Targeted diagnostic applications of tumor stroma using radiolabeled fibroblast activation protein (FAP) inhibitors. Preclinical evaluation of two FAP-based probes labeled with the diagnostic radionuclide gallium-68

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Introduction

Background: Cancer-associated fibroblasts (CAFs) in tumor microenvironment have been implicated to possess a strong tumor-modulating effect in various solid tumors. Fibroblast activation protein (FAP) overexpressed on the CAFs surface is a promising target for cancer diagnosis and therapy due to its non-expression in normal fibroblasts and benign epithelial tumors.

Aim: The current study aims at evaluating two novel FAP-specific inhibitors (a monomer (DOTA.SA.FAPi (**1**)) and a dimer (DOTAGA.$(SA.FAPi)_2$ (**2**)) and verifying their suitability to serve as precursors for the generation of diagnostic radiopharmaceuticals with the potential to be used for non-invasive imaging of FAP-positive tumors.

Methods

Radiolabeling conditions:

Tracer	Buffer	pH	Temp. / Time
[^{68}Ga]Ga-1	0.2 M NaOAc	4.0	95 °C / 5 min
[^{68}Ga]Ga-2	1M HEPES	5.5	95 °C / 5 min

Stability:
The effect of the radioactivity on the tracers (release of gallium-68 or degradation of the radiotracers) was evaluated of a period of 4 h.

Lipophilicity:
- 100 pmol of each tracer were incubated in 1:1 Octanol/PBS solution while shaking for 1 h at RT. The activity distribution in each phase was determined by gamma counter counting.

Protein binding:
- The radiotracers (20 pmol) were incubated in 100 µL of human serum for 30 min. Activity in supernatant and precipitated protein pellet was counted by gamma counter.

Western blot analysis:
- Prostate-derived CAF cells were lysed in RIPA buffer to extract whole-cell proteins.
- Approx. 10 µg of protein was loaded to the gel electrophoresis.
- After electrophoresis, blotting membrane was stained with primary antibodies corresponding to FAP and GAPDH (control) proteins followed by fluorescence-tagged secondary antibodies.

Saturation binding:
- CAF cells were incubated with tracer concentrations (0.1–10 nM) for 2 h on ice.
- Free and cell membrane-bound fractions were collected, and activity was counted by gamma counter.

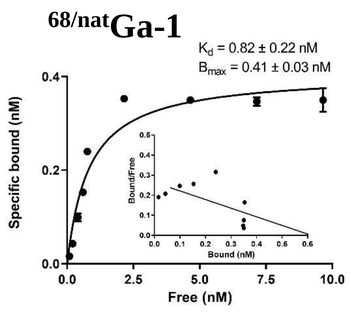
Internalization:
- CAF cells were incubated with 2.5 pmol of radiotracers for 2 h.
- Free, membrane-bound, and internalized fractions were collected and activity was counted by gamma counter.

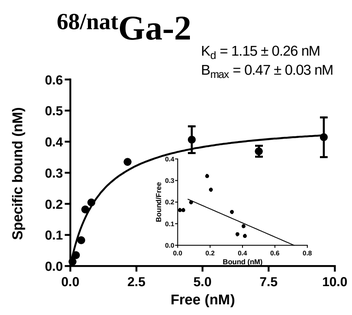
PET Imaging on PC3 xenografts:
- PET imaging studies were performed in PC3 tumor xenografts at 1, 2 and 3 h after the injection of the radiotracers.
- Furthermore, quantitative analysis of the PET images took place.

Results

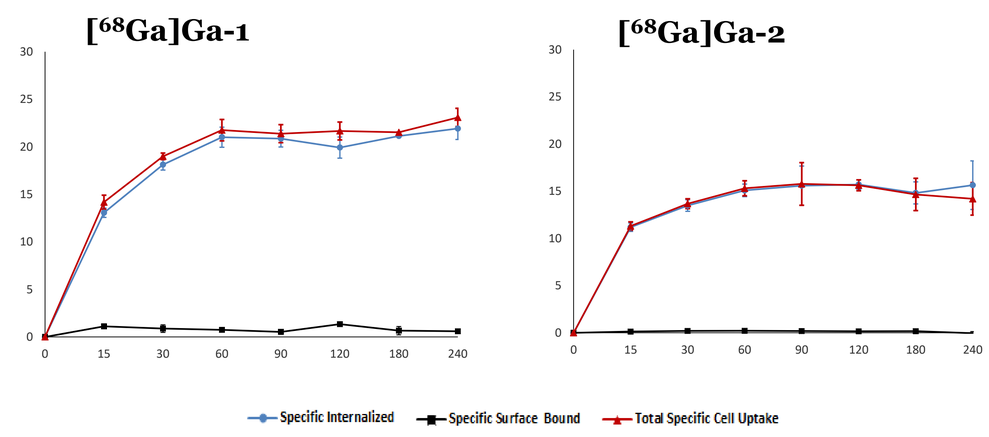
Lipophilicity & Protein binding studies:
[^{68}Ga]Ga-1 and [^{68}Ga]Ga-2 exhibited a logD octanol/PBS of -3.4 and -1.7, respectively.
The % of the radioactivity bound to human serum proteins was 9 for [^{68}Ga]Ga-1 and 18 for [^{68}Ga]Ga-2 after 30 min of incubation.

Saturation binding:
Both radiotracers exhibited high binding affinity to FAP-positive CAF cells with K_d and B_{max} values in the low nM range.

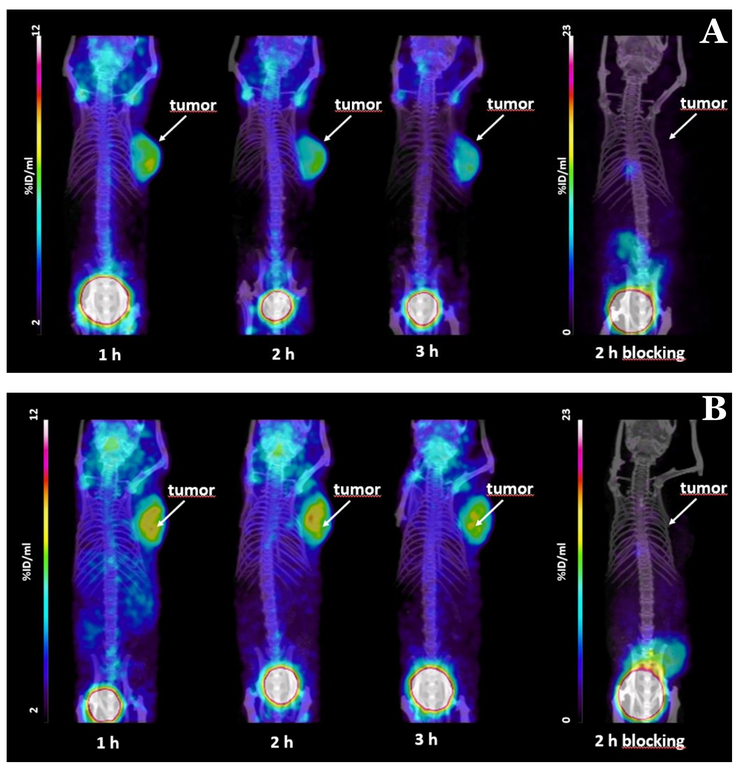




Internalization studies:
Specific internalization was found in cell culture with a maximum of 23% and 15% for [^{68}Ga]Ga-**1** and [^{68}Ga]Ga-**2**, respectively, with 93% of the total cell-associated activity internalized after 1h of incubation with the cells.

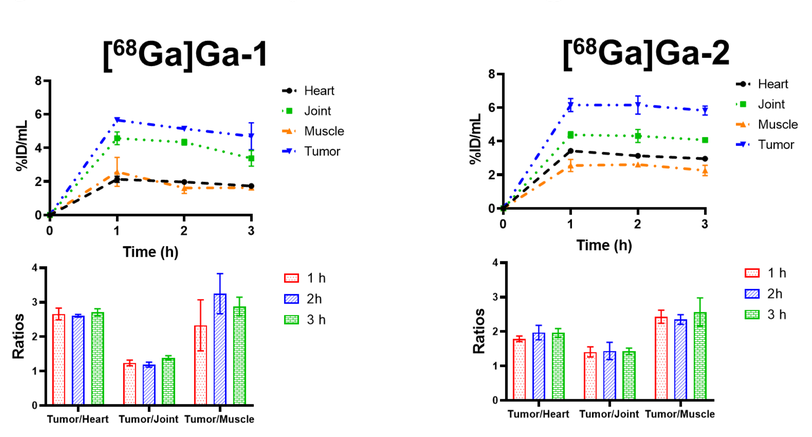


PET Imaging on PC3 xenografts:



Tumors were delineated in both cases, with the ^{68}Ga-labeled dimer (**B**) being superior compared to the ^{68}Ga-labeled monomer (**A**) in terms of radioactivity retention at tumor site in the course of three hours (mice were imaged at 1, 2, and 3 h after the injection of the radiotracers).

Quantitative analysis of PET Images



The quantitative analysis of the PET images showed that tumor uptake of the dimer (2) was higher and retained over time compared to the monomer (1). The higher lipophilicity of the dimer resulted in a somehow higher background activity compared to the monomer.

Conclusions

Results mentioned above provide preliminary evidence for the potential use of [^{68}Ga]Ga-1 and [^{68}Ga]Ga-2 as diagnostic radiotracers for stromal tumor imaging via FAP targeting.

References

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2. 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, Rösch F. *EJNMMI Radiopharm. Chem.* **2020**, *5*, 19.

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