이학석사 학위논문

Phosphoserine aminotransferase1의 구조적 특성을 기반으로 한 저해제 발굴

Screening of Phosphoserine aminotransferase 1 Inhibitor Based on a Structural Feature

2017년 02월

서울대학교 융합과학기술대학원

분자의학 및 바이오제약 전공

김 영 아

A thesis of the Degree of Master of Philosophy

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이 논문을 이학석사 학위논문으로 제출함

2017년 2월

서울대학교 융합과학기술대학원

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김영아의 이학석사 학위논문을 인준함

2017년 2월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

Screening of Phosphoserine aminotransferase 1 Inhibitor Based on a Structural Feature

by

Young Ah Kim

A thesis submitted to the Department of Molecular Medicine and Biopharmaceutical Sciences
in partial fulfillment of the requirements for the Degree of Master of Philosophy in Science at Seoul National University
Graduate School of Convergence Science and Technology, and College of Medicine or College of Pharmacy

February, 2017

Approved by Thesis Committee:

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ABSTRACT

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Phosphoserine aminotransferase 1 (Psat1) is a transaminase involved in *de novo* serine biosynthesis pathway generating α-ketoglutarate (α-KG) (Baek et al., 2003). α-KG is a cofactor of α-KG-dependent dioxygenases including ten-eleven translocation (Tet) family of DNA hydroxylases and Jumanji C-domain-containing histone demethylases (JHDMs) (Kaelin and McKnight, 2013). Specifically Psat1 directs a-KG level thereby regulating epigenetic landscape for maintaining pluripotency in mouse embryonic stem cells (mESCs) (Hwang et al., 2016).

Cancer stem cells (CSCs) are subpopulation of tumor which, like stem cells, can self-renew and differentiate into the cells consisting of the tumor. Eradication of CSCs is critical for fundamental cancer treatment because CSCs can cause cancer recurrence (Nassar and Blanpain, 2016; Reya et al., 2001).

In this study, it was showed that PSAT1 has positive correlation with OCT4, a core pluripotency transcription factor, in breast cancer and specifically is

highly expressed in basal-like subtype which has large enrichment of breast CSCs relative to other subtypes. Based on these, it was expected that inhibition of Psat1 activity can induce differentiation of CSCs, after all, removal of CSCs. Therefore, we tried to identify Psat1 inhibitor which reduces Psat1 activity by blocking active structural formation. First, it was confirmed that Psat1 forms a homo-dimer structure. Screening for identifying Psat1 dimerization inhibitor was performed using 3920 chemicals in mammalian two hybrid system, a powerful tool which can detect protein-protein interaction. As a result, 1 candidate chemical was found. Although screening to more chemicals should be performed, it is anticipated that Psat1 inhibitor can open new era for cancer therapy.

Keywords: Phosphoserine aminotransferase 1 (Psat1), Cancer stem cells (CSCs)

Student number: 2015-26002

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CSCs	Cancer stem cells
Psat1	Phosphoserine aminotransferase 1
mESCs	Mouse embryonic stem cells
α-KG	α-ketoglutarate
Tet DNA hydroxylases	Ten-eleven translocation DNA hydroxylases
JHDMs	Jumanji C-domain-containing histone demethylases
PLP	Pyridoxal phosphate
TCGA	The Cancer Genome Atlas
BPTES	Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide
GFP	Green fluorescent protein

I. INTRODUCTION

1-1. Cancer stem cells

Tumor cells become heterogeneous by accumulating genetic and epigenetic mutation and interaction with microenvironment (Visvader and Lindeman, 2012). Small portion of cells in tumor can self-renew and differentiate into the cells which constitute the tumor, in other words, have tumor-initiating properties. The small portion of cells is called ‘cancer stem cells (CSCs)’ (Reya et al., 2001). Although CSCs are not fully understood, some properties were revealed. Oct4, Sox2, and Nanog, the core pluripotency transcription factors, are important to enhance malignancy and induce stem cell-like properties in tumor (Chiou et al., 2010; Leis et al., 2012). And CSCs existed in dormant state and activation of the dormant CSCs can induce cancer recurrence (Visvader and Lindeman, 2012). These properties make CSCs resistant to current chemo-therapy and surgery for cancer treatment. Because CSCs occupy rare population in whole tumor cells and are long-living cells in dormant state, they can evade many cancer therapies which kill the bulk of fast-dividing tumor cells (Gupta et al., 2009; Visvader and Lindeman, 2012). As a result, a possibility remains that CSCs can regenerate new tumors. Therefore, eradiation of CSCs are critical for ultimate cancer treatment.

1-2. Phosphoserine aminotransferase 1 (Psat1)

Psat1 is a PLP-dependent transaminase which is involved in *de novo* serine biosynthesis. In this biosynthesis pathway, PSAT1 converts phosphohydroxypyruvate to phosphoserine using glutamate as an amine donor and also generates α-KG (Baek et al., 2003).

α-KG is a cofactor of α-KG dependent dioxygenases including Tet family of DNA hydroxylases and JHDMs (Kaelin and McKnight, 2013). Recently, an importance of the α-KG was revealed in stem cell biology. Intracellular α-KG is important to maintain pluripotency by DNA and histone demethylation in mESCs (Carey et al., 2015). And a production of the α-KG is directed by Psat1. Psat1 is a target protein of core pluripotency transcription factors such as Oct4, Sox2 and Nanog. Through the generation of α-KG, Psat1 regulates DNA methylation and histone methylation such as H3K9me3 and H3K36me3 specifically, thereby establishing specific epigenetic landscape to maintain pluripotency in mESCs. When mRNA level of Psat1 was severely down-regulated, dysregulated DNA and histone methylation causes early onset of differentiation in mESCs (Hwang et al., 2016).

Moreover PSAT1 is associated with therapy-resistance in some cancer. Overexpression of PSAT1 is observed in colon cancer patients. Colon cancer cell line overexpressing PSAT1 is resistant to oxaliplatin-induced cell death and G2/M arrest (Vie et al., 2008). Also promoter hypomethylation of PSAT1 and overexpression of PSAT1 is observed in recurrent breast cancer patients despite of tamoxifen therapy (Martens et al., 2005).

1-3. Purpose

Psat1 is a PLP-dependent transaminase which is involved in *de novo* serine biosynthesis. Psat1 generates α-KG catalyzing phosphohydroxypyruvate and glutamate to phosphoserine (Baek et al., 2003). α-KG is a cofactor of DNA and histone modifying enzymes such as Tet DNA hydroxylases and JHDMs thereby playing important role in their enzymatic activities (Kaelin and McKnight, 2013). Recently it was revealed that intracellular α-KG is important to maintain pluripotency by DNA and histone demethylation in mESCs (Carey et al., 2015). And a production of the α-KG is directed by Psat1. Psat1 regulates maintenance of pluripotency by α-KG-mediated epigenetic regulation. It was observed that knock-down of Psat1 ultimately lead to accelerating differentiation (Hwang et al., 2016).

CSCs have strong ability to induce metastasis and regenerate tumor despite chemo-therapy and surgery. Therefore it is essential to eradicate CSCs for fundamental cancer treatment (Reya et al., 2001; Visvader and Lindeman, 2012). Inducing differentiation of CSCs can attenuate stem cell-like properties of CSCs, after all, eradicate CSCs. It is expected that inhibition of Psat1 activity has a potential to promote differentiation of CSCs. Therefore we tried to identify chemicals which can inhibit Psat1 activity.

II. MATERIALS AND METHODS

2-1. Cell culture and transient expression

HEK 293T cells was grown in Dulbecco's Modified Eagle Media (DMEM, GE healthcare) supplemented with 10% (v/v) fetal bovine serum (FBS), 100units/ml penicillin and 100μg/ml streptomycin. Transfections were done using polyethyleneimine (PEI, Polysciences).

2-2. DNA constructs and site-directed mutagenesis

pCAG-Flag-Psat1 plasmid was previously described (Hwang et al., 2016). pCAG-HA-Psat1, pcDNA3.1-Psat1-Myc, pRSETA-Psat1 and pGEX4T1-Psat1 were generated by sub-cloning of full-length Psat1 PCR product. pM-Flag-Psat1 and pVP16-Flag-Psat1 were generated by sub-cloning of full-length Flag-Psat1 PCR product from pCAG-Flag-Psat1 template. To detect Psat1 more easily in western blotting, we amplified *Flag-Psat1* gene sequence in PCR and inserted it into pM or pVP16 vector which can fuse GAL4 DNA binding domain or VP16 activation domain to protein of interest, respectively. So we could identify Psat1 protein with anti-Flag antibody. Mutations were introduced by site-directed mutagenesis using iPfu. For mutant plasmids, PCR products of mutated Psat1 were obtained from pRSETA-Psat1 or pGEX4T1-Psat1 using proper mutagenesis primer sets. The PCR products were treated with DpnI at 37℃ for 1hr and then used for transformation of E.coli (DH5α). The mutant plasmids were obtained from transformants and verified by

sequencing. Other mutant-expressing plasmids were generated by sub-cloning into suitable vectors.

Primers for site-directed mutagenesis were listed below.

Psat1 A99V

Sense : GAAAGCTGGAAGGAGTGTTGACTACGTGGTGACC

Antisense : GGTCACCACGTAGTCAACACTCCTTCCAGCTTTC

Psat1 D100A

Sense : GAAGGAGTGCTGCCTACGTGGTGAC

Antisense : GTCACCACGTAGGCAGCACTCCTTC

Psat1 S179L

Sense : GGTCTGTGACATGTCCTTAAACTTCTTATCCAGG

Antisense : CCTGGATAAGAAGTTTAAGGACATGTCACAGACC

2-3. Western blot analysis

Cells were lysed in 0.5% NP40 lysis buffer (150mM NaCl, 20mM Tris(pH7.4), 0.5% NP40 and protease inhibitor cocktail (GenDEPOT)). Lysates were separated by SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred to nitrocellulose membranes using transfer buffer (25mM Tris-Cl, 250mM Glycine and 15% (v/v) Methanol) at 4℃ for 2 hours. After blocking with blocking buffer (5% non-fat dry milk, 10mM Tris-Clb(pH7.5), 150mM NaCl and 0.1% Tween-20) for 1 hour, membranes were probed with primary antibodies overnight. To get rid of non-specific antibody binding, membranes were washed 3 times with 1XTBST (10mM Tris-Cl(pH7.5), 150mM NaCl and 0.1% Tween-20). Then membranes were probed with HRP (Horse radish peroxidase)-conjugated secondary antibodies at room temperature for 1 hour and washed 3 times with 1XTBST again. For detection, membranes were reacted with ECL solution (Thermo) and exposed to X-ray film.

2-4. Antibodies

Antibodies for western blot analysis were followed. Anti-β-actin (A5441) and anti-Flag (F3165) were from Sigma-Aldrich. Anti-Myc and anti-HA were obtained from Covance. Anti-His were obtained from Cell signaling.

2-5. Immunoprecipitation

PEI was used for transient transfection to HEK 293T cells. 48 hours after transfection, cells were harvested with phosphate-buffered saline (PBS) and lysed in 0.5% NP40 lysis buffer(150mM NaCl, 20mM Tris(pH7.4), 0.5% NP40 and protease inhibitors). Cell lysates were incubated with suitable antibody at 4℃ overnight and then Protein G bead (Santa Cruz) at 4℃ for 2 hours.

2-6. Protein purification

$(His)_6$-tagged proteins and GST-fusion proteins were expressed in BL21(DE3)pLysS or DH5α. Proteins were induced by adding 1mM Isopropyl β-D-1-thiogalactopyranoside(IPTG) at 37℃ for 5 hours. E.coil were suspended in buffer(20mM Tris-Cl(pH8.0), 1mM EDTA, 137mM NaCl, 10% glycerol, 1mM EDTA, 1%(v/v) protease inhibitor cocktails, 1mM PMSF, 1mM DTT) on ice and incubated for 10 min. After incubation, 50mg/ml lysozyme was added and incubated at 4℃ for 30-60 min. Sonication was performed and the cell lysate was incubated with 1% (v/v) Triton X-100. $(His)_6$-tagged proteins and GST-fusion proteins were purified using Ni-NTA agarose bead(Qiagene) or glutathione sepharose 4B bead(GE healthcare), respectively. $(His)_6$-tagged proteins were eluted using 500mM imidazole, 100mM NaCl, 50mM Tris-Cl(pH8.0) and 10% glycerol and GST-fusion

proteins were eluted using 20mM reduced glutathione, 100mM Tris-Cl(pH8.0), 120mM NaCl and 10% glycerol.

2-7. *In vitro* His-pull down assay

GST-fused proteins were incubated with $(His)_6$-tagged proteins in 0.5% NP40 lysis buffer(150mM NaCl, 20mM Tris(pH7.4), 0.5% NP40 and protease inhibitors) at 4℃ overnight. And then, $(His)_6$-tagged proteins were purified using Ni-NTA agarose bead(Qiagene).

2-8. Reporter gene assay

For the reporter gene assays using pG5-luc vector or pG5-GFP vector containing five consensus GAL4 binding sites, TATA box and *luciferase* or *green fluorescent protein (GFP)* gene, HEK 293T cells were transfected with the luciferase or GFP reporter plasmid along with pM-Flag-Psat1 and/or pVP16-Flag-Psat1. Cells were harvested 48hrs after transfection and luciferase activities were measured using infinite M 200 (Tecan) with Magellan^{TM} software. GFP signals were observed using X-Cite series 120 (EXFO).

2-9. Screening of chemical compounds using two hybrid system

pM-Flag-Psat1 and pVP16-Flag-Psat1 were transiently co-transfected in HEK 293T cells which stably express pG5-GFP vector. 24 hours after transfection, cells were seeded onto the 96-well plate at a density of $5x10^4$ cells per well. Next day, chemical compounds (from representative library provided by Korea Chemical Bank) were added at a concentration of 20μM in first screening. DMSO at 1% was used as negative control. Another 24 hours later, GFP signals were observed using X-Cite series 120 (EXFO). The second screening was performed at 10 μM in the same way. Luciferase assay was performed after second screening as readout. HEK 293T cells were transfected with the pG5-luc vector along with pM-Flag-Psat1 and/or pVP16-Flag-Psat1. 24 hours after transfection, chemicals were added at 5 μM concentration. Next day, cells were harvested and luciferase activities were measured using infinite M 200 (Tecan) with Magellan™ software.

Ⅲ. RESULTS

3-1. Psat1 might be associated with properties of CSCs.

Previous studies revealed that core pluripotency transcription factors, OCT4, SOX2, and NANOG can induce more severe malignancy and enhance stem cell-like properties of tumor cells (Chiou et al., 2010; Leis et al., 2012). First, a relationship between PSAT1 and core pluripotency transcription factors was investigated in breast cancer using publically available The Cancer Genome Atlas (TCGA) dataset. It was observed that mRNA level of *PSAT1* had a positive correlation with that of *POU5F1* which encodes OCT4 protein (Figure 3-1-1). Also, *PSAT1* was highly expressed in basal-like subtype relative to the other subtypes (Figure 3-1-2). It is known that breast cancer stem cells are largely enriched in basal-like tumor (Dai et al., 2016). Based on these data it was expected that Psat1 might be involved in regulating properties of CSCs. Therefore, we hypothesized that blocking PSAT1 activity has a potential to promote differentiation of CSCs specifically.

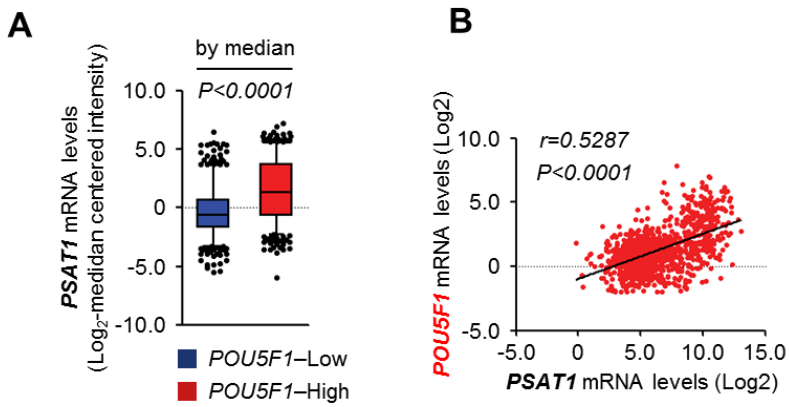

Figure 3-1-1. Relationship between *PSAT1* and *POU5F1* in Breast Cancer

(A) *PSAT1* mRNA levels were elevated in *POU5F1*-High breast cancer relative to *POU5F1*-Low.

(B) Correlation of *PSAT1* and *POU5F1* mRNA expression in human breast cancer.

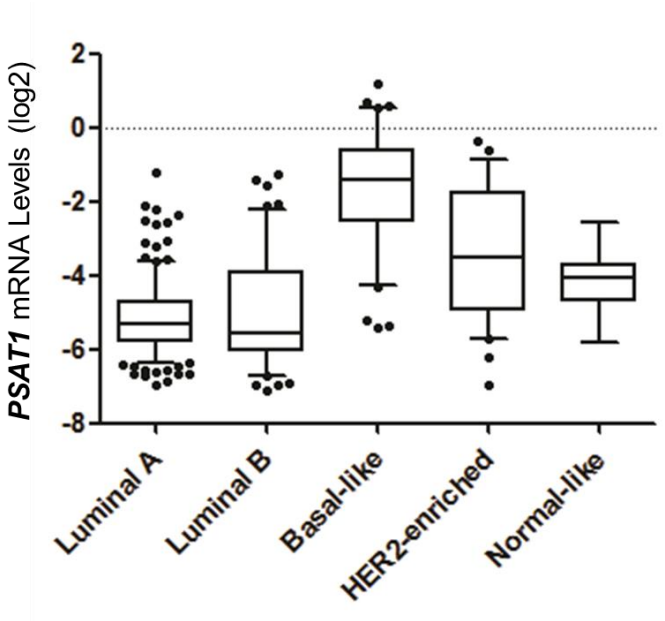

Figure 3-1-2. mRNA levels of *PSAT1* in breast cancer subtypes

mRNA levels of *PSAT1* was investigated in breast cancer molecular subtypes using TCGA dataset. *PSAT1* is highly expressed in basal-like subtype relative to the other subtypes.

3-2. Psat1 forms a homo-dimer structure.

A Strategy for inhibiting Psat1 activity is development of Psat1 allosteric inhibitor. Recently, many studies focused on development of small molecule inhibitor which functions as allosteric inhibition mechanism. Because allosteric inhibitor binds to non-active site which is existed specifically in target protein, it has low risk to affect to other proteins which use the same substrate and high selectivity to target protein. For example, Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES), a glutaminase 1 inhibitor, is an allosteric inhibitor which blocks active tetramer formation (DeLaBarre et al., 2011). Therefore we tried to identify a Psat1 inhibitor which blocks its active structure formation.

First of all, to investigate structure of Psat1, X-ray crystallography was analyzed. As a result, Psat1 consists of a homo-dimer with both monomers contributing to the PLP-containing active site at their interface. (Figure 3-2-1). To confirm the homo-dimerization of Psat1, dimerization test was performed by *in vivo* immunoprecipitation in HEK 293T cells. We transiently overexpressed either Flag-Psat1 and HA-Psat1 or Psat1-Flag and Psat1-Myc in HEK 293T cells and the cell lysate was precipitated with anti-Flag antibody. As a result, Psat1 formed the homo-dimer no matter whether the tag is fused to upstream or downstream of Psat1 (Figure 3-2-2). Next, the homo-dimerization was further confirmed by *in vitro* His pull down assay using bacterially purified $(His)_6$-tagged and GST-fused Psat1 protein (Figure 3-2-3). Psat1 homo-dimerization was also supported using mammalian two

hybrid system. Mammalian two hybrid system is a powerful system which can detect protein-protein interaction (Lievens et al., 2012). In this system, one protein is fused to GAL4 DNA binding domain (GAL4 DNA-BD) and the other to VP16 activation domain (VP16-AD). GAL4 DNA-BD is used to bind a specific DNA sequence. VP16-AD is used to activate transcription of a gene through recruitment of RNA polymerase II. Therefore, interaction between two proteins can activate the gene expression by recruitment of RNA polymerase II to TATA box as shown in Figure 3-2-4. In this system with luciferase reporter gene, luciferase activity was elevated in case of co-transfection of pM-Flag-Psat1 and pVP16-Flag-Psat1 relative to the other cases (Figure 3-2-5) thereby indicating that Psat1 forms the homo-dimer. In addition, when transfection amount of pM-Flag-Psat1 and pVP16-Flag-Psat1 was increased gradually, luciferase activity was also more augmented. In this system with GFP reporter gene, GFP signal was also enhanced in case of co-transfection of pM-Flag-Psat1 and pVP16-Flag-Psat1 (Figure 3-2-6). Thus these data showed that Psat1 forms the homo-dimer.

To investigate whether the disruption of homo-dimerization resulted in reduced reporter activity in mammalian two hybrid system, Psat1 mutants were generated based on PSAT1-dysfunctional patient cases (Table 1 and Figure 3-2-7) (Acuna-Hidalgo et al., 2014). Among them, S179L mutant failed to form the homo-dimer in dimerization test (Figure 3-2-8). As expected, the disruption of homo-dimerization resulted in reduced luciferase activity relative to WT in mammalian two hybrid assay (Figure 3-2-9). Consequently these data confirmed homo-dimerization of Psat1 and suggested availability

of mammalian two hybrid system for screening of chemicals which block Psat1 homo-dimerization.

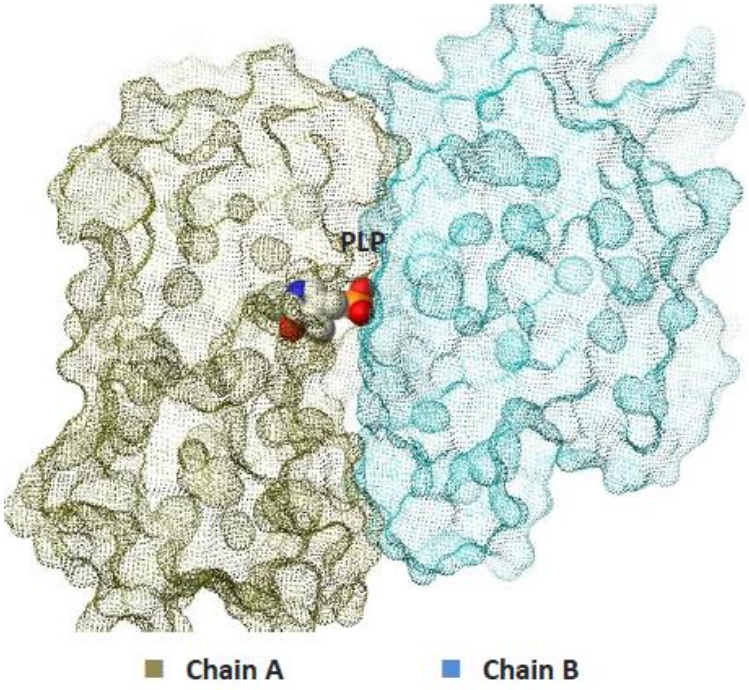

Figure 3-2-1. Analysis of Psat1 structure by X-ray crystallography

Psat1 forms a homo-dimer with both monomers containing PLP-binding active sites at their interfaces. (PDB : 3E77)

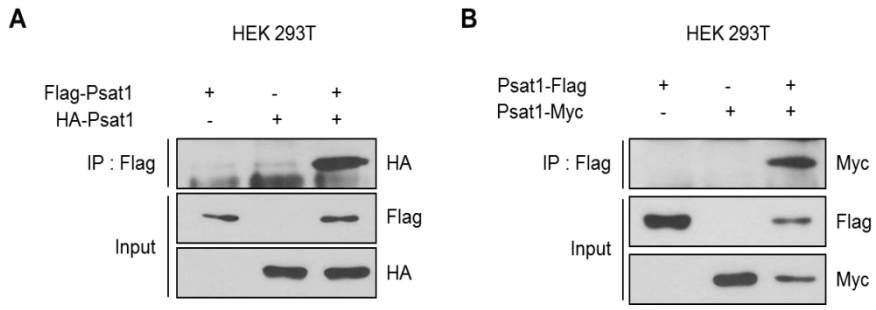

Figure 3-2-2. Psat1 forms the homo-dimer by *in vivo* immunoprecipitation in HEK 293T.

Flag-Psat1, HA-Psat1 (A) or Psat1-Flag, Psat1-Myc (B) constructs were transiently co-transfected in HEK 293T cells. Immunoprecipitaion was done by anti-Flag antibody. Regardless of tag location, Psat1 forms the homo-dimer.

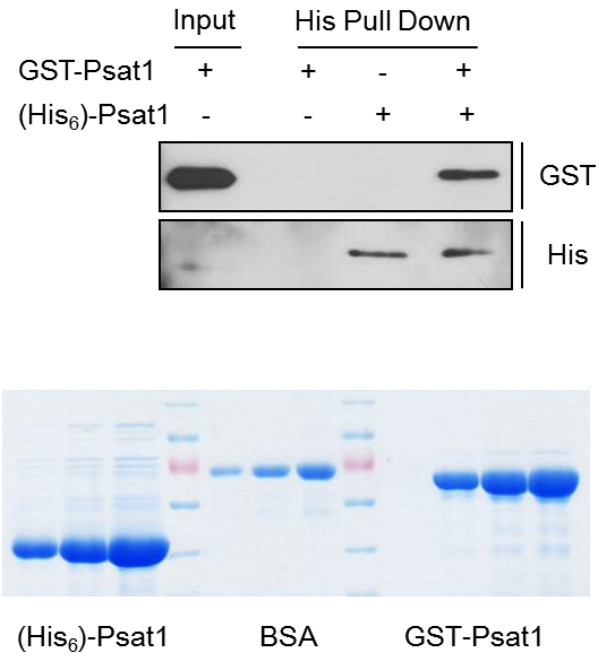

Figure 3-2-3. Psat1 forms the homo-dimer by *in vitro* His pull down assay.

Psat1 forms the homo-dimer by *in vitro* His pull down assay using $(His)_6$-tagged Psat1 and GST-fused Psat1.

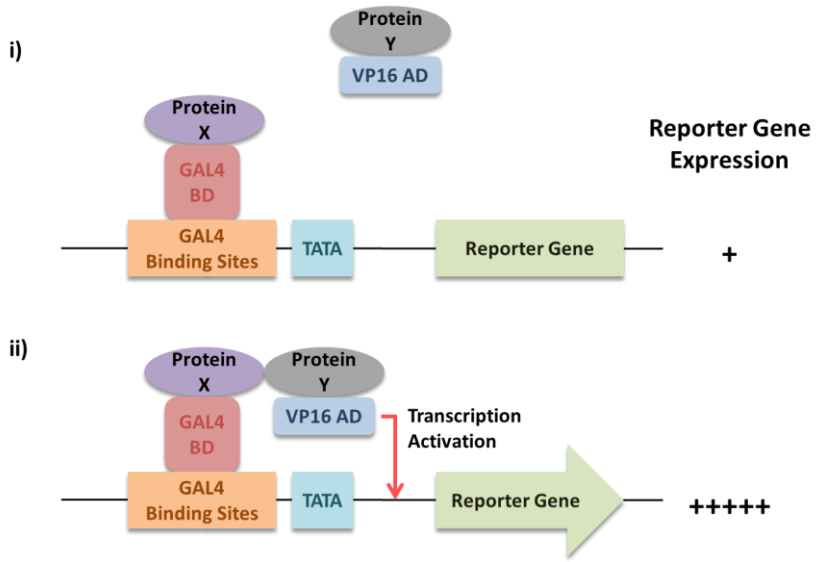

Figure 3-2-4. A schematic model of mammalian two hybrid system

pM vector gene construct which is used to fuse GAL4 DNA-BD and a protein of interest (herein, Psat1), pVP16 vector gene construct which is used to fuse VP16-AD and a protein of interest and reporter gene vector are co-transfected in cells. The reporter vector has GAL4 DNA binding sites, minimal promoter and reporter gene sequentially. If two proteins fail to interact with each other, there is no reporter activity. On the other hand, if two proteins interact with each other, the reporter gene will be expressed.

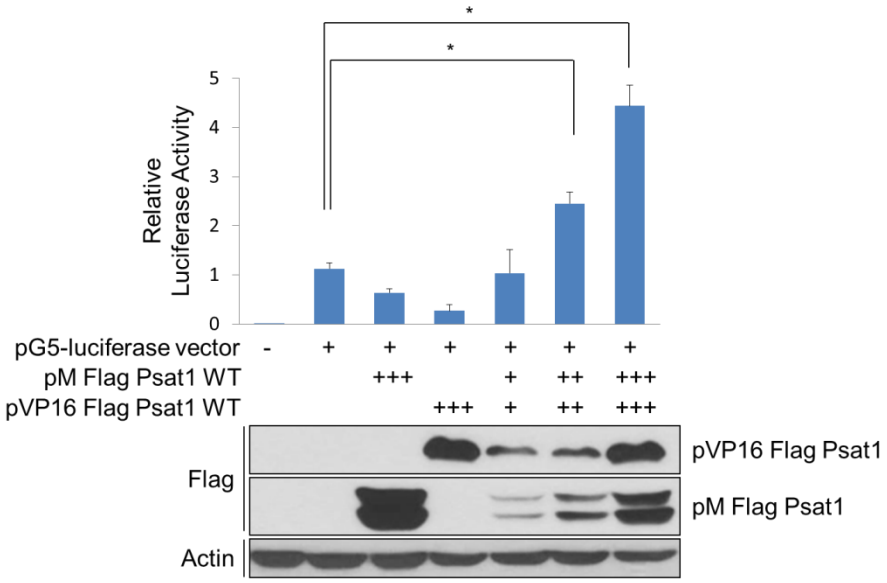

Figure 3-2-5. Psat1 forms the homo-dimer in mammalian two hybrid assay using luciferase reporter.

pG5-luciferase vector was transiently transfected in HEK 293T cells with pM-Flag-Psat1 and/or pVP16-Flag-Psat1. And transfection amount of both Psat1 constructs was increased gradually. Luciferase activity assay was performed. Remaining cell lysates were further analyzed by western blot. *P<0.001(Student's t test).

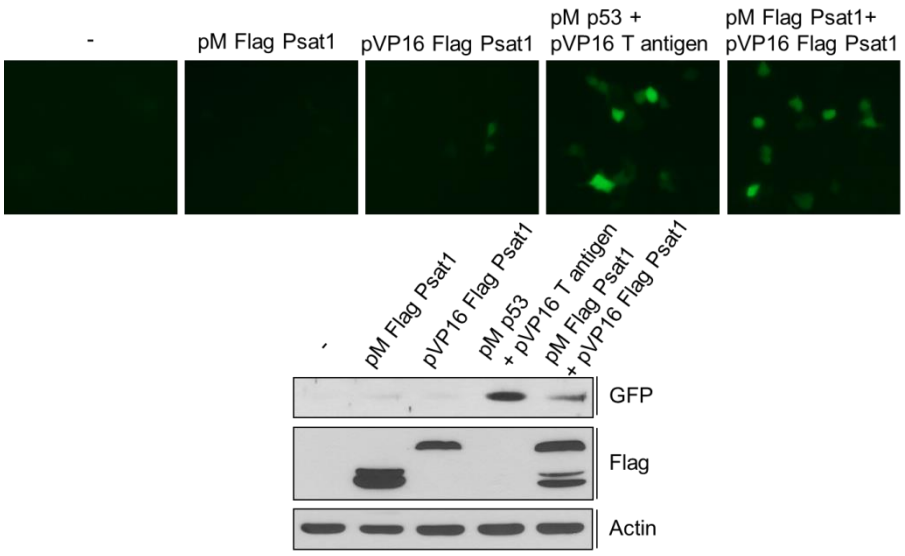

Figure 3-2-6. Psat1 forms the homo-dimer in mammalian two hybrid assay using GFP reporter.

We made HEK 293T cell line which stably expressed pG5-GFP vector. pM-Flag-Psat1 and/or pVP16-Flag-Psat1 were transiently co-transfected in the stable cell. pM-p53 and pVP16-T antigen were used as positive control. Then GFP signals of each case were observed. After observation of GFP signal, remaining cells were lysed and further analyzed by western blot.

Point Mutation	Associated Diseases
A99V	Neu-Laxova syndrome
D100A	Neu-Laxova syndrome
S179L	Neu-Laxova syndrome

Table 1. Point Mutations of Psat1 in patient cases with PSAT1 malfunction.

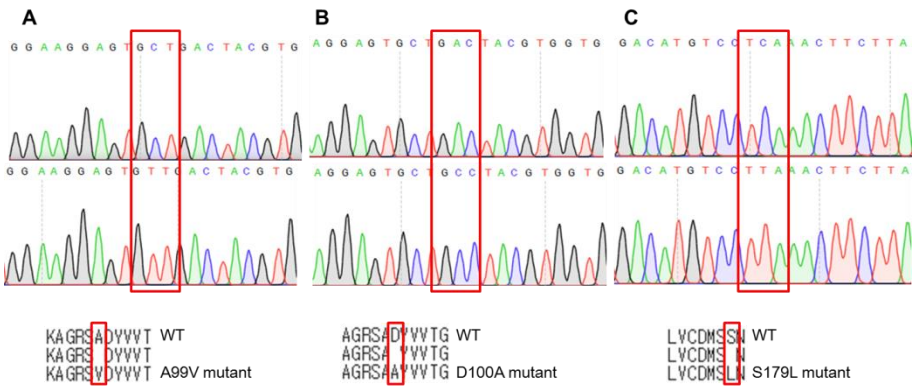

Figure 3-2-7. Sequencing results of Psat1 mutants.

Psat1 mutants were generated according to point mutations reported in Psat1-dysregulated diseases (see Table 1).

(A) In case of A99V mutation, Nucleotides 295 GCT was substituted to GTT. Amino acid 99 Alanine was substituted to Valine.

(B) In case of D100A mutation, Nucleotides 298 GAC was substituted to GCC. Amino acid 100 Asparagine was substituted to Alanine.

(C) In case of S179L mutation, Nucleotides 535 TCA was substituted to TTA. Amino acid 179 Serine was substituted to Leucine.

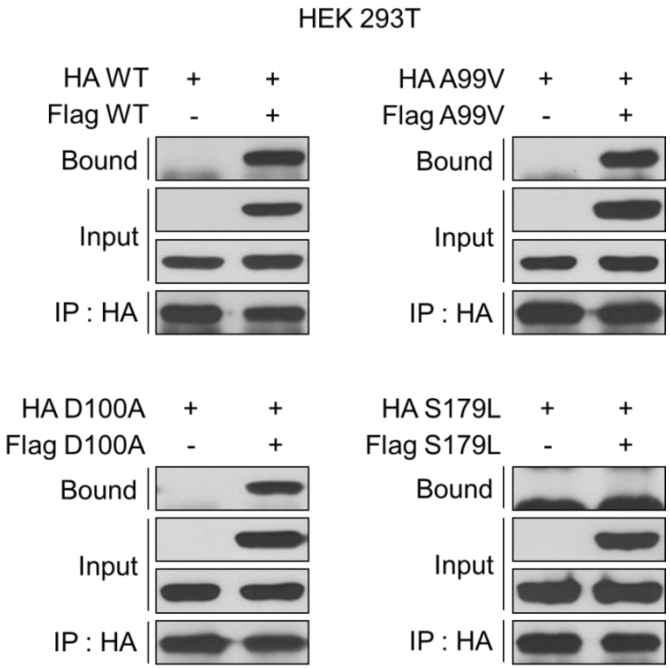

Figure 3-2-8. S179L mutant fails to form the homo-dimer in HEK 293T.

HA-Psat1 WT and mutants were co-transfected transiently with Flag-Psat1 WT and mutants in HEK 293T cells. Dimerization test was performed by immunoprecipitaion using anti-HA antibody. Among mutants, S179L mutant failed to dimerize with WT.

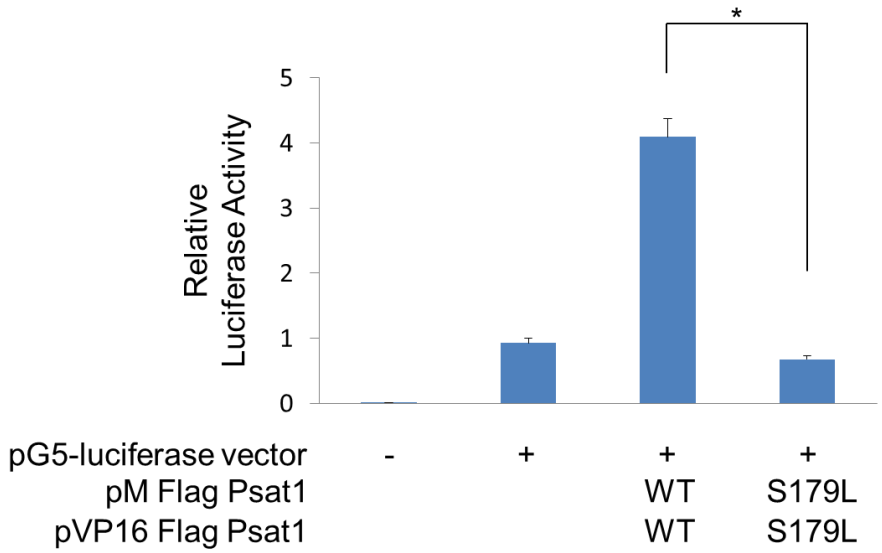

Figure 3-2-9. Reduced luciferase activity caused by disruption of homo-dimerization in mammalian two hybrid assay.

pG5-luciferase vector was transiently co-transfected with pM-Flag-Psat1 WT and pVP16-Flag-Psat1WT or pM-Flag-Psat1 S179L and pVP16-Flag-Psat1 S179L in HEK 293T cells. Luciferase activity assay was performed. *P<0.001(Student's t test).

3-3. Screening of a Psat1 inhibitor which blocks homo-dimerization

Using these approaches, we tried to identify a Psat1 inhibitor which blocks formation of active homo-dimer structure. A procedure of screening is shown in Figure 3-3-1. Representative library containing 8400 chemicals was provided from Korea Chemical Bank. The screening was performed in mammalian two hybrid system with GFP reporter gene. Inhibition of dimerization was detected by the decrease of GFP signals. Among 3820 chemicals screened in first screening, 21 chemicals decreased GFP signal. Because the reduced GFP signal by 21 chemicals occurred by cell death, the second screening was performed at lower concentration in same system. As a result, 3 chemicals still decreased GFP signals (Figure 3-3-2). The 3 chemicals were further analyzed by mammalian two hybrid system with luciferase reporter as readout. As a result, only 239 P19 chemical reduced luciferase activity (Figure 3-3-3).

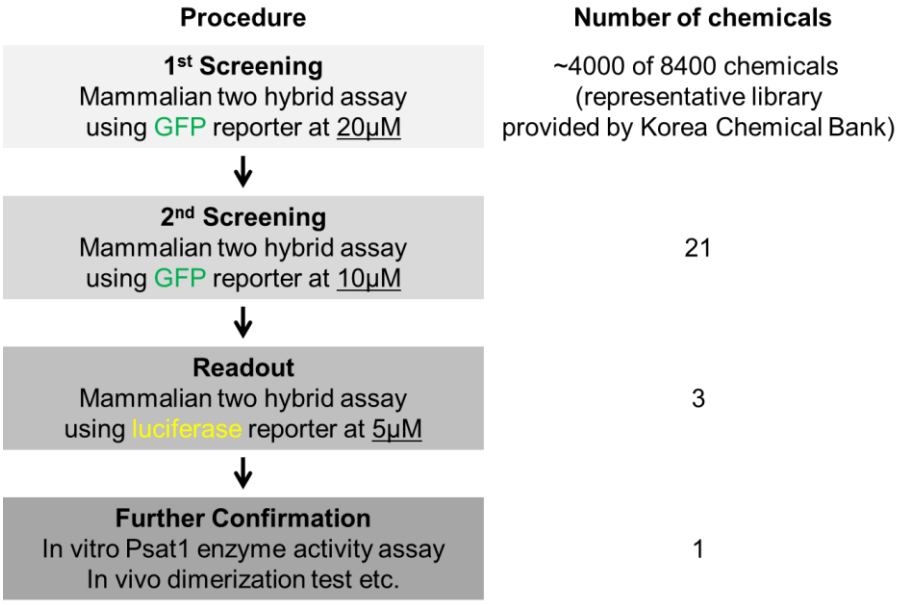

Figure 3-3-1. A Scheme for screening of a Psat1 inhibitor

The screening was performed as shown in this figure.

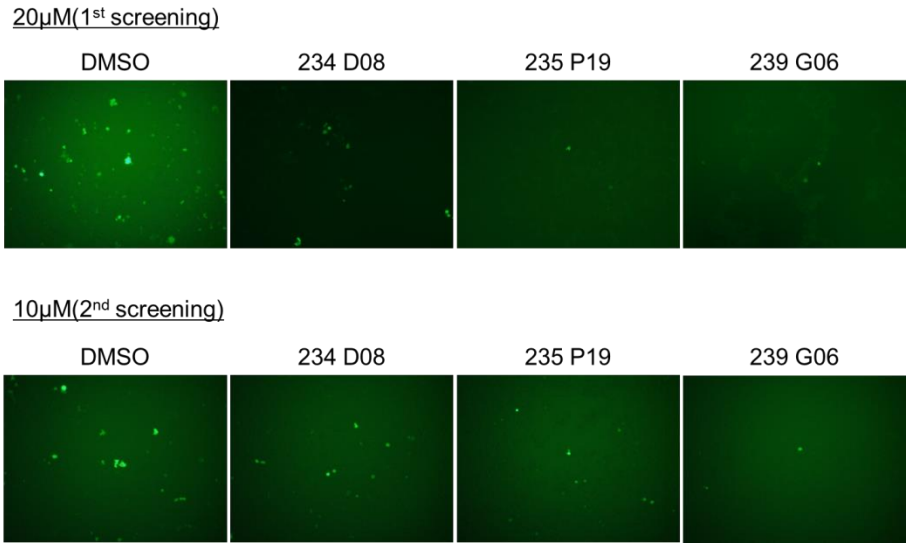

Figure 3-3-2. GFP Signals in Screening of a Psat1 Inhibitor

HEK 293T cell line was generated which stably expressed a pG5-GFP vector. pM-Flag-Psat1 and pVP16-Flag-Psat1 were transiently co-transfected in the stable cell. In 24 hours later after transfection, the cells were seeded on 96 well plates. Next day, chemicals were treated to the cells. In 24 hours later after treatment of chemicals, GFP signals were observed. DMSO was used as negative control. As a result, 234 D08, 235 P19, and 239 G06 still decreased GFP signal in 2nd screening.

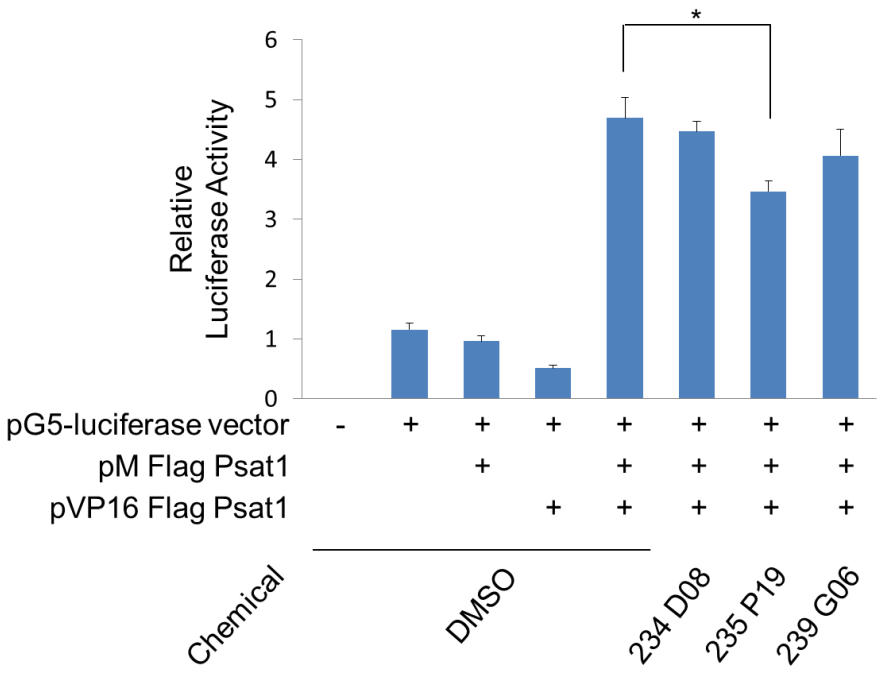

Figure 3-3-3. 235 P19 chemical reduced luciferase activity in mammalian two hybrid system.

pG5-luciferase vector was transiently co-transfected with pM-Flag-Psat1 and pVP16-Flag-Psat1 in HEK293T cells. The 3 chemicals were treated at 5μM. DMSO was used as negative control. Only 235 P19 chemicals decreased luciferase activity significantly. *P<0.001(Student's t test).

IV. DISCUSSION

Recently it was revealed that Psat1 is important to maintain pluripotency through a-KG mediated epigenetic regulation. a-KG functions as a cofactor of a-KG dependent dioxygenases including Tet DNA hydroxylases and JHDMs. When Psat1 was severely knock-downed, dysregulated histone and DNA methylation resulted in failure of maintaining pluripotency (Hwang et al., 2016).

CSCs, which have stem cell-like properties, can induce metastasis and recurrences of cancers. Therefore CSCs should be eradicated (Reya et al., 2001; Visvader and Lindeman, 2012). This study showed that PSAT1 has positive correlation with OCT4 in breast cancer and overexpressed in basal-like type which is most aggressive and enriched with CSCs. Therefore, we hypothesized that inhibition of Psat1 activity can promote differentiation of CSCs, thus, screening for Psat1 inhibitor which blocks homo-dimer formation was performed. In mammalian two hybrid system, a powerful system to detect protein-protein interaction, screening for 3920 chemicals was performed and, as a result, 1 candidate chemical was found. More chemicals should be screened for additional candidates.

In addition to identifying the Psat1 inhibitor, delivery of Psat1 inhibitor to CSCs is another critical point in treatment. Importance of Psat1 function can be different depending upon tissue or organ types. For example, PSAT1 defect during developmental process causes Neu-Laxova syndrome, a rare disorder that manifests with severe neulorological malformations leading to prenatal or early postnatal lethality (Acuna-Hidalgo et al., 2014; El-Hattab et al., 2016). Moreover, serine is involved in synthesis of glycine which acts

neurotransmitter in brain. Therefore inhibition of Psat1 in normal brain cells can cause abnormality of nervous system. To prevent interference to normal cell metabolism, delivery of Psat1 inhibitor should target CSCs tightly. Also properties of CSCs are various in each tissue types. It will be worthwhile for effective cancer treatment to study CSCs derived from which tissue show high sensitivity to Psat1 inhibitor.

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Ⅵ. ABSTRACT IN KOREAN

국문 초록

Phosphoserine aminotransferase 1 (Psat1)은 세린 생합성 과정에 관여하는 transaminase이다. Psat1은 이 과정에서 a-ketoglutarate를 생산해낸다. a-KG는 Tet DNA hydroxylases와 JHDMs를 포함한 a-KG-dependent dioxygenases의 조효소이다. 특히 Psat1은 a-KG의 양을 조절함으로써 줄기 세포에서 전분화능 유지에 중요한 후성 유전체를 조절한다.

한편 종양 내에서 줄기세포처럼 자가증식이 가능하고 종양을 이루는 세포들로 분화하여 종양을 만들어 낼 수 있는 세포 소집단을 암 줄기세포라 한다. 암 줄기세포는 암의 재발을 일으킬 우려가 있으므로. 온전한 암 치료를 위해서는 암 줄기세포의 제거가 필수적이다

본 연구에서는 PSAT1이 유방암에서 전분화능 핵심 조절 인자인 OCT4와 양의 상관관계를 가지며 특히 유방암 줄기세포가 가장 많이 존재한다고 알려진 basal-like subtype에서 더 많이 발현되고 있는 것을 보았다. 그러므로 Psat1의 효소 활성 억제는 암 줄기세포의 분화를 유도 및 촉진하여 궁극적으로 이를 제거할 수 있을 것으로 기대된다. 따라서 정상적인 구조 형성을 방해하여 효소 활성을 감소시키는 Psat1 저해제를 찾고자 하였다. 우선 Psat1의 구조를 알아본 결과 이량체를 형성하는 것을 확인하였다. 특별히 단백질

간 상호작용을 잘 탐지할 수 있는 mammalian two hybrid system 상에서 Psat1의 이량체 형성을 방해하는 저해제를 찾고자 3920개의 화합물을 스크리닝 하였으며 최종적으로 1개의 후보물질을 도출하였다. 아직 더 많은 화합물이 스크리닝 되어야 하지만 Psat1 저해제는 암 치료에 새로운 시대를 열어줄 것이라 기대된다.

주요어 : Phosphoserine Aminotransferase 1 (Psat1), 암 줄기세포

학번 : 2015-26002