A Peptide Mimicking a Region in Proliferating Cell Nuclear Antigen (PCNA) Specific to Key Protein Interactions is Cytotoxic to Breast Cancer

Shanna J. Smith, Long Gu, Elizabeth A. Phipps, Lacey E. Dobrolecki, Karla S. Mabrey, Pattie Gulley, Kelsey L. Dillehay, Zhongyun Dong, Gregg B. Fields, Yun-Ru Chen, David Ann, Robert J. Hickey, and Linda H. Malkas

Molecular Pharmacology

Supplemental Information

Figure S1

	50 uM R9-caPep						100 uM R9-caPep						100 uM R9-scrambled					
	Annexin			PI			Annexin			PI			Annexin			PI		
	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h	4 h	8 h	24 h
Average (%)	6.2667	5.8667	31.2667	1.2667	2.5000	5.3667	11.3667	18.3667	35.9667	3.8000	12.8333	22.8333	3.1000	2.4333	3.7000	2.6667	2.7667	1.8667
Std Dev (%)	0.4041	1.8175	2.2502	0.2082	0.3000	1.3013	1.0970	1.7616	2.4420	0.3606	2.6026	1.1719	0.1732	0.3215	0.6557	0.8963	0.3512	0.2517
# Observations	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Summary of flow cytometry data from Figure 4A. Exponentially growing ($1x10^6$) MDA-MB-436 cells were treated with increasing concentrations of R9-cc-caPeptide or 100 μM R9-cc-scrambled up to 24 hours. Annexin V staining was then evaluated using flow cytometry, as detailed in the Materials and Methods section. The mean and standard deviation (Std Dev) of the %Annexin V and %Annexin V +PI positive cells, and number of observations for each time point are provided to supplement Figure 4A.

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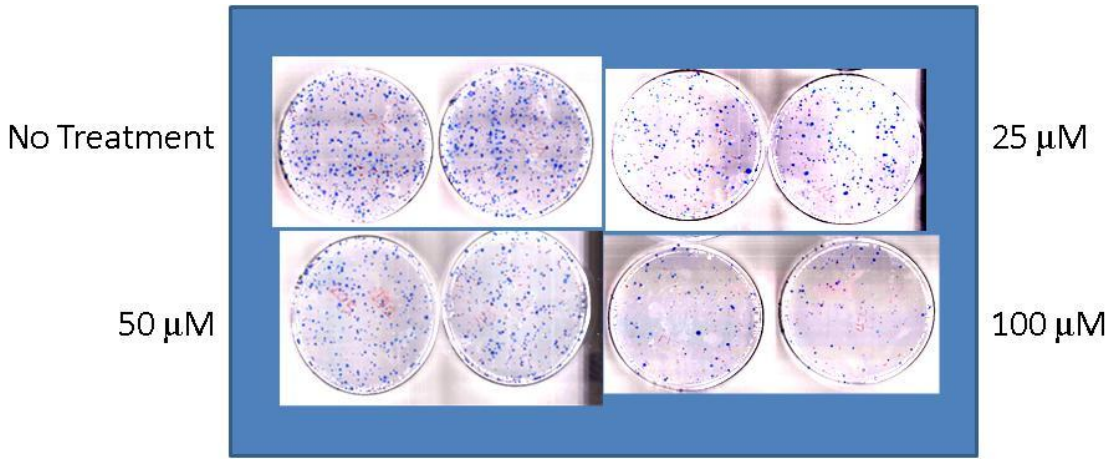
Figure S2



Representative images of colony formation assay. MDA-MB-436 ($3x10^5$) cells were treated with increasing concentrations of R9-cc-caPeptide for one hour, removed from the flask, and plated at 750 cells per 10 cm dish, and incubated for 14 days. Colonies were then counted, as detailed in the Materials and Methods section. Representative plates for each R9-cc-caPeptide treatment are shown.

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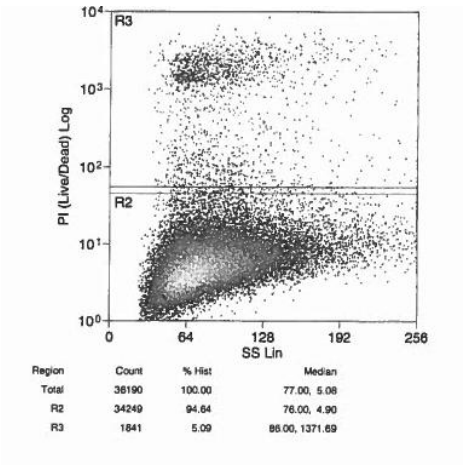
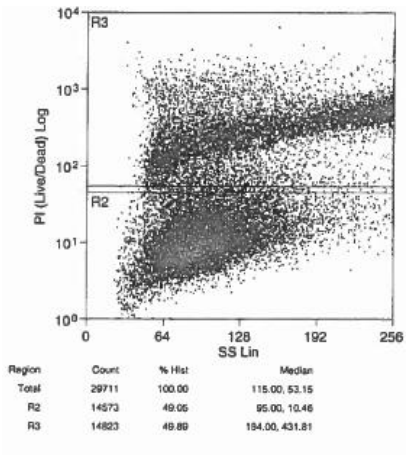
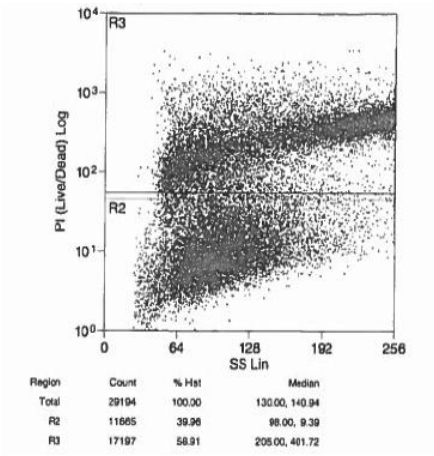
Figure S3

control – no treatment

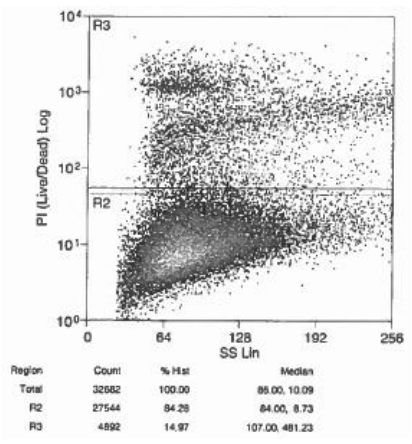


75 μM R9-cc-caPeptide

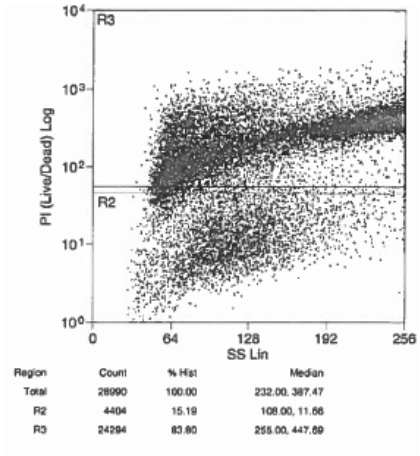




similar response



decreased cytotoxicity



increased cytotoxicity

Representative flow cytometry data for alanine scanning experiment. Exponentially growing ($1x10^6$) MDA-MB-436 cells were incubated with 75 μM of R9-caPeptide or R9-cc-alanine substituted caPeptides for 24 hours. The cells were then analyzed by flow cytometry. % cell death was calculated in reference to control cells (no peptide treatment), with standard deviation displayed from an average of 5 separate experiments. A decrease of cell death indicates the amino acid substituted is critical to the cytotoxicity of the peptide sequence.