THE ROLE OF PTEN IN HUMAN CANCER

Jaimie Michelle Gendron

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Master of Science
in the Department of Biochemistry and Molecular Biology
Indiana University

May 2015

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Master of Science

Master's Thesis Committee

_____
Lindsey D. Mayo, PhD, Chair

_____
Mark Goebl, PhD

_____
Maureen Harrington, PhD

_____
Samisubbu Naidu, PhD

ACKNOWLEDGMENTS

Thank you to my committee, Lindsey D. Mayo, PhD; Mark Goebl, PhD; Maureen Harrington, PhD; and Sami Naidu, PhD for their guidance.

To Kristin Bredhold, thank you for allowing me to learn to love teaching through you.

To my family, thank you for your unconditional love and continued support.

To my love, Allen Jarboe thank you for your patience and unconditional love.

Jaimie Michelle Gendron

THE ROLE OF PTEN IN HUMAN CANCER

Phosphatase and tensin homolog, PTEN, is a key tumor suppressor. Mutation of PTEN is associated with both sporadic cancers and a cluster of familial cancer predisposition syndromes called PTEN hamaratoma syndromes. These germline mutations span the length of the PTEN gene with a mutational hot spot localized in exon 5. This exon encodes the catalytic domain of PTEN, which is critical for its tumor suppressor activity. PTEN function is most commonly attributed to lipid phosphatase activity on Phosphatidylinositol (3,4,5)-trisphosphate (PIP3) that leads to inhibition of a cascade with downstream pro-survival effectors including Akt, but PTEN also has phosphatase activity on a small number of proteins. Recently, a mutation, G129E, has been described as a gain of function (GOF) mutation in PTEN knockin mice. This mutant only retains protein phosphatase activity while it completely lacks lipid phosphatase activity. Collectively (in the mouse and *in vitro* studies), there is no clear mechanism to explain the GOF nature of this mutant. Understanding how mutants of PTEN function in the cells to provide a growth advantage will provide insight into what pathway to therapeutically target. Our central hypothesis is that mutations of PTEN promote tumorigenesis through gain of function activities that result in cell cycle progression. We will determine the signaling pathways that are affected by the gain of function mutant PTEN G129E to better understand the mechanism by which mutants of PTEN confer a growth advantage.

Lindsey D. Mayo, PhD, Chair

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

293T	Human embryonic kidney cell line with SV40 T-antigen
Akt	Protein kinase B
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3-related protein
BL-21	Chemically competent e. coli cell line
bpV(Hopic)	Bisperoxo oxovanadate dipotassium, inhibitor of PTEN
C	Celcius
C124S	PTEN mutation at cysteine 124 to serine
Cdc25C	M-phase inducer phosphatase 3
CDK1	Cyclin dependent kinase 1
Chk1	Checkpoint 1 kinase
Chk2	Checkpoint kinase 2
CO2	Carbon dioxide
CREB	cAMP response element-binding protein
CSK	C-terminal Src Kinase
DMEM	Dulbecco’s modified Eagle’s medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E1	Sumoylation/ubiquitination activating enzyme
E2	Sumoylation/ubiquitination conjugating enzyme
E2F	Transcription factor E2F
E3	Sumoylation/ubiquitination ligase

ECL	Enhanced chemiluminescence
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
ERBB2	Receptor tyrosine-protein kinase HER2
ERK	Extracellular-signal-regulated kinases
FAK	Focal adhesion kinase
FBS	fetal bovine serum
FGF	Fibroblast growth factors
Flag	polypeptide protein tag
G0	Growth phase 0
G1	Growth phase 1
G129E	Glycine to glutamic acid mutation at amino acid 129 on PTEN
G2	Growth phase 2
GAP	GTPase-Activating Proteins
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factors
GST	Glutathione S-transferase
GTP	Guanosine triphosphate
HA	Human influenza hemagglutinin, polypeptide protein tag
HER2	Receptor tyrosine-protein kinase erbB-2
His	polyhistidine tag
hr	hours

IGEPAL	octylphenoxypolyethoxyethanol, non-denaturing detergent
IKK	IκB kinase
IP	Immunoprecipitation
IPTG	Isopropyl-β-D-thio-galactoside
JAK	Janus Kinase
KCl	Potassium chloride
kDa	Kilo Dalton
KO	Knock out, PTEN knockout mice
LB	Luria broth
M	Molar
M	Metaphase
MAPK	Mitogen-activated protein kinases
Mdm2	Mouse double minute 2 homolog
MEF	Mouse embryonic fibroblasts
MEK	Mitogen-activated protein kinase kinase
MET	mesenchymal–epithelial transition
ml	milliliter
mM	millimolar concentration
mm	millimeter
MMAC	Mutated in Multiple Advanced Cancers
MPC	Mitosis promoting complex
mV	millivolt
NEMO	NF-kappa-B essential modulator

NFkB	Rel or NF-kappaB
Ni-NTA	high capacity nickel resin column for affinity purification
OD	Optical density
p21	Cyclin-dependent kinase inhibitor 1
p27	Cyclin dependent kinase inhibitor 1B
p53	Tumor suppressor p53
p57	Cyclin-dependent kinase inhibitor 1C
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween-20
PC3	Human prostate cancer cell line
PEI	Polyethylenimine
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PIAS	E3 ligase, Protein Inhibitor of Activated STAT
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PKC	Protein Kinase C
PP2A	Protein phosphatase 2A
PTEN	Phosphatase and tensin homolog
PVDF	Polyvinylidene fluoride
RAD51	Recombinase Rad51
Ras	Ras protein, small GTPase
RPM	Rotations per minute
RSV	Rous Sarcoma Virus

RTK	Receptor tyrosine kinase
SDS	Sodium dodecyl sulfate
SENP1	Sentrin-specific protease 1
SENP2	Sentrin-specific protease 2
SH2	Src homology domain 2
SH3	Src homology domain 3
Shc	Src homology 2 domain containing transforming protein 1
SHIP	Tyrosine-protein phosphatase non-receptor type 11
Src	Proto-oncogene tyrosine-protein kinase Src
STAT	Signal transducer and activator of transcription
SUMO	Small ubiquitin-related modifier
SUMO1	Small ubiquitin-related modifier 1
SUMO2	Small ubiquitin-related modifier 2
SUMO3	Small ubiquitin-related modifier 3
TNF	Tumor Necrosis Factor
TX-100	Triton X-100, t-octylphenoxypolyethoxyethanol
U87	Human glioblastoma-astrocytoma cell line
UBC9	SUMO-conjugating enzyme, Ubiquitin-conjugating enzyme E21
VEGF	Vascular endothelial growth factor
WB	Western blot
µg	Microgram
µl	Microliter
µM	micromolar

INTRODUCTION

Cancer is a disease in which our very own genes become mutated in a way that give an evolutionary advantage through various means over the surrounding normal cells. This advantage gives cells the ability to grow uncontrollably in unique ways to each and every cancer. In the mid 1800s John Bennett and Rudolf Virchow independently discovered the increased numbers of white blood cells in two patients. Bennett attributed the increase in white blood cells to an infection while Virchow developed a different theory. Virchow proposed, knowing that we are all made of cells that these cells must arise from cells. Furthermore, the increase in white cells he was seeing must be either from an increase in size that he termed hypertrophy or an increase in cell number termed hyperplasia. Virchow named the illness leukemia and was the first to define cancer as an abnormal replication of cells. This new realization could be applied to solid tumors as well (Degos, 2001).

Although abnormal cell growth can be simply said, cells are complex machines that have many means of both regulating growth and senescence. Naturally, cells have genes that produce proteins that function to suppress growth and division as a means of protecting the cell when conditions are not favorable, and these are called tumor suppressors. Conversely, proto-oncogenes are genes that upon mutation or other means of regulation produce proteins that cause the cell to grow and divide despite signaling that it should not. In the mutated state these are referred to as oncogenes (Varmus, Pao, Politi, Podsypanina, & Du, 2005).

There are a couple of ways normal genes become oncogenes and similarly ways in which tumor suppressors no longer guard cells against cancer. Hermann J. Muller helped to define these means and named them morphs (Muller, Carlson, & Schalet, 1961) . Oncoproteins or oncogenes are generally hypermorphs, antimorphs, or neomorphs. Hypermorphs are ways in which genes gain function either through gene dose, expression, or constitutive activity. Antimorphs are genes that are expressed as dominant negative proteins, and neomorphs are genes that express proteins that have completely different functions than the wild type protein. Tumor suppressors are affected by mutations usually to hypomorphs or amorphs. Hypomorphs are cases where genes lose partial function, and amorphs are where genes completely lose function (Muller et al., 1961).

In an attempt to clarify way in which cancer maintains abnormal growth and to define cancer, Hanahan and Weinberg delineated six pathways by which cells maintain carcinogenic signaling and deemed it the hallmarks of cancer (Hanahan & Weinberg, 2000).

1. “Self sufficiency in growth signals”

In normal homeostatic states, cells respond to growth signaling with proliferation. These forms of growth signaling can include ligand growth factors, cell-cell adhesions, or intracellular matrix interactions. A cancerous cell avoids the need for these growth signals by autocrine feedback loops (Alimandi et al., 1997), overexpression of receptors (Slamon et al., 1987; Yarden & Ullrich, 1988), mutated to constitutively active receptors (Alimandi

et al., 1997), and switching to more growth prone extracellular matrix proteins (Lukashev & Werb, 1998).

2. “Evading apoptosis”

Cancerous cells avoid programmed cell death by downregulation or inhibition of cell sensors or effectors involved in recognition or initiation of cell death signaling. Normal cells regulate apoptosis, or programmed cell death, by both internal signals as well as external signals. For instance cell death signals are the Fas ligand/receptor, and the TNFα (Tumor Necrosis Factor) and the TNF receptor and activate apoptosis under unfavorable extracellular conditions (Ashkenazi & Dixit, 1999). Conversely, signaling imbalances, DNA damage, and hypoxia can also initiate apoptosis (Evan & Littlewood, 1998).

3. “Insensitivity to antigrowth signals”

Cells use anti-growth signaling to inhibit proliferation in states where growth is not favored. For example, the cell is heavily regulated by the cell cycle. Most notably, pRB or retinoblastoma protein is a tumor suppressor that is heavily regulated by phosphorylation from Cyclin Dependent Kinases (CDK) when conditions are met for cell cycle to progress. When pRb is hyperphosphorylated, it releases E2F, which is a transcription factor that allows for the production of cell cycle machinery including cyclins E and A. In certain cancers pRb is lost genetically or by mutation and is therefor unable to bind E2F or prevent transcription of necessary cyclins to pass through the cell cycle (Weinberg, 1995)(Figure 1).

4. “Sustained angiogenesis”

Considering the necessities that blood vessels are to carry oxygen and nutrients and provide a system for waste removal, it is essential for a tumor to maintain close proximity to functioning vasculature. Tumors are known to modulate vasculature formation by increasing transcription factors of pro-angiogenic factors like Vascular Endothelial Growth Factor (VEGF) and Fibroblast growth factor (FGF) (Cheung, Singh, Ebaugh, & Brace, 1995; Gately et al., 1997; Volpert, Dameron, & Bouck, 1997).

5. “Limitless replicative potential”

Hayflick first observed that cells have a limited number of divisions and the point at which this limit is reach he deemed the Hayflick limit (Hayflick, 1997). Furthermore, this limit is mediated by telomeres, or nucleotide repeats at the ends of telomeres that shorten with each division as a means of protecting genes from incomplete replication at the ends of chromatids and engaging senescence when the telomeres have become too short. In cancer, an enzyme known as Telomerase becomes activated and lengthens telomeres such that they may continue dividing (Hayflick, 1997).

6. “Tissue evasion and Metastasis”

Metastasis is the leading cause of mortality in human cancer. In order for cancer to metastasize, it must undergo certain changes to mobilize from the primary tumor. Epithelial to mesenchymal transition (EMT) is a process in which cells lose polarity, and cell-cell adhesions to gain motility. Metastatic cancers often express SNAIL, Twist, or ZEB1, which are drivers of EMT. Additionally, when a tumor cell reaches its secondary site, it must undergo

mesenchymal to epithelial transition (MET) to regain polarity and cell-cell contacts to form a secondary or metastatic tumor (Grivennikov, Greten, & Karin, 2010).

Once cancer is characterized, particular pathways begin to emerge. The cell cycle is an essential regulator of growth and division, and in many cases is manipulated in cancer. The cell cycle combines extracellular and intracellular signaling to determine if the cell has the resources and necessary conditions to divide. Cell cycle regulators sense the amount of DNA damage, replication machinery, growth factors, mitotic machinery, and the availability of oxygen and translate those signals into appropriate transcription of proteins to carry out the cell cycle.

During the mammalian cell cycle, growth factors induce cells to leave the quiescent stage (G0) to growth stage one (G1). In the early stage of G1, Rb becomes hyperphosphorylated and looses affinity for the transcription factor, E2F. When E2F is responsible for induction of many gene including cyclin E and cyclin A, which are involved in the progression to synthesis (S) and growth stage two (G2). During S phase, E2F is then phosphorylated and degraded (Mudryj et al., 1991). Rb remains hyperphosphorylated through S, G2, and metaphase (M) (Lin & Wang, 1992). Cyclin B levels are increased in M, cyclin D and E are increased in G1, and cyclin A levels are increased in G2 and S (Breeden, 2000). These cyclins are active when bound to appropriate cyclin dependent kinases (Zhu, Ohtsubo, Bohmer, Roberts, & Assoian, 1996). Aside from regulation by transcription, and binding partner cyclin dependent kinases, additional regulation

exists. Cdc25 is a phosphatase involved in the activation of CDK1/Cyclin B complexes by dephosphorylation of the tyrosine 14 and 15, after Cyclin-dependent activating kinase (CAK) phosphorylates threonine 161 on the CDK. This allows the CDK1/Cyclin B cell to pass from interphase to mitosis (Gabrielli, Lee, Walker, Piwnica-Worms, & Maller, 1992). Cell cycle inhibitors fall into two classes: the CIP/KIP tertiary family inhibitors and the INK4 family binary inhibitors. Within the class of CIP/KIP inhibitors is p21, p27, and p57 (Hengst & Reed, 1998). Loss of p27 is more commonly associated with cancer than the p21 and p57 (Esposito et al., 1997). As an inhibitor of the cell cycle p27 is lost under growth conditions and is also often lost in cancer. Src phosphorylates p27 at tyrosine 74. An additional phosphorylation by cyclin E or A/CDK2 targets p27 for proteolytic degradation (Chu et al., 2007)(Figure 1).

The cell cycle is just one pathway in a network of pathways involved in human cancer. p53 is one protein that bridges many types of stress signaling with the cell cycle. As the most commonly lost tumor suppressor in human cancer, it is pivotal in pausing the cell cycle for moderate damage so that DNA repair can occur (Berger, Vogt Sionov, Levine, & Haupt, 2001). If the DNA damage is beyond the threshold of repair p53 can initiate apoptosis (Chiarugi, Magnelli, & Cinelli, 1997). Under normal growth conditions, activated PI3K pathway leads to and activated Akt. Akt phorphorylates Mdm2 allowing it to translocate to the nucleus bind p53 and facilitate its nuclear export (Hu, Feng, & Levine, 2012). Ubiquitinated p53 that is cytoplasmic localized renders p53 to be

polyubiqutinated and subsequently targeted for proteasomal degradation (Honda, Tanaka, & Yasuda, 1997; Mayo & Donner, 2001).

Among the most commonly altered pathways in human cancer are the receptor tyrosine kinase (RTK), Mitogen-activated protein kinase (MAPK), PIP3 kinases, Ras, and p53 pathways. It becomes clear from the initial overview that kinase signaling is key in cancer pathways.

Beginning at the membrane, RTKs are transmembrane proteins that exist as monomers but upon activation by growth factors, hormones, or cytokines, dimerize with nearby monomers. RTKs have a highly conserved extracellular N-terminus that binds ligands, a transmembrane region, and a C-terminal region containing the kinase domain. Dimerization initiates a transphosphorylation event whereby both monomers become tyrosine phosphorylated. These phosphorylation events then prime the receptor for binding with the Src homology domain 2 (SH2) of binding partners, and activate the kinase for signal transduction through many pathways within the cell. HER2 or ERBB2 is overexpressed in some human breast cancers as a result a modest level of epidermal growth factor (EGF), may have a robust effect on downstream signal transduction (Marshall, 1995).

Within the cell, other kinases take over signaling. Src activated by RTKs. v-Src, or viral Src, was first discovered by Peyton Rous in cell free chicken extracts, that could induce cancer when injected into healthy chickens. Peyton Rous had isolated the Rous Sarcoma Virus (RSV), and thought that it could be the origin of all of cancer (Rous, 1960). Unfortunately, many other oncoproteins

exist and this was only the beginning of the understanding of cancer. Later it was isolated by John Michael Bishop and Harold E. Varmus who determined that Src was a human gene that had been hijacked and mutated by the Rous Sarcoma virus (Varmus et al., 2005). Src can be activated by many receptors, these include RTKs, Insulin-like growth factor receptor 1 (IGFR-1), Human epidermal growth factor receptor (HER2), and Epidermal growth factor receptor (EGFR) (S. Zhang et al., 2011). Src, unlike RTKs, is an intracellular tyrosine kinase composed of three main functional domains. The protein structure of Src contains: Src homology 2 domain (SH2); the Src homology 3 domain (SH3); and the kinase domain. The SH2 domain is a docking domain that binds to multiple other proteins like RTKs when they are tyrosine phosphorylated (Lammers et al., 1993). The SH3 domain is an adapter domain necessary for mediating complex formation. Finally, the kinase domain is the functional tyrosine kinase. The activation and inactivation of Src has been well defined. When inactivated, the SH3, SH2 and kinase domain are bound in a closed conformation with a phosphorylation at tyrosine 527 on the c-terminal tail. Upon docking to a receptor at the SH2 domain, Protein tyrosine phosphatase non-receptor type 11 (Shp2) dephosphorylates tyrosine 527. This dephosphorylation triggers a conformational change and an autophosphorylation results in a phosphorylation at tyrosine 416 (Irby & Yeatman, 2000)(Figure 2). This combination of events results in an active kinase that induces signal transduction on many targeted pathways including Focal Adhesion kinase (FAK), Protein kinase C (PKC), Signal transducer and activator of transcription 3 (STAT3), and Mitogen-activated protein kinase

(MAPK) (S. Zhang et al., 2011). Considering the robust signals that can result from Src, especially in the cases of cancer where receptors are overexpressed or Src is constitutively active it is critical to understand the inactivation of Src. When Src is in the active conformation, C-terminal Src Kinase (CSK) docks at the c-terminus, and allows a protein tyrosine phosphatase to bind and dephosphorylate tyrosine 416. The protein tyrosine phosphatase then unbinds and then CSK phosphorylates tyrosine 527. The c-terminal tail returns to the closed conformation on the SH2 domain. At this point the CSK unbinds and Src is completely inactive (Ayrapetov et al., 2006)(Figure 3).

The Ras subfamily is a group of small GTPases that is an activator of proliferation. It was discovered similarly to Src, but from Rat Sarcoma virus. Ras is a G-protein involved in converting guanosine triphosphate (GTP) to guanosine diphosphate (GDP). When GTP is bound, Ras is in the active “on” confirmation and “off” when GDP is bound. In the active conformation Ras can activate Rapidly accelerating fibrosarcoma (Raf) and Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K). Ras generally has low intrinsic activity for hydrolyzing GTP to GDP, so it recruits guanosine activating proteins (GAPs) under normal conditions. Conversely, guanosine exchange factors (GEFs) are necessary for swapping GDP for GTP and restoring Ras to the active conformation. In cancer, Ras can be mutated to be constitutively active or Ras-GAPs can be lost and Ras is less frequently inactivated (Bos, 1989).

In cancer an active Ras activates Raf, MEK, and ERK a serine/threonine kinase cascade that protects cell survival through activation of transcription of pro-survival genes (Bos, 1989).

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathways can both be activated by Ras. Canonical function of the PI3K pathway involves activation of a receptor tyrosine kinase by an extracellular ligand. PI3K actively converts PIP2 to PIP3. The increase in PIP3 near the membrane causes an increase in recruitment of Protein Kinase B (Akt) and Protein Dependent Kinase (PDK) localization to the membrane. PDK is the activating kinase for Akt, thus their co-localization allows for activation of Akt. Akt is a commonly activated in human cancers as it has robust pro-growth and angiogenic downstream pathways including: VEGF, Mdm2, and mTOR.

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) or Mutated in Multiple Advanced Cancers (MMAC) was named for being lost in advanced cancers and its homology to Protein Tyrosine Phosphatase (PTP) (Li et al., 1997; Steck et al., 1997)(Figure 4). PTEN acts to dephosphorylate PIP3 to PIP2 at the D3 phosphate thereby inhibiting the subcellular localization of PDK and Akt and subsequent activation. Activation of the Akt pathway is usually under control of growth factors and growth factor receptors but can be hijacked in cancer cells when the inhibitory activity of PTEN is lost (Maehama, Okahara, & Kanaho, 2004; Myers et al., 1998)(Figure 5).

PTEN is known to bind p53 and is a transcriptional target of p53(refs Stamblolic work and Mayo in Jbc) The PTEN-p53 complex can targets gene

expression that may induce cell cycle arrest or apoptotic genes in response to DNA damage (Chiarugi et al., 1997; Haapajarvi, Kivinen, Pitkanen, & Laiho, 1995). Similarly, PTEN complexes with p73 inducing Puma and Bax, which initiates apoptosis (Lehman et al., 2011)(Figure 6).

In addition to being lost in many advanced cancers, PTEN was found mutated in cancer predisposition syndromes such as Cowden's syndrome (Liaw et al., 1997).

PTEN is a 403 amino acid protein composed of an N-terminus, a phosphatase domain, a C2 domain, a C-terminal tail, and a PDZ binding domain. There is only a single catalytic site known to exist in the phosphatase domain. The PDZ binding domain is believed to be involved in membrane targeting. The C2 domain is highly conserved and considered to be an important location for post-translational modifications (Lee et al., 1999)(Figure 4).

PTEN is also a heavily regulated protein, posttranslationally. It has 7 phosphorylation sites on the c-terminal tail that when phosphorylated have been shown to give stability to PTEN but also inhibits PTEN activity. These sites are phosphorylated by casein kinase 2 (CK2), ATM, and glycogen synthase kinase 3 beta (GS3Kβ) (Adey et al., 2000; Al-Khouri, Ma, Togo, Williams, & Mustelin, 2005; Okamura et al., 2006)(Figure 4).

Additionally the C2 domain can be phosphorylated by Rho associated protein kinase (ROCK) and the tyrosine kinase Rak which act to dissociate PTEN from the membrane (Fragoso & Barata, 2014)(Figure 4).

Sumoylation and ubiquitination of PTEN is still being delineated but it is clear that multiple sites are sumoylated and at least on is ubiquitinated. These large posttranslational modifications are involved with nuclear targeting (Lang et al., 2014)(Figure 4).

Lysine acetyltransferase 2B (PCAF) acetylates PTEN on lysine 125 and lysine 128 and this reduced catalytic activity. An additional acetylation site is at lysine 402 is acetylated by CREB binding protein (CBP) and it may affect how it binds with partners in the PDZ binding domain (Ikenoue, Inoki, Zhao, & Guan, 2008; Okumura et al., 2006)(Figure 4).

By determining both the regulation of PTEN subcellular localization and its phosphatase activity, we may begin to understand the role of PTEN in human cancers. PTEN was initially determined to function by counteracting the Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway by dephosphorylating Phosphatidylinositol (3,4,5)-trisphosphate (PIP3)(Figure 5).

Initial experiments with recombinant protein and phosphatase assays by Myers et al. show that wild type PTEN has activity on small acidic peptides in addition to lipid phosphatase activity (Myers et al., 1997). This opened up the possibility for functions of PTEN. Gradually discoveries of protein targets occurred, as endogenous phosphatase activity was seen on FAK, and Shc in the cytoplasm. Shc and FAK were found physically interacting with PTEN, and both had decreased phosphotyrosine phosphorylation in the presence of PTEN. Downstream function of activity PTEN on these substrates led to decreased cell migration (J. Gu et al., 1999; Tamura et al., 1998).

In addition to having protein targets, it has become clear that PTEN not only localizes to the nucleus but is functional in the nucleus. Nuclear targets include CREB, which has been shown in complex with and dephosphorylated by PTEN at a site phosphorylated by active Akt, serine 133 (RRP**S**YRK) (T. Gu et al., 2011)(Figure 9).

Furthermore, PTEN has been found in complex with the Anaphase promoting complex/cyclosome (APC/C) and is thought to be involved in allowing APC/C to bind with CDH1 allowing ubiquitin-mediated degradation of kinases and cyclins facilitating inhibition of the cell cycle. Interestingly this function is not dependent on phosphatase activity. (Manchado, Eguren, & Malumbres, 2010). PTEN has also been found in complex with centromere binding protein C, and has been shown to be important in double stranded break repair as it can cause the induction of RAD51. It is unknown if RAD51 induction or function involving centromere binding protein C are the cause, but MEFs lacking PTEN have chromosomal instability. Thus PTEN is involved in chromosomal instability through an unknown means.

While the lipid phosphatase activity on PIP3 has been delineated into a clear mechanism, considerably less is known about how PTEN regulates proteins or how regulation of PTEN localization dictates its action on protein targets. The PTEN mutant $PTEN^{G129E}$ has been shown to act specifically on proteins and not on lipids. Thus, it does not act on PIP3 to downregulate the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway because it is believed to have a shallower pocket in its phosphatase domain (Lee et al., 1999).

Similarly, it has been shown to have a gain of function (GOF) phenotype (Wang et al., 2010). While G129E isn't the only PTEN mutant that has protein phosphatase activity, it is the only mutant that no longer retains lipid phosphatase activity. $PTEN^{M134L}$ has increased protein phosphatase activity with respect to wild type PTEN but also retains lipid phosphatase activity. $PTEN^{G129E}$ is a valuable tool because it lacks the canonical PTEN lipid phosphatase function allowing us to study functions independently. To date, no mutant has been discovered that retains lipid phosphatase activity but not protein phosphatase activity (Myers et al., 1997)(Figure 7 and 8).

G129E is an interesting PTEN mutant that may gain tumorigenic potential through increased phosphatase activity on a target that it shares in common with wild type PTEN or a completely novel target. We wish to determine the pathways by which mutant $PTEN^{G129E}$ gains a growth advantage over wild type PTEN as well as how regulation of PTEN is altered by post-translational modifications.

Cancer predisposition syndromes and sporadic cancers are well-defined clinically, but generally poorly understood biochemically. Having a PTEN mutation until now has been viewed as having merely a passenger mutation or loss of a tumor suppressor in the context of another driver mutation. In certain tumors, mutated PTEN may actually be the driver, leaving little meaning to its known role as a "tumor suppressor". Both PTEN function (as a phosphatase) and subcellular localization are important in preventing tumorigenesis, but little is known about how signaling arises to change PTEN subcellular localization or how PTEN catalytic activity is regulated toward particular targets.

Recently a mutant PTEN, $PTEN^{G129E}$, has been defined as a gain of function (GOF) mutation based upon its phenotype in knockin mice. These mice develop more tumors than those expressing catalytically dead PTEN, or mice lacking PTEN. Only mutant $PTEN^{G129E}$ mice had mammary tumors. Interestingly, this finding has been validated, as human patients with the $PTEN^{G129E}$ Cowden's syndrome, are more likely than other patients with catalytically dead PTEN to have breast cancers (Bassi et al., 2013). The gain of function theory was first hypothesized when certain p53 mutations seemed to have more tumorigenic potential than when there was no p53 at all. This is considered to be due to a dominant negative homodimerization that has activity on an increased number of cell cycle transcriptional targets. Similarly, wild type PTEN has recently been shown to dimerize in its wild type state and with $PTEN^{G129E}$ (Papa et al., 2014). The mutant $PTEN^{G129E}$ specifically retains protein phosphatase activity but no lipid phosphatase activity. Therefore this phosphatase activity could be increased or be operating on different targets compared to wild type PTEN that then promotes proliferation and cell cycle progression.

We believe that PTEN does not always function as a tumor suppressor but an oncoprotein, and elucidating a mechanism by which mutant $PTEN^{G129E}$ overcomes cell cycle checkpoints to propagate cancer may reveal a reasonable treatment approach with PTEN inhibitors that are already in development (De Paula, Cui, Hossain, Antel, & Almazan, 2014; Schmid, Byrne, Vilar, & Woscholski, 2004; Walker & Xu, 2014). Similarly, understanding what roles

nuclear translocation plays in cell cycle may open up new treatment approaches for individuals with mutations outside of the PTEN catalytic domain.

MATERIALS AND METHODS

Cell Culture

Cell culture was performed in Dulbecco's modified Eagle's medium (DMEM)(Life Technologies) with 10% fetal bovine serum (FBS) and 1,000 units/ml penicillin-streptomycin (HyClone) solution at 37°C. The incubator was humidified with 5% CO_2 (Darnell, Levintow, & Scharff, 1970).

Transfections

Cells were transfected using the Polyethylenimine (PEI) method or Lipofectamine (Invitrogen)(Dalby et al., 2004). PEI transfections were performed by mixing PEI, obtained from Clark Wells, in a 1 µg PEI: 1 µg plasmid DNA in 300µl serum free media, followed by a 5 minute incubation period at room temperature. The PEI mixture was then added to the cells and incubated overnight and harvested for protein (Werth et al., 2006). Plasmid DNA was dependent on the size of the plate transfected, 5-6µg plasmid DNA was used for 60mm plates and 10-12µg was used for 100mm plates. Lipofectamine transfections were done according to manufacturer's protocol.

Purification of recombinant proteins

Escherichia coli (BL-21 or Rosetta) were transformed using manufacturer's guidelines, and grown overnight in 50ml culture for Luria broth (LB) under chloramphenicol selection. From this a 10% glycerol stock was made in a 1ml cryo-tube and stored at -80°C for later use. A 200ml culture of LB was

inoculated with 50μg/ml ampicillin and grown overnight at 150RPM at 37°C. In the morning, the culture was diluted with LB to 0.2 OD and then grown to between 0.4 OD to 0.6 OD. The culture was induced to product proteins with the addition of 1mM of Isopropyl-β-D-thio-galactoside (IPTG). Induction continued for 4-6 hours at the same temperature and RPM. Cells were spun down at 6000 RPM for 20 minutes and resuspended in 1X Phosphate Buffered Saline (PBS), 1% TritonX-100, 5mM DTT, and sonicated on ice. Debris was pelleted and the supernant was collected. Proteins were then subjected to glutathione sepharose using the Duo-Flow chromatography system from Bio-Rad. Protein was eluted from the column using 1mm glutathione. The eluted protein fractions were run on an SDS PAGE gel. The gel was stained with Coomassie Brilliant Blue R-250 stain (50%methanol:10% glacial acidic) for 20 minutes then destained in 50% methanol:10 glacial acidic acid). Fractions containing the corresponding bands were then collected and dialyzed overnight at 4°C in dialysis buffer (50mM Tris titrated to pH 7.5, 50mM NaCl, and 1mM DTT).

Protein analysis, immunoprecipitation, and Western blotting

Urea lysis buffer (20mM Tris, pH 6.8, 100mM sodium pyrophosphate, 6M urea) and a 2 second sonication on ice was used to lyse cells for whole cell extracts. A different lysis buffer was used for immunoprecipitation or GST pulldown. IGEPAL lysis buffer (25mM Tris, pH 8.0, 150mM NaCl, 1% IGEPAL, Protease Cocktail (Sigma) 1:100, 1mM sodium orthovanadate), and followed by 2 seconds of sonication on ice. The supernatant collected after centrifugation.

Protein concentration was determined using the supernatant and the use of Bradford Reagent (BioRad) per manufacturer's instructions.

For immunoprecipitation assays 500µg of lysates were incubated for 2 hours at 4 °C with 2µg of antibody in 1ml of lysis buffer and 25µl of Protein A or Protein G Agarose (Pierce). The precipitates spun out of solution by centrifugation, the supernate was removed and the pellet was resuspended in lysis buffer. The pellets were subjected to three rounds of this procedure. Laemmli dye was added and boiled for 10 minutes and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE).

Polyacrylamide gel was transferred overnight onto polyvinylidene fluoride (PVDF) membrane at 100mA. Upon transfer, PVDF was blocked with 5% nonfat dry milk in PBST (phosphate buffered saline with 0.5% Tween 20 for one hour while rocking. Primary antibody was added at the defined dilution for one hour. The membrane was washed with PBST 3 times. Secondary antibody, specific to species, was applied to the membrane in PBST at 1:10,000 dilution for 45 minutes followed by 3 washes, 10 minutes each. Enhanced chemiluminescence (ECL) was then applied and subjected to autoradigraph.

Cell cycle analysis

U87 cells were subjected to a 24 hour serum starvation, followed by varying treatments of 10% serum replacement, harvested and resuspended in PBS. Following a wash in PBS the cells were resuspended in 300 µl of 70% ethanol and 10µl Muse Cell Cycle Reagent from Millipore. Fixation was allowed

to take place overnight at 4 °C in the dark and the samples were run on the Muse Cell Analyzer according to manufacturer's instruction.

RESULTS

PTEN is a dual specificity phosphatase with activity on lipids and proteins (Myers et al., 1997). Early in the history of cancer research, PTEN was determined to have significant effects on tumorigenesis through its phosphatase activity on phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to phosphatidylinositol (3,5)-bisphosphate (PIP2), which antagonizes the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway that activates protein kinase B (Akt) with numerous downstream pro-growth, pro-angiogenic, and anti-apoptotic downstream effectors (Li et al., 1998)(Figure 5). However, PTEN has been shown to act on multiple protein targets (T. Gu et al., 2011). The PTEN mutant $PTEN^{G129E}$ has been shown to be null for lipid phosphatase activity but retain protein phosphatase activity (Myers et al., 1997)(Figure 7 and 8). This provides a model to study the protein phosphatase activity independently of the lipid phosphatase activity. A $PTEN^{G129E}$ knockin mouse has significantly more tumors in diverse organs than the catalytically dead PTEN mutant (Wang et al., 2010). In addition, the mutant $PTEN^{G129E}$ mice develop mammary tumors. Clinicially the $PTEN^{G129E}$ mutant was found to associate with breast cancer (Wang et al., 2010). However, *in vitro* or *in vivo* studies did not demonstrate how $PTEN^{G129E}$ was mechanistically promoting tumor progression. We show here that expression of $PTEN^{G129E}$ in cells leads to a growth advantage through specific modulation of cell cycle checkpoints.

Considering PTEN mutation or loss is a common aberration in human cancers, especially cancers with low survival rates such as glioblastoma multiforme, we sought to determine the role of the PTEN mutation $PTEN^{G129E}$ in the U87MG glioblastoma model (Liu, James, Frederick, Alderete, & Jenkins, 1997). A wild type PTEN, catalytically dead $PTEN^{C124S}$, and $PTEN^{G129E}$ retroviral construct were used to generate U87MG and $PTEN^{-/-}$ MEFs cell lines. Wild type PTEN has functioning lipid phosphatase and protein phosphatase activity. $PTEN^{C124S}$ encodes a PTEN mutant in which the catalytic cysteine is converted to a non-functioning serine, therefore rendering it completely lipid and protein phosphatase dead (Myers et al., 1997). Conversely, the $PTEN^{G129E}$ mutant has a glycine to glutamic acid mutation, which makes it lipid phosphatase dead, but maintains protein phosphatase activity (Figure 7) (Myers et al., 1997).

cAMP response element-binding protein (CREB) is phosphorylated at serine 133 (RRP**S**YRK) by Akt and has shown to be dephosphorylated by PTEN (T. Gu et al., 2011). Similarly we tested if the Akt phosphorylation sites on Mdm2 might also be dephosphorylated by PTEN (Figure 9). Using a phospho-antibody to serine 166 (RAI**S**TEE) on Mdm2 the Akt phosphorylation site, which was observed in the phosphorylated state in response under Akt activation (Mayo & Donner, 2001). We also tested another phosphorylation site on Mdm2, threonine 218, to show PTEN was acing specifically at serine 166 on Mdm2 (T. Zhang & Prives, 2001). Surprisingly, $PTEN^{G129E}$ actually caused a robust increase in the phosphorylation at threonine 218 of Mdm2 in both U87 cells as well as in PC3 cells, a prostate cancer cell line, while PTEN and PTENC124S did not show a

robust increase in the phosphorylation status at threonine 218 of Mdm2 (Figure 10).

Threonine 218 of Mdm2 has been reported to be phosphorylated by the Cyclin B/cdk1, mitosis-promoting complex (MPC) in murine cells (T. Zhang & Prives, 2001). The MPC complex is downregulated by Cdc25c and p27. When Cdc25c is dephosphorylated it is in its active conformation. Examination of phosphorylation state of Cdc25c showed dephosphorylation in the presence of $PTEN^{G129E}$. Examination by western blot of p27, a cycle inhibitor, whose levels are important for blockade of cell cycle movement, were decreased in $PTEN^{G129E}$ expressing cells (Figure 11 and 12, respectively)(Chu et al., 2007).

To determine how $PTEN^{G129E}$ may be influencing these cell cycle regulators we examine upstream regulator of Cdc25c, Checkpoint kinase 1(Chk1). Chk1 inactivates Cdc25c through the phosphorylation of serine 216. Examination of the active state of Chk1 through the phosphorylation of serine 317 showed that Chk1 was active in the $PTEN^{G129E}$ cells (Figure 13)(Sanchez et al., 1997). We next determined if protein phosphatase 2A (PP2A), which is known to dephosphorylate Cdc25c, was active (Forester, Maddox, Louis, Goris, & Virshup, 2007). PP2A had no change in phosphorylation at tyrosine 307 (Figure 14), when G129E was expressed. In addition we were unable to detect complex formation of G129E-PP2A complex. From these data, we concluded that G129E is regulating the cell cycle through other proteins and re-examined the role of p27 (Figure 15).

To assess if the cell cycle was active in G129E cells since they did have activated Cdc25c, flow cytometry cell cycle analyses were used. Cells reconstituted with G129E show an increased percentage of cells in G2/M versus PTEN null cells, 45.6% and 15.4%, respectively (Figure 16). This observation suggests that cells with G129E fail to accumulate in G1/G0, and that loss of p27 as an inhibitor of G1 could be causing this change. A time course study was conducted to examine the levels of cyclins in association with cell cycle movement. Western blots of Cyclin A show rapid increases with G129E when serum is added back which was not evident in PTEN. The other cyclins did not show such robust increases (Figure 17). Therefore, the cells may be continuously dividing rather than pausing at G1/G0. G129E has a distinctly different cell cycle profile than wild type PTEN, catalytically dead C124S, and vector control in U87 cells.

With the decreased levels of p27 shown by western blot in U87 cells transduced with G129E, and an increased proportion of cells in G2/M we investigated the regulation of p27 levels. As an inhibitor G1, it is heavily regulated by the tyrosine kinase Src. Under normal growth conditions, Src is an active kinase that can target p27 for phosphorylation, which inhibits its ability to bind cyclin dependent kinases, and eventually leads to p27 proteasomal degradation (Chu et al., 2007). Src is known for having both inhibitory and activating phosphorylation. Upon growth signaling, Src is targeted to the membrane where it is dephosphorylated at tyrosine 527, the inhibitory phosphorylation site. Then the activating autophosphorylation occurs at tyrosine

416, which accompanies a conformational change allowing Src to become an active kinase (Cooper, Gould, Cartwright, & Hunter, 1986; Irby & Yeatman, 2000; Okada & Nakagawa, 1988, 1989).

First we determined that when G129E is present, p27 levels are decreased by western blot and decreases further under growth conditions (Figure 18). Furthermore, the presence of G129E is associated with a decrease in the inhibitory phosphorylation of Src, but had no effect on the Src. Similarly expression of wild type PTEN was also associated with a moderate decrease in inhibitory phosphorylation Src levels. Thus protein phosphatase activity and Src inactivating phosphorylation levels are associated (Figure 19). In addition bpV(HOpic) (a potent PTEN inhibitor) reversed the inhibitory phosphorylation that had been decreased to levels comparable to cells without G129E (Schmid et al., 2004)(Figure 20). As similarly shown by Zhang et al who were able to precipitate a PTEN-Src interaction, we showed an immunoprecipitation of transduced Flag-G129E by the flag tag resulted in precipitation of Src in both MEFs and U87 cells (Figure 21)(S. Zhang et al., 2011).

Thus, G129E promotes cell cycle movement and may be considered a gain of function mutant through the downregulation of p27, and the activation of Cdc25. The modulation of cell cycle through Src and p27 may result in the increased tumorigenesis seen in mouse models and human patients.

TABLES

Protein	Phosphorylation	Sequence
Cdc25C	S216	RSP**S**MPE
Chk1	S317	YSS**S**QPE
Mdm2	T218	STG**T**PSN
PP2A	Y307	TPD**Y**FL
Src	Y416	DNE**Y**TAR
Src	Y527	EPQ**Y**QPG

Table 1: Phosphorylation sites and sequences

FIGURES

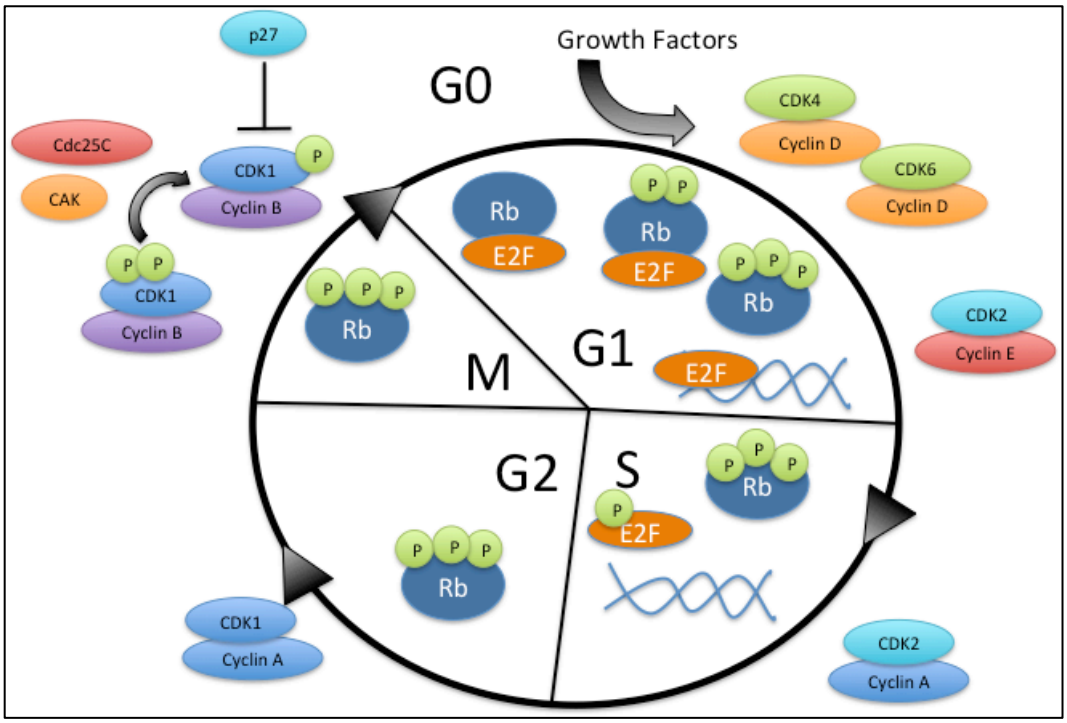

Figure 1: Cell cycle and regulators
As growth factors increase, Rb begins to be hyperphosphorylated, and loses affinity to bind E2F. E2F is then free to transcribe cyclins necessary for the cell cycle. Particular cyclins and their cyclin dependent kinases increase levels then promptly decrease as the cell cycle progresses. Cyclin D and cyclin E are high in G1, Cyclin A is high in G2/S, and Cyclin B is high in M. CAK phosphorylates tyrosine 161 on CDK1 and he regulatory phosphatase Cdc25C dephosphorylates tyrosine 14 and 14 on CDK1 to initiate mitosis after interphase. The cell cycle inhibitor may inhibit CDK1/Cyclin B activity.

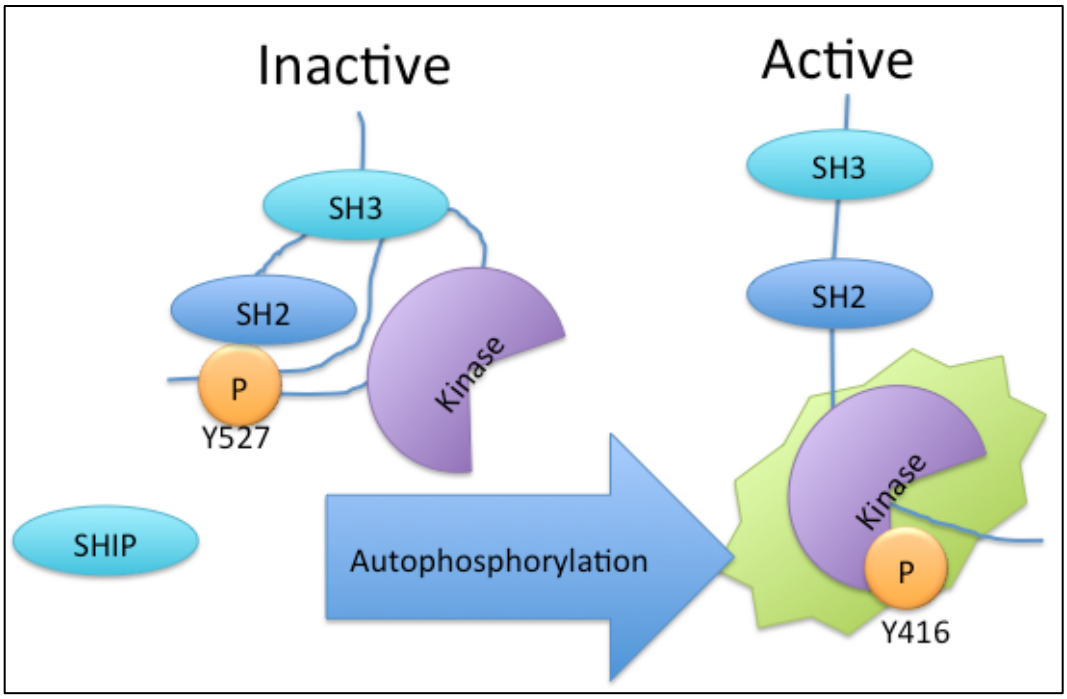

Figure 2: Src activation
Src becomes activated when properly docked and dephosphorylated at tyrosine 416 by SHIP. A conformational change of the SH2, SH3, and kinase domain causes an autophosphorylation at tyrosine 416 that renders the kinase domain active.

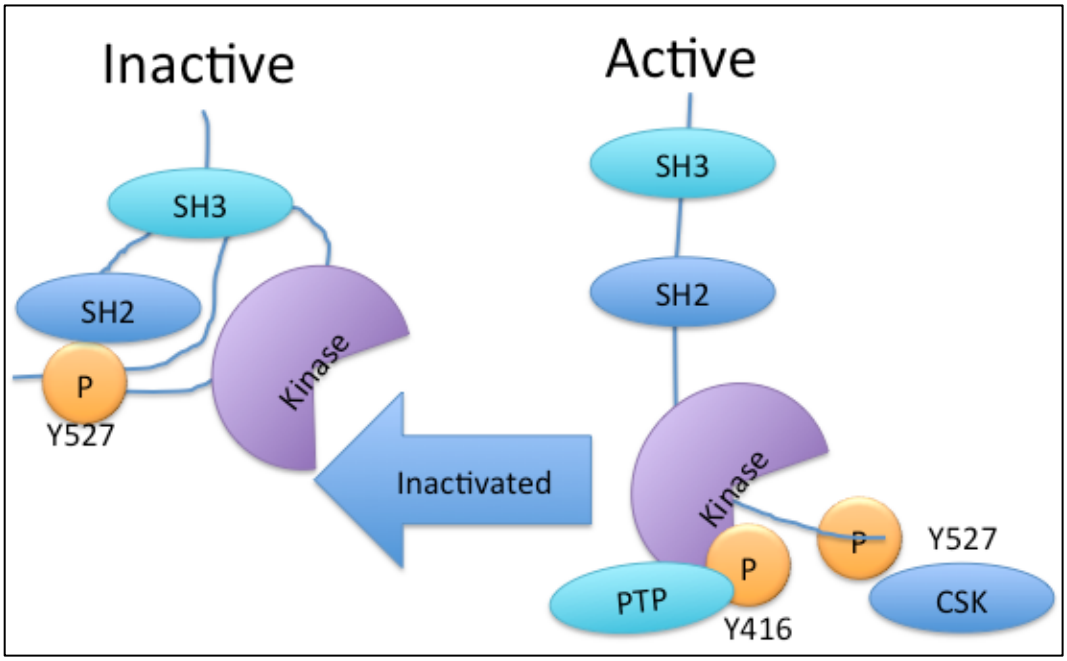

Figure 3: Src Inactivation
In order for Src to be inactivate, CSk must bind the Src kinase domain. Then PTP can dephosphorylate tyrosine 416 and CSK can phosphorylate tyrosine 527. Src is then inactivated and returned to the closed conformation.

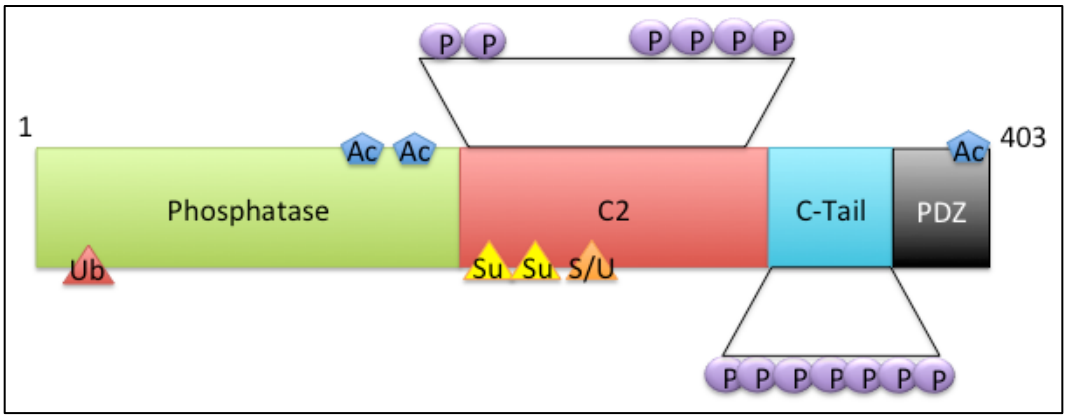

Figure 4: PTEN posttranslational modifications
Ubiquitin can be conjugated onto Lysine 13. Sumoylation conjugation can happen at lysine 254 and 256. Either SUMO or Ubiquitin can be conjugated onto lysine 289. Acetlyation by PCAF can occur at lysine 125 and 128. CBP can acetylate PTEN at lysine 402. Phosphorylation at the C-terminal tail can occur at threonine 336 by GS3Kβ. CK2 can phosphorylate the C-terminal tail at serine 370, serine 380, threonine 382, threonine 383, and serine 385. ATM can phosphorylate PTEN at serine 398. ROCK can phosphorylate PTEN at serine 229, threonine 232, threonine 319, and threonine 321. Rak can phosphorylate PTEN at tyrosine 336.

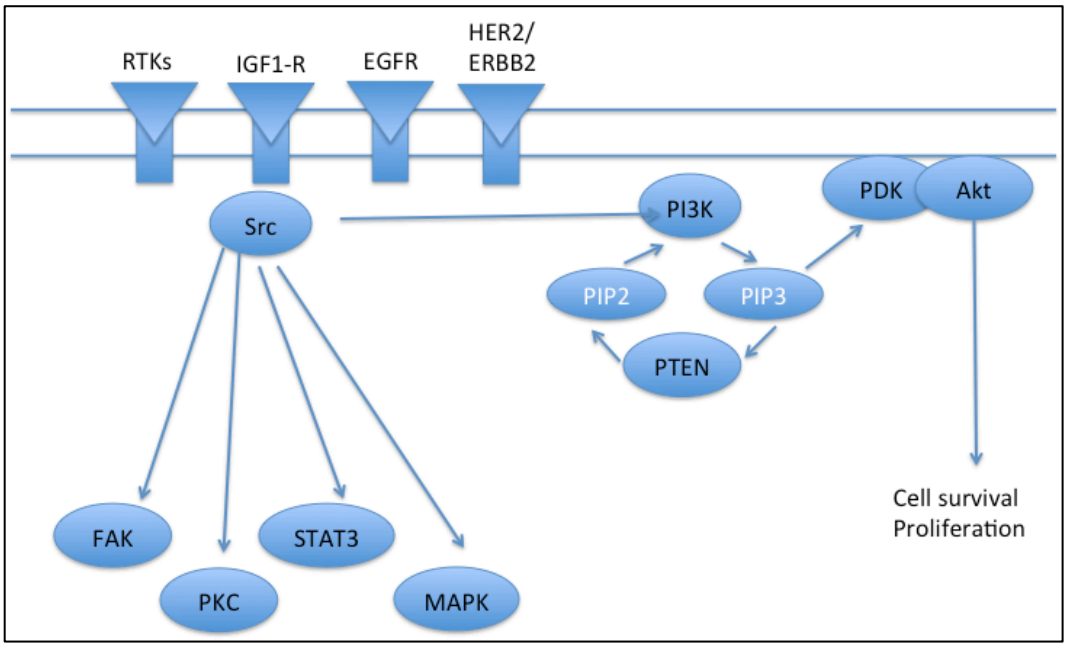

Figure 5: Src, PI3K, and PTEN canonical pathway
Src is a tyrosine kinase that can be activated by many membrane receptors including RTKs, IGF1-R, EGFR, and HER2/ERBB2. Src activates PI3K to phosphorylate PIP2 to PIP3. High concentrations of PIP3 allows recruitment of PDK and Akt to the membrane where they are activated to phosphorylate downstream survival and proliferative pathways. However, this pathway is inhibited by PTEN that dephosphorylates PIP2 leading to a decreased concentration of PIP3 at the membrane, and decreased active Akt at the membrane.

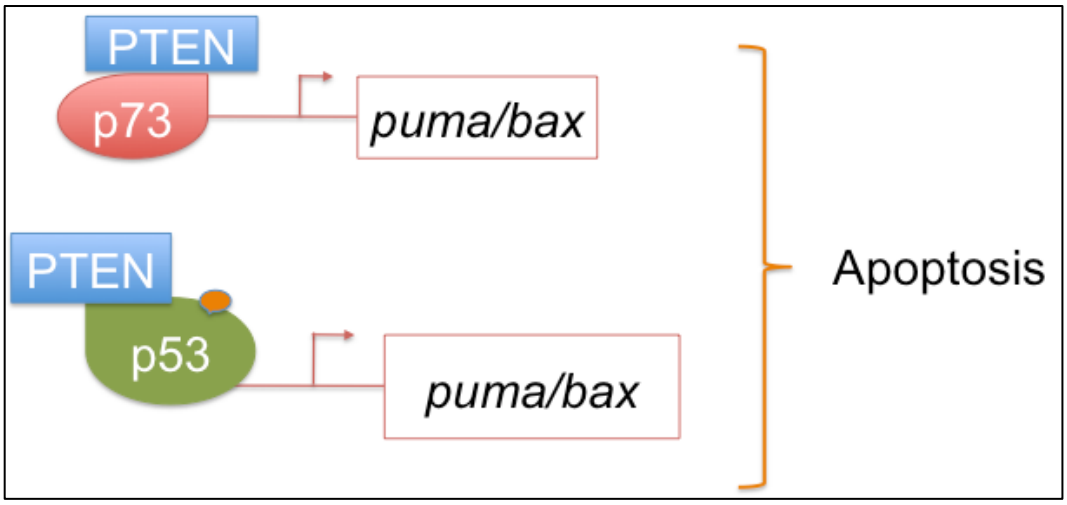

Figure 6: PTEN non-canonical functions
PTEN under DNA damage conditions, can complex with p53 or p73 to transcribe apoptotic genes like puma and bax.

Figure 7: Mutations of the phosphatase domain
Catalytically inactive PTEN^{C124S} and PTEN^{G129E} are located in the catalytic pocket of the phosphatase domain.

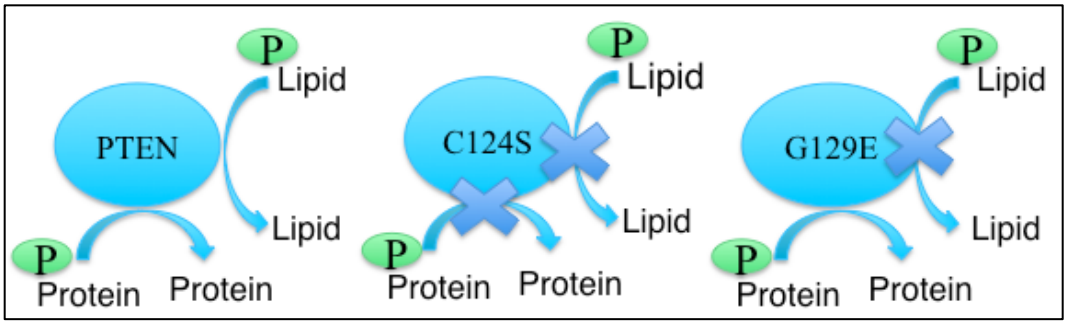

Figure 8: PTEN and mutants
Phosphatase and tensin homolog (PTEN) is both a lipid and protein phosphatase. Mutant PTEN^{C124S} conversely has lost both protein and lipid phosphatase activity as a result of the mutation of the catalytic cysteine to serine. Mutant PTEN^{G129E} has retained protein phosphatase activity but lost lipid phosphatase activity.

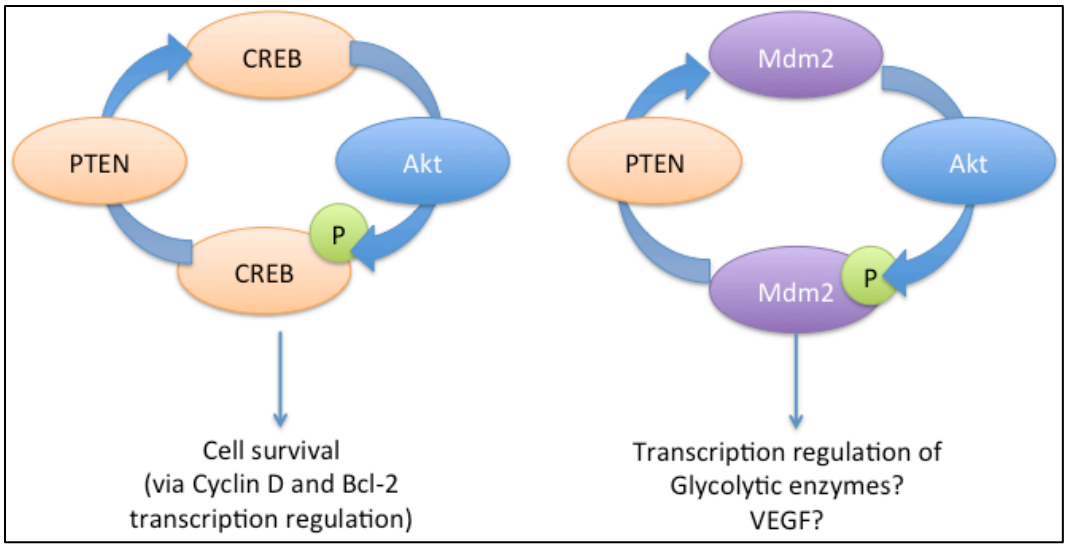

Figure 9: PTEN targets CREB, but not Mdm2
Akt phosphorylates CREB on serine 133, and PTEN dephosphorylates that site. Mdm2 has a similar Akt phosphorylation site on serine 166. PTEN does not dephosphorylate this site.

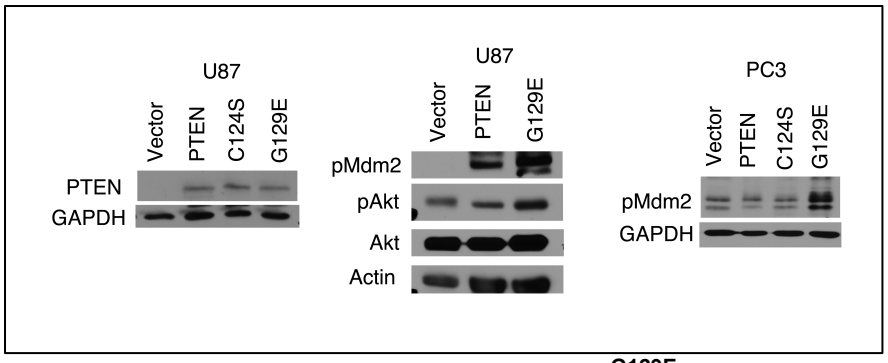

Figure 10: Mdm2 modulated when PTEN^{G129E} is expressed
U87MG glioblastoma cells transduced with either null for PTEN (vector control), PTEN^{wt}, and PTEN^{G129E} were subjected to western blotting for PTEN, phospho-Mdm2 Serine 218, activating phospho-Akt serine 473, total Akt and loading control GAPDH. PC3 Cells transduced with vector control (null for PTEN), PTEN^{C124S}, or PTEN^{G129E} were subjected to western blotting for phospho-Mdm2 serine 218.

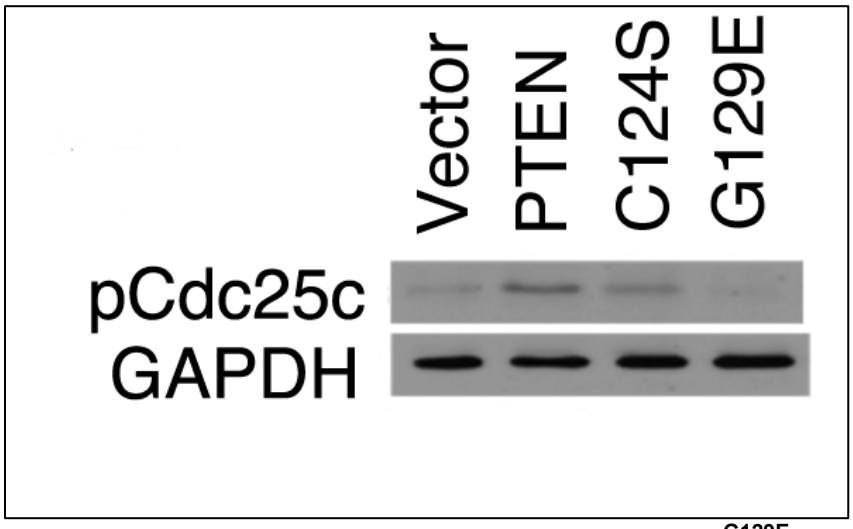

Figure 11: Cdc25c activity modulation by PTEN ^{G129E}
U87 glioblastoma cells transduced with vector control, PTEN^{wt}, PTEN^{C124S}, or PTEN^{G129E} were subjected to western blotting for phospho-Cdc25c at serine 216 levels and loading control GAPDH.

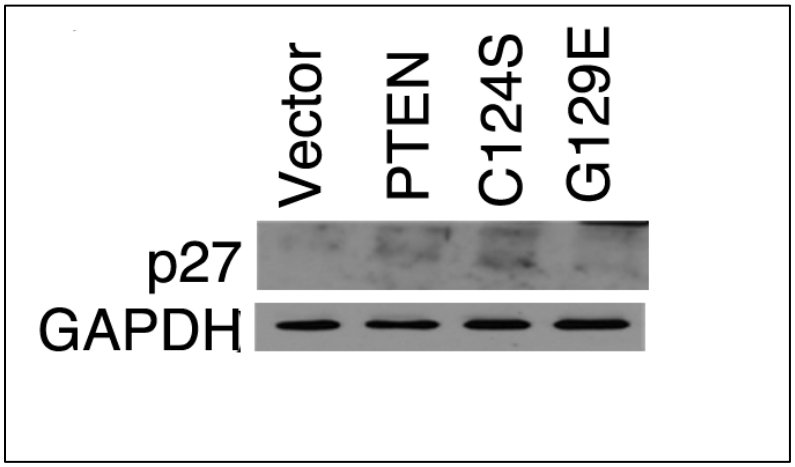

Figure 12: p27 levels in U87 cells with PTEN and mutants
U87 glioblastoma cells transduced with vector control, $PTEN^{wt}$, $PTEN^{C124S}$, and $PTEN^{G129E}$ were subjected to western blotting with p27 and loading control GAPDH.

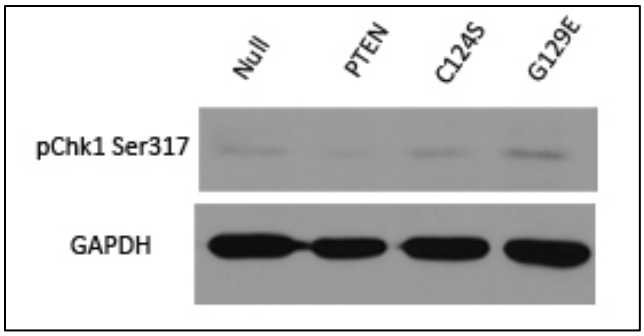

Figure 13: Analysis of active Chk1
U87 cells transduced with vector control, $PTEN^{wt}$, $PTEN^{C124S}$, or $PTEN^{G129E}$ were subjected to western blotting for phospho-Chk1 at serine 317, and GAPDH.

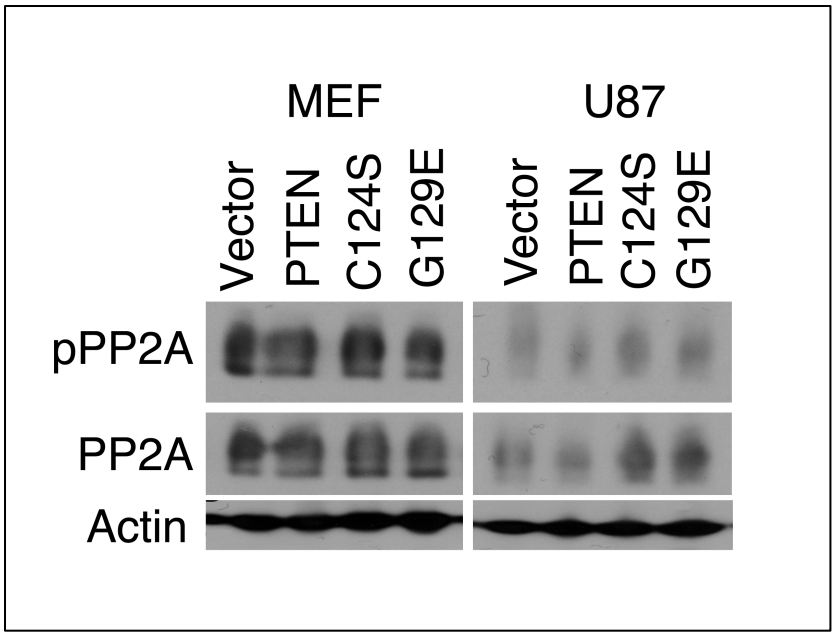

Figure 14: PP2A phosphorylation and levels
PTEN knockout MEFs and U87 cells were virally transduced with vector control, $PTEN^{wt}$, $PTEN^{C124S}$ or $PTEN^{G129E}$. Lysates were subjected to western blotting for both phospho-PP2A at tyrosine 307, total PP2A, and loading control Actin.

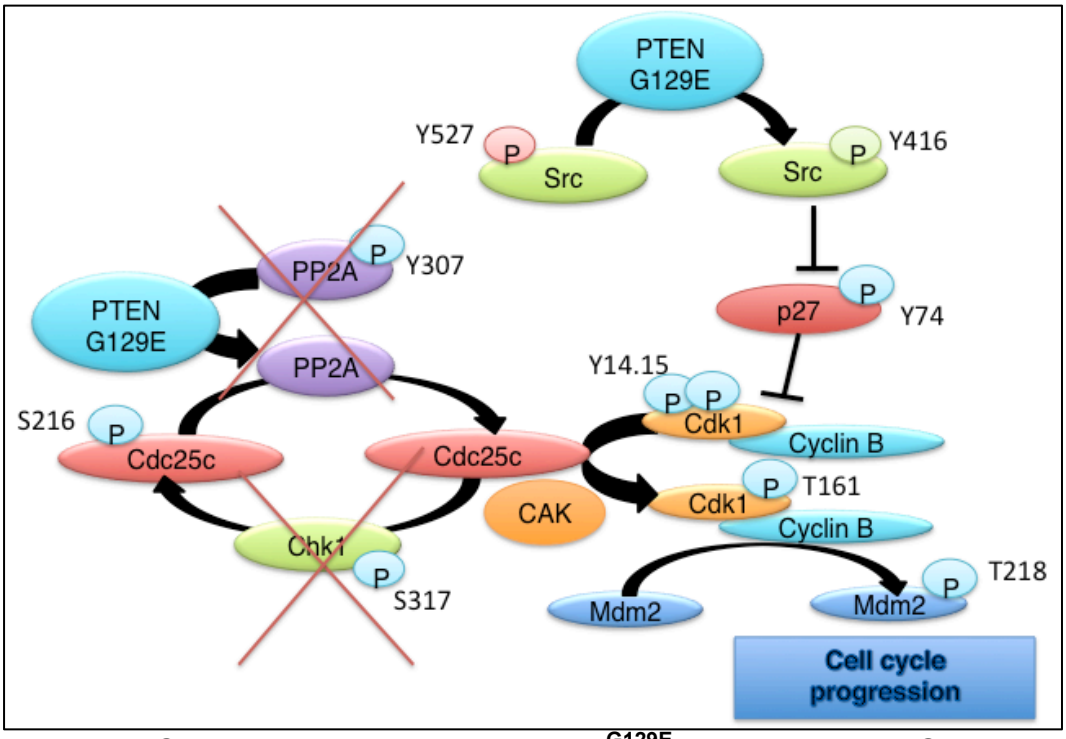

Figure 15: Cell cycle regulation by PTEN^{G129E} through p27 or Cdc25c
Schematic of mutant PTEN^{G129E} acting to propagate cell cycle through activity through two means: Direct activity on PP2A, leading to activation of Cdc25c, which directly activates the Cdk1/Cyclin B complex and then downstream phosphorylation of Mdm2 or an interaction associated with Src activation. Src phosphorylates p27 targeting it for further modification and eventual degradation at the 26S proteasome.

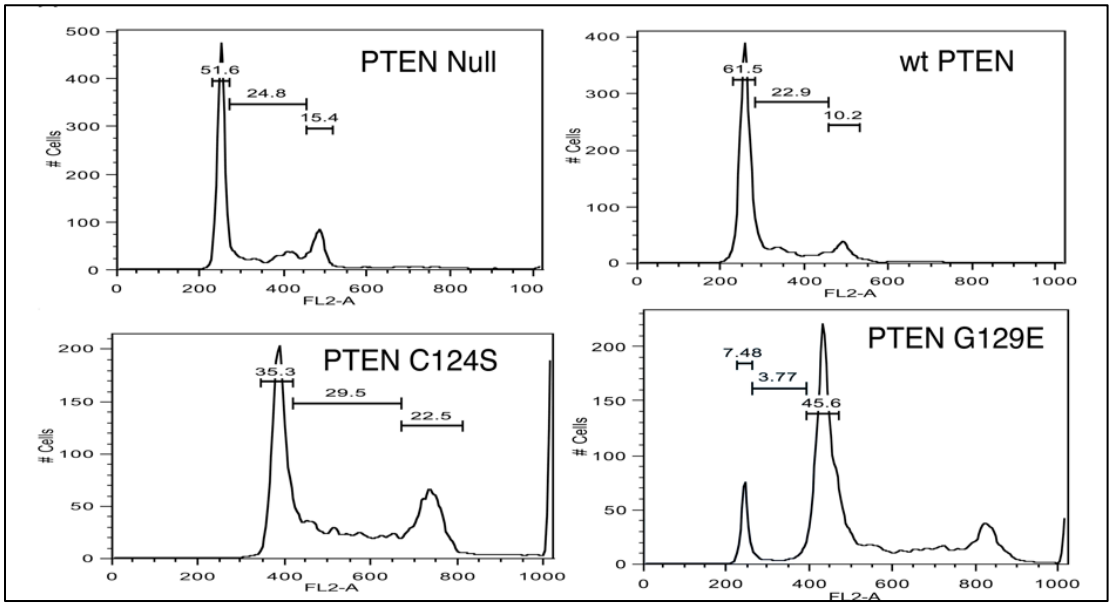

Figure 16: Cell cycle analysis of U87 cells
Cell cycle analysis was performed on U87 glioblastoma cells transduced with vector control, $PTEN^{wt}$, $PTEN^{C124S}$, or $PTEN^{G129E}$.

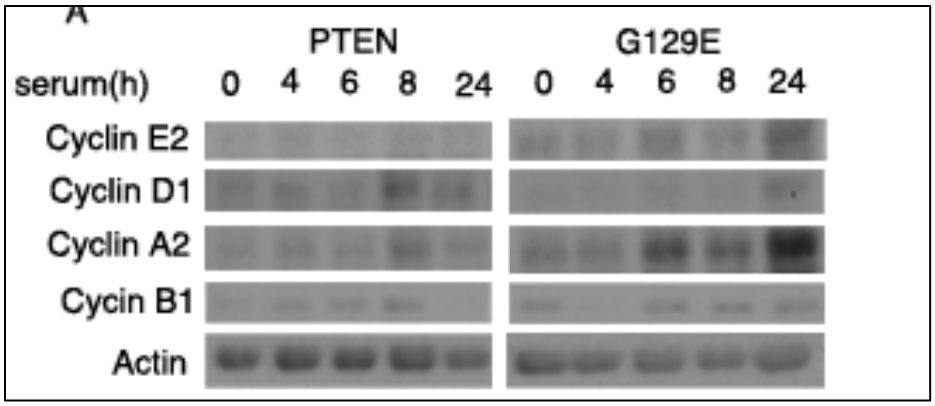

Figure 17: Analysis of cyclin levels over a time course
U87 cells transduced with $PTEN^{wt}$ or $PTEN^{G129E}$ were serum starved for 24 hours then subjected to replacement of serum for a time course of 0,4,6,8, and 24 hours. Lysates were subjected to western blotting for Cyclin E2, Cyclin D1, Cyclin A2, Cyclin B1, and Actin.

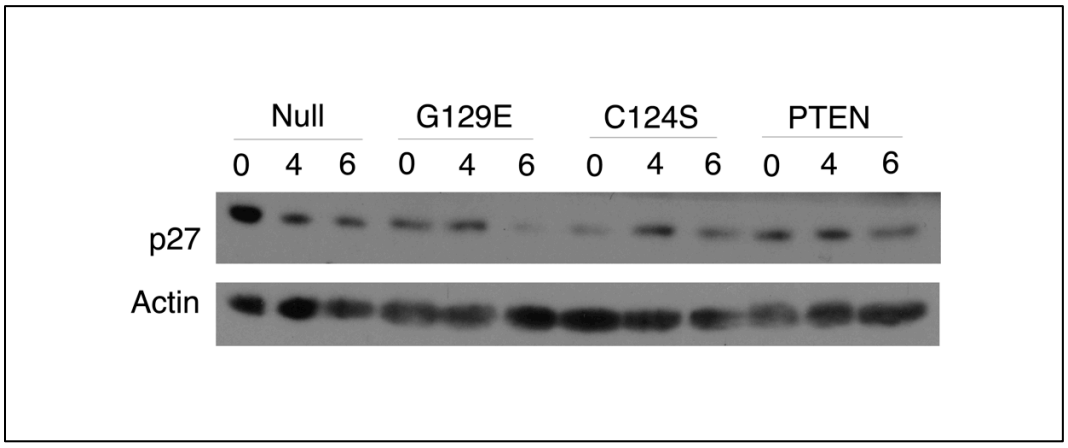

Figure 18: Serum time course of p27 levels in U87 cell lines
U87 cells transduced with vector control (null for PTEN), $PTEN^{G129E}$, $PTEN^{C124S}$, or $PTEN^{wt}$ were subjected to a 24 hour serum starvation then serum was replaced for a time course of 0,4, or 6 hours. Lysates were then subjected to western blotting or p27 levels and loading control Actin.

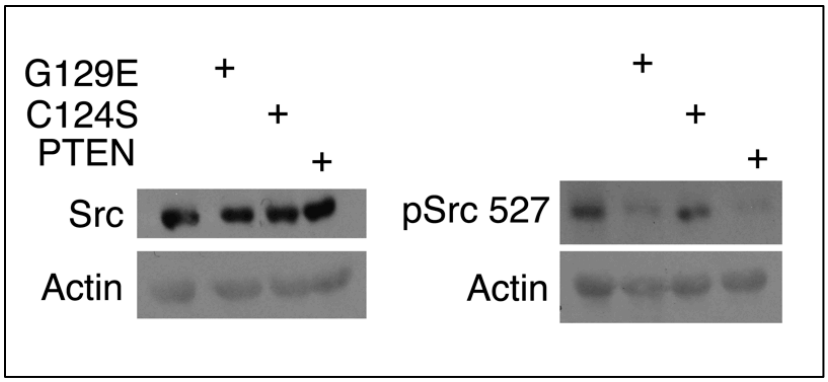

Figure 19: Src levels and phosphorylation in PTEN mutants
U87 cells transduced with vector control, $PTEN^{G129E}$, $PTEN^{C124S}$, or $PTEN^{wt}$ were lysed and subjected to western blotting for total Src levels as well as the inhibitory phosphorylation of tyrosine 527 as well as the loading control Actin.

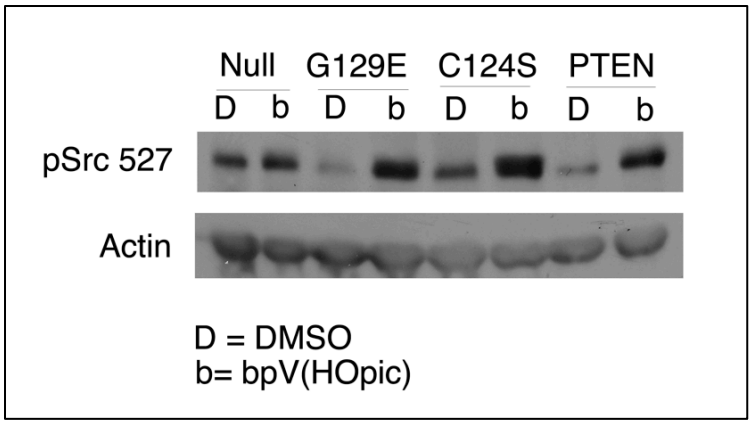

Figure 20: Analysis of Src phosphorylation with selective PTEN inhibitor
U87 cells were treated with either DMSO or 2μM PTEN inhibitor, bpV(HOpic) for 2 hours. Lysates were then subjected to western blotting for the inhibitory phosphorylation at tyrosine 527 and Actin.

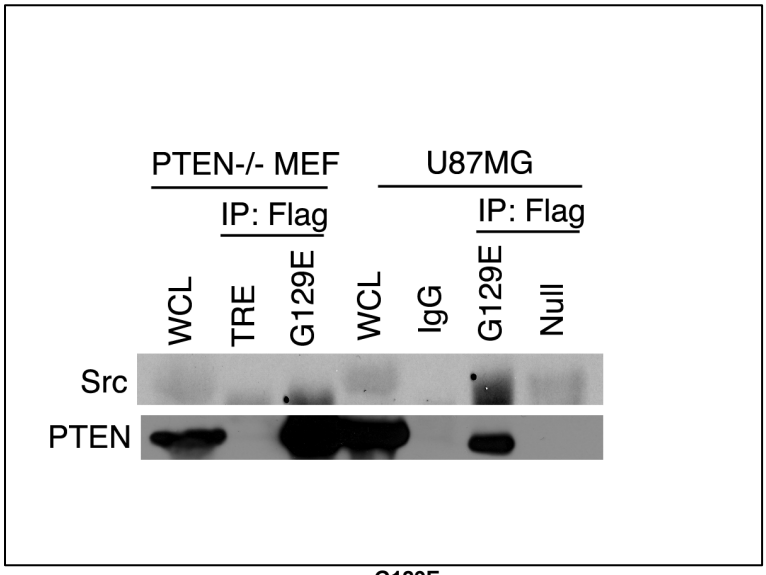

Figure 21: Src and PTEN^{G129E} complex
PTEN KO MEFs were transduced with Flag-PTEN^{G129E} and subjected to immunoprecipitation by Flag. Precipitates and whole cell lysates were subjected to western blotting for total Src and PTEN.

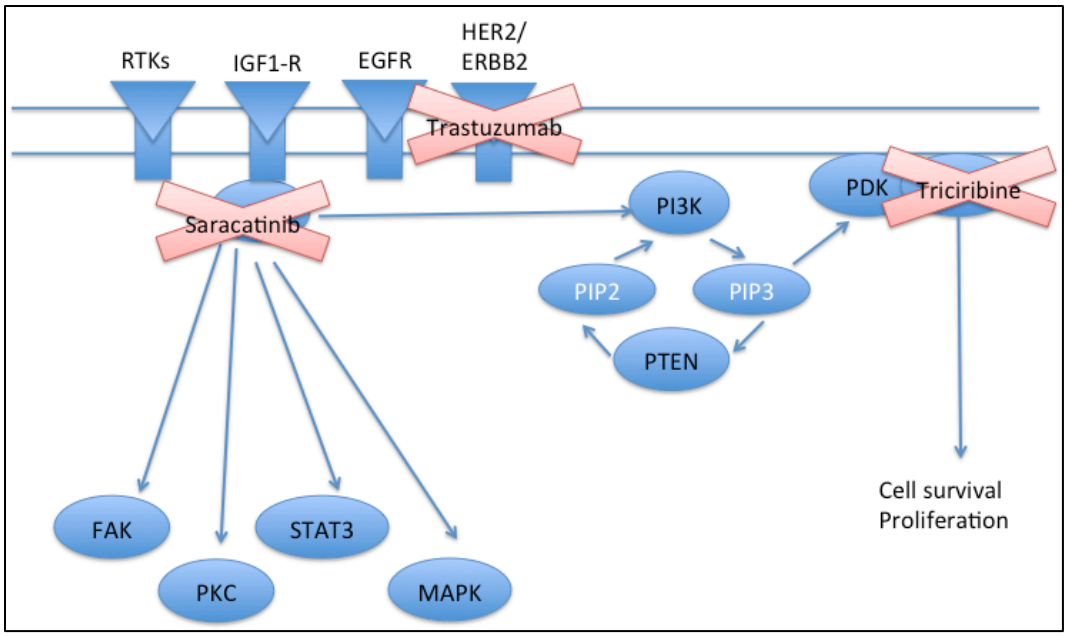

Figure 22: Combinational therapy approaches to combat resistance
High resistance to trastuzumab (HER2 inhibitor) alone and in combination with tricibine (Akt inhibitor), lead to approaches of trastuzumab in combination with saracatinib (Src inhibitor. These approaches lead to the discovery of a PTEN and Src complex.

DISCUSSION

Although PTEN has been classified as a tumor suppressor and its role as such is evident in disease states such as Cowden's syndrome, we show here that PTEN could be considered an oncoprotein with a specific mutation in the catalytic domain, G129E. Previous literature has shown the protein phosphatase activity by $PTEN^{G129E}$ (Myers et al., 1997). Knowledge of $PTEN^{G129E}$ function was further expanded by Wang et al who showed increased tumor incidence in $PTEN^{G129E}$ knockin mice (Wang et al., 2010). Furthermore we intended to gain a mechanistic understanding of signaling regulated by the gain of function $PTEN^{G129E}$.

Due to the nature of the mutation being known for being lipid phosphatase dead but retaining protein phosphatase activity, it was hypothesized that $PTEN^{G129E}$ could be functioning as a gain of function mutation through an additional protein target (Wang et al., 2010). The tumor suppressor mutation and association with tumor incidence gave clues as to how to investigate $PTEN^{G129E}$ as a tumor suppressor with a gain of function mutation. Literature gives one example of an important gain of function mutation in p53. Certain mutations in p53 are associated with increased tumor incidence greater than that of merely loss of p53. It is hypothesized that these mutations allow p53 to transcribe additional targets that increase growth and chemotherapeutic resistance (Dittmer et al., 1993; Runnebaum, Kieback, Tong, & Kreienberg, 1993). Although p53 is a transcription factor, and PTEN is a phosphatase, we drew from these findings

that additional targets could be a means of a mutation gaining tumorigenic potential.

Though efforts to deem Mdm2 or PP2A as direct protein targets were not possible, distinct cell cycle profile deviations pointed us into the direction of p27, a cell cycle inhibitor that had decreased levels in cells expressing $PTEN^{G129E}$. The cell cycle inhibitor, p27 has been considered a player in cancer and regulation of the cell cycle since the early 90's. In addition it is inversely associated with poor prognosis in cancer patients (Esposito et al., 1997). Considering the cell cycle profile indicated that cells containing $PTEN^{G129E}$ were not pausing in G1, modulation through a G1 inhibitor was plausible (Toyoshima & Hunter, 1994). Furthermore, p27 is regulated by the tyrosine kinase Src, and Cyclin E/CDK2 (Chu et al., 2007; Sheaff, Groudine, Gordon, Roberts, & Clurman, 1997).

Since Src has long been known to be an important kinase in cancers. Src is a kinase that is activated upon growth signaling. When tyrosine 527 is phosphorylated and tyrosine 436 is not, Src is considered inactive. Conversely, when tyrosine 436 is phosphorylated and tyrosine 527 is not, the kinase is considered active (Dehm & Bonham, 2004). To determine if Src was playing an active role in the ability for $PTEN^{G129E}$ to modulate the cell cycle we subjected to western blotting for Src phosphorylation using the phosphotyrosine 527 antibody, and saw decreased phosphorylation when G129E and wild type PTEN were present. Furthermore, when the PTEN inhibitor was used, the phosphorylation of inhibitory phospho-tyrosine increased. Conversely, Zhang et al showed that

PTEN could dephosphorylate Src at tyrosine 416 (activating site), while we show PTEN and PTEN^{G129E} modulating tyrosine 527 (inhibiting site)(S. Zhang et al., 2011). Thus the functional implications of these data are opposing.

Through immunoprecipitation of PTEN^{G129E} by Flag, Src was precipitated, which was corroborated with the finding by Zhang et al where wild type PTEN was precipitated with Src (S. Zhang et al., 2011). It is clear that an interaction is occurring both from our own findings in MEFs and U87 glioblastoma cells and from Zhang et al with BT474 breast cancer cells, but it is unclear what those interactions are doing and if a particular unknown condition also plays a role. Due to the resistance seen to trastuzumab both acquired and de novo, Zhang et al intended to find combinations of therapies that could combat this phenomena. Initially, the group tried to inhibit Akt since multiple receptors could be activating the Src/PI3K pathway. However, little effect was seen so the group inspected Src activity levels and realized that PTEN may be capable of inactivating Src. Through this discovery, the group inhibited Src in combination with trastuzumab to more effectively turn off the Src/PI3K pathway. Although they discovered the same interaction of PTEN and Src, they found Src modulating differently in response to the interaction. They show western blots with each PTEN mutant transduced inactivating Src when we show activation of Src. While our experiments were done in U87 glioblastoma cells in our laboratory, The Zhang laboratory did theirs in MDA-MB-468 cells. Therefor cell type specific signaling may be occurring. Furthermore Src is not in completely inactive state with respect to phosphorylation in the publication. Activity of PTEN in the Zhang lab

only modulated the phosphorylation at tyrosine at 416, and the phosphorylation at tyrosine 527 was not returned to the inactive conformation. Further soft agar transformation experiments were done with Src^{Y527F}(CA) and Src^{K295R}(DN) when they were never able to show this phosphorylation site's state change with/without PTEN. These did show transformation, but don't model the action of PTEN on Src, only Src on transformation(S. Zhang et al., 2011)(Figure 22).

From our findings we conclude that $PTEN^{G129E}$ forms a complex with Src and that may be responsible for the activation of Src. Subsequent decreases in p27 levels thereby allow cancerous cells to pass through the G1 checkpoint and continue such that FLOW cytometry captures G129E cells in G2/M. This modulation of the cell cycle through Src and p27 could be responsible for the increased number of tumors in mice with G129E and the increased cancer risk in human patients with the G129E mutation. Biochemically, in literature with the vast numbers of kinases responsible for oncogenic activity, it is unusual and unique to find a phosphatase that could be acting as an oncoprotein.

In future experiments we intend to investigate further why our data differs from the Zhang et al, group examining in our own hands the phosphorylation levels at both sites on Src under expression of PTEN mutants in breast cancer cell lines, as well as in MEFs. We intend to see if PTEN inhibition will also increase p27 expression when G129E is expressed, showing a dependence of the cell cycle on G129E.

REFERENCES

Adey, N. B., Huang, L., Ormonde, P. A., Baumgard, M. L., Pero, R., Byreddy, D. V., . . . Bartel, P. L. (2000). Threonine phosphorylation of the MMAC1/PTEN PDZ binding domain both inhibits and stimulates PDZ binding. *Cancer Res, 60*(1), 35-37.

Al-Khouri, A. M., Ma, Y., Togo, S. H., Williams, S., & Mustelin, T. (2005). Cooperative phosphorylation of the tumor suppressor phosphatase and tensin homologue (PTEN) by casein kinases and glycogen synthase kinase 3beta. *J Biol Chem, 280*(42), 35195-35202. doi: 10.1074/jbc.M503045200

Alimandi, M., Wang, L. M., Bottaro, D., Lee, C. C., Kuo, A., Frankel, M., . . . Pierce, J. H. (1997). Epidermal growth factor and betacellulin mediate signal transduction through co-expressed ErbB2 and ErbB3 receptors. *EMBO J, 16*(18), 5608-5617. doi: 10.1093/emboj/16.18.5608

Ashkenazi, A., & Dixit, V. M. (1999). Apoptosis control by death and decoy receptors. *Curr Opin Cell Biol, 11*(2), 255-260.

Ayrapetov, M. K., Wang, Y. H., Lin, X., Gu, X., Parang, K., & Sun, G. (2006). Conformational basis for SH2-Tyr(P)527 binding in Src inactivation. *J Biol Chem, 281*(33), 23776-23784. doi: 10.1074/jbc.M604219200

Bassi, C., Ho, J., Srikumar, T., Dowling, R. J., Gorrini, C., Miller, S. J., . . . Stambolic, V. (2013). Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. *Science, 341*(6144), 395-399. doi: 10.1126/science.1236188

Berger, M., Vogt Sionov, R., Levine, A. J., & Haupt, Y. (2001). A role for the polyproline domain of p53 in its regulation by Mdm2. *J Biol Chem, 276*(6), 3785-3790. doi: 10.1074/jbc.M008879200

Bos, J. L. (1989). ras oncogenes in human cancer: a review. *Cancer Res, 49*(17), 4682-4689.

Breeden, L. L. (2000). Cyclin transcription: Timing is everything. *Curr Biol, 10*(16), R586-588.

Cheung, C. Y., Singh, M., Ebaugh, M. J., & Brace, R. A. (1995). Vascular endothelial growth factor gene expression in ovine placenta and fetal membranes. *Am J Obstet Gynecol, 173*(3 Pt 1), 753-759.

Chiarugi, V., Magnelli, L., & Cinelli, M. (1997). Complex interplay among apoptosis factors: RB, p53, E2F, TGF-beta, cell cycle inhibitors and the bcl2 gene family. *Pharmacol Res, 35*(4), 257-261. doi: 10.1006/phrs.1997.0140

Chu, I., Sun, J., Arnaout, A., Kahn, H., Hanna, W., Narod, S., . . . Slingerland, J. (2007). p27 phosphorylation by Src regulates inhibition of cyclin E-Cdk2. *Cell, 128*(2), 281-294. doi: 10.1016/j.cell.2006.11.049

Cooper, J. A., Gould, K. L., Cartwright, C. A., & Hunter, T. (1986). Tyr527 is phosphorylated in pp60c-src: implications for regulation. *Science, 231*(4744), 1431-1434.

Dalby, B., Cates, S., Harris, A., Ohki, E. C., Tilkins, M. L., Price, P. J., & Ciccarone, V. C. (2004). Advanced transfection with Lipofectamine 2000

reagent: primary neurons, siRNA, and high-throughput applications. *Methods, 33*(2), 95-103. doi: 10.1016/j.ymeth.2003.11.023
Darnell, J. E., Levintow, L., & Scharff, M. D. (1970). Harry Eagle. *J Cell Physiol, 76*(3), 241-252. doi: 10.1002/jcp.1040760304
De Paula, M. L., Cui, Q. L., Hossain, S., Antel, J., & Almazan, G. (2014). The PTEN inhibitor bisperoxovanadium enhances myelination by amplifying IGF-1 signaling in rat and human oligodendrocyte progenitors. *Glia, 62*(1), 64-77. doi: 10.1002/glia.22584
Degos, L. (2001). John Hughes Bennett, Rudolph Virchow... and Alfred Donne: the first description of leukemia. *Hematol J, 2*(1), 1. doi: 10.1038/sj/thj/6200090
Dehm, S. M., & Bonham, K. (2004). SRC gene expression in human cancer: the role of transcriptional activation. *Biochem Cell Biol, 82*(2), 263-274. doi: 10.1139/o03-077
Dittmer, D., Pati, S., Zambetti, G., Chu, S., Teresky, A. K., Moore, M., . . . Levine, A. J. (1993). Gain of function mutations in p53. *Nat Genet, 4*(1), 42-46. doi: 10.1038/ng0593-42
Esposito, V., Baldi, A., De Luca, A., Groger, A. M., Loda, M., Giordano, G. G., . . . Giordano, A. (1997). Prognostic role of the cyclin-dependent kinase inhibitor p27 in non-small cell lung cancer. *Cancer Res, 57*(16), 3381-3385.
Evan, G., & Littlewood, T. (1998). A matter of life and cell death. *Science, 281*(5381), 1317-1322.
Forester, C. M., Maddox, J., Louis, J. V., Goris, J., & Virshup, D. M. (2007). Control of mitotic exit by PP2A regulation of Cdc25C and Cdk1. *Proc Natl Acad Sci U S A, 104*(50), 19867-19872. doi: 10.1073/pnas.0709879104
Fragoso, R., & Barata, J. T. (2014). Kinases, tails and more: Regulation of PTEN function by phosphorylation. *Methods*. doi: 10.1016/j.ymeth.2014.10.015
Gabrielli, B. G., Lee, M. S., Walker, D. H., Piwnica-Worms, H., & Maller, J. L. (1992). Cdc25 regulates the phosphorylation and activity of the Xenopus cdk2 protein kinase complex. *J Biol Chem, 267*(25), 18040-18046.
Gately, S., Twardowski, P., Stack, M. S., Cundiff, D. L., Grella, D., Castellino, F. J., . . . Soff, G. A. (1997). The mechanism of cancer-mediated conversion of plasminogen to the angiogenesis inhibitor angiostatin. *Proc Natl Acad Sci U S A, 94*(20), 10868-10872.
Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell, 140*(6), 883-899. doi: 10.1016/j.cell.2010.01.025
Gu, J., Tamura, M., Pankov, R., Danen, E. H., Takino, T., Matsumoto, K., & Yamada, K. M. (1999). Shc and FAK differentially regulate cell motility and directionality modulated by PTEN. *J Cell Biol, 146*(2), 389-403.
Gu, T., Zhang, Z., Wang, J., Guo, J., Shen, W. H., & Yin, Y. (2011). CREB is a novel nuclear target of PTEN phosphatase. *Cancer Res, 71*(8), 2821-2825. doi: 10.1158/0008-5472.CAN-10-3399
Haapajarvi, T., Kivinen, L., Pitkanen, K., & Laiho, M. (1995). Cell cycle dependent effects of u.v.-radiation on p53 expression and retinoblastoma protein phosphorylation. *Oncogene, 11*(1), 151-159.

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell, 100*(1), 57-70.
Hayflick, L. (1997). Mortality and immortality at the cellular level. A review. *Biochemistry (Mosc), 62*(11), 1180-1190.
Hengst, L., & Reed, S. I. (1998). Inhibitors of the Cip/Kip family. *Curr Top Microbiol Immunol, 227*, 25-41.
Honda, R., Tanaka, H., & Yasuda, H. (1997). Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett, 420*(1), 25-27.
Hu, W., Feng, Z., & Levine, A. J. (2012). The Regulation of Multiple p53 Stress Responses is Mediated through MDM2. *Genes Cancer, 3*(3-4), 199-208. doi: 10.1177/1947601912454734
Ikenoue, T., Inoki, K., Zhao, B., & Guan, K. L. (2008). PTEN acetylation modulates its interaction with PDZ domain. *Cancer Res, 68*(17), 6908-6912. doi: 10.1158/0008-5472.CAN-08-1107
Irby, R. B., & Yeatman, T. J. (2000). Role of Src expression and activation in human cancer. *Oncogene, 19*(49), 5636-5642. doi: 10.1038/sj.onc.1203912
Lammers, R., Bossenmaier, B., Cool, D. E., Tonks, N. K., Schlessinger, J., Fischer, E. H., & Ullrich, A. (1993). Differential activities of protein tyrosine phosphatases in intact cells. *J Biol Chem, 268*(30), 22456-22462.
Lang, V., Aillet, F., Da Silva-Ferrada, E., Xolalpa, W., Zabaleta, L., Rivas, C., & Rodriguez, M. S. (2014). Analysis of PTEN ubiquitylation and SUMOylation using molecular traps. *Methods*. doi: 10.1016/j.ymeth.2014.09.001
Lee, J. O., Yang, H., Georgescu, M. M., Di Cristofano, A., Maehama, T., Shi, Y., . . . Pavletich, N. P. (1999). Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. *Cell, 99*(3), 323-334.
Lehman, J. A., Waning, D. L., Batuello, C. N., Cipriano, R., Kadakia, M. P., & Mayo, L. D. (2011). Induction of apoptotic genes by a p73-phosphatase and tensin homolog (p73-PTEN) protein complex in response to genotoxic stress. *J Biol Chem, 286*(42), 36631-36640. doi: 10.1074/jbc.M110.217620
Li, J., Simpson, L., Takahashi, M., Miliaresis, C., Myers, M. P., Tonks, N., & Parsons, R. (1998). The PTEN/MMAC1 tumor suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. *Cancer Res, 58*(24), 5667-5672.
Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., . . . Parsons, R. (1997). PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science, 275*(5308), 1943-1947.
Liaw, D., Marsh, D. J., Li, J., Dahia, P. L., Wang, S. I., Zheng, Z., . . . Parsons, R. (1997). Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet, 16*(1), 64-67. doi: 10.1038/ng0597-64

Lin, B. T., & Wang, J. Y. (1992). Cell cycle regulation of retinoblastoma protein phosphorylation. *Ciba Found Symp, 170*, 227-241; discussion 241-223.
Liu, W., James, C. D., Frederick, L., Alderete, B. E., & Jenkins, R. B. (1997). PTEN/MMAC1 mutations and EGFR amplification in glioblastomas. *Cancer Res, 57*(23), 5254-5257.
Lukashev, M. E., & Werb, Z. (1998). ECM signalling: orchestrating cell behaviour and misbehaviour. *Trends Cell Biol, 8*(11), 437-441.
Maehama, T., Okahara, F., & Kanaho, Y. (2004). The tumour suppressor PTEN: involvement of a tumour suppressor candidate protein in PTEN turnover. *Biochem Soc Trans, 32*(Pt 2), 343-347. doi: 10.1042/
Manchado, E., Eguren, M., & Malumbres, M. (2010). The anaphase-promoting complex/cyclosome (APC/C): cell-cycle-dependent and -independent functions. *Biochem Soc Trans, 38*(Pt 1), 65-71. doi: 10.1042/BST0380065
Marshall, C. J. (1995). Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell, 80*(2), 179-185.
Mayo, L. D., & Donner, D. B. (2001). A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci U S A, 98*(20), 11598-11603. doi: 10.1073/pnas.181181198
Mudryj, M., Devoto, S. H., Hiebert, S. W., Hunter, T., Pines, J., & Nevins, J. R. (1991). Cell cycle regulation of the E2F transcription factor involves an interaction with cyclin A. *Cell, 65*(7), 1243-1253.
Muller, H. J., Carlson, E., & Schalet, A. (1961). Mutation by alteration of the already existing gene. *Genetics, 46*, 213-226.
Myers, M. P., Pass, I., Batty, I. H., Van der Kaay, J., Stolarov, J. P., Hemmings, B. A., . . . Tonks, N. K. (1998). The lipid phosphatase activity of PTEN is critical for its tumor supressor function. *Proc Natl Acad Sci U S A, 95*(23), 13513-13518.
Myers, M. P., Stolarov, J. P., Eng, C., Li, J., Wang, S. I., Wigler, M. H., . . . Tonks, N. K. (1997). P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc Natl Acad Sci U S A, 94*(17), 9052-9057.
Okada, M., & Nakagawa, H. (1988). Protein tyrosine kinase in rat brain: neonatal rat brain expresses two types of pp60c-src and a novel protein tyrosine kinase. *J Biochem, 104*(2), 297-305.
Okada, M., & Nakagawa, H. (1989). A protein tyrosine kinase involved in regulation of pp60c-src function. *J Biol Chem, 264*(35), 20886-20893.
Okamura, A., Iwata, N., Tamekane, A., Yakushijin, K., Nishikawa, S., Hamaguchi, M., . . . Matsui, T. (2006). Casein kinase Iepsilon down-regulates phospho-Akt via PTEN, following genotoxic stress-induced apoptosis in hematopoietic cells. *Life Sci, 78*(14), 1624-1629. doi: 10.1016/j.lfs.2005.07.041
Okumura, K., Mendoza, M., Bachoo, R. M., DePinho, R. A., Cavenee, W. K., & Furnari, F. B. (2006). PCAF modulates PTEN activity. *J Biol Chem, 281*(36), 26562-26568. doi: 10.1074/jbc.M605391200

Papa, A., Wan, L., Bonora, M., Salmena, L., Song, M. S., Hobbs, R. M., . . . Pandolfi, P. P. (2014). Cancer-associated PTEN mutants act in a dominant-negative manner to suppress PTEN protein function. *Cell, 157*(3), 595-610. doi: 10.1016/j.cell.2014.03.027

Rous, P. (1960). The possible role of viruses in cancer. Opening remarks. *Cancer Res, 20*, 672-676.

Runnebaum, I. B., Kieback, D. G., Tong, X. W., & Kreienberg, R. (1993). p53 gain-of-function mutation in codon 175 is a rare event in human breast cancer. *Hum Mol Genet, 2*(9), 1501-1502.

Sanchez, Y., Wong, C., Thoma, R. S., Richman, R., Wu, Z., Piwnica-Worms, H., & Elledge, S. J. (1997). Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. *Science, 277*(5331), 1497-1501.

Schmid, A. C., Byrne, R. D., Vilar, R., & Woscholski, R. (2004). Bisperoxovanadium compounds are potent PTEN inhibitors. *FEBS Lett, 566*(1-3), 35-38. doi: 10.1016/j.febslet.2004.03.102

Sheaff, R. J., Groudine, M., Gordon, M., Roberts, J. M., & Clurman, B. E. (1997). Cyclin E-CDK2 is a regulator of p27Kip1. *Genes Dev, 11*(11), 1464-1478.

Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., & McGuire, W. L. (1987). Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science, 235*(4785), 177-182.

Steck, P. A., Pershouse, M. A., Jasser, S. A., Yung, W. K., Lin, H., Ligon, A. H., . . . Tavtigian, S. V. (1997). Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet, 15*(4), 356-362. doi: 10.1038/ng0497-356

Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R., & Yamada, K. M. (1998). Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science, 280*(5369), 1614-1617.

Toyoshima, H., & Hunter, T. (1994). p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21. *Cell, 78*(1), 67-74.

Varmus, H., Pao, W., Politi, K., Podsypanina, K., & Du, Y. C. (2005). Oncogenes come of age. *Cold Spring Harb Symp Quant Biol, 70*, 1-9. doi: 10.1101/sqb.2005.70.039

Volpert, O. V., Dameron, K. M., & Bouck, N. (1997). Sequential development of an angiogenic phenotype by human fibroblasts progressing to tumorigenicity. *Oncogene, 14*(12), 1495-1502. doi: 10.1038/sj.onc.1200977

Walker, C. L., & Xu, X. M. (2014). PTEN inhibitor bisperoxovanadium protects oligodendrocytes and myelin and prevents neuronal atrophy in adult rats following cervical hemicontusive spinal cord injury. *Neurosci Lett, 573*, 64-68. doi: 10.1016/j.neulet.2014.02.039

Wang, H., Karikomi, M., Naidu, S., Rajmohan, R., Caserta, E., Chen, H. Z., . . . Leone, G. (2010). Allele-specific tumor spectrum in pten knockin mice. *Proc Natl Acad Sci U S A, 107*(11), 5142-5147. doi: 10.1073/pnas.0912524107

Weinberg, R. A. (1995). The retinoblastoma protein and cell cycle control. *Cell, 81*(3), 323-330.

Werth, S., Urban-Klein, B., Dai, L., Hobel, S., Grzelinski, M., Bakowsky, U., . . . Aigner, A. (2006). A low molecular weight fraction of polyethylenimine (PEI) displays increased transfection efficiency of DNA and siRNA in fresh or lyophilized complexes. *J Control Release, 112*(2), 257-270. doi: 10.1016/j.jconrel.2006.02.009

Yarden, Y., & Ullrich, A. (1988). Growth factor receptor tyrosine kinases. *Annu Rev Biochem, 57*, 443-478. doi: 10.1146/annurev.bi.57.070188.002303

Zhang, S., Huang, W. C., Li, P., Guo, H., Poh, S. B., Brady, S. W., . . . Yu, D. (2011). Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med, 17*(4), 461-469. doi: 10.1038/nm.2309

Zhang, T., & Prives, C. (2001). Cyclin a-CDK phosphorylation regulates MDM2 protein interactions. *J Biol Chem, 276*(32), 29702-29710. doi: 10.1074/jbc.M011326200

Zhu, X., Ohtsubo, M., Bohmer, R. M., Roberts, J. M., & Assoian, R. K. (1996). Adhesion-dependent cell cycle progression linked to the expression of cyclin D1, activation of cyclin E-cdk2, and phosphorylation of the retinoblastoma protein. *J Cell Biol, 133*(2), 391-403.

CURRICULUM VITAE

Jaimie Michelle Gendron

Education

Indiana University (2012-2015)

Masters of Science

Department of Biochemistry and Molecular Biology

Major: Biochemistry

Minor: Cancer Biology

Purdue University

Bachelor of Science (2007-2011)

Major: Neurobiology and Physiology

Minor: Psychology

Publications

Mdm2 Switches from a Ubiquitinating to Neddylating Enzyme in Response to Src Phosphorylation

Christopher N. Batuello, Paula M. Hauck, **Jaimie M. Gendron**, Jason A. Lehman, and Lindsey D. Mayo

Published Proc Natl Acad Sci U S A. 2015 Feb 10;112(6):1749-54. doi: 10.1073/pnas.1416656112. Epub 2015 Jan 26.

Serdemetan Antagonizes the Mdm2-HIF1α Axis Leading to Decreased Levels of Glycolytic Enzymes

Jason A. Lehman, Paula M. Hauck, **Jaimie M. Gendron**, Christopher N. Batuello, Jacob A. Eitel, Allan Albig, Madhavi P. Kadakia, Lindsey D. Mayo

Published: Sep 06, 2013 DOI: 10.1371/journal.pone.0074741

Work Experience

Research In Dr. Bryan P. Schneider's Research Laboratory (2011-2012)

Working in SNP selection, DNA quantification, DNA extraction, and learning to contribute as a researcher in a translational science setting

Purdue Honors Research in Dr. Louis Sherman's Laboratory (2008-2011)

Maximizing the production of hydrogen by Cyanobacteria, making solutions, spectroscopy, gas chromatography, microscopy, transformations, and conjugations

Mastered dilutions, cell counting, and gas measurements to collect and analyze data.

Presented preliminary research in St. Louis at Washington University in 2009

Presented a research presentation and original poster at the Purdue Honors Undergraduate Research Fair in 2010.

Purdue University Science Ambassadors (2009-2011)

Representing Purdue University by encouraging and informing prospective and

admitted students to discover the extraordinary educational opportunities offered. I spent time helping students register for classes and know where to find help.

Poster Presentations

Biochemistry and Molecular Biology Research Day (2014)

The Gain of Function of Mutant PTEN

Indiana University Simon Cancer Center Cancer Research Day (2012)

The Association of Single Nucleotide Polymorphisms with Taxane Induced Peripheral Neuropathy in African Americans

Purdue University Biological Sciences Honors Research Day (2011)

The Effect of Colicins E1 and E3 on the Cyanobacterium Synechocystis

Purdue University Biological Sciences Honors Research Day (2010)

Development of a System for H2 Production in *Cyanothece*

Honors/Membership

Fellowship in Indiana Biomedical Gateway Program	(2012-2014)
Honors Research Degree for Honors Research Thesis	(2011)
Recognized for excellence in chemistry by professor	(2008)
Academic Excellence Award AP Biology	(2006-2007)
Bausch & Lomb Honorary Science Award	(2005-2006)

NetworkIN: Networking leadership committee at IUSM	(2014)
Biochemistry Student Data Club	(2013-2015)
Indiana University School of Medicine ASCO Review	(2012)
San Antonio Breast Cancer Symposium Attendee	(2011)