Am J Nucl Med Mol Imaging 2021;11(6):537-541
www.ajnmmi.us /ISSN:2160-8407/ajnmmi0139972

Editorial

Dimeric FAPI with potential for tumor theranostics

Chunxia Qin[1,2], Yangmeihui Song[1,2], Weibo Cai[3], Xiaoli Lan[1,2]

[1]Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China; [2]Hubei Key Laboratory of Molecular Imaging, Wuhan 430022, Hubei, China; [3]Departments of Radiology and Medical Physics, University of Wisconsin-Madison, Madison 53705, WI, USA

Received October 21, 2021; Accepted October 23, 2021; Epub December 15, 2021; Published December 30, 2021

Abstract: Radionuclide-labeled fibroblast activation protein inhibitors (FAPIs) are popular nuclear imaging probes in recent years. It's of great significance for tumor diagnosis and has great potential in tumor treatment. However, optimization of the probes is needed to further increase tumor uptake and prolong tumor retention for improved treatment efficacy and fewer side effects. In this issue of AJNMMI, Moon et al. reported two squaramide coupled FAPI conjugates (DOTA.(SA.FAPi)$_2$ and DOTAGA.(SA.FAPi)$_2$) and labeled them with ^{68}Ga. The resulted tracers showed increased tumor accumulation and persistent retention, which led to an advance in PET imaging. The use of dimeric structures provides a feasible strategy to develop radiotherapeutic analogs of FAP inhibitors.

Keywords: Dimer, fibroblast activation protein inhibitor (FAPI), tumor, imaging, endoradiotherapy

Introduction

Although ^{18}F-FDG was named as the molecule of the 20th century and played important role in the field of tumor imaging, it still has many shortcomings, such as limited sensitivity in some types of tumors; poor specificity; only for diagnosis and but not for treatment. Therefore, scientists have been looking for new molecular probes that can supplement ^{18}F-FDG.

Cancer-associated fibroblasts (CAFs) are the major constituent of tumor stroma which control key tumorigenic activities by participating in immune evasion and suppression, extracellular matrix remodeling, neo-angiogenesis, and drug resistance [1, 2]. Fibroblast activation protein (FAP) is overexpressed on the CAFs but shows little expression in most normal organs. FAP's active involvement in many tumor-promoting activities makes it an attractive target for both tumor imaging and endoradiotherapy [2, 3]. In recent years, a series of quinoline-based fibroblast activation protein inhibitors (FAPIs) were synthesized and labeled with ^{68}Ga. With rapid and almost complete internalization and fast clearance from the circulation, these tracers showed high-contrast imaging even at 10 min after tracer administration, excellent diagnostic efficiency has been reported in a large amount of preclinical and clinical trials [4-6]. ^{68}Ga-labeled FAPIs have become rising stars in the field of molecular imaging with the potential of replacing ^{18}F-FDG and may become the molecule of the 21st century. Recently, several new ligands were also designed for labeling with ^{18}F and ^{99m}Tc, which allowed for widespread clinical routine practice [7-9].

Although FAPI derivatives are promising tracers for cancer imaging as mentioned above, their huge potential for therapeutic applications is more exciting. The DOTA chelator presents an opportunity to introduce therapeutic radionuclides, opening the road to a new family of theranostic radiopharmaceuticals. Therapeutic radionuclides such as ^{177}Lu, ^{90}Y, ^{64}Cu, ^{225}Ac, etc., were linked to FAPI to reach tumors with overexpressed FAP directly and exert internal radiation killing [10-13]. Yet, fast clearance and insufficient tumor retention have hampered FAPI-based tracer further clinical applications for cancer treatment. Since many therapeutic radionuclides have relatively long half-lives, the short retention of ^{68}Ga-FAPI-04 and ^{68}Ga-FAPI-02 in the tumor tissue (half-life 3.0 h and 1.7 h,

correspondingly) [2] presents potential limitation with regard to the theranostic application. To improve the binding ability to FAP and prolong the residence time in tumor stromal tissue and to enhance the tumor-killing efficacy of FAPI-based radiotheranostic agents, radiochemists have engaged in chemical modification of the FAPI framework. Moreover, higher doses of radioactivity can be delivered while minimizing damage to healthy tissue, which may improve therapeutic outcome [14].

In recent years, Roesch's group has used the squaramide (SA) containing bifunctional DATA^{5m} and DOTA chelators to create radiopharmaceutical precursor FAPI (namely DATA^{5m}.SA.FAPI and DOTA.SA.FAPI) [15]. The new FAP ligand, same as the commonly used FAPI-02, FAPI-04 and FAPI-46, has shown promise in oncology diagnostic applications [16]. However, on the way to radiotherapeutic agents, the short blood half-life and poor tumor retention of FAPI remain obstacles for efficient radionuclide therapy. So Roesch and coworkers switched their attention on bivalent structures that have shown desirable effects in increased tumor accumulation and prolonged tumor retention. In addition, ^{177}Lu-DOTA-SA-FAPI radiation therapy was performed in a 30-year-old female with metastatic breast cancer with a single cycle of 3.2 GBq, an approximate absorbed dose was observed in the primary tumor and brain metastasis, indicating primary tumors and brain metastases with uptake doses of approximately 1.48 and 3.46 mGy/MBq [17].

In this issue of *American Journal of Nuclear Medicine and Molecular Imaging* [18], the Roesch group reported a scheme to transform FAPI into a homodimeric system, where a single molecule can have two inhibitor clamps. Building on their mastery of the DOTA homologous synthesis method, they further optimized the structure to develop two homodimeric designs DOTA.$(SA.FAPI)_2$ and DOTAGA.$(SA.FAPI)_2$. They used ^{68}Ga to label two dimeric structures separately and found that the precursor DOTAGA and the buffers NaAc and HEPES could bind better to ^{68}Ga. The chemical modification did not significantly affect the biological activity of FAPI, and the FAPI dimer maintained its high affinity with FAP and high FAP/PREP selectivity. Primary clinical trials were performed in six patients. Compared to FAPI monomers, homodimeric FAPI radiotheranostics presented more excellent affinity to FAP in the tumor stroma, slower rate of renal excretion, and longer blood pool retention, expecting to further improve the target/non-target signal ratio and facilitate visual analysis, may improve the sensitivity of radionuclide labeled FAPI to new levels in the diagnosis of malignancies. In terms of treatment, a longer-lasting blood retention time based on the strengthened affinity may result in a significantly enhanced effect of internal irradiation, which contributes to a substantial step forward to turn radionuclide-labeled FAPI into radiotherapeutics. FAPI-based dimeric derivatives allow the development of a new approach to tumor-targeted radiation therapy that has excellent translational clinical application and is expected to significantly impact the treatment of high FAP-expressed cancer in the future.

However, FAPI-based agents have inherent challenges and limitations. FAPI has always been imperfect with the drawback of low specificity for malignant tumors. The high inter-individual variation in the PET images highlights heterogeneity in FAP expression across different diseases and even within the same disease. This variability represents a challenge for the general application of FAP-targeting theranostic radiopharmaceuticals. Non-cancerous fibrosis conditions, such as Ig G4 related diseases, cirrhosis, and Cron's disease, present another challenge for the general application of FAP-targeting theranostic agents [1, 19]. The modified dimer failed to provide improved FAPI diagnostic specificity, as the uptake and retention of the dimer are also enhanced in some non-neoplastic lesions that expressed FAP. In addition, the blood pool delay of ^{68}Ga-DOTAGA.$(SA.FAPI)_2$ in some patients is of concern, which may have additional radiation to normal organs throughout the body. It can cause relatively high bone marrow toxicity. Of note, radiotherapy is associated with more significant side effects at higher doses and with multiple treatments. Therefore, further optimization and improvement is needed.

For therapeutic purposes, the most important factors to be considered include physical characteristics (physical half-life, type of emissions, energy of the radiations, daughter product, method of production, and radionuclide purity) and biochemical characteristics (tissue targeting, retention of radioactivity in the tumor, in

vivo stability, and toxicity) [20]. A balanced optimal biological and physical half-life should be chosen according to the type of tumor to be treated, method of administration, and uptake mechanism, which results in an optimal effective half-life. Radiations with high-linear energy transfer (LET), such as α- and β-particles, are preferable. For biochemical characteristics, a clinically suitable tracer should provide selective concentrations as high as possible and prolonged retention in the tumor, while maintaining minimum uptake in the normal tissues, high stability, proper size of the tracer particles, low toxicity, the specific gravity for optimal flow and distribution during administration, the appropriate pH, and the optimal clearance rate (except for permanent tracer) [20].

The main challenge for potential therapeutic application of the tracer was in optimizing its tumor uptake and retention time. Increasing the size of the molecule is a common practice to prolong the retention of radiopharmaceuticals in the body, common strategies include multimerization, PEGylation, adding albumin binding moieties such as Evens Blue, and covalent connection of radionuclides. The formation of dimeric derivatives is an approach to improve tumor accumulation as well as retention time. The common problem must overcome is side effects. On the other hand, the physical half-life of the radionuclide has to be adjusted to the retention time, radionuclides with shorter half-lives seem preferable to those with longer half-lives. Thus, the use of ^{188}Re, ^{153}Sm, ^{213}Bi, or ^{212}Pb would be favored for FAPI-based endoradiotherapy [3].

As a proof-of-concept of FAP-targeted endoradiotherapy, FAPI-04 was labeled with ^{90}Y (half-life, 64 h) and first used in a patient with metastatic final-stage breast cancer, a considerably low dose of ^{90}Y-FAPI-04 resulted in a significant reduction in pain symptoms, and no therapy-related side effects was reported [2]. Researchers studied low-dose ^{177}Lu-FAPI-04 dosimetry in four patients with metastatic advanced-stage cancer and concluded that the mean absorbed dose to organs at risk with ^{177}Lu-FAPI-04 is reasonably low, allowing for low tumor-absorbed dose rates by administering a higher dose [21]. However, relatively long half-lives of many therapeutic radionuclides are not match the short retention of ^{68}Ga-FAPI-04 and ^{68}Ga-FAPI-02 in the tumor tissue, presents potential limitation with regard to the theranostic application [1]. Further functionalization of the core FAPI-04 structure yielded ^{68}Ga-FAPI-21 and ^{68}Ga-FAPI-46. Both compounds demonstrated improved tumor uptake and higher tumor-to blood, liver, muscle, and intestine contrast, lower uptake in oral mucosa, salivary glands, and thyroid, as well as favorable dosimetry profile, make FAPI-46 potentially more suitable as a theranostic agent [14, 22]. Initial clinical experience with ^{90}Y-FAPI-46 radioligand therapy has been reported for advanced stage solid tumors in a case series of nine patients, but hematologic G3/G4 toxicities were noted in four patients (44%) [12]. FAP-2286 is the conjugate of a FAP-binding peptide, the first-in-human results using ^{177}Lu-FAP-2286 for peptide-targeted radionuclide therapy (PTRT) reported it was relatively well-tolerated with acceptable side effects and demonstrated long retention of the radiopeptide, it could be applied in a broad spectrum of cancers after prospective clinical studies [23].

Further research on optimizing therapeutic efficacy and using alternative radioisotopes is ongoing. A first preclinical therapeutic application of ^{225}Ac-FAPI-04 was performed on xenograft-implanted FAP-expressing pancreatic PANC-1 cancers, an administration of 34 kBq of ^{225}Ac-FAPI-04 showed excellent tumor growth suppression without significant toxicity [11]. As one of the most representative radioisotopes for imaging and therapeutic applications and an analogue of ^{211}At, ^{131}I was used to label FAPI derivatives and pave the way for the radioastatination of related precursors as well as their in vitro and in vivo biological evaluations in targeted alpha therapy, the results implied that FAP-targeted alpha endoradiotherapy (specific to ^{211}At) should be used to treat tumors in the near future [24]. New FAPI derivatives were designed for labeling with ^{99m}Tc and ^{188}Re, which are available from generators and can be used as a couple for diagnosis and FAP targeted endoradiotherapy [9].

FAPI dimer is a good strategy to optimize the pharmacokinetics. ^{68}Ga-DOTA-2P(FAPI)$_2$ based on FAPI-46 was reported to increase tumor uptake and retention properties compared to ^{68}Ga-FAPI-46, and it could be a promising tracer for both diagnostic imaging and targeted therapy of malignant tumors with positive expression of FAP [25]. Two albumin binder-

conjugated FAPI radiotracers, TEFAPI-06 and TEFAPI-07 were developed, which were derived from FAPI-04 and optimized by conjugating two types of well-studied albumin binders, 4-(p-iodophenyl) butyric acid moiety (TEFAPI-06) and truncated Evans blue moiety (TEFAPI-07), notably improved tumor uptake and retention have been observed compared to the original FAPI tracer. Both ^{177}Lu-TEFAPI-06 and ^{177}Lu-TEFAPI-07 showed remarkable growth inhibition to PDX tumors while the side effect is negligible, showing that they are promising for further clinical translational studies [26].

Overall, Roesch's group reported that two novel PET radiopharmaceuticals DOTA.$(SA.FAPI)_2$ and DOTAGA.$(SA.FAPI)_2$ based on a homodimer system are promising molecular probes for integrated PET imaging and oncology treatment and verified the hypothesis that dimeric FAPi derivatives can function as an approach towards increasing tumor stroma residence times. Showing promising pharmaceutical profiles both *in vitro* and *in vivo*, DOTAGA.$(SA.FAPi)_2$ and related compounds could be a suitable PET imaging probe for diagnostic approaches targeting FAP in the tumor stroma. However, ^{68}Ga-DOTAGA.$(SA.FAPi)_2$ is accompanied by high, delayed, and heterogeneous blood pool uptake across the patients, high retention of the tracer in the blood leads to lower tumor-to-blood ratios, this may lead to increased radiation dose to the non-target organs and higher hematotoxicity during treatment, narrowing the therapeutic window. In future work, concern is expected to be focused on optimization of the dimer to reduce blood retention while increasing its therapeutic efficacy. The physical half-life of the radionuclide has to be adjusted to the retention time, radionuclides with shorter half-lives seem preferable to those with longer half-lives.

Acknowledgements

The authors are grateful for financial support from the National Natural Science Foundation of China (No. 81630049 and 82030052), the University of Wisconsin-Madison, and the National Institutes of Health (P30CA014520).

Disclosure of conflict of interest

Weibo Cai is a scientific advisor, stockholder, and grantee of Focus-X Therapeutics, Inc. All other authors declare no conflict of interest.

Address correspondence to: Xiaoli Lan, Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Avenue, Wuhan 430022, Hubei, China. Tel: +86-278754-3437; E-mail: xiaoli_lan@hust.edu.cn

References

[1] Zhuravlev F. Theranostic radiopharmaceuticals targeting cancer-associated fibroblasts. Curr Radiopharm 2021; 14: 374-393.

[2] Lindner T, Loktev A, Altmann A, Giesel F, Kratochwil C, Debus J, Jäger D, Mier W and Haberkorn U. Development of quinoline-based theranostic ligands for the targeting of fibroblast activation protein. J Nucl Med 2018; 59: 1415-1422.

[3] Altmann A, Haberkorn U and Siveke J. The latest developments in imaging of fibroblast activation protein. J Nucl Med 2021; 62: 160-167.

[4] Sollini M, Kirienko M, Gelardi F, Fiz F, Gozzi N and Chiti A. State-of-the-art of FAPI-PET imaging: a systematic review and meta-analysis. Eur J Nucl Med Mol Imaging 2021; 48: 4396-4414.

[5] Sharma P, Singh SS and Gayana S. Fibroblast activation protein inhibitor PET/CT: a promising molecular imaging tool. Clin Nucl Med 2021; 46: e141-e150.

[6] Hathi DK and Jones EF. (68)Ga FAPI PET/CT: tracer uptake in 28 different kinds of cancer. Radiol Imaging Cancer 2019; 1: e194003.

[7] Toms J, Kogler J, Maschauer S, Daniel C, Schmidkonz C, Kuwert T and Prante O. Targeting fibroblast activation protein: radiosynthesis and preclinical evaluation of an (18)F-labeled FAP inhibitor. J Nucl Med 2020; 61: 1806-1813.

[8] Giesel FL, Adeberg S, Syed M, Lindner T, Jiménez-Franco LD, Mavriopoulou E, Staudinger F, Tonndorf-Martini E, Regnery S, Rieken S, El Shafie R, Röhrich M, Flechsig P, Kluge A, Altmann A, Debus J, Haberkorn U and Kratochwil C. FAPI-74 PET/CT using either (18)F-AlF or cold-kit (68)Ga labeling: biodistribution, radiation dosimetry, and tumor delineation in lung cancer patients. J Nucl Med 2021; 62: 201-207.

[9] Lindner T, Altmann A, Krämer S, Kleist C, Loktev A, Kratochwil C, Giesel F, Mier W, Marme F, Debus J and Haberkorn U. Design and development of (99m)Tc-labeled FAPI tracers for SPECT imaging and (188)Re therapy. J Nucl Med 2020; 61: 1507-1513.

[10] Koustoulidou S, Hoorens MWH, Dalm SU, Mahajan S, Debets R, Seimbille Y and de Jong M. Cancer-associated fibroblasts as players in cancer development and progression and their

role in targeted radionuclide imaging and therapy. Cancers (Basel) 2021; 13: 1100.
[11] Watabe T, Liu Y, Kaneda-Nakashima K, Shirakami Y, Lindner T, Ooe K, Toyoshima A, Nagata K, Shimosegawa E, Haberkorn U, Kratochwil C, Shinohara A, Giesel F and Hatazawa J. Theranostics targeting fibroblast activation protein in the tumor stroma: (64)Cu- and (225)Ac-labeled FAPI-04 in pancreatic cancer xenograft mouse models. J Nucl Med 2020; 61: 563-569.
[12] Ferdinandus J, Fragoso Costa P, Kessler L, Weber M, Hirmas N, Kostbade K, Bauer S, Schuler M, Ahrens M, Schildhaus HU, Rischpler C, Grafe H, Siveke JT, Herrmann K, Fendler W and Hamacher R. Initial clinical experience with (90)Y-FAPI-46 radioligand therapy for advanced stage solid tumors: a case series of nine patients. J Nucl Med 2021; [Epub ahead of print].
[13] Liu Y, Watabe T, Kaneda-Nakashima K, Shirakami Y, Naka S, Ooe K, Toyoshima A, Nagata K, Haberkorn U, Kratochwil C, Shinohara A, Hatazawa J and Giesel F. Fibroblast activation protein targeted therapy using [(177)Lu]FAPI-46 compared with [(225)Ac]FAPI-46 in a pancreatic cancer model. Eur J Nucl Med Mol Imaging 2021; [Epub ahead of print].
[14] Loktev A, Lindner T, Burger EM, Altmann A, Giesel F, Kratochwil C, Debus J, Marmé F, Jäger D, Mier W and Haberkorn U. Development of fibroblast activation protein-targeted radiotracers with improved tumor retention. J Nucl Med 2019; 60: 1421-1429.
[15] Moon ES, Van Rymenant Y, Battan S, De Loose J, Bracke A, Van der Veken P, De Meester I and Rösch F. In vitro evaluation of the squaramide-conjugated fibroblast activation protein inhibitor-based agents AAZTA(5).SA.FAPi and DOTA.SA.FAPi. Molecules 2021; 26: 3482.
[16] Moon ES, Elvas F, Vliegen G, De Lombaerde S, Vangestel C, De Bruycker S, Bracke A, Eppard E, Greifenstein L, Klasen B, Kramer V, Staelens S, De Meester I, Van der Veken P and Rösch F. Targeting fibroblast activation protein (FAP): next generation PET radiotracers using squaramide coupled bifunctional DOTA and DATA(5m) chelators. EJNMMI Radiopharm Chem 2020; 5: 19.
[17] Ballal S, Yadav MP, Kramer V, Moon ES, Roesch F, Tripathi M, Mallick S, ArunRaj ST and Bal C. A theranostic approach of [(68)Ga]Ga-DOTA.SA.FAPi PET/CT-guided [(177)Lu]Lu-DOTA.SA.FAPi radionuclide therapy in an end-stage breast cancer patient: new frontier in targeted radionuclide therapy. Eur J Nucl Med Mol Imaging 2021; 48: 942-944.
[18] Moon ES, Ballal S, Yadav MP, Bal C, Van Rymenant Y, Stephan S, Bracke A, Van der Veken P, De Meester I and Roesch F. Fibroblast activation protein (FAP) targeting homodimeric FAP inhibitor radiotheranostics: a step to improve tumor uptake and retention time. Am J Nucl Med Mol Imaging 2021.
[19] Luo Y, Pan Q, Yang H, Peng L, Zhang W and Li F. Fibroblast activation protein-targeted PET/CT with (68)Ga-FAPI for imaging IgG4-related disease: comparison to (18)F-FDG PET/CT. J Nucl Med 2021; 62: 266-271.
[20] Yeong CH, Cheng MH and Ng KH. Therapeutic radionuclides in nuclear medicine: current and future prospects. J Zhejiang Univ Sci B 2014; 15: 845-63.
[21] Kuyumcu S, Kovan B, Sanli Y, Buyukkaya F, Has Simsek D, Özkan ZG, Isik EG, Ekenel M and Turkmen C. Safety of fibroblast activation protein-targeted radionuclide therapy by a low-dose dosimetric approach using 177Lu-FAPI04. Clin Nucl Med 2021; 46: 641-646.
[22] Meyer C, Dahlbom M, Lindner T, Vauclin S, Mona C, Slavik R, Czernin J, Haberkorn U and Calais J. Radiation dosimetry and biodistribution of (68)Ga-FAPI-46 PET imaging in cancer patients. J Nucl Med 2020; 61: 1171-1177.
[23] Baum RP, Schuchardt C, Singh A, Chantadisai M, Robiller FC, Zhang J, Mueller D, Eismant A, Almaguel F, Zboralski D, Osterkamp F, Hoehne A, Reineke U, Smerling C and Kulkarni HR. Feasibility, biodistribution and preliminary dosimetry in peptide-targeted radionuclide therapy (PTRT) of diverse adenocarcinomas using (177)Lu-FAP-2286: first-in-human results. J Nucl Med 2021; [Epub ahead of print].
[24] Ma H, Li F, Shen G, Cai H, Liu W, Lan T, Yang Y, Yang J, Liao J and Liu N. Synthesis and preliminary evaluation of (131)I-labeled FAPI tracers for cancer theranostics. Mol Pharm 2021; 18: 4179-4187.
[25] Zhao L, Niu B, Fang J, Pang Y, Li S, Xie C, Sun L, Zhang X, Guo Z, Lin Q and Chen H. Synthesis, preclinical evaluation, and a pilot clinical PET imaging study of (68)Ga-labeled FAPI dimer. J Nucl Med 2021; [Epub ahead of print].
[26] Xu M, Zhang P, Ding J, Chen J, Huo L and Liu Z. Albumin binder-conjugated fibroblast activation protein inhibitor radiopharmaceuticals for cancer therapy. J Nucl Med 2021; [Epub ahead of print].