Run for Your Life – An Integrated Virtual Tissue Platform for Incorporating Exercise Oncology into Immunotherapy

Josua Aponte-Serrano(✉) and Amit Hagar

Intelligent Systems Engineering, School of Informatics, Computing and Engineering, Indiana University—Bloomington, Bloomington, IN 47405, USA
joaponte@iu.edu

Abstract. The purpose of this paper is to introduce a novel *in silico* platform for simulating early stage solid tumor growth and anti-tumor immune response. We present the model, test the sensitivity and robustness of its parameters, and calibrate it with clinical data from exercise oncology experiments which offer a natural biological backdrop for modulation of anti-tumor immune response. We then perform a virtual experiment with the model that demonstrate its usefulness in guiding pre-clinical and clinical studies of immunotherapy. The virtual experiment shows how dosage and/or frequency of immunotherapy drugs can be optimized based on the aerobic fitness of the patient, so that possible adverse side effects of the treatment can be minimized.

Keywords: Cancer modeling · Immunotherapy · CompuCell3D

1 Introduction

Computational modeling is playing increasingly important roles in advancing a system-level mechanistic understanding of complex interrelated biological processes. Here we present a computational platform that can interrogate potential mechanisms underlying the effect of aerobic fitness on anti-tumor immune response. These effects, documented in pre-clinical [1] and clinical studies [2] support the inclusion of aerobic fitness as a biological variable in clinical contexts. This platform can contribute to the personalization of immunotherapy by optimizing dosage and frequency of treatment and by reducing the risk other adverse side effects [3].

Our basics assumption is that aerobic fitness acts as a tumor suppressor through a systemic enhancement of anti-tumor immune response. This systemic effect is a result of metabolic and endocrinal modifications, which can be modulated with exercise training. While the exact mechanisms behind this effect are currently under investigation, documented pre-clinical experiments point at two potential candidates: (1) increased trafficking of NK cells into the TME [4] and (2) hypoxia-tolerant suppression of the recruitment of immune inhibitory cells ($CD4^{+}FOXP3^{+}$ Tregs) [5]. The model presented here focuses on the latter mechanism.

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2 Methods

2.1 Model Description

The model is a spatiotemporal representation of a TME of a solid tumor in its early stages (T0 to T1). Tumor cells adopt four different phenotypes: "oxphos" (relying on oxidative phosphorylation), "glycolytic" (elevated glycolysis when the surrounding tissue becomes hypoxic), "necrotic" and "apoptotic". Tumor cells grow, divide and invade their environment. The growth rate of tumor cells is limited by the availability of oxygen (modeled as a field) which cells consume from the environment. The fitness parameter controls the oxygen level at which cell transition metabolic phenotype: as oxygen gets depleted, tumor cells change from "oxphos" to "glycolytic". When oxygen is severely depleted, glycolytic cells become necrotic and die. Glycolytic cells secrete lactate (modeled also as a field) to the TME. Lactate serves as a recruiting signal for the tumor promoter cells.

Our model includes two types of immune cells: $CD8^+$ Lymphocytes tumor suppressors ("CTLs") and $CD4^+FOXP3^+$ tumor promoters ("Tregs"). CTLs are constantly recruited to the tumor site and induce apoptosis in the tumor cells they come into contact with. Upon contact with tumor cells, tumor suppressors also release a IFNγ cytokine signal (modeled as a field) attracting other CTLs. The acidification of the TME by the glycolytic cells results in recruitment of Tregs to the tumor site. Recruited Tregs move through the tissue to areas of higher concentration of lactate. "Tregs" inhibit the "CTLs" they come in close proximity to. This inhibition prevents "CTLs" from inducing apoptosis in cancer cells.

We implemented the model in CompuCell3D (CC3D), an open-source modeling environment that allows specification and simulation of multicellular models, diffusing fields and biochemical networks [6]. Diffusion solvers integrate partial differential equations describing the diffusion of oxygen, lactate and IFNγ across the whole simulation domain. Outcomes of the simulation are dependent on the parameter values associated with aerobic fitness and with the emergent patterns of TME invasion associated with availability of resources and immune response (Fig. 1).

2.2 Parameter Estimation and Calibration

Simulation parameters corresponding to the spatial properties of human solid tumor cells, transport of chemicals and rates of immune response were estimated from the literature. Our model is simulated over 10^{-6} lattice sites representing up to 5×10^4 individual cells. Each lattice site corresponds to 16 um such that the simulation domain represents a 16 mm^2 tissue cross section. We assumed that cancer cells occupy an area of 256 μm^2. When sufficient resources are available, tumor cells grow and divide every 24 h. Conversely, when resources are depleted cells die within 12 h, and when "CTLs" induce apoptosis, cells die within 8 h. We estimated the infiltration rates of "CTLs" (1 cell every 1.5 h) and "Tregs" (1 cell every 1 h) using intramural density data, showing that the "CTL"/"Treg" ratio is 5:1 [7]. The intrinsic random motility and the contact energy were fixed so that tumor cells can detach from each other and invade the surrounding tissue [4]. We assumed that the homeostatic concentration of oxygen in tissue is 4.3 ×

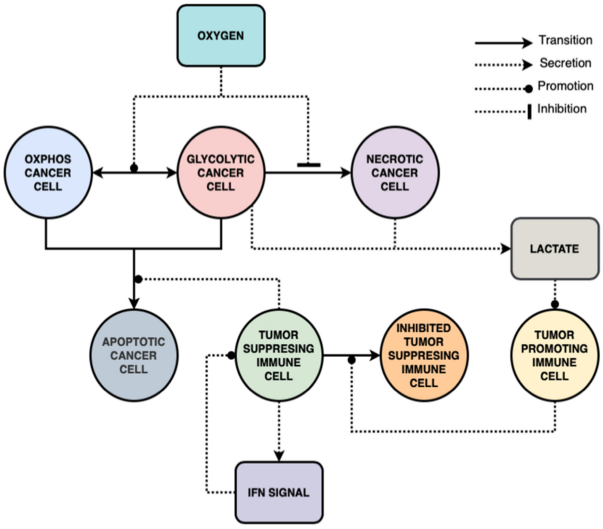

Fig. 1. The model simulates the early stage of solid tumor progression from which a growth rate can be calculated. Tumor cells grow in the TME and become more glycolytic, in a rate that depends on the host's aerobic fitness and tolerance to hypoxia. Tumor cells die through apoptosis or necrosis (lack of oxygen or death by CTLs). CTLs and Tregs react to cytokine and lactate fields secreted by tumor cells. Tumor cells grow until they saturate the grid.

10^{-4} Mol/L [8]. Transport parameters were estimated from the literature. Aerobic fitness was defined as the oxygen concentration threshold at which tumor cells changed from "oxphos" to "glycolytic". We simulated virtual cohort of 200 virtual subjects divided into 10 aerobic fitness levels. Sensitivity analysis on the aerobic fitness parameter show upper and lower bounds below and above which the effects on tumor growth remain constant. To calibrate remaining parameters of the model we matched it to clinical results from breast cancer patients where an aerobic score metric was used [11].

3 Results

3.1 Model Reproduces Key Mechanisms of Immunoregulation by the TME

Immune Suppresors and Immune Promoters Dynamics

Clinical studies have shown that intratumoral CTLs/Treg ratio is a significant prognostic marker for cancer patients and pre-clinical studies have tied this marker to hypoxic conditions in the TME [8]. In our model we introduced two scales of immune cells trafficking (Fig. 2). The first is the seeding rate to the TME; the second is the movement within the TME, implemented with a chemotaxis mechanism. The seeding rates and densities were calibrated using data on respective densities from hot *vs.* cold tumors in humans [6]. "CTLs" migrate towards the "IFNγ" cytokine field, "Tregs" migrate towards the lactate field.

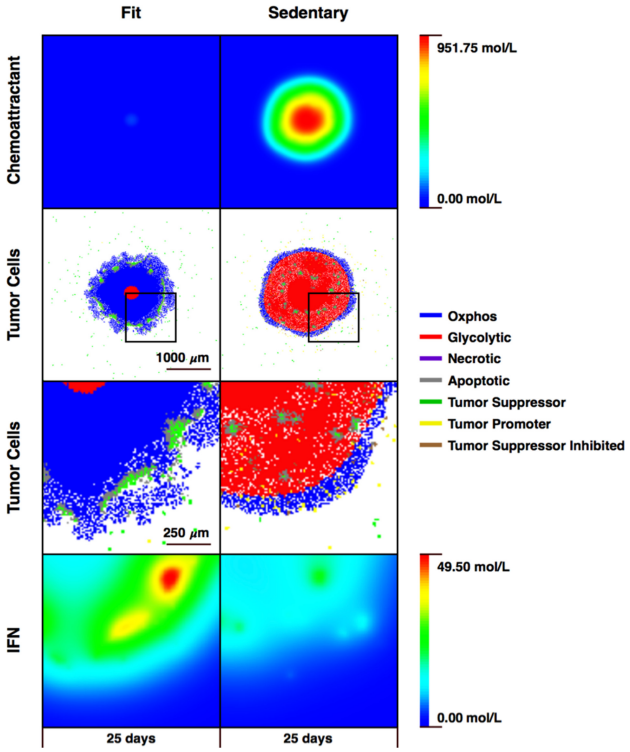

Fig. 2. The more aerobically fit is the host, the less glycolytic its tumor cells are relative to a sedentary host. Consequently, recruitment of Trges that can block CTLs is down regulated relative to a sedentary host, and tumor growth will be relatively suppressed. CTLs move towards the tumor along a cytokine gradient ("INFγ"). Tregs move towards the tumor along the lactate gradient that glycolytic tumor cells secrete. Once infiltrated into the TME, they can block the ability of nearby CTLs to kill tumor cells.

Effect of Aerobic Fitness on Tumor Progression Rate

We simulated a virtual cohort of 200 virtual subjects divided into 10 aerobic fitness levels. The model connects variations in fitness levels to variations in anti-tumor immune response and consequently to variations in tumor growth rates each of which yields a distinct tumor growth curve (Fig. 3A). A similar effect of suppression of tumor growth when inoculation followed endurance exercise was qualitatively demonstrated in pre-clinical studies [7]. The model behaves qualitatively in accordance with a similar plot of tumor doubling times vs. fitness levels from a pilot study in recently diagnosed T1 invasive ductal carcinoma patients (Fig. 3B, C).

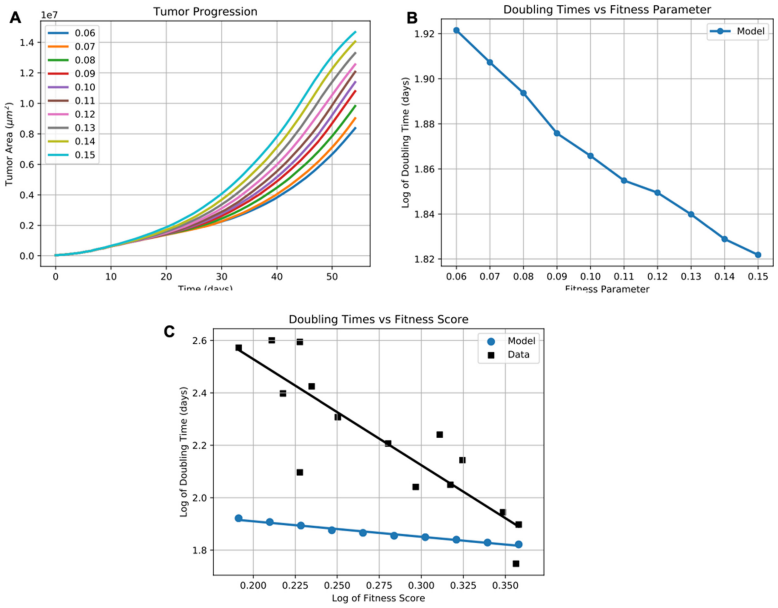

Fig. 3. The model was run on 200 virtual subjects, divided into equal size distinct aerobic fitness levels. Each fitness level generated an average growth rate (3A). These average growth rates where then plotted against the fitness levels on a logarithmic scale (3B). The model behaves qualitatively in accordance with a similar plot of tumor doubling times vs. fitness levels from a pilot study in recently diagnosed T1 invasive ductal carcinoma patients (3C) [14].

3.2 Incorporating Aerobic Fitness into the Personalization of Immunotherapy

While showing remarkable success in patients, immunotherapy treatments can lead to autoimmune adverse effects such as myocarditis, pericardial diseases, and vasculitis [5]. Personalized dosing could mitigate adverse effects. Preclinical studies have shown that aerobically fit patients may require lower dosage of immune check inhibitors (ICI) than sedentary patients [10]. To test this hypothesis, we implemented ICI in our model as an increased efficacy of "CTLs" killing. Cytotoxicity was quantified as additional "IFNγ" cytokine [11]. Performing a virtual experiment on aerobically fit and sedentary virtual subjects treated with ICI, simulations show that without a mitigated dosage, aerobically fit subjects are more prone to adverse effects than their sedentary counterparts (Fig. 4A, B). Lowering the ICI dosage for aerobically fit patients can achieve the same reduction of tumor growth relative to their sedentary counterparts but with lower probability for adverse effects (Fig. 4C, D). In order to translate this result to a clinical setting (Fig. 4E, F). future studies should identify potential markers for aerobic fitness with which such personalization can be accomplished.

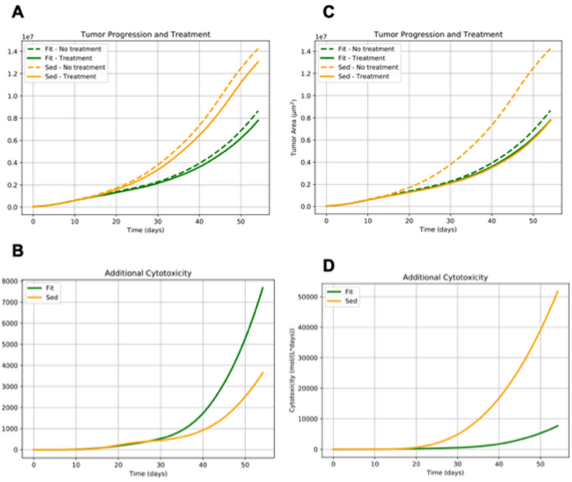

Fig. 4. Arobically fit patients may require smaller dosage of ICI than sedentary patients, which may lead to personalization of treatment and reduction of adverse effects. Without a mitigated dosage, aerobically fit subjects are more prone to adverse effects than their sedentary counterparts (4A, C). Lowering the dosage of ICI for aerobically fit patients can achieve the same reduction of tumor growth relative to their sedentary counterparts but with a lower probability for adverse effects (4B, D)

4 Discussion

We have shown how to generate a time series of TME snapshots during anti-tumor immune response, and how to personalize dosing of ICI for aerobically fit patients in order to lower the risk of adverse effects. In collaboration with cancer biologists and clinicians this platform can be used for improving *in vivo* experimental design and personalization of clinical outcomes.

The hypothesis that underlies the model presented here, connects exercise-induced increased hypoxia-tolerance to more efficient anti-tumor immune response, and requires chronic endurance training (CET) which can be achieved in pre-clinical exercise oncology with forced running wheels [12]. The idea here is that CET induces hypoxia tolerance in the skeletal muscles and in other tissues, and as a result, TMEs are more susceptible to the degradation of HIF1α [13]. This degradation is an upstream factor in a signaling cascade leading to increased anti-tumor immune efficiency, as HIF1α is known to recruit, via cytokine signaling, Trges into the tumor micro-environment, which suppress CTLs [7]. A pre-clinical study detected a twofold decrease in intratumoral Tregs/CTLs ratio in exercised mice relative to their sedentary counterparts [9].

Our platform can perform virtual experiments with no wet-lab or clinical costs, and is proposed here as tool for pre-clinical and clinical researchers. The tool is limited in several ways. First, to obtain simulation results in a reasonable time we must limit the computational cost. Consequently, our grid size is currently bounded by 5×10^{-4} cells. This size allows the simulation to be sensitive to spatiotemporal and stochastic features of the dynamics. Second, specific circumstances may require scaling up to 3D but for

most clinical endpoints, a cross section of the TME may be a good approximation. Third, we introduced only two types of immune cells and three types of fields. From our experience, however, a direct dialogue between model developers and clinicians may help optimize the platform for each specific usage.

Our *in silico* platform is a safe playground for experimentation in dosage scheduling and frequency, as it can easily allow modulation of duration and timing of activation signaling to achieve the most effective treatment. Finally, our platform can easily incorporate and test combination of different types of immunotherapies with other standard-of-care therapies and probe potential synergistic effects. For example, since aerobic exercise promotes oxygenation, it can mimic the effects of antiangiogenic therapy, where different aerobic fitness levels can be calibrated to represent different dosage of such a therapy.

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