Supporting Information

Nanoscale Tools To Selectively Destroy Cancer Cells

Pierre-Luc Boudreault[1], Mathieu Arseneault[1], François Otis[1], Normand Voyer[1]

[1]Département de chimie and Centre de recherche sur la fonction, la structure et l'ingénierie des protéines, Faculté des sciences et de génie, Université Laval, Québec, Canada, G1K 7P4

Biological activity assays

Blood samples were collected from a single healthy donor by venipucture and treated with EDTA solution (di-potassium salt). To collect the erythrocytes, samples were centrifuged at 7500 rpm for 5 min and washed 3 times with ice cold PBS buffer (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4). A 20% hematocrit solution was added to separate samples of the peptide (DMSO stock) in PBS buffer, diH_2O or PBS buffer and incubated for 30 min in a 37°C water bath after which a 50 µL sample from each sample was withdrawn and spun at 1000 rpm for 10 min. In a centrifuge tube containing 980 µL PBS buffer 20 µL of supernanent was added and the absorbance at 414 nm (l_{max}) was measured on a Perkin Elmer UV/Vis Spectrometer Lambda 2 with accompanying software. The percent hemolysis was determined by the equation: % hemolysis = $(100-((A_{100\%}-A)/(A_{100\%}-A_{0\%}))$ x 100.Where $A_{0\%}$ was the absorbance of erythrocytes treated with DMSO, $A_{100\%}$ was the absorbance of erythrocytes incubated in diH_2O and A was the absorbance of erythrocytes incubated with peptide.

Flow cytometry

PC-3 cells were cultured in Ham's media, 10% FCS at 37°C, 5% CO_2 following ATCC protocols. At 90% confluency the cells were trypsinized by first washing the cells twice with PBS buffer then incubating the cells at 37°C, 5% CO_2 with 2 mL of 2.5X trypsin until the cells detached. Fresh media (8 mL) was added and the cell sample was centrifuged for 5 min at 4°C and resuspended in TES buffer to obtain a cell count of 1 x 10^5 cells/mL. The peptide (in a DMSO stock) was added to 0.5 mL of the previously mentioned cell solution and incubated for 2 h at 37°C, 5% CO_2. The samples were pelleted through centrifugation at 1500 rpm for 5 min and resuspended in binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4). Annexin V-FITC (5 µL) and the nuclear stain 7AAD (7-amino-actinomycin D; 500 ng/mL) were added to the samples and then incubated for 15 min at 37°C, 5% CO_2.

Annexin V-FITC binds to PS on the outer membrane plasma surface. Necrotic cells, as well as those cells in the advanced stages of apoptosis, have permeablized membranes and allow 7AAD to stain the cell nucleus. Immediately after staining, flow cytometry was performed on a Beckman Coulter Cytomics FC 500 MPL (Fullerton, CA) flow cytometer.

HeLa cell peptide uptake assays:

HeLa cells were cultivated in a 3 cm Petri dish. The following quantities were used for every cell culture described here: 2ml of Iscove's Modified Dulbecco's Medium (IMDM) from Invitrogen, 0.2ml of Fetal Bovin Serum (FBS) from Hyclone and 75µL of Penicillin-Streptomycin mix from Invitrogen. A sterilized microscope cover glass was in the Petri. Prior to transfer, cells were washed with Phosphate-buffered saline (PBS) hybridome and then Trypsine from Invitrogen was used to transfer the cells from the previous culture dish. The cells were then centrifuged at 1000 rpm for 3 minutes. The supernatant was removed and replaced with 5 mL of IMDM. After shaking this solution thoroughly to suspend the cells, we put 0.2 mL of it in every Petri used. The cells were incubated for 24h. A solution of DMSO containing either Boc-14mer-OH or FITC-caproic acid-OH was dissolved in IMDM. The solution was vigorously shaken for several minutes. An aliquot of this new solution was then put in the Petri to obtain a final concentration of 100µM or 50 µM. Cells were incubated for another 48h.

Fixation procedure started with removal of IMDM and gentle washing of the cells with PBS. Cells were fixed with Formaldehyde (Aldrich) for 20 min. and then washed again with PBS. A solution containing 0.5% of Bovin Serum Albumin (BSA) in PBS was used on the cells for 30 min., followed by another washing with PBS. Nuclei staining was done with a DAPI solution in PBS for 20 minutes. The cells were washed with PBS three times before mounting the cover glasses on microscope glass slides with a mix of 90% glycerol and 10% p-phenylenediamine (Aldrich).

Phase contrast microscopy were performed using a Leitz Aristoplan epifluorescence microscope (Leica Microsystems Canada, Richmond Hill, Ontario, Canada) with a 488-nm filter for FITC. Representative cell fields were captured using a black-and-white digital camera (Dage-MTI, Michigan City, IN) and Bioquant NOVA software (Bioquant-R&M Biometrics, Nashville, TN). All images reported here were captured at 400x zoom.