Contents

Abstract

Development of novel cyclic form of fluorephore- or radiotracer-labeled c-Met peptide to detect high-c-Met expressing lung cancer

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Purpose:

c-Met is a tyrosine kinase receptor for hepatocyte growth factor and have roles in induction of cancer cell growth, reduction of apoptosis, angiogenesis and scatter. c-Met overexpression in non-small cell lung cancer is clinically important, because it causes poor prognosis and leads to acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitor drugs. L5-2 is a novel non-standard macrocyclic peptide which has high affinity to the external domain of the c-Met protein. The purpose of this study was to evaluate specific targeting to c-Met overexpressed lung cancer using FITC- or ^{68}Ga-labeled macrocyclic c-Met peptide for optical or

radionuclide cancer targeting study.

Methods:

L5-2, macrocyclic c-Met binding peptide was synthesized by the Random non-standard Peptide Integrated Discovery (RaPID) system. The c-Met peptide was conjugated with FITC or SCN-DOTA chelator. DOTA-L5-2 c-Met peptide was labeled with ^{68}Ga for 10 minutes (at pH 4.5) at 37 °C. Radiochemical purity was confirmed by radioTLC (0.1 M citric acid/ITLC-SG). Non-small cell lung cancer cell lines, NCI-H441 and NCI-H661 were chosen as c-Met positive and c-Met negative cell lines. Using RT-PCR assay and western blotting, total RNA and protein were prepared to measure c-Met expression level in these lung cancer cell lines. Specific binding of FITC-L5-2 c-Met peptide to cultured NCI-H441 and NCI-H661 cells was identified using high resolution confocal microscopy. *In vitro* binding assay was performed to analyze specific binding of ^{68}Ga-labeled L5-2 c-Met peptide.

Results:

RT-PCR and western blot analysis revealed that NCI-H441 lung cancer cell line, unlike c-Met negative NCI-H661 lung cancer cell line, highly expressed c-Met mRNA and protein. High fluorescence signals level in NCI-H441 was detected after 30 minutes of FITC-L5-2 c-Met peptide treatment. Specific binding of the FITC-L5-2 c-Met peptide to c-Met was found in NCI-H441 cell membrane. No fluorescence intensity was observed in NCI-H661 cells after treatment of FITC-L5-2 c-Met peptide. Radiochemical purity of ^{68}Ga-labeled DOTA-L5-2 c-Met peptide

was more than 90 %. *In vitro* radioligand binding assay exhibited that ^{68}Ga radioactivity was gradually increased when different concentration of ^{68}Ga-labeled DOTA-L5-2 c-Met peptide was treated in NCI-H441 cells. Approximately 3-fold higher radioactivity in NCI-H441 cells was found at 200 nM of ^{68}Ga-labeled DOTA-L5-2 c-Met peptide compared to that in NCI-H661 cells.

Conclusion:

The current *in vitro fluorescence and radioligand binding* study suggests that this novel non-standard macrocyclic peptide, L5-2, has a specific affinity to c-Met in lung cancer cells.. We expect that this successfully radiolabeled novel c-Met peptide will be used to detect the c-Met overexpressed lung cancer specifically *in vivo*.

Keywords: c-Met (hepatocyte growth factor receptor), Cancer targeting study, Radiolabeled macrocyclic c-Met peptide, Lung cancer diagnosis

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List of Figures

Introduction

c-Met is recognized as proto-oncogene encoding c-Met receptor tyrosine kinase. Hepatocyte growth factor (HGF) is generally known to regulate proliferation, motility, mitogenesis, and morphogenesis by stimulating HGF/c-Met signaling pathway. Dysregulation of HGF/c-Met signaling pathway has important roles in the pathophysiology of cancer such as proliferative, survival, invasive and metastatic abilities (1-3). c-Met and HGF is overexpressed in many types of solid tumors including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (1, 4-6). The activation and dysregulation in HGF/c-Met pathway has a clinical association of a poor prognosis in NSCLC, colorectal and gastric cancer (1, 7-10). Therefore, c-Met is regarded as a specific target for anticancer therapy and several small molecular c-Met inhibitors have been developed (11).

Several previous studies have explored *in vivo* cancer targeting study by directly monitoring c-Met expression using radiolabeled monoclonal antibody or small molecule (12-14). However, in the case of using targeting agent such as monoclonal antibody or small molecule, there are many limitations to be solved for diagnostic and therapeutic techniques. For example, monoclonal antibodies have slow clearance in bloodstream owing to relatively large molecular weight (more than 5,000 Da) and undesired immunogenicity (15). Also, small molecule-based *in vivo* targeting study has been challenged to overcome the reduced targeting

efficiency stemming from rapid renal excretion of 500 Da-sized small molecule drugs (16). Therefore, to increase targeting efficacy and minimize immunogenicity, peptide-based cancer targeting study has been developed as an alternative to both using small molecules and monoclonal antibodies, owing to substantial advantages such as high targeting efficiency and fast clearance in bloodstream (11, 17, 18). Despite its recent progress, peptides also have disadvantages such as biological instability and low affinity (17). To overcome this shortcoming, the macrocyclic form of peptide has been exploited for effective cancer targeting, showing excellent merits including high affinity, biological stability, structural diversity and functional versatility (19, 20). Recently, we demonstrated high affinity of a novel macrocyclic peptide named L5-2 to c-Met by *in vivo* FITC imaging study in gastric cancer mouse xenografts (unpublished). Non-standard peptide contains non-proteinogenic amino acids like L-amino acids containing non-canonical side chains, D-amino acids, N-methyl modification, and/or macrocyclic structure. Non-standard peptides are often synthesized by microorganism and a lot of these compounds are used as antibiotics (19).

Lung cancer is the most common cause of cancer death. In the inoperable advanced stage of lung cancer, patients are treated with systemic cytotoxic chemotherapy. The median survival rate of metastatic NSCLC is only about 12 months (21). Recently, molecular targeted therapies by molecular signature identification, such as epidermal growth factor receptor (EGFR) inhibitor, gefitinib

have been developed and have shown improvements in survival of lung cancer (22). Although EGFR targeted therapies initially show good response in EGFR mutant NSCLC, the most of them eventually become resistant to the targeted drugs. c-Met amplification has been recognized to be one of the mechanism of acquired resistance to EGFR inhibitors (23). So it is very important to develop therapeutic strategy to target c-Met.

Optical imaging with fluorophores is a relatively inexpensive tool for real-time detection of cancer and widely used in preclinical cancer research. Kim and colleagues have developed *in vivo* imaging of c-Met expression using Cy5.5-conjugated c-Met binding peptide (24). However, optical imaging using fluorophores have disadvantages for *in vivo* imaging, especially in clinical application, due to the limited tissue penetration (25). Therefore, fluorescence optical imaging has been only used for endoscopic, catheter-based and superficial imaging in clinical field (25). Furthermore, because most of the fluorophores have considerable toxicities, only two compounds such as indocyanine green and fluorescein were approved by the Food and Drug administration (FDA) (26). Radionuclide imaging modality can provide attractive information to overcome the fluorescence image-mediated limitation such as limitless depth of penetration and high sensitivity. Theragnostic radiotracer-based strategy capable of applying for diagnosis and treatment simultaneously, can be feasible by both γ and β particles emitting ^{177}Lu labeling to the peptide of interest (27). Peptide-receptor radionuclide

therapy (PRRT) for neuroendocrine tumors using ^{177}Lu labeled somatostatin analogues has already widely been used (28).

The purpose of this study was to evaluate optical or radionuclide-based cancer targeting of a novel non-standard macrocyclic c-Met peptide using FICT-labeled or ^{68}Ga-labeled macrocyclic c-Met peptide in lung cancer cells.

Materials and methods

Preparation of non-standard macrocyclic c-Met binding peptide

The Random non-standard Peptides Integrated Discovery (RaPID) System rapidly allows for synthesis of c-Met targeted non-standard macrocyclic peptide as follows: First, a non-standard macrocyclic peptide library was constructed from random mRNA library. Puromycin-linker was used to covalently link peptide to translated mRNA. After reverse transcription, peptide which has affinity to c-Met was isolated through affinity selection process. The enriched library was generated from cDNAs encoding the active peptides via polymerase chain reaction (PCR). Active peptide, L5-2 was synthesized from enriched library (19). FITC or tetraazacyclododecane-tetraacetic acid (DOTA) chelator was conjugated to synthesized peptide. Purity was confirmed by high-performance liquid chromatography (HPLC).

Preparation of ^{68}Ga-labeled DOTA-L5-2

^{68}Ga elution with 0.1 N HCl was prepared from ^{68}Ge/^{68}Ga-Generator (Cyclotron Co., Obninsk, Russia) for ^{68}Ga labeling of DOTA-L5-2. Eluted ^{68}Ga (185 MBq in 1 mL of 0.1 N HCl) was added to a mixture of DOTA-L5-2 (70 nM in 0.1 mL of water) and 1 M sodium carbonate buffer (pH 4.5, 50 μL). After incubation for 10 minutes at 37 °C, a small aliquot of the ^{68}Ga-DOTA-L5-2 solution was analyzed

radioactivity distributions on instant thin-layer chromatography silica gel (ITLC-SG, PALL, East Hills, NY, USA), at 0.1 M citric acid for determining radiochemical purity. A Bio-Scan AR-2000 System imaging scanner (Bio-Scan, Washington, DC, USA) was used to analyze RadioTLC plates.

Cell culture and reagent

NCI-H441 and NCI-H661 were selected as c-Met expressed or non-expressed human non-small cell lung cancer cell line. Both these cell lines were purchased from American Type Culture Collection (Rockville, MD, USA). Tumor cells were maintained in RPMI 1640 medium (Welgene, Korea) supplemented with 10 % fetal bovine serum (Invitrogen, NY, USA) and 1 % antibiotic/antimycotic (Life technologies, CA, USA) solution. Tumor cells were cultured at 37 °C and 5 % CO_2 condition. Tumor cells were harvested at 70~80 % confluence to passage the monolayers.

RT-PCR analysis

For total RNA isolation, Trizol agent (Invitrogen, Carlsberg, CA) was used according to manufacturer's instruction. The first-strand cDNA synthesis was done using Superscript II reverse transcriptase (Invitrogen, Carlsberg, CA) in accordance with manufacturer's manuscript. The PCR mix was composed as follows: 10X

PCR Buffer [200 mN Tris-HCl (pH 8.4), 500 mM KCl], 50 mM $MgCl_2$, 10 mM dNTP mix, hMET amplification forward primer (5‘-AAGACTCCTACACC CGAATACTGCCCAGA-3‘, 10 μM), hMET amplification reverse primer (5‘-AAAGAATATCGATGGCCTTTTAAAGGTCAG-3‘, 10 μM), *Taq* DNA polymerase (5 U/μL), cDNA from first-strand reaction and autoclaved distilled water. The optimal concentration of Mg^{++} was determined by repeatitive experiment with various concentraion of Mg^{++}. The reverse transcription (RT)-PCR was performed as following optimized protocol: 94 °C for 5 minutes, followed by 94 °C for 1minute, 60 °C for 0.5 minutes, 74 °C for 1 minute for 40 cycles. After amplication, 20 uL of the reaction mix was analyzed by 2 % agarose gel electrophoresis. PCR amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was also performed.

Western blot analysis

Lung cancer cells were harvested with a scraper and RIPA buffer. The isolated cell lysates were then centrifuged at 4 °C for 5 minutes. The supernatent was added to SDS sample buffer with 1:2 ratio and boiled for 5 minutes. This mixture was loaded in 4-12 % bis-Tris-HCl Gels (Invitrogen, Carlsbad, CA, USA). After transfering proteins to polyvinylidene difluoride (PVDF) membranes (Millipore, MA, USA) by electroblotting, the membrane was blocked with 5 % skim milk in TBS-T buffer. Rabbit polyclonal IgG antibody (c-Met, 1:200, Santa Cruz

Biotechnology, CA, USA) was treated for 24 hour at 4 °C. Secondary antibody (Goat Anti-Rabbit IgG-HRP 1:2000, Cell Signaling Technology, MA, USA) was treated at room temperature for 1 hour. Beta actin antibody (Sigma-Aldrich, MO, USA) was used as loading control. Enhanced chemiluminescence reagents (Roche, Mannheim, Germany) and LAS-3000 device (Fujifilm, Tokyo, Japan) were used to detect band pattern.

Fluorescence-based c-Met targeting study using FITC-c-Met peptide in lung cancer

For *in vitro* c-Met targeting study, NCI-H441 and NCI-H661 were harvested and seeded on the coverslip at 1.0×10^5 cells per coverslip with incubation at 37 °C overnight. 5 μM of FITC-L5-2 was added to the cells on each cover slip. The cover slips were incubated with different reaction time for 10 minutes or 30 minutes. After washing with phosphate buffered saline (PBS), 4 % paraformaldehyde (PFA, USB®, OH, USA) was added to each cover slip for 15 minutes at room temperature. For nuclear staining, the fixed cells were prepared with 4‘,6-diamidino-2-phenylindole (DAPI, Vector Laboratories, CA, USA). Fluorescence signals were imaged by high resolution confocal microscopy (Carl Zeiss, Oberkochen, Germany).

Radioligand binding study in vitro

1.0 x 10^5 NCI-H441 and NCI-H661 cells were seeded in 24 well plate with incubation at 37 °C overnight. ^{68}Ga-DOTA-L5-2 with serially diluted concentrations of 25 nM to 200 nM were added to wells in triplicate. After incubation at 37 °C for 1 hour, the treated cells were washed twice with cold Hank's balanced salt solution (HBSS) buffer (Gibco, Carlsbad, CA, USA). The treated cells were lysated by adding 0.5 mL of 1 % sodium dodecyl dsulphate (SDS) to each well. The radiocounts of the lysates were counted by gamma-scintillation counter (Packard, Meriden, CT) for 0.5 minutes. The lysates were analyzed using total amount of protein.

Results

Synthesis of FITC or DOTA labeled non-standard macrocyclic c-Met binding peptide

Non-standard macrocyclic peptide, L5-2 c-Met peptide potentially bind to c-Met was selected via the RaPID system. L5-2 c-Met peptide has a tyrosine residue which was L-form amino acid with thioether linkage to cysteine residue. FITC was conjugated with lysine residue in L5-2 c-Met peptide. DOTA-NCS has a macrocyclic structure containing 4 nitrogen atoms with 4 acetate chelating arms. DOTA-NCS chelator was also conjugated with lysine residue in L5-2 c-Met peptide and DOTA-conjugated c-Met peptide had sufficiently high purity more than 90 % analyzed by HPLC (Figure 1).

^{68}Ga Radiolabeling of DOTA-L5-2 with high radiochemichal purity

^{68}Ga labeling condition of DOTA-L5-2 c-Met peptide was shown in Figure 2. Labeling efficiency of DOTA-L5-2 c-Met peptide with ^{68}Ga was determined using ITLC-SG on 0.1 M citric acid solvent. The ^{68}Ga labeling efficiency of DOTA-L5-2 c-Met peptide was greater than 99 % when DOTA-L5-2 c-Met peptide was reacted with ^{68}Ga only for 10 minutes at 37 °C.

Differential c-Met expression in NSCLC cell lines

To confirm whether two chosen NSCLC cell lines express c-Met mRNA, RT-PCR was performed from NCI-H441 and NCI-H661 lung cancer. The RT-PCR results showed high level of mRNA expression of c-Met in NCI-H441. In contrast, no mRNA expression of c-Met was detected in NCI-H661 (Figure 3). To observe c-Met expression in protein level, western blot analysis was carried out in NCI-H441 and NCI-H661 cells. The result of western blot analysis in NCI-H441 showed highly expressed c-Met protein. There was no expression of c-Met protein in NCI-H661 cells (Figure 4).

Specific binding of L5-2 c-Met peptide to c-Met positive lung cancer cells

Specific binding of FITC-L5-2 c-Met peptide in NCI-H441 or NCI-H661 lung cancer was examined at different incubation time, 10 minutes and 30 minutes, after peptide treatment. The confocal microscopy results showed no discernible fluorescence signals in both NCI-H441 and NCI-H661 at 10 minutes after treatment of FITC-L5-2 c-Met peptide (Figure 5A). When FITC-L5-2 c-Met peptide was incubated for 30 minutes with each NSCLC cell line, there was a sufficient fluorescence signal level difference betwen two cell lines, showing highly specific targeting of c-Met peptide in the cytoplasmic area of NCI-H441 positve cell line. In contrast, any fluorescence signal was not found in NCI-H661, c-Met negative cell line (Figure 5B). The 3D slice images of NCI-H441 targeted with FITC-L5-2 showed specific intense fluorescence signals along with cell

membrane of NCI-H441 cells (Figure 5C).

In vitro radioligand binding assay

We further evaluated the targeting specificity of ^{68}Ga-labeled DOTA-c-Met peptide in different NSCLC cell lines, NCI-H441 and NCI-H661. After ^{68}Ga labeling of DOTA-c-Met peptide, ^{68}Ga-labeled DOTA-c-Met peptide was treated into the two lung cancer cell lines on dose dependent manner. The radioactivity measured by gamma counter showed gradual increase in ^{68}Ga radioactivity with increasing concentration of ^{68}Ga-labeled DOTA-c-Met peptide in NCI-H441 cells. Three-fold higher radioactivity in NCI-H441 cells at 200 nM was found, compared to radioactivity in NCI-H661 cells (Figure 6).

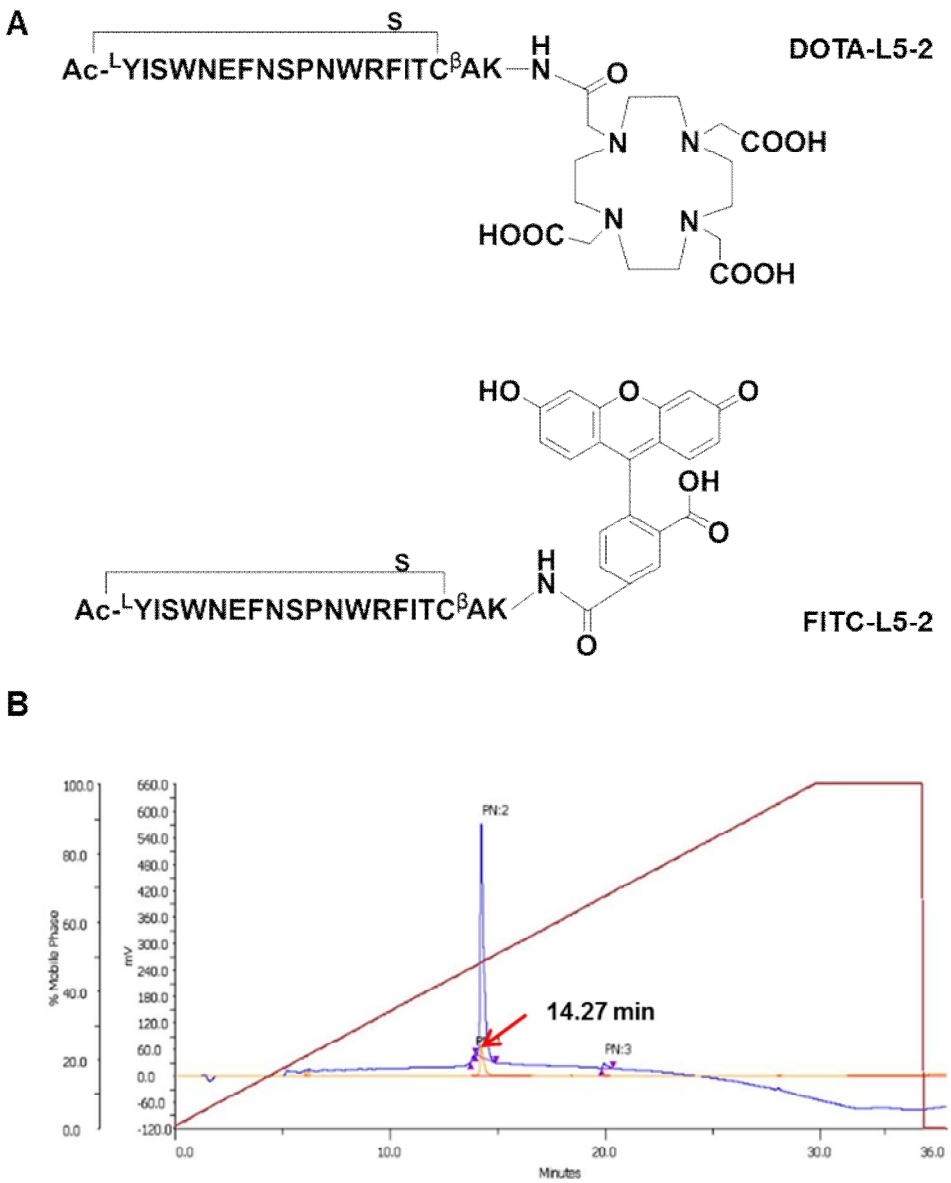


Figure 1. Schematic design of DOTA or FITC labeled L5-2, c-Met binding peptide

L5-2 macrocyclic peptide with high affinity to c-Met was synthesized by the RaPID system. The conjugated product FITC-L5-2 and DOTA-L5-2 were synthesized (A). The conjugated products were purified by HPLC, more than 97 % of purity at DOTA-L5-2 (B).

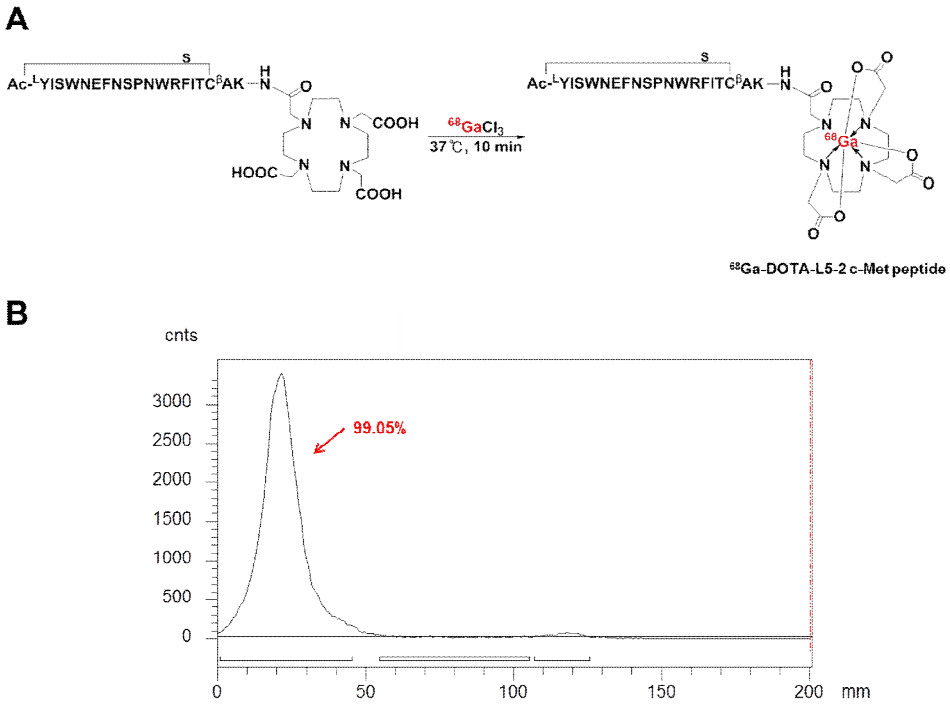


Figure 2. ^{68}Ga Labeling of DOTA-L5-2

^{68}Ga Labeling of DOTA-L5-2 was done by 10 minute incubation at 37 °C at pH 4.5. The radiochemical purity of ^{68}Ga labeled DOTA-L5-2 was 99.05 %, which was checked by ITLC-SG with 0.1 M citric acid.

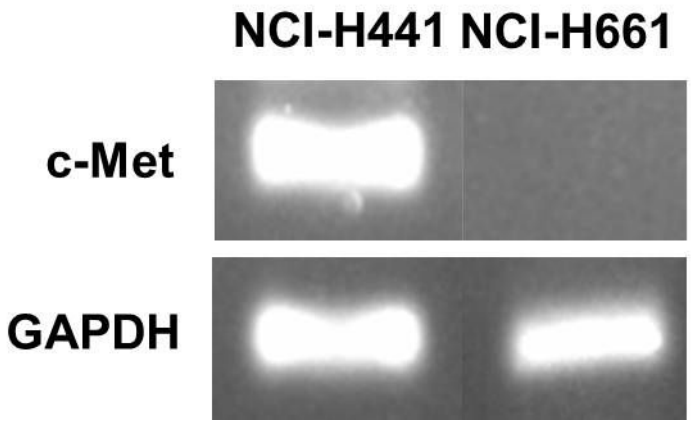


Figure 3. RT-PCR analysis in two NSCLC cell lines, NCI-H441 and NCI-H661

RT-PCR result showed highly expressed c-Met mRNA in NCI-H441, otherwise, unremarkable in NCI-H661.

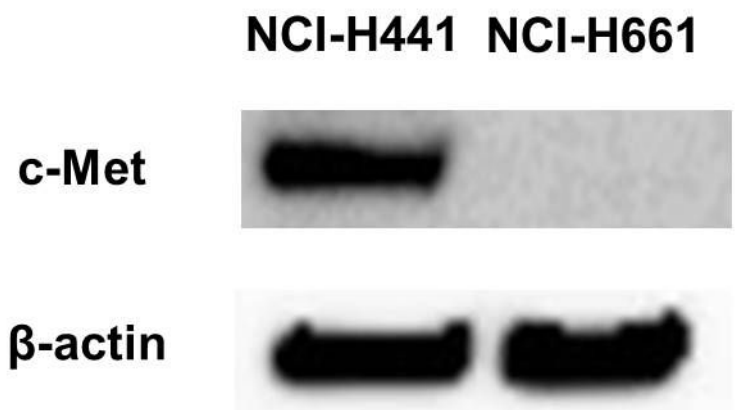
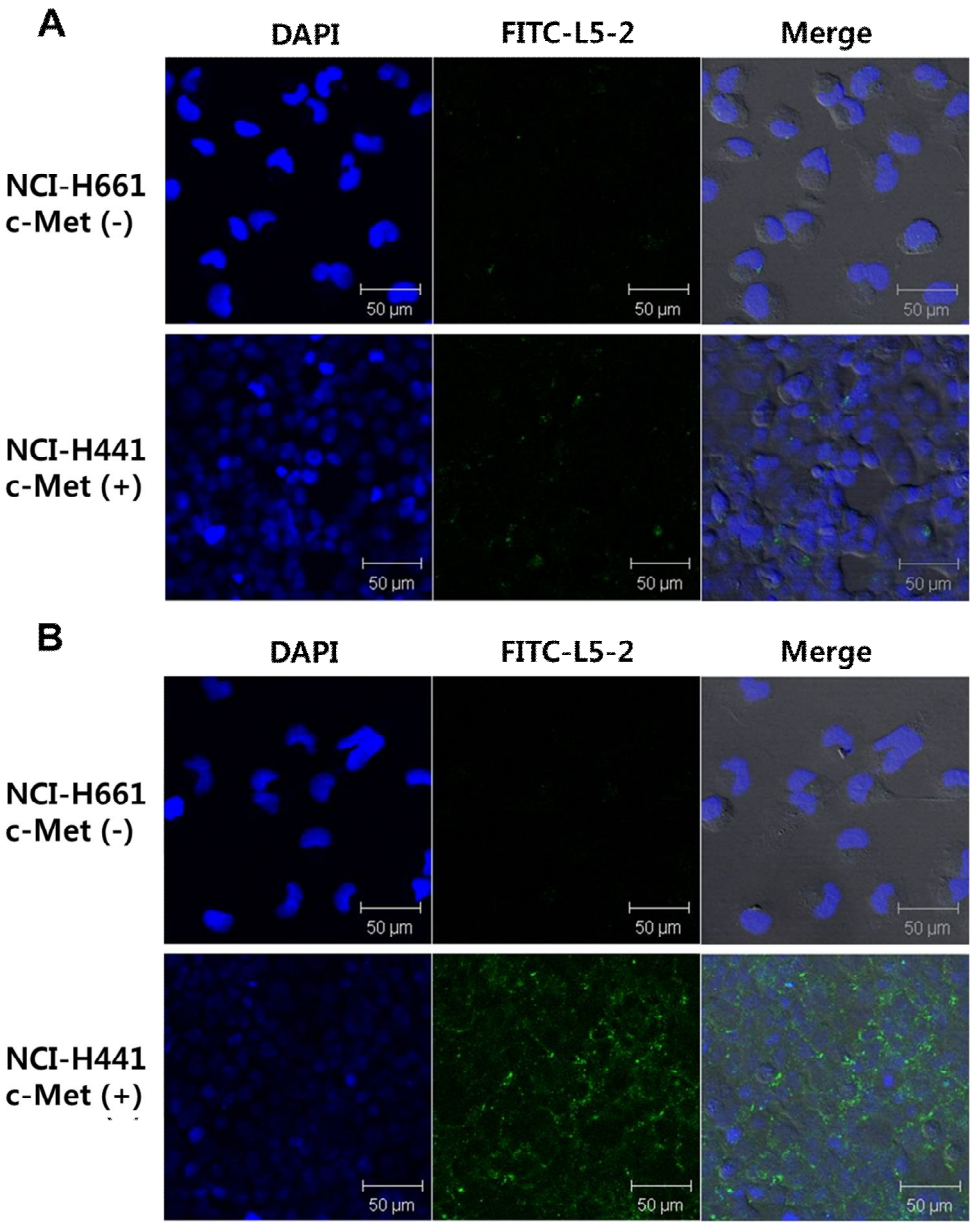


Figure 4. Western blot analysis in two NSCLC cell lines, NCI-H441 and NCI-H661

Western blot analysis demonstrated high level expression of c-Met protein in NCI-H441. In contrast, NCI-H661 did not express detectable level of c-Met protein.


A
DAPI
FITC-L5-2
Merge
NCI-H661
c-Met (-)
NCI-H441
c-Met (+)
50 μm
B
DAPI
FITC-L5-2
Merge
NCI-H661
c-Met (-)
NCI-H441
c-Met (+)
50 μm

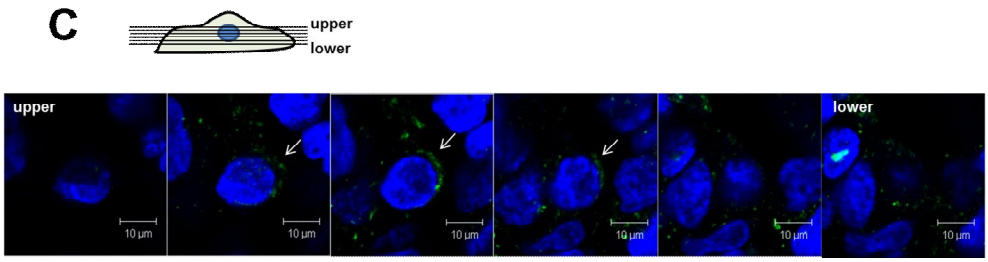


Figure 5. c-Met targeting study using FITC-L5-2 in NCI-H441 and NCI-H661 cell lines

Confocal microscopic fluorescent images were obtained after 5 μM of FITC-L5-2 treatment to NCI-H441 and NCI-H661 cell lines with two different treatment durations. (A) There were no significant binding signal in both NCI-H441 and NCI-H661 cell lines with 10 minutes of treatment duration. (B) NCI-H441 showed significantly higher binding signals of FITC-L5-2 than NCI-H661 with 30 minutes of treatment duration. (C) Serially sliced images revealed specific binding of FITC-L5-2 to cell membrane of c-Met overexpressed cancer cells.

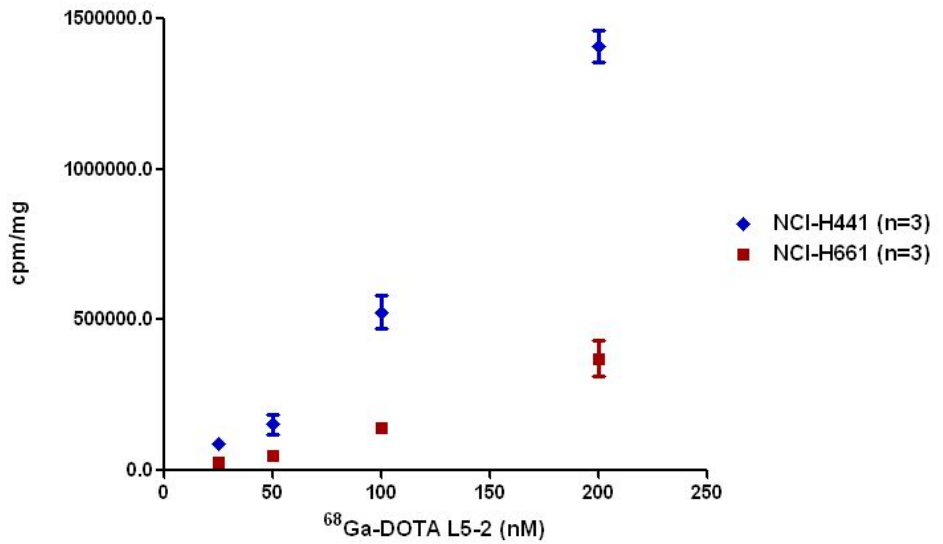


Figure 6. *In vitro* radioligand binding assay, ^{68}Ga-DOTA-L5-2

^{68}Ga-DOTA-L5-2 was treated equally to both NCI-H441 and NCI-H661 cell lines. Higher radioactivity was observed in NCI-H441 than NCI-H661.

Discussion

Peptides have many advantages compared to monoclonal antibodies as targeting molecules, which are rapid clearance, fast tissue penetration, high tumor uptake, and low immunogenicity mainly because of their low molecular weight. Peptides also have merits compared to small molecules in terms of affinity and biosafety. However, most peptides have metabolic instability due to rapid degradation by circulating proteolytic enzymes (16, 17).

Suga and colleagues developed a c-Met targeted non-standard macrocyclic peptide, L5-2 by the RaPID system. The RaPID system is composed of two *in vitro* systems including ribosomal synthesis of a wide variety of non-standard peptides called as the Flexible In-vitro Translation (FIT) system, and puromycin linker-based modified mRNA display system for identifying bioactive non-standard peptides. In the FIT system, genetic code reprogramming is enabled via making vacant codons in the genetic code and reassigning the non-proteinogenic amino acids with the non-proteinogenic aminoacyl-tRNAs by flexizymes. The Cys residue spontaneously reacts with the N-terminal chloroacetyl-amino acid to generate a macrocyclic thioether bond of L5-2 (19). By macrocyclization of peptides, membrane permeability of peptides is improved through elimination of the charged termini and facilitation of intramolecular hydrogen binding (29-31). Cyclization also provides advantages in conjugation with chelator and/or

radioisotope by restricting conformational mobility to reduce risk of a significant loss of its biological activity. A spacer can be positioned between the active site of peptide and imaging moiety to maintain biological activity of peptide. Furthermore, an imaging moiety can be conjugated to a specific site of peptide not to interfere binding to receptor (32).

It is important to select definite c-Met positive and negative tumor cell lines for verifying newly designed c-Met peptide. NCI-H441 is a widely used papillary lung adenocarcinoma cell line which highly expresses c-Met with constitutive phosphorylation of the c-Met receptor tyrosine kinase, and we checked c-Met expression level via RT-PCR and western blotting (33). Furthermore, selection of negative cell line as a control should be done carefully, because c-Met protein is expressed in a large proportion of the available cancer cell lines. In the present study, we confirmed c-Met negative cell line by checking c-Met expression level by RT-PCR and western blotting analysis following screening of a c-Met negative lung cancer cell line.

After 30 minutes of FITC-L5-2 treatment to the c-Met overexpressed lung cancer cells, specific fluorescence signals were observed and the signals were mostly found along the cell membrane. In contrast, there was no discernible fluorescence signal in c-Met negative lung cancer cells. Besides, no significant fluorescence signal was observed after 10 minutes of L5-2 treatment even in c-Met overexpressed lung cancer cells. These results indicated that binding of L5-2 to c-

Met is not an immediate process. Thus, the macrocyclic form of peptides may be a more suitable for *in vivo* imaging than linear form, because cyclization increases the biological stability by lowering structural flexibility (34). In fact, we observed specific binding of FITC-L5-2 in gastric cancer mouse xenografts after 24 hours of the c-Met peptide injection, in the recent past (unpublished). However, fluorescence *in vivo* imaging have critical limitations in clinical translation, such as low tissue penetration and high background signals. In contrast, radioligand *in vivo* imaging has good characteristics for clinical application, which are high tissue penetration, relatively short time resolution and high sensitivity.

In this study, we established ^{68}Ga labeling condition of DOTA-L5-2 at 37 °C for 10 minutes with high purity. ^{68}Ga ($t_{1/2}$ = 67.71 minutes, 89 % β^+ branching) has favorable nuclear decay parameters allowing PET imaging. ^{68}Ga elution process is available from ^{68}Ge/^{68}Ga generator and thus independent from cyclotrons. ^{68}Ge/^{68}Ga generator system has many merits, including inexpensive cost, ease of handling and possibility of multiple elution in a day. (35). Brief ^{68}Ga labeling method with high purity is a very attractive advantage considering possibility of clinical application. *In vitro* radioligand binding assay revealed higher binding of ^{68}Ga-DOTA-L5-2 to c-Met overexpressed lung cancer cell line than c-Met negative lung cancer cell line. However, further *in vitro* radioligand binding assay is needed to obtain saturation curve for calculating Kd value *in vitro*. This *in vitro* study was a preliminary study for *in vivo* imaging using ^{68}Ga-labeled macrocyclic c-Met

peptide in lung cancer. Further positron emission tomography (PET) imaging study using ^{68}Ga-DOTA-L5-2 is warranted to identify affinity of the peptide *in vivo*.

In vivo imaging of c-Met overexpressed tumor using ^{68}Ga-DOTA-L5-2 is clinically important because it may be helpful for treatment strategy using ^{177}Lu-DOTA-L5-2 via pretreatment target localization, radiation delivery and treatment evaluation. Because of its demonstrated roles in cancer proliferation and metastasis, c-Met has been regarded as a suitable target for cancer therapy. Furthermore, c-Met is responsible for acquired resistance to EGFR-tyrosine kinase inhibitor (TKI) approximately 10-20 %. So far, number of therapeutic agents including small molecule inhibitors and monoclonal antibodies against c-Met receptor and HGF, have been developed, but still most of these drugs yet have limited clinical benefits (36). Targeted radionuclide therapy is another option of targeted systemic treatment. Especially, PRRT is being commonly used tool for treatment of neuroendocrine tumor and it has several benefits over conventional chemotherapy, radiotherapy and monoclonal antibody therapy. For example, PRRT enables specific delivery of lethal dose of radiolabeled peptides to target tumor cells and emission of therapeutic radiation like beta-particles from radiolabeled peptides result in a crossfire effect that impinges on both targeted and non-targeted neighboring tumor cells (37). DOTA is a universal chelating agent for metals such as ^{68}Ga, ^{90}Y and ^{177}Lu (38). Feasible ^{177}Lu labeling process of DOTA-L5-2 for following study to evaluate efficacy of ^{177}Lu-DOTA-L5-2, also have been established by our group

(unpublished).

Conclusion

L5-2 is a novel promising candidate of non-standard macrocyclic peptide for c-Met targeting. In this *in vitro* fluorescent and radioligand binding study, we demonstrated a specific affinity of L5-2 to c-Met in lung cancer. Further *in vivo* c-Met imaging study using ^{68}Ga-DOTA-L5-2 is needed in lung cancer mouse xenografts.

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요약 (국문초록)

c-Met 과발현 폐암종을 표적하는 새로운 형광 또는 방사성 추적자 표지 원형구조 펩티드의 개발

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목적:

c-Met은 간세포성장인자 수용체로서, 티로신 키나아제 수용체의 일종이며, 암세포의 세포 성장, 세포 자연사 줄임, 신생혈관생성 및 산포 등에 관여한다. 임상적으로 비소세포성 폐암종에서 c-Met의 과발현은 중요하다. c-Met이 과발현한 폐암종에서는 예후가 좋지 않으며, 표적치료제인 상피 성장 인자 수용체 티로신 키나아제 억제제에 대한 저항성 획득의 한 기작으로 여겨진다. L5-2는 c-Met 단백질의 외부 구역에 높은 친화도를 가지는 거대원형구조 펩티드이다. 이번 연구는 FITC 또는 ^{68}Ga 표지 거대원형구조 c-Met 펩티드를 이용하여 c-Met 과발현 폐암종을 특이적으로 표적함을 평가하는 광학적 또는 방사성 암 표적 연구이다.

방법:

L5-2 거대원형구조 c-Met 펩티드는 무작위성 비표준 펩티드 통합 발견 (RaPID) 방식으로 합성하였고, FITC 또는 SCN-DOTA 킬레이터와 연결하였다. DOTA 표지 c-Met 펩티드의 ^{68}Ga 표지는 pH 4.5 및 37 ° C에서 10분간 진행하였다. ^{68}Ga 표지 c-Met 펩티드의 방사화학적 순도는 방사성 얇은층 크로마토그래피 (0.1 M 시트릭 산/인스턴트 얇은층 크로마토그래피-실리카겔)로 확인하였다. c-Met 발현 및 c-Met 비발현 세포주로 비소세포성 폐암종인 NCI-H441과 NCI-H661를 선정하였고, 역전사 중합효소연쇄반응 및 단백질 발현 분석법을 이용하여 c-Met 발현정도를 평가하였다. NCI-H441과 NCI-H661 세포들에 대한 FITC 표지 c-Met 펩티드의 c-Met에 대한 특이적 결합을 고해상도 공초점 현미경으로 확인하였다. ^{68}Ga 표지 c-Met 펩티드의 c-Met 특이 결합을 확인하기 위해 체외 결합 실험을 수행하였다.

결과:

역전사 중합효소연쇄반응 과 단백질 발현 분석 결과, NCI-H441 세포주에서 c-Met mRNA와 단백질 발현 정도가 높았으며, NCI-H661 세포주에서는 c-Met mRNA와 단백질이 발현되지 않았다. FITC 표지 c-Met 펩티드를 NCI-H441 세포들에 처리한 30분 후에 특이적 결합을 나타내는 형광이 공초점 현미경으로 관찰되었으며, NCI-H441 세포들의 세포막을 따라서 특이적으로 결합함을 확인하였다. 반면, NCI-H661 세포들에서는 FITC-c-Met 펩티드의 특이적 결합을 나타내는 형광이 관찰되지 않았다. DOTA-c-Met 펩티드에 ^{68}Ga의 표지를 진행하였고, 방사화학적 순도는 90 % 이상으로 확인되었다. 체내 방사성 추적자 결합 실험에서, NCI-H441에 결합한 ^{68}Ga-DOTA-c-Met 펩티드의 방사능은 처리해 준 펩티드의 농도에 따라 점차적으로 증가하였으며,

200 nM을 처리시, NCI-H441 세포들에 결합한 방사능이 NCI-H661 세포들에 결합한 방사능보다 약 3배 가량 높았다.

결론:

이번 연구에서 형광 물질 또는 방사성 추적자를 표지 한 새로운 c-Met 펩티드가 c-Met을 발현하는 폐암종에서 특이적으로 결합함을 체외 실험을 통해 확인하였다. 이러한 결과들을 통해, 추후 진행할 체내 영상 실험에서도 c-Met 발현 폐암종에 대해서 특이적으로 표적 할 수 있을 것으로 기대한다.

keywords : c-Met (간세포 성장인자 수용체), 암 표적 연구, 방사성 추적자 표지 거대원형구조 펩티드, 폐암 진단
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