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A cellular Potts model analyzing differentiated cell behavior during *in vivo* vascularization of a hypoxic tissue

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ABSTRACT

Angiogenesis, the formation of new blood vessel networks from existing capillary or post-capillary venules, is an intrinsically multiscale process occurring in several physio-pathological conditions. In particular, hypoxic tissue cells activate downstream cascades culminating in the secretion of a wide range of angiogenic factors, including VEGF isoforms. Such diffusive chemicals activate the endothelial cells (ECs) forming the external walls of the nearby vessels that chemotactically migrate toward the hypoxic areas of the tissue as multicellular sprouts. A functional network eventually emerges by further branching and anastomosis processes. We here propose a CPM-based approach reproducing selected features of the angiogenic progression necessary for the reoxygenation of a hypoxic tissue. Our model is able to span the different scale involved in the angiogenic progression as it incorporates reaction–diffusion equations for the description of the evolution of microenvironmental variables in a discrete mesoscopic cellular Potts model (CPM) that reproduces the dynamics of the vascular cells. A key feature of this work is the explicit phenotypic differentiation of the ECs themselves, distinguished in quiescent, stalk and tip. The simulation results allow identifying a set of key mechanisms underlying tissue vascularization. Further, we provide evidence that the nascent pattern is characterized by precise topological properties. Finally, we link abnormal sprouting angiogenesis with alteration in selected cell behavior.

1. Introduction

Blood vessel formation and development involves two different mechanisms: *vasculogenesis* and *angiogenesis* (for comprehensive reviews, see [6,9,10]). Vasculogenesis consists of the *de novo* formation of a primitive vascular network that emerges from migration, aggregation and organization of typically dispersed populations of endothelial cells (ECs). Angiogenesis refers instead to the formation of new vessels from an existing capillary or post-capillary venule. Although angiogenesis intervenes at the embryonic stage to remodel the initial capillary network into a mature and functional vascular bed (composed of arteries, capillaries, and veins), its main role is played during adult life, when it is involved in many physiological processes, as the vascularization of hypoxic tissues, of ovary and uterus during the female cycle, of mammary gland during lactation, and of granulation tissue during wound healing. However, when the equilibrium of its underlying control mechanisms is disrupted, angiogenesis becomes pathological, as in the case of chronic inflammatory diseases, vasculopathies, degenerative disorders, and tissue injury occurring in ischemia. Finally, angiogenic progression is a pivotal transition phase in the development of cancer: in fact, by providing nutrition and oxygen, it allows malignant cells to grow and remain viable, and, eventually, to spread metastases through invasion of the circulatory system [9,10].

Entering in more details, angiogenesis is a multiscale program which involves mechanisms at both the subcellular and the cellular level. The overall process starts when tissue cells, deprived of oxygen and/or nutrients, accumulate in the nucleus increasing amounts of hypoxic growth factor (HIF-1). By selected binding to DNA, HIF-1 is able to regulate the expression levels of numerous genes, which control the production of a wide range of angiogenic growth factors [13,29], including vascular endothelial growth factors (VEGFs), transforming growth factor β (TGF-β), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF). Such diffusive chemicals in turn stimulate the endothelial cells forming the external walls of preexisting blood vessels, which loosen their adhesive connections, thereby reducing the vascular tonus and increasing both the permeability the interstitial pressure. The activated ECs are then able to migrate chemotactically towards the hypoxic areas of tissues (i.e., those releasing

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angiogenic factors) as multicellular strands. Collective EC movement is facilitated by the production of proteolytic enzymes (serine-proteins, iron-proteins, matrix metalloproteinases) which degrade the basal lamina and the extracellular matrix surrounding the growing branches. An optimal extension of new vascular sprouts is allowed by a phenotypic differentiation of the component ECs, which undergo the so-called *tip cell selection and lateral inhibition*, which is mediated by VEGF-induced delta-notch signaling pathways. This process occurs in the following stages: (i) activation of VEGFR-2 receptors in a tip individual leads to up-regulation of ligand Dll4 [36]; (ii) Dll4, in turn, is able to activate Notch1 receptors in the neighboring cells, whose VEGFR-2 expression is consequently down-regulated. The Notch1-overexpressing individuals are set to assume a *stalk* fate [51,72]. The notch–Dll4 lateral inhibition generates therefore an alternating pattern of cell phenotype, a *salt and pepper* pattern [8], where tip cells are separated by one or two stalk cells. During angiogenic progression, tip individuals lead the way through the extracellular space (also by producing matrix degrading enzymes) while stalk cells, forming the rear of the multicellular strands, sustain branch elongation by repeated divisions along the axis perpendicular to the direction of sprout extension. During growth, vascular sprouts undergo branching or they encounter and merge to form loops, a process called *anastomosis*. From these branches and loops more sprouts form. Eventually, the whole process repeats several times, resulting in the formation of a capillary-like network. In a subsequent maturation stage, the new born vessels are remodeled into a more elaborate and hierarchically spaced vascular tree by pruning events resulting in the loss of some physiologically useless capillaries. The last phase of the angiogenic programme involves the formation and size-adaptation of the capillary lumen, the formation of new perivascular ECM and the arrival of pericytes and sometimes of flat muscle cells.

Experimental studies performed on *in vivo* systems have revealed the role of many different factors driving the formation of vascular networks via sprouting angiogenesis in physiological situations. However innumerable other processes, acting at different spatiotemporal scales, remain far from being completely understood. The complexity of the problem makes in fact it difficult and expensive to study using solely laboratory-based biological methods: indeed, the support and insight gained by using *in silico* approaches is vital. For the sake of completeness, we have to admit that although angiogenesis occurs in a wide range of physio-pathological circumstances and situations, the majority of the theoretical literature focuses on the tumor-induced angiogenesis, one of the most dangerous aspects, as reviewed in [58]. However, in this work, we propose a multilevel mathematical model reproducing an *in vivo* revascularization and reoxygenation of a hypoxic tissue. The endothelial cell population is described at the mesoscopic level with a discrete cellular Potts model (CPM), a lattice-based Monte Carlo technique which follows an energy minimization philosophy and preserves the identity and the behaviors of single individuals [21–24,55,61]. In particular, cell exploratory movements are implemented by stochastic acceptances of domain configuration updates, which depend on sets of cell physical and behavioral rules. Selected reaction–diffusion (RD) equations instead describe the evolution of molecular variables, i.e., angiogenic growth factors and oxygen. These two components are integrated and interfaced together, constituting a hybrid and multiscale simulation environment characterized by a constant flux of information from the different levels, as the kinetics of the microscopic variables strongly influence cell dynamics. Our model appears a possibly new and biologically interesting representative in the class of models of blood vessel development: we here in fact focus on the role played by the dynamical and self-emerging differentiation of phenotypes of both vascular cells (i.e., *quiescent*, *tip*, *stalk*) and of tissue cells (*oxygenated*, *hypoxic*). As we will see in the following, the model results will reproduce with good accuracy the emerging of a functional capillary network, able to provide the reoxygenation of the host tissue. Then, through different sets of numerical realizations, we will dissect selected component mechanisms of the angiogenic progression, in order to link altered newly formed vascular structures with abnormal cell behavior.

The rest of this paper is organized as follows. In Section 2, we clarify the assumptions on which our approach is based and present the model components. The simulation results are then shown in Section 3. Finally, a discussion on possible model improvements as well as a comparison with the existing theoretical literature on these topics is proposed in Section 4.

2. Mathematical model

Model assumptions: The major hypothesis of the model, which account both intracellular biochemical cascades and cell-level behavior, include: (1) we start with a tissue characterized by a preexisting, but insufficient, vasculature. In particular, we neglect the distinction between veins and arteries; (2) oxygen-depleted areas of a tissue become hypoxic by simple thresholding depending on the local partial pressure of oxygen; (3) hypoxic tissue starts to secrete a single long-diffusion-length isoform of VEGF-A, like VEGF-$A_{120,121}$. The molecular HIF-1-dependent cascades resulting in VEGF-A production are not explicitly included in the model; (4) VEGF-A activity induces a dose-dependent activation of the ECs forming the external walls of the preexisting vessels; (5) we include tip cell selection and stalk cell lateral inhibition without explicitly modeling the delta-notch signaling pathways; (6) the tip cells respond to VEGF-A by polarizing and chemotactically migrating, while the stalk individuals respond by proliferating [13]; (7) the tip/stalk cell fate is reversible, in the sense that a tip cell can assume a stalk state after anastomosis and a stalk cell can acquire a tip fate if it is not surrounded by tip individuals. In this last case, we do not consider recovery delays, which are due to the time needed by stalk cell gene expression to return in a normal non-inhibited state; (8) both type of activated vascular endothelial cells secrete and chemotax towards a short-diffusion-length chemoattractant (which can represent for instance VEGF-A_{165} [60]); (9) oxygen is assumed to be secreted both by the functional preexisting vasculature and by emerging loop structures.

Extended cellular Potts model: The physiological angiogenic process is modeled at the mesoscopic level using an extended version of the cellular Potts model, a grid-based stochastic approach, which realistically preserves the identity of single cell-level individuals and describes their behavior and interactions with the environment in energetic terms and constraints. The simulation domain is a two-dimensional regular lattice $\Omega \subseteq \mathbb{R}^2$, formed by identical closed grid sites that, with an abuse of notation, will be identified by their center $\mathbf{x} \in \mathbb{R}^2$. Each site is labeled by an integer number, $\sigma(\mathbf{x}) \in \mathbb{N}$, that can be interpreted as a degenerate *spin* originally coming from statistical physics [27,48]. As classically adopted in CPMs, a neighboring site of $\mathbf{x}$ is denoted by $\mathbf{x}'$, while its overall neighborhood by $\Omega'_{\mathbf{x}}$, i.e., $\Omega'_{\mathbf{x}} = \{\mathbf{x}' \in \Omega : \mathbf{x}' \text{ is a neighbor of } \mathbf{x}\}$. A vascular cell, identified by Σ_σ, consists of a subdomain of contiguous sites with identical spin (i.e., $\Sigma_{\overline{\sigma}} = \{\mathbf{x} \in \Omega : \sigma(\mathbf{x}) = \overline{\sigma}\}$ with $\overline{\sigma} = 1, \ldots, N(t)$, where $N(t)$ is the total number of ECs at time t), and has an associated type $\tau(\Sigma_\sigma)$. In particular, we here distinguish three types of vascular cells: *quiescent*, $\tau = Q$, *tip*, $\tau = T$, and *stalk*, $\tau = S$. Both the tip and the stalk phenotype can be classified as *activated*. We further define a special, generalized cell $\Sigma_{\sigma=0}$ representing the host tissue, which is indeed formed by the part of the domain not occupied by any