Landscape Phage Probes for Breast Cancer Cells

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ABSTRACT

Landscape phage libraries are collections of filamentous phages displaying random foreign peptides on all 4,000 surface domains of the major coat protein. They serve as a rich source of bioselective materials that can be used in different areas of technology and medicine, including gene delivery, drug delivery and imaging of tumor cells. In this study, we used 8 mer and 9 mer landscape libraries for selection of novel highly specific and selective phage probes against breast cancer cells, MCF-7 and ZR-75-1. The selected clones were identified by nucleotide sequencing of their DNA. To confirm the specificity of the phage probes, they were tested in binding assay with the target breast cancer cells and control cell-free serum-treated plates in comparison with a non-related phage. Development of the breast cancer-specific probes and further identification of peptides specific to different cellular compartments of the cells can provide unique tools for enhancing efficacy of anticancer therapeutics and diagnostics.

Keywords: filamentous phage, landscape phage, breast cancer cells.

1 INTRODUCTION

Breast Cancer is the most frequent cancer of women (23% of all cancers) [1]. Its high incidence makes it the most prevalent cancer in the world today: there are estimated 4.4 million women alive who have had breast cancer diagnosed within the last 5 year. Clinical effectiveness of existing non-targeted anti-cancer agents
is compromised due to non-selective delivery of the drug leading to a lower therapeutic response and undesirable side effects. Currently used vectors for drug delivery and gene therapy using antibodies for tumor recognition and drug delivery [2; 3] have proven not so useful because of their immunogenicity and lack of selectivity [4]. Highly tumor selective peptides probably present better opportunities to deliver drugs or their vehicles to tumor sites. Discovery of peptide ligands has become a high throughput procedure with phage display technology. This technology uses filamentous phages which are thread-shaped bacterial viruses with tubular outer coat (capsid) formed by thousands of copies of the major coat protein pVIII [5]. A landscape phage library is a multibillion population of filamentous phage particles displaying random foreign peptides on all the 4000 surface domain of major coat protein pVIII [6]. Landscape phage probes have been used as a substitute for antibodies against different cellular and bacterial antigens receptors [7-8]. Phage probes were adapted for gene delivery devices as well as interfaces in biosensors. In our approach, we used two landscape phage libraries, the f8/8 library (with octapeptide inserts) and the f8/9 library (with nonapeptide inserts), to select highly specific ligands against breast cancer cell lines MCF-7 and ZR-75-1.

2 AFFINITY SELECTIONS OF BREAST CANCER CELL-SPECIFIC PHAGE

Three different selection strategies were used to select breast cancer-specific phage clones from f8/8 and f8/9 libraries:

2.1 Non-biased selection

In this selection protocol, an aliquot of phage library containing 100 billion phage particles was incubated at room temperature with confluent MCF-7 cells. Unbound phage virions were washed away with subsequent washes of cells with medium and the cell-surface bound phage were eluted with mild acid. The eluted phage was amplified in *E.coli* and used as an input in further round of selection. Four rounds of selection were performed altogether and clones selected in different rounds were isolated as individual clones, sequenced and propagated for further characterization. In each round, the enrichment in phage binding to the cells was determined via tittering of input and output phage. The ratio of output to input phage increased from one round to another indicating successful selection for phage clones that bind to the target cells, as shown in Fig. 1.

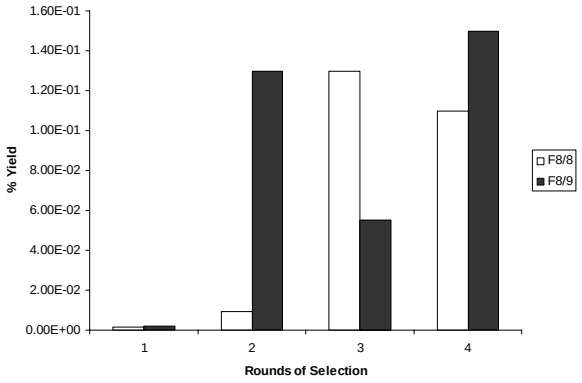

Figure 1. Specific enrichment of MCF-7 cell-binding phage isolated from the f8/8 and f8/9 libraries during four rounds of affinity selection

2.2 Biased selection: acid and detergent extraction of bound phage

To find specific probes against breast cancer that do not cross-react with nonrelated cell receptors and serum components, the initial phage library (f8/8 or f8/9) was depleted against plastic, serum and lung fibroblasts (W1-38). The depleted phage library was then incubated with confluent breast cancer cells

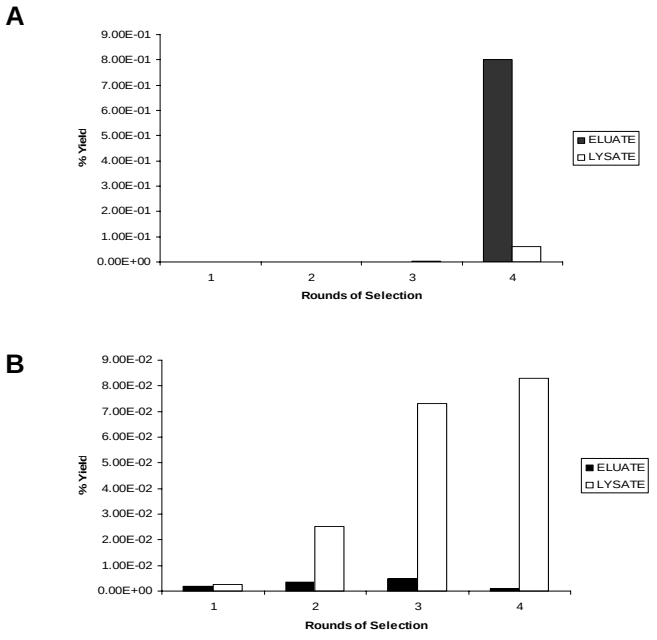

Figure2. Specific enrichment of MCF-7 bound phages in eluate and lysate fractions from the f8/8 (A) and f8/9 (B) libraries.

(MCF-7). The unbound phage were washed away and the bound phage were eluted with acid to get the phage clones that bound to the surface of the cells. Subsequently, cells were lysed with buffer containing deoxycholate to obtain phage clones that have penetrated into (or through) the cell membrane. The fractions of acid- and detergent-extracted phage were amplified separately and used as input in the further round of selection. Four rounds of selection were performed altogether, and individual phage clones selected in different rounds were isolated, sequenced and propagated for further characterization. The ratio of output to input phage increased from one round, as shown in fig 2A for the f8/8 library and 2B for the f8/9 library.

2.3 Biased selection: detergent extraction of cell-interacting phage

The strategy of biased selection of phage probes for breast cancer cells was further modified to simplify the selection procedure and obtain a whole pool of phage that interact with different compartments of breast cancer cells. In this procedure, the depleted phage library was incubated with confluent breast cancer cells (ZR-75-1), as described above, but phage was recovered by lysing the cells with buffer containing deoxycholate, without preliminary treatment of cells with acid. The lysed fraction was amplified and used as input in subsequent round of selection. The ratio of output to input phage increased from one round to another indicating successful selection for phage clones that bind to ZR-75-1 cells as shown in Fig. 3. Four rounds of selection were performed and individual clones from different rounds were selected, sequenced and propagated for further characterization.

The structure of representative peptides displayed on the surface of selected landscape phage, their frequency of occurrence, affinity to either MCF-7 or ZR-75-1 or both and method of extraction from the cells and the selection procedure from all the three selection protocol discussed above is shown in table 1.

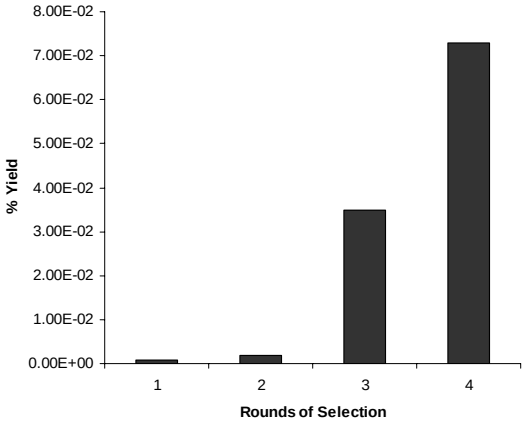

Figure 3. Specific enrichment of ZR-75-1 cell-binding phages isolated from the f8/8 library during four rounds of affinity selection

NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8504-4 Vol. 2

Family	Clones	ZR-75-1	MCF-7	Extraction	Selection
1	ATIF**EPGQ**^{73}	+		D	B
	VTLL**EPG**E^{2}	+		D	B
	APNF**EPGQ**^{3}		+	A	B
	GNPL**EPGQ**		+	A	B
	VPAL**EPGQ**		+	A	B
	VANF**EPGQ**^{2}		+	A	B
	VTAL**EPGQ**^{13}		+	A	B
2	**DGR**AVGSP	+		D	B
	DGRENPLT	+		D	B
	DGRPMANS	+		D	B
	DGRTADTS	+		D	B
	V**DGR**TPPP	+		D	B
	DGRQGLETD		+	A	NB
3	D**DPR**GVMLE		+	A	NB
	D**DPR**LLAS		+	A	NB
	DP**DPR**VNTL		+	A	NB
	EV**DPRY**STA		+	A	NB
4	GPI**DTDYS**		+	A	NB
	VPT**DTDYS**		+	A	B
	VSD**D**R**DYT**	+		D	B
5	AAGPE-**WQGD**		+	A	NB
	ANESA-**WSGD**		+	A	NB
	VEQAS**WTG**		+	A	NB
	DLTGT-**WQGD**		+	A	NB
6	**DPLPS-WQG**		+	D	B
	DS**LPS-WQG**		+	D	B
	DP--SN**WQ**SA		+	D	B
7	**DSDF-FTSQ**^{2}		+	D	B
	DTDF-FTSQ^{21}		+	WD	B
	DSGFLLQSQ		+	D	B
	D-PPP**L**P**TQ**	+		D	B
8	DS**S**GS**WSGD**		+	A	NB
	GNSDA**WSGD**		+	A	NB
	GSEQS**WTGD**		+	A	NB
9	**D**NL**P**S**WSQ**^{12}		+	AWD	B
	DSPRDWSQ^{5}		+	WD	B
	VL**SPRDWSQ**		+	W	B
10	EL**V**S**ME-GLD**		+	A	NB
	GM**V-MEPGLD**		+	A	NB
	AQGGL**PGLD**		+	A	NB
11	**APDP**QPAL	+		D	B
	APEPASQT	+		D	B
12	**DT**GAL**WSS**		+	W	B
	DTVQP**WST**^{2}		+	WD	B
13	**D**LERS**TMQ**	+		D	B
	DTPP-**TMQ**^{2}	+		D	B
14	**DTMY-**SP**M**P		+	W	B
	ESRYLNH**M**	+		D	B
15	**ED**AR**TAAM**A		+	A	NB
	EDHT**TAAM**		+	A	NB
16	**D**AG**G**LGL**GSM**^{2}		+	W	B
	E-GSWT**GSM**^{7}		+	WD	B
17	A**SMEEVSTL**		+	A	NB
	G**SLEEVSTL**		+	A	NB
18	GTGPLD**SYD**		+	A	NB
	VP**SYD**ADPS		+	A	NB
19	**AG**A**N-A-D**YA	+		D	B
	AG-NPAQ**D**S^{2}	+		D	B
20	**DTGQMTVN**		+	A	NB
	DTGQMTVND		+	A	NB
21	**E**G-**Q**SGIA**YD**		+	A	NB
	ESA**Q**LE-G**YD**		+	A	NB

Table1. Structure of peptides displayed on selected phage. Common motifs are indicated by bold letters. Superscripted numbers indicate the iterations of a particular structure among the sequenced clones. D-deoxycholate, A-acid, B-biased selection, NB-non-biased selection, W - wash.

3. SPECIFICITY OF SELECTED PHAGE TOWARD BREAST CANCER CELLS

Representative selected phage clones were characterized for their ability to bind specifically target breast cancer cells in comparison with non-related phage. As a non-related (negative control) target we used a flask with immobilized serum. As seen in Fig. 4, phage clones containing W(S/T/Q/)GD (5th family), GLD (10th family), EQYD (21st family) and SYD (18th family) selected from the f8/9 library appeared to be the best binders as their binding to MCF-7 cells was several folds higher than to serum. Similarly, phage clones from the f8/8 library with DYS (4th family) motifs and several orphan phage clones also demonstrated higher affinity towards MCF-7 cells than to serum. Binding of the control phage to MCF-7 cells was negligible.

4. CONCLUSION

It is commonly believed that efficacy and selectivity of anti-cancer therapeutics can be enhanced by their targeting to tumor-specific cellular receptors. In this study we used landscape phage to identify probes that interact with two breast cancer cell lines, MCF-7 and ZR-75-1. We used two different libraries, f8/8 and f8/9 to increase the chance t find diverse clones with different binding characteristics towards breast cancer cells. Three different selection protocols were developed to obtain phage clones that interacts with breast cancer cells. Modification of selection procedure from non-biased to biased condition lead to identification of unique as well as similar peptide families specific for MCF-7 and ZR-75-1 breast cancer cell lines. Phage clones expressing DGR, DYS motifs were in both non-biased and biased selection. Families with EPG (E/Q), DGR motifs were found in both breast cancer cell lines indicating remarkable specificity of cell recognition resulting in isolation of peptides having similar motifs. Non-biased selection led to identification of several peptide families W(S/T/Q)GD, GLD, SYD and YD

motifs that showed high specificity to MCF-7 cells. Preliminary depletion of libraries against serum and fibroblasts (biased selection) prevented the selection of non-specific phage clones that were common in non-biased selection. These peptides will be further analyzed for their selectivity. In summary, breast cancer cell specific phage probes were identified from two landscape phage libraries. These phage will be used as a source of navigating breast cancer specific fusion proteins for development of targeted nano-particulate drug vehicles.

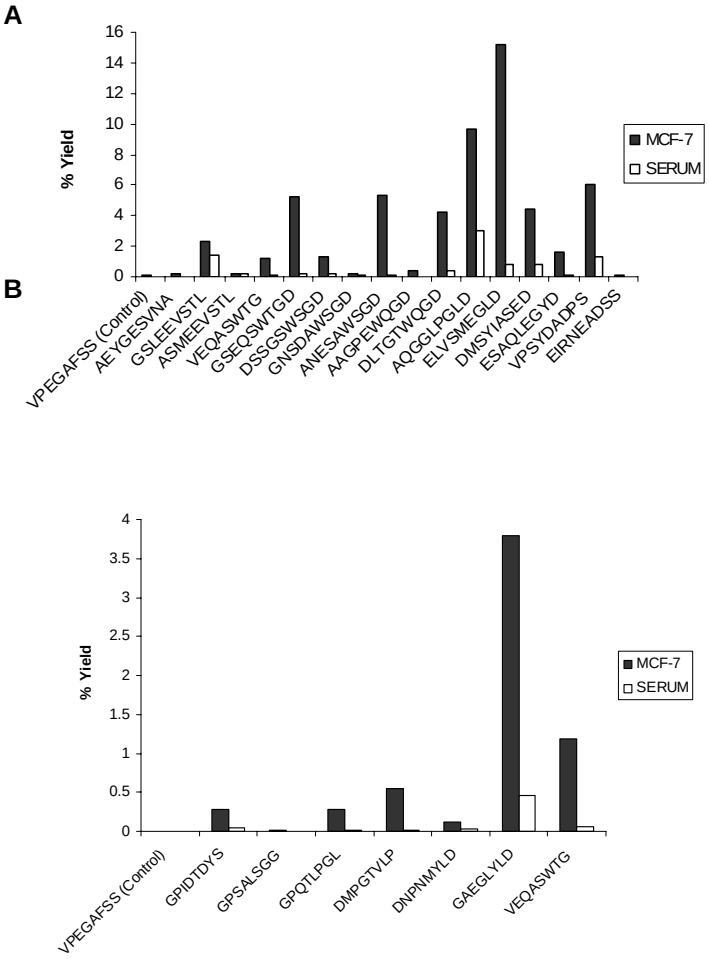

Figure 4. Binding of phage selected from the library f8/8 (A) and f8/9 (B) with breast cancer cells MCF-7 in comparison with their binding to control flasks with immobilized serum.

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