DDT Vol. 7, No. 22 November 2002

Drug targeting to specific vascular sites

Erkki Ruoslahti

The blood vessels of individual tissues are biochemically distinct, and pathological lesions put their own signature on the vasculature. In tumors, both blood and lymphatic vessels differ from normal vessels. New methods, such as *in vivo* screening of phage libraries, have provided peptides and antibodies that recognize these vascular signatures and can be used in targeted delivery of therapeutic agents. Targeting a therapy to the diseased tissue enhances the efficacy of the treatment while reducing the side effects in mouse experiments. Results from drug delivery to tumor vessels have been particularly encouraging.

Erkki Ruoslahti
The Burnham Institute
Cancer Research Center
10901 North Torrey Pines Road
La Jolla, CA 92037, USA
tel: +1 858 646 3125
fax: +1 858 646 3198
e-mail:
ruoslahti@burnham.org

▼ The vasculature of individual tissues expresses molecules that are specific to a particular tissue or tissue type. A well-known example is the blood vessels of lymphoid tissues, in which the high endothelium expresses unique adhesion molecules for lymphocyte homing. Many, perhaps all, other tissues are now also known to put a 'signature' on their vasculature [1].

Pathological tissue processes can also put their own signature on the vasculature. Inflammation and malignancy are known to do this. Tumor vasculature continuously undergoes angiogenesis to provide the blood supply that feeds the growing tumor [2]. The activated endothelial cells and pericytes in this neovasculature express molecules that are characteristic of angiogenic vessels (not expressed or expressed at much lower levels in normal vessels). Moreover, tumor lymphatics can also express their own marker. The heterogeneity in the vasculature might provide new opportunities for targeted delivery of therapies. This review discusses these developments.

Molecular markers in blood vessels and lymphatics

Blood vessel specialization in normal tissues. Recently, an unexpected complexity of tissue-specific molecular individuality in vascular beds was uncovered. Monoclonal antibodies (MAbs) and a new technique using *in vivo* screening of peptide and antibody libraries expressed on the surface of phage or bacteria, have been particularly informative in this regard (reviewed in Ref. [1]). Intravenous injection of mice with peptide libraries displayed on phage, followed by isolation of phage from individual tissues has yielded tissue-specific vascular homing peptides for each normal tissue and organ our laboratory has chosen for targeting so far. Preparation of MAbs against specific membrane fractions from endothelia has yielded additional tissue-specific blood vessel markers [3,4].

Special features of blood vessels in pathological tissues. Tumor blood vessels express specific markers that are not present in the blood vessels of normal tissues. Such marker molecules can be present in the endothelial cells, the pericytes or the extracellular matrix (ECM) of tumor blood vessels. Many of the marker molecules that are selectively expressed in tumor blood vessels are proteins associated with tumor-induced angiogenesis, the sprouting of new blood vessels [5]. These proteins are often functionally important in the angiogenesis process; agents that perturb their function suppress angiogenesis. Tumor blood vessels are prime targets for inhibiting tumor growth. Because these vessels are distinct from normal resting blood vessels, they can be selectively destroyed without significantly affecting normal vessels. Inflammatory lesions such as arthritic synovium are also angiogenic and can be targeted with phage-displayed peptides [6]. Atherosclerotic plaques is another lesion in which specific molecular markers are detectable with *in vivo* peptide library screening [7].

Endothelial marker molecules. The molecular nature of vascular changes that give rise to the individuality of the vessels in various tissues and pathological lesions is partially understood. Several proteases have been identified as markers of the vasculature in individual normal tissues (Table 1). Thus, two peptidases

PII: S1359-6446(02)02501-1

(dipeptidyl peptidase IV [8] and membrane dipeptidase [1]) and a chloride channel are selectively expressed in lung vessels [4]. Another peptidase, aminopeptidase P, is a marker for breast gland vasculature [9].

The molecular markers of angiogenesis include additional peptidases or proteases and integrins. Aminopeptidase N is a marker of angiogenic vessels. It is a membrane-spanning 140-KDa cell surface protein that has previously been linked to cell migration and tumor invasion, but is a new marker of angiogenic endothelium. Antibodies against aminopeptidase N and enzymatic inhibitors of this enzyme block angiogenesis [10].

The cell adhesion receptors, integrins αvβ3, αvβ5 and α5β1 are over-expressed in tumor vasculature [11,12]. Indeed, one of the peptides identified by *in vivo* screening of phage libraries for tumor homing recognizes αvβ3 and αvβ5 [Table 1; 13]. Antibodies and peptides specific for these integrins have been used as receptors for targeted delivery of anti-cancer and anti-angiogenic agents (see below).

Serial analysis of gene expression has been used to survey differences in mRNA expression between endothelial cells from human colon cancers and from adjacent normal tissue [14]. Certain ECM proteins, particularly collagens, were found to be expressed at 10- to 30-fold higher levels in tumor endothelial cells than those from the normal tissue. Some matrix metalloprotease mRNAs were also over-expressed in tumor vasculature. Yet another set of differences included 'new' tumor-specific molecules, related only to expressed sequence tags in the databases. Some of these tumor endothelial markers (TEMs) had apparent transmembrane domains, which suggests that the proteins are expressed on the cell surface. One of the TEMs is endosialin, independently shown by another group to be a marker of angiogenic blood vessels [15].

Genetic programs initiated by angiogenic growth factors produced by tumor tissue (and other tissues that require neovascularization) are likely to be responsible for the production of the angiogenesis-related markers in tumor vasculature. Upregulation of tissue factor (TF) expression in tumor endothelial cells is also likely to occur under the influence of the tumor tissue, as TF is not expressed on endothelial cells of normal vessels [16]. TF binds factor VII to initiate blood clotting, and its expression by tumor endothelia is thought to contribute to the increased incidence of thrombosis seen in cancer patients. Further analyses of tumor vessels are likely to uncover markers that are specific for individual tumor types.

Markers in pericytes and ECM. Molecular markers of angiogenesis are not limited to the endothelium. The supporting mural cells (pericytes and smooth muscle cells) and the ECM also carry distinct markers. NG2 proteoglycan, also known as melanoma-associated chondroitin sulfate proteoglycan, is one such marker. NG2 is a membrane-spanning cell surface protein that is expressed in the neovasculature of tumors, regenerating tissues and in fetal vessels [17]. NG2 expression is limited to pericytes; endothelial cells do not express detectable levels of NG2.

The ECM of blood vessels consists of a sub-endothelial basement membrane and the matrix surrounding the mural cells. The expression of one matrix component, an alternatively spliced form of fibronectin containing an additional type III domain, ED-B, is restricted to tumor vessels and vessels of non-malignant tissues undergoing angiogenesis [18]. Fibronectin promotes cell adhesion, migration, growth and survival; the specific function of its ED-B isoform is not known.

Lymphatics in tumors. The presence and importance of blood vessels in tumors is well-established, but it has only recently been found that lymphatic vessels can also be present within tumors. The abundance of lymphatic vessels in and around a tumor correlates with the propensity of that tumor to metastasize [19]. We have recently reported evidence indicating that lymphatic vessels in tumors can also be specialized. A nonapeptide, LyP-1, isolated by combining phage display *ex vivo* and *in vivo*, directs the homing of the phage to vessel-like structures in certain tumors [20]. These structures are not blood vessels because they stain negative for blood vessel markers. Instead, they stain for various markers of lymphatic vessels. LyP-1 recognizes these apparent lymphatic vessels in most, but not all, tumors. Fluorescein-labeled LyP-1 peptide also homes to tumors after an intravenous injection. The fluorescence appears in tumor lymphatics and accumulates in the nuclei of the lymphatic endothelial cells (Fig. 1). Fluorescent LyP-1 is not detected in normal tissues, indicating that this peptide distinguishes lymphatics in tumors from normal lymphatics of the same animal. In addition to the lymphatic endothelial cells, LyP-1 also binds to the tumor cells, accumulating in their nuclei. Thus, this peptide can selectively transport its payload (fluorescein) all the way from the systemic circulation to the nuclei of the target cells.

Tumor cells can cover a substantial fraction of the luminal surface of tumor blood vessels [21]. These tumor cells might be in the process of transmigrating through the blood vessel wall, rather than being a structural component of the vessel. Tumor cells might also form ECM-lined channels that have been proposed to function as an auxiliary vasculature in highly malignant tumors [22]. These findings suggest that peptides or antibodies that bind to tumor cells, rather than to the conventional vascular cells, might also be capable of targeting tumor vasculature.

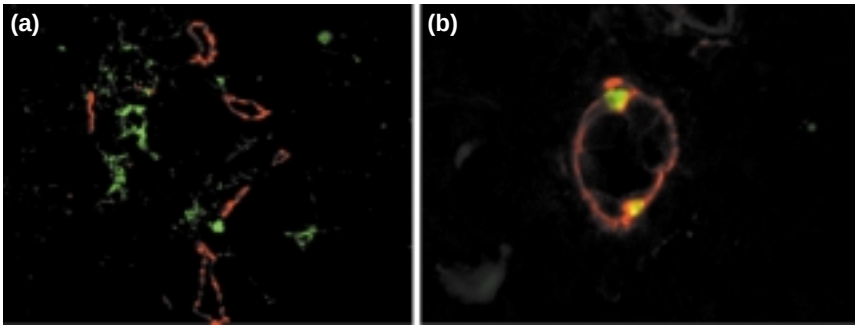

Figure 1. Targeting of tumor lymphatics with a fluorescein-labeled homing peptide. Intravenously injected LyP-1 peptide (sequence, CGNKRTRGC) homes to tumor tissue. **(a)** The peptide fluorescence (green) shows no overlap with blood vessels (red). **(b)** The peptide (green) appears in endothelial cells of a lymphatic vessel identified by podoplanin staining (red). The peptide accumulates in cell nuclei. Figure modified, with permission, from Ref. [20]. Magnification ×200.

Origin of vascular specialization. One can tentatively make two generalizations regarding tissue- and tumor-specific markers of vessels: these markers are likely to be functionally important to the tumor vessels and many of them represent special forms of otherwise common proteins. Integrins illustrate the first point. The αvβ3, αvβ5 and α5β1 integrins are each upregulated in angiogenic vessels and play a role in angiogenesis [11,12]. In the fetus, α5β1 is necessary for the development of the vasculature [23], whereas fetal or adult angiogenesis can take place without the αv integrins [24,25]. However, αvβ3 and αvβ5 are somehow important in adult angiogenesis because peptides and antibodies that perturb their function block angiogenesis [5,11]. Aminopeptidase N is another angiogenesis marker, which appears to be needed for angiogenesis to proceed [10].

The αvβ3 and αvβ5 integrins are essentially absent from the normal tissues of an adult animal and are selectively upregulated in angiogenesis. In contrast, aminopeptidase N is expressed in several epithelial tissues and in macrophages. Two factors might account for the homing specificity of the aminopeptidase N-binding peptides: (1) angiogenic vessels are the only vessels that express aminopeptidase N and (2) these vessels seem to express a specific form of this peptidase, as peptides that bind to aminopeptidase N in tumor vessels do not bind to macrophages [26]. In a similar vein, an antibody that recognizes a splice variant of fibronectin selectively recognizes the ECM of angiogenic blood vessels [18]. A peptide that homes to the vessels in normal breast tissue might similarly recognize a subset of aminopeptidase P molecules [9].

Delivery of therapeutic agents to vascular targets

Drug delivery. Peptides, peptidomimetics and antibodies that home to a specific site in the vasculature are attractive as carriers of therapeutic and diagnostic agents. Specific drug delivery should concentrate the drug at the targeted site, increasing efficacy and also decreasing side effects in other tissues. Targeted delivery is particularly attractive in cancer therapy. The chemotherapeutic drugs currently used to treat cancer are highly toxic, which places a limit on the dose a patient can tolerate. Targeted delivery of high drug-concentrations to tumor tissue might alleviate this problem because normal tissue would be less affected. Moreover, targeting therapeutic agents to the vasculature of tumors, as opposed to the tumor cells themselves, might offer some additional advantages. Tumors are critically dependent on a blood supply; eliminating that supply can profoundly suppress tumor growth [2]. Blood vessels are more readily accessible to intravenously administered therapy than tumor cells. Furthermore, although tumor blood vessels acquire a tumor-associated 'signature', they are composed of normal cells that do not readily acquire mutations leading to drug resistance [27]. Moreover, when a targeted anti-angiogenic agent is also active against the tumor cells, additional gains in efficiency can be expected.

Proof for the vasculature-targeted delivery principle has been obtained in studies with experimental tumors. Researchers have designed compounds that can specifically deliver anti-tumor drugs to tumor vasculature. In one approach, investigators used vascular endothelial growth factor (VEGF) to target diphtheria toxin to VEGF receptors expressed by the tumor endothelial cells [28]. We have used homing peptides to construct drugs that bind to tumor vasculature. The RGD-4C peptide specifically binds to the αvβ3 and αvβ5 integrins [29], and the CNGRC peptide binds to aminopeptidase N [10]. Coupling of doxorubicin to the RGD-4C and CNGRC targeting peptides yielded compounds that were more effective and less toxic than doxorubicin alone [13]. Doxorubicin, like several other cytotoxic chemotherapeutics, inhibits angiogenesis in addition to being toxic to tumor cells [30]. The vascular targeting approach is likely to enhance these anti-angiogenic effects. The targeting of doxorubicin using the RGD-4C and CNGRC peptides reduced the side effects of the treatment in the heart and liver, which are the main sites of doxorubicin toxicity.

We have developed compounds that use a homing peptide for delivery and uptake into target cells, and a proapoptotic peptide as the drug component [31]. A major advantage of

Table 1. Structure and activities of homing peptides identified by phage display[a].

	RGD-4C[b]	NGR[c]	LyP-1[d]	F3[e]	SMS[f]	GFE[g]
Sequence	CCDCRGDCFC	CCNGRC	cCGNKRTRGC	*	SMSIARL	cCGFECVRQCPERC
Cell surface	integrins αvβ3 and αvβ3	amino- peptidase N	not known	not known	not known	membrane dipeptidase
Tissue	Angiogenesis	Angiogenesis	Tumor lymphatics/ tumor cells	Angiogenesis/ vessels	Prostate	Lung
Cellular localization of the peptide	Cell surface/ cytoplasmic	Cell surface/ cytoplasmic	Cell surface/ nuclear	Cell surface/ nuclear	Cell surface/ intracellular	Cell surface

[a]The amino acid sequences of the peptides are given in the single letter code; c denotes cyclic structure formed by a disulfide bond.
[b]This is a cyclic nonapeptide containing two disulfide bonds; the form with the 1-3, 2-4 disuldide bond pattern is active in binding to the integrins [29].
[c]Peptides with the NGR motif bind to aminopeptidase N, which is specifically expressed in angiogenic vessels within the vasculature [10,13].
[d]A cyclic nonapeptide that recognizes tumor lymphatics and tumor cells in certain (but not all) tumors. This peptide is taken up by the cells it binds to and tranlocated into the nucleus [20].
[e]*A 31-amino acid peptide that binds to the endothelial cells in tumor blood vessels and tumor cells and is translocated into the nucleus [33].
[f]A linear heptapeptide that selectively binds to the vasculature of the normal prostate [32].
[g]A cyclic nonapeptide that recognizes membrane dipetidase, which is selectively expressed by lung blood vessels within the vasculature [39].

this class of compounds is that they can be prepared by solid phase peptide synthesis. In contrast, conjugation steps are required in joining a homing peptide with conventional drugs. The proapoptotic peptide is an anti-bacterial peptide that disrupts the membranes of bacteria, but harms mammalian cells only if it is introduced into a cell. Inside a mammalian cell, the proapoptotic peptide disrupts the mitochondria, as their membranes resemble those of bacteria (mitochondria are ancestrally related to bacteria). Leakage of mitochondrial membrane, in turn, is one of the main initiators of apoptosis. The efficacy of this approach has been demonstrated in mice in two diseases associated with angiogenesis, a tumor model [31] and synovial inflammation in arthritis [6]. Moreover, combining the same proapoptotic peptide with a homing peptide that binds to the blood vessels of the normal prostate yielded a compound with a different targeting specificity. Intravenous administration of this compound to male mice resulted in partial destruction of the normal prostate tissue. In a transgenic prostate cancer model (TRAMP mice), administering the prostate-targeted compound before the appearance of the tumors delayed tumor development [32]. That the same non-selective proapoptotic peptide could be used successfully for different targets by changing the homing peptide, dramatically illustrates the potential of the targeting technology.

Specific delivery to subcellular sites. Relatively recently, we have identified peptides that selectively recognize tumor endothelial cells and tumor cells, and are capable of delivering a drug-like payload, such as fluorescein or rhodamine, into the nucleus of these cells. One of these peptides, Lyp-1, recognizes the tumor lymphatics and tumor cells [20], whereas another peptide transports fluorescein into the nuclei of tumor blood vessel endothelial cells and tumor cells ([33]; Table 1). These peptides contain numerous basic amino acid residues, which apparently form a nuclear localization signal. They might prove to be particularly useful for delivering anti-cancer drugs that act in the nucleus. It might also be possible to develop specific targeting probes for other intracellular organelles.

Targeted gene therapy. Viral gene therapy vectors have been genetically modified with homing peptides and antibodies for targeting purposes. Inserting an RGD sequence into an adenovirus surface protein changes the tropism of the virus such that the virus infects cells expressing integrins [34,35]. A non-peptidic compound that binds to αvβ3 integrin has been used to target a nanoparticle-based gene therapy vector to tumor vasculature [36]. The particle consisted of a lipid micelle that carries, on its surface, the αvβ3-binding compound and a DNA-binding cationic lipid. The particle selectively delivered a mutated *Raf-1* gene to the αvβ3-expressing endothelium in tumors. A single intravenous injection of the targeted nanoparticles induced apoptosis in tumor vessels, tumor regression and prolonged survival in mice.

Anti-angiogenic versus anti-vascular targeting. The experimental tumor targeting studies used fast-growing mouse tumors. The blood vessels in these tumors are 'new' and might be more likely to carry angiogenesis markers, such as the αvβ3 integrin, than vessels in slow-growing tumors. Thus, the experimental tumors could be more responsive to anti-angiogenic therapies than human tumors, which grow over years and might have a greater proportion of mature vessels than the experimental tumors. Treating

tumors with well-established vessels is likely to require both anti-vascular and anti-angiogenic approaches. As blood vessels mature, they acquire a pericyte coating and associated ECM. Tumor-specific changes in these vascular elements, such as the NG2 proteoglycan in pericytes [17] and oncofetal fibronectin and altered collagen in the matrix [18,37], might make it possible to develop targeted delivery approaches for anti-vascular therapy. Partial damage to the vessels might suffice, as thrombosis of the affected vessels might be initiated that would further restrict blood flow to the tumor [38]. Targeting through the lymphatic vessels is another potential way of expanding the targeting strategy [20]. In the future, anti-vascular tumor therapies are likely to rely on drug combinations that provide a concerted attack at more than one of the vulnerabilities in tumor vasculature.

Concluding remarks

The homing peptides isolated by phage display have shown the great diversity of blood vessels. Vascular homing peptides for a large number of tissues have already been found, which suggests that it will be possible to identify homing peptides that are specific to blood vessels in most, if not all, tissues. It is likely that additional homing peptides specific to tumor vasculature can also be found by further screening of various tumors. Uncovering the identity of the 'receptors' for homing peptides is a priority. The already known receptors (integrins, proteases and a proteoglycan) in tumor vasculature suggests that the markers of tumor vasculature discovered by phage screening will be a heterogeneous group of proteins, predominantly associated with angiogenesis. Many of these proteins are likely to prove to be functionally significant to the process of angiogenesis. In the future, vascular markers that characterize the vasculature of one tumor type, rather than angiogenesis, might also be found.

Promising results have come from the first attempts to direct drugs to tumors by using drug–peptide conjugates that home to tumor vasculature. As the receptors for the homing peptides are identified, improved versions of these peptides and their drug conjugates can be developed. As the identity of the receptors unfolds, it will also be possible to quantitatively determine the difference between the expression of these receptors within the vasculature of tumors and normal tissues. This information can be used to improve the selectivity of drug targeting by optimizing the affinity and binding valency of the homing peptides (or their mimetics). More efficient delivery of drugs, radioactive compounds and genes into tumors will undoubtedly ensue.

So far, the only pathology extensively targeted with the phage is tumors (angiogenesis). Other pathologies might also cause alterations in the resident vasculature. In fact, it is known that this happens in inflammation and ischemia. Thus, *in vivo* phage screening to target tissues affected by various diseases might prove rewarding. Another attractive possibility is screening for peptides (or proteins) that are able to cross certain barriers, such as the blood–brain barrier.

Translating homing peptide technology developed in the mouse into therapies for human disease should be possible. All of the peptides we have tested to date have recognized the equivalent human vascular sites, suggesting that the peptides already developed might be directly applicable in humans. However, it might be necessary to modify the peptides for optimal activity. The optimization might require converting the peptides into peptidomimetics or non-peptide chemistries, and will benefit from identification of the vascular receptors for the peptides. The available results demonstrate the potential of the homing peptide technology for affinity-based drug targeting. Clearly, what has been done so far represents only the beginning in exploiting its potential.

Acknowledgement

The author's work was supported by the National Institutes of Health grants CA 82713 and Cancer Center Support Grant CA 30199, and the DAMD 17-02-1-0315 Innovator Award from the Department of Defense.

References

1 Ruoslahti, E. and Rajotte, D. (2000) An address system in the vasculature of normal tissues and tumors. *Annu. Rev. Immunol.* 18, 813–827

2 Hanahan, D. and Folkman, J. (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86, 353–364

3 Jacobson, B.S. *et al.* (1996) Identification of endothelial cell-surface proteins as targets for diagnosis and treatment of disease. *Nat. Med.* 2, 482–484

4 Elble, R.C. *et al.* (2002) Molecular and functional characterization of a murine calcium-activated chloride channel expressed in smooth muscle. *J. Biol. Chem.* 277, 18586–18591

5 Ruoslahti, E. (2002) Specialization of tumour vasculature. *Nat. Rev. Cancer* 2, 83–90

6 Gerlag, D.M. *et al.* (2001) Suppression of murine collagen-induced arthritis by targeted apoptosis of synovial neovasculature. *Arthritis Res.* 3, 357–361

7 Houston, P. *et al.* (2001) Homing markers for atherosclerosis: applications for drug delivery, gene delivery and vascular imaging. *FEBS Lett.* 492, 73–77

8 Abdel-Ghany, M. *et al.* (1998) Truncated dipeptidyl peptidase IV is a potent anti-adhesion and anti-metastasis peptide for rat breast cancer cells. *Invasion Metastasis* 18, 35–43

9 Essler, M. and Ruoslahti, E. (2002) Molecular specialization of breast vasculature: a breast-homing phage-displayed peptide binds to aminopeptidase P in breast vasculature. *Proc. Natl. Acad. Sci. U. S. A.* 99, 2252–2257

10 Pasqualini, R. *et al.* (2000) Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis. *Cancer Res.* 60, 722–727

11 Eliceiri, B.P. and Cheresh, D.A. (1999) The role of alphav integrins during angiogenesis: insights into potential mechanisms of action and clinical development. *J. Clin. Invest.* 103, 1227–1230
12 Kim, S. *et al.* (2000) A regulation of angiogenesis *in vivo* by ligation of integrin α5β1 with the central cell-binding domain of fibronectin. *Am. J. Pathol.* 156, 1345–1362
13 Arap, W. *et al.* (1998) Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science* 279, 377–380
14 St Croix, B. *et al.* (2000) Genes expressed in human tumor endothelium. *Science* 289, 1197–1202
15 Christian, S. *et al.* (2001) Molecular cloning and characterization of EndoGlyx-1, an EMILIN-like multisubunit glycoprotein of vascular endothelium. *J. Biol. Chem.* 276, 48588–48595
16 Hu, Z. and Garen, A. (2001) Targeting tissue factor on tumor vascular endothelial cells and tumor cells for immunotherapy in mouse models of prostate cancer. *Proc. Natl. Acad. Sci. U. S. A.* 98, 12180–12185
17 Ozerdem, U. *et al.* (2001) NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Dev. Dyn.* 222, 218–227
18 Halin, C. *et al.* (2002) Enhancement of the antitumor activity of interleukin-12 by targeted delivery to neovasculature. *Nat. Biotechnol.* 20, 264–269
19 Alitalo, K. and Carmeliet, P. (2002) Molecular mechanisms of lymphangiogenesis in health and disease. *Cancer Cells* 1, 219–227
20 Laakkonen, P. *et al.* (2002) Tumor-homing peptide with a lymphatic vessel-related targeting specificity. *Nat. Med.* 8, 751–755
21 Chang, Y.S. *et al.* (2000) Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. *Proc. Natl. Acad. Sci. U. S. A.* 97, 14608–14613
22 Folberg, R. *et al.* (2000) Vasculogenic mimicry and tumor angiogenesis. *Am. J. Pathol.* 156, 361–381
23 Yang, J.T. *et al.* (1993) Embryonic mesodermal defects in alpha 5 integrin-deficient mice. *Development* 119, 1093–1105
24 Bader, B.L. *et al.* (1998) Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell* 95, 507–519
25 Reynolds, L.E. *et al.* (2002) Enhanced pathological angiogenesis in mice lacking β3 integrin or β3 and β5 integrins. *Nat. Med.* 8, 27–34
26 Curnis, F. *et al.* (2002) Differential binding of drugs containing the NGR motif to CD13 isoforms in tumor vessels, epithelia, and myeloid cells. *Cancer Res.* 62, 867–874
27 Kerbel, R.S. (1991) Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anti-cancer therapeutic agents. *BioEssays* 13, 31–36
28 Olson, T.A. *et al.* (1997) Targeting the tumor vasculature: inhibition of tumor growth by a vascular endothelial growth factor–toxin conjugate. *Int. J. Cancer* 73, 865–870
29 Assa-Munt, N. *et al.* (2001) Solution structures and integrin binding activities of an RGD peptide with two isomers. *Biochemistry* 40, 2373–2378
30 Browder, T. *et al.* (2000) Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res.* 60, 1878–1886
31 Ellerby, H.M. *et al.* (1999) Anti-cancer activity of targeted pro-apoptotic peptides. *Nat. Med.* 5, 1032–1038
32 Arap, W. *et al.* (2002) Targeting the prostate vasculature for destruction through a vascular address. *Proc. Natl. Acad. Sci. U. S. A.* 99, 1527–1531
33 Porkka, K. *et al.* (2002) Targeting of peptides to the nuclei of tumor cells and tumor endothelial cells *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 7444–7449
34 Wickham, T.J. (2000) Targeting adenovirus. *Gene Ther.* 7, 110–114
35 Haviv, Y.S. *et al.* (2002) Adenoviral gene therapy for renal cancer requires retargeting to alternative cellular receptors. *Cancer Res.* 62, 4273–4281
36 Hood, J.D. *et al.* (2002) Tumor regression by targeted gene delivery to the neovasculature. *Science* 296, 2404–2407
37 Xu, J. *et al.* (2001) Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth *in vivo*. *J. Cell Biol.* 154, 1069–1080
38 Huang, X. *et al.* (1997) Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. *Science* 275, 547–550
39 Rajotte, D. and Ruoslahti, E. (1999) Membrane dipeptidase is the receptor for a lung-targeting peptide identified by *in vivo* phage display. *J. Biol. Chem.* 274, 11593–11598