Nanofitin as a New Molecular-Imaging Agent for the Diagnosis of Epidermal Growth Factor Receptor Over-Expressing Tumors

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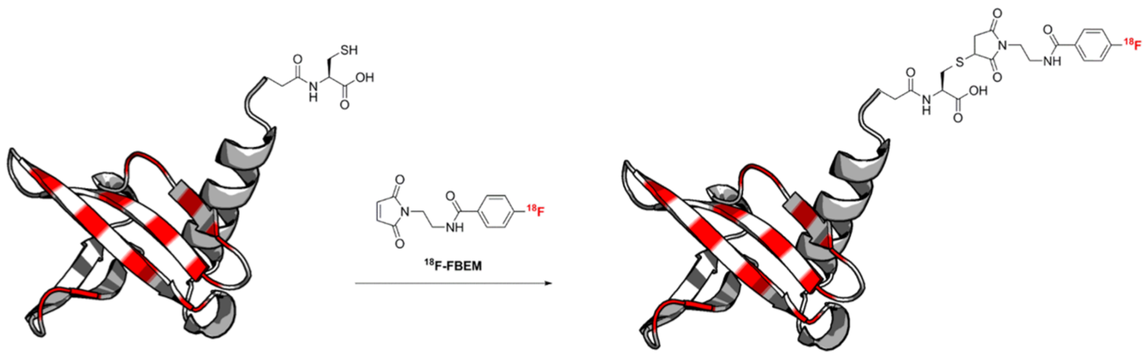
INTRODUCTION & METHODS

Nanofitins are cysteine-free protein scaffolds derived from the hyperstable DNA-binding protein Sac7d (7 kDa, 66 amino acids) of Sulfolobus acidocaldarius [1]. High-affinity nanofitins have been easily engineered by ribosome-display over a wide range of targets by the full randomization of 10 to 14 amino acid residues localized in the DNA-binding site of Sac7d. In this study, the anti-EGFR Nanofitin Cys−B10 was site-specifically labeled with ^{18}F by site-specific conjugation with the prosthetic group ^{18}F−4-fluorobenzamido−N-ethylamino-maleimide (^{18}F− FBEM), using a unique cysteine residue specifically introduced in C-terminus [2].



The resulting probe, ^{18}F−FBEM− Cys−B10, was then injected in a double-bearing tumor model to evaluate the biodistribution and the ability of the radiolabeled protein to specifically target in vivo the EGFR over-expressing A431 tumor.

Radiolabeling

The radioactive ^{18}F−FBEM (molar activity: 830 MBq/nmol) was automatically synthetized on a FastLab Multitracer (GE Healthcare) as previously described [2]. The Nanofitin Cys−B10 was incubated with Ni−nitrilotriacetic acid magnetic beads (GE Healthcare) in the presence of TCEP−HCl (50 equiv, 30 min, 25 °C, pH adjusted at 8). Beads were washed with phosphate buffer (200 mM, pH 7.4) and incubated with ^{18}F−FBEM freshly resuspended in phosphate buffer. Beads were washed with phosphate buffer, and the radiolabeled Nanofitin called ^{18}F−FBEM−Cys−B10 (effective molar activity: 37.0−53.6 MBq/nmol) was eluted with imidazole.

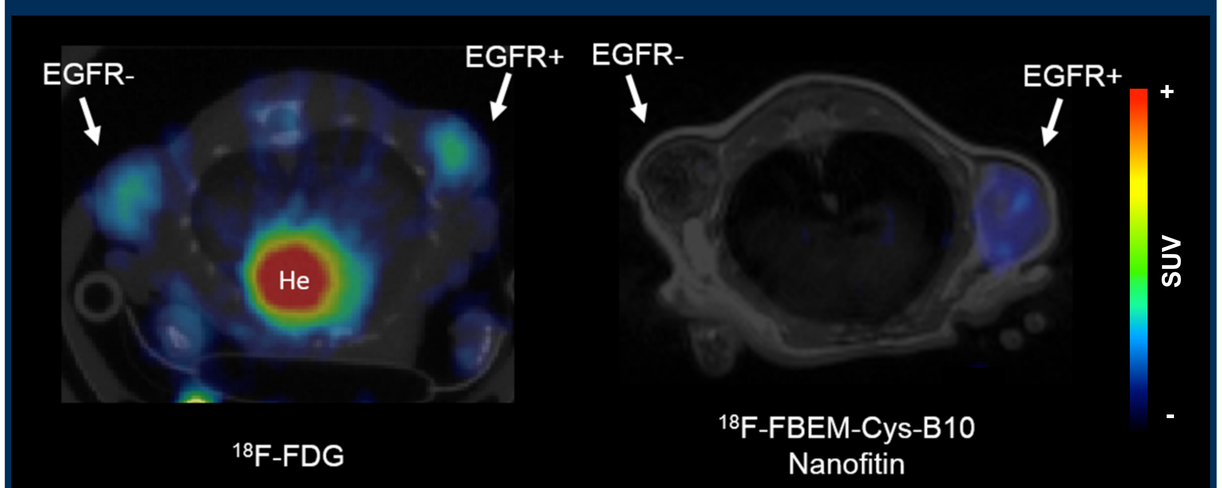
microPET

- Emission: 2 h dynamic scan, Siemens FOCUS 120.
- Transmission: 10 min., 57Co point source.
- ^{18}F-FDG: 10 min. static acquisition, 50 min. after i.p. injection.

Anatomical reference

- MRI anatomical whole body imaging, 9.4 T micro-MRI (Agilent Technologies).
- Micro-CT anatomical whole body imaging (Trifoil).

HIGHLIGHTS

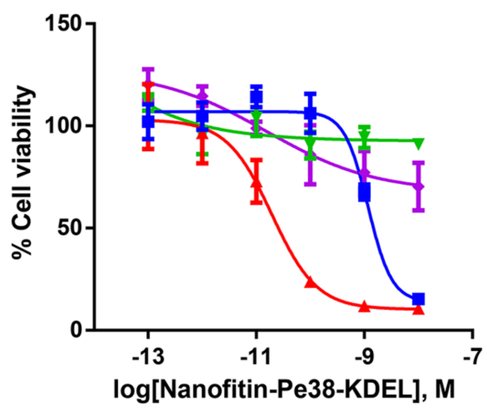


Targeting of the EGFR-positive tumor A431 by the radiolabeled anti-EGFR Nanofitin

A Co-registered transversal sections of PET and CT 1 h after the injection of ^{18}F−FDG (9 MBq) in a xenograft model under isoflurane anesthesia (blood glucose level of 73 mg/dL and weight of 29 g). He: heart. **B** Co-registered transversal sections of PET and MRI 2 h after injection in xenograft model under isoflurane anesthesia of ^{18}F− FBEM−Cys−B10 (19 MBq/100 µL).

RESULTS & DISCUSSION

Anti-EGFR B10 Nanofitin.



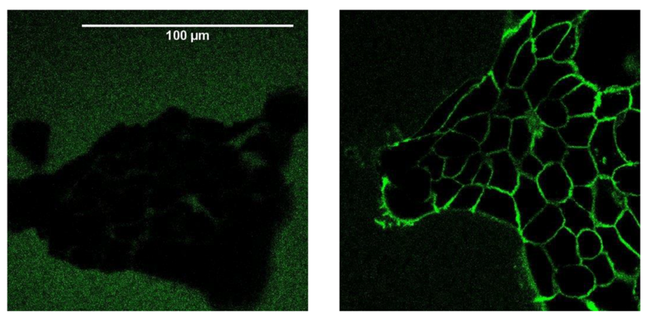
Measurement of A431 cells viability after incubation with Nanofitins fused to the Pe38 toxin.

αEGFR_B10_Pe38-KDEL, anti-EGFR B10 Nanofitin.
αEGFR_NF1- and NF2−Pe38-KDEL, other anti-EGFR Nanofitins.
IrrNF−Pe38_KDEL, irrelevant Nanofitin.

The non-internalizing profile of αEGFR_B10_Pe38-KDEL appeared not to be shared with he two other anti-EGFR Nanofitins NF1.

IrrNF labeled with Alexa Fluor

anti-EGFR B10 Nanofitin labeled with Alexa Fluor



In vitro specificity experiments of the Nanofitin B10 cell surface labeling of A431 cells. Labeling of the A431 cells with a 2 µM solution of an irrelevant Nanofitin conjugated to Alexa Fluor 488 and a 1 µM solution of the anti-EGFR Nanofitin B10 conjugated to Alexa Fluor 488.

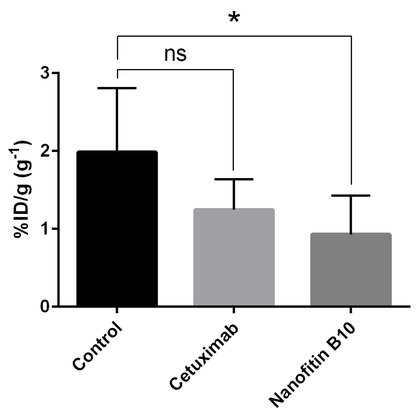
Time-lapse microscopy on A431 cells incubated with either Alexa Fluor 488 labeled anti-EGFR B10 or anti-egg white lysozyme H4 Nanofitin (negative control) revealed a fast accumulation (visible after few seconds) of fluorescence on cells membrane for B10, while no targeting was observed with the irrelevant Nanofitin.

Specificity of Tumor Targeting

Biodistribution of ^{18}F−FBEM−Cys−B1 at 2.5 h Post-Injection.

Tissues	Xenograft model
Blood	0.32 ± 0.07
Brain	0.02 ± 0.01
Bone	0.20 ± 0.01
Liver	1.13 ± 0.52
Kidney	1.55 ± 0.57
Heart	0.17 ± 0.03
Spleen	0.27 ± 0.08
Skin	0.28 ± 0.12
Muscle	0.12 ± 0.03
Lung	0.63 ± 0.31
Tumor A431	1.42 ± 0.18
Tumor H520	0.56 ± 0.10

Uptake ratio	Xenograft model	Balb/c
liver-to-blood	3.90 ± 1.33	4.80 ± 1.11
kidney-to-blood	4.82 ± 0.84	6.74 ± 2.43
A431-to-kidney	0.98 ± 0.29	
A431-to-liver	1.43 ± 0.52	
A431-to-H520	2.53 ± 0.18	
A431-to-lung	2.53 ± 0.89	
A431-to-blood	4.55 ± 0.63	
A431-to-heart	8.56 ± 1.34	



A431 tumor targeting and specificity of ^{18}F-labeled B10 Nanofitin. Uptake in tumors of ^{18}F−FBEM−Cys−B10 (2.1−9.1 MBq) in mice carrying EGFR-expressing A431 tumors without blocking (n = 6) or with blocking amounts of nonlabeled B10 (500 µg, n = 6) or Cetuximab (45 µg, n = 4) injected 48 h post-injection.

SUMMARY

In this study, we provided the first report of the use of the Nanofitin scaffold for generating targeted PET radiotracers, using the anti-EGFR B10 Nanofitin as proof-of-concept. ^{18}F−FBEM−Cys−B10 shows a favorable in vivo profile. The possibility to drive Nanofitins molecular recognition capability, over a fast and tunable in vitro selection system, could facilitate the development of valuable PET-based companion diagnostics.

REFERENCES

[1] Mouratou, B. et al, PNAS 2007. [2] Dammicco, S. et al, Nucl Med Biol, 2017.

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