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Data-driven modeling and characterization of anti-angiogenic molecule effects on tumoral vascular density

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ABSTRACT

Angiogenesis is the phenomenon by which new blood vessels are created from preexisting ones. But this natural process is also involved, in a chaotic way, in tumor development. Many molecules have shown particular efficiency in inhibiting this phenomenon, hopefully leading to either: (i) a reorganization of the neovessels allowing a better tumor uptake of cytotoxic molecules (as chemotherapy) or (ii) a deprivation of the tumor vascular network with the view to starve it. However, characterizing the anti-angiogenic effects of a molecule remains difficult, mainly because the proposed physical modeling approaches have barely been confronted to *in vivo* data, which are not directly available. This paper presents an original approach to characterize and analyze the anti-angiogenic responses in cancerology that allows biologists to account for spatial and dynamical dimensions of the problem. The proposed solution relies on the association of a specific biological *in vivo* protocol using skinfold chambers, image processing and dynamic system identification. An empirical model structure of the anti-angiogenic effect of a tested molecule is selected according to experimental data. Finally the model is identified and its parameters are used to characterize and compare responses of the tested molecule.

1. Introduction

Angiogenesis is a normal and necessary phenomenon consisting in creating new blood vessels from preexisting ones. It concerns many physiological processes during life cycle as, for example, wound healing, development of new tissues, embryonic maturation, menstrual cycles, etc.

Unfortunately, it is also involved in tumor development, especially in tumor neovascularization. As a matter of fact, at the very beginning of tumor development (i.e. before it reaches 1–3 mm^3) oxygen and nutrients uptakes can be done by a simple diffusion process. But beyond a 3 mm^3 threshold, to carry on their development, cancer cells over-express physiological pro-angiogenic factors, as Vascular Endothelial Growth Factor (VEGF), in order to stimulate the angiogenesis process in favor of the tumor [1]. Nearby endothelial cells (blood vessel constituting cells) will then be activated by VEGF and will get out of their quiescent state and start the construction of a new vessel in the angiogenic signal source direction.

Due to the permanent excess of physiological pro-angiogenic factors, the newly created blood vessels have an aberrant architecture, non-stabilized, permeable and tortuous. Therefore the tumor irrigation is not optimal and creates hypoxia areas, leading to a continuous tumor angiogenesis stimulation. Such conditions could make the tumor treatment-resistant to chemo- or radiotherapies since the lack of suitable vascularization prevents a good drug delivery or a good tumor oxygenation [2]. It has also been proved that the start of tumor growth was directly linked to neovascular development, [3], hence, it is now accepted that inhibiting angiogenesis in tumor could increase the efficiency of standard therapies [4].

However, the efficiency and secure use of anti-angiogenic drugs requires to better define their pharmacodynamic effects and consequences on tumor growth. Indeed, it will allow to determine when, how and how long these drugs should be administrated in order to optimize the therapeutic protocols.

On the other hand, the use of mathematical models has been largely democratized for various biological applications, either for characterization, prediction or control purposes. A literature review proposed by Mriouah et al., [5], published in 2012, showed that three main types of mathematical models have

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been used to: (i) simulate and understand complex phenomena involved in angiogenic processes [6–8], (ii) analyze interactions between tumor and vasculature systems [9,10] and (iii) optimize anti-angiogenic therapies [11–15]. This review also emphasizes that the model structures can take various forms: temporal, spatio-temporal or multi-scale. However they were all designed from physical and biological equations, and very few behavioral modeling approaches of angiogenesis have been tested, so far. For instance, Drexler and Kovacs have used in [16–20] a state-space representation derived from a linearization process applied to a nonlinear model initially proposed in [11]. Nevertheless the relevance of this state-space model has never been assessed *in vivo*. Indeed, Mriouah et al. particularly highlight the lack of *in vivo* validation of existing models. In fact, not only very few models were confronted with real data but statistical tests are barely applied to validate the model performance. Yet, without any relevant validation tests based on *in vitro* or *in vivo* data, the credibility and medical application of mathematical models remains unlikely.

One of the main bottleneck preventing empirical modeling and practical validation is the lack of experimental data availability (in terms of quality and quantity).

In such a context, the objective of the present paper is to propose an innovative approach integrating:

- an *in vivo* protocol of experiments using skinfold chambers (real time observation of *in vivo* angiogenesis processes) applied to mice;
- image segmentation technics (access to informative biological data);
- dynamic system identification methods (data-driven modeling);

with the purpose of characterizing and comparing more accurately the anti-angiogenic treatment responses.

This paper is organized as follows. Section 2 describes material and methods performed from *in vivo* experiments to image acquisition and Section 3 presents the automatic image segmentation process by which data are extracted from images. A data-driven model structure selection is then proposed in Section 4 before being identified. Finally results are discussed in Section 4 before drawing conclusion and perspectives.

2. Material and methods

In vivo angiogenesis imaging through skinfold chambers, on nude mice, allows to visualize the creation, functionality and remodeling of blood vessels within tumor during 4–5 weeks [21].

2.1. Skinfold chamber

This chamber model is made of two titanium shields placed on either side of a skinfold on the mouse back. These shields have a central circle hole of 10 mm diameter through which each side of the skinfold is visible. The skin of one side is dissected and removed in front of one of the apertures, which is then hermetically sealed with a thin sterilized cover glass in order to visualize the skin blood vessels of the other side dermis. It is possible, by removing the cover glass then by replacing it, to implant tumor cells or to graft tumors within the visualization chamber.

The main advantages of such a system is that chambers allow repeated (almost daily) observations of both vascular network and tumor growth by *in vivo* intravital microscopy over 4–5 weeks [22]. An example of this model is presented in Fig. 1.

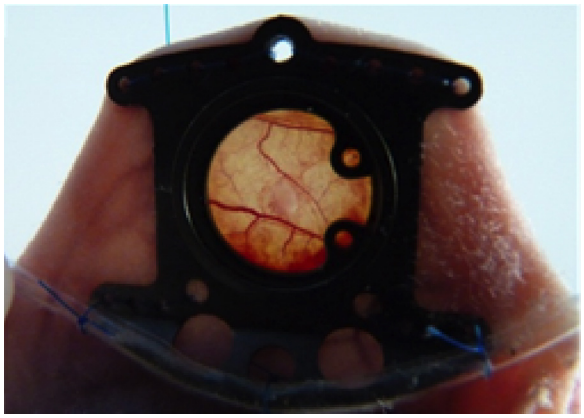

Fig. 1. Example of a xenograft and skin vascular observation from skinfold chamber placed on a nude mouse.

2.2. Animals and tumor xenografted model

The study design was approved (authorization number: CELMEA-2012-0018) by Animal Protection Bureau of the French Ministry for Fishing, Agriculture and Food and the experiments were conducted in accordance with the Guiding Principles for Research Involving Animals. Using human tumor fragments requires to work with immunodeficient mice (nude) to avoid graft rejections.

Experiments were performed on 6–12-weeks-old female nude mice (nu/nu) weighting between 25 and 30 g ($n = 16$). They were provided by Janvier breeding (Le Genest St Isle, France).

Anesthesia was achieved by a single intraperitoneal injection of a Xylazine (8 mg/kg, Rompun 2%, Bayer Health Care, Puteaux, France) and Ketamine (90 mg/kg, Imalgène 500, Merial, Lyon, France) mixture. To prevent post-operative pain and stress, mice were injected subcutaneously with single doses of Buprenorphine (0.05 mg/kg, Buprécare, Axience) and Meloxicam (1 mg/kg, Metacam, Boehringer Ingelheim).

The dorsal skinfold chamber is positioned and xenograft implanted respectively at days D_{-14} and D_{-13}, see Fig. 2. The xenograft consists in placing a non-vascularized 1 mm thick tumor fragment (of 2–4 mm^2) on the vascular network of the skin. We use tumor fragments derived from a human glioblastoma cell line (U87). The cells are injected subcutaneously in the flanks of nude mice to form a tumor which is then cut into small fragments that are placed on the vascular network in the skinfold chamber.

At the end of experiments, mice were killed by a lethal dose of sodium pentobarbital (Pentobarbital sodique®, CEVA Santé Animale, La Ballastière, France).

2.3. Anti-angiogenic drugs and administration protocols

Only one anti-angiogenic drug was tested: bevacizumab (Avastin®, Roche, France). It is a monoclonal antibody targeting VEGF in order to prevent the fixation of VEGF to its receptors on endothelial cells, hence hindering angiogenesis stimulation and in consequence, the angiogenesis stimulation [23,24]. When the tumor vascular network was complete (i.e. when the entire visible part of the tumor is vascularized) the mice were randomized into two batches: one treated and one control. The treated batch was daily injected intraperitoneally with bevacizumab (10 mg/kg).

2.4. Image acquisition

Picture acquisitions were made 3–6 times a week; from anesthetized mice placed on the platform of the microscope (Nikon