Harnessing competing endocytic pathways for overcoming the tumor-blood barrier: MRI and NIR imaging of bifunctional contrast media

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Supplementary Methods

Western blot analysis: MLS cells lysates were prepared in ice-cold RIPA buffer and fractionated by SDS-PAGE. Proteins were eletrophoretically transferred from the SDS-polyacrylamide gel to a nitrocellulose membrane (Protran BA 85, Schleicher & Schuell). The membrane was blocked (in 2% BSA in 10 mM Tris-buffered saline containing 0.05% Tween (TBST); overnight,(4 °C) followed by incubation with rabbit anti-caveolin1 antibody (1μg/ml TBS) for 2h at 25 °C. The membrane was then washed three times in TBST, incubated with an HRP-conjugated goat anti-rabbit antibody (1:10,000 in TBS) and re-washed. The protein bands were visualized by homemade ECL.

Synthesis of Daidzein-BSA-EuDTTA**:** EuDTTA (1 mg dissolved in 200 μl of HPLC water) was added to Daidzein-BSA (2 mg) or BSA (2 mg) in 2 ml carbonate buffer 50mM, pH 9.8. The reaction was stirred overnight. Unreacted europium chelate was removed by centrifugation using Microcon-30 centrifugal filters (Millipore, Bedford,

MA). The labeled proteins were diluted to 1mg/ml PBS and stored at 4^0 C until use. The Europium fluorescence in the conjugates was determined using a time-resolved fluorescence plate reader (Victor; Perkin-Elmer, USA).

In vivo biodistribution: CD-1 nude mice were inoculated subcutaneously with $2.5 \cdot 10^6$ MLS tumor cells. After tumor development the mice were injected with BSA-Eu Chelate (i.v.; 0.34 mg equivalent to 100000 counts of europium in 0.1 ml PBS/mouse; n=3) or daidzein-BSA-Eu chelate (0.24 mg equivalent to 100000 counts of europium in 0.1 ml PBS/mouse; n=5). The Eu chelates were used here due to their chemical similarity to the Gd chelates used for MRI. After 24 and 48h the mice were sacrificed, the tumors and various tissues were removed. The tissues were subsequently homogenized in RIPA buffer [(20 mM Tris, pH 7.4, 137 mM NaCl, 10% glycerol, 0.5% (wt/vol) sodium deoxycholate, 0.1% (wt/vol) sodium dodecyl sulfate (SDS), 1% Triton X-100, 2 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail], and centrifuged in an airfuge. The protein content in the supernatants was determined using Bradford. The supernatants were then used for biodistribution studies.

For biodistribution studies an aliquot (20 μg) from the supernatant was added to microtiter plate. Enhancement solution (200 μl) was then added to each aliquot, and the plate was shaken for 15 minutes on a plate shaker. The Europium signal in the wells was then measured by time resolved fluorescence using the Victor (Perkin-Elmer, USA).

MRI analysis of BSA overload: Tumor bearing mice were injected iv with BSA-GdDTPA (12 mg in 200 µL PBS/mouse) or daidzein-BSA-GdDTPA (12 mg in 200 µL PBS /mouse)) or with combination of daidzein-BSA-GdDTPA and BSA-FAM.

Supplementary Figures:

Figure S1

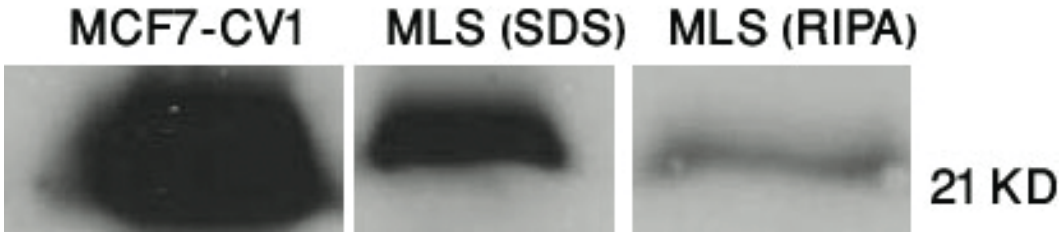

Figure S1: CAV-1 expression by ovarian cancer cells

Western blot analysis of human ovarian carcinoma cells probed with α-cav-1 antibody.

Figure S2

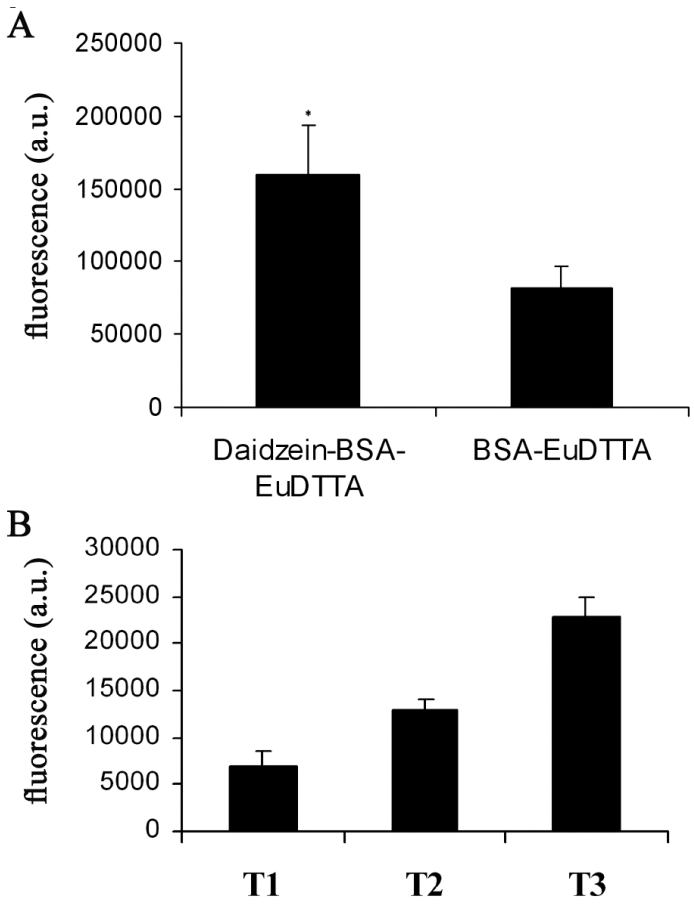

Figure S2: Biodistribution: MLS tumor bearing mice were administered intravenously with BSA-Eu chelate or Daidzein-BSA-Eu chelate. After 24 or 48h the mice were euthanized and the tumors and various organs were removed and processed (as described in Materials and Methods section for biodistribution and Western blot analysis) the fluorescence signal due to Eu in the various extracts was measured by time resolved fluorescence. A) Europium signal 24h after injection (n=3-5). B) Eu fluorescence 48h after injection (T1-injected with BSA- Eu, T2 and T3 – injected with daidzein-BSA-Eu). P=0.015.

Figure S3

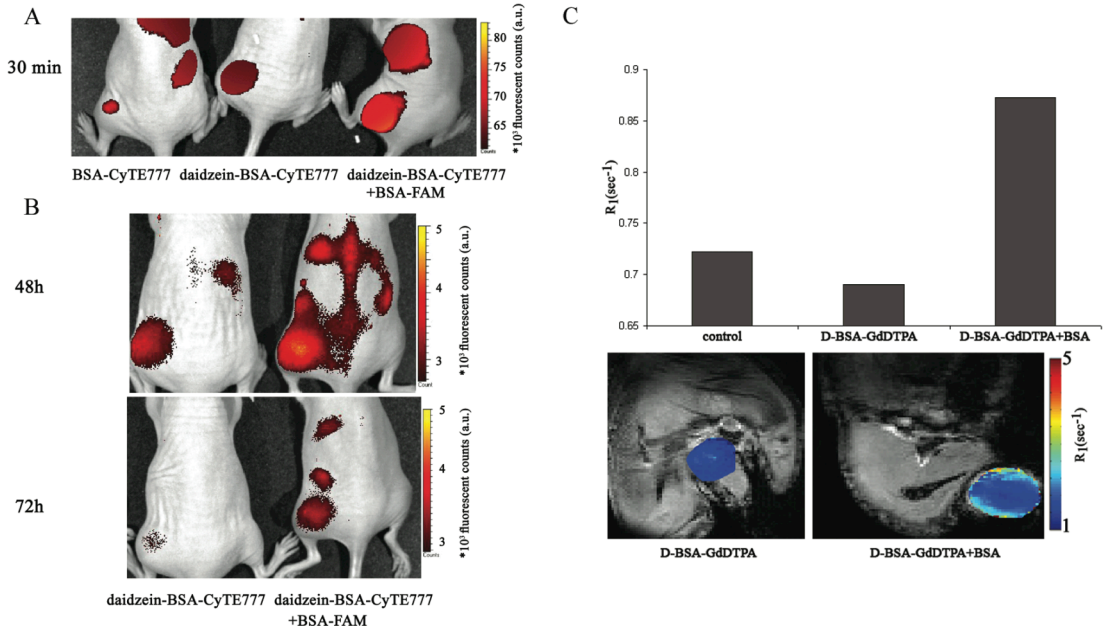

Figure S3: In vivo imaging of the interaction between albumin and daidzein on tumor targeting: MLS tumor bearing mice were administered intravenously with 1) BSA-CyTE-777; 2) daidzein-BSA-CyTE-777; 3) daidzein-BSA-CyTE-777 + BSA-FAM. A) NIR image 30 minutes after injection. B) Comparison between NIR signal distribution in mice administered daidzein-BSA-CyTE-777 and mice administered daidzein-BSA-CyTE-777 + BSA-FAM 48 and 72h after injection. C) MRI analysis of the impact of BSA overload on targeted delivery of daidzein-BSA-GdDTPA. MLS bearing mice were administered intravenously with BSA-FAM (2mg), daidzein-BSA-GdDTPA (4mg) or both. Longitudinal relaxation was monitored by MRI at 9.4T. Top) ROI analysis of MLS tumors in mice 24h after injection of contrast material. Bottom) T1 weighted MRI images (grayscale) and R_1 maps (color overlay) 24h after injection.