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High-throughput identification of putative receptors for cancer-binding peptides using biopanning and microarray analysis

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Abstract

Phage-display peptide biopanning has been successfully used to identify cancer-targeting peptides in multiple models. For cancer-binding peptides, identification of the peptide receptor is necessary to demonstrate mechanism of action and to further optimize specificity and target binding. The process of receptor identification can be slow and some peptides may turn out to bind ubiquitous proteins not suitable for further drug development. In this report, we describe a high-throughput method for screening a large number of peptides in parallel to identify peptide receptors, which we have termed “reverse biopanning,” which can then be selected for further development based on their peptide receptor. To demonstrate this method, we screened a library of 39 peptides previously identified in our laboratory to bind specifically cancers after irradiation. The reverse biopanning process identified 2 peptides, RKFLMTTRYSRV and KTAKKNVFFCSV, as candidate ligands for the protein tax interacting protein 1 (TIP-1), a protein previously identified in our laboratory to be expressed in the cell surface in tumors and upregulated after exposure to ionizing radiation. We used computational modeling as the initial method for rapid validation of peptide-TIP-1 binding. Pseudo-binding energies were calculated to be −360.645 kcal/mol, −487.239 kcal/mol, and −595.328 kcal/mol for HVGGSV, TTRYSRV, and NVFFCSV respectively, suggesting that the peptides would have at least similar, if not stronger, binding to TIP-1 compared to the known TIP-1 binding peptide HVGGSV. We validated peptide *in vitro* via electrophoretic mobility shift assay, which showed strong binding of RKFLMTTRYSRV and the truncated form TTRYSRV. This method allows for the identification of many peptide receptors and subsequent selection of peptides for further drug development based on the peptide receptor.

Keywords

Peptide; Biopanning; Phage Display; Microarray; TIP-1; TAX3BP1

INTRODUCTION

Phage-display peptide biopanning has been used to successfully identify putative targets for peptide-based imaging agents and therapeutics [1]. Phage-display biopanning can be

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performed both *in vitro* and *in vivo* and allows for a large number of random peptides to be screened in a short amount of time, reducing the number of candidate peptides in the library from 10^9 to 10^1 [2]. Additionally, this technique can be performed under a wide variety of conditions to screen peptides that selectively bind to tumors after specific stimuli, such as chemotherapy or irradiation [3, 4].

Once a peptide has been identified, the next step is to identify its binding partner. Routine methods for identification of peptide-protein interactions include techniques such as yeast two-hybrid; however, yeast two-hybrid is time-consuming and labor-intensive. Co-precipitation and tandem affinity purification followed by proteomic analysis are other methods available to identify potential peptide ligands [5-7]. While these techniques can be scaled to allow for parallel processing of multiple samples, individual conditions for each protein-peptide pair need to be optimized. Additionally, the output from these methods must then be sent for proteomic analysis, which adds another level of complexity and cost. In contrast, phage-displayed protein libraries have been developed using cDNA libraries from cancer cell lines. Phage libraries allow for proteins to be displayed on a bacteriophage and then panned against a candidate peptide ligand. This method has been successfully used to identify protein-binding partners; however, serial identification of peptide-binding partners using this method is time consuming and can become costly [8].

We have used phage-display biopanning to identify peptides that bind specifically to cancers in the past [9-13]. We have found that the bottleneck with phage-displayed peptide technology is the step of identifying target protein-binding partners to the peptides discovered through phage display. In an effort to develop an efficient method for the identification of putative binding proteins following phage-display peptide biopanning, we used a combination of “reverse biopanning” and microarray analysis. Using reverse biopanning, we are able to screen many peptides found to have desirable properties in parallel with the purpose of identifying putative binding partners. Here we discuss this method and demonstrate the methodology through the identification of two putative novel cancer-targeting peptides, RKFLMTTRYSRV and KTAKKNVFFCSV, that bind to the protein tax interacting protein 1 (TIP-1).

METHODS

Peptide and Protein Generation

Cancer-targeting peptides were identified through *in vivo* biopanning as described previously [8, 12-14]. The peptides for reverse biopanning were purchased in the PepScreen format from Sigma-Aldrich (Saint Louis, MO). Peptides (>95% HPLC purified) for electrophoretic mobility shift assay (EMSA) were purchased from China Peptide (Shanghai, China).

A bacterial codon-optimized DNA sequence for TIP-1, including an N-terminal Strep-II tag and a C-terminal 6xHis tag (Supplemental Data 1), was synthesized in the bacterial expression vector pJexpress411 (DNA2.0 Inc., Menlo Park, CA). BL21 DE3 star *E. coli* competent cells (Invitrogen, CA) were transformed with 5ng of pJexpress411 using standard methods. A single colony was selected to inoculate a 100 ml LB miller culture containing 50 mg/L kanamycin as a starter culture and grown overnight. The following day, 0.2% of starter culture was used to inoculate 1L LB miller containing 50 mg/L kanamycin. The cultures were grown at 37°C with 220 RPM agitation. When the cultures reached an optical density of ~0.4, 1M IPTG was added to the growing cultures at final concentration of 0.5mM to induce protein expression. The cultures were incubated at 37°C with agitation for an additional 3 hours. The cells were centrifuged at 4000 RCF for 30 minutes and the cell pellet was resuspended in 25ml of 50 mM NaPO₄, 300 mM NaCl, 0.25% NP-40, pH 8.0.

Lysozyme, DNase I and phenylmethanesulfonylfluoride (PMSF) were added to give final concentrations of 6.5 mg/ml, 0.01mg/ml, 0.1mg/ml, respectively. The cells were lysed by the freeze thawing followed by sonication. Talon CellThru beads (Clontech Laboratories, Mountain View, CA) equilibrated in 50 mM NaPO₄, 300 mM NaCl, pH 8.0 were added to the lysate and gently rocked for 2 hours at 4°C to allow binding of the target protein to the beads. The beads were loaded onto 10 ml CellThru columns and washed with 30 bed volumes of 50 mM NaPO₄, 300 mM NaCl, pH 8.0, followed by a 10 bed volumes of 50 mM NaPO₄, 300 mM NaCl, 10 mM imidazole, pH 8.0. The protein was eluted using 50 mM NaPO₄, 300 mM NaCl, 200 mM imidazole, pH 8.0. The protein eluent was then loaded onto a 5ml Strep-Tactin affinity column (IBA, Gottingen, Germany) pre-equilibrated with 100 mM Tris-Cl, 150 mM NaCl, 1mM ethylenediaminetetraacetic acid (EDTA), pH 8.0. The column was washed with 5 bed volumes of 100 mM Tris-Cl, 150 mM NaCl, 1mM EDTA, pH 8.0. TIP-1 was eluted using 100 mM Tris-Cl, 150 mM NaCl, 1mM EDTA, 2.5 mM desthiobiotin, pH 8.0. This eluent was buffer exchanged into phosphate buffered saline (PBS) using centrifugal ultrafiltration.

Reverse Biopanning

N-terminal biotinylated peptides were immobilized on Dynal® MyOne™ Streptavidin magnetic beads (1.05 µm diameter, Invitrogen, Carlsbad, California) pre-equilibrated in PBS by adding 100 µg of peptide in DMSO to 2 mg of beads in 100 µl of PBS and gently rocking for two hours at room temperature. The beads were then washed five times in 200 µL of PBS +0.1% Tween 20 (PBST). The immobilized peptide beads were incubated with 1 ml of 1.0×10^{10} pfu/ml T7 select human lung cancer and breast cancer phage display libraries obtained from Novagen (Gibbstown, NJ). Biopanning against the phage-display libraries was performed similar to the manufacturer's instructions. Briefly, the mixture of phage and immobilized peptide beads was rocked overnight at 4°C. The beads were then washed 5 times with PBST to remove non-specific and unbound phage. The target specific phage was isolated by adding 200uL PBS containing 1.0% sodium dodecyl sulfate (SDS) and rocked for 15 minutes at room temperature to remove the phage from the peptide-bead complex, followed by removal of the immobilized peptide beads by magnetic separation.

The target phages were amplified in *E.coli* BLT5615 in 35 mL LB containing 100 mg/L ampicillin at 37°C with Isopropyl -D-1-thiogalactopyranoside (IPTG) induction per manufacturer's instructions. Following bacteriophage lysis of the *E.coli*, 5M NaCl was added to each culture to achieve a final concentration of 1.25 M in order to stabilize the phage and the cultures were centrifuged at 5,000 x *g* to remove cell debris. The phage-containing supernatants were stored at 4°C. The input phage for the next round of biopanning was prepared by diluting 100 µL of the culture broth tenfold with PBS. Freshly prepared immobilized-peptide magnetic beads were used for each round of screening. A total of 5 rounds of biopanning were performed for each sample.

Extraction of Phage DNA and Amplification of Insert Sequences Specific to Peptide

After the final round of biopanning, DNA from the amplified phage was obtained by processing the lysed culture with the QIAmp blood minikit (Qiagen, Valencia, CA). DNA was eluted in 75 µL of nuclease-free water. The cDNA inserts contained in the phage DNA were amplified in 50 µL reaction volumes by PCR using the primer pair GGAGCTGTCGTATCCAGTC and TGGATTGACCGGAAGTAGAC. DNA from the PCR reactions was purified using the QIAquick PCR purification kit (Qiagen, Carlsbad, California). Purified DNA was eluted in 70 µL Tris Cl (10 mM, pH 8.5). A master library of all inserts biopanned in this experiment was created by combining 300ng of each amplified insert. Equivalent amounts of the DNA from the two control phage inserts were pooled in a

separate micro-centrifuge to serve as a control. These two samples were used for analysis on microarray.

Microarray Analysis

DNA concentration and purity was determined using UV spectroscopy. DNA sizes were determined using an Agilent 2100 bioanalyzer DNA 12000 assay kit (Agilent Technologies, Santa Clara CA) according to manufacturer's recommendations. To prepare for the microarray, DNAs were chemically labeled using a Kreatech ULS labeling kit (Kreatech Diagnostics, Durham, North Carolina). For each reaction, 2µg of DNA was mixed with Kreatech 10X labeling buffer and Kreatech cy5-ULS. The reactions were incubated at 85°C for 15 minutes in the dark and placed on ice for 3 minutes. Labeled DNAs were purified with QIAquick PCR purification columns (Qiagen Sciences) and then quantified on a Nanodrop spectrophotometer.

Labeled DNA (1µg) was suspended in Agilent 2X Gene Expression hybridization buffer, Agilent 10X Blocking agent, and Kreatech Kreablock. The hybridization solutions were applied to Agilent Human 4×44Kv2 microarrays. Hybridization was carried out at 65° C for 20 hours. Washing procedures were carried out according to Agilent gene expression protocols. Slides were scanned on an Agilent SureScan microarray scanner to detect Cy5 fluorescence. Gridding and analysis of images was performed using Agilent Feature Extraction v10.7.3.1.

Raw data from the microarray was analyzed using Partek Genomics Suite (St. Louis, MO, USA). The processed signal was logarithm base 2 transformed and subsequently quantile normalized to allow for comparisons of signal intensities among both probe runs and control runs. Signal intensities for the same gene probe for the phage genetic insert runs as well as the control insert runs were separately averaged and subtracted from one another. The signal intensities were then ordered from highest signal intensity following subtraction of background. Signal intensities that were 4 standard deviations above the mean were investigated further.

Real-Time PCR Validation—The TIP-1 forward primer GTCACACGGGTGTCTGAAGGAGG and the reverse primer TCCAATCTGCAGCCCAGCG were used for Quantitative Polymerase Chain Reaction (qPCR). A 10 µL reaction consisting of 5µL of 2x Applied Biosystems SYBR Green PCR Master Mix, 1 µL of each forward and reverse primer (final concentration 300nm), 2 µL of H₂O, and 1 µL of purified DNA from each peptide, diluted 1:100 in H₂O. Each sample was plated in triplicate on an Applied Biosystems Prism 384-well reaction plate, sealed with Applied Biosystems MicroAmp adhesive film and then centrifuged. qPCR was run on an Applied Biosystems 9700HT using the SDS 2.4 software package. The following PCR conditions were used: 50°C for 2 minutes, an incubation step at 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The samples were analyzed for Ct values using RQ Manager 1.2.1.

Molecular Modeling

The peptides of interest were modeled into the peptide-binding site of TIP-1 taken from the crystal structure 3DIW [15]. The crystal structure contains the bound peptide QLAWFDTDL, the last 6 residues of which fit into the binding site of the PDZ domain. We modeled the HVGGSV peptide into the WFDTDL portion of the bound peptide. While our new sequence is one residue longer, the deep binding pockets for Trp and Leu in the crystal structure lend themselves well to His and Val replacement. A small kink in the bound peptide is introduced between the Gly to accommodate the extra residue. The subsequent

peptides TTRYSRV and NVFFCSV were modeled in the same fashion, and the structures were energy minimized with molecular dynamics using the NAMD package [16] and the CHARMM36 forcefield [17]. A pseudo-binding energy was determined as the difference in total energy between the peptide bound and unbound complexes.

Peptide Validation

The interaction of peptide and TIP-1 protein was determined by EMSA performed by the use of Panomics EMSA kit (Panomics, Fremont, CA), as per manufacturer's instructions. Briefly, each biotin labeled peptide probe was incubated with TIP-1 in the presence of binding buffer (20 mM Hepes, pH 7.6, 100 mM KCl, 10% (vol/vol) glycerol, 0.2mM EDTA, and 4mM dithiothreitol (DTT)) for 1 hour at 37°C. Protein/peptide complexes were separated on a non-denaturing polyacrylamide gel, transferred to a PVDF membrane with 0.5 X TBE buffer, and detected using streptavidin-HRP and a chemiluminescent substrate. The bands were visualized after exposure to a Hyperfilm-MP autoradiography film (Amersham, Piscataway, NJ) with a Konica Minolta SRX 101A Processor.

RESULTS

Biopanning and Microarray Analysis

The “reverse biopanning” process was designed to identify target proteins that interact with peptides previously identified through phage-displayed peptide biopanning. This is accomplished by using a second phage-display system containing a cDNA library constructed from the total RNA from specific cancer cell lines. In order to increase the diversity of proteins expressed by the phage-display library, we chose to use two cDNA libraries, from human lung and human breast carcinomas. The libraries were combined after initial expansion and titration in a 1:1 fashion.

Thirty-nine peptides were identified from previous *in vivo* phage display biopanning experiments, explained in detail in *Jaboin et. al.* [14]. Each peptide was constructed with an N-terminal biotin that served as a linking moiety to magnetic streptavidin beads. As a negative control, the streptavidin beads alone (no added peptide) were brought through the reverse biopanning process in duplicate. After five rounds, enriched phage were collected and stored at 4°C. In order to preserve the heterogeneous nature of the enriched phage, we did not select individual colonies as detailed in many phage-display protocols. Instead, DNA was extracted from each of the enriched samples, and PCR amplification of the cDNA insert region of the phage genome was performed. The amplified product was pooled and then analyzed for DNA size in preparation for microarray analysis (Figure 1).

To identify the specific genes encoded and displayed in the enriched phage samples, the amplified and pooled PCR products were hybridized to Agilent Human 4×44Kv2 microarrays. The controls served to identify any displayed proteins that bound non-specifically, or to components of the biopanning system (beads, plastic, etc.). Signal generated from the streptavidin bead controls was subtracted from the phage samples and the final series of genes was ordered by intensity of signal above background (Figure 2). The average signal for all genes was 1.647×10^{-7} with a standard deviation of 0.866. We limited the list of “putative” hits to genes with normalized signal 4 standard deviations above the mean, resulting in a list of 113 “true” hits for the 39 peptides. We made the assumption that each peptide may bind 2-3 different proteins during the biopanning process, and a cutoff of 4 standard deviations fit well with our expected possible true hits of 80-120. At this point, the list of genes was analyzed for proteins that were of interest to our laboratory based on known characteristics of the encoded proteins. One of the genes identified by the microarray was TAX3BP1, which encodes TIP-1, a protein previously identified to translocate to the

cell surface after exposure to ionizing radiation [3, 8]. This protein contains a PDZ binding domain and had been shown to bind the peptide HVGGSSV *in vitro* and *in vivo* [12].

To identify which of the 39 peptides bound to TIP-1 during the reverse biopanning process, primers to TAX1BP3 were constructed based on the DNA fragment sequence on the microarray. Each phage sample, including controls, was analyzed for the presence of TAX1BP3 DNA (Figure 3) using qPCR. The average threshold cycle value for all samples analyzed was 29.6 (SD = 4.49). The control samples had an average threshold cycle value of 26.0 (SD = 0.388). The peptide RKFLMTTRYSRV had an average threshold cycle value of 15.0 (SD=0.237) and the peptide KTAKKNVFFCSV had an average threshold cycle value of 19.8 (SEM = 0.188). Each of these peptides demonstrated a significantly lower threshold cycle number compared to the other samples in aggregate (ANOVA, $p < 0.001$), suggesting the presence of TAX3BP1 DNA in these two samples, further suggesting that these peptides bind to TIP-1-expressing phage during the biopanning process.

Peptide Validation

In order to validate if the peptides identified by qPCR were potential TIP-1 binding peptides, we modeled these peptides in the peptide-binding domain of TIP-1 and compared computational modeling to our previously identified TIP-1 binding peptide HVGGSSV. Based on previous work with PDZ binding peptides, and TIP-1 specifically, peptides bind to TIP1 via the terminal 6-8 amino acids and the c-terminal carboxylate with canonical binding sequence of the terminal 4 amino acids being X-S/T-X-V. For computational modeling, the two identified peptides were truncated from RKFLMTTRYSRV and KTAKKNVFFCSV to TTRYSRV and NVFFCSV, respectively.

Computational modeling indicated that these peptides bind favorably in the PDZ binding domain of TIP-1 (Figure 4). While NVFFCSV contains a cysteine in place of a serine or threonine in the canonical binding site, it did not appear to cause any sterically disallowed conformations of the peptide. Predicted pseudo-binding energies were calculated to be -360.645 kcal/mol, -487.239 kcal/mol, and -595.328 kcal/mol for HVGGSSV, TTRYSRV, and NVFFCSV respectively. While we do not expect these pseudo-binding energies to exactly match the experimental values, we expect the relative values to be correct. These values therefore suggest that the two newly identified peptides may bind TIP-1 more favorably than the previously identified HVGGSSV peptide.

We qualitatively tested the ability of TTRYSRV and NVFFCSV to bind TIP-1 *in vitro* through the use of an EMSA. HVGGSSV was used as a positive control and as expected HVGGSSV did associate with TIP-1. The peptide RKFLMTTRYSRV and the truncated peptide TTRYSRV showed increased association to TIP-1 when compared to HVGGSSV and very low signal was detected for the peptide KTAKKNVFFCSV (Figure 5). Despite the favorable binding predicted by computational modeling, we were unable to detect binding of the truncated form of the peptide NVFFCSV (data not shown).

DISCUSSION

Phage-display peptide biopanning is a powerful screening tool for the identification of small peptides that bind in cancer *in vivo*. However, identification of peptide ligands can give rise to obstacles in the discovery process. Many peptides may bind ubiquitous targets that are not very specific, or bind targets that are inappropriate for use in later applications. Recently, *in vitro* and *in vivo* phage-displayed peptide biopanning have resulted in the identification of peptides that target specific diseases. One example is the peptide EHMALTYPRPP, identified by Zang *et al.* [18]. This peptide was discovered by biopanning against NCI-H1299 cells, and was found to bind specifically to NCI-H1299, A549 cells, and lung tumor

biopsy specimens, but not normal lung tissue. Similarly, the peptide SATTHYRLQAAN was identified by biopanning against NG4TL4-tk tumor cells and was found to bind *in vivo* to sarcoma tumors in mouse models [19]. In both cases, as with many other examples in the literature, tumor specificity of the peptide is demonstrated; yet, the mechanism for binding to the tumor is not known.

Established methods for identifying the receptor for cancer specific peptides discovered by biopanning have been a bottleneck in the process of prioritizing lead peptides for translation from discovery to pre-clinical testing in our laboratory. In many cases, a single lead peptide must be brought through the process of ligand identification, which can be time and labor intensive. Additionally, further validations may reveal a target that is inevitably not of interest, resulting in loss of time and resources. The method described in this paper allows for a large number of peptides to be tested in parallel followed by rational selection of putative target proteins based on their characteristics such as cell localization and expression profiles (Figure 6). This method is easily scalable and lead peptides do not need to be selected early in the process. Additionally, because a ranking of putative receptors is generated, one can prioritize candidates based on these results.

We demonstrated the use of this method by identifying two peptides that bind the radiation-inducible target TIP-1. TIP-1 is a 124 amino acid PDZ domain protein. It has been shown to interact strongly with β -catenin, and because it has a single domain, it is thought to have PDZ scaffold antagonist activity [20]. Overexpression of TIP-1 has also been shown to inhibit the growth of colorectal cells, presumably through a novel regulatory pathway of the WNT/ β -catenin signaling pathway [21]. Moreover, in a study of human breast cancer cell lines, knock-down of TIP-1 expression was shown to suppress cell proliferation [22]. TIP-1 has also been implicated in the activation of the Rho protein Cdc42 by binding the guanine exchange factor ARHGEF16 [23]. A recent report also suggests that TIP-1 may be alternatively spliced in some cancers, altering the PDZ binding site, and leading to a cancer-specific variant with altered function [24].

It is likely that the peptide KTAKKNVFFCSV did not bind TIP-1 well enough *in vitro* due to the cysteine in the canonical binding sequence of the peptide. If the peptide remained a monomer, the cysteine did appear to be able to interact favorably with the binding site of TIP-1, as demonstrated by computational modeling; however, the peptide is able to dimerize through the cysteines by the formation of a disulfide bond. In this case, binding to TIP-1 would be impaired. EMSA assays, showed peptide signal developed between the area of the TIP-1-peptide complex and free peptide. This may represent dimerized peptide, suggesting that KTAKKNVFFCSV dimerized under the EMSA conditions. During the initial phage-display process, KTAKKNVFFCSV might dimerize due to steric constraints as it was displayed on the surface of the phage as a terminal portion of a chimeric protein. In the reverse biopanning process, even a small amount of monomeric peptide would be sufficient to prioritize a lead protein. We did not test KTAKKNVFFCSV further, as the propensity of this peptide to dimerize rendered it non-useful for further development as a clinical targeting agent. Modifications of this peptide, specifically at the cysteine thiol, may eliminate dimerization and retain binding to TIP-1.

While this method of reverse biopanning can greatly increase the speed of identification of target proteins, there are some limitations. First, reverse biopanning is based on a phage-display system using the T7 bacteriophage. We chose this system as it is well described and commercial cDNA libraries from human tumors are available for this platform. Since this system relies on bacteria to produce the proteins displayed on the surface of the bacteriophage hence large proteins are not produced efficiently and may be excluded early in the biopanning process whether or not they are candidate receptors for the peptide. Codon

bias and lack of glycosylation may also cause some of the proteins to be misfolded, poorly expressed, or not expressed by the bacteria, also biasing the discovery process. Membrane proteins in particular may be difficult targets to identify using this technique; however, some membrane proteins, such as neuromodulin, have been demonstrated to display on the surface of phage [25]. Additionally, this process only identifies the presence of peptide-target interactions and gives no information on the specificity of the peptide for the target. Additional studies need to be performed to confirm peptide selectivity once a suitable peptide candidate is identified.

CONCLUSION

The process of identification of putative binding partners for tumor targeting peptides can be the bottleneck in the transition from discovery to target validation. The method of reverse biopanning can successfully identify peptide receptors despite the limitations of a bacteriophage display system for the display of proteins from a tumor cDNA library. This method allows for parallel processing of peptides and allows researchers to select the lead peptides based on the targeted molecule, rather than forcing the identification of a lead peptide early in the discovery process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Analysis of DNA specific to proteins that bind cancer-specific peptides using phage display technology. DNA was analyzed from phage inserts of peptide-specific phage DNA and non-peptide specific phage DNA. Phage insert DNA was obtained from sample specific phage DNA from the fifth round of biopanning following PCR amplification and purification. The DNA was analyzed on the Agilent 2100 Bioanalyzer using the DNA 12000 assay.

A.) DNA from phage inserts of peptide-specific phage DNA and non-peptide specific phage DNA. Lane 1 shows DNA from pooled peptide-specific phage inserts. Lane 2 shows control phage inserts from non-peptide specific phage inserts.

B.) Electropherogram of phage insert DNA specific to cancer-targeting peptides. This DNA was obtained from equal amounts of phage insert DNA from cancer-targeting peptides biopanned in this study pooled together.

C.) Electropherogram of phage insert DNA obtained from control samples without cancer-targeting peptides. DNA was obtained from amplified phage of the fifth round of biopanning with beads lacking a cancer-targeting peptide.

Figure 2.

Distribution of genes based on normalized microarray signal. A cutoff of 4 standard deviations above the mean signal (Log signal of 3.464) was used to prioritize putative binding proteins. A total of 113 genes were identified as encoding putative binding proteins based on the microarray analysis.

Figure 3.

Real Time PCR of phage DNA. Real-time PCR for all peptides was run in triplicate using primers for TIP-1 (A). Threshold cycles for TTRYSRV (peptide 4) and NVFFCSV (peptide 9) were significantly lower than those of all other peptides and controls with values of 15.0 and 19.8, respectively (B), suggesting the presence of TIP-1 DNA in the phage recovered from these peptides.

Figure 4.

Computational models of HVGGSV, TTRYSRV, and NVFFCSV to the PDZ binding site of TIP-1. HVGGSV binds in the PDZ binding site of TIP-1 (A). The peptides NVFFCSV (B) and TTRYSRV (C) are also predicted to favorably interact and bind to the PDZ binding site of TIP-1. When the modeled peptides are overlaid (D), minimal change in the peptide backbone is predicted.

Figure 5.

RKFLMTTRYSRV and truncated form TTRYSRV strongly bind TIP-1. The EMSA assay demonstrates that the peptides RKFLMTTRYSRV and TTRYSRV bind strongly to TIP-1. The peptide KTAKKNVFFCSV demonstrated minimal binding to TIP-1. The positive control peptide HVGGSV also binds TIP-1. The signal between the region of high molecular weight (representing TIP-1) and low molecular weight (representing free peptide) may represent dimerized peptide, likely through the free cysteine.

Figure 6.

Flow chart of the reverse biopanning process. This schematic demonstrates the steps of the reverse biopanning process followed by microarray analysis. In the process, individual peptides are processed separately through the step of PCR amplification of DNA. After that point in the process, DNA is pooled and analyzed as a group by microarray.