Supplementary Information

Novel linear peptides with high affinity to αvβ3 integrin for precise tumor identification

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Supplementary Figure S1. 3D view of the stable conformation between peptide and integrin αvβ3 after 20 ns dynamic simulation. (αv domain: yellow; β3 domain: purple; peptides: green)
Supplementary Figure S2. Mass spectrum and high performance liquid chromatography of synthesized peptides (RWr (A) and RWrNM (B)). The molecular weight of RWr was 516.5 and the purity was up to 98.79%. The molecular weight of RWrNM was 761.91 and the purity was up to 99.39%.
Supplementary Figure S3. Targeting ability of the peptides in MDA-MB-231 cells. (A) Laser confocal imaging of cellular uptake of the peptides conjugated with RhB and the cellular uptake after c(RGDyK) blocking. (B) Comparison of average fluorescent intensity in MDA-MB-231 cells in the experiment and blocking groups.
Supplementary Figure S4. Time course of the cellular uptake of negative control peptide (NWMrR) in U87MG cells, and the cellular uptake of the negative control peptide after c(RGDrK) blocking. Scale bar: 40 µm
Supplementary Figure S5. The relative avβ3 integrin expression in U87MG, MDA-MB-231, A549, HepG2 and L02 cells by western blot.
**Supplementary Figure S6.** The cellular uptake of RWtNM in (a) U87MG cells, (b) U87MG cells pre-incubated with mAbs against αvβ5 and (c) U87MG cells pre-incubated with mAbs against α5β1; The cellular uptake of c(RGDyK) in (d) U87MG cells, (e) U87MG cells pre-incubated with mAbs against αvβ5 and (f) U87MG cells pre-incubated with mAbs against α5β1.
Supplementary Figure S7. Inhibition effects of RWrNM, RWr, c(RGDyK) peptides, PTX and Sunitinib on the migration of MDA-MB-231, HepG2 and L02 cells, respectively.
Supplementary Figure S8. Inhibition effects of RWrNM, RWr, c(RGĐyK) peptides, PTX and Sunitinib on the invasion of MDA-MB-231, HepG2 and L02 cells, respectively.
Supplementary Figure S9. Serum stability of the three peptides (RWr, RWrNM and c(RGĐyK)) in 24 hours examined by liquid chromatography. Mobile phases: Buffer A:0.1% TFA in 100% water(v/v), Buffer B:0.1% TFA in 80% acetonitrile + 20% water(v/v). The gradients of Buffer B in 20 minutes: 25-45% (RWr), 30-50% (RWrNM) and 49-69% (c(RGĐyK)). Retention time: 9.076 minutes (RWr), 10.827 minutes (RWrNM) and 8.537 minutes (c(RGĐyK)).
**Supplementary Figure S10.** Mass spectrum (A) and high performance liquid chromatography (B) of peptide-Dox conjugation.
Supplementary Figure S11. Antitumor efficacy of RWr, c(RGDyK) and RWrNM conjugated with Dox on HepG2-tumor-bearing mice. (A) Images of the tumors in different treatment groups obtained after the 15-days therapy. (B) Tumor growth curves of the HepG2-tumor-bearing mice after intravenous injection with saline, Dox, RWr-Dox, c(RGDyK)-Dox and RWrNM-Dox. (C) Body weight of mice during different peptide-Dox treatments in 15 days. (D) Survival rate of mice in different peptide-Dox treatment groups.
Supplementary Figure S12. In vitro cytotoxicity of Dox, c(RGDyK)-Dox, RWr-Dox and RWrNM-Dox toward U87MG cells and HepG2 cells. Data points represent mean ± SD (n=6).