Function and Molecular Mechanism of Tumor-Targeted Peptides for Delivering Therapeutic Genes and Chemical Drugs

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Abstract: Tumor-targeted chemo- or gene-therapies is a new form of treatment which provides the benefits of reducing systemic toxicity, increasing the tolerance, and enhancing therapeutic efficacy. This review will discuss the discovery, function, application, and mechanism by which the short peptides (5-9) work for tumor-targeted gene or drug delivery.

Keywords: Peptide, tumor-targeting, tumor vessel, gene therapy, gene delivery, drug delivery, adhesion molecule, phage display.

INTRODUCTION

One of the most daunting tasks facing the field of cancer therapy today is finding an effective means of transporting therapeutic agents to the site of tumors. The accomplishment of this goal would allow for eradication of specific cancerous cells without damaging healthy tissue of critical organs. The most attractive method would be attacking the cell surface proteins that are altered on cancer cells or in the tumor vasculature, given that cancer escapes the immune system through genetic changes in protein expression that occurs on those cells. These proteins could be growth factor receptors [1], cell adhesion molecules [2], integrins [3], or surface markers on endothelial cells. Targeting DNA or viral vector-containing therapeutic genes to areas expressing tumor-specific proteins would allow researchers to localize the delivery of their treatments. Tumor-targeted minipeptides, are an attractive group of peptides that can increase survival time [4], instill a memory immune response [5], and limit metastasis with great potential to be used for tumor-targeted gene delivery [6]. An obvious advantage of peptides alone over viral or liposomal vectors for tumor targeting is that most peptides do not elicit an aggressive immune response [7]. In contrast to antibodies, multiple treatments can be given without causing systemic toxicity while increasing efficacy. The designs for targeting peptides to tumors are practically limitless. Researchers have used everything from membrane permeable peptides [8] to viruses coated with tumor targeting peptides [9] to immunoliposomes with tumor targeted antibody fragments [10]. These peptide-based gene delivery strategies are becoming extremely valuable for tumor-specific gene therapy.

With the recent increase in discoveries of functional peptide:ligand pairs, researchers are now able to target to specific organs or tissue types [11]. The main interest for the field of cancer research lies in targeting the tumor vasculature, including tumor blood as well as tumor lymphatic vessels [12]. The extensive diversity of molecules expressed in the vasculature provides numerous potential targets for directing gene vectors to tumors in different organs. Endothelial cells line blood vessels and serve as "gateways" to tumors. They contain surface proteins that function as vascular receptors able to transduce growth or angiogenic signals [13]. The main advantage of targeting to endothelial cells is that they are highly accessible from the blood stream, thus simplifying the experimental design. Several peptides express high affinity for endothelial markers, such as RRKRRR (VEGF) [14], RGD-4C (integrins) [15], TAASGVRSMH (proteoglycans) [16], or NGR (aminopeptidase N/CD13) [13]. For a more complete list of targeted peptides, see Table **1**.

Targets for peptide therapy have also been found in most vital organs. Data shows that small peptide sequences can home in to colon (RPMC) [17], prostate (AGG) [18], lungs (CGFE) [19], pancreas (CVSNPRWKC) [20], bone marrow (GGG) [18], and lymphatic vessels (CGNKRTRGC) [12]. These studies also use different methodologies. Some use mini-peptides to bind and stabilize intracellular proteins, thus rescuing the denatured mutant proteins, as shown through the use of peptide stabilization for restoring the tumor suppressor function of mutated p53 [21]. Others attach chemotherapeutic drugs [4] or DNA molecules [22] to the peptides so that internalization takes place. Hong and Clayman found that the sequence TSPLNIHNGQKL allowed its complex to be internalized in human head and neck squamous cell carcinoma [23]. In 2002, De Groot *et al.* developed a doxorubicin prodrug conjugated to an integrin binding peptide [24]. The molecule had two peptide sequences, the first for integrin targeting and other for activation of doxorubicin by plasmin cleavage. An aminocaproyl residue was inserted between the two peptide sequences. In addition, a self-eliminating 4-aminobenzyl alcohol spacer was added between the doxorubicin and the plasmin substrate (Figs. (**1, 2**)). Since plasmin plays a key role in tumor invasion, metastasis, and angiogenesis, it was used to convert the prodrug to its active form in the tumor vasculature. These organ-specific peptides that are explored for delivery of anti-tumor agents can also be used to target viral or non-viral vectors to specific organs. It is possible that through gene therapy, tumor targeting oncolytic peptides could directly induce tumor cell death.

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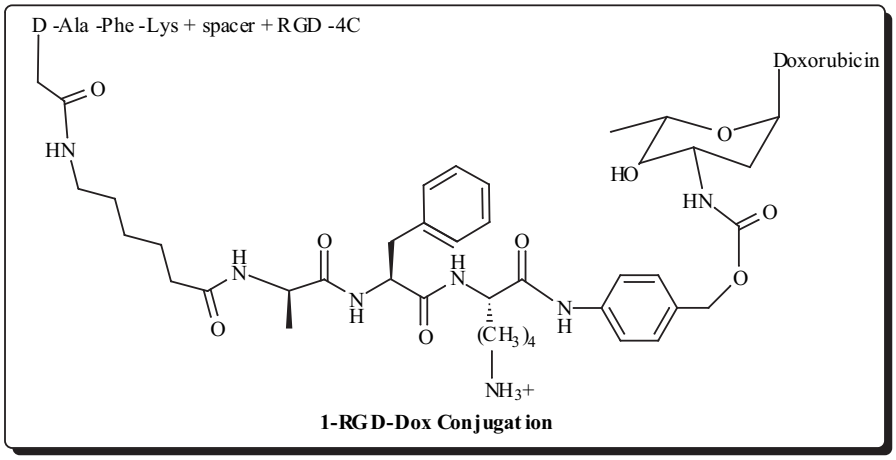
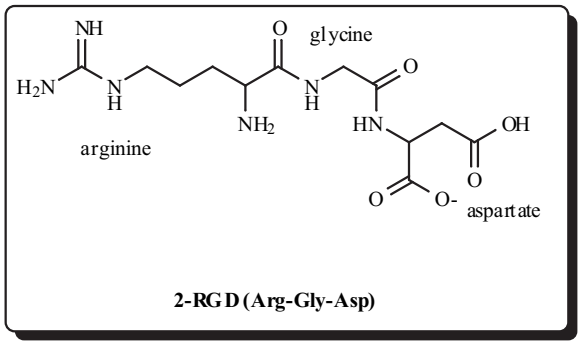


1-RGD-Dox Conjugation



2-RGD (Arg-Gly-Asp)

Walensky *et al.* found that providing the stability to these peptides would be the key for developing effective tumor peptide therapies [25]. To overcome the instability problem, they described a helical, protease resistant, cell permeable peptide on a hydrocarbon-stapled BH3 domain of the Bid protein. BH3 was investigated because their structural studies of members of the apoptotic pathway show that the α-helix of BH3 is critical for interaction with Bcl-2 and Bcl-X_L. Interaction of BH3 with Bcl-2 and Bcl- X_L could inhibit the formation of Bcl-2 dimers, thereby inducing apoptosis. Ellerby *et al.* devised a method of attaching an additional peptide sequence to the tumor homing sequence CNGRC motifs in order to target mitochondrial membranes and induce apoptosis [26]. They reported that the pro-apoptotic domain of this peptide $(KLAKLAK)_2$ could disrupt the membrane and activate caspase-3 without causing toxicity or immunogenic response. These oncolytic peptides are great candidates for targeting toxins or therapeutic drugs into the local tumor area although they may not be the best candidates for delivering DNA vectors, because the targeted tumor cells may be killed without expressing the desired therapeutic gene the vector delivered.

One key technique that contributes to the progress of discovering tumor-targeted peptides is phage display. In the last decade, phage libraries have been widely used to create peptides that bind with high affinity to their tumor associated proteins. The majority of these peptides generated this way will bind to functionally important domains of their target proteins [27]. George Smith described the first phage library screening technique in 1985 [28]. Since then, many improvements have been made to increase yield and specificity, as well as decrease time and cost. Originally, the phage libraries were carried out *in vitro*; however, in recent years the trend has moved toward *in vivo* panning to isolate ligands that localize to the vasculature of tumor tissue [29].

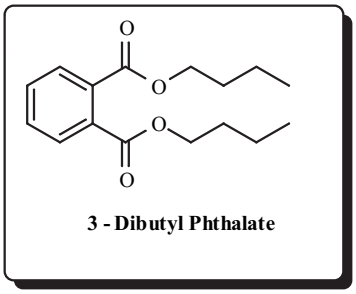
Another method of discovering tumor-targeted peptides takes the reverse approach. Studies have been done which compare the gene expression of endothelial cells isolated from a tumor to cells from adjacent normal tissue, and the differentially expressed proteins emerge as possible targets. Then those proteins are analyzed to find binding sites, and anti-sense peptides are designed to bind in those pockets [30]. These methods have drastically increased the speed at which new peptides are discovered, and they have led to many new ideas for effective treatments. It is expected that this method will be more dynamic than ever due to rapid progress in gene expression profiling through the use of proteomics and microarrays.

BRASIL SCREENING

In order to begin targeting peptides to tumors, it is necessary to elucidate both the mechanisms and participants. There are several methods for determining exact peptide sequences that bind with high affinity to their target receptors. The most common is screening through a phage library, as seen in the work of Ward, *et al.* [31]. For this, a ligand library is displayed on the surface of the phage. The phage then binds antigen and is subsequently eluted by acidic or enzymatic cleavage. Finally, the augmented phage population is re-amplified through rounds of selective propagation. Phage libraries can screen many types of molecules from large antibodies to short peptide fragments.

A more recent technique developed is referred to as BRASIL, or biopanning and rapid analysis of selective interactive ligands, where cell suspensions incubated with phage are centrifuged through a single step organic phase separation. This method has proven faster, more sensitive, and more specific than common phage selection and sorting methods [32]. It may also lead to the future discovery of new cell surface protein families resulting in more effective targeting peptides. Giordano *et al.* first used BRASIL to screen phage display random peptide libraries on endothelial cells stimulated with VEGF. They found the most effective organic phase combination to be dibutyl phthalate: cyclohexane (Fig. (**3**)) (9:1 [v:v]; d=1.03g ml^{-1}), but found

other phthalate combinations with comparable densities gave the similar results. The upper aqueous phase contains the cell suspension in MEM with 1% BSA at $1x10^6$cells/ml.



3 - Dibutyl Phthalate

Centrifugation followed by snap freezing in liquid nitrogen allows for the bottom of the tube to be sliced off, and the cell pellet transferred without cross-contamination of phage in aqueous layer. The quickness and consistency of this method could easily lead to high-throughput screening for clinical applications.

A related method used to target molecules to cancer cells involves monoclonal antibodies (Mabs). Although Mabs have been shown to target to tumors, the high molecular weight of the antibody results in low tissue penetration and low cellular uptake when used *in vivo* [33], raising the question of whether an upper size limit exists for molecular therapeutics. Antibody fragment-cytokine fusion proteins known as immunocytokines have been shown to reduce tumor volume as well as establish a memory immune response due to activation of effector cells by the cytokines in the tumor microenvironment [5].

Halin *et al.* used a slightly modified version of this to target interleukin-12 to neovasculature by fusing it to an antibody fragment [34]. The antibody fragment – scFv(L19), has been shown to home to an isoform of fibronectin (ED-B) that is expressed in angiogenic tissues, most commonly found in tumors. Immunocytokines can stimulate the innate immune response to eliminate antigens, which in turn, activates the adaptive system to establish a memory response. This synergism between the innate and adaptive responses presents great potential for eradicating tumors while sustaining long-term protection against recurrence or metastasis.

RGD-4C : INTEGRIN TARGETING

Integrins are a family of at least 25 heterodimeric, transmembrane, cell surface receptors mediating cell adhesion and migration [35]. They are composed of different α and β subunits with each combination resulting in a distinctive ligand binding specificity. Of the 25 known integrins, 8 bind to RGD sequences peptides, which serve as the main integrin recognition site in the extracellular matrix proteins [36]. One type of integrin – $\alpha_v\beta_3$, is commonly isolated on platelets, osteoclasts, dendritic cells, and endothelium [37]. It is highly expressed in endothelial cells of angiogenic vessels and exhibits high affinity for matrix metalloproteinase-2 [38]. The $\alpha_v\beta_3$ receptor has a wide range of ligands to which it binds, including but not limited to, fibronectin, vitronectin, osteopontin, and fibrinogen. The properties and three-dimensional structure of $\alpha_v\beta_3$ suggests that this molecule is a great candidate for tumor-targeting.

Experiments by Li *et al.* show that the RGD sequence is effective in binding multiple integrin types. They found peptides of various lengths with the RGD sequence included bind $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_{II}\beta_3$ [39]. One unique discovery related to $\alpha_v\beta_6$ integrins, which are involved in carcinoma proliferation through cell adhesion events, confirms that peptides with the sequence DL*XX*L exhibit specific binding affinities [40]. By limiting binding to only one integrin type, this gives researchers the added benefit of controlling the side effects and limiting toxicity when using this molecule in cancer treatment.

Assa-Munt *et al.* produced a cyclic RGD-4C peptide that showed high affinity for $\alpha_v\beta_5$ and $\alpha_v\beta_3$ integrins after screening with phage library [41]. Their peptide – A**C**D**C****RGD****C**F**C**G, contains four cysteine residues that produced several isomers of disulfide linked cyclic peptides. They found that the most effective peptide for binding $\alpha_v\beta_3$ was the form with 1-4, 2-3 disulfide bonds. These bonds force the Cys2 residue away from the interior, creating a distorted type I β-turn. In addition to the cysteine residues, the phenyalanine's hydrophobic ring position plays an important role in the formation of a pocket. The carbonyl group from the Cys2 is exposed to solvent and might facilitate integrin binding through hydrogen bonds. Most favorable binding occurs when Asp and Arg are less than 6.7Å apart, and this RGD peptide is within that range.

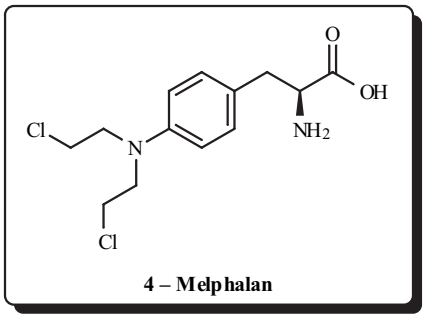
All these factors lead to a highly restricted RGD peptide yet confer high selectivity for $\alpha_v\beta_5$ and $\alpha_v\beta_3$ integrins. The specificity of RGD peptide and the endothelial cell localized expression makes this peptide a competitive candidate for targeting DNA vectors to tumors. In fact, this peptide has been integrated as part of adenovirus surface protein and has been heavily explored to target the virus into $\alpha_v\beta_3$ expressing endothelial and tumor cells via the RGD-4C and $\alpha_v\beta_3$ interaction, instead of using the natural CAR receptors that are often lost in highly malignant tumor cells [42]. The success *in vitro* does not translate well *in vivo* because Ad vector is heavily accumulated in the liver. This strategy still needs to be improved before it finds its niche in a clinical setting.

Many peptide sequences target integrins on endothelial cells in blood vessels. These peptides localize in the tumor vasculature which has highly angiogenic activity. There are several classes of integrin receptors that show specificity for certain organs. One type of integrin receptor $\alpha_5\beta_1$, is highly expresses in the colon. According to Kelly *et al*, they found a seven-fold increase in binding and internalization with cyclic peptides containing the sequence RPMC in colon cancers in a murine model [17].

Richards *et al.* found that a peptide sequence of RGDWXE integrated with high affinity into the highly flexible loop connecting the F and G β-strands (FG loop) of $\alpha_v\beta_3$ integrins [43]. They also discovered that this form is more heat stable than a monoclonal antibody, and unlike most integrin-targeted molecules, it binds with high specificity to only $\alpha_v\beta_3$. Analysis of the crystal structure of the RGD: $\alpha_v\beta_3$ complex resulted in the discovery of several key factors. First, the F residue is highly exposed, suggesting that the F and W residue (RGD**W**XE) form a binding pocket. It also contains a small hydrophobic pocket near the side chain that might impart the high selectivity to

this peptide. These small RGD peptides have many advantages over common techniques used today in that they are more stable, can be incorporated into gene delivery vectors, target multiple cell types (dendritic and endothelial), and may even be useful in cancer imaging because of its ability to recognize angiogenic vessels in tumors.

Fusing anticancer molecules to RGD peptides that target tumor vasculature seems potentially useful. Recently, Curnis *et al.* showed that coupling TNF-α to $\alpha_v\beta_3$ ligands improves its anti-angiogenic activity [44]. They reported that subnanogram doses are enough to induce antitumor effects when TNF is fused with A**C**D**C**R**GD**C**F**C**G (RGD-4C) and co-administered with chemotherapeutic drugs like melphalan (Fig. (**4**)). Additionally, they found that this complex can also bind to TNF receptors and induce cytotoxic death signals. These synergistic effects confer greater antineoplastic activity and target the molecules to tumors better, with less toxicity and smaller doses, than each method alone. They proposed a mechanism that relates TNF's ability to alter endothelial barriers and reduce interstitial tumor pressure to increased chemotherapeutic drug penetration [45].



4 – Melphalan

CNGRC : CD13 TARGETING

CNGRC, containing the core peptide NGR (asparagine-glycine-arginine), is a novel peptide and is effective in delivering anti-tumor compounds to angiogenic blood vessels as an aminopeptidase N ligand [13]. A common cyclic NGR peptide, CNGRC, homes much more effectively than linear NGR motifs, proving that tertiary structure plays an important role in receptor:ligand binding [4]. Immunohistochemical analysis shows that different isoforms of aminopeptidase N, also known as CD13, are expressed in normal epithelium, tumor-associated vessels, and myeloid cells. Studies show that CD13 isoforms in other tissues express different epitopes [46]. However, NGR selectively binds only to tumor vessel-related isoforms that may be expressed on connective tissue, fibroblasts, or mastocytes in the tumor stroma [47].

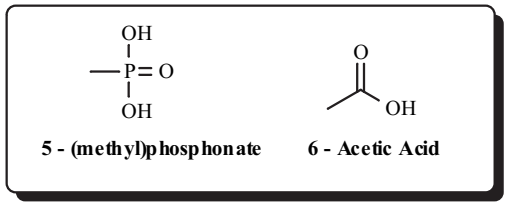
CD13 isoforms range from 150 to 240 kDa as mature cell surface protein. Its molecular weight is composed of 25-30% carbohydrate. By differential utilization of the *O*-glycosylation sites, at least five isoforms can be formed [48]. The biological activity of CD13 varies depending on the microenvironment, but normally it is activated by angiogenic signals to catalyze the removal of NH_2-terminal residues from small peptides [49], perhaps for antigen presentation [50], cytokine [51] or extracellular matrix degradation [52], cell cycle control [53], or tumor invasion [54].

Recent work proves that in vascularized tumors, NGR-TNF-α is 10 to 30 times more efficient than TNF-α alone [55]. This efficiency lowers the necessary dose, and thus the toxicity, for tumor eradication, making this an interesting possibility for future treatment. The anti-tumor activity of TNF relies on damage of tumor vessels and activation of the adaptive response rather than directly killing cancer cells [56]. The immune response mechanism is crucially important in activating a T-cell-dependent response.

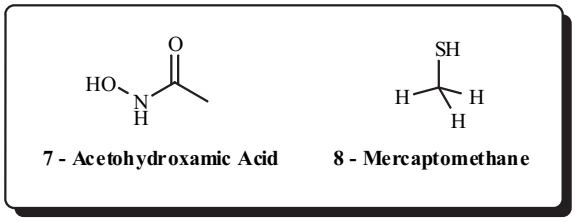
Curnis *et al.* produced a functional CNGRCG-TNF molecule by recombinant DNA technology, with the glycine added as a spacer between TNF and the NGR peptide. Both the peptide and TNF were purified by ammonium chromatography, ion exchange, and gel filtration chromatography resulting in a 40-50 kDA homotrimeric protein joined by disulfide bridges. Electrospray mass spectrometry found a relative molecular mass of 17,843.7 +/- 2.5 for NGR-TNF monomers [55]. In 2002, Colombo *et al.* reported that cyclic CNGRC is more effective at targeting *in vivo* than linear CNGRC, or GNGRC. The Gly^3-Arg^4 creates critical bend geometry and the two cysteines are required for the disulfide bonds that stabilize the bent conformation [57]. This short peptide, only five amino acids, is very attractive for use in targeting DNA vectors to tumors. It is also interesting to know that both the RGD peptides and these NGR peptides contain twin amino acid residues of RG, which may prove that Arg-Gly are key amino acids for tumor targeting.

HWG: MATRIX-METALLOPROTEASE TARGETING

Matrix metalloproteases (MMPs) are a family of enzymes involved in degrading the extracellular matrix and the basement membrane, which lead to tissue remodeling and cell migration through the action of collagenases, gelatinases, and stromelysins [58]. MMPs, particularly gelatinase A(2) and B(9), are upregulated in tumors to augment metastasis and invasion by degrading collagen in the extracellular matrix. Current therapy attempts to inhibit specific MMPs through peptide aptamers without altering the action of the other families. Peptide aptamers are short peptide sequences that are selected to recognize a predetermined target protein domain and are able to interfere with its function [59]. Molecules with zinc-chelating groups, such as thiols (Fig. (**8**)) or hydroxamates (Fig. (**7**)), or phosphonates (Fig. (**5**)) and acetic acid (Fig. (**6**)), are among the common compounds used to inhibit MMPs [60]. Grams *et al.* reported that the hydroxamate acts as a ligand for each oxygen at an optimum distance of 1.9 – 2.3Å from the zinc(II)-ion in the active site [61]. They also found that the position of the hydroxamate nitrogen suggests that it is protonated and hydrogen bonds with the carbonyl oxygen of the enzyme backbone.



5 - (methyl)phosphonate **6 - Acetic Acid**



7 - Acetohydroxamic Acid **8 - Mercaptomethane**

Koivunen *et al* recently proposed a mechanism by which HWGF-derived peptides target gelatinase to inhibit the growth of tumor cells and migration of tumor and endothelial cells [62]. Specifically, they found the cyclic disulfide bonded decapeptide CTTHWGFTLC homed and bound to gelatinases with the greatest affinity. One benefit of specific MMP targeting is the apparent lack of toxicity that can be attributed to its specificity for only one type of MMP. HWGF peptides have a twofold mechanism of action. First, they explicitly inhibit MMP-2 and MMP-9. Second, they home in on tumors because of the overexpression of the MMPs in the tumor vasculature. The tumor targeting, specific inhibition of MMPs, anti-angiogenic and anti-invasive properties all make the HWGF motif an enticing compound for anticancer treatment.

RRKRRR: VEGFR TARGETING

Vascular Endothelial Growth Factor (VEGF) plays a role in angiogenesis and metastasis of tumors as well as the proliferation and migration of endothelial cells through remodeling of the extracellular matrix [63]. VEGF signals are transduced through the human tyrosine kinase domain receptor (KDR) and the Fms-like tyrosine receptor (Flt-1), which are overexpressed in tumors [64]. These receptors are composed of extracellular Ig-like domains, single membrane spanning regions, and an intracellular tyrosine kinase domain. The two proteins dimerize and signal upon ligand binding. Several peptides have been found to bind to VEGFR and to induce anti-tumor effect *in vitro* and *in vivo*, such as HTMYYHHYQHHL reported by Hetian *et al.* [6]. In addition to Hetian's peptide, Binetruy-Tournaire *et al.* found a peptide – ATWLPPR, which abolished VEGF-induced tumor angiogenesis [65]. Their ATWLPPR motif acts as an agonist to the KDR region of the VEGF receptor.

Since most tumorigenic effects are mediated by VEGF through KDR-mediated endothelial cell development, it is the most appropriate target for interrupting angiogenesis in tumors [66]. Bae *et al.* discovered an arginine rich sequence that homed to VEGF receptors in the colon [14]. The RRKRRR motif inhibited both tumor growth and metastases. A novel technique used by Veenendaal *et al.* targets the bacterial toxin gelonin (rGel) to tumors by fusing it to $VEGF_{121}$ [67]. Gelonin is a single chain *N*-glycosidase plant toxin, with similar actions to ricin A chain [68]. VEGF brings the toxin to the tumor where it can activate KDR and destroy tumor vasculature. Although not a peptide targeted treatment, it is ingenious in its exploitation of the tumor microenvironment.

One problem is the stability of the peptides, as mentioned in the introduction. El-Mousawi *et al.* attempted to overcome the lack of binding *in vivo* between peptide and cognate receptor by stabilizing the peptide with conjugated proteins like β-galactosidase or peroxidase [69]. Their results showed that the conjugates bound to Flt-1 act with considerably higher potency when attached to the 16-mer peptide **N**GY**EIE**W**Y**S**W**VTHGM**Y** (**bold** letters denote those amino acids involved in binding). The idea of increasing stability by chaperoning peptides with proteins might serve as a possible therapy in the future.

CGNKRTRGC: LYMPHATIC VESSEL TARGETING

Just as blood vessels in tumors express unique proteins and antigens, lymphatic vessels in tumors have their own specific expression patterns. Laakkonen *et al* discovered that a nine amino acid peptide named Lyp-1 accumulated only in lymphatic vessels of certain tumors and not in normal lymphatic tissue [70]. Since many cancers spread through the lymphatics, this is a vital area of metastatic studies. Lyp-1 has a proapoptotic and cytotoxic effect on tumor cells, and when administered systemically it inhibits breast cancer xenografts in mice. Given that lymphatic vessels near the center of tumors are usually obstructed by tumor cells, those areas are usually hypoxic. Lyp-1 takes advantage of this by binding to tumor cells near the hypoxic areas of the lymphatic vessels. This mechanism predicts how Lyp-1 can inhibit metastases by preventing those cells from escaping. In a separate article, Laakkonen characterizes the peptide known as Lyp-1 [12]. The sequence, CGNKRTRGC, causes internalization of the molecule and transportation to the nucleus. Lyp-1 is effective in several cancers including breast, osteosarcoma, and prostate. Noticeably, this peptide also contains the twin RG residues.

FUTURE DIRECTIONS

The future of peptide targeting is not only restricted to tumor eradication. Some researchers are focusing on using peptides to deliver imaging molecules to the site of tumors in order to enhance diagnostic capabilities. One very interesting application uses contrast-enhanced ultrasound microbubbles targeted to the endothelial inflammatory marker, ICAM-I. This allows for non-invasive *in vivo* imaging through ultrasound to activate microbubbles and locate tumor neovascularization [71]. Another imaging technique was recently published by Thumshim *et al*. which proposed conjugating *para*-trimethylstannylbenzaldehyde to *cyclo*(RGDfE-) peptides [72]. *Para*-trimethylstannylbenzaldehyde is a precursor for radioiododestannylation, which might be useful in imaging tumors that express αvβ3 integrins. The polyvalency of this molecule allows for enhanced integrin targeting and versatile ligation. Yet another approach utilizes radiolabelled RGD peptides to bind to αvβ3 integrin targets in tumor tissues [73]. Systemic delivery followed by imaging allows for site-specific accumulation and diagnosis of cancer. The potential imaging function of these peptides will be valuable for viral vector therapy in which the peptide is integrated in the genome of these vectors in such that the peptide is expressed on the surface of viral particle. The presence of these peptides on the surface of viral particles will make the image possible, which will be a step forward for tracking the safety and distribution of viral vectors.

The tumor targeting revolution has also spurred other avenues of research. Besides peptides, bacteriophages are used to transport anti-tumor agents to the site of malignancy. Recently, studies have indicated that the bacteriophage from peptide screening libraries may have more uses than originally thought. Chen *et al.* developed a bifunctional bacteriophage that not only targets tumors but also delivers an anti-tumor agent [74]. They modified the screening process to genetically transform the phage to a bioconjugate that is able to use RGD motifs to deliver anticancer agents to tumors. In a recent article in *Nature Immunology* it was shown that peptide fragments with a chaperone protein, not the intact proteins themselves, are necessary and sufficient source of antigen presentation to CD8+ T cells [75]. This finding illustrates the possibility to control immune response by using peptide targeted tumor antigens.

Finally, a method that was once believed to be the answer for delivering genes to target tissues but has performed poorly in most trials is viral vector therapy. Viral vectors have not achieved the benefits they were thought to be capable of producing. However, as peptide targeting emerges, researchers are merging the two fields to create highly specific tumor targeted viral vectors. These vectors would be of great benefit because they could transport large molecules such as DNA or functional proteins to specific areas in the body. Using a NSSRDLG sequence, Mueller *et al*. targeted adeno-associated virus particles to coronary endothelial cells in the heart [76]. This peptide-targeted viral vector approach shows potential not only for cancer therapies but also for curing cardiovascular diseases.

Within the last decade, major progress has been made in methods of developing peptide screening, creating peptides, imaging and analyzing the localization of peptides, targeting peptides to tumors, eradicating those tumors through peptide-mediated anti-tumor agent delivery, and novel, effective targeted therapies. The future holds infinite potential and outstanding benefit for peptide targeted treatment, especially with respect to the bioinformatics, molecular mapping, and nanotechnology prospects [77]. Nanoscience is investing heavily in nanoparticle formation and design to act as nanocarriers that deliver therapeutics to targets such as mononuclear phagocytes, dendritic cells, endothelial cells, and cancers [78]. Hopefully, these peptides and related methods hold the key to safe, successful, and less toxic tumor treatment and prevention.

Table 1. Tissue-Targeted Peptides

Peptide	Target	Authors
	Organs/Tissues	
LMLPRAD	Adrenal gland	Rajotte, D. 1998 [19]
CKGGRAKDC	Adipose Tissue	Kolonin, M. 2004 [79]
SRl	Brain	Brown, C. 2000 [11]
CLSSRLDAC	Brain endothelium	Pasqualini, R. 1996 [3]
GGG	Bone Marrow (BMP-3B)	Arap, W. 2002 [18]
GFS	Bone Marrow	Arap, W. 2002 [18]

(Table 1). contd.....

Peptide	Target	Authors
LWS	Bone Marrow	Arap, W. 2002 [18]
LTVxPWx	Breast cancer cell lines	Shadidi, M. 2003 [7]
LTVxPWY	Breast cancer *erbB2* receptor	Mendoza, F. 2005 [79]
RPMC	Colon	Kelly, K. 2004 [17]
NSVRDL(G/S)	Coronary artery endothelia	Muller, O. 2003 [80]
NSVSSx(S/A)	Coronary artery endothelia	Muller, O. 2003 [80]
VGLPEHTQ	Glioma cells	Samoylova, T. 2003 [81]
TSPLNIHNGQKL	Head and Neck Squamous Cell Cancer	Hong, F. 2000 [23]
YSGKWGW	Intestine	Rajotte, D. 1998 [19]
CGFELETC	Lung vasculature	Rajotte, D. 1999 [82]
CGNKRTRGC (Lyp-1)	Lymphatic Vessels	Laakkonen, P. 2002 [12]
GVL	Multiple Organs	Arap, W. 2002 [18]
EGRx	Multiple Organs (MMP-9)	Arap, W. 2002 [18]
xFG(G/V)	Multiple Organs (CDO)	Arap, W. 2002 [18]
CVSSNPRWKC	Pancreatic Islets	Yao, V. 2005 [20]
CHVLWSTRC	Pancreatic Islets	Yao, V. 2005 [20]
SWCEPGWCR	Pancreas	Rajotte, D. 1998 [19]
AGG	Prostate (IL-11 or Smad6)	Arap, W. 2002 [18]
DPRATPGS	Prostate	Romanov, V.I. 2001 [83]
SMSIARL	Prostate	Arap, W. 2002 [80]
CGRRAGGSC	Prostate IL-11R	Zurita, A. 2004 [84]
GVL	Prostate and Bone Marrow	Arap, W. 2002 [18]
VPWMEPAYQRFL	Neuroblastoma	Zhang, J. 2001 [85]
RDV	Retina	Brown, C. 2000 [11]
CSCFRDVCC	Retina	Rajotte, D. 1998 [19]
TPKTSVT	Teratogen ligand	Kolonin, M. 2002 [86]
LLGPYELWELSH	Trastuzumab mimotope (HER-2 Ab)	Jiang, B. 2005 [87]
GLSGGRS	Uterus	Rajotte, D. 1998 [19]
	Integrins (RGD)	
CRRETAWAC	αvβ1 integrin	Koivunen, E. 1994 [88]
CDCRGDCFC (RGD-4C)	αvβ3 integrin	Arap, W. 1998 [4]

(Table 1). contd.....

Peptide	Target	Authors
RGDWXE	αvβ3 integrin	Richards, J. 2003 [43]
TRGDTF	αvβ5 integrin	Li, R. 2003 [39]
RGDLxxL or xxDLxxL	αvβ6	Kraft, S. 1999 [40]
SRGDM	αIIβ3	Li, R. 2003 [39]
VVISYSMPD	annexin V mimic for αvβ5	Cardo-Vila, M. 2003 [89]
	Endothelium	
IELLQAR	E-selectin	Fukuda, M. 2000 [2]
CNGRC-GG-(KLAKLAK)2	Endothelial cell mitochondria	Ellerby, H.M. 1999 [26]
CVSNPRWKC	Ephrin-A2 and Ephrin-A4	Yao, V. 2005 [20]
CHVLWSTRC	Ephrin-A2 and Ephrin-A4	Yao, V. 2005 [20]
CWDDGWLC	Fibronectin	Pasqualini, R. 1995 [90]
CPCFLLGCC (LLG-4C)	ICAM-I or von Willebrand factor	Koivunen, E. 2001 [91]
DFKLFAVY	lamin-1	Lourdes Ponce, M. 2003 [92]
EWVDV	P-selectin	Molenaar, T. 2002 [93]
(D/E)(D/E)(G/L)W	MMP-9:integrin complex	Stefanidakis, M. 2003 [94]
CTTHWGFTLC	MMP-9 and MMP-2 (gelatinases)	Koivunen, E. 1999 [62]
N-Ac-CHAVC-*NH2*	Type I cadherin on endothelium	Mendoza, F. 2005 [95]
	VEGF Receptor	
NxxEIExYxxWxxxxxY	Flt-1 region of VEGF	El-Mousawi, M. 2003 [69]
HTMYYHHYQHHL	KDR region of VEGF	Hetian, L. 2002 [6]
ATWLPPR	KDR region of VEGF	Binetruy-Tournaire, R. 2000 [65]
WHSDMEWWYLLG	VEGF receptor	Mendoza, F. 2005 [95]
RRKRRR	VEGF receptor	Bae, D.G. 2000 [14]
	CD13:NGR	
NGR	Aminopeptidase N/CD13	Pasqualini, R. 2000 [13]
TAASGVRSMH	NG2 proteolgycan	Burg, M. 1999 [16]
LTLRWVGLMS	NG2 proteolgycan	Burg, M. 1999 [16]
	Apoptosis	
CEFESC	Apoptosis inhibitor (XIAP) in organs	Tamm, I. 2003 [96]
KKLSECLKRIGDELDS	BH3 domain of Bax	Mendoza, F. 2005 [95]
AVPIAQK	Procaspase-3	Mendoza, F. 2005 [95]

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