

# Tumor cell-targeting by phage-displayed peptides

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We isolated cancer cell-specific phages by subtracting and selecting complex peptide display phage libraries on cultured human cancer cells. The best candidate was selected by performing three rounds of subtraction before each of five selections on the human colorectal WiDr cell line. The phage showed more than 1000-fold higher binding efficiency for WiDr cells when compared to five other human cancer cell lines, including two of colorectal origin, and when compared to wild-type M13 phage. Fifty-fold higher binding efficiency was also seen for a human breast cancer cell line. We show that the WiDr cell binding of the selected phage was efficiently competed by the synthetic peptide HEWSYLAPYPWF, predicted from the phage sequence. This confirms that the specificity of the peptide is independent of the display by the phage coat proteins. The identified peptide may target biomarkers linked to colorectal cancer, and thus be useful for designing gene transfer vectors as well as diagnostic and prognostic tools for this disease.

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**Keywords:** targeting; peptide display; phage display; subtraction; human cancer cells; WiDr cells

Further development and general application of gene therapy will depend on the ability to target delivery vectors to tissues of interest and limit uptake by healthy tissues. The commonly used experimental vectors (adenoviral and synthetic) target the liver and lung after intravenous (i.v.) injections, respectively. Redirecting these vectors to other tissues has not yet been successful; therefore, targeting ligands are needed. Targeting may in some cases be done by an *ex vivo* procedure, but direct i.v. injection of organ-homing complexes would be far less invasive and elaborate. Our aim was to identify peptide sequences that would allow targeting of human tumor tissues with gene delivery vectors or with diagnostic probes.

It has been shown that endothelial cells in the vasculature of tumors are different from normal endothelial cells, and that tumor blood vessels express proteins that are not produced in quiescent vascular endothelium.<sup>1–3</sup> Extravasation of complexes may also be possible in growing tumors.<sup>4</sup> Phage display libraries have been used extensively to select peptides that bind to specific receptors or epitopes. Strategies for panning cells *in vitro*<sup>5–8</sup> or tissues *in vivo*<sup>3,9–12</sup> with complex phage libraries have been described to yield phages with organ- or tumor-binding specificity. Screening phage display libraries against specific target tissues would therefore seem a direct and fast method to identify novel peptide sequences to be used for targeting of gene delivery vectors. Selected peptides could be integrated into synthetic or viral vectors, and after i.v. administration, the complex would

home to the tumor vascular endothelium or tumor cells after extravasation. Such a strategy would hold hope for eliminating cancers by i.v. injections, provided the tumor cells (including metastatic cells) persistently express the receptor that was targeted, and by including an apoptotic gene or a cell-killing compound in the vector.

We focused on two common human cancers, colorectal and breast, for selecting specific peptide displaying phages from complex peptide display phage libraries. Phages, that showed high binding efficiency to the selection cells, could be selected from *in vitro* screenings and binding of a colorectal cell-specific phage could be competed by the free peptide.

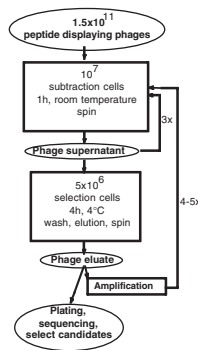
## Materials and methods

### Cells and phage libraries

The human cell lines WiDr, 293, HeLa, MDA-MB-231, MDA-MB-435, Colo205, and LOVO were obtained from ATCC (American Type Culture Collection, Manassas, VA). They were all grown in DMEM (Gibco BRL, Life Technologies, Paisley, Scotland) containing 3 g glucose, 3.7 g bicarbonate, 40 mg gentamycin per liter, 2 mM glutamine, and 10% fetal calf serum, except for LOVO cells, which were grown in NUT.MIX.F-12(HAM) (Gibco BRL) and 10% fetal calf serum. Generally, the cells were propagated twice per week by trypsinization and dilution. None were kept in continuous culture for more than 2 months. Phage libraries were obtained from New England Biolabs (Ozyme, Montigny-le-Bretonneux, France). Phages from the PhD-12 library displayed 12 random amino acids, and phages from PhD-C7C displayed seven random amino acids, flanked by cysteines, on the N-terminal of the pIII coat protein. The titer of the libraries varied from 0.5 to  $2 \times 10^{13}$  pfu/mL, and the complexity varied from 2 to  $4 \times 10^9$  transformants.

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**Figure 1** Flowchart of the phage selection protocol. Three rounds of subtractions were done before each of the selections. The entire cycle was repeated four to five times.

*In vitro selections*

A subtraction/selection protocol<sup>5,13</sup> was optimized and used for our phage selections. Before subtraction, the cells were detached with 10 mM EDTA in PBS, washed two times in PBS, counted and resuspended at 10<sup>7</sup> cells/mL in PBS, 1% BSA, containing 1.5×10<sup>11</sup> phages/mL. The mix was incubated 1 hour at room temperature with slow shaking, the cells were pelleted in a microfuge at 1500 rpm for 1 minute, and the subtracted supernatant recovered and used to resuspend another 10<sup>7</sup> subtraction cells. This subtraction cycle was repeated three times in total. Selection was done by resuspending 5×10<sup>6</sup> selection cells with the subtracted phage supernatant at 4°C and incubating with slow shaking for 4 hours at 4°C. The cells were then washed five times in cold PBS, 1% BSA, and 0.1% Tween-20, changing tubes between washes (microfuge, 1500 rpm, 1 minute). The bound phages were eluted from the selection

cells by adding 100 μL 0.1 M glycine–HCl, pH 2.2, for 10 minutes on ice. The cells were pelleted as above and the supernatant recovered and neutralized by 10 μL 2 M Tris–HCl, pH 8. The recovered pool of phages was titered, amplified (using the supplied host strain, and following the supplier’s protocol) and titered again before the next round of subtraction/selection. The protocol is outlined in Figure 1. Three rounds of subtraction were done before each round of selection, and four to five rounds of selections were done in total. Different subtraction methods and cells were tested and the results are shown in Table 1. Subtraction method (a) included three subtractions with HeLa cells before the first selection on WiDr cells, and no further subtractions were done before the next four WiDr cell selections. In method (b), three subtractions with HeLa cells were done before the first selection on WiDr or MDA-MB-231 cells, and three subtractions with 293 cells were done before each of the subsequent four selections on the WiDr or MDA-MB-231 cells, respectively. Method (c) included three subtractions with 293 cells before each of the four selections on MDA-MB-231 cells.

*Determination of phage binding efficiency by titration and competition with free peptides*

After four to five rounds of subtraction/selection, single plaques were selected for preparation of purified stocks and for sequencing. Phage binding efficiency was determined by incubating 1.5×10<sup>11</sup> phages with 5×10<sup>6</sup> target or control cells at 4°C. Incubation, washings, and elution conditions were performed as above. After titering, the phage binding efficiency was calculated by dividing the number of recovered plaque-forming phages (output) by the input number of plaque-forming phages. After the selections, a sequence was chosen for further characterization (see *Results*), and the corresponding free peptides were synthesized (Neosystem, Strasbourg, France). The peptides

**Table 1** *In vitro* selection of cell-binding phages

Phage library	PhD-12	PhD-12	PhD-12	PhD-C7C
Subtraction method	(a)	(b)	(b)	(c)
Selection cells	WiDr	WiDr	MDA-MB-231	MDA-MB-231
Round 1	2.3	1.6	3.2	0.7
2	2.3	8.8	7.7	23
3	6.3	13	29	8,750
4	17	80	35	43,000
5	607	1240	830	
Frequency of selected sequence	24/24	13/24	4/6	11/14
Peptide sequence	QIDRWFDVAVQWL	HEWSYLAPYPWF	YQATPARFYTNT	CGWWMGLELC

The numbers represent phage binding efficiencies of the phage pools (output/input) after each subtraction/selection round (×10<sup>-6</sup>). “Frequency of selected sequence” indicates the representation of the most frequently appearing sequence per total number of phages sequenced.

(a) Three subtractions with HeLa cells before the first selection on WiDr cells. No further subtractions were done before the four next selections.

(b) Three subtractions with HeLa cells before the first selection on WiDr cells (shown in the second column and in Figure 2), or MDA-MB-231 cells (third column), and three subtractions with 293 cells before each of the subsequent four selections on the WiDr or MDA-MB-231 cells, respectively.

(c) Three subtractions with 293 before each of the four subsequent selections on MDA-MB-231 cells.

contained the WiDr cell-specific phage sequence HEWSY-LAPYPWF (HEW) at the N-terminal end, and a cationic DNA-condensing peptide at the C-terminal end, either poly-L-lysine (K<sub>16</sub>)<sup>14</sup> or C1q.<sup>15</sup> Unspecific control peptides contained the GHLPLRQPSHQ sequence (GHL), a sequence that was found in a phage with no selectivity to any cells or organ. Thus, the following peptides were used: HEW-K<sub>16</sub>: HEWSYLAPYPWFK<sub>16</sub>, HEW-C1q: HEWSY-LAPYPWFCRAPDGKKGEAGRPRRRGRPLK, and the controls: GHL-K<sub>16</sub>: GHLIPLRQPSHQK<sub>16</sub>, and GHL-C1q: GHLIPLRQPSHQCRAPDGKKGEAGRPRRRGRPLK. All peptides were reconstituted in H<sub>2</sub>O. Binding assays as described above were performed with increasing amounts of free peptide added to the cells 15 minutes before the purified phages. Assuming five molecules of pIII-displayed peptide on each phage, molar excess from 1 to 10<sup>4</sup> of each of the free peptides was tested. Purified M13 wild type (wt) or the phage displaying the GHL sequence was used as negative (background level) control phages. Binding is considered to be specific when competition with the free peptide could be demonstrated on the phage binding efficiency. Phage selectivity refers to a higher than background binding efficiency to some cells compared to other cells.

#### Immunocytochemistry and internalization assay

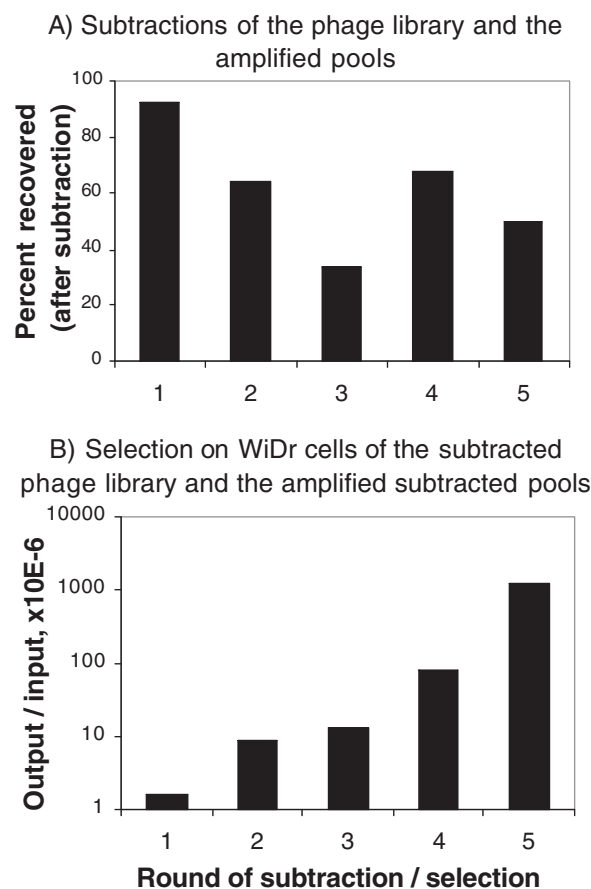
The protocols of Poul *et al*<sup>6</sup> were followed with slight modifications. Cells were plated and grown to about 80% confluency on Lab-Tek chamber slides (eight wells; Nunc, Naperville, IL). The wells were washed two times with PBS, and 100  $\mu$ L PBS, 1% BSA, 2 $\times$ 10<sup>10</sup> purified phage was added to each well, and the slides were incubated 2 hours at 4°C or at 37°C. The slides were washed five times in cold PBS, 1% BSA, and 0.1% Tween-20, before addition of rabbit anti-fd bacteriophage IgG fraction (Sigma-Aldrich, Saint Quentin Fallavier, France) 1/500 (vol/vol) in PBS, 1% BSA, for 45 minutes at 4°C. Slides were washed four times in cold PBS, 1% BSA, and incubated with the second antibody, goat F(ab')<sub>2</sub> anti-rabbit IgG-FITC (Southern Biotechnology Associates, Birmingham, AL) 1/200 (vol/vol) in PBS, 1% BSA, for 45 minutes at 4°C. The slides were then washed four times in cold PBS and 1% BSA, fixed in 2% formaldehyde in PBS, and rinsed twice in PBS. The slides were mounted in Mowiol (Calbiochem/Novabiochem, La Jolla, CA), and viewed in the fluorescence microscope at 500 $\times$  magnification using a FITC filter set. For visualisation of internalized phages, the cells were stripped of phages by washing the slide three times in 100 mM glycine, 0.5 M NaCl, pH 2.5 at room temperature, fixing for 5 minutes in PBS, 2% formaldehyde, and permeabilization for 30 seconds in ice-cold acetone, before continuation with the antibody incubations.

## Results

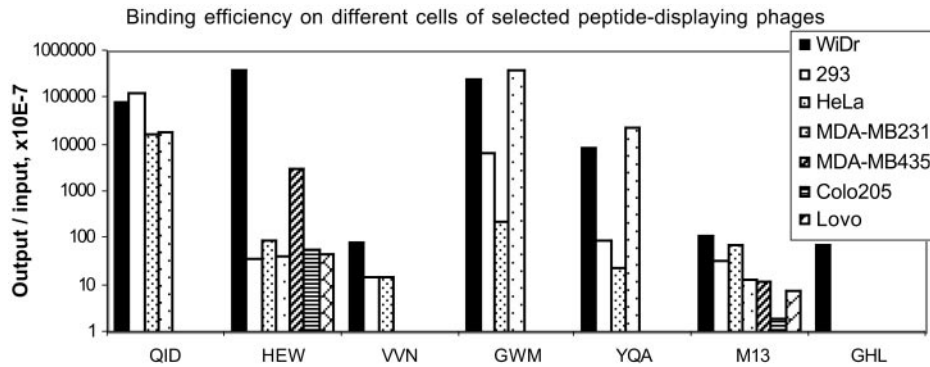
### In vitro selection of phages

Figure 1 shows a flowchart of the general protocol that was used for phage selection and identification. Four to five subtraction/selection rounds on cultured cells yielded phage

pools that exhibited significantly higher binding efficiency than the nonselected starting pool or control phages. For the subtractions we used human cells, either HeLa (cervical cancer), or 293 (embryonic kidney) cells. For the selections, the human colorectal (WiDr) or breast (MDA-MB-231) cancer cells were used. Titrations before and after subtraction showed that three consecutive subtractions of an unselected phage library reduced the phage pool on average 7% when HeLa cells were used, and up to 24% when 293 cells were used. Figure 2A shows the decrease in phage-pool size after three subtractions with HeLa cells (column 1) and the decrease after subsequent subtractions with 293 cells of WiDr-selected and amplified phage pools (columns 2 to 5). A substantial subtraction ( $\sim$ 50%) was still obtained after four rounds of subtraction/selection/amplification (column 5), indicating the importance of subtraction steps before each selection. The increase in binding efficiency of the WiDr-selected phage pools (Fig 2B, and column 2 in Table 1), served as a good indicator for a successful selection because



**Figure 2** Selection of the PhD-12 library on human colorectal cancer cells. A total of  $1.5 \times 10^{11}$  phages were subtracted on  $10^7$  HeLa cells, three times. The percentage of remaining phages after the subtractions are shown in column 1 (A). The subtracted pool of phages was then selected on WiDr cells, and binding efficiency of the recovered phage pool is shown in column 1 (B). After amplification, the cycle of three subtractions/one selection was repeated four more times (columns 2 to 5 in A and B), but 293 cells were used for the subtractions. Subtraction method (b), Table 1.



**Figure 3** Binding efficiency and selectivity of candidate phages. Selected purified phages were assayed for cell-binding efficiency and selectivity. The candidate phages are QID, HEW, GWM, and YQA (referring to the first three amino acid letters only); see Table 1. VVN was coselected with HEW, but was less frequently represented in the final pool (5/24). Mean values for two or more independent experiments are shown. M13, without any peptide insert (wt), and GHF were used as negative controls.

each round of selection should decrease the number of nonbinding phages.

Table 1 shows the enrichment of cell-binding phages (increase in binding efficiency) following four different subtraction/selection protocols. After four or five selection rounds, an aliquot of the phage pool was plated out, and plaques were picked for purification and sequencing. The phages that appeared most frequently were selected as candidates, and binding efficiency and selectivity of the purified phages to different cells were then compared to the negative control phages M13 wt and GHF. These results showed that enrichment varied widely between the protocols, and strong enrichment did not necessarily correlate with selection of a single cell-specific phage.

*Binding efficiency of isolated phage candidates*

Figure 3 shows the binding efficiency and selectivity of the four isolated phage candidates presented in Table 1. Of all phages the HEW phage, which was selected by following protocol (b), appeared to be the most promising candidate: It showed more than 1000-fold higher binding efficiency and selectivity for the colorectal WiDr cells than for the subtraction cells (HeLa or 293), and when compared to the M13 wt or GHF controls. In addition, about 50-fold higher selectivity for the breast cancer MDA-MB-435 cells was also seen, showing that the HEW receptor (or binding molecule) is not uniquely WiDr specific. The VVN phage (VVNQRPLAPGW) was coselected with the HEW phage at a lower frequency (5/24), it contained also the sequence LAP-W, but it showed no binding or selectivity for WiDr cells (see Fig 3). Other coselected phages showed no homology or WiDr cell binding. The QID phage showed binding to all four cell lines (including HeLa) (Fig 3); it was also selected on WiDr cells, but by subtracting with HeLa cells only before the first selection method (a). This suggests that subtractions before each selection round are critical for the isolation of cell-selective phages. The GWM phage showed high binding efficiency for MDA-MB-231, on which it was selected, but also some binding to the 293 subtraction cells. It also bound WiDr cells efficiently, indicating that it did not recognize a unique receptor. The three remaining phages from the sequenced pool of

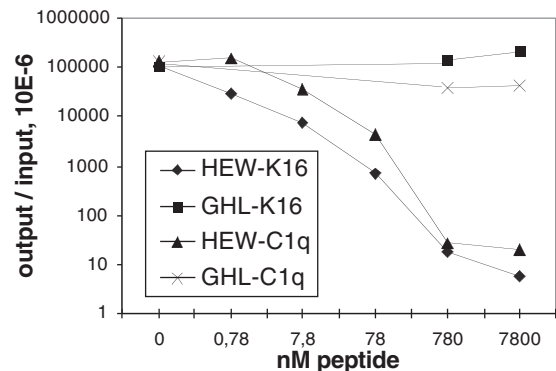
14 showed no homology to the GWM phage, or binding to MDA-MB-231 cells. The YQA phage was also not uniquely MDA-MB-231 selective and the overall binding was 10-fold lower (Fig 3). Thus, the HEW and the GWM phages showed the highest binding efficiencies to their respective selection cells ( $>10^3$ ), and because the HEW phage was more cell specific than the GWM phage it was further characterized.

A BLAST search on swissprot+trembl with the selected candidates did not reveal homology to any known polypeptide.

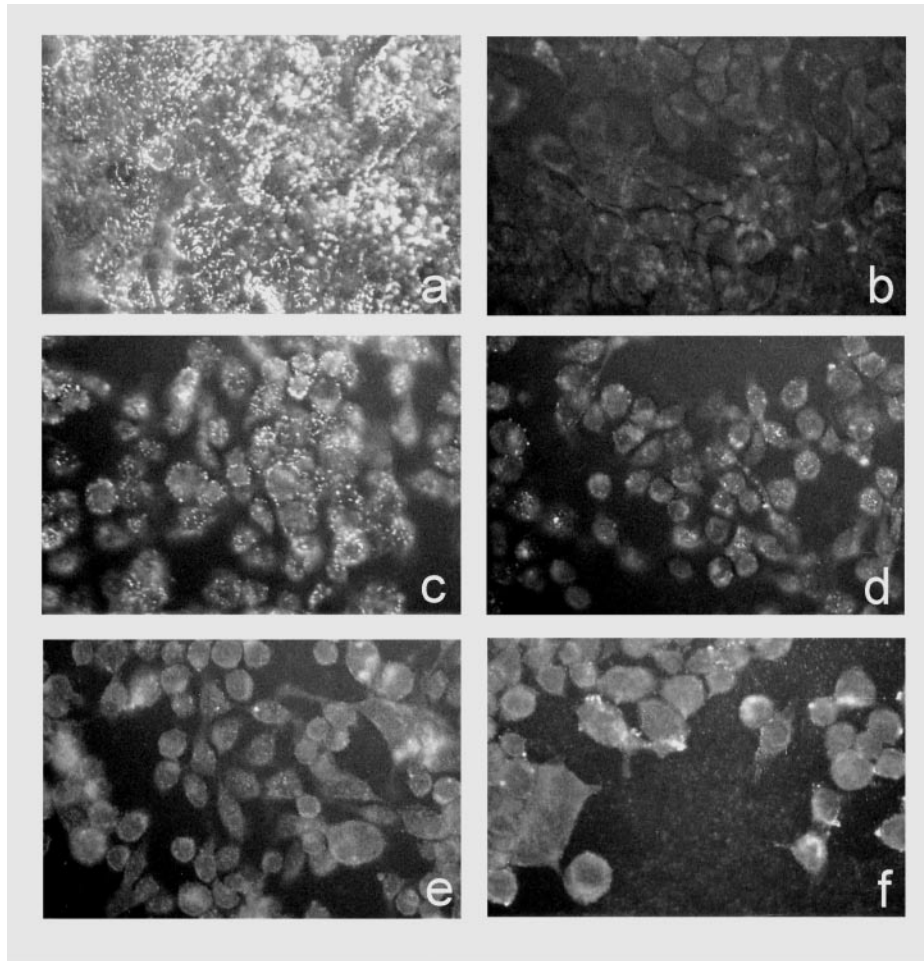
*Specificity of selected HEW peptide*

Peptides containing the HEW sequence were obtained to test whether the free peptide could compete with phage binding. Competition by free peptide would indicate that the binding to WiDr cells is not restricted to an M13-induced conformation, but the sequence may be

*Competition of phage binding with free peptides on WiDr cells*



**Figure 4** Competition of HEW phage binding to WiDr cells with free peptides. Increasing amounts of peptide (HEW-K<sub>16</sub>, HEW-C<sub>1q</sub>, or control GHL6-K<sub>16</sub> or GHL-C<sub>1q</sub>) were added to  $5 \times 10^6$  WiDr cells before addition of  $1.5 \times 10^{11}$  HEW phages and determination of binding-efficiencies. Mean values are shown for two (HEW-C<sub>1q</sub>) or three (HEW-K<sub>16</sub>) independent experiments.



**Figure 5** Immunocytochemistry of WiDr cells using anti-phage IgG, and FITC-conjugated secondary antibodies. The cells were incubated with the HEW phage (**a, c, e**) or the M13 wt phage (**b, d, f**) at either 4°C (**a, b**) or at 37°C (**c, d, e, f**). After incubation at 37°C and “stripping” of phages bound to the cell surface, cells were fixed and permeabilized before the antibody incubations (**e, f**). Phages were visible as discrete fluorescent spots at the cell surface.

incorporated in another context and still target WiDr cells. We tested peptides with the specific HEW sequence and with either of two different cationic peptide sequences (K<sub>16</sub> or C1q) at the C-terminal end. The cationic peptides have DNA-condensing abilities and might be useful for transfections. The unspecific GHL sequence was chosen as the negative control. As shown in Figure 4, competition by the HEW peptide started to become visible at equimolar concentration (780 pM), it was complete at a 10,000-fold molar excess (7.8 μM), and the concentration for 50% competition was at about 100-fold molar excess. The control GHL peptide did not compete with phage binding. Thus, the free HEW peptide binds to the same WiDr receptor as the HEW phage.

Immunocytochemistry was performed to confirm the HEW-phage binding to adhered WiDr cells, and to test for presence of intracellular phages after incubation at 37°C. Figure 5 shows binding of the HEW phage at 4°C and 37°C, but not of the M13 wt phage. The signal from the HEW phage after stripping and permeabilization was similar to the negative M13 wt control background, indicating that the

HEW phage is not being internalized during the 37°C incubation.

#### Transfections

The K<sub>16</sub> polylysine and the C1q sequence have been reported to condense DNA and mediate transfection.<sup>14,15</sup> Therefore, we tested whether the HEW-K<sub>16</sub> or HEW-C1q fusion peptides would target transfections to WiDr cells after condensing a luciferase expression plasmid. Transfections in WiDr cells were compared with transfections in 293 cells, using the two HEW fusion peptides, or the two negative control GHL fusion peptides. After optimizing the condensation conditions and following the protocols of Harbottle *et al*,<sup>14</sup> we did not see luciferase expression in WiDr cells. Adding lipofectin resulted in transfection with the HEW peptide, but this was also the case in 293 cells when no washings were done (not shown), indicating that the lipofectin-mediated transfection is unspecific. Thus, further development of complexes containing DNA and the HEW peptide will be necessary to obtain a suitable gene transfer vector for targeting.

## Discussion

Here we have described a method to select peptide displaying phages with high binding efficiency and selectivity to the human colorectal WiDr cells. We compared different methods and cells, and the protocol that yielded the best phage candidate involved three subtraction rounds before each selection step. Although detaching cells from the surface of the culture vessel may change the composition/repertoire of some exposed cell receptors, we chose to include a detaching step and to perform selections on cell suspensions. We tested the protocol on both attached and suspended cells, and we found that adherent cells detached during the incubations. In addition, incubations could be performed with higher cell concentrations using cell suspensions. Also, it was easier to standardize the protocol (same number of cells and phages in the same volume for each subtraction/selection), and to compare the results when suspension cells were used. Binding of selected phages on attached cells was then verified by immunocytochemistry. Subtractions were first done on HeLa or 293 cells before each subsequent selection. The readily available human HeLa and 293 cells were chosen for practical reasons as the subtraction cells. However, for future selections it would be more relevant to use normal colon epithelial cells for the subtractions, to avoid targeting of normal colon epithelium-related receptors, and to optimize selection for tumor-related epitopes.

We selected a phage candidate displaying the peptide sequence HEWSYLAPYPWF. It showed more than 1000-fold higher specificity for WiDr cells when compared to negative control phages, and when compared to five other human cancer cell lines. Synthetic peptides containing the phage-displayed sequence competed efficiently for the phage binding on the WiDr cells. Thus, this sequence is specific for a receptor on WiDr cells, and independent of the display by phage coat protein pIII. The immunohistochemistry confirmed the WiDr cell binding on attached cells, and suggests that the peptide may be used in designing reagents for diagnostic purposes. Specificity is described here only in the context of *in vitro* selections, and binding to the cells used. A colorectal cancer targeting therapeutical may also be developed by combining the sequence with a cytotoxic compound.<sup>11,16</sup> However, because therapeutic treatment implies *in vivo* use, it will be important to know the *in vivo* specificity of the selected peptide sequence by determining the binding efficiencies to different normal human tissues. Because any given receptor may be expressed in several cell types at different levels and times, one has to exclude any unwanted binding to healthy tissue.

The WiDr cell-specific HEW peptide may also be used as a targeting ligand on gene transfer vectors, or other vehicles, which could deliver genes or small toxic molecules to colon tumors. However, a simple fusion of the HEW peptide to a DNA-condensing peptide did not result in transfection of cells. This is most likely due to the inability of the HEW peptide to be internalized via the cell surface receptor to which it binds. Harbottle *et al*<sup>14</sup> demonstrated transfection of cells using RGD-containing peptides fused to DNA-condensing peptides. The RGD motif targets integrin

receptors, which are found in many tissues, healthy as well as cancerous, and they are known to be internalized by phagocytosis. Thus, an optimized transfection complex needs to contain an internalizing component in addition to the targeting peptide. For example, the HEW peptide could be grafted onto an adenovirus fiber knob in which the CAR binding site has been mutated. Such a viral vector may acquire a HEW-directed tropism by binding specifically to colon cancer cells, and internalize via the adenovirus-specific mechanism involving the RGD motif in the penton base.<sup>17</sup>

As shown, specific phages can be readily isolated from complex libraries by *in vitro* selections. A similar protocol has also been used to select for phage-displayed peptides that bind specifically to malignant glioma cells.<sup>18</sup> However, for the purpose of targeted gene delivery, internalization is necessary, and if the candidate peptide sequence is not internalized via the cell surface receptor to which it binds, an internalization component has to be added together with the DNA of interest.

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## References

1. St Croix B, Rago C, Velculescu V, *et al*. Genes expressed in human tumor endothelium. *Science*. 2000;289:1197–1202.
2. Pasqualini R. Vascular targeting with phage peptide libraries. *Q J Nucl Med*. 1999;43:159–162.
3. Pasqualini R, Koivunen E, Ruoslahti E. Alpha v integrins as receptors for tumor targeting by circulating ligands. *Nat Biotechnol*. 1997;15:542–546.
4. Yip YL, Hawkins NJ, Smith G, *et al*. Biodistribution of filamentous phage-Fab in nude mice. *J Immunol Methods*. 1999;225:171–178.
5. Nicklin SA, White SJ, Watkins SJ, *et al*. Selective targeting of gene transfer to vascular endothelial cells by use of peptides isolated by phage display. *Circulation*. 2000;102:231–237.
6. Poul MA, Becerril B, Nielsen UB, *et al*. Selection of tumor-specific internalizing human antibodies from phage libraries. *J Mol Biol*. 2000;301:1149–1161.
7. Ridgway JB, Ng E, Kern JA, *et al*. Identification of a human anti-CD55 single-chain Fv by subtractive panning of a phage library using tumor and nontumor cell lines. *Cancer Res*. 1999;59:2718–2723.
8. Barry MA, Dower WJ, Johnston SA. Toward cell-targeting gene therapy vectors: selection of cell-binding peptides from random peptide-presenting phage libraries. *Nat Med*. 1996;2:299–305.
9. Johns M, George AJ, Ritter MA. *In vivo* selection of sFv from phage display libraries. *J Immunol Methods*. 2000;239:137–151.
10. Samoylova TI, Smith BF. Elucidation of muscle-binding peptides by phage display screening. *Muscle Nerve*. 1999;22:460–466.

11. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science*. 1998;279:377–380.
12. Rajotte D, Arap W, Hagedorn M, et al. Molecular heterogeneity of the vascular endothelium revealed by *in vivo* phage display. *J Clin Invest*. 1998;102:430–437.
13. Van Ewijk W, de Kruif J, Germeraad WT, et al. Subtractive isolation of phage-displayed single-chain antibodies to thymic stromal cells by using intact thymic fragments. *Proc Natl Acad Sci USA*. 1997;94:3903–3908.
14. Harbottle RP, Cooper RG, Hart SL, et al. An RGD-oligolysine peptide: a prototype construct for integrin-mediated gene delivery. *Hum Gene Ther*. 1998;9:1037–1047.
15. Jiang H, Cooper B, Robey FA, et al. DNA binds and activates complement *via* residues 14–26 of the human C1q A chain. *Biol Chem*. 1992;267:25597–25601.
16. Park BW, Zhang HT, Wu C, et al. Rationally designed anti-HER2/neu peptide mimetic disables P185<sup>HER2/neu</sup> tyrosine kinases *in vitro* and *in vivo*. *Nat Biotechnol*. 2000;18:194–198.
17. Peng KW, Russell SJ. Viral vector targeting. *Curr Opin Biotechnol*. 1999;10:454–457.
18. Spear MA, Breakefield XA, Beltzer J, et al. Isolation, characterization, and recovery of small peptide phage display epitopes selected against viable malignant glioma cells. *Cancer Gene Ther*. 2001;7:506–511.