Journal of Theoretical Biology 419 (2017) 211–226

Contents lists available at ScienceDirect

Journal of Theoretical Biology

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

How tumour-induced vascular changes alter angiogenesis: Insights from a computational model

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ARTICLE INFO

Keywords:
Angiogenesis
Hybrid model
Hypoxia
Vascular changes
Quiescence

ABSTRACT

A computational model was developed to describe experimentally observed vascular changes induced by the introduction of a tumour on a mouse equipped with a dorsal skinfold chamber. The vascular structure of the host tissue was segmented from *in vivo* images and transposed into the computational framework. Simulations of tumour-induced vascular changes were performed and include the destabilizing effects of the growth factor VEGF on the integrity of the vessels walls. The integration of those effects, that include alteration of the vessel wall elasticity and wall breaching, were required to realistically reproduce the experimental observations. The model was then used to investigate the importance of the vascular changes for oxygen delivery and tumour development. To that end, we compared simulations obtained with a dynamic vasculature with those obtained with a static one. The results showed that the tumour growth was strongly impeded by the constant vascular changes. More precisely, it is the angiogenic process itself that was affected by vascular changes occurring in bigger upstream vessels and resulting in a less efficient angiogenic network for oxygen delivery. As a consequence, tumour cells are mostly kept in a non-proliferative hypoxic state. Tumour dormancy thus appears as one potential consequence of the intense vascular changes in the host tissue.

1. Introduction

Over the recent years, more and more theoretical models of tumour growth take explicitly into account the vasculature surrounding the tumour. *Explicitly* means that the spatial and structural properties of the vascular network are integrated into the modelling framework. This prove very useful to understand how the nutrients, oxygen and drugs are delivered to the tumour (Cai et al., 2015; Sefidgar et al., 2015; Welter and Rieger, 2013). Such studies highlighted why in some cases drugs could bypass the tumour and lead to therapy failure (Stéphanou et al., 2005). The vascular network is one of the most essential element of the tumour microenvironment since it conditions the mode of tumour development in terms of growth rate and invasivity. Metastatic spreading is highly depending on angiogenesis, *i.e.* on the way the tumour acquires new vessels. The processes by which the tumour cells are able to enter the vessels, to be carried by the bloodstream and to extravase to form distal metastases are now well documented (Nguyen et al., 2009). It was also recently observed that tumour cells could crawl along the exterior wall of the vessels using vessels as rails that allow them to spread over very long distances (Bentolila et al., 2016). This emerges as an other *softer* mode for spreading metastases, *i.e.* without breaching the vascular walls.

Most studies are theoretically grounded with simulations based on artificial vascular structures (Cai et al., 2016; Goldman and Popel, 2000; Pons-Salort et al., 2012) often using regular grids of vessels (Alarcòn et al., 2003; Bartha and Rieger, 2006; Owen et al., 2009; Welter et al., 2008) and/or one or two main parent vessels to initiate angiogenesis (Stéphanou et al., 2005). Very few studies integrate tumour vascular networks directly extracted from images (Caraguel et al., 2016; Stamatelos et al., 2014). The main reason is that it is not easy to observe experimentally the developing tumour vasculature. To this end, the most adapted technique is represented by *in vivo* microscopy using a dorsal skinfold chamber (Baron et al., 2011; Duansak and Chatpun, 2013; Lavigne et al., 2002). In the present study, we use this observation setup to follow the evolution of the skin tissue vasculature in which a piece of tumour is implanted. The vascular changes induced by the introduction of the tumour are numerous and varied and evolve continuously along the two weeks of the observation period. The vascular changes include: (i) variations in the vessels diameters that can either increase (vessel dilation) and

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http://dx.doi.org/10.1016/j.jtbi.2017.02.018
Received 12 September 2016; Received in revised form 22 January 2017; Accepted 15 February 2017

decrease (vessel constriction), (ii) the appearance of new blood vessels through the well-known angiogenic process, (iii) the disappearance of blood vessels due to vessels collapse (induced by the reduced flow) or vessels breaching (gap-junctions related).

We developed a computational model to describe the experimentally observed vascular changes induced by the introduction of the tumour. The tissue vasculature was first segmented from the experimental images and transposed into the computational framework. The vessels were then functionalised by computing the effects of the blood flow through the vascular network. The blood rheology and hemodynamics generate some constraints on the vessels that respond by adapting their diameters through dilation or constriction. Moreover angiogenesis creates some new vessels where the blood can flow and can dynamically modify the constraints state throughout the entire network. Previous studies have already successfully introduced those mechanisms to describe the adaptation of tumour vasculatures (Alarcòn et al., 2003; McDougall and Anderson, 2006; Stéphanou et al., 2006). However, feedbacks from the tumour to the vasculature were not fully considered. Although vessels shrinkage, regression and degeneration were already considered in previous models (Macklin et al., 2009; Welter et al., 2009; Welter and Rieger, 2010), no model directly takes into account the effect of VEGF on the integrity of the vessels beyond its effect on vessels sprouting (*i.e.* angiogenesis). A first VEGF-related perturbation on vascularity was proposed by Alarcòn et al. (2005). The authors chose to introduce the VEGF modulation on the metabolic term responsible for vessel radius adaptation. One originality of our approach is to integrate the VEGF regulation on the vessel wall elasticity instead since loosening of tight junctions, induced by VEGF, has a direct impact on this mechanical property and can also lead to wall breaching (Suarez and Ballmer-Hofer, 2001).

The major novelty brought by our model is the introduction of these effects, that include alteration of the vessel wall elasticity and wall breaching, to account for the salient features of the experimental observations made using the dorsal skinfold chamber. We then made use of this model to investigate the importance of the vascular changes for oxygen delivery and tumour development. Although angiogenesis is clearly required for tumour growth, the new vascularisation is known to be inefficient for perfusing the tumour homogeneously in space and time (Palmer et al., 2010). The defective blood flow leads to chronic or cyclic states of hypoxia for the tumour cells (Michiels et al., 2016) with consequences for radiotherapy, where treatment efficacy is reduced, and also for cell phenotypic and genotypic alterations that are greatly enhanced (Bedessem and Stéphanou, 2014) and associated to a poor prognosis for patients (Eales et al., 2016; Harris, 2002).

Antiangiogenic treatments are developed with the primary aim to prevent the formation of new vessels (Folkman, 1971). Alternatively this treatment can potentially normalize the tumour vasculature and improve tumour perfusion for a more efficient drug delivery (Jain, 2005; Sitohy et al., 2012). In this context, we expected that freezing the vascular changes could have a similar positive impact to improve tumour perfusion. In other words, we expected that chronic hypoxia would be reduced. To test this assertions, we ran simulations with (*i.e.* dynamic) and without (*i.e.* static) vascular changes to assess the differences between the two cases. As expected, the results showed that the tumour growth was strongly impeded by the constant vascular changes. More precisely, it is the angiogenic process itself that was affected by vascular changes occurring in bigger upstream vessels. As a consequence, tumour cells were less subjected to changing oxygen conditions and were mostly kept non-proliferative compared to the case without vascular changes. Our simulations suggest that tumour dormancy could appear as one potential consequence of the intense vascular changes in the host tissue.

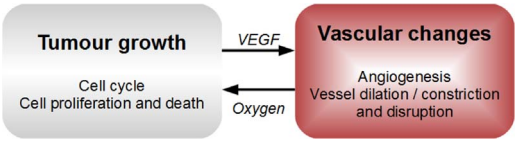

Fig. 1. The two modules of the computational model.

2. Material and methods

2.1. The dorsal skinfold chamber model

Dorsal skinfold chambers (small dorsal kit SM100, APJ Trading Co., Ventura, CA, USA) are implanted on the back of nude mice. During surgery and imaging, mice are anaesthetized by a continuous inhalation of 1.5% isoflurane in a gas mixture with 30% oxygen and 70% nitrogen. Core temperature is maintained at approximately 37 °C. The chamber is made of two symmetrical titanium frames with central holes (1 cm diameter) which are placed each at on side of the dorsal skin fold of animals in order to sandwich the extended double layer of skin which consists of one layer of striated muscle, subcutaneous tissue and epidermis. A small excised part of a kidney tumour (RENCA, *ATCC®CRL* – 2947™), grown on a donor nude mouse, is cut into small pieces of 1–2 mm^3. The pieces are then implanted on receiver mice bearing the dorsal chambers. The observation window is covered with a glass to allow for *in vivo* microscopic observations (Baron et al., 2011; Duansak and Chatpun, 2013). One day after surgery, mice are checked on the presence of infection. At the absence of infection, the tumour and vascular developments can then be followed. Pictures are taken every 3 days approximately using a Nikon AZ100 Multizoom.

2.2. The computational model

The computational framework consists of two interacting modules (Fig. 1). The first *tumour growth* module is based on a cellular automaton to describe the tumour cell states evolution. It takes into account elements of the cell cycle that can either lead to cell proliferation or death depending on the environmental context. The second *vascular changes* module is a hybrid discrete-continuous model that describes angiogenesis and vessels structural changes in response to blood rheological constraints and hemodynamics. The coupling between these two modules is mainly driven by two molecules: VEGF released by hypoxic tumour that stimulates vascular growth and reciprocally oxygen delivered by the vessels that allows the tumour to grow.

The vascular model of angiogenesis with vessels adaptation is described in full details in Stéphanou et al. (2006) and the coupling with the cellular automaton for tumour growth is detailed in Lesart et al. (2012) and Pons-Salort et al. (2012). Consequently, we limit here the description of the model to the novelties and adaptations made from those previous source models. These novelties specifically concern:

- extension of the computational grid to its diagonal elements for a more flexible description of the vascular network and to avoid the occurrence of vascular nodes with 4 branches,
- variation in cell cycle durations and inheritance rules from mother cell to daughter cells,
- integration of passive displacements of the tumour cells induced by the pressure of the dividing cells,
- integration of the submicrovascular (capillary) network as a field of oxygen source to ensure homogeneous and physiological oxygenation of the healthy tissue,
- integration of vascular changes under the effects of both hemodynamical and tumour-induced constraints.