PET of Follicle-Stimulating Hormone Receptor: Broad Applicability to Cancer Imaging

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Materials and methods

Cell Internalization and Efflux Assays

The radioactivity was measured using a Wizard^2 gamma-counter (Perkin-Elmer; energy window: 400 - 600 keV). FSHR-positive CAOV-3 cells (5×10^6/mL) were incubated at 4 ºC for 30 min with 10^5 cpm/mL of ^{64}Cu-NOTA-FSHR-mAb. For cell internalization study, the cells were washed three times with cold HBSS and resuspended in complete medium at 4×10^6/mL. At serial time points (0.25, 0.5, 1, 1.5, 2, 2.5, and 3 h), 1 mL of the cell suspension was removed from the culture and centrifuged at 14,000×g for 15 s. The cell pellets were then resuspended in 0.5 mL of 0.01 M sodium citrate, 0.14 M NaCl, pH 2 buffer for 2 min at room temperature and then centrifuged at 14,000×g for 15 s. The amount of internalized proteins was calculated by the radioactivity that remained associated with the cells after the wash with pH 2 buffer. All data were presented as mean ± SD using triplicate samples.

For cellular efflux studies, CAOV-3 cells (5×10^6/mL) were incubated at 37 ºC for 2 h with 10^5 cpm/mL of ^{64}Cu-NOTA-FSHR-mAb. The cells were washed three times with cold HBSS and resuspended in complete medium at 4×10^6/mL. At serial time points (0.25, 0.5, 1, 1.5, 2, 2.5, and 3 h), 1 mL of the cell suspension was removed from the culture and centrifuged at 14,000×g for 15 s. The radioactivity in the supernatant represents the efflux of ^{64}Cu-NOTA-FSHR-mAb from CAOV-3 cells at different time points. All data were presented as mean ± SD using triplicate samples.

Results

^{64}Cu-NOTA-FSHR-mAb can be internalized rapidly by the cells at earliest time point (0.25 h) but the efflux rate is also significant after 0.5 h (**Figure S1**). More than 40% of intaken ^{64}Cu-NOTA-FSHR-mAb was excreted from the CAOV-3 cells at 3 h post-incubation. Only less

than 30% of total ^{64}Cu-NOTA-FSHR-mAb was taken inside the CAOV-3 (**Figure S1**), indicating that most of ^{64}Cu-NOTA-FSHR-mAb stayed bound to the surface of CAOV-3 cells.

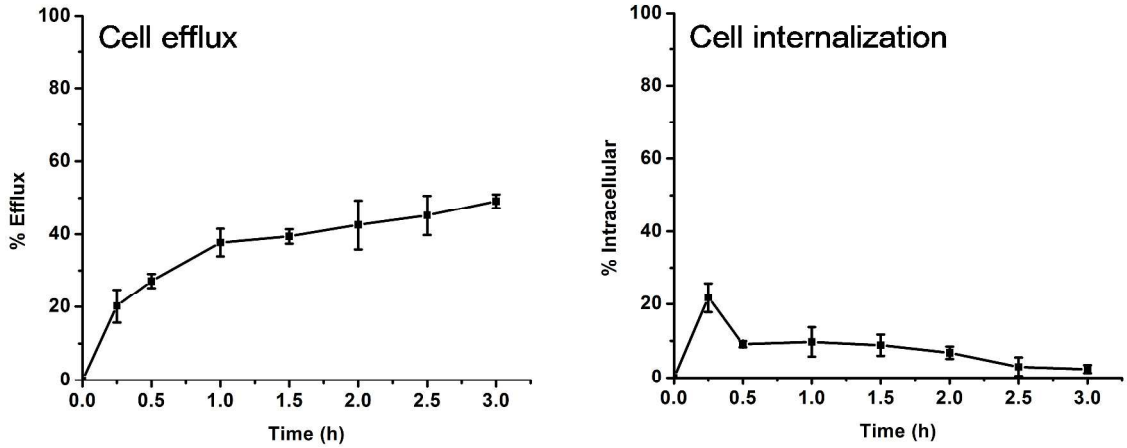

Figure S1 Internalization and efflux of ^{64}Cu-NOTA-FSHR-mAb in FSHR-positive CAOV-3 cells.