이학석사 학위논문

소세포폐암에서 CDK7 표적억제제를 활용한 항암 효과 규명 연구

Anti-cancer effects of CDK7 inhibitors in small cell lung cancer (SCLC)

울산대학교 대학원

의 과 학 과

이 현 정

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지 도 교 수　노 진 경

이 논문을 이학석사학위 논문으로 제출함

2022 년 2 월

울산대학교 대학원

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감사의 글

설레는 마음을 한가득 품고 포닥을 하고 싶다며 당차게 인터뷰했던 게 엊그제 같은데 벌써 석사학위논문을 쓰게 되니 시간이 참 빠르다는 것을 느꼈습니다. 먼저, 석사과정을 시작하게 도와주신 그동안 많은 지도와 진솔한 조언을 해 주신 저의 지도교수님 노진경 교수님께 큰 감사를 드립니다. 어려웠던 논문, 10 개중 9 개가 실패가 일상인 실험을 통해 실패를 딛고 일어날 수 있는 힘, 다양한 경험들을 통해서 무엇이든 할 수 있다는 희망과 자신감을 배울 수 있었습니다. 항상 풍요로운 좋은 환경에서 연구할 수 있게 끔 배려해주시고 도와주신 덕분에 무사히 석사생활을 마칠 수 있었습니다. 그리고 바쁘신 와중에도 저의 석사학위 논문 심사해주신 언제나 자신 있는 모습으로 더욱 더 성장하기를 응원해주신 김인기 교수님, 백찬기 교수님께도 감사의 말씀을 드립니다. 또한 첫 단추를 끼우는 일이 가장 중요한데 아낌없는 따가운 채찍질과 절벽에서 스스로 올라올 수 있도록 끈기, 집념 그리고 연구자의 자세를 가르쳐 주신 지금까지도 앞으로도 변함없는 저의 영원한 롤 모델 최윤정 박사님 정말 사랑하고 감사하다는 말씀을 드리고 싶습니다. 또한 어두운 동굴속에서 헤매고 있을 때 하하 웃으며 랜턴 빛을 비춰 주시던 김동하 박사님, 언제나 호탕하게 껄껄 웃으시며 괜찮다 응원해주시고 격려해주신 임경택 박사님, 석사과정 동안 제가 도움을 청할 때마다 도와주고 힘이 되어 준 동물실험실 메이트 다솜언니, 언제나 스마일 펑펑

과즙미 넘치는 효정이, 화한 매력의 이채원 선생님, 온화한 제주도 바다 같은 공윤정 선생님께 고맙다는 말씀 전하고 싶습니다. 그리고 인턴시절부터 석사 생활까지 짧다면 짧고 길다면 긴 시간을 보내면서 많이 힘들었지만 울고 웃고 버티면서 할 수 있다며 함께해주던 은진이가 있었기에 지금의 제가 있었다고 생각하고, 이에 감사의 말을 전합니다. 마지막으로 하늘 나라에서 항상 저를 지켜봐 주시며 큰 사람이 되길 응원해주시는 외할아버지, 늘 내편 기를 팍팍 넣어 주시는 든든한 나의 뿌리 외할머니, 언제나 딸의 선택을 믿어주고 후회 없는 삶을 살길 바라며 하고 싶은 건 다 할 수 있도록 물심양면으로 지지해주신 부모님, 먼 미국에 계시지만 지금껏 버틸 수 있도록 도와주신 든든한 ZOOM 력자, 삼촌의 사랑이 있었기에 해낼 수 있었습니다. 이외에도 미처 적지 못했지만, 많은 도움을 주신 교수님들과 다른 지인분들께도 감사의 인사를 드립니다.

Abstract

In contrast to non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC) has not been significant progress in the development of therapies for the disease for decades. Although various targeted agents including immunotherapy are recently being developed into a drug for testing in clinical trials, the development of new therapeutic agents are needed for SCLC. We developed a potent inhibitor of cyclin-dependent kinase 7 (CDK7), designated YPN-005, and searched anti-cancer effects in SCLC cells, cisplatin or etoposide-resistant cells and organoids derived from SCLC patients. In a panel of kinases assay, YPN-005 was highly selective for CDK7 and showed antiproliferative effects in SCLC as well as cells with acquired resistance to conventional anti-cancer drugs. Consistent with other CDK7 inhibitor, YPN-005 treatment significantly decreased the phosphorylation of the carboxyl-terminal domain of RNA polymerase II. In consistent with in vitro results, YPN-005 treatment showed a significant inhibition of tumor growth though the suppression of RNA polymerase II phosphorylation. Lastly, we found that YPN-005 showed potent anticancer effects in organoids derived from SCLC patients compared to other CDK7 inhibitor, THZ1. Therapeutic targeting of CDK7 in SCLC might be suitable for clinical investigation, and YPN-005 may be a promising therapeutic option for primary SCLC and SCLC with acquired resistance to conventional therapy.

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Introduction

Lung cancer remains the most prevalent cancer and the leading cause of cancer-related death globally. Small cell Lung cancer (SCLC) which is a very aggressive disease that accounts for approximately 15% of all lung cancer. [1-4] SCLC patients initially respond to platinum-etoposide (EP) chemotherapy. However, most of SCLC patients nearly recur tumors within 6-12 month, resulting in a 5-year survival rate of less than 7%.[5-8] In addition, the EP-resistance in SCLC patient's mechanism remain undetermined. [9-12]

The general transcription factor TFIIH is recruited to start the transition from transcription initiation to early elongation, which is a key step for transcription by RNA polymerase II (Pol II). [13-16] Other papers suggested that targeting TFIIH subunits, such as CDK7, has been a promising strategy in cancer treatment. [17-22] CDK7 phosphorylates the C-terminal domain (CTD) of the poll II subunit RPB1 at serine 2, 5, and 7, which is indispensable for productive transcription elongation.[15, 20, 23-29]

Cyclin dependent kinases (CDKs) are a large group of serine/threonine protein kinase playing central roles in controlling the cell cycle and RNA transcription. [30-34] Of them, inhibition of CDK7 activity is expected to suggest a therapeutic window by regulating both of transcription and cell cycle progression. The importance of targeting CDK7 is highlighted by

the discovery of novel CDK7 inhibitors including THZ1, QS1189, YKL-5-124 and BS-181. [35-45] However, the first generation CDK7 inhibitors showed modest activity because of specificity and unfavorable pharmacology. [46-49] Hence the discovery of more selective and potential CDK7 inhibitors are necessary for future cancer research.

Here, we investigated the anti-cancer therapeutic efficacy of novel CDK7 inhibitor, YPN-005 for pre-clinical evaluation.

Material and Methods

Cell lines and reagents

Human SCLC (H209, H82, and H69) cell lines were purchased from American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA) at 37°C in an atmosphere of 5% CO_2. THZ1, cisplatin, and etoposide were purchased from Selleck Chemicals (Houston, TX). YPN-005 was kindly provided by Yungjin Pharmaceutical (Suwon, Korea).

Kinase profile assay

Kinase selectivity was assessed by the scanMAX® Kinase Assay (Eurofins, San Diego, CA) consisting of 468 protein kinases for YPN-005 at a single concentration (1 μM) using KM value of ATP (https://www.discoverx.com).

Kinase IC_{50} profiler assay

CDK7, CDK12, CDK13, and CDK16 inhibition were evaluated by a radiometric-binding assay using myelin basic protein as a substrate (Eurofins, New Taipei City, Taiwan) according to the manufacturer's protocols (https://www.eurofinsdiscoveryservices.com).

MTT assay

Cells (1×10^4) were seeded in 96-well sterile plastic plates overnight and then treated with the indicated agents. After 72 h, 15 μL of MTT solution (5 mg/mL) was added to each well and the plates were incubated for 4 h. Crystalline formazan was solubilized by 100 μL of a 10% (w/v) SDS solution for 24 h. Absorbance at 595 nm was read spectrophotometrically using a microplate reader. The results represent at least three independent experiments, and the error bars signify the standard deviation (SD) from the mean. The IC_{50} values were determined using GraphPad Prism software (GraphPad Software, La Jolla California USA,).

Immunoblotting

Whole-cell lysates were prepared using EBC lysis buffer (50 mM Tris–HCl [pH 8.0], 120 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 0.3 mM phenylmethylsulfonylfluoride, 0.2 mM sodium orthovanadate, 0.5% NP-40, and 5 U/mL aprotinin), and then centrifuged. Proteins were separated using SDS-PAGE and transferred to PVDF membranes (Invitrogen,Carlsbad,CA) for immunoblot analysis. The membranes were probed using primary antibodies against RNAPII CTD p-Ser2 (#13499), RNAPII CTD p-Ser5 (#13523), RNAPII CTD p-Ser7 (#13780), RNAPII CTD (#2629; all from Cell Signaling Technology, Danvers, MA), CDK7 (sc-7344),

and β-actin (sc-47778; from Santa Cruz Biotechnology, Dallas, TX). The membranes were then treated with a horseradish peroxidase-conjugated secondary antibody. All membranes were developed using an enhanced chemiluminescence system (Thermo Scientific, Waltham, MA).

Xenograft studies

To establish xenograft models, female SCID mice (18–20 g, 6 weeks of age) were purchased from JA Bio Inc. (Suwon, Korea). All experimental procedures were performed in accordance with the National Institutes of Health (NIH, Bethesda, MD) guidelines and with an approved protocol (2019-04-253) from the Institutional Animal Care and Use committee of Asan Institute for Life Sciences. Tumors were cultured by implanting cells (1–5 × 10^6 cells/0.1 mL) in 50% Matrigel (BD Biosciences, San Jose, CA) and subcutaneously injected into the right flank of animals. Drug treatment was initiated when the tumors reached a volume of 50–100 mm^3. To measure the tumor size, the length (L) and width (W) of each tumor were measured using calipers, and the tumor volume (TV) was calculated using the formula: TV = (L × W2)/2. YPN-005 was dissolved in 5% dextrose and administered via intravenous tail vein injection twice per week (2 x).

Immunohistochemical staining

Each tumor was harvested from tumor-bearing mice 24 h after 2 treatments of YPN-005. Resected tumors were fixed in 10% formaldehyde and embedded in paraffin. Immunohistochemical staining was performed using a specific primary antibody (Ki-67; DakoCytomation, Los Angeles, CA), the EnVision Plus Staining kit (DakoCytomation, Los Angeles, CA), and an APP-Direct terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) Assay Kit (Millipore, Burlington, MA) according to the supplier's instructions. Quantitative analysis of section staining was performed by counting immunopositive cells in five arbitrarily selected fields.

SCLC organoid culture

SCLC-derived tumor organoids were established in previous study (Choi et al., 2021). All studies involving human participants were conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects. For culturing human SCLC-derived tumor organoids, SCLC cells (1×10^3) were seeded in 25 μl of growth factor-reduced Matrigel (Corning, NY, USA) onto 48-well plates. The cells were allowed to polymerize at 37°C for 1 h, before adding advanced DMEM/F12 medium containing 100 ng/ml of EGF (Peprotech, Cranbury, NJ), 50 ng/ml

of bFGF, 100 ng/ml of noggin (Peprotech, Cranbury, NJ), 10% R-spondin-1 conditioned medium, 1× N2 (Sigma-Aldrich, St. Louis, MO) 1.25 mM N-acetyl cysteine (Sigma-Aldrich, St. Louis, MO, USA), 10 mM nicotinamide (Sigma-Aldrich, St. Louis, MO, USA), and 50 nM A83–01 (Sigma-Aldrich, St. Louis, MO, USA). The growth medium was refreshed every 2 days, and the cells were passaged by mechanical disruption every 10–14 days at a 1:5 ratio.

Organoid proliferation test

Patient-derived tumor organoids (PDTOs, #7 and #10) were used for the organoid cell viability assay. After dissociation of SCLC organoids into a single cell suspension using TrypLE Express (Thermo Scientific, IL, USA), 400 cells in 10 μl of Matrigel were dispensed onto 96-well microplates and allowed to polymerize at 37°C for 1 h. After 2 days, cisplatin, etoposide, THZ1, and YPN-005 were added at concentrations of 0.01, 0.1, 1, or 10 μM for 8 days. Tumor organoid growth was measured by the Cell Titer-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI) according to the manufacturer's instructions. Brightfield images were captured using a TE-2000U microscope (Nikon, Tokyo, Japan).

Statistical analysis

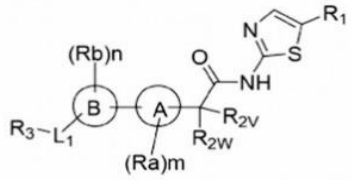
P-values were determined with unpaired t-tests between comparator groups using GraphPad software (La Jolla, CA).

Result

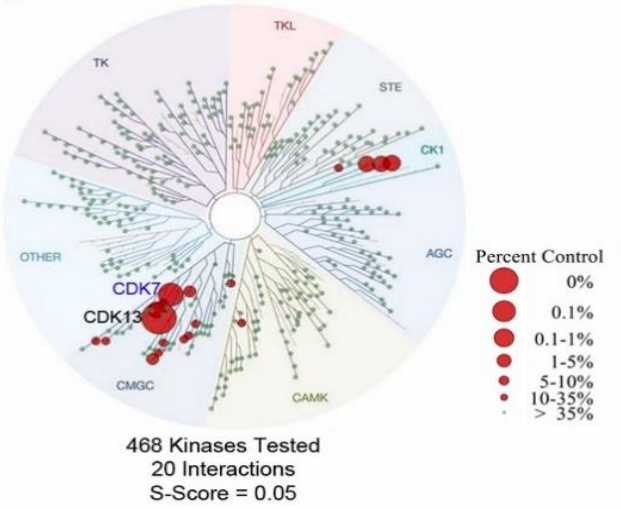
1. YPN-005 is a novel selective inhibitor of CDK7.

The chemical structure of YPN-005(C26H28N6O2S) was shown in Fig. 1A. To investigate *in vitro* profiles of YPN-005 for various targets, we firstly performed the YPN-005 target kinase profile by KINOMEscan, an indirect binding assay that provides kinome-wide assessment of inhibitor specificity. YPN-005 was analyzed at 1 μM against 468 kinase targets, and showed remarkable overall selectivity with CDK8, CDK12, CDK13, CDK19, and CDK7 (Fig.1B-C).

A



B



C

CDKs	1)% control at 1 μM	2)IC_{50} (nM)	CDKs	1)% control at 1 μM	2)IC_{50} (nM)
CDK1	100	NC	CDK11B	100	NC
CDK2	80	NC	CDK12	11	NC
CDK3	95	NC	CDK13	0	> 1000
CDK4	97	NC	CDK14	73	NC
CDK5	70	NC	CDK15	30	NC
CDK6	100	NC	CDK16	16	53
CDK7	0.25	31	CDK17	52	NC
CDK8	21	NC	CDK18	60	NC
CDK9	82	NC	CDK19	1.4	NC
CDK10	63	NC			

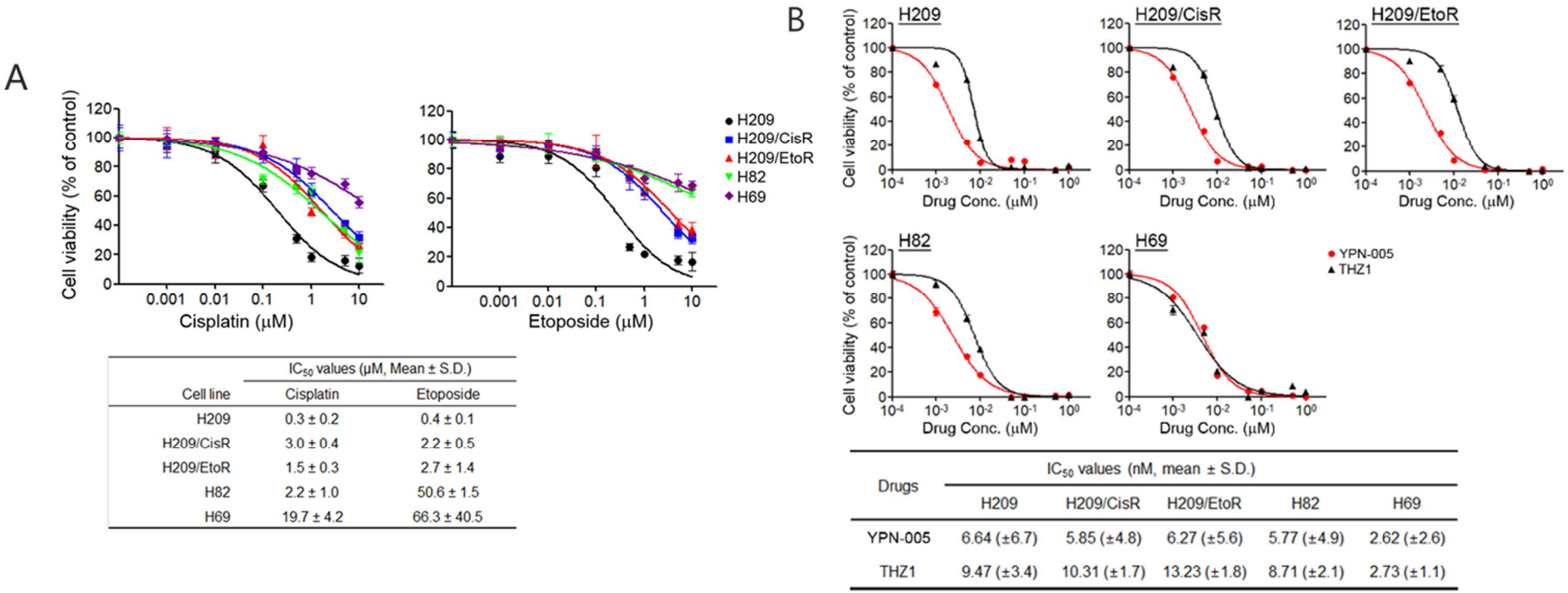
Fig.1 Kinome profiling for the CDK7 Kinase inhibitor, YPN-005.

(A) Chemical structure of the CDK7 inhibitor, YPN-005. (B) KINOMEscan profile of YPN-005 tested against 468 kinases at a final concentration of 1 μM. TreeSpot diagram shows all test kinases on a circular dendrogram of the human kinome, with interacting kinases shown as red circles; non-interacting kinases are represented as small green dots. Overall, 20 kinase interactions were observed for an S-Score of 0.05, indicate of a very selective inhibitor. (C) IC_{50} values of YPN-005 from a screening of 20 against 468 kinases.

2. YPN-005 shows potential therapeutic agent in SCLC cells.

Before evaluating the efficacy of CDK7 inhibitors in SCLC cells, firstly we established cisplatin and etoposide resistance H209 cell line. We identified sensitivity with cisplatin or etoposide in the used SCLC cells. Our MTT data demonstrated that the H209 cells were more sensitive to chemotherapy than H82 and H69 cells (Cisplatin IC_{50}: 0.3 μM in H209, 2.2 μM in H82, and 19.7 μM in H69; etoposide IC_{50}: 0.4 μM in H209, 50.6 μM in H82, and 66.3 μM in H69). Moreover, two resistant cell lines (H209/CisR and H209/EtoR) showed over 5–10-fold higher resistance to each drug than the parental cells (Cisplatin IC_{50}: 3.0 μM in H209/CisR and 0.3 μM in H209; etoposide IC_{50}: 2.7 μM in H209/EtoR and 0.4 μM in H209) (Fig.2A).

To verify the effect of YPN-005 on the cell viability of SCLC cell lines such as H209, H209/CisR, H209/EtoR, H82 and H69 cells, all cells were treated with various concentrations of THZ1 and YPN-005 (0, 0.01,0.1,1,5, and 10 μg/ml) for 72 h. The results revealed that YPN-005 treatment inhibited the proliferation of the five SCLC cells in a concentration-dependent manner. The IC_{50} values were 9.47±3.4, 10.31±1.7, 13.23±1.8 and 8.71±2.1, 2.73±1.1 μg/ml for H209, H209/CisR, H209/EtoR, H82 and H69 cells, respectively, in addition, the IC_{50} values were 6.64±6.7, 5.85±4.8, 6.27±5.6 and 5.77±4.9, 2.62±2.6 μg/ml for YPN-005 in H209, H209/CisR, H209/EtoR, H82 and H69 cells, respectively (Fig.2B). These results indicated that YPN-005 showed comparable of better efficacy than THZ1 in SCLC.



Cell line	IC_{50} values (μM, Mean ± S.D.) Cisplatin	Etoposide
H209	0.3 ± 0.2	0.4 ± 0.1
H209/CisR	3.0 ± 0.4	2.2 ± 0.5
H209/EtoR	1.5 ± 0.3	2.7 ± 1.4
H82	2.2 ± 1.0	50.6 ± 1.5
H69	19.7 ± 4.2	66.3 ± 40.5

Drugs	IC_{50} values (nM, mean ± S.D.) H209	H209/CisR	H209/EtoR	H82	H69
YPN-005	6.64 (±6.7)	5.85 (±4.8)	6.27 (±5.6)	5.77 (±4.9)	2.62 (±2.6)
THZ1	9.47 (±3.4)	10.31 (±1.7)	13.23 (±1.8)	8.71 (±2.1)	2.73 (±1.1)

Fig. 2 Cytotoxic efficacy of YPN-005 in SCLC cells.

(A) Establishment of cisplatin and etoposide resistant H209 cell lines. H209 cell line were exposed H209 cell to cisplatin and etoposide with rising levels of concentration for over 1 year. The drug exposing process was repeated until there was certain resistance demonstrated IC_{50} values. (B) SCLC cell lines were treated with various drug concentrations of YPN-005 for 72 h. The IC_{50} values were obtained by using MTT assay and the graphs generated using GraphPad Prism software for IC_{50} calculations based on MTT data. The mean values of three independent experiments performed in quadruplicates are presented with standard deviation (n=3) ±S.D.

3. YPN-005 inhibits RNAP II signaling in SCLC cells.

CDK7 regulates RNA Pol II-mediated transcriptional initiation and pausing, in addition to its effects on transcript elongation through its CAK activity working via other transcriptional CDKs. To examine transcriptional reduction in SCLC cell lines, we examined phosphorylation levels of residues Ser 2, Ser 5 and Ser 7 on RNAPII CTD after 6h incubation of THZ1 and YPN-005 at final concentrations of 0.01, 0.1 μM. As shown in the fig. 3, H209, H82 and H69 cell lines showed that phosphorylation status of residues Ser 2, Ser 5 and Ser 7 were significantly reduced by YPN-005 incubation at 0.01 μM concentration. In addition, H209/CisR and H209/EtoR cell lines more effectively inhibited than parent H209 cell line. THZ1 treatment showed that phosphorylation status of residues Ser 2, Ser 5 and Ser 7 were inhibited with 0.01 μM concentration in the H209, H82, H69, H209/CisR and H209/EtoR cell lines. These data indicated that YPN-005 is more effectively inhibits RNAPII CTD phosphorylation compared with THZ1 in SCLC.

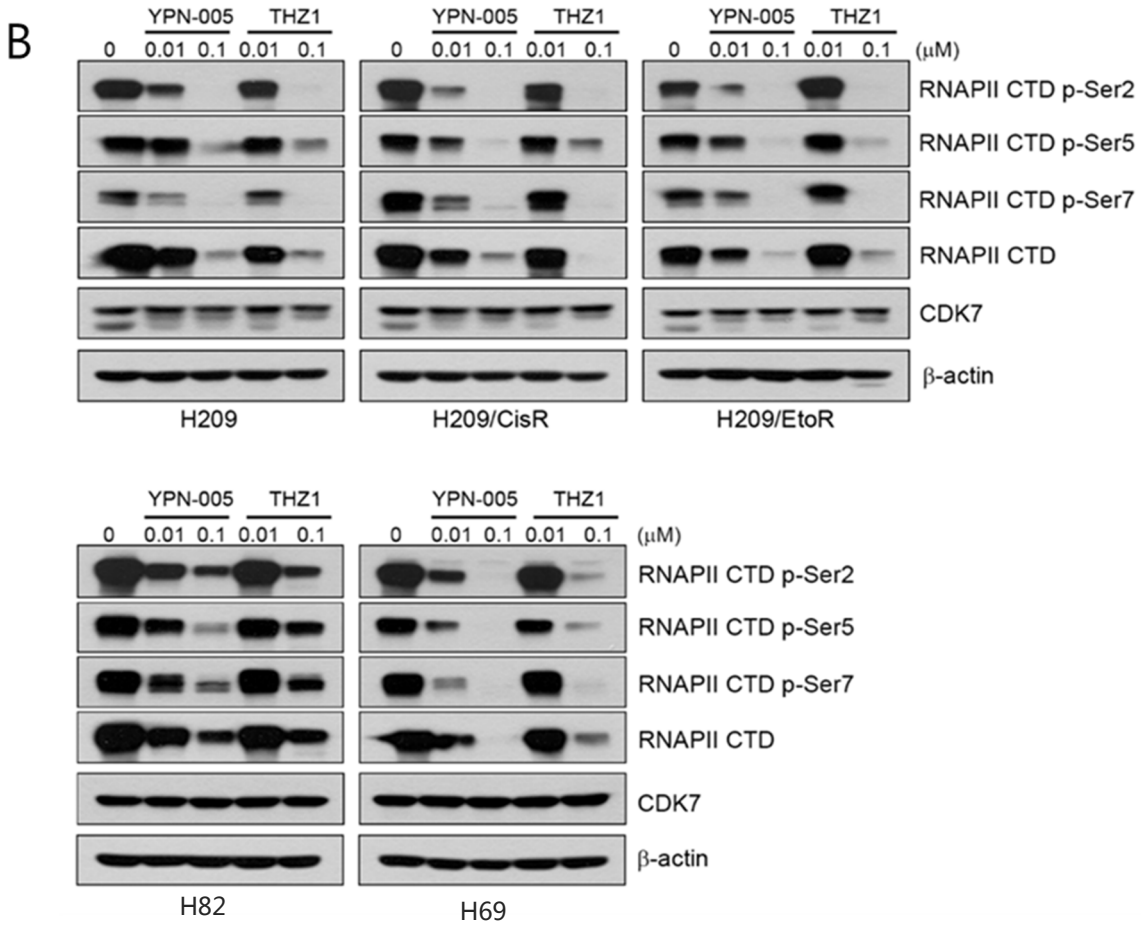


Fig. 3 Transcriptional inhibition by YPN-005 treatment in vitro.

(A) Western blot analysis of proteins shown after treatment of SCLC cells with THZ1 and YPN-005 at 0.01 μM, 0.1 μM, 1 μM concentrations for 6 h.

4. YPN-005 suppresses tumor growth in SCLC xenograft model.

SCLC xenograft studies were performed to test whether YPN-005 could inhibit the growth of SCLC cancer. We used H209-, H209/CisR-, and H209/EtoR-tumor xenograft models and SCLC cells were subcutaneously injected into female SCID mice. The groups were then randomly grouped and treated YPN-005 for 4 weeks and YPN-005 was intravenously injected for 2 weeks (2 times/week). All mice sacrificed and the tumors were excised for analysis. In dose-response experiments, maximum antitumor activity was achieved at 2 mg/kg in xenograft models. The results demonstrated that YPN-005 significantly inhibited tumor growth (Fig. 4). The effect of YPN-005 was slightly higher in parental H209 cell line xenografts than in H209/CisR and H209/EtoR resistant cell xenografts, but it also effective in H209/CisR and H209/EtoR resistant cell lines. These antitumor effects of YPN-005 occurred without substantial overt toxicity as assessed by changes in body weights.

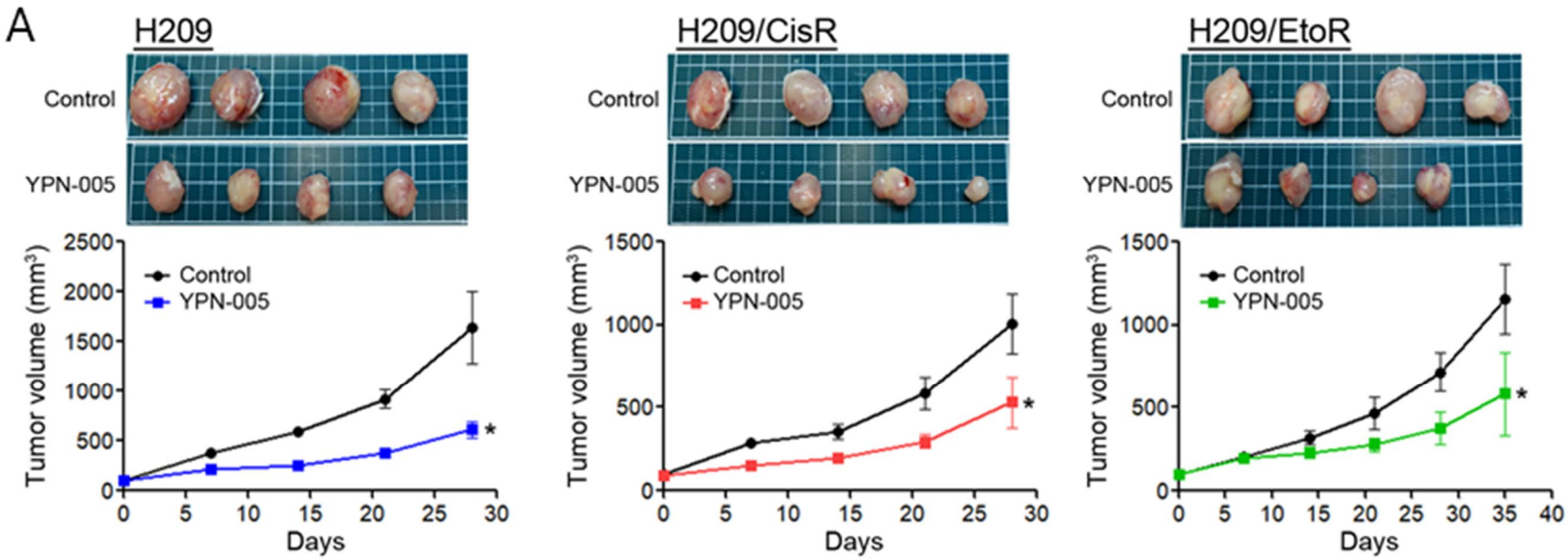


Fig. 4 Therapeutic regression efficacy of YPN-005 in SCLC xenografts.

(A) Tumor volumes were measured once a week with calipers, and tumor volumes were calculated using the formula: Volume = (width)2 × length/2. After the tumors had established, Subcutaneous tumor-bearing mice were treated with vehicle(control) and YPN-005 at 2mg/kg (n=6 each) or vehicle over 14 days (2 times/week for 2 weeks). YPN-005 inhibitor showed inhibition of H209, H209/CisR, H209/EtoR tumor growth. The data preseed ± S.D.

5. YPN-005 shows inhibition of RNAP II signaling in SCLC xenograft model.

To understand the molecular mechanisms underlying YPN-005-induced tumor inhibition, we examined the tumor protein levels of RNAP II CTD, RNAP II CTD Ser-2, RNAP II CTD Ser-5 and RNAP II CTD Ser-7 in SCLC xenografts by western blot analysis. As shown in the fig. 5A and B, YPN-005 was reduced phosphorylation of RNAPII at the serine 2, the serine 5 and the serine 7 sites in H209 parent cell and H209/EtoR- cell lines. In addition, in H209/CisR cell line showed substantially inhibition of RNAPII at the serine 2, the serine 5 and the serine 7 sites. These results suggested that YPN-005 can be potential inhibitor.

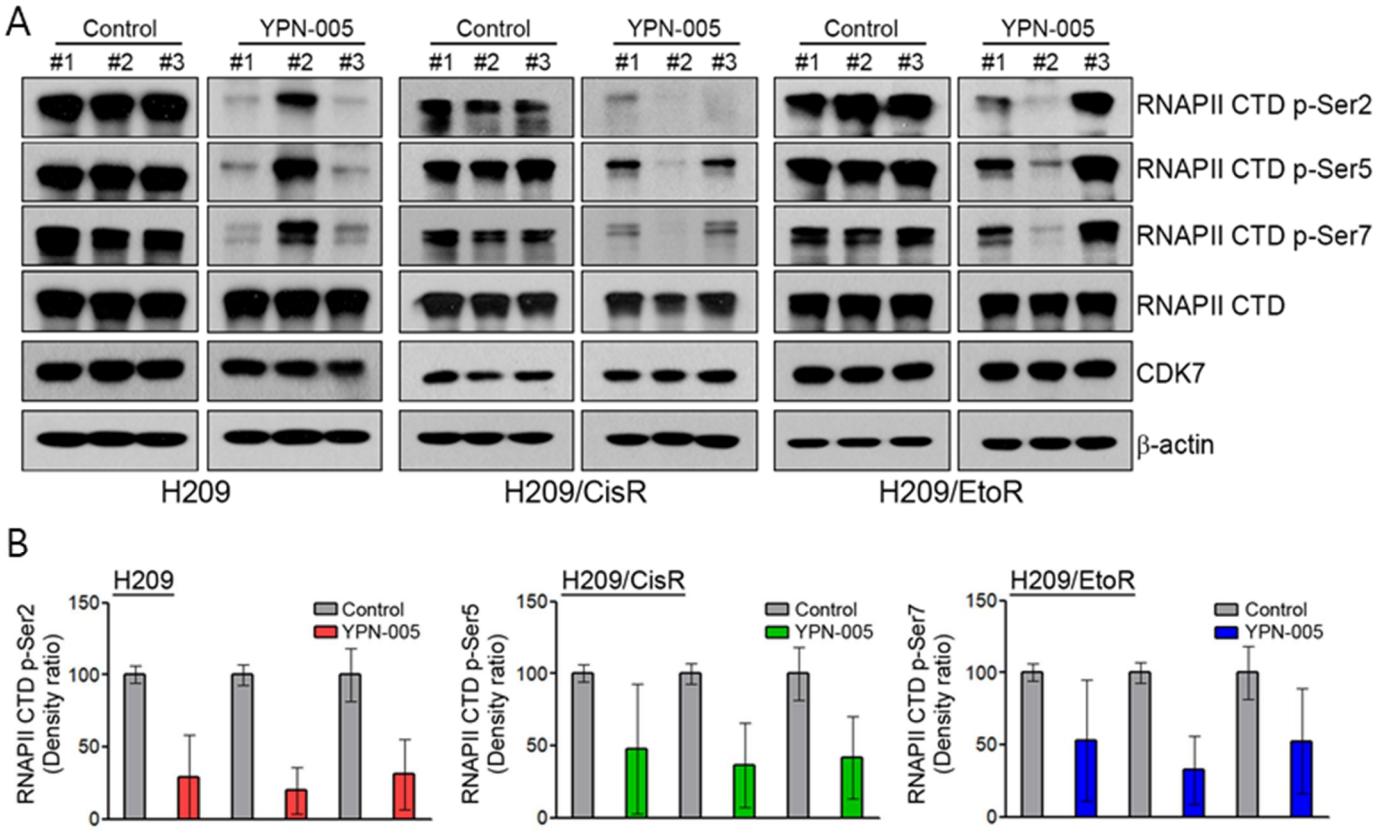


Fig. 5 Western blot analysis of SCLC xenografts tumor proteins.

(A) RNAP II CTD transcriptional signals were determined by using western blot analysis in SCLC xenograft. (B) Western blot analysis of protein level of RNAP II CTD p-Ser2, RNAP II CTD p-Ser5 RNAP II CTD p-Ser7. Western blot densitometry was quantified (ImageJ) and the level of the indicated protein was normalized to β-actin.

6. YPN-005 shows anticancer effects in SCLC xenograft model.

The effects of YPN-005 treatment on signaling were accompanied by decreased proliferation (as measured by quantitative analysis of Ki-67 staining in the tumor cells using immunohistochemistry [IHC]). The data showed that YPN-005 decreased proliferation in H209, H209/CisR and H209/EtoR cell lines. Moreover, in resistant cell lines indicated slightly effective compared with parent cell line. (Fig. 6A) We detected apoptosis in SCLC xenografts using a TUNEL assay and examined by immunohistochemistry. We observed significantly higher TUNEL+ signals level in SCLC-treated tumor samples, when compared to samples from the control or vehicle group (Fig. 6B). These results suggest that YPN-005, by targeting the kinase activity of CDK7, induces apoptosis of tumor cells in vivo (as measured by quantitative analysis of TUNEL staining in the tumor cells using IHC).

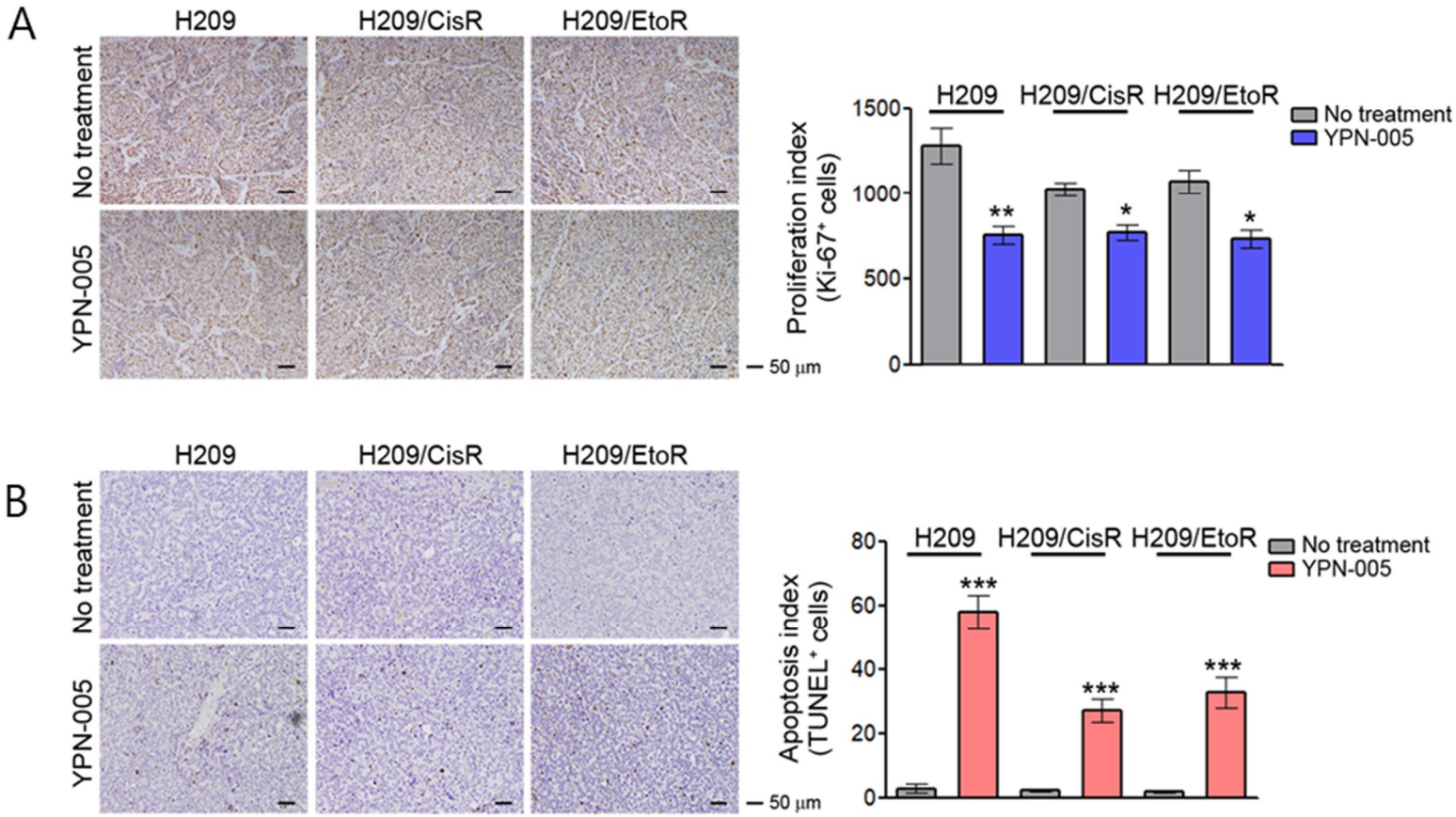


Fig. 6 The effect of YPN-005 on apoptosis and cell proliferation in SCLC xenografts.
(A and B) Immunohistochemical analysis measuring each indicated pharmacodynamics biomarker (A, Ki-67; B, TUNEL staining) in representative control-treated or YPN-005-treated tumors harvested from tumor-bearing mice at 24 h after 2 times treatment. The right graphs show quantification of the Ki-67 and TUNEL staining. $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$ compared to the control group.

7. YPN-005 inhibited organoid model derived from SCLC patient.

Previously, we established the SCLC PDTOs (patient-derived organoids). [50]Above all, we chose PDTO #7 and #10 because they were non-responsive to initial standard EP doublet therapy. In consistent with clinical data, two PDTOs showed resistance to cisplatin and etoposide (Cisplatin IC50: >10 μM in PDTO #7 and #10; etoposide IC50: 607 nM in PDTO #7 and 212.8 nM in PDTO #10), while two CDK7 inhibitors were sensitive in two PDTOs (Fig. 7A and B). In addition, YPN-005 treatment demonstrated higher efficacy than THZ1 treatment. (THZ1 IC50: >189.4 nM in PDTO #7 and 109.8 nM PDTO #10; YPN-005 IC50: 20.4 nM in PDTO #7 and 15.7 nM in PDTO #10). Accordingly, YPN-005 has the potential to be used to treat patients with SCLC who are non-responsive to EP chemotherapy.

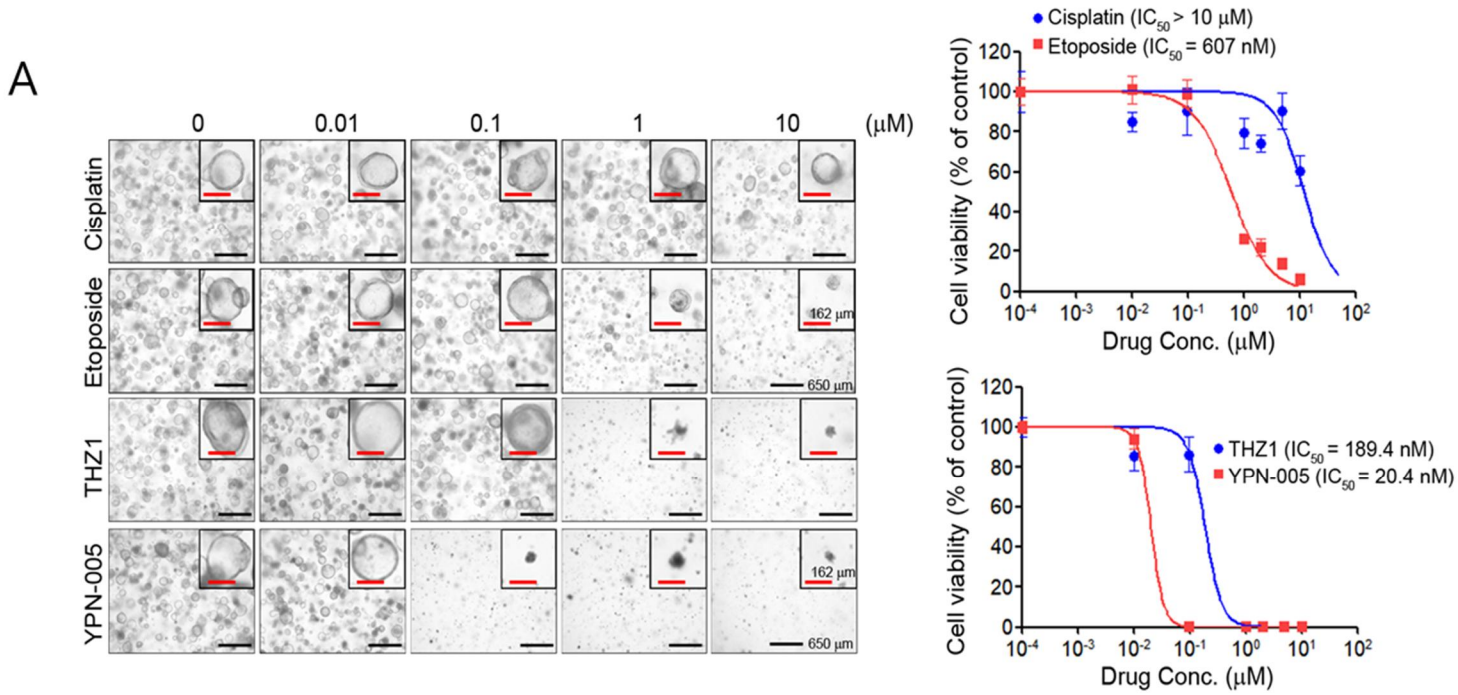
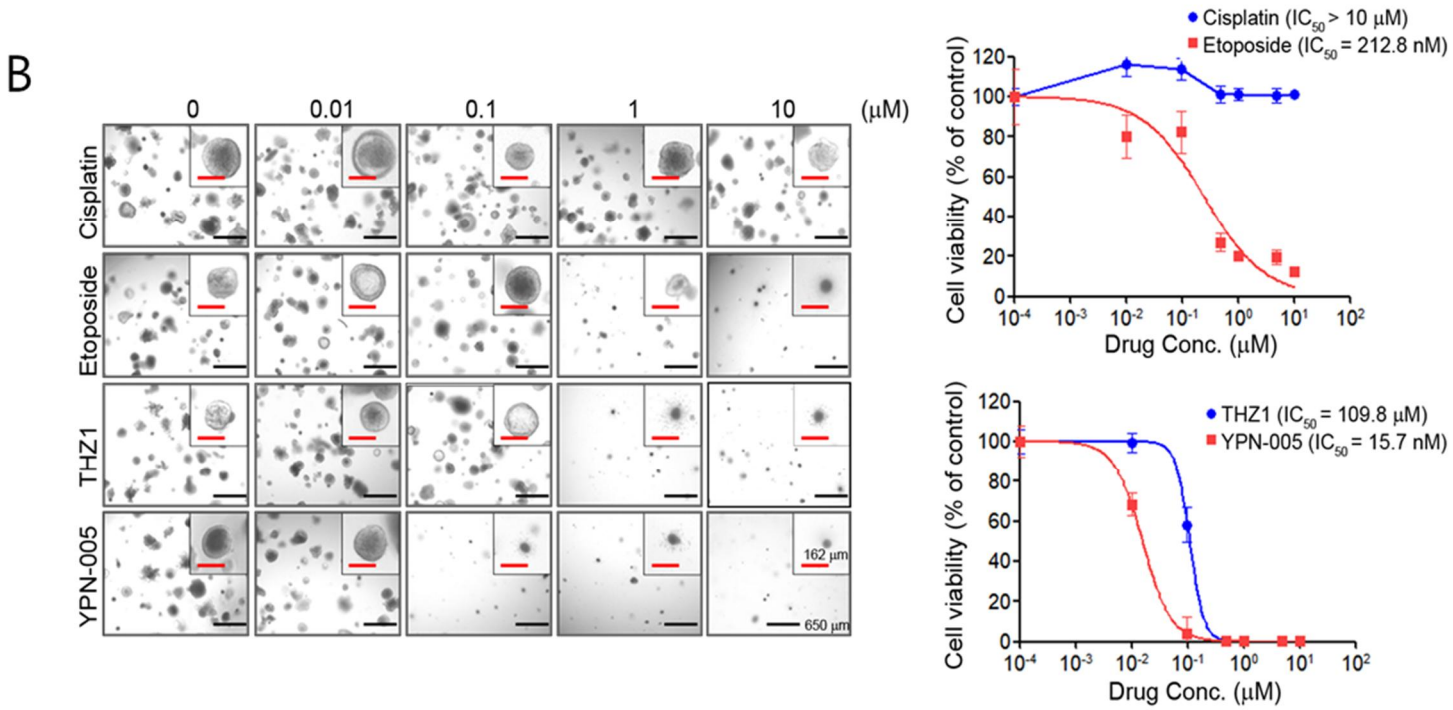


Fig. 7 Therapeutic effects of YPN-005 on SCLC-derived organoids.

The SCLC cells were seeded in 25 μl of matrigel and cultured with advanced DMEM/F12 containing 50 ng/ml of bFGF, 100 ng/ml of human EGF, 100 ng/ml noggin, 10% R-spondin1 conditioned medium, 50 nM of A8301 and 10 μM of Y27632. The indicated drugs were treated to the SPDTO #7 (**A**) or #10 (**B**). The left panels show the bright field images of SPDTO #7 and #10 with indicated treatments of cisplatin, etoposide, THZ1 or YPN-005. The right graphs show that the measurements of tumor organoid growth were performed by the Cell Titer-Glo® Luminescent Cell Viability Assay according to the manufacturer's instructions.



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Discussion

SCLC accounts for 10%–15% of lung cancers and leads to grow rapidly and metastasizes more easily. [1, 51, 52 53] Although most SCLC patients (60%–70%) are responsive to the current standard EP therapy, most patients recur quickly. [10, 54] The EP therapy has proven to be effective in controlling cancer cells, but it causes side effects that impact the quality of life. [1 55] Therefore, the development of other anticancer agents for SCLC patients are urgently needed.

CDKs are a family of serine-threonine kinases that regulate cell proliferation and control the cell cycle. [56-58] Thus, CDKs are considered highly validated anticancer drug targets. [59 60] Among these CDKs, especially CDK7 has recently concerned interest as an attractive target for anticancer target because of its dual functions as an essential component of the general transcription factor Transcription Factor II Human, and as a component of the CDK-activating kinase that is responsible for phosphorylating other CDKs on their stimulatory, T-loop sites. [61-63] Other papers have shown that the inhibition of CDK7 can be useful in the treatment of various cancers. [64-73] Previous studies have suggested that the CDK7 inhibitor THZ1 is a promising drug for SCLC. [74] Indeed, it has been demonstrated that THZ1 shows sensitivity in SCLC by targeting transcriptional addictions. However, THZ1 also inhibits the activities of several other kinases, including CDK12 and CDK13, and therefore may contribute to side effects. [73, 75]Although the THZ1-derived CDK7 inhibitor SY-1365 was developed by Syros Pharmaceuticals to improve on the potency, selectivity, and metabolic stability of THZ1, novel CDK7 inhibitors are still urgently required. [76]

Here we generated YPN-005, a novel CDK7 inhibitor that effectively inhibits SCLC and refractory SCLC with nanomolar potency in various cellular assays. Although YPN-005 completely inhibited CDK13 in the KINOMEscan Kinase Assay, the IC50 values of YPN-005 on CDK13 were over 1 μM. We demonstrate that the KINOMEscan Kinase assay can evaluate the binding affinity between drugs and kinases at indicated doses of drugs, while the KinaseProfiler assay evaluates the biding affinity between drugs and kinases in conditions including cyclins and ATP. Thus, we believe that YPN-005 does not indeed inhibit CDK13 in cellular level, is potent and selective on CDK7.

Our data shows clinical meaning as YPN-005 shows potent efficacy in refractory SCLC, including in cells with acquired resistance to cisplatin or etoposide, and EP-refractory SCLC organoids. Thus, we consider that YPN-005 may be successfully applied in clinical trials in patients with SCLC or refractory SCLC after EP therapy. As the clinical trials of YPN-005, the strategy of combined with EP chemotherapy and low dose of YPN-005 can be superior to monotherapy because many CDK7 inhibitors have issue of cytotoxicity. In addition, a recent report suggested that CDK7 inhibition potentiates genome instability, which triggers antitumor immunity in SCLC. [77].Although we will confirm whether YPN-005 treatment can induce genome instability, YPN-005 may also have potential as an immunotherapy-based combination treatment. We believe that YPN-005 is a potential candidate to enter clinical trials, and only well-controlled clinical trials will be able to answer these questions.

Several limitations of this study should be considered. Firstly, while YPN-005 shows potent anticancer efficacy, the mechanisms are currently unclear, although YPN-005 completely inhibits RNAPII activity in a similar manner to other CDK7 inhibitors. Further research is required to identify the specific mechanisms of the action involved. Secondly, YPN-005 is not effective in all cases of SCLC. Thus, more studies are needed to establish predictive biomarkers for drug response by using SCLC cells that are sensitive or resistant to YPN-005.

In conclusion, we developed the novel CDK7 inhibitor YPN-005 as an attractive strategy for efficiently suppressing SCLC cells. YPN-005 may be applicable for treating refractory SCLC after EP therapy.

Conclusion

In conclusion, our study demonstrates that YPN-005, the selective inhibitor of CDK7, shows cell growth inhibition both in vitro and in vivo, induces the cell apoptosis and inhibits cell.

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국문 요약

비소세포폐암(NSCLC)과는 달리, 소세포폐암은 수십 년 동안 이 질병에 대한 치료법 개발에 큰 진전이 없었다. 최근 면역요법을 비롯한 다양한 표적제들이 임상시험용 의약품으로 개발되고 있지만 SCLC 를 위한 새로운 치료제 개발이 필요하다. Cyclin dependent kinase 7 (CDK7)의 잠재적 억제제인 YPN-005 로 SCLC Cisplain 또는 Etoposide 내성 세포 및 SCLC 환자에서 파생된 Organoid 의 항암 효과를 조사했다. Kinase 분석 패널에서 YPN-005 는 CDK7 에 대해 매우 선별적이었으며 SCLC 뿐만 아니라 기존 항암제에 대한 후천적 내성을 가진 세포에서 항증강 효과를 보였다. 다른 CDK7 억제제와 일관되게 YPN-005 약물은는 RNA 중합효소 II 의 카복실 말단 도메인의 인산화를 상당히 감소시켰다. In-vitro 결과와 일관되게 YPN-005 약물은 RNA 중합효소 II 인산화 억제에도 불구하고 종양 성장의 상당한 억제 효과를 보였다. 마지막으로 YPN-005 는 다른 CDK7 억제제인 THZ1 에 비해 SCLC 환자로부터 파생된 Organoid 에서 강력한 항암 효과를 보였다. SCLC 에서 CDK7 의 Targeted therapy 는 임상 조사에 적합할 수 있으며, YPN-005 는 기존 치료에 대한 후천적인 내성을 가진 1 차 SCLC 및 SCLC 의 유망한 치료 옵션이 될 수 있음을 시사한다.

중심단어 : 소세포폐암(SCLC), CDK7, Targeted Therapy, Kinase inhibitor, SCLC-Clinical data