Progress in Biophysics and Molecular Biology 106 (2011) 450–462

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

Review

A multiscale hybrid approach for vasculogenesis and related potential blocking therapies

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ARTICLE INFO

Article history:
Available online 5 February 2011

Keywords:
Cellular Potts model
Vascular endothelial growth factor
Tubulogenesis
Calcium

ABSTRACT

Solid tumors must recruit and form new blood vessels for maintenance, growth and detachments of metastases. Discovering drugs that block malignant angiogenesis is thus an important approach in cancer treatment and has given rise to multiple *in vitro* and *in silico* models. The present hybrid individual cell-based model incorporates some underlying biochemical events relating more closely the classical Cellular Potts Model (CPM) parameters to subcellular mechanisms and to the activation of specific signaling pathways. The model spans the three fundamental biological levels: at the extracellular level a continuous model describes secretion, diffusion, uptake and decay of the autocrine VEGF; at the cellular level, an extended lattice CPM, based on a system energy reduction, reproduces cell dynamics such as migration, adhesion and chemotaxis; at the subcellular level, a set of reaction-diffusion equations describes a simplified VEGF-induced calcium-dependent intracellular pathway. The results agree with the known interplay between calcium signals and VEGF dynamics and with their role in malignant vasculogenesis. Moreover, the analysis of the link between the microscopic subcellular dynamics and the macroscopic cell behaviors confirms the efficiency of some pharmacological interventions that are currently in use and, more interestingly, proposes some new therapeutic approaches, that are counter-intuitive but potentially effective.

Contents

1. Introduction

Blood vessel formation and development is a multiscale process, driven by the activation of endothelial cells (ECs) and occurring both in the embryo and in the adult (for a review, see Bussolino et al., 2003). In the adult, vascular progression plays a key role under several physiological conditions, such as in ovary and uterus during the menstrual cycle, in mammary glands during lactation and in granulation tissue after wound healing. It is a complex and highly regulated phenomenon, controlled by coordinated molecular and cellular events operating at different levels. When this equilibrium is disrupted vascularization becomes pathological, as in the cases of

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doi:10.1016/j.pbiomolbio.2011.01.004

chronic inflammatory diseases, vasculopathies, degenerative disorders, tissue injury occurring in ischemia, and cancer progression (Carmeliet, 2005a). In particular, tumor-derived vessels, which differ from their "normal" counterpart in morphology (with irregular diameters, high tortuosity, random branching and absence of a hierarchical arrangement), permeability and blood flow (Baluk et al., 2003; Bussolino et al., 2003; Jain, 2003), provide the nutrition and oxygen needed by malignant cells to grow, and give them an access into the circulation, eventually causing metastases (Bussolino et al., 2003; Carmeliet, 2005a). The switch to the vascular phenotype is thus a pivotal transition in cancer development, leading to a fast progression and to a potentially fatal stage of tumors beyond which they become extremely difficult to treat. Existing therapies are in fact rarely effective and, consequently, the survival rates decrease (Carmeliet and Jain, 2000; Straume et al., 1999). The discovery of efficient anti-angiogenic therapies represents therefore a fundamental issue in biomedical research (Carmeliet, 2005b) and has given rise to multiple experimental models (see reviews Ambrosi et al., 2005; Bussolino et al., 2003; Munaron et al., 2008), whose aim is to understand the key mechanisms involved in malignant vascularization and to identify interventions potentially able to disrupt them.

Among others, classic *tubulogenic* assays analyze the ability of tumor-derived endothelial cells (TECs), cultured in Matrigel (a commercial product mimicking the extracellular matrix), to autonomously organize in a bidimensional network, which resembles a primitive *in vivo* capillary-like plexus (Fiorio Pla et al., 2008). This process, also called *in vitro vasculogenesis*, is largely mediated by the activity of diffusible chemical morphogens (such as VEGF isoforms (Carmeliet, 2005b)). They in fact initiate a series of calcium-dependent downstream pathways, which involve a number of intracellular messengers (such as nitric oxide (NO) and arachidonic acid (AA) (Boonstra and Van Rossum, 2003; Fiorio Pla and Munaron, 2001; Fleming and Busse, 1999; Kimura and Esumi, 2003; Mottola et al., 2005; Munaron, 2006)) and regulate fundamental biophysical properties of TECs, such as motility, adhesion and elongation (Berridge et al., 2003; Munaron, 2002; Munaron et al., 1997, 2004b; Munaron and Fiorio Pla, 2000a; Santella et al., 2005), see Fig. 1. Indeed, the complexity of these multilevel mechanisms presents a number of components that could be interfered in multiple ways to inhibit malignant vascular progression. This large combinatorial space of possible therapies is obviously unfeasible to search using only laboratory-based biological methods, but can be efficiently analyzed with computational simulations realistically reproducing the experimental system. With this aim, we propose a multilevel and hybrid search-based model replicating the in vitro TEC *tubulogenesis*. As shown in Fig. 2, it integrates:

- At the cellular level, an extended Cellular Potts Model (CPM), a lattice-based Monte Carlo technique which follows an energy minimization philosophy (Glazier et al., 2007a,b; Graner and Glazier, 1992; Marée et al., 2007), is the core of the simulation system and describes the phenomenology of TEC population, capturing the mechanisms of cell migration, polarization and adhesion. With respect to previous applications of the CPM, the model presented here is characterized by an innovative and realistic compartmentalized cell approach;
- At the molecular level a continuous model describes the extracellular VEGF profile, dealing with its production, diffusion and degradation, while a set of reaction-diffusion equations reproduces simplified intracellular VEGF-induced cascades, which regulate cytosolic calcium entry and homeostasis.

The interface of these multiple submodels is another novel feature of this work. In fact, differently from previous similar individual cell-based approaches (Bauer et al., 2007; Merks and Glazier, 2006; Merks et al., 2006, 2008), cells properties, behaviors and mutual interactions are in this case regulated by the VEGF-induced calcium-dependent molecular dynamics, via reasonable simplified intracellular cascades, and the whole capillary network thus emerges as a consequence of realistic and relevant biochemical and biomechanical mechanisms.

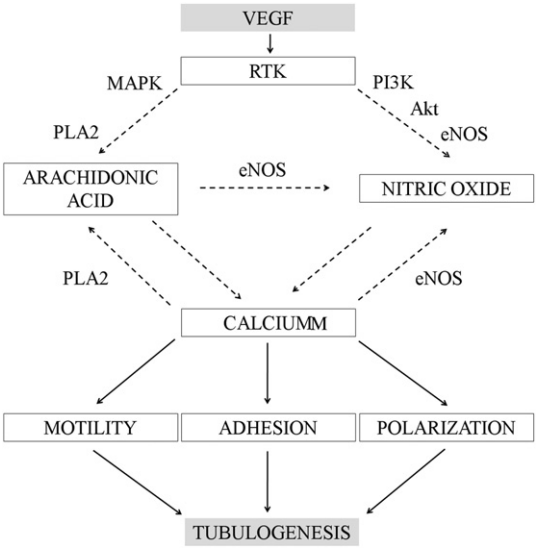

Fig. 1. Simplified schematic representation of VEGF signaling cascades in the control of tumor-derived endothelial cell tubulogenesis. VEGF tyrosine kinase receptors activate a series of intracellular events inducing the recruitment of phospholipases A2 (PLA2) and eNOS and the subsequent release of arachidonic acid (AA) and nitric oxide (NO) respectively. Both intracellular messengers are able to activate plasmamembrane calcium channels. Increases in cytosolic calcium levels trigger motility, adhesion and cytoskeletal reorganization of TECs, crucial events for the capillary-like network formation. The dashed arrows stand for indirect pathways not completely included in the model.

The rest of this paper is organized as follows. We first clarify in Section 2 the assumptions on which our cell-based approach is based. We then show, in Section 3, the model capability to realistically reproduce selected features of *tubulogenic* assays. Then, after confirming the efficiency of some currently available therapies, we turn to suggest novel and experimentally testable strategies, which have the potential to disrupt TEC capillary formation. Finally, in Section 4, we discuss our results and propose some interesting extensions and improvements of the work.

2. Mathematical model

Our multilevel model is based on the following set of assumptions, see again Fig. 1 for a diagrammatic representation:

- The TECs release VEGF in the extracellular medium, where it diffuses and degrades at constant rate (Bauer et al., 2007; Gamba et al., 2003; Merks and Glazier, 2006; Merks et al., 2008; Serini et al., 2003; Tosin et al., 2006);
- VEGF acts as a chemoattractant for the cells, which move in the direction of increasing chemical concentrations: in particular, empirical data suggest that, because of the high viscosity of the experimental Matrigel, their motion is overdamped and the force required is proportional to the velocity, and thus to the chemical gradient (Ambrosi et al., 2004; Merks et al., 2006; Tosin et al., 2006);
- During migration TECs remodel their cytoskeleton and polarize, differentiating in a leading and a trailing surface. The