Agent-based models and structured equations for evolutionary dynamics in heterogeneous cell populations: resistance to stress conditions and cross-talk

Background. Increasing evidence suggests that tumors are extremely heterogeneous and composed of different cell subpopulations [2]. It is commonly thought that intra-tumor heterogeneity results mainly from genetic modifications (e.g. mutations) leading, through a Darwinian-like process, to the selection of cancer cells expressing phenotypes adapted to their local micro-environment [3]. However, tumor cell heterogeneity can also be generated via non-genetic processes. Indeed, in a single tumor cell population, heterogeneity can result from either stochastic events (e.g. biological noise) or epigenetic modifications [1]. In agreement, many reports have shown that distinct phenotypes can be observed in genetically homogeneous cell populations grown in culture medium [4, 10, 11]. Moreover, phenotype equilibrium can be influenced by changes modifying the cellular environment, which lead to increasing invasiveness, or resistance to exogenous aggressions [4, 10].

In this respect, Agent-Based models (A-B models) and Partial Differential Equations (PDEs) for the dynamics of populations structured by phenotypical traits [5, 6, 7] provide a promising research framework. In fact, both classes of models can be used as *in-silico* laboratories to highlight stylized facts, and uncover mechanisms that underlie emergent features of solid tumors. A-B models allow an intuitive and flexible description of the system at hand, while PDEs make possible a study of the system in terms of qualitative analysis and less computationally expensive numerical simulations.

Aims. In this thesis project we will develop specific mathematical models to study how phenotypic heterogeneity and cross-talk between cells characterized by different phenotypes, can influence the emergence of resistance to environmental stresses and epithelial to mesenchymal transition (EMT). A-B formalism and PDE for phenotype structured populations will be used. Both the modeling part and the result analysis will be performed in collaboration with biologists, with the aim of designing validated models, which can be used both to reproduce qualitative behaviors and to produce quantitative forecasts.

The student will mainly work on the mathematical models, but is also expected to participate in the experimental work - at least in some simple tasks and, above all, to be in very close contact with the LBTC researchers conducting the experiments so that there is a permanent bi-directional feedback between experimental program conception and mathematical modeling.

Model Design. A-B and PDE models for phenotypic evolution in cancer cell populations have been recently developed at the LJLL, yielding encouraging preliminary results in agreement with the experimental data presented in [9]. Based on these models, suitable PDE and A-B models will be designed in the context of the present project. We provide here a prototype PDE model that will be improved during the thesis.

Let us consider a well-mixed sample composed of an epithelial and a mesenchymal population of cancer cells structured by two non-negative real variables: $x \in [0,1]$, which stands for the normalized level of a survival potential phenotype (i.e. the level of robustness towards life-threatening events in extreme conditions), and $y \in [0,1]$, which models the normalized level of a proliferative potential phenotype. The density of epithelial and mesenchymal cells with phenotypic expressions (x,y) at time $t>0$ are modeled by functions $n_E(x,y,t) \geq 0$ and $n_M(x,y,t) \geq 0$, respectively. Interactions amongst the two cell populations, cell-oxygen interactions and, in presence of cytotoxic drugs, cell-drug interactions are explicitly taken into account. The total densities of epithelial and mesenchymal cells inside the system are modeled by functions $\varrho_{E,M}(t) = \int\int n_{E,M}(x,y,t)dxdy$, while the concentrations of oxygen and nutrients are described, respectively, by functions $s(t) \geq 0$ and $c(t) \geq 0$, which are supposed to be given. We assume that the evolution of functions $n_{E,M}$ is ruled by the equations below

$$\begin{cases} \partial_t n_E(x,y,t) + \underbrace{\nabla \cdot (v_E(x,y,t) n_E(x,y,t))}_{\substack{\text{stress induced}\\ \text{phenotype adaptation}}} = \underbrace{R_E(x,y,t) n_E(x,y,t)}_{\substack{\text{natural}\\ \text{selection}}} + \underbrace{D_E \Delta n_E(x,y,t)}_{\substack{\text{stochastic variation}\\ \text{in phenotype}}} + \underbrace{K_E(n_M(x,y,t)) n_E(x,y,t)}_{\text{cross-talk}}, \\ \partial_t n_M(x,y,t) + \underbrace{\nabla \cdot (v_M(x,y,t) n_M(x,y,t))}_{\substack{\text{stress induced}\\ \text{phenotype adaptation}}} = \underbrace{R_M(x,y,t) n_M(x,y,t)}_{\substack{\text{natural}\\ \text{selection}}} + \underbrace{D_M \Delta n_M(x,y,t)}_{\substack{\text{stochastic variation}\\ \text{in phenotype}}} + \underbrace{K_M(n_E(x,y,t)) n_M(x,y,t)}_{\text{cross-talk}}, \end{cases}$$

where the following notations and assumptions are used:

• The parameters $D_{E,M}$ represent the average rate of stochastic phenotypic modifications.

• The functions $v_{E,M}(x,y,t) = v_{E,M}(x,y,s(t),c(t))$ model the rate of epimutations that lead cells to adapt their phenotype (x,y) to local environmental conditions at time t.

• The functions $R_{E,M}(x,y,t) = R_{E,M}(x,y,\varrho(t),s(t),c(t))$ are the net proliferation rates of epithelial and mesenchymal cells with phenotypic expression (x,y) at time t. They satisfy the following natural assumptions:

$$\partial_{\varrho_{E,M}} R_{E,M}(x,y,\cdot,s,c) \leq 0, \qquad \partial_s R_{E,M}(x,y,\varrho_{E,M},\cdot,c) \geq 0, \qquad \partial_c R_{E,M}(x,y,\varrho_{E,M},s,\cdot) \leq 0.$$

• The functions $K_{E,M}(n_{M,E}(x,y,t))$ model the cross-talk between the two cell populations.

A key step of the project will be the definition of functions $v_{E,M}$, $R_{E,M}$ and $K_{E,M}$ according to suitable assumptions, which will rely on the results of biological experiments. As a collateral topic, we mention the development of more refined strategies to model the effect of stochastic phenotypic modifications and biological noise.

Let us note that the above model can be easily extended to describe the dynamics of, and the cross-talking between, more cell populations. The effects of more environmental variables or more phenotypes can be included and cell dynamics can be also studied in this setting from the point of view of optimal control.

Main Biological Model and specific questions addressed using it. Although the type of model developed is intended for wider applications, in this thesis we will start by focusing on specific biological questions to develop and validate the model. Our main experimental models will be three HT29 colorectal cancer cell lines studied in LBTC:
• E (epithelial) line is made of HT-29 cells, a classical colorectal cancer cell line. These cells are very epithelial, easy to grow and show a high level of natural resistance to bevacizumab, a VEGF-targeted monoclonal antibody.
• M (mesenchymal) line is made of HT-29/Snail HT-29 cells stably transfected with the Snail transcription factor that promotes the epithelial to mesencymal transition (EMT).
• VKD (VGEFA knock-down) line studied at LBTC is made of HT-29 shVEGFA cells, which are HT-29 cells stably transfected with short hairpin RNA that downregulates the expression of VEGFA.

The first two are our E and M model cell lines and the third one will be used to understand better the role of VEGFA in the system. In fact, it has been recently shown that VEGFA plays an important role in the expression of cell resistance phenotype to bevacizumab through a positive feed-back loop [8]. We will start by focusing on two ways of generating stress:
• Hypoxia Hypoxia through the use of a cell culture incubator available at LBTC that provides nitrogen gas in addition to carbon dioxide to achieve hypoxic conditions.
• Drug therapy using nintedanib, which was shown to overcome autocrine VEGF signaling resistance [8] (other anti-cancer drugs can also be considered).

We will first characterize these individual cell lines - E, M and VKD - with experimental data gathered under comparable conditions, in order to provide information for the development, and the validation, of mathematical models. Then, in the spirit of [9], we will start by submitting each of our individual cell lines to a cycle of intense stress (e.g. prolonged 1% hypoxia, IC90 nintedanib), with the aim of verifying if we can obtain TP (tolerant persister) cells and, subsequently TEP (tolerant expanded persister) cells. At this stage, we will also be interested in obtaining detailed information on the cellular dynamics, through a characterization of the population at many time points along the evolution. In particular, we will determine whether phenotypically characterized TP and TEP cells can be seen in the initial untreated cell population as a pre-existing heterogeneous minor subpopulation and how it evolves during the course of the treatment. Moreover, we will define whether hypoxia-selected TEP are cross-resistant to nintedanib and vice-versa. This will allow us to determine whether both stress-induced resistance phenotype share common feature. Finally, will then remove the stress to study the evolution of the resistance phenotype. In each stress setting, different behaviors between E and VKD lines would be a first indication of the role of VEGFA in the development of resistance to the source of stress at hand. The M line will allow us to address the importance of the mesenchymal character in the stress resistance (hypoxia and drug, in these particular experiments).

Likewise, by looking at mesenchymal as a phenotype of the cell population that evolves in time (which is also a natural point of view in our models), these experiments enable us to study EMT dependence on initial cell line (which will allow us to see the role of VEGFA) and on stress conditions. We recall that EMT is a reversible transition and that when we talk about EMT dynamics it also includes MET.

We will then enter the second part of our study, where we will look for data to develop the mathematical models describing the interaction among different cell lines (like the one presented above for E and M cells). We will do co-cultures of all combinations of two cell lines at a time. We will characterize the populations at different time points, with the aim of determining what changes in the dynamics are actually due to the cross-talk between cell populations. In the cases where there will be interesting changes indicating commensality between the two populations in presence, we will measure secreted factors in the intercellular medium through ELISA assay. This will be useful to calibrate the cross-talk terms of our models ($K_{E,M}(n_{M,E}(x,y,t))$).

Finally, the three cell lines will be co-cultured and their population dynamics in presence of different stress factors will be followed in time. This should allow us to determine what phenotypes are associated with stress

resistance, as well as to clarify whether or not heterogeneous populations behave as homogenous ones. If not, this would suggest the existence of strong functional interactions between different cell types that might shed some light on the interest of having heterogeneous populations in real tumors. In particular, this approach will allow us to determine whether commensalism (where one cell benefits from the other without affecting it) or mutualism (in which both cell types benefit from the functional interaction) occur in our system and act on the emergence rate of TP and subsequently TEP cells. As above, these interactions will then be further investigated by measuring secreted factors through ELISA assay and taken into account in the model cross-talk terms.

Concerning the role of VEGFA, it would be particularly interesting if we would observe that co-culture interaction with E or M cells can rescue specific phenotypes observed in VKD cells when cultured alone.

We remark that using hypoxia and drugs should allow us to test two very different way of stressing the cells since the way they are affected by hypoxic conditions should be much more gradual in time than being submitted to massive drug doses like in [9]. This should induce very different dynamics and gives us some insight on the processes involved in resistance development (in particular the role played by selection drift or biological noise in the establishment of the resistant phenotype or, alternatively, if it is just selection of an initially existent population). Whats more, understanding the mechanisms of cell dynamics in hypoxic stress has a much wider interest beyond tumor growth and EMT - it also plays a very important role in other traumas like heart attacks, strokes (CVA), limb amputations and embryogenesis (where the first stages are done in hypoxic conditions, until a functional vascular system is established).

Model Validation. The properties of the models will be rigorously characterized through qualitative analysis aimed at studying the behavior of the solutions. In particular, asymptotic analysis will be developed to identify key parameters and to verify the existence of equilibrium configurations. Numerical simulations will be implemented, focusing on the critical parameters highlighted by qualitative analysis, and the obtained results will be compared with the ones provided by laboratory experiments, in order to verify their biological consistency.

Final remarks.
This thesis work will be developed as part of a wider project of mathematical modeling of tumor growth currently pursued at LJLL. This dynamic group will be an ideal environment for the student to pursue a thesis taking advantage of the discussion opportunities with other researchers and numerous talks by world leading specialists of this field. The versatility of the models developed opens the opportunity to consider spatial dependence (which enables detailed study of drug of hypoxia induced effects in colospheres) and similar population evolution and differentiation dynamics in different biological settings like, for instance, development or tissue repair.

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