MicroPET imaging of CD13 expression using a ^{64}Cu-labeled dimeric NGR peptide based on sarcophagine cage

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Experimental Section:

Results:

Western blot analysis

HT-1080 or MCF-7 cells grown in a 75-cm^2 culture flask were suspended in lysis buffer (Beyotime, China) supplemented with complete, mini protease inhibitors (Roche, Canada). The cell debris was then removed by centrifugation (10,000 rpm at 4°C for 10 min) and the protein concentration was determined with the Coomassie (Bradford) Protein Assay Kit (Thermo Fisher Scientific, IL, USA). Samples of cell extracts containing 40 μg of protein were loaded on SDS–PAGE gels and transferred to polyvinylidene fluoride membrane filters (Life Technologies, NY, USA). CD13 protein was detected with anti-CD13 antibody (1:100, Santa Cruz Biotechnology, CA, USA) and peroxidase-conjugated secondary antibody (1:400, Life Technologies, NY, USA). The antigen–antibody complexes on the membranes were visualized with ECL Western Blotting Detection System (Thermo Fisher Scientific, IL, USA) with ChemiDOC XRS+ (Bio-Rad, CA, USA). Beta-actin was detected with anti-β-actin as an internal loading control.

Immunofluorescence staining

HT-1080 or MCF-7 cells were plated into a 24-well plate at a density of 5×10^4cells/well. After overnight incubation, cells were fixed with 4% of paraformaldehyde for 10 min, and washed with PBS three times. The cells were then incubated in 3% BSA for 30 min to block nonspecific

binding, followed by overnight incubation at 4°C with anti-CD13 polyclonal antibody (Santa Cruz Biotechnology, CA, USA) diluted to 1:50 in PBS (pH 7.4) containing 1% BSA. On the following day, the cells were washed with PBS three times, and incubated for 45 min with a secondary goat anti-rabbit IgG fluorescein isothiocyanate (FITC) conjugated antibody (1:400, Life Technologies, NY, USA) diluted 1:400 in PBS (pH 7.4) containing 1% BSA. After washing with PBS three times, the cells were mounted in a mounting medium containing DAPI. The cells were then visualized by an Olympus IX71 fluorescence microscope (Olympus, Japan).

Western blot and immunofluorescence staining

The western blot analysis of CD13 expression in HT-1080 and MCF-7 cells is shown in Figure S1a. For HT-1080 cells, there was a clear band at 150 KDa which belongs to CD13 expression, whereas no band was identified at 150 KDa for MCF-7 cells, suggesting CD13 is indeed overexpressed in HT-1080 cells but not in MCF-7 cells. In addition, immunofluorescence staining of HT-1080 and MCF-7 cells showed that strong green fluorescence signal can be observed on the cell membrane of HT-1080 cells but not MCF-7 cells (Figure S1b). This result further demonstrated that HT-1080 is a positive cell line for CD13 expression while MCF-7 cell line is negative.

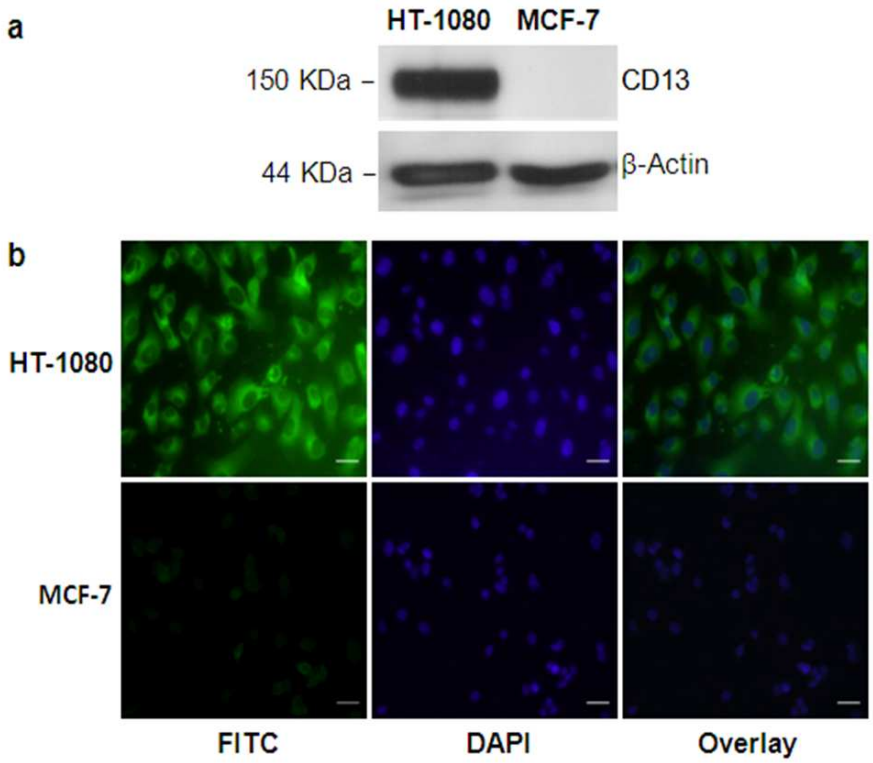

Figure S1. (a) Representative Western blot analyses of HT-1080 and MCF-7 cell lysates for CD13 receptor expression (150 KDa). Beta-actin was used as a loading control. **(b)** Immunofluorescence staining of CD13 receptor expression in HT-1080 and MCF-7 cells with FITC–goat anti-rabbit IgG (green). The cells were co-stained with DAPI (blue) for nuclei presentation. Magnification, 20×; Scale bar = 20 μm. Both of Western blot and immunofluorescence staining data demonstrated that CD13 receptors are overexpressed in HT-1080 cells, but not in MCF-7 cells.