Supplemental Information

Cancer Cell-Specific Oligopeptides Selected by an Integrated Microfluidic System from a Phage Display Library for Ovarian Cancer Diagnosis

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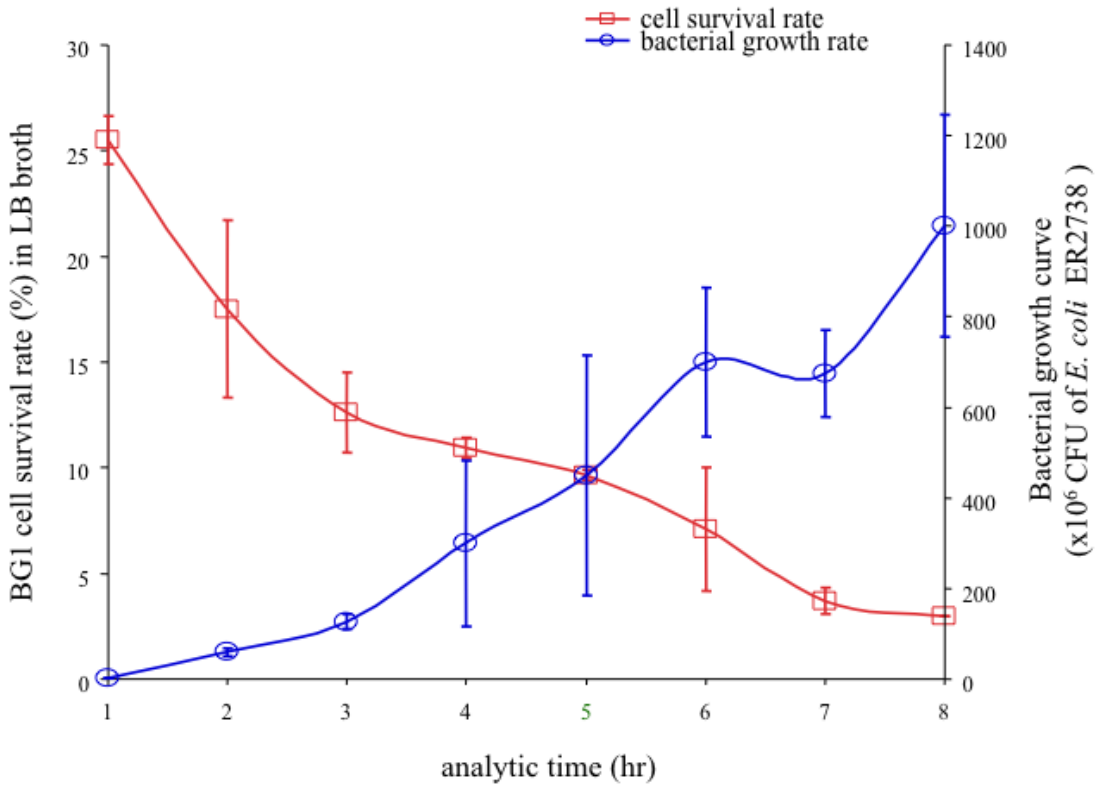
(A)



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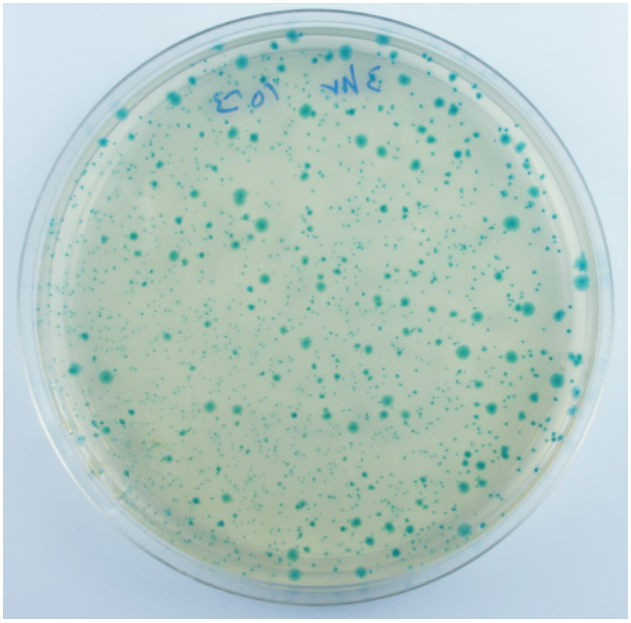

Figure S1. The determination of the optimal culture time in the phage multiplication step in the microfluidic system. (A) The relationship between the ratio of BG1 cell death and the growth rate of *E. coli* ER2738 in LB broth. Most BG1 cells were

disrupted in the bacterial growth medium in the absence of serum and growth factors within a short period of time. The captured phages were released from the disrupted BG1 cells, anchored and invaded into cytoplasm of *E. coli*, phage DNA amplified and assembled as a whole phage, Finally, the phage was budded out to the culture broth. The red line showed the survival rate of BG1 cells in LB broth. The cell number was counted after 0.4% tryphan blue staining. The dead cell was presented as blue color after tryphan blue staining and viable cell was observed as transparent and clear by optical microscope. The survival rate of BG1 cells was determined as viable cell number/total original cell number. The blue line indicated the growth rate of *E. coli* ER2738 in LB broth. The total bacterial number in each hour was counted to the CFU (colony forming unit) of *E. coli* on a LB plate. (B) Ten-fold serial dilutions of collected phages from each infection period in LB broth. The mixture was incubated with *E. coli* suspension until the mid-log phase of *E. coli* ER2738. The reactant was poured with a soft top agar onto the IPTG (Isopropyl β-D-1-thiogalactopyranoside)/Xgal(5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside)/LB plate for overnight incubation at 37℃. Then approximately 10^2 blue plaques were counted for a relative diluted factor to calculate the original phage titer as plaque forming unit (PFU). The PFU reflected the multiplicity of infected phages within *E. coli*. The PFU/CFU ratio in each culture period was determined for the optimal operating period for the maximum phage amplification in *E. coli* by the microfluidic system.

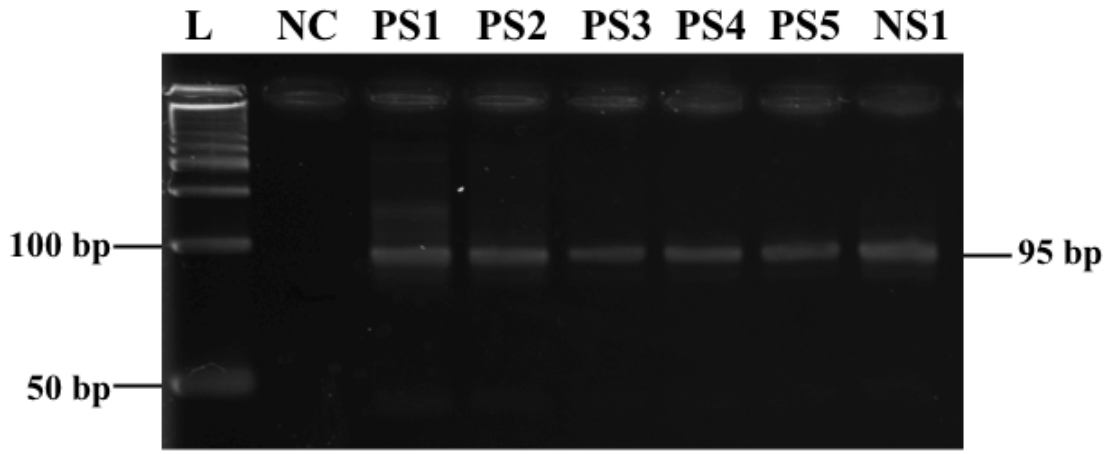

Figure S2. The phage enrichment from five continuous positive selections and one negative selection in the developed microfluidic system. To ensure that the selected phage existed in each panning round, the PCR products with a length of 95 bp (base pairs) were amplified from each round. The PCR primer pairs were designed on the outside of the M13 cpIII region that contained an insertion site for nucleotide sequence of putative oligopeptides. The PCR results were observed in the electrophoretic photographs. Lane L indicated 50-bp DNA ladders; NC indicated the negative control that used ddH_2O to replace reactants from each panning process; PS1, PS2, PS3 and PS4 showed the PCR result of the collected reactant from the first, second, third and fourth positive panning rounds. NS1 indicated the PCR result of the collected phage from the final negative panning round.

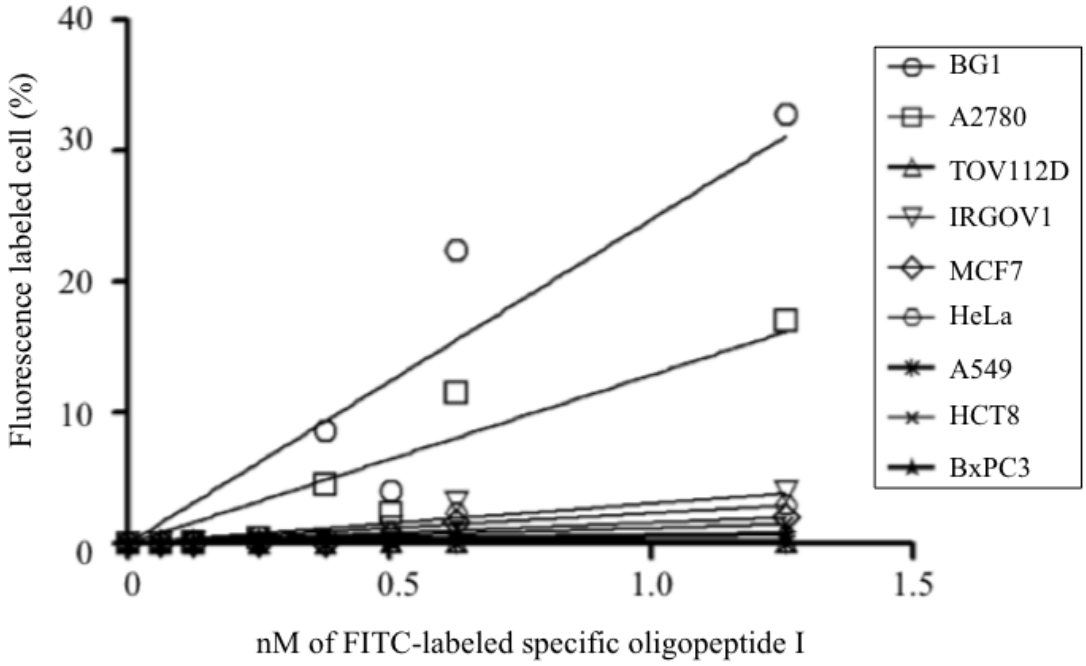

Figure S3. The binding capacity analysis of oligopeptide I with various cancer cells by using a flow cytometry assay. The low concentration of oligopeptide I was used from 0 to 1.25 nM. The ratio between the FITC-labeled cells and total number of the cells was measured from the gated fluorescence intensity and was determined by using an Accuri® C6 flow cytometer (BD Biosciences, USA). The flow cytometric data were analyzed by the manufacturer's software.

Table S1. Binding capacity of the oligopeptides I for various cell types. Note that 1 nM FITC-labeled oligopeptide I was used in this study. Three consecutive experiments were performed.

organ	cell type	binding capacity (%)*
ovary	BG1/serous	25.42 ± 3.11
	A2780/serous	13.52 ± 1.47
	TOV112D/endometrioid	1.08 ± 0.03
	IRGOV1/endometrioid	4.08 ± 0.31
cervix	HeLa	2.33 ± 0.72
breast	MCF7	1.58 ± 0.27
lung	A549	0.03 ± 0.01
colon	HCT8	1.16 ± 0.05
pancreas	BxPC3	0.58 ± 0.10

* ratio of fluorescence labeled cells with 1 nM FITC-labeled oligopeptide I by using the flow cytometry assay.

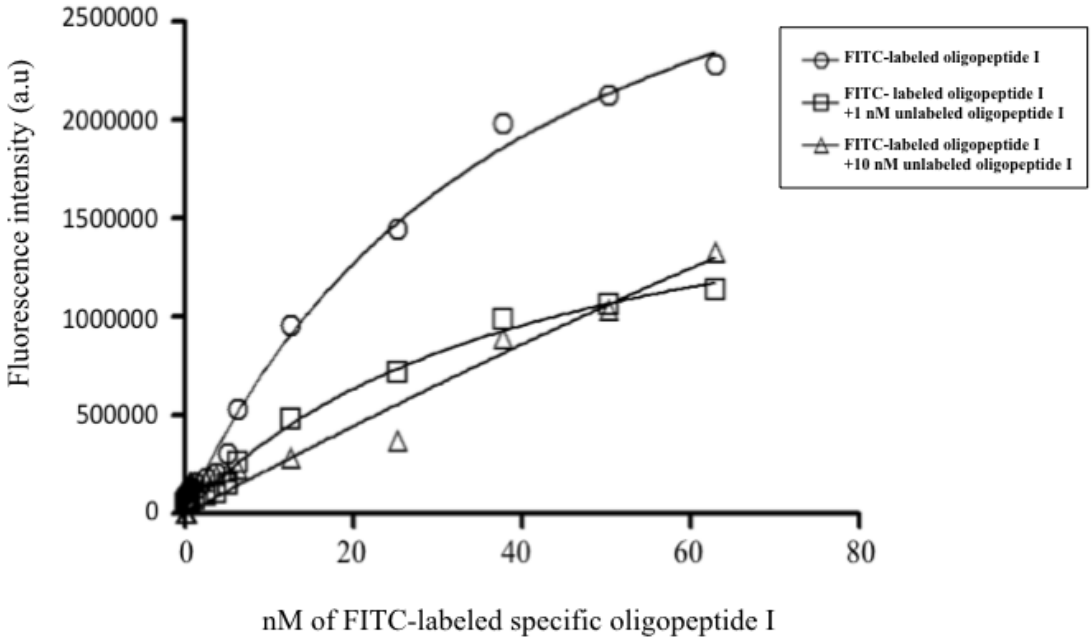

Figure S4. The competition test of FITC-labeled oligopeptide I with unlabeled oligopeptide I by using a flow cytometry assay. Serial dilutions of FITC-labeled oligopeptide I (○), 1 nM (□) and 10 nM (△) of unlabeled oligopeptide I were added to test FITC-labeled oligopeptide I which competed with FITC-labeled oligopeptide I for cell binding. The fitted curve of the dissociation constant (K_d) was then measured by using the gated fluorescence intensity and then determined with GraphPad Prism 6 software.