135 Selecting vectors to image apoptosis by phage display technique

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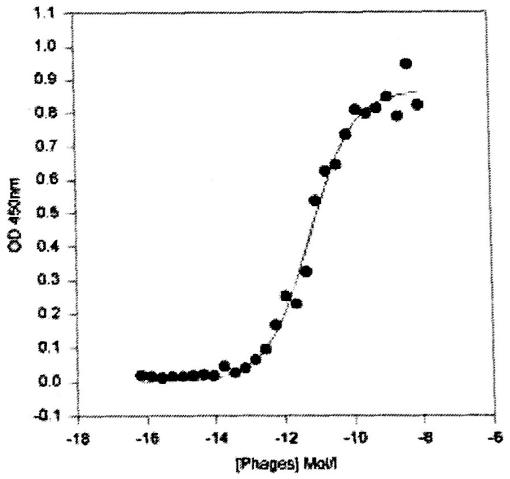
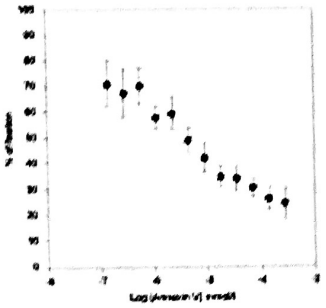
The Abstract

Introduction: Apoptosis is a physiological process that becomes pathologic either by overactivity or inhibition. A dedicated contrast agent evidencing pathologies where apoptosis takes place or would be useful to monitor antitumor therapies. Phage display is a new and powerful method to select peptides with high affinity for a given target like phosphatidylserine (PS) in this study. Subsequent coupling of the selected peptides with a magnetically active species would produce selective MRI contrast agents.
Methods: Biopanning was performed ex vivo, with a linear 6-mer library, on livers from a anti-fas-treated mice. One peptide (3E), was obtained by solid phase synthesis. Binding experiments were performed by ELISA to determine its apparent affinity constant. Competition ELISA was also carried out with annexin V. The new contrast agent obtained by grafting the synthetic peptide to USPIO was tested on cells culture. Apoptosis was induced on JURKAT cells by treatment with camptothecin. Treated and untreated cells were incubated with the vectorized USPIO ([Fe] = 4 mM) during 2h at 37°C. The image acquisition was performed at 4,7 T, with a spin echo sequence (TR/TE: 3000/15 ms, 24 echos).
Results: The Ka of clone called (3^E) obtained from the curve fixation (Fig. 1A) is 1.6 10^{11} mol/l. The competition between the synthetic peptide and the 3E-displaying phages bound to PS, as expressed by the half-maximal inhibition (IC 50), is equal to 217.7 nM (Fig. 1B). The same index in the case of annexin V is 31.6 nM. The in vitro MRI shows that the contrast agent specifically targets the apoptotic cells (fig.1D) demonstrating that the peptide does not loose its affinity for PS after coupling with USPIO.





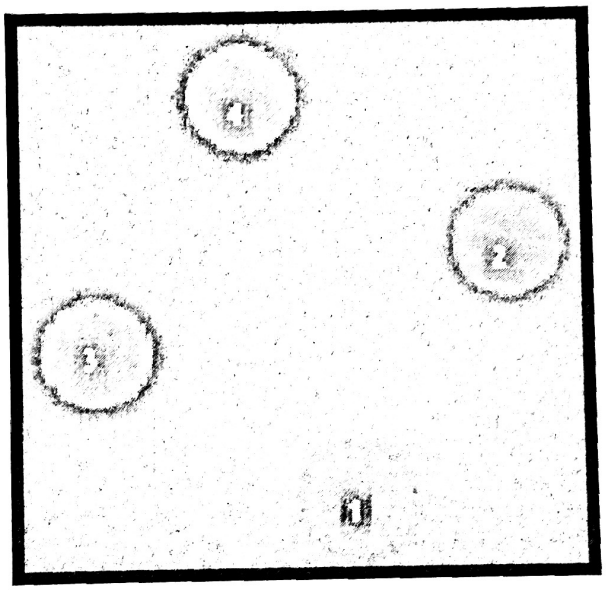


Fig. 1: Fixation curve of clone 3E (A), competition curves with annexinV (B)
Apoptotic (1) and healthy (2) cells incubated with USPIO-peptide, healthy (3) and apoptotic cells (4) incubated with USPIO (C).

Discussion and Conclusions: The phage display technique is a very promising strategy to select proper vectors to be branched to magnetic reporters in order to produce specific contrast agents for molecular imaging. In this study we have selected a hexapeptide to target apoptosis by MRI. This peptide was synthesized and branched to USPIO to give a new contrast agent which successfully distinguished apoptotic cells from healthy ones.