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THE ROLE OF TYPE-I INTERFERON IN LIMITING SPREAD AND KILLING OF AN ONCOLYTIC RNA VIRUS IN PROSTATE CELLS

by

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B.Tech Vellore Institute of Technology, 2014

A thesis submitted in partial fulfilment of the requirements
for the degree of Master of Science
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Major Professor: Griffith D. Parks

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ABSTRACT

Prostate cancer is the second most prevalent cancer amongst men and there is an urgent need to address viable therapeutic options for its treatment. Development of viruses which target and kill cancer cells has gained momentum due to the first FDA approved oncolytic virus for treating human cancer patients. Our previous work with the RNA virus, Parainfluenza Virus 5 (PIV5), has led to the generation of mutants that are potential candidates for oncolytic viruses: 1) the hyperfusogenic (P/V/F) mutant has a mutated P/V and fusion gene which activates anti-viral responses and causes massive cell-cell fusion respectively, and 2) the Leader mutant has a mutated viral genomic promoter which kills cells due to overactive viral gene expression. The P/V/F mutant has shown effectiveness in reducing prostate tumor burden in a mouse model system, however, the specificity of these viruses is unclear, i.e. targeting cancerous prostate cells while leaving uninvolved cells unaffected. In this study, we addressed how these PIV5 mutants replicate in and killed tumor versus benign human prostate cells. Flow cytometry demonstrated that the mutants are able to infect and replicate in prostate tumor cells (22Rv1), resulting in effective cell killing. However, these mutants showed highly restricted spread in benign prostatic hyperplasia cells (BPH-1). Upon further exploration, it was determined that the restriction observed in the BPH-1 cells is due to the induction and signaling of type-I Interferon (IFN). This was confirmed upon treatment with an IFN-β neutralizing antibody, which relieved restricted spread of mutants in benign cells. BPH-1 cells infected with the mutants also showed upregulation of key anti-viral, IFN-induced genes such as TLR3, IFIT1, and OAS2. Upon characterization of the mutant viruses

in an additional metastatic prostate cancer cell line (C4-2B), a restriction in viral spread was observed. The restricted spread did not correlate with production of high levels of type-I IFN, suggesting that other cytokines or intracellular factors can limit replication in tumor cells. Therefore, these studies lay the groundwork for further improving the specificity of oncolytic PIV5 mutants by exploiting type-I IFN pathways as well as other anti-viral factors.

To my family and friends

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TABLE OF CONTENTS

LIST OF FIGURES ix

LIST OF ACRONYMS x

CHAPTER ONE: INTRODUCTION 1

Prostate Cancer 1

Oncolytic Viruses 2

Parainfluenza Virus 5 5

Viral Life Cycle 7

Activation of Anti-Viral Immune Responses. 10

Type-I IFN Induction and Signaling. 11

Activation of Anti-Viral Genes 12

PIV5 Counteracting Anti-Viral Responses. 13

CHAPTER TWO: MATERIALS AND METHODS 15

Cells and Viruses 15

IFN treatment and IFN antibody neutralization. 16

Flow Cytometric Analysis 16

Death Assays 16

Type-I IFN Detection Assay. 17

Reverse Transcription and Real Time PCR. 17

Fluorescence Microscopy. 18

Figures and Statistics. 18

CHAPTER THREE: RESULTS 19

Defining spread and growth properties of PIV5 WT and mutant viruses in benign and prostate cancer cell lines. 19

Cytopathic effect of PIV5 WT and mutant viruses in benign and prostate cancer cells at high MOI. 22

Type-I IFN production and signaling in benign and prostate cancer cells 25

IFN-β is a key contributing factor to restriction of PIV5 mutant viruses in benign cells. 28

Expression of key anti-viral genes upon PIV5 WT and mutant virus infection in benign cells. 30

Characterization of PIV5 mutants in additional prostate cancer cell line 32

CHAPTER FOUR: CONCLUSION 34

LIST OF REFERENCES 39

LIST OF FIGURES

Figure 1. Genomic representation of the P/V/F Mutant. 9

Figure 2. Genomic representation of the Le mutant 10

Figure 3. Low MOI spread and growth of PIV5 mutant viruses in benign and prostate cancer cells.. 21

Figure 4. Cytopathic effect of PIV5 mutant viruses in benign and prostate cancer cells 24

Figure 5. Type-I IFN production and response in benign and prostate cancer cells upon PIV5 mutant virus infection.. 27

Figure 6. Neutralization of IFN-β. 29

Figure 7. Relative expression levels of anti-viral genes in PIV5 mutant infected benign cells ... 31

Figure 8. Characterization of PIV5 mutants in metastatic prostate cancer cell line 33

LIST OF ACRONYMS

AIPC – Androgen Independent Prostate Cancer

AR – Androgen Receptor

BSA – Bovine Serum Albumin

CPE – Cytopathic Effect

DMEM - Dulbecco Modified Eagle Medium

dpi – days post infection

dsRNA – double stranded RNA

FACS – Fluorescence Activated Cell Sorting

FBS – Fetal Bovine Serum

FDA – United States Food and Drug Administration

Fig. - Figure

GFP – Green Fluorescence Protein

GM-CSF – Granulocyte-Macrophage Colony-stimulating Factor

HCl – Hydrochloric Acid

hpi – hours post infection

HSV – Herpes Simplex Virus

IFIT1 – Interferon-induced protein with tetratricopeptide repeats 1

IFN – Interferon

IFN-β – Interferon - beta

IRF-9 - Interferon Regulatory Factor-9

ISGF3 - Interferon Stimulatory Gene Factor 3

ISGs – Interferon Stimulated Genes

ISRE - Interferon Stimulatory Response Elements

Le – Leader

mda-5 - Melanoma differentiation-associated protein 5

MTS - (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)

NaOH – Sodium Hydroxide

NDV – Newcastle Disease Virus

OAS – 2'-5'-Oligoadenylate Synthetase

PAMPs – Pathogen-associated Molecular Patterns

PBS – Phosphate-buffered Saline

PFU – Plaque Forming Units

PI – Propidium Iodide

PIV5 – Parainfluenza Virus 5

PRRs - Pattern Recognition Receptors

RIG-I - Retinoic Acid-inducible Gene 1

SEAP - Secreted Alkaline Phosphatase

SV5 – Simian Virus 5

TIR – Toll-Interleukin-1 Receptor

TLR – Toll-like Receptor

TNF-α – Tumor Necrosis Factor - alpha

WT – Wild Type

CHAPTER ONE: INTRODUCTION

Since the discovery of viruses, virologists have always been interested in the various interactions that take place between the host and the virus. Viruses, by nature, require the presence of host cells to grow and replicate. They can either be detrimental to the host they infect or remain undetected in them for years. However, scientists have recently begun exploring and determining ways by which viruses can be exploited as effective therapy for various diseases.

My thesis focuses on two mutant PIV5 viruses that have the potential to specifically target and kill tumor cells. This work describes the characterization of these two mutants in benign and prostate tumor cell lines. It also gives us insight about the specificity of the mutants in a biological system (healthy/normal vs tumor).

Prostate Cancer.

Prostate cancer is the second most prevalent cancer amongst men. According to the American Cancer Society (last reviewed in 2016), 1 in 7 men will be diagnosed during his lifetime. Prostate cancer can be treated successfully in benign stages, however, advanced stage prostate cancer can develop tumors that become resistant to treatment. Therapy is limited for these aggressive tumors as it is possible to merely control the cancer but not cure it (Hutchinson, L., 2014). One common treatment for advanced staged prostate cancer is androgen ablation therapy, which causes regression of the tumor. Since prostate tumors require high levels of androgen to grow and proliferate, withdrawal or deprivation of androgen would cause these tumors to die out. In most

cases, this regression is temporary and the cancer progresses into AIPC. Few common mechanisms that lead to AIPC are mutations in the AR gene, sensitization of the AR receptor to low levels of androgen, triggering of ARs by cytokines and growth hormones, etc. (Brian J. Feldman and David Feldman, 2001). These mechanisms ensure that the tumor would be able to grow, proliferate, and metastasize without the required levels of androgen, thereby resulting in ultimate death of the patient. Therefore, there is an urgent requirement to address the need for viable therapeutic options for this lethal form of prostate cancer.

Oncolytic Viruses.

Oncolytic viruses are viruses that lack pathogenicity and are therapeutic in nature. They aid in overcoming one of the main hurdles of cancer therapy, i.e. targeting tumor cells while leaving the healthy/normal cells unaffected. Oncolytic viruses can either be naturally occurring or genetically modified (Chiocca A.E and Rabkin D. S, 2014). Naturally occurring oncolytic viruses are inherently not pathogenic to the host and preferentially replicate in tumor cells. Some common examples include NDV, reovirus, and myxoma virus. Pathogenic viruses can also be genetically engineered or modified to replicate in tumor cells; for example HSV, measles virus, polio virus, vaccinia virus etc. The FDA recently approved IMLYGIC™ (Talimogene Laherparepvec), a genetically modified HSV type 1 that can be used to treat melanoma tumors. The virus selectively replicates in tumors and also encodes GM-CSF which, when expressed, leads to activation and recruitment of dendritic cells to the tumor site (Andtbacka et al., 2015; Liu, B. L, et al., 2003). These dendritic cells then present tumor antigens to cytotoxic T cells to help target and kill the

tumor. There are many advantages to oncolytic viral therapy. Oncolytic viruses can be modified to replicate in cells that have an abnormal cellular environment or disrupted signaling pathways (Chiocca, A E. 2002). They are capable of replicating to high titers within the tumor. They can also target the entire cell, as opposed to a drug which can only target specific signaling pathways within the cell (Bell, C. J. et al., 2003). Additionally, they can also generate immune responses that will aid in clearing the tumor from the host (Chiocca, A. E and Rabkin D.S. 2014, Bateman, A. R et al., 2002, Melcher, A at al., 1999, Errington, F et al., 2006).

While this is true in theory, there are challenges associated with controlling an oncolytic virus in a natural biological system. Since these viruses are infectious in nature, it is possible that the oncolytic virus could spread to a healthy person. It could result in an overactive immune response; for example, some strains of influenza can cause cytokine storms which are potentially fatal in nature (Liu et al., 2015). Oncolytic viruses are also capable of establishing a persistent infection in the host (Chia et al., 2014, Boldogh I et al., 1996). In theory, oncolytic viruses may only be effective during their first administration. The host, upon exposure to the virus, can develop neutralizing antibodies against it. This is could affect the efficacy of the oncolytic virus during additional rounds of administration with the same virus. If not engineered suitably, they could have the potential of infecting and causing death in healthy/normal cells. Therefore, it is crucial to add additional layers of specificity to ensure that these viruses only target tumor cells. These layers of specificity will act as safety features to ensure that the virus remains tumor-specific. Examples of these layers of specificity are: 1) Ensuring that the oncolytic virus targets receptors found on tumor cells but not on healthy cells (Uchida et al., 2012, Anderson B D et al., 2004) 2) Triggering of viral fusion proteins by tumor specific proteases (Shobana, R et al., 2013) 3) Developing an oncolytic

virus that can induce IFN so that they can replicate in IFN-defective tumor cells but not in healthy cells, which have functioning IFN machinery (Stojdl, D. F et al., 2003, Dunn, G. P et al., 2005).

Certain viruses from the *Paramyxoviridae* family show great potential as oncolytic viruses. Some of these include mumps virus, NDV and measles virus which have been developed for various cancer therapies. One of the earliest studies with mumps showed that various modes of administration to 90 patients (with various tumor types) showed a decrease in tumor size or complete disappearance of tumors in 37 of the 90 patients. Toxicity studies reported that only 7 of the 90 showed adverse effects to the virus (Asada, T. 1974).

NDV is another popular oncolytic virus due to its natural ability to replicate in tumor cells, while being restricted in non-tumorigenic cells (Fiola, C et al., 2006). A study by Fiola et al. shows that NDV is restricted in non-tumorigenic cells due to the establishment of an anti-viral state in response to the virus infection. However, the tumor cells investigated were defective in anti-viral pathways and were therefore susceptible to NDV infection. Phase I trial studies have also shown that a replication competent oncolytic NDV strain was well tolerated in humans with solid cancers (Pecora A.L et al., 2002).

Measles virus is another paramyxovirus member that shows promise as an oncolytic candidate; one of the reasons being that the non-pathogenic vaccine strain of the measles virus has a very strong safety profile (Myers, R. et al., 2005). A live attenuated strain of this measles vaccine has been shown to have anti-tumor activity in ovarian tumor mouse xenograft models (Peng KW et al., 2002). It also shows selective CPE in tumor cells by massive fusion of neighboring tumor cells, also known as syncytia formation. The oncolytic virus in this study has undergone Phase I clinical

trials and has shown effective tolerance in patients with recurring ovarian cancer (Galanis, E et al., 2010). Although a lot of work has been done with many members of the *Paramyxoviridae* family, recent work has shown that PIV5 has great promise as an agent for cancer therapy

Parainfluenza Virus 5.

PIV5 is a member of the *Rubulavirus* genus of the *Paramyxoviridae* family and has a single stranded, negative sense RNA genome that is ~15 kb in size (Lamb and Parks, 2007). It has been studied as a prototype for negative strand RNA viruses since its discovery in the 1950's by Hull et al. In this study, PIV5 was isolated from monkey kidney tissue and was originally coined as a simian virus, SV5. However, it was later found that natural isolates of the virus could be derived from a wide variety of animal tissue sources: canine, human and porcine (McCandlish, I. A. et al., 1978; Goswami et al., 1984; Tong et al., 2002; Robbins et al., 1981). WT PIV5 has proved to be mostly non-cytopathic in nature in fibroblast and epithelial cell types (Sun M et al., 2004, Lamb, R. A., and G. D. Parks. 2007, Parks G D., 2002).

The PIV5 virion contains an envelope that is composed of a lipid bilayer along with an internalized ribonucleocapsid complex. The internal nucleocapsid complex consists of the RNA genome which is encapsidated by multiple units of a viral protein called N or NP. Two major glycoproteins project from the surface of the virion which help with attachment and fusion to the host cell. The virus also codes for an RNA-dependent RNA polymerase that aids in viral transcription and replication. All these elements of the virion are encoded by eight major genes present in the RNA genome in the order of 3'-NP-P/V-M-F-SH-HN-L-5'. The NP gene codes for the NP protein or the

nucleocapsid protein, which encapsidates the RNA genome tightly to form a stable, helical structure. It also prevents the viral genome from being degraded by host cellular nucleases. The P/V gene encodes for two proteins: P and V. The phosphoprotein (P) is the non-catalytic subunit of the viral polymerase. Studies have shown that it also helps increase the fidelity and reduces error rates of the viral polymerase, so as to reduce dsRNA production by the virus (Dillon, P. J., and G. D. Parks. 2007). PIV5 encodes for an accessory V protein that helps evade the host immune response (Lamb, R. A., and G. D. Parks. 2007). The V protein is also known for slowing down cell growth and delaying the cell cycle (Lin and Lamb, 2000). It delays the progression from at the G1/S boundary of the cell cycle so that the cell can remain in the S phase for an extended period of time. The M protein or the matrix protein is the most abundant protein in a PIV5 virus particle and acts as a scaffold for the lipid bilayer envelope of the virus. The F protein is a glycoprotein that is projected on the surface of the virion and aids in fusion of the virion lipid bilayer of PIV5 with the plasma membrane of the host cell. In addition, an infected cell is able to express the F protein on its cell surface, and as a result, the infected cell is capable of fusing with its neighboring cells to form a multinucleated body (syncytia). The precursor for the F protein is F_o, which is then glycosylated in the ER and subsequently transported to the Gogli apparatus for further processing. Here, a host furin like cellular protease cleaves F_o at a stretch of five arginine residues to form F1 and F2 subunits, which are attached by disulfide bonds. This cleavage into F1 and F2 results in the fusogenicity of the virus. The SH gene codes for a small hydrophobic protein whose function still remains unknown. The HN gene codes for the HN protein, or the hemagglutinin-neuraminidase protein, which is also a glycoprotein that is projected on the surface of the virion. This is an attachment protein that enables the virus to attach to the host cell via the sialic acid receptor. The

neuraminidase activity of this protein helps in the release of the virus from the host cell, by cleaving sialic acid from the surface of the host cell, and also preventing aggregation with other virus particles. The L gene encodes the large (L) protein which is the catalytic subunit of the viral polymerase. The PIV5 also encodes a genomic promoter (Le) in the 3' end of the genome. The 5' end encodes a trailer (tr) sequence which directs replication of anti-genomic RNA to make negative-strand genome progeny.

Viral Life Cycle

The viral genes mentioned above are separated by inter-genomic junctions that have a varying number of nucleotides. These regions are cis-acting and direct capping and polyadenylation of resulting nascent mRNA, produced during transcription. The Le genomic promoter directs transcription of the RNA genome via the viral polymerase. The polymerase employs "stop-start" transcription, wherein it transcribes a viral gene and after encountering the inter-genomic sequence can either re-initiate to transcribe the next gene or fall off the genome to initiate from the first gene (NP). This results in a gradient of viral transcripts, with an abundance of NP mRNA and reducing amounts of subsequent viral transcripts. PIV5 also displays the phenomenon of 'RNA editing' which occurs for the P/V gene. As mentioned before, the P/V gene encodes for both the P and V proteins. During viral transcription of this gene, the polymerase undergoes slippage within the P/V gene and inserts two non-template guanosine (G) residues. This slippage results in a downstream frame shift mutation and as a result the P transcript contains two additional G residues. Consequently, the P and V proteins have similar N terminal residues but differ in their C terminal

domains. During early time points in PIV5 infections, the incoming viral genome undergoes primary transcription. This is a growth phase in which the genome is processed by viral polymerase to synthesize and accumulate viral transcripts and subsequently proteins. Once viral proteins are accumulated (late in viral infection), a switch from primary transcription to replication occurs. Upon reaching a certain threshold level of NP, a signal from the NP protein directs the viral polymerase to completely transcribe the genome in its entirety. This leads to the generation an anti-genome, in the 5'-3'orientation. This switch to viral replication enables the polymerase to produce negative strand RNA genome progeny by utilizing the tr sequence as a promoter and the anti-genome as a template. The resulting copies of the genome can now be used as templates for a secondary transcription growth phase, in which higher levels of viral transcripts and proteins can be produced. This enables successful assembly and budding of viral progeny. The progeny genomes are packaged by appropriate viral proteins (that are produced by host cellular translational machinery), and are assembled at the plasma membrane of the host cell. The virus then buds out of the cell, taking the assembled glycoproteins on the cell surface along with it.

Previous work by the Parks lab with PIV5 has resulted in the development of PIV5 mutant viruses that are oncolytic in nature. Studies have shown that six naturally occurring amino acid substitutions in the P/V gene lead to virus induced apoptosis of tumor cells and induction of type-I - IFN (Wansley, E. K., and G. D. Parks. 2002). Another study showed that the substitutions in the P/V gene, along with a single Gly-to-Ala substitution in the fusion peptide of WT PIV5, have resulted in a mutant virus that is massively hyperfusogenic (Gainey et al., 2008) (Fig 1). This mutant is also capable of inducing a strong anti-viral response (particularly IFN-β production). CPE mediated by virus infection is observed in tumor cells by formation of syncytia, which

subsequently leads to necrosis. Additionally, *in vivo* studies showed that prostate tumor burden was reduced upon treatment with the mutant virus (Gainey et al., 2008). These properties lead to terming the virus as the hyperfusogenic (P/V/F) mutant.

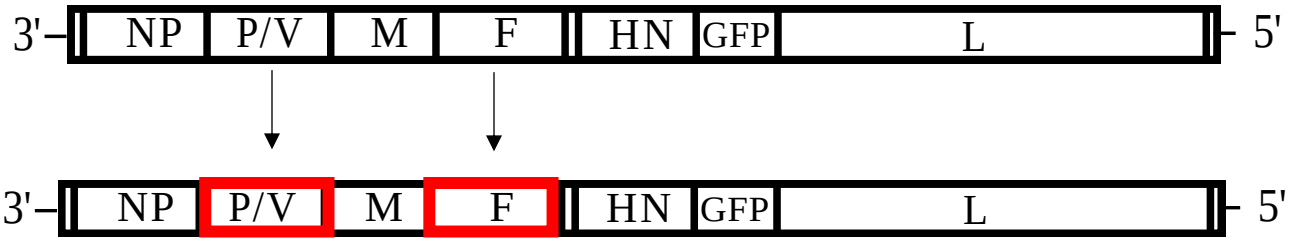

Figure 1. Genomic representation of the P/V/F Mutant.

In another study, two nucleotide changes in the highly conserved PrE-I element (U5C and A14G) resulted in the Leader (Le) mutant (Manuse and Parks, 2009) (Fig 2). This mutant showed increased rates of viral transcription and translation, although the number of genomic RNA remain the same as compared to that of a WT PIV5 infection. It induced increased production of dsRNA, which then activated anti-viral responses to trigger cell death. It was also able to induce high levels of proinflammatory cytokine IL-6 in tumor cells. This mutant shows great potential as an oncolytic virus as it is capable of inducing CPE in tumor cells (albeit slower than the P/V/F mutant), despite having a functional V protein.

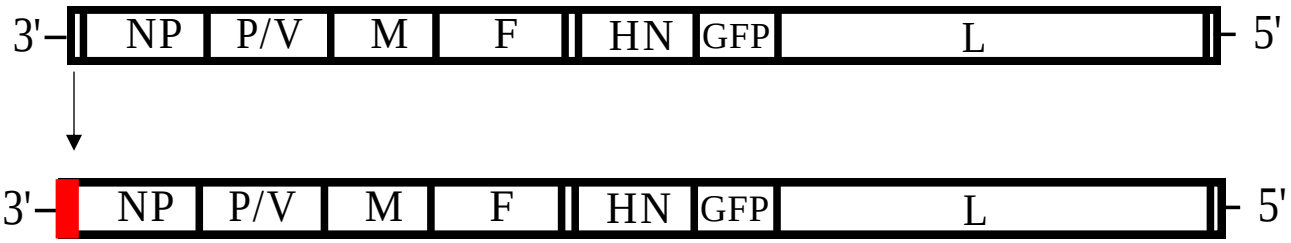

Figure 2. Genomic representation of the Le mutant

In summary, the previous studies on the properties of the P/V/F and Le mutants in tissue culture led to the suggestion that either of these mutants could be used as potential oncolytic viruses for tumor therapy (Manuse and Parks, 2009 and Gainey et al., 2008).

Activation of Anti-Viral Immune Responses.

RNA viruses are able to be detected by host cell RNA sensors called pattern recognition receptors (PRRs) (Parks, G. D and Alexander-Miller, M. A., 2013). These host cell receptors are able to recognize distinct signals called Pathogen Associated Molecular Patterns (PAMPs). One example of this is dsRNA that is produced by replicating RNA viruses. Different viruses are able to induce different immune pathways through the synthesis of these PAMPs (Randall, R. E and Goodbourn, S., 2008). Two common sensors of dsRNA are mda-5 and RIG-I. These sensors are able to identify specific structures of viral RNA; mda-5 can bind to and recognize dsRNA structures, whereas RIG-I can recognize 5'pppRNA molecules that are improperly capped as well as dsRNA

structures. These sensors typically activate the induction and signaling of the type-I IFN pathway, which then puts the cell and its neighbors in an anti-viral state.

Host immune responses can also be activated via the TLR signaling pathway. TLR or Toll-like Receptors are a family of 11 distinct receptors expressed by different cells that commonly possess common structural traits, such as leucine-rich repeats (LRRs) and a cytoplasmic signaling domain called TIR (Toll/Il-1 receptor) (Boehme, K. W and Compton, T., 2004). Most relevant to RNA virus replication is TLR3, which acts as a dsRNA sensor that can induce IFN – β secretion.

Type-I IFN Induction and Signaling.

Type-I IFN is a common anti-viral cytokine which is synthesized in response to virus infections. Upon production and signaling, the IFN response activates a range of different genes that inhibit viral infection and/or replication in a host cell (Parks, G. D and Alexander-Miller, M. A., 2013; Randall, R. E and Goodbourn, S., 2008). According to their amino acid sequence, IFNs can be classified into three categories: Type I, II, and III. Type I IFN has 13 IFN-α genes, 1-3 IFN-β genes and other genes like IFN- δ, -τ, -ω, and -ϵ. Viral infection mainly triggers IFN-α and β. Upon infection of a cell with a virus, sensors like RIG-I and mda-5 are activated upon recognizing viral structures like dsRNA or improperly capped 5'pppRNA molecules. This results in the activation of MAVS, a mitochondria associated factor, which then leads to subsequent activation of TBK-1 and IKK-ε, which is a kinase complex. IRF-3 (a cytoplasmic, latent transcription factor) is then phosphorylated by this kinase complex and homo-dimerizes with itself. This complex then hetero-dimerizes with IRF-7 (another latent transcription factor), and translocates into the nucleus to

activate IFN-α and β promoters, to ultimately produce type-I IFN. TLR3 also mediates the activation of various IFN genes. TLR3 is triggered upon dsRNA interaction which results in sending signals via TRIF (TIR domain-containing adaptor inducing IFN-β). This in turn activates transcription factors such as IRF-3, AP-1, and NF-κB, which then leads to production of type-I IFN and other cytokines (Matsumoto, M and Seya, T., 2008).

Irrespective of the pathway of activation, the IFN molecules produced bind to dimeric IFN receptors on the infected cell or on neighboring cells, which then leads to the IFN signaling mechanism. Binding to IFN receptors induces dimerization of STAT1 and STAT2 which are cytoplasmic, latent transcription factors. This dimer complex is then phosphorylated by Jak1 and Tyk2 kinases. This phosphorylated complex then interacts with IRF-9 to form ISGF3 complex. ISGF3 is then translocated into the nucleus to activate promoters with ISREs, which leads to the expression of multiple ISGs.

Activation of Anti-Viral Genes.

ISGs are anti-viral genes which help in blocking viral transcription, viral translation or degrading viral RNA. Some common ISGs include OAS, PKR, and IFIT1. OAS2 specifically identifies dsRNA and activates a latent RNaseL enzyme, which is initially in a monomeric form, into an active dimeric enzyme that is capable of degrading viral mRNA (Sadler, A. J and Williams, B. R., 2008). PKR is a protein kinase that is induced by type-I and -III IFN. Upon interacting with viral mRNA, PKR (in its inactive monomer form) phosphorylates and dimerizes into its active form. Activated PKR is then able to phosphorylate EIF2α, an important initiation factor for host cellular

translation, which subsequently inhibits viral and host translation. IFIT1 is induced by type-I IFN and is a member of a large family of proteins that have one common structural feature: multiple tetratricopeptide repeats that aid in several protein interactions. IFIT1 has been known to inhibit translation by binding to eIF3, a translation initiation factor. It also recognizes improperly capped viral tri-P RNA and prevents their subsequent translation (Andrejeva, J et al., 2012).

PIV5 Counteracting Anti-Viral Responses.

As mentioned earlier, the V protein is crucial in inhibiting the host immune response. The V protein binds to and blocks mda-5, thereby preventing expression of the IFN-β gene (Didcock, L et al., 1999, Lamb, R. A., and G. D. Parks. 2007). The cysteine-rich zinc binding domain of the V protein also blocks type-I IFN signaling by targeting STAT1 for degradation via ubiquitination. STAT2 is not degraded, but is essential for the V protein mediated degradation of STAT1 (Parks, G. D and Alexander-Miller, M. A., 2013). However, the substitutions in the P/V gene of the P/V/F mutant result in the disruption of this ability of the V protein to circumvent the host immune response. Specifically, the mutant is unable to block mda-5 or target STAT1 for degradation. Additionally, the mutations in the P gene also result in an overactive viral polymerase which leads to high dsRNA production. As a result, the mutant is able to induce a strong IFN response, (Gainey, M. D. et al., 2008; Wansley, E. K., and G. D. Parks. 2002; Dillon, P. J., and G. D. Parks. 2007). Here, we have exploited the type-I IFN response for specifically targeting tumor cells. The rationale behind this is that many tumors have dysregulated type-I IFN machinery, while healthy

cells have functional IFN responses. Therefore, the P/V/F would be able to target, infect and kill tumor cells, but would be restricted in benign/healthy cells.

The PIV5 mutants, P/V/F, and Le, are yet to be characterized in benign, non-tumor forming cell lines, and their anti-viral responses are yet to be explored in these specific cells. Therefore, the main goal of my thesis is to characterize and define the properties of these PIV5 mutant viruses in benign and prostate cancer cell lines. We have found that type-I IFN plays a major role in contributing to the specificity of these mutants in different prostate cell lines. These studies lay the groundwork for further improving the specificity of oncolytic PIV5 mutants by exploiting type-I IFN pathways.

CHAPTER TWO: MATERIALS AND METHODS

Cells and Viruses.

BPH-1, 22Rv1, and C4-2B cells were kindly provided by R.Chakrabarti (University of Central Florida). BPH-1 and A549 cells were grown in DMEM supplemented with 10% heat inactivated FBS. 22Rv1 and C4-2B cells were grown in RPMI-1640 medium supplemented with 10% heat inactivated FBS. HEK-Blue™ IFN-α/β cells (InvivoGen) were maintained in DMEM supplemented with 10% heat inactivated FBS, 30ug/ml Blasticidin and 10ug/ml Zeocin.

PIV5 expressing GFP was recovered from a cDNA plasmid as previously described (He et al., 1997). Generation of the PIV5 hyperfusogenic mutant P/V/F (P/V-CPI⁻-G3A), PIV5 Le mutant [Le-(U5C, A14G)] and P/V mutant (P/V-CPI-) have been described previously (Gainey et al., 2008; Manuse and Parks, 2009; Wansley and Parks, 2002). Stocks of WT PIV5 expressing GFP were grown in MDBK cells. Stocks of the P/V/F and Le mutants were grown in Vero cells, which are defective in IFN production. Infections were carried out by incubating virus and cells in DMEM supplemented with 10% BSA. After one hour of incubation, virus was removed, cells were washed with PBS, and media was replaced with DMEM supplemented with 2% heat inactivated FBS. Viral titers were determined by plaque assays.

IFN treatment and IFN antibody neutralization.

Type–I IFN (IFN-α A/D; Sigma Aldrich catalog # I4401) was used to treat cells. In the IFN neutralization experiments performed, a neutralizing antibody against IFN-β (Millipore, catalog # AB1431) was used at a dilution of 1:20 while TNF-α antibody (Biosource ,catalog #AHC3812) was used at a final concentration of 10ug/ml. Neutralizing antibodies were added at the indicated concentrations directly into the replacement media.

Flow Cytometric Analysis.

Mock or virus infected 22Rv1 and C4-2B cells were trypsinized, while BPH-1 cells were detached using Accutase™ (Millipore). Cells were then quenched in DMEM supplemented in 10% FBS and washed with PBS. Flow cytometry was performed using the FACS Canto (BD Biosciences) or the CytoFLEX (Beckman Coulter), and 10,000 independent events were recorded. Results were analyzed using Flowlogic software (for FACS Canto) or CytExpert (for CytoFLEX).

Death Assays.

MTS assays were carried out in 96 well plates using CellTiter 96® Aqueous One Solution reagent (Promega) as described previously (Gainey et al., 2008). Final values are expressed as percentage of mock infected cells, which are analyzed in parallel. PI stain (BD Pharmingen™) was used to

quantify non-viable cells. 1ul of PI dye (stock concentration of 50 ug/ml) was added to cell suspension prior to performing flow cytometry.

Type-I IFN Detection Assay.

Supernatant from infected cells was treated with 1N HCl for half an hour at room temperature and subsequently titrated with 1N NaOH, until a pale pink color is achieved. Resulting neutralized media was used to treat the reporter cell line HEK-Blue™ IFN-α/β overnight, as per manufacturer's instructions. A standard curve of treatment of these cells with known concentrations of type-I IFN was also generated. SEAP produced by the cells in response to IFN stimulation was measured by addition of QUANTI-Blue™ reagent. Absorbance was read at 650nm during 15 minute intervals for an hour. Amounts of type-I IFN in the collected media were determined utilizing the standard curve and expressed as units/ml.

Reverse Transcription and Real Time PCR.

Total RNA from at least 10^5 cells was isolated from mock or virus infected cells using TRIzol (Invitrogen). 1 ug of total RNA was used to produce cDNA by utilizing the TaqMan® Reverse Transcription Reagents (Applied Biosystems) 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′.

OAS2 forward: 5'-AGAAGCTGGGTTGGTTTATC-3', and OAS2 reverse 5'-GACGTCACAGATGGTGTTC-3'. IFIT1 forward and reverse primers were obtained from a study by Madigan, A. et al., 2012. TLR3 forward and reverse primers were obtained from a study performed by González-Reyes, S et al., 2010. Relative gene expression was determined by utilizing RT^2 Profiler PCR array analysis software (SABiosciences) as described previously (Li et al., 2013).

Fluorescence Microscopy.

Before imaging, at indicated times post infection, infection media was replaced with PBS. Fluorescence microscopy was then performed using a Zeiss fluorescence microscope and a 10x lens (Carl Zeiss Microscopy, LLC, United States). Exposure times were adjusted to be constant between samples.

Figures and Statistics.

Averages and standard deviations were generated using Microsoft® Excel®. P values were generated using two-tail, students t-test, and are statistically significant for $p<0.05$. Graphs were generated using GraphPad® Prism 6 software.

CHAPTER THREE: RESULTS

Defining spread and growth properties of PIV5 WT and mutant viruses in benign and prostate cancer cell lines.

WT and mutant viruses were engineered to encode GFP and all cells expressing GFP represent virus infected cells (previously described in He et al., 1997, Gainey et al., 2008 and Manuse et al., 2009). To define the spread of PIV5 WT and mutant viruses, benign prostate cells (BPH-1) and prostate cancer cells (22Rv1) were mock infected or infected at an MOI of 0.05 with PIV5 WT, P/V/F or Le mutants. Bright field and fluorescence images were taken 1, 2, and 3 dpi (days post infection) for the 22Rv1 cells and 1, 2, 3, and 4 dpi for the BPH-1 cells. As shown in Fig 3A, WT and mutant viruses were able to infect and spread through most of the 22Rv1 cell population by day 3 pi. Infected 22Rv1 cells were then quantified using flow cytometry and it was determined that ~70-80% of the cells were infected with WT or mutant viruses day 3 pi (Fig 3C). No significant difference was observed in the spread of the viruses through the 22Rv1 cells. In contrast, WT and mutant viruses were restricted for spread in the BPH-1 cells across the time course, as shown in Fig 3B. Upon quantification with flow cytometry it was found that only ~20% of the cells were infected with WT and Le mutant while only ~10% of the cells were infected with the P/V/F mutant by day 4 pi (Fig 3D).

Multi-step growth properties of WT and mutant viruses were then determined by infecting 22Rv1 and BPH-1 cells with WT, P/V/F, and Le mutants at an MOI of 0.01 to 0.05. Media was harvested at indicated time points to determine infectious viral units by performing plaque assays. As shown in Fig 3E, WT and Leader mutant viruses were able to reach similar titers in 22Rv1 cells, while

the P/V/F mutant achieved a titer ~2 log higher day 4 pi. Strikingly, in the BPH-1 cells (Fig 3F), WT and Le mutant viruses reach similar titers day 4 pi, although there is an initial burst of Le mutant viral growth during the earlier time points. The P/V/F mutant, however, shows a reduction in viral growth across the time course. Taken together, these data indicate the WT and mutant viruses are capable of spreading and growing in the 22Rv1 cells, whilst the viruses are restricted for spread and growth in the BPH-1 cells, at a low MOI.

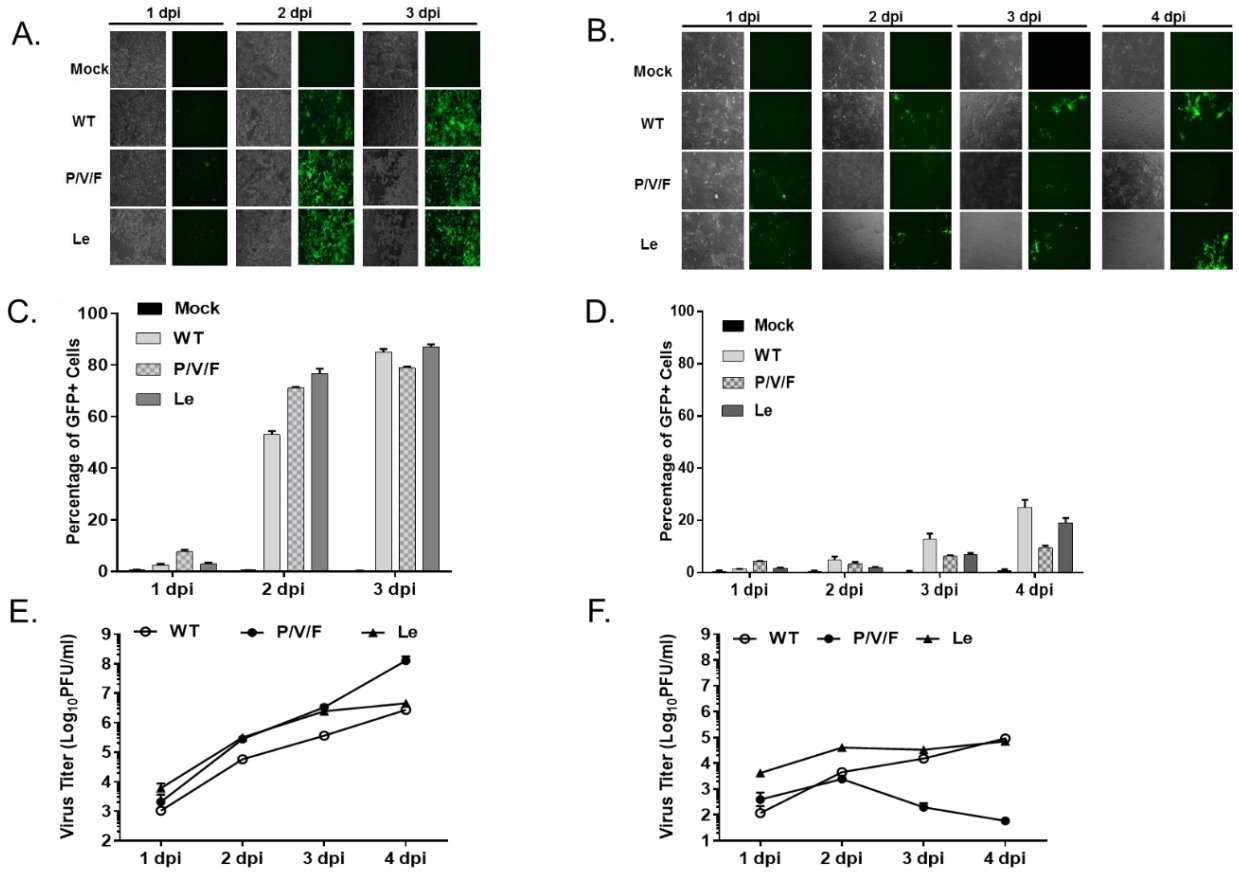

Figure 3. Low MOI spread and growth of PIV5 mutant viruses in benign and prostate cancer cells. 22Rv1 cells (panels A, C, and E) and BPH-1 cells (panels B, D, and F) were mock infected or infected with WT PIV5 or PIV5 mutant viruses at a low MOI (0.01 to 0.05). At the indicated time points, bright field and fluorescence microscopy images were taken (panel A and B) and virus infected cells were quantified using flow cytometry (panel C and D). Media from infected cells was harvested at the indicated time points and titers of infectious virus units were determined using plaque assays (panel E and F).

Cytopathic effect of PIV5 WT and mutant viruses in benign and prostate cancer cells at high MOI.

We hypothesized that the restriction of WT and mutant viruses in the BPH-1 cells was caused either due to a) an intracellular restriction in viral gene expression or b) the culture being in an anti-viral state, which prevents viral spread from infected to non-infected cells. To test the first hypothesis, 22Rv1 and BPH-1 cells were mock infected or infected at a high MOI of 10 with WT or mutant viruses, and examined for GFP and CPE, at the times indicated in Fig 4. Bright field and fluorescence microscopy images in Fig 4A and 4D show that WT and mutant viruses were able to infect most of the cell population in 22Rv1 and BPH-1 cells respectively. P/V/F and Le mutants also exhibited CPE in the 22Rv1 cells, which was evident by cell rounding, loss of intact cells and increased cell debris.

To quantitate CPE, flow cytometry and PI stain was utilized to measure non-viable cells. Fig 4B shows that a high MOI infection (of 10) with P/V/F and Le mutant in 22Rv1 cells resulted in ~90% of cell population being PI positive (PI+) on day 3 pi. When the same experiment was performed with BPH-1 cells, an obvious CPE was observed only late times post infection by the P/V/F mutant, while the Le mutant did not exhibit visible CPE (as shown in microscopy images in Fig 4D). Upon quantifying PI+ BPH-1 cells using flow cytometry, it was found that P/V/F mutant infection resulted in ~90% of PI+ cells, while Le mutant infection lead to ~25% of PI+ cells, on day 3 pi.

Cell metabolism assays were then performed to confirm flow cytometry data. 22Rv1 and BPH-1 cells were infected at an MOI of 10 with WT and mutant viruses and MTS assays were carried out. Fig 4C shows a time dependent loss of cell viability in 22Rv1 cells infected with P/V/F and

Le mutant viruses, whereas WT infected cells showed no loss of viability. Fig 4F reveals that BPH-1 cells experienced loss of viability upon infection with the P/V/F mutant. The Le mutant infected cells showed less extensive loss of viability than P/V/F mutant. These data indicate that the WT, P/V/F, and Le mutant viruses express viral genes and are able to grow and replicate within the BPH-1 cells, and that restriction in viral spread is not due an inability of the viruses to express their genes. Importantly, the mutant viruses exhibit significant CPE in 22Rv1 cells, whereas only the P/V/F mutant induces CPE in BPH-1 cells.

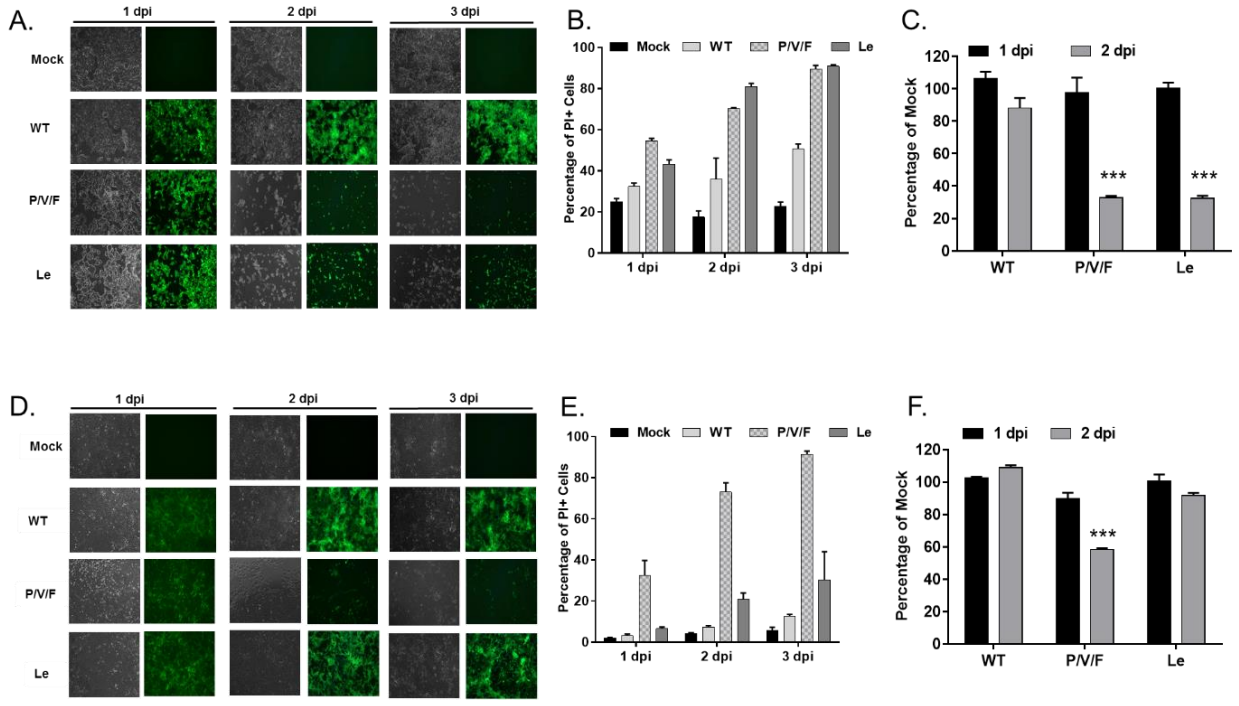

Figure 4. Cytopathic effect of PIV5 mutant viruses in benign and prostate cancer cells. 22Rv1cells (panels A, B, and C) and BPH-1 cells (panels D, E, F) were mock infected or infected with WT or PIV5 mutant viruses at a high MOI of 10. At the indicated time points, bright field and fluorescence microscopy images were taken (panel A and D) and non-viable cells were quantified with PI stain using flow cytometry (panels B and E). Cell viability of infected cells was measured using MTS assays, 1 and 2 dpi (panels C and F).

Type-I IFN production and signaling in benign and prostate cancer cells.

Given that all viruses were capable of growth in benign and cancer cells, we next tested the hypothesis that the restriction of mutant viruses we observe in BPH-1 cells is due to triggering of anti-viral responses in the culture. Type-I IFN production and signaling is a common anti-viral mechanism against viral infection, and we hypothesized that this mechanism was a contributing factor towards the restriction of mutant viruses observed in BPH-1 cells. First, type-I IFN production in response to mutant virus infection was quantified. Both 22Rv1 and BPH-1 cells were mock infected or infected at a high MOI (of 10) with P/V/F and Le mutant viruses. Infection media was then collected 24hpi and type-I IFN production was assayed using the HEK-Blue™ IFN-α/β cells, which are indicator cells that respond to type-I IFN by producing alkaline phosphatase. This is subsequently detected with QUANTI-Blue™ reagent (as described in Materials and Methods). As shown for the positive control in Fig 5A, A549 cells infected with the P/V mutant produced type-I IFN, which was shown previously in our published work (Wansley et al., 2002). However, when assayed in parallel, 22Rv1 cells did not produce type-I IFN upon mutant virus infection. By contrast, as seen in Fig 5B, infection of BPH-1 cells with P/V/F mutant resulted in ~2,500 units/ml of type-I IFN, whereas the Le mutant infection produced ~1,000 units/ml.

In addition to producing type I IFN, many cancer cells are defective in responding to IFN to enter an antiviral state (Stojdl, D. F et al., 2003, Dunn, G. P et al., 2005). To test this, we treated cells with type-I IFN and then measured their ability to enter an antiviral state, by assaying GFP production following infection, via flow cytometry. Here, 22Rv1 and BPH-1 cells were first treated with 0, 1 or 10 units of type-I IFN for 24 hours and subsequently infected with P/V/F or Le

mutant viruses for 24 hours. Fig 5C indicates that the number of GFP+ 22Rv1 mutant virus infected cells remained relatively similar across increasing concentrations of IFN treatment. In contrast, there was a dose dependent decrease in GFP+ mutant virus infected BPH-1 cells across increasing concentrations of IFN treatment (Fig 5D). These data indicate that 22Rv1 cells are incapable of producing type-I IFN upon mutant virus infection. In addition, these cells do not respond to exogenous treatment of type-I IFN. By contrast, the BPH-1 cells produce and respond to type-I IFN.

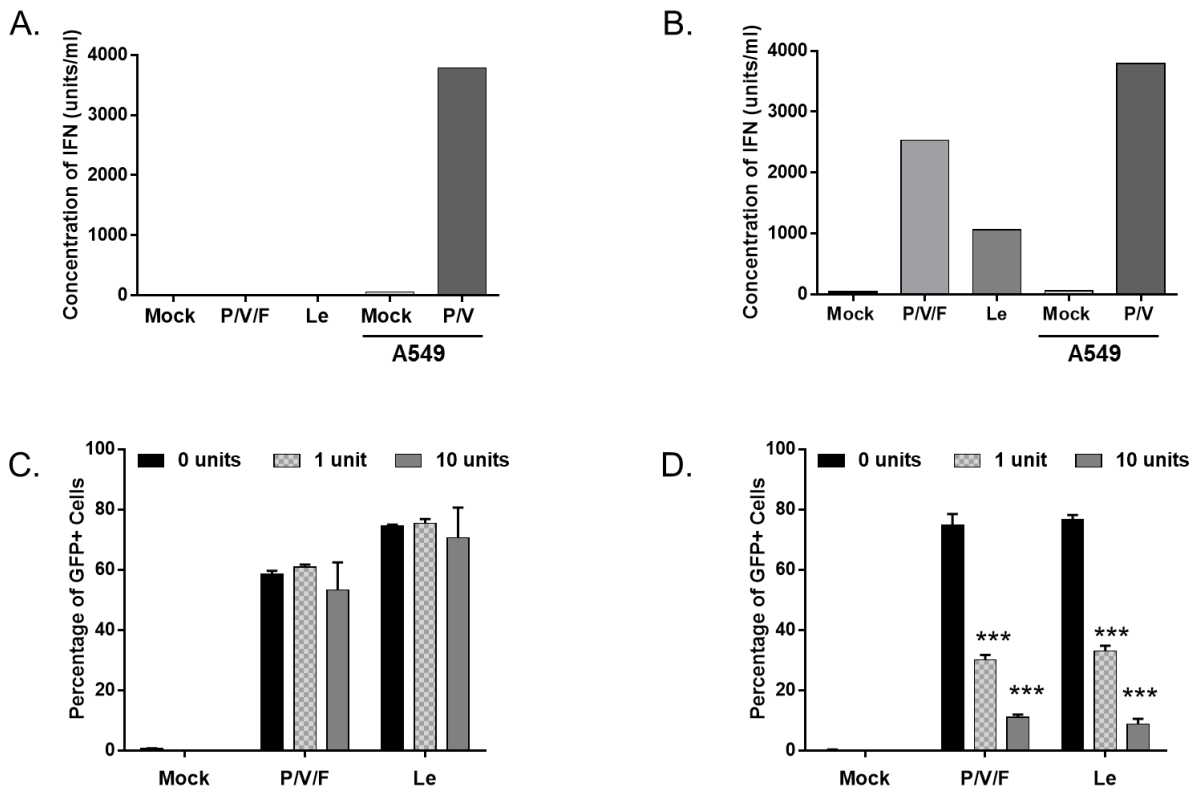

Figure 5. Type-I IFN production and response in benign and prostate cancer cells upon PIV5 mutant virus infection. Panel A and B. 22Rv1 cells (A) and BPH-1 cells (B) were infected at a high MOI of 10 with PIV5 mutant viruses and type –I IFN detection assay was carried out to quantify the production of type-I IFN (as described in Materials and Methods). A549 cells were used as a control cell line, as they produce type-I IFN in upon infection with the P/V mutant. Panel C and D. 22Rv1 cells (C) and BPH-1 cells (D) were pretreated with indicated concentrations of type-I IFN for 24 hours, followed by a high MOI infection of 10 with the PIV5 mutant viruses for another 24 hours. Infected cells were then quantified using flow cytometry.

IFN-β is a key contributing factor to restriction of PIV5 mutant viruses in benign cells.

To determine if type-I IFN contributed to the restriction of mutant virus infection in BPH-1 cells, an IFN-β neutralization experiment was performed. BPH-1 cells were either mock infected or infected with P/V/F or Le mutant at an MOI of 0.05. Infected cells were then treated for 3 days with or without an antibody that neutralizes either TNF-α, as a negative control, or IFN-β. Flow cytometry was then performed to determine the percentage of GFP+ cells. Bright field and fluorescence microscopy images in Fig 6A show that in the absence of antibody treatment, few cells were infected (as shown above in Fig. 3B). Upon TNF-α antibody treatment, there was no visible change in number of P/V/F or Le mutant infected cells observed compared to untreated samples. However, in the presence of IFN-β antibody treatment, ~90% of the mutant infected BPH-1 cells were GFP+ (Fig. 6B). These data indicate that IFN-β is a contributing factor to the restriction of mutant viruses in BPH-1 cells.

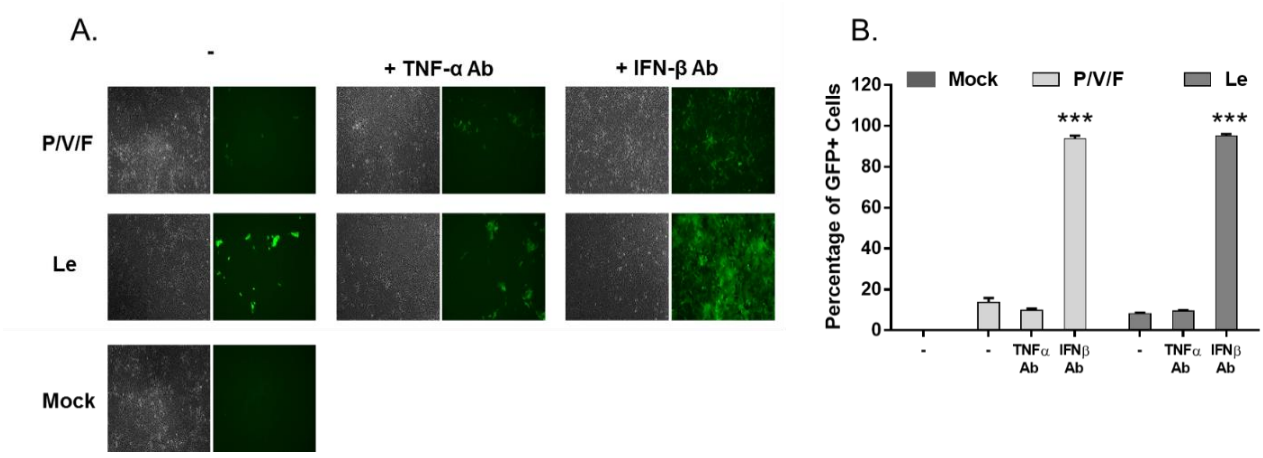

Figure 6. Neutralization of IFN-β. BPH-1 cells were infected with PIV5 mutants at a low MOI of 0.05 and either left untreated or treated with antibodies against TNF-α or IFN-β (as described in Materials and Methods). Day 3 pi, bright fields and fluorescence images were taken (A) and infected cells were quantified using flow cytometry (B).

Expression of key anti-viral genes upon PIV5 WT and mutant virus infection in benign cells.

To determine if key anti-viral genes are expressed upon virus infection, quantitative RT-PCR was performed upon mock infected and infected BPH-1 cells. We chose to focus on OAS2, IFIT1, and TLR3 as these are prototypic IFN-induced genes, and have been shown to be important for restriction of many RNA viruses (Andrejeva, J. et al., 2012; Manuse, M. J., & Parks, G. D. 2010). BPH-1 cells were mock infected or infected with WT or mutant viruses at an MOI of 10 for 14 hours. Data was expressed as fold increase in level of RNA compared to mock infection. Fig 7A shows that relative expression levels of OAS2 was slightly decreased by WT infection and slightly increased by Le mutant infection. However, there was ~40% fold upregulation of OAS2 upon P/V/F mutant virus infection. Fig 7B indicates that P/V/F mutant also upregulated expression levels of IFIT1 by ~350 fold, while there was a smaller upregulation seen by Le mutant and WT virus. Data in Fig 7C shows that P/V/F mutant upregulated TLR3 expression by ~15 fold and there was a slight upregulation seen with WT and Le mutant virus infection as well. Together, these data indicate that the P/V/F mutant upregulates expression of three key antiviral genes (OAS2, IFIT1, and TLR3) by a large fold difference. The Le mutant also shows upregulation of these genes, however, it is not as high as that with the P/V/F mutant.

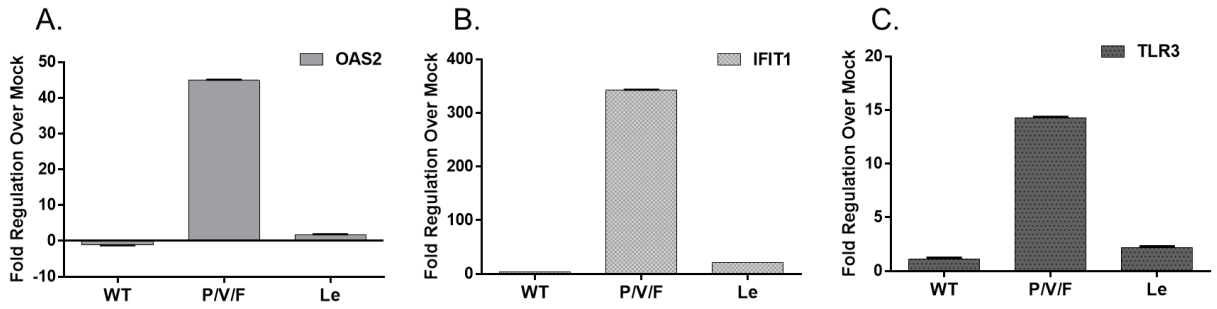

Figure 7. Relative expression levels of anti-viral genes in PIV5 mutant infected benign cells. BPH-1 cells were infected with PIV5 mutants at a high MOI of 10 for 14 hours and total RNA was used to determine relative expression levels of OAS2 (A), IFIT1 (B), and TLR3 (C) in PIV5 mutant infected cells via quantitative RT-PCR.

Characterization of PIV5 mutants in additional prostate cancer cell line.

To determine if the results obtained previously were consistent in multiple prostate cancer cell lines, C4-2B cells were either mock infected or infected at low MOI of 0.05 with WT, P/V/F, or Le mutants. Bright field and fluorescence images were taken days 1, 2 and 3 p.i and flow cytometry was performed, to determine percentage of GFP+ cells. As observed in Fig 8A, WT and mutant viruses were restricted for spread. Flow cytometry data showed that <10% of the cells were GFP+ by day 3 pi (Fig 8B). To determine if the viruses were capable of inducing CPE, C4-2B cells were infected at a high MOI of 10 with WT, P/V/F, and Le mutants for days 1, 2, and 3 pi. Bright Field and fluorescence images (Fig 8C) show that visible CPE was observed day 3 pi in P/V/F and Le mutant infected cells, but not in WT infected cells. Flow cytometry results showed that ~80% of the C4-2B cells were PI+ upon P/V/F mutant infection while ~75% of Le mutant infected cells were PI+, by day 3 pi (Fig 8D). These data indicate that the mutant viruses are restricted for spread in C4-2B prostate tumor cells but are capable of inducing CPE.

To determine if the observed restriction of mutant viruses in C4-2B cells (as seen in Fig 8A and B) was due to production of type-I IFN, C4-2B cells were infected with P/V/F and Le mutants for 24 hpi. Infected media was then collected and type-I IFN detection assay was carried out as described for Fig. 5 above. As shown in Fig 8E, only ~22 units/ml of IFN was produced in response to mutant virus infection. This was very low compared to the positive control, which is IFN produced from P/V infected A549 cells (Wansley et al, 2002). These data indicate that the restriction for spread in C4-2B cells may either be due to factors other than type-I IFN production, or that the low level of IFN produced is sufficient to restrict spread.

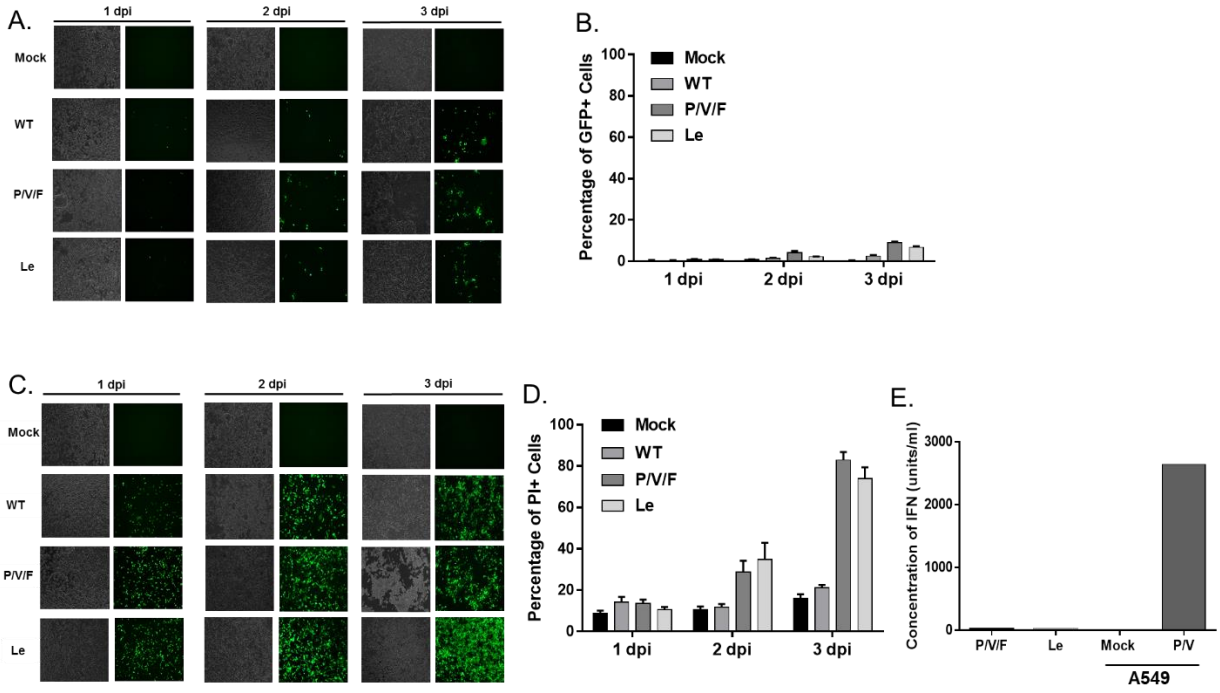

Figure 8. Characterization of PIV5 mutants in metastatic prostate cancer cell line. Panel A and B. C4-2B cells were either mock infected or infected with WT, P/V/F, or Le mutants (panel A and B) at MOI of 0.05. At the indicated time points, bright field and fluorescence microscopy images were taken (panel A) and virus infected cells were quantified using flow cytometry (panel B). Panel C and D. C4-2B cells were either mock infected or infected with WT, P/V/F or Le mutants (panel C and D) at a high MOI of 10. At the indicated time points, bright field and fluorescence microscopy images were taken (panel C) and non-viable cells were quantified with PI stain using flow cytometry (panel D). Panel E. C4-2B cells were infected at with PIV5 mutant viruses at MOI 10 and production of type-I IFN was determined using type-I IFN detection assay (as described in the Materials and Methods section). A549 cells were used as a control, as they produce type-I IFN in upon infection with the P/V mutant.

CHAPTER FOUR: CONCLUSION

Previous studies with the P/V/F mutant (Gainey et al., 2008) showed that the virus is capable of infecting and inducing CPE in prostate tumor cell lines. This virus was also efficient in reducing prostate tumor burden in a xenograft mice model. These data indicate that the P/V/F mutant could be a potential oncolytic virus for prostate tumor therapy. Studies with the Le mutant in tissue culture have shown that it induces CPE in lung carcinoma cells and is able to mediate an anti-viral response despite having a functional V protein (Manuse et al., 2009). However, the Parks lab has not yet tested the Le mutant in tumors *in vivo*. Importantly, characterization of these mutants was not performed in benign prostate cell lines and the restriction of the mutants in normal prostate cells was not previously addressed.

In this project, I have characterized the P/V/F and Le mutant in benign prostate cells (BPH-1) as well as two metastatic prostate cancer cell lines (22Rv1 and C4-2B). The WT and mutant viruses are able to spread and grow in the 22Rv1 cells, while they show restriction in the BPH-1 cell line. Both mutants are capable of inducing significant CPE in the 22Rv1 cells, while only the P/V/F mutant can induce CPE in the BPH-1 cells, at a high MOI. Upon further investigation, it was found that the restriction of the mutants observed in the BPH-1 cells was due to the triggering of anti-viral responses, at least in part, due to production and signaling of type-I IFN. More importantly, IFN-β plays a large contributing role in causing this restriction. It was also found that three key anti-viral genes (OAS2, TLR3 and IFIT1) were upregulated in the BPH-1 cells upon mutant virus infection. In summary, the mutant viruses showed preferential replication and spread in the prostate cancer cells (22Rv1) while being restricted in the benign cells (BPH-1). Interestingly, the

same results did not hold true for an additional prostate tumor cell line that was investigated (C4-2B cells). It was found that the WT and mutants were also restricted in this cell line, and were able to cause significant CPE at a high MOI. The restriction of mutant virus spread in this cell line however, did not correlate with significant amount of type-I IFN production.

This leads us to the question of what could be responsible for the restriction of virus spread in the C4-2B cells. It is possible that there could be anti-viral factors other than type-I IFN that are causing this restriction. For example, it is known that TNF-α can restrict spread of other RNA viruses in cell culture (Seo, S. H., and Webster, R. G; 2002). Alternatively, it is possible that the C4-2B cells are extremely sensitive to IFN, and that the low levels of the cytokine, which are produced by infection with the P/V/F mutant, could induce a strong anti-viral response. This possibility is confounded by the finding that WT PIV5 was also restricted for spread in C4-2B cells, and this virus is known to produce very little IFN compared to the strong induction by the P/V/F mutant (Wansley et al, 2002). Therefore, future studies need to be dedicated to exploring this observation in the C4-2B cell line.

This project also drew out comparisons between the P/V/F and Le mutant viruses. Both mutants are capable of inducing CPE in 22Rv1 cells and both show restriction in benign cells. However, the P/V/F mutant is more restricted in the BPH-1 cells than the Le mutant. The P/V/F mutant is also restricted for growth in the BPH-1 cells at a low MOI, while the Le mutant has similar growth properties as the WT PIV5. In addition, a stronger anti-viral response is generated upon P/V/F mutant infection in the BPH-1 cells. The P/V/F mutant produces a larger amount of type-I IFN compared to the Le mutant. It also induces larger upregulation of OAS2, IFIT1 and TLR3 compared to the Le mutant. Previous studies with the P/V/F mutant also show that the CPE induced

is necrotic in nature (Gainey, M. D et al., 2008). Necrosis-induced tumor death is more favorable than apoptosis, as it recruits various immune factors to aid in tumor-clearance (Melcher, A. et al., 1999). However, it is to be noted that the P/V/F mutant is also capable of inducing a CPE in the benign cells at a high MOI, while the Le mutant does not. Overall, we can conclude that these PIV5 derived mutants can make for effective oncolytic viruses, although the P/V/F mutant appears to be a better candidate than the Le mutant.

There are many advantages to using a PIV5 virus for cancer therapy.1) It has a small genome and has no constraints or complex viral genome packaging mechanisms. 2) It is possible to add foreign genes into the PIV5 genome. 3) It is capable of infecting non-dividing cells and can grow to very high viral titers ($>10^{10}$ pfu/ml). 4) The genome does not integrate itself into host DNA as it replicates in the cytoplasm of the host cell, and 5) when PIV5 infects human hosts, it does not induce adverse symptoms or diseases. Importantly, there is no pre-existing humoral immunity to PIV5 in the human population which could prevent effective administration of the virus to patients.

Another major advantage of PIV5, is that the F protein is able to function independently from the viral attachment protein. Therefore, mutating the F viral protein to create the hyperfusogenic mutant does not compromise viral attachment and subsequent budding and release of progeny viruses. However, there are questions that need to be addressed with regard to the P/V/F and Le mutants. Previous studies with PIV5 and anti-viral proteins showed that IFIT1 is capable of selectively inhibiting PIV5 translation via the IFN pathway (Andrejeva, J. et al., 2012). Data generated from this project also indicates that relative gene expression of IFIT1 is induced to high levels upon P/V/F mutant infection. Therefore, it would be interesting to explore how IFIT1 contributes to the restriction of the P/V/F mutant in BPH-1 cells. A study has also shown that

substitutions in the P/V gene of the PIV5 (which are present in the P/V/F mutant) activates PKR, which subsequently leads to shutdown of viral translation (Gainey, M. D et al., 2007). Future work would focus on determining any relation between PKR and IFIT1 during P/V/F mutant virus infection.

Since the tumor suppressing effects of the P/V/F mutant have been observed in prostate tumor xenograft nude mouse models (Gainey, M. D et al., 2008), eventual studies could be performed in mice with intact immune systems. This would help us determine how the immune system is able to aid or restrict mutant virus infection and investigate its subsequent effect on prostate tumors in a biological system. It is important to remember that there are various components in the immune system that work in concert to help mediate an immune response against viruses. Previous findings have shown that a G3A mutation in the F gene of PIV5 results in neutralization of the mutant virus, either via antibody mediated neutralization or by activating a specific pathway of complement (Johnson, J. B et al., 2013). Therefore, it is possible that the complement pathway could play a role in safe clearance of the mutant virus. However, it is important to explore and strike a balance between safety of the mutant virus and the possibility of it being cleared from the system before it can have an anti-tumor effect.

Overall, this study has supported the pre-clinical implications of utilizing these mutant viruses for tumor therapy. However, it is still unclear if the mutants are suitable for different kinds of tumors. The P/V/F mutant could be effective for prostate tumors but may not be suitable for breast tumors. From these findings it is implied that these mutants are selective for tumors that have dysfunctional IFN machinery. Many tumors have mutations in their IFN response mechanism. They may be unable to produce IFN, unable to respond to IFN or unable to produce and respond to IFN.

Therefore, it would be important to screen tumors for IFN competence before administration of these mutant viruses. In the future, individual patients will need to be thoroughly tested for tumor type and the oncolytic virus to be used will need to be tailored according to tumor type and properties.

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