Comparison of linker length in compounds for nuclear medicine targeting Fibroblast activation protein as molecular target†

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Novel nuclear medicine therapeutics are being developed by labeling medium-molecular-weight compounds with short-lived alpha-emitting radionuclides. Fibroblast activation protein α (FAPα) is recognized as a highly useful molecular target, and its inhibitor, FAPI, is a compound capable of theranostics, both therapeutic and diagnostic, for cancer treatment. In this study, we compared the functions of two compounds that target FAPα: ^{211}At-FAPI1 (FAPI1) and ^{211}At-FAPI2 (FAPI2). First, *in vitro* screening procedures are generally accepted because of the low endogenous expression of FAPα. We suggest the usefulness of this 3D culture system for *in vitro* screening. Second, when FAPIs are used therapeutically, the expected therapeutic effects are often not achieved. Thus, we compared the accumulation and excretion in tumor tissues and the antitumor effects based on the length of the linker in the compounds. The compounds were rapidly labeled using the *Shirakami reaction*.

Figure 2 compares monolayer (2D) and sphere (3D) cultures, confirming that 3D culture increased FAPα expression and uptake. Similar trends were observed in the two cell lines, but the selectivity of the compounds varied by cell line. This is because in PANC-1 cells, FAPI1 uptake increased around 10-fold and FAPI2 uptake increased around 15-fold, whereas in BxPC3 cells, FAPI1 uptake was 10-fold higher than that in PANC-1 cells, but FAPI2 uptake was around three-fold higher. The difference between 3D and 2D cultures has different values depending on the state (size) of the sphere in 3D culture, but the uptake is significantly higher than that in 2D culture.

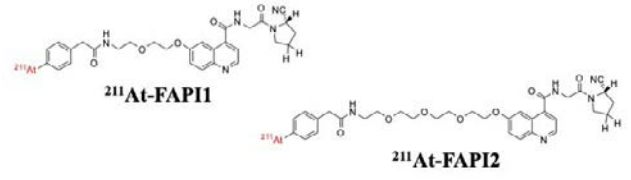

Fig. 1. Structures of ^{211}At-FAPI1 and ^{211}At-FAPI2. The only difference between these compounds was the length of the linker. In FAPI2, the number of PEG molecules was twice that in FAPI1. These were rapidly labeled using the *Shirakami reaction*[1)] based on previously reported precursors[2)] using ^{211}At produced based on previous reports.[3)]

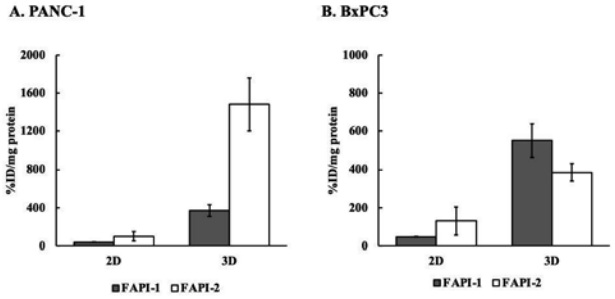

Fig. 2. Comparison of intracellular uptake between 2D and 3D cultures of (A) PANC-1 and (B) BxPC3 cells. The cells were then treated with FAPI1 or FAPI2. Gray histograms: FAPI1 uptake, a white histograms: FAPI2 uptake.

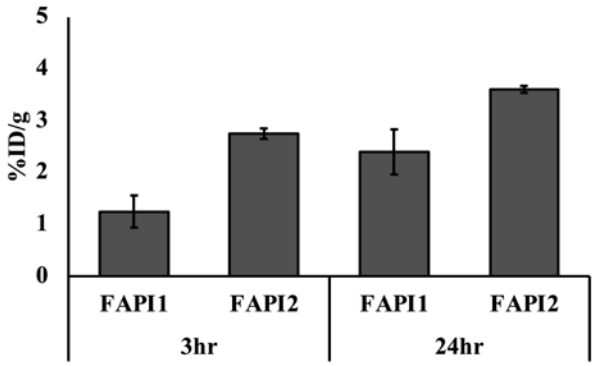

Fig. 3. %ID/g in tumor *in vivo*. FAPI1 and FAPI2 were administered to the BxPC3 tumor model and dissected 3 or 24 hours after administration. No significant differences were there.

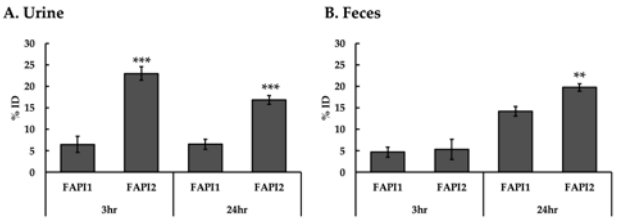

Fig. 4. Amount of excretion *in vivo*. FAPI1 and FAPI2 were administered to the BxPC3 tumor model and dissected 3 or 24 hours after administration. (A) % ID in urine. (B) % ID in feces. There were significant differences between FAPI1 and FAPI2. $^{**}p < 0.01$, $^{***}p < 0.001$.

Doubling the linker length increased tumor retention (Fig. 3). Additionally, the excretion pathway was

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altered, suggesting a potential reduction in toxicity (Fig. 4). Although no significant differences were observed in the anti-tumor effects of FAPI1 and FAPI2, it was confirmed that the linker length affects the biological half-life.

References

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