Selective membrane poration by the anticancer peptide *killer*FLIP-E: a spectroscopic study

Zahra Vaezi,[a] Sara Bobone,[a] Daniela Roversi,[a] and Lorenzo Stella[a,*]

[a] Department of Chemical Sciences and Technologies, University of Rome Tor Vergata, 00133 Rome, Italy

* E-mail: stella@stc.uniroma2.it

Keywords: membrane active peptides, membrane permeabilization, cancer cells, fluorescence

*Killer*FLIP-E is a recently synthesised artificial peptide, originally designed to interfere with apoptosis signalling by joining a short sequence derived from the C-terminal domain of c-FLIP (an anti-apoptotic protein) and an N-terminal TAT sequence, added to facilitate cell uptake (in bold in the following sequence): **GRKKRRQRRR**FFWSLCTA [1]. The peptide was demonstrated to be strongly active and selective, killing cancer cells in the low micromolar range, while not being toxic. However, its mechanism of action was surprisingly not linked to apoptosis. The observation of peptide-induced loss in plasma membrane integrity, and the similarity of the peptide physico-chemical properties to those of membranolytic antimicrobial peptides (cationic charge, amphiphilicity) [2] led to the hypothesis that its mechanism of action might be related to membrane perturbation [1].

To verify if membrane disruption is indeed the first effect of peptide interaction with the cell, or is just a secondary event of cell death caused by another mechanism, we studied peptide permeabilization of artificial membranes (Figure 1). The peptide was able to cause leakage of liposome contents, supporting the view that membrane perturbation is indeed its mechanism of action. These experiments confirmed also *killer*FLIP-E selectivity, since leakage was significant only in membranes containing anionic phosphatidyl-serine lipids, which are present on the external surface of cancerous cells [3]. Peptide membrane association studies, exploiting the intrinsic fluorescence of the peptide, showed that this selectivity was due to a higher affinity of the peptide for charged than for neutral lipid bilayers (Figure 2). However, previous studies performed in our laboratory, demonstrated that charge is not sufficient to determine the selectivity of membrane-active peptides [4]. Due to its amphipathic structure, *killer*FLIP-E is likely to aggregate in water. This is indeed the case, as demonstrated by light scattering experiments (Figure 3), indicating a threshold concentration between 0.1 μM and 0.5 μM. Leakage experiments performed at different peptide concentrations showed that selectivity is much higher at [*killer*FLIP-E] = 1 μM than at 0.1 μM (Figure 4). This finding indicates that aggregation is an important determinant of peptide selectivity. A possible explanation is that in the peptide micelles the apolar residues are screened from the solvent, reducing the hydrophobic driving force for binding to the neutral membranes of normal cells. On the other hand, the aggregates still tend to associate to the anionic membranes of cancer cells, driven by electrostatic attraction. If confirmed, these preliminary results will pave the way to a new principle in the rational design of selective anticancer peptides.

Acknowledgements: we gratefully acknowledge MIUR for financial support (PRIN project 2010NRREPL: Synthesis and biomedical applications of tumor-targeting peptidomimetics). We thank Dr. R. Khosravi-Far and B. Pennarun (Harvard Medical School, USA) for providing us with the *killer*FLIP-E peptide.

[1] B. Pennarun, G. Gaidos, O. Bucur, A. Tinari, C. Rupasinghe, T. Jin, R. Dewar, K. Song, M.T. Santos, W. Malorni, D. Mierke and R. Khosravi-Far, Cell Death Dis. 4 (2013) e894.

[2] G. Bocchinfuso, A. Palleschi, B. Orioni, G. Grande, F. Formaggio, C. Toniolo, Y. Park, K.S. Hahm and L. Stella. J. Pept. Sci. 15 (2009) 550-558.

[3] D. W. Hoskin, and A. Ramamoorthy. Biochim. Biophys. Acta 1778 (2008) 357–375.

[4] S. Bobone, G. Bocchinfuso, Y. Park, A. Palleschi, K. -S. Hahm, and L. Stella. J. Pept. Sci. 19 (2013) 758–769.

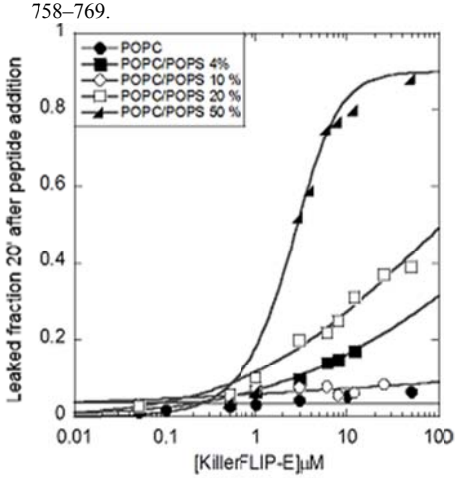

Figure 1. Peptide-induced leakage of carboxyfluorescein (CF) entrapped into liposomes of varying composition [Lipid]=50 μM.

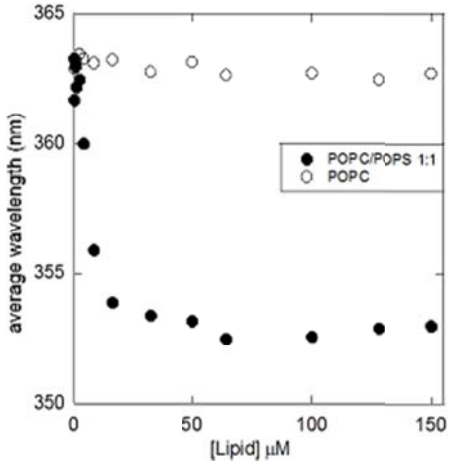

Figure 2. Peptide water-membrane partition as measured from the blue-shift in the emission spectrum of the Trp residue [Peptide] = 1.0 μM.

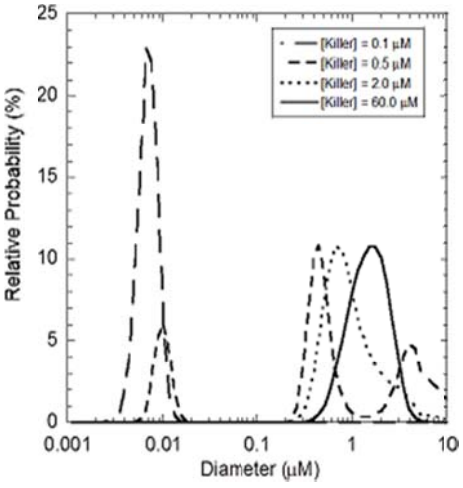

Figure 3. Light scattering measurements of the size of peptide aggregates.

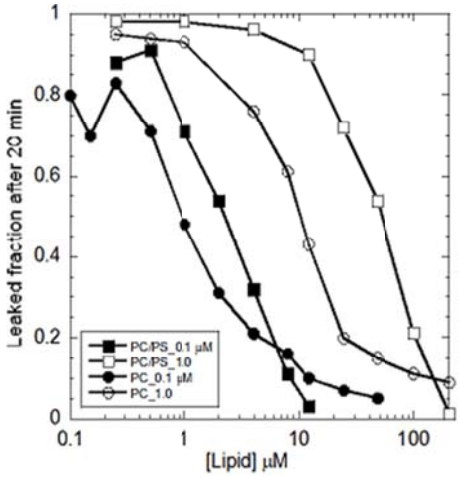

Figure 4. Effect of peptide concentration on its selectivity for membranes of different compositions.