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Anti-angiogenic peptides for cancer therapeutics

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Abstract

Peptides have emerged as important therapeutics that are being rigorously tested in angiogenesis-dependent diseases due to their low toxicity and high specificity. Since the discovery of endogenous proteins and protein fragments that inhibit microvessel formation (thrombospondin, endostatin) several peptides have shown promise in pre-clinical and clinical studies for cancer. Peptides have been derived from thrombospondin, collagens, chemokines, coagulation cascade proteins, growth factors, and other classes of proteins and target different receptors. Here we survey recent developments for anti-angiogenic peptides with length not exceeding 50 amino acid residues that have shown activity in pre-clinical models of cancer or have been tested in clinical trials; some of the peptides have been modified and optimized, e.g., through L-to-D and non-natural amino acid substitutions. We highlight technological advances in peptide discovery and optimization including computational and bioinformatics tools and novel experimental techniques.

Keywords

Angiogenesis; animal model; computational biology; inhibitor; in vitro model; peptidomimetics; tumor vasculature; tumor

INTRODUCTION

Angiogenesis process

Angiogenesis, or neovascularization, is the formation of new microvessels from an established vascular network, and was first proposed in 1971 by Judah Folkman as a therapeutic target for cancer [1]. He postulated that tumors cannot grow past a size of 1 mm [2] without developing their own blood supply. Later one of the key factors responsible for angiogenesis, the Vascular Endothelial Growth Factor (VEGF) was identified [3, 4]. Since this and subsequent discoveries of the molecular determinants of vascular growth, angiogenesis research has come center stage with an estimated 500 million people predicted to benefit from pro- and anti-angiogenic therapies in the next decades [5]. The process of angiogenesis is governed by a delicate balance between multiple endogenous pro- and anti-angiogenic factors. An imbalance in these factors leads to the development or progression of pathological conditions. The angiogenic process involves interactions among multiple cell types including: endothelial cells (EC) and circulating endothelial progenitor cells, pericytes, vascular smooth muscle cells, stromal cells, including stem cells, and parenchymal cells. These interactions occur through secreted factors such as VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and angiopoietins, as well as through cell-

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extracellular matrix (ECM) interactions [5–7]. The interaction between EC and the extracellular matrix is critical and influences cellular processes such as EC migration, proliferation, differentiation and apoptosis which are essential to the angiogenesis process. The interactions among these molecular factors and cells have been essential to research into understanding of this process. The focus of this review is on anti-angiogenic factors with application in cancer therapeutics and more specifically on peptides with length not exceeding 50 amino acid residues derived from naturally occurring proteins and peptides.

Anti-angiogenic agents in cancer

Angiogenesis is crucial to the progression of cancer and particularly in metastasis [8]. There is abundant evidence of angiogenesis being involved in cancer in pre-clinical and clinical settings. For example, increased intratumoral microvascular density and VEGF expression correlate with shorter relapse-free intervals and overall survival [9–11]. Important differences exist in pathological versus physiological angiogenesis. In pathological angiogenesis a large excess of pro-angiogenic factors over anti-angiogenic factors leads to abnormal vasculature in tumors and poor perfusion contributing to limited efficacy of anti-cancer agents [12]. Anti-VEGF therapies showed promise in clinical trials and agents like an anti-VEGF monoclonal antibody, bevacizumab, and small molecule VEGFR tyrosine kinase inhibitors (TKIs) sorafenib (Nexavar, Bayer/Onyx, Wayne, NJ) and sunitinib (Sutent, Pfizer, New York, NY) have been FDA-approved for treatment of several cancers in combination with chemotherapeutic agents or, in some cases, as monotherapy. Yet, the increase in overall survival of patients with these drugs has been modest [13, 14]. It has been shown that multiple anti-angiogenic agents may be more efficient in sustained inhibition of tumor angiogenesis [15]. One approach to implement this multimodal approach would be to utilize multiple short peptides each of which targets a different signaling pathway and inhibits angiogenesis by a different mechanism.

Anti-angiogenic peptides as therapeutics for cancer

An editorial devoted to angiogenesis inhibitors suggested that “Angiogenesis inhibitors, particularly polypeptides or endogenous peptides, may become the safest and least toxic therapy for diseases associated with abnormal angiogenesis” [16]. The number of peptides as approved drugs and drug candidates has increased significantly in recent years. The reasons are that generally peptides have low toxicity and high specificity for their receptor targets and their success rate in clinical trials is 2 to 3 times higher compared to small molecules [17, 18]. Also, the technology for solid-phase synthesis of therapeutic peptides has progressed significantly in the last five years. As a result, currently there are over 300 therapeutic peptides under development for different disease applications, with over 20 FDA-approved peptide drugs in the U.S. [17, 18]. However, none of these approved peptide drugs are anti-angiogenic. Peptides have attracted attention because in addition to low toxicity and high specificity they possess certain desirable characteristics such as good penetration of tissues due to their small size, the ability to be easily “functionalized” to improve their properties (e.g. pegylation, encapsulation in liposomes and nanoparticles) or to produce combination therapies (e.g. conjugation with chemotherapeutic or radio-labeled molecules) [19]. Most of the endogenous anti-angiogenic proteins are too large and complex so they have difficulty penetrating tissues and are also too costly to be produced in large quantities necessary for mainstream therapies. As a result, significant effort is being placed into developing small peptide fragments that possess similar anti-angiogenic properties but overcome the issues of their parent proteins.

Peptides with demonstrated ability to suppress tumor angiogenesis in pre-clinical models and those that have made it through the pre-clinical stages and are currently in clinical stages will be discussed in this review. Many challenges remain, specifically how to design and

optimize peptides as anti-angiogenic agents for cancer applications. Several excellent reviews summarize findings of peptide pharmaceutical advancement in various diseases [20], and specifically in angiogenesis-dependent diseases not limited to cancer [21].

We present a comprehensive overview of the anti-angiogenic peptides in application to cancer currently in pre-clinical or clinical studies and focus on their development as they emerge as pharmaceutical agents targeting angiogenic receptors. The term peptide has been used loosely in the literature to describe polymeric structures with amino acids linked by amide bonds; for the purpose of this review we will focus on structures that are less than 50 amino acids. This length of peptide chain is the current upper limit of sequence that can be produced successfully via solid-phase synthesis which is the method of choice for producing large quantities of product necessary for clinical applications. We discuss various technological advances in peptide discovery and optimization including computational and bioinformatics tools and novel experimental techniques. We classify the peptides based on the classes of proteins from which they were derived as shown in Table 1.

CLASSIFICATION OF ANTI-ANGIOGENIC PEPTIDES WITH KNOWN ACTIVITY IN TUMORS

Peptides derived from extracellular matrix proteins

The extracellular matrix (ECM) is a network of fibrous proteins that surrounds cells and provides a conduit for cellular communication. Also, the ECM serves as a signaling hub for cells, via direct interaction with cells or through different soluble factors trapped in the network. Hence, protein components of this network have been investigated for potential smaller peptides that can elicit or interfere with specific cellular responses, such as cellular migration, proliferation or survival.

A well-studied integrin-binding motif, the tri-amino acid peptide RGD, is derived from fibronectin and is capable of controlling cellular behavior, particularly adhesion and migration. Integrins are heterodimeric membrane receptors composed of non-covalently associated subunits α and β , and demonstrating cationic-dependent interaction with multiple ECM proteins. The integrin family is an extensive group of cellular receptors, generated by the association of one of the 18 α subunits with one of the 8 β subunits generating 24 unique heterodimers, involved in attachment and migration of cells to the surrounding ECM. Some integrins such as $\alpha_5\beta_1$ can recognize single ligands while others such as $\alpha_v\beta_3$ can bind multiple ligands. Binding of a ligand to the extracellular domain leads to integrin clustering and subsequent intracellular signal transduction activation. Unlike growth factors, integrin signaling is not based on intrinsic enzymatic activation; rather, it is dependent on co-clustering with kinases and adaptor proteins within focal adhesion complexes. *In vitro* and *in vivo* experiments have identified a number of endothelial cell integrins involved in cell growth, survival and migration during angiogenesis. These integrins include fibronectin-binding ($\alpha_5\beta_1$, $\alpha_4\beta_1$, $\alpha_6\beta_4$, $\alpha_9\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$) and laminin-binding ($\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$) [22]. $\alpha_v\beta_3$ was the first integrin shown to regulate angiogenesis; it is present on tumor blood vessels but not on normal tissues and antagonists have been shown to inhibit angiogenesis and tumor growth in a variety of animal models of cancer, probably by inducing endothelial cells apoptosis and balancing opposing signals in the tumor microenvironment [22].

Cyclization and modifications of the RGD peptide fragment led to the development of an optimized sequence (c-[Arg-Gly-Asp-DPhe-(NMeVal)]), cilengitide, which demonstrates increased potency and specificity towards endothelial cells making it a therapeutic drug candidate. Data on cilengitide and other peptides derived from ECM are presented in Table 2, where *in vivo* doses are included. In pre-clinical studies it reduces EC proliferation and migration [23]. The targets for cilengitide are the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ and since these

integrins are also present on some tumor cells, this anti-angiogenic treatment also showed a direct anti-tumorigenic effect specifically in glioma cells [23]. Besides identifying the target, understanding the mode of action (MOA) of the peptide becomes a very important component in developing successful treatment plans based on the activation or inhibition of particular pathways [23, 24]. Cilengitide has also been tested *in vivo* in several phase II trials of different cancers including prostate cancer [25], chemotherapy refractory renal, colorectal, melanoma cancers [26], and glioblastoma [27]. The treatment was well tolerated and showed a 6-month progression-free survival (PFS) of 15% of the patients and median overall survival (OS) of 9.9 months in the glioblastoma study. Although microvascular density (MVD) was intended as a biomarker it was eliminated due to high tumor variability. In other cancer models, such as prostate cancer, the treatment was well tolerated but the treatment arms did not demonstrate a significant improvement [25]. This partial failure could be due to the redundancy in signaling pathways present in tumors and high tumor-to-tumor variability influenced by different tumor microenvironments.

RGD modifications have also been undertaken to create targeting molecules. For example, coupling the RGD molecule to a heparin derivative which has been shown to target dividing tumor endothelial cells resulted in an increase in potency in comparison to the unmodified cyclic-RGD. This new construct showed potent inhibition of proliferation, migration, tube formation of EC and of tumor growth in a mouse colon cancer model, accompanied by a reduction in MVD [28].

ATN-161, a peptide derived from the synergy region of fibronectin that binds $\alpha_v\beta_3$ and $\alpha_v\beta_1$, has been demonstrated to inhibit growth and metastasis in a breast cancer model accompanied by a decrease in MVD (43%) along with inhibition of tumor cell growth via an integrin mediated signaling [29]. Furthermore, ATN-161 was evaluated *in vivo* in several therapy-refractive cancer models. The treatment was well tolerated, and 23% of the patients demonstrated stable disease for >4 months [30]. The effect of ATN-161 was also investigated in combination with standard chemotherapy treatment in a murine model of liver metastasis. The treatment showed a decrease in tumor burden; a decrease in the number of metastatic lesions and in microvascular density [31]. A labeled analog of ATN-161, ATN-453, was shown to bind selectively to the neovasculature and not to the pre-existing vasculature in a syngeneic mouse model of adenocarcinoma [32]. Several clinical trials are investigating the potency of this agent as a monotherapy or in a combination therapy in advanced renal cell carcinoma and recurrent glioma.

In contrast with the RGD-inspired peptides, which were developed by starting with a known peptide sequence by adding structural modifications, such as cyclization and non-natural amino acids, there are peptides that were derived by truncations of longer ECM proteins. Tumstatin, a 28 kDa fragment from the noncollagenous domain of type IV collagen, has been demonstrated to exhibit anti-angiogenic properties [33]. Further study of this fragment led to the identification of shorter anti-angiogenic fragments [34, 35]. Structure activity studies resulted in the identification of amino acids responsible for the anti-angiogenic characteristics of the short tumstatin peptide. This novel 22 amino acid peptide demonstrated potency in inhibiting proliferation and migration of EC along with strong inhibition of tumor growth in animal models (Lewis lung carcinoma, SCC-PSA1 teratocarcinoma, and human renal clear cell carcinoma) [36].

Thevenard *et al.* demonstrated that a shorter fragment of the tumstatin sequence, YSNS, forms a β -sheet which is important for biological activity and that a cyclized version of YNSG formed a very stable and active structure [37, 38]. This compound showed activity in inhibiting adhesion and migration of endothelial and melanoma cells. Also this cyclized peptide reduced tumor size, growth and tumor microvasculature in a melanoma model.

Karagiannis and Popel created a bioinformatics-based methodology to identify novel putative anti-angiogenic fragments based on homology to known anti-angiogenic sequences. This effort was based on the identification of common motifs present in known anti-angiogenic peptides which were then used to identify other endogenous proteins that contained the motif. Thus, multiple peptide fragments were identified, in particular 10 peptides belonging to the class of collagen IV. One candidate which was derived from the $\alpha 5$ fibrils of type IV collagen and is termed pentastatin-1 showed significant activity inhibiting proliferation and migration of EC cells [39]. This peptide has been tested in mouse xenograft models of breast cancer [40] and small cell lung cancer [41] and showed significant inhibition of tumor growth and MVD.

Endostatin, which was first isolated in 1997, is a 20 kDa protein fragment of the extracellular matrix protein collagen XVIII that successfully inhibited Lewis lung cancer metastases [42]. As an early-identified endogenous inhibitor of angiogenesis with strong potential, endostatin was rapidly moved to clinical trials; however poor solubility and difficulty purifying the molecule through recombinant expression made it inappropriate for the clinic [43]. Endostar, a modified form of the molecule, containing a MGGSHHHHH tag added to the N-terminal, was approved in 2005 in China for non-small cell lung cancer (NSCLC) [44]. Endostar has been tested in tumor xenografts of human nasopharyngeal carcinoma and human lung adenocarcinoma each in combination with radiation, where it significantly increased endothelial cell apoptosis and limited tumor growth [45]. Endostar is a large polypeptide, and must be produced recombinantly in E-coli for quantities sufficient for clinical trials.

Shorter fragments of endostatin have also been identified and tested in pre-clinical cancer models. A 27-amino acid zinc binding fragment of endostatin from the N-terminal called mP1 was isolated and shown to retain the anti-tumor, anti-migratory, and anti-permeability activities of endostatin. This peptide fragment was tested in BxPC-3 and Lewis lung carcinoma models where it demonstrated significant tumor suppression [46]. Morbidelli *et al.* segmented endostatin into five fragments named I(1–39), II(40–89), III(90–134), IV(135–184) and IVox(135–184), which contains a disulfide bond between C135–C165. Fragment IV and IVox exhibited anti-angiogenic activity similar to native endostatin, and inhibited tumor growth in an A4-431 xenograft model [47]. Similarly, Cattaneo *et al.* created fragments of endostatin corresponding to residues I(6–49), II(50–92), III(93–133), IV(134–178). They found fragment I to be a potent inhibitor of angiogenesis *in vitro* and *in vivo* in Matrigel plug assays [48]. Olsson *et al.* demonstrated the minimal active epitope of endostatin to be a heparin-binding motif of 20 amino acids (180–199, FLSSRLQDLYSIVRRADRAA), which was effective in human tumor xenografts [49]. Using crystal structure analysis, Wickstrom *et al.* created five endostatin-derived sequences of 11–13 amino acids, and found a minimal epitope (IVRRADRAAVP) that inhibited endothelial cell migration and tube formation. However, its activity *in vivo* is unknown [50].

Other ECM-derived peptides include laminin-derived peptides, many of which are pro-angiogenic. Two peptides of interest are A13 (RQVFQVAYIIKA) and C16 (KAFDITYVRLKF), derived from the alpha 1 and gamma 1 domains of laminin. For unknown reasons a scrambled sequence of C16, termed C16S peptide (DFKLFAVTIKYR) inhibited angiogenesis and a further mutation from T to Y termed C16Y (DFKLFAVYIKYR) had stronger effects than C16S in cell adhesion assays and inhibited tumor growth in breast xenograft model [51].

Peptides derived from growth factors and growth factor receptors

Vascular endothelial growth factor (VEGF) is one of the most important modulators of angiogenesis. Inhibiting its interaction with the receptor via antagonistic peptides could

present an effective anti-angiogenic therapy. Studying the binding interaction of VEGF₈₋₁₀₉ with VEGF receptor-1 (VEGFR1), one of the VEGF-A binding partners, resulted in the identification of critical residues responsible for binding. Coupling this information with Alanine-scan analysis aided the development of several potent drug candidates. Data on peptides derived from VEGF and other growth factors are presented in Table 3. Recently, the short sequence CPQPRPLC was identified through a subtractive-phage display screening approach as a sequence targeting VEGFR1 and neuropilin-1. The shorter epitope RPL was identified as the minimal sequence required for activity [53]. This sequence was retro-inverted Δ (LPR), and displayed activity in a syngeneic mouse mammary cancer model [54]. This may be the only known compound that targets both VEGFR1 and neuropilin-1.

A peptide derived from the 6a-exon domain of the VEGF gene demonstrated activity in binding to HUVEC surface by competing binding of the VEGF₁₆₅ to cell surface. The 20 amino acid peptide inhibited cell migration and tumor growth in lung cancer model by reducing microvascular density [55].

Fibroblast growth factor (FGF), another modulator of angiogenesis, is involved in angiogenesis and anti-angiogenic therapies. Contrary to initial expectations that anti-angiogenic therapies would not be susceptible to drug resistance, resistance to anti-VEGF treatment is observed, notably due to upregulation of FGF2. Therefore, antagonists, such as peptides derived from long-pentraxin-3 (PTX3) which bind FGF2 and prevent it from binding to its receptor are being pursued as anti-angiogenic agents. Several peptides derived from PTX3 have been investigated and found to inhibit the proliferation and adhesion of EC. They also inhibit angiogenesis in the chick embryo chorioallantoic membrane assay (CAM) assay and tumor xenograft growth in zebrafish [56, 57].

Transforming growth factor β (TGF β) is a pleiotropic agent and its effects are context driven. In early phases of tumor development TGF β exhibits anti-angiogenic activity, in later stages it appears to mainly have pro-angiogenic activity. Several peptides have been studied, however a 14 amino acid peptide (P144) derived from TGF β type III receptor displaying a high affinity for soluble TGF β has been shown to reduce skin fibrosis and colonization of the bone by lung cancer cells [58]. Serratti *et al.* has investigated its anti-angiogenic potential and demonstrated it to be capable of inhibiting tube formation of EC *in vitro* and *in vivo* in the Matrigel sponge assay. P144 appears to exert its anti-angiogenic effect by inhibiting the signaling and downregulation of the pro-angiogenic response initiated by TGF β [59].

Peptides derived from coagulation cascade proteins

The coagulation cascade along with angiogenesis and homeostasis are among the host responses associated with cancer [60]. Several proteins involved in the coagulation cascade have been shown to exhibit potent anti-angiogenic and anti-neoplastic activity; anti-angiogenic peptides derived from these proteins are presented in Table 4.

High molecular weight kininogen (HK) is a multifunctional protein that plays a role in multiple pathophysiological conditions such as thrombosis and inflammation. It also binds to EC where it can be cleaved by plasma kallikrein. The cleaved HK has been shown to inhibit migration and proliferation of EC [61]. Proteolytic cleavage of HK releases a vasoactive 9 amino acid (nonamer) peptide called bradykinin that appears to be involved in the coagulation cascade and have pro-angiogenic activity. B9870, a peptide antagonist of bradykinin has been investigated as a therapeutic agent in combination with chemotherapy in lung and prostate cancer. Treated tumors showed a reduction in microvasculature and increased number of apoptotic cells indicating this compound has application in cancer therapy by inhibition of angiogenesis [62].

Histidine-proline-rich glycoprotein (HPRG) is a potent anti-angiogenic protein evolutionarily related to high molecular-weight kininogen [63]. HRGP330 is a 35 amino acid sequence first identified by Dixelius *et al.* from the histidine/proline-rich domain, and is a potent inhibitor of angiogenesis for pancreatic carcinoma [64]. A synthetic peptide (HHPHG)₄ binds to tropomyosin and inhibits angiogenesis *in vitro* and *in vivo* in syngeneic mouse tumor models [65]. The peptide functions by targeting focal adhesions in endothelial cells and disrupting cytoskeletal organization [66].

Angiotensin-(1-7), Ang[1-7], is a biologically vasoactive peptide. Substitution of D-Ala at position seven created [D-Alanine7]-Ang-(1-7), A-779, a compound that inhibits EC tube formation and results in microvascular density reduction in treated lung xenografts [67]. In Phase I clinical trials, subcutaneous administration of 400µg/kg daily for 5 days in 3 cycles of treatment lead to stable disease for >3 months in most patients. Measuring the efficacy of treatment via a biomarker is very useful in clinical settings and in most cases has not been analyzed. The clinical trial discovered that plasma levels of placental growth factor (PlGF) can serve as a biomarker for treatment responsiveness [68].

Kringle are large protein domains stabilized by disulfide bonds important in the blood coagulation cascade. KV11 a 12-mer (dodecamer) derived from Kringle domain 5 has been shown to inhibit angiogenesis *in vitro* by inhibiting EC migration and microtubule formation. The peptide has been shown to inhibit capillary network formation *in vivo* and limit tumor growth in breast cancer xenografts by decreasing microvascular density through the c-SRC/ERK pathway [69].

Urokinase plasminogen activator system (uPA system) is comprised of 4 members, urokinase plasminogen activator, its receptor, and two plasminogen activator inhibitors. It has been demonstrated that the uPA system is involved in cancer progression, in particular in the remodeling of ECM by modulating cell adhesion. Clinical studies showed that either inhibition of uPA activity or its binding to the receptor reduced tumor growth, angiogenesis and metastasis [70]. Å6, an octamer capped peptide derived from the urokinase plasminogen activator, has been demonstrated to inhibit migration of EC while having no effect on proliferation. However, *in vivo* in combination with cisplatin, Å6 demonstrated significant tumor growth reduction in a subcutaneous glioma model [71], and in combination with tamoxifen demonstrated strong activity in orthotopic breast cancer xenografts [72].

Fibrinogen, a glycoprotein composed of the pairs of non-identical chains, gets cleaved by thrombin to form fibrin, the main component of blood clots, upon vascular injury. Various cleavage products of fibrinogen are involved in regulating cell adhesion and spreading. A 20 amino acid peptide derived from the β chain of the E-fragment of fibrinogen has been reported to inhibit the binding of EC to collagen IV via the $\alpha_v\beta_3$ integrin. This peptide does not inhibit proliferation or migration of EC. However, it significantly inhibits the adhesion of EC cells to collagen IV and disrupts vessels formation; in a breast cancer xenograft model the peptide showed activity in reducing tumor growth [73].

Peptides derived from chemokines

Platelet growth factor 4 (PF4) is a cytokine and part of the CXC chemokine family. When the ELR motif precedes the CXC pattern the chemokine is usually pro-angiogenic and if the motif is absent it is usually anti-angiogenic, the exception being the growth related protein β which contains the ELR motif but has been shown to possess anti-angiogenic properties *in vitro* and *in vivo* [75]. We present peptides derived from several chemokines in Table 5. Replacement of the DLQ amine terminal motif with the ELR motif and its consequent DLR mutation in the PF4⁴⁷⁻⁷⁰ fragment generated peptides with anti-angiogenic properties that abrogated VEGF or FGF2 induced proliferation, and inhibited capillary network formation

in CAM assays. Also, intratumoral delivery of these peptides via Alzet pumps in an intracranial glioma model strongly inhibited tumor growth [75]. Another variant of the same PF4 fragment was shown to inhibit melanoma xenografts at low doses (7 µg overall treatment) [76]. Sustained delivery via nanospheres delivered intratumorally in orthotopic glioma models showed nanoparticles homed around the tumor with considerable amount of peptide detected even after 14 days after a single intratumoral injection [77].

Using a bioinformatics methodology, a group of 7 anti-angiogenic peptides derived from ELR positive CXC chemokines has been identified. They inhibit migration and proliferation of EC [78]. One peptide from the group, CXCL1 derived chemokinstatin-1, was also tested in breast cancer xenografts where it exhibited tumor growth inhibition accompanied by a decrease in microvascular density [40].

An analysis of the 3D structure of PF4, an anti-angiogenic protein, revealed the importance of a β -sheet structure which appeared to be critical for activity. Based on this information, a 33 amino acid peptide, anginex, was developed to capture the critical conformation. This peptide showed significant inhibition of EC proliferation, migration, and capillary network formation in the CAM assay [79–81]. Treatment of ovarian tumor xenografts with anginex (10mg/kg/day) via a pump significantly inhibited tumor growth. The peptide was efficacious in both preventing tumors from forming and inhibiting the growth of pre-existing tumors and it also resulted in a decrease in intratumoral microvasculature [80, 82]. Anginex-conjugated liposomes have also been shown to be capable of targeting fluorescently labeled paramagnetic liposomes to activated endothelium [83]. Moreover, treatment with anginex proved to be efficient in delaying tumor growth post radiation therapy [84].

Peptides derived from Type I Thrombospondin domain containing proteins

Thrombospondin (TSP) was the first identified endogenous inhibitor of angiogenesis [86]; peptides derived from its family are presented in Table 6. The TSP family is composed of five different polypeptides named thrombospondin 1–5 with TSP1 and TSP2 being similar in structure [87]. The anti-angiogenic activity of TSP1 is localized to three type I repeats in the pro-collagen domain, and several peptides derived from these regions have been identified as angiogenesis inhibitors. The use of full-length TSP1 has been prohibitive due to its size and other multiple biological functions. Substitution of D-enantiomers of any of 3 L-amino acids of the non-active 19-mer peptide from the Mal II sequence conferred activity approaching that of the full length TSP-1 protein. The peptide containing D-Ile at position 15 could be shortened to a seven amino acid sequence without loss of activity [88]. A more solubilized form of this molecule, DI-TSPa, demonstrated tumor growth inhibition in a human bladder cancer model, and inhibition of melanoma metastases *in vivo* [89].

Further developed thrombospondin-derived molecules include ABT-526 and ABT-510. Based on a heptapeptide of the known anti-angiogenic thrombospondin-1 repeat, various amino acid substitutions were made to improve the PK/PD profile. ABT-526 and ABT-510 were selected from hundreds of variations, and both inhibit angiogenesis *in vitro* and *in vivo* [90]. Further toxicity data in dogs with naturally occurring cancers showed both ABT-526 and ABT-510 were well tolerated [91]. Clinically, ABT-510 has been tested in renal cell carcinoma [92], soft tissue sarcoma [93], and glioblastoma [94]. The drug was well tolerated but in phase II trials for renal carcinoma and soft tissue sarcoma the overall efficacy of the monotherapy was not significant.

Properdin is a plasma protein active in the alternative complement pathway of the innate immune system and contains a conserved TSP1 domain [95]. Properdistatin, a bioinformatically-identified peptide derived from the TSP1 domain of properdin, has been

demonstrated to exhibit potent anti-angiogenic properties *in vitro* [95, 96], and in breast cancer xenografts [40].

Peptides derived from serpins

Serpins are a group of proteins with similar structure able to inhibit proteases. Proteins from this family exhibit anti-tumor activity [97]; peptides derived from this family have been tested in cancer as anti-angiogenic agents as shown in Table 7.

Pigment epithelium-derived factor (PEDF) is a potent anti-angiogenic, non-inhibitory serpin protein first identified in 1991 as having potent anti-angiogenic attributes greater than those of endostatin [98]. Although a significant extent of PEDF research has focused on ocular applications, PEDF loss has been correlated with the growth of several types of tumors including prostate cancer, pancreatic cancer, osteosarcomas, breast, neuroblastomas, melanomas and gliomas [99]. Overexpression of the native PEDF molecule delays the growth and progression of solid tumors through p53 mediated apoptosis [100]. Its anti-angiogenic properties have been localized to an N-terminal fragment TGA (16–26) and an overlapping 34 amino acid peptide (24–57) that suppresses PC-3 cell prostate tumor growth and microvascular density. A separate 44 amino acid epitope (58–101) is responsible for neurotrophic activity, but it contains a region called the ERT segment (78–94) which is anti-angiogenic [101].

The 34-mer anti-angiogenic peptide contained in native PEDF was further truncated to identify the minimal active epitope for anti-angiogenic activity. A P18 fragment blocked angiogenesis *in vivo* in renal cell carcinoma and prostate cancer xenografts, even more effectively than the 34 amino acid epitope and native PEDF [102]. Ek *et al.* created four synthetic 25-mer PEDF peptides termed StVOrth-1, -2, -3, and -4 with StVOrth-2 (residues 78–102) and StVOrth-3 (residues 90–114) inhibiting osteosarcoma growth and pulmonary metastases [103]. Other serpin-derived proteins such as maspin are also potent inhibitors of angiogenesis; however, to our knowledge there are no peptides from this family tested in cancer models.

Peptides derived from other proteins

Troponin I is an intracellular protein responsible for binding actin in myofilaments. A 19 amino acid sequence derived from bovine and shark cartilage has demonstrated anti-angiogenic properties including limiting endothelial cell tube formation, and preventing liver metastases after injection of CAPAN-1 pancreatic cancer cells in mice [104]; data about this peptide along with other peptides that possess anti-angiogenic potential in cancer models are presented in Table 8. The HPRG derived peptide and the 19-mer derived from troponin I are unique as they both have intracellular targets while the other peptides discussed in this review are thought to work by binding to cell membrane receptors or extracellular pro-angiogenic factors.

Bombesin, a tetradecamer which stimulates gastrin release has also been shown to possess anti-neoplastic properties. Bajo *et al.* also demonstrated that a shorter fragment, RC-3940 II, a modified 11-mer has anti-angiogenic properties. Daily administration of 10µg significantly inhibited tumor growth accompanied by a decrease in tumor mRNA levels of FGF2, IFGF2, VEGF-A and a reduction in tumor vasculature [105, 106].

PAMP, a 20 amino acid peptide derived from the amine terminus of Proadrenomedullin exhibits very strong angiogenic activity (at concentration six orders of magnitudes lower than VEGF). Interestingly, a fragment of PAMP, the amino acids 12–20 generates a peptide that exhibits anti-angiogenic activity. Martinez *et al.* demonstrated activity in inhibition of

capillary network formation in CAM assay and microvasculature formation in DIVAA. Furthermore, the peptide demonstrated strong activity in inhibiting tumor growth [107].

IM-862 is a di-amino-acid peptide (EW) that has anti-angiogenic and immunostimulatory activity in renal cancer carcinoma. Phase II studies of intranasally delivered peptide showed no significant toxicity. The anti-angiogenic effects were measured by monitoring the plasma levels of VEGF and showed a significant decrease as the result of the treatment. However, the efficacy was modest with 7 patients showed no disease progression while 17 progressed on treatment.

A β , a dodecameric peptide derived from full length β -amyloid peptide was demonstrated to be effective in tumor suppression in breast xenografts of MCF-7 cells. The peptide showed no cytotoxic or antiproliferative effects on MCF-7 cells *in vitro*, however the reduction in proliferation *in vivo* is attributed to the anti-angiogenic effects of the peptide supported by a reduction in tumor microvasculature.

COMPUTATIONAL METHODOLOGIES FOR ANTI-ANGIOGENIC PEPTIDE DISCOVERY

Leveraging angiogenesis-associated protein domains

While there are many effective computational approaches for drug discovery in general, this section will focus on computational methodologies that have been used to produce anti-angiogenic peptides. A large fraction of the anti-angiogenic peptides discussed in this review were derived or designed from conserved domains of angiogenesis-associated proteins. This approach has proven successful in identifying peptides with anti-angiogenic activity. A recent study bioinformatically identified approximately 120 peptides and reported results of *in vitro* screening for 72 peptides derived from diverse angiogenesis-associated protein domains; the authors found that nearly 80% of the 72 peptides had significant anti-angiogenic activity [96]. The rationale is that evolutionarily conserved protein domains are often responsible for protein-protein interactions. One hypothesis is that peptide fragments from the conserved domain of whole protein will competitively inhibit the activity of the whole protein. Furthermore, the computational approach is easily applied to newly discovered angiogenesis-associated protein domains. For example, given a newly discovered angiogenesis-associated protein domain as a short amino acid sequence, one could identify evolutionarily-related sequences that exist in the proteome using the protein basic local alignment search tool (BLAST [110]). The peptides identified using this approach may be from the same domain or an evolutionarily-related domain. This sequence alignment approach is powerful in the sense that it can identify cryptic fragments in whole proteins that have anti-angiogenic activity when the whole protein does not have activity or may even be pro-angiogenic.

Many cryptic fragments exist throughout the human proteome with potential anti-angiogenic activity. Small leucine rich proteoglycans (SLRP) were discovered based on sequence conservation, were characterized by leucine-rich repeat (LRR) regions, and include a class of secreted proteoglycans, such as decorin and biglycan [111]. Decorin, a glycoprotein closely associated with collagen type I, plays a role in matrix assembly. A 26 amino acid leucine rich repeat, LRR5, derived from full decorin has been demonstrated to possess anti-angiogenic properties by abrogating VEGF and FGF2-induced angiogenesis. *In vitro*, LRR5 inhibits VEGF induced migration, tube formation, adhesion to fibronectin without significantly affecting proliferation. Further analysis of the domains revealed two fragments of LRR5M, which inhibits endothelial tube formation: LRR5N involved in the cell attachment to fibronectin and LRR5C that inhibits VEGF induced migration [112, 113].

These peptides have the potential to treat angiogenesis-dependent disease, however, to our knowledge have not been tested in cancer models.

Computational approaches for peptide structural analysis

Short peptide fragments may have simplistic secondary structures, while larger peptides form more complex arrangements of α -helices and β -sheets. Structural analysis of the peptide candidate can be useful to gain a better understanding of interaction and stability. In most cases a crystal structure or other high-resolution structure of the peptide candidate is unavailable. However, homology modeling can be used to identify a potential secondary or tertiary structure. Homology modeling is a technique where the structure of a peptide is determined by comparison to a high-resolution structure or structures with sequence homology. The strategy has also been used to construct peptides with structures similar to conserved domains with anti-angiogenic activity. For instance, the structure of the D5 domain of high-molecular-weight kininogen (HK) was identified using homology modeling to a known homologous protein hisactophilin [114]. Further structural analysis of kininogen revealed surface loops, and synthetic peptides containing these surface loop structures were subsequently found to have anti-angiogenic activity.

Griffioen *et al.* used a combination of structural information from basic folding principles along with the conserved domain of anti-angiogenic proteins PF4 to design an anti-angiogenic peptide, anginex [79]. This 33-mer peptide combines a conserved angiogenesis-associated domain with additional structural components (i.e. amphipathic β -sheet forming peptides) [79] to improve binding to its target receptor galectin-1. The design of anginex illustrates a method for combining the conserved anti-angiogenic domains of multiple proteins to create a multimodal anti-angiogenic peptide with unique properties.

Another design consideration in converting an angiogenesis-associated protein to a peptide candidate is the exposed surface of the protein. Structural analysis of the whole protein can reveal if the conserved interaction domain is exposed or cryptic. Through crystal structure analysis, Wickstrom *et al.* created five short synthetic peptides of 11–13 amino acids termed ES-1 to ES-5 from the exposed surface of endostatin [50]. The work resulted in an 11 amino acid peptide, ES-2 that inhibited migration and tube formation of EC [50].

In Silico peptide-target docking

In cases where the putative receptor or binding partner is known, computational screening via docking is a viable method of peptide discovery. A high-resolution structure (e.g. obtained from X-ray or NMR) of the target is often needed to produce high quality results from computational models. Due to the complexity of computational protein docking, a virtual screen of an entire combinatorial peptide library can be prohibitively expensive. However, using conserved domains of angiogenesis-associated proteins can drastically reduce the candidate search space and render the problem tractable. Computational docking is relatively new in the area of anti-angiogenic peptide discovery. Chandrasekaran *et al.* [115] used computational docking to identify anti-angiogenic peptide candidates. The extracellular domain of SPARC/osteonectin was found to bind VEGF by computational docking. Knowing that SPARC blocks VEGF induced phosphorylation of VEGFR1, the authors predicted and verified that SPARC contains structural homology with VEGFR1. The SPARC domain competes with the VEGFR1 domain for VEGF binding [115]. To carry out the docking simulation the authors used the FTDock and RPScore components of the 3D-Dock program suite from the Biomolecular Modeling Laboratory [116, 117]. Computational docking will produce a ranked list of docked conformations. To identify the correct docked structure, it is important to consider the area of surface contact, extent of interactions present, and stability of the model in addition to any domain-specific knowledge.

Computational docking can also be used for peptide side-chain analysis. Alanine scanning (Ala-scan) is commonly used in experimental screens to reveal amino acids crucial for target binding. Chandrasekaran *et al.* [115] identified the key amino acids in the SPARC peptide using a computational Alanine scan. The process involves replacing each amino acid in the peptide with an Alanine in turn and identifying the change in free energy using the virtual docking tools such as FTDock and RPScore. A computational Ala-scan may be significantly less expensive than an experimental Ala-scan. The reduced cost may enable exquisite experiments such as combinatorial Ala-scans for shorter peptides, or pairwise Ala-scans for longer peptides. As the predictive accuracy of computational Ala-scans improves, so will the ability to design peptides with optimized sequences and greater stability at a fraction of the experimental cost.

Molecular dynamics of the peptide-target complex

Molecular dynamics (MD), a computational simulation technique, provides an explicit model of how the molecules such as a peptide binding to its target interact over a given period of time. The information gained from MD comes at a high computational cost, where single molecules are documented, often requiring high-performance computing resources. As a result there are fewer reports of angiogenesis inhibiting peptides being optimized using MD. Chandrasekaran *et al.* [115] refined their SPARC peptide through the use of MD simulations. The SPARC-VEGF complex was first optimized using a conjugate gradient minimization method with the Kollman all-atom force field according to the method by Weiner *et al.* [118]. This initial procedure can be used to eliminate steric clashes and other high energy conformations. The optimized SPARC-VEGF was used as an initial conformation for MD simulation using Amber 7.0 software [119]. Molecular dynamics and energy minimization were used in combination for the optimization of kininostatin [114]. Colman *et al.* used alternating rounds of energy minimization and molecular dynamics with the help of the Biopolymer module from the Sybyl computational chemistry suite (Tripos and Assoc, St Louis, MO) to optimize the structure of the peptide-target interaction [114].

CHALLENGES AND POTENTIAL SOLUTIONS FOR THERAPEUTIC PEPTIDE DEVELOPMENT

Peptides have two distinct advantages over small molecules in terms of having potential superior specificity and affinity for biological targets. Peptides also have an advantage over antibodies in terms of size and relative ease of manufacturing. However, the majority of peptides suffer from short serum half-life resulting from fast elimination from systemic circulation because of renal clearance and enzymatic degradation and a lack of oral bioavailability, which has hampered their transition from discovery into viable clinical products. The advent of novel technologies over the past decades such as non-natural amino acids and peptidomimetics, pegylation, polymer supported formulation, and macromolecule conjugation however, has enabled a number of recent clinical entries and commercialization of peptide based therapeutics. Novel drug delivery technologies and recent advancement in cost effective solid-phase synthesis have also certainly contributed to a flurry of new peptide therapeutics. The overall development scheme for a peptide therapeutic with anti-angiogenic activity in tumors is illustrated in Figure 1.

Chemical synthesis of non-natural amino acids has seen great advances over the past decade. Today, varieties of non-natural amino acids are readily available from commercial sources for product development. Thrombospondin mimetic peptide (ABT-510) is an example of a successful integration of a single non-natural amino acid into a peptide scaffold. ABT-510 is a subcutaneously administered nonameric peptide thrombospondin analogue developed for treatment of advanced malignancies. ABT-510 benefits from a modest improvement in half-

life and decreased clearance parameters compared to the parent TSP1 endogenous fragment from which it was derived [90]. These improved pharmacokinetic parameters contributed to the relative ease of transition for this product from discovery to the clinic. However, the recent phase II study of ABT-510 for treatment of metastatic melanoma failed to reach its primary endpoint resulting in early termination of the study [120].

Recent work by a number of investigators in the area of peptidomimetics has also contributed greatly to the understanding and development of methods for improving serum half-life of peptides. Peptidomimetic principles in drug design are now routinely implemented in generation of drugs with improved pharmacokinetic profile. Continued research in peptide and protein structural chemistry, coupled with the development of novel scaffolds and templates that can predictably mimic the stereo-structural properties of peptides continue to provide the tools for successful *de novo* peptidomimetic design [121]. These developments have allowed many peptidomimetic therapeutic molecules based on the natural RGD tri-peptide scaffold with improved druggability profiles to be developed [122]. Examples include small cyclic peptides and peptidomimetics designed based on the RGD tri-peptide sequence developed to inhibit cell adhesion and cell-matrix interactions. Most notable is the cyclic peptide cilengitide, which has undergone multiple clinical trials as an angiogenesis inhibitor with excellent clinical safety and pharmacokinetic profile. Cilengitide monotherapy is well tolerated and exhibits modest antitumor activity among recurrent glioblastoma multiform patients [27]. Proteins smaller than 40kDa are subject to renal filtration and excretion resulting in their rapid systemic clearance. The anti-angiogenic peptides being discussed here would fall in this category. Rapid renal clearance can be overcome by conjugating the peptides with water-soluble polymers resulting in molecules larger than 40kDa [123]. Conjugation can also increase resistance against enzyme degradation and lower immunogenicity both of which can result in rapid elimination of the peptide from systemic circulation. Another advantage of conjugation to increase the molecular weight is that the conjugated peptide tends to accumulate preferentially in solid tumors via the enhanced permeability and retention (EPR) effect. The EPR effect occurs because tumor vessels tend to be much leakier than vessels from healthy tissue and they tend to be permeable to macromolecules with a MW of 50kDa or higher. The macromolecules enter the tumor interstitial space. Also the lymphatic vessels in tumors are thought to have decreased function in tumor [124] so that the macromolecules in the tumor interstitium tend to get trapped. Of the various polymers tested poly (ethylene glycol) (PEG) is the most commonly used polymer for modification.

Pegylation was originally described in early 1970s, which enabled biopharmaceutical company Enzon Inc (Bridgewater, N.J.), to advance many of the initial pegylated therapeutics to the market. Pegylation is commonly achieved by covalent attachment of Polyethylene glycol polymer chains to therapeutic peptides. Additional technologies have since been developed to perform site-specific pegylation of peptides. Most recently, Ambrx Inc. (San Diego, CA) has developed a novel long-acting human growth hormone product using site specific pegylation technology that is currently in phase II clinical trials.

Another strategy used to enhance circulation time of drugs is by conjugating with polymers that are not large enough to prevent renal clearance themselves, but which can attach together with their cargo to natural long-circulating blood plasma components such as serum albumin or lipoproteins. A good example of such a polymer is poly(styrene-co-maleic acid anhydride) (SMA) [124]. This polymer is only 1.5 kDa but it binds to plasma albumin which results in enhanced circulation times of anti-cancer proteins and peptides [125] and increases its antimetastatic effect on lung metastasis of B16-BL6 melanoma cells. As with pegylation, conjugation with SMA protects peptides from enzymatic degradation and decreases immunogenicity.

Recent years has also seen advancement of peptide conjugation to a suitable antibody or antibody fragment that has resulted in improved stability and higher specificity in delivery to the site of disease [126]. Peptides can be also be attached to each other by either chemical or recombinant means, however, best results are achieved by conjugation of peptides to large molecular weight molecules. The earliest examples of enzyme-antibody conjugates using chemical cross-linkers, such as maleimide, to couple thiol moieties between cysteine residues on each partner were published more than a decade ago [127]. However, initial nonspecific approaches resulted in multiple products, with one or both partners inactivated by the coupling reaction. Alternative methodologies have since been developed to induce specific conjugation of particular residues in the protein species using site specific cross-linking reagents [128]. Site specific conjugation technologies combining chemically modified peptides with monoclonal antibodies are under development by a number of biopharmaceutical companies that have advanced promising products in clinical trials. These companies include Seattle Genetics (Bothell, WA) and CovX (part of Pfizer, NY). Most recently, however, a convenient methodology became available that uses recombinant DNA techniques to fuse the antibody open-reading frame to that of the therapeutic peptide, giving rise to an antibody-peptide fusion after translation. This approach to conjugation is amenable to the manufacture of a uniform protein species for therapeutic application [129].

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Abbreviations

CAM	Chorioallantoic membrane
CRC	Colorectal cancer
DIVAA	Directed in vivo angiogenesis assay
EC	Endothelial cell
ECM	Extracellular matrix
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
HK	High molecular weight kininogen
HPRG	Histidine-proline-rich glycoprotein
LLC	Lewis lung carcinoma
MVD	Microvascular density
OS	Overall survival
PAMP	Proadrenomedullin peptide
PC	Prostate cancer
PDGF	Platelet-derived growth factor
PEDF	Pigment epithelium-derived factor
PEG	Poly (ethylene glycol)
PF	Platelet factor
PFS	Progression-free survival

RCC	Renal cell carcinoma
SCC	Squamous cell carcinoma
SCLC	Small Cell Lung Cancer
SLRP	Small leucine rich proteoglycan
SMA	Styrene-co-maleic acid anhydride
SPARC	Secreted protein acidic and rich in cysteine
TGF	Transforming growth factor
TKI	Tyrosine kinase inhibitor
TSP	Thrombospondin
uPA	Urokinase plasminogen activator
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

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Figure 1. Anti-angiogenic peptide discovery and progression paradigm

Shown are typical steps for development of anti-angiogenic peptides in cancer. The steps involved in the process are iterative and dependent on how the peptide was identified. Knowledge of target or receptor will guide peptide optimization through *in vitro* and *in vivo* experiments. The formulation step is used to increase peptide stability and availability *in vivo*.

Table 1
Peptides with anti-angiogenic activity in tumors classified according to the protein or endogenous peptide of origin

EXTRACELLULAR MATRIX (ECM)	GROWTH FACTORS & RECEPTORS	COAGULATION CASCADE	CXC CHEMOKINES	THROMBOSPONDIN DOMAIN CONTAINING PROTEINS	SERPIN	OTHERS
Collagens Laminin Fibronectin	VEGF VEGFR1 FGF TGFR β receptor	Bradykinin HPRG Angiotensin Kringle Urokinase plasminogen activator Fibrinogen	PF4 CXCL1	Thrombospondin Properdin	PEDF	Troponin Bombesin IM 862 PAMP β -amyloid

Peptides derived from extracellular matrix proteins

Table 2

Peptide Name	Sequence(# aa)	Modification	Origin	Target	In vivo status	Delivery method	Cancer model	Ref
Clengitide (EMD 121974)	c-[RGD-DF-NMEV] (5)	Cyclization Non-natural amino acid	RGD Fibronectin	$\alpha\beta 3$, $\alpha\beta 1$	Phase I	IV Infusion	Prostate, RCC, CRC, Glioma	[26, 52]
Targeting RGD	cRGD-HL (3)	Attachment to heparin, Cyclization	Collagen IV	$\alpha\beta 3$, $\alpha\beta 1$	Pre-clinical	IV 0.5mg/kg every 3 days	SCC	[28]
ATN-161	Ac-PHSCN-NH2 (5)	Acetylation	Fibronectin	β_1	Phase II	IV, 0.2–200µg. kg 3x/ week	LLC	[32]
Tumstatin Peptide	TLPFAYCNIHQV CHYAQRNDRSY WL (25)	Non-natural amino acid	Collagen IV	$\alpha\beta 3$	Pre-clinical	IP & IV (30µg/daily)	LLC, SCC-PCa1, RCC	[36]
Tumstatin fragment	YSNSG (5)	Cyclization	Collagen IV	β_1		IP (10 mg/kg) on days 6,8,14	Melanoma	[37]
Pentastatin-1	LRRFSTMPFMFCNINNVCNF (20)	None	Collagen IV	$\alpha\beta 3$, $\alpha\beta 1$	Pre-clinical	10 mg/kg IP daily	Breast	[40]
Endostatin peptide	HTHQDFQPVLLHLVALNTPLSGGMR GIR (28)	None	Collagen XVIII	Unknown	Pre-clinical	20 mg/kg daily	BxPC-3 tumors, LLC	[46]
Endostatin fragment IV, IVox	CETWRTETTGTATGQASSLLSGRLLLEQKAASCH NSYIVLCIENSFMTSFSK (50)	None	Collagen XVIII	Unknown	Pre-clinical	62.5 µg/kg/day	A-431	[47]
Endostatin peptide fragment I (180–199)	FLSSRLQDLYSIVRRADRAA (20)	None	Collagen XVIII	Unknown	Pre-clinical	25 mg/kg/day	T241 sarcoma	[49]
C16Y	DFKLFAVYIKYR (12)	T-Y substitution	Laminin	$\alpha\beta 3$, $\alpha 5\beta 1$	Pre-clinical	1.0 mg/kg daily	Breast	[51]
C16S	DFKLFAVTIKYR (12)	None	Laminin	$\alpha\beta 3$, $\alpha 5\beta 1$	Pre-clinical	1.0 mg/kg	Breast	[51]

Table 3

Peptides derived from Growth Factors or their receptors

Peptide Name	Sequence (# aa)	Modification	Origin	Target	In vivo status	Delivery method	Cancer model	Ref
VEGF derived peptide	D(LPR) (3)	Retro-inversion	VEGF-B	VEGFR1 NRP-1	Pre-clinical	50mg/kg Daily/5 days	Mouse mammary carcinoma	[54]
VEGFderived peptide	KSVRGKGKGQKRKRKKSRYK (20)	None	Exon 6a of VEGF gene	VEGFR2	Pre-clinical	Subcutaneous 20mg/kg/day twice/day	LLC	[55]
FGF-derived peptide	Ac-ARPCA (5)	Acetylation	PTX3		Pre-clinical		Zebrafish xenograft	[56, 57]
P144	TSLDASIIWAMMQN (14)	None	TGFβ Receptor	TGFβ	Pre-clinical	Dermal application	Skin fibrosis	[58, 59]

Table 4

Peptides derived from proteins involved in the coagulation cascade

Peptide Name	Sequence (# aa)	Modification	Origin	Target	In vivo status	Delivery method	Cancer model	Ref
B-9870	SUIM-DR-RP-Hyp-G-Igl-S-DIgl-Oic-R (10)	Non-natural amino acid, dimerization	Bradykinin	Unknown	Pre-clinical	IP (10mg/kg every 4 th day)	SCLC, PC	[62]
HPRG derived	(HHPHG) ₄ (20)	Multimerization	HPRG protein	Tropomyosin	Pre-clinical	IV (25–75 mg/kg)	Melanoma	[32, 63–66]
A-779	ARCTIH-DA (7)	Non-natural amino acids	Angiotensin	AT1, AT2	Pre-clinical Phase I	SC 1000µg/kg 5 days on 2 days off for 6 cycles	Lung xenografts	[68, 74]
KV11	YTMNPRKLFDY (11)	None	Kringle 5	cSrc ERK	Pre-clinical	Intra tumor 50 µg/kg daily	Breast	[69]
Á6	KPSPPE (7)	Capped	uPA		Pre-clinical	IP twice day/21 days	Glioma	[71, 72]
Fibrinogen derived	ARPAKAAATQK KVERKAPDA (20)	None	Fibrinogen	αβ3	Pre-clinical	IP daily 0.084mg/kg for 10 days	Breast	[73]

Table 5

Peptides derived from chemokines

Peptide Name	Sequence (#aa)	Modification	Origin	Target	In vivo status	Delivery method	Cancer model	Ref
PF4 derived	NGRKISL ~~DL~~ RAPLYKKIKKILLES (24)	None	PF4	VEGF FGF2	Pre-clinical	Alzet pump 0.25, 0.5 or 1 mg/ml	Glioma	[75–77]
Chemokinstatin-1	NGREACLDPEAPMVQKIVQKMLKG (24)	None	CXCL1	CXCR3	Pre-clinical	IP 20mg/kg daily	Breast	[40, 78]
Anginex	ANIKLSVQMKLFRHLKWKIIVKLNDRGRELSDA (33)	None	PF4	Galectin 1	Pre-clinical	10 mg/kg Subcutaneous pump	Ovarian, Melanoma	[79–82, 85]

Table 6

Peptides derived from TSP1 domain containing proteins

Peptide Name	Sequence (# aa)	Modification	Target	In vivo status	Delivery method	Application	Ref
DL-TSPa	GVTRIR (7)	D-Ile amino acid substitution	CD36	Pre-clinical	Subcutaneous 10, 50, 100 mg/kg	Human bladder cancer, Melanoma metastases	[89]
ABT-510	Ac-Sar-GV-DalloI-T-NorV-IRP-NHEt (9)	Non-natural amino acid, End modifications	CD36	Phase I, Phase II clinical trials	Subcutaneous 100 mg/kg	Renal, Glioblastoma Ovarian	[90]
ABT-526	Ac-Sar-GV-DL-T-NorV-IRP-NHEt (9)	Non-natural amino acid, End modifications	CD36	Pre-clinical		Murine melanoma	[91–94]
Properdistatin	GPWEPCSVTCSKGTRTRRR (19)	None	CD36	Pre-clinical	Subcutaneous 20 mg/kg	Breast	[40]

Table 7

Peptides derived from serpin proteins

Peptide Name	Sequence (# aa)	Modification	Origin	Target	In vivo status	Delivery method	Cancer model	Ref
PEDF-TGA fragment	TGALVEEEDPF (11)	None	PEDF	Unknown	Pre-clinical	IP 10mg/kg every 2 days	Prostate	[99]
PEDF 34-mer fragment	DPEFKVPVNKLAAVSNFGYDLYRVRSSMSPTTN (34)	None	PEDF	Unknown	Pre-clinical	IP 10mg/kg every 2 days	Prostate	[102]
PEDF P18	NFGYDLYRVRSSSTPTTN (18)	None	PEDF	Unknown	Pre-clinical	Subcutaneous (SC) 10, 50, 100 mg/kg	Prostate, Renal	[102]
SvOrth-2	NVLLSPLSVATALSALSLGAEQRTES (26)	None	PEDF	Unknown	Pre-clinical	Intra tumor 25 nM	Osteosarcoma	[103]

Table 8

Peptides of various origin with anti-angiogenic activity in tumors

Peptide Name	Sequence (# aa)	Modification	Origin	Target	In vivo status	Delivery method	Cancer model	Ref
pTnI	EDMNQKLFDLRG KFKRPPLRRVRMS ADAML (30)	None	Troponin I	Unknown	Pre-clinical	I.P. 3.5mg/kg for 5 days	Pancreatic cancer cells	[104]
RC-3940-II	D-Tpi-QWAVGL- ϕ (CH ₂ NH)L (8)	Non-natural amino acids	Bombesin	Bombesin receptor	Pre-clinical	Subcutaneous 20 μ g/day	Breast, Glioma	[105, 106]
PAMP₁₂₋₂₀	KWNKWALSR (9)	None	PAMP	Unknown	Pre-clinical	Intra tumor 1 μ mol/L Every 3 rd day	Adenocarcinoma	[107]
IM862	EW (2)	None	NA	Unknown	Phase II (Europe)	Intranasal (3x/daily) 20mg	Renal, Prostate	[108]
Aβ	EVHHQKLVFF (10)	None	β -amyloid	Unknown	Pre-clinical	I.P. 50mg/kg	Breast	[109]