Chapter 2
Simulating Therapeutics Using Multiscale Models of the VEGF Receptor System in Cancer

Feilim Mac Gabhann, Marianne O. Stefanini, and Aleksander S. Popel

1 Angiogenesis as a Therapeutic Target in Cancer

Exploration of antiangiogenic cancer therapeutics began when Dr. Judah Folkman postulated that tumors must depend on angiogenesis (neovascularization) for their growth and metastasis, and therefore that angiogenesis is a therapeutic target (Folkman 1971). His pioneering research paved the way to the discoveries of the primary angiogenic cytokine, Vascular Endothelial Growth Factor (VEGF) (Dvorak 2006; Leung et al. 1989), and subsequent development of therapeutic agents targeting VEGF and its receptors (Gaur et al. 2009; Mac Gabhann and Popel 2008). In the last two decades numerous other molecular families have been identified and extensively studied as potential targets for antiangiogenic therapeutics including: integrins; angiopoietins; the delta-notch system; semaphorins; ephrins; platelet-derived growth factors (PDGF); hepatocyte growth factor (HGF); transforming growth factor beta (TGFβ); matrix metalloproteinases (MMP); and the transcription factors, hypoxia-inducible factors (HIF). These are variously involved in initiation, propagation, or stabilization of sprouting angiogenic neovessels. Many of these families interact with one another directly or at the level of intracellular signaling, and thus quantitative systems biology approaches are required to unravel this complexity and to design novel approaches to antiangiogenic therapeutics.

In this chapter we will outline approaches to multiscale molecular-detailed computational modeling of the VEGF family, with particular emphasis on

F. Mac Gabhann
Institute for Computational Medicine and Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
e-mail: feilim@jhu.edu

M.O. Stefanini • A.S. Popel (✉)
Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
e-mail: stefanini@jhmi.edu; apopel@jhu.edu

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pharmacokinetic-pharmacodynamic (PK/PD) modeling of current or potential therapeutic interventions. At the molecular and cellular scales, we use a biophysically accurate kinetic model of ligand–receptor interactions and/or intracellular signaling. Models of other molecular families important for angiogenesis have also been formulated and can be incorporated into integrative models: fibroblast growth factor-2 (Filion and Popel 2004; Forsten-Williams et al. 2008); MMPs (Karagiannis and Popel 2004; Karagiannis and Popel 2006; Vempati et al. 2007); PDGF (Park et al. 2003); and HIF1α (Qutub and Popel 2008). Along with these molecular and kinetic details, multiscale models also incorporate cellular and tissue information, to simulate molecular trafficking and tissue responses to drugs. Combined molecular-cellular models have sought to delineate the role of the Delta-Notch family in endothelial tip cells and stalk cells in the development of nascent capillary sprouts (Bentley et al. 2008, 2009). At the tissue scale, models have been developed to simulate the processes of growing vasculature; the vessels may be treated as discrete objects (Bauer et al. 2007; Owen et al. 2009; Sun et al. 2005; Milde et al. 2008; Qutub and Popel 2009; Das et al. 2010; Macklin et al. 2009), or as a continuum in terms of vascular density (Levine et al. 2001). Molecular-detailed and integrative models of angiogenesis have been reviewed in (Owen et al. 2009; Macklin et al. 2009; Anderson and Quaranta 2008; Byrne 2010; Chaplain et al. 2006; Qutub and Popel 2009; Frieboes et al. 2010; Peirce 2008; Stefanini et al. 2011). Some of these models describe not only the vasculature but also the growing tumor, which further increases the level of complexity. In assembling these multiscale models, processes at different scales may be simulated using different modeling methodologies; ordinary differential equations (ODEs), partial differential equations (PDEs), stochastic Monte Carlo simulations, and agent-based modeling (ABM) are all used as appropriate; examples are given later.

Systems biology, and specifically computational modeling and simulations, is becoming mainstream in drug discovery (Laubenbacher et al. 2009). There are several examples of pharmacokinetic and pharmacodynamic computational predictions from angiogenesis models. A PDE-based continuum model that describes temporal and spatial aspects of endothelial cell migration, proliferation, apoptosis and cell–cell contact, as well as tumor cell cycle was applied to model endostatin gene therapy (Billy et al. 2009). A compartment ODE-based model was formulated to describe temporal variation of spatially-averaged vessel density, growth, maturation, and regression, as well as tumor growth (Arakelyan et al. 2003); the model was applied to simulate administration of a chemotherapeutic drug in combination with an antiangiogenic drug bevacizumab, a monoclonal antibody to VEGF (Gorelik et al. 2008). A model with a similar structure was applied to study a combination of chemotherapeutic and antiangiogenic agents (d'Onofrio and Gandolfi 2010). A single-compartment ODE-based model describing ligand–receptor interactions for VEGF isoforms and their receptors simulated administration of agents disrupting the association of VEGF receptor-2 (VEGFR2) with neuropilin-1 (NRP1) (Mac Gabhann and Popel 2006). A multi-compartment model describing VEGF transport among blood, tumor, and normal tissue compartments simulated bevacizumab

administration, as a single bolus or metronomic treatment (Stefanini et al. 2011). An ODE-based signal transduction model of Bcl-2 protein downstream from VEGFR2 made predictions for inhibition of Bcl-2 by a small-molecule inhibitor (Jain et al. 2009).

Complementing experimental studies of important molecular factors and cellular and tissue processes in tumor angiogenesis, multiscale mathematical modeling is prepared to move toward translational applications, such as antiangiogenic drug discovery and development. The models can be used to analyze the results of clinical trials and application of approved antiangiogenic agents as a monotherapy or in combination with other agents (antiangiogenic or chemotherapeutic), and also to design novel therapeutics.

2 The VEGF Receptor System

The VEGF family in humans includes five genes encoding cell-secreted protein ligands: VEGF-A, -B, -C, -D, and placental growth factor (PlGF), comprising important cytokines involved in angiogenesis and lymphangiogenesis (Ferrara et al. 2004; Takahashi and Shibuya 2005). VEGF-A is commonly referred to as VEGF; this convention is used later. VEGF has been shown to have several effects on the vasculature. For example, VEGF increase is often correlated with vascular density, vessel tortuosity, and increased microvascular permeability (Lee et al. 2005; Nagy et al. 2009; Nagy et al. 2007). Human VEGF family mRNA splice variants give rise to VEGF isoforms including: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, $VEGF_{206}$, $VEGF\text{-}B_{167}$, $VEGF\text{-}B_{186}$ (Robinson and Stringer 2001). The VEGF family of ligands has multiple cell-membrane receptors: homodimers of VEGFR1 (Flt-1), VEGFR2 (Flk-1 or KDR), and VEGFR3 (Flt-4); the heterodimers VEGFR1/2 and VEGFR2/3; and the coreceptors neuropilin-1 (NRP1) and neuropilin-2 (NRP2) (Ferrara et al. 2003). Soluble (nonmembrane-based) forms of these receptors, such as sFlt-1, also bind VEGF isoforms.

The set of exons encoding each VEGF isoform determines the specific binding of that isoform to the receptors (Fig. 2.1a). For example, $VEGF_{165}$, which contains exon 7, can bind to NRP1, and bridge VEGFR2 and NRP1 (Soker et al. 2002). $VEGF_{121}$ does not contain exon 7 and was thought not to be able to bind NRP1 (Neufeld et al. 2002; Pan et al. 2007). Recently, however, it was experimentally shown that, although unable to bridge VEFGR2 and NRP1, $VEGF_{121}$ does bind NRP1 (Pan et al. 2007). NRP1 also serves as a receptor to PlGF-2 and $VEGF_{145}$, while NRP2 serves as a receptor to $VEGF_{165}$, PlGF-2, $VEGF_{145}$, and VEGF-C (Neufeld et al. 2002; Gluzman-Poltorak et al. 2000). Both NRP1 and NRP2 are thought to enhance $VEGF_{121}$-stimulated signal transduction by the VEGFR-2 receptor (Pan et al. 2007; Shraga-Heled et al. 2007). Because of its involvement in cancer, neuropilin receptors are potential targets for antiangiogenic treatments (Geretti and Klagsbrun 2007).

As with neuropilins, class 3 semaphorins (SEMA3) were originally discovered for their involvement in the nervous system but have shown promising results

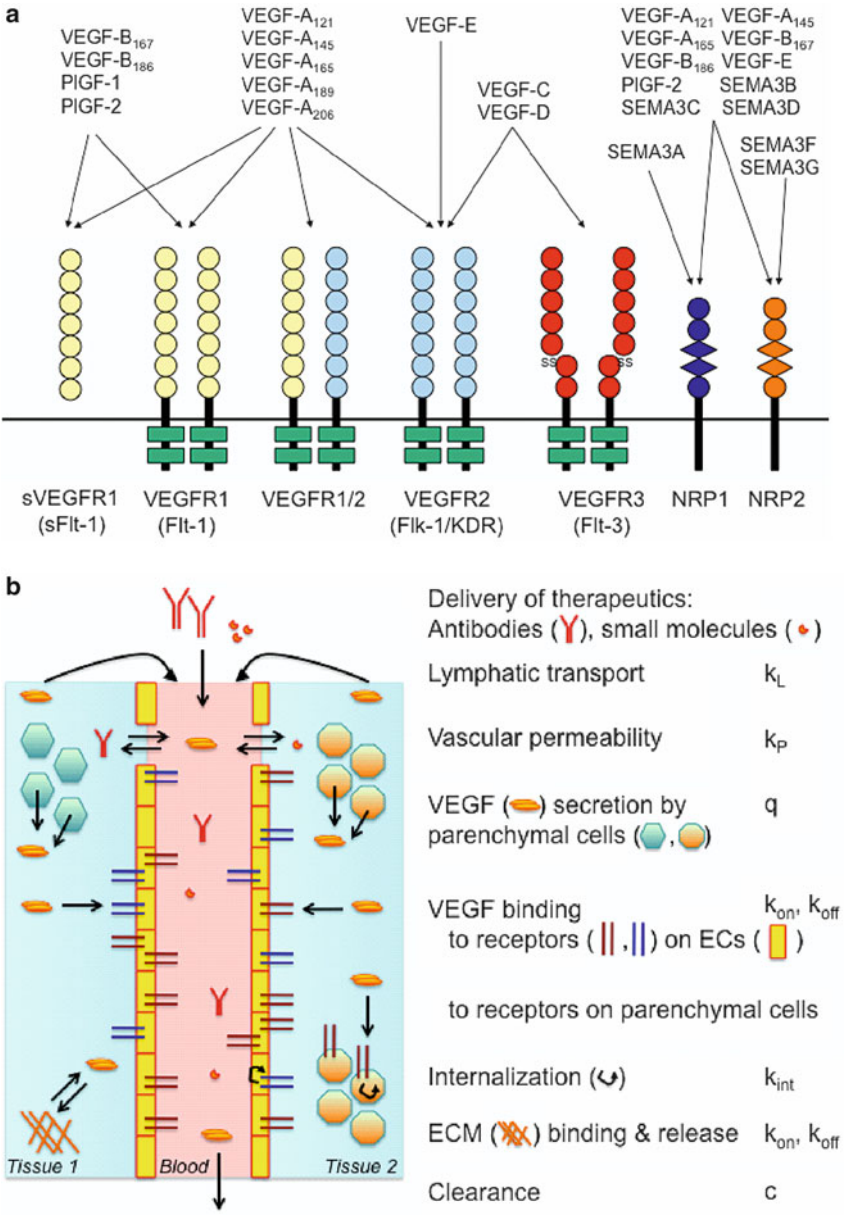

Fig. 2.1 Schematics of the VEGF-VEGFR interactions and pharmacokinetic-pharmacodynamic (PK/PD) models. (**a**) At the heart of the coupled-ODE model is a reconstruction of the kinetic interaction network between the various VEGF ligands and their receptors. Simulating the pharmacodynamics of VEGF-targeting agents requires including the interactions of the agent with these VEGF and VEGFR molecules. (**b**) The pharmacokinetic model includes the transport of VEGF, and any therapeutics for testing, within and between compartments (tissues). We combine this model with the kinetics of VEGF (**a**) and pharmacodynamics of therapeutic agents

in tumor angiogenesis (Gaur et al. 2009; Geretti et al. 2008; Rizzolio and Tamagnone 2007). The inhibitory effects of SEMA3 may be due to competitive binding to VEGF receptors and coreceptors. SEMA3A binds to NRP1, whereas SEMA3F and SEMA3G bind to NRP2. Other members (SEMA3B, SEMA3C, SEMA3D) bind to both neuropilins. Because of this competition with VEGF isoforms, exogenously administered class 3 semaphorins could serve as potential anti-VEGF therapy.

3 Multiscale Models of the VEGF Receptor System

In order to look at tumor angiogenesis and the effects of different angiogenic targets, multiscale computational models have been developed. These models are classified in two categories: spatial and nonspatial (compartment) models.

Spatial multiscale models focus on the spatial VEGF distribution within tissues and how the tumor microenvironment plays a role in drug delivery; they include diffusion and possibly convection terms for diffusible VEGF ligands and therapeutic molecules (Qutub et al. 2010; Wu et al. 2009). Matrix metalloproteinases (MMPs) are key factors in the matrix degradation, facilitate the tip cell migration into the tissue, and cleave VEGF molecules into smaller isoforms. Some spatial models therefore include the interactions between VEGF and MMPs (Small et al. 2008; Vempati et al. 2010).

In compartment models, each compartment can represent a tissue volume, an organ or an organ system; compartments are characterized by their total volume, the volumes and surface areas of the cell types that comprise the tissue, the vascular volume, and the concentrations of the molecules of interest. An ensemble of these compartments can therefore model an organ system or the whole body, and are thus more suitable for a pharmacokinetic-pharmacodynamic approach, applied to the human body or to animal disease models with species-specific parameters.

As an example, a compartmental model was used to investigate VEGF distribution in the whole body in healthy subjects and cancer patients. This design was motivated by a meta-analysis that showed that VEGF concentration was several-fold higher in cancer patients as compared to healthy subjects on average (Kut et al. 2007). This model was a necessary step for simulating the administration of antiangiogenic drugs. The model is divided into: the vascular system; the tissue of interest (in this case, a tumor; in the case of peripheral arterial disease, ischemic calf muscle); and the rest of the body (Fig. 2.1b, Stefanini et al. 2011; Wu et al. 2009; Stefanini et al. 2008). The models retain tissue characteristics (cell geometry, basement membranes, extra-cellular matrix). The molecular interaction of VEGF with its receptors, as described earlier, as well as VEGF secretion, receptor internalization, and binding to the extracellular matrix are included. Diffusible molecules travel between the compartments via microvascular permeability and lymphatic drainage and are cleared from the system by plasma clearance (Fig. 2.1b). Ligands in the system, such as VEGF, are described by nonlinear ODEs in the form:

$$\frac{\mathrm{d}[V_i]_N}{\mathrm{d}t} = q_i^N + \sum_j \left(k_{\mathrm{off},VR}[V_i R_j]_N - k_{\mathrm{on},V,R}[V_i]_N[R_j]_N\right) + \sum_j \left(k_{\mathrm{off},VM}[V_i M_j]_N - k_{\mathrm{on},V,M}[V_i]_N[M_j]_N\right) + \frac{S_{NB}}{U_N}\left(k_{pV}^{BN}\frac{[V_i]_B}{K_{AV,B}} - k_{pV}^{NB}\frac{[V_i]_N}{K_{AV,N}}\right) - \frac{k_L}{U_N}\frac{[V_i]_N}{K_{AV,N}}. \tag{2.1}$$

On the right-hand side, q represents the secretion of VEGF isoform i by the parenchymal cells in the normal tissue compartment (denoted N); the second and third terms are the binding interactions of VEGF with receptors (R) or matrix components (M); the fourth and fifth terms correspond to intercompartmental transport (microvascular permeability and lymphatic drainage, respectively) between the tissue (N) and the blood (B). $K_{AV,i}$ represents the ratio of the fluid volume accessible to VEGF and the total volume of the same tissue U_i, while S_{NB} represents the tissue–blood interface area. The kinetic parameters k_{off}, k_{on}, k_{pV}^{BN} ,and k_L are the dissociation and association rates of VEGF with the receptors or the matrix components, the microvascular permeability rate for VEGF (from the blood B to the tissue N) and the lymphatic drainage rate of VEGF, respectively. A similar equation governs the temporal variation of the VEGF concentration in the tumor (denoted T), with the additional assumption that the lymph flow rate is assumed to be negligible in this compartment, as tumor lymphatics are thought not to properly function because of the interstitial pressure exerted on the vessels (Fukumura and Jain 2007; Jain and Fenton 2002; Ji 2006). Thus, the equation reads:

$$\frac{\mathrm{d}[V_i]_T}{\mathrm{d}t} = q_i^T + \sum_j \left(k_{\mathrm{off},VR}[V_i R_j]_T - k_{\mathrm{on},V,R}[V_i]_T[R_j]_T\right) + \sum_j \left(k_{\mathrm{off},VM}[V_i M_j]_T - k_{\mathrm{on},V,M}[V_i]_T[M_j]_T\right) + \frac{S_{TB}}{U_T}\left(k_{pV}^{BT}\frac{[V_i]_B}{K_{AV,B}} - k_{pV}^{TB}\frac{[V_i]_T}{K_{AV,T}}\right). \tag{2.2}$$

Finally, the blood communicates with both other compartments and the temporal variation of plasma VEGF concentration is governed by:

$$\frac{\mathrm{d}[V_i]_B}{\mathrm{d}t} = -c_V[V_i]_B + \frac{S_{NB}}{U_B}\left(k_{pV}^{NB}\frac{[V_i]_N}{K_{AV,N}} - k_{pV}^{BN}\frac{U_B}{U_p}[V_i]_B\right) + \frac{k_L}{U_B}\frac{[V_i]_N}{K_{AV,N}} + \frac{S_{TB}}{U_B}\left(k_{pV}^{TB}\frac{[V_i]_T}{K_{AV,T}} - k_{pV}^{BT}\frac{U_B}{U_p}[V_i]_B\right), \tag{2.3}$$

where c_V represents the clearance of VEGF from the plasma, and U_p is the volume of plasma.

The earlier equations are replicated for each of the isoforms of VEGF included in the model, with isoform-specific parameters for binding and transport. The addition of therapeutic molecules (such as antibodies to VEGF – see Sect. 4) requires additional equations to describe transport of those proteins, and terms describing the interaction of these proteins with VEGF isoforms are added to the VEGF equations.

4 Targeting VEGF Ligands

Several molecules targeting VEGF and its receptor tyrosine kinases have been under development in the past decade (Hsu and Wakelee 2009). These drugs may be antibodies, short peptides, fusion proteins, or small molecules and they vary in isoform specificity.

We have extended our compartment model for VEGF outlined earlier by adding equations describing the kinetics and transport of a VEGF-sequestering agent A in the blood, normal and tumor tissue compartments; e.g., in the blood compartment (Stefanini et al. 2011):

$$\frac{\mathrm{d}[A]_B}{\mathrm{d}t} = q_A - c_A[A]_B + \sum_i \left(k_{\mathrm{off},VA}[V_i A]_B - k_{\mathrm{on},V,A}[V_i]_B[A]_B\right) - k_{pV}^{BN}\frac{S_{NB}}{U_p}[A]_B + \left(\frac{k_L + k_{pV}^{NB}S_{NB}}{U_B}\right)\frac{[A]_N}{K_{AV,N}} - k_{pV}^{BT}\frac{S_{TB}}{U_p}[A]_B + k_{pV}^{TB}\frac{S_{TB}}{U_B}\frac{[A]_T}{K_{AV,T}}. \quad (2.4)$$

VEGF-sequestering molecules include humanized monoclonal antibodies (such as bevacizumab or HuMV833), and fusion proteins (such as aflibercept, a fusion of VEGF-binding domains of VEGFR1 and VEGFR2; Holash et al. 2002; Tew et al. 2010; Lockhart et al. 2010; Jayson et al. 2005; Jayson et al. 2002).

Using parameters specific for bevacizumab, Fig. 2.2 illustrates predictions of the computational model for the response of VEGF in plasma and tumor interstitium following drug infusion. Tumor VEGF concentrations do not appear to decline if the bevacizumab is restricted to the plasma. In addition, free VEGF concentration in plasma is predicted to decrease if the anti-VEGF agent is confined in the blood but will increase if the anti-VEGF agent extravasates into the tissue interstitium in accordance with its molecular-weight dependent permeability (Fig. 2.2b). This last result is in agreement with observations in several clinical studies (Gordon et al. 2001; Segerstrom et al. 2006; Willett et al. 2005; Yang et al. 2003) These simulations also reveal that one of the modes of action of the anti-VEGF agent is to deplete the tumor interstitium of free VEGF concentration.

While most antiangiogenic therapies target one or multiple isoforms of VEGF-A, evidence is mounting that other ligands of the VEGF family may also be useful targets (Fischer et al. 2008). PlGF-2 ligates and activates VEGFR1 but not VEGFR2 (Fig. 2.1a), and appears to be pro-angiogenic and synergistic with VEGF-A (Autiero et al. 2003). An antibody to mouse PlGF-2 was reported to decrease

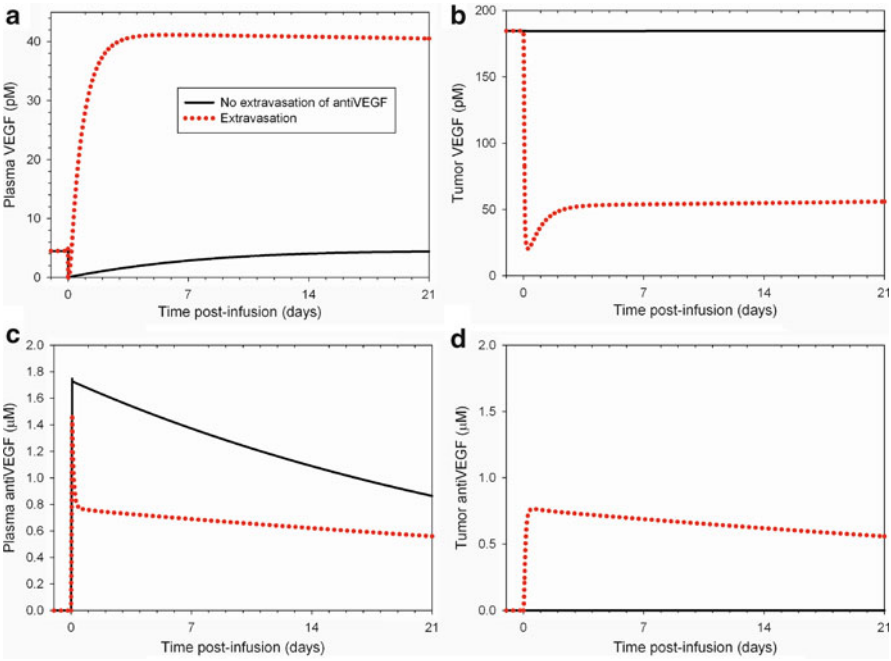

Fig. 2.2 Simulations of antiVEGF treatment: transport and effect on VEGF. The impact on VEGF in the plasma (**a**) or in the interstitial space of a tumor (**b**) of infusing a VEGF-sequestering agent depends on the ability of that agent to extravasate (**c**–**d**). Extravasation (*red lines*) is required for decrease in VEGF within the tissue, but not in the blood. The decrease in the tissue is also long-lasting, despite a rebound of VEGF in the blood. Simulations based on three-compartment model of VEGF transport (Stefanini et al. 2011)

the growth and metastasis of certain pancreatic, colon, and melanoma syngeneic tumors, and enhanced response to anti-VEGFR2 antibodies (Fischer et al. 2007). However, different PlGF-2 antibodies with similar characteristics were reported to fail to reduce growth and inhibit angiogenesis in many tumors, including some of the same tumor lines tested for the first antibody (Bais et al. 2010). Further studies with additional anti-PlGF antibodies reported significant differences among these molecules in the inhibition of neovascularization (Van de Veire et al. 2010). This variability in response between groups of investigators may reflect the complexity in controlling the VEGF system in vivo, with subtle changes in approach resulting in altered efficacy. Computational modeling of these individual antibodies may allow for effective therapeutic design. In single-compartment simulations, the expression of either PlGF isoform can impact the efficacy of VEGF-targeted therapy (Fig. 2.3a), while inhibition of both VEGF and PlGF is dependent on the local receptor expression profile (Fig. 2.3a, b). An antibody to VEGF-B, which like PlGF binds VEGFR1 but not VEGFR2, has also been shown to regress neovascular growth in corneal models (Zhang et al. 2009). None of the anti-PlGF or anti-VEGF-B antibodies has yet entered human clinical trials.

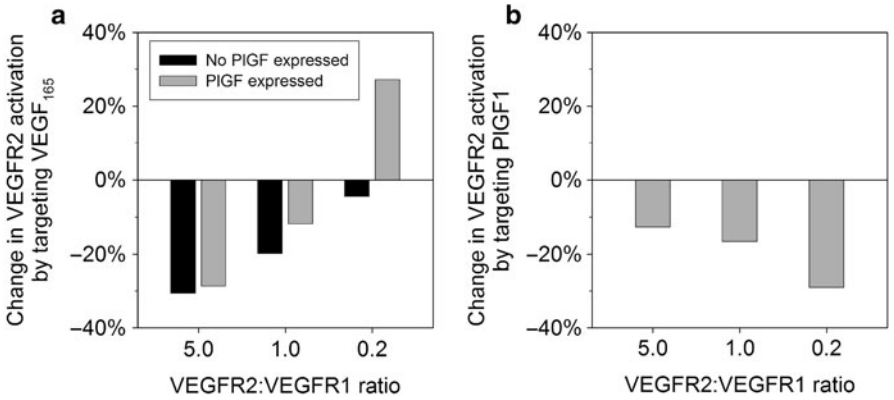

Fig. 2.3 Simulations of therapies targeting VEGF ligands. (**a**) The ability of anti-VEGF targeting agents to alter VEGFR2 activation depends on the relative expression of receptors and of competing ligands, such as PlGF. (**b**) Anti-PlGF targeting has a different dependence on receptor expression. Simulations based on steady inhibition of the ligands in a single-compartment model

5 Targeting VEGF Receptors

The ability of VEGF to initiate and sustain angiogenesis signaling is mediated by binding to VEGFRs, and VEGFR targeting is a possible route for anti-angiogenic therapies (Lyons et al. 2010; Shibuya 2006; Shibuya and Claesson-Welsh 2006). Such therapeutic interventions can also be described by computational models. Equations governing VEGF receptors already utilized in the developed models are of the form:

$$\frac{\mathrm{d}[R_j]_N}{\mathrm{d}t} = s_{Rj} - k_{\mathrm{int},R}[R_j]_N + \sum_i \left(k_{\mathrm{off},VR}[V_i R_j]_N - k_{\mathrm{on},V,R}[V_i]_N[R_j]_N\right) + \sum_{k\neq j}\left(k_{\mathrm{dissoc},RjRk}[R_j R_k]_N - k_{c,Rj,Rk}[R_j]_N[R_k]_N\right), \tag{2.5}$$

where s_R and k_{int} represent the insertion and internalization rates of the receptor R_j. The second term illustrates the binding of VEGF to VEGF receptors and the third term corresponds to the coupling of neuropilins and VEGFRs. Incorporating anti-VEGFR antibodies A into these equations requires additional terms:

$$\frac{\mathrm{d}[R_j]_N}{\mathrm{d}t} = s_{Rj} - k_{\mathrm{int},R}[R_j]_N + \sum_i \left(k_{\mathrm{off},VR}[V_i R_j]_N - k_{\mathrm{on},V,R}[V_i]_N[R_j]_N\right) + k_{\mathrm{off},AR}[AR_j]_N - k_{\mathrm{on},V,A}[A]_N[R_j]_N + \sum_{k\neq j}\left(k_{\mathrm{dissoc},RjRk}[R_j R_k]_N - k_{c,Rj,Rk}[R_j]_N[R_k]_N\right). \tag{2.6}$$

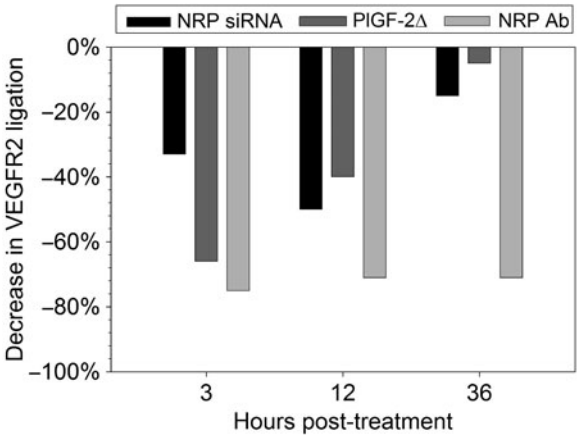

Fig. 2.4 Targeting neuropilin to inhibit VEGFR2 signaling. Three methods for targeting neuropilin – decreasing expression with siRNA (NRP siRNA), blocking VEGF-NRP binding with a fragment of PlGF (PlGF-2Δ), or blocking VEGFR-NRP coupling with a NRP antibody (NRP Ab), differ significantly in their ability to create and sustain VEGFR2 inhibition. Simulations based on single-compartment model (Mac Gabhann and Popel 2006)

Most antibodies and short-peptide drugs compete with ligands for the ligand-binding site on VEGFR1 (Schwartz et al. 2010; Wu et al. 2006; Hattori et al. 2002), VEGFR2 (Youssoufian et al. 2007; Witte et al. 1998; Krupitskaya and Wakelee 2009; Spratlin et al. 2010), or NRP1 (von Wronski et al. 2006; Barr et al. 2005). Some interfere with the coupling reaction or dimerization (Mac Gabhann and Popel 2006; Kolodkin et al. 1997); these behaviors are incorporated into the equations for the antibody and for the antibody–receptor complex.

Thus, an antibody to the VEGF-binding domain of NRP competes with (and has a similar mathematical formulation to) a VEGF isoform; while an antibody to the NRP dimerization domain does not compete with ligand binding, but creates an antibody–NRP complex that has different VEGFR-coupling characteristics. The outcomes of these approaches are quite different, as shown in Fig. 2.4 for neuropilin-targeting drugs; blocking VEGF binding displaces VEGF from neuropilin to the other VEGF receptors, while blocking coupling allows the internalization of VEGF by neuropilin without the involvement of VEGFRs, thus decreasing overall VEGF binding to VEGFRs (Mac Gabhann and Popel 2006).

Tyrosine kinase inhibitors (TKIs) are small molecule ATP competitors, less specific than antibodies, inhibiting signaling of several VEGFRs and related receptors. In the mathematical models, small molecules such as TKIs are transported differently to proteins due to their ability to cross cell membranes, and their binding does not interfere with ligand binding.

Neuropilin or the VEGFRs are expressed on the tumor cells themselves in several cancers (Ellis 2006). Thus a NRP-targeting drug can result in inhibition of NRP on both endothelial cells and tumor cells, a potentially beneficial impact; however it

reduces the impact of the inhibitor on the endothelial cell specifically, thus blunting any vascular-specific effects. Mathematically, the expression of VEGF receptors on parenchymal or other cells is incorporated as a distinct receptor population that competes for the binding of interstitial VEGF and other ligands or inhibitors (Fig. 2.1b).

6 Lymphangiogenesis, Angiogenesis, and Targeting VEGFR3

VEGF receptors are present on the endothelial cells lining both blood vessels and lymphatic vessels (Xu et al. 2010). VEGFR3 has been used as a marker for lymphatic vessels, however recent evidence supports expression of both VEGFR2 and VEGFR3 on both vascular and lymphatic cells (Nilsson et al. 2010; Nilsson et al. 2004; Dixelius et al. 2003), and blockade of VEGFR3 has been reported to reduce neovascularization in embryoid body models of vascular development in vitro (Nilsson et al. 2010). A VEGFR3 antibody is in development for oncology applications, possibly in combination with anti-VEGFR2 antibodies, along with a diabody of anti-VEGFR2 and anti-VEGFR3 molecules (Jimenez et al. 2005). Neuropilin may also be involved in VEGFR3 signaling, increasing the complexity further (Xu et al. 2010).

VEGFR3 blockade also inhibits the lymphangiogenesis (Bock et al. 2008; Tammela and Alitalo 2010) that contributes to tumor growth and metastasis. The ability to inhibit both blood and lymphatic vascular formation and the fact that expression of VEGFR3 appears to be specifically associated with active angiogenic sprouts (Nilsson et al. 2010; Tammela et al. 2008), make it a useful target for pathological growth.

The impact of VEGFR3 on angiogenesis appears to be via VEGFR2/VEGFR3 heterodimers (Nilsson et al. 2010), which demonstrate different phosphorylation profiles than VEGFR2 homodimers. Mathematically, dimerization of VEGFRs has been modeled using surface-restricted receptor-coupling (Mac Gabhann and Popel 2007), as described earlier for the neuropilin-VEGFR interactions; activation of VEGFRs requires VEGF to bind two receptor monomers simultaneously. The expression of VEGFR3 on both vascular and lymphatic endothelial cells can be modeled as described earlier for parenchymal expression of receptors; the two receptor populations will compete for binding of ligands and antibodies. Therapies targeting VEGFR3 can therefore be included similarly to those targeting other receptors.

7 DLL4-NOTCH

VEGF receptors are internalized, recycled, degraded, and new receptors inserted into the membrane continuously. The expression of VEGFR1, 2, and 3 is an opportunity for external control of the system by therapeutics, and is controlled both

by VEGF signaling itself within a single cell, and by a cell–cell communication mechanism mediated by Dll4 and Notch (Jakobsson et al. 2009); typically, this permits the repression of VEGFRs on neighboring cells to suppress close sprouting (Tammela et al. 2008; Suchting et al. 2007), and inhibition of this Dll4 axis has been shown to inhibit tumor growth (Noguera-Troise et al. 2006; Ridgway et al. 2006). Simulation of this dynamic control requires the first term in (2.5) to be dependent on the activation of receptors on adjacent cells (with or without explicit simulation of the Dll4-Notch interaction). Models of these dynamics can result in realistic sprout simulation (Bentley et al. 2008, 2009; Qutub and Popel 2009), allowing molecules that interfere with VEGFR production to be simulated.

8 Conclusion and Future Studies

We have systematically outlined existing and prospective mathematical models to describe the VEGF family. We included both compartment and spatial models that describe VEGF ligands and their receptors and some of the important molecules that are associated with this family. Multiple extracellular and intracellular molecular interactions define a complex web that will increasingly require the power of systems biology (computational models, bioinformatics, high-throughput experiments) to unravel this complexity and to predict therapeutic approaches that can successfully control the behavior of the system (Laubenbacher et al. 2009; Edelman et al. 2010). Important translational information can be derived from compartment models in which various pharmacological agents targeting angiogenic factors are introduced. The agents can be small molecules, peptides, oligonucleotides, or macromolecules; the targets can be growth factors, cell-membrane or intracellular receptors, enzymes, signaling molecules, or genetic elements. In parallel with these developments, complex spatial models of vascular and tumor growth will continue to evolve. Significant progress has already been achieved, but emerging temporal and spatial data from animal models and humans using different imaging methodologies should contribute significantly to the progress. ABM appears to be an appropriate methodology, combined with PDE-based methods. Integrative models of this kind describe the phenomena at multiple scales and comprise models that can be formulated autonomously (e.g., ligand–receptor interactions; transcriptional control of angiogenic genes; cell proliferation, migration, and apoptosis; oxygen transport, blood flow; capillary sprout formation; microvascular network maturation; antiangiogenic drug pharmacokinetics) and then combined computationally as interacting modules (Qutub 2009). To incorporate an increasing number of modules, likely from different laboratories, a computational systems biology infrastructure is required, e.g., markup languages to formulate models in standardized form, model repositories, parameter databases, and effective simulation tools such as for ABM and PDEs (Popel and Hunter 2009). These developments addressing the complexity of the disease at the multiple levels, from gene to organism, will eventually lead to novel effective agents and procedures for cancer therapeutics.

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