

Review

Systemically administered, target-specific, multi-functional therapeutic recombinant proteins in regenerative medicine

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Abstract: Growth factors, chemokines and cytokines guide tissue regeneration after injuries. However, their applications as recombinant proteins are almost non-existent due to the difficulty of maintaining their bioactivity in the protease-rich milieu of injured tissues in humans. Safety concerns have ruled out their systemic administration. The vascular system provides a natural platform for circumvent the limitations of the local delivery of protein-based therapeutics. Tissue selectivity in drug accumulation can be obtained as organ-specific molecular signatures exist in the blood vessels in each tissue, essentially forming a postal code system (“vascular zip codes”) within the vasculature. These target-specific “vascular zip codes” can be exploited in regenerative medicine as the angiogenic vasculature forming in the regenerating tissues has a unique molecular signature. The identification of vascular homing peptides capable of finding these unique “vascular zip codes” after their systemic administration provides an opportunity for the target-specific delivery of therapeutics to tissue injuries. Therapeutic proteins can be “packaged” together with homing peptides by expressing them as multi-functional recombinant proteins. These multi-functional recombinant proteins provide an example how molecular engineering gives a compound an ability to home to regenerating tissue and enhance its therapeutic potential. Regenerative medicine has been dominated by the locally applied therapeutic approaches despite these therapies are not moving to clinical medicine with success. There might be a time to change the paradigm towards systemically administered, target organ-specific therapeutic molecules in future drug discovery and development for regenerative medicine.

Keywords: vascular homing peptide; cell penetrating peptide; angiogenesis; vascular heterogeneity; fibrosis; targeted delivery; decorin; transforming growth factor- β (TGF- β), bystander effect, CendR peptide, tissue regeneration, regenerative medicine, hypoxia, neuropilin-1

Abbreviations

- AAA Abdominal aorta aneurysm
- BMP-4 Bone morphogenetic protein-4
- BGN Biglycan
- CTGF/CCN2 Connective tissue growth factor
- DCN Decorin
- EGFR Epidermal growth factor receptor
- ECM Extracellular matrix
- GAG Glycosaminoglycan
- HSPG Heparan sulphate proteoglycan
- KO Knockout
- NRP-1 Neuropilin-1
- PAH Pulmonary arterial hypertension
- PDGF Platelet derived growth factor

- TGF- β Transforming growth factor- β

1. Drug delivery in regenerative medicine

Intensive research during the past few decades has led to the identification of the key molecules, growth factors, cytokines and chemokines, for tissue regeneration after injuries [1,2]. As most of the top-selling drugs are recombinant proteins in the modern world, it was anticipated that these powerful molecules could be expressed as recombinant proteins and used as drugs in humans to augment the repair of tissue injuries. Unfortunately, their clinical application in aiding tissue regeneration in humans has been scarce [1,3,4], as the molecules have failed in clinical trials. There are several biological reasons for the failure: the rapid degradation of locally administered proteins in the protease-rich environment present in the injured tissue, the inability to retain small (growth factor, chemokines and cytokines are very small in size) recombinant proteins at the site of injury, poor tissue penetration, and side effects [4-6]. The biological issues illustrate the major roadblocks that need to be solved for biological protein-based therapeutics to be successful as a clinical therapy: (i) to reduce or eliminate the degradation of the recombinant protein-drugs in the inflammatory milieu of the injured tissue and (ii) to extend their retention time at the wound site [7]. Besides to these issues, most injuries are inaccessible for local delivery or involve multiple tissues injured simultaneously. They naturally call for a systemic administration.

In this regard it is actually quite stunning that almost all efforts with recombinant proteins aimed to enhance tissue regeneration are based on their local administration [4-6,8]. Although systemic drug administration of both recombinant proteins and conventional drugs is the only drug delivery mode used for the vast majority of human diseases, systemic administration has not been considered as a viable option in the treatment of tissue injuries due to the lack of efficiency and safety. These concerns are justified as only a small fraction of drug administered reaches its desired location in the body and the side effects such as the increased cancer risk are valid [7]. Moreover, sizable drugs such as antibodies have poor tissue penetration and therefore do not reach the intended target [9-12]. Target organ-specific drug delivery by vascular homing peptides and functional protein domains, such as cell penetrating peptides proficient in penetrating cells and tissues, could solve these problems. They could provide the means for the selective accumulation of the therapeutics in the injured tissue [12-17].

Vascular Heterogeneity – Zip code-system in vasculature enables tissue-specific drug delivery

One of the goals of the modern pharmaceutical treatment is to be as target specific as possible; drugs should be highly active against the disease, while having as few side effects in the healthy parts of the body as possible [18]. This goal is usually obtained by developing drugs that act on molecules selectively over-expressed in the diseased organ. However, any drug can be converted to a target-specific one by a targeted delivery to the desired location. The vascular system provides a natural platform for doing that. The expanding understanding of the molecular composition of blood vessels has shown that the blood vessels in each tissue have a molecular structure unique for that given tissue, essentially forming a system that is very similar to postal codes (“vascular zip codes”) [19,20] (Fig. 1). Moreover, various diseases induce the expression of disease-specific, unique molecular signatures in the vasculature of diseased tissue, providing an appealing target for the disease specific delivery of systemically administered drugs [19,20]. This is particularly evident in diseases like cancer and tissue injuries; both of them are associated with tissue hypoxia, which induces new blood vessel growth by a process called angiogenesis [20]. The angiogenic blood vessels are structurally and molecularly different from the dormant blood vessels elsewhere in the body and provide an accessible and abundant target for the organ-specific delivery of therapeutics [19,20] (Fig. 2). These organ- and disease-specific vascular zip codes can be engaged by systemically administered affinity ligands that seek out, i.e. “home”, to their target by binding to the corresponding vascular zip code receptor [19-21] (Fig. 2). A particularly useful method for discovering affinity ligands (vascular homing peptides or antibodies) for vascular zip codes has been *in vivo* phage display.

Ligands targeting injured tissues

The injured tissue provides an excellent target for the ligand-mediated delivery of systemically administered therapeutics [18]. The first actual sign of tissue regeneration after an injury and a

subsequent inflammatory period is the massive angiogenesis induced by the hypoxia to the injured area. The injured area is hypoxic due to the disrupted circulation in the damaged tissue [18,22,23]. Hypoxia triggers the production of angiogenic growth factors, which induce angiogenesis to the injured tissue. The abundance of angiogenic blood vessels in the granulation tissue, the loose connective tissue produced in the early phase of the healing period, is well illustrated by the fact that the word “granulation tissue” is actually derived from the granular appearance of the angiogenic capillaries. The thick microvascular bed essentially fills up the injured area and presents large number of sprouting angiogenic blood vessels expressing an overwhelming number of angiogenesis-related “molecular fingerprints” for systemically administered homing ligands to bind and deliver therapeutics to the regenerating tissue in target-specific fashion (Fig. 2). To obtain a “vehicle” for targeting tissues undergoing repair and regeneration, we screened a random peptide library (1.0×10^9) by *in vivo* phage display for injury-homing peptides against wounds and injuries in skin and tendon at the peak of the angiogenesis [24]. The screening yielded a number of peptides that homed to the angiogenic blood vessels in the newly formed granulation tissue [24]. The most potent homing peptide among them has the sequence CARSKNKDC, and was dubbed “CAR”. The CAR sequence contains a classical heparin-binding motif that shows high homology with the heparin binding domain of bone morphogenetic protein-4 (BMP-4), a well-established angiogenic growth factor. CAR peptide accumulates in the skin and tendon wounds/injuries at levels that are as much as 100-200 times higher than the levels of control peptides, and the homing is injury-specific; no CAR is detected in normal tissues. Importantly, CAR is a potent cell/tissue penetrating peptide since it effectively accumulates deep in the tissue parenchyma [24]. CAR binds to the cell surface heparan sulfate proteoglycans (HSPGs) and uses the HSPGs as receptors for cell binding and penetration into cells and target tissues [24]. The subsequent research has demonstrated that in addition to angiogenesis, CAR also recognizes vasculature in inflammatory diseases. It shows remarkable homing specificity (no homing to respective normal tissue) to pulmonary arterial hypertension (PAH), myocardial infarction, abdominal aorta aneurysm (AAA) and dystrophic muscle lesion in muscular dystrophies [25-29].

Although the target site-specific delivery of the therapeutics is an emerging field in regenerative medicine, quite many different practical applications for vascular homing peptides are already emerging. Some of them are more general, i.e. can be applied to all injuries, such as identifying peptides capable of binding to the angiogenic or inflammatory blood vessels forming at the injured tissues [24], or peptides binding to the extracellular matrix (ECM) collagen exposed in inflammatory conditions [30]. Additional targeting peptides have been described towards more specific clinical molecular targets such as blood clots after an injury [31], angiogenic blood vessels at early [32] and late stage of wound healing when granulation tissue matures to scar tissue [24], ruptured blood vessels [33], transected nerves [34], intestine with burn injury [35,36], brain injury after a traumatic breakage of the blood-brain barrier [37,38], bone tissue [39-41], bacterial infections [42] or simply illuminating neural structures during surgery [43,44] (Table 1).

Systemically administered anti-fibrotic molecule, CAR-decorin

To make use of the disease-homing properties of CAR, we generated a multi-functional therapeutic recombinant protein, in which the CAR peptide is expressed together with the decorin (DCN) core protein (CAR-DCN) [45,46] (Fig. 3). CAR functions (1) as a homing device in the fusion protein that delivers the therapeutic molecule to the target, (2) mediates cell and tissue penetration, and (3) attaches its payload to the target cells through HSPGs [45] (Fig. 3). DCN, in turn, functions as a therapeutic molecule in the multi-functional fusion molecule. DCN is a member of the small leucine-rich proteoglycan (SLRP) family of ECM proteins. Owing to its physical interactions with the collagen fibers in the ECM, i.e. DCN decorates collagen fibers, the proteoglycan was named decorin [47]. In addition to being a structural component of the ECM, DCN influences cellular functions such as proliferation, spreading, migration, differentiation and regulates inflammation [48-50]. DCN has received much attention as a therapeutic agent because of its anti-fibrotic, -inflammatory and -cancer effects [51-53]. The anti-fibrotic function of DCN is related to its property of being a natural inhibitor of

TGF- β , a growth factor responsible for scarring and fibrosis [48,50,54,55]. The scar-inducing activities of TGF- β 1 are mediated by the connective tissue growth factor (CTGF/CCN2) and the epidermal growth factor (EGF) family receptors (ERBBs) [56]. DCN also neutralizes CCN2 [57], ERBBs [58-60] and myostatin [61,62], an important contributor to scarring in several organs. Thus, a single DCN molecule can neutralize multiple fibrosis-inducing growth factors simultaneously as the binding sites for these growth factors exist in different parts of DCN molecule [52].

Systemically administered CAR-DCN homes to skin wounds, abdominal aorta aneurysms and dystrophic muscle lesions substantially better than the native DCN. This can be considered as a remarkable feat in the light of studies demonstrating that the DCN core protein homes to angiogenesis [63,64] and has been used as a delivery vehicle for other therapeutics [65]. Most recently a collagen binding peptide derived from the DCN sequence was used as a delivery vehicle for anti-inflammatory and -fibrotic therapeutics to inflammatory diseases [30]. Furthermore, naturally occurring glycosaminoglycan (GAG) in DCN can bind to α 2 β 1-integrins on angiogenic endothelial cells [66]. Taken together, CAR-DCN illustrates the advantages offered by a vascular homing peptide even for a therapeutic protein that has an inherent ability to home to target organ.

Systemically administered CAR-DCN is substantially more active than native DCN in scar prevention and tissue regeneration [45], attenuating the progression of the aorta aneurysm progression [28], reducing the severity of the muscular dystrophy (Fig. 3). The molecular mechanism behind the enhanced biological activity of CAR-DCN is not only related to homing, i.e. the better accumulation of the therapeutic molecule at the site on the injury or the disease. We have demonstrated that CAR-DCN is substantially more active than the native DCN in suppressing TGF- β bioactivity [45]. Whereas DCN binds to all isoforms of TGF- β [55], CAR-DCN is significantly more active than DCN in neutralization of TGF- β 1 and - β 2, but shows no activity against TGF- β 3 [45]. The differential inhibitory activity of CAR-DCN against the TGF- β isoforms could be clinically highly relevant; TGF- β 1 is the growth factor responsible for scar formation, while TGF- β 2 enhances the profibrotic activity of TGF- β 1 [67]. TGF- β 3, in turn, could have a substantial anti-scarring activity. A molecular basis for this selectivity could be the different HSPG-binding properties of CAR-peptide and the TGF- β isoforms. It has become apparent that some isoforms of TGF- β bind HSPG [68-70]. CAR-peptide, TGF- β 1 and - β 2 bind HSPG, but TGF- β 3 does not. This effect may enhance the biological activity of CAR-DCN and provide unique selectivity to the molecule by bringing CAR-DCN to the proximity of scar-inducing isoforms of TGF- β , namely - β 1 and - β 2, that seek HSPG binding [69] (Fig. 3). Interestingly, a large number of naturally occurring TGF- β /bone morphogenetic protein (BMP) antagonists are also heparin binding proteins [69]. Thus, CAR peptide may have converted DCN into a selective inhibitor of scar-inducing isoforms of TGF- β through the selectivity obtained by HSPG binding [45,52]. The re-engineering of the therapeutic proteins by providing them with an additional heparin binding domain could be an emerging theme in tissue engineering as a similar approach to the one used in the engineering of CAR-DCN, has been utilized more recently in generating super growth factors [8,71]. The super growth factors were created by fusing a growth factor with a heparin binding domain from placental growth factor (PLGF). Whereas the CAR peptide has homology with the heparin binding domain of BMP4 [24], the addition of the heparin binding domain of PLGF makes the chimeric super growth factors more active than the native ones by affording them an enhanced heparan sulfate-dependent cell binding and presentation to the growth factor receptor [8]. Furthermore, it was recently demonstrated that the addition of HSPG, i.e. syndecan-binding domain, to growth factors induces tonic growth factor signaling [71]. In line with the syndecan-binding domain enhancing growth factor signaling, it has been reported that the use of locally applied, fibrin-conjugated peptides with heparin binding domains enhance wound healing [72,73]. The authors attributed the beneficial effects of the biomaterial on wound healing on growth factor retention in the wounds by binding to the heparin-binding domain [72,73]. On the other hand, it was recently demonstrated that some of the heparin-binding growth factors bound too strongly to the HSPGs in the ECM, i.e. their biological activity was restricted by the ECM binding. Tissue regeneration was enhanced by the application of an enzyme, sulfatase, that cleaves growth factor docking sites from HSPGs and released the trapped growth factors to increase their bioavailability [70]. In line with that study, a chimeric fibroblast growth factor 21 (FGF21) was generated by substituting thermally labile and low

receptor affinity core of an FGF hormone (FGF21) with an HS binding-deficient endocrinized core derived from a stable high receptor affinity paracrine FGF [74]. The chimeric FGF21 is metabolically more potent and longer acting than native FGF21 [74,75].

There are large number of systemically administered, target-specific multi-functional recombinant fusion proteins that contain both targeting and therapeutic domains developed against cancer or autoimmune diseases. Some of these could also be applied to regenerative medicine with a very specific indication. Large number of such molecules carry either anti- or pro-inflammatory protein in hopes of deciphering immune response [30,76-82]. Recently, a homing peptide targeting tumor ECM delivered tumor necrosis factor- α (TNF α) to ECM rich regions in tumors and caused immune cell infiltration and subsequent immune cell-induced ECM breakage [77]. Theoretically, similar approach could be applied to situations where scarring or fibrosis has ensued, and the breakage of the fibrosis would be desirable. Selective vascular stabilization of the angiogenic blood vessels with poor perfusion by targeted therapeutic [83,84] could be also a viable option for regenerative medicine to improve blood perfusion and nutrient delivery, not only for acute injuries, but also chronic injuries where hypoxia and angiogenic, nonfunctional blood vessels persist [85].

Combining a short vascular homing peptide as part of a large recombinant fusion protein makes it possible to circumvent two major pharmacological disadvantages of peptides, the short half-life in circulation and the low binding affinity towards the target molecule [18]. Thus, multiple therapeutic multi-functional recombinant fusion proteins consisting of vascular targeting element and a therapeutic molecule have been described in the biomedical literature and the most advanced ones have already moved to clinical trials (Phase III) in humans. Most of these are in the field of cancer research. A concern when a vascular homing peptide is used as a targeting domain for a therapeutic protein is the potential immunogenicity of the resulting fusion protein. The vascular homing peptides are generally short (<10 amino acids), and as such, are unlikely to be immunogenic. For example, they are shorter than the complementary determining regions inserted into therapeutic antibodies, which usually do not cause immune reaction and are considered safe as drugs. Furthermore, vascular homing peptides sometimes share sequence homology with parts of human and mammalian proteins, as is the case with CAR peptide, which is also likely to reduce immunogenicity as the native domain exists in the host body [18].

Bystander effect – a novel approach to drug delivery

The most remarkable breakthrough in the field of targeted therapeutics are the demonstrations that the systemically administered drugs can also be converted to target organ/disease specific by co-administering the drug simultaneously with certain homing peptides without a need to bind or chemically couple the peptide to the drug [19,86] (Fig. 4). The mechanism is the activation of a trans-tissue transport pathway by the vascular homing peptide [19,87,88]. The drug is essentially “sucked” into this pathway and transported into the target tissue of the homing peptide (bystander effect) (Fig. 4). This effect was first discovered with an integrin-binding vascular homing peptide that has tumor-penetrating properties [86,89]. In addition to the actual homing peptide sequence, the tumor-specific cell and tissue penetrating homing peptides contain a consensus (cryptic) motif R/KXXR/K, with an arginine (or rarely lysine) residue at the C-terminus, thus called the C-end Rule (CendR) sequence (Fig. 5). The CendR-peptide binds to its integrin receptor, which is specific for tumor endothelium, and becomes proteolytically processed to expose a cryptic CendR motif. Once CendR motif is exposed in the C-terminus it can bind to another receptor, neuropilin-1 (NRP-1) [86,89]. The NRP-1 binding by CendR-motif activates the transport pathway (CendR pathway), which transports the peptide and a co-administered (or coupled) drug out of vessels and deep into the target tissue [19,88] (Figs. 4 & 5). Even large molecules such as antibodies and nanoparticles can be transported in a tumor-specific fashion using the tumor-penetrating peptides without coupling them to the peptide [86,90].

So far, several tumor homing peptides with CendR domain that are capable of inducing the bystander effect have been described. In addition to the CendR peptides, CAR peptide has homing and cell penetration properties resembling those of CendR-peptides and is the only known peptide without the CendR domain to be capable of inducing a bystander effect on drugs administered

simultaneously ^[18,26] (Fig. 6). The main difference is that the internalization of heparin-binding CAR peptide is mediated by HSPGs and not by NRP-1 ^[91].

The CAR peptide induced bystander effect has been demonstrated in pulmonary arterial hypertension (PAH) ^[26] (Fig. 6). A fundamental problem with the pharmaceutical treatment of PAH is that the disease is very resistant to vasodilation induced by blood pressure lowering drugs. As no anti-hypertensive drug selective for the pulmonary circulation exists, the anti-hypertensive drugs need to be used in high doses in PAH. This causes side-effects related to high drug doses such as an unwanted drop in systemic blood pressure (hypotensive shock) and limits the utility of these drugs in the treatment of the lethal disease. Co-administering CAR peptide with blood pressure lowering drugs converts these drugs to tissue specific in their action, i.e. they only lower blood pressure in lung vasculature ^[26]. CAR peptide homes to the inflammatory pulmonary blood vessels in PAH (but not to normal lung vessels) and penetrates deep into the vessel wall in experimental models of PAH ^[26]. It induces the bystander effect in PAH, i.e. it increases the accumulation of the co-administered drugs in the vessels affected by PAH and induces pulmonary vasodilation without affecting blood pressure on the systemic circulation ^[25,26] (Fig. 6). Drug doses as low as 10 % of the dose used when drugs are administered alone were effective in lowering pulmonary blood pressure in PAH, while having little or no effect on the blood pressure on the systemic circulation ^[26] (Fig. 6).

The activation of the endocytic transcytosis and trans-tissue transport pathway by the CendR and CAR peptides that triggers the bystander effect for drug delivery reveals the importance of developing targeting devices that not only deliver the therapeutic molecules to the target organ but also provide a mechanism for cell and tissue penetration. Proper cell penetration guarantees that the drug indeed accumulates at the site of the disease and does not only stick to the endothelium in blood vessels. Cell penetration afforded by CendR-pathway also solves targeting restrictions related to low number of receptors in the blood vessels of the target organ ^[10] as the targeting peptide is constantly shuttled to the parenchyma rendering the receptor available to binding again. Another similar innovative ways to deliver molecules deep in tissue parenchyma are antibodies that bind to caveolae and are pumped into the target organ ^[92,93] and having both cell binding and internalization domains in one molecule ^[17].

Targeted nanoparticles and stem cells for regenerative medicine

A sustained drug release can be obtained by packaging large amounts of therapeutic molecules into nanoparticles. Any particle can be called as a nanoparticle if its diameter is 1000 nm or less. Various nanoparticles have been introduced as potential drug carriers: organic, liposomes, micelles, nanoerythrocytes, dendrimers, carbon-based, fullerenes, or inorganic nanoparticles, silicon nanoparticles, metal or quantum dots ^[16,19]. These particles can be loaded with drugs to offer advantages like prolongation of drug release, deep tissue deposition for efficient drug absorption and simultaneous delivery of multiple therapeutic drugs ^[94-99]. Nanoparticles offer an option to modify them on their surface, by applying targeting elements to the surface of the particles. The modified particles can be targeted to the diseased vasculature ^[13,16,19,95,97,98,100-102]. This could be especially important because many nanoparticles (especially the ones made from inorganic materials) are rapidly removed from the circulation by the reticuloendothelial system ^[19]. Thus, the delivery of the nanoparticle to the target organ by a vascular homing peptide could substantially increase the amount of the nanoparticle-loaded drug accumulation in the desired target ^[19].

Selective vasodilation in PAH has also been achieved with various types of nanoparticles coated with CAR peptide for targeting and loaded with an anti-hypertensive drug that is released slowly from the nanoparticles ^[95,96,98,103]. The nanoparticle approach eliminates a major limitation of the CAR peptide co-administration ^[26], short half-life of the peptide and the drug. CAR peptide delivered nanoparticles containing anti-hypertensive drugs induce selective vasodilation in pulmonary vasculature (no concomitant drop of blood pressure in the systemic circulation) and extend the duration of selective vasodilation up to 15 x from one obtained by conventional drug administration ^[95,97,98,100,101,103,104]. Recent examples of targeted nanoparticles are the demonstration of targeting nanoparticles loaded with antibiotics to infection foci for an enhanced anti-bacterial activity ^[42] and

macrophage-specific nanoparticles that introduce an oligonucleotide directly to the cytosol for gene knockdown to enhance the macrophage capacity to combat staphylococcus aureus infection [105]. Placenta targeting nanoparticles carrying insulin-like growth factor 2 (IGF2) improved fetal weight in a model of fetal growth restriction [106] and a selective delivery of nanoparticle carrying nitric oxide releasing drug to uteroplacental vasculature rescued impaired placental perfusion [107].

In addition to applying vascular homing peptide technology to delivery of nanoparticles, the stem cells have been targeted with them as well [27,108]. Different homing peptides were used to deliver mesenchymal stem cells, which were “painted” with multiple copies of the peptides, to obtain accumulations of therapeutic stem cells in the target organ [27,29].

2. Future perspectives

Ligand-mediated targeting of systemically administered pharmaceutical agents shows a promise in the treatment of different diseases. The approach is also emerging in the field of regenerative medicine for tissue injuries, where numerous practical applications of vascular homing peptides have been described very recently. The homing peptide CAR has demonstrated that a target-specific delivery of therapeutic molecules can be achieved as a part of multi-functional recombinant protein, painting nanoparticles or stem cells with the homing peptide, as well as without attachment of the drug to the peptide through the so-called bystander effect. The outcome of the targeted delivery is the improved specificity and efficacy of the targeted drug, but numerous studies indicate that the homing peptide can enhance the biological activity of the therapeutic recombinant protein itself. Although the field of regenerative medicine has been dominated by the locally applied therapeutic advantages, these therapies have not moved to clinical medicine. There might be a time to change the paradigm towards systemically administered, target organ-specific therapeutic molecules in future drug discovery and development for regenerative medicine.

Acknowledgements: The work was funded by the Academy of Finland, Päivikki and Sakari Sohlberg Foundation, Pirkanmaa Hospital District Research Foundation, Tampere Tuberculosis Foundation, and Emil Aaltonen Foundation.

Conflict of interest: The authors have nothing to declare.

References

References

1. Eming, S. A.; Martin, P.; Tomic-Canic, M. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci. Transl. Med.* **2014**, *6*, 265sr6.
2. Martin, P.; Nunan, R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. *Br. J. Dermatol.* **2015**, *173*, 370-378.
3. Gurtner, G. C.; Werner, S.; Barrandon, Y.; Longaker, M. T. Wound repair and regeneration. *Nature* **2008**, *453*, 314-321.
4. Martino, M. M.; Briquez, P. S.; Maruyama, K.; Hubbell, J. A. Extracellular matrix-inspired growth factor delivery systems for bone regeneration. *Adv. Drug Deliv. Rev.* **2015**, *94*, 41-52.
5. Geer, D. J.; Swartz, D. D.; Andreadis, S. T. Biomimetic delivery of keratinocyte growth factor upon cellular demand for accelerated wound healing in vitro and in vivo. *Am. J. Pathol.* **2005**, *167*, 1575-1586.

6. Zhao, Y.; Shimizu, T.; Nishihira, J.; Koyama, Y.; Kushibiki, T.; Honda, A.; Watanabe, H.; Abe, R.; Tabata, Y.; Shimizu, H. Tissue regeneration using macrophage migration inhibitory factor-impregnated gelatin microbeads in cutaneous wounds. *Am. J. Pathol.* **2005**, *167*, 1519-1529.
7. Mitchell, A. C.; Briquez, P. S.; Hubbell, J. A.; Cochran, J. R. Engineering growth factors for regenerative medicine applications. *Acta Biomater.* **2016**, *30*, 1-12.
8. Martino, M. M.; Briquez, P. S.; Guc, E.; Tortelli, F.; Kilarski, W. W.; Metzger, S.; Rice, J. J.; Kuhn, G. A.; Muller, R.; Swartz, M. A.; Hubbell, J. A. Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. *Science* **2014**, *343*, 885-888.
9. Arap, W.; Pasqualini, R.; Ruoslahti, E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science* **1998**, *279*, 377-380.
10. Hussain, S.; Rodriguez-Fernandez, M.; Braun, G. B.; Doyle, F. J., 3rd; Ruoslahti, E. Quantity and accessibility for specific targeting of receptors in tumours. *Sci. Rep.* **2014**, *4*, 5232.
11. Ruoslahti, E.; Rajotte, D. An address system in the vasculature of normal tissues and tumors. *Annu. Rev. Immunol.* **2000**, *18*, 813-827.
12. AlDeghaither, D.; Smaglo, B. G.; Weiner, L. M. Beyond peptides and mAbs--current status and future perspectives for biotherapeutics with novel constructs. *J. Clin. Pharmacol.* **2015**, *55 Suppl 3*, S4-20.
13. Ruoslahti, E.; Bhatia, S. N.; Sailor, M. J. Targeting of drugs and nanoparticles to tumors. *J. Cell Biol.* **2010**, *188*, 759-768.
14. Teesalu, T.; Sugahara, K. N.; Ruoslahti, E. Mapping of vascular ZIP codes by phage display. *Methods Enzymol.* **2012**, *503*, 35-56.
15. Huang, R.; Ma, H.; Guo, Y.; Liu, S.; Kuang, Y.; Shao, K.; Li, J.; Liu, Y.; Han, L.; Huang, S.; An, S.; Ye, L.; Lou, J.; Jiang, C. Angiopep-conjugated nanoparticles for targeted long-term gene therapy of Parkinson's disease. *Pharm. Res.* **2013**, *30*, 2549-2559.
16. Morgan, C. E.; Wasserman, M. A.; Kibbe, M. R. Targeted Nanotherapies for the Treatment of Surgical Diseases. *Ann. Surg.* **2016**, *263*, 900-907.
17. Dixon, J. E.; Osman, G.; Morris, G. E.; Markides, H.; Rotherham, M.; Bayoussef, Z.; El Haj, A. J.; Denning, C.; Shakesheff, K. M. Highly efficient delivery of functional cargoes by the synergistic effect of GAG binding motifs and cell-penetrating peptides. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E291-9.
18. Järvinen, T. A. H.; Rashid, J.; May, U.; Valmari, T.; Ahsan, F. Systemic targeted delivery of multi-functional recombinant proteins and nanoparticles in regenerative medicine. *ACS Biomaterials Science Engineering* **2017**, *3*, 1273-1282.

19. Ruoslahti, E. Tumor penetrating peptides for improved drug delivery. *Adv. Drug Deliv. Rev.* **2017**, *110-111*, 3-12.
20. Ruoslahti, E. Vascular zip codes in angiogenesis and metastasis. *Biochem. Soc. Trans.* **2004**, *32*, 397-402.
21. Pasqualini, R.; Ruoslahti, E. Organ targeting in vivo using phage display peptide libraries. *Nature* **1996**, *380*, 364-366.
22. Barker, H.; Aaltonen, M.; Pan, P.; Vähätupa, M.; Kaipainen, P.; May, U.; Prince, S.; Uusitalo-Järvinen, H.; Waheed, A.; Pastorekova, S.; Sly, W. S.; Parkkila, S.; Järvinen, T. A. Role of carbonic anhydrases in skin wound healing. *Exp. Mol. Med.* **2017**, *49*, e334.
23. Järvinen, T. A.; Järvinen, T. L.; Kääriäinen, M.; Kalimo, H.; Järvinen, M. Muscle injuries: biology and treatment. *Am. J. Sports Med.* **2005**, *33*, 745-764.
24. Järvinen, T. A.; Ruoslahti, E. Molecular changes in the vasculature of injured tissues. *Am. J. Pathol.* **2007**, *171*, 702-711.
25. Urakami, T.; Järvinen, T. A.; Toba, M.; Sawada, J.; Ambalavanan, N.; Mann, D.; McMurtry, I.; Oka, M.; Ruoslahti, E.; Komatsu, M. Peptide-directed highly selective targeting of pulmonary arterial hypertension. *Am. J. Pathol.* **2011**, *178*, 2489-2495.
26. Toba, M.; Alzoubi, A.; O'Neill, K.; Abe, K.; Urakami, T.; Komatsu, M.; Alvarez, D.; Jarvinen, T. A.; Mann, D.; Ruoslahti, E.; McMurtry, I. F.; Oka, M. A novel vascular homing peptide strategy to selectively enhance pulmonary drug efficacy in pulmonary arterial hypertension. *Am. J. Pathol.* **2014**, *184*, 369-375.
27. Kean, T. J.; Duesler, L.; Young, R. G.; Dadabayev, A.; Olenyik, A.; Penn, M.; Wagner, J.; Fink, D. J.; Caplan, A. I.; Dennis, J. E. Development of a peptide-targeted, myocardial ischemia-homing, mesenchymal stem cell. *J. Drug Target.* **2012**, *20*, 23-32.
28. Shen, Y.; Russo, V.; Zeglinski, M. R.; Sellers, S. L.; Wu, Z.; Oram, C.; Santacruz, S.; Merkulova, Y.; Turner, C.; Tauh, K.; Zhao, H.; Bozin, T.; Bohunek, L.; Zeng, H.; Seidman, M. A.; Bleackley, R. C.; McManus, B. M.; Ruoslahti, E.; Jarvinen, T. A. H.; Granville, D. J. Recombinant Decorin Fusion Protein Attenuates Murine Abdominal Aortic Aneurysm Formation and Rupture. *Sci. Rep.* **2017**, *7*, 15857-017-16194-8.
29. Schneider, M.; Angele, P.; Järvinen, T. A. H.; Docheva, D. Rescue plan for Achilles: Therapeutics steering the fate and functions of stem cells in tendon wound healing. *Adv. Drug Deliv. Rev.* **2017**, *129*, 352-375.
30. Katsumata, K.; Ishihara, J.; Mansurov, A.; Ishihara, A.; Raczy, M. M.; Yuba, E.; Hubbell, J. A. Targeting inflammatory sites through collagen affinity enhances the therapeutic efficacy of anti-inflammatory antibodies. *Sci. Adv.* **2019**, *5*, eaay1971.

31. Pilch, J.; Brown, D. M.; Komatsu, M.; Järvinen, T. A.; Yang, M.; Peters, D.; Hoffman, R. M.; Ruoslahti, E. Peptides selected for binding to clotted plasma accumulate in tumor stroma and wounds. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 2800-2804.
32. Yanez, C. O.; Morales, A. R.; Yue, X.; Urakami, T.; Komatsu, M.; Jarvinen, T. A.; Belfield, K. D. Deep vascular imaging in wounds by two-photon fluorescence microscopy. *PLoS One* **2013**, *8*, e67559.
33. Morgan, C. E.; Dombrowski, A. W.; Rubert Perez, C. M.; Bahnson, E. S.; Tsihlis, N. D.; Jiang, W.; Jiang, Q.; Vercammen, J. M.; Prakash, V. S.; Pritts, T. A.; Stupp, S. I.; Kibbe, M. R. Tissue-Factor Targeted Peptide Amphiphile Nanofibers as an Injectable Therapy To Control Hemorrhage. *ACS Nano* **2016**, *10*, 899-909.
34. Sellers, D. L.; Bergen, J. M.; Johnson, R. N.; Back, H.; Ravits, J. M.; Horner, P. J.; Pun, S. H. Targeted axonal import (TAXI) peptide delivers functional proteins into spinal cord motor neurons after peripheral administration. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 2514-2519.
35. Costantini, T. W.; Eliceiri, B. P.; Putnam, J. G.; Bansal, V.; Baird, A.; Coimbra, R. Intravenous phage display identifies peptide sequences that target the burn-injured intestine. *Peptides* **2012**, *38*, 94-99.
36. Costantini, T. W.; Putnam, J. G.; Sawada, R.; Baird, A.; Loomis, W. H.; Eliceiri, B. P.; Bansal, V.; Coimbra, R. Targeting the gut barrier: identification of a homing peptide sequence for delivery into the injured intestinal epithelial cell. *Surgery* **2009**, *146*, 206-212.
37. Mann, A. P.; Scodeller, P.; Hussain, S.; Joo, J.; Kwon, E.; Braun, G. B.; Molder, T.; She, Z. G.; Kotamraju, V. R.; Ranscht, B.; Krajewski, S.; Teesalu, T.; Bhatia, S.; Sailor, M. J.; Ruoslahti, E. A peptide for targeted, systemic delivery of imaging and therapeutic compounds into acute brain injuries. *Nat. Commun.* **2016**, *7*, 11980.
38. Joo, J.; Kwon, E. J.; Kang, J.; Skalak, M.; Anglin, E. J.; Mann, A. P.; Ruoslahti, E.; Bhatia, S. N.; Sailor, M. J. Porous silicon-graphene oxide core-shell nanoparticles for targeted delivery of siRNA to the injured brain. *Nanoscale Horiz.* **2016**, *1*, 407-414.
39. Wang, Y.; Newman, M. R.; Ackun-Farmmer, M.; Baranello, M. P.; Sheu, T. J.; Puzas, J. E.; Benoit, D. S. W. Fracture-Targeted Delivery of beta-Catenin Agonists via Peptide-Functionalized Nanoparticles Augments Fracture Healing. *ACS Nano* **2017**, *11*, 9445-9458.
40. Liang, C.; Guo, B.; Wu, H.; Shao, N.; Li, D.; Liu, J.; Dang, L.; Wang, C.; Li, H.; Li, S.; Lau, W. K.; Cao, Y.; Yang, Z.; Lu, C.; He, X.; Au, D. W.; Pan, X.; Zhang, B. T.; Lu, C.; Zhang, H.; Yue, K.; Qian, A.; Shang, P.; Xu, J.; Xiao, L.; Bian, Z.; Tan, W.; Liang, Z.; He, F.; Zhang, L.; Lu, A.; Zhang, G. Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA interference-based bone anabolic strategy. *Nat. Med.* **2015**, *21*, 288-294.

41. Jiang, T.; Yu, X.; Carbone, E. J.; Nelson, C.; Kan, H. M.; Lo, K. W. Poly aspartic acid peptide-linked PLGA based nanoscale particles: potential for bone-targeting drug delivery applications. *Int. J. Pharm.* **2014**, *475*, 547-557.
42. Hussain, S.; Joo, J.; Kang, J.; Kim, B.; Braun, G. B.; She, Z. G.; Kim, D.; Mann, A. P.; Molder, T.; Teesalu, T.; Carnazza, S.; Guglielmino, S.; Sailor, M. J.; Ruoslahti, E. Antibiotic-loaded nanoparticles targeted to the site of infection enhance antibacterial efficacy. *Nat. Biomed. Eng.* **2018**, *2*, 95-103.
43. Whitney, M. A.; Crisp, J. L.; Nguyen, L. T.; Friedman, B.; Gross, L. A.; Steinbach, P.; Tsien, R. Y.; Nguyen, Q. T. Fluorescent peptides highlight peripheral nerves during surgery in mice. *Nat. Biotechnol.* **2011**, *29*, 352-356.
44. Glasgow, H. L.; Whitney, M. A.; Gross, L. A.; Friedman, B.; Adams, S. R.; Crisp, J. L.; Hussain, T.; Frei, A. P.; Novy, K.; Wollscheid, B.; Nguyen, Q. T.; Tsien, R. Y. Laminin targeting of a peripheral nerve-highlighting peptide enables degenerated nerve visualization. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 12774-12779.
45. Järvinen, T. A.; Ruoslahti, E. Target-seeking antifibrotic compound enhances wound healing and suppresses scar formation in mice. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 21671-21676.
46. Järvinen, T. A. Design of target-seeking antifibrotic compounds. *Methods Enzymol.* **2012**, *509*, 243-261.
47. Krusius, T.; Ruoslahti, E. Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 7683-7687.
48. Yamaguchi, Y.; Mann, D. M.; Ruoslahti, E. Negative regulation of transforming growth factor-beta by the proteoglycan decorin. *Nature* **1990**, *346*, 281-284.
49. Yamaguchi, Y.; Ruoslahti, E. Expression of human proteoglycan in Chinese hamster ovary cells inhibits cell proliferation. *Nature* **1988**, *336*, 244-246.
50. Border, W. A.; Noble, N. A.; Yamamoto, T.; Harper, J. R.; Yamaguchi, Y.; Pierschbacher, M. D.; Ruoslahti, E. Natural inhibitor of transforming growth factor- β protects against scarring in experimental kidney disease. *Nature* **1992**, *360*, 361-364.
51. Jarvinen, T. A.; Prince, S. Decorin: A Growth Factor Antagonist for Tumor Growth Inhibition. *Biomed. Res. Int.* **2015**, *2015*, 654765.
52. Jarvinen, T. A. H.; Ruoslahti, E. Generation of a multi-functional, target organ-specific, anti-fibrotic molecule by molecular engineering of the extracellular matrix protein, decorin. *Br. J. Pharmacol.* **2019**, *176*, 16-25.

53. Sainio, A. O.; Järveläinen, H. T. Decorin-mediated oncosuppression - a potential future adjuvant therapy for human epithelial cancers. *Br. J. Pharmacol.* **2018**.
54. Border, W. A.; Ruoslahti, E. Transforming growth factor- β in disease: the dark side of tissue repair. *J. Clin. Invest.* **1992**, *90*, 1-7.
55. Hildebrand, A.; Romaris, M.; Rasmussen, L. M.; Heinegard, D.; Twardzik, D. R.; Border, W. A.; Ruoslahti, E. Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor β . *Biochem. J.* **1994**, *302* (Pt 2), 527-534.
56. Andrianifahanana, M.; Wilkes, M. C.; Gupta, S. K.; Rahimi, R. A.; Repellin, C. E.; Edens, M.; Wittenberger, J.; Yin, X.; Maidl, E.; Becker, J.; Leof, E. B. Profibrotic TGF β responses require the cooperative action of PDGF and ErbB receptor tyrosine kinases. *FASEB J.* **2013**, *27*, 4444-4454.
57. Vial, C.; Gutierrez, J.; Santander, C.; Cabrera, D.; Brandan, E. Decorin interacts with connective tissue growth factor (CTGF)/CCN2 by LRR12 inhibiting its biological activity. *J. Biol. Chem.* **2011**, *286*, 24242-24252.
58. Csordas, G.; Santra, M.; Reed, C. C.; Eichstetter, I.; McQuillan, D. J.; Gross, D.; Nugent, M. A.; Hajnoczky, G.; Iozzo, R. V. Sustained down-regulation of the epidermal growth factor receptor by decorin. A mechanism for controlling tumor growth in vivo. *J. Biol. Chem.* **2000**, *275*, 32879-32887.
59. Iozzo, R. V.; Moscatello, D. K.; McQuillan, D. J.; Eichstetter, I. Decorin is a biological ligand for the epidermal growth factor receptor. *J. Biol. Chem.* **1999**, *274*, 4489-4492.
60. Moscatello, D. K.; Santra, M.; Mann, D. M.; McQuillan, D. J.; Wong, A. J.; Iozzo, R. V. Decorin suppresses tumor cell growth by activating the epidermal growth factor receptor. *J. Clin. Invest.* **1998**, *101*, 406-412.
61. Miura, T.; Kishioka, Y.; Wakamatsu, J.; Hattori, A.; Hennebry, A.; Berry, C. J.; Sharma, M.; Kambadur, R.; Nishimura, T. Decorin binds myostatin and modulates its activity to muscle cells. *Biochem. Biophys. Res. Commun.* **2006**, *340*, 675-680.
62. Zhu, J.; Li, Y.; Shen, W.; Qiao, C.; Ambrosio, F.; Lavasani, M.; Nozaki, M.; Branca, M. F.; Huard, J. Relationships between transforming growth factor-beta1, myostatin, and decorin: implications for skeletal muscle fibrosis. *J. Biol. Chem.* **2007**, *282*, 25852-25863.
63. Goldoni, S.; Seidler, D. G.; Heath, J.; Fassan, M.; Baffa, R.; Thakur, M. L.; Owens, R. T.; McQuillan, D. J.; Iozzo, R. V. An antimetastatic role for decorin in breast cancer. *Am. J. Pathol.* **2008**, *173*, 844-855.
64. Seidler, D. G.; Goldoni, S.; Agnew, C.; Cardi, C.; Thakur, M. L.; Owens, R. T.; McQuillan, D. J.; Iozzo, R. V. Decorin protein core inhibits in vivo cancer growth and metabolism by hindering

- epidermal growth factor receptor function and triggering apoptosis via caspase-3 activation. *J. Biol. Chem.* **2006**, *281*, 26408-26418.
65. Ma, H. I.; Hueng, D. Y.; Shui, H. A.; Han, J. M.; Wang, C. H.; Lai, Y. H.; Cheng, S. Y.; Xiao, X.; Chen, M. T.; Yang, Y. P. Intratumoral decorin gene delivery by AAV vector inhibits brain glioblastomas and prolongs survival of animals by inducing cell differentiation. *Int. J. Mol. Sci.* **2014**, *15*, 4393-4414.
66. Fiedler, L. R.; Schonherr, E.; Waddington, R.; Niland, S.; Seidler, D. G.; Aeschlimann, D.; Eble, J. A. Decorin regulates endothelial cell motility on collagen I through activation of insulin-like growth factor I receptor and modulation of alpha2beta1 integrin activity. *J. Biol. Chem.* **2008**, *283*, 17406-17415.
67. Järvinen, T. A.; Ruoslahti, E. Targeted Antiscarring Therapy for Tissue Injuries. *Adv. Wound Care. (New Rochelle)* **2013**, *2*, 50-54.
68. Iwao, K.; Inatani, M.; Matsumoto, Y.; Ogata-Iwao, M.; Takihara, Y.; Irie, F.; Yamaguchi, Y.; Okinami, S.; Tanihara, H. Heparan sulfate deficiency leads to Peters anomaly in mice by disturbing neural crest TGF- β 2 signaling. *J. Clin. Invest.* **2009**, *119*, 1997-2008.
69. Rider, C. C.; Mulloy, B. Heparin, Heparan Sulphate and the TGF-beta Cytokine Superfamily. *Molecules* **2017**, *22*, 10.3390/molecules22050713.
70. Korf-Klingebiel, M.; Reboll, M. R.; Grote, K.; Schleiner, H.; Wang, Y.; Wu, X.; Klede, S.; Mikhed, Y.; Bauersachs, J.; Klintschar, M.; Rudat, C.; Kispert, A.; Niessen, H. W.; Lubke, T.; Dierks, T.; Wollert, K. C. Heparan Sulfate-Editing Extracellular Sulfatases Enhance VEGF Bioavailability for Ischemic Heart Repair. *Circ. Res.* **2019**, *125*, 787-801.
71. Mochizuki, M.; Guc, E.; Park, A. J.; Julier, Z.; Briquez, P. S.; Kuhn, G. A.; Muller, R.; Swartz, M. A.; Hubbell, J. A.; Martino, M. M. Growth factors with enhanced syndecan binding generate tonic signalling and promote tissue healing. *Nat. Biomed. Eng.* **2019**.
72. Ishihara, J.; Ishihara, A.; Starke, R. D.; Peghaire, C. R.; Smith, K. E.; McKinnon, T. A. J.; Tabata, Y.; Sasaki, K.; White, M. J. V.; Fukunaga, K.; Laffan, M. A.; Lutolf, M. P.; Randi, A. M.; Hubbell, J. A. The heparin binding domain of von Willebrand factor binds to growth factors and promotes angiogenesis in wound healing. *Blood* **2019**, *133*, 2559-2569.
73. Ishihara, J.; Ishihara, A.; Fukunaga, K.; Sasaki, K.; White, M. J. V.; Briquez, P. S.; Hubbell, J. A. Laminin heparin-binding peptides bind to several growth factors and enhance diabetic wound healing. *Nat. Commun.* **2018**, *9*, 2163-018-04525-w.
74. Zhao, L.; Niu, J.; Lin, H.; Zhao, J.; Liu, Y.; Song, Z.; Xiang, C.; Wang, X.; Yang, Y.; Li, X.; Mohammadi, M.; Huang, Z. Paracrine-endocrine FGF chimeras as potent therapeutics for metabolic diseases. *EBioMedicine* **2019**, *48*, 462-477.

75. Flippo, K. H.; Potthoff, M. J. Chronicles of an FGF chimera: The odyssey continues. *EBioMedicine* **2019**, *49*, 15-16.
76. Schmid, A. S.; Neri, D. Advances in antibody engineering for rheumatic diseases. *Nat. Rev. Rheumatol.* **2019**, *15*, 197-207.
77. Yeow, Y. L.; Kotamraju, V. R.; Wang, X.; Chopra, M.; Azme, N.; Wu, J.; Schoep, T. D.; Delaney, D. S.; Feindel, K.; Li, J.; Kennedy, K. M.; Allen, W. M.; Kennedy, B. F.; Larma, I.; Sampson, D. D.; Mahakian, L. M.; Fite, B. Z.; Zhang, H.; Friman, T.; Mann, A. P.; Aziz, F. A.; Kumarasinghe, M. P.; Johansson, M.; Ee, H. C.; Yeoh, G.; Mou, L.; Ferrara, K. W.; Billiran, H.; Ganss, R.; Ruoslahti, E.; Hamzah, J. Immune-mediated ECM depletion improves tumour perfusion and payload delivery. *EMBO Mol. Med.* **2019**, e10923.
78. Sasaki, K.; Ishihara, J.; Ishihara, A.; Miura, R.; Mansurov, A.; Fukunaga, K.; Hubbell, J. A. Engineered collagen-binding serum albumin as a drug conjugate carrier for cancer therapy. *Sci. Adv.* **2019**, *5*, eaaw6081.
79. Schwager, K.; Kaspar, M.; Bootz, F.; Marcolongo, R.; Paresce, E.; Neri, D.; Trachsel, E. Preclinical characterization of DEKAVIL (F8-IL10), a novel clinical-stage immunocytokine which inhibits the progression of collagen-induced arthritis. *Arthritis Res. Ther.* **2009**, *11*, R142.
80. Gutbrodt, K. L.; Schliemann, C.; Giovannoni, L.; Frey, K.; Pabst, T.; Klapper, W.; Berdel, W. E.; Neri, D. Antibody-based delivery of interleukin-2 to neovasculature has potent activity against acute myeloid leukemia. *Sci. Transl. Med.* **2013**, *5*, 201ra118.
81. Morrison, A. R.; Yarovinsky, T. O.; Young, B. D.; Moraes, F.; Ross, T. D.; Ceneri, N.; Zhang, J.; Zhuang, Z. W.; Sinusas, A. J.; Pardi, R.; Schwartz, M. A.; Simons, M.; Bender, J. R. Chemokine-coupled beta2 integrin-induced macrophage Rac2-Myosin IIA interaction regulates VEGF-A mRNA stability and arteriogenesis. *J. Exp. Med.* **2014**, *211*, 1957-1968.
82. Franz, M.; Doll, F.; Grun, K.; Richter, P.; Kose, N.; Ziffels, B.; Schubert, H.; Figulla, H. R.; Jung, C.; Gummert, J.; Renner, A.; Neri, D.; Berndt, A. Targeted delivery of interleukin-10 to chronic cardiac allograft rejection using a human antibody specific to the extra domain A of fibronectin. *Int. J. Cardiol.* **2015**, *195*, 311-322.
83. Johansson-Percival, A.; Li, Z. J.; Lakhiani, D. D.; He, B.; Wang, X.; Hamzah, J.; Ganss, R. Intratumoral LIGHT Restores Pericyte Contractile Properties and Vessel Integrity. *Cell. Rep.* **2015**, *13*, 2687-2698.
84. He, B.; Jabouille, A.; Steri, V.; Johansson-Percival, A.; Michael, I. P.; Kotamraju, V. R.; Junckerstorff, R.; Nowak, A. K.; Hamzah, J.; Lee, G.; Bergers, G.; Ganss, R. Vascular targeting of LIGHT normalizes blood vessels in primary brain cancer and induces intratumoural high endothelial venules. *J. Pathol.* **2018**, *245*, 209-221.

85. Jarvinen, T. A. Neovascularisation in tendinopathy: from eradication to stabilisation? *Br. J. Sports Med.* **2019**.
86. Sugahara, K. N.; Teesalu, T.; Karmali, P. P.; Kotamraju, V. R.; Agemy, L.; Greenwald, D. R.; Ruoslahti, E. Coadministration of a tumor-penetrating peptide enhances the efficacy of cancer drugs. *Science* **2010**, *328*, 1031-1035.
87. Liu, X.; Lin, P.; Perrett, I.; Lin, J.; Liao, Y. P.; Chang, C. H.; Jiang, J.; Wu, N.; Donahue, T.; Wainberg, Z.; Nel, A. E.; Meng, H. Tumor-penetrating peptide enhances transcytosis of silicasome-based chemotherapy for pancreatic cancer. *J. Clin. Invest.* **2017**, *127*, 2007-2018.
88. Pang, H. B.; Braun, G. B.; Friman, T.; Aza-Blanc, P.; Ruidiaz, M. E.; Sugahara, K. N.; Teesalu, T.; Ruoslahti, E. An endocytosis pathway initiated through neuropilin-1 and regulated by nutrient availability. *Nat. Commun.* **2014**, *5*, 4904.
89. Teesalu, T.; Sugahara, K. N.; Kotamraju, V. R.; Ruoslahti, E. C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 16157-16162.
90. Nel, A.; Ruoslahti, E.; Meng, H. New Insights into "Permeability" as in the Enhanced Permeability and Retention Effect of Cancer Nanotherapeutics. *ACS Nano* **2017**, *11*, 9567-9569.
91. Pang, H. B.; Braun, G. B.; Ruoslahti, E. Neuropilin-1 and heparan sulfate proteoglycans cooperate in cellular uptake of nanoparticles functionalized by cationic cell-penetrating peptides. *Sci. Adv.* **2015**, *1*, e1500821.
92. Oh, P.; Testa, J. E.; Borgstrom, P.; Witkiewicz, H.; Li, Y.; Schnitzer, J. E. In vivo proteomic imaging analysis of caveolae reveals pumping system to penetrate solid tumors. *Nat. Med.* **2014**, *20*, 1062-1068.
93. Oh, P.; Borgstrom, P.; Witkiewicz, H.; Li, Y.; Borgstrom, B. J.; Chrastina, A.; Iwata, K.; Zinn, K. R.; Baldwin, R.; Testa, J. E.; Schnitzer, J. E. Live dynamic imaging of caveolae pumping targeted antibody rapidly and specifically across endothelium in the lung. *Nat. Biotechnol.* **2007**, *25*, 327-337.
94. Gupta, V.; Gupta, N.; Shaik, I. H.; Mehvar, R.; Nozik-Grayck, E.; McMurtry, I. F.; Oka, M.; Komatsu, M.; Ahsan, F. Inhaled PLGA particles of prostaglandin E(1) ameliorate symptoms and progression of pulmonary hypertension at a reduced dosing frequency. *Mol. Pharm.* **2013**, *10*, 1655-1667.
95. Gupta, N.; Ibrahim, H. M.; Ahsan, F. Peptide-micelle hybrids containing fasudil for targeted delivery to the pulmonary arteries and arterioles to treat pulmonary arterial hypertension. *J. Pharm. Sci.* **2014**, *103*, 3743-3753.

96. Nahar, K.; Absar, S.; Gupta, N.; Kotamraju, V. R.; McMurtry, I. F.; Oka, M.; Komatsu, M.; Nozik-Grayck, E.; Ahsan, F. Peptide-coated liposomal fasudil enhances site specific vasodilation in pulmonary arterial hypertension. *Mol. Pharm.* **2014**, *11*, 4374-4384.
97. Gupta, N.; Al-Saikhan, F. I.; Patel, B.; Rashid, J.; Ahsan, F. Fasudil and SOD packaged in peptide-studded-liposomes: Properties, pharmacokinetics and ex-vivo targeting to isolated perfused rat lungs. *Int. J. Pharm.* **2015**, *488*, 33-43.
98. Gupta, N.; Patel, B.; Nahar, K.; Ahsan, F. Cell permeable peptide conjugated nanoerythrocytes of fasudil prolong pulmonary arterial vasodilation in PAH rats. *Eur. J. Pharm. Biopharm.* **2014**, *88*, 1046-1055.
99. Wang, Q.; Zhang, H.; Xu, H.; Zhao, Y.; Li, Z.; Li, J.; Wang, H.; Zhuge, D.; Guo, X.; Xu, H.; Jones, S.; Li, X.; Jia, X.; Xiao, J. Novel multi-drug delivery hydrogel using scar-homing liposomes improves spinal cord injury repair. *Theranostics* **2018**, *8*, 4429-4446.
100. Keshavarz, A.; Alobaida, A.; McMurtry, I. F.; Nozik-Grayck, E.; Stenmark, K. R.; Ahsan, F. CAR, a Homing Peptide, Prolongs Pulmonary Preferential Vasodilation by Increasing Pulmonary Retention and Reducing Systemic Absorption of Liposomal Fasudil. *Mol. Pharm.* **2019**, *16*, 3414-3429.
101. Rashid, J.; Nahar, K.; Raut, S.; Keshavarz, A.; Ahsan, F. Fasudil and DETA NONOate, Loaded in a Peptide-Modified Liposomal Carrier, Slow PAH Progression upon Pulmonary Delivery. *Mol. Pharm.* **2018**, *15*, 1755-1765.
102. Agemy, L.; Sugahara, K. N.; Kotamraju, V. R.; Gujratty, K.; Girard, O. M.; Kono, Y.; Mattrey, R. F.; Park, J. H.; Sailor, M. J.; Jimenez, A. I.; Cativiela, C.; Zanuy, D.; Sayago, F. J.; Aleman, C.; Nussinov, R.; Ruoslahti, E. Nanoparticle-induced vascular blockade in human prostate cancer. *Blood* **2010**, *116*, 2847-2856.
103. Gupta, N.; Rashid, J.; Nozik-Grayck, E.; McMurtry, I. F.; Stenmark, K. R.; Ahsan, F. Cocktail of Superoxide Dismutase and Fasudil Encapsulated in Targeted Liposomes Slows PAH Progression at a Reduced Dosing Frequency. *Mol. Pharm.* **2017**, *14*, 830-841.
104. Gupta, N.; Patel, B.; Ahsan, F. Nano-engineered erythrocyte ghosts as inhalational carriers for delivery of fasudil: preparation and characterization. *Pharm. Res.* **2014**, *31*, 1553-1565.
105. Kim, B.; Pang, H. B.; Kang, J.; Park, J. H.; Ruoslahti, E.; Sailor, M. J. Immunogene therapy with fusogenic nanoparticles modulates macrophage response to *Staphylococcus aureus*. *Nat. Commun.* **2018**, *9*, 1969-018-04390-7.
106. King, A.; Ndifon, C.; Lui, S.; Widdows, K.; Kotamraju, V. R.; Agemy, L.; Teesalu, T.; Glazier, J. D.; Cellesi, F.; Tirelli, N.; Aplin, J. D.; Ruoslahti, E.; Harris, L. K. Tumor-homing peptides as tools for targeted delivery of payloads to the placenta. *Sci. Adv.* **2016**, *2*, e1600349.

107. Cureton, N.; Korotkova, I.; Baker, B.; Greenwood, S.; Wareing, M.; Kotamraju, V. R.; Teesalu, T.; Cellesi, F.; Tirelli, N.; Ruoslahti, E.; Aplin, J. D.; Harris, L. K. Selective Targeting of a Novel Vasodilator to the Uterine Vasculature to Treat Impaired Uteroplacental Perfusion in Pregnancy. *Theranostics* **2017**, *7*, 3715-3731.
108. Kean, T. J.; Lin, P.; Caplan, A. I.; Dennis, J. E. MSCs: Delivery Routes and Engraftment, Cell-Targeting Strategies, and Immune Modulation. *Stem Cells Int.* **2013**, *2013*, 732742.

Table 1. Vascular homing peptides described for regenerative purposes in tissue injuries.

Peptide	Sequence	Target	Receptor	Therapeutic application	Ref. #
CAR	CARSKNKDC	angiogenesis (early state) and inflammatory vasculature	heparan sulfate proteoglycans	recombinant proteins	24, 28, 45
				stem cells	27
				unconjugated drug delivery (bystander effect)	26
				nanoparticles	24,94-98, 100-101, 103-104
CARG	CARGGLKSC	<i>Staphylococcus aureus</i> infected tissue		nanoparticles	42
c-RGDfK	CRGDFC	Angiogenesis	$\alpha_v\beta_3$ integrin	nanoparticles	32
CAQK	CAQK	acute brain injury	chondroitin sulfate	siRNA	37, 38
CH6	see ref. 40	osteoblasts		lipid nanoparticle	40
CLT1	CGLIIQKNEC	blood dots	chloride intracellular channel 3 and integrin $\alpha_5 (\beta_1)$		31
CRK	CRKDKC	angiogenesis (late stage)		stem cells	27
				nanoparticles	24, 102
Np41	NTQTLAKAP EHT	nerves	laminins α_2, α_4	identification of nerves during surgery	43, 44
poly-Asp	DDDDDDDC	bone	hydroxyapatite	nanoparticles	41
RTL	RTLAFVRFK	ruptured blood vessel	tissue factor (TF)	nanofibers	33
TAxI	SACQSQSQM RCGGG	transected nerves		recombinant proteins	34
TBP	TPLSYLKGLV TVG	bone, fracture	tartrate-resistant acid phosphatase (TRAP)	nanoparticles	39
4-11	SFKPSGLPAQ SL	burn-injured intestine			35, 36
CBP	LRELHLNNN C	Inflammation	Collagen	recombinant proteins	30

Legends for Figures.

Figure 1. The vascular zip codes. Each tissue has its own unique molecular structure in the luminal side of its vessels. These molecules function as receptors for tissue homing molecules such as peptides. Combining a homing peptide with another molecule creates a compound that can home to its target tissue. Homing diminishes the amount of the drug needed and helps to avoid side effects of the medication.

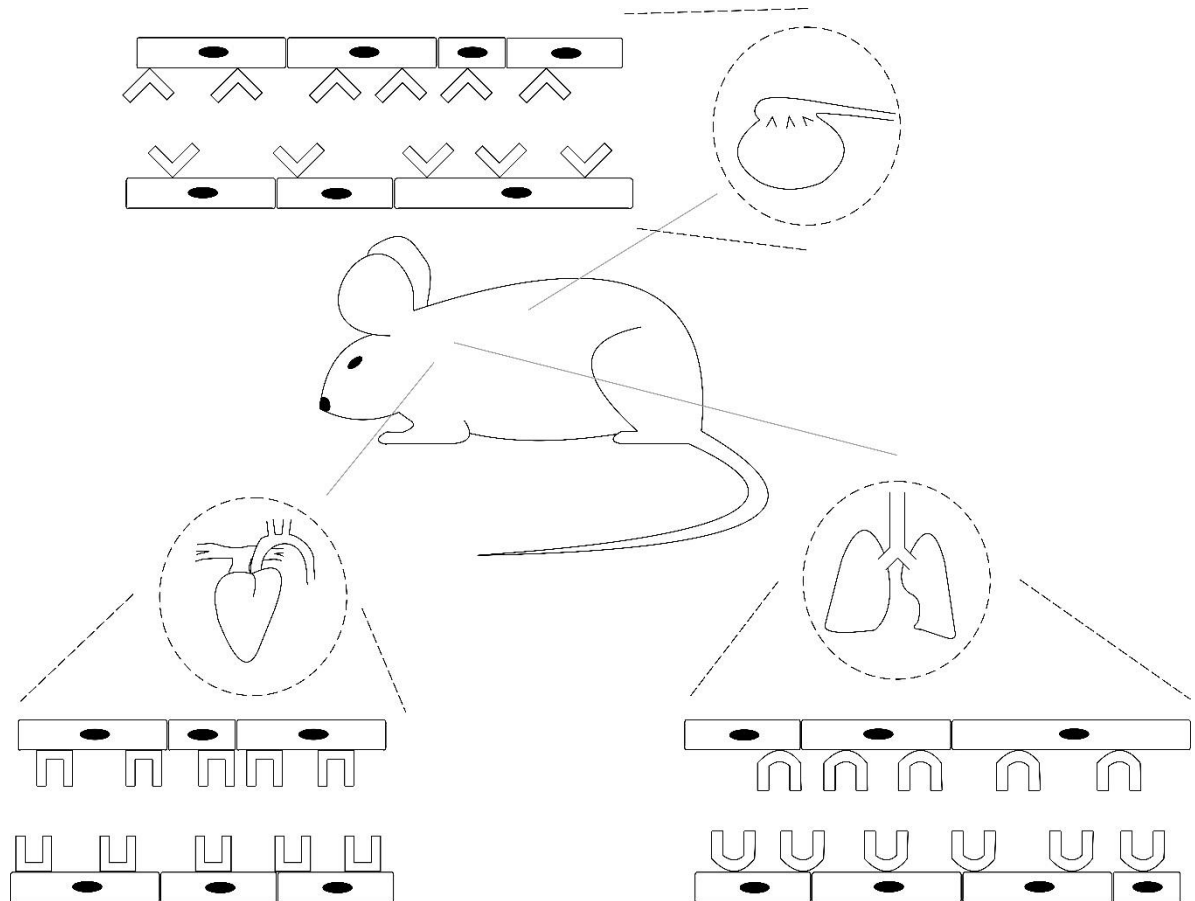


Figure 2. Target organ-specific delivery of therapeutics in regenerative medicine. The angiogenic blood vessels that form in regenerating tissues after injury are structurally and molecularly different from the dormant blood vessels elsewhere in the body. They provide an accessible and abundant target for the organ-specific delivery of therapeutics during the repair of the tissue injuries. They can be targeted by vascular homing peptides that bind to their receptor selectively expressed only in angiogenic blood vessels. Drugs administered systemically can be converted to target tissue-specific by coupling them to the vascular homing peptide.

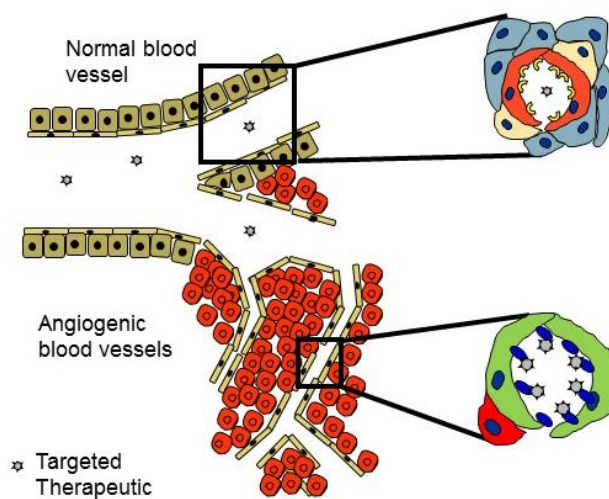


Figure 3. Mechanism of action of multi-functional re-engineered decorin in inhibition of scar formation. Schematic presentation on recombinant fusion protein consisting of decorin (DCN) and the vascular homing and cell penetrating peptide CAR. CAR-decorin ① is a multifunctional biotherapeutic that inhibits numerous growth factor signaling pathways involved in fibrosis. The systemically administered molecule is targeted by CAR peptide to the inflammatory or angiogenic vasculature in any organ of the body ②. The CAR homing peptide binds to its receptor (“zip code”) in angiogenic or inflammatory vasculature ② and as cell penetrating peptide, it delivers the fusion molecule deep in the target organ parenchyme ③. The CAR peptide then binds to heparin sulfate proteoglycans on the cell surface of the stromal cells ③. This binding facilitates the neutralization of scar forming isoforms TGF- β 1 and TGF- β 2 by the therapeutic part of the molecule, DCN ④. This mechanism results in a therapeutic response, i.e. reduction of scar formation in skin wound. Picture by Helena Schmidt; reproduced with permission from Finnish Medical Journal Duodecim (originally published in Duodecim 2011;127:50–51).

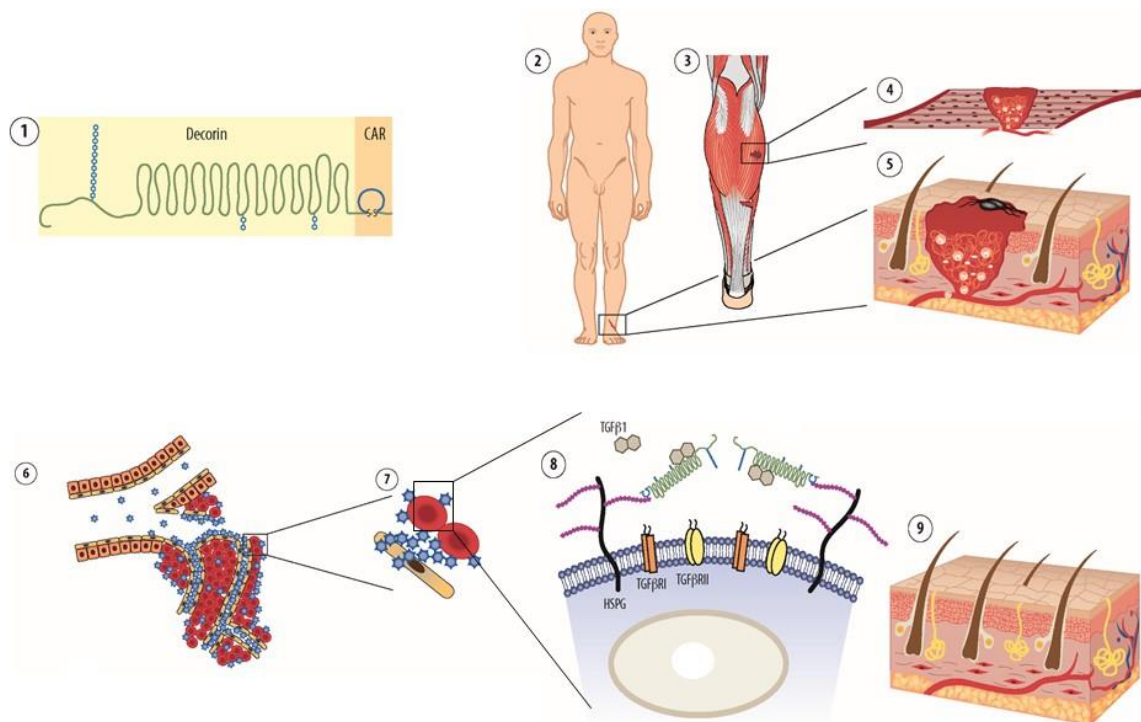


Figure 4. The bystander effect enables delivering a therapeutic into the tissue without a covalent link between the therapeutic and the homing peptide. The drawing represents the homing of a peptide (diamond) to its target tissue (cuboidal cells) and the bystander effect created by the peptide. The tissue on the right has the receptor for the homing peptide (Y-shape). When the peptide binds to its receptor, it opens a pathway for the therapeutic molecule (triangle) to pass through the vascular wall endothelium and enter the tissue parenchyma.

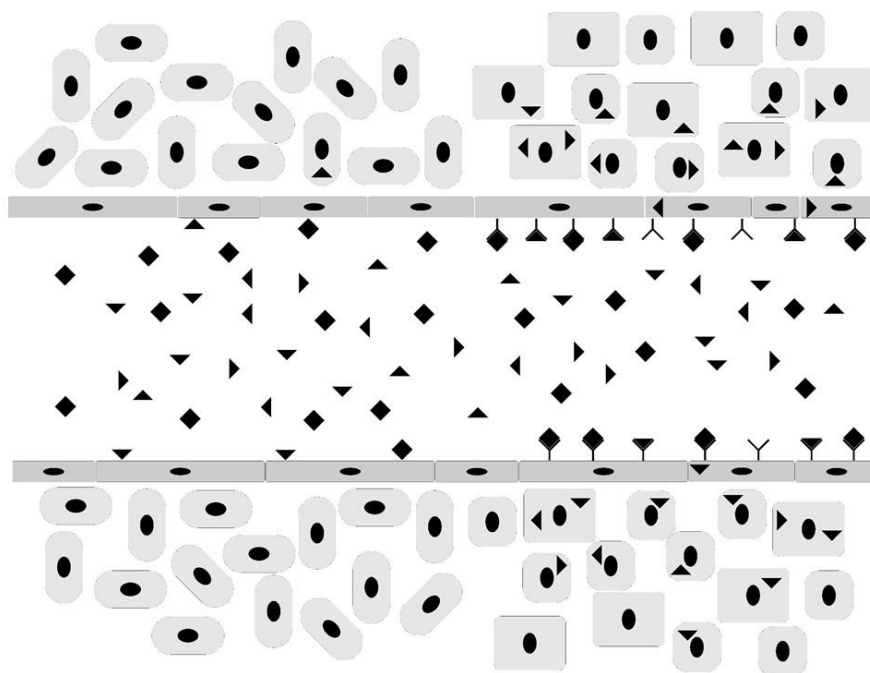


Figure 5. The function of the C-end Rule sequence. Peptides containing the C-end Rule (CendR) sequence are capable of penetrating to tissues and delivering cargo. Typically, the CendR sequence is cryptic and needs to be exposed. The receptor specific for the target tissue (fork) first binds the homing peptide enabling its cleavage by an enzyme (gray), which results in the exposure of the CendR sequence (RXXR). The CendR sequence then binds to another receptor (half a circle), neuropilin-1 (NRP-1), which triggers the cell membrane to form a vesicle containing the peptide and other molecules close to it. The vesicle is then transferred through the cell to let its contents enter to the tissue parenchyma.

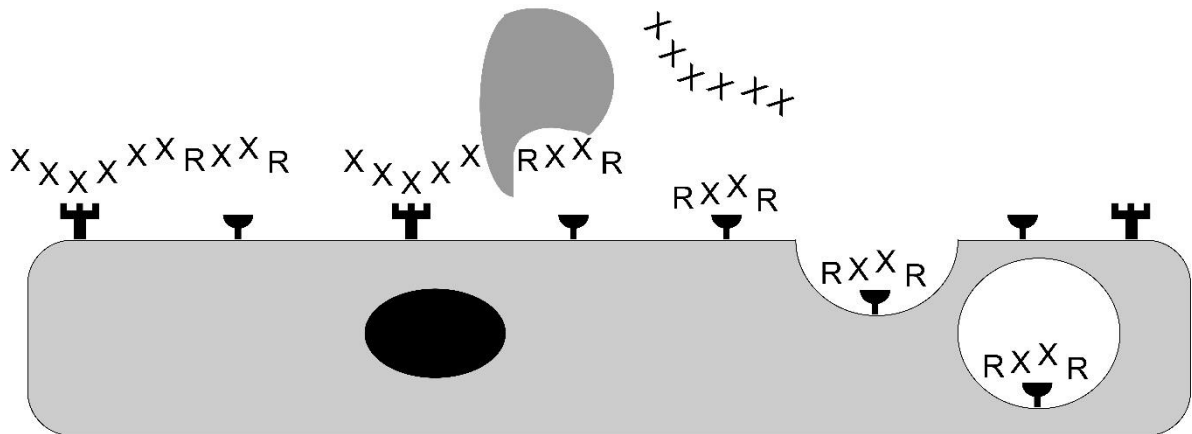


Figure 6. Vascular homing peptide induced tissue-selective vasodilation in pulmonary arterial hypertension (PAH). (A) Vascular homing and cell penetrating peptide CAR homes to pulmonary arteries in disease-specific fashion in experimental models of PAH [25,26]. A little bit of control peptide (mutant CAR peptide) binding can be seen to similar blood vessel in PAH. (B) Effect of CAR (0.3 mg/kg) and of Rho-kinase inhibitor Y27632 (1 mg/kg) mixture on right ventricle (RVSP, right ventricle systolic pressure) and left ventricle (SAP, systemic arterial pressure) systolic pressure in PAH. The CAR/Y27632 combination treatment induced a marked pulmonary-specific vasodilation RVSP with only a minimum effect on SAP in PAH. (C) Schematic presentation of the “bystander effect”, i.e. target organ-specific drug delivery, in PAH [26].

