SUPPORTING INFORMATION

Enhanced Cellular Uptake of Short Polyarginine Peptides through Fatty Acylation and Cyclization

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TITLE RUNNING HEAD. Fatty Acylated Cyclic Polyarginine as Molecular Transporter

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Cytotoxicity Assays of W_4-[R_5] and W-dodecanoyl-[R_5]

The cytotoxicity assays of cyclic peptide [R_5] having tryptophan residues were carried out against CCRF-CEM, SK-OV-3, and HEK 293T cell lines. The assay protocol was the same as described in the experimental section. The cytotoxic assay results are shown in Figure S1. The tryptophan modified peptides, W_4-[R_5] and W-dodecanoyl-[R_5], showed ≥89% cell viability up to concentration of 10 μM against CCRF-CEM, ≥74% viability up to 15 μM against SK-OV-3, and ≥ 86% viability up to 10 μM against HEK 293T cell lines.

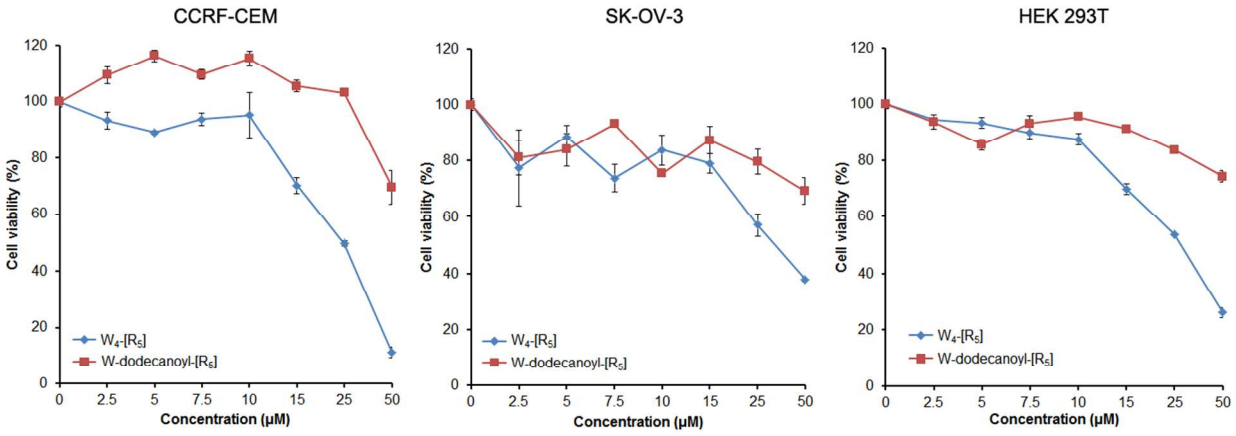

Figure S1. Cytotoxicity assay of W_4-[R_5] and W-dodecanoyl-[R_5] having tryptophan residues at various concentrations against CCRF-CEM, SK-OV-3, and HEK 293T after 24 h.

Comparison of Serum Stability of Linear and Cyclic Acylated Polyarginine Peptides. Serum stability assay was performed with linear dodecanoyl-(R_5) and cyclic dodecanoyl-[R_5] in mammalian serum. The assay protocol was adapted from serum stability study of peptides.[1,2] Fetal bovine serum (FBS, 25%) was prepared in water and centrifuged at 13,000 rpm for 10 min, and the supernatant was collected and used for sample preparation. Peptide stock solution (1

mg/mL) was prepared in water, and 10 μL of this aqueous stock solution was added to 100 μL of 25% FBS. The samples were incubated at 37 °C. Samples were taken at 0, 1, 2, 4, and 9 h, and serum protein were precipitated by addition of 20 μL of 15% trichloroacetic acid aqueous solution. The samples were kept at 4 °C for at least 15 min before centrifugation at 13,000 rpm for 10 min. The supernatant was analyzed by an HPLC equipped with a reverse-phase C_{18} column (5 μm, 4.6 × 150 mm) at UV 215 nm wavelength. Water with 0.1% trifluoroacetic acid (TFA) was used as mobile phase A and acetronitrile with 0.1% TFA was used as mobile phase B. A gradient with 0-100% acetonitrile with 0.1% TFA was applied to the HPLC system with a flow rate at 1.2 mL/min. The relative % peptide remaining amount was calculated by setting 0 h sample as 100%. The stability assay results were presented in Figure S2. The cyclic peptide was remained stable by 92% after 9 h incubation with 25% FBS, but the linear peptide was degraded completely even after 1 h. Thus, cyclic acylated polyarginine peptide is significantly more stable than the linear acylated polyarginine peptide against mammalian serum.

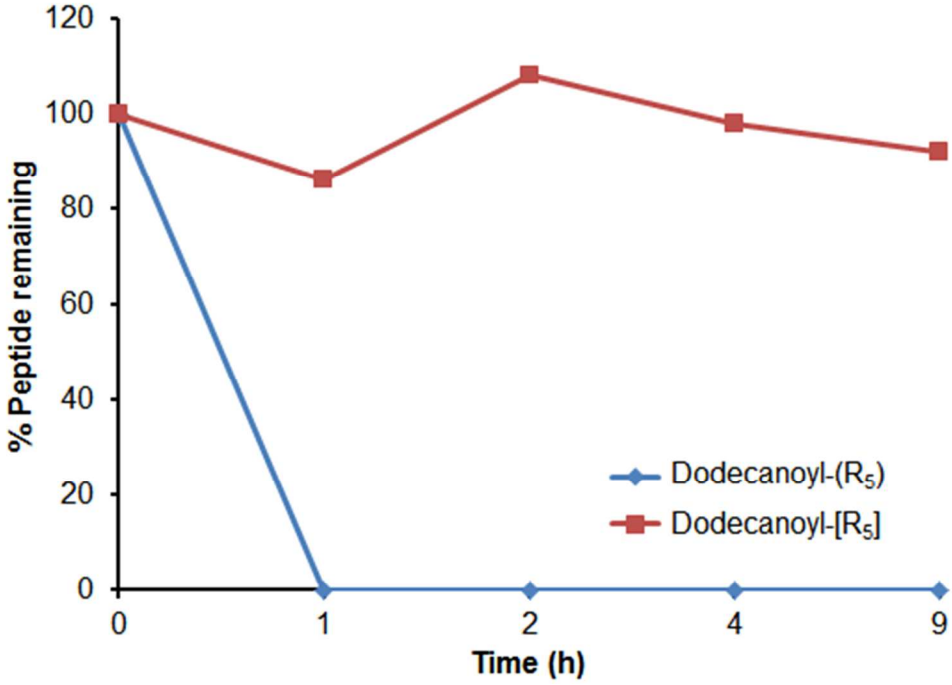

Figure S2. Serum stability of linear dodecanoyl-(R_5) and cyclic dodecanoyl-[R_5] peptides in fetal bovine serum. Samples were incubated at 37 °C and taken at 0, 1, 2, 4, and 9 h.

Cell Culture. Human ovarian adenocarcinoma (SK-OV-3), breast (MCF-7), leukemia (CCRF-CEM), and embryonic kidney (HEK 293T) cells were purchased from American Type Culture Collection. The SK-OV-3 and HEK 293T cells were grown in eagle's minimum essential medium (EMEM), and RPMI-1640 medium (ATCC, Manassas, VA) was used for CCRF-CEM cells. MCF-7 cells were grown in DMEM medium (Sigma). All medium were supplemented with fetal bovine serum (FBS, 10%) and penicillin-streptomycin solution (1%, 10,000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2 at 37 °C.

Time-Dependent Antiproliferative Assay. The inhibitory potency of Dox against MCF-7 cells proliferation was evaluated in the presence and absence of [R_6]-C_{12} by MTS assay. All cells were plated 24 h prior to the experiment in 96-well plates (5000 cells in 0.1 mL of appropriate growth medium per well) at 37 °C. Cells were incubated with Dox alone (5 μM) and Dox-loaded [R_6]-C_{12} (5 μM) for 1 h. After 1 h incubation, the treatments were replaced by fresh complete medium.

The cells were kept in an incubator for 24−72 h. The cells with no treatment were used as a control in each experiment. After 24 h, 48 h, and 72 h incubation, 20 μL of MTS solution was added and incubated for 2 h. The absorbance of the formazan product was measured at 490 nm using a microplate reader. The percentage of cell viability was calculated as (OD value of untreated cells − OD value of treated cells)/OD value of untreated cells × 100%.

References

(1) Cudic, M.; Condie, B. A.; Weiner, D. J.; Lysenko, E. S.; Xiang, Z. Q.; Insug, O.; Bulet, P.; Otvos, L., Jr. *Peptides* **2002**, *23*, 2071–2083.

(2) Nguyen, L. T.; Chau, J. K.; Perry, N. A.; de Boer, L.; Zaat, S. A. J.; Vogel, H. J. *PLoS ONE* **2010**, *5*, e12684.