

Computational models of VEGF-associated angiogenic processes in cancer

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[Received on 16 October 2009; revised on 14 November 2010;
accepted on 21 December 2010]

Tumour angiogenesis allows a growing mass of cancer cells to overcome oxygen diffusion limitation and to increase cell survival. The growth of capillaries from pre-existing blood vessels is the result of numerous signalling cascades involving different molecules and of cellular events involving multiple cell and tissue types. Computational models offer insight into the mechanisms governing angiogenesis and provide quantitative information on parameters difficult to assess by experiments alone. In this article, we summarize results from computational models of tumour angiogenic processes with a focus on the molecular-detailed vascular endothelial growth factor-associated models that have been developed in our laboratory, spanning multiple scales from the molecular to whole body.

Keywords: computational biology; systems biology; angiogenesis.

1. Introduction

When diffusion is not sufficient to sustain oxygen delivery to a tumour, new capillaries may arise from nearby pre-existing blood vessels. This phenomenon, known as tumour angiogenesis, has its origin within the cancer cells where transcription factors stimulate secretion of growth factors into the extracellular space. The binding of these growth factors to their receptors on the endothelial cells lining the blood vessels induce signalling cascades which initiate the growth of the new vasculature from the existing vessels.

Several research groups have modelled tumour angiogenesis by focusing on the structural or mechanistic aspects of the newly formed blood vessels, while grouping the growth factors into a generic ‘tumour angiogenic factor’ (Chaplain *et al.*, 2006). However, each molecule plays a specific role in this process and ideally these molecular details would be maintained in tumour angiogenic models (Mantzaris *et al.*, 2004). In particular, multiscale models of tumour angiogenesis are of high interest as they provide insights into tissue and organ level effects while keeping the molecular level detail.

General recent reviews on the mathematical modelling of angiogenesis are available (Anderson & Quaranta, 2008; Byrne, 2010; Peirce, 2008). In this article, we specifically focus on the effects of vascular endothelial growth factor (VEGF) in tumour angiogenesis. We refer to the models formulated by our laboratory and other researchers, spanning multiple levels, from transcription factor to whole body, and summarize their main results. This includes the intracellular, cellular, tissue and whole-body levels. At the tissue level, we consider both spatial and non-spatial models.

2. Models of transcription factor HIF1 α pathways

As tumours grow beyond ~ 1 mm, they no longer can rely purely on diffusion to provide them with a sufficient oxygen supply. Hypoxia is a major stimulus for the recruitment of local host vasculature and the growth of capillaries within tumours. As such, some of the key molecules involved in tumour angiogenesis are transcription factors mediated by intracellular oxygen levels.

While numerous transcription factors and growth factors are involved in angiogenesis and cellular hypoxic response (e.g. protein 53, nuclear factor κ B, phosphoinositide 3-kinase, insuline-like growth factor, fibroblast growth factor), for the purposes of this article, we focus on one key transcription factor hypoxia-inducible factor (HIF1) and one key HIF-activated growth factor—VEGF.

HIF1 α is a transcription factor that has been called both an ‘angiogenic switch’ (Semenza, 2000) and a requirement for sustained angiogenesis (Dewhirst *et al.*, 2007). HIF1 α is omnipresent—a factor all nucleated cells in the human body are thought to be capable of expressing. However, in a basal normoxic state, HIF1 α is rarely detected. This is the result of HIF1 α ’s very rapid hydroxylation and subsequent degradation in the presence of oxygen. Conversely, when oxygen levels drop, HIF1 α cannot be hydroxylated. Instead, it enters the cell’s nucleus, where it binds to its constitutively expressed partner, HIF1 β /aryl hydrocarbon receptor nuclear translocator. This α - β dimer then activates genes through their hypoxic response element. The large number of genes that HIF1 regulates makes its pathway a prime target for both pro- and anti-angiogenic therapies.

To help characterize such a complex network, computational modelling has emerged as a prominent tool (Dayan *et al.*, 2009; Kohn *et al.*, 2004; Qutub & Popel 2006, 2007, 2008; Yu *et al.* 2007; Yucel & Kurnaz 2007). All the models have focused on characterizing the dynamics of HIF1’s intracellular signalling pathway. One of the first computational models of the HIF1 pathway tested therapeutic strategies to enhance hydroxylation—a potential mechanism to lower HIF1 α levels in cancer cells (Qutub & Popel, 2006). Enzymes called the prolyl hydroxylase domain-containing proteins (PHDs) require the cofactors iron, deoxoglutarate and oxygen, prior to binding and hydroxylating HIF1 α . The model predicted the degree to which supplementing with ascorbate (vitamin C) could rescue iron from its ferric form, freeing it to bind to the PHDs, and increase HIF1 α hydroxylation. Subsequent experiments have shown ascorbate can shrink tumours in mice (Gao *et al.*, 2007), and the PHD pathway is a target of anti-angiogenic drug development. Another HIF1-related model predicted how factor-inhibiting HIF1 can lead to the differential regulation of genes over a gradient of oxygen levels (Dayan *et al.*, 2009).

Along with hypoxia being a stimulus for tumour angiogenesis, the ability of some cancer cells to adjust—even thrive—in a hypoxic microenvironment is an equally intriguing driving force behind

computational models of the HIF1 pathway. High expression of HIF1 α has been linked to a tumour's resistance to radiation and chemotherapy (Moeller *et al.*, 2004; Zhou *et al.*, 2006). One hypothesized mechanism is through the generation of reactive oxygen species. An intracellular computational model predicted the effects of reactive oxygen species on HIF1 α expression levels, and showed quantitatively how hydrogen peroxide and moderate HIF1 α levels in cancer cells could allow for sustained cell growth (Qutub & Popel, 2008). The hypoxic microenvironment may also provide a fertile niche for 'cancer stem cells'—self-renewing cells that are not yet fully differentiated and that are prone to become cancerous.

Understanding the crosstalk between cancer cells and their microenvironment as well as cancer cells and endothelial cells in the presence of hypoxia requires going beyond intracellular models of HIF1. Extending these models to the tissue level and combining them with mechanistically detailed models of upstream regulators and downstream growth factors will further advance anti-angiogenic therapeutic development.

3. Single-compartment tissue models describing VEGF ligand–receptor interactions

Homeostatic regulation of VEGF is vital for physiological angiogenesis and dysregulation can result in disease (Ferrara, 2005; Folkman, 1990) and has been studied in computational modelling (Mac Gabhann & Popel, 2008; Qutub *et al.*, 2009). Thus another method to limit oxygen delivery to the vascularized tumour is to diminish or abrogate VEGF signalling, which results in reduction or even regression of neovascularization. To determine optimal strategies for vascular endothelial growth factor inhibition, we developed a computational model of VEGF distribution in tissue applicable to many diseases and used the model to compare multiple methods of targeting neuropilin-1 (NRP1), a VEGF co-receptor, in breast tumours (Mac Gabhann & Popel, 2006). The model describes kinetic ligand–receptor interactions between VEGF isoforms (VEGF₁₂₁, VEGF₁₆₅) and endothelial cell surface receptors (VEGFR1, VEGFR2 and NRP1), as well as extracellular matrix (ECM) binding sites for VEGF₁₆₅. VEGFR1 binding to NRP1 and forming VEGFR2–VEGF₁₆₅–NRP1 triplets are considered. This model does not describe 3D tissue geometry explicitly; instead, it comprises spatially integrated components (e.g. capillaries, endothelial cells, interstitial space and basement membranes) and within its components, concentrations of molecular species are defined. Several of our observations from this model are generalizable to other ligand–receptor systems.

First, we noted that direct interference with the binding of a ligand to one receptor, e.g. a decrease in receptor expression or blockade of the ligand-binding site on the receptor, does not impact the steady-state receptor activation unless there is an alternative receptor competing for the ligand to bind. Due to the constant production of new ligand by the parenchymal cells, the ligand will passively overcome a barrier such as this by increasing its concentration (due to reduction in internalization) unless it is diverted to a different internalization route. This also introduces a characteristic time—some therapies reduce signalling for longer than others.

Second, as a consequence of the first observation, the outcome of therapeutic interventions depends not only on the expression of the target molecule but also on other ligands and receptors within the same family. Thus, efficacy varies based on differences in both tumour and host (e.g. receptor expression on normal vs. pathological blood vessels and on tumour vs. parenchymal cells).

Third, one effective method for the reduction in efficacy of a ligand is the conversion of a cell surface receptor to a 'dummy' receptor, i.e. a receptor that binds the ligand and internalizes it, without initiating a signal. The example from our study is an antibody that binds to NRP1, allowing VEGF to bind but sterically preventing the subsequent presentation of VEGF to the signalling VEGFR2 receptor. By providing a novel pathway for the removal of ligand from the interstitial space, this takes advantage

of the competing ligand–receptor system to simultaneously reduce VEGF concentration and signalling efficiency.

In our study, inhibition of VEGFR2 signalling was effected by blocking NRP1 binding only when VEGFR1 was also present; whereas the conversion of NRP1 to a dummy receptor was shown to be effective in both VEGFR1-expressing and non-expressing tissues. Thus, the model predicts the efficacy of therapies and also suggests tests that should be carried out to determine those characteristics of the tissues that will most determine which approaches should be used.

More generally, the models revealed the importance of the competition between multiple pathways for VEGF binding and internalization in assessing therapeutic potential. This becomes even more important in understanding the impact of new broad- and narrow-spectrum tyrosine kinase inhibitors that can target some or all the signal-initiation complexes that receptors form due to homo- and heterodimerization (Mac Gabhann & Popel, 2007).

4. Multi-compartment models of VEGF distribution

In current therapeutic anti-angiogenic strategies targeting VEGF, an anti-VEGF agent (e.g. bevacizumab—a humanized monoclonal antibody, or aflibercept [VEGF Trap]—a fusion protein [Gaur *et al.*, 2009]) is administered into the blood that subsequently binds and neutralizes VEGF. To investigate the distribution and transport of VEGF and potentially exogenous therapeutic agents between the normal tissue, blood and tumour, we have developed a three-compartment model (Fig. 1).

The model was motivated by our meta-analysis of elevated free (unbound) plasma VEGF in cancer patients compared to healthy subjects (Kut *et al.*, 2007). One of the aims of the model was to understand the underlying mechanisms that could explain this increase in VEGF when the cancer develops (Stefanini *et al.*, 2008). Molecular interactions in each compartment included predominant VEGF isoforms (VEGF₁₂₁ and VEGF₁₆₅), VEGF receptors (VEGFR1 and VEGFR2) and co-receptor NRP1.

The system is described by non-linear ordinary differential equations (ODEs) that include VEGF secretion and binding to its receptors, VEGF isoform binding to the ECM and basement membranes, receptor insertion and internalization and VEGF microvascular permeability and plasma clearance. The full system of equations can be found in Stefanini *et al.* (2008, 2009). As a sample, the VEGF₁₆₅ isoform variations in time in the available interstitial fluid and the blood are given by the following equations. The subscripts/superscripts N , B and T represent the normal tissue, the blood and the tumour, respectively. The definition of each term can be found in Table 1:

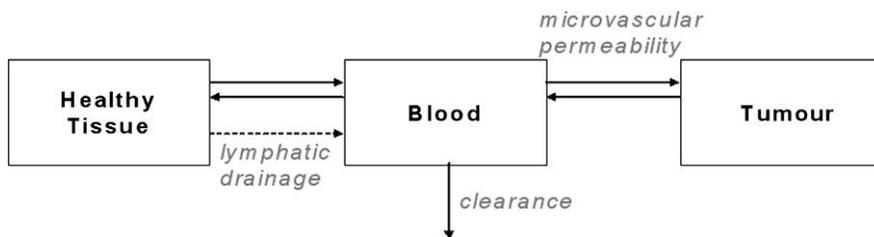


FIG. 1. Schematic of a tumour angiogenesis compartment model.

TABLE 1 *Glossary of terms used in (4.1) and (4.2)*

Concentrations and densities	
$[M_{ECM}]$	Density of VEGF binding sites in the ECM,
$[M_{EBM}]$	in the endothelial basement membrane (EBM) and
$[M_{PBM}]$	in the parenchymal basement membrane (PBM)
$[V_{165}]$	Concentration of unbound VEGF ₁₆₅ in the available interstitial fluid
$[R_1], [R_2]$	Density of the unoccupied receptor tyrosine kinases VEGFR1 and VEGFR2
$[N_1]$	Density of the unoccupied co-receptor NRP1
$[R_1 N_1]$	Density of the VEGFR1-NRP1 complex
$[V_{165} R_j]$	Concentration of VEGF ₁₆₅ bound to VEGF receptor j
$[V_{165} N_1]$	Concentration of VEGF ₁₆₅ bound to co-receptor NRP1
Kinetic parameters	
q_{V165}	Secretion rate of VEGF ₁₆₅
s_R	Rate at which the receptors are inserted into the cell membrane
k_{on}	Kinetic rate for binding
k_{off}	Kinetic rate for unbinding
k_{int}	Internalization rate of the receptors
k_{pV}^{ij}	Microvascular permeability k_p for VEGF (denoted as V) from compartment i to j ($N = \text{tissue}$; $B = \text{blood}$; $T = \text{tumour}$)
k_L	Lymph flow rate
c_{V165}	Clearance of VEGF ₁₆₅ in the blood
Geometric parameters	
U_i	Volume of the compartment i ($N = \text{tissue}$, $B = \text{blood}$, $T = \text{tumour}$; $p = \text{plasma}$)
S_{iB}	Total surface of the microvessels at the interface of the tissue (i) and the blood (B)
$K_{AV,i}$	Available volume fraction in the tissue i , i.e., ratio of available fluid volume to total tissue volume U_i

$$\begin{aligned}
 \frac{d[V_{165}]_N}{dt} = & q_{V165}^N - k_{on,V165,MEBM}[V_{165}]_N[M_{EBM}]_N + k_{off,V165,MEBM}[V_{165}M_{EBM}]_N \\
 & - k_{on,V165,MECM}[V_{165}]_N[M_{ECM}]_N + k_{off,V165,MECM}[V_{165}M_{ECM}]_N \\
 & - k_{on,V165,MPBM}[V_{165}]_N[M_{PBM}]_N + k_{off,V165,MPBM}[V_{165}M_{PBM}]_N \\
 & - k_{on,V165,R1}[V_{165}]_N[R_1]_N + k_{off,V165,R1}[V_{165}R_1]_N - k_{on,V165,R2}[V_{165}]_N[R_2]_N \\
 & + k_{off,V165,R2}[V_{165}R_2]_N - k_{on,V165,N1}[V_{165}]_N[N_1]_N + k_{off,V165,N1}[V_{165}N_1]_N \\
 & - \left(\frac{k_L + k_{pV}^N B S_{NB}}{U_N} \right) \frac{[V_{165}]_N}{K_{AV,N}} + k_{pV}^{BN} \frac{S_{NB}}{U_N} \frac{U_B}{U_p} [V_{165}]_B, \tag{4.1}
 \end{aligned}$$

$$\begin{aligned}
 \frac{d[V_{165}]_B}{dt} = & c_{V165}[V_{165}]_B - k_{on,V165,R1}[V_{165}]_B[R_1]_B \\
 & + k_{off,V165,R1}[V_{165}R_1]_B - k_{on,V165,R2}[V_{165}]_B[R_2]_B \\
 & + k_{off,V165,R2}[V_{165}R_2]_B - k_{on,V165,N1}[V_{165}]_B[N_1]_B + k_{off,V165,N1}[V_{165}N_1]_B
 \end{aligned}$$

$$\begin{aligned}
& - \frac{k_{pV}^{BN} S_{NB}}{U_p} [V_{165}]_B + \left(\frac{k_L + k_{pV}^{NB} S_{NB}}{U_B} \right) \frac{[V_{165}]_N}{K_{AV,N}} \\
& - \frac{k_{pV}^{BT} S_{TB}}{U_p} [V_{165}]_B + \frac{k_{pV}^{TB} S_{TB}}{U_B} \frac{[V_{165}]_T}{K_{AV,T}}.
\end{aligned} \tag{4.2}$$

A sensitivity analysis was performed and we found that an increase in tumour VEGF secretion and/or an increase in microvascular permeability in the tumour could not explain a 4–5 fold increase of VEGF concentration in plasma seen in breast cancer patients. Thus, additional mechanisms need to be incorporated into the design. Such a model allows testing of biological hypotheses and also provides insights into the VEGF distribution and the VEGF receptors and co-receptor occupancy.

These computational models can also serve to predict the system behaviour in response to pro- or anti-angiogenic treatments. For example, (1) a compartmental model was used to examine how soluble VEGF receptor-1 can trap VEGF (Wu *et al.*, 2009) and (2) the increase of plasma VEGF observed after intravenous administration of bevacizumab was reproduced by a pharmacokinetic model (Stefanini *et al.*, 2010).

5. Multi-scale 3D spatial models of angiogenesis

The designs discussed above (intracellular transcription factor pathways, growth factor receptor–ligand binding interactions and compartment models) are described by ODEs as they do not include explicit spatial representation. However, the process of tumour angiogenesis is highly dependent on spatial cues. In vivo, an endothelial cell on a quiescent microvessel can be activated by VEGF secreted by a tumour. Once activated, the leading cell (or tip cell) can start to migrate through the surrounding ECM in a heterogeneous, 3D microenvironment. This migration requires degrading the matrix as the cell migrates. The degree of endothelial cell migration and proliferation in a growing capillary sprout is a function of chemical and mechanical stimuli.

To characterize angiogenesis both in a quantitative and in a spatial way, 2D and 3D models have evolved addressing different parts of the process: microvascular blood flow with vessel growth (McDougall *et al.*, 2006) or oxygen transport to tissue (Ji *et al.*, 2006); effects of fibronectin in the ECM (Levine *et al.*, 2002); VEGF spatial gradients (Ji *et al.*, 2007; Mac Gabhann *et al.*, 2007; Mac Gabhann & Popel, 2006); tip cell migration and cell–matrix interactions via matrix metalloproteinases (Karagiannis & Popel, 2006); the sequence of cell elongation, migration and proliferation during capillary sprout formation (Qutub & Popel, 2009) and capillary anastomosis Milde *et al.* (2008). Two methods used to represent 3D spatial models include partial differential equations (PDEs) and agent-based models (ABMs). PDEs are frequently employed at the molecular level to describe time-dependent or steady-state diffusion and convection of molecular species, while ABMs have been applied to heuristically describe both molecular and cellular components. These methods and related models were recently reviewed in detail (Peirce, 2008; Qutub *et al.*, 2009; Sefcik *et al.*, 2010). 3D spatial models of angiogenesis have wide applications. As examples, they have been employed to study capillary sprout formation accounting for key proteins involved in angiogenesis (Bentley *et al.*, 2008, 2009; Peirce, 2008; Qutub & Popel, 2009) and tumour angiogenesis (Bearer *et al.*, 2009; Billy *et al.*, 2009; Owen *et al.*, 2009) (Fig. 2).

One 3D agent-based spatial model describes endothelial cell sprouting at the onset of angiogenesis (Qutub & Popel, 2009). Agents are computational datastructures that can respond to and be modified by their environment (Bonabeau, 2002). In turn, agents can modify each other and their surroundings. Logical rules determine how agents respond, and what events occur in a model. The rules that govern

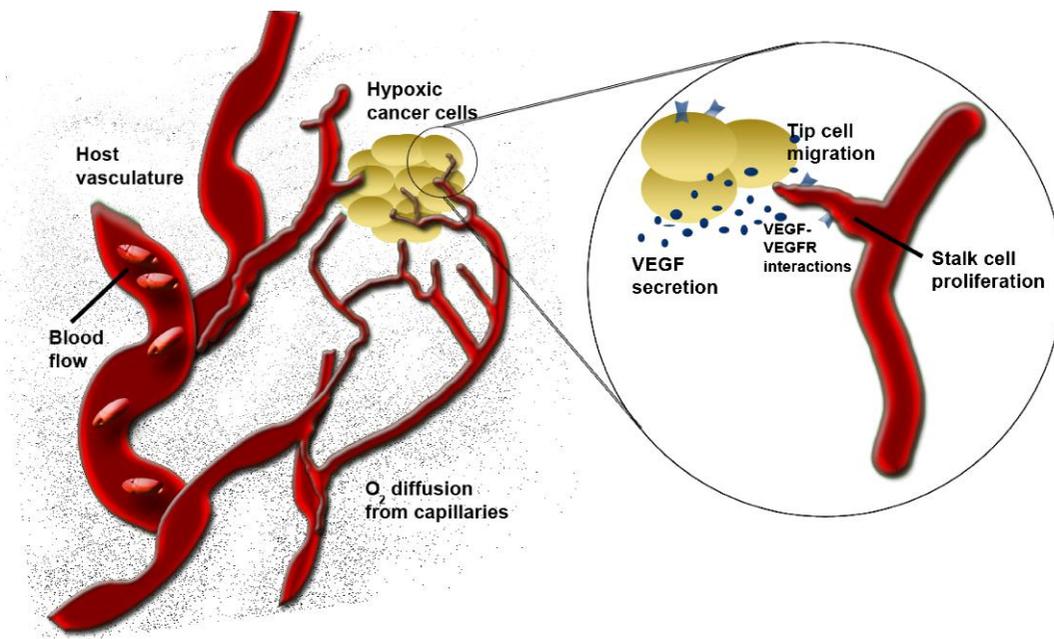


FIG. 2. Representation of the angiogenic processes by 3D models. These have included blood flow, O₂ diffusion, VEGF–VEGFR interactions, endothelial cell activation, migration and proliferation, capillary sprout and vascular network formation.

agents can be articulated as equations, Boolean queries, or probabilities, as well as numerous other computational methods. In building the model, we relied on experimental work compiled from extensive literature research and formulated the logical rules to represent biological processes consistent with experiments.

We first applied the model to conditions that might occur in a 3D *in vitro* setting. Physiological changes are represented at the cell level. These include endothelial cell activation, migration, elongation, proliferation and branching. We then tested cell-level behaviour in response to different stimuli, first a threshold change in VEGF and then changes in delta-like ligand 4 (Dll4). From the combined, combinatorially complex interactions of single cells, a new capillary network emerges. This capillary network can then be characterized phenotypically and spatially—by branching, multiple sprouting, anastomosing, looping and regression. We used this model to evaluate the hypothesis that the characteristics of a growing capillary network are dependent on a set sequence of cellular events: a combination of elongation, migration and proliferation between the leading tip cell and its adjacent stalk cell. Additionally, we produced *in silico* knockouts of the Dll4 ligand, and cell-level knockouts of individual cellular processes, and observed the resulting vascular network in each case—with the goal of eventually guiding therapy aimed at particular processes.

The ABM we described here can be used as a framework for integration of angiogenesis models in our laboratory (e.g. HIF1 α , VEGF, matrix metalloproteinase), and also could include contributions from multiple laboratories; the models spanning multiple scales, from molecular, to cellular, tissue, organ and organ system or whole body (Qutub *et al.*, 2009). The development involves creation of a computational framework that enables the computer simulation of multiple models with different mathematical representations (e.g. ODEs, PDEs and ABMs). A key point is that each model—spatial or non-spatial—plays a unique role (e.g. focuses on a unique molecule; new receptor–ligand interaction,

different spatial scale or cellular process). Through this means, each model individually helps elucidate the mechanisms underlying proteins and other molecular factors in angiogenesis, while the simulation resulting from the integration of the models is one step closer to being used for clinical and therapeutic applications.

6. Conclusions

In this article, we have shown that computational models of angiogenesis can provide useful insights, whether it is at the nano- to microscopic level (molecules/cells), at the mesoscopic level (microvascular/tissue) or at the macroscopic level (organ and organ system). The focus of our approach is to analyse complex processes in tumour angiogenesis while preserving the molecular details. This systems biology approach is complementary to other approaches, experimental and theoretical, used to study tumour angiogenesis.

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