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The Cytotoxic Molecule Ansamitocin P-3 Suppresses Cell Proliferation and Tumor Growth in Lung Carcinoma

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Abstract

Lung cancer, mainly including small cell lung cancer (SCLC) and non-small lung cancer (non-SCLC, nSCLC), is a malignant and aggressive one and the leading cause of cancer-related death worldwide. Patients at late stages of lung cancers are usually treated with radiotherapy and chemotherapy. These traditional therapies displayed very limited benefits, with a poor five-year survival rate. The receptor-targeting therapy is becoming a new hot topic. Our previous studies demonstrated the receptor-targeting drug conjugates could enhance the anti-tumor efficacy of the free molecules via linking them to peptide vehicles. Presently, three chemical molecules camptothecin, AP-3 and colchicine were pre-tested for their cytotoxic activities in SCLC A549 cells and non-SCLC NCI-H69 cells. All these molecules displayed their potent effects on cell proliferation and cell apoptosis in both. Especially, AP-3 was extremely more potent than the other two. Our further in-vivo assay showed that AP-3 suppressed NCI-H69 tumor growth, but had a limited ability. A new strategy may be needed for AP-3 in SCLC treatment. Meanwhile, We found that somatostatin receptor type II (SSTR2) was highly expressed in SCLC cells, not non-SCLC cells. These findings may provide a golden opportunity to develop a SSTR2-targeting AP-3 somatostatin conjugate for SCLC treatments.

Keywords: Lung cancer; SCLC; non-SCLC; Chemical molecules; Ansamitocin P-3 (AP-3); Cell proliferation; Apoptosis; Tumor growth; Receptor targetingClinical; Manufacturing process; Quality; Stability

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Introduction

Lung cancer is a type of malignant and lethal cancers and the leading cause of cancer-related death worldwide. The incidence and mortality in male lung cancer patients are ranked top one in all malignant cancers [1,2]. A large amount of evidences document that lung cancers are tightly connected to their smoking histories. Long-term heavy smokers and early smokers have much higher incidence of lung cancers than non-smokers. Besides, other environmental factors such as air pollutants, dusts and carcinogens are also the causes of high incidence of lung cancers [3,4]. There are two major types of lung cancers including small cell lung cancer (SCLC) and non-small lung cancer (non-SCLC, nSCLC), accounting for approximately 15% and 80% of all cases, respectively [1,2,5]. In all non-SCLC cases, most of them belong to large cell lung cancer, squamous cell cancer and adenocarcinoma. According to internationally recognized TNM staging system (T: Tumor, N: Node, M: Metastasis), lung cancers were classified as four stages (I, II, III and IV). Lung cancers at stages I and II belong to early stage, with less symptoms. At stage III and IV, lung cancers become late stages, occurred with tumor metastasis [6-8]. Surgery is eligible when patients are at early stages while patients at late stages are usually treated with radiotherapy, chemotherapy or immunotherapy [4,5-9].

Although molecular mechanisms are not completely disclosed, scientists found that many genes such as VEGFR, VEGF, EGFR (HER1/ERBB1), HER2, and the associated signal pathways were involved in lung cancer progression [10-13]. Via targeting these genes and the signaling cascades, scientists have successful achievements in the fields of drug research and development. Nowadays, Food and Drug Administration (FDA) have been approved many drugs for the treatments of lung cancers. For instances, Lorlatinib (Pfizer) targets to ALK and ROS1, with Necitumumab (Lilly), Everolimus (Novartis), Bevacizumab (Roche) acting on the targets EGFR, mTOR and VEGFR, respectively [14-

17]. However, most of them are small molecules. Besides various side effects, patients could get limited benefits from most current treatments, with a poor five-year survival rate [18,19]. A more effective and specific therapeutic is needed.

Drug conjugating technology has been applied via linking small molecules to drug delivery vehicles in order to enhance the anti-cancer efficacy of these small molecules and reduce their side effects [20]. Monoclonal antibodies, unique proteins and short synthetic peptides are the generally used drug delivery vehicles [20-22]. Reportedly, certain membrane receptors are aberrantly expressed in lung cancers [23]. This provides a potential opportunity for developing a receptor targeting therapy. Our previous studies also demonstrated that small molecules could be delivered to cancer-specific sites via coupled to peptides that interacted with specific receptors expressed and appeared on cancer cell surfaces [24,25]. In the present study, we identified that somatostatin receptors (SSTRs) were highly expressed in SCLC cells and also identified that the chemical molecule ansamitocin P-3 (AP-3) was the potent anti-cancer cytotoxic agent compared to other tested ones. This provides a good chance to develop an AP-3 peptide conjugate to target SCLCs.

Materials and Methods

Materials

The three molecular compounds ansamitocin P-3 (AP-3), camptothecin and colchicine were purchased from MCE (MedChemExpress, NJ, USA), with their purity over 98%. The sequences of primers used were commercially synthesized as shown in **Table 1.**

Cell culture

Both SCLC NCI-H69 cell line and non-SCLC A549 cell line were purchased from BNCC (Beijing Nightingale Consultation of Culture, China). NCI-H69 cells were maintained in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS) and 1% penicilin/streptomycin, with A549 cells in F12 medium. All cells were cultured in a humidified incubator (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C in a 5% CO_2 atmosphere.

Cell proliferation assay

Cell viability assay was performed with the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. Briefly, cells were seeded in 96-well cell culture plates at a cell density of 8×10^3 cells/well, with different concentrations of compounds AP-3, colchicine and camptothecin added to each well, respectively. The content in wells were mixed well. The plates were incubated at 37°C for 72 h. Then 10 µL of CCK-8 solution was added and the plates were incubated for another 1-4 h. The OD values were measured at 450 nm by a microplate reader (BioTek, USA).

Table 1 The sequences of primers designed for qPCR analysis of SSTR genes.

Primer		5'---3'
SSTR1	F	GGAGCCGGTTGACTATTACG
	R	CAGGTTCTCAGGTTGGAAGTC
SSTR2	F	GCCGTACTATGACCTGACAAG
	R	TCTTCATCTTGGCATAGCGG
SSTR3	F	CCCTTCAGTCACCAACGTCT
	R	TGGTGAACTGGTTGATGCCA
SSTR4	F	GCATGGTCGCTATCCAGTG
	R	GCGAAGGATCACGAAGATGAC
SSTR5	F	TGTTTGCGGGATGTTGGCT
	R	CTGTTGGCGTAGGAGAGGA

Cell apoptosis analysis

Cells at a density of 2×10^5 cells/well were seeded into 6-well plates, and treated respectively with AP-3, colchicine and camptothecin at different concentrations for 48 h. Then cell apoptosis assay was carried out according to the manufacturer's protocol. Briefly, the treated cells were collected by centrifugation at 1500 rpm for 5 min. After washed twice with PBS, the cells were suspended in 100 µL binding buffer and were incubated with 5 µL FITC-AV and 5 µL PI for 15 min in the dark place at room temperature. Subsequently, an additional 400 µL binding buffer was added and analysis was performed by a CytoFLEX flow cytometer.

Cell cycle analysis

Cells at a density of 2×10^5 cells /well were cultured in 6-well plates and incubated with different concentrations of AP-3, colchicine and camptothecin for 24 h. After digested with 0.25% non-EDTA trypsin solution, the cell suspension was collected and centrifuged at 1500 rpm for 5 min. The cells were harvested, washed twice with PBS, fixed with 70% cold ethanol at -20°C for 24 h. After centrifuged at 4000 rpm for 2 min, the supernatants were removed, cells were suspended in 500 µL PBS with 0.25% Triton-X 100 and incubated for 15 min on ice. After centrifuged, the supernatant was discarded, each tube was added with 500 µL PBS containing 10 µg/mL RNase A and 20 µg/mL PI, and incubated in the dark place at room temperature for 30 min. Finally, the cell samples were placed in Falcon tubes and analyzed by a CytoFLEX flow cytometer.

Quantitative polymerase chain reaction (qPCR) assay

Cells were harvested and the total RNAs were extracted using HP Total RNA Kit (OMEGA). RNAs were reversely transcribed into complementary DNA (cDNA). Following cDNA synthesis, qPCR reactions were performed in triplicate for each of the individual samples using the SYBR Green (Genecopoeia, USA) detection method by a StepOne PCR machine (Bio-Rad). The sequences of the pair primers (SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5) are shown in Table 1. RNA expression was normalized with the internal control actin purchased from Genecopoeia. The relative RNA expression was calculated through the $2^{-\Delta\Delta Ct}$ method.

Xenograft mouse model

Female BALB/c nude mice aged 4-6 week-old were purchased and reared in SPF animal house for one week to adapt to the environment. SCLC NCI-H69 cells at a total amount of 5×10^6 cells/200 µl were inoculated into the right flank of each mouse

by subcutaneous injection. After tumors grown up with the tumor sizes reached 100-300 mm^3 (Tumor volume=0.5 × Length × $Width^2$), tumor-carrying mice were separated into two groups with 8 mice in each group, and given by tail vein injection with 200 µl of the tested compound AP-3 at the dose of 0.4 mg/kg in the experimental group, with 200 µl of normal saline given to each mouse in the control group. Mice were treated once a week for total four weeks. Tumor volumes were measured and bodyweights were weighted twice a week since the first administration.

Results

Our previous studies showed that the chemical molecules will usually lose part of its *in vitro* anti-cancer activities by cell-based assays after coupled to drug delivery vehicles. Therefore, to find the suitable molecules, we first selected certain cytotoxic molecule candidates and tested their possibility of serving for drug conjugates. Here, such molecular compounds as ansamitocin P-3 (AP-3), camptothecin and colchicine were evaluated for their anti-cancer efficacy in SCLC NCI-H69 cells and non-SCLC A549 cells.

The inhibitory effects of the cytotoxic molecules on cell growth

Three chosen cytotoxic molecules were evaluated for their inhibitory activity of lung cancer cell growth. They showed their potent activities against cell proliferation in both tested cell models as shown in **Figure 1**. The IC_{50} values for camptothecin were 49.30 ± 13.00 nM in A549 cells and 20.58 ± 5.20 nM in NCI-H69 cells. And the IC_{50} values for AP-3 were 0.33 ± 0.13 nM in A549 cells and 0.69 ± 0.04 nM in NCI-H69 cells, along with 25.63 ± 9.17 nM in A549 cells and 10.10 ± 2.11 nM in NCI-H69 cells for colchicine. Among them, Ap-3 demonstrated its much stronger potency in both SCLC and non-SCLC cell lines in comparison with the other two, displaying its possibility to be applied for drug conjugates.

The effects of the cytotoxic molecules on cell apoptosis

Three cytotoxic molecules were further investigated for their activity to induce cell apoptosis via flow cytometry analysis. They induced either early cell apoptosis or late cell apoptosis in both SCLC NCI-H69 cells and non-SCLC A549 cells in a dose-dependent manner. In non-SCLC A549 cells, AP-3 at the concentrations of 0, 0.4 and 1.6 nM increased the total apoptosis rates from 10.22 ± 1.90% (control) to 23.94 ± 2.58 % (0.4 nM) and 44.19 ± 3.17% (1.6 nM), respectively. And camptothecin at the concentration of 0, 50 and 200 nM increased the apoptosis rates from 10.22 ± 1.90% (control) to 16.53 ± 5.95% (50 nM) and 29.92 ± 4.88% (200 nM), with the apoptosis rates induced by colchicine at the concentrations of 0, 30 and 120 nM increased from 10.22 ± 1.90% (control) to 29.22 ± 6.64% (30 nM) and 51.76 ± 1.72% (120 nM), respectively **(Table 2)** and **(Figure 2).** In SCLC NCI-H69 cells, the similar results were observed in SCLC NCI-H69 cells. All three cytotoxic agents displayed their efficacy to induce cell apoptosis in a dose-dependent manner.

The effects of the cytotoxic molecules on cell cycle progression

Besides investigating the effects on cell growth and cell apoptosis, we further evaluated whether these molecules affected cell cycle progression and which phase to arrest cell cycles in both SCLC NCI-H69 cells and non-SCLC A549 cells. AP-3 mainly induced cell cycle arrest at G2/M phase of both tested cells. In non-SCLC A549 cells, AP-3 induced an increase at G2/M phase from 16.18 % ± 1.03% (control) to 53.34 ± 8.06% (0.4 nM) and 62.40 ± 4.97% (1.6 nM), respectively. Similar results were observed in SCLC NCI-H69 cells. AP-3 at the concentrations of 0, 0.25, 0.5 and 1.0 nM induced cell arrest at G2/M phase from 32.97 % ± 1.05% (control) to 58.48 ± 8.37% (0.25 nM), 77.34 ± 3.19% (0.5 nM) and 73.86 ± 5.52% (1.0 nM), respectively **(Table 3)** and **(Figure 3).** Colchicine was also found to arrest cell cycle at G2/M phase in both SCLC NCI-H69 cells and non-SCLC A549 cells, with the rates being from 16.18 % ± 1.03% (control) to 49.84 ± 4.89% (30 nM) and

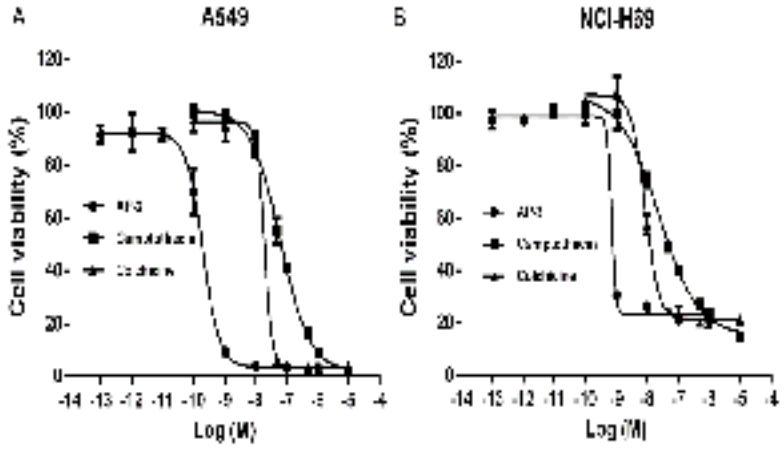

Figure 1 The cell viability assay. Both non-SCLC A549 cells and SCLC NCI-H69 cells were treated with AP-3, camptothecin and colchicine for 72 hours. The concentrations of AP-3 were set from10^{-6} M to 10^{-13} M. The concentrations of camptothecin and colchicine were set from 10^{-5} M to 10^{-10} M.

Table 2 The chemical molecules induced cell apoptosis in a dose-dependent manner.

Cell types	Compounds	Concentration (nM)	Apoptosis (%)		
			Early stage	Late stage	Total
A549	Control		5.74 ± 2.77	4.48 ± 0.88	10.22 ± 1.90
	AP-3	0.4	21.65 ± 2.47	2.29 ± 0.11	23.94 ± 2.58
		1.6	41.9 ± 3.39	2.29 ± 0.22	44.19 ± 3.17
	Camptothecin	50	11.08 ± 5.97	5.45 ± 0.01	16.53 ± 5.95
		200	24.85 ± 5.87	5.07 ± 0.99	29.92 ± 4.88
	Colchicine	30	26.75 ± 6.43	2.47 ± 0.21	29.22 ± 6.64
		120	48.25 ± 3.46	3.51 ± 1.75	51.76 ± 1.72
NCI-H69	Control		28.53 ± 3.30	3.97 ± 1.14	32.50 ± 2.89
	AP-3	0.25	34.70 ± 3.82	3.73 ± 0.86	38.43 ± 3.14
		0.5	41.93 ± 9.09	4.55 ± 0.35	46.49 ± 9.37
		1.0	42.20 ± 11.41	4.50 ± 0.59	46.70 ± 10.97
	Camptothecin	10	38.70 ± 4.07	4.39 ± 1.00	43.09 ± 3.32
		20	46.03 ± 7.86	5.42 ± 1.83	51.46 ± 6.07
		40	53.63 ± 5.38	5.46 ± 2.97	59.10 ± 3.99
	Colchicine	10	30.03 ± 2.01	3.47 ± 1.20	33.50 ± 2.20
		20	41.97 ± 14.18	4.03 ± 0.76	46.00 ± 13.71
		40	47.03 ± 14.09	3.13 ± 0.30	50.16 ± 14.22

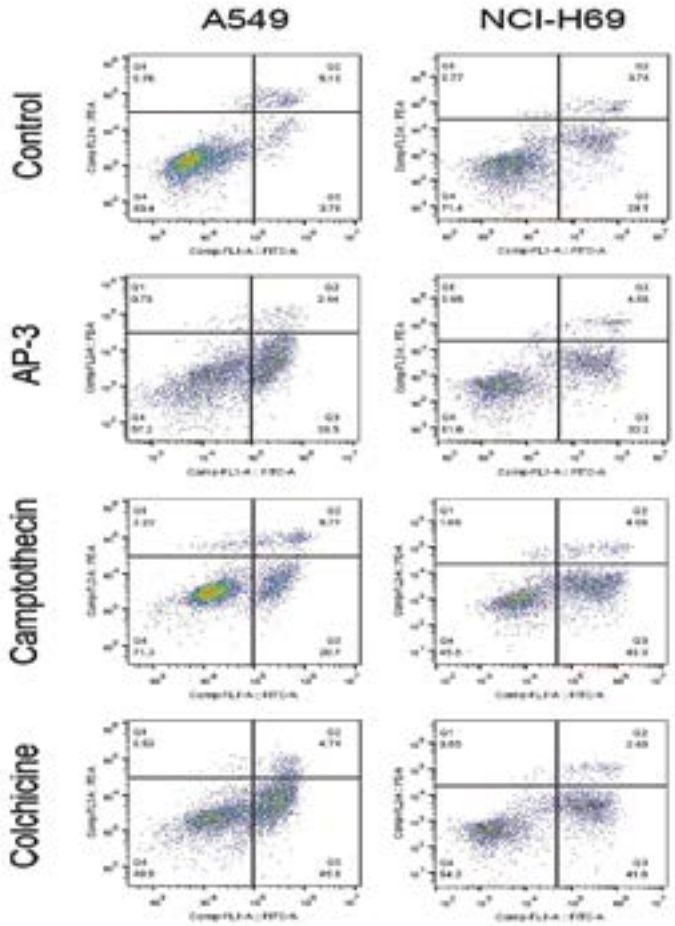

Figure 2 The molecule compounds AP-3, camptothecin and colchicine induced cell apoptosis in both tested non-SCLC A549 cells and SCLC NCI-H69 cells. Cells were treated with these compounds for 48 hours. Q1, Q2, Q3, and Q4 in each image represent dead cells, late apoptosis, early apoptosis and alive cells, respectively.

Table 3 The chemical molecules induced cell cycle arrest at different phases and in a dose-dependent manner.

Cell types	Compounds	Concentration (nM)	Different phase (%)		
			G0/G1	S	G2/M
A549	Control	0	52.91 ± 1.24	30.91 ± 2.28	16.18 ± 1.03
	AP-3	0.4	8.30 ± 1.61	38.37 ± 6.46	53.34 ± 8.06
		1.6	2.57 ± 1.00	35.04 ± 5.98	62.40 ± 4.97
	Camptothecin	50	21.28 ± 5.98	54.07 ± 2.03	24.66 ± 3.95
		200	6.82 ± 5.85	73.65 ± 4.20	19.53 ± 1.65
	Colchicine	30	30.48 ± 10.94	19.69 ± 6.05	49.84 ± 4.89
		120	12.97 ± 5.60	17.18 ± 9.82	69.86 ± 4.22
NCI-H69	Control	0	42.42 ± 2.82	24.62 ± 3.87	32.97 ± 1.05
	AP-3	0.25	20.28 ± 6.38	21.24 ± 1.99	58.48 ± 8.37
		0.5	9.14 ± 0.64	13.53 ± 2.55	77.34 ± 3.19
		1.0	8.37 ± 0.31	17.78 ± 5.84	73.86 ± 5.52
	Camptothecin	10	32.00 ± 1.77	39.34 ± 22.05	28.66 ± 23.82
		20	23.92 ± 0.16	48.61 ± 7.17	27.48 ± 7.00
		40	20.13 ± 1.04	54.67 ± 7.64	25.20 ± 8.68
	Colchicine	10	42.88 ± 6.63	21.18 ± 0.29	35.95 ± 6.34
		20	18.55 ± 3.13	14.75 ± 5.29	66.70 ± 8.41
		40	15.00 ± 3.97	12.18 ± 3.03	72.82 ± 0.95

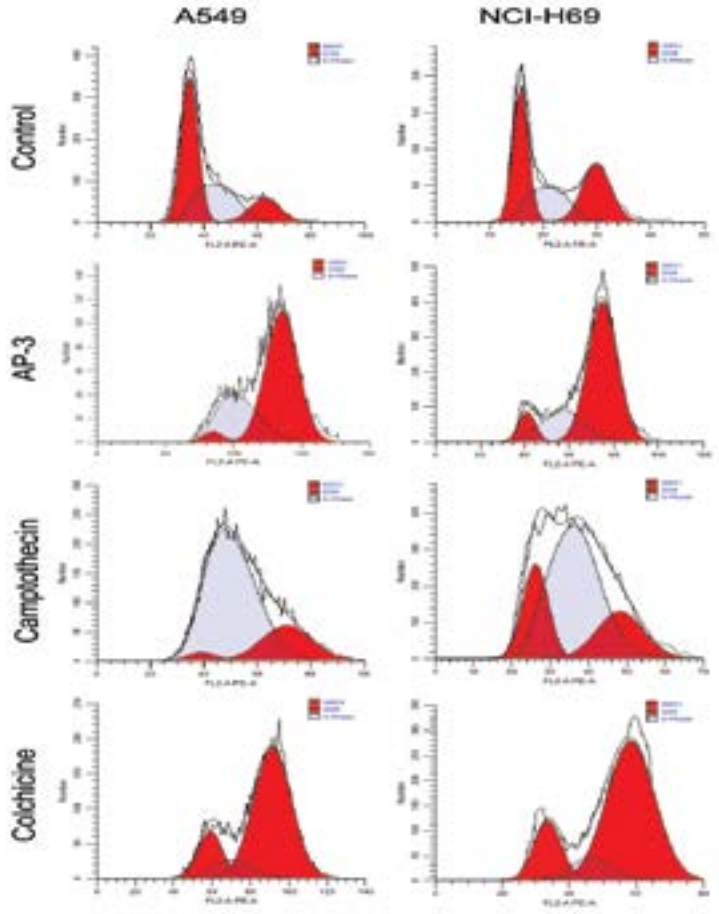

Figure 3 The molecule compounds AP-3, camptothecin and colchicine induced cell cycle arrests in both tested non-SCLC A549 cells and SCLC NCI-H69 cells. The two compounds AP-3 and colchicine arrested cell cycle at G2/M phases in A549 and NCI-H69 cells, but camptothecin did at S phases.

69.86 ± 4.22% (120 nM) in A549 cells, and from 32.97 % ± 1.05% (control) to 35.95 ± 6.34% (10 nM), 66.70 ± 8.41% (20 nM) and 72.82 ± 0.95% (40 nM) in NCI-H69 cells, respectively. However, different from other two, camptothecin mainly induced cell cycle arrest at S phase in both A549 cells and NCI-H69 cells and in a dose-dependent manner. In non-SCLC A549 cells, camptothecin arrested cell cycle at S phase from 30.91 % ± 2.28% (control) to 54.07 ± 2.03% (50 nM) and 73.65 ± 4.20% (200 nM), with the cell cycle arrest rates at S phase being from 24.62 % ± 3.87% (control) to 39.34 ± 22.05% (10 nM), 48.61 ± 7.17% (20 nM) and 54.67 ± 7.64% (40 nM) in SCLC NCI-H69 cells, respectively Table 3 and Figure 3.

The suppressive effects of the potent cytotoxic molecule AP-3 on tumor growth

The *in-vitro* assays above have demonstrated that AP-3 had extremely suppressive activity in lung cancer cells and was the most potent one among all three molecules. We further did *in-vivo* assay and investigated AP-3's anti-tumor efficacy in the xenograft mouse models. Tumors grown from SCLC NCI-H69 cells in nude mice were treated with AP-3 at the dose of 0.4 mg/kg and by tail vein injection. As shown in **Figure 4A**, the average tumor volumes in the control group increased from 126.93 ± 9.76 mm^3 (day 0) to 2030.71 ± 104.12 mm^3 (day 35), with an increased rate of 1499.87%. However, the average tumor volumes in the tested group decreased from 126.95 ± 10.37 mm^3 (day 0) to 1090.68 ± 100.33 mm^3 (day 35), with an increased rate of 759.14%, showing Ap-3 at a low dose resulted in a tumor growth suppression, with a suppressive rate of 49.38% in compared to the control. Meanwhile, we did not find obvious side effects from AP-3 treatment by monitoring bodyweights **(Figure 4B)** and observing mouse behaviors. Our further *in-vivo* anti-tumor assays showed that AP-3 resulted in severe side effects, even mouse deaths when we attempted to increase the dose to 1 mg/kg, indicating AP-3 itself has a small dose window that limits its anti-cancer drug ability. Thus, it is necessary to develop a new technological strategy for AP-3's clinical application.

The potential application of receptor-specific cytotoxic drug conjugates

The characteristics that certain membrane surface receptors are aberrantly expressed in many types of cancer tissues have been successfully applied for receptor-targeting cancer therapy [20,21]. Somatostatin (SST) receptors (SSTRs), particularly the subtype SSTR2, have been demonstrated highly expressed in lung cancers. We investigated the expression profiles of SSTRs in different lung cancer cells and identified that there were mainly SSTR1 and SSTR2 in these cells. The further comparison in both SCLC NCI-H69 cells and non-SCLC A549 cells showed that abundant SSTR2 particularly existed in SCLC NCI-H69 cells **(Figure 5).** This finding supported a new potential strategy to develop a SSTR2-targeting AP-3-SST conjugate for SCLC treatments. Actually, our preliminary data showed that the non-specific AP-3 after conjugated to SST displayed its SSTR2-targeting specificity and enhanced its anti-tumor efficacy while reducing side effects (data not shown).

Discussion

Lung cancer is one type of the aggressive and malignant human cancers. The SCLC and non-SCLC patients account for over 90 % of all lung cancer cases [1, 2, 9]. Moreover, the new cases were observed to quickly rise up in China and other developing countries while dropping down in the developed countries [2, 4, 26]. The precise molecular mechanism of lung cancers is not completely disclosed although various pathological factors and signalling pathways are reportedly associated with lung carcinogenesis and pathogenesis. The drugs currently commercial available for lung cancer treatments mostly are traditional chemical molecules that limited patients' benefits with a poor five-year survival rate and usually resulted in severe side effects [9, 17-19]. And it is more difficult to find a brand new chemical molecule that is likely druggable. Scientists from industries and academic are working

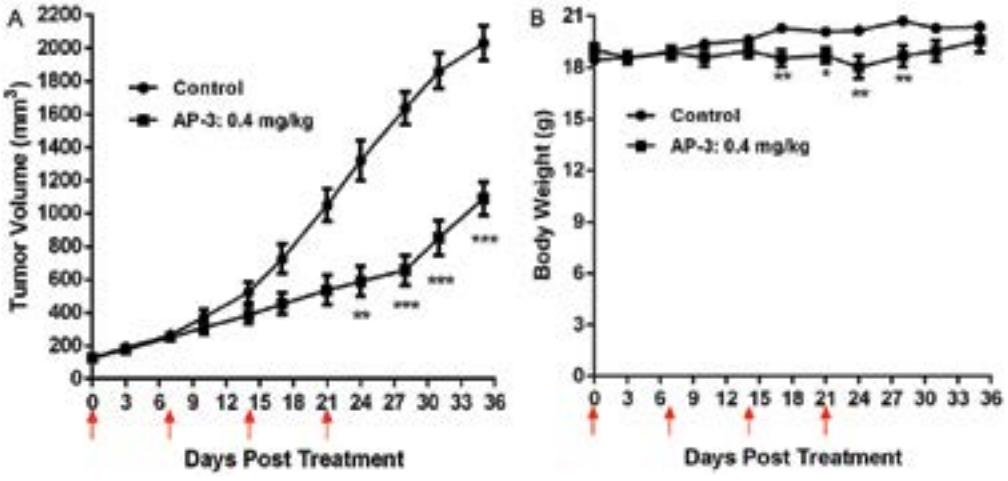

Figure 4 The suppressive effect of the cytotoxic molecule AP-3 on SCLC NCI-H69 tumor growth in nude mice. AP-3 was administrated by tail vein injection at a dose of 0.4 mg/kg and with a frequency of once a week for total four weeks (red arrows). A: tumor growth (Day 0: Control, 126.93 ± 9.76 mm^3, AP-3: 126.95 ± 10.37 mm^3. Day 35: Control, 2030.71 ± 104.12 mm^3, AP-3: 1090.68 ± 100.33 mm^3); B: bodyweight.

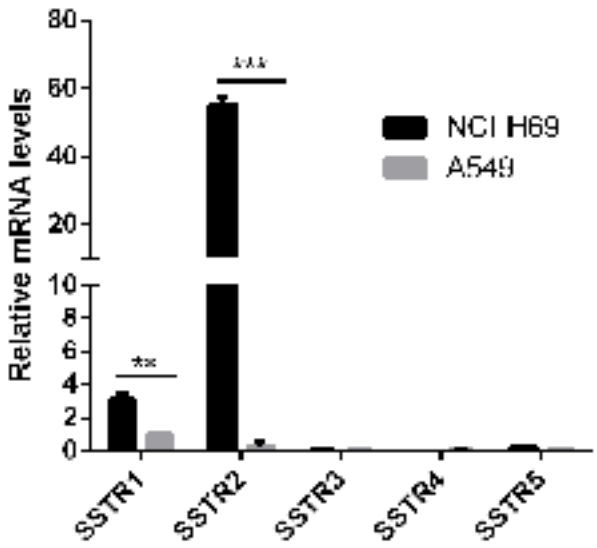

Figure 5 qPCR analysis showed the expression of the five SSTR subtypes SSTR1, SSTR2, SSTR4 and SSTR5 at the mRNA levels in both non-SCLC A549 cells and SCLC NCI-H69 cells.

on various effective strategies and attempt to promote efficacy of current drugs or to develop new technologies [27,28].

Receptor-targeting cancer therapy has been investigated for several decades and very recently, promising progress has been made in this field. Particularly, there are certain peptide ligands highly binding to their receptors that are abundantly expressed in cancers and appeared on cancer cell surfaces [20, 21]. These support that these peptides possibly serve as potential drug delivery vehicles to carry small molecules to receptor-specific cancer sites and to improve anti-cancer efficacy of these free molecules. Decades ago, Dr. Schally and his colleagues firstly developed the novel peptide-drug conjugates (PDCs) via linking small molecules to terminus of the suitable peptides. They conjugated the anti-cancer agents such as cisplatin, transbis, melphalan and chlorambucil (Chl), to the modified luteinizing hormone-releasing hormone (LH-RH) analogs. These PDCs displayed the enhanced anti-cancer cell and anti-tumor activities [29, 30]. Our previous studies also proved that our new PDCs could effectively increase anti-cancer ability of the small molecules and decrease their side effects [21, 22, 31, 32].

Presently, we pre-tested three small molecules camptothecin, AP-3 and colchicine for their cytotoxic activities. All these molecules displayed their potent effects on cell proliferation and cell apoptosis in both SCLC and non-SCLC cells. Especially, AP-3 was extremely more potent than the other two (Figure 1). However, AP-3 displayed limited anti-tumor activity (Figure 4). And in our other assays, we attempted to increase AP-3's doses in tumor treatments, but AP-3 at higher dose resulted in severe toxic side effects (data not shown). As for camptothecin, actually, there are two of its analogs, irinotecan and topotecan, that have been approved for clinical applications [33, 34]. However, both drugs have limited clinical applications due to their non-stability and severe side toxicity. Thus, a new technology is urgent need for many of such cytotoxic agents as AP-3 and camptothecin in order to improve their clinical applications and reduce side effects. Our previous studies have demonstrated that the new camptothecin peptide conjugates such as JF-10-81 had much better anti-tumor efficacy than the free molecule. And also in our ongoing projects, our preliminary data supported that the AP-3 peptide conjugates could enhance the effects of AP-3 on tumor growth while cutting off doses in use and reducing side effects (data not shown). Receptor-targeting cancer therapy is becoming a hot topic and is also believed to be a great opportunity for scientists to successfully dig out new drugs and for patients to get better benefits.

Acknowledgements

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References

1 Gazdar AF, Bunn PA, Minna JD (2017) Small-cell lung cancer: what we know, what we need to know and the path forward. Nat Rev Cancer 17: 725-737.

2 Wang S, Tang J, Sun T, Zheng X, Li J, et al. (2017) Survival changes in patients with small cell lung cancer and disparities between different sexes, socioeconomic statuses and ages. Sci Rep 7: 1339.

3 Rahal Z, Nemr SE, Sinjab A, Chami H, Tfayli A, et al. (2017) Smoking and Lung Cancer: A Geo-Regional Perspective. Front Oncol 7: 194.

4 Maghfoor I, Perry MC (2005) Lung cancer. Ann Saudi Med 25: 1-12.

5 Bunn Jr PA, Carney DN (1997) Overview of chemotherapy for small cell lung cancer. Semin Oncol 24: S 7-69-S 7-74.

6 Zhang YH, Lu Y, Lu H, Zhou YM (2020) Development of a Survival Prognostic Model for Non-small Cell Lung Cancer. Front Oncol 10: 362.

7 Marel M, Padr R, Fila L, Rakita D, Mendez FC, et al. (2020) Biopsy of lung lesions under CT control. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.

8 Peng Y, Sun Y (2020) Development and validation of nomograms for predicting overall and cancer-specific survival in young patients with non-small cell lung cancer. J Thorac Dis 12: 1404-1416.

9 Santabarbara G, Maione P, Rossi A, Palazzolo G, Gridelli C (2016) Novel immunotherapy in the treatment of advanced non-small cell lung cancer. Expert Rev Clin Pharmacol 9: 1571-1581.

10 Ricciardi GRR, Russo A, Franchina T, Ferraro G, Zanghì M, et al. (2014) NSCLC and HER 2: between lights and shadows. J Thorac Oncol 9: 1750-1762.

11 Marks JL, McLellan MD, Zakowski MF, Lash AE, Kasai Y, et al. (2007) Mutational analysis of EGFR and related signaling pathway genes in lung adenocarcinomas identifies a novel somatic kinase domain mutation in FGFR 4. PLoS One 2: 426.

12 Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, et al. (2008) PIK 3CA mutations and copy number gains in human lung cancers. Cancer Res 68: 6913-6921.

13 Desai A, Menon SP, Dy GK (2016) Alterations in genes other than EGFR/ALK/ROS 1 in non-small cell lung cancer: trials and treatment options. Cancer Biol Med 13: 77-86.

14 Blair HA (2021) Sotorasib: First Approval. Drugs.

15 Syed YY (2021) Amivantamab: First Approval. Drugs 81: 1349-1353.

16 Wang L, Sheng Z, Zhang J, Song J, Teng L, et al. (2021) Comparison of lorlatinib, alectinib and brigatinib in ALK inhibitor-naive/untreated ALK-positive advanced non-small-cell lung cancer: a systematic review and network meta-analysis. J Chemother pp: 1-10.

17 Sakai K, Yokote H, Murakami-Murofushi K, Tamura T, Saijo N, et al. (2007) Pertuzumab, a novel HER dimerization inhibitor, inhibits the growth of human lung cancer cells mediated by the HER 3 signaling pathway. Cancer Sci 98: 1498-1503.

18 Chen P, Kuang P, Wang L, Li W, Chen B, et al. (2020) Mechanisms of drugs-resistance in small cell lung cancer: DNA-related, RNA-related, apoptosis-related, drug accumulation and metabolism procedure. Transl Lung Cancer Res 9: 768-786.

19 Jafri NF, Ma PC, Maulik G, Salgia R (2003) Mechanisms of metastasis as related to receptor tyrosine kinases in small-cell lung cancer. J Environ Pathol Toxicol Oncol 22: 147-65.

20 Reubi JC (2003) Peptide receptors as molecular targets for cancer diagnosis and therapy. Endocr Rev 24: 389-427.

21 Sun LC, Coy DH (2011) Somatostatin receptor-targeted anti-cancer therapy. Curr Drug Deliv 8: 2-10.

22 Sun L, Fuselier JA, Coy DH (2004) Effects of camptothecin conjugated to a somatostatin analog vector on growth of tumor cell lines in culture and related tumors in rodents. Drug Deliv 11: 231-238.

23 Kaloudi A, Lymperis E, Kanellopoulos P, Waser B, de Jong M, et al. (2019) Localization of (99m)Tc-GRP Analogs in GRPR-Expressing Tumors: Effects of Peptide Length and Neprilysin Inhibition on Biological Responses. Pharmaceuticals 12.

24 Sun LC, Mackey VL, Luo J, Fuselier JA, Coy DH (2008) Targeted chemotherapy using a cytotoxic somatostatin conjugate to inhibit tumor growth and metastasis in nude mice. Clin Med Oncol 2: 491-499.

25 Sun L, Morris LM, Luo J, Mackey LV, Leslie JS, et al. (2011) Application of human pancreatic carcinoid BON cells for receptor-targeted drug development. J Drug Target 19: 666-674.

26 Ramalingam SS, Owonikoko TK, Khuri FR (2011) Lung cancer: New biological insights and recent therapeutic advances. CA Cancer J Clin 61: 91-112.

27 de Mello RA, Neves NM, Tadokoro H, Amaral GA, Castelo-Branco P, et al. (2020) New Target Therapies in Advanced Non-Small Cell Lung Cancer: A Review of the Literature and Future Perspectives. J Clin Med 9: 3543.

28 Serzan MT, Farid S, Liu SV (2020) Drugs in development for small cell lung cancer. J Thorac Dis 12: 6298-6307.

29 Bajusz S, Janaky T, Csernus VJ, Bokser L, Fekete M, et al. (1989) Highly potent analogues of luteinizing hormone-releasing hormone containing D-phenylalanine nitrogen mustard in position 6. Proc Natl Acad Sci U S A 86: 6318-6322.

30 Bajusz S, Janaky T, Csernus VJ, Bokser L, Fekete M, et al. (1989) Highly potent metallopeptide analogues of luteinizing hormone-releasing hormone. Proc Natl Acad Sci U S A 86: 6313-6317.

31 Moody TW, Sun LC, Mantey SA, Pradhan T, Mackey LV, et al. (2006) In-vitro and in-vivo antitumor effects of cytotoxic camptothecin-bombesin conjugates are mediated by specific interaction with cellular bombesin receptors. J Pharmacol Exp Ther 318: 1265-1272.

32 Zhu B, Sun L, Luo W, Li M, Coy DH, et al. (2017) Activated Notch signaling augments cell growth in hepatocellular carcinoma via up-regulating the nuclear receptor NR 4A 2. Oncotarget 8: 23289-23302.

33 Bailly C (2019) Irinotecan: 25 years of cancer treatment. Pharmacol Res 148: 104398.

34 Bailly C (2003) Homocamptothecins: potent topoisomerase I inhibitors and promising anticancer drugs. Crit Rev Oncol Hematol 45: 91-108.