Supplement materials

Materials and methods

Assessment of cell-surface phosphatidylserine exposure and plasma membrane integrity using FITC-annexin V and propidium iodide (PI) staining

To evaluate the effects of temporin-1CEa on cell-surface phosphatidylserine (PS) exposure and plasma membrane integrity, A375 cells were seeded in a 24-well plate and incubated with various concentrations of temporin-1CEa (20–40 μM) or were left untreated (control) for 60 min. After treatment with peptides, the cells were stained with FITC-annexin V and PI according to manufacturer's instructions (FITC-Annexin V Apoptosis Detection Kit, BD Biosciences, USA). The cell-surface phosphatidylserine (PS) exposure and plasma membrane integrity were analyzed using FACSCanto flow cytometer (BD Biosciences). Cells that are considered viable are FITC-annexin V and PI negative (lower-left quadrant, Q3); cells with membrane lipid asymmetry and PS exposure are FITC-annexin V positive (lower-right quadrant, Q4); and cells with interrupted membrane integrity are both FITC-annexin V and PI positive (upper-right quadrant, Q2).

Cell membrane permeability assay using calcein AM and ethidium homodimer (EthD-1) staining (Live/Dead assay)

The cell membrane integrity and permeability were determined using the LIVE/DEAD® Viability/Cytotoxicity Assay Kit (Molecular Probes, Inc., USA), which is a two-color fluorescence assay with two probes that measure recognized parameters of cell viability, including intracellular esterase activity and plasma membrane integrity. An increased fluorescence intensity of EthD-1 or a decreased fluorescence intensity of calcein means enhanced membrane permeability and interrupted membrane integrity. A375 cells were seeded into 96-

well plates at 5×10^4 cells/mL. After treatment with temporin-1CEa (20–40 μM) or were left untreated (control) for 60 min, the medium was removed, and 20 μL of dye containing 2 μM calcein AM and 4 μM EthD-1 was then added and incubated for 30 min in the dark. The fluorescence intensity was distinguished by FACS analysis with Ex485nm/Em530nm for calcein and Ex530nm/Em645nm for EthD-1.

Morphological observation using electronic microscope

In order to assess the possible impacts of temporin-1CEa on cell membranes of A375 melanoma cells, the morphological changes of A375 cells after one hour temporin-1CEa treatment were examined by scanning electronic microscopy (SEM, KYKY-1000B, China) and transmission electron microscope (TEM, JEM-200EX, Japan) using standard protocols [Lin et al., 2003].

Peptides 3-D structure simulation

The peptide's 3-D structure was simulated using online server Mobyle@RPBS v1.5.1 (http://mobyle.rpbs.univ-paris-diderot.fr/) , which is a platform developed jointly by the Institut Pasteur Biology IT Center and the Ressource Parisienne en Bioinformatique Structurale (Thévenet et al. 2012).

Reference

V.C. Lin, R. Jin, P.H. Tan, S.E. Aw, C.T. Woon, B.H. Bay, Progesterone induces cellular differentiation in MDA-MB-231 breast cancer cells transfected with progesterone receptor complementary DNA, Am. J. Pathol. 162 (2003) 1781-1787.

P. Thévenet, Y. Shen, J. Maupetit, F. Guyon, P. Derreumaux, P. Tufféry, PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides, Nucleic Acids Res. 40 (2012) W288-293.

Figures legends

Suppl. Fig. 1. Temporin-1CEa induced loss of membrane integrity and phosphatidylserine exposure in A375 cell lines. Cells were incubated with various concentrations of temporin-1CEa for one hour and then were stained with Annexin-V-FITC/PI. Fluorescence intensity was determined using flow cytometry.

Suppl. Figu. 2. Temporin-1CEa induces enhancement of membrane permeability in A375cells using calcein AM/EthD-1 staining. Cells were exposed to various concentrations of temporin-1CEa or were left untreated for 60 min, then the medium was removed, and 20 µl of dye containing 2 µM calcein AM and 4 µM EthD-1 was added for 30 min in the dark. The fluorescence intensity was distinguished by FACS analysis. Each bar represents the mean value from three determinations with the standard deviation (SD).

Suppl. Fig. 3. Morphological changes of A375 cells upon one-hour exposure to temporin-1CEa. Scanning Electronic Microscopy (A) and Transmission Electronic Microscopy (B) evaluation of human melanoma A375 cells treated with temporin-1CEa.

Suppl. Fig. 4. Three-Dimensional (3-D) structure prediction of temporin-1CEa. The modelling was performed using online server Mobyle@RPBS v1.5.1. A. Color scheme of 3-D structure displayed by residues. B. Color scheme of 3-D structure displayed by second-structure (Red in α-helix).