약학석사학위논문

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Prolyl-tRNA Synthetase를 표적으로 하는 암 억제 연구

2014년 8월

서울대학교 융합과학기술대학원

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지도교수 김성훈

이 논문을 약학석사학위논문으로 제출함

2014년 4월

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박민영의 석사학위논문을 인준함

2014년 7월

위 원 장 _____ (인)

부 위 원 장 _____ (인)

위 원 _____ (인)

ABSTRACT

Lung cancer is the most common cancer in terms of both incidence and mortality. Despite intensive investigation, effective therapeutic target and reliable compounds are still limited. Here, I discovered bifunctional glutamyl-prolyl-tRNA synthetase (EPRS) included in protein synthesis as a potential cancer target and its specific inhibitors using validated assay systems. EPRS is highly expressed in a variety of cancers including lung cancer and also involved in cancer metastasis and angiogenesis. Recently, halofuginone (HF) was identified as a compound binding with EPRS concomitant with inhibiting prolyl-tRNA synthetase (PRS) catalytic activity although HF has a poor drug-likeness and high cytotoxicity. In order to find novel compound which has improved druggability, I have screened PRS inhibitors using HF as a reference compound. Hundreds of compounds were synthesized by inherent synthetic strategy to maximize druggability and selected by analysis systems such as *in vitro* enzyme assay, proliferation, migration and cell death. Finally I identified effective 3 hit compounds controlling PRS catalytic activity as well as cell viability. Taken together, this study suggested the validation of anti-cancer targets and plausible anti-cancer therapeutic tools.

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Keywords : Prolyl-tRNA Synthetase (PRS), Lung Cancer, Inhibitor, Halofuginone

***Student Number* : 2012 – 24144**

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INTRODUCTION

Mammalian cells contain a battery of cytoplasmic and mitochondrial aminoacyl-tRNA synthetases (ARSs) that are in charge of cellular protein synthesis. They catalyze the ligation of amino acids to their cognate transfer ribonucleic acids (tRNAs) with a high fidelity. As the molecular adaptors that translate messenger RNA (mRNA) to protein, ARSs exist in the most primitive prokaryotes and are thus both ancient in evolutionary terms and of interest to biologists who are creating synthetic organisms (1). Mammalian ARSs carry out the same catalytic function as their prokaryotic counterparts but they contain other domains in addition to their catalytic domains, which confer the ability to form diverse complexes with each other or with other cellular regulatory factors. This structural complexity seems to be linked to a functional versatility that has only recently been uncovered, and which implicates ARSs in a variety of human diseases (2, 3). It seems that the aberrant expression, mutation and variant formation of ARSs can cause pathologies through multiple routes. Many ARSs function in signaling pathways that are linked to the control of tumorigenesis (1). Human glutamyl-prolyl-tRNA synthetase (EPRS) is a bifunctional enzyme in which the two catalytic domains are linked by three tandem repeats (4). In response to interferon-γ (IFNγ), EPRS forms a multi-component complex with other regulatory proteins at a 3′ UTR region that is involved in the translational silencing of target transcripts (5), many of which function during an inflammatory response. The translation of

vascular endothelial growth factor A (VEGFA), which is a crucial factor for angiogenesis, is also controlled by EPRS through a similar mechanism (6).

The small molecule halofuginone (HF), which is a racemic halogenated derivative of the plant alkaloid febrifugine (FF) (7) and is synthesized for less toxic form (8), was reported that it selectively activates the amino acid starvation response (AAR) (9). It is determined that HF binds EPRS and inhibits prolyl-tRNA synthetase (PRS) activity, which quenched by addition of an excess amount of proline (10). One part of HF mimics bound proline and the other mimics the 3' end of bound tRNA, so that HF occupies two different substrate binding sites on PRS in ATP-dependent manner (11). It was identified the residues, Phe1097 and Arg1152, of PRS play a key role in HF inhibition mechanism (12). During the roughly 2,000 years of FF's therapeutic usage, its molecular mechanism in animal tissues has remained unknown. Historically recognized for its antiprotozoal activity, this herbal extract was used as an antimalarial remedy in traditional Chinese medicine. In the last two decades, HF has gained attention and progressed to clinical trial phase 2 for its potential as a therapeutic in cancer and fibrotic disease (13-15). However, HF has the bromide residue known for its toxicity and carcinogenicity to rats (16) and has difficulty of synthesizing for its structural isomers. For all these reasons, we started to find novel PRS specific inhibitor with better drug-likeness.

In this study, I found hit compounds, which were designed PRS specific inhibitor on the basis of human PRS structure, expected to have similar or better effects to HF on cancer (17), and confirmed by *in vitro* aminoacylation assay and cell-based research .

MATERIALS AND METHODS

Materials

Halofuginone hydrobromide (Sigma Aldrich) which is known as a PRS specific inhibitor was used for aminoacylation assay and treated to cells. 249 compounds were synthesized as PRS specific inhibitors after the model of halofuginone from febrifugine and considered of PRS structure by Daewoong Pharmaceutical Co., Ltd (14).

Aminoacylation assay

His-tagged human PRS was expressed in *E. coli* strain Rosetta 2 (DE3) pLysS, harvested in phosphate-buffered saline (PBS), lysed with lysis buffer (20 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol pH 8.0) and purified using Ni–NTA affinity chromatography. His-tagged human PRS was eluted 200 mM imidazole pH 6.0 and dialyzed against PBS. Fractions were analyzed using SDS–PAGE and those containing protein were pooled. The protein was further purified by ion-exchange chromatography in buffer (20 mM Tris, 5 mM $MgCl_2$, 5 mM DTT pH 8.0). The protein was concentrated using Amicon Ultra centrifugal filters (Millipore) as a final purification step. To ensure that all of the material was dimeric, a size-exclusion chromatography step was performed using Superdex 200 26/60 column (GE Healthcare) equilibrated in the final buffer (20 mM Tris, 100 mM NaCl, 5 mM MgCl2, 1% glycerol, 5 mM DTT pH 8.0) (12).

PRS Aminoacylation assay was carried out in a reaction buffer containing 20 mM Tris pH 7.4, 20 mM Potassium Phosphate pH 7.5, 6 mM Magnesium Acetate, 0.5 mM DTT, 5 mM ATP (Sigma Aldrich), 5 mg/mL tRNA (Baker's yeast total, Roche), and 3 μCi [^{3}H] Proline (1 mCi/mmol, American Radiolabeled Chemicals). The enzyme reactions were initiated with adding 100nM of PRS enzyme into the buffer and conducted in a 37 ℃ heat block in the presence of various concentration of HF and other compounds. 15 μL of Aliquots were taken at 5 min and quenched on 23 mm filter papers (Whatman) that were presoaked with 5 % trichloroacetic acid (TCA, Sigma Aldrich) and dried. The filter papers were washed three times for 10 min each with cold 5 % TCA and once with cold 100 % ethanol. The washed filter papers were then dried. Radioactivity of synthesized $tRNA^{Pro}$ on the filter paper was quantified by liquid scintillation counter (Perkin Elmer) soaked in LSC cocktail (18).

Cell culture

A549 and H460, human non-small cell lung cancer cell were cultured in RPMI-1640 medium (with 24 mM HEPES and L-Glutamine, Hyclone) with 10 % fetal bovine serum (FBS, Hyclone) and 100 μg/mL of penicillin and streptomycin at 37 ℃ in 5 % CO_2 incubator.

Cell proliferation assay

A549 and H460 cells were seeded 5×10^3 ~ 6×10^3 per well in a 96-well plate (TPP) and the plate was placed within a microplate tray into the IncuCyte™ FLR (Essen Bioscience). When cell confluency monitored by the equipment every 2 hours was 20 ~ 30 %, various concentration of HF or its derivatives were treated to cells. Growth Curve was drawn using cell confluency at different time.

Cell death assay

A549 cells were seeded 5×10^3 ~ 6×10^3 per well in a 96-well plate (TPP) and the plate was placed within a microplate tray into the IncuCyte™ FLR (Essen Bioscience). When cell confluency monitored by the equipment every 2 hours was 20 ~ 30 %, various concentration of HF or its derivatives were treated to cells with 50nM YOYO® -1 iodide reagent (Life Technologies). As a cell impermeant cyanine dimer stain that binds to double-stranded DNA (dsDNA) of membrane compromised cells, it allows for the kinetic evaluation of cytotoxicity using the IncuCyte™ FLR live cell imaging systems. This membrane integrity based assay measures the uptake of a fluorescent dye, normally excluded from intact cells.

Wound healing assay

96-well plate (Essen ImageLock™) was coated with 0.5 mg/mL collagen type-1 (Corning) dissolved in 20 mM acetic acid and incubated overnight. After removing the collagen, A549 cells were plated 3×10^4 per well on top of thin-layer

matrix, and incubated to allow them to adhere for several hours. Wound area created by 96-well WoundMaker™ (Essen Bioscience) in just second. When cell confluency monitored by IncuCyte™ FLR every 2 hours was over 95 %, various concentration of HF or its derivatives were treated to cells. Cell migration was monitored and quantified by IncuCyte™ FLR.

RESULTS

The EPRS is highly expressed in lung cancer.

It is already known that EPRS overexpression is related to cancer. In order to determine cancer target, I checked its expression level in several cancer types (Figure 1). According to both previous research data (1) and this expression profile, lung cancer is the most significant disease target on EPRS gene among cancer types, so that I used lung cancer cell line in later cell-based research

Strategy for the development of novel PRS specific inhibitor

First of all, the compounds which were designed to bind the catalytic site of PRS and inhibit its activity were synthesized in collaboration with pharmaceutical company. With the compounds, *in vitro* aminoacylation assay was performed to find novel PRS inhibitor retaining better drug-likeness. The PRS enzyme is needed three substrates; ATP, $tRNA^{Pro}$ and proline. With substrates, PRS plays a role in ligation of proline to its cognate tRNA. Meanwhile, if the specific inhibitor is added to the reaction, the compound occupies substrate binding site so that the reaction is blocked (Figure 2A). After compound screening by *in vitro* assay, phenotype-based research was performed with hit compounds in non-small cell lung cancer cells.

PRS aminoacylation assay for specific inhibitor screening

At first time, single concentration (1μM) of compounds was treated to compare inhibition effect of HF with synthesized compounds. Several compounds were shows catalytic inhibition effect (Figure 2B) so that they were given dose-dependent treatment to measure 50 % inhibition concentration (IC_{50} value). As a result, 13 compounds which have under 100nM IC_{50} were selected because IC_{50} of the reference compound, HF was 94.8nM with the assay condition (Figure 2C). When I took a closer look, there was a structural similarity. Finally three structural representative compounds; BC-PI-DW-0112, -0159 and -0176 were chosen, but the detailed structural formula could not be revealed because of confidentiality. BC-PI-DW-0159 was most effective of inhibiting PRS catalytic activity (Figure 2D). The whole screening process was summarized in the scheme (Figure 2E).

The hit compounds inhibit cell proliferation

After *in vitro* screening, the selected hit compounds were treated to A549, typical non-small cell lung cancer cell line at several concentrations to determine their effect of cell proliferation control. BC-PI-DW-0159 and 0176 showed cell proliferation inhibition at 10 μM as much as HF. However, BC-PI-DW-0112 was not effective at 10 μM (Figure 3A). The compounds were also treated to H460, which is another typical non-small cell lung cancer cell line, at equal condition (Figure 3B). BC-PI-DW-0159 and 0176 were effective on cell growth inhibition as before. As a result, the hit compounds could inhibit lung cancer cell proliferation.

The effect of the hit compounds on cell migration

To investigate the effect of the hit compounds on tumor invasion and metastasis, wound healing assay was carried out because cell migration is an essential prerequisite for invasion and metastasis. After wound made on A549 cells, each compound was treated and 12 hour's relative cell migration were observed. 10 μM of BC-PI-DW-0159 and 0176 showed migration inhibition effect as much as HF (Figure 4A). They inhibit cancer cells from narrowing wound width (Figure 4B). In conclusion, the hit compounds inhibit cancer cell migration so that they could control tumor invasion and metastasis.

The hit compounds lead to cell death

To investigate how to the compounds control cell growth and cell migration, cytotoxicity reagent was added with each compound to A549 cells to measure cell death with fluorescence dye. 10 μM of BC-PI-DW-0159 and 0176 showed as much as 1 μM of HF, which induced cell death. However BC-PI-DW-0112 was not effective as before (Figure 5A, C). Cytotoxicity of the hit compounds was confirmed by not only the number of fluorescence dye but also cell image (Figure 5B). To investigate at molecular level, western blot was performed. After 12 hours of serum starvation, each compound was treated with several doses to A549 cells and the cells were harvested 12 hours later. The induced cell death was identified

that it was related to poly ADP-ribose polymerase (PARP) pathway (Figure 5D). In conclusion, the hit compounds lead to cancer cell death via PARP pathway.

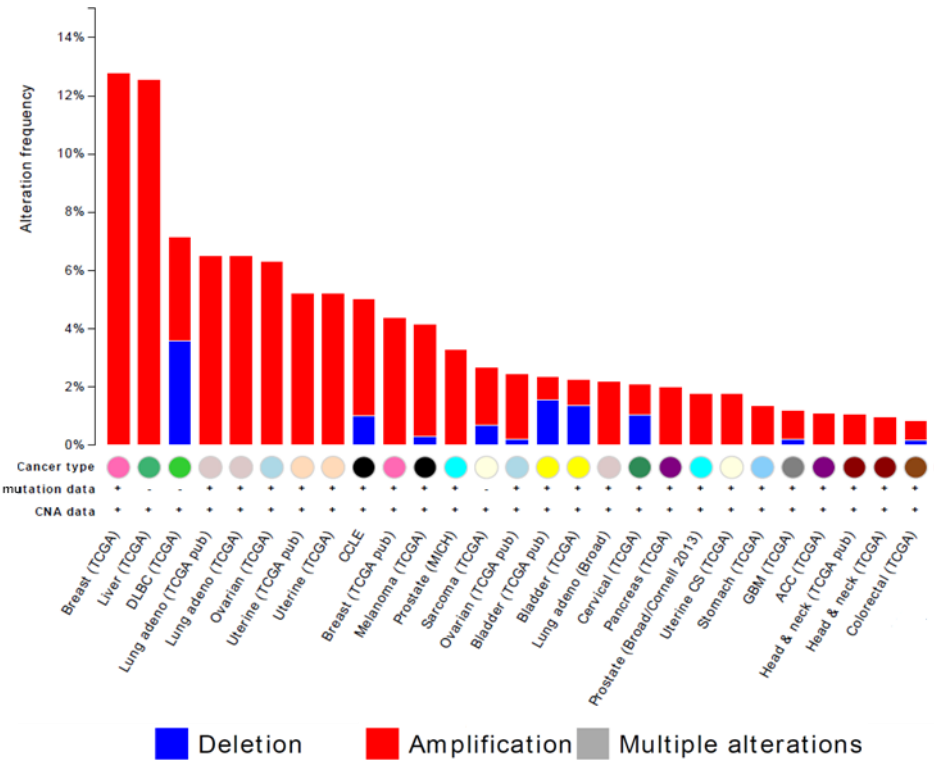
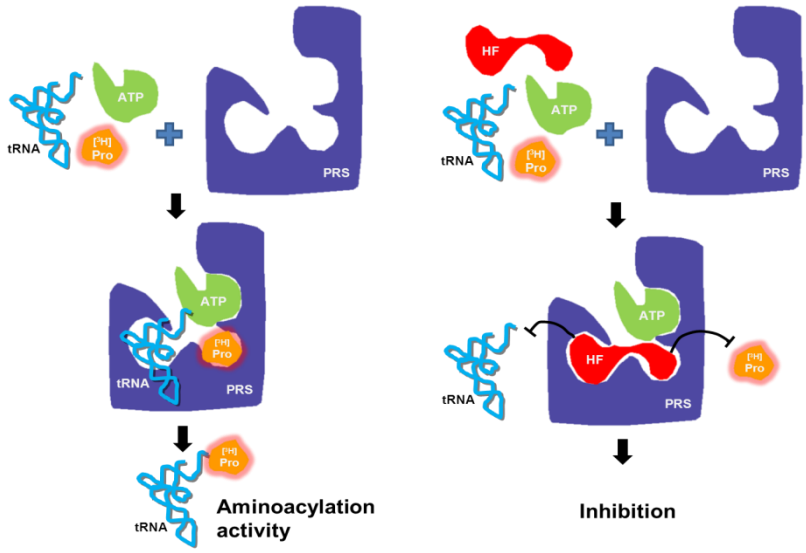


Figure 1. EPRS is highly overexpressed in cancers.

The height of the bars represents the relative alteration frequency. EPRS gene is amplified in each indicated cancer type. EPRS gene is amplified in cancers (red bars), significantly in Lung adenocarcinoma. (cBioPortal)

A

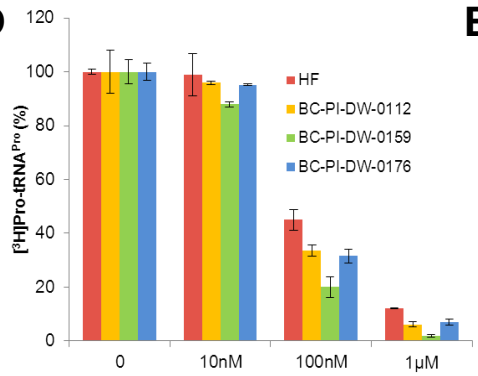


B

C

compounds	IC_{50} (nM)
BC-PI-DW-0112	62
BC-PI-DW-0134	89.7
BC-PI-DW-0135	39.2
BC-PI-DW-0147	54
BC-PI-DW-0149	83
BC-PI-DW-0152	97
BC-PI-DW-0153	97
BC-PI-DW-0159	34
BC-PI-DW-0163	44
BC-PI-DW-0171	44
BC-PI-DW-0173	96
BC-PI-DW-0176	62
BC-PI-DW-0179	90

D



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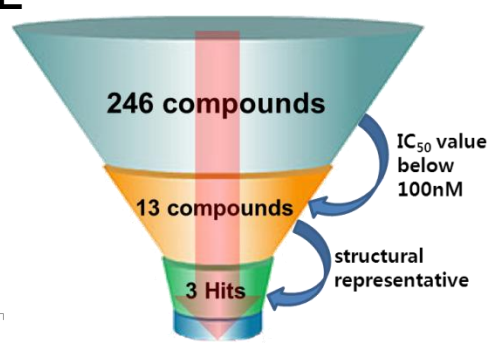
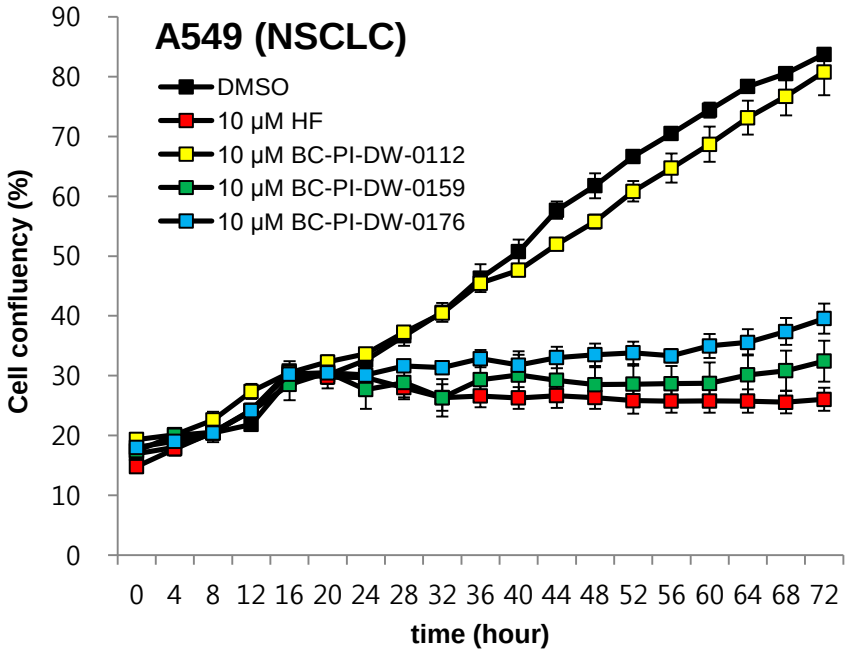


Figure 2. Compounds screening by PRS aminoacylation assay

(A) Schematic diagram for the compound screening principal based on PRS aminoacylation assay. The PRS enzyme is needed three substrates; ATP, $tRNA^{Pro}$ and proline. With substrates, PRS plays a role in ligation of proline to its cognate tRNA (left). Meanwhile, if the specific inhibitor; HF is added to the reaction, the compound occupies substrate binding site so that the reaction is blocked (right) (11). **(B)** Heat map of the primary screening data. Inhibitory effects of 246 compounds, which were treated 1μM of working concentration, on the aminoacylation level were examined by PRS aminoacylation assay and the results are presented. Several compounds inhibited PRS aminoacylation activity (yellow color). **(C)** The selected 13 compounds showed below 100nM IC_{50} value. **(D)** The aminoacylation assay result of HF and 3 hit compounds for calculation of IC_{50}. **(E)** Schematic diagram of compound screening process.

A


A549 (NSCLC)
DMSO
10 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
Cell confluency (%)
0 10 20 30 40 50 60 70 80 90
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
time (hour)

B

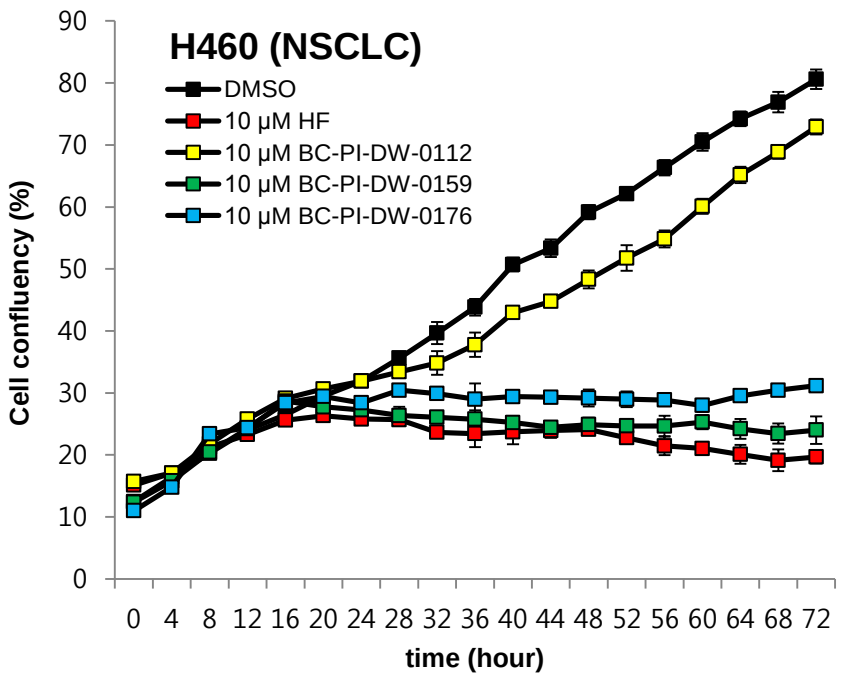
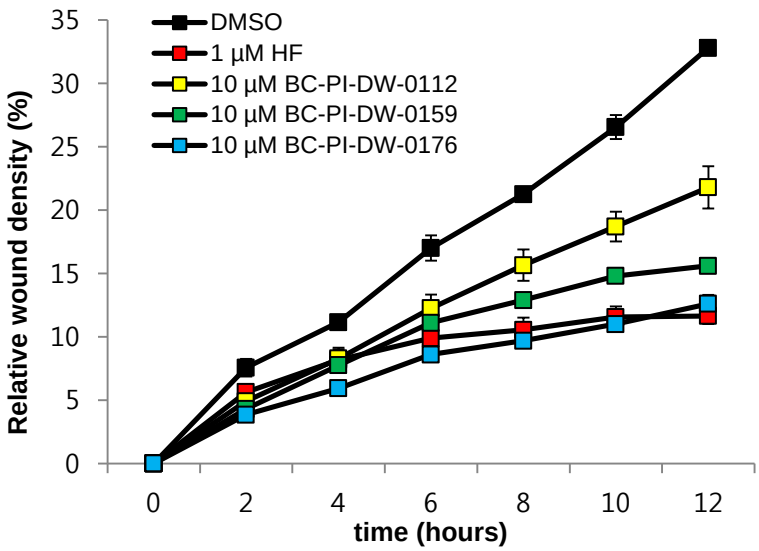

H460 (NSCLC)
DMSO
10 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
Cell confluency (%)
0 10 20 30 40 50 60 70 80 90
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
time (hour)

Figure 3. The hit compounds inhibit cell proliferation

(A) The selected 3 compounds were treated to A549 cells, typical non-small cell lung cancer cell line, at indicated concentration to determine their effect of cell proliferation control. BC-PI-DW-0159 and 0176 showed as much as HF effect. However, BC-PI-DW-0112 was not effective. DMSO were used a negative control because those compounds dissolved in DMSO **(B)** Same experiment was carried out with H460 cells, which is another typical non-small cell lung cancer cell line.

A


DMSO
1 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
Relative wound density (%)
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
time (hours)

B

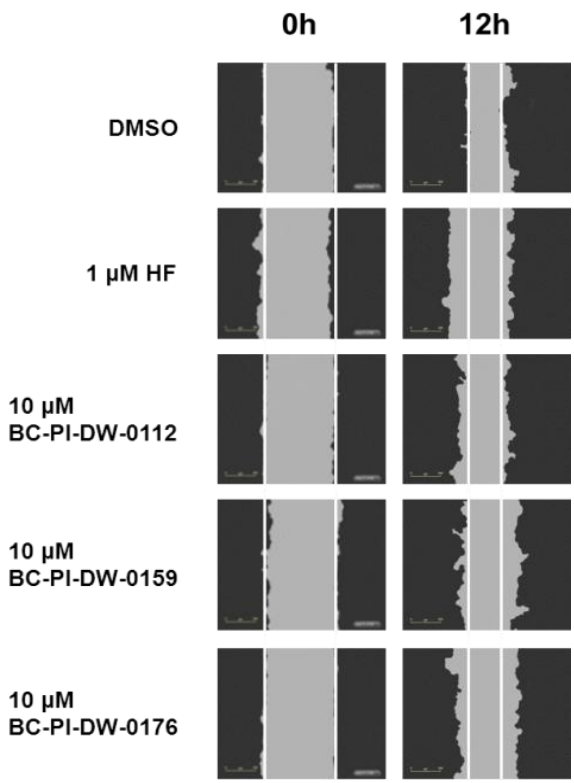
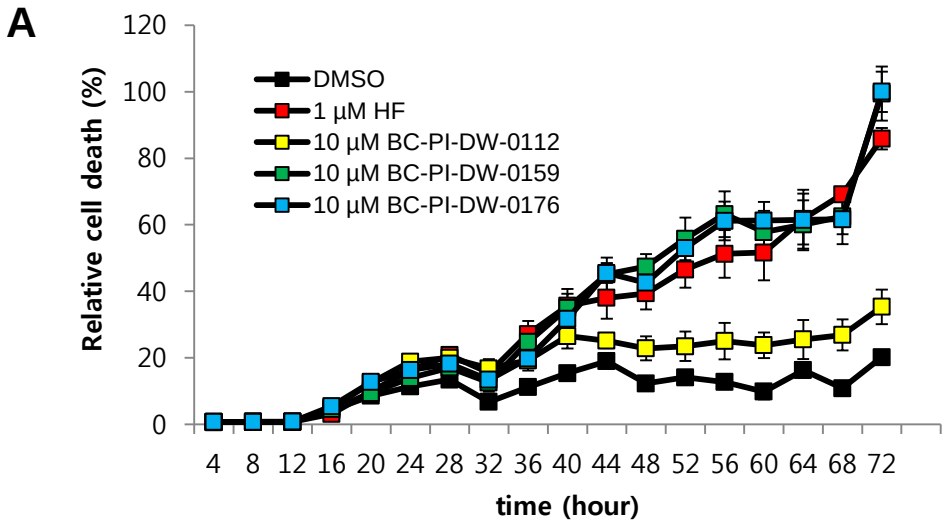
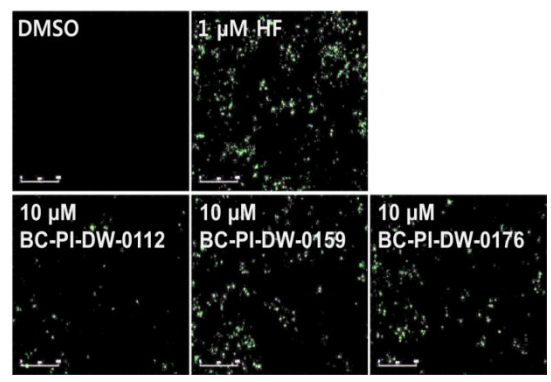
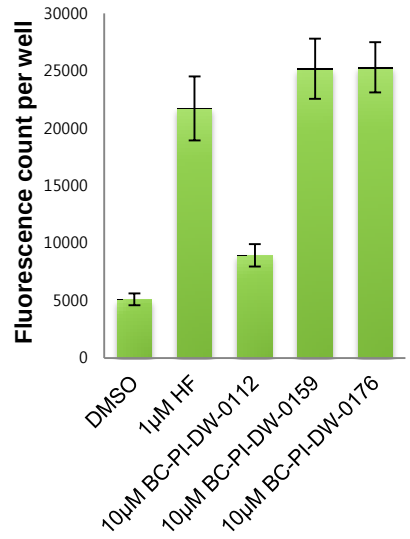

0h
12h
DMSO
1 μM HF
10 μM
BC-PI-DW-0112
10 μM
BC-PI-DW-0159
10 μM
BC-PI-DW-0176

Figure 4. The effect of hit compounds on cell migration

(A) The result of wound healing assay with 3 hit compounds. After wound made on A549 cells, each compound was treated and 12 hour's relative cell migration were observed. BC-PI-DW-0159 and 0176 showed as much as HF controls cell migration. **(B)** Comparison of narrowed wound width among treated compounds at 12 hour.


A
Relative cell death (%)
120
100
80
60
40
20
0
DMSO
1 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
time (hour)


B
DMSO
1 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176


C
Fluorescence count per well
30000
25000
20000
15000
10000
5000
0
DMSO
1μM HF
10μM BC-PI-DW-0112
10μM BC-PI-DW-0159
10μM BC-PI-DW-0176

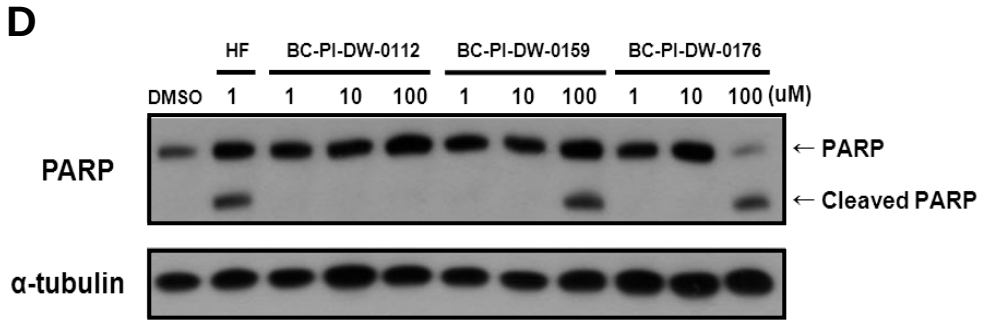

D
HF
BC-PI-DW-0112
BC-PI-DW-0159
BC-PI-DW-0176
DMSO 1 1 10 100 1 10 100 1 10 100 (uM)
PARP
← PARP
← Cleaved PARP
α-tubulin

Figure 5. The hit compounds induce cell death

(A) To investigate how to the compounds control cell growth and cell migration, YoYo-1 reagent was added with each compound to A549 cells to measure cell death by fluorescence dye. 10 μM of BC-PI-DW-0159 and 0176 showed as much as 1 μM HF induced cell death and BC-PI-DW-0112 barely affected. **(B)** The image of the cells with each compound at 72 hour. Green fluorescence means dead cells. **(C)** Comparison of cytotoxicity among 3 hit compounds by the number of fluorescence. **(D)** To investigate at molecular level, western blot was performed. After 12 hours of serum starvation, each compound was treated with several doses to A549 cells and it was harvested 12hrs later. The induced cell death mechanism was identified that it is related to poly ADP ribose polymerase (PARP) pathway.

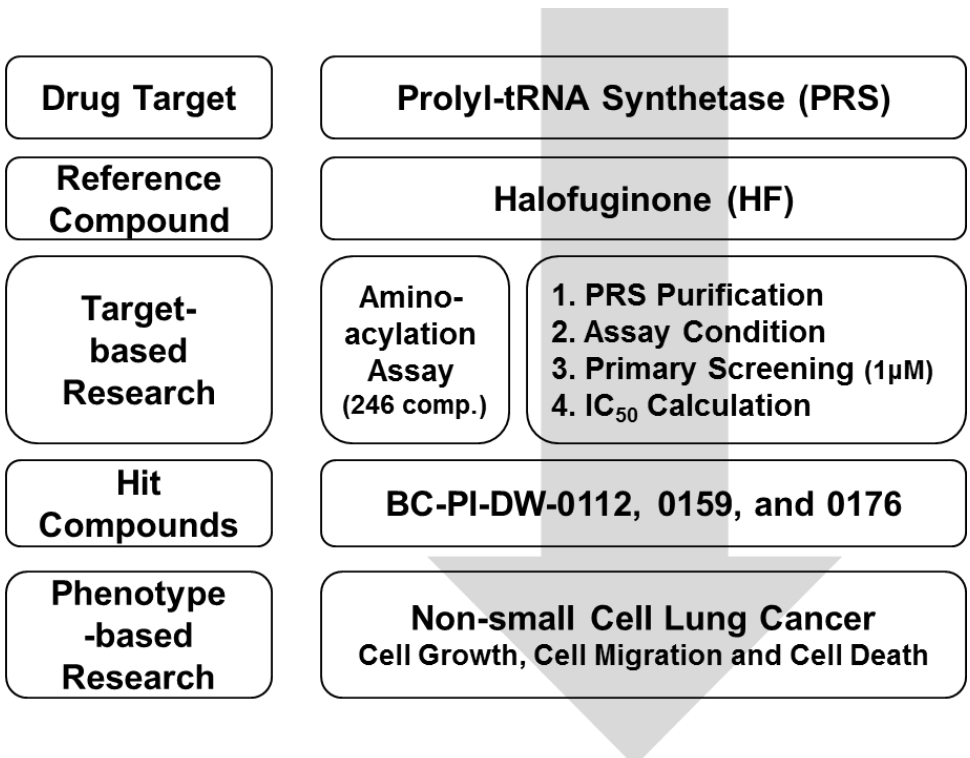


Figure 6. PRS specific inhibitor screening process

The diagram explains novel compound screening process from targeting PRS to phenotype-based research.

Table 1. Summary of hit compounds

Compound	Molecular weight (kDa)	Inhibition of PRS aminoacylation activity	Cell proliferation	Cell migration	Cell death
		IC_{50} (nM)	EC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)
Halofuginone	414.68	94.8	<0.1	<0.1	1
BC-PI-DW-0112	308	34	<100	<100	<100
BC-PI-DW-0159	353.22	62	<10	10	10
BC-PI-DW-0176	342.22	62	<10	<10	10

DISCUSSION

PRS is one of the ARS enzymes that play a role of ligation of amino acids to cognate tRNA in protein synthesis. Besides, PRS is highly expressed in cancer, especially non-small cell lung cancer (NSCLC), so it would be a cancer target. Even though there was a HF as a PRS specific inhibitor, it has fatal side effect for human drug, so I started to find novel compound.

First, various PRS inhibitor compounds were synthesized with better drug-likeness. With those compounds, I check their PRS catalytic inhibition effect by aminoacylation assay. Throughput of the assay was very low, so it was very time-consuming process. At the beginning, none of compounds was effective but, later some started to appear inhibition. Finally I got three hit compounds.

After compound screening, I treated those compounds to A549 and H460 cells, which are typical NSCLC cells. I investigate their effect of cell proliferation, migration and death by using real-time cell imaging tool, IncuCyte^{TM} FLR. Western blot was also carried out to check at molecular level. In activity test, the hit compounds had better effect than HF, but in cell, they were less than HF (Table 1). I thought that HF might be related to known or unknown factor on controlling cancer in addition to PRS dependent manner. The whole compound screening process is described in a diagram (Figure 6).

The mode of action mechanism study of HF and hit compounds are still needed for further steps and more optimized lead compound are also needed for drug candidate. Moreover, *in vivo* study like xenograft mouse model is also

necessary to be a real drug development.

In conclusion, I suggest that PRS would be a novel target for anti-NSCLC drug development and the hit compounds were found.

REFERENCES

1. Kim S, You S, Hwang D. Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping. Nature reviews Cancer. 2011;11(10):708-18.
2. Park SG, Ewalt KL, Kim S. Functional expansion of aminoacyl-tRNA synthetases and their interacting factors: new perspectives on housekeepers. Trends in biochemical sciences. 2005;30(10):569-74.
3. Park SG, Schimmel P, Kim S. Aminoacyl tRNA synthetases and their connections to disease. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(32):11043-9.
4. Jeong E-J, Hwang G-S, Kim. Kyung H. Structural Analysis of Multifunctional Peptide Motifs in Human Bifunctional tRNA Synthetase. Biochemistry. 2000;39(51):15775-82.
5. Sampath P, Mazumder B, Seshadri V, Gerber CA, Chavatte L, Kinter M, et al. Noncanonical function of glutamyl-prolyl-tRNA synthetase: gene-specific silencing of translation. Cell. 2004;119(2):195-208.
6. Arif A, Jia J, Moodt RA, DiCorleto PE, Fox PL. Phosphorylation of glutamyl-prolyl tRNA synthetase by cyclin-dependent kinase 5 dictates transcript-selective translational control. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(4):1415-20.
7. Coatney GR, Cooper WC, Culwell WB, White WC, Imboden CA, Jr. Studies in human malaria. XXV. Trial of febrifugine, an alkaloid obtained from Dichroa febrifuga lour., against the Chesson strain of Plasmodium vivax.

Journal National Malaria Society (US). 1950;9(2):183-6.

8. Ryley JF, Betts MJ. Chemotherapy of chicken coccidiosis. Advances in pharmacology and chemotherapy. 1973;11:221-93.
9. Sundrud MS, Koralov SB, Feuerer M, Calado DP, Kozhaya AE, Rhule-Smith A, et al. Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. Science. 2009;324(5932):1334-8.
10. Keller TL, Zocco D, Sundrud MS, Hendrick M, Edenius M, Yum J, et al. Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase. Nature chemical biology. 2012;8(3):311-7.
11. Zhou H, Sun L, Yang XL, Schimmel P. ATP-directed capture of bioactive herbal-based medicine on human tRNA synthetase. Nature. 2013;494(7435):121-4.
12. Son J, Lee EH, Park M, Kim JH, Kim J, Kim S, et al. Conformational changes in human prolyl-tRNA synthetase upon binding of the substrates proline and ATP and the inhibitor halofuginone. Acta crystallographica Section D, Biological crystallography. 2013;69(Pt 10):2136-45.
13. Pines M, Nagler A. Halofuginone: a novel antifibrotic therapy. General pharmacology. 1998;30(4):445-50.
14. Elkin M, Ariel I, Miao HQ, Nagler A, Pines M, de-Groot N, et al. Inhibition of bladder carcinoma angiogenesis, stromal support, and tumor growth by halofuginone. Cancer research. 1999;59(16):4111-8.
15. Koon HB, Fingleton B, Lee JY, Geyer JT, Cesarman E, Parise RA, et al. Phase II AIDS Malignancy Consortium trial of topical halofuginone in AIDS-related Kaposi sarcoma. Journal of acquired immune deficiency syndromes (1999).

2011;56(1):64-8.

16. Mitsumori K, Maita K, Kosaka T, Miyaoka T, Shirasu Y. Two-year oral chronic toxicity and carcinogenicity study in rats of diets fumigated with methyl bromide. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 1990;28(2):109-19.
17. Kikuchi H, Yamamoto K, Horoiwa S, Hirai S, Kasahara R, Hariguchi N, et al. Exploration of a New Type of Antimalarial Compounds Based on Febrifugine. Journal of Medicinal Chemistry. 2006;49(15):4698-706.
18. Francklyn CS, First EA, Perona JJ, Hou YM. Methods for kinetic and thermodynamic analysis of aminoacyl-tRNA synthetases. Methods (San Diego, Calif). 2008;44(2):100-18.

요약(국문초록)

Prolyl-tRNA Synthetase를 표적으로 하는 암 억제 연구

박 민 영
의약생명과학전공
분자의학 및 바이오제약학과
융합과학기술대학원
서울대학교

폐암은 발병률과 사망률이 가장 높은 암 중 하나이다. 집중적인 연구에도 불구하고, 효과적인 치료 표적과 믿을 만한 치료제가 여전히 부족하다. 여기, 나는 단백질 합성과정에 포함된 두 기능을 가진 글루타민 – 프롤린 – 트랜스퍼RNA 합성효소 (glutamyl−prolyl−tRNA synthetase, 이하 EPRS)를 잠재적인 암 표적으로 발견하고, 유효성이 입증된 검사법으로 그것의 특정 저해제를 발견하였다. EPRS는 폐암을 포함한 여러 암에서 과발현되어 있으며, 암의 전이와 신혈관생성에 관련되어 있다. 최근에 할로푸지논(halofuginone, 이하 HF) 이라는 화합물이 약 유사성이 낮고 세포독성이 높음에도 불구하고, EPRS에 결합하여 프롤린−트랜스퍼RNA 합성효소 (prolyl−tRNA synthetase, 이하 PRS)의 촉매 활성 저해를 수반한다는 것이 밝혀졌다. 약효성이 개선된 새로운 화합물을 찾기 위해서, HF를 참고 화합물로 하여 PRS 저해제를 선별하였다. 약효성을 최대화하기 위해서 고유의 합성전략을 가지고 수백 개의 화합물을

합성하였고, in vitro 효소 검사법, 세포 성장, 이동 그리고 사멸 분석으로 선별하였다. 결과적으로 PRS의 효소 활성뿐만 아니라 세포 생존까지 억제하는 효과적인 세 개의 화합물을 찾아내었다. 종합해보면, 이 연구는 항암 표적을 확인하고, 타당한 항암 치료 도구를 제시하였다는데 의의가 있다.

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주요어 : 프롤린-트랜스퍼 RNA 합성효소, 폐암, 저해제, 할로푸지논

학 번 : 2012 - 24144

약학석사학위논문

Targeting Prolyl-tRNA Synthetase to Control Cancer

Prolyl-tRNA Synthetase를 표적으로 하는 암 억제 연구

2014년 8월

서울대학교 융합과학기술대학원

분자의학 및 바이오제약학과

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지도교수 김성훈

이 논문을 약학석사학위논문으로 제출함

2014년 4월

서울대학교 융합과학기술대학원

분자의학 및 바이오제약학과

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박 민 영

박민영의 석사학위논문을 인준함

2014년 7월

위 원 장 _____ (인)

부 위 원 장 _____ (인)

위 원 _____ (인)

ABSTRACT

Lung cancer is the most common cancer in terms of both incidence and mortality. Despite intensive investigation, effective therapeutic target and reliable compounds are still limited. Here, I discovered bifunctional glutamyl-prolyl-tRNA synthetase (EPRS) included in protein synthesis as a potential cancer target and its specific inhibitors using validated assay systems. EPRS is highly expressed in a variety of cancers including lung cancer and also involved in cancer metastasis and angiogenesis. Recently, halofuginone (HF) was identified as a compound binding with EPRS concomitant with inhibiting prolyl-tRNA synthetase (PRS) catalytic activity although HF has a poor drug-likeness and high cytotoxicity. In order to find novel compound which has improved druggability, I have screened PRS inhibitors using HF as a reference compound. Hundreds of compounds were synthesized by inherent synthetic strategy to maximize druggability and selected by analysis systems such as *in vitro* enzyme assay, proliferation, migration and cell death. Finally I identified effective 3 hit compounds controlling PRS catalytic activity as well as cell viability. Taken together, this study suggested the validation of anti-cancer targets and plausible anti-cancer therapeutic tools.

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Keywords : Prolyl-tRNA Synthetase (PRS), Lung Cancer, Inhibitor, Halofuginone

***Student Number* : 2012 – 24144**

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INTRODUCTION

Mammalian cells contain a battery of cytoplasmic and mitochondrial aminoacyl-tRNA synthetases (ARSs) that are in charge of cellular protein synthesis. They catalyze the ligation of amino acids to their cognate transfer ribonucleic acids (tRNAs) with a high fidelity. As the molecular adaptors that translate messenger RNA (mRNA) to protein, ARSs exist in the most primitive prokaryotes and are thus both ancient in evolutionary terms and of interest to biologists who are creating synthetic organisms (1). Mammalian ARSs carry out the same catalytic function as their prokaryotic counterparts but they contain other domains in addition to their catalytic domains, which confer the ability to form diverse complexes with each other or with other cellular regulatory factors. This structural complexity seems to be linked to a functional versatility that has only recently been uncovered, and which implicates ARSs in a variety of human diseases (2, 3). It seems that the aberrant expression, mutation and variant formation of ARSs can cause pathologies through multiple routes. Many ARSs function in signaling pathways that are linked to the control of tumorigenesis (1). Human glutamyl-prolyl-tRNA synthetase (EPRS) is a bifunctional enzyme in which the two catalytic domains are linked by three tandem repeats (4). In response to interferon-γ (IFNγ), EPRS forms a multi-component complex with other regulatory proteins at a 3′ UTR region that is involved in the translational silencing of target transcripts (5), many of which function during an inflammatory response. The translation of

vascular endothelial growth factor A (VEGFA), which is a crucial factor for angiogenesis, is also controlled by EPRS through a similar mechanism (6).

The small molecule halofuginone (HF), which is a racemic halogenated derivative of the plant alkaloid febrifugine (FF) (7) and is synthesized for less toxic form (8), was reported that it selectively activates the amino acid starvation response (AAR) (9). It is determined that HF binds EPRS and inhibits prolyl-tRNA synthetase (PRS) activity, which quenched by addition of an excess amount of proline (10). One part of HF mimics bound proline and the other mimics the 3' end of bound tRNA, so that HF occupies two different substrate binding sites on PRS in ATP-dependent manner (11). It was identified the residues, Phe1097 and Arg1152, of PRS play a key role in HF inhibition mechanism (12). During the roughly 2,000 years of FF's therapeutic usage, its molecular mechanism in animal tissues has remained unknown. Historically recognized for its antiprotozoal activity, this herbal extract was used as an antimalarial remedy in traditional Chinese medicine. In the last two decades, HF has gained attention and progressed to clinical trial phase 2 for its potential as a therapeutic in cancer and fibrotic disease (13-15). However, HF has the bromide residue known for its toxicity and carcinogenicity to rats (16) and has difficulty of synthesizing for its structural isomers. For all these reasons, we started to find novel PRS specific inhibitor with better drug-likeness.

In this study, I found hit compounds, which were designed PRS specific inhibitor on the basis of human PRS structure, expected to have similar or better effects to HF on cancer (17), and confirmed by *in vitro* aminoacylation assay and cell-based research .

MATERIALS AND METHODS

Materials

Halofuginone hydrobromide (Sigma Aldrich) which is known as a PRS specific inhibitor was used for aminoacylation assay and treated to cells. 249 compounds were synthesized as PRS specific inhibitors after the model of halofuginone from febrifugine and considered of PRS structure by Daewoong Pharmaceutical Co., Ltd (14).

Aminoacylation assay

His-tagged human PRS was expressed in *E. coli* strain Rosetta 2 (DE3) pLysS, harvested in phosphate-buffered saline (PBS), lysed with lysis buffer (20 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol pH 8.0) and purified using Ni–NTA affinity chromatography. His-tagged human PRS was eluted 200 mM imidazole pH 6.0 and dialyzed against PBS. Fractions were analyzed using SDS–PAGE and those containing protein were pooled. The protein was further purified by ion-exchange chromatography in buffer (20 mM Tris, 5 mM $MgCl_2$, 5 mM DTT pH 8.0). The protein was concentrated using Amicon Ultra centrifugal filters (Millipore) as a final purification step. To ensure that all of the material was dimeric, a size-exclusion chromatography step was performed using Superdex 200 26/60 column (GE Healthcare) equilibrated in the final buffer (20 mM Tris, 100 mM NaCl, 5 mM MgCl2, 1% glycerol, 5 mM DTT pH 8.0) (12).

PRS Aminoacylation assay was carried out in a reaction buffer containing 20 mM Tris pH 7.4, 20 mM Potassium Phosphate pH 7.5, 6 mM Magnesium Acetate, 0.5 mM DTT, 5 mM ATP (Sigma Aldrich), 5 mg/mL tRNA (Baker's yeast total, Roche), and 3 μCi [^{3}H] Proline (1 mCi/mmol, American Radiolabeled Chemicals). The enzyme reactions were initiated with adding 100nM of PRS enzyme into the buffer and conducted in a 37 ℃ heat block in the presence of various concentration of HF and other compounds. 15 μL of Aliquots were taken at 5 min and quenched on 23 mm filter papers (Whatman) that were presoaked with 5 % trichloroacetic acid (TCA, Sigma Aldrich) and dried. The filter papers were washed three times for 10 min each with cold 5 % TCA and once with cold 100 % ethanol. The washed filter papers were then dried. Radioactivity of synthesized $tRNA^{Pro}$ on the filter paper was quantified by liquid scintillation counter (Perkin Elmer) soaked in LSC cocktail (18).

Cell culture

A549 and H460, human non-small cell lung cancer cell were cultured in RPMI-1640 medium (with 24 mM HEPES and L-Glutamine, Hyclone) with 10 % fetal bovine serum (FBS, Hyclone) and 100 μg/mL of penicillin and streptomycin at 37 ℃ in 5 % CO_2 incubator.

Cell proliferation assay

A549 and H460 cells were seeded 5×10^3 ~ 6×10^3 per well in a 96-well plate (TPP) and the plate was placed within a microplate tray into the IncuCyte™ FLR (Essen Bioscience). When cell confluency monitored by the equipment every 2 hours was 20 ~ 30 %, various concentration of HF or its derivatives were treated to cells. Growth Curve was drawn using cell confluency at different time.

Cell death assay

A549 cells were seeded 5×10^3 ~ 6×10^3 per well in a 96-well plate (TPP) and the plate was placed within a microplate tray into the IncuCyte™ FLR (Essen Bioscience). When cell confluency monitored by the equipment every 2 hours was 20 ~ 30 %, various concentration of HF or its derivatives were treated to cells with 50nM YOYO® -1 iodide reagent (Life Technologies). As a cell impermeant cyanine dimer stain that binds to double-stranded DNA (dsDNA) of membrane compromised cells, it allows for the kinetic evaluation of cytotoxicity using the IncuCyte™ FLR live cell imaging systems. This membrane integrity based assay measures the uptake of a fluorescent dye, normally excluded from intact cells.

Wound healing assay

96-well plate (Essen ImageLock™) was coated with 0.5 mg/mL collagen type-1 (Corning) dissolved in 20 mM acetic acid and incubated overnight. After removing the collagen, A549 cells were plated 3×10^4 per well on top of thin-layer

matrix, and incubated to allow them to adhere for several hours. Wound area created by 96-well WoundMaker™ (Essen Bioscience) in just second. When cell confluency monitored by IncuCyte™ FLR every 2 hours was over 95 %, various concentration of HF or its derivatives were treated to cells. Cell migration was monitored and quantified by IncuCyte™ FLR.

RESULTS

The EPRS is highly expressed in lung cancer.

It is already known that EPRS overexpression is related to cancer. In order to determine cancer target, I checked its expression level in several cancer types (Figure 1). According to both previous research data (1) and this expression profile, lung cancer is the most significant disease target on EPRS gene among cancer types, so that I used lung cancer cell line in later cell-based research

Strategy for the development of novel PRS specific inhibitor

First of all, the compounds which were designed to bind the catalytic site of PRS and inhibit its activity were synthesized in collaboration with pharmaceutical company. With the compounds, *in vitro* aminoacylation assay was performed to find novel PRS inhibitor retaining better drug-likeness. The PRS enzyme is needed three substrates; ATP, $tRNA^{Pro}$ and proline. With substrates, PRS plays a role in ligation of proline to its cognate tRNA. Meanwhile, if the specific inhibitor is added to the reaction, the compound occupies substrate binding site so that the reaction is blocked (Figure 2A). After compound screening by *in vitro* assay, phenotype-based research was performed with hit compounds in non-small cell lung cancer cells.

PRS aminoacylation assay for specific inhibitor screening

At first time, single concentration (1μM) of compounds was treated to compare inhibition effect of HF with synthesized compounds. Several compounds were shows catalytic inhibition effect (Figure 2B) so that they were given dose-dependent treatment to measure 50 % inhibition concentration (IC_{50} value). As a result, 13 compounds which have under 100nM IC_{50} were selected because IC_{50} of the reference compound, HF was 94.8nM with the assay condition (Figure 2C). When I took a closer look, there was a structural similarity. Finally three structural representative compounds; BC-PI-DW-0112, -0159 and -0176 were chosen, but the detailed structural formula could not be revealed because of confidentiality. BC-PI-DW-0159 was most effective of inhibiting PRS catalytic activity (Figure 2D). The whole screening process was summarized in the scheme (Figure 2E).

The hit compounds inhibit cell proliferation

After *in vitro* screening, the selected hit compounds were treated to A549, typical non-small cell lung cancer cell line at several concentrations to determine their effect of cell proliferation control. BC-PI-DW-0159 and 0176 showed cell proliferation inhibition at 10 μM as much as HF. However, BC-PI-DW-0112 was not effective at 10 μM (Figure 3A). The compounds were also treated to H460, which is another typical non-small cell lung cancer cell line, at equal condition (Figure 3B). BC-PI-DW-0159 and 0176 were effective on cell growth inhibition as before. As a result, the hit compounds could inhibit lung cancer cell proliferation.

The effect of the hit compounds on cell migration

To investigate the effect of the hit compounds on tumor invasion and metastasis, wound healing assay was carried out because cell migration is an essential prerequisite for invasion and metastasis. After wound made on A549 cells, each compound was treated and 12 hour's relative cell migration were observed. 10 μM of BC-PI-DW-0159 and 0176 showed migration inhibition effect as much as HF (Figure 4A). They inhibit cancer cells from narrowing wound width (Figure 4B). In conclusion, the hit compounds inhibit cancer cell migration so that they could control tumor invasion and metastasis.

The hit compounds lead to cell death

To investigate how to the compounds control cell growth and cell migration, cytotoxicity reagent was added with each compound to A549 cells to measure cell death with fluorescence dye. 10 μM of BC-PI-DW-0159 and 0176 showed as much as 1 μM of HF, which induced cell death. However BC-PI-DW-0112 was not effective as before (Figure 5A, C). Cytotoxicity of the hit compounds was confirmed by not only the number of fluorescence dye but also cell image (Figure 5B). To investigate at molecular level, western blot was performed. After 12 hours of serum starvation, each compound was treated with several doses to A549 cells and the cells were harvested 12 hours later. The induced cell death was identified

that it was related to poly ADP-ribose polymerase (PARP) pathway (Figure 5D). In conclusion, the hit compounds lead to cancer cell death via PARP pathway.

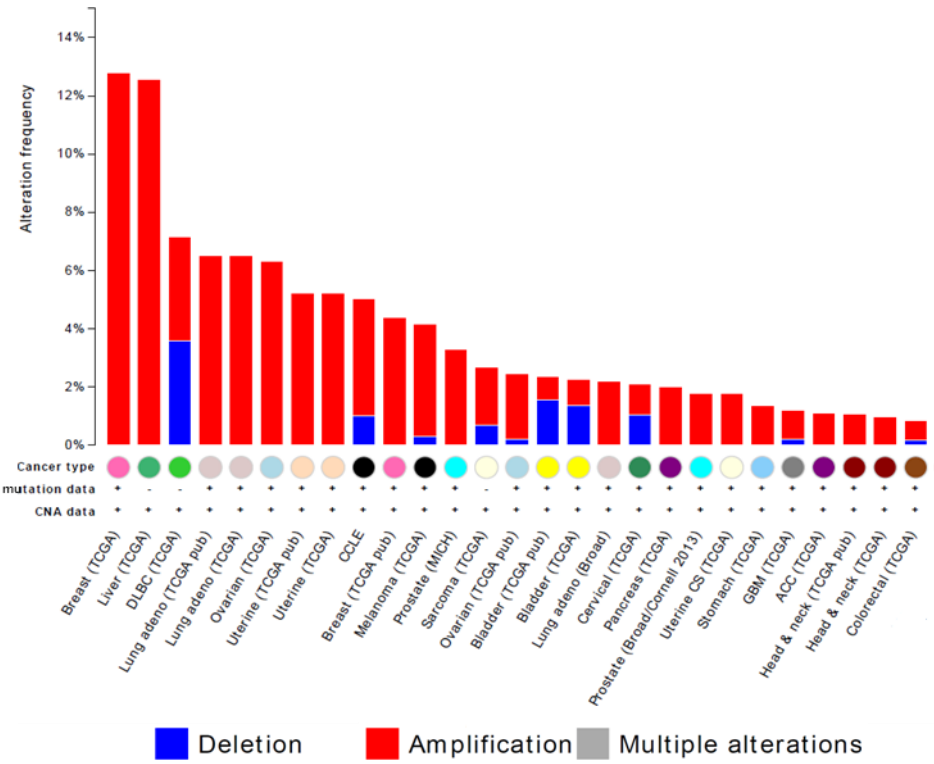
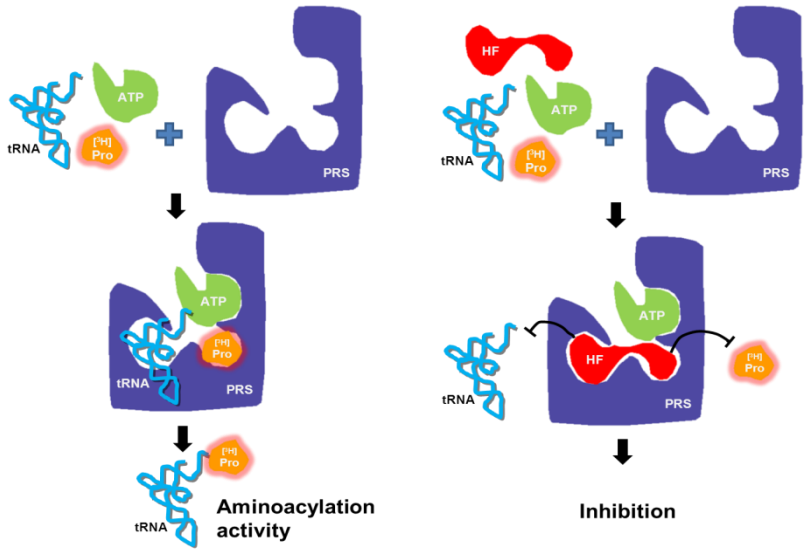


Figure 1. EPRS is highly overexpressed in cancers.

The height of the bars represents the relative alteration frequency. EPRS gene is amplified in each indicated cancer type. EPRS gene is amplified in cancers (red bars), significantly in Lung adenocarcinoma. (cBioPortal)

A

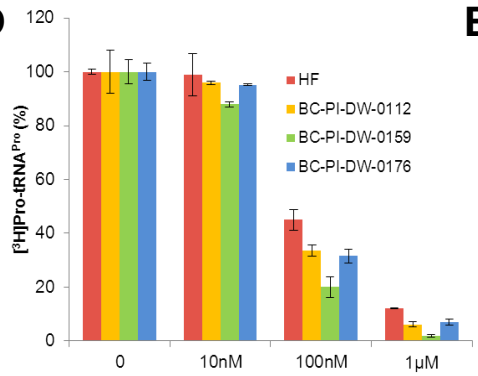


B

C

compounds	IC_{50} (nM)
BC-PI-DW-0112	62
BC-PI-DW-0134	89.7
BC-PI-DW-0135	39.2
BC-PI-DW-0147	54
BC-PI-DW-0149	83
BC-PI-DW-0152	97
BC-PI-DW-0153	97
BC-PI-DW-0159	34
BC-PI-DW-0163	44
BC-PI-DW-0171	44
BC-PI-DW-0173	96
BC-PI-DW-0176	62
BC-PI-DW-0179	90

D



E

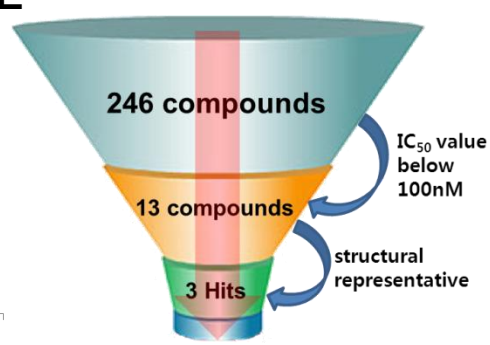
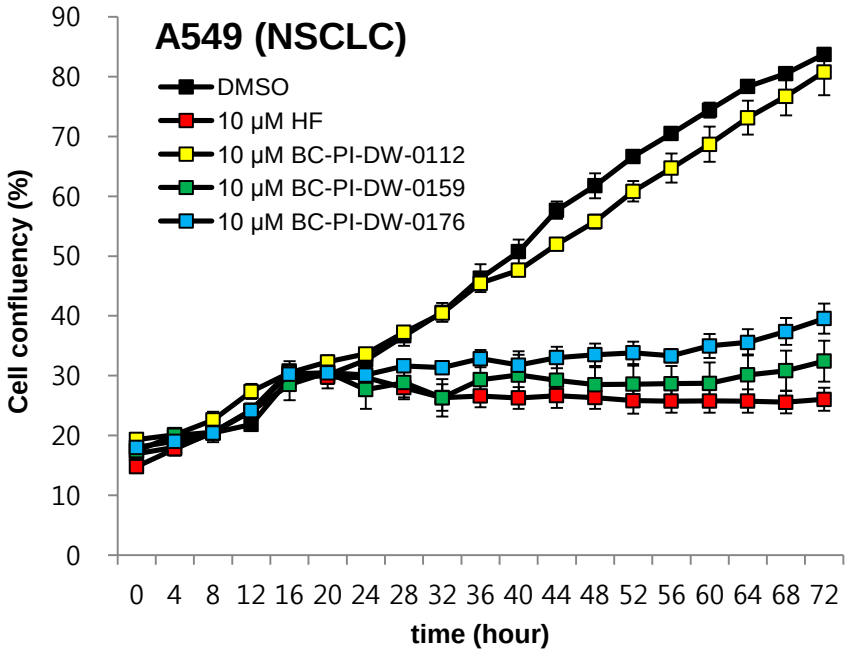


Figure 2. Compounds screening by PRS aminoacylation assay

(A) Schematic diagram for the compound screening principal based on PRS aminoacylation assay. The PRS enzyme is needed three substrates; ATP, $tRNA^{Pro}$ and proline. With substrates, PRS plays a role in ligation of proline to its cognate tRNA (left). Meanwhile, if the specific inhibitor; HF is added to the reaction, the compound occupies substrate binding site so that the reaction is blocked (right) (11). **(B)** Heat map of the primary screening data. Inhibitory effects of 246 compounds, which were treated 1μM of working concentration, on the aminoacylation level were examined by PRS aminoacylation assay and the results are presented. Several compounds inhibited PRS aminoacylation activity (yellow color). **(C)** The selected 13 compounds showed below 100nM IC_{50} value. **(D)** The aminoacylation assay result of HF and 3 hit compounds for calculation of IC_{50}. **(E)** Schematic diagram of compound screening process.

A


A549 (NSCLC)
DMSO
10 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
Cell confluency (%)
0 10 20 30 40 50 60 70 80 90
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
time (hour)

B

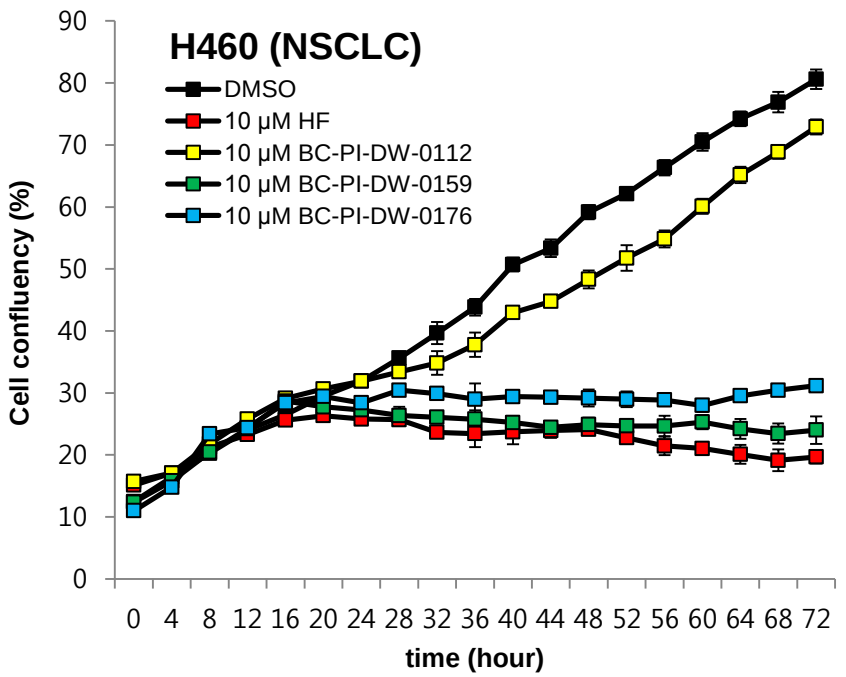
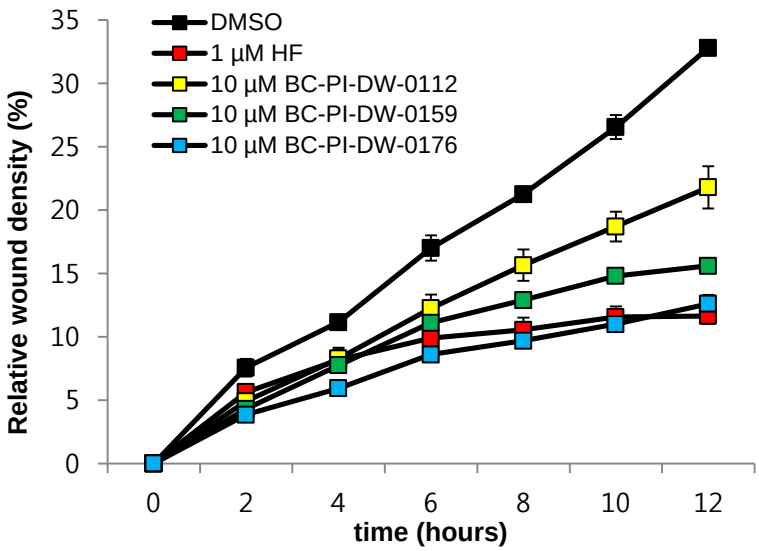

H460 (NSCLC)
DMSO
10 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
Cell confluency (%)
0 10 20 30 40 50 60 70 80 90
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
time (hour)

Figure 3. The hit compounds inhibit cell proliferation

(A) The selected 3 compounds were treated to A549 cells, typical non-small cell lung cancer cell line, at indicated concentration to determine their effect of cell proliferation control. BC-PI-DW-0159 and 0176 showed as much as HF effect. However, BC-PI-DW-0112 was not effective. DMSO were used a negative control because those compounds dissolved in DMSO **(B)** Same experiment was carried out with H460 cells, which is another typical non-small cell lung cancer cell line.

A


DMSO
1 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
Relative wound density (%)
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
time (hours)

B

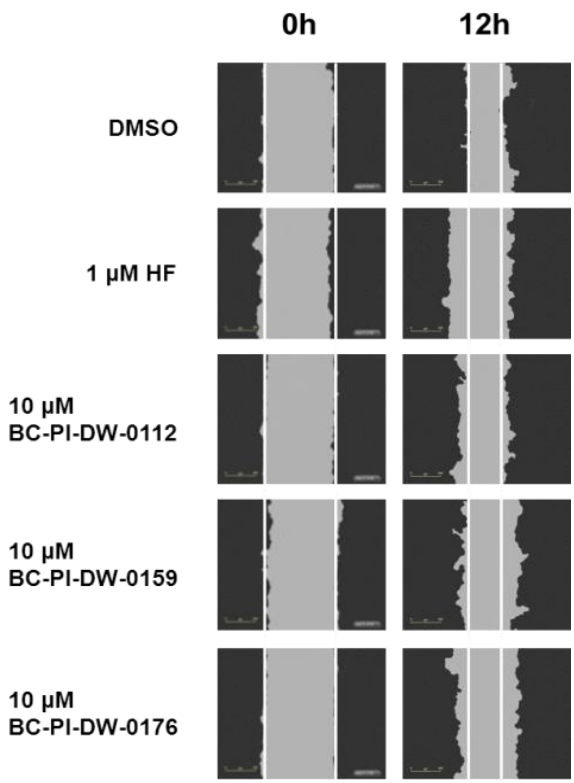
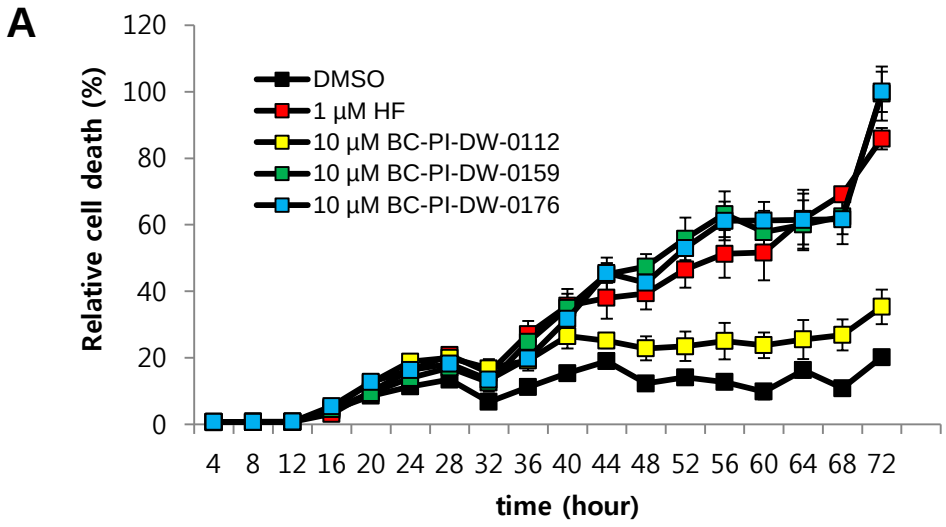
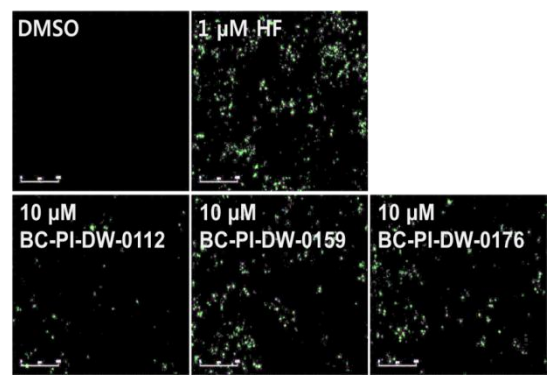
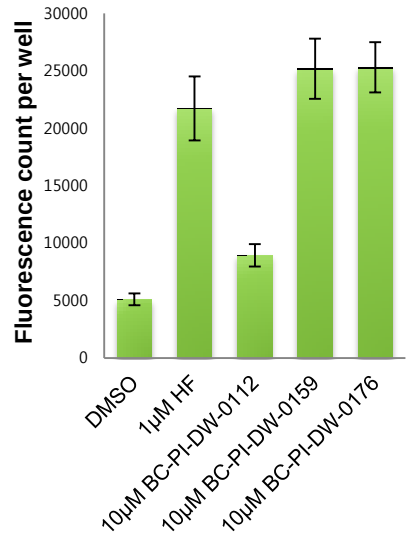

0h
12h
DMSO
1 μM HF
10 μM
BC-PI-DW-0112
10 μM
BC-PI-DW-0159
10 μM
BC-PI-DW-0176

Figure 4. The effect of hit compounds on cell migration

(A) The result of wound healing assay with 3 hit compounds. After wound made on A549 cells, each compound was treated and 12 hour's relative cell migration were observed. BC-PI-DW-0159 and 0176 showed as much as HF controls cell migration. **(B)** Comparison of narrowed wound width among treated compounds at 12 hour.


A
Relative cell death (%)
120
100
80
60
40
20
0
DMSO
1 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
time (hour)


B
DMSO
1 μM HF
10 μM BC-PI-DW-0112
10 μM BC-PI-DW-0159
10 μM BC-PI-DW-0176


C
Fluorescence count per well
30000
25000
20000
15000
10000
5000
0
DMSO
1μM HF
10μM BC-PI-DW-0112
10μM BC-PI-DW-0159
10μM BC-PI-DW-0176

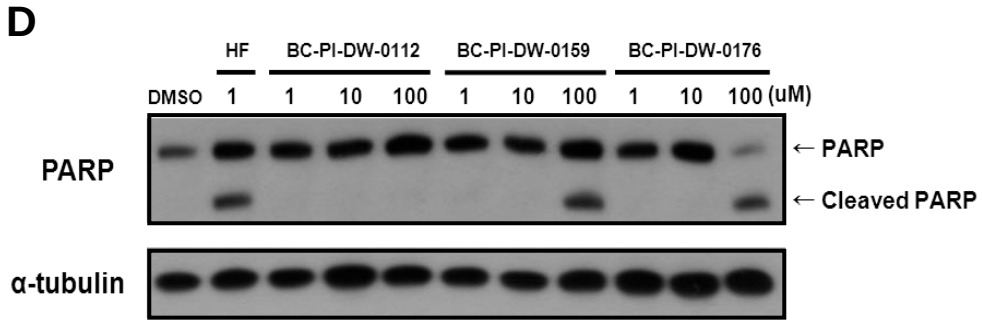

D
HF
BC-PI-DW-0112
BC-PI-DW-0159
BC-PI-DW-0176
DMSO 1 1 10 100 1 10 100 1 10 100 (uM)
PARP
← PARP
← Cleaved PARP
α-tubulin

Figure 5. The hit compounds induce cell death

(A) To investigate how to the compounds control cell growth and cell migration, YoYo-1 reagent was added with each compound to A549 cells to measure cell death by fluorescence dye. 10 μM of BC-PI-DW-0159 and 0176 showed as much as 1 μM HF induced cell death and BC-PI-DW-0112 barely affected. **(B)** The image of the cells with each compound at 72 hour. Green fluorescence means dead cells. **(C)** Comparison of cytotoxicity among 3 hit compounds by the number of fluorescence. **(D)** To investigate at molecular level, western blot was performed. After 12 hours of serum starvation, each compound was treated with several doses to A549 cells and it was harvested 12hrs later. The induced cell death mechanism was identified that it is related to poly ADP ribose polymerase (PARP) pathway.

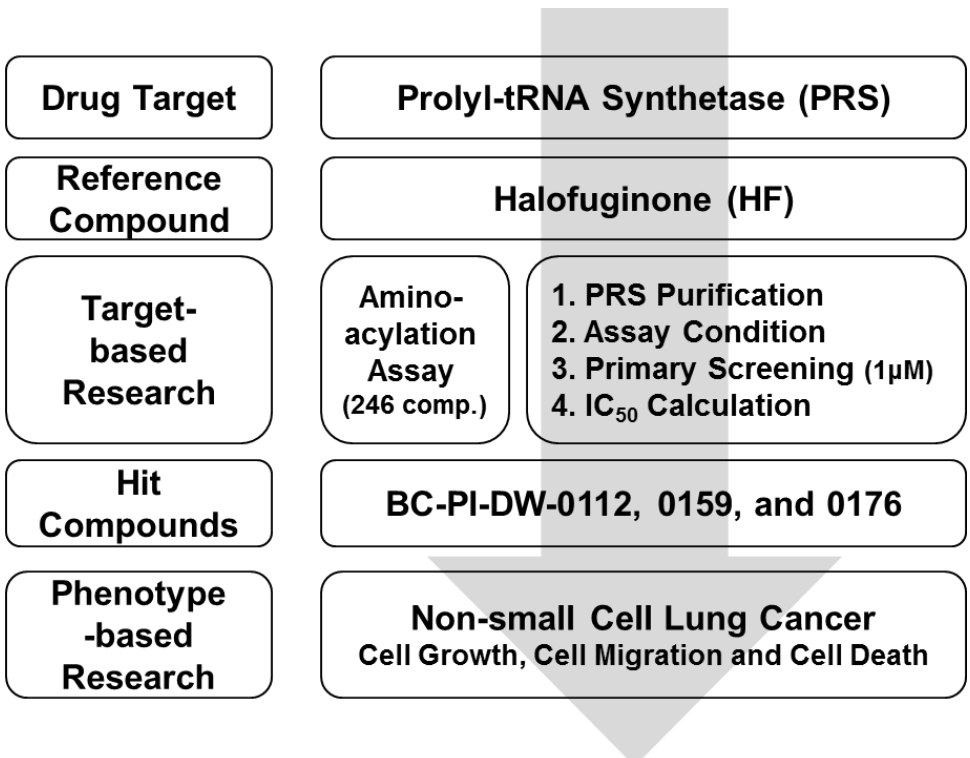


Figure 6. PRS specific inhibitor screening process

The diagram explains novel compound screening process from targeting PRS to phenotype-based research.

Table 1. Summary of hit compounds

Compound	Molecular weight (kDa)	Inhibition of PRS aminoacylation activity	Cell proliferation	Cell migration	Cell death
		IC_{50} (nM)	EC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)
Halofuginone	414.68	94.8	<0.1	<0.1	1
BC-PI-DW-0112	308	34	<100	<100	<100
BC-PI-DW-0159	353.22	62	<10	10	10
BC-PI-DW-0176	342.22	62	<10	<10	10

DISCUSSION

PRS is one of the ARS enzymes that play a role of ligation of amino acids to cognate tRNA in protein synthesis. Besides, PRS is highly expressed in cancer, especially non-small cell lung cancer (NSCLC), so it would be a cancer target. Even though there was a HF as a PRS specific inhibitor, it has fatal side effect for human drug, so I started to find novel compound.

First, various PRS inhibitor compounds were synthesized with better drug-likeness. With those compounds, I check their PRS catalytic inhibition effect by aminoacylation assay. Throughput of the assay was very low, so it was very time-consuming process. At the beginning, none of compounds was effective but, later some started to appear inhibition. Finally I got three hit compounds.

After compound screening, I treated those compounds to A549 and H460 cells, which are typical NSCLC cells. I investigate their effect of cell proliferation, migration and death by using real-time cell imaging tool, IncuCyte^{TM} FLR. Western blot was also carried out to check at molecular level. In activity test, the hit compounds had better effect than HF, but in cell, they were less than HF (Table 1). I thought that HF might be related to known or unknown factor on controlling cancer in addition to PRS dependent manner. The whole compound screening process is described in a diagram (Figure 6).

The mode of action mechanism study of HF and hit compounds are still needed for further steps and more optimized lead compound are also needed for drug candidate. Moreover, *in vivo* study like xenograft mouse model is also

necessary to be a real drug development.

In conclusion, I suggest that PRS would be a novel target for anti-NSCLC drug development and the hit compounds were found.

REFERENCES

1. Kim S, You S, Hwang D. Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping. Nature reviews Cancer. 2011;11(10):708-18.
2. Park SG, Ewalt KL, Kim S. Functional expansion of aminoacyl-tRNA synthetases and their interacting factors: new perspectives on housekeepers. Trends in biochemical sciences. 2005;30(10):569-74.
3. Park SG, Schimmel P, Kim S. Aminoacyl tRNA synthetases and their connections to disease. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(32):11043-9.
4. Jeong E-J, Hwang G-S, Kim. Kyung H. Structural Analysis of Multifunctional Peptide Motifs in Human Bifunctional tRNA Synthetase. Biochemistry. 2000;39(51):15775-82.
5. Sampath P, Mazumder B, Seshadri V, Gerber CA, Chavatte L, Kinter M, et al. Noncanonical function of glutamyl-prolyl-tRNA synthetase: gene-specific silencing of translation. Cell. 2004;119(2):195-208.
6. Arif A, Jia J, Moodt RA, DiCorleto PE, Fox PL. Phosphorylation of glutamyl-prolyl tRNA synthetase by cyclin-dependent kinase 5 dictates transcript-selective translational control. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(4):1415-20.
7. Coatney GR, Cooper WC, Culwell WB, White WC, Imboden CA, Jr. Studies in human malaria. XXV. Trial of febrifugine, an alkaloid obtained from Dichroa febrifuga lour., against the Chesson strain of Plasmodium vivax.

Journal National Malaria Society (US). 1950;9(2):183-6.

8. Ryley JF, Betts MJ. Chemotherapy of chicken coccidiosis. Advances in pharmacology and chemotherapy. 1973;11:221-93.
9. Sundrud MS, Koralov SB, Feuerer M, Calado DP, Kozhaya AE, Rhule-Smith A, et al. Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. Science. 2009;324(5932):1334-8.
10. Keller TL, Zocco D, Sundrud MS, Hendrick M, Edenius M, Yum J, et al. Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase. Nature chemical biology. 2012;8(3):311-7.
11. Zhou H, Sun L, Yang XL, Schimmel P. ATP-directed capture of bioactive herbal-based medicine on human tRNA synthetase. Nature. 2013;494(7435):121-4.
12. Son J, Lee EH, Park M, Kim JH, Kim J, Kim S, et al. Conformational changes in human prolyl-tRNA synthetase upon binding of the substrates proline and ATP and the inhibitor halofuginone. Acta crystallographica Section D, Biological crystallography. 2013;69(Pt 10):2136-45.
13. Pines M, Nagler A. Halofuginone: a novel antifibrotic therapy. General pharmacology. 1998;30(4):445-50.
14. Elkin M, Ariel I, Miao HQ, Nagler A, Pines M, de-Groot N, et al. Inhibition of bladder carcinoma angiogenesis, stromal support, and tumor growth by halofuginone. Cancer research. 1999;59(16):4111-8.
15. Koon HB, Fingleton B, Lee JY, Geyer JT, Cesarman E, Parise RA, et al. Phase II AIDS Malignancy Consortium trial of topical halofuginone in AIDS-related Kaposi sarcoma. Journal of acquired immune deficiency syndromes (1999).

2011;56(1):64-8.

16. Mitsumori K, Maita K, Kosaka T, Miyaoka T, Shirasu Y. Two-year oral chronic toxicity and carcinogenicity study in rats of diets fumigated with methyl bromide. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 1990;28(2):109-19.
17. Kikuchi H, Yamamoto K, Horoiwa S, Hirai S, Kasahara R, Hariguchi N, et al. Exploration of a New Type of Antimalarial Compounds Based on Febrifugine. Journal of Medicinal Chemistry. 2006;49(15):4698-706.
18. Francklyn CS, First EA, Perona JJ, Hou YM. Methods for kinetic and thermodynamic analysis of aminoacyl-tRNA synthetases. Methods (San Diego, Calif). 2008;44(2):100-18.

요약(국문초록)

Prolyl-tRNA Synthetase를 표적으로 하는 암 억제 연구

박 민 영
의약생명과학전공
분자의학 및 바이오제약학과
융합과학기술대학원
서울대학교

폐암은 발병률과 사망률이 가장 높은 암 중 하나이다. 집중적인 연구에도 불구하고, 효과적인 치료 표적과 믿을 만한 치료제가 여전히 부족하다. 여기, 나는 단백질 합성과정에 포함된 두 기능을 가진 글루타민 – 프롤린 – 트랜스퍼RNA 합성효소 (glutamyl−prolyl−tRNA synthetase, 이하 EPRS)를 잠재적인 암 표적으로 발견하고, 유효성이 입증된 검사법으로 그것의 특정 저해제를 발견하였다. EPRS는 폐암을 포함한 여러 암에서 과발현되어 있으며, 암의 전이와 신혈관생성에 관련되어 있다. 최근에 할로푸지논(halofuginone, 이하 HF) 이라는 화합물이 약 유사성이 낮고 세포독성이 높음에도 불구하고, EPRS에 결합하여 프롤린−트랜스퍼RNA 합성효소 (prolyl−tRNA synthetase, 이하 PRS)의 촉매 활성 저해를 수반한다는 것이 밝혀졌다. 약효성이 개선된 새로운 화합물을 찾기 위해서, HF를 참고 화합물로 하여 PRS 저해제를 선별하였다. 약효성을 최대화하기 위해서 고유의 합성전략을 가지고 수백 개의 화합물을

합성하였고, in vitro 효소 검사법, 세포 성장, 이동 그리고 사멸 분석으로 선별하였다. 결과적으로 PRS의 효소 활성뿐만 아니라 세포 생존까지 억제하는 효과적인 세 개의 화합물을 찾아내었다. 종합해보면, 이 연구는 항암 표적을 확인하고, 타당한 항암 치료 도구를 제시하였다는데 의의가 있다.

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주요어 : 프롤린-트랜스퍼 RNA 합성효소, 폐암, 저해제, 할로푸지논

학 번 : 2012 - 24144