their tyrosine phosphorylation, which then phosphorylates tyrosine residues on the cytoplasmic tail of the receptor (Schindler, 1999). Latent transcription factors, called signal transducers and activators of transcription (STAT1 and STAT2), are then phosphorylated, heterodimerize, and associate with IRF-9 to form the transcription factor interferon stimulated gene factor 3 (ISGF3; Horvath, 2000). ISGF3 then translocates to the nucleus, where it binds to interferon sensitive response elements (ISRE) located in the promoter region of many IFN-stimulated genes (ISGs). In a third phase, the ISG IRF-7 can activate transcription of "late" response genes, including the IFN-α genes (reviewed in Levy et al., 2002). Most importantly, many ISGs have antiviral properties and can contribute to the inhibition and clearance of viral infections (reviewed in Sen, 2000; Biron and Sen, 2007).

Members of the paramyxovirus family utilize a wide range of mechanisms to counteract IFN, for example limiting IFN induction, blocking IFN signaling pathways, or both processes (reviewed in Conzelmann, 2005; Garcia-Sastre, 2001; Goodbourn et al., 2000; Horvath, 2004). Many of the mechanisms involved to circumvent IFN pathway activation have been attributed to products of the P/V gene (or sometimes P/V/C gene), which encodes both the phosphoprotein P subunit of the vRdRp and the accessory V protein (Didcock et al., 1999; Garcin et al., 1999; Kubota et al., 2001; Parisien et al., 2001). PIV5 V protein can block activation of IFN-β promoter by transfected dsRNA (Poole et al., 2002) or following infection (He et al., 2002). This inhibition of IFN-β synthesis is proposed to be due to V protein targeting IFN-inducible RNA helicase mda-5 (Childs et al., 2007), through binding of the cysteine-rich region of the V protein (Andrejeva et al., 2004).

In addition to blocking IFN synthesis, paramyxoviruses can also block IFN signaling pathway as an additional method to evade the IFN response, mainly by interference with STAT proteins. As examples, NDV infection can induce IFN synthesis, but the virus can suppress IFN responses by inactivation of STAT1 through action of the V (Huang et al., 2003) and C proteins (Garcin et al., 1999; Takeuchi et al., 2001), respectively. The measles P protein is required for blocking STAT1 phosphorylation (Devaux et al., 2007). Like NDV, V proteins of PIV5 and MuV inhibit IFN signaling by targeting STAT1 for proteasome-mediated degradation (Didcock et al., 1999; Kubota et al., 2001), whereas hPIV2 targets STAT2 (Andrejeva et al., 2002b; Parisien et al., 2001). Importantly for this thesis project, the PIV5 V protein hijacks the host protein damage-specific-DNA-binding-protein-1 (DDB1) as demonstrated in fig. 2. In virus infected cells, DDB1 becomes part of a cytoplasmic “V degradation complex” (VDC), which

induces the degradation of STAT1 or STAT2, resulting in inhibition of type I IFN signaling (Andrejeva et al., 2002a; Didcock et al., 1999; Ulane et al., 2005).

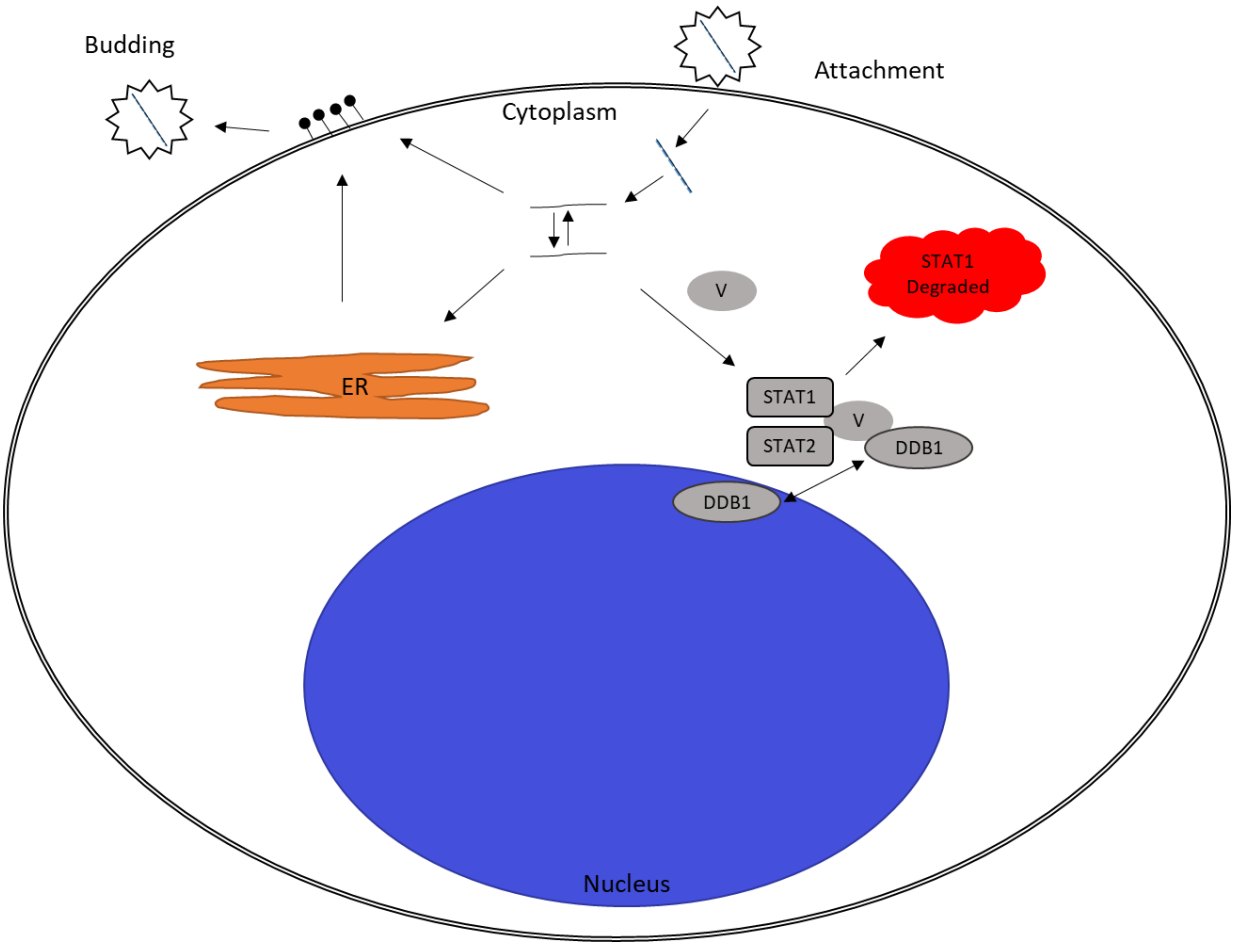

Figure 2: Innate immunity pathway inactivation by PIV5.

Through the actions of the viral V protein hijacking DDB1, the VDC forms and targets STAT1 for proteasome-mediated degradation. The IFN pathway signaling phase is largely deactivated due to STAT1 degradation.

PIV5 P/V-CPI- mutant

For reasons that are not fully understood, WT PIV5 replicates to very high levels in epithelial cells (>10^9 infectious units per ml), but is largely noncytopathic to most cell types in vitro. Interestingly, alterations to the PIV5 P/V gene can convert the noncytopathic WT virus into a highly cytopathic mutant (P/V-CPI-) (Sun et al., 2004; Wansley and Parks, 2002; Wansley et al., 2005; Young et al., 2006; Dillon et al., 2006; Gainey et al., 2008a). The PIV5 P/V gene encodes the phosphoprotein P and the accessory protein V, which share an identical 164 residue amino-terminal domain (the shared P/V region), but have unique C terminal domains. The P protein is an essential subunit of the viral RNA-dependent RNA polymerase (Lamb and Parks, 2007). V protein is thought to function in the regulation of viral RNA synthesis (Lin et al., 2005), but also has additional roles in blocking IFN signaling (Didcock et al., 1999; Ulane et al., 2005), disrupting apoptosis pathways (Sun et al., 2004) and inhibiting IFN-beta gene expression through binding to mda-5 (Childs et al., 2007). In the case of the P/V-CPI- mutant, the introduction of six amino acid changes in the shared P/V region (listed in Materials and Methods) results in a mutant with properties very different from WT PIV5, including the activation of IFN and proinflammatory cytokine responses (Wansley and Parks, 2002; Young and Parks, 2003), over-expression of viral RNA and proteins (Wansley and Parks, 2002; Dillon and Parks, 2007), and induction of massively cytopathic effects to most cancer cell lines tested so far (Wansley and Parks, 2002; Dillon et al., 2006; Gainey et al., 2008a).

The PIV5 P/V-CPI- mutant is currently being developed as a novel oncolytic vector. This mutant is restricted for growth in normal cells, but is fully capable of growth and spread through a population of tumor cells *in vitro* (Wansley et al., 2005; Gainey et al., 2008b). These differences in cell type restriction is proposed to be in part due to effective IFN responses

following P/V-CPI- infection of normal cells but not cancer cells (Gainey et al., 2008b). During the transformation process, tumor cells can accumulate specific defects in IFN pathways that contribute to resistance to the anti-proliferative effects of IFN (e.g., Wong et al., 1997; Xu et al., 1994). It is proposed that these alterations can also confer increased susceptibility to viral infection (Russell et al., 2012; Bell et al., 2003) particularly in the case of mutants such as P/V-CPI- which is defective in blocking IFN.

The mechanism of cell killing by the P/V-CPI- mutant is not completely understood at this time, however it could be tied mechanistically to the high induction of dsRNA during replication and the shut off of host and viral protein synthesis through Protein Kinase R (PKR) pathways (Gainey et al., 2008a). Similarly, cell killing by the P/V-CPI- mutant involves activation of caspase dependent death pathways (Wansley et al., 2003; He et al., 1997).

Viral Persistence

A majority of the cell population die following an acute P/V-CPI- virus infection. However unexpectedly, at later timepoints a substantial percentage of viabile cells remained. Cells that constitute this persistent infection (PI) could be passaged, and still retained viral protein expression. The mechanisms which lead to the transition of a cytopathic acute infection to a non-cytopathic PI are not completely understood (reviewed in Rima and Martin, 1976). This can involve apparently unrelated changes in cell morphology, such as with a PI with Foot and Mouth Disease Virus (FMDV) where there are alterations in cell shape and increased growth characteristics, as well as an acquired resistance to acute FMDV infection (de la Torre et al., 1988). In the case of MuV, previous studies have shown that PI cells *in vitro* continually express MuV antigen and are resistant to the cytopathic effects of parental MuV (Walker and Hinze, 1962). Host cell type can also be key in developing a paramyxovirus PI (Holmes and Choppin,

1966). Consistent with this, an NDV PI was successfully established in one type of ovarian cancer cell line OVCAR3, while other cells lines such as OAW28, CAL27, FaDu, and PE/CA PJ15 were unable to establish a PI (Rangaswamy et al., 2017).

IFN induction could also play a role in lower virus production from PI cells compared to an acute infection, but other possibilities include slower cell growth rate, elevated cell stress responses (e.g., translation arrest) or the accumulation of mutations in the virus population. PI cells can have an altered antiviral state induced by low levels of IFN production and the presence of IFN stimulated genes as seen in the case of NDV infection (Rangaswamy et al., 2017; Schneider et al., 2014). The P/V-CPI- virus is a potent inducer of IFN (Wansley and Parks, 2002), and work is in progress to understand the role this cytokine plays in differential ability to establish P/V-CPI- PI cells in different cancer cell lines.

The observation that the cytopathic P/V-CPI- virus established a persistent infection raised numerous questions on the mechanisms by which the cells are surviving while harboring a cytopathic virus. One explanation is the virus could acquire mutations to become a less cytopathic virus than the parental P/V-CPI- virus. Consistent with this, virus derived from MeV PI cells leading to subacute sclerosing panencephalitis (SSPE) had mutations in the M, H and F genes due to polymerase errors and hypermutation events (Billeter et al., 1994). An additional possibility is the PI cells could evolve with altered apoptotic pathways to prevent cell death normally activated by the P/V-CPI-. We performed challenge experiments to determine how the PI cells would respond to external inducers of death. While testing the hypothesis that PI cells have altered apoptotic pathways, we found that PI cells and cells acutely infected with P/V-CPI-

virus show enhanced DNA damage and cell death induced by chemotherapy agents such as cisplatin.

Chemotherapy and DNA Damage Response (DDR)

Surgery, radiation, chemotherapies, specific targeted therapies, and immunotherapies are the treatments available for non-small cell lung cancer (NSCLC). NSCLC chemotherapy treatment most often utilizes a combination of two drugs. Chemotherapy used for the treatment of NSCLC can include; platinum-based DNA damage inducers, like cisplatin or carboplatin; microtubule-targeting cytoskeletal drugs, taxol and docetaxel as examples; the DNA damage inducer gemcitabine; topoisomerase inhibitors such as etoposide and irinotecan; and pemetrexed, an inhibitor of enzymes needed for nucleotide synthesis (PDQ, 2019). Histone deacetylase inhibitors are another class of chemotherapy being investigated to treat lung cancer in clinical trials (reviewed by Suraweera et al., 2018). NSCLC tumors that contain specific types of epidermal growth factor receptor (EGFR) tyrosine kinase domain gene mutations can be targeted through drugs like erlotinib or gefitinib. Immunotherapies such as monoclonal antibodies targeting tumor antigens, checkpoint inhibitors, therapeutic vaccines, and adoptive T-cell transfer, are also NSCLC treatment options. Two immunotherapies have been approved by FDA for the treatment of NSCLC: targeting programmed death-1 (PD-1) and it's ligand PD-L1 interactions using nivolumab and pembrolizumab (PDQ, 2019).

Many chemotherapies elicit a DDR that can lead to cancer cell death. Approved by the FDA in 1978 as cancer treatment, cis-diammine-dichloroplatinum (II) or cisplatin's mode of action is to bind to guanine and induce DNA adducts (Dasari and Tchounwou, 2014). Damaged DNA can be repaired through five DNA repair pathways: 1) nucleotide excision repair (NER), 2) base excision repair, and 3) mismatch repair, which can recognize single base alterations,

whereas 4) nonhomologous end joining and 5) homologous recombination can recognize double-strand breaks. The DNA damage induced by cisplatin is mainly recognized by the nucleotide excision repair (NER) pathway, which can lead to cell cycle arrest to efficiently repair the DNA or cell death based on the extent of the DNA damage (Siddik, 2003). NER pathways can repair bulky DNA lesions that cause distortions in its structure, for example UV-induced lesions, intrastrand cross-links, and DNA adducts, such as those elicited by cisplatin (as reviewed in Goldstein and Kastan 2015). NER occurs through two pathways, transcription-coupled NER (TC-NER) and global genome-NER (GG-NER) and requires 20-30 proteins (Fousteri and Mullenders, 2008). The NER pathways are composed of steps - the damaged DNA is recognized by members of the xeroderma pigmentosum (XP) protein family, DNA is unwound, the lesion is excised, the DNA gap is filled, and DNA is sealed by ligase I (Christmann et al., 2003).

The NER pathway is composed of sensors that transduce signals to downstream effectors mediated through post-translational modifications, such as protein phosphorylation on serine or threonine residues, and modifications by ubiquitin. Upon DNA damage, DDB1 binds to DDB2 forming the DNA damage binding (DDB) complex that can then translocate to the nucleus from the cytoplasm. The DDB complex can form an E3 ubiquitin ligase complex with CUL4A as a scaffold, RBX1/ROC1/Hrt1 acts to recruit E2, and DDB2 for substrate recognition (Angers et al., 2006). Once localized to the DNA damage site, the CUL4A-RBX1-DDB1-DDB2 complex induces XPC polyubiquitination, which does not result in its degradation, but instead results in enhanced DNA affinity (Sugasawa et al., 2005). XPC promotes denaturation bubble formation opened by XPB and XPD helicases of transcription factor II H (TFIIH) to allow the DNA lesion to be excised. Additional CUL4A-DDB1 substrates include histones H2A, H3, and H4 near the

DNA lesion site, resulting in an open chromatin environment to allow the NER proteins to properly assemble (Kapetanaki et al., 2006; Wang et al., 2006).

p-H2A.X acts as a platform for recruitment of DNA repair proteins and can be phosphorylated by the DNA damage sensors ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and rad3-related (ATR). Downstream of sensor activation, effectors such as checkpoint kinases (Chk1/2) are phosphorylated and activated as reviewed by De Zio et al., (2013). Once activated, the effectors can interact with downstream proteins p53 and cdc25 proteins to initiate G2/M cell cycle arrest to allow for DNA repair to occur or apoptosis depending on the extent of DNA damage.

Cisplatin is a relatively effective chemotherapy used to treat numerous malignancies. However as with many chemotherapies, cisplatin has many disadvantages; such as severe kidney problems, allergic reactions, gastrointestinal disorders, hearing loss, hemorrhage and tumor recurrence (Dasari and Tchounwou, 2014). Although cisplatin is the gold standard for numerous cancers, chemoresistance is a major concern. For example, cisplatin treatment for ovarian cancer patients is initially very effective, but tumor recurrence occurs in up to 75% of cases, resulting in chemotherapy resistant tumors (Agarwal and Kaye, 2003). Previous studies have shown that cisplatin resistance can result when cells increase DNA repair capabilities (Siddik, 2003; Parker et al., 1991; Masuda et al., 1988; De Pooter et al., 1996; Ferry et al., 2000). Conversely, cisplatin sensitivity has been associated with lower capacity of cells in their DNA repair pathways (Rosell et al., 2003; Welsh et al., 2004; Calsou et al., 1993; Hill et al., 1994).

Cancer development and tumor progression are hallmarked by genetic and epigenetic DNA alterations, such as hypoacetylation of histone tails (Ropero and Esteller, 2007). We extended our study to investigate if the P/V-CPI- virus would act synergistically with a

chemotherapy that modifies DNA. There are 18 known human histone deacetylase (HDAC) enzymes that remove acetyl groups from lysine residues on histone tails and are involved in a number of other cellular processes. HDAC expression levels have been found to be associated with differing prognosis in various cancers. For example, high expression of HDAC1 and HDAC2 has been correlated with a poor prognosis in patients with lung or oral cancers (Minamiya et al., 2011; Chang et al., 2009). As such, there has been a recent focus on developing HDAC inhibitors for cancer therapy. These chemicals can have pleotropic effects on cancer cells such as altering the cell cycle, inducing senescence or autophagy, altering signaling pathways and immune responses, or regulating apoptosis. Importantly however, many HDAC inhibitors have been shown to have little cytotoxic effect on normal cells (reviewed by Eckschlager et al., 2017). To date, four HDAC inhibitors are approved by the FDA for use in humans (Vorinostat, Romidepsin, Belinostat, and Panobinostat), and a large number of other inhibitors are in clinical trials for cancer therapy (Suraweera et al., 2018).

Oncolytic Viruses and Chemotherapy

Our studies raise the possibility of using combination therapies with paramyxovirus oncolytic viruses and chemotherapies, as have been studied with other viruses. For example, a phase II clinical trial has shown the combination of the adenovirus oncolytic virus ONYX-015, cisplatin, and 5-fluorouracil to be more effective than the therapies alone in patients with recurrent head and neck cancer (Khuri et al., 2000). A phase I/II study investigated the combination of oncolytic virus T-VEC, radiotherapy, and cisplatin to treat patients with head and neck cancer. These clinical trials have found combination therapy with an oncolytic virus and chemotherapy to improve disease progression, rate of relapse-free, and overall survival (Khuri et al., 2000; Harrington et al., 2010). Our studies differ from prior work in that our oncolytic virus

is a cytoplasmic replicating RNA virus with no requirement for the nucleus for infection and growth.

Combination studies have investigated oncolytic viruses and HDAC inhibitors as reviewed by Nakashima et al., (2015). HDAC inhibitors may lead to amplification of tumor-specific lytic effects by immune suppression and enhanced viral replication. Combination studies of HDAC inhibitors and the oncolytic vesicular stomatitis virus (VSV) have shown increases in caspase activation, mitochondria membrane potential loss, blocking IFN-1 response, activation of NF-κB and NF-κB- mediated autophagy (Shulak et al., 2014). Additional immune responses can be altered in combination of HDAC inhibitors and oncolytic viruses, such as preventing NK cell activation and enhanced immunogenic responses (Alvarez-Breckenridge et al., 2012; Bridle et al., 2013). Combination of HDAC inhibitors and viruses have also altered cell cycle processes by causing G1/S arrest and cyclin D down-regulation (Liu et al., 2008; Katsura et al., 2009). Our work is unique because we are investigating how a cytoplasmic-replicating RNA virus can act in cooperation with chemotherapy that targets DNA to enhance cancer cell death and immune responses.

Summary of Thesis Work

While the P/V-CPI- virus is inherently cytopathic to cancer cells, we have recently shown that P/V-CPI- infection can sensitize cancer cells to further killing by treatment with cisplatin (Fox and Parks, 2018), a DNA adduct-inducing chemotherapy drug. Remarkably, infection with the cytoplasmic-replicating P/V-CPI- virus followed by cisplatin treatment led to increased damage to cellular DNA, along with enhanced caspase activation and death compared to treatment with the virus or drug alone. The ability of P/V-CPI- to sensitize cells to cisplatin-

induced death correlated with virus-induced defects in the cell's ability to repair damaged DNA, which may be related to the V protein retention of DDB1 in the cytoplasm as demonstrated in Fig. 3. These results with DNA-damaging agents, such as cisplatin, raise the question of whether the P/V-CPI- virus would be more effective at killing cancer cells when coupled with other chemotherapeutic agents that alter DNA metabolism and stress responses.

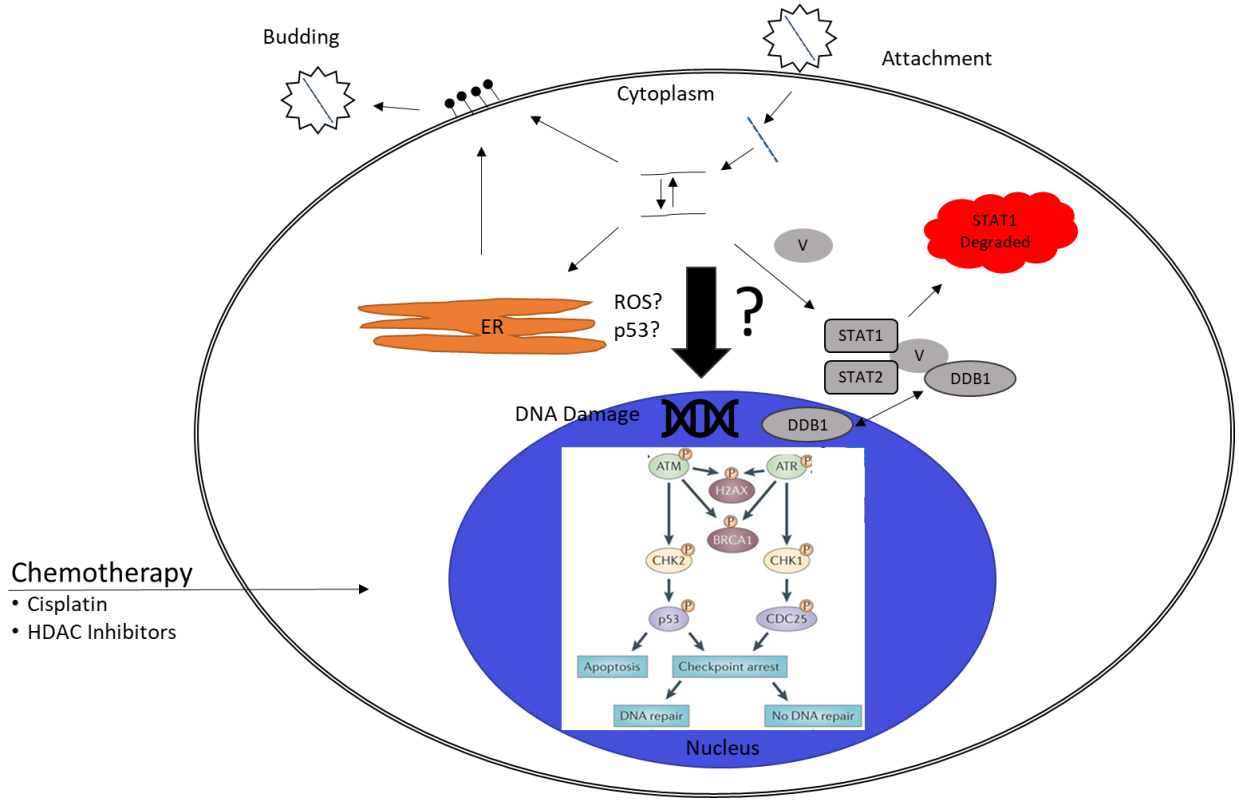

Figure 3: Model of DNA damage sensitivity by PIV5.

Following infection, DDB1 is complexed with the viral V protein to form the VDC, which targets STAT1 for proteasome-mediated degradation. Upon DNA damage such as those induced by chemotherapy, DDB1 normally translocates from the cytoplasm to the nucleus, where it can aid in DNA repair pathway activation. However in PIV5 infected cells, we hypothesize DDB1 is unable to translocate to the nucleus because DDB1 is in the VDC. Additional sources of DNA

damage could be production of reactive oxygen species and activation of p53. Due to decreased DNA repair capabilities, PIV5 infected cells are more sensitive to DNA damage, such as that induced by the chemotherapy cisplatin. Adapted from: Gabriele Sulli, Raffaella Di Micco & Fabrizio d'Adda di Fagagna. Crosstalk between chromatin state and DNA damage response in cellular senescence and cancer Nature Reviews Cancer 12, 709-720 (October 2012).

We have also shown that treatment of human airway cancer cells with HDAC inhibitors increased cell death and caspase activation induced by P/V-CPI- mutant infection, through pathways that involved at least in part recognition of dsRNA (Fox and Parks, 2019). In addition, HDAC inhibitors reduced IFN-β production by P/V-CPI- infection by blocking phosphorylation and nuclear localization of the key transcription factor interferon regulatory factor-3 (IRF-3), resulting in enhanced P/V-CPI- spread through a population of human airway cancer cells. Our studies support a common property by which a cytoplasmic-replicating RNA virus sensitizes cancer cells to chemotherapeutic agents and suggest combined paramyxovirus and chemotherapy as promising future approaches to treat cancer.

CHAPTER TWO: MATERIALS AND METHODS

Cells, Viruses, and Infections

The H1299 cell line was obtained from Annette Khaled (University of Central Florida, Orlando, FL, USA). A549, HEp-2, Vero, MDBK, and CV-1 cell lines were provided by Robert Lamb (Northwestern University, Evanston, Illinois, USA). Cultures of H1299 non-small cell lung carcinoma cells were grown in Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 10% heat inactivated fetal calf serum (HI FBS, Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Cultures of A549 alveolar adenocarcinoma cells, HEp-2 laryngeal carcinoma cells, Vero cells, MDBK cells, and CV-1 cells were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 10% HI FBS. Previously described A549 cells that constitutively express reovirus type 3 Dearing sigma3 protein (Manuse and Parks, 2009) were generated by transfection with pCXN-S4T3D (Kobayashi et al., 2007) followed by selection in DMEM containing 0.5 mg/mL G418.

Wild type (WT) PIV5 was grown in MDBK cells and titered on CV-1 cells. The P/V mutant rPIV5-P/V-CPI− (P/V-CPI-) encoding Green Flourescence Protein (GFP) as an additional gene between HN and L was generated and grown in Vero cells as described previously (Wansley and Parks, 2002) using a cDNA plasmid (He et al., 1997) kindly provided by Robert Lamb (Northwestern University, Evanston, Illinois, USA) and Biao He (University of Georgia, Athens, Georgia, USA). P/V-CPI- encodes six naturally-occurring mutations in the amino-terminal region of the P/V gene, resulting in amino acid changes at: Y26H, V32I, T33I, L50P, L102P, and S157F (Dillon et al., 2006). Vesicular stomatitis virus (VSV), human parainfluenza virus type 2 (hPIV2), and Zika virus (ZIKV, MR766, BEI resources, Manassas,

VA, USA) were grown in Vero cells. La Crosse virus (LACV; a kind gift from Andy Pekosz, Johns Hopkins University, Baltimore, Maryland, USA) were grown in C636 cells and stocks were titered by plaque assay on Vero cells.

Infections were performed by incubating virus and cells in DMEM or RPMI supplemented with 10% BSA. After one hour of incubation, cells were washed, and media was replaced with DMEM or RPMI supplemented with 2% HI FBS and various HDAC inhibitors as indicated in the figure legends.

To obtain persistently infected (PI) cell lines, HEp-2 cells were infected with the P/V-CPI− mutant at an MOI of 10, and media was replaced every 3 days post infection (pi) for 2 weeks. P/V mutant infected cells were sorted for high GFP expression using FACS Calibur flow cytometer (BD Bioscience, San Diego, CA) and were designated as the HEp-2 PI cells.

Chemical Preparation

YM155 was purchased from EMD Millipore and was reconstituted in sterile DMSO at a stock concentration of 3 mM. Embelin was purchased from Tocris and was reconstituted in sterile DMSO at a stock concentration of 5 mM. Cisplatin was purchased from Sigma-Aldrich and was reconstituted in sterile water at a stock concentration of 5 mM. WIP1 inhibitor (GSK 2830371) was purchased from Tocris and was reconstituted in sterile DMSO at a stock concentration of 5 mM. PI and naïve cells cultured in 24 well plates (diameter, 2 cm) were challenged as indicated in figure legends with drugs that were diluted in DMEM containing 10% HI FBS. Tunicamycin was purchased from Fisher and was reconstituted in DMSO.

HDAC inhibitors were purchased from Sigma-Aldrich (St. Louis, MO, USA). Scriptaid, CI-994, Apicidin, Panobinostat (LBH589), Suberoylanilide hydroxamic acid (SAHA, also known as Vorinostat), Tubacin, and Trichostatin A were reconstituted in sterile dimethyl

sulfoxide (DMSO). Sodium 4 Phenylbutuyrate, Suberoyl bis-hydroxamic acid (SBHA), and Valproic Acid were reconstituted in sterile water.

Cell Viability and Caspase Assays

[3–5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt] MTT cell viability assays were performed in 96-well dishes using Cell Titer 96 Aqueous One solution reagent (Promega, Madison, WI, USA) according to the manufacturer's instructions. Data are expressed as a percentage of mock-infected cells analyzed in parallel.

Alternatively, cells cultured in 24-well plates (2 cm diameter) were treated as indicated in each figure legend (concentration of drug and time). Media and trypsinized adherent cells were centrifuged and analyzed for annexin V binding (BD Bioscience, San Jose, CA, USA) and propidium iodide (BD Bioscience) staining as described by the manufacturer. Cells were analyzed by flow cytometry using the CytoFLEX (Beckman Coulter, Brea, CA, USA) and 10,000 independent events were analyzed using CytExpert software (Beckman Coulter).

Cytotoxicity assays were performed in 96-well white plates (Corning, Corning, NY, USA) using CytoTox-Glo reagent (Promega) according to the manufacturer's instructions. Data are expressed as a fold change of mock-infected cells analyzed in parallel. Functional caspase assays were performed in 96-well white plates (Corning) using Caspase-Glo 9, 8, or 3/7 assays (Promega) according to the manufacturer's instructions. Data are expressed as a fold change of mock-infected cells analyzed in parallel.

Western Blotting

As described in the figure legends, 6-well dishes (60 mm diameter) of cells were treated, followed by lysis in 1X protein lysis buffer (Cell Signaling Technology, Danvers, MA, USA).

Cell lysate was resolved on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (Bio-Rad, Hercules, CA, USA) and transferred to nitrocellulose membranes. Samples were probed with antibodies indicated in the figure legends (Cell Signaling Technology), anti-β-actin antibody (A5316, Sigma-Aldrich, St. Louis, MO, USA), rabbit polyclonal antisera to the PIV5 NP, HN and P proteins (Goodbourn et al., 2000) or IFIT-1 antibody (Novus Biologicals, Centennial, CO, USA). Blots were visualized by horseradish peroxidase-conjugated antibodies (Cell Signaling Technology) and chemiluminescence (Thermo Fisher Scientific).

Immunostaining and Terminal Deoxynucleotidyltransferase-mediated dUTP-biotin Nick End Labeling (TUNEL) Staining, Immunostaining, and Fluorescence Microscopy

Cells grown on 8 chamber slides (ThermoFisher) were treated with cisplatin as indicated in the figure legends and analyzed by staining with Anti-DDB1 (1:250 dilution, Zymed, Invitrogen) followed by goat anti-rabbit Alexa Fluor 568 (1:2000 dilution, Invitrogen). 4;6-Diamidino-2-pherylindole (DAPI) was included to stain for nuclei. To visualize DNA damage, cells were treated with cisplatin as indicated in the figure legend and analyzed by the Click-iT TUNEL Alexa Fluor 647 assay kit as described by the manufacturer (Invitrogen).

Cells were grown on glass bottom 48-well plates (MatTek, Ashland, MA, USA) and treated as indicated in the figure legends. Live imaging microscopy was performed using the Perkin Elmer Ultraview microscope with 20X objective lens. IRF-3 immunostaining was performed as previously described (Dillon and Parks, 2007) using a primary antibody against IRF-3 at 1:400 dilution (BD PharMingen, clone SL-12.1). Slides were imaged on a Ziess710 confocal microscope with 40X objective lens.

Nuclear Extraction

Cells were grown in a 6 well dishes and treated with cisplatin as indicated in the figure legends. Nuclear and cytoplasmic extracts were obtained using a kit according to manufacturer's guidelines (Active Motif). 10 ug of extracts were lysed in 1X protein lysis buffer (Cell Signaling Technology) and analyzed by western blotting.

Human IFN-β ELISA

As described in the figure legends, 6 -well dishes (60 mm diameter) of cells were treated and supernatants were evaluated using a VeriKine Human IFN-β ELISA kit as described by the manufacturer (PBL Assay Science, Piscataway, NJ, USA). ELISA results were normalized to 10^6 cells.

Reverse Transcription and Real Time PCR

As described in the figure legends, 6-well dishes (60 mm diameter) of cells were treated, followed by RNA extraction using TRIzol (Invitrogen, Carlsbad, CA, USA). To produce cDNA, 1 ug of total RNA was used by utilizing TaqMan® Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA) as per the manufacturer's instructions. Quantitative real-time PCR was performed using Bio-Rad CFX Connect Real-Time and Fast SYBR® FAST Green Master Mix (Applied Biosystems). Primers used include: β-actin forward 5′-GATCATTGCTCCTCCTGAGC-3′, and β-actin reverse 5′-ACTCCTGCTTGCTGATCCAC-3′ and OAS2 forward: 5'-AGAAGCTGGGTTGGTTTATC-3', and OAS2 reverse 5'-GACGTCACAGATGGTGTTC-3'. IFIT1 and TLR3 forward and reverse primers were obtained from studies by (Madigan et al., 2012) and (Gonzalez-Reyes et al., 2010), respectively. Relative gene expression was determined using CFX Manager 3.1 Software (Bio-Rad).

Statistical Analyses

Values are the mean of three replicates and experiments were performed at least twice. Statistical analysis was performed using Prism GraphPad, students T test or a two-way ANOVA. In all figures, * indicates p-value < 0.05, ** indicates p-value < 0.01, and *** indicates p-value < 0.001.

CHAPTER THREE: PARAINFLUENZA VIRUS INFECTION SENSITIZES CANCER CELLS TO DNA DAMAGING AGENTS: IMPLICATIONS FOR ONCOLYTIC VIRUS THERAPY

The Cytopathic PIV5 P/V-CPI- Mutant is Capable of Establishing a Persistent Infection

The PIV5 P/V-CPI- mutant is highly cytopathic to a number of cancer cell lines (Wansley and Parks, 2002; Dillon et al., 2006; Gainey et al., 2008a). This is illustrated in Fig. 4A, where human laryngeal cancer HEp-2 cells were mock infected or infected with WT PIV5 or P/V-CPI- mutant. Cell viability was followed over 72 hpi using MTT assays. In contrast to WT PIV5, the P/V-CPI- mutant induced ~70% cell death at 48 and 72 hpi. Unexpectedly, at these later timepoints a substantial percentage of viability remained. Cells that survived this infection could be passaged, and retained GFP expression (Fig. 4B), thus establishing these cells as persistently infected (PI). Western blotting confirmed that the PI cells expressed all viral proteins examined so far (Fig. 4C). The PI cells produced infectious virions (Fig. 4D), albeit at ~3 logs lower yield in PFU/ml compared to an acute P/V-CPI- infection. These data indicate that in some cell types the cytopathic P/V-CPI- mutant can establish a PI cell line that still expressed viral proteins and produced virus.

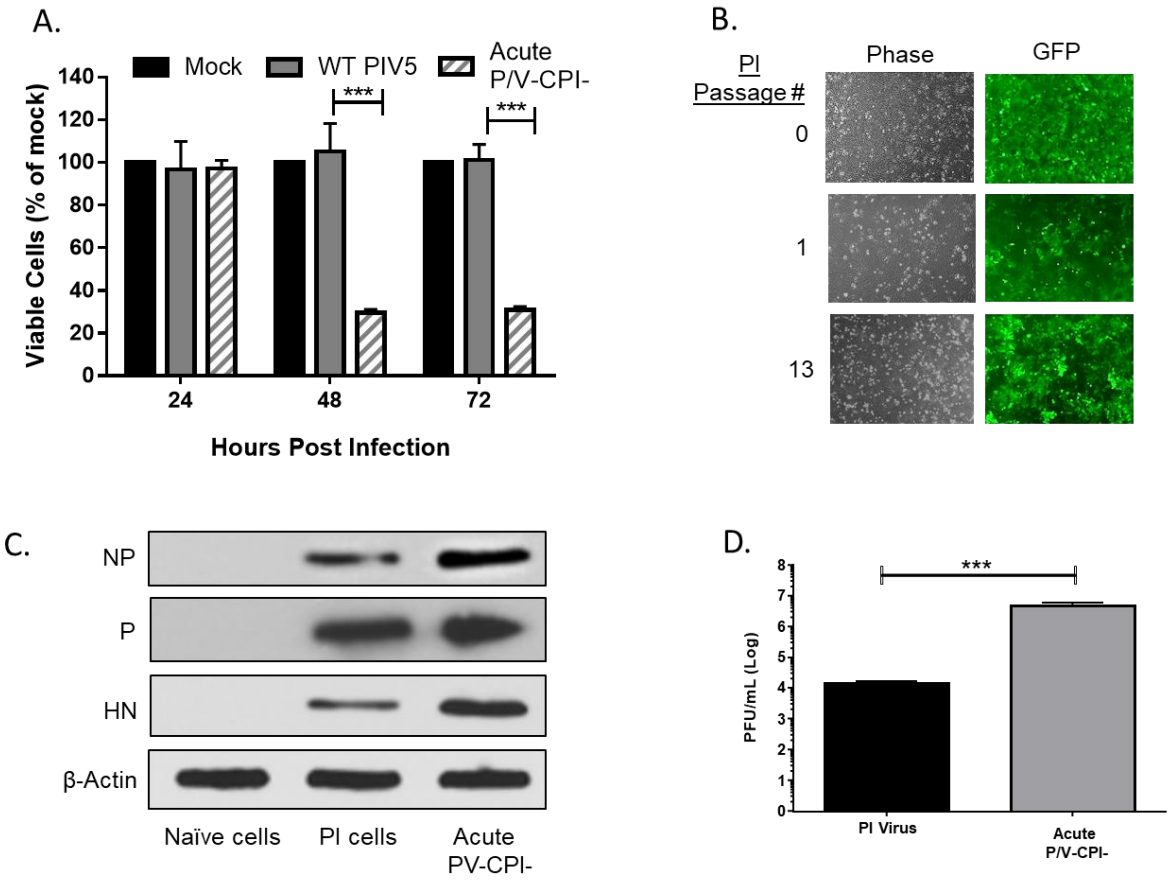

Figure 4: Persistent infection with a cytopathic oncolytic virus.

Naïve HEp-2 cells were mock infected or infected with rPIV5-WT or the P/V-CPI- mutant at an MOI of 10 PFU/cell. Cell viability was determined by MTT assay at the indicated hr pi. Values are the mean of three samples with *** indicating P-value < 0.001 comparing WT versus P/V-CPI- infections. B) Cells that survived the P/V mutant infection were examined by fluorescence microscopy for GFP expression. C) Lysates from naïve, PI and acute P/V-CPI- infected cells (24 hr pi) were analyzed by western blotting for the indicated viral proteins. D) Virus released from PI HEp-2 cells or after 24 hr acute infection with P/V-CPI- were titered. *** indicates P-value < 0.001.

PI Cells have Elevated Basal Levels of Apoptotic Markers and are Highly Dependent on Inhibitors of Apoptosis for Survival

The above findings raised the question of whether the PI cells had downregulated apoptotic pathways in order to survive P/V-CPI- infection. To determine levels of steady state stress, naïve and PI HEp-2 cells were examined for cell viability and apoptotic effector proteins. As shown in Fig. 5A (top panels), ~10 and ~20% of PI cells stained positive for annexin V and propidium iodide under steady state conditions, respectively, as compared to 2-3% of the naïve HEp-2 cells. The levels of steady state propidium iodide staining of PI cells varied between experiments but were always in the range of ~10-20%, a variability which may reflect differences in cell passage number, confluence, time after plating or other differences in cell culture conditions. In addition, a higher fraction of the PI cells showed cleaved caspase 3 and active caspase 3/7 as compared to naïve cells (Fig. 5A bottom panels; and Fig. 5B). The PI cells and acute P/V-CPI- showed similar levels of cleaved caspase 3 (Fig. 5B). By contrast, PI cells did not contain substantially higher levels of the downstream apoptotic markers such as cleaved PARP (Fig. 5B). Together, these data suggest the PI cells have high basal levels of cell stress indicated by caspase activation, however this stress does not lead to substantial levels of cell death.

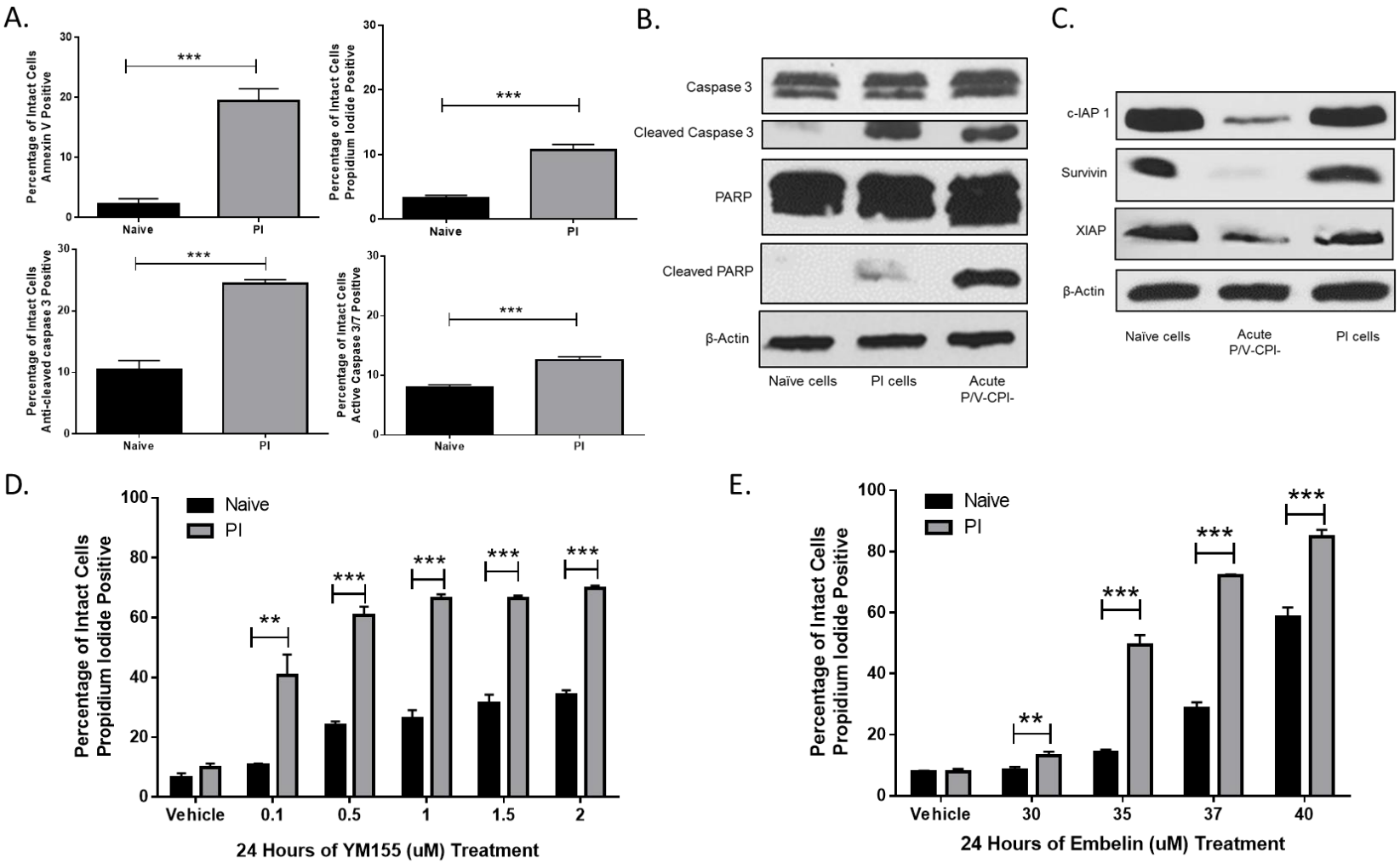

Figure 5: PI cells have elevated basal levels of apoptotic markers and are highly dependent on inhibitors of apoptosis for survival.

A) Naïve HEp-2 and PI HEp-2 cells were stained with Annexin V and propidium iodide, or with antibodies to cleaved caspase 3 or active caspase 3/7 before analysis by flow cytometry. Values are the mean of three samples with *** indicating P-value < 0.001. B and C) Lysates from naïve HEp-2 cells, PI cells or acute P/V-CPI- infected cells were analyzed by western blots the indicated apoptotic proteins (panel B) or for cellular inhibitors of apoptosis (panel C). D and E) Naïve and PI Hep-2 cells were treated with survivin inhibitor YM155 (panel D) or with XIAP inhibitor Embelin (panel E) for 24 hours. Cells were stained with propidium iodide before analysis by flow cytometry. Values are the mean of three samples with **, and *** indicating P-values of < 0.01 and < 0.001, respectively.

Cellular Inhibitors of Apoptosis Proteins (IAPs) have been found to have distinct mechanisms of action to block apoptosis. For example, XIAP can directly bind and inhibit cleaved caspases 3, 7, and 9 from activating their substrates, whereas Survivin binds to and inhibits cleaved caspases 3 and 7 (Deveraux et al., 1997; Tamm et al., 1998). Our finding of high levels of caspase activation and low levels of cell death in the PI cells suggested that IAPs may be important for survival of the P/V-CPI- infection. To test this hypothesis, levels of IAPs were determined by western blotting. As shown in Fig. 5C, levels of c-IAP1, surviving and XIAP expression in PI cells were very similar to that of naïve HEp-2 cells. By contrast acute infection with P/V-CPI- led to reduced levels of all three of these of these IAPs, a result which may be due to Protein Kinase R (PKR) activation and a global reduction of protein synthesis (Gainey et al., 2008a).

To determine the functional importance of IAPs in PI cell survival, naïve and PI cells were treated with increasing concentrations of a Survivin inhibitor (Fig. 5D) or a XIAP inhibitor

(Fig. 5E) for 24 hrs before determining viability by propidium iodide staining. The viability of PI cells was more sensitive to IAP inhibition as compared to naïve HEp-2 cells, suggesting that the PI cells are highly dependent on the IAPs in order to survive the high levels of cell stress under steady state conditions.

PI Cells Have Increased Sensitivity to DNA Damaging Agents

Given the survival of PI cells harboring the cytopathic P/V-CPI- and the increased sensitivity of PI cells to IAP inhibitors, we hypothesized that PI cells have increased resistance to inducers of cell death. To test this, naïve and PI cells were challenged with the DNA damaging agent cisplatin and cell viability was measured by annexin V and propidium iodide staining. As shown in Fig. 6, annexin V staining of PI cells showed enhanced sensitivity to cisplatin compared to naïve cells, and this sensitivity was both dose (Fig. 6A) and time dependent (Fig. 6B). This was also observed with propidium iodide staining, where by 24 hours of cisplatin treatment resulted in about 60% propidium iodide positive PI cells compared to ~10% of naïve cells (Fig. 6B). Although P/V-CPI- is defective at blocking IFN signaling, IFN treatment of naïve Hep2 cells did not alter sensitivity to cisplatin (data not shown).

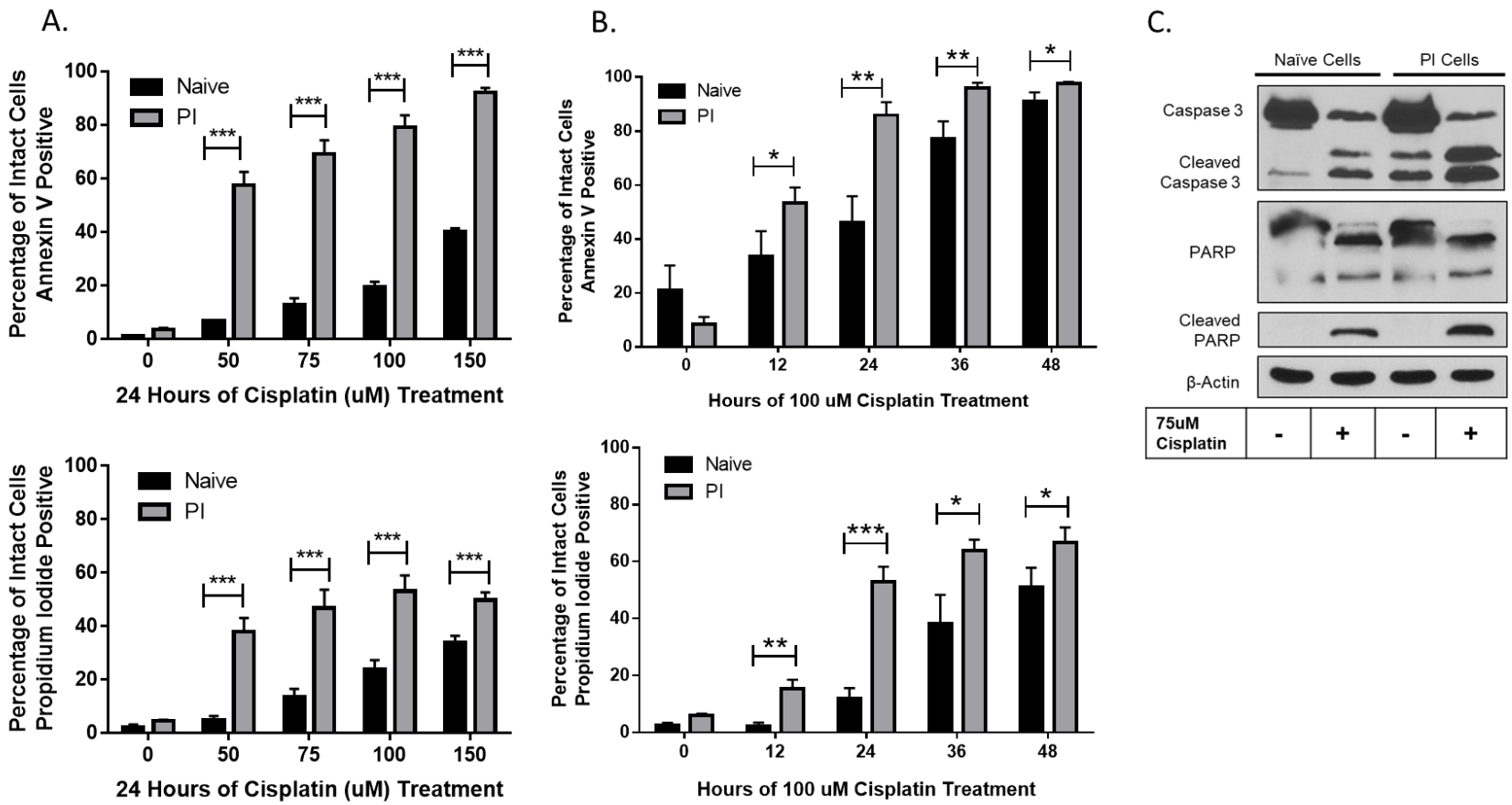

Figure 6: PI cells have increased sensitivity to DNA damaging agents.

A and B) Naïve and PI Hep-2 cells were treated with the indicated concentrations of cisplatin for 24 hours (panel A) or with 100 uM cisplatin for the indicated times (panel B). Remaining cell viability was determined by Annexin V and Propidium Iodide staining followed by flow cytometry. Values are the mean of three samples with *, **, and *** indicating P-values of < 0.05, <

0.01, and < 0.001, respectively. C) Naïve and PI Hep-2 cells were treated with 75 uM Cisplatin for 24 hours, and lysates were analyzed by western blotting for levels of caspase 3 and PARP.

Western blotting was carried out to confirm that cisplatin activated apoptotic pathways in PI cells. As shown in Fig. 6C, cisplatin treatment of PI cells resulted in higher levels of cleaved caspase 3 and cleaved PARP than was detected in the untreated PI cells. Higher levels of cleaved PARP were also detected in the cisplatin treated PI cells compared to the naïve cisplatin treated cells (Fig. 6C). Levels of cleaved caspase and PARP were similar between PI and naïve cells. Taken together, these data indicate the PI cells are more sensitive to cisplatin-induced cell death compared to naïve cells.

To test whether cisplatin treatment resulted in more DNA damage in the PI cells, naïve and PI cells were treated with increasing concentrations of cisplatin, and in situ TUNEL assays were used to visualize DNA fragmentation by microscopy. As shown in Fig. 7A, naïve cells did not show substantial DNA damage until treatment with 10 uM cisplatin (left column). By contrast, PI cells treated with the lowest tested cisplatin concentration (2 uM) resulted in the majority of cells staining TUNEL positive (Fig. 7B). These data indicate that PI cells have enhanced sensitivity to cisplatin-induced cell death and DNA damage.

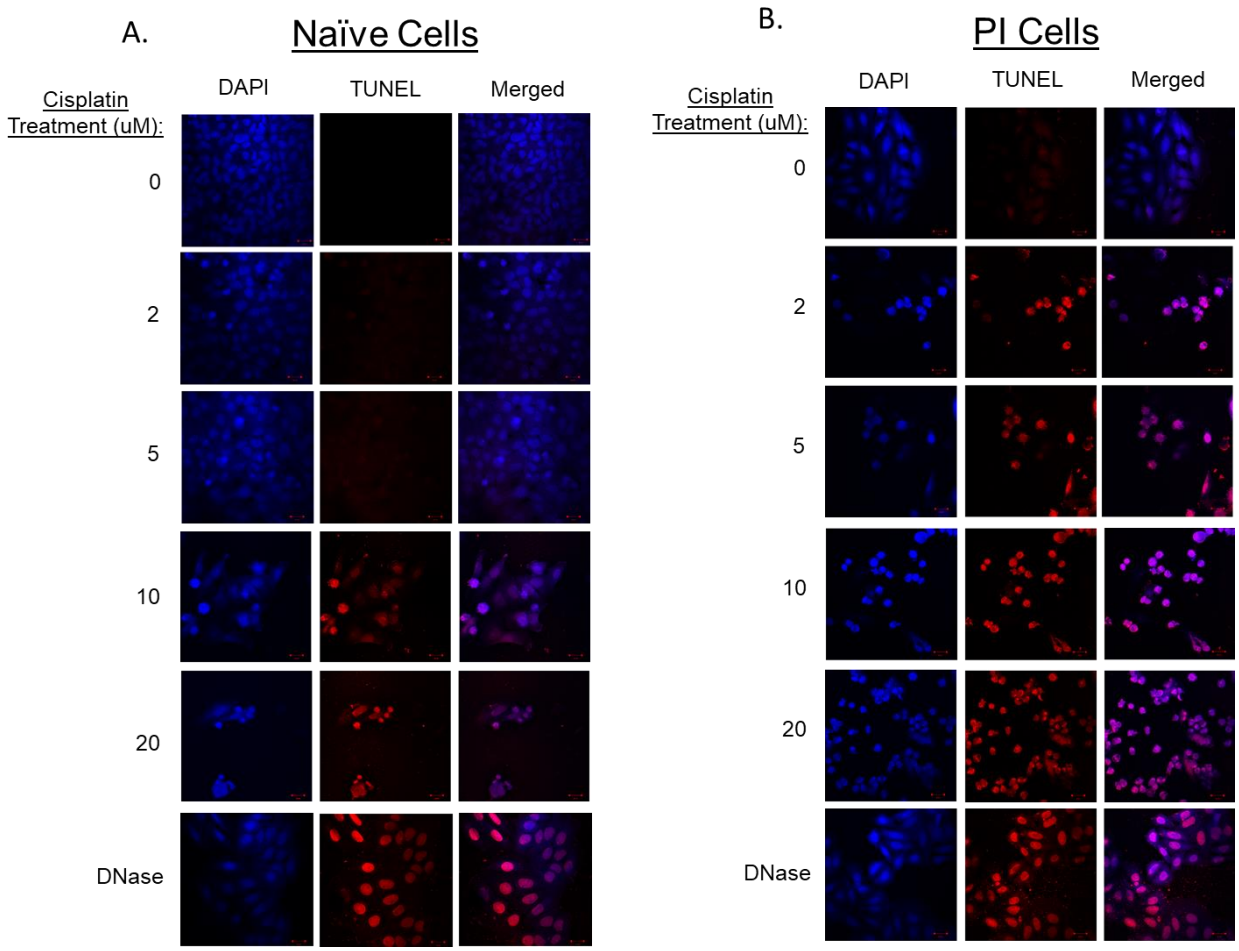

Figure 7: PI cells have enhanced DNA damage following cisplatin treatment.

Naïve (panel A) and PI Hep-2 (panel B) cells were treated with the indicated concentrations of cisplatin for 18 hrs. Alternatively, cells were treated with DNAse as a positive control (bottom). All samples were also treated with DAPI to visualize DNA. Cells were analyzed by an in situ TUNEL assay to determine levels of DNA fragmentation.

PI Cells Have Altered DNA Damage Repair Pathways

DNA adducts induced by cisplatin are mainly recognized by the NER pathway (Siddik, 2003), which can lead to cell cycle arrest to efficiently repair the DNA or can lead to cell death

based on the extent of the DNA damage. To examine DNA damage repair pathways, naïve and PI cells were treated with and without cisplatin and examined by western blotting for the phosphorylation of upstream sensors of DNA damaged such as ataxia telangiectasia- and Rad3-related (ATR) protein and repair mediator protein gamma-histone H2A.X. As shown in Fig. 8A, both naïve and PI cells responded in similar fashion to cisplatin treatment, as evidenced by similar levels of phosphorylation of ATR. Phosphorylation of H2A.X was slightly lower in PI cells treated with cisplatin than that detected in the naïve cells treated with cisplatin.

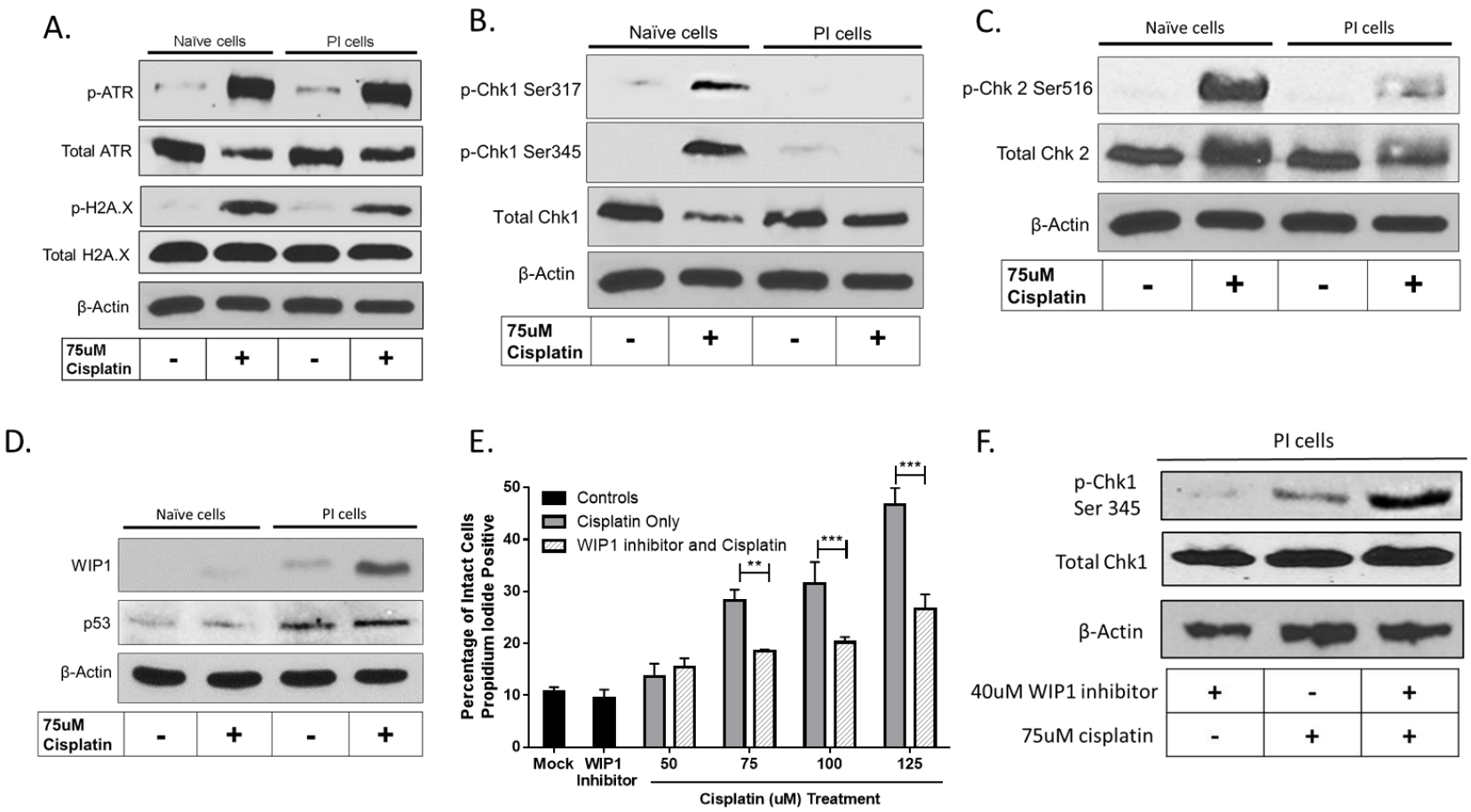

Figure 8: PI cells have altered DNA repair pathways.

Naïve and PI Hep-2 cells were treated with 75 uM Cisplatin for 24 hours, and lysates were analyzed by western blotting for DNA damage repair sensors ATR and H2A.X (panel A), effector proteins Chk1 (panel B) and Chk2 (panel C) or p53 and WIP1 (panel D). E) PI Hep-2 cells were treated with or without WIP1 inhibitor for 24 hrs, followed by an additional 24 hr cisplatin treatment

with or without WIP1 inhibitor before analysis by flow cytometry. Values are the mean of three samples with *** indicating P-value < 0.001. F) PI HEp-2 cells were treated with or without WIP1 inhibitor for 24 hrs, followed by an additional 24 hr cisplatin treatment with or without WIP1 inhibitor, and lysates were analyzed by western blotting for DNA damage repair effector protein Chk1.

Downstream of H2A.X, checkpoint kinases 1 and 2 (Chk1 and Chk2) are two DNA repair effector proteins which, when activated by phosphorylation, can lead to cell cycle arrest and repair of damaged DNA (Hurley and Bunz, 2007). As shown in Fig. 8B, phosphorylation of Chk1 at sites Ser317 and Ser345 was detected in naïve cells treated with cisplatin. Importantly, phosphorylation at these sites was not detected in PI cells treated with cisplatin. Similarly, phosphorylation of Chk2 at Ser516 was detected in the naïve cisplatin treated cells, but was greatly diminished in PI cells (Fig. 8C). Taken together, these data indicate DNA damage is sensed in the PI cells as evidenced by phosphorylation of H2A.-X, however PI cells are altered in downstream Chk1 and Chk2 signaling, resulting in a defect in DNA repair.

Wild-type p53 inducible protein 1 (WIP1) phosphatase is a stress responsive PP2C phosphatase that acts as a negative feedback loop to terminate the DNA repair pathways (Lu et al., 2008) through dephosphorylation of signaling proteins including Chk1 and Chk2 (Shreeram et al., 2006; Cha et al., 2010; Lu et al., 2005; Olivia-Trastoy et al., 2007). As shown in the western blot in Fig. 8D, levels of p53 and WIP1 were elevated in cisplatin-treated PI cells compared to naïve cells. This finding raised the hypothesis that WIP1 activity against DNA repair proteins contributed to enhanced sensitivity of PI cells to cisplatin. A prediction of this hypothesis is that WIP1 inhibition should result in decreased cell killing by cisplatin treatment.

To test this, PI cells were pre-treated with a WIP1 inhibitor, followed by increasing concentrations of cisplatin treatment and cell viability assays. As shown in Fig. 8E, treatment of PI cells with 125 uM cisplatin resulted in ~45% of the population being positive for propidium iodide staining. In the presence of the WIP1 inhibitor, cisplatin treatment resulted in ~25% of cells staining positive for propidium iodide. Consistent with a decrease in phosphatase activity, WIP1 inhibition of the PI cells followed by cisplatin treatment restored phosphorylation of Chk1 (Fig. 8F). These data are consistent with the hypothesis that an elevated expression of WIP1 in the PI cells results in alterations in DNA repair pathway effector proteins which are unable to effectively repair damaged DNA.

Acute Infection with P/V-CPI- PIV5 Sensitize Cancer Cells to DNA Damage-Induced Death

In the above studies we sought to understand how PI cells were sensitized to stress pathways leading to cell death. Our results raised the question of whether the phenotype of enhanced sensitivity to cisplatin-induced death was limited to P/V-CPI-derived PI cells or was also seen during acute infections with P/V-CPI- virus. To test this, naïve cells were mock infected or infected with the P/V-CPI- mutant, followed by treatment for 24 hrs with increasing doses of cisplatin and cell viability assays. As shown in Fig. 9A, treatment of mock infected cells with 75 uM cisplatin resulted in ~12% annexin V-positive cells and ~18% propidium iodide positive cells. By contrast, acute infection with the P/V-CPI- mutant followed by 75 uM cisplatin resulted in >50% annexin V-positive cells and ~40% propidium iodide positive cells. Under similar conditions, DNA damage repair pathway proteins Chk1 and Chk2 were phosphorylated in mock infected cisplatin-treated cells as expected, but not in the case of cells acutely infected with P/V-CPI- or in PI cells (Fig. 9B). These data indicate an acute infection with the P/V-CPI-

mutant can sensitize cancer cells to cisplatin-induced death and alter DNA damage response pathways similarly to that seen with the PI cells.

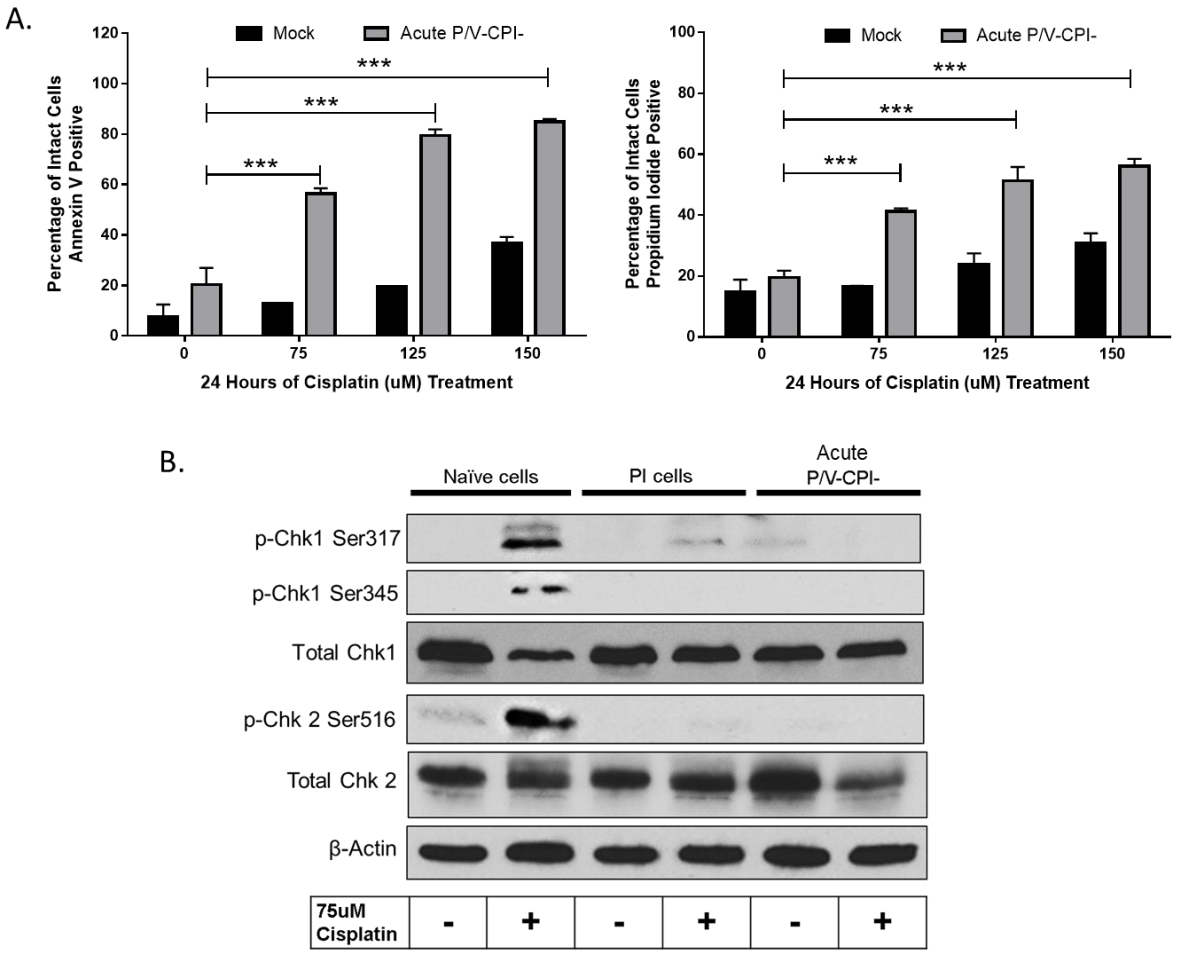

Figure 9: Acute P/V mutant infection sensitizes cells to a DNA damaging agent and results in deficient DNA repair signaling.

A) Naïve HEp-2 cells were mock infected or infected with the P/V-CPI- mutant for 18 hr. Cells were treated with the indicated concentrations of cisplatin for 24 hr before analysis for annexin V and propidium iodide staining by flow cytometry. B) Naïve cells, PI Hep-2 cells or P/V-CPI- acutely infected cells were treated with 75 uM Cisplatin for 24 hours. Cell lysates were analyzed by western blotting for the indicated DNA repair proteins.

PI Cells Show a Difference in Distribution of DDB1 Protein Following Treatment with a DNA Damaging Agent

Upon DNA damage, DDB1 binds to DDB2 forming the DNA damage binding (DDB) complex that can then translocate to the nucleus. DDB1 is also known to function in a cytoplasmic complex with the *rubulavirus* V protein to induce STAT degradation (Didcock et al., 1999; Ulane et al., 2005; Parisien et al., 2001; Young et al., 2000). As such, we hypothesized that the DDB1 protein in PI cells would be localized to the cytoplasm and altered in its translocation to the nucleus in response to DNA damage. To test this hypothesis, naïve and PI cells were mock treated or treated with 100 uM cisplatin before immunostaining for DDB1. As shown in Fig. 10A for naïve cells, DDB1 appeared in both nucleus and cytoplasm, but had exclusive nuclear staining after cisplatin treatment. While untreated PI cells had a similar staining as untreated naïve cells, cisplatin treatment of PI cells resulted in a peri-nuclear localization with very little nuclear accumulation. Thus, these data suggest that DDB1 is unable to enter the nucleus when the PI cells are treated with cisplatin.

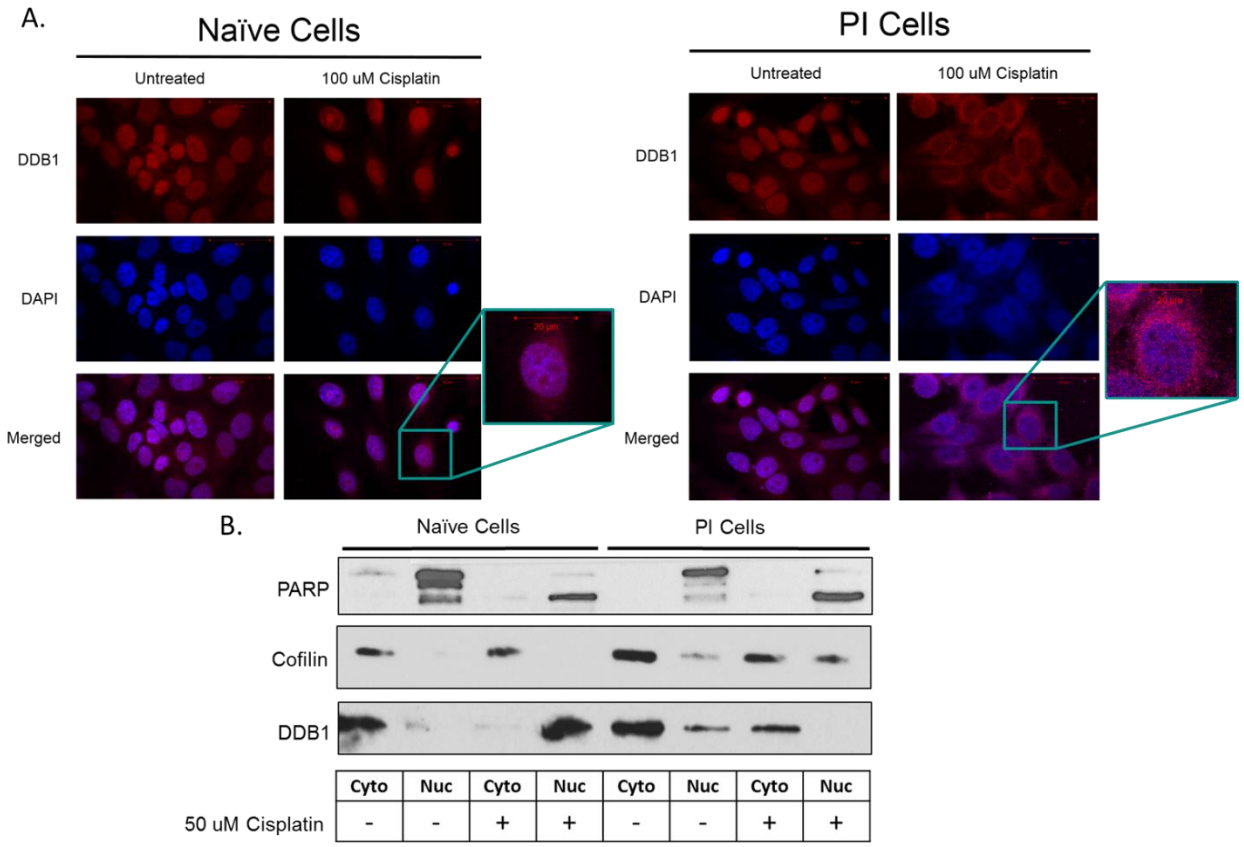

Figure 10: PI cells show a difference in distribution of DDB1 protein following treatment with DNA damaging agent.

A) Naïve and PI Hep-2 cells were treated with 150 uM cisplatin for 18 hrs and stained with antibody to DDB1 and with DAPI. Representative pictures are shown. B) Naïve and P/V PI Hep2 cells were treated with 50 uM cisplatin for 18 hrs prior to cell lysis and separation into nuclear (Nuc) and cytoplasmic (Cyto) fractions. Samples were analyzed for the indicated proteins by western blotting.

As an alternative approach to localize DDB1 after DNA damage, naïve and PI cells were treated with 50 uM cisplatin, and cell extracts were fractionated into nuclear and cytoplasmic

fractions followed by western blotting for a nuclear control protein PARP, a cytoplasmic control protein cofilin, and DDB1. As shown in Fig. 10B, PARP localized to the nucleus as a high molecular weight protein in untreated cells and a cleaved smaller form in cisplatin-treated cells. Cofilin largely localized to the cytoplasm, although PI cells had some detected in the nucleus. Most importantly, DDB1 was detected in the nuclear extract in the cisplatin-treated naïve cells, but was not detected in the nuclear extract in the cisplatin treated PI cells. Taken together, these data support the proposal that DDB1 is defective in translocating to the nucleus upon DNA damage in the PI cells.

Acute Infections with WT PIV5 and hPIV2 Sensitize Cancer Cells to DNA Damage-Induced Death

We tested the hypothesis that WT viruses in the *Rubulavirus* family could also sensitize cancer cells to DNA damaging agents. Naïve HEp-2 cells were mock infected or infected with WT PIV5 or hPIV2, followed by 24 h treatment with doses of cisplatin and cell viability assays. Treatment of WT PIV5 infected cells with 100 uM cisplatin resulted in about 90% annexin V positive cells and 65% propidium iodide positive cells, whereas the mock infected cells were about 20% annexin V positive (Fig. 11A). As shown in Fig. 11B, treatment of cells after acute hPIV2 infection with 50 uM cisplatin resulted in ~80% annexin V positive and 75% propidium iodide positive cells, much higher than that seen with cisplatin-treated mock infected cells or in infected cells that did not receive cisplatin treatment. Taken together, these data indicate that acute infections with at least two WT *Rubulaviruses* can sensitize cells to DNA damage-induced cell killing.

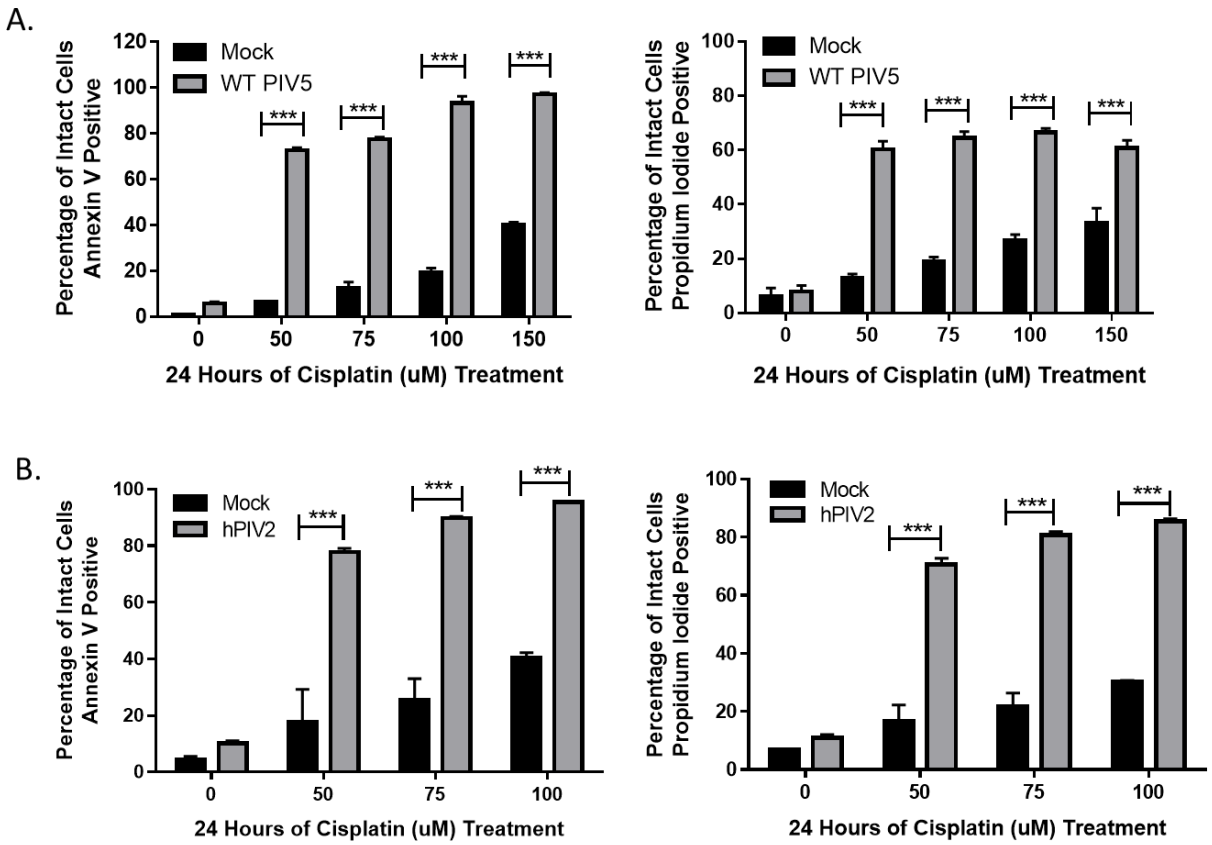

Figure 11: Acute WT PIV5 and hPIV2 infections sensitize cells to a DNA damaging agent.

Naïve HEp-2 cells were mock infected or infected with WT PIV5 (panel A) or hPIV2 (panel B) at an MOI of 10 for 18 hrs. Cells were treated with the indicated concentrations of cisplatin for 24 hrs, followed by analysis for annexin V (left panels) and propidium iodide (right panels) staining by flow cytometry. Values are the mean of three samples with *** indicating P-value < 0.001.

By Contrast to Death Induced by DNA Damaging Agents, PI Cells are More Resistant to Death Induced by ER Stress than Naïve Cells

To better understand how the PI cells can survive harboring a cytopathic RNA virus, we extended our study to determine how the PI cells respond to endoplasmic reticulum (ER) stress. ER stress can lead to either the unfolded protein response (UPR) or apoptosis through the action of sensors and effectors. The UPR can upregulate protein chaperones, foldases, ER-associated protein degradation, and autophagy activity (as reviewed by Oslowski and Urano, 2011). Protein chaperones and foldases can alleviate ER stress by properly folding misfolded proteins, whereas ER-associated protein degradation and autophagy pathways degrade proteins. The UPR can return the cell to homeostasis or if the ER stress is too extensive, then the cell undergoes apoptosis.

Tunicamycin is a naturally occurring antibiotic that blocks the first step in N-linked oligosaccharide biosynthesis, preventing protein glycosylation in the ER (Heifetz et al., 1979). Tunicamycin treatment results in unfolded glycoproteins accumulation in the ER, leading to ER stress, UPR activation and ultimately cell death. To determine if the PI cells were more resistant to ER stress induction, naïve and PI cells were treated with various concentrations of tunicamycin and stained with annexin V or propidium iodide. Contrasting to the previous observation with cisplatin treatment, the PI cells were more resistant to death induced by ER stress. For example, 75 ug/mL tunicamycin treatment in naïve HEp-2 cells results ~90% annexin V and ~60% propidium iodide positive, whereas the PI HEp-2 cells stain ~40% annexin V and ~30% propidium iodide positive (Fig. 12 A and B). A similar tread can be observed with the naïve and PI A549 cells (Fig. 12 C and D). These results indicate the PI cells are more resistant to death induced by ER stress as compared to the naïve cells.

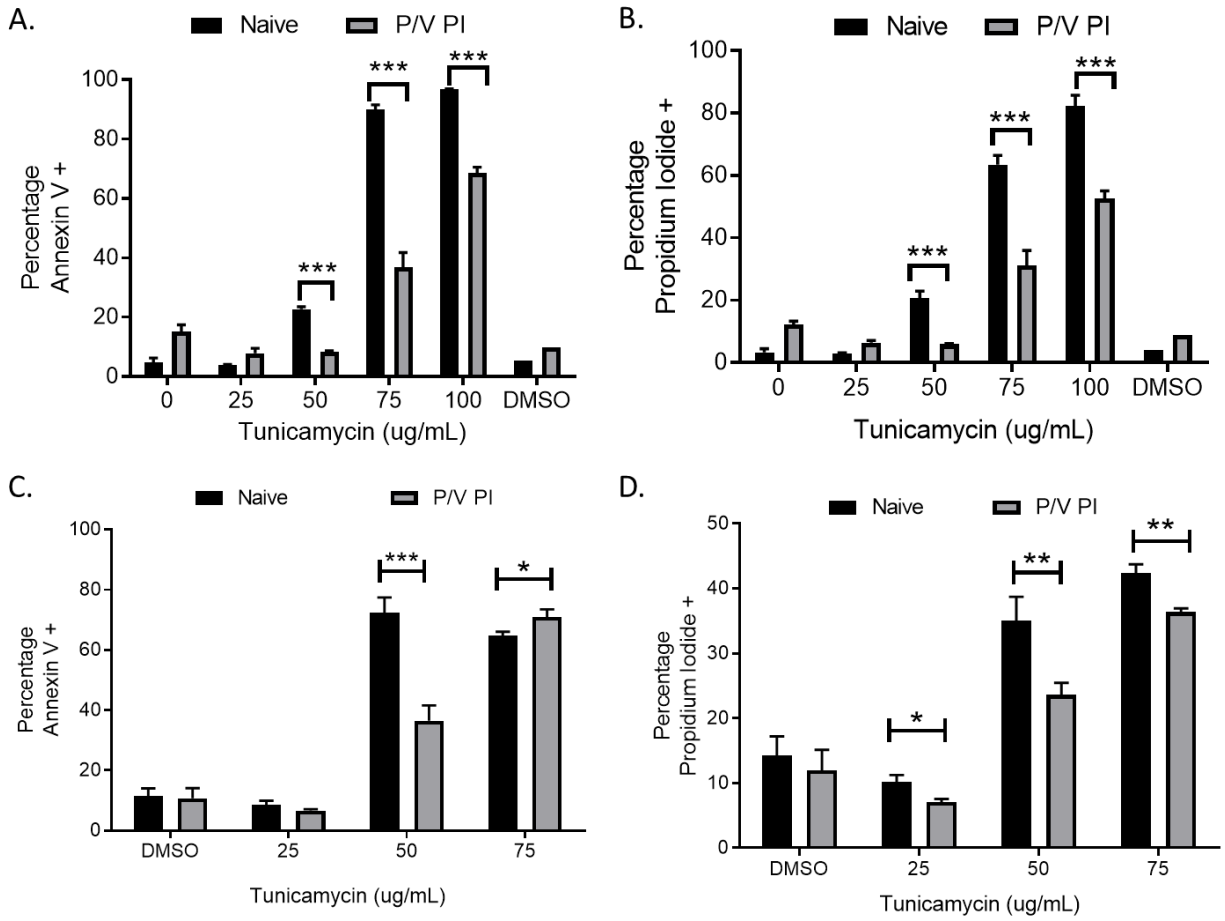

Figure 12: PI cells are more resistant to an ER stress inducer than naïve cells.

(A and B) Naïve and PI Hep-2 cells were treated with the indicated concentrations of tunicamycin for 24 hours. (C and D) Naïve and PI A549 cells were treated with the indicated concentrations of tunicamycin for 24 hours. For all conditions, remaining cell viability was determined by annexin V and propidium iodide staining followed by flow cytometry. Values are the mean of three samples with *, **, and *** indicating P-values of < 0.05, < 0.01, and < 0.001, respectively.

The UPR is composed of sensors and effectors that influence cell fate in response to ER stress. Three of the ER transmembrane UPR sensors are Inositol requiring 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6). A hypothesis that could explain why the PI cells are more resistant to tunicamycin is the PI cells have altered UPR proteins that could act to prime the cells to better cope with ER stress. To determine if proteins in the UPR are altered in the PI cells, naïve and PI HEp-2 cells were treated with 75 ug/mL tunicamycin for 24 hours, followed by protein detection through western blot (Fig. 13A). The sensors IRE1 and ATF6 were reduced in the treated PI cells (lane 4) as compared to the treated naïve cells (lane 2).

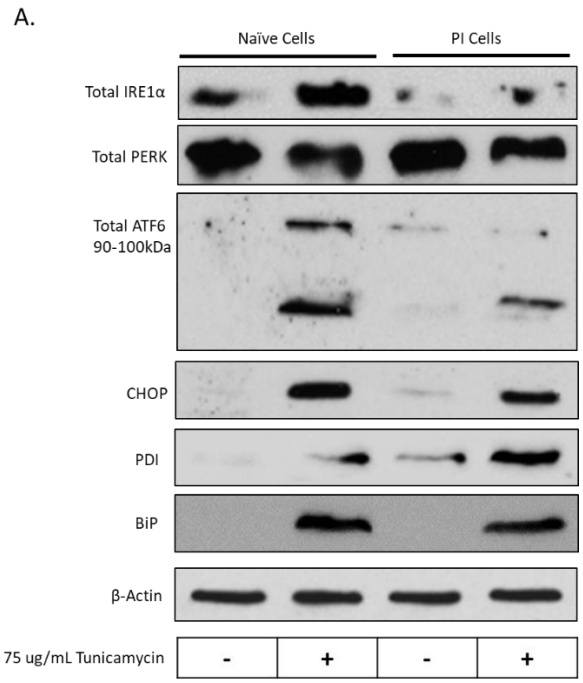

Figure 13: ER stress response to tunicamycin in naive and PI cells.

A) Naïve and PI Hep-2 cells were treated with 75 ug/mL tunicamycin for 24 hours, and lysates were analyzed by western blotting for levels of ER stress response sensors, effector, and protein chaperones.

One of the UPR effectors, C/EBP homologous protein (CHOP), is involved in ER stress induced apoptosis (Harding et al., 2000). CHOP expression was slightly reduced in the treated PI cells (lane 4) as compared to treated naïve cells (lane 2). Interestingly, protein disulfide isomerase (PDI), a protein chaperone, was elevated in both the untreated and treated PI cells (lanes 3 and 4) as compared to naïve cells. Another protein chaperone, immunoglobin binding protein (BiP), had similar levels in the treated naïve and PI cells. Taken together, these data suggest the PI cells have an altered ER stress response, which may allow the PI cells to be more resistant to ER stress induced death.

To determine if acutely infected cells were also more resistant to ER stress, naïve HEp-2 cells were infected with the P/V-CPI- mutant. At 18 HPI, cells were treated with various concentrations of tunicamycin for 24 hrs, followed by annexin V and propidium iodide staining. Contrary to the PI cells, the acutely infected cells were more sensitive to death induced by ER stress. As a representative, 100 ug/mL tunicamycin treated, mockly infected cells stained ~20% annexin V positive, whereas the acutely infected cells stained ~60% positive (Fig. 14A). Propidium iodide staining also suggests the acutely infected cells were more sensitive to ER stress induced death than the mockly infected cells (Fig. 14B). These data indicate the acutely infected cells respond differently to death induced by ER stress than the PI cells and proteins involved in the UPR may be altered in the PI cells, attributing to the difference in sensitivity.

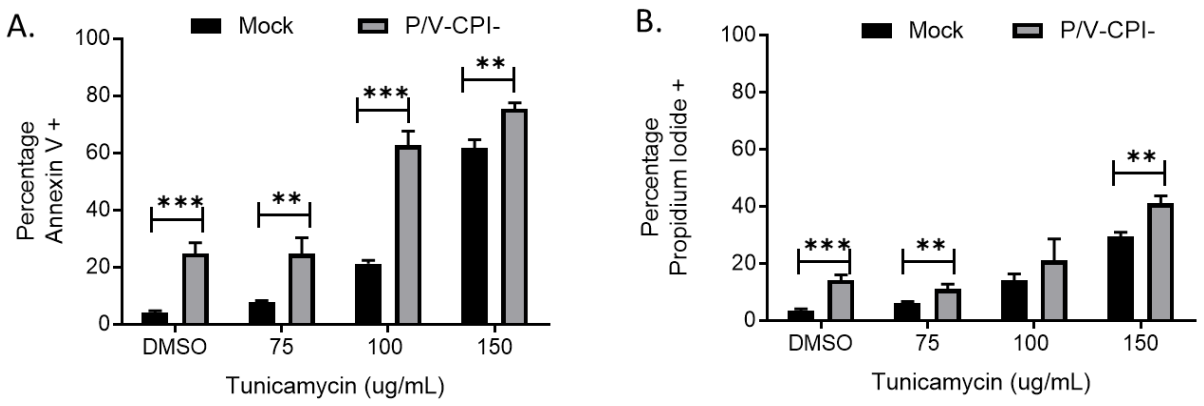

Figure 14: Acute P/V-CPI- infected cells are more sensitive to tunicamycin than mock.

A and B) Naïve HEp-2 cells were mock infected or infected with the P/V-CPI- mutant for 18 hr. Cells were treated with the indicated concentrations of tunicamycin for 24 hr before analysis for annexin V (panel A) and propidium iodide (panel B) staining by flow cytometry.

CHAPTER FOUR: HISTONE DEACETYLASE INHIBITORS ENHANCE CELL KILLING AND BLOCK INTERFERON-BETA SYNTHESIS ELICTED BY INFECTION WITH AN ONCOLYTIC PARAINFLUENZA VIRUS

HDAC Inhibitors Enhance Killing of Lung Cancer Cells by the P/V-CPI- Mutant through Increases in Caspase Activity

To determine the effects on cell viability of combining HDAC inhibitors with P/V-CPI- mutant infection, human non-small cell lung cancer H1299 cells were pretreated for 12 h with DMSO as a vehicle control, or with various concentrations of either the HDAC inhibitor SAHA or scriptaid. SAHA was the first HDAC inhibitor approved by the FDA and scriptaid is a structurally related novel pan-HDAC inhibitor (Suraweera et al., 2018). Cells were then mock infected or infected with the P/V-CPI- mutant at a multiplicity of infection (MOI) of 10 plaque forming units (PFU)/cell for 24 h. Cell viability was determined using MTT assays. As shown in Figure 15A, pretreatment with either HDAC inhibitor enhanced death induced by the P/V-CPI- mutant. For example, mock infected cells that were pretreated with 100 uM SAHA retained roughly 70% viability (left panel, Figure 15A), whereas cells pretreated with 100 uM SAHA and then infected with P/V-CPI- virus showed only ~35% viability. A similar enhancement of cell killing was observed with scriptaid pretreatment (Figure 15A, right panel).

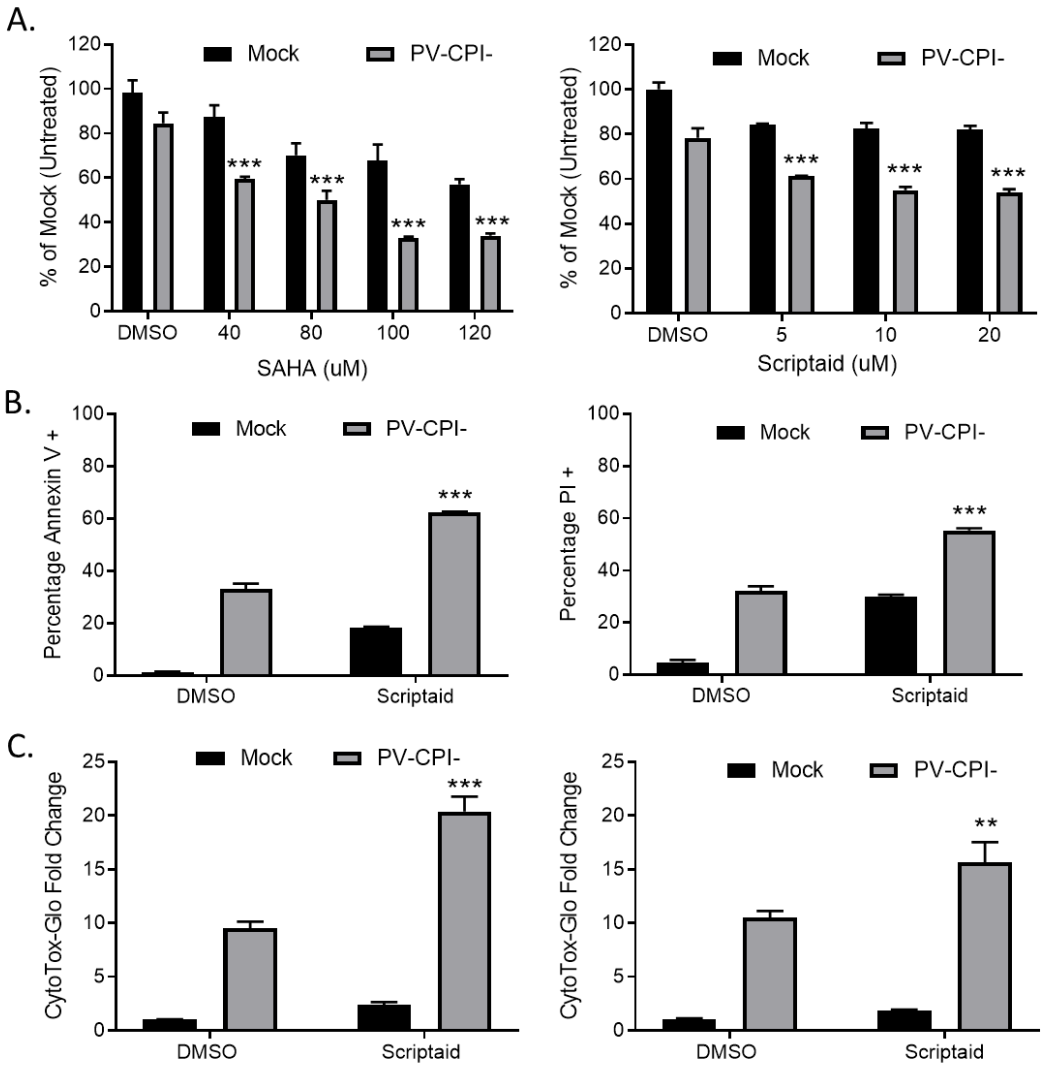

Figure 15: Pretreatment with HDAC inhibitors enhances cell death by the P/V-CPI- mutant virus.

(A) H1299 cells were pretreated with the indicated concentrations of SAHA (left panel), scriptaid (right panel), or DMSO as a vehicle control for 12 h. Cells were mock infected or infected with the P/V-CPI- mutant at an MOI of 10, and cultured in the presence of the indicated concentration of drugs. After 24 h, cell viability was determined by MTT assay as described in Methods section. (B) A549 cells were pretreated with 5 μM scriptaid or DMSO as a vehicle

control for 12 h. Cells were mock infected or infected with the P/V-CPI- mutant at an MOI of 10, and cultured in the presence of 5 μM scriptaid or DMSO. Cell viability was determined by annexin V (left panel) and propidium iodide staining (right panel) at 24 hpi. (C) H1299 (left panel) or A549 (right panel) cells were pretreated with 20 μM scriptaid or DMSO for 12 h before mock infection or P/V-CPI- infection at an MOI of 10. After culturing in the presence of 1 μM scriptaid or DMSO for 24 h, cytotoxicity was determined by CytoTox-Glo assay. Values in all panels are the mean of three replicates normalized to DMSO pretreated mock infected samples with error bars indicating standard deviation. *** indicates *p*-value of < 0.001 comparing DMSO versus HDAC inhibitor pretreated infected samples.

We extended this study to another human non-small cell lung cancer cell line. A549 cells were pretreated with either DMSO as a control or with 5 uM scriptaid, followed by mock infection or P/V-CPI- infection. At 24 h post infection (hpi), cell viability was determined by staining with annexin V and propidium iodide followed by flow cytometry. As shown in Figure 15B, infected control cells showed ~35% and ~30% of the cell population being positive for annexin V (left panel) and propidium iodide staining (right panel), respectively. By contrast, scriptaid pretreated cells that were infected with P/V-CPI- virus showed ~60% and ~55% annexin V and propidium iodide positive staining.

To directly measure cytotoxicity, H1299 (Figure 15C, left panel) or A549 (Figure 15C, right panel) cells were pretreated with either vehicle control or scriptaid for 12 h, followed by mock infection or infection with P/V-CPI- virus. At 24 hpi, cytotoxicity was determined using a CytoTox-Glo assay which measures overall cell death. As shown in Figure 15C, P/V-CPI- infection of H1299 cells resulted in a ~9 fold increase in cell killing compared to control mock

infected samples. By contrast, scriptaid pretreatment followed by P/V-CPI- infection resulted in a ~20-fold change in cytotoxicity. Similarly, in A549 cells (Figure 15C, right panel) control P/V-CPI- mutant infection resulted in a ~10-fold increase in cytotoxicity compared to mock infected samples, and this was enhanced to ~15-fold increase by scriptaid pretreatment.

To measure cellular caspase activity, H1299 cells were pretreated with 20 uM Scriptaid for 12 h followed by mock infection or infection at high MOI with P/V-CPI-. At 24 hpi, caspase activity was determined using caspase-glo assays. As shown in Figure 16A, effector caspases 3/7 were increased ~5-fold by Scriptaid treatment of mock infected cells, but were increased ~20-fold compared to control samples when Scriptaid pretreated was combined with P/V-CPI- infection. In these same samples, activities of the extrinsic pathway initiator caspase 8 and intrinsic pathway initiator caspase 9 were increased ~9-fold and ~15-fold, respectively, in cells treated with scriptaid and infected with P/V-CPI- virus compared to mock infected control samples (Figure 16B, C).

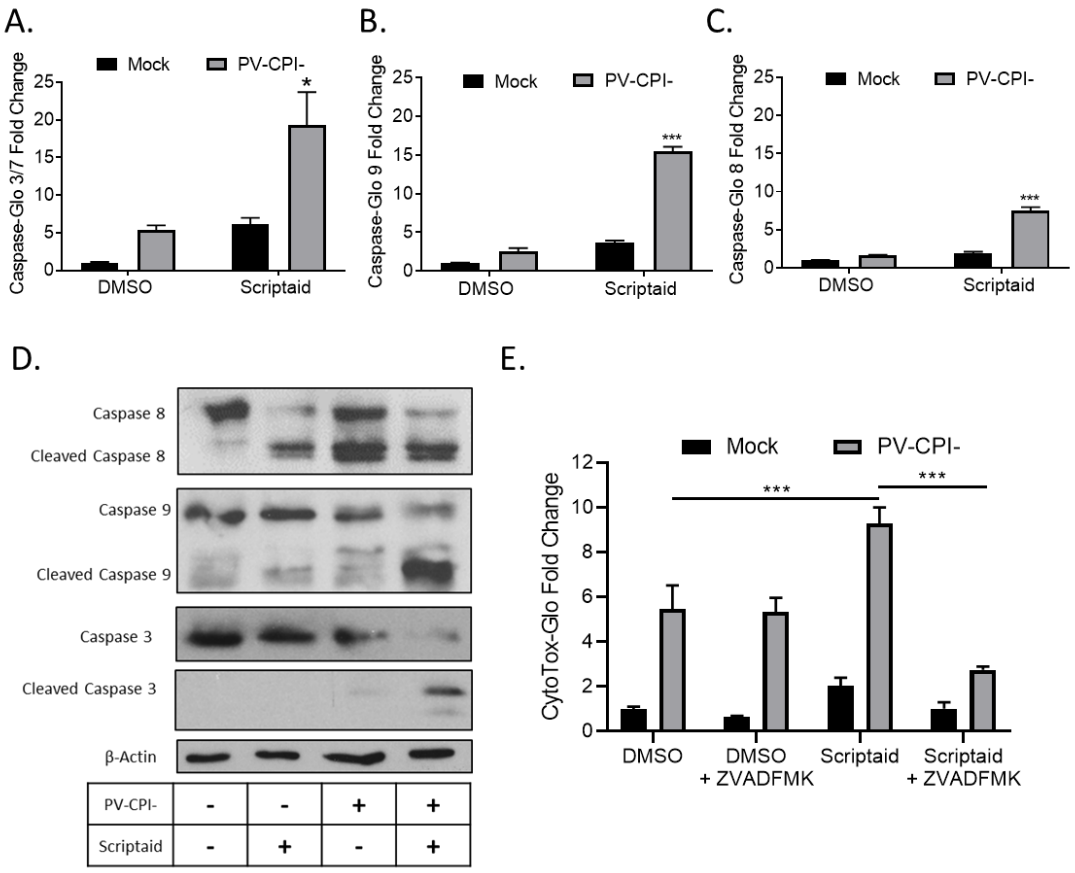

Figure 16: HDAC inhibitor enhances caspase activation in P/V-CPI- infected cells.

(A–C) H1299 cells were pretreated with 20 μM Scriptaid or DMSO as a control for 12 h. Cells were then mock infected or infected with P/V-CPI- at an MOI of 10, and cultured for 24 h in media containing either 1 μM Scriptaid or DMSO. At 24 hpi, caspase activity was determined by Caspase-Glo-3/7 (A), -9 (B) or -8 (C) assays. Values are the mean of three replicates normalized to DMSO pretreated mock infected samples with * and *** indicating *p*-values of <0.05 and <0.001, respectively, comparing DMSO versus HDAC inhibitor pretreated infected samples. (D) A549 cells were treated as described for panels A-C. At 24 hpi, cells were harvested, and lysates were analyzed by western blotting for β-actin or for caspase-8, -9, and -3 cleavage products. (E) H1299 cells were pretreated with 20 μM scriptaid or DMSO for 12 h, and then mock infected or

infected with P/V-CPI- at an MOI of 10. Cells were then cultured in media containing either 1 μM scriptaid or DMSO, with and without 50 μM of the pan-caspase inhibitor Z-VAD-FMK. At 24 hpi, cytotoxicity was determined by CytoTox-Glo assay. Values are the mean of three replicates normalized to DMSO pretreated mock infected samples with error bars indicating standard deviation and *** indicating *p*-values of < 0.001.

To confirm the above results, western blots were used to measure caspase cleavage products in lysates from A549 cells that were pretreated with scriptaid, followed by P/V-CPI- infection. As shown in Figure 16D, scriptaid pretreatment followed by virus infection induced higher levels of cleaved caspases-9 and -3 compared to treatment with inhibitor alone or P/V-CPI- alone. Increases in caspase-8 cleavage products following combined treatment was less clear, consistent with the lower induction of enzyme levels shown in Figure 16B. The increase in caspase activity after scriptaid pretreatment was responsible at least in part for the increases in cell death. This is evident in Figure 16E, where culturing cells with a pan-caspase inhibitor Z-VAD-FMK greatly reduced the level of killing in scriptaid-pretreated cells infected with P/V-CPI- virus.

Taken together, these data from two airway cancer cell lines and three different cytotoxicity assays indicate that scriptaid pretreatment followed by P/V-CPI- infection leads to increases in caspase-9 and -3/7 activity and to increases in cell killing through caspase-dependent pathways.

Double Stranded RNA Contributes to Scriptaid-Mediated Enhancement of Cell Killing by the P/V-CPI- Virus

We have previously shown that strong induction of proinflammatory cytokines by the P/V-CPI- virus is inhibited in a A549 cell line that was engineered to constitutively express the reovirus sigma 3 protein – a viral protein known to specifically bind to and sequester double stranded RNA (dsRNA; Manuse and Parks, 2009; Yue and Shatkin, 1997). Given that viral dsRNA can be an inducer of apoptosis (Zhao et al., 2012), we tested the hypothesis that if dsRNA was involved in scriptaid-mediated enhancement of P/V-CPI- killing, cell death would be reduced in sigma 3-expressing cells. Parental A549 cells and A549-sigma 3 cells were pretreated with scriptaid for 12 h before mock infection or high MOI infection with P/V-CPI- virus. Cells were analyzed for Annexin V and propidium iodide staining at 24 hpi. As shown in Figure 17, both Annexin V (panel A) and propidium iodide (panel B) staining was enhanced in P/V-CPI-infected parental A549 cells by prior treatment with scriptaid. Most importantly, staining for both of these cytotoxicity markers was significantly reduced in A549 cells expressing sigma 3 protein, indicating dsRNA plays a role in the scriptaid-mediated enhancement of P/V-CPI- cell death.

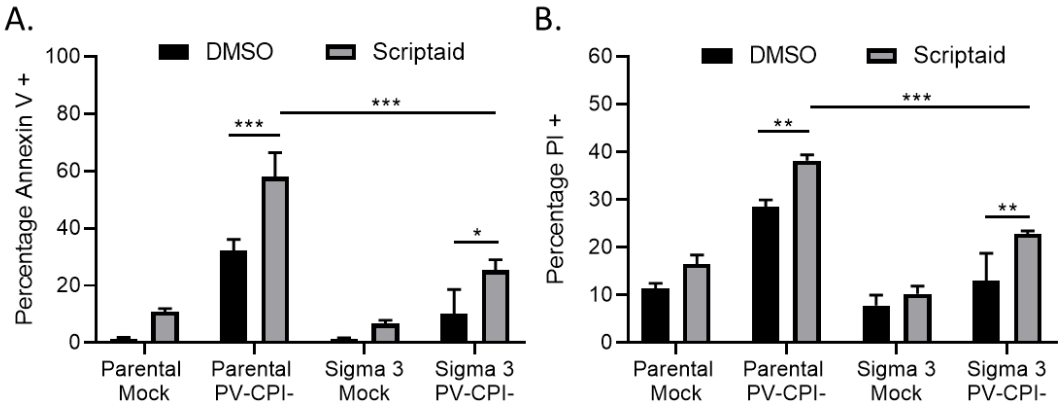

Figure 17: dsRNA contributes to enhanced death in cells treated with HDAC inhibitor and P/V-CPI- infection.

(A, B) Parental A549 cells and A549 cells expressing reovirus sigma 3 protein were pretreated with 20 μM Scriptaid or DMSO for 12 h. Cells were mock infected or infected with P/V-CPI- virus at an MOI of 10, and cultured in the presence of 1 μM Scriptaid or DMSO control. Cell viability was determined by Annexin V (A) and propidium iodide (B) staining at 24 hpi. Values are the mean of three replicates with error bars indicating standard deviation. ** and *** indicating *p*-values of < 0.01 and < 0.001, respectively.

Scriptaid Pretreatment Enhances Killing of Lung Cancer Cells Infected with LACV and VSV

We tested the hypothesis that HDAC-mediated enhancement of P/V-CPI- cell killing would extend to other cytoplasmic-replicating RNA viruses. A549 or H1299 cells were pretreated with Scriptaid for 12 h prior to mock infection or high MOI infection with hPIV2, ZIKV, VSV, WT PIV5 or LACV. Due to the large virus-induced cytopathic effects seen at late times pi, the cells infected with hPIV2, ZIKV and VSV were analyzed at 16 hpi by CytoTox-Glo assay. For WT PIV5 and LACV, cells were analyzed at 24 hpi. As shown in Figure 18, Scriptaid pretreatment enhanced killing of both H1299 and A549 cells by VSV (panels A and C) and LACV (panels B and D) infections, but this was not seen after infection with hPIV2, ZIKV or WT PIV5. Microscopic analysis of VSV and LACV infected cells showed relatively little cytopathic effect (e.g., cell rounding) in control treated cultures, but this was greatly enhanced in scriptaid-pretreated cells (Figure 18E). The scriptaid-mediated enhancement of VSV and LACV cell killing was confirmed by analysis of Annexin V and propidium iodide (Figure 18F,G).

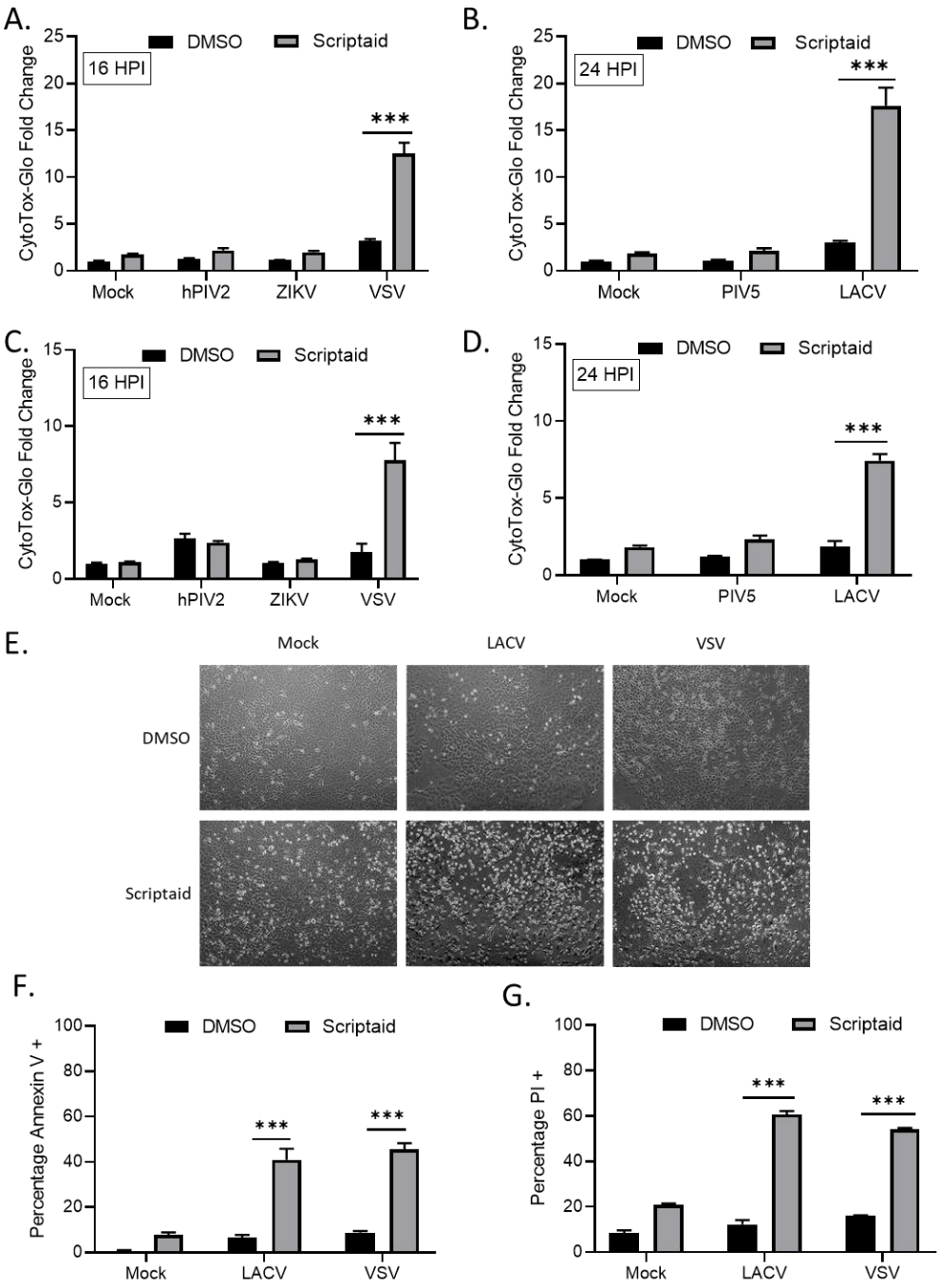

Figure 18: HDAC inhibitor pretreatment enhances cell death by other RNA viruses.

(A–D) H1299 (A,B) or A549 (C,D) cells were pretreated with 20 μM scriptaid or DMSO for 12 h. Cells were then either mock infected or infected with the indicated viruses at an MOI of 10.

After culturing in the presence of 1 μM scriptaid or DMSO for either 16 (A,C) or 24 (B,D) h, cytotoxicity was determined by CytoTox-Glo assay. hPIV2, human parainfluenza virus 2; ZIKV, Zika virus; VSV, vesicular stomatitis virus. LACV, La Crosse virus. Values are the mean of three replicates normalized to DMSO pretreated mock infected samples, with error bars indicating standard deviation. ** and *** indicating *p*-values of < 0.01 and < 0.001, respectively. (E–G) A549 cells were pretreated with 20 μM scriptaid or DMSO and either mock infected or infected with LACV or VSV at MOIs of 10. After culturing in the presence of 1 μM scriptaid or DMSO for 16 h, cells were imaged at 10X magnification, and representative bright field images are shown (E). Cell viability was determined by annexin V (F) and propidium iodide (G) staining.

HDAC Inhibitor Pretreatment Downregulates IFN-β Production and Enhances Spread of the P/V-CPI- Mutant

During the course of our studies, we observed that infected cells pretreated with scriptaid had higher GFP expression derived from the P/V-CPI- genome compared to infected control untreated cells. This is evident in Figure 19A, where microscopy analysis of infected H1299 cells showed brighter green fluorescence in cultures of scriptaid pretreated P/V-CPI- infected cells compared to the DMSO pretreated P/V-CPI- infected cells. When analyzed by flow cytometry, 10 uM scriptaid pretreatment reproducibly resulted in ~1.5-fold increase in GFP intensity during high MOI P/V-CPI- infections compared to vehicle treated cells (Figure 19B).

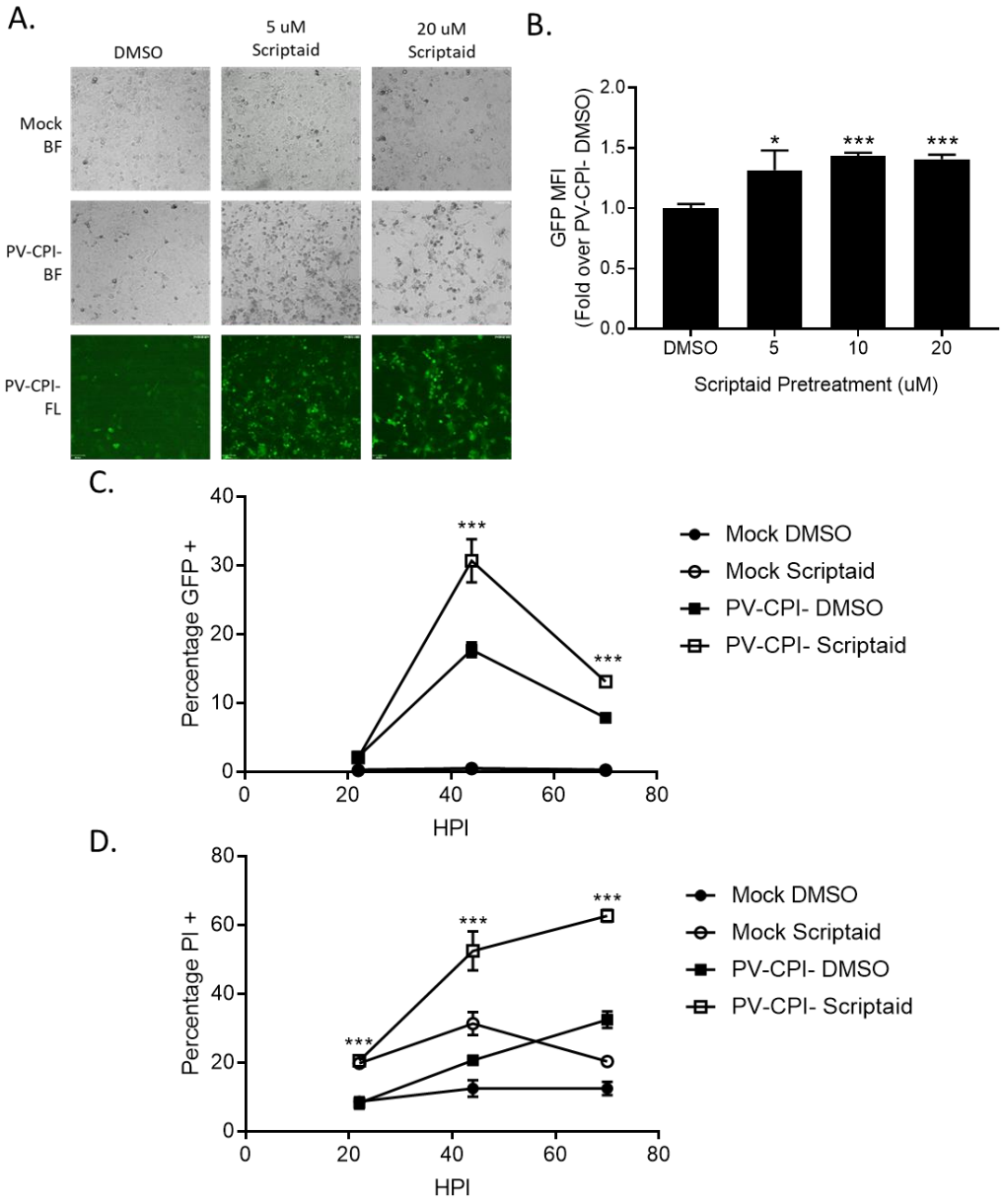

Figure 19: Pretreatment of cells with an HDAC inhibitor increases P/V-CPI- GFP expression and promotes low MOI virus spread.

(A,B) H1299 cells were pretreated with the indicated concentrations of scriptaid or control DMSO for 12 h. Cells were then mock infected or infected with P/V-CPI- at an MOI of 10. After 24 h of culture in media containing either 1 μM scriptaid or DMSO, 20X bright field (BF) or

fluorescence (FL) images of cells were captured (A). Alternatively, cells were analyzed by flow cytometry for GFP expression (B). Values are expressed as fold change over DMSO treated virus infected cells set at 1.0. (C,D). A549 cells were pretreated with 20 μM scriptaid or DMSO for 12 h. Cells were then mock infected or infected with the P/V-CPI- mutant at an MOI of 0.05, and cultured in media containing 1 μM scriptaid or DMSO. At the indicated hpi, cells were harvested and analyzed by flow cytometry for GFP expression (C) or propidium iodide staining (D). For all panels, error bars indicate standard deviation. * and *** indicates P-values of <0.05 and <0.001, respectively.

To determine if scriptaid pretreatment alters P/V-CPI- spread through a population of cells, A549 cells were infected at an MOI of 0.05 and cells were analyzed over time for GFP expression (Figure 19C) and propidium iodide staining (Figure 19D). At 45 hpi, control cultures of P/V-CPI- infected cells showed GFP expression and propidium iodide staining in ~15% and 20% of cells, respectively. By contrast, scriptaid pretreatment resulted in GFP expression and propidium iodide staining in 30% and 50% of the cells. At later times after infection, GFP expression in the cell population decreased in both control and scriptaid treated samples, likely due to increased cell death. These data indicate that scriptaid pretreatment relieves a restriction on low MOI spread of P/V-CPI- through a cell population.

Given our previous findings that P/V-CPI- is a potent inducer of IFN-β and is defective in blocking IFN signaling (Wansley and Parks, 2002), we tested the hypothesis that scriptaid pretreatment enhanced virus spread by altering IFN responses. H1299 and A549 cells were pretreated with DMSO as a control or scriptaid, and then mock infected or infected with P/V-CPI- at an MOI of 10. At 24 hpi, media was collected and IFN-β levels were determined by

ELISA. As shown in Figure 20A and B, mock infected cells produced minimal IFN-β levels, which were largely unaltered by scriptaid. As shown previously (Wansley and Parks, 2002), P/V-CPI- infected control cells produced high amounts of IFN-β (Figure 20A,B). Most importantly, virus-induced IFN-β secretion was effectively eliminated from cells that had been pretreated with scriptaid.

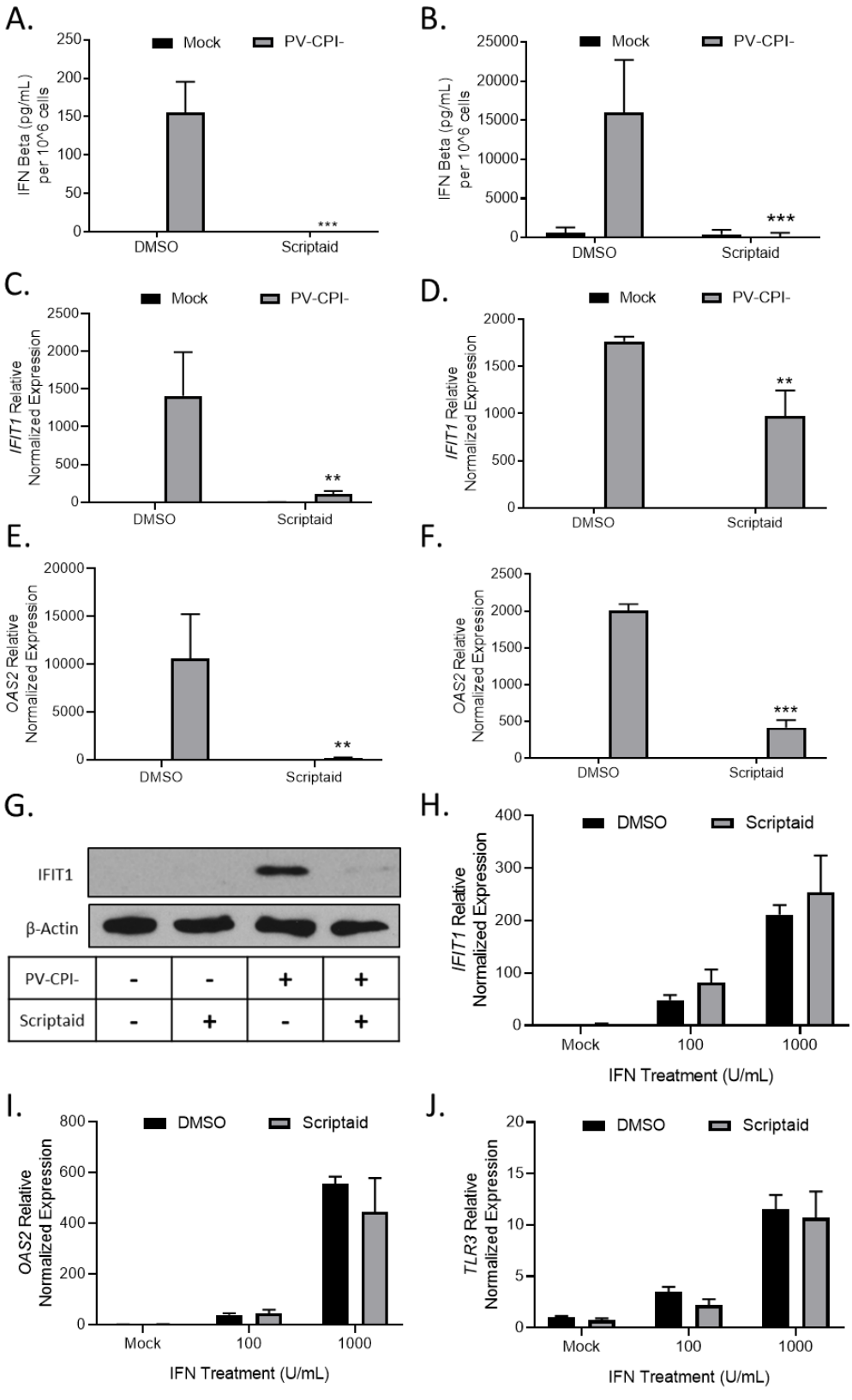
A.
Mock
PV-CPI-
IFN Beta (pg/mL) per 10^6 cells
250
200
150
100
50
0
***
DMSO
Scriptaid
B.
Mock
PV-CPI-
IFN Beta (pg/mL) per 10^6 cells
25000
20000
15000
10000
5000
0
***
DMSO
Scriptaid
C.
Mock
PV-CPI-
IFIT1 Relative Normalized Expression
2500
2000
1500
1000
500
0
**
DMSO
Scriptaid
D.
Mock
PV-CPI-
IFIT1 Relative Normalized Expression
2000
1500
1000
500
0
**
DMSO
Scriptaid
E.
OAS2 Relative Normalized Expression
20000
15000
10000
5000
0
**
DMSO
Scriptaid
F.
OAS2 Relative Normalized Expression
2500
2000
1500
1000
500
0
***
DMSO
Scriptaid
G.
IFIT1
β-Actin
PV-CPI- - - + +
Scriptaid - + - +
H.
DMSO
Scriptaid
IFIT1 Relative Normalized Expression
400
300
200
100
0
Mock
100
1000
IFN Treatment (U/mL)
I.
DMSO
Scriptaid
OAS2 Relative Normalized Expression
800
600
400
200
0
Mock
100
1000
IFN Treatment (U/mL)
J.
DMSO
Scriptaid
TLR3 Relative Normalized Expression
20
15
10
5
0
Mock
100
1000
IFN Treatment (U/mL)

Figure 20: HDAC inhibitor pretreatment decreases P/V-CPI- induction of IFN-β and ISG expression.

(A–G) H1299 (A,C,E) or A549 (B,D,E) cells were pretreated with 20 μM scriptaid or DMSO for 12 h. Cells were then mock infected or infected with P/V-CPI- at an MOI of 10 and cultured in media containing either 1 μM scriptaid or DMSO. At 24 hpi, media was collected and analyzed for IFN-β amounts by ELISA (A,B). Total cellular RNA was extracted and evaluated for *IFIT1* (C,D) and *OAS2* (E,F) expression levels by RT-qPCR. (G) H1299 cell lysates were analyzed for levels of IFIT protein by western blotting. (H–J) H1299 cells were pretreated with 20 μM scriptaid or DMSO for 12 h. Cells were then either mock treated or treated with 100 or 1000 U/mL of universal type 1 IFN. At 24 hpi, total cellular RNA was extracted and evaluated for *IFIT1* (H), *OAS2* (I), and *TLR3* (J) expression levels by RT-qPCR. For all panels, error bars indicate standard deviation. ** and *** indicates *p*-values of < 0.01 and < 0.001, respectively.

To determine if scriptaid pretreatment altered IFN stimulated gene (ISG) expression in P/V-CPI-infected cells, H1299 and A549 cells were pretreated with DMSO or scriptaid and then infected at an MOI of 10 with P/V-CPI-. Expression of two ISGs was analyzed by qPCR at 24 hpi. As shown in Figure 20C and D, *IFIT1* and *OAS2* genes were induced by P/V-CPI- infection of control cells. Scriptaid pretreatment significantly reduced the expression of these ISGs after P/V-CPI- infection. Western blotting confirmed scriptaid pretreatment reduced IFIT1 protein levels in H1299 cells (Figure 20G).

The above described scriptaid-mediated reduction in ISG expression could be due to a direct altering of IFN signaling, or alternatively, be primarily due to the loss of IFN-β production which indirectly reduces ISG expression due to loss of autocrine/paracrine signaling. In the

absence of virus infection, control and scriptaid-pretreated H1299 cells were induced with increasing levels of IFN and ISG expression was assayed by qPCR. As shown in Figure 20H–J, scriptaid pretreatment did not significantly alter the induction of *IFIT1*, *OAS2* or *TLR3* genes by exogenously-added IFN.

Taken together, these data support the conclusion that scriptaid pretreatment directly reduced IFN-β production, which in turn indirectly reduced ISGs expression, contributing to enhanced P/V-CPI- spread and cell death.

Scriptaid Treatment Reduces P/V-CPI-Induced Nuclear Localization of IRF-3

Following virus infection, IFN-β synthesis requires the phosphorylation and translocation of IRF-3 to the nucleus to initiate transcription of the *IFN-β* gene (Hiscott et al., 1999). To determine if scriptaid treatment altered IRF-3 nuclear translocation, A549 cells were treated with DMSO or scriptaid and then infected at high multiplicity with P/V-CPI-. At 22 hpi, IRF-3 location was examined by immunofluorescence. As seen in the representative images in Figure 21A, mock infected cells showed diffuse cytoplasmic IRF-3 staining which was largely unaltered by scriptaid treatment. Consistent with previous results (Manuse and Parks, 2009; Dillon and Parks, 2007) and the strong induction of IFN-β synthesis by P/V-CPI-, nearly all P/V-CPI- infected cells showed intense nuclear IRF-3 staining. Most importantly, in the case of most cells pretreated with scriptaid, P/V-CPI- infection did not produce intense IRF-3 nuclear staining, but rather the staining was seen in a pattern resembling mock infected samples. Quantification of multiple microscopy images showed that ~70%–80% of P/V-CPI- infected cells showed nuclear IRF-3 staining at either 14 or 22 hpi, which was reduced to ~10% by scriptaid pretreatment.

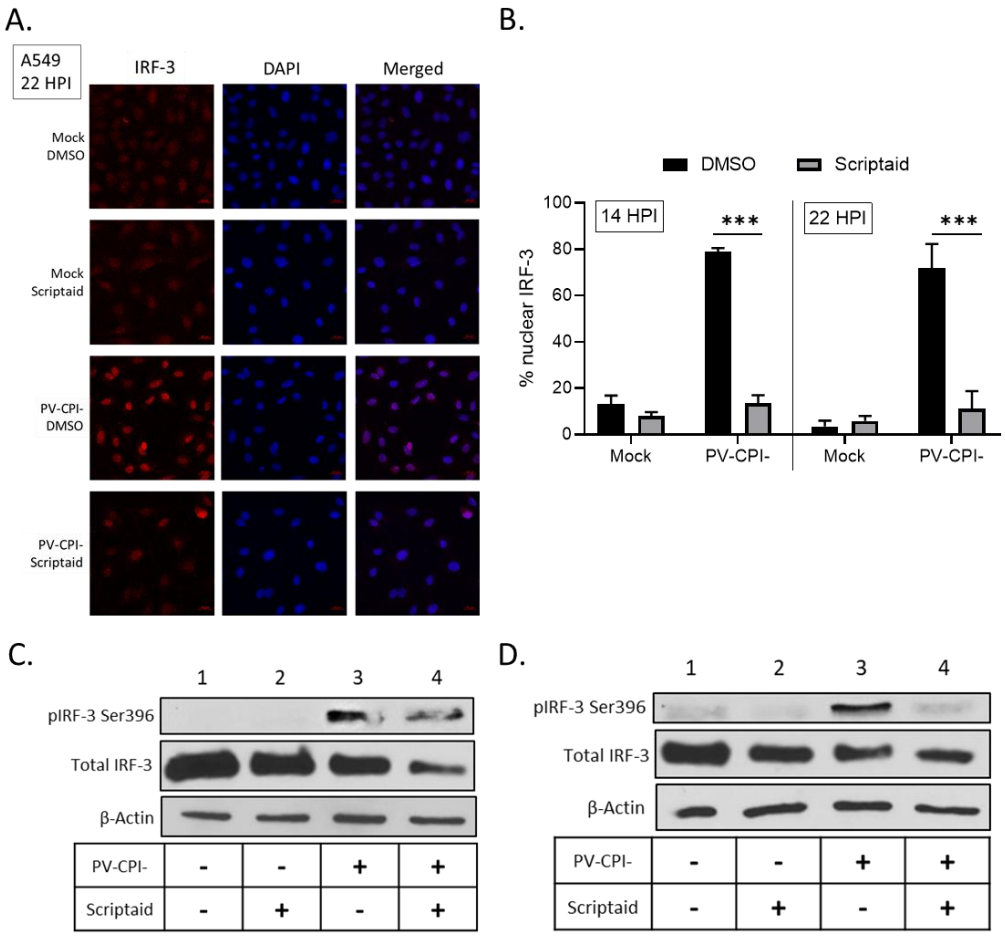

Figure 21: Effect of scriptaid treatment on P/V-CPI-induced IRF-3 nuclear localization and phosphorylation.

(A,B) A549 cells were pretreated with 20 µM scriptaid or DMSO for 12 h. Cells were then mock infected or infected with P/V-CPI- at an MOI of 10 and cultured in media containing either 1 µM scriptaid or DMSO. IRF-3 immunostaining and DAPI nuclear staining was performed at 22 hpi and imaged at 40X magnification (A). Samples from the experiment displayed in panel A were used to determine the number of cells displaying intense nuclear staining as a percentage of the population (B). For each sample, five random fields were counted and averaged, with error bars denoting standard deviations. (C,D) A549 (C) and H1299 (D) cells were treated as described in

panel A. At 24 hpi, cell lysates were evaluated for IRF-3 phosphorylated at Ser396, total IRF-3 and β-actin by Western blotting.

IRF-3 is phosphorylated in the cytoplasm prior to nuclear translocation to activate the *IFN-β* gene (Hiscott et al., 1999). Western blotting was carried out to determine if scriptaid treatment altered P/V-CPI-induced phosphorylation of IRF-3 at residue Ser396. As shown in Figure 21C and D, P/V-CPI- infection induced phosphorylation of IRF-3 (lane 3), consistent with strong induction of IFN-β synthesis. In lysates from scriptaid-treated P/V-CPI- infected cells, there was a reduction in IRF-3 phosphorylation but this was typically not completely removed.

Post-Infection Treatment of P/V-CPI-Infected Cells with a Panel of HDAC Inhibitors Reveals Two Cell Killing Profiles

We extended our analysis of the effect of 10 HDAC inhibitors on P/V-CPI- killing of HEp-2 cells, a human laryngeal cancer cell line. In addition, we tested the hypothesis that treating cells with these inhibitors after P/V-CPI- infection would enhance cell death. Cells were mock infected or infected at high MOI with P/V-CPI-, and at 12 hpi were treated with a range of concentrations of 10 different HDAC inhibitors shown in Figure 22. At 24 h post treatment, cell viability was determined by MTT cell viability assay. The results shown in Figure 22 are organized into two groups which reflect the differential effect of HDAC inhibitors on the viability of mock infected versus P/V-CPI-infected cells. Figure 22 panel A shows examples of 5 HDAC inhibitors which enhance P/V-CPI- killing but also greatly reduce the viability of mock infected control cells. For example, treatment of cells with 50 μM Tubacin or Panobinostat

resulted in ~10% and 40% viability when coupled with P/V-CPI infection, respectively. However, mock infected cell viability was also reduced to ~50%–60% by 50 μM of the HDAC inhibitors (Figure 22A). By contrast, Figure 8 panel B shows examples of drugs such as SBHA, SAHA, and Trichostatin A which had a very modest effect on the viability of mock infected control cells even at high concentrations of the drug (e.g., 100 μM), but showed strong loss of viability in P/V-CPI- infected cells. These data suggest that not all HDAC inhibitors will function optimally with P/V-CPI- infections, and the relative effectiveness of each drug may differ between different human airway cancer cells.

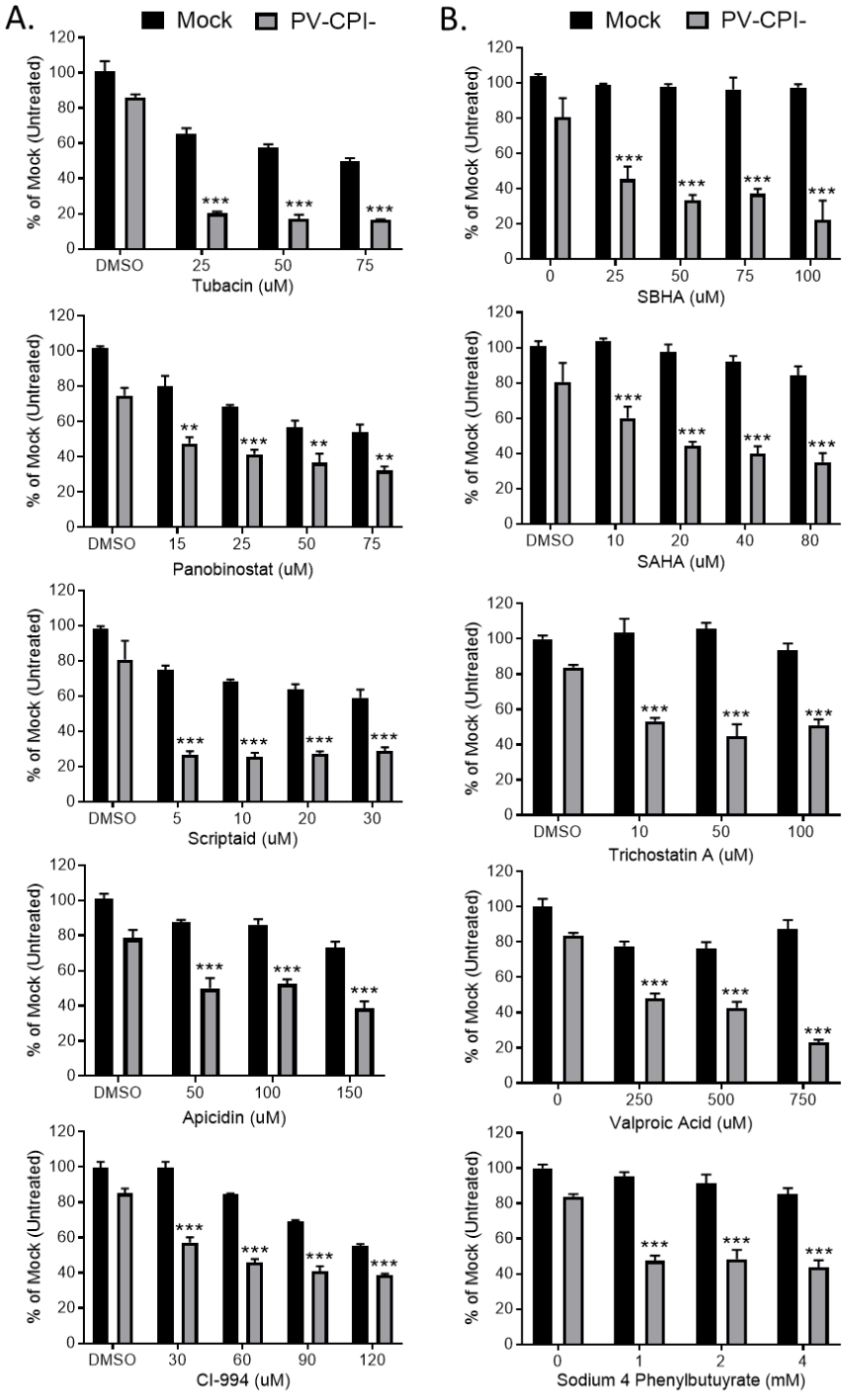

Figure 22: Differential effect of various HDAC inhibitors on killing of mock infected and P/V-CPI- infected human laryngeal cancer cells.

(A,B) HEp-2 cells were mock infected or infected with P/V-CPI- at an MOI of 10 PFU/cell. At 12 hpi, cells were treated with indicated concentrations of HDAC inhibitors or solvent control. At 24 h post treatment, cell viability was determined by MTT assay. Graphs were organized based if HDAC inhibitors reduced viability of mock infected cells (panel A) or if the inhibitors did not reduce viability of mock infected cells (panel B). Values are the mean of three replicates with ** and *** indicating *p*-values of < 0.01 and < 0.001, respectively, comparing DMSO versus HDAC inhibitor treated infected samples. Error bars indicate standard deviation.

CHAPTER FIVE: DISCUSSION

During our analysis of the capacity of the P/V-CPI- mutant to kill a range of cancer cell lines, we discovered that P/V-CPI- infection results in the majority of the cells dying, but in the case of some cell lines, there is an emergence of a population of cells which survive as a P/V-CPI- PI. One of the goals of the work described here was to understand how cells are altered to survive harboring a cytopathic virus. Our most striking finding, which emerged from challenge experiments aimed at testing resistance to apoptosis, is that cells either acutely or persistently infected with P/V-CPI- have an enhanced sensitivity to DNA damaging agents. This was evidenced in PI cells by increased cisplatin-induced DNA damage and death relative to naïve cells. This was also reflected in alterations in PI cell DNA damage response signaling pathways in the nucleus (e.g., Chk1), as well as in alterations to translocation of key DNA damage response factors from the cytoplasm to the nucleus (e.g., DDB1). Based on these findings, we present a model below for how a cytoplasmic-replicating RNA virus such as PIV5 alters the cellular responses to DNA damage.

The finding that the P/V-CPI- could sensitize cancer cells to DNA damaging agents led us to test the hypothesis that P/V-CPI- infection would also sensitize cancer cells to treatment with other chemotherapeutic agents that alter gene expression, DNA metabolism and stress responses. Here we show that airway cancer cells treated with a variety of HDAC inhibitors show dose-dependent enhanced cell killing by the P/V-CPI- mutant virus as well as two other negative strand RNA viruses (HPIV2 and LACV). This enhanced killing is due to the upregulation of caspase-dependent death pathways that include, at least in part, the response to dsRNA. In addition, treatment with the HDAC inhibitor scriptaid repressed IFN-β production which is normally potently induced by P/V-CPI- infection and led to increased viral spread

through the cell population. Taken together, these results with PI cells and acute infections have implications for the design of oncolytic RNA virus-based vectors and possible use of combinatorial virus and chemical approaches to cancer therapy.

P/V-CPI- PI Cell Survival

Under standard cell culturing conditions, the P/V-CPI- PI cells survive with elevated levels of stress markers (e.g., annexin V) and cleaved caspases compared to naïve cells. This survival was at least partially dependent on the activities of IAPs, since inhibition of Survivin and XIAP dramatically increased cell death in PI cells relative to naïve cells. These findings support a model where the continuous production of viral procducts which are inducers of cell death during low level P/V-CPI- replication (e.g., dsRNA) is countered by IAP activity. Once IAP activity is inhibited, PI cells are induced to die. This raises the attractive hypothesis that screening tumors for IAP expression could provide a tool to determine their susceptibility to establishing a PI by an oncolytic virus. In addition, the findings that PI cells are sensitized to IAP inhibitors which are currently in various phases of clinical trials (Fulda and Vucic, 2012), raises the possibility of their repurposing as antivirals and as combined chemotherapy agents in conjunction with oncolytic viruses.

Since P/V-CPI- PI cells survive while harboring a cytopathic virus, we anticipated that they would be more resistant to cell killing after challenge with external inducers of apoptosis. Unexpectedly, cisplatin-treated PI cells had more DNA damage compared to naïve cells and showed dose- and time-dependent enhancement of cisplatin-induce cell death. While phosphorylation of “upstream” sensor molecules such as H2A.X appeared normal, PI cells were defective in phosphorylation of “downstream” molecules such as Chk1 and 2. The phosphatase WIP1 was upregulated in PI cells by cisplatin treatment, and WIP1 inhibitors decrease cisplatin-

induced cell death. WIP1 has also been reported to be upregulated and have enhanced activity by Human T cell leukemia virus type 1 Tax protein. Here, WIP1 upregulation results in suppressed DNA repair capabilities and cell cycle progression in the presence of DNA damage, although the exact mechanism has yet to be elucidated (Dayaram et al., 2013). We have found the combination of P/V-CPI- and cisplatin to be effective in HEp-2 (Fig 3) and human non-small cell lung carcinoma A549 cells (data not shown), both of which have wild-type p53 (Abdulkarim et al., 2002; Yao et al., 2016). Future studies will determine roles of wild type vs mutated p53 and WIP1 in P/V-CPI- enhanced sensitivity to cisplatin.

PIV5 Influences the DDR

How does an RNA virus which replicates in the cytoplasm sensitize cells to DNA-damaging agents and enhanced cell killing? The cellular protein DDB1 normally shuttles from the cytoplasm to the nucleus in response to DNA damage and acts to assemble sensors and effectors to efficiently repair DNA damage (Iovine et al., 2011). This includes DNA repair signaling proteins such as Chk1 and 2, which are activated by phosphorylation (reviewed by Sulli et al., 2012). Once DNA repair is completed, WIP1 can be induced to deactivate the DNA damage signaling pathways by de-phosphorylation for continued cell cycle progression and return the cell to homeostasis (Lu et al., 2008). PIV5 (and other Rubulaviruses) hijack DDB1 as part of the "V degradation complex" which targets cytoplasmic degradation of STAT proteins to inhibit IFN signaling pathways (Andrejeva et al., 2002a; Didcock et al., 1999; Ulane et al., 2005; Parisien et al., 2001; Young et al., 2000). Our microscopy and cell fractionation data on P/V-CPI- PI cells show DDB1 is localized largely to the cytoplasm in structures distinct from that of naïve cells, and it is altered in accumulating in the nucleus in response to cisplatin treatment. We propose a model whereby treatment of PI cells with cisplatin induces a typical DNA damage

response including phosphorylation of ATR and H2A.X (Fig. 5A). Retention of DDB1 in the cytoplasm of PI cells results in reduced sensing of the extent of DNA damage, in effect telling the cell that DNA damage is not extensive. The finding that WIP1 is induced in cisplatin-treated PI cells is consistent with our finding of un-phosphorylated Chk1 and Chk2, and with a model that PI cells shut down signaling pathways due to lack of DDB1-mediated ability to sense the extent of DNA damage. In our model, ultimately the cell is unable to recognize the extent of DNA damage which is then not repaired and cells undergo apoptosis.

Oncolytic Virus and Chemotherapy Combination Therapy

Our studies raise the possibility of using combination therapies with paramyxovirus oncolytic viruses and chemotherapies, as have been studied with other viruses. For example, a phase II clinical trial has shown the combination of the adenovirus oncolytic virus ONYX-015, cisplatin, and 5-fluorouracil to be more effective than the therapies alone in patients with recurrent head and neck cancer (Khuri et al., 2000). A phase I/II study investigated the combination of oncolytic virus T-VEC, radiotherapy, and cisplatin to treat patients with head and neck cancer. These clinical trials have found combination therapy with an oncolytic virus and chemotherapy to improve disease progression, rate of relapse-free, and overall survival (Harrington et al., 2010).

The finding that acute infection with the oncolytic virus P/V-CPI- sensitizes cells to DNA damaging agents raises a practical application for our work in terms of resistance of some cancer cells to DNA damaging agents. Although cisplatin is the gold standard for numerous cancers, chemoresistance is a major concern (Dasari and Tchounwou, 2014). For example, cisplatin treatment for ovarian cancer patients is initially very effective, but tumor recurrence occurs in up to 75% of cases, resulting in chemotherapy resistant tumors (Agarwal and Kaye, 2003). Previous

studies have shown that cisplatin resistance can result when cells increase DNA repair capabilities (Siddik, 2003; Parker et al., 1991; Masuda et al., 1988; De Pooter et al., 1996; Ferry et al., 2000). Conversely, cisplatin sensitivity has been associated with lower capacity of cells in their DNA repair pathways (Rosell et al., 2003; Welsh et al., 2004; Calsou et al., 1993; Hill et al., 1994). Thus, an attractive property of an oncolytic virus would be in its ability to impair DNA damage repair pathways and increase cisplatin sensitivity, especially in the case of drug resistant tumors that have up-regulated DNA repair pathways. This is in line with what we report here with the ability of P/V-CPI- to reduce DNA repair capacities.

HDAC Inhibitors Mediate Antiviral Response to Oncolytic Viruses

HDAC inhibitors have shown promise as chemotherapy agents. While four of these chemical inhibitors have been approved by the FDA for hematologic cancers, there are disadvantages on their use as single agents such as less success in solid tumor therapies (Robey et al., 2011). Therefore, numerous ongoing clinical trials are investigating HDAC inhibitors and additional forms of cancer therapies, such as other chemotherapies, radiation, and immunotherapies (reviewed by Suraweera et al., 2018). One such promising combination therapy is HDAC inhibitors and oncolytic viruses. Due to the pleiotropic effects of HDAC inhibitors on various pathways, their mechanism of action is often complex when coupled with oncolytic viruses. For example, valproic acid was shown to act synergistically with the DNA viruses adenovirus, herpes virus, and vaccinia virus; however, it antagonized the potency of a different strain of adenovirus (Otsuki et al., 2008; Sasaki et al., 2008; Goldsmith et al., 2007; MacTavish et al., 2010; Hoti et al., 2006). The proposed mechanisms of enhanced cell death with HDAC inhibitors and DNA oncolytic viruses are very diverse, such as amplified viral replication (including increased viral entry due to increased receptor expression), reduced antiviral

responses, increased apoptosis or autophagy, increased NF-kB activity, increased cell cycle arrest, and increased oxidative stress (reviewed by Nakashima et al., 2015; Marchini et al., 2016; Nguyen et al., 2010).

Our study was prompted by an interest in how HDAC inhibitors could be used with a novel oncolytic virus based on a cytoplasmic replicating parainfluenza mutant. Shulak et al., 2014 previously showed in prostate cancer cell lines that the combination of HDAC inhibitors with VSV infection resulted in an increase in virus replication and caspase dependent death, as well as a decrease in IFN-α and ISG expression. HDAC inhibitors were shown to induce NF-κB-regulated genes and increased NF-κB dependent autophagy, which led to enhanced death. In pancreatic cancer cell lines, Ellerhoff and co-workers (Ellerhoff et al., 2016) found synergistic effects of HDAC inhibitors and measles virus infection on cell death, however there was no alterations in virus growth or in IFN signaling pathways. Our results with P/V-CPI- and airway cancer cells differs from these two prior reports by showing that scriptaid pre-treatment: (1) enhanced killing through caspase-dependent pathways (versus autophagy for VSV; Shulak et al., 2014), (2) promoted virus spread through a cell population, and (3) reduced IFN-β production and ISGs expression through a block at or upstream of IRF-3 nuclear translocation.

Our studies show the effect that an HDAC inhibitor can have on RNA virus activation of IFN pathways. P/V-CPI- is a potent inducer of IFN-β, due to alterations in both the V protein and the P protein component of the viral polymerase (Wansley and Parks, 2002; Dillon and Parks, 2007), and it is restricted in spread due to the paracrine and autocrine effects of IFN-β (Wansley et al., 2003). As reviewed by Eckschlager et al., 2017 and Suraweera et al., 2018, HDAC inhibitors have a tumor cell specificity and show minimal effects on healthy cells. Therefore, healthy cells should be able to produce IFN and induce an anti-viral state upon P/V-CPI-

infection, thereby maintaining tumor specificity of our proposed combination therapy. Here we show that Scriptaid treatment reduced IFN-β production from P/V-CPI-infected cells to background levels, and enhanced low MOI growth and killing of a cell population. While prior work has shown that HDAC inhibition can in some cases alter IFN signaling (e.g., Nusinzon and Horvath, 2003), we showed that with this virus/cell system, HDAC inhibitors directly limited IFN induction but did not directly affect ISG expression. Virus induced IRF-3 nuclear translocation was blocked by scriptaid treatment, suggesting an alteration in cytoplasmic sensing or signaling versus more distal effects on nuclear transcription of the *IFN-β* gene. While IRF-3 phosphorylation at a key regulatory site was reproducibly decreased by scriptaid treatment, it is not clear that this was sufficient to account for the nearly complete loss of IFN-β production. Future work will address the specific step in IFN-β induction altered by HDAC inhibition.

HDAC Inhibitors Enhance Virus-Induction of Cell Death

HDAC inhibitors can regulate expression of proteins involved in apoptosis, including an increase in pro-apoptotic protein family members, e.g., Bid, Bim, Bmf, Bad, and Noxa (Chen et al., 2009; Xargay-Torrent et al., 2011; Inoue et al., 2007; Terui et al., 2003). Conversely, HDAC inhibitors can in some cases result in a decrease in anti-apoptosis proteins such as Bcl-2 and Bcl-w, as well as in select caspase inhibitors, such as X-linked inhibitor of apoptosis (XIAP), survivin, and cellular FLICE-like inhibitory protein (c-FLIP; Aron et al., 2003; Rosato et al., 2007; Sanda et al., 2007). Signals that activate the extrinsic apoptosis pathway have also been shown to increase following HDAC inhibitor treatment (Glick et al., 1999; Insinga et al., 2005; Nebbioso et al., 2005). For example, Nakata et al., 2004 found levels of death receptor 5 to be upregulated following HDAC inhibitor treatment, leading to increased caspases -8, -10, -9, and -3 activation and apoptosis induced by Tumor Necrosis Factor (TNF) related apoptosis ligand

(TRAIL). While at this point it is unclear how HDAC inhibitors promote P/V-CPI-mediated increases in caspases, there is a potential mechanism linked to scriptaid-induced decreases in cellular inhibitors of apoptosis. This is supported by our previous findings that expression of cIAP-1, XIAP, and survivin are all decreased by P/V-CPI- infection and that chemical inhibition of survivin (with YM155) or XIAP (with Embelin) enhanced killing of a P/V-CPI- persistently infected cell line (Fox and Parks, 2018).

dsRNA produced during the course of virus infection can be a potent inducer of cell death (Zhao et al., 2012), through pathways that can involve both caspase-8 and -9 (Garcia et al., 2002). Here we show that P/V-CPI-induced killing is decreased in an A549 cell line engineered to express the reovirus sigma 3 protein which sequesters dsRNA. This decreased killing is seen in both untreated and scriptaid treated A549-sigma 3 cells. This suggests that scriptaid does not activate alternative death pathways, but rather amplifies death pathways already activated by dsRNA produced during replication of P/V-CPI-.

Scriptaid treatment promoted airway cancer cell killing by P/V-CPI-, but also by two other negative strand RNA viruses we tested – LACV and VSV. While the mechanism by which WT PIV5 can replicate to very high levels in most cell types but not induce a cytopathic effect is currently unknown, it was nonetheless expected that HDAC inhibitors would not alter this phenotype in airway cancer cell lines. Scriptaid treatment had a very strong effect on LACV cytopathic effect. What was surprising, however, was our finding that HDAC inhibitors did not accelerate killing by infection with hPIV2 or ZIKV, both of which are associated with strong cytopathic effects. Since a number of groups are proposing the use of ZIKV as an oncolytic vector (for example Mazar et al., 2018), this result may warrant further in-depth study to investigate variables such as HDAC inhibitors other than scriptaid, other cancer cell types, or the

length of treatment and infection. The timing of HDAC inhibitor treatment can play a role in the effectiveness of the combination treatment. For example, pretreatment before viral infection can improve viral yields, however concurrent treatment eliminated this effect (Otsuki et al., 2008; Flemington, 2001).

Implications

A number of paramyxo- and rhabdoviruses are being developed as oncolytic vectors for tumor therapy, including MeV, MuV, SeV, and NDV (Elankumaran et al., 2006; Kinoh et al., 2004; Lorence et al., 1994; Myers et al., 2005; Peng et al., 2003; Yu et al., 2015; Matveeva et al., 2015). Our finding that the highly cytopathic PIV5 P/V mutant can in some cells establish a PI raises a concern on the potential use of RNA viruses for oncolytic therapy. Future work will focus on the extent to which establishment of PI cells occurs during treatment of tumors in animal model systems, as well as the potential for combinations of cisplatin and P/V-CPI- for reducing tumor burden.

Due to the multifaceted nature of HDAC inhibitors, our studies showcase the necessity of personalized medicine by characterizing tumors to better indicate the proper treatment options. Which therapies to combine, the timing and length of treatments are imperative factors that need to be considered when utilizing a combination approach with HDAC inhibitors and oncolytic viruses.

By combining oncolytic viruses and chemotherapies, such as cisplatin and HDAC inhibitors, we can overcome numerous hurdles in cancer treatment. Ideally the combination will reduce the dose of chemotherapy needed, thus reducing side effects. The first treatment combination should be effective with no cells surviving, thus reducing the emergence of chemoresistant cancer. By combining oncolytic viruses and chemotherapy, an immune response

would be elicited that can aid in tumor clearance. Cancer specificity can still be maintained in the combination therapy due to the nature of an oncolytic virus and by utilizing chemotherapy targeting cancer cells.

One of the main concerns with using oncolytic virus is the tumor cells' ability to activate the antiviral response and limit the virus' effectiveness. This obstacle can be overcome by combining OVs with chemotherapy, specifically HDAC inhibitors. HDAC inhibitors can prime the tumor cells to allow for optimal virus replication. Future work will determine the best combination of P/V mutant with which chemotherapy for cancer treatment.

REFERENCES

Abdulkarim, B., Sabri, S., Deutsch, E., Chagraoui, H., Maggiorella, L., Thierry, J., . . . Bourhis, J. (2002). Antiviral agent Cidofovir restores p53 function and enhances the radiosensitivity in HPV-associated cancers. *Oncogene, 21*(15), 2334-2346. doi:10.1038/sj.onc.1205006

Agarwal, R., & Kaye, S. B. (2003). Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer, 3*(7), 502-516. doi:10.1038/nrc1123

Alexopoulou, L., Holt, A. C., Medzhitov, R., & Flavell, R. A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature, 413*(6857), 732-738. doi:10.1038/35099560

Alvarez-Breckenridge, C. A., Yu, J., Price, R., Wei, M., Wang, Y., Nowicki, M. O., . . . Chiocca, E. A. (2012). The histone deacetylase inhibitor valproic acid lessens NK cell action against oncolytic virus-infected glioblastoma cells by inhibition of STAT5/T-BET signaling and generation of gamma interferon. *J Virol, 86*(8), 4566-4577. doi:10.1128/jvi.05545-11

Andrejeva, J., Childs, K. S., Young, D. F., Carlos, T. S., Stock, N., Goodbourn, S., & Randall, R. E. (2004). The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc Natl Acad Sci U S A, 101*(49), 17264-17269. doi:10.1073/pnas.0407639101

Andrejeva, J., Poole, E., Young, D. F., Goodbourn, S., & Randall, R. E. (2002a). The p127 subunit (DDB1) of the UV-DNA damage repair binding protein is essential for the targeted degradation of STAT1 by the V protein of the paramyxovirus simian virus 5. *J Virol, 76*(22), 11379-11386.

Andrejeva, J., Young, D. F., Goodbourn, S., & Randall, R. E. (2002b). Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: consequences for virus replication in the presence of alpha/beta and gamma interferons. *J Virol, 76*(5), 2159-2167. doi:10.1128/jvi.76.5.2159-2167.2002

Angers, S., Li, T., Yi, X., MacCoss, M. J., Moon, R. T., & Zheng, N. (2006). Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. *Nature, 443*(7111), 590-593. doi:10.1038/nature05175

Aron, J. L., Parthun, M. R., Marcucci, G., Kitada, S., Mone, A. P., Davis, M. E., . . . Byrd, J. C. (2003). Depsipeptide (FR901228) induces histone acetylation and inhibition of histone deacetylase in chronic lymphocytic leukemia cells concurrent with activation of caspase 8-mediated apoptosis and down-regulation of c-FLIP protein. *Blood, 102*(2), 652-658. doi:10.1182/blood-2002-12-3794

Bell, J. C., Lichty, B., & Stojdl, D. (2003). Getting oncolytic virus therapies off the ground. *Cancer Cell, 4*(1), 7-11.

Billeter, M. A., Cattaneo, R., Spielhofer, P., Kaelin, K., Huber, M., Schmid, A., . . . ter Meulen, V. (1994). Generation and properties of measles virus mutations typically associated with subacute sclerosing panencephalitis. *Ann N Y Acad Sci, 724*, 367-377.

Biron, C. A. & Sen, G. C. (2007). Innate responses to viral infections. In B. N. Fields, D. N. Knipe, & P. M. Howley (Eds.), *Fields virology: Fifth Edition* (5 ed., pp. 249-278). Lippincott, Williams, and Wilkins.

Blumberg, B. M., Leppert, M., & Kolakofsky, D. (1981). Interaction of VSV leader RNA and nucleocapsid protein may control VSV genome replication. *Cell, 23*(3), 837-845. doi:10.1016/0092-8674(81)90448-7

Bridle, B. W., Chen, L., Lemay, C. G., Diallo, J. S., Pol, J., Nguyen, A., . . . Wan, Y. (2013). HDAC inhibition suppresses primary immune responses, enhances secondary immune responses, and abrogates autoimmunity during tumor immunotherapy. *Mol Ther, 21*(4), 887-894. doi:10.1038/mt.2012.265

Calsou, P., Barret, J. M., Cros, S., & Salles, B. (1993). DNA excision-repair synthesis is enhanced in a murine leukemia L1210 cell line resistant to cisplatin. *Eur J Biochem, 211*(3), 403-409.

Cha, H., Lowe, J. M., Li, H., Lee, J. S., Belova, G. I., Bulavin, D. V., & Fornace, A. J., Jr. (2010). Wip1 directly dephosphorylates gamma-H2AX and attenuates the DNA damage response. *Cancer Res, 70*(10), 4112-4122. doi:10.1158/0008-5472.Can-09-4244

Chang, H. H., Chiang, C. P., Hung, H. C., Lin, C. Y., Deng, Y. T., & Kuo, M. Y. (2009). Histone deacetylase 2 expression predicts poorer prognosis in oral cancer patients. *Oral Oncol, 45*(7), 610-614. doi:10.1016/j.oraloncology.2008.08.011

Chen, S., Dai, Y., Pei, X. Y., & Grant, S. (2009). Bim upregulation by histone deacetylase inhibitors mediates interactions with the Bcl-2 antagonist ABT-737: evidence for distinct roles for Bcl-2, Bcl-xL, and Mcl-1. *Mol Cell Biol, 29*(23), 6149-6169. doi:10.1128/mcb.01481-08

Childs, K., Stock, N., Ross, C., Andrejeva, J., Hilton, L., Skinner, M., . . . Goodbourn, S. (2007). mda-5, but not RIG-I, is a common target for paramyxovirus V proteins. *Virology, 359*(1), 190-200. doi:10.1016/j.virol.2006.09.023

Christmann, M., Tomicic, M. T., Roos, W. P., & Kaina, B. (2003). Mechanisms of human DNA repair: an update. *Toxicology, 193*(1-2), 3-34. doi:10.1016/s0300-483x(03)00287-7

Conzelmann, K. K. (2005). Transcriptional activation of alpha/beta interferon genes: interference by nonsegmented negative-strand RNA viruses. *J Virol, 79*(9), 5241-5248. doi:10.1128/jvi.79.9.5241-5248.2005

Curran, J., Boeck, R., & Kolakofsky, D. (1991). The Sendai virus P gene expresses both an essential protein and an inhibitor of RNA synthesis by shuffling modules via mRNA editing. *Embo j, 10*(10), 3079-3085.

Curran, J., Marq, J. B., & Kolakofsky, D. (1995). An N-terminal domain of the Sendai paramyxovirus P protein acts as a chaperone for the NP protein during the nascent chain assembly step of genome replication. *J Virol, 69*(2), 849-855.

Dasari, S., & Tchounwou, P. B. (2014). Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol, 740*, 364-378. doi:10.1016/j.ejphar.2014.07.025

Dayaram, T., Lemoine, F. J., Donehower, L. A., & Marriott, S. J. (2013). Activation of WIP1 phosphatase by HTLV-1 Tax mitigates the cellular response to DNA damage. *PLoS One, 8*(2), e55989. doi:10.1371/journal.pone.0055989

de la Torre, J. C., Martinez-Salas, E., Diez, J., Villaverde, A., Gebauer, F., Rocha, E., . . . Domingo, E. (1988). Coevolution of cells and viruses in a persistent infection of foot-and-mouth disease virus in cell culture. *J Virol, 62*(6), 2050-2058.

De Pooter, C. M., Van Oosterom, A. T., Scalliet, P. G., Maes, R. A., & de Bruijn, E. A. (1996). Correlation of the response to cisplatin of human ovarian cancer cell lines, originating from one tumor but with different sensitivity, with the recovery of DNA adducts. *Biochem Pharmacol, 51*(5), 629-634.

De Zio, D., Cianfanelli, V., & Cecconi, F. (2013). New insights into the link between DNA damage and apoptosis. *Antioxid Redox Signal, 19*(6), 559-571. doi:10.1089/ars.2012.4938

Devaux, P., von Messling, V., Songsungthong, W., Springfeld, C., & Cattaneo, R. (2007). Tyrosine 110 in the measles virus phosphoprotein is required to block STAT1 phosphorylation. *Virology, 360*(1), 72-83. doi:10.1016/j.virol.2006.09.049

Deveraux, Q. L., Takahashi, R., Salvesen, G. S., & Reed, J. C. (1997). X-linked IAP is a direct inhibitor of cell-death proteases. *Nature, 388*(6639), 300-304. doi:10.1038/40901

Didcock, L., Young, D. F., Goodbourn, S., & Randall, R. E. (1999). The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. *J Virol, 73*(12), 9928-9933.

Dillon, P. J., & Parks, G. D. (2007). Role for the phosphoprotein P subunit of the paramyxovirus polymerase in limiting induction of host cell antiviral responses. *J Virol, 81*(20), 11116-11127. doi:10.1128/jvi.01360-07

Dillon, P. J., Wansley, E. K., Young, V. A., Alexander-Miller, M. A., & Parks, G. D. (2006). Exchange of P/V genes between two non-cytopathic simian virus 5 variants results in a recombinant virus that kills cells through death pathways that are sensitive to caspase inhibitors. *J Gen Virol, 87*(Pt 12), 3643-3648. doi:10.1099/vir.0.82242-0

Eckschlager, T., Plch, J., Stiborova, M., & Hrabeta, J. (2017). Histone Deacetylase Inhibitors as Anticancer Drugs. *Int J Mol Sci, 18*(7). doi:10.3390/ijms18071414

Elankumaran, S., Rockemann, D., & Samal, S. K. (2006). Newcastle disease virus exerts oncolysis by both intrinsic and extrinsic caspase-dependent pathways of cell death. *J Virol, 80*(15), 7522-7534. doi:10.1128/jvi.00241-06

Ellerhoff, T. P., Berchtold, S., Venturelli, S., Burkard, M., Smirnow, I., Wulff, T., & Lauer, U. M. (2016). Novel epi-virotherapeutic treatment of pancreatic cancer combining the oral histone deacetylase inhibitor resminostat with oncolytic measles vaccine virus. *Int J Oncol, 49*(5), 1931-1944. doi:10.3892/ijo.2016.3675

Ferry, K. V., Hamilton, T. C., & Johnson, S. W. (2000). Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: role of ERCC1-XPF. *Biochem Pharmacol, 60*(9), 1305-1313.

Flemington, E. K. (2001). Herpesvirus lytic replication and the cell cycle: arresting new developments. *J Virol, 75*(10), 4475-4481. doi:10.1128/jvi.75.10.4475-4481.2001

Fousteri, M., & Mullenders, L. H. (2008). Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. *Cell Res, 18*(1), 73-84. doi:10.1038/cr.2008.6

Fox, C. R., & Parks, G. D. (2018). Parainfluenza Virus Infection Sensitizes Cancer Cells to DNA-Damaging Agents: Implications for Oncolytic Virus Therapy. *J Virol, 92*(7). doi:10.1128/jvi.01948-17

Fox, C. R., & Parks, G. D. (2019). Histone Deacetylase Inhibitors Enhance Cell Killing and Block Interferon-Beta Synthesis Elicited by Infection with an Oncolytic Parainfluenza Virus. *Viruses, 11*(5). doi:10.3390/v11050431

Fukuhara, H., Ino, Y., & Todo, T. (2016). Oncolytic virus therapy: A new era of cancer treatment at dawn. *Cancer Sci, 107*(10), 1373-1379. doi:10.1111/cas.13027

Fulda, S., & Vucic, D. (2012). Targeting IAP proteins for therapeutic intervention in cancer. *Nat Rev Drug Discov, 11*(2), 109-124. doi:10.1038/nrd3627

Gainey, M. D., Dillon, P. J., Clark, K. M., Manuse, M. J., & Parks, G. D. (2008). Paramyxovirus-induced shutoff of host and viral protein synthesis: role of the P and V proteins in limiting PKR activation. *J Virol, 82*(2), 828-839. doi:10.1128/jvi.02023-07

Gainey, M. D., Manuse, M. J., & Parks, G. D. (2008). A hyperfusogenic F protein enhances the oncolytic potency of a paramyxovirus simian virus 5 P/V mutant without compromising sensitivity to type I interferon. *J Virol, 82*(19), 9369-9380. doi:10.1128/jvi.01054-08

Garcia-Sastre, A. (2001). Inhibition of interferon-mediated antiviral responses by influenza A viruses and other negative-strand RNA viruses. *Virology, 279*(2), 375-384. doi:10.1006/viro.2000.0756

Garcia, M. A., Guerra, S., Gil, J., Jimenez, V., & Esteban, M. (2002). Anti-apoptotic and oncogenic properties of the dsRNA-binding protein of vaccinia virus, E3L. *Oncogene, 21*(55), 8379-8387. doi:10.1038/sj.onc.1206036

Garcin, D., Latorre, P., & Kolakofsky, D. (1999). Sendai virus C proteins counteract the interferon-mediated induction of an antiviral state. *J Virol, 73*(8), 6559-6565.

Glick, R. D., Swendeman, S. L., Coffey, D. C., Rifkind, R. A., Marks, P. A., Richon, V. M., & La Quaglia, M. P. (1999). Hybrid polar histone deacetylase inhibitor induces apoptosis and CD95/CD95 ligand expression in human neuroblastoma. *Cancer Res, 59*(17), 4392-4399.

Goldsmith, M. E., Aguila, A., Steadman, K., Martinez, A., Steinberg, S. M., Alley, M. C., . . . Fojo, T. (2007). The histone deacetylase inhibitor FK228 given prior to adenovirus infection can boost infection in melanoma xenograft model systems. *Mol Cancer Ther, 6*(2), 496-505. doi:10.1158/1535-7163.Mct-06-0431

Goldstein, M., & Kastan, M. B. (2015). The DNA damage response: implications for tumor responses to radiation and chemotherapy. *Annu Rev Med, 66*, 129-143. doi:10.1146/annurev-med-081313-121208

Gonzalez-Reyes, S., Marin, L., Gonzalez, L., Gonzalez, L. O., del Casar, J. M., Lamelas, M. L., . . . Vizoso, F. J. (2010). Study of TLR3, TLR4 and TLR9 in breast carcinomas and their association with metastasis. *BMC Cancer, 10*, 665. doi:10.1186/1471-2407-10-665

Goodbourn, S., Didcock, L., & Randall, R. E. (2000). Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. *J Gen Virol, 81*(Pt 10), 2341-2364. doi:10.1099/0022-1317-81-10-2341

Grdzelishvili, V. Z., Smallwood, S., Tower, D., Hall, R. L., Hunt, D. M., & Moyer, S. A. (2005). A single amino acid change in the L-polymerase protein of vesicular stomatitis virus completely abolishes viral mRNA cap methylation. *J Virol, 79*(12), 7327-7337. doi:10.1128/jvi.79.12.7327-7337.2005

Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., & Ron, D. (2000). Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell, 6*(5), 1099-1108.

Harrington, K. J., Hingorani, M., Tanay, M. A., Hickey, J., Bhide, S. A., Clarke, P. M., . . . Nutting, C. M. (2010). Phase I/II study of oncolytic HSV GM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. *Clin Cancer Res, 16*(15), 4005-4015. doi:10.1158/1078-0432.Ccr-10-0196

Haus, O. (2000). The genes of interferons and interferon-related factors: localization and relationships with chromosome aberrations in cancer. *Arch Immunol Ther Exp (Warsz), 48*(2), 95-100.

He, B., Lin, G. Y., Durbin, J. E., Durbin, R. K., & Lamb, R. A. (2001). The SH Integral Membrane Protein of the Paramyxovirus Simian Virus 5 Is Required To Block Apoptosis in MDBK Cells. *Journal of Virology, 75*(9), 4068-4079. doi:10.1128/jvi.75.9.4068-4079.2001

He, B., Paterson, R. G., Stock, N., Durbin, J. E., Durbin, R. K., Goodbourn, S., . . . Lamb, R. A. (2002). Recovery of paramyxovirus simian virus 5 with a V protein lacking the conserved cysteine-rich domain: the multifunctional V protein blocks both interferon-beta induction and interferon signaling. *Virology, 303*(1), 15-32. doi:10.1006/viro.2002.1738

He, B., Paterson, R. G., Ward, C. D., & Lamb, R. A. (1997). Recovery of infectious SV5 from cloned DNA and expression of a foreign gene. *Virology, 237*(2), 249-260. doi:10.1006/viro.1997.8801

Heifetz, A., Keenan, R. W., & Elbein, A. D. (1979). Mechanism of action of tunicamycin on the UDP-GlcNAc:dolichyl-phosphate Glc-NAc-1-phosphate transferase. *Biochemistry, 18*(11), 2186-2192. doi:10.1021/bi00578a008

Hercyk, N., Horikami, S. M., & Moyer, S. A. (1988). The vesicular stomatitis virus L protein possesses the mRNA methyltransferase activities. *Virology, 163*(1), 222-225. doi:10.1016/0042-6822(88)90253-x

Hill, B. T., Scanlon, K. J., Hansson, J., Harstrick, A., Pera, M., Fichtinger-Schepman, A. M., & Shellard, S. A. (1994). Deficient repair of cisplatin-DNA adducts identified in human testicular teratoma cell lines established from tumours from untreated patients. *Eur J Cancer, 30a*(6), 832-837.

Hiscott, J., Pitha, P., Genin, P., Nguyen, H., Heylbroeck, C., Mamane, Y., . . . Lin, R. (1999). Triggering the interferon response: the role of IRF-3 transcription factor. *J Interferon Cytokine Res, 19*(1), 1-13. doi:10.1089/107999099314360

Holmes, K. V., & Choppin, P. W. (1966). On the role of the response of the cell membrane in determining virus virulence. Contrasting effects of the parainfluenza virus SV5 in two cell types. *J Exp Med, 124*(3), 501-520.

Horikami, S. M., Smallwood, S., & Moyer, S. A. (1996). The Sendai virus V protein interacts with the NP protein to regulate viral genome RNA replication. *Virology, 222*(2), 383-390. doi:10.1006/viro.1996.0435

Hornung, V., Ellegast, J., Kim, S., Brzozka, K., Jung, A., Kato, H., . . . Hartmann, G. (2006). 5'-Triphosphate RNA is the ligand for RIG-I. *Science, 314*(5801), 994-997. doi:10.1126/science.1132505

Horvath, C. M. (2000). STAT proteins and transcriptional responses to extracellular signals. *Trends Biochem Sci, 25*(10), 496-502. doi:10.1016/s0968-0004(00)01624-8

Horvath, C. M. (2004). Weapons of STAT destruction. Interferon evasion by paramyxovirus V protein. *Eur J Biochem, 271*(23-24), 4621-4628. doi:10.1111/j.1432-1033.2004.04425.x

Hoti, N., Chowdhury, W., Hsieh, J. T., Sachs, M. D., Lupold, S. E., & Rodriguez, R. (2006). Valproic acid, a histone deacetylase inhibitor, is an antagonist for oncolytic adenoviral gene therapy. *Mol Ther, 14*(6), 768-778. doi:10.1016/j.ymthe.2006.07.009

Huang, Z., Krishnamurthy, S., Panda, A., & Samal, S. K. (2003). Newcastle disease virus V protein is associated with viral pathogenesis and functions as an alpha interferon antagonist. *J Virol, 77*(16), 8676-8685. doi:10.1128/jvi.77.16.8676-8685.2003

Hurley, P. J., & Bunz, F. (2007). ATM and ATR: components of an integrated circuit. *Cell Cycle, 6*(4), 414-417. doi:10.4161/cc.6.4.3886

Inoue, S., Riley, J., Gant, T. W., Dyer, M. J., & Cohen, G. M. (2007). Apoptosis induced by histone deacetylase inhibitors in leukemic cells is mediated by Bim and Noxa. *Leukemia, 21*(8), 1773-1782. doi:10.1038/sj.leu.2404760

Insinga, A., Monestiroli, S., Ronzoni, S., Gelmetti, V., Marchesi, F., Viale, A., . . . Pelicci, P. G. (2005). Inhibitors of histone deacetylases induce tumor-selective apoptosis through activation of the death receptor pathway. *Nat Med, 11*(1), 71-76. doi:10.1038/nm1160

Iovine, B., Iannella, M. L., & Bevilacqua, M. A. (2011). Damage-specific DNA binding protein 1 (DDB1): a protein with a wide range of functions. *Int J Biochem Cell Biol, 43*(12), 1664-1667. doi:10.1016/j.biocel.2011.09.001

Jacobs, B. L., & Langland, J. O. (1996). When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. *Virology, 219*(2), 339-349. doi:10.1006/viro.1996.0259

Kapetanaki, M. G., Guerrero-Santoro, J., Bisi, D. C., Hsieh, C. L., Rapic-Otrin, V., & Levine, A. S. (2006). The DDB1-CUL4ADDB2 ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. *Proc Natl Acad Sci U S A, 103*(8), 2588-2593. doi:10.1073/pnas.0511160103

Karin, M., & Ben-Neriah, Y. (2000). Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol, 18*, 621-663. doi:10.1146/annurev.immunol.18.1.621

Katsura, T., Iwai, S., Ota, Y., Shimizu, H., Ikuta, K., & Yura, Y. (2009). The effects of trichostatin A on the oncolytic ability of herpes simplex virus for oral squamous cell carcinoma cells. *Cancer Gene Ther, 16*(3), 237-245. doi:10.1038/cgt.2008.81

Kaufman, H. L., Kohlhapp, F. J., & Zloza, A. (2015). Oncolytic viruses: a new class of immunotherapy drugs. *Nat Rev Drug Discov, 14*(9), 642-662. doi:10.1038/nrd4663

Khuri, F. R., Nemunaitis, J., Ganly, I., Arseneau, J., Tannock, I. F., Romel, L., . . . Kirn, D. H. (2000). a controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med, 6*(8), 879-885. doi:10.1038/78638

Kinoh, H., Inoue, M., Washizawa, K., Yamamoto, T., Fujikawa, S., Tokusumi, Y., . . . Hasegawa, M. (2004). Generation of a recombinant Sendai virus that is selectively activated and lyses human tumor cells expressing matrix metalloproteinases. *Gene Ther, 11*(14), 1137-1145. doi:10.1038/sj.gt.3302272

Kobayashi, T., Antar, A. A., Boehme, K. W., Danthi, P., Eby, E. A., Guglielmi, K. M., . . . Dermody, T. S. (2007). A plasmid-based reverse genetics system for animal double-stranded RNA viruses. *Cell Host Microbe, 1*(2), 147-157. doi:10.1016/j.chom.2007.03.003

Kubota, T., Yokosawa, N., Yokota, S., & Fujii, N. (2001). C terminal CYS-RICH region of mumps virus structural V protein correlates with block of interferon alpha and gamma signal transduction pathway through decrease of STAT 1-alpha. *Biochem Biophys Res Commun, 283*(1), 255-259. doi:10.1006/bbrc.2001.4764

Lamb, R. A., & Parks, G. D. (2007). Paramyxoviridae: the viruses and their replication. In B. N. Fields, D. N. Knipe, & P. M. Howley (Eds.), *Fields virology: Fifth Edition* (5 ed., pp. 1449-1496). Lippincott, Williams, and Wilkins.

Levy, D. E., Marie, I., Smith, E., & Prakash, A. (2002). Enhancement and diversification of IFN induction by IRF-7-mediated positive feedback. *J Interferon Cytokine Res, 22*(1), 87-93. doi:10.1089/107999002753452692

Lin, G. Y., & Lamb, R. A. (2000). The paramyxovirus simian virus 5 V protein slows progression of the cell cycle. *J Virol, 74*(19), 9152-9166. doi:10.1128/jvi.74.19.9152-9166.2000

Lin, Y., Horvath, F., Aligo, J. A., Wilson, R., & He, B. (2005). The role of simian virus 5 V protein on viral RNA synthesis. *Virology, 338*(2), 270-280. doi:10.1016/j.virol.2005.05.014

Liu, T. C., Castelo-Branco, P., Rabkin, S. D., & Martuza, R. L. (2008). Trichostatin A and oncolytic HSV combination therapy shows enhanced antitumoral and antiangiogenic effects. *Mol Ther, 16*(6), 1041-1047. doi:10.1038/mt.2008.58

Lorence, R. M., Reichard, K. W., Katubig, B. B., Reyes, H. M., Phuangsab, A., Mitchell, B. R., . . . Peeples, M. E. (1994). Complete regression of human neuroblastoma xenografts in

athymic mice after local Newcastle disease virus therapy. *J Natl Cancer Inst, 86*(16), 1228-1233.

Lu, X., Nannenga, B., & Donehower, L. A. (2005). PPM1D dephosphorylates Chk1 and p53 and abrogates cell cycle checkpoints. *Genes Dev, 19*(10), 1162-1174. doi:10.1101/gad.1291305

Lu, X., Nguyen, T. A., Moon, S. H., Darlington, Y., Sommer, M., & Donehower, L. A. (2008). The type 2C phosphatase Wip1: an oncogenic regulator of tumor suppressor and DNA damage response pathways. *Cancer Metastasis Rev, 27*(2), 123-135. doi:10.1007/s10555-008-9127-x

MacTavish, H., Diallo, J. S., Huang, B., Stanford, M., Le Boeuf, F., De Silva, N., . . . Bell, J. C. (2010). Enhancement of vaccinia virus based oncolysis with histone deacetylase inhibitors. *PLoS One, 5*(12), e14462. doi:10.1371/journal.pone.0014462

Madigan, A. A., Sobek, K. M., Cummings, J. L., Green, W. R., Bacich, D. J., & O'Keefe, D. S. (2012). Activation of innate anti-viral immune response genes in symptomatic benign prostatic hyperplasia. *Genes Immun, 13*(7), 566-572. doi:10.1038/gene.2012.40

Manuse, M. J., & Parks, G. D. (2009). Role for the paramyxovirus genomic promoter in limiting host cell antiviral responses and cell killing. *J Virol, 83*(18), 9057-9067. doi:10.1128/jvi.01055-09

Marchini, A., Scott, E. M., & Rommelaere, J. (2016). Overcoming Barriers in Oncolytic Virotherapy with HDAC Inhibitors and Immune Checkpoint Blockade. *Viruses, 8*(1), 9. doi:10.3390/v8010009

Masuda, H., Ozols, R. F., Lai, G. M., Fojo, A., Rothenberg, M., & Hamilton, T. C. (1988). Increased DNA repair as a mechanism of acquired resistance to cis-diamminedichloroplatinum (II) in human ovarian cancer cell lines. *Cancer Res, 48*(20), 5713-5716.

Matveeva, O. V., Guo, Z. S., Senin, V. M., Senina, A. V., Shabalina, S. A., & Chumakov, P. M. (2015). Oncolysis by paramyxoviruses: preclinical and clinical studies. *Mol Ther Oncolytics, 2*. doi:10.1038/mto.2015.17

Mazar, J., Li, Y., Rosado, A., Phelan, P., Kedarinath, K., Parks, G. D., . . . Westmoreland, T. J. (2018). Zika virus as an oncolytic treatment of human neuroblastoma cells requires CD24. *PLoS One, 13*(7), e0200358. doi:10.1371/journal.pone.0200358

Minamiya, Y., Ono, T., Saito, H., Takahashi, N., Ito, M., Mitsui, M., . . . Ogawa, J. (2011). Expression of histone deacetylase 1 correlates with a poor prognosis in patients with adenocarcinoma of the lung. *Lung Cancer, 74*(2), 300-304. doi:10.1016/j.lungcan.2011.02.019

Myers, R., Greiner, S., Harvey, M., Soeffker, D., Frenzke, M., Abraham, K., . . . Peng, K. W. (2005). Oncolytic activities of approved mumps and measles vaccines for therapy of ovarian cancer. *Cancer Gene Ther, 12*(7), 593-599. doi:10.1038/sj.cgt.7700823

Nakashima, H., Nguyen, T., & Chiocca, E. A. (2015). Combining HDAC inhibitors with oncolytic virotherapy for cancer therapy. *Oncolytic Virother, 4*, 183-191. doi:10.2147/ov.S66081

Nakata, S., Yoshida, T., Horinaka, M., Shiraishi, T., Wakada, M., & Sakai, T. (2004). Histone deacetylase inhibitors upregulate death receptor 5/TRAIL-R2 and sensitize apoptosis induced by TRAIL/APO2-L in human malignant tumor cells. *Oncogene, 23*, 6261. doi:10.1038/sj.onc.1207830

Nebbioso, A., Clarke, N., Voltz, E., Germain, E., Ambrosino, C., Bontempo, P., . . . Altucci, L. (2005). Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells. *Nat Med, 11*(1), 77-84. doi:10.1038/nm1161

Nguyen, T. L.-A., Wilson, M. G., & Hiscott, J. (2010). Oncolytic viruses and histone deacetylase inhibitors—A multi-pronged strategy to target tumor cells. *Cytokine & Growth Factor Reviews, 21*(2), 153-159. doi:https://doi.org/10.1016/j.cytogfr.2010.03.002

Nusinzon, I., & Horvath, C. M. (2003). Interferon-stimulated transcription and innate antiviral immunity require deacetylase activity and histone deacetylase 1. *Proc Natl Acad Sci U S A, 100*(25), 14742-14747. doi:10.1073/pnas.2433987100

Ogino, T., Kobayashi, M., Iwama, M., & Mizumoto, K. (2005). Sendai virus RNA-dependent RNA polymerase L protein catalyzes cap methylation of virus-specific mRNA. *J Biol Chem, 280*(6), 4429-4435. doi:10.1074/jbc.M411167200

Oliva-Trastoy, M., Berthonaud, V., Chevalier, A., Ducrot, C., Marsolier-Kergoat, M. C., Mann, C., & Leteurtre, F. (2007). The Wip1 phosphatase (PPM1D) antagonizes activation of the Chk2 tumour suppressor kinase. *Oncogene, 26*(10), 1449-1458. doi:10.1038/sj.onc.1209927

Oslowski, C. M., & Urano, F. (2011). Measuring ER stress and the unfolded protein response using mammalian tissue culture system. *Methods Enzymol, 490*, 71-92. doi:10.1016/b978-0-12-385114-7.00004-0

Otsuki, A., Patel, A., Kasai, K., Suzuki, M., Kurozumi, K., Antonio Chiocca, E., & Saeki, Y. (2008). Histone Deacetylase Inhibitors Augment Antitumor Efficacy of Herpes-based Oncolytic Viruses. *Mol Ther, 16*(9), 1546-1555. doi:10.1038/mt.2008.155

Parisien, J. P., Lau, J. F., Rodriguez, J. J., Sullivan, B. M., Moscona, A., Parks, G. D., . . . Horvath, C. M. (2001). The V protein of human parainfluenza virus 2 antagonizes type I interferon responses by destabilizing signal transducer and activator of transcription 2. *Virology, 283*(2), 230-239. doi:10.1006/viro.2001.0856

Parker, R. J., Eastman, A., Bostick-Bruton, F., & Reed, E. (1991). Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. *J Clin Invest, 87*(3), 772-777. doi:10.1172/jci115080

Paterson, R. G., Harris, T. J., & Lamb, R. A. (1984). Analysis and gene assignment of mRNAs of a paramyxovirus, simian virus 5. *Virology, 138*(2), 310-323. doi:10.1016/0042-6822(84)90354-4

PDQ® Adult Treatment Editorial Board. PDQ Non-Small Cell Lung Cancer Treatment. Bethesda, MD: National Cancer Institute. Updated <06/13/2019>. Available at: https://www.cancer.gov/types/lung/hp/non-small-cell-lung-treatment-pdq. Accessed <09/05/2019>. [PMID: 26389304]

Peng, K. W., Donovan, K. A., Schneider, U., Cattaneo, R., Lust, J. A., & Russell, S. J. (2003). Oncolytic measles viruses displaying a single-chain antibody against CD38, a myeloma cell marker. *Blood, 101*(7), 2557-2562. doi:10.1182/blood-2002-07-2195

Pichlmair, A., Schulz, O., Tan, C. P., Naslund, T. I., Liljestrom, P., Weber, F., & Reis e Sousa, C. (2006). RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. *Science, 314*(5801), 997-1001. doi:10.1126/science.1132998

Poole, E., He, B., Lamb, R. A., Randall, R. E., & Goodbourn, S. (2002). The V proteins of simian virus 5 and other paramyxoviruses inhibit induction of interferon-beta. *Virology, 303*(1), 33-46. doi:10.1006/viro.2002.1737

Rangaswamy, U. S., Wang, W., Cheng, X., McTamney, P., Carroll, D., & Jin, H. (2017). Newcastle Disease Virus Establishes Persistent Infection in Tumor Cells In Vitro: Contribution of the Cleavage Site of Fusion Protein and Second Sialic Acid Binding Site of Hemagglutinin-Neuraminidase. *J Virol, 91*(16). doi:10.1128/jvi.00770-17

Rima, R. K., & Martin, S. J. (1976). Persistent infection of tissue culture cells by RNA viruses. *Med Microbiol Immunol, 162*(2), 89-119.

Robertson, H. D., & Mathews, M. B. (1996). The regulation of the protein kinase PKR by RNA. *Biochimie, 78*(11-12), 909-914. doi:10.1016/s0300-9084(97)86712-0

Robey, R. W., Chakraborty, A. R., Basseville, A., Luchenko, V., Bahr, J., Zhan, Z., & Bates, S. E. (2011). Histone deacetylase inhibitors: emerging mechanisms of resistance. *Mol Pharm, 8*(6), 2021-2031. doi:10.1021/mp200329f

Ropero, S., & Esteller, M. (2007). The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol, 1*(1), 19-25. doi:10.1016/j.molonc.2007.01.001

Rosato, R. R., Almenara, J. A., Kolla, S. S., Maggio, S. C., Coe, S., Gimenez, M. S., . . . Grant, S. (2007). Mechanism and functional role of XIAP and Mcl-1 down-regulation in

flavopiridol/vorinostat antileukemic interactions. *Mol Cancer Ther, 6*(2), 692-702. doi:10.1158/1535-7163.Mct-06-0562

Rosell, R., Taron, M., Barnadas, A., Scagliotti, G., Sarries, C., & Roig, B. (2003). Nucleotide excision repair pathways involved in Cisplatin resistance in non-small-cell lung cancer. *Cancer Control, 10*(4), 297-305. doi:10.1177/107327480301000404

Russell, S. J., Peng, K. W., & Bell, J. C. (2012). Oncolytic virotherapy. *Nat Biotechnol, 30*(7), 658-670. doi:10.1038/nbt.2287

Sanda, T., Okamoto, T., Uchida, Y., Nakagawa, H., Iida, S., Kayukawa, S., . . . Ueda, R. (2007). Proteome analyses of the growth inhibitory effects of NCH-51, a novel histone deacetylase inhibitor, on lymphoid malignant cells. *Leukemia, 21*(11), 2344-2353. doi:10.1038/sj.leu.2404902

Sasaki, Y., Negishi, H., Idogawa, M., Suzuki, H., Mita, H., Toyota, M., . . . Tokino, T. (2008). Histone deacetylase inhibitor FK228 enhances adenovirus-mediated p53 family gene therapy in cancer models. *Mol Cancer Ther, 7*(4), 779-787. doi:10.1158/1535-7163.Mct-07-0395

Schindler, C. (1999). Cytokines and JAK-STAT signaling. *Exp Cell Res, 253*(1), 7-14. doi:10.1006/excr.1999.4670

Schneider, W. M., Chevillotte, M. D., & Rice, C. M. (2014). Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol, 32*, 513-545. doi:10.1146/annurev-immunol-032713-120231

Sen, G. C. (2000). Novel functions of interferon-induced proteins. *Semin Cancer Biol, 10*(2), 93-101. doi:10.1006/scbi.2000.0312

Shreeram, S., Demidov, O. N., Hee, W. K., Yamaguchi, H., Onishi, N., Kek, C., . . . Bulavin, D. V. (2006). Wip1 phosphatase modulates ATM-dependent signaling pathways. *Mol Cell, 23*(5), 757-764. doi:10.1016/j.molcel.2006.07.010

Shulak, L., Beljanski, V., Chiang, C., Dutta, S. M., Van Grevenynghe, J., Belgnaoui, S. M., . . . Hiscott, J. (2014). Histone deacetylase inhibitors potentiate vesicular stomatitis virus oncolysis in prostate cancer cells by modulating NF-κB-dependent autophagy. *Journal of virology, 88*(5), 2927-2940. doi:10.1128/JVI.03406-13

Siddik, Z. H. (2003). Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene, 22*(47), 7265-7279. doi:10.1038/sj.onc.1206933

Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., & Schreiber, R. D. (1998). How cells respond to interferons. *Annu Rev Biochem, 67*, 227-264. doi:10.1146/annurev.biochem.67.1.227

Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., . . . Hanaoka, F. (2005). UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell, 121*(3), 387-400. doi:10.1016/j.cell.2005.02.035

Sulli, G., Di Micco, R., & d'Adda di Fagagna, F. (2012). Crosstalk between chromatin state and DNA damage response in cellular senescence and cancer. *Nat Rev Cancer, 12*(10), 709-720. doi:10.1038/nrc3344

Sun, M., Rothermel, T. A., Shuman, L., Aligo, J. A., Xu, S., Lin, Y., . . . He, B. (2004). Conserved cysteine-rich domain of paramyxovirus simian virus 5 V protein plays an important role in blocking apoptosis. *J Virol, 78*(10), 5068-5078.

Suraweera, A., O'Byrne, K. J., & Richard, D. J. (2018). Combination Therapy With Histone Deacetylase Inhibitors (HDACi) for the Treatment of Cancer: Achieving the Full Therapeutic Potential of HDACi. *Front Oncol, 8*, 92. doi:10.3389/fonc.2018.00092

Takeuchi, K., Komatsu, T., Yokoo, J., Kato, A., Shioda, T., Nagai, Y., & Gotoh, B. (2001). Sendai virus C protein physically associates with Stat1. *Genes Cells, 6*(6), 545-557.

Tamm, I., Wang, Y., Sausville, E., Scudiero, D. A., Vigna, N., Oltersdorf, T., & Reed, J. C. (1998). IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res, 58*(23), 5315-5320.

Taniguchi, T., Ogasawara, K., Takaoka, A., & Tanaka, N. (2001). IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol, 19*, 623-655. doi:10.1146/annurev.immunol.19.1.623

tenOever, B. R., Servant, M. J., Grandvaux, N., Lin, R., & Hiscott, J. (2002). Recognition of the measles virus nucleocapsid as a mechanism of IRF-3 activation. *J Virol, 76*(8), 3659-3669. doi:10.1128/jvi.76.8.3659-3669.2002

Terui, T., Murakami, K., Takimoto, R., Takahashi, M., Takada, K., Murakami, T., . . . Niitsu, Y. (2003). Induction of PIG3 and NOXA through acetylation of p53 at 320 and 373 lysine residues as a mechanism for apoptotic cell death by histone deacetylase inhibitors. *Cancer Res, 63*(24), 8948-8954.

Thomas, S. M., Lamb, R. A., & Paterson, R. G. (1988). Two mRNAs that differ by two nontemplated nucleotides encode the amino coterminal proteins P and V of the paramyxovirus SV5. *Cell, 54*(6), 891-902. doi:10.1016/s0092-8674(88)91285-8

Ulane, C. M., Kentsis, A., Cruz, C. D., Parisien, J. P., Schneider, K. L., & Horvath, C. M. (2005). Composition and assembly of STAT-targeting ubiquitin ligase complexes: paramyxovirus V protein carboxyl terminus is an oligomerization domain. *J Virol, 79*(16), 10180-10189. doi:10.1128/jvi.79.16.10180-10189.2005

Vidal, S., & Kolakofsky, D. (1989). Modified model for the switch from Sendai virus transcription to replication. *J Virol, 63*(5), 1951-1958.

Walker, D. L., & Hinze, H. C. (1962). A carrier state of mumps virus in human conjunctiva cells. I. General characteristics. *J Exp Med, 116*, 739-750.

Wang, H., Zhai, L., Xu, J., Joo, H. Y., Jackson, S., Erdjument-Bromage, H., . . . Zhang, Y. (2006). Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. *Mol Cell, 22*(3), 383-394. doi:10.1016/j.molcel.2006.03.035

Wansley, E. K., Dillon, P. J., Gainey, M. D., Tam, J., Cramer, S. D., & Parks, G. D. (2005). Growth sensitivity of a recombinant simian virus 5 P/V mutant to type I interferon differs between tumor cell lines and normal primary cells. *Virology, 335*(1), 131-144. doi:10.1016/j.virol.2005.02.004

Wansley, E. K., Grayson, J. M., & Parks, G. D. (2003). Apoptosis induction and interferon signaling but not IFN-beta promoter induction by an SV5 P/V mutant are rescued by coinfection with wild-type SV5. *Virology, 316*(1), 41-54.

Wansley, E. K., & Parks, G. D. (2002). Naturally occurring substitutions in the P/V gene convert the noncytopathic paramyxovirus simian virus 5 into a virus that induces alpha/beta interferon synthesis and cell death. *J Virol, 76*(20), 10109-10121.

Welsh, C., Day, R., McGurk, C., Masters, J. R., Wood, R. D., & Koberle, B. (2004). Reduced levels of XPA, ERCC1 and XPF DNA repair proteins in testis tumor cell lines. *Int J Cancer, 110*(3), 352-361. doi:10.1002/ijc.20134

Wilson, R. L., Fuentes, S. M., Wang, P., Taddeo, E. C., Klatt, A., Henderson, A. J., & He, B. (2006). Function of small hydrophobic proteins of paramyxovirus. *J Virol, 80*(4), 1700-1709. doi:10.1128/jvi.80.4.1700-1709.2006

Wong, L. H., Krauer, K. G., Hatzinisiriou, I., Estcourt, M. J., Hersey, P., Tam, N. D., . . . Ralph, S. J. (1997). Interferon-resistant human melanoma cells are deficient in ISGF3 components, STAT1, STAT2, and p48-ISGF3gamma. *J Biol Chem, 272*(45), 28779-28785.

Xargay-Torrent, S., Lopez-Guerra, M., Saborit-Villarroya, I., Rosich, L., Campo, E., Roue, G., & Colomer, D. (2011). Vorinostat-induced apoptosis in mantle cell lymphoma is mediated by acetylation of proapoptotic BH3-only gene promoters. *Clin Cancer Res, 17*(12), 3956-3968. doi:10.1158/1078-0432.Ccr-10-3412

Xu, B., Grander, D., Sangfelt, O., & Einhorn, S. (1994). Primary leukemia cells resistant to alpha-interferon in vitro are defective in the activation of the DNA-binding factor interferon-stimulated gene factor 3. *Blood, 84*(6), 1942-1949.

Yao, C. J., Chow, J. M., Lin, P. C., Hu, T. S., Kuo, H. C., Huang, J. S., . . . Lai, G. M. (2016). Activation of p53/miR-34a Tumor Suppressor Axis by Chinese Herbal Formula JP-1 in

A549 Lung Adenocarcinoma Cells. *Evid Based Complement Alternat Med, 2016*, 5989681. doi:10.1155/2016/5989681

Yoneyama, M., Kikuchi, M., Matsumoto, K., Imaizumi, T., Miyagishi, M., Taira, K., . . . Fujita, T. (2005). Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J Immunol, 175*(5), 2851-2858. doi:10.4049/jimmunol.175.5.2851

Young, D. F., Didcock, L., Goodbourn, S., & Randall, R. E. (2000). Paramyxoviridae use distinct virus-specific mechanisms to circumvent the interferon response. *Virology, 269*(2), 383-390. doi:10.1006/viro.2000.0240

Young, V. A., Dillon, P. J., & Parks, G. D. (2006). Variants of the paramyxovirus Simian virus 5 with accelerated or delayed viral gene expression activate proinflammatory cytokine synthesis. *Virology, 350*(1), 90-102. doi:10.1016/j.virol.2006.01.006

Young, V. A., & Parks, G. D. (2003). Simian virus 5 is a poor inducer of chemokine secretion from human lung epithelial cells: identification of viral mutants that activate interleukin-8 secretion by distinct mechanisms. *J Virol, 77*(12), 7124-7130.

Yu, N., Puckett, S., Antinozzi, P. A., Cramer, S. D., & Lyles, D. S. (2015). Changes in Susceptibility to Oncolytic Vesicular Stomatitis Virus during Progression of Prostate Cancer. *J Virol, 89*(10), 5250-5263. doi:10.1128/jvi.00257-15

Yue, Z., & Shatkin, A. J. (1997). Double-stranded RNA-dependent protein kinase (PKR) is regulated by reovirus structural proteins. *Virology, 234*(2), 364-371. doi:10.1006/viro.1997.8664

Zhao, X., Ai, M., Guo, Y., Zhou, X., Wang, L., Li, X., & Yao, C. (2012). Poly I:C-induced tumor cell apoptosis mediated by pattern-recognition receptors. *Cancer Biother Radiopharm, 27*(9), 530-534. doi:10.1089/cbr.2012.1226