

Cell surface profiling with peptide libraries yields ligand arrays that classify breast tumor subtypes

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Abstract

Cancer heterogeneity renders risk stratification and therapy decisions challenging. Thus, genomic and proteomic methodologies have been used in an effort to identify biomarkers that can differentiate tumor subtypes to improve therapeutic outcome. Here, we report a generally applicable strategy to generate tumor type-specific peptide ligand arrays. Peptides that specifically recognize breast tumor-derived cell lines (MDA-MB-231, MCF-7, and T47-D) were identified using cell-displayed peptide libraries carrying an intrinsic fluorescent marker allowing for sorting and characterization with quantitative flow cytometry. Tumor cell specificity was achieved by depleting libraries of ligands binding to normal mammary epithelial cells (HMEC and MCF-10A). Although integrin binding RGD motifs were favored by some cell lines, screening with RGD competitors yielded several novel consensus motifs exhibiting improved tumor specificity. The resultant peptide array contained multiple consensus motifs exhibiting strong similarity to breast tumor-associated proteins. Profiling a panel of breast cancer cell lines with the peptide array revealed receptor expression patterns distinctive for luminal or basal tumor subtypes. In addition, peptide displaying bacteria and peptide functionalized microparticles enabled fluorescent labeling of tumor cells and frozen tumor tissue sections. Our results indicate that cell surface profiling using highly specific breast tumor cell binding ligands may provide an efficient route for tumor subtype classification, biomarker identification, and for the development of targeted diagnostics and therapeutics. [Mol Cancer Ther 2009;8(5):1312–8]

Received 12/16/08; accepted 2/24/09; published OnlineFirst 5/5/09.

Grant support: NIH -National Cancer Institute grant R21- CA107481, University of California-Cancer Research Coordinating Committee grant CRCC 02-03, the U.S. Army Research Office- Institute for Collaborative Biotechnologies (DAAD19-03-D-0004).

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doi:10.1158/1535-7163.MCT-08-1105

Introduction

Given the immense challenges associated with tumor heterogeneity, the effective classification of cancers and their subtypes using genomic, proteomic, and systems biology methods will substantially effect cancer diagnosis and therapy. Breast cancer classification is particularly important because classic histologic approaches provide limited prognostic and predictive value (1). Genomic profiling of breast tumors has resulted in the identification of five distinct molecular subtypes: luminal subtypes A and B, basal-like, ERBB2 positive, and normal breast like (2). Subtype identification has enabled improved risk stratification because the identified subtypes are associated with significantly different survival times (3) but unfortunately do not adequately predict patient responses to therapeutic regimens (4). The development of economical proteomic analysis methodologies would substantially augment diagnostic and therapeutic capabilities enabling the selection of targeted therapies and the elucidation of disease-associated protein networks, posttranslational modifications, and aberrant protein localization (5).

Proteomic methods have used protein, antibody, and tissue arrays, and mass spectrometry to characterize tumor heterogeneity (6). Cell surface characterization using proteomic approaches is of particular interest to identify tumor-specific receptors. More recently, protein display technologies have been applied to identify ligands that recognize tumor cell receptors using library panning on whole cells, thereby targeting cell surface receptors in their native environment (7). For example, a cell surface receptor profiling approach was applied to a panel of tumor cell lines from the National Cancer Institute (NCI-60) using peptide libraries displayed on bacteriophage (8). The presence of cell surface receptors was suggested by the enrichment of particular tripeptide motifs during library selection. Bacterial display libraries have also been used to identify peptide ligands specific for tumor cell surfaces using fluorescence-activated cell sorting. Highly tumor-specific binding peptides were identified by screening for breast tumor cell line recognition and for nonbinding to normal mammary epithelial cells (9). Given the potential utility of tumor cell receptor profiling, we sought to develop an efficient alternative for obtaining quantitative molecular signatures of tumor cells. Here, we applied bacterial display to identify multiple families of breast tumor cell-specific ligands and show the utility of the resulting ligand array for generating tumor cell signatures that enable molecular classification into luminal and basal subtypes.

Materials and Methods

Cell Lines, Vectors, and Reagents

Human breast tumor cell lines (MDA-MB-231, MCF-7, T47-D, ZR-75-1, and Hs578T) and the immortalized human mammary cell line (MCF-10A) were purchased from American

Type Culture Collection and cultured as per manufacturers' instructions. The tumor cell line MDA-MB-435 was a gift from Erkki Ruoslahti (Burnham Institute—UCSB, Santa Barbara, CA). Human mammary epithelial cells (HMEC) were obtained from Lonza.

Library Screening

Fluorescent bacterial surface display libraries with fully random 15-mers (X_{15}) or constrained 7-mers ($X_2CX_7CX_2$) tethered to the circularly permuted outer membrane protein *OmpX* (CPX) scaffold were generated and propagated as described (9). Selections were done with the following modifications: 2 mmol/L EDTA in PBS was used for detachment of tumor cells, and selection steps were done in cell culture media. Sorting was done using a FACS Aria flow cytometer (Becton Dickinson). For MCF-7 and T47-D, the third round of selection was repeated with the addition of 1 mg/mL RGD-4C (Anaspec).

Analysis of Peptides for Specificity and Cross-Reactivity

Tumor cell binding was quantified using flow cytometry, and sequence characterization was done as described (9). For analysis of individual peptides, 2.5 to 5×10^5 mammalian cells in 50- μ L medium were incubated with 50-fold excess bacteria for 1 h on an inversion shaker at 37°C. Cells were washed twice in PBS (1 mL) and analyzed by flow cytometry. Competitive binding assays were done in the presence of 50 μ mol/L soluble RGD-4C. Clustered image maps were generated using CIMminer.⁵

Fluorescence Microscopy of Peptide Binding to Tumor Cells and Frozen Tissue Sections

To assay bacterial binding to adherent tumor cells, tumor cells were seeded in culture dishes with 0.17-mm bottom thickness (Bioprotech Δ T, Fisher) as described (9). To enhance binding avidity, MDA-MB-231 tumor binding peptides were subcloned into the eCPX scaffold (10). To assay bacteria binding to frozen tumor tissue sections, 1×10^7 MDA-MB-231 cells were injected s.c. into the flanks of severe combined immune-deficient mice and harvested at the size of 200 mm³. Tumor and normal organ tissues were excised, frozen in embedding medium (O.C.T. compound, VWR) on an isopentane/dry ice slurry, and stored at -80°C. Frozen tissue sections were cut (6 μ m) onto teflon coated multiwell slides (Knittel). Tissues were fixed in cold acetone for 10 min and incubated with bacteria (80 μ L, 10^9 /mL) in PBS with 10% fetal bovine serum and 1% bovine serum albumin. Slides were incubated at 37°C for 1 h and washed 6 \times with PBS-Tween. Luria-Bertani medium was added to the wells to allow bound bacteria to amplify on the tissues. Bacteria were washed 6 \times and sealed with Permount mounting medium (Fisher) under 0.17-mm coverslips.

Binding of Peptide-Coated Microparticles to Breast Tumor Cells

Synthetic cyclic peptides (Anaspec) were modified through the addition of a COOH-terminal linker with biotin.

PepC3 [Ac-EWCGIVRVGYCLGGGKK-K(biotin)-NH₂] was dissolved in water (2.5 mg/mL). Peptide pepT7 recognized by the anti-T7 antibody was used as a nonbinding control: Ac-MASMTGGQQMGGK(LC-Biotin)-NH₂. For binding assays, 1 μ m Neutravidin-coated yellow FluoSpheres (Invitrogen) were diluted 1:50 in water and centrifuged (5,000 \times g) for 25 min. Peptide was added at 15-fold excess of biotin binding capacity of the beads, and incubated in a blocking solution (30% fetal bovine serum and 2% bovine serum albumin in water) at 0.5% solids for 1 h at room temperature. Beads were washed again and incubated at 50-fold excess with 5×10^5 mammalian cells for 1 h at 4°C. Cells were washed 3 \times and analyzed by flow cytometry. The number of beads per cell was calculated from: $(FL_{\text{mammalian cells incubated with beads}} - FL_{\text{mammalian cells without beads}}) / (FL_{\text{beads}})$. For internalization assays, 1×10^5 MCF-7 cells were grown overnight on 0.17-mm coverslips. Microparticles were added to the coverslips at 50-fold excess and were incubated for 1.5 h at 37 °C, washed 3 \times in PBS, and fixed with 2% paraformaldehyde in PBS (EMS). Cells were counterstained with anti-human E-cadherin antibody (R&D Systems), followed by anti-mouse IgG-quantum dots (QD655; Invitrogen). Coverslips were mounted in fluorescence mounting medium (DAKO) on multiwell slides (Knittel) and imaged on a laser scanning confocal microscope (Olympus IX81/FV500 Fluoview System). Images were taken with a 60 \times oil immersion objective, using filter settings for FITC and Cy3.5. XYZ stacks with 1- μ m step size were recorded.

Flow Cytometric Analysis of Permeabilized Breast Cancer Cells with Soluble Peptides

To investigate the cellular localization of the target of pepC3, 2 μ mol/L peptide (pepC3 or pepT7) was incubated with 500 nmol/L streptavidin-phycoerythrin for 1 h at room temperature. For surface staining, 30 μ L of the peptide-streptavidin-phycoerythrin mixtures were incubated with MCF-7, MCF-10A, and HMEC cells for 45 min at 4°C, washed once with PBS (1 mL), and analyzed by flow cytometry. For intracellular staining, cells were permeabilized with 70% ethanol for 2 h before incubation with peptides.

Results

Tumor cell-specific peptides were identified by screening for binding to human breast cancer cell lines (MDA-MB-231, MCF-7, and T-47D; Table 1), and for nonbinding to normal cell lines (MCF-10A and HMEC). To facilitate efficient screening and rapid characterization of binding peptides, a fluorescent *Escherichia coli* surface display library was used as described (9). Ligands specific for MDA-MB-231 (M peptides) exhibited strong consensus motifs (Table 2). For example, two clones shared seven identities, including five consecutive residues (LMSLE, M14, and M15; Table 2). Overall, peptides were highly specific for MDA-MB-231

⁵ <http://discover.nci.nih.gov/cimminer>

⁶ Supplementary material for this article are available at Molecular Cancer Therapeutics Online (<http://mct.aacrjournals.org/>).

Table 1. Characteristics of cell lines used for screening and ligand array analysis

Cell line	Histologic subtype	Site of origin	Receptor status*	Molecular subtype [†]
ZR-75-1 [‡]	Ductal adenocarcinoma	Distant metastasis (ascites)	ER+ PR- ErbB2+	Luminal
T-47D	Ductal adenocarcinoma	Distant metastasis (pleural effusion)	ER+ PR+ ErbB2+	Luminal
MCF-7	Adenocarcinoma (not further specified)	Distant metastasis (pleural effusion)	ER+ PR+ ErbB2(+)	Luminal
MDA-MB-231	Adenocarcinoma (not further specified)	Distant metastasis (pleural effusion)	ER- PR- ErbB2-	Basal
Hs 578T	Carcinosarcoma	Breast	ER- PR- ErbB2-	Basal
MDA-MB-435 [§]	Adenocarcinoma, reclassified as melanoma	Distant metastasis (pleural effusion)	ER- PR- ErbB2-	"Basal"
HMEC	Normal breast epithelium	Breast	ER- PR- ErbB2-	Normal
MCF10A	Fibrocystic breast epithelium	Breast	ER- PR- ErbB2-	Normal

* ER, estrogen receptor; PR, progesterone receptor; ErbB2 (HER-2/neu), erythroblastic leukemia viral oncogene homologue 2.

[†] Neve RM et al.: A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell* 2006, 10:515-527.

[‡] Screened in Dane KY, Chan LA, Rice JJ, Daugherty PS: Isolation of cell specific peptide ligands using fluorescent bacterial display libraries. *J Immunol Methods* 2006;309:120-9.

[§]Rae JM, Creighton CJ, Meck JM, Haddad BR, Johnson MD: MDA-MB-435 cells are derived from M14 melanoma cells—a loss for breast cancer, but a boon for melanoma research. *Breast Cancer Res Treat* 2007, 104:13-19.

and did not bind to the normal cell lines MCF-10A and HMEC (Supplementary Fig. S1),⁶ as measured by quantitative flow cytometric binding assays.

Selections with MCF-7 and T-47D (Table 2) cells were initially dominated by peptides with integrin binding RGD motifs (11). Interestingly, several peptides exhibited DGRP motifs resembling RGD ligands but with inverse orientation. Notably, RGD/DGRP motifs were not identified in selections against ZR-75-1 (9) or MDA-MB-231. Selected clones with RGD (C9- C12 and T3) or DGRP motifs (C13, C14, and T1) exhibited differing specificities for tumor versus normal cells (Supplementary Figs. S1 and S2).⁶ Binding of peptides with both RGD and DGRP motifs was blocked by preincubation of cells with RGD-4C (data not shown), suggesting that the DGRP motif also recognizes members of the integrin family.

To identify nonintegrin binding ligands specific for MCF-7 and T-47D, selections were repeated in the presence of an excess of soluble RGD-4C peptide. Five novel ligands conferring high specificity for MCF-7 were identified (Supplementary Fig. S1;⁶ Table 2). Among the MCF-7-specific peptides, two novel consensus motifs were evident as follows: CGIxxVGYC (C3-C4) and RxCTWNxxWxC (C5-C8). Peptide consensus motifs exhibited a high degree of similarity with a diverse group of tumor-associated proteins (Table 3), but even strong consensus motifs composed of six amino acid identities were insufficient to unambiguously identify corresponding tumor-specific ligands. For example, peptide T2 was highly similar to the hemochromatosis protein known to bind the transferrin receptor (Table 3), which is overexpressed in rapidly dividing cells and many types of

cancer (12). However, functional assays are required to prove the specificity of the peptide for this receptor.

To generate cell surface fingerprints of 6 tumor and 2 normal cell lines, 24 tumor cell specific ligands were assayed for cross-reactivity with each cell line using flow cytometry (Supplementary Figs. S1-S3).⁶ Each cell line exhibited a unique cell surface fingerprint that enabled identification of basal and luminal cell types (Fig. 1) that corresponded to their molecular subtyping (13). The ligand array fingerprint of basal cell subtypes (Hs578T and MDA-MB-231) was highly similar; likewise, luminal cell types (ZR-75-1 and T-47D) clustered into a single group (Fig. 1). In contrast, a cell line thought to be derived from a breast tumor (MDA-MB-435) but recently reclassified as a melanoma (14) did not react appreciably with 23 of 24 array ligands. In addition, the use of RGD and DGRP motif containing ligands for fingerprinting did not reveal distinct patterns that distinguished cell types (Supplementary Fig. S2;⁶ Fig. 1). In fact, four of five RGD-containing array ligands bound to normal breast cells.

To investigate whether array ligands identified by this approach could be used for biopsy analysis or *in vivo* targeting, their ability to label adherent cells and tissue sections using fluorescence microscopy was assessed. In tissue culture, bacteria expressing M10 effectively labeled MDA-MB-231 tumor cells, whereas control bacteria that did not express a peptide were readily washed away (Fig. 2A). Similarly, M10 effectively labeled tumor tissue sections prepared from murine xenografted MDA-MB-231 tumors. Additionally, fluorescent signals were amplified by allowing the bacteria to proliferate on the slide

Table 2. Sequences of peptides binding to MDA-MB-231, MCF-7, and T-47D cells

Name	Freq*	Sequence [†]	Name	Freq*	Sequence [†]
MDA-MB-231			MCF-7		
M1	1	MSCLMNSNSFCSEI	C1	1	VECDPVRNNFCWW
M2	1	WACLMMYSFCSSE	C2	1	LECHRLRLTNMCFLE
M3	1	LRCLTTLDNFCTI	C3	1	EWCGIVRVGYCLG
M4	2	LIICLHRIDRFCSV	C4 [‡]	1	DACGIIHVGYCKV
M5	2	MECLKSMFTYCDI	C5 [‡]	1	RVCTWNWSWICKKE
M6	1	LSCLYSMYSYCDV	C6 [‡]	1	RMCTWNLEWVCDL
M7	1	LWCLTDLMGWCTV	C7 [‡]	1	RLCVWDWEWLCRD
M8	1	LGCLLDVQSWCIV	C8 [‡]	1	RVCTWRMVVWCDY
M9	1	LWCLLDLMSWCEI	C9	1	NLCRGDLEKLCMK
M10	1	LDCFRNIYGFCNI	C10	1	YACRGDAYYLCAE
M11	1	LKCLWEMRGFCEI	C11	1	HSCRGDMLLCCWL
M12	1	VLCLLDITNRFCEI	C12 [‡]	2	FACRGDRWVLCNS
M13	1	VDCLFHTDRFCYI	C13	1	GLCVADGRPRCLE
M14	1	WRCLMSLETWCMV	C14	2	GWCFRDGRPMCSY
M15	1	LACLMSLEQWCAV	T-47D		
M16	1	WSCLWDLISQFCNF	T1	16	FWCMGDGRPRCTG
M17	1	PSCLFNLDSECFE	T2	2	VWCYLWKYGYCVY
			T3	1	PICRGDRDWRCRD
			T4 [‡]	1	GQIWKGEWVKLWRDV

*Frequency of occurrence among peptides sequenced from the round threepopulations.

[†]For alignment, amino acid consensus is shown in dark gray, whereas similarities are in light gray and are defined as follows: A/G/V/I/L/M, F/Y/W/M, S/T, D/E, N/Q, and R/H/K.

[‡]Peptides isolated in the presence of 1 mg/mL soluble RGD-4C.

after nonbound bacteria had been washed away (Fig. 2B). In contrast, fluorescent bacteria that did not display a peptide were removed from tumor sections with stringent wash conditions (Fig. 2B). These results indicate the peptides identified may be useful for profiling biopsy tissues.

To assess ligand function independent of the bacterial display scaffold, fluorescent microparticles were functionalized with arbitrarily chosen peptides pepM12 or pepC3. PepM12 functionalized microparticles did not bind to tumor cells, suggesting that this peptide may be scaffold dependent. However, pepC3-conjugated fluorescent microparticles bound to MCF-7 cells and did not bind MCF-10A and HMEC (Supplementary Fig. S4A).⁶ These functionalized microparticles were not internalized by MCF-7, as determined by confocal microscopy (Supplementary Fig. S4B).⁶ Microparticles that were unlabeled or labeled with an unrelated control peptide (an anti-T7 antibody tag) did not bind tumor or normal cells (Supplementary Fig. S4A).⁶ Finally, tetravalent complexes of pepC3 prepared with streptavidin-phycoerythrin also bound specifically to MCF-7 and did not bind HMEC or MCF-10A as measured by flow cytometry (Supplementary Fig. S4C).⁶ Interestingly, when the same cell types were permeabilized, these tetravalent pepC3 ligands labeled all three cell types, indicating that

the target receptor of pepC3 is intracellular in normal cell lines (Supplementary Fig. S4C).⁶

Discussion

Methodologies for molecular characterization of heterogeneous tumor-derived cells are poised to effect diagnostic and therapeutic decision making (15). For this study, we chose to profile breast tumor cell lines because genomic expression studies have shown that these cell lines mirror the aberrations found in primary tumors and can be divided into two groups, namely luminal and basal subtypes (13). It has also been suggested that the biology of primary tumors can be better represented by a panel, rather than individual tumor derived cell lines (16). In accordance, we generated a tumor cell binding ligand array from peptides selected against four cell lines. Probing of the peptide array yielded cell surface fingerprints that are distinctive for breast tumors, enabling correct categorization of the subtypes of five breast tumor cell lines examined. Importantly, distinctive luminal and basal cell type signatures were only obtained with non-RGD array peptides.

Interestingly, MCF-7 exhibited expression of receptors present on both luminal and basal cell types, as well as

a separate group of receptors not present in other cell lines. In agreement with this finding, MCF-7 has been shown to exhibit an unusually high-level of genomic amplifications and genetic abnormalities (13). Moreover, subclones of MCF-7 exhibited characteristics of cancer stem cells (17, 18). Additionally, although MDA-MB-435 was originally profiled as a basal breast cancer cell line (13), its array reactivity pattern did not match that of other basal subtypes. Comprehensive studies have confirmed that MDA-MB-435 is a melanoma cell line rather than a breast tumor cell line (14). The array fingerprints generated here are consistent with the revised classification of MDA-MB-435; only a single binding clone exhibited reactivity with MDA-MB-435.

Analysis of degenerate ligand motifs for similarity with human proteins identified numerous candidate proteins potentially interacting with receptors localized on tumor cell surfaces. Consensus motifs obtained in this study exhibited similarity to cancer-related proteins, including some that are ordinarily intracellular. However, despite previous suggestions that native ligands can be identified from tripeptide motifs (8), even strong consensus motifs of six amino acids identified here did not enable identification of corresponding

biological ligands. In addition, given that peptide consensus motifs sharing five to six identities were noncontiguous, similarity analysis methods limited to three consecutive residues seem unlikely to reveal biological ligands, or even to identify critical peptide binding motifs. Nevertheless, the consensus motifs reveal a variety of candidate biomarkers that warrant further investigation (Table 3).

Although peptide selectivity for breast cancer subgroups is desirable for profiling applications, a broader reactivity pattern toward many subgroups would be beneficial for diagnostic or therapeutic targeting. One of the peptides, T2, bound to all malignant cell lines used in this study, including MDA-MB-435 but not to nonmalignant cells. Given that the receptor targeted by T2 seems to be present in all six of the profiled tumor cell lines, but not in the normal cell lines, T2 warrants further examination as a potential targeting ligand.

Transposition of normally intracellular components to the surface of cancer cells is a well-known phenomenon in cancer (19, 20), which is not readily detected using genomic approaches. Indeed, one of the peptides, pepC3, bound to a receptor that was detected intracellularly in all cell lines assayed, but only in the cancer cell line MCF-7 was it also

Table 3. Alignment of human proteins with peptide consensus motifs found to be specific for breast cancer cells

Motif input (no. of proteins identified)		Sequence alignment of peptides with human protein	Human protein (Swiss Prot/ TrEMBL accession numbers)	Comments
LMNxxSF (4)	M1-M2 Q9NRK6	CLMNxxSFC 728 KLMNKQSFIS 737	ATP-binding cassette subfamily B member 10, mitochondrial (Q9NRK6)	ABC-Transporters (subgroup G) are up-regulated in cancer stem cells and confer multidrug resistance
LLDLMS (3)	M7-M9 P30419	CLLDLMSWC 430 PLLDLMSDAL 439	Glycylpeptide Ntetradecanoyltransferase 1 (P30419) or Glycylpeptide Ntetradecanoyltransferase 2 (O60551)	Adds a myristoyl group to the NH ₂ -terminal glycine residue of certain cellular and viral proteins in cytoplasm
LMSLE (15)	M14-M15 P49815	CLMSLExWC 1207 SWLMSLENPL 1216	Tuberin (Tuberous sclerosis 2 protein) (P49815)	Implicated as a tumor suppressor. May have a function in vesicular transport but may also play a role in the regulation of cell growth arrest and in the regulation of transcription mediated by steroid receptors
IVRVG (8)	C3 P10721	CGIVRVGYC 44 SDLIVRVGDE 53	Mast/stem cell growth factor receptor precursor (SCFR) (P10721)	Proto-oncogene tyrosineprotein kinase kit (c-kit) (CD117 antigen)
IVRVxY (1)	C3 Q96T92	CGIVRVGYC 284 CSRIVRVYR 293	Insulinoma-associated protein 2 (Q96T92)	May function as a growth suppressor or tumor suppressor
GIxxVGY (4)	C3-C4 Q99835	CGIxxVVC 387 SGICFVGYKN 396	Smoothed homologue precursor (SMO) (Q99835)	Tumor-suppressor gene patched encodes a membrane protein candidate receptor for Sonic hedgehog
TWNWxW (1)	C5-C8 Q96E22	CTWNWxWVC 31 GTWNWIWRRC 40	Nogo-B receptor precursor (NgBR) (Nuclear undecaprenyl PPI synthase 1 homologue) (Q96E22)	Acts as a specific receptor for the NH ₂ terminus of Nogo-B, a neural and cardiovascular regulator. Able to regulate vascular remodeling and angiogenesis
DGRPR (13)	C13,T1 P22105	CxxDGRPRC 1494 KDRDGRPRAV 1503	Tenascin-X precursor (P22105)	Substrate-adhesion molecule; may play a role in supporting the growth of epithelial tumors
WKYGY (2)	T2 Q30201	CYLWKYGYC 133 EGYWKYGYDG 142	Hereditary hemochromatosis protein precursor (HLA-H) (Q30201)	Binds to transferrin receptor and reduces its affinity for iron-loaded transferrin

NOTE: Sequences were aligned using <http://ca.expasy.org/tools/scanprosite>.

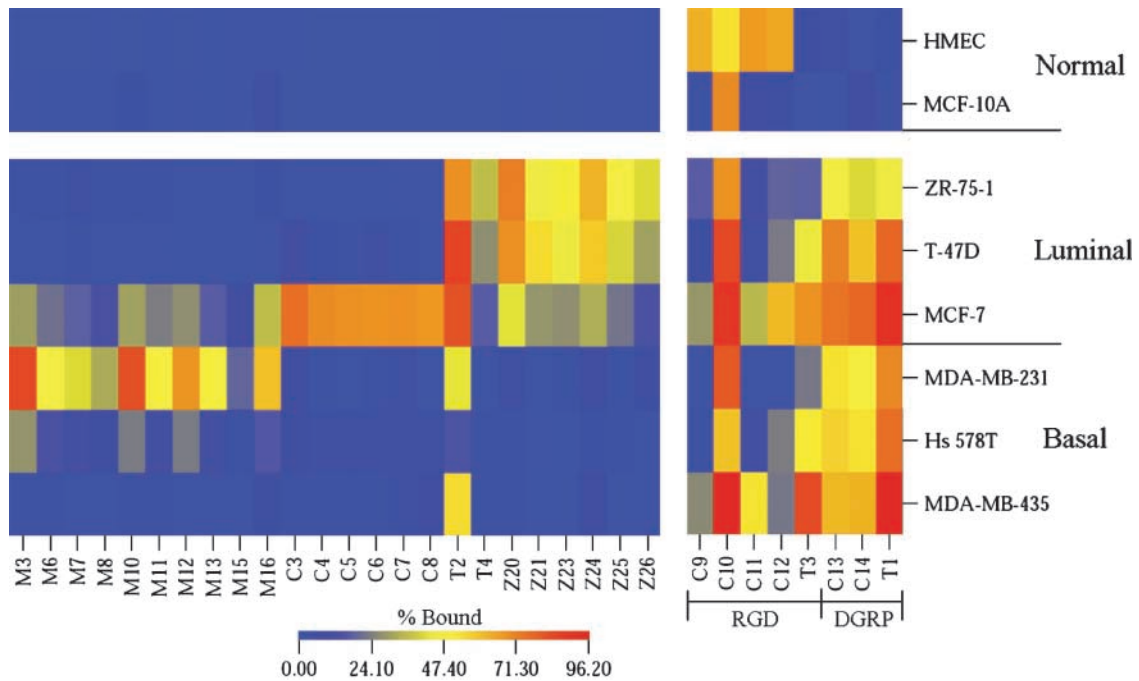


Figure 1. Clustered image map of specific tumor cell binding peptides used to profile the surface of breast cancer cells. Profiling peptides displayed on the surface of fluorescent bacteria were subjected to quantitative, flow cytometric binding assays to determine the percentage of tumor cells with bacteria bound (colored scale bar). Peptides were selected for binding to MDA-MB-231 (M peptides), MCF-7 (C peptides), T47-D (T peptides), and ZR-75-1 (Z peptides) breast tumor cells and nonbinding to HMEC and MCF-10A cells. Cell lines Hs578T and MDA-MB-435 not used in selections were also profiled. Specific peptides identified distinguish basal and luminal subtypes, whereas isolated motifs containing RGD or DGRP do not.

present on the cell surface. Another motivation for the identification of specific binding ligands to breast cancer cells is the possibility of using them as targeting moieties for diagnosis and therapy. PepC3 bound independently of its scaffold both

as a tetravalent complex and after coupling to the surface of microparticles.

In conclusion, the combination of fluorescent bacterial display libraries with quantitative fluorescence-activated

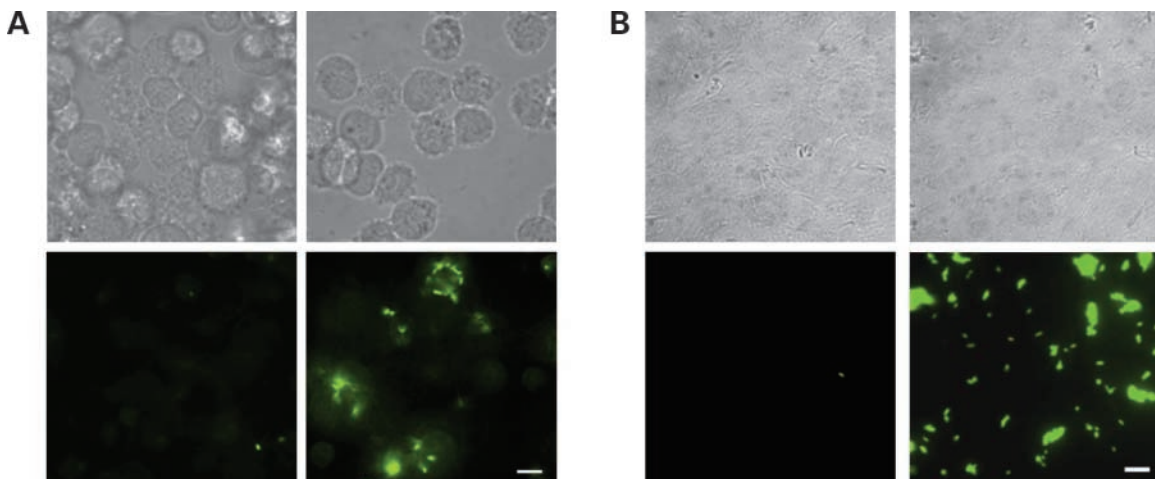


Figure 2. Bacteria displaying tumor-specific peptides bind both live cells and tumor tissue sections enabling facile detection. **A**, brightfield (top) and fluorescence (bottom) images of bacteria binding to living MDA-MB-231 cells. Control bacteria not displaying a peptide are removed through washing (left), whereas bacteria expressing MDA-MB-231 binding peptide M10 (right) remain tightly bound to the tumor cells. **B**, brightfield (top) and fluorescence images (bottom) of fixed tissue sections from murine xenografted MDA-MB-231 cells. Control bacteria not displaying a peptide (left) are removed through stringent washing, whereas bacteria expressing M10 (right) bind and amplify on the sections. Scale bars, 10 μ m.

cell sorting screening enabled high throughput surface profiling of tumor cells, effectively differentiating breast cancer subgroups with different prognosis. This approach is applicable for any cell type including primary tumors, and peptides identified using these screens could be used in a variety of diagnostic and therapeutic applications. Furthermore, the receptors targeted by these peptides could likely be determined using antibody competition assays or affinity chromatography approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Erkki Ruoslahti for helpful discussions and Kathy Kamath for critically reading the manuscript.

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