The effect of the attachment of a penetration accelerating sequence and the influence of hydrophobicity on octaarginine-mediated intracellular delivery

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Supporting Information

Peptide synthesis

All of the peptides used were chemically synthesized by Fmoc (9-fluorenylmethyloxycarbonyl) solid-phase peptide synthesis on a Rink amide resin as already reported.[16] Deprotection of the peptide and cleavage from the resin were conducted by treatment with a trifluoroacetic acid/ethanedithiol mixture (95:5) at room temperature for 3 h followed by reverse-phase high performance liquid chromatography (RP-HPLC) purification. Fluorescent labeling of the peptides was conducted by treatment with Alexa 488 C_5 maleimide sodium salt (Invitrogen) in a dimethylformamide (DMF)/methanol mixture (1:1) for 1.5 h followed by HPLC purification. For the preparation of the PEG5000-PasΔPKR8, PEG10000-PasΔPKR8, and PEG30000-PasΔPKR8 conjugates, Alexa-labeled PasΔPKR8 peptides were treated with bis(sulfosuccinimidyl)suberate (BS^3) cross-linker (Pierce) as previously reported[23] and conjugated to propylamine-functionalized polyethylene glycols (amino-PEGs, NOF). The structure of the products was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS). Actual sequences of the synthesized peptides are as follows (*italics*: D-amino acids): R8, RRRRRRRRC-amide; PasR8, FFLIPKGRRRRRRRRC-amide; F4R8(Alexa), FFFFGRRRRRRRRC-amide; R8(Alexa), RRRRRRRRC(Alexa)-amide; PasR8(Alexa), FFLIPKGRRRRRRRRC(Alexa)-amide; LILIR8(Alexa), LILIGRRRRRRRRC(Alexa)-amide; F2R8, FFGRRRRRRRRC(Alexa)-amide; F4R8(Alexa), FFFFGRRRRRRRRC(Alexa)-amide; F6R8(Alexa),

FFFFFFGRRRRRRRRGC(Alexa)-amide; K8(Alexa), KKKKKKKKGC(Alexa)-amide; F4K8(Alexa), FFFFGKKKKKKKKGC(Alexa)-amide; PasΔPKR8, FFLIGRRRRRRRRGC(Alexa)-amide; p53C', *KKHRSTSQ*GKKSKLHSSHARSG-amide; dR8-p53C', *RRRRRRRR*GKKHRSTSQGKKSKLHSSHARSG-amide; dPasR8-p53C', *FFLIPKGRRRRRRRR*GKKHRSTSQGKKSKLHSSHARSG-amide; dF4R8-p53C', *FFFFGRRRRRRRR*GKKHRSTSQGKKSKLHSSHARSG-amide; dR12-p53C', *RRRRRRRRRRRR*GKKHRSTSQGKKSKLHSSHARSG-amide; $p27^{Kip1}$C, KKPGLRRRQT-amide; R8-$p27^{Kip1}$C, RRRRRRRRGGKKPGLRRRQT-amide; PasR8-$p27^{Kip1}$C, FFLIPKGRRRRRRRRGGKKPGLRRRQT-amid; F4R8-$p27^{Kip1}$C, FFFFGRRRRRRRRGGKKPGLRRRQT-amide; PAD, *KLAKLAKKLAKLAK*-amide; R8-PAD, RRRRRRRRGG*KLAKLAKKLAKLAK*-amide; PasR8-PAD, FFLIPKGRRRRRRRRGG*KLAKLAKKLAKLAK*-amide; F4R8-PAD, FFFFGRRRRRRRRGG*KLAKLAKKLAKLAK*-amide. MALDI-TOF MS: R8, 1427.6 [calcd. for $(M+H)^+$: 1427.7]; PasR8, 2230.5 [calcd. for $(M+H)^+$: 2230.7]; F4R8, 2073.2 [calcd. for $(M+H)^+$: 2073.5]; R8(Alexa), 2125.6 [calcd. for $(M+H)^+$: 2125.4]; PasR8(Alexa), 2929.5 [calcd. for $(M+H)^+$: 2928.4]; LILIR8(Alexa), 2635.5 [calcd. for $(M+H)^+$: 2635.1]; F2R8(Alexa), 2476.9 [calcd. for $(M+H)^+$: 2476.8]; F4R8(Alexa), 2771.5 [calcd. for $(M+H)^+$: 2771.1]; F6R8(Alexa), 3065.9 [calcd. for $(M+H)^+$: 3065.5]; K8(Alexa), 1901.2 [calcd. for $(M+H)^+$: 1901.3]; F4K8(Alexa), 2547.3 [calcd. for $(M+H)^+$: 2547.0]; PasΔPKR8, 2703.1 [calcd. for $(M+H)^+$: 2703.1]; p53C', 2432.0 [calcd. for $(M+H)^+$: 2432.8]; dR8-p53C', 3738.8 [calcd. for $(M+H)^+$: 3739.3]; dPasR8-p53C', 4542.3 [calcd. for $(M+H)^+$: 4542.3]; dF4R8-p53C', 4384.7 [calcd. for $(M+H)^+$: 4385.0]; dR12-p53C', 4364.0 [calcd. for $(M+H)^+$: 4363.4]; $p27^{Kip1}$C, 1238.9 [calcd. for $(M+H)^+$: 1239.5]; R8-$p27^{Kip1}$C, 2603.2 [calcd. for $(M+H)^+$: 2603.1]; PasR8-$p27^{Kip1}$C, 3405.7 [calcd. for $(M+H)^+$: 3406.1]; F4R8-$p27^{Kip1}$C, 3248.3 [calcd. for $(M+H)^+$: 3248.8]; PAD, 1524.0 [calcd. for $(M+H)^+$: 1524.0]; R8-PAD, 2887.7 [calcd. for $(M+H)^+$: 2887.6]; PasR8-PAD, 3690.8 [calcd. for $(M+H)^+$: 3690.6]; F4R8-PAD, 3533.3 [calcd. for $(M+H)^+$: 3533.3].

Cell culture

The human cervical cancer-derived HeLa cells were maintained in α-minimum essential medium with 10% heat-inactivated calf serum [α-MEM(+)]. A subculture was performed every 3-4 days. The human malignant glioma cell line T98G (expressing the M237I mutant p53)[36] were provided by Health Science Research Resources Bank (Osaka, Japan), and were maintained in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum [DMEM(+)], 100 U/mL penicillin and 100 μg/mL streptomycin. All cell lines were grown on 100-mm dishes and incubated at 37 °C under 5% CO_2 to approximately 70% confluence.

Confocal microscopy

HeLa cells (2.0×10^5) were plated on 35-mm glass-bottomed dishes (Iwaki) and cultured in α-MEM(+) for 48 h. After complete adhesion, the culture medium was exchanged, and the cells were then incubated with 150 μL of α-MEM(+) on a micro chamber (5% CO_2, 37 °C) attached on the stage of a confocal microscope for 10 min. Then the fluorescently labeled peptides (final concentration, 10 μM) were added to the medium and the distribution of peptides was analyzed over time using a confocal laser scanning microscope (CLSM) FV1000 (Olympus) equipped with a 40× objective (dry, NA 0.95).

Reference

[36] Wischhusen, J.; Naumann, U.; Ohgaki, H.; Rastinejad, F.; Weller, M. CP-31398, a novel p53-stabilizing agent, induces p53-dependent and p53-independent glioma cell death. *Oncogene* **2003**, *22*, 8233-8245.

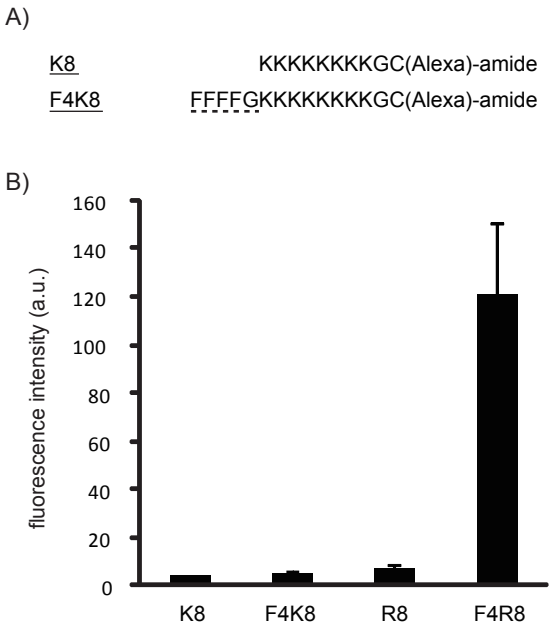

Figure S1. (A) Structures of Alexa488-labeled K8 and F4K8. (B) Cellular uptake of K8(Alexa) and F4R8(Alexa) in comparson with the corresponding R8 derivatives analyzed by flow cytometry. Cell line, HeLa; peptides, 1 μM; incubation, for 15 min at 37°C in PBS. Means ± standard deviation (s.d.) of three experiments are shown.

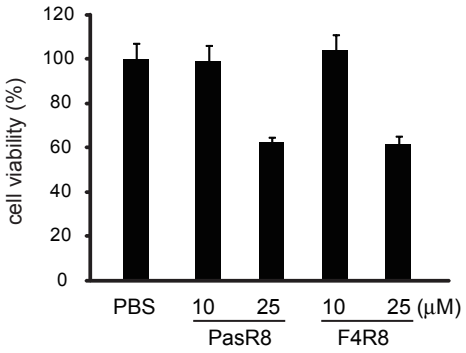

Figure S2. Cell viability of HeLa cells treated with PasR8 and F4R8 for 24 h at 37°C in α-MEM(+). Means ± s.d. of three experiments are shown.