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Computational systems biology approaches to anti-angiogenic cancer therapeutics

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Abstract

Angiogenesis is an exquisitely regulated process that is required for physiological processes and is also important in numerous diseases. Tumors utilize angiogenesis to generate the vascular network needed to supply the cancer cells with nutrients and oxygen, and many cancer drugs aim to inhibit tumor angiogenesis. Anti-angiogenic therapy involves inhibiting multiple cell types, molecular targets, and intracellular signaling pathways. Computational tools are useful in guiding treatment strategies, predicting the response to treatment, and identifying new targets of interest. Here, we describe progress that has been made in applying mathematical modeling and bioinformatics approaches to study anti-angiogenic therapeutics in cancer.

Keywords

mathematical model; bioinformatics; signaling networks; monoclonal antibodies; peptides; miRNA

Introduction

Angiogenesis, the formation of new blood capillaries from pre-existing vessels, enables tissues to obtain the oxygen and nutrients needed to maintain homeostasis and support growth. Angiogenesis is required during development and in the adult animal, and it is also crucial in many pathological conditions, including cancer, retinopathies, peripheral and coronary artery diseases, and pre-eclampsia. In the case of many cancer types, angiogenesis enables solid tumors to grow beyond oxygen diffusion limits and also provides a route for metastasis. Given the prominent role of angiogenesis in cancer, many cancer therapies aim to block angiogenesis and thereby inhibit tumor growth. These anti-angiogenic therapeutics

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target the numerous angiogenic pathways that occur in different cell types and microenvironments.

The growth of new blood vessels includes several distinct steps that occur across multiple time and spatial scales [1], and systems biology approaches (e.g., quantitative and high-throughput experimental techniques combined with computational models) enable a deeper understanding of the mechanisms involved in cancer development [2], including the process of neovascularization [1]. Computational systems biology, in particular, offers powerful tools with which to study complex biological processes, such as tumor angiogenesis. Mathematical modeling and bioinformatics can aid the discovery of new anti-angiogenic agents, provide insight into the effects of these drugs, and identify patient populations that will benefit from the drugs.

Mathematical models of angiogenesis are reviewed in [1,3–5]. These models provide a platform to study anti-angiogenic therapies and identify new targets. However, the application of modeling to investigate anti-angiogenic therapies has only been briefly summarized previously [1,6] or focused on VEGF pathway [7]. In this review article, we begin with a description of anti-angiogenic cancer therapeutics, and then summarize results from mathematical models and computational analyses of anti-angiogenic agents. We also present bioinformatics approaches used to understand angiogenesis signaling networks. We describe how these tools have been applied to study anti-angiogenic therapies and consider new opportunities and challenges for the application of computational systems biology approaches in the area of tumor angiogenesis.

Description of anti-angiogenic therapeutics

Anti-angiogenic therapeutics can be classified based on their targets. For example, angiogenesis inhibitors can directly target endothelial cells (ECs) and inhibit their ability to proliferate, migrate, survive, and form new vessels. Other inhibitors disrupt the production of angiogenic factors or downstream angiogenesis signaling pathways. Additionally, there are anti-angiogenic agents whose mechanism of action is either not specific or unknown. Figure 1 illustrates the various mechanisms of anti-angiogenic agents. In total, nine anti-angiogenic agents [8], which target various growth factors and cellular receptors, are currently approved by the US Food and Drug Administration (FDA).

One anti-angiogenic strategy is to target ECs by blocking proliferation and migration leading to the formation of new blood vessels. Endogenous inhibitors of angiogenesis have been explored as suitable anti-angiogenic agents, and many of these inhibitors target ECs [9]. One such compound is endostatin, which inhibits EC migration and proliferation. Although the mechanism of action is not completely understood, the anti-angiogenic action of endostatin is mediated in part by its binding to integrins, which are receptors present on the surface of ECs that mediate cell–cell and cell–matrix interactions, as well as heparin sulfate on the surface of ECs [10]. Endostatin is derived from the extracellular matrix (ECM) collagen XV or collagen XVIII proteins, but it proved difficult to synthesize in adequate amounts, limiting its use. Therefore, the anti-angiogenic effects of a modified form, called Endostar, and peptides containing short fragments of endostatin have been

investigated [11]. Endostar is currently approved for the treatment of non-small cell lung cancer in China. Blocking the action of integrins is another strategy used to target ECs, and peptides, monoclonal antibodies, and peptidomimetics are being explored as potential anti-integrin agents [12].

Other classes of angiogenesis inhibitor block neovascularization by targeting the signaling pathways of numerous factors, including VEGF, EGF, PDGF, Ang/Tie, IGF, FGF, TSP1, DLL4/Notch, and HGF/c-Met signaling (see Table 1 for definitions of these and other molecules discussed here). Additionally, anti-angiogenic agents can target transcription factors involved in angiogenesis. Anti-angiogenic therapies that target signaling pathways and transcription factors are briefly described below. Although most of the efforts to develop anti-angiogenic therapies have focused on VEGF, targeting other pathways is also important and could circumvent tumor resistance or recurrence following inhibition of a single pathway.

Inhibiting VEGF signaling has been a major focus in the development of angiogenesis inhibitors. VEGF is a potent regulator of angiogenesis in physiological conditions, such as exercise, wound healing, and reproduction, as well as in pathological conditions, such as cancer, preeclampsia, and peripheral and coronary diseases. Therapeutic agents can bind VEGF or its receptors (VEGFRs) extracellularly or inhibit intracellular signaling [13]. For example, bevacizumab is a monoclonal antibody that binds to VEGF and prevents it from binding to its receptors and promoting intracellular signaling leading to new vessel growth. Bevacizumab was the first anti-VEGF agent approved by the FDA, and it is used in the treatment of metastatic colorectal and kidney cancers, glioblastoma, and non-small cell lung cancer. At the time of publication, there were nearly 400 open clinical trials investigating the use of bevacizumab for the treatment of cancer (www.clinicaltrials.gov; 1 August, 2014). Another FDA-approved inhibitor of VEGF is aflibercept, a soluble decoy receptor approved for the treatment of metastatic colorectal cancer. Aflibercept is also being investigated for its efficacy in treating other cancer types. Agents that prevent VEGF-mediated signaling by blocking VEGF binding to its receptors are also in preclinical and clinical development. For example, icrucumab, a monoclonal antibody for VEGFR1, is in Phase I trials [14], ramucirumab is a monoclonal antibody for VEGFR2 being investigated for the treatment of metastatic gastric cancer, as well as other types of cancer [15], and monoclonal antibodies for VEGFR1 [14] and NRP1 [16] have results from Phase I trials.

An alternative approach to specifically blocking VEGF–VEGFR interactions includes inhibiting intracellular signaling through tyrosine kinase receptors. Small molecule tyrosine kinase inhibitors (TKIs) can block the action of these receptors. Tyrosine kinase receptors are involved in multiple angiogenesis pathways; therefore, TKIs disrupt the activation of multiple receptors. FDA-approved anti-angiogenic TKIs include sunitinib, sorafenib, pazopanib, axitinib, vandetanib, cabozantinib, and regorafenib, which target VEGF, PDGF, EGF, and FGF receptors, as well as c-Kit, Met, Raf kinase, Ret, and Tie2 [17].

It is also possible to inhibit tumor angiogenesis by inhibiting the signaling of other growth factor and protein families involved in angiogenesis. For example, there are efforts to target the diverse signaling pathways mediated by PDGF, FGF, IGF, HGF, angiopoietins, and

DLL4/Notch [18,19]. These factors mediate the complex, interconnected pathways that lead to tumor angiogenesis, and targeting multiple pathways could reduce tumor evasion and resistance. Recently, researchers have focused on the PI3K/AKT/mTOR signaling pathway, which is involved in cell survival. Targeting this pathway could aid in preventing tumor resistance [20], and the FDA has approved two mTOR inhibitors, everolimus and temsirolimus. Other agents that inhibit PI3K/AKT/mTOR signaling are being investigated [20,21]. There is also growing interest in mimicking the action of TSP1, an endogenous inhibitor of angiogenesis, and TSP1-derived peptides and peptidomimetics are in clinical trials [22].

Another mechanism of action of angiogenesis inhibitors is to target the products of proliferating ECs. During angiogenesis, the newly formed sprout must break through the ECM to migrate through the tissue and create a vessel network. ECs secrete enzymes such as matrix metalloproteinases (MMPs) that are able to proteolyze the ECM. MMPs are key proteases that contribute to the breakdown of the ECM and tumor angiogenesis. Therefore, targeting these enzymes is a promising anti-angiogenic and anticancer therapeutic approach. Strategies to inhibit MMPs include small molecules, antibodies, peptidomimetics, and endogenous compounds that sequester, compete with, or inhibit the expression of MMPs [23]. However, to date, MMP inhibitors have not been successful in clinical trials. Other protease inhibitors, such as serpins, are also being investigated as anti-angiogenic therapies [11].

Targeting and exploiting features of the ECM is another strategy used by angiogenesis inhibitors. The ECM is a dynamic microenvironment that provides structural support for sprouting ECs and contains many endogenous promoters and inhibitors of angiogenesis. In this case, anti-angiogenic compounds aim to inhibit the production and release of pro-angiogenic factors localized in the ECM and block the interactions between pro-angiogenic factors and the ECM. Inhibiting the production of pro-angiogenic factors can be achieved by modulating genetic regulation or utilizing gene therapy, and these approaches are being investigated [24]. MMP inhibitors are a strategy used to inhibit the release of promoters of angiogenesis. Developing mimetics of endostatin and TSP1 is an example of exploiting the anti-angiogenic factors that reside in the ECM, and other compounds that mimic the action of endogenous ECM angiogenesis inhibitors are undergoing preclinical and clinical evaluation [11,24].

It is also possible to inhibit angiogenesis by targeting miRNAs, small noncoding RNAs that can suppress mRNA translation. miRNAs regulate genes involved in many different biological processes, including tumor angiogenesis [25]. Several mechanisms by which miRNAs regulate gene expression have been reported [26], and anti-angiogenic therapies involving miRNAs can exploit these proposed mechanisms. One cluster of miRNAs shown to be highly expressed on ECs and in tumors is miR-17-92 [27]. The action of miR-92a is of particular interest, because experimental evidence demonstrates that it controls angiogenesis [27].

Lastly, angiogenesis inhibitors can target transcription factors involved in angiogenesis. Transcription factors influence cellular processes such as proliferation and survival, as well

as expression of angiogenic genes. Thus, transcriptional regulation is an important means of controlling angiogenesis. HIFs are a widely studied family of transcription factors that act in response to hypoxic conditions, leading to activation of numerous genes, including VEGF. HIF-1 in particular has been shown to mediate tumor angiogenesis and metastasis [28], and substantial research efforts have gone toward the development of HIF-1 inhibitors. In addition, experimental evidence shows several other transcription factors are important in angiogenesis, including FoxC2 [29], SP1 [30], NF- κ B [31], and NFAT [32]. The experimental studies provide the basis for computational modeling of transcriptional regulation of angiogenesis.

Computational models of anti-angiogenic therapeutics

Anti-angiogenic therapies targeting VEGF and other signaling pathways have been studied extensively, both in the pre-clinical and clinical settings [17]; however, it is difficult to predict the patient population that can benefit most from these drugs. Therefore, it is of interest to identify biomarkers that predict the patients who will respond favorably to anti-angiogenic treatment [33], and this is an area where computational modeling can contribute [34]. Additionally, modeling can be applied to identify new therapeutic targets. Here, we review computational modeling used to study angiogenesis inhibitors. Several of the computational studies described below are highlighted in Table 2. These modeling efforts provide a framework to test different hypotheses regarding the mechanism of action of angiogenesis inhibitors, predict the effect of single- and multi-modal therapeutic strategies and identify promising new targets. We focus on tumor angiogenesis involving ECs; however, the contribution of endothelial progenitor cells (EPCs) to angiogenesis is also important, and has been studied computationally [35,36].

Targeting endogenous inhibitors

The action of endogenous angiogenesis inhibitors has been explored as an anti-angiogenic therapy, and mathematical modeling can contribute to this research. Billy *et al.* developed a model of tumor growth that includes inhibitors (endostatin and Ang2) and promoters (VEGF and Ang1) of angiogenesis [37]. The multiscale model incorporates molecular interactions leading to tumor angiogenesis, which influences oxygen concentration gradients, and subsequently, cell proliferation, apoptosis, or quiescence. The model was used to investigate the effect of endostatin gene therapy, where adenoviruses encoding the endostatin gene are injected into the tumor to stimulate overexpression of endostatin by tumor cells. Model results indicate that it might be more effective to increase the duration of endostatin overproduction rather than the degree of overproduction (i.e., dose) when aiming to inhibit tumor growth. Additionally, the model predicts a threshold value for the dose, above which increasing the duration of treatment improves the efficacy. However, increasing the duration of treatment for doses below the threshold value has an adverse effect, because it triggers an angiogenic rebound that enables the tumor to grow more quickly following treatment. These results demonstrate the utility of the model in investigating the effect of endostatin therapy on tumor growth. Earlier work by Sleeman and coworkers [38] investigated the effect of angiostatin, another endogenous inhibitor of angiogenesis. A recent study using a model that combined angiogenesis and hemodynamic simulations in metastatic tumors, predicted that

treatment with angiostatin affects tumor vessels such that they more closely resemble normal vessels [39]. This process, called ‘vessel normalization’, results in reduced hypoxia and interstitial fluid pressure (IFP). Vascular normalization leads to improved treatment outcomes by increasing perfusion of cancer therapeutics in the tumor [8]. The simulation results show that IFP is decreased and perfusion is improved, demonstrating the efficacy of angiostatin treatment.

Inhibiting angiogenesis signaling pathways

As described above, blocking angiogenesis signaling is a major mechanism of anti-angiogenic therapies. Inhibiting integrin signaling is an approach used to target ECs. Bauer and coworkers constructed a Boolean signal transduction network that included VEGF, integrin, and cadherin receptor signaling and crosstalk between the pathways [40]. The network can be used to predict how inhibition of a single molecular species, or combinations of species, in the network influences cellular phenotype (i.e., apoptosis, proliferation, motility, or quiescence). Loss of integrin signaling is predicted to produce an apoptotic response. Additionally, the receptors that mediate integrin and VEGF crosstalk determine cellular phenotype and are predicted to be important targets for anti-angiogenic therapy.

Antibodies have been utilized because they are able to target specifically ligands and receptors that mediate angiogenesis. The antibodies must be optimized to bind selectively to and inhibit their targets, and computational modeling is useful in understanding the factors that influence the efficacy of antibody therapeutics. Thurber and coworkers developed a model to study antibody uptake in tumors [41]. The mechanistic model predicted antibody concentration in a tumor over time, as a function of measured or estimated parameters, such as binding affinity, internalization rate, permeability, and clearance. The model can be applied to identify specific drug properties that directly influence uptake to maximize tumor localization. Harms *et al.* developed a kinetic model of antibody–antigen binding based on experimental measurements of monovalent antibody binding kinetics and affinity curves of antibody–antigen binding [42]. The model predicts the avidity of an antibody, which contributes to its potency. Thus, the model can be used to investigate antibody binding and aid in designing antibodies with optimal potency.

A major focus of anti-angiogenic therapy has been on the VEGF signaling pathway. VEGF is an important promoter of angiogenesis and initiates intracellular signaling by binding to, and activating, its cellular receptors. Compartmental models have been developed to study the VEGF/VEGFR pathway. The models include interactions of two major VEGF-A isoforms involved in tumor angiogenesis (VEGF₁₂₁ and VEGF₁₆₅), receptors (VEGFR1 and VEGFR2), and co-receptors neuropilins (NRP1 and NRP2). The models have been applied to investigate how therapeutics targeting the VEGF pathway influence the distribution of VEGF in the body [43–46].

A model of VEGF interactions in tumors was used to test alternate hypotheses regarding the efficacy of inhibiting neuropilin coupling to VEGFR2, blocking NRP1 expression, or preventing VEGF–NRP binding in tumor tissue [43]. The model predicted that inhibiting NRP–VEGFR2 coupling is most effective in decreasing VEGF–VEGFR2 binding and signaling. In another example, a whole-body compartment model was used to study the

effect of anti-VEGF treatment [44]. The pharmacokinetic/pharmacodynamics (PK/PD) model, which includes normal tissue, plasma, and tumor, predicts and explains the mechanism by which free VEGF in the plasma increases following anti-VEGF treatment. Interestingly, the model showed that free VEGF in plasma increases with anti-VEGF treatment because of the shuttling of the anti-VEGF/VEGF complex between the compartments. Although evidence for the increase of plasma-free VEGF has been disputed (see summary of previous studies by Loupakis *et al.* [47]), an increase in total plasma VEGF after treatment has been observed clinically and is primarily attributed to longer half-life of bevacizumab-bound VEGF [48]. To further investigate the relations between the plasma VEGF levels and those in the tumor and normal tissue interstitia, the compartment model was significantly expanded to include VEGF receptor densities based on quantitative experimental measurements, VEGF degradation, and VEGF secretion by tumor cells. The resulting model was then applied to determine how various model parameters, drug-specific characteristics, and properties of the tumor microenvironment influence the response to anti-VEGF treatment [49] and to predict the effect of anti-VEGF agents that target specific VEGF isoforms [45]. The model predicted that the density of VEGFRs on tumor cells, as well as the rate at which VEGF is secreted by tumor cells, determines the response to treatment. Additionally, specifically targeting VEGF₁₂₁ is most effective in reducing free VEGF in the tumor and VEGF–VEGFR2 complexes in the blood. Thus, the models are useful in identifying salient drug design parameters and understanding how tumor-specific properties influence the response to anti-VEGF treatment.

Recently, the compartmental model was further expanded to include endothelial cell secretion of VEGF and key soluble factors sVEGFR1 (sFlt-1) and α -2-macroglobulin. The extended model was applied to investigate the dynamic VEGF levels during anti-VEGF treatment [46]. The concentration of free VEGF in the tumor was predicted to be seven to 13 times higher than plasma VEGF and predominantly in the form of VEGF₁₂₁ (>70%). These model predictions, which are validated by experimental data, indicate that depleting tumor VEGF might be an effective anti-angiogenic strategy, and the isoform specificity of the drug should be considered. Additionally, the model reveals that anti-VEGF treatment can increase free VEGF in the tumor, depending on the tumor microenvironment. Thus, the response to treatment depends on properties of the tumor, such as the secretion ratio of specific VEGF isoforms and expression of cell surface VEGF receptors, pointing to the importance of personalized medicine. The significance of specific VEGF isoforms is supported by experimental data regarding mRNA expression of VEGF isoform in tumor tissues [50]. In another study, the molecular-detailed model of VEGF was applied to study the effect of aflibercept [51]. The model quantified the contributions of specific transport processes and molecular interactions of the drug that influence the distribution of aflibercept in the body. In this way, the model can estimate PK properties of the drug. Importantly, given the molecular detail of the model, it can be used to study the mechanism of action of the drug. The predictions generated by these models have important clinical applications that are relevant to the development and optimization of anti-VEGF therapies. In addition to predicting VEGF concentration following anti-VEGF treatment, mathematical modeling has also been applied to predict the effect on tumor size and tumor vasculature, using several

computational techniques, such as partial differential equation-based, agent-based modeling, and hybrid modeling [52–55].

Anti-VEGF treatment also influences properties of the tumor microenvironment, and has been shown to result in vascular normalization. Jain and coworkers developed a model to predict changes in tumor microenvironment (i.e., IFP and interstitial fluid volume) following vessel normalization as a result of anti-VEGF treatment [56]. The model predicted that there are multiple ways by which vascular normalization can reduce tumor IFP: decreasing the tumor size, reducing vascular hydraulic permeability, decreasing the surface area per unit tissue volume of tumor vessels, or increasing hydraulic conductivity in the tumor interstitium. The model also described a mechanism by which normalization reduces edema and lymphatic metastasis, whereby fluid convection within the tumor increases and convection at the tumor margin simultaneously decreases. Another model estimated the 'angiogenic activity of a solid tumor, which is, a measure of the relative levels of pro- and anti-angiogenic compounds, and how it is influenced by IFP [57]. This model can be applied to predict the effect of anti-angiogenic agents by varying certain model parameters that are directly affected by anti-angiogenic drugs, such as hydraulic permeability and vessel wall surface area per unit volume of tumor.

Computational approaches that incorporate tumor-specific measurements are of interest in the development of personalized medicine. Titz and coworkers combined image-based modeling of tumor growth with PK/PD modeling of tumor angiogenesis [58]. Data from PET and CT imaging provide patient-specific parameters that are used to predict tumor oxygenation and proliferation [59]. A PK/PD model of bevacizumab was incorporated into the image-based model of tumor growth, and the integrated model was applied to predict the effect of anti-VEGF treatment on tumor proliferation and oxygenation for specific patients. The authors investigated the sensitivity to patient-specific input data and different treatment regimens. For example, the level of tumor hypoxia was predicted to be 50% lower in tumors with low VEGF expression when bevacizumab was administered at a dose of 10.5 mg/kg compared with a 15 mg/kg dose. Thus, the model can be used to optimize personalized anti-VEGF treatment strategies. In another example, Stamatelos *et al.* used bioimage informatics approaches to analyze and reconstruct tumor microvasculature, obtained via *ex vivo*, high-resolution micro-CT imaging [60]. The reconstructed vascular network served as an input to a computational model that estimates blood flow in the vessel segments. This model of blood flow, based on imaging of tumor tissue, can be used as a framework to study the effects of anti-angiogenic therapies, including anti-VEGF agents.

Mathematical modeling has also enabled the study of agents that target VEGF receptors. As described above, VEGF promotes angiogenesis by activating its cellular receptors, VEGFRs. Thus, the density and availability of these receptors influences the pro-angiogenic action of VEGF. Alarcon and coworkers developed a model of VEGFR association with VEGF, dimerization, and internalization to investigate how these processes influence the response to anti-angiogenic therapy [61]. The model predicted the effect of anti-VEGF therapy when VEGFRs are overexpressed, which has been observed in tumor vasculature. Overexpression of VEGFRs is predicted to lead to resistance to treatment, and the model can be used to understand the mechanisms of resistance.

Recently, a novel model of VEGFR intracellular signaling has been developed and validated against experimental data [62,63]. Tan and coworkers developed a molecular-detailed, mass-action model of VEGFR2 trafficking and signaling leading to Akt activation. The model included scaffolding proteins Gab1 and Gab2, which are positive and negative regulators of Akt phosphorylation, respectively. The model predicted that Gab1 and Gab2 influence VEGFR2 recruitment and, therefore, Akt regulation, to different extents. Additionally, by performing an extensive sensitivity study, the authors showed that the ratios of certain molecular species (Gab1/Gab2 and PI3K/Shp2) determine Akt activation, rather than their individual concentrations. This is the first model to stimulate explicitly molecular interactions in VEGFR2 signaling, and it provides a useful framework to study TKIs that target VEGFRs.

In addition to its role in promoting angiogenesis, VEGF has been shown to upregulate pro-survival signals in endothelial cells, specifically through Bcl-2, an anti-apoptotic protein. Therefore, another anti-angiogenic strategy is to inhibit Bcl-2 signaling. The effects of targeting the VEGF-Bcl-2 signaling axis have been studied computationally [64,65], generating predictions for the optimal timing and dosing of potential anti-apoptotic drug candidates.

Mathematical modeling and computational analysis have also been applied to study signaling pathways beyond VEGF. Recent examples include models of EGF [66], angiopoietins [67], FGF [68], and DLL4/Notch [69], which provide a framework to investigate anti-angiogenic therapies. For example, a multiscale model of tumor growth in brain cancer was expanded to include an angiogenesis module and an EGFR signaling pathway and utilized to investigate the action of TKIs [70]. The model predicted that, although TKI treatment reduces cell survival and tumor invasion in the early stages of tumor growth, ongoing tumor angiogenesis enables the tumor to obtain the oxygen and glucose needed to increase cell survival at later stages. Thus, it is proposed that TKI therapy alone is not sufficient to inhibit tumor development. Another example is the work in simulating IGF signaling, where Iadevaia and colleagues combined mass-action kinetic modeling with particle swarm optimization, a stochastic modeling technique, to develop a trained model that fits experimental data from the MDA-MB-231 breast cancer cell line; the model included signal transduction through IGF and its receptor, leading to the MAPK, PI3K, and mTOR pathways [71]. The model predicted the effect of targeting specific signaling proteins, as well as combinations of proteins. Inhibition of the PI3K/AKT and MAPK pathways is predicted to be the optimal strategy required to disrupt aberrant signaling, and the prediction was validated experimentally. Additionally, combined MEK and mTOR inhibition, predicted to be a nonoptimal strategy, was shown experimentally to increase the viability of breast cancer cells. Thus, the model can be used to compare therapeutic strategies. A model of the uptake and transport of IGF and its diffusible binding partner, IGFBP3, has been developed for cartilage tissue [72]. This model predicts mechanisms by which diffusible binding molecules regulate protein uptake in tissue, and could be adapted for tumor tissue to investigate therapies targeting IGF.

Interest in alternative pathways involved in angiogenesis has also been increasing. A relatively new target for anti-angiogenesis therapies is the Wnt signaling pathway. Wnts are

glycoproteins that regulate many cellular processes, including proliferation, survival, adhesion, and proliferation, by binding to the Frizzled (Fz) receptor. Wnt signaling has been shown to be important in angiogenesis and, thus, is a potential target for anti-angiogenic therapies [73]. Antagonists of Wnt signaling include secreted Frizzled-related protein (sFRP), Wnt inhibitory factor 1 (WIF-1), and Dickkopf (Dkk). Mathematical models have been used to investigate the effect of inhibiting Wnt via its antagonists and, in one example, modeling was applied to investigate Wnt inhibition via Wnt3a, sFRP, and Dkk [74]. The effect of inhibiting Wnt signaling was quantified by estimating the accumulation of β -catenin, which leads to the transcription of various genes involved in EC proliferation, migration, and other processes. The model was validated by comparing the predicted effect of Wnt3a and sFRP to published experimental data. Additionally, the model simulated the effect of Dkk alone and in combination with sFRP. Interestingly, Dkk and sFRP were predicted to have a synergistic effect on inhibiting β -catenin accumulation [74], and this work could lead to a new anti-angiogenic treatment strategy.

Tools that enable identification of novel therapeutic targets are of particular interest. Wang and coworkers developed a multiscale model of tumor growth that incorporates the signaling pathways of EGF and TGF- β in a 3D model of tumor cell growth [75]. These signaling pathways mediate cell proliferation and migration and are involved in angiogenesis. The authors identified novel anti-angiogenic targets by performing a sensitivity study on the model parameters to determine how changes in the molecular signaling pathways influence two tumor outcomes: tumor cell number and the rate of tumor expansion [76]. The analysis predicted, for example, that inhibiting MEK activity by 0.5-fold is the optimal inhibition strategy, because it reduces the number of tumor cells and inhibits tumor expansion toward the nutrient source. Upregulation of ERK activity is also an optimal therapeutic strategy. The analysis can be applied to identify additional promising anti-angiogenic strategies.

Targeting products of growing ECs

ECs move through the endothelial basement membrane and ECM as they sense the pro-angiogenic mechanical and biochemical stimuli. Proteases enable ECs to move through structural barriers in the basement membrane and ECM. Additionally, these enzymes catalyze reactions that release pro-angiogenic factors from the ECM. Therefore, inhibiting the action of the proteases, a product of proliferating ECs, is considered to be a viable anti-angiogenic strategy. Interestingly, ECs release MMPs as they move through the ECM; however, ECs also secrete MMP inhibitors, called tissue inhibitors of metalloproteinases (TIMPs). Thus, mathematical models of these reaction systems are useful in understanding how MMPs and other proteases can be exploited in anti-angiogenic therapies [50]. Popel and coworkers developed kinetic models of the biochemical reactions whereby collagen I is degraded by MT1-MMP and MMP2 [77,78], as well as a model for activation, inhibition, and deactivation of MMP9 [79]. These models were used to investigate synergy between MMP inhibitors and described mechanisms by which MMP activation occurs, predictions that can aid in the development of anti-angiogenic drugs. In more recent work, Vempati *et al.* studied VEGF patterning in tissues [80,81], which is important in controlling vascular networks. One model predicted that the spatial distribution of VEGF can be attributed to isoform-specific degradation and is mediated by MMPs [81]. Thus, controlling MMP

activity can modulate the localization of VEGF isoforms and regulate EC response to VEGF.

Targeting miRNA regulation

Targeting gene regulation by miRNAs is another anti-angiogenic strategy. One miRNA can target multiple genes, one gene can be targeted by several miRNAs, and regulation by miRNA involves feedback and feed forward loops [82]. Therefore, systems biology approaches to study anti-angiogenic therapies involving miRNAs are useful in understanding the complexities of miRNA regulation and possibly for therapeutic applications.

Recently, mathematical modeling has been applied to investigate miRNA regulation networks [82], the mode of action of miRNAs [83], and therapeutics that target miRNAs [84]. Although miRNA regulation has not been studied computationally within the context of angiogenesis, these existing mathematical models of miRNAs provide a template for studying therapeutic treatments targeting miRNAs. For example, one model studied miRNA regulation in chronic myeloid leukemia (CML) [84], which is characterized by expression of the tyrosine kinase BCR-ABL. The model was applied to predict the effect of the TKI imatinib combined with miRNA treatment on BCR-ABL phosphorylation in CML cells, and whether the drugs act synergistically.

Targeting transcription factors

Transcription factors regulate many different steps involved in angiogenesis. Additionally, transcription factors themselves are regulated by signaling pathways. Therefore, targeting transcriptional regulation is an approach to inhibit angiogenesis. The HIF-1 pathway has been studied computationally [85–87], and the models provide insight into the effects of inhibiting HIF-1. For example, the prolyl and asparaginyl hydroxylases are predicted to be possible targets for anti-angiogenic treatment [87]. Other transcription factors are also involved in angiogenesis, as described above, but have not been incorporated into mathematical models. Thus, there are numerous opportunities for computational studies of these important angiogenic regulatory mechanisms.

Bioinformatics approaches to drug discovery

Bioinformatics tools can increase the efficiency of the drug development pipeline, both in terms of time and financial investment [88]. Specifically, bioinformatics studies can identify new targets for various pathological conditions and predict new therapeutic agents using high-throughput data sets. For example, analysis of genome-wide association studies (GWAS) results revealed that 21% of GWAS-associated genes are druggable by small molecules compared with 17% of genes derived from the entire genome, a difference that is statistically significant [89]. Here, we explore specific applications of bioinformatics for identification of anti-angiogenic targets and anti-angiogenic drugs.

Genome studies

The Cancer Genome Atlas (TCGA) provides a platform to search, download, and analyze data sets in various types of cancer, including breast cancer and glioblastoma. These data sets include DNA copy number arrays, DNA methylation, exome sequencing, mRNA arrays, and miRNA sequencing, providing a wealth of information to study cancer therapeutics in specific subclasses of a particular type of cancer. Based on these data, breast cancer was subtyped into four classes: basal-like, HER2, Luminal A, and Luminal B [90]. Only three genes (*p53*, *PIK3CA* and *GATA3*) occurred at >10% incidence across the four breast cancer subtypes. Lehmann *et al.* [91] further clustered the basal-like subtype, which is often referred to as triple-negative breast cancer (TNBC), into seven subtypes by using 21 publicly available data sets. Vaske *et al.* developed the methodology called Pathway Recognition Algorithm using Data Integration on Genomic Models (PARADIGM) to infer patient-specific genetic activities incorporating curated pathway interactions among genes in breast cancer and glioblastoma from TCGA data sets [92]. Masica *et al.* developed the algorithm called Multivariate Organization of Combinatorial Alterations (MOCA) using Boolean set operations coupled with optimization to identify simultaneous genetic alterations and translate them into biomarkers for drug response [93]. These various machine-learning tools could facilitate the prediction and identification of angiogenesis-related drug targets in various cancer types.

Proteome studies

Missing gene annotations related to angiogenesis, which can be obtained from numerous sources of biological data, can be used to identify novel drug targets for angiogenesis. Proteins that physically interact are more likely to participate in the same biological process, and proteins that share similar domains are more likely to have similar molecular functions. The integration of these and other sources of data can improve the annotation of proteins. To facilitate the understanding of angiogenesis-associated protein interactions, Chu *et al.* constructed a global protein–protein interaction network (PIN) of angiogenesis, called the angiome, by combining information from Gene Ontology (GO), GeneCards, and a commercial website, SABiosciences. The authors then applied a GeneHits algorithm developed for the study [94]. The angiome constructed using this analysis comprised 1233 proteins and 5726 protein–protein interactions. It provides the foundation for the analysis of various pathways and proteins known to be involved in angiogenesis, including VEGF, FGF, MMP and Notch pathways. Importantly, the angiome reveals proteins, protein clusters and their relations that might be novel targets for angiogenic therapies. The analysis also identifies growth factor networks that drive pro- and anti-angiogenic signaling; these could be used for pro- and anti-angiogenic drug discovery or repurposing.

Identification of anti-angiogenic peptides

In drug development, peptides have several advantages compared with small molecules and full proteins [11]. A bioinformatics approach was proposed to identify efficiently numerous endogenous peptide inhibitors of endothelial cell proliferation and migration [95]. First, amino acid sequences of 40 known potent anti-angiogenic fragments were compiled from an extensive search of the relevant literature; the protein families containing these fragments

included somatotropins, CXC chemokines, type IV collagens, serpins, and type 1 thrombospondin repeat-containing proteins. Multiple sequence alignment algorithms were performed to calculate the similarity among amino acid sequences of the conserved domains of various proteins in the human proteome. Selected bioinformatically identified peptides from these active domains have been experimentally tested for their ability to inhibit endothelial cell proliferation, migration and capillary tube formation *in vitro*. The serpin-, collagen IV-, CXC chemokine-, thrombospondin-, and somatotropin-derived peptides have also been tested in *in vivo* mouse models to investigate their ability to inhibit angiogenesis in breast, brain, and lung cancer [96,97]. Additionally, Koskimaki *et al.* tested synergy effects of a collagen IV-derived mimetic peptide and a somatotropin domain-derived peptide on human umbilical vein endothelial cells (HUVECs), microvascular endothelial cells (MECs), and lymphatic endothelial cells (LECs), and also *in vivo* using subcutaneous matrigel plugs [98]. Quantification of synergy by the Chou-Talalay method was used, as is common in drug combination studies [99]. Interestingly, administration of both peptides enhances inhibition of angiogenesis and lymphangiogenesis, compared with treatments with each peptide alone. The authors also identified the mechanism of action responsible for the synergistic effects. This and other studies [97,100] utilize anti-angiogenic peptides identified by the bioinformatics approach, demonstrating the relevance of these tools in the development of anti-angiogenic therapeutics.

Quantitative structure-activity relationships

Endogenous peptides do not always have characteristics desirable in a drug; thus, modifications are necessary to optimize their properties. To guide the design of biomimetic peptides, computer-aided quantitative structure-activity relations (QSAR) can be generated. Rivera *et al.* generated QSAR based on the Least Absolute Shrinkage and Selection Operator (LASSO) to optimize the activity of collagen IV-derived anti-angiogenic peptides [101]. The method is based on *in vitro* experimental data that associate peptide features with a quantitative activity score (e.g., endothelial cell proliferation inhibition activity). Peptides were assigned a unique sparse vector of features where each feature uniquely identifies an amino acid at a single position. The convex optimization was used to select features that differentiate active and inactive peptides and reach global optimality.

Concluding remarks

Computational systems biology tools provide a framework to study the complexity of the angiogenesis process and the therapeutic interventions needed to inhibit angiogenesis in cancer. Mathematical modeling and bioinformatics approaches aim to test different anti-angiogenic treatment strategies, predict the effects of the therapeutic agents, identify diagnostic and prognostic biomarkers, and stratify patient populations to identify those that might benefit best from the therapies. These approaches can be used together; for example, the interactions between nodes in a signaling network predicted through bioinformatics studies and the consequences of targeting those interactions can be examined in detail using mechanistic mathematical modeling [102]. Thus, bioinformatics results can be used as inputs for mechanistic mathematical models, and recent efforts have focused on how to integrate effectively large data sets into predictive mathematical models. Importantly,

computational systems biology approaches complement preclinical and clinical studies, and could make the drug development process more efficient (i.e., result in fewer failed drug candidates) and less costly.

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Research Highlights

- Systems biology approaches support drug development efforts.
- Models of angiogenesis aid in testing anti-angiogenic therapeutic strategies.
- Models provide novel insight into effects of targeting VEGF-mediated angiogenesis.
- Angiogenesis-associated protein interaction networks help identify novel targets.
- Bioinformatics analyses have identified anti-angiogenic peptide sequences.

Figure 1.

Schematic of anti-angiogenic targets. Angiogenesis inhibitor (AIs) agents aim to disrupt angiogenesis in cancer in multiple ways. **(A)** Endogenous AIs bind directly to endothelial cells via multiple cellular targets; **(B)** AIs can bind to secreted growth factors or **(C)** tyrosine kinase receptors to inhibit angiogenesis signaling pathways leading to cellular processes involved in vascularization, such as proliferation, migration, and differentiation; **(D)** AIs target secreted factors that permit migration through the extracellular matrix or basement membrane, such as MMPs; **(E)** AIs target components of the extracellular matrix (ECM) to regulate the release of angiogenic factors; and **(F)** AIs such as miRNAs and transcription factors influence gene expression involved in angiogenesis. For definitions of abbreviations used in figure, see Table 1 (main text).

Table 1

Definitions of abbreviations used in the article

Abbreviation	Definition
Ang	Angiopoietin
Bcl	B cell lymphoma 2
CXCL8	
DLL4	Delta-like ligand 4
EGF	Epidermal growth factor
ERK	Extracellular signal-regulated kinase
FGF	Fibroblast growth factor
FOXC2	Forkhead box protein C2
Gab	Growth factor receptor-bound protein 2-associated binder
HGF	Hepatocyte growth factor
HIF	Hypoxia-inducible factor
IGF	Insulin-like growth factor
MAPK	Mitogen-activated protein kinase
MEK	MAPK/ERK kinase
mTOR	Mammalian target of rapamycin
NF- κ B	Nuclear factor κ -light-chain-enhancer of activated B cells
NFAT	Nuclear factor of activated T cells
NRP	Neuropilin
ODE	
PDE	
PDGF	Platelet-derived growth factor
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
Shp	Src homology-2 domain-containing phosphatase
SP1	Specificity protein 1
TGF- β	Transforming growth factor β
TSP	Thrombospondin
VEGF(R)	Vascular endothelial growth factor (receptor)

Table 2

Summary of computational systems biology studies of anti-angiogenesis therapies

Anti-angiogenic strategy and targets	Highlighted computational studies (model type)	Refs
Endogenous inhibitors		
Endostatin and Ang2	Multiscale reaction–diffusion PDE model of angiogenesis and tumor growth	[37]
Angiostatin		[39]
Endogenous peptides	Bioinformatics-based identification of anti-angiogenic peptide sequences	[95]
Inhibiting angiogenesis signaling pathways		
VEGF, integrin, and cadherin receptor	Boolean network model of crosstalk in endothelial cell signaling	[40]
VEGF binding	Multicompartment, reaction ODE model of VEGF distribution in body	[43–46]
VEGF and tumor vasculature	Hybrid cellular automaton of tumor growth in vascular environment	[52]
VEGF secretion	Multiscale agent-based model of vasculature with intracellular, intercellular, and tissue levels	[53]
VEGF secretion	Image-based model of tumor growth combined with pharmacokinetic/pharmacodynamics model	[58]
VEGFR signaling	Reaction ODE model of intracellular signaling network	[62,63]
Bcl-2 signaling	Reaction ODE model of VEGF, CXCL8, and Bcl-2	[64,65]
EGFR signaling	Multiscale agent-based model of EGFR signaling, tumor growth, and angiogenesis	[70]
IGF signaling	Reaction ODE model combined with particle swarm optimization	[71]
Wnt signaling	Reaction ODE model of Wnt and its receptors	[74]
EGF and TGF- β signaling	Multiscale agent-based model of signaling and tumor cell growth	[76]
Protein interactions	Bioinformatics-based identification of pathways and proteins known to be involved in angiogenesis	[94]
Targeting products of growing ECs		
MMP9 activation	Reaction ODE model of MMP9 activation and inhibition	[77–79]
MMP9 proteolysis	Reaction–diffusion PDE models of MMP9 proteolysis of VEGF isoforms	[80,81]
Targeting miRNA regulation		
mRNA translation	ODE model of protein translation	[83]
	Steady-state model of miRNA repression	[84]
Targeting transcription factors		
HIF-1	Reaction–diffusion PDE models of HIF regulation and signaling	[85–87]