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Use of in vivo phage display to engineer novel adenoviruses for targeted delivery to the cardiac vasculature

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ABSTRACT

We performed in vivo phage display in the stroke prone spontaneously hypertensive rat, a cardiovascular disease model, and the normotensive Wistar Kyoto rat to identify cardiac targeting peptides, and then assessed each in the context of viral gene delivery. We identified both common and strain-selective peptides, potentially indicating ubiquitous markers and those found selectively in dysfunctional microvasculature of the heart. We show the utility of the peptide, DDTRHWG, for targeted gene delivery in human cells and rats in vivo when cloned into the fiber protein of subgroup D adenovirus 19p. This study therefore identifies cardiac targeting peptides by in vivo phage display and the potential of a candidate peptide for vector targeting strategies.

1. Introduction

A greater understanding of the pathophysiological mechanisms of cardiovascular disease has aided the development of intervention strategies. Gene therapy could correct debilitating and costly disorders such as coronary heart disease and reduce the damage caused by ischemia after myocardial infarction by delivering cardioprotective genes. Major hurdles exist before these disorders can be treated routinely in the clinical setting. For example, the therapeutic gene must be delivered safely and efficiently to its target site in vivo, ideally in a site-specific manner. Concerns over currently available vectors include their native tropism and the inability to efficiently target to the heart after *iv* injection. It is possible to engineer novel tropism into viral vectors through vector engineering strategies [1]. For this to be achieved effectively in the context of heart disease, there is a need for "ligands" which are specific and efficient at targeting the heart. To this end, ligands have been identified by screening random peptide presenting libraries, in vitro/ex vivo, that are able to bind specifically to other cell types [2–5] as well as the heart vasculature [6]. Targeting vectors to specific vascular beds in vivo is possible due to the phenomenon termed "the vascular address system" [4].

Molecular signatures exist in blood vessels of different organs and tissues as well as between normal and angiogenic or remodelled blood vessels [5,7–9] providing a rational basis for developing targeted gene therapy vectors. Phage display of peptide libraries is based on the concept that insertion of a random oligonucleotide encoding a peptide within a gene encoding one of the structural proteins of the phage will lead to its display on the surface of the phage. Aside from different peptide lengths, random peptide libraries can be presented differently on the phage surface, e.g. in linear or constrained forms, the latter created via the incorporation of two additional cysteine residues flanking the peptide sequence and consequently enabling formation of a disulfide bond in non-reducing conditions. Over 1×10^9 permutations of 7mer peptides are possible, with each clone represented several times in the library.

Recipient vectors for targeting peptides include non-viral systems as well as those based on viral vectors, including retroviruses, adenoviruses and adeno-associated viruses. Targeting of adenovirus serotype 5 vectors (Ad5) has proven highly efficient in vitro but difficult in vivo, likely due to previously unknown mechanisms regarding liver transduction by Ad5. We recently identified that the Ad5 hexon is responsible for liver delivery through recruitment of host FX to the hypervariable region on the capsid surface [10,11]. Furthermore, we recently created a potentially useful vector, Ad5/19p, a vector based on Ad5 but the fiber swapped for that of the subgroup D prototype fiber 19p [12], for targeted gene delivery using peptides [13,14]. We show that basal transduction of

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hepatocytes in culture was very low compared to Ad5 and that the HI loop of the 19p fiber was permissive for peptide insertions and targeted gene delivery [14].

Here, we use in vivo phage display to identify targeting peptides selective for the heart in two rat strains, the normotensive Wistar Kyoto rat (WKY) and the stroke prone spontaneously hypertensive rat (SHRSP), a model of human essential hypertension with a propensity for left ventricular hypertrophy. We demonstrate their selectivity for the heart and utility in targeted vector delivery using a novel Ad5/19p-based vector targeting strategy.

2. Materials and methods

2.1. Animals

Twelve-week old male WKY and SHRSP were used. Animal experiments were in accordance with the Animals Scientific Procedures Act 1986.

2.2. In vivo phage selections

For in vivo phage display (Fig. 1A), the femoral vein was exposed and 2×10^{11} pfu phage (7mer M13 Phage Display Library; New England Biolabs, UK) injected. Immediately prior to cardiac perfusion at physiological pressure with saline 150 μL of heparin was injected via the vena cava. Animals were perfused until the perfusate was clear and the organs appeared free from blood. Organs and tissues were placed in DMEM-PI (DMEM, 1% BSA, 1 μg/mL leupeptin, 2 μg/mL aproprotin, 1 mM Phenylmethylsulfonylfluride) on ice before being snap frozen through isopentane for storage at −70 °C.

2.3. Phage recovery

Tissues were thawed on ice, weighed, and homogenized in DMEM-PI using a Ribolyser as previously described [15]. Briefly, samples were homogenized via repeated cycles on the Ribolyser

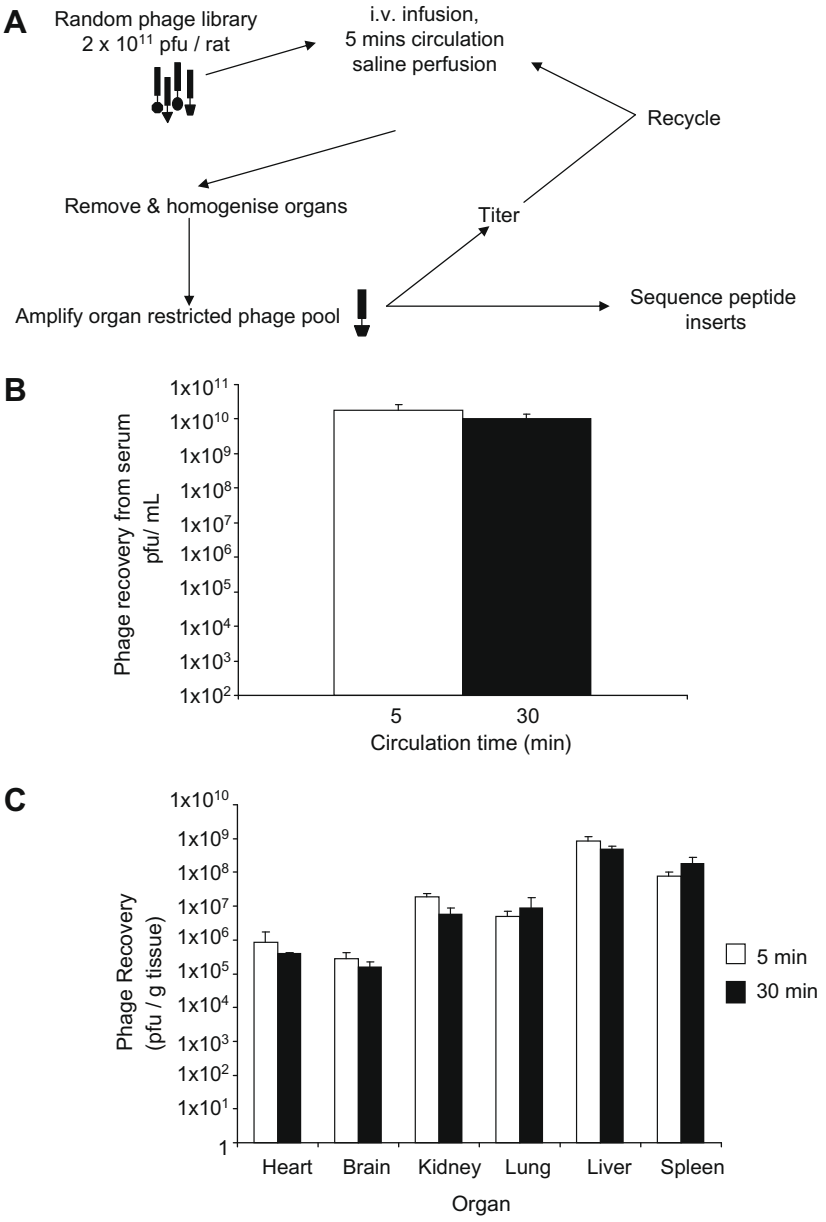

Fig. 1. Optimisation of in vivo phage display. (A) Schematic representation of phage display. (B) Levels of phage associated with serum at 5′ and 30′ post *iv* injection in rats. (C) Levels of phage associated with organs at 5′ and 30′ post *iv* injection in rats.

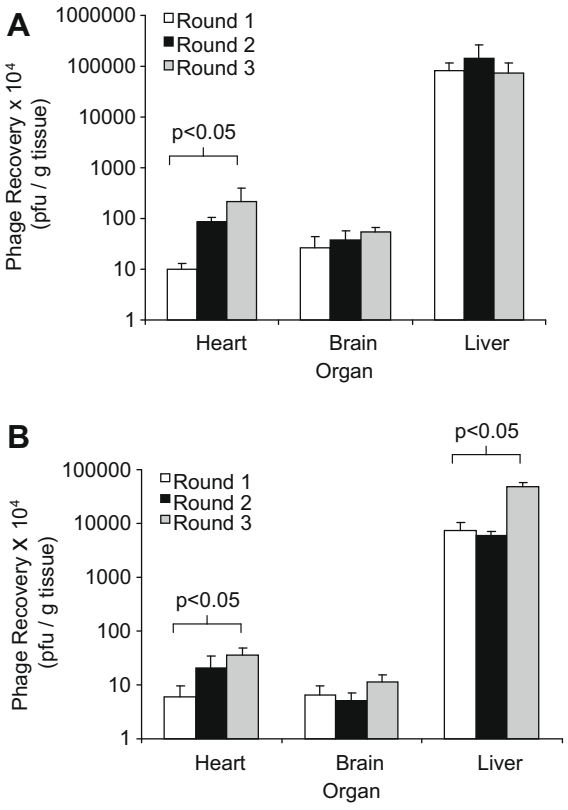

Fig. 2. Analysis of phage enrichment over successive rounds of in vivo phage display. Phage libraries were injected into (A) WKY and (B) SHRSP rats (n = 3/strain/round) and hearts harvested following saline perfusion 5 min post-injection. Heart-bound phage were extracted, amplified and recycled through subsequent rounds. The recovery in each target organ (heart) and control non-target organs (brain, liver) were quantified.

at a speed of 5.5 for 45 s per run (3 times) before the homogenate was centrifuged and cleared lysate harvested. The organ tissue lysate was stored at 4 °C before phage titering according to manufacturer's instructions (New England Biolabs, UK). Titers were normalized per gram of homogenized starting tissue. For subsequent rounds of biopanning, 500 μL of tissue lysate containing phage from the previous round was amplified by infection into bacteria and purified according to manufacturer's instructions. Three rounds of phage display to identify heart targeting peptides was performed in each rat strain (n = 3 per round). The phage was titred and the same input phage dose of 2×10^{11} PFU was used for each round of panning. The values for the total phage recovered do not represent the total for the whole organ rather they represent the total number of phage obtained from homogenized tissue normalized to input weight of tissue homogenized thus phage per gram of tissue and for serum calculated per milliliter.

2.4. Evaluating phage enrichment

The M13 antisense primer (5′-CCC TCA TAG TTA GCG TAA CG-3′), and M13 sense primer (5′-GCA ATT CCT TTA GTG GTA CC-3′), were designed based on the pIII gene sequence presented in the Ph.D.-7 Phage Display instruction manual (New England BioLabs, Hertfordshire, UK). The following PCR conditions were used: 94 °C 1 min; denaturing: 94 °C, 1 min; annealing: 54 °C, 1 min; extending: 72 °C, 1 min (35 cycles), 72 °C for 15 min. The PCR product, was then sequenced to determine the peptide insertion.

2.5. Biodistribution analysis of individual candidate phage in vivo

Peptides were selected from in vivo biopanning based on frequency of identification and/or selectivity for the heart. WKY and SHRSP rats (n = 3) were predosed with 2×10^{11} PFU of non-peptide expressing (control) M13 phage without LacZ α-complementation via the femoral vein to saturate non-specific reticulo-endothelial system sequestration. Five minutes later, a second infusion of a homogeneous population of individual candidate peptide-expressing phage with LacZ α-complementation (5×10^{10} PFU) was infused. After a further 5 min, the rats were killed and blood and tissues harvested. The levels of candidate peptide-expressing phage located in the heart were compared to that obtained in animals infused with a control phage which displayed no peptide by infection into bacteria followed by plating serial dilutions onto LB:X-Gal plates and calculating the ratio of blue:white plaques following 16 h incubation.

2.6. Construction of targeted Ad5/19p vectors

The Ad19p fiber cDNA in the plasmid pDV145 and the cloning plasmid Ad19p-*Eco*47III, which contains a unique restriction site in the Ad19p HI loop were constructed as previously described [14]. Oligonucleotides encoding the peptide DDTRHWG were purchased from MWG (Milton Keynes, UK) and ligated into *Eco*47III digested pDV145-*Eco*47III and sequenced to ensure correct orientation. The following plasmid was produced pDV145-DDTRHWG and used to generate Ad19p-DDTRHWG virus. Stocks of recombinant pseudotyped adenoviruses Ad19p-DDTRHWG and Ad19p-*Eco*47III were generated by transfection of 293T cells (ATCC) with the modified plasmids described above followed by superinfection with a fiber-deleted Ad5 vector [16,17]. Briefly, 293T cells were transfected with the appropriate fiber-expressing plasmid. 16 h later cells were superinfected with an E1, E3, fiber-deleted rAd5 (Ad5ΔF) (β-galactosidase) at 2000 virus particles (VP)/cell. Virus particles were purified by CsCl ultracentrifugation and dialysed into 10 mM TRIS (pH 8.1), 150 mM EDTA and 10% glycerol. Virus particles were quantified by BCA protein assay with bovine serum albumin (BSA) standards according to the conversion: 1 μg protein = 4×10^9 VP [18].

3. Results

3.1. Selection of optimal phage harvest time point by in vivo analysis in rats

For in vivo phage display in rats (Fig. 1A) we first injected 2×10^{11} plaque forming units (PFU) of phage peptide library into the femoral vein of WKY rats for either 5 or 30 min. A blood sample was taken prior to saline perfusion and organ harvest. The levels of phage in serum after 30 min of circulation fell to 55.8% of input at 5 min (Fig. 1B). In harvested organs at 5 min post-injection, the brain had the lowest level of phage per gram of tissue (2.69×10^5 PFU), followed by heart (8.86×10^5 PFU), lung (5.06×10^6 PFU), kidney (1.95×10^7 PFU), spleen (7.82×10^7 PFU), and liver (8.26×10^8 PFU) (Fig. 1C). After a 30 min circulation time, the brain remained the organ with the lowest mean phage levels and the pattern of phage distribution remained essentially the same as that at 5 min (Fig. 1C). The 5 min circulation time was therefore selected for all further experiments.

3.2. In vivo phage display

We first ensured the heterogeneity of the input library by plating and sequencing 91 individual plaques. As shown in Supplementary Table 1 all 91 sequences were different. We therefore proceeded to in vivo phage display. Phage titers in organs from normotensive

WKY rats and hypertensive SHRSP were compared over three individual rounds of phage display following sacrifice and tissue harvesting 5 min post injection (Fig. 1A; Fig. 2). In WKY rats, phage levels in the heart increased significantly over the rounds of phage display while levels in the two control organs (liver and brain; representing organs with and without a reticulo-endothelial system, respectively) were not altered. As expected, through each individual round of phage display, the liver remained the predominant tissue for phage accumulation, yet elevated heart-homing phage pools were clearly identified (Fig. 2A). Similarly, in the SHRSP a statistically significant increase in heart-homing phage was observed by round 3, with no accompanying elevation in the brain homing phage, although an elevation in the phage in the liver was noted (Fig. 2B).

3.3. Identification of peptide sequence enrichment for heart-homing phage

To identify candidate heart-homing peptide motifs, we sequenced the peptide inserts in individual phage clones and calculated their frequency in the phage pool (based on the total number of phage sequenced at each round). Following phage display, clones isolated from the hearts of perfused WKY and SHRSP in phage display rounds 2 and 3 were sequenced. In WKY animals (Supplementary Table 2), although a number of peptides appeared relatively frequently (e.g. SPTQELF and VQASNSN) we noted remaining heterogeneity in the phage pool. We therefore performed an additional round of biopanning to enrich the pool further (Supplementary Table 2). Following this additional round a powerful enrichment of the peptide VQASNSN (from 3% to 43% of the pool) was observed with IPTHIRP (7% of the pool) being the second most frequent peptide (Supplementary Table 2). Analysing peptide structure via side chain characteristics of individual amino acids highlighted further conserved structure amongst peptides (Supplementary Table 2). In SHRSP (Supplementary Table 3) identical and different peptides to those in WKY, with substantial enrichment of the pool by round 3 were also identified. In agreement with WKY rats VQASNSN (47%), LPPPPNP (24%) and LPLTPLP (3%) were recovered. However, other different peptides including DDTRHWG (7%) and

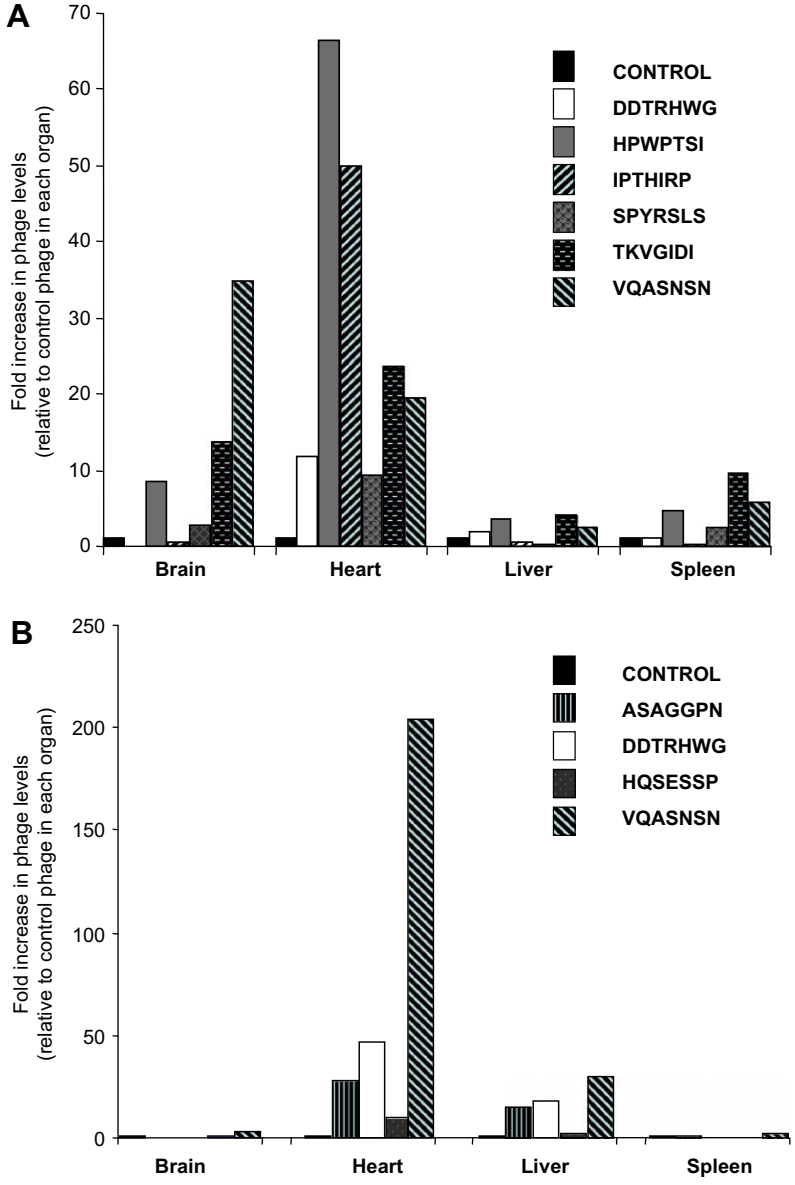

Fig. 3. Analysis of heart homing of individual populations of individual candidate peptide-expressing phage. About 5×10^{10} phage (for each individual peptide) were infused into (A) WKY or (B) SHRSP rats (3/group) using a double-infusion protocol and homing capacity for the heart and control, non-target organs (brain, liver and spleen) quantified and expressed as a fold change vs. control phage (also $n = 3$/group).

LPPPPNP (3%) were also identified. Since both strains yielded a pool of phage with VQASNSN being very predominant we further analysed the pool sequenced from rounds 3 and 4 in the WKY and 2 and 3 in the SHRSP for related peptides. Remarkably, we identified an array of closely related peptide sequences, particularly enriched in the final round of biopanning in each strain (representing 70% of the pool for WKY and 52% for SHRSP; Supplementary Table 4).

3.4. Evaluation of candidate peptide-expressing phage in vivo

From all the sequencing data, several candidate peptides were selected for further analysis in the two rat strains. Pure populations of each candidate peptide-expressing phage were injected individually into rats (3/peptide) via a double phage infusion strategy (see Section 2). In the WKY rat, we assessed DDTRHWG, HPWPTSA, IPTHIRP, SPYRSLS, TKVGIDI and VQASNSN and compared homing to brain, heart, liver and spleen compared to insertless phage (Fig. 3A; $n = 3$ animals/group). For WKY, all peptides examined homed to the heart at levels at least 9-fold-higher than control phage. For example, IPTHIRP phage was 49.8-fold higher and HPWPTSI 66.3-fold higher than control phage. VQASNSN, the most frequently identified peptide (Supplementary Table 2) homed to the heart and the brain (Fig. 3A). The profiles of each selected peptide were all different yet all possessed a degree of heart homing capacity (Fig. 3A). In the SHRSP, the heart-homing capabilities of phage displaying ASAGGPN, DDTRHWG, HQSESSP and VQASNSN peptides were compared to control phage. VQASNSN homed to the heart at levels 200-fold higher than control phage (Fig. 3B). In addition, VQASNSN also homed to the lung, liver and kidney at levels more than 10-fold higher than control phage. Both ASAGGPN and DDTRHWG homed to the heart considerably more than control phage (by 27.4- and 46.4-fold, respectively). DDTRHWG therefore showed 46-fold enhancement in SHRSP compared to 11-fold in WKY further supporting its potential selectivity for the SHRSP heart.

3.5. Assessment of phage binding to vascular endothelium and hepatocytes

Based on the heart selectivity and heart homing capacity we focused on DDTRHWG for further studies as a candidate for targeted gene delivery. In order to evaluate targeted virus-mediated gene delivery we further screened phage displayed-peptide in vitro in endothelial (RGE and Y-PEN) and non-endothelial (hepatocyte-derived – ARL6) cell lines from the rat. Exposure of each cell line to control phage and phage expressing DDTRHWG was performed for 30 min at 4 °C and phage binding quantified following extensive washing to remove unbound or weakly bound phage. Both rat endothelial lines showed high level binding for the peptide compared to control phage, an effect that was not apparent in the hepatocyte cell line (Fig. 4). This confirms the selectivity of this peptide for endothelial cell targeting.

3.6. Analysis of peptides in the context of targeted adenovirus-mediated gene delivery

Therapeutic avenues for peptides that home to defined tissues include both delivery of biologically active peptides and small molecules as well as their utility in driving targeted gene therapy

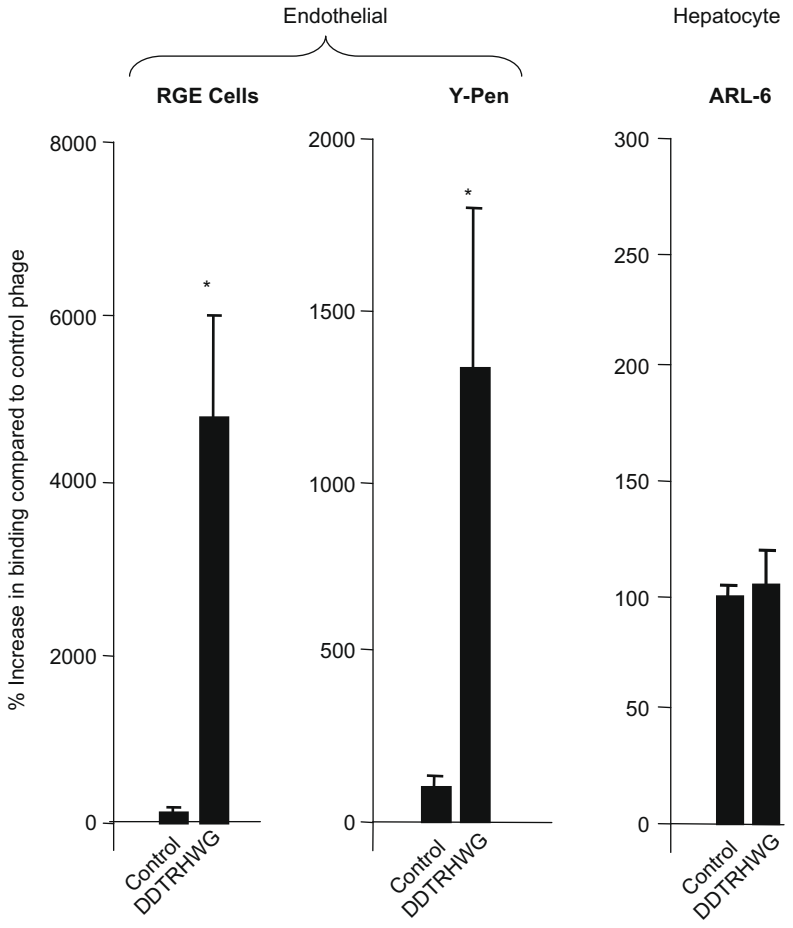

Fig. 4. Analysis of phage binding to cells in vitro. RGE, Y-PEN and ARL6 rat cell lines were plated and incubated with control phage or DDTRHWG-expressing phage for 30 min at 4 °C. Bound phage was quantified by titration following harvest. $^{*}P < 0.05$ vs. control phage.

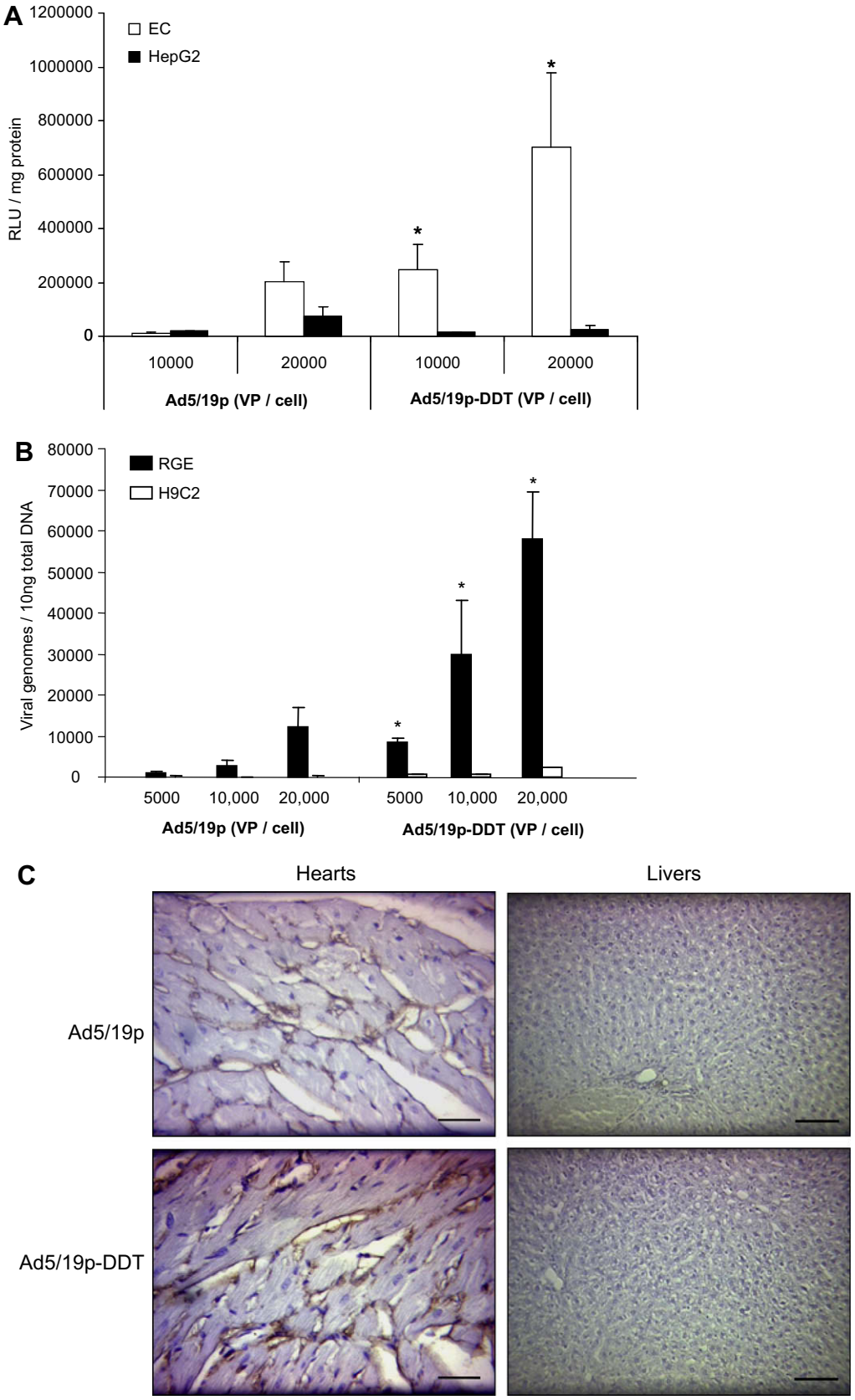

Fig. 5. Analysis of transgene expression from modified viruses in vitro and in vivo. (A) Human endothelial cells and HepG2 cells were infected with Ad5/19p or Ad5/19p-DDT for 3 h, cells washed and transgene expression quantified at 48 h post-infection. Values on the *x*-axis represent VP/cell used for infection. $^{*}P < 0.05$ vs. Ad5/19p. (B) Rat endothelial cells (RGE) and rat cardiac cells (H9c2) were exposed to increased concentrations of Ad5/19p or Ad5/19p-DDT at 4 °C for 1 h, subsequently washed and total DNA harvested. Bars represent levels of cell bound virus in vector genomes/10 ng recovered DNA quantified by Taqman RT-PCR. $^{*}P < 0.05$ vs. Ad5/19p. (C) Viruses (1×10^{12} VP/kg) were infused intravenously into SHRSP rats, killed at 48 h post infection and assessed for transgene (LacZ) expression by immunohistochemistry.

approaches. For the latter, we have previously used both adenovirus and adeno-associated viruses as vectors for assessment of targeted gene delivery evoked by phage display-derived peptides. However, due to the spatial constraints on peptides dictated by the site in which they are inserted into the viral capsids, not all peptides maintain their targeting potential in the context of viral delivery. We therefore cloned DDTRHWG into the HI loop of the novel adenovirus vector Ad5/19p [14]. We have previously shown that Ad5/19p has reduced tropism for hepatocytes compared to Ad5 and that the HI loop can be modified by targeting peptides to achieve modified tropism *in vitro* and in vivo [14]. Insertion of DDTRHWG in this study was well tolerated as evidenced by equivalent titers for both non-modified and peptide-modified vectors (not shown). We next assessed gene delivery to human endothelial cells. For Ad5/19p-DDT we observed significantly improved gene delivery to endothelial cells compared to control Ad5/19p (Fig. 5A) which was not observed in the human hepatocyte cell line HepG2. This indicates the efficiency of the peptide for mediating selective endothelial targeting across species. We also assessed the peptide-modified virus for binding to endothelial vs. cardiomyocyte specificity using RGE rat endothelial cells and H9c2 rat cardiomyocyte cells. Ad5/19p-DDT showed strong selectivity for RGE cells (Fig. 5B). In order to assess if binding to cells was increased in the presence of cytokines, we stimulated rat and human endothelial cells with interleukin-1α, TNF-α or a combination of the two and assessed the resulting transgene expression levels. Neither cytokine, nor the combination resulted in elevated endothelial cell transduction (data not shown). Finally, we injected 1×10^{12} VP/kg of Ad5/19p or Ad5/19p-DDT expressing LacZ into SHRSP rats and assessed cardiac targeting (Fig. 5C). Ad5/19p-DDT virus showed efficient transduction of the cardiac vasculature at levels clearly higher than that achieved with control Ad5/19p (Fig. 5C). Livers for both viruses showed no transduction (Fig. 5C).

4. Discussion

In this study we performed extensive in vivo phage display in normotensive Wistar WKY and hypertensive SHRSP rats to identify phage-displayed peptides which possess homing capacity for the heart in vivo. We report a number of peptides that were identified from hearts in both strains and some peptides found selectively in SHRSP. We further show the heart selectivity of phage by infusion of individual candidate peptide-expressing phage. Based on this we selected the peptide DDTRHWG for further studies in vitro and in the context of targeted gene delivery using a novel adenovirus vector, Ad5/19p. We demonstrate the selectivity of these peptides for vascular endothelial cells in the context of phage and once engineered into a novel adenovirus targeting vector Ad5/19p in vitro and in vivo. Importantly, the peptide showed the ability to mediate targeted gene delivery across species as efficiency could be shown in both rat and human cells, supporting its translational promise.

The ability to identify heart-homing peptides offers significant improvements in developing selective therapies, to enable their use as targeting moieties within the context of liposomes or viral gene delivery systems. Thus, phage display offers broad potential in this context. Our phage display highlighted several important points worthy of discussion. First, enrichment of selected phage was clear over sequential rounds of phage display since we sequenced the peptide insert from the phage recovered from the heart at multiple rounds. The enrichment for certain phage displayed-peptides (VQASNSN and derivatives – Supplementary Tables 2–4) was marked. In future studies it will be important to isolate the cognate receptors for such peptides and assess their in vivo biodistribution to correlate peptide homing and receptor expression. This is particularly important since injection of high quantities of individual candidate peptide-expressing phage populations (Fig. 3) showed, importantly, that all peptides possessed heart homing capacity. However, increased targeting to certain other tissues was also evident, and in a peptide-dependent manner. Based on the combination of sequencing to identify the encoded peptide and profiling by injection of individual candidate peptide-expressing phage we were able to identify efficient peptides for the targeted gene delivery approach.

In the context of gene delivery we have used a novel virus (Ad5/19p) that showed reduced liver tropism compared to Ad5 and can be targeted to alternate tissues when efficient peptides are included in the HI loop of the fiber [14]. Here, we show the inclusion of our cardiac homing peptides and show its efficiency for cardiac targeting following *iv* delivery. This novel vector will therefore have utility for future applications for in vivo gene delivery to the heart.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.05.037.

References

[1] Baker, A.H., Kritz, A., Work, L.M. and Nicklin, S.A. (2005) Cell-selective viral gene delivery vectors for the vasculature. Exp. Physiol. 90, 27–31.
[2] Pasqualini, R., Koivunen, E. and Ruoslahti, E. (1995) A peptide isolated from phage display libraries is a structural and functional mimic of an RGD-binding site on integrins. J. Cell Biol. 130, 1189–1196.
[3] Pasqualini, R. and Ruoslahti, E. (1996) Organ targeting in vivo using phage display peptide libraries. Nature 380, 364–366.
[4] Rajotte, D., Arap, W., Hagedorn, M., Koivunen, E., Pasqualini, R. and Ruoslahti, E. (1998) Molecular heterogeneity of the vascular endothelium revealed by in vivo phage display. J. Clin. Invest. 102, 430–437.
[5] Koivunen, E. et al. (1999) Tumor targeting with a selective gelatinase inhibitor. Nat. Biotechnol. 17, 768–774.
[6] Zhang, L., Hoffman, J.A. and Ruoslahti, E. (2005) Molecular profiling of heart endothelial cells. Circulation 112, 1601–1611.
[7] Arap, W., Pasqualini, R. and Ruoslahti, E. (1998) Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 279, 377–380.
[8] Pasqualini, R., Koivunen, E. and Ruoslahti, E. (1997) Alpha v Integrins as receptors for tumor targeting by circulating ligands. Nat. Biotechnol. 15, 542–546.
[9] Laakkonen, P., Porkka, K., Hoffman, J.A. and Ruoslahti, E. (2002) A tumor-homing peptide with a targeting specificity related to lymphatic vessels. Nat. Med. 8, 751–755.
[10] Kalyuzhniy, O., Di Paolo, N., Silvestry, M., Hofherr, S., Barry, M., Stewart, P. and Shayakhmetov, D. (2008) Adenovirus serotype 5 hexon is critical for virus infection of hepatocytes in vivo. PNAS 105, 5483–5488.
[11] Waddington, S. et al. (2008) Adenovirus serotype 5 hexon mediates liver gene transfer. Cell 132, 397–409.
[12] Von Seggern, D.J. et al. (2003) In vivo transduction of photoreceptors or ciliary bodies by intravital injection of pseudotyped adenoviral vectors. Mol. Ther. 7, 27–34.
[13] Denby, L., Work, L.M., Graham, D., Hsu, C.-L., Von Seggern, D.J., Nicklin, S.A. and Baker, A. (2004) Adenoviral serotype 5 vectors pseudotyped with fibers from subgroup D show modified tropism in vitro and in vivo. Hum. Gene Ther. 15, 1–15.
[14] Denby, L., Work, L.M., von Seggern, D.J., Wu, E., McVey, J., Nicklin, S.A. and Baker, A.H. (2007) Development of renal targeted vectors through combined in vivo phage display and capsid engineering of adenoviral fibers from serotype 19p. Mol. Ther. 15, 1647–1654.
[15] Work, L.M., Nicol, C.G., Denby, L. and Baker, A.H. (2005) In vivo biopanning: A methodological approach to identifying novel targeting ligands for delivery of biological agents to the vasculature. Methods Mol. Med. 108, 395–413.

[16] Von Seggern, D.J., Huang, S., Kaye-Fleck, S., Stevenson, S.C. and Nemerow, G.R. (2000) Adenovirus vector pseudotyping in fiber expressing cell lines: improved transduction of epstein-barr virus-transformed B cells. J. Virol. 74, 354–362.
[17] Jakubczak, J.L., Rollence, M.L., Stewart, D.A., Jafari, J.D., Von Seggern, D.J., Nemerow, G.R., Stevenson, S.C. and Hallenbeck, P.L. (2001) Adenovirus type 5 viral particles pseudotyped with mutagenized fiber proteins show diminished infectivity of coxsackie B-adenovirus receptor-bearing cells. J. Virol. 75, 2972–2981.
[18] Von Seggern, D.J., Chiu, C.Y., Fleck, S.-K., Stewart, P.L. and Nemerow, G.R. (1999) A helper-independent adenovirus vector with E1, E3 and fiber deleted: structure and infectivity of fiberless particles. J. Virol. 73, 1601–1608.