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研究課題名（和文）New strategy of diagnostic imaging probes prior to administrating molecular target drugs

研究課題名（英文）New strategy of diagnostic imaging probes prior to administrating molecular target drugs

研究代表者
Ｅｆｆｅｎｄｉ　Ｎｕｒｍａｙａ（Effendi, Nurmaya）

金沢大学・新学術創成研究機構・研究協力員

研究者番号：６０８４２９１１

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研究成果の概要（和文）：PDGFRβイメージングプローブとしての7つの化合物を合成、インビトロとインビボで評価した。その結果、[67Ga]Ga-DOTA-EG2-IPLPPPRRPFFKと[67Ga]Ga-DOTA-EG4-IPLPPPRRPFFKは、優れた結果を示した。L858R/T790M変異NSCLCのイメージングのために、125Iと77Br標識化合物を合成した。インビトロとインビボで評価した結果、腫瘍への蓄積は、血液や筋肉よりも高かった。変異を伴うEFGRを可視化するために、酵素で切断可能なリンカーを含む新規プローブペプチド-オシメルチニブ結合化合物を現在合成、評価中である。

研究成果の学術的意義や社会的意義
Overexpression of PDGFRβ and EGFR as the subfamilies of TKRs associated with various human cancer. Imaging agents targeting PDGFRβ and mutated-EGFR can help determine their expression level via imaging to optimize and evaluate the effectiveness of treatment outcomes.

研究成果の概要（英文）：Tyrosine kinase receptors (TKRs) are transmembrane proteins consisting of the extracellular domain for ligand binding and the intracellular part for signaling. PDGFRβ and EGFR are the subfamilies of RTKs. Seven radiotracers, 67Ga-DOTA-linker-IPLPPPRRPFFK peptides, were synthesized. Their feasibility as PDGFRb imaging agents were evaluated in vitro and in vivo. [67Ga]Ga-DOTA-EG2-IPLPPPRRPFFK and [67Ga]Ga-DOTA-EG4-IPLPPPRRPFFK showed promising result than others. Two radiohalogenated Osimertinib, 125I-Osimertinib & 77Br-Osimertinib, were synthesized. Their feasibilities as imaging agents of NSCLC with L858R/T790M mutation were evaluated in vitro and in vivo. Their accumulation in tumor was higher than in blood and muscle. Due to high lung uptake than tumor, the probe's structure needs to be modified. To visualize EFGR with mutation, a novel probe peptide-osimertinib-conjugated compound containing an enzyme-cleavable linker is currently being synthesized and evaluated.

研究分野：Radiological sciences-related

キーワード：Imaging　Osimertinib　Peptide　EGFR　PDGFRb　TKI　NSCLC

１．研究開始当初の背景

Tyrosine kinase receptors (TKRs) are transmembrane proteins consisting of the extracellular domain for ligand binding and the intracellular part for signaling. Platelet-derived growth factor receptor β (PDGFRβ) and Epidermal growth factor receptor (EGFR) are the subfamilies of RTKs.

The overexpression of PDGFRβ has been associated with tumor progression features such as cell migration, metastasis, angiogenesis, and proliferation (Ostman et al., Adv. Cancer Res. 2007). Therefore, PDGFRβ is one of the molecular targets for diagnosis and therapy in oncology. Previously, we explored radioiodinated and radiobrominated quinoline derivatives as probes targeting the ATP binding site of PDGFRβ (Effendi et al., Bioorganic Med. Chem, 2017; Effendi et al., Sci Rep, 2018; Effendi et al., Bioorganic Med. Chem, 2019). These radiolabeled probes found a high affinity for PDGFRβ and sufficient stability; however, their tumor accumulations were insufficient as tumor imaging agents. Therefore other potential PDGFRβ-targeted radiopharmaceuticals were required. Peptides are attractive carriers in an attempt to visualize the molecular target due to their chemical and biological properties. Due to the small molecular sizes of peptides compared to those of antibodies and antibody fragments, peptides can be synthesized and modified easily, have high transitivity into the target tissue, show fast blood clearance, and possess less immunogenicity. Askoxylakis et al. identified linear dodecapeptide IPLPPPSRPFFK (PDGFR-P1) targeting PDGFRβ by the biopanning technology with a high affinity for PDGFRβ (IC50 = 0.48 μM). The chelating agent for trapping metal radionuclides placed too close to the pharmacophore may decrease the binding affinity of the peptides to the target molecules. An appropriate spacer insertion between the chelator and the pharmacophore could improve its binding affinity. The linker insertion can affect the in vitro and in vivo peptide properties of the peptide toward the molecular target and their pharmacokinetics. Therefore the influence of linker in peptide's affinity to PDGFRβ needs to be studied to explore other potential PDGFRβ-targeted radiopharmaceuticals.

Epidermal growth factor receptor (EGFR), a transmembrane RTK, is overexpressed in various human cancers. EGFR upregulation has been considered a potential biomarker for predicting the response of tumor to therapy in non-small cell lung cancer (NSCLC) (Salomon et al., Crit Rev Oncol Hematol 1995). NSCLC is the most prevalent type of lung cancer that affects approximately 83% of all lung cancer patients. Moreover, more than 40% of Japanese lung cancer patients have activating mutations in the EGFR (Dearden et al., Ann Oncol 2013). Therefore, owing to the availability of targeted therapies, determining the mutation status of target molecules in NSCLC patients is now a key component of diagnosis to optimize treatment outcomes because the therapeutic effects of drugs vary depending on the mutation status. Currently, biopsy is the chief method for assessing EGFR mutations in NSCLC. However, analyses from biopsy samples cannot reflect the whole tumor status. In addition, re-biopsy is invasive and harmful to the physical and psychological condition of the patients. In contrast, nuclear molecular imaging is an alternative to biopsy because imaging can noninvasively assess the molecular target's expression level and mutation status. Thus, imaging target molecules in nuclear medicine can contribute to personalized medicine. Previously, it has been reported that imaging agents that were used for assessing the mutation status (L858R) of EGFR-tyrosine kinase (TK) can predict the therapeutic effects of a first-generation EGFR-tyrosine kinase inhibitor (TKI) (Gefitinib, IRESSA®) (Yeh et al., PNAS 2011). Recently, the FDA and PMDA in Japan approved the third generation of EGFR-TKI, osimertinib (TAGRISSO®) (Fig. 1), to treat NSCLC with L858R/T790M mutation that is resistant to Gefitinib. Radiolabeled Osimertinib analog can be helpful in predicting the therapeutic effect of osimertinib in NSCLC cancer patients with specific mutations.

２．研究の目的

This study aims to develop radiolabeled probes for imaging and therapy targeting TKRs, namely PDGFRβ and EGFR. In the first study, peptide-based probes targeting plateletderived growth factor receptor β (PDGFRβ) with different types of linkers were designed and synthesized, then the influence of the length and types of linkers in peptide-based probes as candidates of PDGFRb imaging agents were analyzed. In addition, the feasibility of osimertinib-based probes, namely ^{125}I-Osimertinib and ^{77}Br-Osimertinib, targeting EGFR with L858R/T790M mutation, were synthesized and evaluated as well. Moreover, peptide-osimertinib conjugated compounds with different linkers were designed and synthesized to develop new probes for visualization of EFGR with L858R/T790M mutation.

３．研究の方法

1. Seven probes, ^{67}Ga-labeled peptides with different lengths and types of linkers inserted between the N-terminus of the IPLPPPRRPFFK peptide and the Ga-DOTA complex, namely aaa [(D-alanine)$_3$], aaaaa [(D-alanine)$_5$], ββ [(β-alanine)$_2$], βββ [(β-alanine)4], EG$_2$ [(ethylene glycol)$_2$], or EG$_4$ [(ethylene glycol)$_4$] were synthesized using SPPS method. The lipophilicity and stability of probes in buffer and murine plasma were examined. The probe accumulations in PDGFRβ positive cells were evaluated as well. The biodistribution study of some probes that showed high stability and high uptake in PDGFRβ positive cell was conducted using tumor-bearing mice.

2. Two radiohalogenated Osimertinib, namely ^{125}I-Osimertinib and ^{77}Br-Osimertinib, were synthesized, and their potential as imaging agents for detecting NSCLC with L858R/T790M mutation were performed in vitro (lipophilicity, cytotoxicity, kinase enzymatic activity, and stability in buffer and murine plasma) and in vivo (biodistribution study in normal and tumor-bearing mice).

3. Peptide-Osimertinib conjugated molecules with enzyme cleavable linkers were being developed. I-Osimertinib, a small-molecule specific for EGFR with L858R/T790M mutation, peptide targeting EGFR, and the cathepsin-B cleavable linkers were synthesized.

４．研究成果

1. Seven radiotracers, ^{67}Ga-DOTAlinker-IPLPPPRRPFFK peptides with different lengths and types of linkers, were designed, synthesized, and evaluated as PDGFRβ imaging agents. Two radiotracers, namely [^{67}Ga]GaDOTA-EG$_2$-IPLPPPRRPFFK and [^{67}Ga]Ga-DOTA-EG$_4$-IPLPPPRRPFFK (Fig. 1a), showed high in vitro stability in murine plasma and high uptake in BxPC3-luc cells. The accumulation of [^{67}Ga]Ga-DOTAEG$_2$-IPLPPPRRPFFK in BxPC3-luc cells is higher than [^{67}Ga]GaDOTA-EG4-IPLPPPRRPFFK, and their accumulations can be significantly reduced by an excess amount of IPLPPPRRPFFK, indicating their specific uptake (Fig. 1b). In the biodistribution study of [^{67}Ga]Ga-DOTA-EG2-IPLPPPRRPFFK and [^{67}Ga]Ga-DOTAEG4-IPLPPPRRPFFK, the tumor-to-blood ratios were 2.61±0.75 and 2.05±0.77, respectively at 1h post-injection. Co-injection of [^{67}Ga]Ga-DOTA-EG2- IPLPPPRRPFFK and an excess IPLPPPRRPFFK peptide as a blocking agent can significantly decrease this ratio (Fig. 1c). However, tumor accumulation was not considered sufficient. Therefore, further probe modification is required to assess tumor accumulation for in vivo imaging.

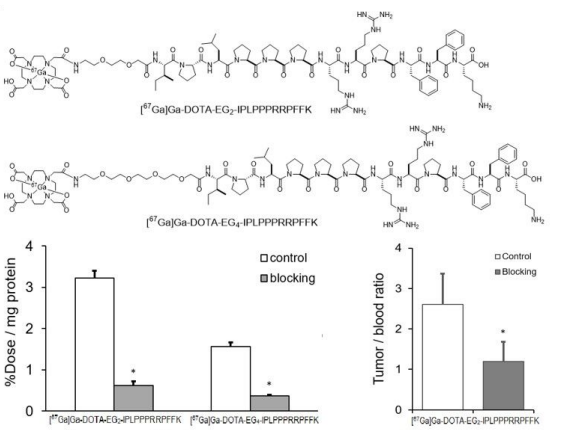

Figure 1. (a) Structure of [^{67}Ga]Ga-DOTA-EG$_2$-IPLPPPRRPFFK and [^{67}Ga]Ga-DOTA-EG$_4$-IPLPPPRRPFFK; (b) Uptake of [^{67}Ga]Ga-DOTA-EG$_2$-IPLPPPRRPFFK and [^{67}Ga]Ga-DOTA-EG$_4$-IPLPPPRRPFFK into BxPC3-*luc* cells at 1 h with or without IPLPPPRRPFFK as a blocking agent; (c) Tumor-to-blood ratio of [^{67}Ga]Ga-DOTA-EG$_2$-IPLPPPRRPFFK in BxPC3-*luc*-tumor-bearing mice with or without blocking agent

2. Two radiohalogenated Osimertinib derivatives, [^{125}I]I-Osimertinib and [^{77}Br]Br-Osimertinib, and their halogenated compounds, I-Osimertinib and Br-Osimertinib (Figure 2), were synthesized. Halogenated compounds were evaluated in vitro to access their potency as the inhibitors of EGFR L858R/T790M. Meanwhile radiohalogenated compounds were evaluated in vitro and in vivo for NSCLC imaging with EGFR mutation. The WST-8 assay exhibited that I-Osimertinib and Br-Osimertinib have a specifically high activity toward EGFR L858R/T790M double mutation and are comparable with osimertinib (Table 1). The kinase assay showed that I-Osimertinib and Br-Osimertinib have a significantly higher affinity toward EGFR L858R/T790M than osimetrinib (Table 2). These in vitro studies suggested that I-Osimertinib and Br-Osimertinib have high in vitro potency against the EGFR L858R/T790M double mutation than EGFR wild-type. In biodistribution experiments, [^{125}I]I-Osimertinib and [77Br]Br-Osimertinib showed high accumulation in tumors with mutations. However, the accumulations of [^{125}I]I-Osimertinib and [^{77}Br]Br-Osimertinib in the lung were much higher than those in the tumors, which can interfere with the visualization of mutation NSCLC patients. Therefore, structural modification of radiohalogenated Osimertinib is still needed.

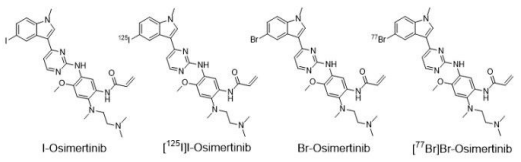

Figure 2. Structure of I-Osimertinib, [^{125}I]I-Osimertinib, Br-Osimertinib, [^{77}Br]Br-Osimertinib

Table 1. IC_{50} Values (nM) after Exposure of Gefitinib, Osimertinib, I-Osimertinib, or Br-Osimertinib to Different Mutation Statuses of NSCLC Cell Lines by the WST-8 Assay

	H411 (wild type)	H3255 (L858R)	H1975 (L858R/T790M)
Gefitinib	29850.0 ± 8456.4	8.5 ± 1.4	20386.7 ± 4506.8
Osimertinib	957.5 ± 238.4	11.0 ± 2.0	52.7 ± 12.0
I-Osimertinib	2230.0 ± 247.0	13.7 ± 1.9	122.3 ± 20.5
Br-Osimertinib	2659.3 ± 257.4	24.7 ± 3.4	44.2 ± 18.0

Table 2. IC_{50} Values (nM) of Activities of Gefitinib, Osimertinib, I-Osimertinib, or Br-Osimertinib to EGFR Tyrosine Kinase (Wild-Type, L858R Mutation, and L858R/T790M Mutations

	wild type	L858R)	L858R/T790M
Gefitinib	4.2 ± 1.5	6.0 ± 0.9	2274.7 ± 409.1
Osimertinib	140.3 ± 29.2	13.2 ± 3.7	0.98 ± 0.27
I-Osimertinib	39.4 ± 13.6	0.056 ± 0.019	0.0026 ± 20.5
Br-Osimertinib	4.3 ± 2.1	0.052 ± 0.018	0.0019 ± 0.001

3. Peptide-Osimertinib conjugated molecules with different linkers which are easy to be cleaved by Cathepsin-B enzyme in the tumor environment, were being developed. These Peptide-Osimertinib conjugated molecules consist of three parts, namely (1) I-Osimertinib, a small-molecule specific for EGFR with L858R/T790M mutation, (2) the cathepsin-B cleavable linkers (3) peptide targeting EGFR, were synthesized. Their structures were analyzed and confirmed by 1H-NMR and/or ESI-MS.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Effendi Nurmaya, Mishiro Kenji, Shiba Kazuhiro, Kinuya Seigo, Ogawa Kazuma	４．巻 26
２．論文標題 Development of Radiogallium-Labeled Peptides for Platelet-Derived Growth Factor Receptor β (PDGFRβ) Imaging: Influence of Different Linkers	５．発行年 2020年
３．雑誌名 Molecules	６．最初と最後の頁 1～13
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/molecules26010041	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Kenji Mishiro, Ryuichi Nishii, Izumi Sawazaki, Tomoki Sofuku, Takeshi Fuchigami, Hitomi Sudo, Nurmaya Effendi, Akira Makino, Yasushi Kiyono, Kazuhiro Shiba, Junichi Taki, Seigo Kinuya, and Kazuma Ogawa	４．巻 65
２．論文標題 Development of Radiohalogenated Osimertinib Derivatives as Imaging Probes for Companion Diagnostics of Osimertinib	５．発行年 2022年
３．雑誌名 Journal of Medicinal Chemistry	６．最初と最後の頁 1835 ～ 1847
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.jmedchem.1c01211	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関