Electronic Supplementary Information

A novel Buthus martensii Karsch chlorotoxin derivative for glioma SPECT imaging

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Part of the experimental details

Preparation of peptides

According to the amino acid sequence of BmK CT-his that is CGPCFTTDANMARKCRECCGGIGKCFGPQCLCNRIHHHHHH (Cys1-Cys19, Cys4-Cys26, Cys16-Cys31 and Cys20-Cys33), the disulfide bridges were formed using four different methods, respectively. The first one (Cys1-Cys19) was prepared by air oxidation. The cysteine side chain was protected using triphenylmethyl (Trt) and deprotected via acetic acid. After deprotection, clean air was fed to oxidize the cysteine for the disulfide bridge formation. The second one (Cys4-Cys26) was synthesized through iodine oxidation. The cysteine was protected by acetamidomethyl (Acm), and the disulfide bridge was formed during the Cys deprotection. Similarly, tert-Butyl (tBu) and dibenzosuberyl (Dbs) protection strategies were adopted to the remaining two disulfide bridges (Cys16-Cys31, and Cys20-Cys33), respectively, and trifluoroacetic (TFA) and TFA containing 10% DMSO were used as corresponding deprotection strategies. The BmK CT-D peptide was also synthesized using the same method. Purification was needed in each step to remove the excess reagent and impurities. The final products were characterized using mass spectrometry and high performance liquid chromatography.

Animal model

The C6 glioma models were established by subcutaneous injection of 5×10^6 cells (100 μL) in the right flanks of each mouse. About 3 weeks post-injection, the tumor nodules reached a diameter of 0.8-1.2 cm, and could be used for animal experiments.

Radiolabeling

In this study, TLC plates were employed to test the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex using the methanol containing 1% HCl as the mobile phase. The colloidal ^{99m}Tc and free $^{99m}TcO^{4-}$ have retention factors (R_f) of 0-0.2 and 0.8-1.0, respectively, while the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex exhibits a broad peak with an average R_f = 0-0.4. Meanwhile, the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex was evaluated by ITLC-SG papers using the citrate buffer as the mobile phase. In this system, the colloidal ^{99m}Tc has a R_f of 0-0.2, while both the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex and $^{99m}TcO^{4-}$ have a R_f of 0.8-1.0. Then the RCP of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex could be defined as a percentage of total ^{99m}Tc radioactivity on the plates and papers. When its RCP was above 95%, the formed $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex was applied for the radiolabeling of BmK CT. Similarly, the RCP of ^{99m}Tc-BmK CT was analyzed by ITLC-SG papers using the citrate buffer mobile phase. The ^{99m}Tc-BmK CT had a R_f of 0-0.2, while the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ complex has a R_f of 0.8-1.0. If its RCP was above 95%, the ^{99m}Tc-BmK CT could be used for further studies directly.

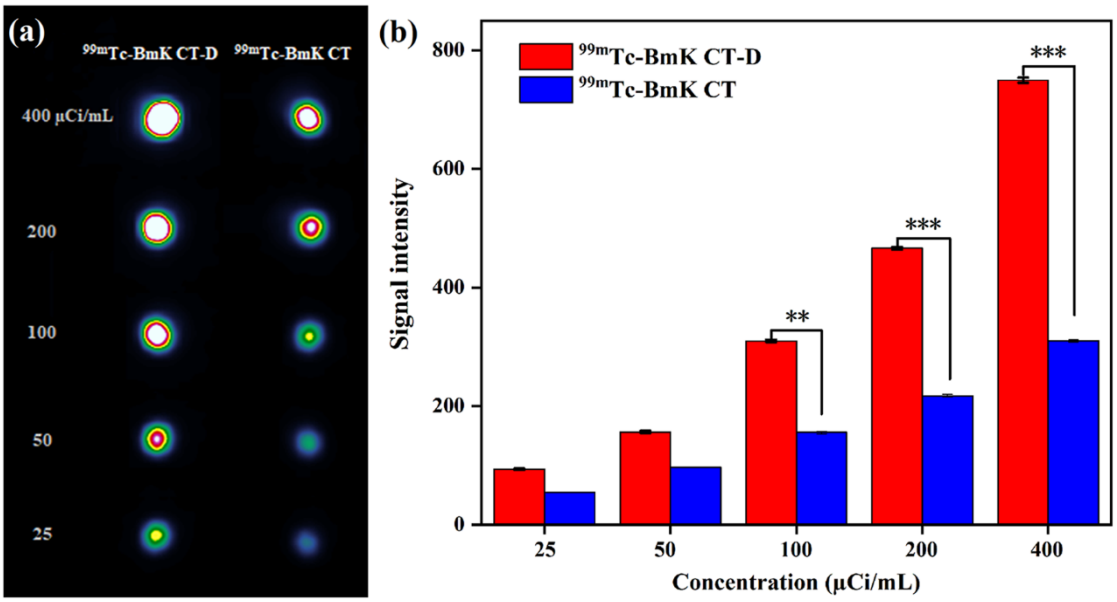

Fig. S1 SPECT images of C6 cell incubated with ^{99m}Tc-BmK CT and ^{99m}Tc-BmK CT-D at the different radioactivity concentrations (25, 50, 100, 200 and 400 μCi/mL) for 4 h (a), and the SPECT signal intensities were quantitative analyzed by the regions of interest (ROIs) for comparsion (b).