Viperin upregulates PD-L1 through PI3K/AKT/mTOR/HIF-1α signaling pathway in Gastric Cancer Cells

Ku Sul Kim

Department of Medical Science

The Graduate School, Yonsei University

Viperin upregulates PD-L1 through PI3K/AKT/mTOR/HIF-1α signaling pathway in Gastric Cancer Cells

Ku Sul Kim

Department of Medical Science

The Graduate School, Yonsei University

Viperin upregulates PD-L1 through PI3K/AKT/mTOR/HIF-1α signaling pathway in Gastric Cancer Cells

Directed by Professor Jun-Young Seo

The Master's thesis
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Master of Medical Science

Ku Sul Kim

June 2019

This certifies that the Master's Thesis of Ku Sul Kim is approved

Seo

Thesis Supervisor: Jun-Young Seo

Thesis Committee Chair: Ki Taek Nam

Thesis Committee Member: Je-Wook Yu

The Graduate School
Yonsei University

June 2019

TABLE OF CONTENTS

LIST OF FIGURES LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Viperin upregulates PD-L1 through PI3K/AKT/mTOR/HIF-1α signaling pathway in Gastric Cancer Cells

Ku Sul Kim

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Jun-Young Seo)

Viperin, a multifunctional interferon (IFN)-inducible protein, normally localizes to endoplasmic reticulum (ER) and plays a role as an antiviral protein. However, recent studies show that human cytomegalovirus infection (HCMV) causes re-localization of viperin from ER to mitochondria. Mitochondria-localized viperin interacts with trifunctional protein and modulates cellular metabolism including lipogenesis and glycolysis. Previous studies from our laboratory have shown that viperin regulates metabolic alteration in cancer cells as well as in virus-infected cells. It has been also shown that viperin expression is highly upregulated under tumor microenvironment condition such as hypoxia, serum starvation, and cytokines including IFNs through activation of PI3K/AKT/mTOR/HIF-1α pathway or JAK/STAT pathway. This induction mechanism of viperin expression

is similar to that of PD-L1 expression in cancer cells. Furthermore, like viperin function in cancer cells, PD-L1 regulates cancer metabolism to compete against T cell. Here, I hypothesized that viperin interacts with PD-L1, which may have synergic effect on metabolic regulation of cancer cells. Using human cancer cell lines, I demonstrated that viperin positively regulates PD-L1 gene and protein expression through PI3K/AKT/mTOR/HIF-1α pathway and vice versa. In addition, the interaction between viperin and PD-L1 modulates expression of glycolytic and lipogenic enzymes in cancer cells. The data suggest that viperin and PD-L1 can be mutual regulators and their interplay controls cancer metabolism. Therefore, dual targeting of viperin and PD-L1 may provide an exciting new avenue for treatment of cancers.

Key words: viperin, PD-L1, gastric adenocarcinoma, metabolism, signaling pathway

Viperin upregulates PD-L1 through PI3K/AKT/mTOR/HIF-1α signaling pathway in Gastric Cancer Cells

Ku Sul Kim

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Jun-Young Seo)

I. INTRODUCTION

Viperin (virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible), also known as RSAD2 (radical S-adenosyl methionine domain-containing protein 2) was identified in human macrophages as the products of an interferon (IFN)-gamma inducible gene.[1,2] Viperin as an interferon (IFN)-inducible protein, is evolutionary highly conserved. It is composed of 361-amino acid protein with approximately molecular mass of 42kDa.[3] It is composed of three distinct domains, N-terminal domain, central domain containing a radical SAM enzymes domain and a C-terminal domain.[3] The N-terminal domain has length and sequence variability between species and contains an amphipathic α helix sequence, which is responsible for viperin association with the cytosolic face

of the endoplasmic reticulum (ER) and lipid droplets.[4,5] The conserved central domain that has three cysteine residues organized in a CxxxCxxC motif, which is responsible for Fe-S cluster.[6,7] In hepatitis C virus (HCV) and HCMV infection, this motif is essential for function of viperin.[8,9] Although the exact function of C-terminal domain is unknown, it is highly conserved and required for viperin antiviral activity against HCV and dengue virus (DENV).[10,11]

Viperin is induced by various type of cells by type I, II, and III IFNs, double-stranded (ds) B-form DNA, lipopolysaccharides (LPS), the dsRNA analog poly I:C, and viral infections. In primary macrophages, viperin is highly induced by IFN-gamma. But in the majority of cell types such as fibroblasts, hepatocytes, dendritic cells and T cell, viperin is effectively induced by IFN-alpha and -beta.[1] Viperin induced by both classical IFN-mediated pathway and IFN-independent pathway. B-form DNA, LPS, Poly I:C and viruses such as Sindbis virus, Snedai virus, Pseudorabies virus (PrV) induce viperin by IFN-stimulated gene (ISG) induction pathway. Other viruses, such as HCMV and vesicular stomatitis virus (VSV), directly induce viperin by IFN-independent pathway independently of IFN production.[3]

Viperin is a multifunctional protein. It has function as an anti-viral protein and a regulator of cell signal pathways or cellular metabolism. Viperin can inhibit DNA and RNA viruses such as herpesvirus (HCMV), flaviviruses (HCV, Dengue virus and West Nile virus), alphavirus, orthomyxovirus (influenza A virus), paramyxovirus (Sendai virus), rhabdovirus (VSV), retrovirus (HIV-1).[2] Pre-

expression of viperin inhibits HCMV infection by attenuating the viral structural protein, including pp65, gB and pp28, which are essential for virus mutation and release.[1] Over-expression of viperin also inhibits budding and release of influenza A virus by altering lipid raft microdomains on plasma membrane.[12] While HCV and Dengue virus infection, replication was occurred on lipid droplets. Thus, viperin localized to lipid droplets to suppress viral replication.[10] Moreover, viperin can regulate Toll-like receptor (TLR) 7 and 9-mediated type I IFN production in plasmacytoid dendritic cells.[13] Viperin also can regulate T cell activation by facilitating T cell-mediated GATA-3 activation and optimal Th2 cytokine production by modulating NF-κB (nuclear factor-kappa B), AP-1 (activator protein 1) activities.[14]

During HCMV infection, expression of viperin also can act as a pro-viral protein by modulating cellular energy and lipid metabolism. Viperin is re-localized from ER to viral assembly compartment (AC) to inhibit replication of virus by blocking secretion of soluble viral proteins critical for virion maturation and assembly. However, if expression of viperin is induced directly by HCMV infection, it interacts with viral mitochondrial inhibitor of apoptosis (vMIA) and translocated from ER to mitochondria. Translocated viperin interacts with mitochondrial trifunctional protein (TFP) that catalyzes fatty acid beta-oxidation to generate ATP. Following viperin interacts with TFP, decrease cellular ATP level and thus AMP-activated protein kinase (AMPK) is activated. The activation of AMPK induces expression and translocation to cell surface of glucose transporter

GLUT4, which increase glucose uptake. By activating the glucose regulated transcription factor ChREBP, lipogenic enzymes are induced and enhance lipid synthesis and accumulate lipid droplets that are used for formation of the viral envelope. These metabolic changes are not only induced by HCMV infection, but also induced by directly targeted viperin to mitochondria.[15]

Unlike normal differentiated cells, cancer cells change their metabolism to promote proliferation, growth and survival. In cancer cell, increasing glucose uptake and fermentation of glucose to lactate, even in the presence of oxygen is known as Aerobic glycolysis or the Warburg Effect. This is observed even in the presence of completely functioning mitochondria.[16,17] Glucose as an essential source of metabolic energy regulates cellular function and homeostasis. Differentiated cells produce about 8-90% of the ATP through mitochondrial oxidative phosphorylation and only 10% is derived from the metabolism of glucose to pyruvic acid. But, in rapidly growing cancer cell, even in the presence of oxygen, about 60% of the total cell ATP was derived from glycolysis and 40% from oxidative phosphorylation.[18] Through the Warburg effect, cancer cell can adapt to hypoxic conditions which frequently develop during their growth.[19]

Fatty acid is essential for various cellular processes. In normal human tissues, fatty acid is acquired from the circulation. However, lipogenesis and expression of lipogenic enzymes is highly induced in cancer cells.[20] Fatty acid function as building blocks for membranes, they are used as anchors to target proteins to membranes and as precursors in the synthesis of lipid second messengers, they

function as a medium to store energy.[21] A role of fatty acid synthase in tumor cells demonstrated a large number of studies is activation of cell cycle and inhibition of apoptosis. Other lipogenic genes, including the acetyl CoA carboxylase (ACC) and the stearoyl CoA desaturase 1 (SCD1) are highly expressed in primary tumors and also appear to play a role in their development. Hence high lipogenesis is associated with cancer progression and metastasis.[20]

The immune system also plays an important role in the protection of the human or animal against not only pathogens but also cancers. When the immune system mounts a cell-mediated response to foreign antigens, three signals are required for T- cell activation. First, T-cell receptors must engage specific peptides presented by MHCs on the cancer cell or antigen-presenting cells (APC). Second, specific receptors on T cells must bind ligands expressed on cancer cells or APCs to prevent anergy, which refers to failure to mount the response against an antigen. Third, signals provided by cytokines play a critical role in regulating the strength and type of immune responses.

Multiple costimulatory or coinhibitory interactions among APCs and T cells, providing a key checkpoint in the regulation of T-cell immunity and maintenance of immune homeostasis. Programmed death ligand-1 (PD-L1), also called CD274, one of the critical checkpoint, inhibits the immune response through interaction with receptor programmed cell death 1 PD-1.[22] PD-L1 is a type I transmembrane protein with an extracellular N-terminal domain. In physiological conditions, PD-L1 expression can be detected on hematopoietic cells including T cells, B cells,

macrophages, dendritic cells (DCs), and mast cells, and non-hematopoietic healthy tissue cells including vascular endothelial cells, keratinocytes, pancreatic islet cells, astrocytes, placenta syncytiotrophoblast cells, and corneal epithelial and endothelial cells and shown to be overexpressed with immune activation, such as inflammations. Because PD-L1/PD-1 axis maintains self-tolerance and limit collateral tissue damage during anti-microbial immune responses, and thus deficiency function of it can lead to many auto-immune diseases.[23,24] PD-L1 express in 5–40% tumor cells, can disrupt the immune response.[25,26] Through interaction with PD-1 on T cell help cancer to evade anti-cancer immune response.[27] Thus by suppressing immune-inhibitory checkpoint proteins can enhance immune responses, prevent cancer progression, and improve patient survival. Blockade of these inhibitory checkpoint proteins has been intensely pursued in recent years as a strategy to enhance T cell infiltration and effector functions in cancer. Blocking antibodies against PD-L1/PD-1 has shown promising efficacy in advanced melanoma, non–small cell lung carcinoma, renal cell carcinoma, bladder cancer, and lymphoma.[22,28]

Even though immune checkpoint blockade therapy seems to be positive clinical applications in certain cancer patients, the efficacy rate and profits of usage in general patients remain at a modest level and thus impeding the application of immune checkpoint blockade therapy. The tumor immunogenicity is a multilevel and delicately controlled process. Therefore, accumulation of mutations may lead to dysregulation of immunogenicity and create an

immunosuppressive microenvironment, causing intrinsic resistance to immune checkpoint blockade therapy.[29] Moreover, expression of PD-L1 is upregulated following chemotherapy and nivolumab treatment.[30] To possible more patients to benefit from immune checkpoint blockade therapy, the mechanisms of induction pathway such as inflammatory signaling, oncogenic signaling and regulation at the protein level have been proposed. IFN-γ as a pro-inflammatory cytokine, that is abundantly produced by activated T cells and also produced by NK cells. Binding between IFN-γ and Interferon gamma receptor (IFNGR) results in signaling through the classical JAK/STAT pathway, increased expression of a series of transcription factors, the interferon-responsive factors (IRFs). IRF1 has been shown to play a role in the IFN-γ-mediated induction of PD-L1.[31] Not only IFN-γ, including macrophages, monocytes, primary bone marrow-derived dendritic cells, and with lipopolysaccharide (LPS), Poly I:C leads to increased PD-L1 expression.[24] Genetic or pharmacological inactivation of MYC has been shown to result in reduced PD-L1 expression in melanoma, NSCLC, leukemia, lymphoma, and HCC.[32-34] As transcription factors, such as STAT3, NF-κB, AP-1 also can induce expression of PD-L1 binding to PD-L1 promoter.[35-38] Expression of PD-L1 has been shown to be regulated by HIF-1α in mouse melanoma, human breast cancer, prostate cancer, NSCLC cells, and myeloid-derived suppressor cells (MDSCs).[39-42] By activating mutations in EGFR and EGF, induce PD-L1 expression in bronchial epithelial cells, NSCLC, head and neck cancer (HNC), and breast cancer cells.[43,44] Through EGFR activation increased PD-L1 expression can

be blocked by rapamycin and by an ERK inhibitor, suggesting mTOR- and ERK-dependent regulatory mechanisms.[44,45] Activation of oncogenic pathways, including RAS/RAF/MAPK and PI3K signaling, also associate with PD-L1 expression.[46] Recently, the role of miRNAs as posttranscriptional regulators, for example miR-513, -155, -34a has been revealed in regulating PD-L1 expression.[47] Because N-glycosylation is a critical protein modification that determines protein structure and function, especially the function of membrane proteins, posttranslational regulation is final mechanism by which the level of PD-L1 expression is modulated. CMTM6, CMTM4 as positive regulators bind to PD-L1 and thereby increase its half-life, presumably by preventing ubiquitination and lysosomal degradation during protein recycling.[48,49] And when bound to non-glycosylated PD-L1, GSK3β leads to phosphorylation and resultant ubiquitination of PD-L1.[50] CSN5 induced by tumor necrosis factor alpha (TNF-α) inhibits the ubiquitination and degradation of PD-L1.[51]

Among the mechanisms of expression of PD-L1, especially PI3K/AKT/mTOR pathway can be important for PD-L1 induction pathway. PI3K/AKT signaling can regulate both IFN-γ-induced and-independent PD-L1 expression. Inhibition of PI3K/AKT signaling suppressed IFN-γ-induced PD-L1 expression. [52,53] In NSCLC, CRC, glioma, breast cancer, and melanoma cells and in melanoma and breast cancer cells, Loss of PTEN enhances PI3K/AKT pathway regulated PD-L1 expression in both IFN-γ-induced and-independent pathway.[45,52,54-56] Recently studies demonstrate a competition between tumor cells

and tumor-infiltrating T lymphocytes (TIL) for glucose in tumor microenvironment (TME) that can drive cancer progression through metabolic competition. Tumor PD-L1 expression promoted glycolysis and AKT/mTOR activation in tumor cells whereas suppressing mTOR activity in T cells through glucose competition. Blockade with anti-PD-L1 antibodies inhibited tumor progression and glucose uptake in tumor cells and increased mTOR activity and glucose uptake of T cells.[57]

Expression of immune checkpoint proteins can inhibit immune cells by altering cellular and microenvironmental metabolism. But the underlying mechanism of metabolic reprogramming in cancer cells and immune cells by immune checkpoint proteins have yet to be revealed. On the basis of metabolic changes in cells by viperin, previously studies demonstrated the roles of viperin in gastric cancer cells. In cancer cells, viperin expression is highly upregulated under tumor microenvironment condition such as hypoxia, serum starvation, and cytokines including IFNs through activation of PI3K/AKT/mTOR/HIF-1α pathway or JAK/STAT pathway. Induced viperin changes metabolism in cancer cells, thus cancer cells effectively survive and promote proliferation and tumorigenic capacity. Furthermore, recently studies reported that expression of PD-L1 is associated with IFN-γ signaling pathway, AKT/mTOR pathway, and HIF-1α induced by hypoxia, similar with viperin induction pathway and tumor-expressed PD-L1 promotes glycolysis and AKT/mTOR activation. To references the previous study, I identified that between viperin and PD-L1 have interaction

with not only expression but also function each other within tumor microenvironment (TME). Generating viperin knockdown stable cell lines and PD-L1 knockdown cell lines, I assessed viperin positively regulates PD-L1 gene and protein expression through PI3K/AKT/mTOR/HIF-1α pathway and vice versa. Moreover, this interaction results in enhancement lipogenesis as well as glycolysis in tumor metabolism aspect. I suggest that both viperin and PD-L1 can be therapeutic targets in antitumor therapy and dual targeting of viperin and PD-L1 may provide an exciting new avenue for treatment of cancers.

II. MATERIALS AND METHODS

1. Cell culture

Human gastric cancer cell line MKN28 and MKN1 were cultured in RPMI-1640 media supplemented with 10% FBS (Hyclone, USA) and 1% penicillin/streptomycin. Human lung cancer cell line A549 was cultured in RPMI-1640 media supplemented with 10% FBS (Hyclone, USA) and 1% penicillin/streptomycin (Hyclone, USA). Cells were incubated at 37℃ in a humidified incubator containing 5% CO_2.

2. Antibodies and chemicals

Antibodies used in this study are as follows: mouse polyclonal anti-Viperin (Map.Vip); PD-L1 (Cell signaling technology, #13684); Akt (Cell signaling technology, #4691); Phospho-Akt (Ser473) (Cell signaling technology, #9271); S6 Ribosomal protein (Cell signaling technology, #2217); Phospho-S6 Ribosomal protein (Ser235/236) (Cell signaling technology, #4858); Grp94 (Enzo, ADI-SPA-850); α-tubulin (Sigma, T6199). To induce viperin 100ng/ml Recombinant Human Interferon-γ (IFN-γ) (gibco, PHC4031) were treated.

3. RNA extraction, cDNA preparation, and quantitative real-time PCR

RNeasy mini kit (Qiagen) was used to isolate total RNA from cells. Prime script 1st strand cDNA synthesis kit was used to synthesis cDNA according to the manufacturer's instructions (Takara Bio, Japan). Real-time PCR was conducted using SYBR Green PCR Kit (Applied Biosystems). The reaction included 95℃ for 10 minutes, which was followed by a three-step PCR program of 95℃ for 30 seconds, 55℃ for 1 minute, and 72℃ for 30 seconds repeated for 40 cycles. The mRNA levels of each of genes were compared with control (Luc shRNA or Control siRNA). Primer sequences are listed in Table 1.

Table 1. Primer sequences used for real -time PCR

Target gene	Primer sequence(5'-3')	
	Forward	Reverse
Viperin	TAGAGTCGCTTTCAAGATA	TTCAGATCAGCCTTACTCC
PD-L1	TATGGTGGTGCCGACTACAA	TGGCTCCCAGAATTACCAAG
GLUT4	CTCAGCAGCGAGTGACTGG	AGCCACGTCTCATTGTAGCTC
ACL	TGTAACAGAGCCAGGAACCC	CTGTACCCCAGTGGCTGTTT
ACC2	GACCACAGGTGAAGCTGAGA	GTGTTCCCGTCCCCTCCTC
LDHA	GCACGTCAGCAAGAGGGAGAAAG	AGGTAACGGAATCGGGCTGAATC
HK2	ACCTTTGTGAGGTCCACTCC	TGTCCGTTACTTTCACCCAA
PDK1	CACCAAGACCTCGTGTTGAG	AGCTTCAGGTCTCCTTGGAA
β-Actin	GCTCCGGCATGTGCAA	TAAGCCGGCTGAGATCTTGT

4. Western blotting

Cells were washed with PBS after harvest and lysed 1x TBS containing 1% Triton X-100 (AMRESCO, USA) and protease inhibitor (cOmplete). The concentrations of protein were examined by using BSA assay. After sample buffer (60mM Tris-HCl pH 6.8, 10% Glycerol, 5% β-mercaptoethanol, 2% SDS, 0.01% Bromophenol blue) was added to cell lysates. Quantitative proteins were separated by SDS-PAGE and transferred to PVDF membranes, which blocked in PBS-T containing 5% non-fat milk to block the non-specific binding for 1 hour. Primary antibodies were incubated overnight at 4℃. After the incubation, the blots were washed 3 times and secondary antibodies conjugated with HRP were incubated for 1hour at Room temperature. Followed by incubation with enhanced chemiluminescence (ECL) reagents (Thermo Scientific) after 3times washing. Grp94 and α- tubulin were used as a loading control to confirm the amount of protein.

5. Reverse transfection

Lipofectamine 2000 (Invitrogen, USA) PD-L1 siRNAs were used for knockdown PD-L1. Dilute siRNA in Opti-MEM (gibco, USA) in the well of the 6well plate and mix gently. Lipofectamine is diluted in Opti-MEM in tube and incubate for 5 minutes at Room temperature. Then lipofectimine complexes were gently mixed to each well containing the diluted siRNA molecules and incubated

for 20 minutes at Room temperature. Cells were diluted in RPMI without antibiotics and FBS so that the appropriate number of cells to give 30-50% confluence 24 hours after plating. Diluted cells were plated in each well that contains siRNA-lipofectamine complexes and leaved 3 hours at 37℃ in a humidified incubator containing 5% CO_2. Then cells were added RPMI containing 30% FBS without antibiotics up to a third of the final volume. The cells were incubated for 72 hours at 37℃ in a humidified incubator containing 5% CO_2.

6. Statistical analysis

The data are presented as mean ± standard error of the mean (SEM). Statistical significance was determined using unpaired Student's t test. $p < 0.05$ was considered significant.

III. RESULTS

1. Viperin and PD-L1 expressions are highly induced in gastric cancer cells under interferon-γ, serum starvation, and hypoxia

As it is known, viperin is induced by interferon(IFN)-γ. Interestingly, PD-L1 also has been shown promoted on tumor cells by interferon-γ, endogenous antitumor immunity factors in the tumor microenvironment which secreted by immune cells.[58] Therefore, I treated IFN-γ to gastric cancer cell lines (MKN1, MKN28, MKN45 and SNU484) to assess induction of viperin and PD-L1. The MKN1 and MKN28 which expressed viperin and PD-L1 in basal level were significantly upregulated expression of viperin and PD-L1 but MKN45 and SNU484 which doesn't express viperin was only increase expression of PD-L1 following IFN-γ treatment (Fig. 1A). As a previously study, we demonstrated that viperin induced by tumor environment condition such as nutrition deprivation and hypoxia.[59] I observed that under serum starvation condition not only MKN1, MKN28 viperin expressed cells but also MKN45 which doesn't express viperin were time-dependently increase expression of viperin and PD-L1 (Fig. 1B). Furthermore, hypoxic condition upregulates PD-L1 expression by HIF-1α was already reported.[41,42] Thus, I screened that hypoxic condition (1% O_2, 5% CO_2 and 94% N_2) increased expression levels of viperin and PD-L1 in MKN28 cell (Fig. 1C).

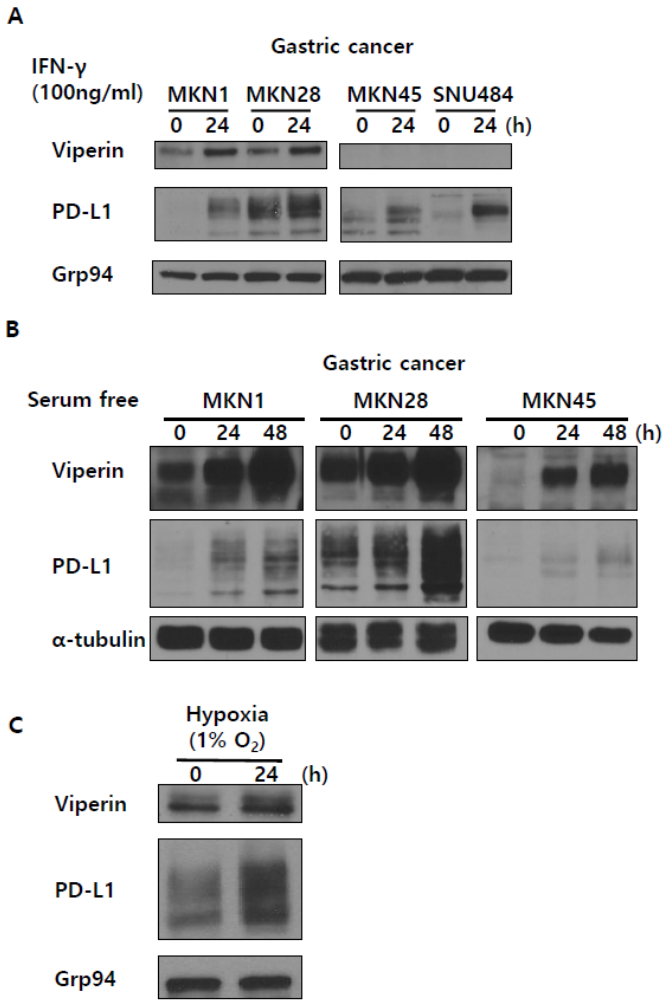


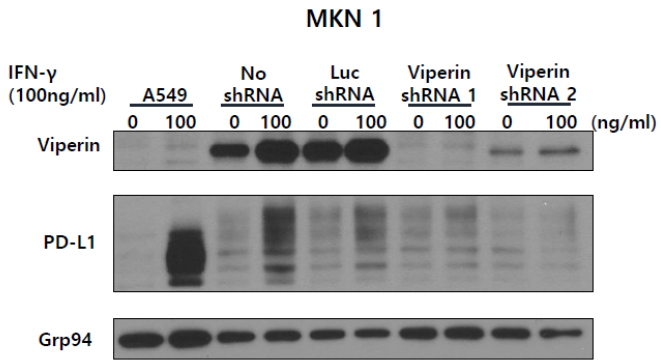
Figure 1. Viperin and PD-L1 expressions are highly stimulated in gastric cancer cells under interferon-γ, serum starvation, and hypoxia. (A) Immunoblots of viperin and PD-L1 in IFN-γ treated gastric cancer cell lines. Gastric cancer cell lines (MNK1, MKN28, MKN45 and SNU484) were treated with 100ng/ml IFN-γ for 24 hours and cell lysates were analyzed by western blot. GRP94 was used as a loading control. (B) Immunoblots of viperin and PD-L1

under serum starvation condition. Gastric cancer cell lines (MNK1, MKN28, MKN45) were incubated in complete media (10% FBS) and no FBS media. Cell lysates of each cell line were analyzed at each indicated time points and analyzed by western blot. α-tubulin was used as a loading control. (C) The MKN28 cell line was incubated under hypoxia (1% O_2, 5% CO_2 and 94% N_2) for 24hours.

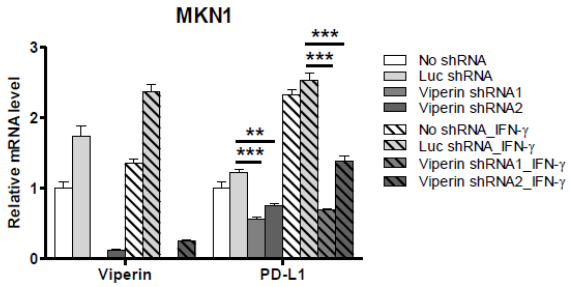
2. Viperin-dependent expression of PD-L1 in cancer cells

Viperin and PD-L1 were induced through similar pathway within tumor environment condition. Thus, I wondered whether between two molecules associated with each other. Therefore, first I generated cell lines with viperin knockdown in MKN1 and MKN28 which highly express viperin. To identify relations between viperin and PD-L1 under tumor environment condition, I treated IFN-γ to stable shRNA knockdown of viperin cell lines MKN1 and MKN28. A549 was used as a positive control about IFN-γ. In normal condition, viperin knockdown cell lines slightly diminished protein expression of PD-L1. The differences of PD-L1 expression depends on viperin were maximized when treated with IFN-γ (Fig. 2A and 2C). Expression mRNA levels of PD-L1 as well as Protein levels were also reduced by viperin knockdown in normal condition. When viperin was induced by IFN-γ, vipeirin-dependent mRNA levels of PD-L1 were also increased (Fig. 2B and 2D). In addition, under another tumor environment condition, serum starvation, I confirmed that the differences both expression mRNA levels (Fig. 3B and 3D) and protein levels (Fig. 3A and 3C) of PD-L1 depend on viperin in MKN28, MKN1 viperin knockdown cell lines.

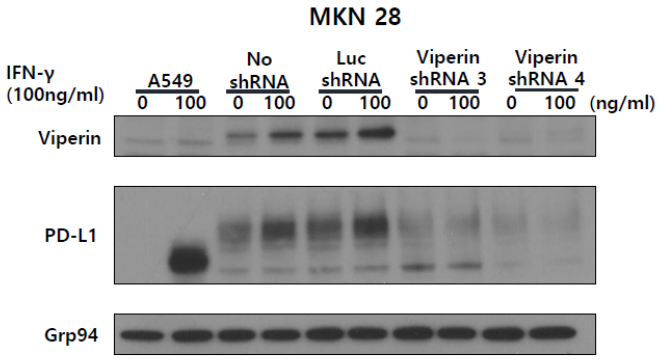
A



B



C



D

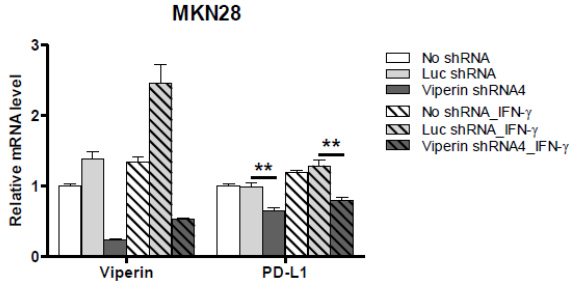


Figure 2. Viperin-dependent PD-L1 expression under interferon-γ stimulation. Viperin knockdown cell lines were generated from MKN1 and MKN28 by lentivirus-based vector system. The stable shRNA knockdown of viperin cell lines and A549 cell line were treated with 100ng/ml IFN-γ for 24 hours. A549 cell was used as a positive control about IFN-γ. Protein expression levels of viperin and PD-L1 were assessed by western blot in (A) MKN1 and (C) MKN28 knockdown of viperin cell lines. GRP94 was used as a loading control. mRNA expression levels of viperin and PD-L1 were detected by RT-qPCR in (B) MKN1 and (D) MKN28 knockdown of viperin cell lines. $^{**}p < 0.01$ and $^{***}p < 0.001$.

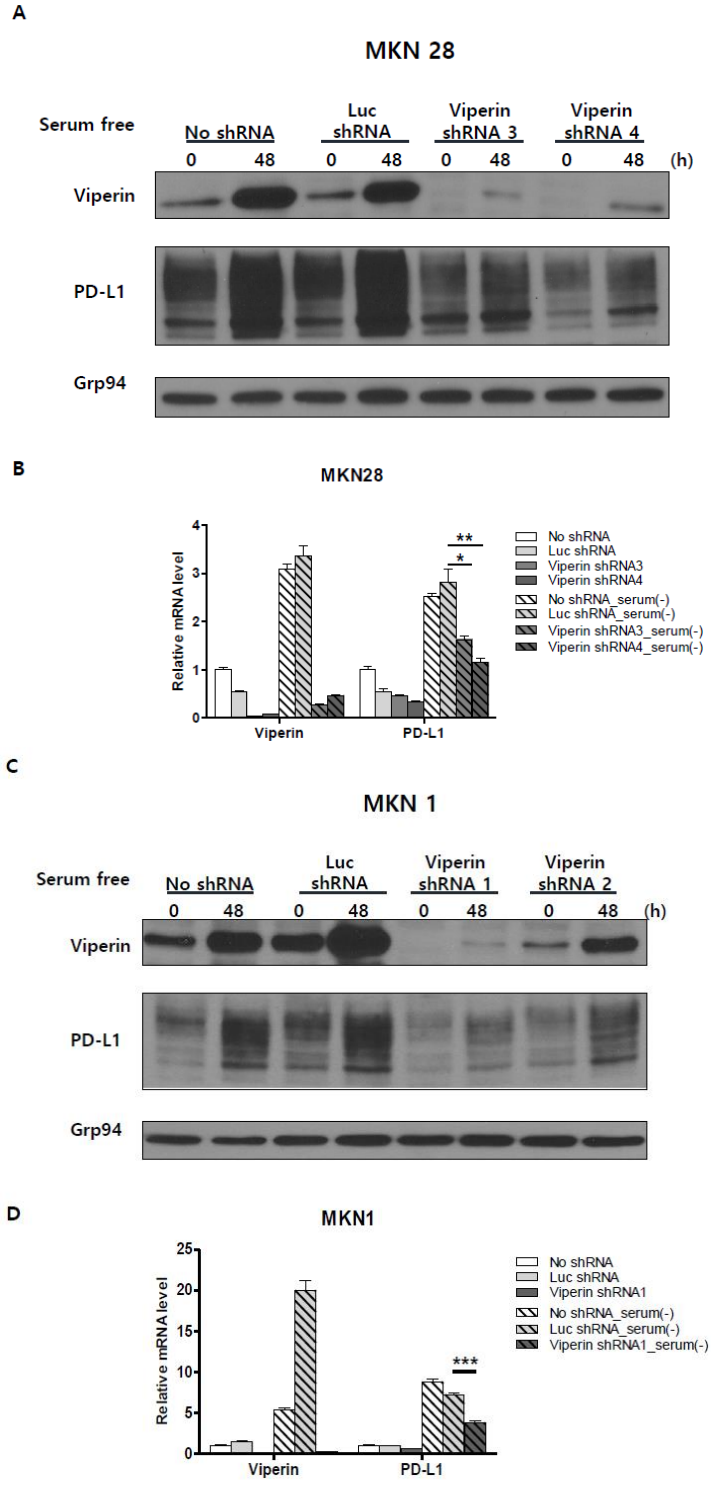


Figure 3. Viperin-dependent PD-L1 expression under serum starvation. The stable shRNA knockdown of viperin cell lines were cultured in serum starvation condition for 48 hours. Protein expression levels of viperin and PD-L1 were assessed by western blot in (A) MKN28 and (C) MKN1 viperin knockdown of viperin cell lines. GRP94 was used as a loading control. mRNA expression levels of viperin and PD-L1 were detected by RT-qPCR in (B) MKN28 and (D) MKN1 knockdown of viperin cell lines. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

3. PD-L1 expression is regulated by viperin through PI3K/AKT/mTOR pathway

By promoting PI3K/AKT/mTOR pathway glycolysis and fatty acid synthesis increase and involved in cell growth. Under serum starvation, in normal cells inactivate PI3K/AKT/mTOR pathway, however various of cancer cells were dysregulated PI3K/AKT pathway and PTEN (negative regulator of PI3K) to stimulate high levels of signaling under minimal dependence on extrinsic stimulation by growth factors.[60] Under hypoxia, HIF1-α, as a downstream of mTOR is induced and binds to HIF1-β in the nucleus.[61] Hence, we identified that viperin is induced by PI3K/AKT/mTOR/HIF-1α pathway under serum starvation and hypoxic condition. Consequently, expressed viperin can regulate cancer cell metabolism. Similar with viperin induction pathway and functions, PD-L1 is induced by AKT/mTOR pathway and by binding HIF1-α to PD-L1 promoter.[41] Besides, expressed PD-L1 promotes AKT/mTOR and glycolysis to compete nutrition in tumor microenvironment.[57] Thus, I investigated expression levels of viperin, PD-L1 and AKT and S6 in MKN28 viperin knockdown cell lines under serum starvation. As shown in figure 4, under serum starvation, viperin expressed cell lines by activating AKT and mTOR enhanced expression of viperin and PD-L1. Contrary to expression of viperin cell lines, knockdown of viperin reduced or have no change of activated levels of AKT and S6 by serum starvation and expressions of PD-L1 and viperin were slightly induced.

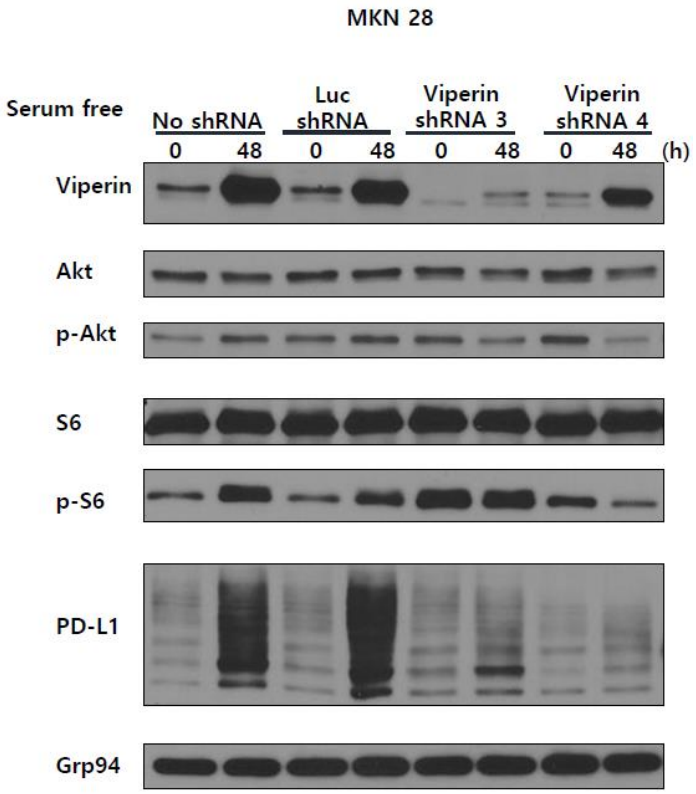


Figure 4. Viperin regulates PD-L1 expression through PI3K/AKT/mTOR signaling pathway. The stable shRNA knockdown of viperin cell lines were cultured in serum starvation condition for 48 hours. (A) Protein expression levels of PD-L1, viperin, AKT, S6 and the forms of activated by phosphorylation p-AKT, p-S6 were examined by western blot. GRP94 was used as a loading control.

4. PD-L1 positively regulates viperin expression through PI3K/AKT/mTOR pathway

Since PD-L1 is reported that can promote AKT/mTOR pathway. Thus, to investigate effect of PD-L1 on viperin expression through AKT/mTOR pathway, MKN28 cells were transfected with PD-L1 siRNA or negative control siRNA. As shown in figure 5, activated levels of AKT and S6 by serum starvation in PD-L1 knockdown cell lesser than PD-L1 expressed cell and reduce expression of viperin. These data suggest that PD-L1 also can effect on expression of viperin by regulating AKT/mTOR pathway. Hypoxia upregulates viperin and PD-L1 expression by HIF-1α was already reported. In addition, HIF-1α is a downstream of mTOR. Thus, I treated 2-methoxyestradiol (2-ME) to cells which were incubated under hypoxic condition. Under hypoxic condition (1% O_2, 5% CO_2 and 94% N_2) increased viperin and PD-L1 expression but when inhibits HIF-1α by treating 2-methoxyestradiol (2-ME) does-dependently diminished expression of viperin and PD-L1 (Fig. 6A). This pattern is also similar in serum starvation condition inhibited HIF-1α by treating 2-methoxyestradiol (2-ME) (Fig. 6B).

Taken together, viperin and PD-L1 were induced by interferon-γ, serum starvation, hypoxia and when stimulated by serum starvation and hypoxia they were regulated by HIF-1α, downstream of PI3K/AKT/mTOR pathway.

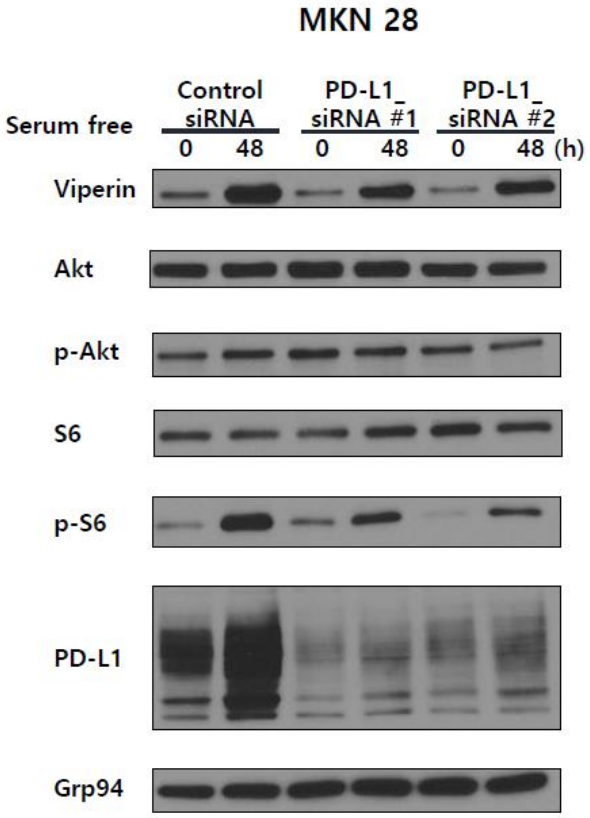


Figure 5. PD-L1-dependent viperin expression in cancer cells. To knockdown expression of PD-L1, the MKN28 cell line was transfected with siRNA and lipofectamine 2000. After 24 hours of transfection, transfected cells were incubated under serum starvation condition for 48 hours. (A) Protein expression levels of PD-L1, viperin, the forms of activated by phosphorylation p-AKT, p-S6 were examined by western blot. GRP94 was used as a loading control.

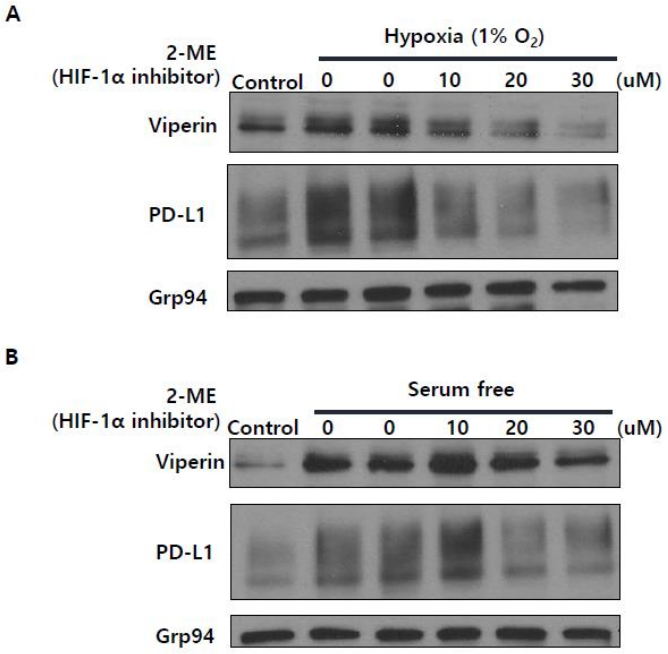


Figure 6. Viperin and PD-L1 are expressed as a downstream of HIF-1α pathway. (A) The MKN28 cell line was incubated under hypoxia (1% O_2, 5% CO_2 and 94% N_2) for 24hours. 2-methoxyestradiol (2-ME) was treated in indicated does for 8 hours before harvest. Protein expressions of viperin and PD-L1 were analyzed by western blot. GRP94 was used as a loading control. (B) The MKN28 cell line was incubated under serum starvation condition for 8 hours. 2-methoxyestradiol (2-ME) was treated in indicated does for 8 hours. Protein expressions of viperin and PD-L1 were analyzed by western blot. GRP94 was used as a loading control.

5. Interaction between viperin and PD-L1 regulates metabolism in cancer cells

To determine interaction of viperin and PD-L1 expression can effect on cancer metabolism, viperin knockdown cell lines and PD-L1 knockdown cells were cultured in serum starvation condition. I measured mRNA levels of lipogenesis-related genes and glycolysis-related genes by RT-qPCR. In viperin knockdown cell, lipogenic enzymes (ACL, ACC2, SREBP, DGAT1), GLUT4, GLUT1 were downregulated and slightly induced by serum starvation. In contrast, these genes were highly induced in viperin expressed cell under serum starvation (Fig. 7). PD-L1 knockdown cell also downregulated lipogenic enzymes (ACL, ACC2), glycolysis related genes (GLUT4, LDHA, HK2, PDK1) (Fig. 8). Taken together, the interplay viperin with PD-L1 can regulate lipogenesis and glycolysis in cancer cells.

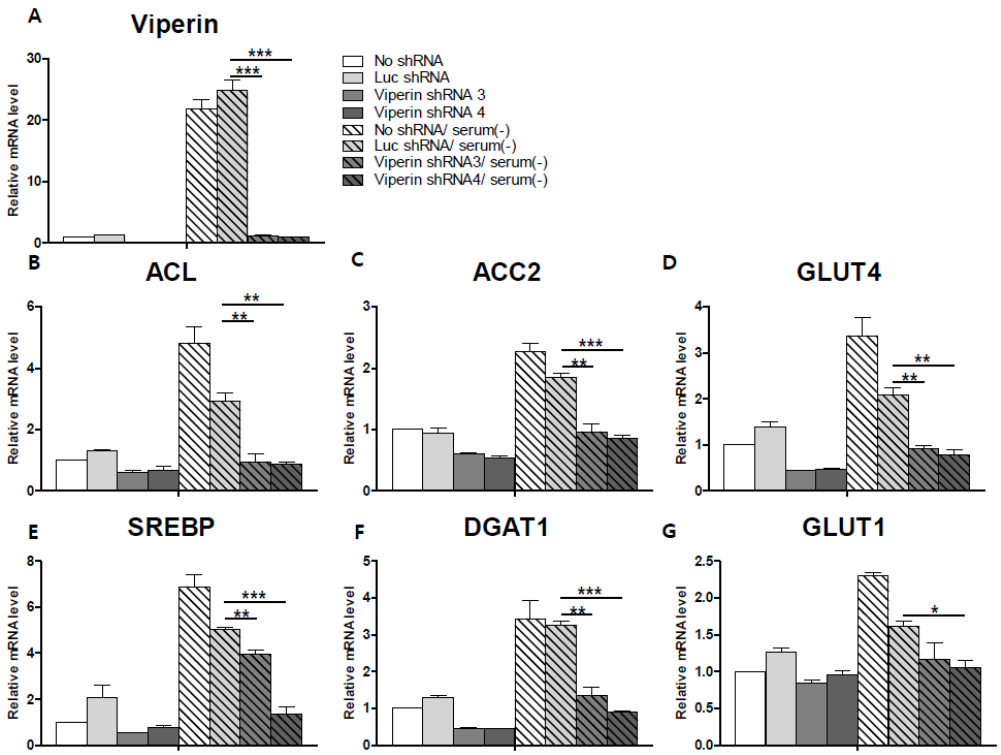


Figure 7. Viperin promotes lipogenesis and glycolysis in cancer cells. To assess metabolism effects of viperin and PD-L1, stable shRNA knockdown of viperin MKN28 cell lines were cultured in serum starvation condition for 48 hours. Cells were examined by RT-qPCR about mRNA levels of lipogenesis genes and glycolysis genes. *p < 0.05, **p < 0.01 and ***p < 0.001.

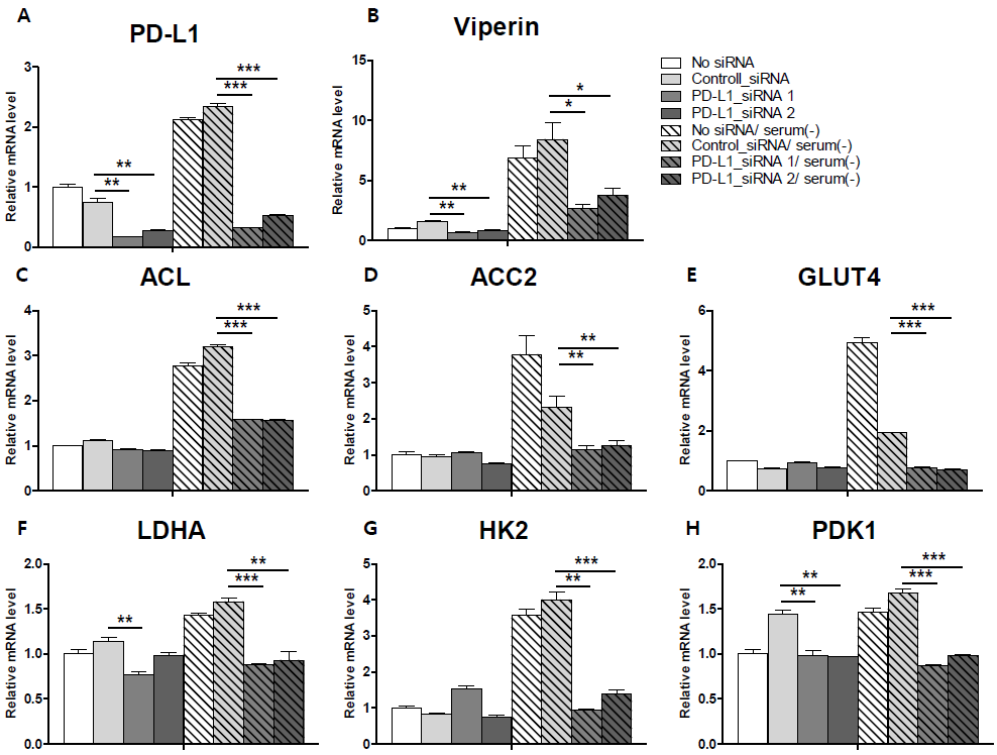


Figure 8. PD-L1 promotes viperin-mediated lipogenesis and glycolysis in cancer cells. To assess metabolism effects of viperin and PD-L1, siRNA knockdown of PD-L1 MKN28 cell lines were incubated under serum starvation condition for 48 hours. Cells were examined by RT-qPCR about mRNA levels of lipogenesis genes and glycolysis genes. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

IV. DISCUSSION

Viperin is an interferon (IFN)-inducible protein that identified in human macrophages as the products of an interferon (IFN)-gamma inducible gene. It is induced by various type of cells by type I, II, and III IFNs, double-stranded (ds) B-form DNA, lipopolysaccharides (LPS), the dsRNA analog poly I:C, and viral infections. Function of viperin is normally acting as anti-viral protein but by HCMV infection re-localized to mitochondria, viperin plays role as a regulator of cell signal pathways or cellular metabolism. By interacting with vMIA viperin is translocated to mitochondria and interacts with trifunctional protein (TFP), an essential enzyme for fatty acid beta-oxidation. As a result, viperin modulates cellular metabolism that increase lipid synthesis and accumulation of lipid droplets. On the basis of metabolic changes by viperin, we previously demonstrated that under serum starvation, hypoxia and IFN treatment through PI3K/AKT/mTOR/ HIF-1α pathway expressed viperin plays role as a regulator in cancer metabolism. Similar with viperin, PD-L1 also induced by hypoxia and IFN treatment and activates AKT/mTOR pathway and glycolysis to dampen T cell by competing glycolysis.

In this study, I identified viperin and PD-L1 have interaction through PI3K/AKT/mTOR/ HIF-1α pathway and result in regulation of cancer metabolism. Using viperin knockdown cell lines, I determined expression of PD-L1 depends on expression of viperin. Viperin previously demonstrated induced by PI3K/AKT/mTOR/ HIF-1α pathway under serum starvation. Interestingly, viperin

knockdown cells have no change or reduce activation levels of AKT and S6 under serum starvation. The result suggests that viperin may play a role of feedback on the pathway by serum starvation. PD-L1 is previously reported that promote AKT/mTOR and glycolysis. PD-L1 knockdown cell showed that diminished activation levels of AKT and S6 and consequently reduced expression of viperin. Moreover, despite of decrease of AKT/mTOR activation levels, viperin and PD-L1 were induced slightly in viperin knockdown cell lines. Its suggest that viperin and PD-L1 may be induced through another pathway as well as PI3K/AKT/mTOR /HIF-1α. In cancer metabolism, PD-L1 is reported that contribute to glycolysis but not lipogenesis. I indicated that interplay of viperin and PD-L1 regulates not only glycolysis but also lipogenesis. However, whether mechanism of interactions between viperin and PD-L1 are direct or indirect is still unclear and will need to be further elucidated.

In PD-L1/PD-1 immune checkpoint blockade therapy aspect, pretreatment showed that tumor PD-L1 expression correlates with response to anti-PD-L1/PD-1 therapies. But, the significant number of patients with PD-L1-positive do not respond to PD-1 pathway blockade. Recently studies identified additional intratumoral factors, including not only associated with immune functions but also associated with metabolic were involved in failure of PD-1–targeted therapies patients.[62] Therefore, these studies suggest that dual targeting of viperin and PD-L1 may provide an exciting new avenue for treatment of cancers.

V. CONCLUSION

The study shown that expression and function of viperin and PD-L1 have interaction with between each other in cancer cells. Two molecules share PI3K/AKT/mTOR/HIF-1α pathway and regulates mutual expression. In serum starvation, viperin positively regulates PD-L1 gene and protein expression through PI3K/AKT/mTOR/HIF-1α pathway and vice versa. Besides, the interaction between viperin and PD-L1 results in modulation of glycolytic and lipogenic enzymes expression in cancer cells. To sum up, viperin and PD-L1 interact each other and this interaction is involved in cancer metabolism. Altogether, these data suggest that viperin and PD-L1 can be potential therapeutic targets in treatment of cancers and dual targeting of viperin and PD-L1 may provide an exciting new avenue for treatment of cancers.

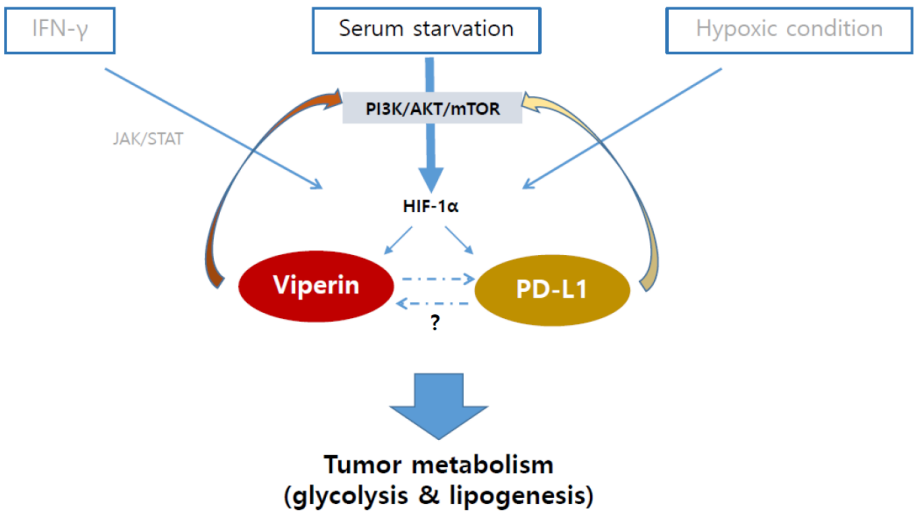


Figure 9. Working model for the interplay of viperin and PD-L1.

REFERENCES

1. Chin KC, Cresswell P. Viperin (cig5), an IFN-inducible antiviral protein directly induced by human cytomegalovirus. Proc Natl Acad Sci U S A 2001;98:15125-30.
2. Helbig KJ, Beard MR. The role of viperin in the innate antiviral response. J Mol Biol 2014;426:1210-9.
3. Seo JY, Yaneva R, Cresswell P. Viperin: a multifunctional, interferon-inducible protein that regulates virus replication. Cell Host Microbe 2011;10:534-9.
4. Hinson ER, Cresswell P. The antiviral protein, viperin, localizes to lipid droplets via its N-terminal amphipathic alpha-helix. Proc Natl Acad Sci U S A 2009;106:20452-7.
5. Hinson ER, Cresswell P. The N-terminal amphipathic alpha-helix of viperin mediates localization to the cytosolic face of the endoplasmic reticulum and inhibits protein secretion. J Biol Chem 2009;284:4705-12.
6. Duschene KS, Broderick JB. The antiviral protein viperin is a radical SAM enzyme. Febs Letters 2010;584:1263-7.
7. Shaveta G, Shi JH, Chow VTK, Song JX. Structural characterization reveals that viperin is a radical S-adenosyl-L-methionine (SAM) enzyme. Biochemical and Biophysical Research Communications 2010;391:1390-5.
8. Jiang D, Guo HT, Xu CX, Chang JH, Gu BH, Wang LJ, et al. Identification of three interferon-inducible cellular enzymes that inhibit the replication of hepatitis C virus. Journal of Virology 2008;82:1665-78.
9. Seo JY, Yaneva R, Hinson ER, Cresswell P. Human cytomegalovirus directly induces the antiviral protein viperin to enhance infectivity. Science 2011;332:1093-7.
10. Helbig KJ, Eyre NS, Yip E, Narayana S, Li K, Fiches G, et al. The antiviral protein viperin inhibits hepatitis C virus replication via interaction with nonstructural protein 5A. Hepatology 2011;54:1506-17.
11. Helbig KJ, Carr JM, Calvert JK, Wati S, Clarke JN, Eyre NS, et al. Viperin

Is Induced following Dengue Virus Type-2 (DENV-2) Infection and Has Anti-viral Actions Requiring the C-terminal End of Viperin. Plos Neglected Tropical Diseases 2013;7.

12. Nasr N, Maddocks S, Turville SG, Harman AN, Woolger N, Helbig KJ, et al. HIV-1 infection of human macrophages directly induces viperin which inhibits viral production. Blood 2012;120:778-88.
13. Hinson ER, Joshi NS, Chen JH, Rahner C, Jung YW, Wang XY, et al. Viperin Is Highly Induced in Neutrophils and Macrophages during Acute and Chronic Lymphocytic Choriomeningitis Virus Infection. Journal of Immunology 2010;184:5723-31.
14. Qiu LQ, Cresswell P, Chin KC. Viperin is required for optimal Th2 responses and T-cell receptor-mediated activation of NF-kappa B and AP-1. Blood 2009;113:3520-9.
15. Seo JY, Cresswell P. Viperin regulates cellular lipid metabolism during human cytomegalovirus infection. PLoS Pathog 2013;9:e1003497.
16. Devic S. Warburg Effect - a Consequence or the Cause of Carcinogenesis? J Cancer 2016;7:817-22.
17. Icard P, Poulain L, Lincet H. Understanding the central role of citrate in the metabolism of cancer cells. Biochim Biophys Acta 2012;1825:111-6.
18. Nakashima RA, Paggi MG, Pedersen PL. Contributions of glycolysis and oxidative phosphorylation to adenosine 5'-triphosphate production in AS-30D hepatoma cells. Cancer Res 1984;44:5702-6.
19. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 2003;91 Spec No:179-94.
20. Mounier C, Bouraoui L, Rassart E. Lipogenesis in cancer progression (review). Int J Oncol 2014;45:485-92.
21. Abramson HN. The lipogenesis pathway as a cancer target. J Med Chem 2011;54:5615-38.
22. Lim S, Phillips JB, da Silva LM, Zhou M, Fodstad O, Owen LB, et al. Interplay between Immune Checkpoint Proteins and Cellular Metabolism. Cancer Research 2017;77:1245-9.

23. Wang Y, Wang H, Yao H, Li C, Fang JY, Xu J. Regulation of PD-L1: Emerging Routes for Targeting Tumor Immune Evasion. Front Pharmacol 2018;9:536.
24. Sun C, Mezzadra R, Schumacher TN. Regulation and Function of the PD-L1 Checkpoint. Immunity 2018;48:434-52.
25. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Molecular Cancer Therapeutics 2015;14:847-56.
26. Xie QK, Zhao YJ, Pan T, Lyu N, Mu LW, Li SL, et al. Programmed death ligand 1 as an indicator of pre-existing adaptive immune responses in human hepatocellular carcinoma. Oncoimmunology 2016;5.
27. Topalian SL, Drake CG, Pardoll DM. Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. Cancer Cell 2015;27:450-61.
28. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations. Journal for Immunotherapy of Cancer 2018;6.
29. Zhao XD, Subramanian S. Intrinsic Resistance of Solid Tumors to Immune Checkpoint Blockade Therapy. Cancer Research 2017;77:817-22.
30. Haratake N, Toyokawa G, Tagawa T, Kozuma Y, Matsubara T, Takamori S, et al. Positive Conversion of PD-L1 Expression After Treatments with Chemotherapy and Nivolumab. Anticancer Res 2017;37:5713-7.
31. Lee SJ, Jang BC, Lee SW, Yang YI, Suh SI, Park YM, et al. Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274). FEBS Lett 2006;580:755-62.
32. Atsaves V, Tsesmetzis N, Chioureas D, Kis L, Leventaki V, Drakos E, et al. PD-L1 is commonly expressed and transcriptionally regulated by STAT3 and MYC in ALK-negative anaplastic large-cell lymphoma. Leukemia 2017;31:1633-7.
33. Casey SC. MYC regulates the antitumor immune response through CD47 and PD-L1 (vol 352, aaf7984, 2016). Science 2016;353:229-.

34. Wang J, Jia Y, Zhao S, Zhang X, Wang X, Han X, et al. BIN1 reverses PD-L1-mediated immune escape by inactivating the c-MYC and EGFR/MAPK signaling pathways in non-small cell lung cancer. Oncogene 2017;36:6235-43.
35. Bu LL, Yu GT, Wu L, Mao L, Deng WW, Liu JF, et al. STAT3 Induces Immunosuppression by Upregulating PD-1/PD-L1 in HNSCC. Journal of Dental Research 2017;96:1027-34.
36. Marzec M, Zhang Q, Goradia A, Raghunath PN, Liu XB, Paessler M, et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). Proceedings of the National Academy of Sciences of the United States of America 2008;105:20852-7.
37. Gowrishankar K, Gunatilake D, Gallagher SJ, Tiffen J, Rizos H, Hersey P. Inducible but Not Constitutive Expression of PD-L1 in Human Melanoma Cells Is Dependent on Activation of NF-kappa B. Plos One 2015;10.
38. Green MR, Rodig S, Juszczynski P, Ouyang J, Sinha P, O'Donnell E, et al. Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy. Clin Cancer Res 2012;18:1611-8.
39. Barsoum IB, Smallwood CA, Siemens DR, Graham CH. A Mechanism of Hypoxia-Mediated Escape from Adaptive Immunity in Cancer Cells. Cancer Research 2014;74:665-74.
40. Koh J, Jang JY, Keam B, Kim S, Kim MY, Go H, et al. EML4-ALK enhances programmed cell death-ligand 1 expression in pulmonary adenocarcinoma via hypoxia-inducible factor (HIF)-1alpha and STAT3. Oncoimmunology 2016;5:e1108514.
41. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1 alpha., and its blockade under hypoxia enhanced MDSC-mediated T cell activation. Journal of Experimental Medicine 2014;211:781-90.
42. Ruf M, Moch H, Schraml P. PD-L1 expression is regulated by hypoxia

inducible factor in clear cell renal cell carcinoma. Int J Cancer 2016;139:396-403.

43. Akbay EA, Koyama S, Carretero J, Altabef A, Tchaicha JH, Christensen CL, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov 2013;3:1355-63.

44. Chen N, Fang W, Zhan J, Hong S, Tang Y, Kang S, et al. Upregulation of PD-L1 by EGFR Activation Mediates the Immune Escape in EGFR-Driven NSCLC: Implication for Optional Immune Targeted Therapy for NSCLC Patients with EGFR Mutation. J Thorac Oncol 2015;10:910-23.

45. Lastwika KJ, Wilson W, 3rd, Li QK, Norris J, Xu H, Ghazarian SR, et al. Control of PD-L1 Expression by Oncogenic Activation of the AKT-mTOR Pathway in Non-Small Cell Lung Cancer. Cancer Res 2016;76:227-38.

46. Zhao XD, Subramanian S. Oncogenic pathways that affect antitumor immune response and immune checkpoint blockade therapy. Pharmacology & Therapeutics 2018;181:76-84.

47. Wang Q, Lin W, Tang X, Li S, Guo L, Lin Y, et al. The Roles of microRNAs in Regulating the Expression of PD-1/PD-L1 Immune Checkpoint. Int J Mol Sci 2017;18.

48. Burr ML, Sparbier CE, Chan YC, Williamson JC, Woods K, Beavis PA, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. Nature 2017;549:101-5.

49. Mezzadra R, Sun C, Jae LT, Gomez-Eerland R, de Vries E, Wu W, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. Nature 2017;549:106-+.

50. Li CW, Lim SO, Xia WY, Lee HH, Chan LC, Kuo CW, et al. Glycosylation and stabilization of programmed death ligand-1 suppresses T-cell activity. Nature Communications 2016;7.

51. Lim SO, Li CW, Xia WY, Cha JH, Chan LC, Wu Y, et al. Deubiquitination and Stabilization of PD-L1 by CSN5. Cancer Cell 2016;30:925-39.

52. Song M, Chen D, Lu B, Wang C, Zhang J, Huang L, et al. PTEN loss increases PD-L1 protein expression and affects the correlation between PD-L1 expression and clinical parameters in colorectal cancer. PLoS One

2013;8:e65821.

53. Zhang XH, Zeng YY, Qu QX, Zhu JJ, Liu ZY, Ning WW, et al. PD-L1 induced by IFN-gamma from tumor-associated macrophages via the JAK/STAT3 and PI3K/AKT signaling pathways promoted progression of lung cancer. International Journal of Clinical Oncology 2017;22:1026-33.
54. Atefi M, Avramis E, Lassen A, Wong DJL, Robert L, Foulad D, et al. Effects of MAPK and PI3K Pathways on PD-L1 Expression in Melanoma. Clinical Cancer Research 2014;20:3446-57.
55. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nature Medicine 2007;13:84-8.
56. Xu C, Fillmore CM, Koyama S, Wu H, Zhao Y, Chen Z, et al. Loss of Lkb1 and Pten leads to lung squamous cell carcinoma with elevated PD-L1 expression. Cancer Cell 2014;25:590-604.
57. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. Cell 2015;162:1229-41.
58. Mandai M, Hamanishi J, Abiko K, Matsumura N, Baba T, Konishi I. Dual Faces of IFNgamma in Cancer Progression: A Role of PD-L1 Induction in the Determination of Pro- and Antitumor Immunity. Clin Cancer Res 2016;22:2329-34.
59. DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv 2016;2:e1600200.
60. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene 2008;27:5497-510.
61. Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol 2013;15:555-64.
62. Ascierto ML, McMiller TL, Berger AE, Danilova L, Anders RA, Netto GJ, et al. The Intratumoral Balance between Metabolic and Immunologic Gene Expression Is Associated with Anti-PD-1 Response in Patients with Renal Cell Carcinoma. Cancer Immunology Research 2016;4:726-33.

ABSTRACT (IN KOREAN)

위암세포주에서 바이페린의 PI3K/AKT/mTOR/HIF-1α 신호 경로에 의한 PD-L1 발현 조절

<지도교수 서 준 영>

연세대학교 대학원 의과학과

김 구 슬

바이페린은 인터페론에 의해 유도되는 다기능의 단백질로서 일반적으로 소포체에 위치하며 항 바이러스 작용을 한다. 그러나 최근 연구에 따르면 Human cytomegalovirus (HCMV) 감염에 의해 이 단백질은 소포체에서 미토콘드리아로 이동하게 된다. 미토콘드리아로 이동된 바이페린은 삼중기능단백질과의 결합을 통해 세포내 지방 대사와 해당 작용을 조절한다. 바이페린이 바이러스 감염 세포에서만 아니라 암세포에서도 대사 작용의 변화를 조절에 있어 산소와 영양분이 결핍되거나 인터페론과 같은 시토카인이 존재하는 종양 미세 환경에서 PI3K/AKT/mTOR/HIF-1α 또는 JAK/STAT 경로를 통해 바이페린의 발현이 유도된다는 사실을 선행 연구를 통해 확인하였다. 또한, PD-L1

의 발현 경로와 발현이 유도되는 조건이 바이페린과 유사하고 암에서 발현된 PD-L1이 T 세포와의 대사 경쟁을 위해 해당 과정을 활성화 시킨다고 보고를 기반으로, 바이페린과 PD-L1이 유사한 발현 경로에 있어 공동 상호작용을 통해 암세포의 대사 작용을 조절할 것이라는 가설을 세웠다. 가설을 증명하기 위해 위암 세포 주에 Lentivirus를 통해 바이페린이 발현하지 않는 세포 주를 제작하고, 짧은 간섭RNA를 이용해 PD-L1이 발현되지 않는 세포를 제작하여 두 단백질이 같은 경로를 통해 발현하며 서로의 전달RNA와 단백질 발현에 영향을 끼침을 확인하였다. 또한 상호작용을 통해 발현된 두 단백질은 암세포의 지방 대사와 해당 작용과 같은 암 대사에서 역할을 하고 있음을 확인하였다. 결론적으로 바이페린과 PD-L1은 상호작용을 통해 서로의 발현 조절 인자로서 역할을 할 수 있고, 이러한 상호작용을 통해 암의 대사 작용을 조절 할 수 있음을 밝혔다. 따라서 항암치료에서 PD-L1과 바이페린은 이중 표적이 될 수 있음을 제시하는 바이다.

핵심되는 말: 바이페린, PD-L1, 위암, 대사, 해당작용, 지방 대사, 정보 전달 경로