Evaluation of ^{211}At-labeled fibroblast activation protein inhibitor (FAPI): comparison of different linkers with polyethylene glycol and piperazine†

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Cancer tissues are heterogeneous, where cancer cells coexist with various other cell types. Therefore, it is difficult to deliver drugs to. By targeting fibroblast-activated protein (FAP), which is a marker for stromal cells, we may be able to efficiently target cancer tissues. The usefulness of ^{211}At should also be demonstrated using FAPI. In 2022, Ma *et al.* first reported the antitumor activity of ^{211}At-FAPI in U87MG xenograft mice.[1] They also claimed that no toxicity was observed in the kidneys, liver, stomach, or thyroid tissue. We also synthesized ^{211}At-FAPI(s) using dihydroxyboryl astatine substitution reaction.[2] We have previously established an astatination method that does not require toxic reagents.[2,3] In this study, we investigated the usefulness of ^{211}At-FAPI(s) synthesized using an established safe method and a linker in the FAPI structure.

During the preparation of ^{211}At-FAPI with polyethylene glycol (PEG) linker, the radiochemical yields (RCYs) of ^{211}At-FAPI1 and 2 were high (100% and 99%, respectively). When producing ^{211}At-FAPI5, a byproduct derived from the amino group of piperazine (PIP) was observed (∼10%). However, PEG-linked FAPI suppressed the byproduct production. Therefore, non-PIP linkers are suitable for drug manufacturing applications. The RCYs of ^{211}At-FAPI3 and 4 were not high (45% and 15%, respectively), because astatination was at 50°C to prevent production of a byproduct caused by fluorides (Fig. 1(a)). Additionally, the presence of glucosamine units decreased the astatination reactivity. Based on these results, we presume that ^{211}At-FAPI1 is suitable for a stable drug supply. The results of our investigation indicated that FAPα selectivity and cellular uptake were the same for ^{211}At-FAPI1 and ^{211}At-FAPI5.

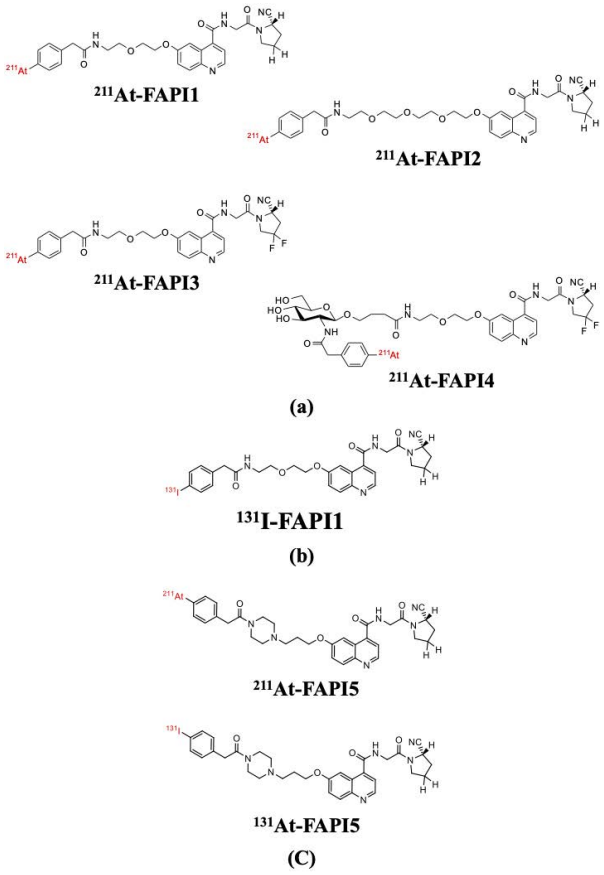

Fig. 1. Structure of ^{211}At-FAPI and ^{131}I-FAPI compounds. (a) ^{211}At-labeled PEG linker FAPI(s) were numbered from 1 to 4 according to their structural complexity. (b) ^{131}I-labeled PEG linker compound. (c) PIP linker compound synthesized based on the reported compound for comparison with the PEG linker compound.

Considering the molecular size and efficiency of astatination, we employed ^{211}At-FAPI1 for comparison with ^{211}At-FAPI5 in *in vitro* experiments. We compared the biodistribution of ^{211}At-FAPI1 and ^{211}At-FAPI5. At 1 hour after injection, tumor accumulation of ^{211}At-FAPI1 and ^{211}At-FAPI5 was 2.15 ± 0.24% ID and 1.40 ± 1.14% ID, which surpassed the accumulation of ^{225}Ac-FAPI-04.[4] After 3 hours, the accumulation in tumor cells increased for ^{211}At-FAPI1.

Based on the results of cellular uptake, nuclide labeling efficiency, and *in vivo* pharmacokinetics, sim-

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ple PEG-linker compounds (FAPI1) exhibited the best properties (Fig. 2).

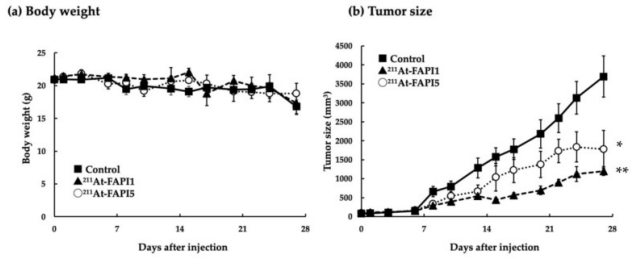

Fig. 2. Anticancer effect in PANC-1 xenograft mice after administration of ^{211}At-labeled FAPI (approximately 1 MBq). (a) Body weight of mice. (b) Tumor sizes of the experimental mice. Means ± S.E. Filled squares are the control group, filled triangles are the ^{211}At-FAPI1 group, and white circles are the ^{211}At-FAPI5 group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $\#p < 0.05$.

References

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